Fundamentals of molecular virology 2nd edition pdf free book download

Altered parts are shown in orange. May play a role in the inhibition of host cell DNA replication and in exclusion of superinfecting phage. T7: a model phage for DNA replication, transcription, and RNA processing Bacteriophage T7 is one of seven phages selected by a group of pioneer molecular biologists, headed by Max Delbrück, in the early 1940s to serve as prototypes on which the phage research effort should be focused. The nsP4 protein has a Gly-Asp-Asp motif common to the active site of a number of viral RNA polymerases. It is detected only in the presence of hepatitis B virus (see Chapter 30), and it uses the hepatitis B virus envelope protein (S antigen) to package its RNA genome. It is common to achieve expression levels so high that the desired protein product represents 25% to 50% of the total cell protein. In this family, both plus- and minusstrand viroid RNAs have the ability to self-cleave their RNA multimers (see below). It is not clear if the protein 3a-viral RNA complex contains any replicase components or other host factors. the singly spliced 4-kb class of RNAs, which encode Vif, Vpr, Vpu, or Env 3. It is closely related to the virus that causes rinderpest, a disease of cattle, and it may have jumped from cattle herds to humans. Virions are spherical particles with a star-shaped density corresponding to pentamers of the 2 protein at the fivefold axes and flower-like hexameric rings corresponding to the 3 protein covering the remainder of the surface. Although latently infected cells do not directly contribute to disease, they do represent a significant hurdle to treatment and a cure. (b) Monomeric precursors can interact with each other, weakly and reversibly, until a stable infective seed is formed (shown as a bracketed structure). Its mysteries are only now being unraveled. EBNA-2 and EBNA-LP together are responsible for transcriptional activation of viral LMP genes and a number of cellular genes. During conversion of virions to ISVPs, the attachment protein 1 undergoes a conformational change 1. Antiviral chemotherapy is a comparatively recent science that has progressed rapidly with the discovery of effective agents against HIV, once thought to lead inexorably to disease and death. These viruses infect numerous organisms including bacteria, archaea, fungi, algae, higher plants, invertebrates, and vertebrates. Some time later (in this case, starting at 18–20 hours after infection), new progeny virus begins to appear. • Togavirus (alphavirus) expression vectors have promising potential uses as vaccines or to express useful or toxic proteins. Mast cells: cells that reside in tissues and contain granules loaded with histamine and heparin; they respond to stimulation by degranulation, inducing inflammation. One mechanism of insertional activation of cellular proto-oncogenes is called "promoter insertion". The SARS coronavirus (SARS-CoV) was isolated from people of all age groups who presented with fever and progressive pneumonia, leading to hypoxemia and death. tauri have been sequenced in their entirety: strains OtV-1 and OtV-5 infect algae found in high light conditions, and strain OtV-2 infects algae found in low light conditions. Oncogenes are indicated in orange. The U3 region contains transcriptional enhancers that interact with cellular transcription factors and determine in what cell type and to what extent transcription takes place. How does large T antigen subsequently help to initiate viral DNA replication? This led to the demonstration that PrPSc is encoded by a cellular gene. In contrast, Pospiviroidae require cellular enzymes for all three steps: polymerization, cleavage, and ligation. They share no sequence similarity with cucumber mosaic virus RNAs. No translational products of these satellite RNAs have been found in infected plants, and they contain only short open reading frames, which are most likely without function as they are not conserved. What are the most important factors determining which species and cell types can be infected by a given virus? In contrast, some retroviruses that have lost their envelope glycoprotein genes can form "bald" particles that contain nucleocapsids wrapped in membranes lacking envelope proteins. Most physical maps are now compared with that of AcMNPV (Figure 25.3), whose complete nucleotide sequence (133,894 bp) has been determined. The G protein can be seen extending beyond the envelope, and the coiled nucleocapsid is visible underneath the envelope. Hepatitis A virus is a picornavirus that causes acute liver disease, which is usually self-limiting, but occasional fatalities do occur. 16/05/11 8:17 PM 392 CHAPTER 32 Prions In an alternative proposed mechanism, the PrPSc form is postulated to be inherently more stable than PrPC, but its formation is highly improbable because PrPC folds in a relatively stable conformation upon its synthesis in the cell. Unlike other viral glycoproteins and many cellular membrane proteins, which protrude outward from membranes, the long axis of the E molecule lies parallel to the lipid bilayer and has a convex shape that follows the curved surface of the viral envelope. Invariably fatal. It is also possible that reovirus infects cells in the central nervous system using alternative receptors that have not yet been discovered. Formed from 72 capsomers, pentamers of L1. The following chapter focuses on sentinel cells involved in the innate immune response, and on the deployment of the adaptive immune response. vEGF has amino acid sequence homology to epidermal growth factor (EGF) and transforming growth factor alpha (TGF-␣). An alternative approach is to insert a second promoter to control transgene expression (Figure 37.4c). This domain contains 52 repeats of a seven–amino acid sequence that is rich in serines and threonines, amino acids that can be phosphorylated by specialized protein kinases. Presently, variants of H3N2 and H1N1 viruses continue to circulate in human populations along with influenza type B strains. The B-cell receptor, consisting of surface immuglobulin, associates with membrane proteins that recruit tyrosine kinases such as Lyn and Syk following activation. Yet the study of the mimivirus from a virological perspective got off to a slow start. In the second model, the packaging signal of RNA 3 interacts first with RNA 4, resulting in an initiation complex for co-packaging. Decrease in the surface expression of CD4 and MHC 1. Battista, Louisiana State University Karen Beemon, Johns Hopkins University Martha Brown, University of Toronto Craig E. The following section gives an outline and analysis of the replication pathway starting with the initial infection of a cell and progressing to the release of progeny virions (Figure 1.6). Entry and uncoating 3. Viruses have developed numerous strategies to evade the interferon response Viruses fight back against the host interferon response by making proteins specifically designed to interfere with these host defense systems. These VLPs have been used as vaccines against viruses such as human papillomaviruses, Ebola virus, and influenza viruses. Upon entry into the cell nucleus, parvoviruses must first make a double-stranded version of their genome, using cellular DNA polymerases, because only then can cellular RNA polymerase II recognize and transcribe their genome. This phenomenon, known as silencing, is most likely caused by methylation of DNA sequences located in the LTR, leading to loss of transcriptional activity. The encapsidated RNA is highly condensed, with much of its stem-loop secondary structure retained during assembly. CH012.indd 145 16/05/11 8:03 PM 146 CHAPTER 12 Flaviviruses Introduction of West Nile virus into North America In the summer of 1999, residents of Queens, New York, experienced an outbreak of an encephalitic disease reminiscent of a flavivirus infection. This virus has been very useful for experimental studies, as it grows to high titers in cultured cells and its molecular biology and pathogenesis can be readily studied. 9.9c: Courtesy of Phillipe Lemercier; Fig. Therefore, beginning with one adenovirus DNA molecule, these two pathways together produce two double-stranded progeny molecules. They migrate to the nucleus, where they enter via the 16/05/11 8:08 PM Parvoviruses 241 B 60 T C C A G C G G G C T G C G G G C C C G A T A T G G T C G A C 20 1 C 40 C G G C C T C A G T G A G C G A G C G A G C G C G C A G A G A GG G A G T GG C C A A 3' C C GG A G T C A C T C G C T C G C T C G C G C G T C T C T C C C T C A C C G G T T G A G G T A G T G A T C C C C A A GG A G C 120 145 100 C C 80 A' G G G C A C Figure 20.3 DNA hairpin at the 3' end of the adeno-associated virus genome. (c) (d) Figure 32.7 A mechanism for prion propagation. What three features make T7 and related phages of special interest to biologists? Class III ("late") genes, encoded in the right half of the genome, code for phage structural proteins and proteins needed for DNA maturation and packaging. • Protein 3a binds to viral RNAs and forms a ribonucleoprotein complex that helps transport viral RNAs through plasmodesmata, facilitating movement of viral genomes and spread of the infection throughout the plant. HTLV-1 associated myelopathies (HAM): a disease similar to tropical spastic paraparesis. In both cases, interaction of virus surface components with elements of the complement system triggers a cascade of complement reactions that results ultimately in phagocytosis of the virus particle. Natural killer (NK) cells: lymphocytes that attack and kill abnormal cells, particularly cells infected with pathogens and cancer cells; part of the innate immune response. 24.6: Reprinted from Fig. 1 in Molineux, I.J., "No Springs Please, Ejection of Phage T7 DNA from the Viron is Enzyme Driven," Molecular Microbiology 40 (2001):1–8, with permission of Blackwell Publishing Co. Chapter 9 Fig. Mice deficient for IPS-1 are much more susceptible than wild-type mice to infection with a variety of RNA and DNA viruses, suggesting that this pathway plays a critical role in defending against virus infection. In a comparison of the first three phycodnavirus genomes to be fully sequenced (PBCV-1, EhV86, and EsV-1), a mere 14 genes out of a total of more than 1000 genes were found to be common to all three viruses. Dissociation of the nuclear membrane during mitosis. Second, they demonstrated that mosquitoes are capable of transmitting the virus after obtaining a blood meal from an infected host. The A protein plays a critical role in genome replication and interacts with many host cell proteins. Selectivity of inhibition by acyclovir triphosphate can occur at any of these steps. (b,c) Higher-magnification images of particles undergoing DNA packaging (green arrowheads) at a position opposite the stargate structure, seen clearly in c (red arrowheads). Apoptosis, an important process in both development and differentiation of multicellular organisms, is controlled by signals that monitor cell-cycle progression, metabolism, growth regulation and stress. CH003.indd 40 Genome Genome size (kb) segments and topology Icosahedral Viroids do not code for proteins, but replicate independently of other viruses Viroids (Table 3.7) are small, virus-like RNAs that are similar to satellite nucleic acids, but viroids are able to replicate and cause disease in a variety of plants in the absence of helper viruses. The RNA genome contains six to 10 genes, depending on the virus strain. CII is inactivated by host cell proteases, and has a half-life of only a few minutes. One of the polypeptide products made by a self-replicating RNA, perhaps a mutant of the RNAdependent RNA polymerase or the reverse transcriptase, could then have acquired the ability to act as a primitive 16/05/11 7:52 PM The Evolutionary Origin of Viruses DNA-dependent RNA polymerase. In a new twist, virus vectors have recently been used to combat cancers by expressing proteins that specifically kill tumor cells. Antibody neutralization and hemagglutination inhibition sites have been mapped to both virion glycoproteins Gn and Gc encoded by phleboviruses, suggesting that for these viruses both Gn and Gc are involved in attachment to cells. Viroid RNAs have a high degree of base pairing throughout the molecule. The prophage makes a repressor that turns off expression of the lytic genes. These outlines are designed to serve as study aids that will help students understand and remember common features of the viruses they study. Brome mosaic virus has been used as a model for the study of packaging of segmented viral genomes. Viruses spread between hosts by direct transmission, contaminated food or water, or arthropod vectors. Consider a growing plus-strand RNA being made by polymerase from a minus-strand RNA template. Some type 3 reovirus strains infect the epithelial lining of bile ducts within the liver, leading to ductal obliteration and obstructive jaundice. Microvilli greatly increase the cell surface and play an important role in absorption of substances from the extracellular medium. Lymph nodes are a major site of interaction between antigens and cells of the immune system. The two major E1A mRNAs are shown; introns removed by splicing are shown as inverted V's. Few other viruses have so many overlapping functions within such a compact genome. What role might the chaperone protein and fatty acids play? Because of genome replication, there can be many templates 16/05/11 7:46 PM 16 CHAPTER 1 Introduction to Virology for late messenger RNA synthesis, and these mRNAs may therefore be abundant. During cell division the nuclear envelope disintegrates, allowing the preintegration complexes to gain access to the nucleus when the nuclear envelope reforms. • The capacity of HIV-1 to mutate rapidly and to establish latent infections makes curing this infection extremely difficult. This novel pathogen consists solely of a small, unencapsidated single-stranded RNA, for which the term "viroid" ("virus-like") was proposed. Viral movement proteins (shown as small spheres) can enlarge the diameter of the plasmodesma. Cells expressing the corrected gene are then reintroduced into the body where they can differentiate and proliferate. RNA INTERFERENCE Small interfering RNAs are involved in combating virus infections in plants and invertebrates In mammals, the primary response to virus infection is the production of interferon. This generates a direct repeat of host DNA (4–6 bp depending on the virus) and results in the loss of the terminal 2 bp of viral DNA. This virus is still being tested in clinical trials. This system, outlined in Figure 17.6, involves the transfection of cells with a set of plasmids containing cDNAs of the L, M, and S genome segments flanked by bacteriophage T7 promoters and hepatitis delta virus ribozyme sequences (see Chapter 31). The Reoviridae family presently includes 12 genera of double-stranded RNA viruses that infect a wide variety of animals, from mammals to insects, as well as plants and fungi (Table 19.1). The Vif protein increases virion infectivity by counteracting a cellular deoxycytidine deaminase Vif (viral infectivity factor) is a 193-amino acid protein found in the cytoplasm of infected cells and incorporated at low levels in virions via an interaction with viral genome RNA. The unique vertex with its spike structure is at the top. Intrinsic cellular defenses are rapid responses by infected cells to invading pathogens. Thus a replication fork is born. This organizational scheme has been used in a number of successful virology textbooks, including Salvador Luria's classic 1953 book, General Virology. The two largest viral proteins, 1a and 2a, together constitute the viral RNA replicase that includes the RNA polymerase, a capping enzyme and a helicase, along with host factors. Alternative splicing occurs between two possible 5' splice sites, at nt 411 and 748, and two possible 3' splice sites, at nt 797 and 811 (Figures 21.4 and 21.7). Amyloid: insoluble proteins that accumulate in microscopic fibers and macrocrystalline arrays called plaques, usually resulting from a conformational change in a normal cellular protein. DNA is delivered into the cell through spikes on the virion surface. The other families in the order include viruses of fish and bivalves, respectively. All mechanisms for activating complement result in the proteolytic cleavage of the critical third complement component, C3, into fragments C3a and C3b, and the attachment of C3b to the target cell or pathogen. • All viral structural proteins are made from a 3'-coterminal nested set of subgenomic messenger RNAs. • These subgenomic messenger RNAs are copies of subgenomic RNAs transcribed from genome RNA by an unusual discontinuous transcription mechanism. Live vaccines may also be subject to inactivation by preformed maternal antibodies when administered in early childhood. This is partly due to the lack of expression of any viral genes by these gutless vectors. • Activated IRFs and NFB induce the production and secretion of cytokines, chemokines, and interferons by virus-infected cells. Zevenhoven-Dobbe, Yvonne van der Meer, Abraham J. CH035.indd 432 The recently introduced cold-adapted influenza A virus vaccine is a "second-generation" live attenuated virus that was designed with specific characteristics. CH002.indd 26 16/05/11 7:49 PM Viral Envelopes 27 (Figure 13.1), have virions with essentially perfect icosahedral symmetry. Depending on the virus, this "uncoating" step may occur simultaneously with the first step of entry, or it may only come after a complex series of membrane fusion and transport steps as outlined above. Plant viruses, which are usually injected into host cells by their insect vectors, do not require a springloading mechanism. This divergence is most likely a reflection of the ancient origins of the phycodnaviruses and the highly diverse nature of their hosts. (adj.: chimeric.) Chitin: long-chain polymer of N-acetylglucosamine (a glucose derivative) found in cell walls and exoskeletons. It is not presently known what signals at the ends of genome and antigenome RNA specify transcription. What is the role of the core proteins that some DNA viruses package along with their genomes? Note that all three reading frames are used between nt 811 and 831. Because most of the cellular enzymes required for DNA replication and for RNA synthesis and processing are located in the nucleus, poxviruses must provide virus-coded enzymes to carry (a) (b) Lateral bodies (protein) Core wall (protein) Tube (DNA, protein?) Membrane (lipid, protein) Figure 26.4 Model of the structure of vaccinia virus virions a. High degree of internal base pairing. When an integrated prophage is induced, N-antiterminated mRNA from PL produces Int and Xis, the latter a protein that binds to sites only in the P arm of attR. 354 CH029.indd 354 16/05/11 8:15 PM Human Immunodeficiency Virus Human immunodeficiency virus type 1 (HIV-1) and acquired immunodeficiency syndrome (AIDS) In 1981, the term acquired immunodeficiency syndrome (AIDS) was coined to describe a condition in a group of previously healthy young males within the Los Angeles/ San Francisco area who showed a marked depletion of their immune CD4-positive T lymphocytes, rendering them immune-incompetent. West Nile virus caused a mini-epidemic as it spread from the East coast to the West coast of North America over a five-year period via infected birds. Purple arrowheads: inverted terminal repeated sequences. 16/05/11 8:05 PM 196 CHAPTER 16 Filoviruses VP40 of both Marburg and Ebola viruses binds strongly to membranes via its C-terminal region and forms oligomers when membrane-bound. Non-nucleoside inhibitors: DNA polymerase inhibitors that are not nucleoside analogs. Proteolytic processing yields 10 mature virus proteins (Figure 12.4 and Table 12.2). Prion: pathogen that replicates and causes prion diseases, presumably a conformational variant PrPsc of a normal cell protein, PrPc. The term prion stands for "proteinaceous infectious" particle. DNA gyrase uses energy to catalyze the reverse reaction, resulting in a supercoiled DNA molecule. Neuraminidase was named for its ability to cleave terminal N-acetyl neuraminic acid (sialic acid) from oligosaccharides located on mucoproteins, cell-surface glycoproteins, and glycolipids. 16/05/11 8:21 PM Antiviral Chemotherapy can stabilize the capsid and prevent the conformational change that releases the viral RNA into the cell, thereby preventing uncoating and genome entry. Once integrated, the proviral DNA becomes part of a host cell chromosome and is replicated along with host DNA, just like any cellular U3 AA 1. Endoplasmic reticulum–Golgi intermediate compartment (ERGIC): a membranous cytoplasmic organelle that is continuous with the endoplasmic reticulum and the Golgi apparatus, and is involved in transport, processing, and modification of proteins. Within each table, these lists are divided into sections based on host organism, starting with the simplest (prokaryotes) and ending with the most complex (vertebrates). Cleaves plus-strand viral DNA at a specific site, initiating rolling circle replication. By the time nt 123 is added, all nucleotides needed to build the cloverleaf shown in Figure 5.8a have been incorporated. Center: virions in the extracellular space. This may be related to their mechanism of transcription. The vector transmits the virus to a vertebrate when taking a blood meal. Viruses can be engineered to prevent and cure disease. Some of 3CD is eventually cleaved to form 3C, which, along with 3CD, is able to carry out the remaining cleavages of P2 and P3 to form their constituent mature proteins. Chapter 29 Chapter 34 Fig. Once this packaging sequence is bound to the capsid proteins, the helical structure can form like a zipper around the remainder of the RNA molecule. Ganciclovir: nucleoside analogue used for treatment of human cytomegalovirus infections. Armed with this information, they were able to demonstrate that a series of injections with spinal cord material dried for variable periods of time could protect dogs. Their approach was to study how the proteins and nucleic acids of viruses interact with cellular molecules and organelles, transforming the cell into a factory that can produce many new progeny virus particles. The 40 kb T7 genome is shown with the positions of selected genes immediately above the map. Top left and bottom: filamentous particles being extruded from the cell surface. An alternative is conjugation of the fiber protein with small-molecularweight ligands that will bind to specific cellular receptors. This is a sequence of 118 nucleotides that significantly increases the transduction efficiency of lentivirus vectors in both dividing and nondividing cells. fragment (PrP 27–30) that retains infectivity. Membrane penetration Sialic acid (T3 strains) JAM-A λ2 σ3/μ1 Virion δ/φ ISVP Core β1 Integrin Figure 19.4 Stepwise disassembly of reovirus. • Assembly of flavivirus virions takes place on membranes of the endoplasmic reticulum, and virions mature by cleavage of the premembrane protein as they pass through the trans-Golgi network. Susceptible host cells are strains of Pyrobaculum and Thermoproteus isolated from terrestrial hot springs in the USA, Iceland, and Indonesia. Retroviruses can only infect certain species and types of cells, a property based mainly on the interaction of SU with receptor proteins on the cell surface. In addition, understanding their mechanism of action could identify new targets for the development of drugs that mimic the action of the viral protein. CH004.indd 47 16/05/11 7:52 PM 48 CHAPTER 4 Virus Entry non-productive binding, these viruses carry neuraminidases in their envelopes. Bronchitis: inflammation of the bronchi that results in coughing and excess sputum production. Early endosome: intermediate vesicle to which molecules bound to receptors during receptor-mediated endocytosis are first delivered. In this mode (Figure 11.5a), the eIF-4F complex, consisting of eukaryotic initiation factors eIF-4A, eIF-4E, and eIF-4G, recruits a 40S ribosomal subunit to the capped 16/05/11 8:02 PM Picornaviruses VPg 3' NCR VPg 5' NCR P1 L VP4 VP2 P3 P2 VP3 VP1 2A 2B 129 2C 3A 3C 3D AAAAA(A) n Leader protein 5' Noncoding Region Capsid Proteins • Internal ribosome entry site • Binding site for replicase complex Nonstructural Proteins 3' Noncoding Region • Protein processing • Host shutoff • RNA replication • Binding site for replicase complex Figure 11.4 Picornavirus genome organization. The modern era of vaccine development was foreshadowed by the introduction of the serum-based hepatitis B surface antigen (HBsAg) vaccine in 1981, and was fully inaugurated with the cloning and expression of the HBsAg gene in yeast and mammalian cells and the licensing of a recombinant vaccine in 1986. All viral transcripts have a copy of near their 3⬘ ends, just upstream of the polyadenylation site; however, 1. In contrast to vertebrate cells, infection of mosquito cells by wild-type virus is often not cytopathic. HIV-1 infected cells treated with protease inhibitors continue to make viral proteins, but the Gag polyprotein is not cleaved. This suggests that prion infectivity depends on sequence similarity between donor and recipient prion proteins. The largest open reading frame in the genome encodes the hepatitis B DNA polymerase protein (P, or HBp). 6–8 capsid proteins. The prolonged transgene expression observed after gene transfer in vivo using AAV vectors was first thought to be a consequence of their integration into the host chromosome. This capped RNA fragment, still bound to PB2, is then used as a primer to begin copying the genome RNA. In 1967, Werner and Gertrude Henle, working in Philadelphia, discovered that Epstein–Barr virus is the agent responsible for infectious mononucleosis. Are they two extremes of the same superfamily? • Single-stranded DNA viruses must produce a double-stranded DNA before host cell RNA polymerase can transcribe viral genes. Viruses causing respiratory infections, gastroenteritis, and diarrhea in young children lead to millions of deaths each year in less-developed countries. This facilitates the entry of a second cytotoxic protein, granzyme B, which activates apoptosis, or programmed cell death. In 1983, the complete sequence (39,937 base pairs) of T7 DNA was published along with identification of the major functional landmarks on the genome. Distinct naming conventions and classification schemes have developed in different domains of virology Research on viruses has historically been carried out by scientists who study viruses that infect only one category of organism; for example, bacteria, fungi, algae, plants, insects, fish, domesticated animals, or humans. Examples: flaviviruses, togaviruses, rhabdoviruses, hepadnaviruses. Hemocoel: in arthropods, an open body cavity in which blood flows and bathes the tissues and organs. This is an important advantage if the product of gene X is toxic or even mildly detrimental to the growth of the host bacterium (in which case there is selection against plasmid retention and/or gene X functionality). Upon cell lysis, the influx of divalent cations probably dissociates protein D, yielding the virion. We will mainly focus on unique morphotypes of these viruses, which are not encountered among doublestranded DNA viruses of the other two domains of life, the Bacteria and the Eukarya. The P protein then makes a complete (minus-strand) copy of the remainder of the pregenome RNA by extending the DNA chain to the 5⬘ end of the RNA template. The DNA polymerase and helicase make their way around the circular double-stranded DNA, eventually passing through the site where the A protein originally cleaved the plus-strand (step 3). Uptake of virions occurs in vertebrate cells via receptor-mediated endocytosis. A linear representation of the circular genome of papillomaviruses, based on information from human papillomavirus types 16 and 31. M2 is an essential component of the virus envelope because of its ability to form a highly selective transmembrane ion channel that allows H⩽ ions to penetrate the membrane. It is recommended that influenza vaccine be administered yearly to all individuals with significant chronic respiratory disease as well as all individuals over the age of 65. The surface of the particle is completely covered by proteins, and the underlying lipid bilayer is not visible. Retinoblastoma protein (pRb) controls cell cycle and S-phase gene expression. 2.5b: from VIPERdb2, op. 3–6 nonstructural proteins. A number of newly emerging human diseases are caused by viruses. Severe diarrhea is often misleading, since it is also found in many bacterial enteric infections. Molecules can move between the connected cells, but the size of the molecules is restricted. This makes sense: there should be no selective pressure to mutate the p53 gene in these cancers, since the p53 protein is eliminated by the activity of the viral E6 protein. Fibers make contact with cellular receptor proteins to initiate infection Adenoviruses attach to specific cellular receptors via the knob of the fiber protein. Cytotoxic T cells are generated upon interaction of Tc cells with MCH I-bound peptides EFFECTS OF INTERFERONS ON THE IMMUNE RESPONSE Cell-mediated immune reactions are carried out by T lymphocytes and target host cells infected by intracellular bacteria and viruses. Many of these evolved spontaneously in cells infected with the phage or in amplification reactions carried out in vitro. A different assembly model proposes that budding progresses through "shell interactions" of the glycoproteins. Histone acetyl transferases: enzymes that acetylate nucleosomal histones and other proteins, reducing their binding affinity for DNA and resulting in a more relaxed chromatin structure that is more accessible to specific transcription factors and is more easily transcribed. Signaling pathways promote changes in cell architecture and induce macropinocytosis and caveolar/lipid raft-dependent endocytosis, enabling crucial steps in the entry of certain viruses (see below). The Rep78/68 proteins of adeno-associated virus also have antiproliferative effects on cells. Unlike myocarditis caused by several other viruses, reovirus-induced myocarditis is not mediated by immune responses. By contrast, the protein coat of the filamentous bacteriophage fd forms a sleeve that surrounds the circular, single-stranded DNA, without a specific interaction between each subunit and a particular number of nucleotides in the DNA. The bridge between the membranes is thus established, and further structural changes induce bilayer contact and fusion. They can therefore spread within the cell genome, but do not code for proteins that allow them to escape the cell. 1879 Galtier succeeds in passaging rabies from rabbit to rabbit. How is the terminal nucleotide replaced? Pleconaril: a capsid-binding drug active against rhinoviruses and other picornaviruses. Nicked circular DNA or linear DNA will also bind more of the fluorescent dye ethidium bromide than covalently closed circular DNA. SARS coronavirus may have originated from related bat coronaviruses Since SARS had not been observed in humans before the 2002–2003 pandemic, the virus causing this disease was thought to be either an avirulent human coronavirus that mutated to become more virulent, or a virus newly introduced into the human population. HIV-1 was probably transmitted to humans from chimpanzees infected with SIVcpz Many species of African monkeys and apes are hosts for specific strains of simian immunodeficiency virus (SIV), closely related to HIV. A noncoding region of ~400 nt, bounded by the divergent promoters for early and late transcription (PE and PL), contains sequences that control transcription and DNA replication. Virions are shown as cross-sections, revealing the capsids or nucleic acid genomes within. • Large T antigen also binds to the replication origin on viral DNA, recruiting a set of DNA replication proteins that result in replication of the viral genome. These include the production of virus or noninfectious virus-like particles in tissue culture cells. Until recently this was difficult to accomplish because, unlike positivestrand RNA viruses, expression of the RNA genome of a negative-strand virus from a transfected plasmid will not by itself generate virions. Infect humans (reoviruses types 1–3, rotaviruses, and Colorado tick fever virus), mammals, birds, fish, mollusks, plants, insects, and fungi. Required for virion formation. 1.2b: Courtesy of John Finch, Cambridge University; Fig. Affected animals develop behavioral changes, tremor (especially of head and neck), and uncoordinated movements that progress to prostration and death without associated fever. BASIC CONCEPTS OF VIRUS STRUCTURE Virus particles are molecular structures that package virus genomes in infected cells and transmit them to new host cells. With far-reaching implications for the function of the global ecosystem, these understudied giants of the virus world have only recently begun to reveal their tantalizing secrets. Thus, like RNA polymerases of many positive- and negative-stranded RNA viruses, the L proteins are by far the largest viral proteins made by bunyaviruses. Battisti, Valorie D. KEY TERMS Bronchitis Class I fusion proteins Class II fusion proteins Coiled-coil Discontinuous transcription Encephalitis Endoplasmic reticulum– Golgi intermediate compartment (ERGIC) Gastroenteritis Hepatitis Hypoxemia Lectin Metalloproteinases Nested set Papain-like cysteine proteinases Peritonitis Picornavirus 3C proteinases Polyprotein Pseudoknot Replication complexes Replicative intermediate Reservoir Reverse genetics Sialic acid Subgenomic mRNA Syncytium Tropism 16/05/11 8:04 PM Coronaviruses 171 genome 1. Proteolytic cleavage is the most common way in which an assembled virion is "primed" for the delivery program. Table 28.1 Retrovirus genera Genus Examples of virus Host Alpharetrovirus Rous sarcoma virus Chickens Betaretrovirus Mouse mammary tumor virus Mice Gammaretrovirus Murine leukemia virus Mice Deltaretrovirus Human T-cell leukemia virus type 1 Humans Epsilonretrovirus Walleye dermal sarcoma virus Fish Lentivirus Human immunodeficiency virus type 1 Humans Simian immunodeficiency virus Monkeys Feline immunodeficiency virus Cats Simian foamy virus Monkeys Spumavirus Transmembrane protein (TM) Surface protein (SU) Matrix protein (MA) tRNA Capsid protein (CA) Nucleocapsid protein (NC) Genome RNA (2 copies) Reverse transcriptase (RT) Protease (PR) Integrase (IN) Figure 28.1 A typical retrovirus virion. There is a significant depletion of CD4-positive T cells in this lymphoid tissue during acute infection, which is not restored even after resolution of the initial viremia. The other three DNAs contain the coding sequences of the three vesicular stomatitis virus proteins (P, L, and N) required for viral genome replication, each cloned downstream from a T7 RNA polymerase promoter. Map units divide the viral genome into 100 equal divisions of approximately 50 nt. Finally, there are agents that inhibit uncoating. DNA must become double-stranded before being transcribed. The next step probably involves formation of "replicase particles" engaged in minus-strand synthesis. Chapter 35 discusses the history and the technology of vaccine development, and outlines the advantages and problems of different kinds of viral vaccines: live attenuated viruses, killed whole viruses, subunit vaccines, DNA vaccines, and recombinant vaccines. Chapter 4 describes how viruses that infect eukaryotic organisms bind to and enter host cells. Mortality was highest (up to 44%) in individuals over 65 years of age. (b) Structure of influenza virus particle, showing HA and M2 proteins in envelope, matrix protein, and viral ribonucleoprotein (RNP). Furthermore, by changing the binding sequence between the ribozyme and its substrate, it is possible to create a ribozyme with a new substrate specificity. Therefore these virus-like particles are replication-deficient and not pathogenic, but can be used to study a variety of aspects of virus replication in cultured cells under lower biocontainment levels (BSL-2). Assembly 7. NF-B complexes activated by reovirus are comprised of NF-B subunits p50 and p65. At the right are shown possible origins of different kinds of viruses. 27–32 kb. Some viroids, as well as hepatitis delta virus RNA and the small, circular single-stranded RNA satellites, have the ability to self-cleave and self-ligate. The nucleocapsids are subsequently transported into the nucleus via nuclear localization signals on the NP and polymerase proteins. CH017.indd 204 The S RNAs of phleboviruses (Figure 17.2) and tospoviruses employ an ambisense coding strategy. The human papillomavirus (HPV) L1 protein is produced using recombinant technologies and is expressed in either yeast or transfected insect cells, where it also spontaneously forms virus-like particles. 14.1 in Siegl, G. • A number of nucleoside analogues, beginning with azidothymidine, inhibit HIV reverse transcriptase by premature termination of growing DNA chains. Interferon-stimulated genes: a collection of genes that encode antiviral and anti-proliferative properties and are induced in cells treated with interferon. In addition to its proteinase activity, nsp3 is likely involved in the formation of replication complexes, in viral RNA synthesis, and in suppression of interferon activity. Perforin: a cytolytic protein produced by cytotoxic T lymphocytes and natural killer cells. Association with the receptors can occur sequentially because the binding sites for the coreceptor are exposed only after interaction of the virion with the primary receptor. It can always be replaced by a new transcript. (c) Joining of growing DNA strands. Among the most important were the discovery and intensive study of DNA tumor viruses (polyomaviruses, papillomaviruses, and adenoviruses; see Chapters 21–23) and RNA tumor viruses (retroviruses; see Chapters 28–29). Herpesviruses are important human pathogens Human herpesviruses are best known as the cause of discomfort from recurring "cold sores", lesions of the mucosa of the mouth and lips. Thus the 5' end of the new minus-strand starts with a G residue. Each virus was named (using the Latin binomial) according to the insect from which it was isolated, leading to the description of hundreds of different baculoviruses isolated from as many species of insects (see Table 25.1). Can be formed by cell-cell fusion. Flavivirus E protein directs both binding to receptors and membrane fusion The E protein is a type I transmembrane protein of approximately 500 amino acids, anchored in the membrane via two C-terminal hydrophobic regions. STIV-1 has elaborate turret-like structures at the fivefold vertices (Figure 9.14a). Protein 3a has been shown to bind to protein 2a in a yeast two-hybrid system. Among these are nine distinct herpesviruses that infect humans (Table 24.1). Most genes (~80%) are of unknown function. CH002.indd 20 16/05/11 7:49 PM Capsids with Icosahedral Symmetry The rigorous definition of symmetry involves spatial operations such as rotations, that when applied to an object give a result that appears identical to the original object. The association of CH012.indd 144 NS2B with NS3 provides enhanced cleavage site specificity to the proteinase. molecule of protein H and five each of proteins F, G, and B. Over 100 types of human papillomaviruses have been identified (Table 22.1). N acts by a mechanism analogous to that of the Tat protein of human immunodeficiency virus (see Chapter 29). SEC-II.indd 58 12/05/11 5:02 PM C H A P T E R 5 Single-Stranded RNA Bacteriophages Jan van Duin Leviviridae From Latin levis (light), referring to small size of phage VIRION Naked icosahedral capsid made from 180 copies of coat protein (T 3). • Expression cassettes used in DNA virus vectors are designed for maximal levels of expression by use of effective transcriptional enhancers, splice sites, and polyadenylation signals. Noncoding leader and trailer sequences comparable to those found in paramyxoviruses and rhabdoviruses are found at the 3' and 5' ends of filovirus genomes. T7 DNA polymerase can then extend the new primer toward the left, setting up a second replication fork and establishing bidirectional replication. They can reduce the duration of fever by one or two days, but only if administered within 48 hours after the appearance of the first symptoms of infection. Fivefold axes Scaffolding proteins have a flexible structure Several lines of evidence suggest that scaffolding proteins have structures that allow a high degree of adaptability. GENES AND PROTEINS Two transcriptional promoters and two polyadenylation signals (early and late) on the same DNA strand. At left, a diagram showing structural elements. • Tat stimulates elongation by RNA polymerase II molecules that have initiated transcription of HIV-1 proviral DNA. These enzymes may reside in a small pocket between the spike and the internal membrane. Plants respond to virus infection by RNA silencing, and cucumber mosaic virus protein 2b suppresses silencing RNA silencing (see Chapter 33) is a gene inactivation mechanism mediated by small interfering RNAs (siRNAs). Gene gun Accelerates gold particles carrying plasmid DNA to high velocities for penetration of skin to the desired depth, targeting dermal layers or muscle. Garrett, "Viruses of the Archaea: a unifying view," Nature Reviews Microbiology 4 (2006), 837-848; Fig. DISEASES Smallpox: high fatality rate, now eradicated. The cleavage pathways vary among picornaviruses in different genera. For human T-cell leukemia virus and members of the lentivirus subfamily (Chapter 29), viral RNA can undergo multiple splicing events to generate mRNAs encoding regulatory proteins. Table 7.2 Selected class II gene products Gene CH007.indd 80 Function 1.3 DNA ligase, joins lagging-strand DNAs 2 Inhibits E. However, antibodies still have a major role in protecting against viral infections. Synthesis of minus-strand strong-stop DNA. Sequential expression of "immediate-early", "early", and "late" genes. Reovirus plus-strand RNAs are assembled into particles called early replication complexes, which contain only one copy of each of the 10 different transcripts. Arrest occurs at the transition between the G2 stage of the cell cycle and mitosis. The discovery of reverse transcriptase independently in the laboratories of Harold Temin and David Baltimore in 1971 demonstrated that retroviruses 342 CH028.indd 342 16/05/11 8:15 PM Retroviruses integrate a DNA copy of their RNA genome into the chromosomes of infected cells. REVIEW QUESTIONS 1. AAV DNA can integrate at random sites into the cell chromosome. The envelope forms around the coiled nucleocapsid during budding from the plasma membrane, giving rise to the bullet shape. Rhinoviruses release their RNA by a similar mechanism, but it is induced by low pH within endosomes. Other viruses have sequence elements adjacent to the polyadenylation signal near the 5' end that repress recognition of that AAUAAA. Poxviruses replicate in the cytoplasm Unlike other eukaryotic DNA-containing viruses covered in this book, poxviruses replicate exclusively in the cytoplasm of infected cells. However, instead of an attP site and Int protein, N15 has a telRL site and a telomerase enzyme. One of the two recently licensed vaccines against oncogenic human papillomaviruses contains VLPs produced in insect cells infected by baculoviruses expressing human papillomavirus L1 capsid proteins. Paul Ahlquist's laboratory showed that translation, replication, and encapsidation of brome mosaic virus RNAs can be carried out in yeast transfected with viral genome RNAs. This system facilitated detailed genetic and molecular studies of the mechanism of viral RNA replication. Cultured cells also facilitated production of numerous antiviral vaccines (see Chapter 35), starting with the vaccines against poliovirus that were developed in the 1950s. As it is copied into DNA, most of the genome RNA is digested by RNAse H. THE ADAPTIVE IMMUNE RESPONSE Antigens are macromolecules that give rise to specific immune responses that lead to production of: • • antibodies cytotoxic T lymphocytes B and T lymphocytes arise in the bone marrow and thymus (primary lymphoid organs). cos: cohesive end site; sib: site encoding a transcription termination sequence, or if antiterminated, a site for RNAse III attack; attP : phage attachment site for integration; ori: origin of DNA replication. Lambda DNA replication is directed by the early genes O and P, but what is the role of host cell proteins? This ability to integrate has stimulated interest in using these viruses as vectors for the transfer of genes into human cells. The E3L gene product binds to and sequesters dsRNA, interrupting both the PKR and the parallel 2', 5'-oligoadenylate synthetase antiviral pathways. Transport and release of viral DNA into the nucleus appears to be a two-step process. Class I fusion proteins: envelope glycoproteins that form long alpha-helical coiled-coils, which undergo major conformational changes during fusion to bring the virus envelope and the target cell membrane in close proximity. Upon entering cells, nucleoside analogues must first be activated by phosphorylation so that they mimic the natural substrates of DNA polymerases, deoxynucleoside 5'triphosphates. As a result, public health authorities have great interest in maintaining high levels of vaccine coverage to protect the small number of individuals who are missed by the program, who have failed to respond to vaccination, who respond initially to vaccination but subsequently lose protection, who have a valid contraindication to vaccination, or who simply refuse vaccination. A cell is coinfected with two closely related bunyavirus strains (parent 1 and parent 2). ORF 0 Ebola virus uses RNA editing to make two glycoproteins from the same gene In addition to the envelope glycoprotein, all Ebola viruses (but not Marburg virus) produce a second protein from the GP gene. • Virus replication begins in the nucleus using host RNA polymerase II, which produces viral mRNAs and a full-length pre-genome RNA template from circularized viral DNA. Note that our Milky Way galaxy measures approximately 100,000 light years edge to edge, and the furthest visible galaxies in the universe are approximately 10 billion (10 109) light years distant. Although the resulting complex is drawn as a linear molecule in Figure 28.4, it could remain circular. Genomes of satellite viruses encode their own capsid proteins, while genomes of satellite nucleic acids either contain no coding regions or encode only non-capsid proteins; their genomes are encapsidated by structural proteins of the helper virus. The large number of identical protein subunits will not form a tight and enclosed package if they are bound to each other by random contacts. What type of RNA polymerase is NS5 and why does it contain a methyltransferase? (a) Negative contrast electron micrographs of virions. Its design began with one of the natural substrates of HIV-1 protease (Leu165-Asn-Phe-Pro-Ile), a region of the Gag-Pol polyprotein that is cleaved to release reverse transcriptase. The virions consist of a tubelike superhelix formed by the DNA and a single basic, DNA-binding protein, which for the Sulfolobus islandicus rod-shaped virus 1 (SIRV-1) has a mass of 14.4 kDa. At both ends of the virions are plugs about 50 nm long into which three tail fibers are anchored. Reprinted with permission Fig. Rossmann; Fig. Conserved nucleotide sequences Open reading frames: 1095–3867 nucleotides 5' GCUA 5' nontranslated region 12–32 nt UCAUC 3' 3' nontranslated region 32–83 nt Figure 19.3 General characteristics of a mammalian reovirus gene segment. As diploids, E. A large number of plant viruses have postive-stranded RNA genomes. On the other hand, sheep homozygous for a PrP gene encoding substitution of arginine for glutamine at codon 171 (R171Q) are entirely resistant to scrapie. Usually expressed as PFU/ml. One member of this subfamily, caspase-1, can be part of a large protein complex, known as the "inflammasome." Caspase-1 is rapidly activated following recognition of viral RNA or double-stranded DNA by the inflammasome components AIM2 and NLRP3 (Figure 33.5). These three relatively mild but often annoying human diseases are caused, respectively, by herpes simplex virus types 1 and 2, and by varicella-zoster virus. Proteins J, K, I, H, M, and L assemble into the coneshaped initiator, and then hexameric disks of V, the major tail subunit, stack on the initiator. Refers to a disease or pathogen that is present and circulates among hosts in a specific location, but does not necessarily infect a large segment of the population. Adenovirus produces high levels of VA I RNA, which is a small (160 nt) RNA transcribed from the adenovirus genome by RNA polymerase III. This has been likened to an iris that can change its diameter by contracting or expanding. When a plaque assay is used to measure the infectious titer of a virus suspension, the results are usually expressed as plaque-forming units (PFU) per mL of suspension. Budding: the process of forming an enveloped virus particle by the folding of a cellular membrane around the virus capsid as it is extruded through the membrane. promoter elements of the right-hand LTR, which generates self-inactivating vectors as described above. In the future, the system will be exploited for vaccine design by making attenuating mutations in the genome, either by mutating or deleting virulence genes or by mutating noncoding control sequences. Most latently infected individuals continually produce small amounts of virus throughout their lifetime. CH015.indd 176 Louis Pasteur developed an effective rabies vaccine in the mid-nineteenth century (see Chapter 35), and a modern, safer version is used to immunize veterinarians and other people at risk. Early work by the groups of Wolfgang Joklik and Angus Graham showed that the isolated viral core contains all of the enzymes required to synthesize messenger RNA molecules from the double-stranded genome templates. Self-cleavage occurs by nucleophilic attack by the 2'-hydroxyl of the nucleotide at the cleavage site, generating a 2', 3'-cyclic phosphate at one side of the cleavage site, and a 5'-hydroxyl terminus at the other side (Figure 31.3d). Cellular targets include a number of genes that are important for host immune responses and cell survival. NK cells also express several dominant inhibitory receptors that bind CH034.indd 418 Inhibitory receptor MHC cell survives NK cell Normal cell; surface MHC Activating receptor NK cell Virus-infected cell: no surface MHC Apoptosis, cell dies Figure 34.2 Cell killing by natural killer (NK) cells. (d) Human parainfluenza virus (a paramyxovirus; in this image, the helical nucleocapsid is revealed by penetration of stain inside the particle). P proteins serve as chaperones that help to assemble N proteins onto the growing helical nucleocapsid. M is a highly conserved, triple membrane-spanning 16/05/11 8:04 PM 164 CHAPTER 14 Coronaviruses protein containing three hydrophobic domains separated by short hydrophilic regions. In initial studies, proteins derived from influenza virus, herpes virus, and human immunodeficiency virus were expressed. Strand transfer. Promoter/Regulatory sequences TIF SP1 SP1 SP1 ICP4 SP1 TIF SP1 TATAA ICP4 300 1 Second distal TK(UL23) β (E) First distal 105 80 61 47 CCATT, SP1 SP1 SP1 UL19 γ1 (E/L) 48 TATA 30 27 TTTAAA Inr UL38 γ2 (L) 29 24 1 Proximal 29 12 TATA 1 Inr 1 DAS 20 33 Figure 24.6 Structure of promoter regions of different classes of herpes simplex virus genes. Tobacco mosaic virus movement protein also interacts with many other host factors, including the cell wall protein pectin methylesterase, several transcriptional factors, and cytoskeleton proteins. Emiliania huxleyi virus-86 (EhV-86): 472 genes. xxv flast.indd xxv 13/05/11 2:23 PM flast.indd xxvi 13/05/11 2:23 PM flast.indd xxvii 13/05/11 2:23 PM flast.indd xxviii 13/05/11 2:23 PM SECTION I INTRODUCTION TO VIROLOGY C H A P T E R 1 Introduction to Virology C H A P T E R 2 Virus Structure and Assembly C H A P T E R 3 Virus Classification: The World of Viruses C H A P T E R 4 Virus Entry Virus particles, or virions, consist of an RNA or DNA genome packaged within a protein coat, and in some cases a lipid envelope. During virus infection, these responses are mediated primarily by cytotoxic CD8⩽ T cells but also include CD4⩽ T cell responses. If there is not sufficient N protein to initiate encapsidation within the newly formed leader or trailer RNAs, the RNA polymerase terminates some 40 to 60 nt downstream from its start site and releases a short 16/05/11 8:05 PM 186 CHAPTER 15 Paramyxoviruses and Rhabdoviruses RNA molecule. These new genome RNAs can either be translated, replicated, or packaged with new virion structural proteins. Peritonitis: inflammation of the peritoneum (the lining surrounding the abdominal cavity). Instead of producing cytokines, cells respond to entry of enveloped viruses directly by inducing a small subset of interferon-stimulated genes, most of which encode intracellular proteins that block virus transcription and translation. The L proteins contain motifs characteristic of all RNA polymerases, and can both transcribe viral genome RNA, making viral messenger RNAs, and replicate the genome, making full-length genome copies. Papillomavirus genomes are circular, double-stranded DNA Papillomavirus virions are small, non-enveloped particles with T 7 icosahedral symmetry (Figure 22.1). DNA is packaged as a "minichromosome" with cellular nucleosomal histones. However, RNAs that have between one and five additional nucleotides will not properly bind to the last N protein subunit, leaving some RNA sequences at the 3' end unpackaged. A related virus emerged in 1976, when two epidemics occurred simultaneously in Zaire (now Democratic Republic of Congo) and Sudan. Mx proteins inhibit the activity of the viral RNA polymerase, thus blocking viral mRNA production. Adeno-associated virus makes use of an additional promoter (p19) within the left open reading frame to generate mRNAs encoding shorter versions of its replication proteins. The initial cleavages of the nascent polyprotein are carried out by the uncleaved 3C polypeptide in the intact polyprotein molecule; that is, 3C can properly fold and act as a proteinase while part of the polyprotein. NK cells constitute a minority population of circulating large, granulated lymphocytes. 17.4: Courtesy of Esa Kuismanen, bcreidt.indd 486 Chapter 19 Fig. Genome replication begins when free NP protein enters the nucleus. Following cleavage of P1, five protomers, each containing one molecule of VP0, VP1, and VP3, self-assemble to form a 14S pentamer structure (Figure 11.8). The adenovirus genome map showing major early (E) and late (L) transcription units is shown at top. In the early 1960s, Peter Gomatos and Igor Tamm noticed that inclusion bodies in reovirus-infected cells fluoresce greenish-yellow when stained with acridine DISEASES Members of genus Orthoreovirus cause little or no disease in humans. In addition, the carboxy-terminal domain (CTD) of RNA polymerase II is phosphorylated by a reaction mediated by cdc9, ICP22, and both US2 and UL13 protein kinases. Transmission of infection in this way is obviously limited to cells located next to each other; spread of infection between organisms cannot occur by this mechanism unless there is exchange of cells. Since then, various species of bats from around the world have been examined, resulting in the discovery of a greater number of coronaviruses than had previously been anticipated. The microalga may respond to coccolithovirus attack by switching from its usual diploid life stage (where it is susceptible to infection) to a haploid cell, changing its physical appearance and making it impenetrable ("invisible") to the coccolithovirus. Plasmodesmata are membrane-lined channels with defined size exclusion limits that interconnect adjacent plant cells (Figure 10.7). Four introductory chapters in Section I cover the history of virology and the virus life cycle, virus structure, virus classification, and the entry of viruses into animal cells. Antibodies acquired from the mother near the end of pregnancy are critically important for the protection of infants between birth and 6 to 9 months of age. Considering the dynamics of virus assembly, some inherent flexibility is probably required. Filoviruses cause hemorrhagic fever Infections with Marburg and Ebola viruses cause severe viral hemorrhagic fever. Nonspecific phagocytosis and cell-mediated cytotoxicity during an inflammatory response are important resistance mechanisms. Rhabdoviruses: • • • Humans: rabies virus Cattle: vesicular stomatitis virus Numerous rhabdoviruses infecting bats, fish, insects, plants DISEASES Paramyxoviruses: several important childhood diseases, including measles, mumps, respiratory diseases in humans; canine distemper, rinderpest. Model of poliovirus virion determined by x-ray crystallography. Top: viral RNA polymerase initiates transcription at the 3' end of genome RNA and progresses (below) to one of six transcription regulatory sequences (TRS: 5'-UCUAAC-3' for murine hepatitis virus), where it pauses and then interacts with the TRS at the 3' end of the leader sequence. This choice is dictated partly by the precise sequence elements in the end/start region. The incubation period for viral infection averages 2 to 3 months but can be as long as 6 months, depending upon the level of virus exposure. For example, vaccine-associated paralytic polio due to reversion of Sabin polio vaccine-strain virus to neurovirulence is understood in terms of the mutations required to maintain attenuation (see above). • The herpesvirus capsid is surrounded by an amorphous region called "tegument" that contains numerous viral proteins that function upon entry of the virion into the cell. However, the contribution of aerosol transmission to the course of human outbreaks seems to be low. Crescent formation is directed by viral proteins that are synthesized on endoplasmic reticulum, which may be present within factories. 11.2: Reproduced from virusworld/ictv8/p1m-human-poliovirus-1-ictv8.jpg, courtesy of Jean-Yves Sgro, University of Wisconsin; Fig. The ability of the AAV genome to form concatemers by recombination across the inverted terminal repeats has been employed to increase its capacity for carrying foreign DNA. Some of the most widespread and dangerous human diseases are caused by negative-strand RNA viruses; among these are influenza, measles, rabies encephalitis, and Ebolavirus hemorrhagic fever. When the template is genome RNA, the RNA polymerase can then produce a set of viral mRNAs by the transcription mechanism described above; these mRNAs do not interact with N protein. • A virus-coded RNA polymerase packaged in the virion synthesizes viral mRNAs by transcribing the RNA in the nucleocapsid after it enters the cell. Bacteriophage lambda (Chapter 8) is a temperate virus that can integrate its DNA into the host cell and remain latent until the conditions are right to make new virus particles. Shown here are schematized diagrams of electrophoretic profiles of prototype strains type 1 Lang (T1L) and type 3 Dearing (T3D) along with some reassortant progeny. The E6-E6AP complex then binds to p53, stimulating the ubiquitination of p53 and its subsequent degradation in proteasomes. Shortly afterward, Maurice Hilleman and colleagues identified the same agent as the cause of a respiratory epidemic in army recruits in the United States. Protein 2a may also be involved in virus movement Table 10.1 Proteins encoded by cucumber mosaic virus Viral protein Translated from M. Primary and secondary organs of the immune system harbor B and T lymphocytes The cells responsible for producing the immune response are lymphocytes found in blood, lymph nodes, spleen, bone marrow, and thymus (Figure 34.3). USA 105 (2008), 8008-8013, with permission from PNAS, Copyright (2008) National Academy of Sciences, U.S.A. Chapter 10 Fig. How virions are released without killing the archaeal cell remains unclear. Comparison of properties of viruses of archaea, bacteria, and eukaryotes will help to reconstruct scenarios of virus evolution and will contribute to deeper understanding of the mechanisms of virus–host interactions. The biological effects of p53 are largely due to sequence-specific transcriptional activation of target genes whose products mediate cell-cycle arrest or apoptosis (Figure 22.6). A single-stranded DNA binding protein (ssDBP) is produced from the E2A region, while both a viral DNA polymerase and an 80-kDa preterminal protein (pTP) are encoded by E2B (Figure 23.4). However, translation cannot yet start because ribosome binding needs the start codon and sequences downstream of the initiator codon, up to about nts 140–145. (c) Influenza virions in endosomes undergo fusion with the endosomal membrane upon a drop in pH induced by an endosomal proton pump. Furthermore, apoptosis is blocked in reovirus-infected cells deficient in the expression of the p50 or p65 NF-B subunits. Interferons. Inflammatory response: induction of inflammation, mediated by cytokines and white blood cells; this response functions to remove injurious stimuli and to initiate the healing process. In contrast, a lot of information exists on the biogeochemical impact on the marine ecosystem by members of the genus Phaeocystis. Capsids with larger T numbers require additional "glue" proteins to stabilize the interactions between subunits. Although virions are not transported through plasmodesmata, they are found in sieve elements. This structure is so named because it resembles the head of a hammer. Apoptosis induced by a mutant lacking the gene encoding ICP4 is blocked by a protein kinase encoded by the US3 gene. As described above, such an unfortunate event happened in some children who have undergone retrovirus gene therapy to cure severe immunodeficiencies. APOBEC3G is incorporated into virions during assembly, and can induce deamination of multiple deoxycytidine residues in the DNA product of reverse transcription made during a subsequent infection. RESPONSE OF THE CELL TO VIRUS INFECTION Virus-mediated signal transduction leads to activation of cellular transcription factors When cellular detector proteins recognize viral components, they initiate signal transduction pathways that activate a variety of transcription factors, which in turn activate transcription of many cellular response genes (Figure 33.3). Rhabdoviruses: bullet-shaped rods, 180 75 nm. Expression of the DNA-binding protein EBNA-1 permits the replication of circular DNA containing the latent origin of DNA replication (oriP) in dividing cells, thus maintaining the DNA in daughter cells. Synthesis and capping of viral mRNAs occurs within these capsids, and the mRNAs are extruded through channels in the "turrets" that lie at the axes of fivefold symmetry (see Chapter 19). Many different molecules located in the plasma membrane can serve as attachment factors and receptors for viruses (Figure 4.2). The RNA helicase, retinoic acid-inducible gene I (RIG-I), and a related protein, melanoma differentiationassociated gene 5 (MDA5), play important roles in intrinsic antiviral responses induced by double-stranded RNA. 27.13, 14a-c: Images kindly provided courtesy of Avi Minsky, reprinted from Figures 3A, 6A, B, C in Zauberman, N. This book is also a valuable, up-to-date source of information for graduate students, postdoctoral fellows and research scientists working with viruses. Under these conditions, B lymphocytes undergo rapid cell division, and the B-lymphocyte population expands. These include stressinduced natural killer (NK) cell ligand (MICB), which is important for recognition and killing by NK cells; interferon-inducible T-cell attracting chemokine (CXCL-11/I-TAC), which regulates the host interferon response; and p53 up-regulated modulator of apoptosis (PUMA), which is a pro-apoptotic protein. nsp2–3 Intermediate product, timing of exponential phase growth in culture. Acheson Filoviridae From Latin filum (filament or thread), referring to shape of virion VIRION Filamentous enveloped particles. These oncogenes are 16/05/11 7:46 PM Detection and Titration of Viruses Virus vectors can replace defective genes, serve as vaccines, and combat cancer Advances in molecular cloning have allowed the construction of numerous virus vectors (see Chapter 37). One of the consequences of having a tubular nucleocapsid is the potential for packaging DNA longer than the viral genome. Although reoviruses are mostly nonpathogenic in humans, these viruses have served as very productive experimental models for studies of viral pathogenesis. Most RNA- and DNAcontaining viruses induce interferon synthesis; however, double-stranded RNA viruses, such as reovirus and blue tongue virus, are the best inducers. Generally speaking, such a delay can be caused only by a "kinetic trap". Th2 cells synthesize interleukins-4, 5, 6, and 10, and thereby facilitate antibody production by B cells (Figure 34.6). After release of these complexes in the cytoplasm by fusion with the endosome membrane, they interact with the importins, and are rapidly transported to and through the nuclear pore complexes (Figure 4.5b). A distinct reovirus strain was isolated from a mouse in the Cameroon; this virus may represent a fourth mammalian reovirus serotype. VIRUSES AND HOSTS Human immunodeficiency virus types 1 and 2 (HIV-1, HIV-2). Mutation, recombination, reassortment, and genetic bottlenecks are involved in the evolution of cucumber mosaic virus Cucumber mosaic virus has been very successful in adapting to new hosts and new environments. huxleyi V-86 (EhV-86) is the largest algal virus genome sequenced to date

(Figure 27.9), but a virus that infects Pyramimonas orientalis, a marine microalga, has an even larger genome, estimated at 560 kb. Thus herpes simplex viruses contain at least 84 different genes. Below the genome map are ribbon diagrams for each of the flavivirus proteins whose atomic structures have been determined. human papillomavirus type 16 genome contains six early genes (E1, E2, E4, E5, E6, E7) and two late genes (L1, L2). Chapter 32 Chapter 36 Fig. nsP1 protein contains a methyltransferase domain and functions as a capping enzyme, adding a methylated GTP cap to the 5' end of positive-strand viral RNAs (for details on enzymes involved in RNA capping, see Chapters 19 and 26). Triangulation number: T = h2 + hk + k2, where h and k are integers; the number of protein subunits on the surface of viral capsids with icosahedral symmetry is 60 x T. Tail formation starts with J, the attachment fiber. Thus, the stem-loop structure at the beginning of the NP gene inhibits viral RNA polymerase from initiating mRNA synthesis, and VP30 activates transcription by reversing this inhibition. Dura mater: the tough outer membrane covering the brain and spinal cord (from Latin words for "hard" and "mother"). These changes have been studied in most detail for the trimeric influenza virus HA (Figure 4.4). The cap-binding and endonuclease activities of the influenza PB2 and PB1 proteins lead to the degradation of many cellular pre-mRNAs in virus-infected cells. Thus virus-infected cells that display viral peptides on their surface in a complex with MHC type I proteins will be killed when they encounter cytotoxic T lymphocytes that recognize those peptides. However, with more widespread use it became apparent that this vaccine induced serious autoimmune demyelination of the central nervous system in as many as four per thousand vaccinees. Finally Z is added and the tail is complete. Chromatin remodeling: alteration of nucleosome structure and/or position, thus changing their binding affinities to DNA and the access of chromatin to transcription factors. Table 11.1 Selected members of the picornavirus family Genus Members Characteristics Enterovirus Poliovirus Coxsackie virus (A, B) Replicate in gastrointestinal tract; can cause meningitis, encephalitis, rashes, cardiac and muscular disease Rhinovirus Human rhinovirus Over 100 serotypes; cause common cold; sensitive to low pH Cardiovirus Encephalomyocarditis virus Theiler's murine encephalomyelitis virus Infect mice and other mammals; cause cardiac or neurologic disease Aphthovirus Foot-and-mouth disease virus Epidemics in domestic ruminants; sensitive to low pH Hepatovirus Hepatitis A virus Causes acute hepatitis in humans Parechovirus Human parechovirus (Ljungan virus Cause gastroenteritis and respiratory diseases Note: There are presently three additional picornavirus genera that are not listed here. LMP-2A binds to the cellular tyrosine kinases Lyn and Syk, as does an activated B-cell receptor (Figure 24.12b). One of the major late transcripts, T2, codes for the major capsid protein, VP1. PENETRATION THROUGH CELLULAR MEMBRANES Membrane penetration is mediated by conformational changes of virion proteins. A major outbreak of Sudan ebolavirus disease occurred in Gulu, northern Uganda, in 2000/2001 with 425 cases (53% case fatality rate). Often budding takes place at the (external) plasma membrane, but some viruses use the membranes of the nucleus, the endoplasmic reticulum, the intermediate compartment, endosomes, or the Golgi complex. Plants use RNA-silencing mechanisms (see Chapter 11 and Figure 33.11) to protect themselves against virus infection. Viral structural proteins encapsidate the newly replicated genomes to form progeny virus particles. Interleukin-1: a cytokine, secreted early in the immune response, that activates both T and B lymphocytes. DISTINCTIVE CHARACTERISTICS Proviral DNA can enter nucleus without requirement for cell division. Plants infected with one viroid will not allow replication and pathogenesis by a related viroid. May involve risks because foreign genes are expressed in the body. Somatic mutation of these regions during immune responses further increases the specificity and binding affinity of the antibodies. Thus HIV-1 has the ability to evolve rapidly to overcome barriers to its replication, be they an immune response or drug treatment. In fact, RNA phages share the same habitat and the same inactivation characteristics in sewage treatment processes as numerous pathogenic enteroviruses (polioviruses, hepatitis A virus, etc). An empty virus capsid is left attached to the cell wall (Figure 27.5b). Treatment: pegylated interferon, entecavir, telbivudine, tenofovir, adefovir, lamivudine. When the basal cells become committed to the pathway toward terminally differentiated epithelial cells (keratinocytes), there is a burst of viral DNA synthesis, known as "vegetative replication". Dependoviruses: adeno-associated viruses (human, bovine, canine). To view an online dynamic model of vaccinia virus virions, see www.vacciniamodel.com. How do adeno-associated virus (AAV) vectors circumvent this problem? Finally, I would like to thank my wife, Françoise, for enduring the seemingly endless task of writing, editing, and correcting the text and figures for this book, through two editions. Note that refolding and priming can happen at either end, or at both ends simultaneously, in which case two single-stranded genomes are formed. Nucleocapsid: viral capsid containing the viral nucleic acid genome. Genome RNAs with a CCA 3'-terminus are substrates for tyrosine-tRNA synthetase, which covalently links tyrosine residue to the 3'-terminal A residue. Table 11.2 Cell receptors and coreceptors used by some picornaviruses CH011.indd 127 Virus Receptor or coreceptor Characteristics Poliovirus Poliovirus receptor (Pvr or CD155) Immunoglobulin superfamily Coxsackievirus Decay accelerating factor (CD55) Vitronectin receptor (_v 3) Coxsackievirus-adenovirus receptor (CAR) Intercellular adhesion molecule-1 (ICAM-1) Integrin _v 6 Complement cascade Integrin Immunoglobulin superfamily Immunoglobulin superfamily Integrin Human rhinoviruses ICAM-1 Low-density lipoprotein receptor Immunoglobulin superfamily Foot-and-mouth disease virus Heparan sulfate Vitronectin receptor (_v 3) Glycosaminoglycan Integrin Encephalomyocarditis virus Vascular cell adhesion molecule-1 (VCAM-1) Sialylated glycophorin A Immunoglobulin superfamily Carbohydrate 16/05/11 8:02 PM 128 CHAPTER 11 Picornaviruses Genome RNA VPg VP1 N-terminii NN VP4 Capsid Poliovirus receptor VP1 VP1 VP1 VP1 VP4 Cell membrane Cytoplasm N N VP1 N-terminii VPg Figure 11.3 Model for entry of poliovirus RNA into the cytoplasm. The nascent RNA therefore serves as a primer, allowing the RNA polymerase to extend the nascent negative-strand RNA through the leader sequence up to the 5' end of the genome. See also Chapter 11. CH033.indd 413 The RNA interference machinery is also present in vertebrates, including mammals, but instead of destroying viral RNAs, it generally regulates gene expression via either degradation of mRNA or inhibition of mRNA translation. These four families have been grouped into the order Mononegavirales (for "single segment, negative-strand RNA viruses"). Spherical nucleocapsid (25–30 nm diameter) with icosahedral symmetry (T = 3). Pestiviruses and hepaciviruses have an internal ribosome entry site (IRES) for initiation of protein synthesis. These proteins are involved in regulating cell polarity and cell proliferation control, targeting of which may be involved in the later stages of malignant progression. Envelope (E) Virion structural protein; envelope formation and stability. Concatemeric DNAs accumulate in cells infected with T7 phage mutated in gene 3 (endonuclease) or genes 18 or 19 (DNA maturation). Replicating genomes and antigenomes are assembled into nucleocapsids during elongation of RNA chains. During synthesis and transport to the plasma membrane via the endoplasmic reticulum and the Golgi membranes, a 22-amino acid N-terminal signal peptide and 24 C-terminal amino acids are cleaved from the precursor 253-amino acid polypeptide. explained by the existence of a large library of variable region exons within the genes that code for both the heavy chain and light chains. Hemorrhagic fever with renal syndrome: a disease caused by various hantaviruses involving internal bleeding and kidney pathology, which can be mild or severe with a high fatality rate. When monocytes move from the blood into tissues, they differentiate into macrophages or dendritic cells. A volcanic crater in the Campo Flegrei near Naples is named Solfatara, and is the original source of the archaeon, Sulfolobus solfataricus. Induction is the switch from lysogeny to lytic growth. T7 allows in vitro evolution in a reasonable time frame and yields results that can be analyzed at both the molecular and biological level. Ribosomes translating this mRNA make a protein that contains the same first 191 amino acids as small T antigen, but they continue in a different reading frame (–1) downstream of the splice junction. Recently X has been shown to associate with mitochondria and to inhibit membrane potential, causing the generation of reactive oxygen species that can lead to mutations in the liver. VIRUSES AND HOSTS Nine genera, including Enterovirus (poliovirus, Coxsackieviruses), Rhinovirus (common cold viruses), Cardiovirus, Hepatovirus (hepatitis A virus). Numerous insect and plant diseases. No signs of a host immune response. (a, b) Top and side views, respectively, of PrP 27–30 modeled with the N-terminal portion of the protein as a left-handed -helix (ribbon arrows displayed in a triangular barrel). GENES AND PROTEINS Codes for up to 50 proteins. Virus replication begins with at least partial disintegration of the virus particle, and release (uncoating) of the viral genome within the cell. Below are examples of helical capsids: tobacco mosaic virus, 12 300 nm; and pseudomonas phage Pf1, 7 2000 nm. Larger and more complex capsids are made from more than one kind of protein subunit. Quasi-equivalent: in virus capsid structure, describes the symmetrical distribution of protein subunits on the surface of a capsid in such a way that they form similar but not identical bonds with each other. Aaron Shatkin and colleagues were the first to describe the methylated cap structure that adorns most eukaryotic messenger RNA molecules and is required for normal translation; this work was done with RNAs synthesized in vitro from reovirus cores. Picornavirus proteins 2B, 2C, and 3A participate in the induction of these vesicles. Enzymes in the viral core synthesize and cap messenger RNAs The core particles released into the cytoplasm contain all of the machinery needed by the virus to make and cap messenger RNAs. These enzymes are activated on removal of the outer capsid, and ribonucleoside triphosphates required for RNA synthesis diffuse into the core from the cytoplasm. Virions are constructed from 72 capsomeres, each containing 5 molecules of the major capsid protein L1 as well as one molecule of the minor capsid protein L2. The concentration of these molecules is usually too small to be detected by conventional techniques. Sulfolobus spindle-shaped virus 1 (SSV-1) genomes have the property of being positively supercoiled when isolated from virions, and were one of the first examples of such DNA found in nature. EBNA mRNAs are generated by alternative splicing and 3' processing of long transcripts that initiate at promoters Cp or Wp. LMP-1 and LMP-2A genes are transcribed from a bidirectional promoter. Although and genes are spread throughout the genome of herpes simplex virus, it is notable that all six genes are localized within or near the boundaries of the inverted repeat sequences b and c (Figure 24.5). Novel ribozymes with catalytic properties different from those of the naturally occurring molecules have been made by designing oligonucleotides synthesized in vitro or by 3' 5' 3' G C G C G C U A C G C G C G U A G G C G C A C G C U A C U A þ G C G C G C CUC U G UG C GC G A U U S G C C G 5' GC G CU 3' 1 G G C U G G G 5' mutagenesis of existing ribozymes. Both of these DNA sensors contain pyrin domains, allowing for interaction with the pyrin domain of the adaptor molecule, apoptosis-associated speck-like protein (ASC). All of these viruses have capsids with icosahedral symmetry, in contrast to the helical nucleocapsids of viruses with negative-strand RNA genomes. Total genome length 11–19 kb. To replicate in resting cells, the virus must target the growth regulatory pathways described above. Assembly of virions and egress at the plasma membrane Two models have been proposed to explain virus assembly and release from the plasma membrane. Enhancement of virus infectivity. Release Zanamivir (Influenza A & B) Figure 36.1 Inhibitors useful at different stages in the virus replication cycle. Viral proteins are named with the Greek letters (lambda), (mu), and (sigma), corresponding to the size classes (L, M, and S) of their respective gene segments. As we have seen, productive infection of permissive cells by polyomaviruses leads to cell death and release of progeny virions. Coating the inner surface of the membrane is the viral matrix protein (MA). N protein is a transcriptional antiterminator that, with a recruited CH008.indd 87 A recently discovered class of genetic elements found in roughly one-half of sequenced bacterial genomes provides resistance to phage infection. The misreading process is stimulated by a particular secondary structure called a pseudoknot, located just beyond the termination codon in the mRNA. ssRNA Early proteins DNA-dependent RNA polymerase 4. A key insight was provided by the crystal structure of HIV-1 protease. This DNA is called proviral DNA. Besides "autoregulating" synthesis of their own proteins, viral miRNAs target cellular mRNAs that encode proteins involved in apoptosis or host antiviral immune responses. GPI interacts with cellular receptors and is exposed to the extracellular medium. Whether we define them as "living" or "nonliving" or as an "infectious enzyme," we do know the following about them: (1) they have arisen in organisms during evolution; (2) they are able to propagate themselves and the diseases they cause; and (3) they appear to be able to evolve and to adapt themselves to different hosts. What is the purpose of the cellular tRNA incorporated into every retrovirus particle? Cutting the second cos sequence requires recognition of cosQ, a site located just upstream of the nicking site cosN, and this completes packaging of a chromosome. The classification of viruses into species and into a hierarchy of related groups of species (genera, families, and orders) helps virologists to compare viruses and to analyze and understand many aspects of virus structure, replication pathways, interactions with host cells, and disease transmission. This could reflect inefficient infection of the host, as with some viruses. Prions 387 SECTION VI: LARGER DNA VIRUSES OF EUKARYOTES 23. No naturally occurring satellite RNA has been found associated with tomato aspermy virus, but this cucumovirus does support replication of cucumber mosaic virus satellite RNAs. Cucumber mosaic virus satellite RNAs are from 332 to 405 nucleotides in length. The additional ethidium bromide in these samples renders them less dense than complexes with supercoiled DNA, and the two forms can be separated when centrifuged in cesium chloride density gradients. Another approach involves the use of the human papillomavirus L2 protein, which may provide protection against a wider range of virus types. Originally (and incorrectly) postulated to be a cause of the common cold, adenoviruses were subsequently shown to be responsible for a number of other respiratory syndromes including pneumonia; they can also cause eye and gastrointestinal infections. This approach also will allow rational design of stably attenuated viruses as possible vaccine candidates. These two proteins have functions similar to those of the cellular proteins DNA polymerase and PCNA (see Chapter 21). Very likely, host cytoskeleton components are involved. Watson, Nancy H. In the absence of Vpu, virions accumulate on the cell surface in a partially budded state. • Some coronaviruses also appear to have an additional spherical capsid. b. Chapter 5 Fig. Although antibodies can be effective in destroying some invading viruses, a cellular response mediated by cytotoxic T cells is usually required. The protein subunits that make up the capsids of many simple viruses exhibit this property. Mammalian double-stranded RNA-dependent protein kinase (PKR) is activated by potato spindle tuber viroid, as its secondary structure resembles that of double-stranded RNA. For example, EhV-86 contains 21 of the core set of 40 to 50 conserved virus genes found in NCLDVs; these genes encode some of the principal features of virion structure, genome replication and expression. (b) Three-dimensional image reconstruction of virions derived from cryoelectron microscopy analysis. An inactivated vaccine protective against hepatitis A virus infection is available. The maturation cleavage of prM, carried out by furin (thick black arrow), releases the C-terminal fragment as mature M protein. There are multiple alternative ways to produce a large number of mRNAs (not shown), many of which contain multiple open reading frames. • Parvovirus replication depends on the cell entering S phase. 16/05/11 8:01 PM Cucumber Mosaic Virus A U A C A AG ACG CGC GGAGAGGC GGC U U ||| ||| |||| ||| || A UGU GUG CCUC UCG UCG U AA U G U A A Figure 10.11 The stem-loop structure of the D satellite RNA. The first known communitybased outbreak of Marburg virus in Africa occurred in 1998–2000 in the eastern part of the Democratic Republic of Congo, close to the border with Uganda. Therefore, multiple doses are usually required to achieve protection, and immunity often fades with time, requiring booster doses. bursaria. This property has led to speculation that viruses that infect Chrysochromulina only require a low host density for propagation and that these viruses may even prevent bloom formation. This discovery shook the scientific world, because it placed viruses at the edge between living organisms and simple chemical compounds like sodium chloride. RNA polymerases can make errors in incorporation of nucleotides into genome RNAs during replication, and do not have the proofreading mechanisms possessed by many DNA polymerases. Some engineered vaccinia viruses as well as viruses such as myxoma virus, a rabbit poxvirus, have been found to replicate preferentially in human tumor cells and are effective in selectively killing these tumors in animal models. These considerations led to the concept of multiplicity of infection (m.o.i.). Cellular messenger RNAs are synthesized in exactly the same way, and therefore all cells contain such enzymes. Clonal selection theory: a model of how B and T lymphocytes recognize and respond to antigens to protect the host from infections. Other picornaviruses inhibit cap-dependent translation by binding and sequestration of eIF-4E, the protein subunit of the eIF-4F complex that binds directly to the 5' cap structure of eukaryotic mRNAs. Infection of cultured mammalian cells by most picornaviruses causes a shutoff of host cell RNA synthesis. See also individual entry large T antigen (LTAg), 254–257 middle T antigen (MTAg), 254 small T antigen, 254–255 T lymphocytes, 395, 416, 417, 419, 420, 464 cytotoxic, 296, 298, 356, 399, 416, 417, 420, 422 in coronavirus infections, 163 in herpesvirus infections, 286, 296, 298, 299 in HIV infection, 355, 356, 357, 362 in measles virus infection, 179 interferon production, 406, 411 Tank-binding kinase 1 (TBK-1), 402–403 Target-based high throughput screen, 445 Tat protein, 357, 360, 361 Tat-responsive element (TAR), 360 TATA-binding protein (TBP), 241–242, 253, 256, 257, 278 Taxonomy, 31 T-cell receptors, 360, 417, 420, 421, 422 Tegument proteins, 288, 294, 303, 304 in cellular mRNA degradation, 289–290, 291 in viral gene expression activation, 289–290, 296 Telomerase, 93, 242 Temperate viruses, 85, 99, 335, Terminal protein (adenovirus), 276, 280, 281 Therapeutic index (TI), 448 Thermophiles, 98 Thermoproteus tenax spherical virus 1 (TTSV-1), 104 Thermoproteus tenax virus 1 (TTV-1), 102–103 Threefold rotation axes, 21 Thrips, 201 Thymidine kinase, 449 Thymidylate kinase, 451 499 Tick-borne encephalitis virus, 50, 138, 140, 430 Tight junctions, 228 Tobacco mosaic virus (TMV), 119–120 Togaviruses, 148–157 alphaviruses, 149 entry by low pH-induced fusion, 150 expression vectors, 155–157 discovery, 148 genome RNA, 151 nonstructural proteins, 151 pathogenesis, 155 polyprotein, 151 replication, 151–153 representative togaviruses, 149 rubella virus, 149 structural proteins, 152 subgenomic mRNA, 153 transcription, 153 transmission, 148 viral polymerase, 151 virion assembly, 154–155 virion structure, 148–150 Toll-like receptors (TLRs), 399–401, 402, 437, 438 Tonoplast, 117 Topoisomerase, 249, 250, 257, 259, 292, 318, 330, 335, 338 Toroidal coil, 249 Transcriptional enhancer, 252, 259, 260, 277, 281, 351, 367, 368, 407, 459, 463, 464 Transfer construct, 462, 464 Transmembrane anchor, 27 Transmissible mink encephalopathy (TME), 389 Transovarial transmission, 201 Triangulation number, 20, 21 Tripartite leader (adenovirus mRNAs), 281 Tropism, 13, 170, 204, 234, 241, 286, 463, 464, 468 Tumor necrosis factor (TNF), 289, 322, 363, 370, 418 Tumor suppressor protein, 267, 370 Tumorigenesis studies, polyomaviruses as model for, 248 2', 5'-Oligo(A) synthetase, 231, 322, 410 Twofold rotation axes, 21 Type 1 T helper lymphocytes (Th-1 cells), 420, 437 Type 2 T helper lymphocytes (Th-2 cells), 420, 436 Type I integral membrane protein, 27, 178 Type I transmembrane protein, 50, 204, 214 Type II integral membrane protein, 27, 178 Type II transmembrane protein, 220 Ubiquitin, 307, 328 Ubiquitin ligase, 196, 279–280, 282, 291, 295, 299 Ubiquitination, 196, 268, 362 Ubiquitin-mediated degradation, 267, 279 Uncoating, 3, 13, 14, 29, 53, 54, 204, 240–241, 252, 289, 317, 338, 446–448 Upstream complementary sequence (UCS), 68 Uukuniemi virus, 50, 207 Vaccine-associated neurological disease, 313 Vector development, recent progress in, 468 12/05/11 4:08 PM 500 Subject Index Venezuelan equine encephalitis virus, 149 Vesicle transport, 46, 154 Veterinary diseases by coronaviruses, 160 Viral capsids, size variation of, 24 Viral epidermal growth factor (vEGF), 423 Viral evolution factor (VIF), 361 Viral interference, 405 Viral RNA recognition, cellular proteins in, 401 Virion host shutoff (Vhs) protein, 290, 295 Virion protein R (Vpr), 362 Virion protein unique to HIV-1 (Vpu), 362 Virions with icosahedral symmetry, 21 Viroceptors, 423 Virogenic stroma, 306 Viroids, 40, 378–385 ancient RNA world and, 41–41, 384–385 asymmetric replication, 380 bindsub.indd 500 classification of, 379 cleavage, 381 discovery, 379 disease, 382 enzymatic activities needed for replication, 380–382 hepatitis delta virus (HDV), 383–384. 5a from Juan Ramón Rodriguez, Cristina Risco, José L. The majority of these genes are of unknown function and the direction of transfer is yet to be determined, although to date the vast majority 05/05/11 11:24 AM Pyornaviruses and Raphidoviruses of horizontal gene transfers between the phycodnaviruses and their hosts are believed to have gone in the host-to-virus direction. Brain pathology is spongiform encephalopathy—large vacuoles in cortex and cerebellum give brain a sponge-like appearance. (a) Electron micrograph of negatively stained murine hepatitis virions shows spikes protruding from the envelope, providing its crown-like appearance. Some viral surface protein molecules can be denatured and therefore unable to bind to the cell receptor. The icosahedral capsid is enclosed in an envelope along with tegument proteins The herpes simplex virus genome is wrapped in a bilayered capsid constructed from six proteins, of which the major one, VP5 (150 kDa), makes up the 162 capsomers (Figure 24.3). Unfortunately, the numbering schemes used for genes and proteins, based on their relative migration rates in polyacrylamide gels, do not perfectly correspond. The circular form of the genome template would allow for multiple rounds of transcription. Interferons are most effective in reducing viral spread during the initial stages of virus infection, before most symptoms are detected, but are less effective if administered late during an acute infection. Growing RNA chains are shown as blue lines trailing the RNA polymerases, and completed mRNAs are labeled. 7. A third variation on budding is carried out by some retroviruses, such as HIV-1. 16/05/11 7:52 PM 34 CHAPTER 3 Virus Classification: The World of Viruses Understanding these and other conclusions that are derived from our knowledge of virus classification will be an interesting future challenge for virologists. Clathrin: a protein involved in the generation of endocytic vesicles; lines the cytoplasmic side of coated pits. • Most flaviviruses (but not hepatitis C virus) are transmitted by mosquitoes or ticks. There is a 60S ribosomal subunit joins to form an 80S complex, and protein synthesis begins. Above: a map of the integrated proviral DNA framed by two long terminal repeats, and the reading frames for the virion proteins. Leukopenia and enteritis in dogs, cats, mink. Where there were known index cases for Marburg virus outbreaks located, and how does the location of additional outbreaks differ from those for Ebola virus? This may seem plausible when the activity of coccolithoviruses is taken into consideration. In contrast, the only viruses known to infect algae are doublestranded DNA viruses (Phycodnaviridae), and these are some of the largest known viruses (many of their genomes are 300 kb or larger). At some point, these RNAs and their associated polypeptides may have become localized and concentrated within lipid droplets. In fact, in a topological sense, the capsid never leaves the cytoplasmic compartment. Progeny virions can be detected as early as 5 minutes after infection. Only LINEs encode reverse transcriptase. Ubiquitin: a 76-amino acid polypeptide that is covalently joined to lysine residues on proteins, which are then degraded in proteasomes. The N-terminal amino acid of VP2 is linked to myristate, a C14 saturated fatty acid. Consequently, spread of the infection within an animal can be achieved either by infection of new cells with progeny virus or by multiplication of cells already containing proviral DNA. However, the conservation of the reading frames for these proteins in most clinical isolates of the virus suggests that they play important roles during infection within the host. So far, there is no evidence that sGP is involved in activation of macrophages or endothelial cells, which are thought to lead to capillary leakage and coagulation defects. These viral proteins either sequester the double-stranded RNA and do not allow it to come in contact with PKR, so that activation cannot occur. (P1 Precursor Polypeptide) VP0 VP3 VP1 Proteolytic cleavage by 3C/3CD proteinase 5S Protomer (VP0, VP3, VP1) 160S Virion (VP2, VP4, VP3, VP1)60 VP2 VP4 VP0 () Progeny RNA r ny oge P () 14S Pentamer (VP0, VP3, VP1)5 A RN 150S Provirion (VP0, VP3, VP1)60 80S Procapsid (VP0, VP3, VP1)60 Figure 11.8 Assembly of the picornavirus virion. These drugs inhibit virus release from infected cells. When expressed in cells infected by wild-type virus, these minigenomes can replicate, because they contain the essential binding sequences for the viral RNA polymerase at both ends. The viral RNA polymerase contains four virus-coded subunits. The term was generalized by Louis Pasteur in 1881, in honor of Jenner, to include artificial immunization against any disease. Herpesviruses are known for their ability to remain latent in the host organism until an external trigger induces lytic growth, and the mechanisms of latency are being intensively studied. The three other proteins in the complex are cellular proteins: the S1 protein of the small ribosomal subunit, and two translational elongation factors called EF-Tu and EF-Ts (Table 5.1). Vpr can also arrest infected cells in the G2 stage of the cell cycle. Virions at different stages of assembly can be seen (arrows). Polyomaviruses are models for studying DNA virus replication and tumorigenesis Because they can transform cells in vitro and cause tumors in experimental animals, both mouse polyomavirus and simian virus 40 have been studied intensively as models for understanding tumorigenesis. Vectors are propagated in cell lines that express the deleted viral genes and can therefore provide the essential replication and packaging components normally provided by the intact virus. Some laboratories have been working on generating an edible hepatitis B virus vaccine by expressing HBs proteins in potatoes, carrots, and bananas. Parvoviruses 238 Peter Beard, Swiss Institute for Experimental Cancer Research 21. Binding of these glycoproteins to TLR-2 or TLR-4 begins a signaling cascade that eventually leads to production of cytokines, including interferons, which help to inhibit virus replication and spread. C. The cis-acting replication element. T-cell lymphoma: a T-cell malignancy in which the cancer cells are localized mostly in lymphoid tissues rather than in the blood. Although many such institutions have programs to promote immunization, there has been no attempt to legislate or mandate annual influenza vaccination for healthcare workers. Some filamentous virions, not shown here in their entirety, are 1000 nm or more in length. Conjugates A variety of molecules, such as cytokines, monoclonal antibodies, and lectins can be conjugated to antigens to enhance immune reactions. Splicing removes either a small or a large intron, starting at the same 5' splice site but cutting at two possible 3' splice sites. See also Orthomyxoviruses 1918 H1N1 pandemic influenza A virus, 221 2009 H1N1 pandemic influenza A virus strain, 222–223 alternative splicing of proteins, 216–217 antigenic drift, 207, 220, 224 antigenic shift, 207, 220, 221, 222 avian influenza A H5N1 strains, 221–222 Asian flu, 221, 221 export of nucleocapsids from nucleus, 218 genome replication, 217–218 genome RNAs and proteins, 213 helical nucleocapsid, 215 hemagglutinin protein (HA), 212, 213, 214 Hong Kong flu, 211, 221 NS1 protein interferes with polyadenylation of cellular mRNAs, 218–219 NS1 protein suppresses host cell antiviral response, 219 nucleocapsids enter nucleus, 215 PB1-F2 in host immune response suppression, 219 respiratory tract infections, 211 secondary bacterial infections, 211 serious acute disease in humans by 210–211 Spanish flu, 210 spherical and filamentous influenza virus A particles, 212 'stuttering' transcription, 216 transport between nucleus and cytoplasm, 215, 216 vaccination against, 223 viral envelope protein assembly, 219 viral mRNA production by cap-stealing, 217 viral mRNAs undergo splicing in nucleus, 216–217 virions of, 213 Innate immune responses, 398, 399, 403, 415–425 cells involved in, 417 complement proteins, 416–417 inflammatory response mediated by cytokines and migrating leukocytes, 417–418 macrophages localized in tissues, 418 mediated by circulating specialized cell types, 416 viruses evading, 424 Intasome, 91 Integrase, 101, 102, 106, 343, 347, 452 Integrins, 47, 48, 51, 52, 127, 193, 204, 229, 240, 265, 276 495 Interferons, 403, 405–412 antiviral activities induced by, 409–411 in animal drug vs. kingdom, although hepatitis A virus, a viroid-like RNA, replicates in humans. Sexual transmission has been suspected for Marburg and Ebola viruses, and neonatal transmission was reported for the 1976 outbreak in the Democratic Republic of Congo. B and T lymphocytes circulate in the blood and populate the peripheral or secondary lymphoid organs (spleen, lymph nodes, gut), where the immune responses subsequently occur. Patients are dehydrated, apathetic, and disoriented, and they may develop a characteristic stare and expressionless face with peeling skin by day 5 or 7 of the illness. These viruses have been targets for vaccine development and use, and have served as models for the study of coronavirus biology. • Neuraminidase inhibitors suppress release of influenza virions from sialic acid residues on the surface of infected cells. The external domain (above) is responsible for binding to cell receptors and for mediating fusion between the viral envelope and cell membranes. Section IX includes the two new chapters on host responses to virus infection, with important new information on detection of virus infection, intrinsic fpref.indd xx cellular responses to virus infection, and innate and adaptive immune responses. CH015.indd 178 Genomes of paramyxoviruses contain from 15 to 18 kb of single-stranded, negative-sense RNA. NLRP3 is one of over 20 human NLR proteins. Hemagglutinin, neuraminidase, M2 ion channel, and matrix protein are associated with lipid bilayer membrane. • The paramyxovirus measles causes a highly contagious disease that is often fatal in children in developing countries. prM 164 Binds to E glycoprotein, inhibits membrane fusion during transit through Golgi. The recombinant virus or plasmid is inoculated into the host, where the viral protein is expressed and induces an immune response. (a) gp120 on the virion binds to CD4 on the cell surface. Right: expanded view of the first 16 N subunits, showing (in white) nucleotide sequences required for initiation of replication and transcription. Interestingly, mutant coronaviruses lacking E are viable, indicating that this protein is not required for virion formation. This description of virus groups is not meant to be exhaustive; the periodical reports on virus taxonomy published by the International Committee on Virus Taxonomy (ICTV) are the definitive source on known viruses and their classification. Viruses of vertebrates and invertebrates are more evenly represented in all genome categories; they are currently grouped into 28 distinct families and only 3 unassigned genera. Independent experiments examining transcription in vitro with disrupted virions showed that mRNAs closest to the 3' end of the genome appear first, and mRNAs closest to the 5' end appear last, a result also consistent with the single-entry model. Recent work has determined that Vpu induces degradation of tetherin, a host cell protein located on the plasma membrane that is believed to interact with budding virions and promote their endocytosis. Just in case you lost track, the cap guanylyltransferase participates in mRNA capping, early mRNA transcription termination, and intermediate gene transcription initiation; and the cap ribose-methyltransferase participates in mRNA capping and polyadenylation, and in postreplicative gene transcription elongation or termination. Result is a mixed viral/cellular mRNA that expresses the proto-oncogene under control of viral promoter/enhancer. Green shaded regions indicate self-cleaving motifs on the genome RNA (nt 688–771) and antigenome RNA (nt 819–902); arrowheads indicate the cleavage sites. The chance observation of such plaques played an important role in the discovery of bacteriophages. This is sufficient to carry cDNAs from 16/05/11 8:22 PM 468 CHAPTER 37 Eukaryotic Virus Vectors Retargeting virus vectors. Viral proteins and peptides derived from them are recognized by receptors on B and T lymphocytes, activating those cells to differentiate into plasma cells, which produce antibodies, or into cytotoxic T cells, which kill virus-infected cells. The host cell rep protein acts together with the viral A protein as a helicase to unwind the plus-strand from its complementary minus-strand (step 2). (a) A typical prion rod or fibril (black bar 100 m). An interesting mechanism called retroregulation limits Int expression when the late promoter of lambda is active. Nucleocapsids are seen in the virus factories, and are being enveloped via the budding of membranes of the endoplasmic reticulum into the lumen. Therefore, the normal coding sequences of the nonstructural proteins are reconstructed and translated to produce, in yeast, positive-strand genome and subgenomic RNAs are made. • Study of cancer-inducing viruses led to the isolation of numerous cellular oncogenes and to the understanding that cancer is caused by their unregulated expression. Simultaneously, Emile Roux and colleagues in Pasteur's group had been able to "fix" rabies virus (i.e., establish consistent biological properties) by serial passage in rabbit brains. Because bacteriophages are small and simple and can be propagated easily in bacterial cultures, they would be a fertile terrain for scientific discovery. N-antiterminated transcription from PL leads to expression of both Xis (for "excision") and Int (for "integration"), which in turn sponsor excision of the prophage DNA from the bacterial chromosome, and the lytic transcription pattern ensues. A region thought to be responsible for membrane fusion is shown. David Baltimore first recognized that all viruses can be divided into six (now seven) groups based on the pathways leading to mRNA and protein synthesis; this has come to be known as the Baltimore classification system (see Figure 1.7). 16/05/11 8:06 PM Bunyaviruses 5' Cap 205 3' mRNA 3' 5' 5' Cap Transcription 3' 5' Cap 5' Genome RNA 3' 5' 5' Replication 3' 5' 5' 3' Antigenome RNA Transcription 3' mRNA Cap 5' Figure 17.3 Transcription and replication of genome segments. 16/05/11 7:46 PM Why Study Viruses? The nucleocapsids (not shown in the figure) are now exposed, and can move from the vacuole into the cytoplasm. Structure: It is a screening method, not a viral diagnostic test. However, the details of the molecular mechanisms of pathogenesis are still unknown. phagotrophs, which then become the scavenging debris and detritus from their environment. DISTINCTIVE CHARACTERISTICS Polyomaviruses are dependent on cellular enzymes for DNA synthesis and processing, and DNA replication. CH028.indd 345 6. These highly reactive oxygen species can oxidize envelope lipids and cause strand breaks in viral nucleic acids. By limiting expression of highly immunogenic viral proteins, the virus is able to avoid immune surveillance and therefore can survive within these latently infected cells. The sequence of preS1 varies among different strains and it is likely involved in host cell attachment. Thanks also to Joan Longo and Mei Lee of the Department office for their help. This is perhaps a reflection of the low importance with which they have been regarded in the past. • Coccolithoviruses can affect the weather by causing the demise of coccolithophore blooms and triggering the release of climate-active gases into the atmosphere. Once activated, these transcription factors induce the production of cytokines and interferons, which are then secreted and act on neighboring cells. The envelope surrounds the nucleocapsid or core, which is composed of 180 molecules of hepatitis B core (C) protein arranged with T = 3 or T = 4 icosahedral symmetry. This barrier is overcome by inducing the ribosome to shift its reading frame at a precise position within the capsid. It should be noted that some viruses lack a capsid. Since the receptor binding site is located in the virion, virions are delivered into early endosomes, as are other substances taken up by clathrin-coated vesicles. However, large T antigen is known to increase transcription of many other genes, probably via its interactions with the machinery that assembles RNA polymerase II at promoters. Int produced from CII-activated Pint inserts the chromosome into the bacterial chromosome. These viruses can be thought of as pure messenger RNA molecules packaged in a protein coat. Efficient recruitment of RNAs into the replication complex requires a host protein, LSM1, which functions in host mRNA turnover. The mechanisms controlling genome packaging are unknown; as with other viruses that have segmented genomes, the question of how viruses package at least one of each genome segment into the virion is not resolved. This gives rise to five families of late mRNAs, named L1 through L5. Man in center with paralysis of right leg, perhaps from poliomyelitis. In the absence of viral genomes, these viruses can package segments of histone-associated, non-viral DNA to form "pseudovirions." Also affect domestic or wild animals and cause economically important diseases: swine fever, bovine viral diarrhea. 16/05/11 7:49 PM C H A P T E R 3 Virus Classification: The World of Viruses Nicholas H. E. coli chaperone proteins DnaJ and DnaK are needed to release it from the O-some. Nucleotide sequence conservation extends inward from both nontranslated regions into the protein-coding region. Fulminant hepatitis: severe and rapidly progressive hepatitis often leading to hepatic failure and death. Virions are released from the host cell. As the plus-strand is freed, it is coated with the single-stranded DNA binding protein. The sequence of the first 60 to 100 nucleotides starting from their capped 5' ends is identical to the same region at the 5' end of genome RNA. 40S and 60S: small and large ribosomal subunits. The occlusion bodies are very characteristic of baculovirus infections and appear as an obvious cytopathic effect easily seen by light microscopic examination of infected insect cells. What are the advantages and disadvantages of the packaging strategies used by such viruses? Methyltransferase: an enzyme that transfers a methyl group from S-adenosyl-methionine to an acceptor molecule (e.g., a nucleotide base or ribose during RNA cap formation) Micelle: small, water-soluble droplets of polar lipids. Viruses with RNA genomes may also have arisen from primitive RNA-based life forms. The prion protein is shown inserted into the plasma membrane via the glycolipid phospholipid anchor. The bat may have been infected originally by migratory wildfowl, increasing the likelihood of generation of mutants or reassortant viruses that will acquire the ability to be transmitted among humans. Chapter 3 concludes with some speculation on their origin, perhaps billions of years ago. However, for many viruses the ratio of physical particles to infectious particles can be 10, 100, or even 1000! There are several possible reasons for the low infectivity of virus preparations: CH001.indd 11 11 • Not all virus particles may be infectious. The human viruses are further classified into six subgroups (A to F) based on their ability to agglutinate red blood cells. Virus Classification: The World of Viruses 31 VIRUS CLASSIFICATION 31 Many different viruses infecting a wide variety of organisms have been discovered 31 Virus classification is based on molecular architecture, genetic relatedness, and host organism 31 Viruses are grouped into species, genera, and families 32 Distinct naming conventions and classification schemes have developed in different areas of virology 33 MAJOR VIRUS GROUPS 33 Study of the major groups of viruses leads to understanding of shared characteristics and replication pathways 33 Viruses with single-stranded DNA genomes are small and have one or two genes 34 Viruses with double-stranded DNA genomes include the largest known viruses 35 Most plant viruses and many viruses of vertebrates have positive-strand RNA genomes 35 Viruses with negative-strand RNA genomes have helical nucleocapsids; some have fragmented genomes 38 Viruses with double-stranded RNA genomes have capsids with icosahedral symmetry 38 Viruses with a reverse transcription step in their replication cycle can have either RNA or DNA genomes 39 Satellite viruses and satellite nucleic acids require a helper virus to replicate 40 Viroids do not code for proteins, but replicate independently of other viruses 40 THE EVOLUTIONARY ORIGIN OF VIRUSES 40 The first steps in the development of life on Earth: the RNA world 40 Viroids and RNA viruses may have originated in the RNA world 41 The transition to the DNA-based world 42 Retroviruses could have originated during the transition to DNA-based cells 43 Small- and medium-sized DNA viruses could have arisen as independently replicating genetic elements in cells 43 Large DNA viruses could have evolved from cellular forms that became obligatory intracellular parasites 43 These hypotheses about the origin of viruses are only speculations 44 4. The virus is carried in the wild by a number of small mammals, including foxes and raccoons. Significant alterations of subcellular structure have been observed in flavivirus-infected cells. GENES AND PROTEINS Herpes simplex virus type 1: 90 different transcriptional units. Both cap analogues and capped mRNAs appear to act as primers for transcription. The large number of endogenous retroviruses and retrotransposons present in the genome have enormous mutagenic potential. Because cell monolayers are too thin to deflect light well, plaques in cultured animal or insect cells are usually visualized by staining the cells. USA 85 (1988): 3304–3308; Fig. The 5♠ end of the minusstrand is attached to the viral polymerase protein (orange sphere) and the 5♠ end of the plus strand is attached to a short, capped RNA (blue line). Synthesis proceeds to the end of the linear DNA, producing a double-stranded progeny DNA containing a parental and a daughter strand, and a displaced parental single-stranded DNA molecule (step 3). Amino acids near the binding site are labeled. BGLOSS.indd 482 Tegument: region between the envelope and the nucleocapsid in virions of certain viruses, notably baculoviruses and herpesviruses. Although the details of this remarkable multifunctionality remain to be resolved, it appears that each protein has multiple independent activities rather than multiple uses of a single enzymatic active site. Venkataram Prasad, Baylor College of Medicine; Fig. These "agglutinated" red blood cells, when allowed to settle, form a light pink hemispherical shell in the bottom of a tube or plastic well. Antitermination sponsored by N leads to early gene expression that includes CII and CIII, along with recombination and replication proteins. Matrix proteins of a number of other enveloped viruses contain short, conserved amino acid sequences, called "late domains", which are required for production of virus-like particles in vitro. At least in either case, the weakening of the cell wall causes an increased sensitivity to osmotic pressure, which leads to cell lysis. Polymorphonuclear Leukocytes Participate in inflammatory response, phagocytosis. A deletion is made within the U3 region of the right-hand LTR of the transfer construct, thus eliminating its transcriptional activity (Figure 37.4c). coli can have two possible outcomes. Although it was originally isolated as a poxvirus that infected cows and/or dairy workers, vaccinia virus is distinct from cowpox and is now found in the wild only as "escaped" vaccine virus that has recently emerged in buffaloes in India and in cattle in Brazil, in both cases spilling back over to humans. However, a year or two after the virus became established in a given area, the number of human cases of encephalitis declined. However, selectivity at these two steps is not as great for ganciclovir as it is for acyclovir: While dependence on the host cell nucleus carries with it many advantages, it also poses a problem: the incoming virus must deliver its genome and secondary structure and self-repair cleavage sites. The right terminal domain (about 360 nt) of the genome RNA of hepatitis delta virus is reminiscent of viroids, and includes self-cleaving motifs used in both genome and complementary antigenome RNAs (delta motif; see Figure 31.5c). The extrinsic pathway (upper right) begins by binding of a ligand such as TNF ₊ to a cell surface receptor; adaptor proteins form a "death-induced signaling complex" (DISC) with procaspase-8, leading to the activation of caspase-8. Numerous full-color figures focus on individual steps in virus replication. Some viruses incorporate polyamines such as spermidine into their capsids to neutralize the charge of phosphate groups on their nucleic acid genomes. The structural protein of retroviruses could cause cancer, their presence in preparations of retrovirus vectors intended for gene therapy applications is intensively scrutinized. • New nucleoside analogue polymerase inhibitors are effective in reducing viral loads and reversing the symptoms of hepatitis B. Thus these proteins reverse the transcriptional repressive effects of the histone deacetylases referred to above. One contains the wild-type adenovirus genome cleaved within the E1 region to remove the left-hand inverted terminal repeat and packaging signals. These include smallpox, influenza, poliomyelitis, yellow fever, measles, and AIDS (acquired immunodeficiency syndrome). C. (d) The self-cleavage reaction involving a Mg2+-catalyzed phosphoryl transfer reaction that cleaves the RNA, producing 2'-3' cyclic phosphate (top right) and 5'-hydroxyl (bottom right) terminal. Chapter 2 Fig. The pathogenicity of influenza virus strains is not yet completely understood, but appears to be caused by a constellation of sequence variations in multiple genes. Gp0.7 inactivates the host cell RNA polymerase by phosphorylation of a specific tyrosine residue, limiting the amount of class I mRNAs synthesized and interrupting further class I gene expression. Thus mosquito control in Panama overcame the most significant impediment to the construction of the Panama Canal. Replication complex: for RNA viruses, a factory for viral RNA synthesis consisting of viral and cellular proteins associated with membranes in the cytoplasm of an infected cell. Harrison; Fig. Middle T antigen, bound to plasma membrane via hydrophobic residues near its C-terminus, associates with c-Src. An example of the latter: closely related phages (a member of the Siphoviridae that grows in E. The released virus can infect the oral epithelium, giving rise to infectious virus that can spread to other individuals via saliva. The Gaia hypothesis states that the Earth is a self-regulating organism. Viral proteins derived from the gag, pol, and env genes are incorporated in virions Retroviruses are roughly spherical and approximately 100 nm in diameter (Figure 28.1). • The influenza virus hemagglutinin (HA) protein binds to cell receptors and mediates fusion of the viral envelope with the endosomal membrane after endocytic entry into cells. Infection can have some secondary effects, such as nausea and fever, which make this treatment • • • • • • You should never use someone else's toothbrush. Are poxvirus RNAs spliced, and if so, where? These cell types interact with each other, and both antibody production and cytotoxic lymphocytes are maintained by long-lived "memory" cells that in many cases provide lifelong immunity against reinfection by a pathogen. These two phenomena limit viral infection to a small local lesion zone on infected plants (Figure 10.9). In addition, virus-like particles (VLPs) generated by transient expression of the viral proteins VP40, NP, and GP have shown promise as vaccine candidates. Viral double-stranded RNAs are cleaved into small interfering RNAs, which direct cleavage and therefore inactivation of viral mRNAs or genome RNAs. Immune responses are mediated by specialized cells that circulate in the blood or lymph and that are localized in the thymus, lymph nodes, spleen, and gut. However, development efforts are not limited to these viruses. From there, viral DNA must be transported to the nucleus. It is possible that signals allowed the production of the poly(A)+ mRNA functionally substitute for the poly(A) tail by binding to cellular proteins. The receptor-binding domain (RBD) is found within S1 in most coronaviruses. Budding involves curvature of the membrane as it is wrapped around the viral nucleocapsids. NF-jB (nuclear factor-jB): a heterodimeric transcription factor that regulates many aspects of immune responses, apoptosis, and a variety of signaling pathways. Cell death results from the activation of caspases, which are members of a family of cysteine proteases that cleave after aspartate residues. Icosahedral capsid, T = 4. Types that infect skin are likely transmitted through skin 263 CH022.indd 263 16/05/11 8:10 PM 264 CHAPTER 22 Papillomaviruses Table 22.1 Sites of infection of selected human papillomaviruses Virus type Site of infection Clinical manifestation 1 Soles of feet Deep plantar warts 2, 4, 7 Hands Common warts 13, 32 Oral cavity Focal epithelial hyperplasia 3, 8, 9, 10, 12, 14, 15, 17, 19–25, 36, 38 Arms, trunk Flat warts, skin cancer 6, 11 Genital tract, larynx, nasal cavity Cervical warts, condyloma 16, 18, 31, 33, 35, 39, 41–45, 52, 56, 58, 59 Genital tract Cervical cancer, atypical condyloma contact with an infected individual or through contact with a virus-containing surface. Consequently, withdrawal from therapy results in reemergence of the virus and continued disease progression. These 16/05/11 8:06 PM 204 CHAPTER 17 Bunyaviruses glycoproteins are cleaved during synthesis by a cellular protease at relative positions in the precursor polyprotein. Gn lies toward the N-terminus and Gc lies toward the C-terminus. A complementary sequence in the 5' end of the genome is involved in the cyclization of the RNA, and as such is also involved in RNA replication. The virus-induced membrane changes have been described as a "reticulovesicular network" of interconnecting and evolving structures over the course of 16/05/11 8:04 PM 166 CHAPTER 14 Coronaviruses Table 14.2 Coronavirus proteins and functions Protein Functions nsp1 Unknown functions in replication; may mediate cell-cycle arrest during over-expression in culture; SARS nsp1 is an interferon antagonist. This may be due in part to the sequence of the polyadenylation signal, UAUAAA, which differs from the consensus AAUAAA found in most genes in vertebrate cells. In contrast, Nef-induced loss of MHC 1 from the cell surface is due to a block in trafficking of MHC 1 from the Golgi apparatus to the plasma membrane. C3b bound to a virus particle is recognized by receptors on the surface of inflammatory cells such as macrophages, increasing the efficiency of phagocytosis and leading to engulfment and destruction of the virus particle. The 5' noncoding region of hepatitis C virus RNA shares little primary sequence or structure homology with that of other picornaviruses, and this virus has been suggested to have a Type III IRES element. Hershey and Chase's paper was followed a year later by the proposal by James Watson (a student of Luria) and Francis Crick of the doublehelical model of DNA, which galvanized thinking and research throughout biology, but in particular in virology and molecular biology. To date, more than 10 million people have been inoculated with rabies vaccines grown in cultured cells, with seroconversion and protection rates approaching 100% and without a single proven case of vaccine-associated neurological damage. Subsequent biochemical and electrophysiological experiments showed that the M2 protein is a channel for H+ ions and that amantadine blocks this channel. Microvirus J proteins are small, basic polypeptides ranging from 20 to 40 amino acids. The 72 capsomers, each composed of pentamers of VP1 and a single molecule of VP2 or VP3, are organized with icosahedral symmetry in a T 4 lattice. CH028.indd 353 16/05/11 8:15 PM C H A P T E R 2 9 Human Immunodeficiency Virus Alan Cochrane Lentivirus From Latin lentis (slow), for slow progression of disease VIRION Spherical enveloped particle. These proteins are both synthesized from the same complementary-sense mRNA but from different, overlapping reading frames, as is the result of alternative initiation at different AUG codons. Interferon production in plasmacytoid dendritic cells does not require virus replication. The sizes of the genome RNAs are shown for the Fny strain. (a1–c1) Scanning electron micrographs; (a2–c2) transmission electron micrographs (Scale bars: 200 nm). Enveloped and non-enveloped viruses have distinct penetration strategies To move from cell to cell, the viral genome has to cross two or more cellular membranes. See also flaviviruses and alphaviruses (Chapters 12 and 13) are arranged with icosahedral symmetry, and these viruses could therefore also be considered to have an external "capsid" embedded in an internal lipid membrane. One set of cellular targets of PP2A are mitogen-activated protein kinases (MAP kinases). A wide variety of RNAs can be targeted for cleavage by such engineered ribozymes. Antibody-dependent enhancement: enhanced entry of certain viruses when the virions are complexed with antibody, resulting in an increased variety of host cells that can be infected. The two polypeptide chains are linked by a S-S bond between cysteines. As the title implies, it concentrates on the molecular mechanisms of virus replication, and on the interactions between viruses and the cells in which they replicate. Poxviruses and herpesviruses also have G protein-coupled receptors that bind to a variety of chemokines and prevent the recruitment of immune cells to the site of infection. In this way, viruses can take advantage of the cell while remaining virtually invisible to the antiviral defenses of the organism. It was noticed that the M2 protein resembles known channel-forming transmembrane proteins. Bovine papillomavirus, which causes warts in cattle, originally served as a prototype for the study of the molecular biology and transforming genes of papillomaviruses because bovine warts are easily available, and because this virus can transform cultured rodent cells in vitro. 16/05/11 7:46 PM 12 CHAPTER 1 Introduction to Virology Nearly all cells in a culture are infected simultaneously and there remain very few uninfected cells. Chikungunya virus, an alphavirus related to Ross River virus, erupted in epidemic form in the Indian Ocean region in 2005, resulting in significant disease and death. 9.14b: Fig 4D reprinted from: Jäälinoja HT, Roine E, Laurinmäki P, Kivelä HM, Bamford DH, & Butcher SJ, "Structure and hostcell interaction of SH1, a membrane-containing, halophilic euryarchaeal virus," Proc. Genetic shift: see Antigenic shift. Certain viral proteins function to retard cell death, prolonging the period of virus replication, and other viral proteins can kill the host cell, leading to virus release or dissemination. EBV is present in high amounts in the saliva of seropositive individuals. Unlike many other viral glycoproteins, Gn and Gc do not migrate to the plasma membrane but are retained in the Golgi membranes where virus assembly takes place (see below). This strand is then called the "first strand transfer", since the strong-stop DNA strand is transferred from the 5♠ end to the 3♠ end of the genome RNA. Most of these enzymes are encoded by early genes and many have overlapping functions in viral DNA replication, recombination, and repair since these three processes have numerous steps in common. 5.3: Courtesy of A.B. Jacobson, Reprinted from Fauquet, C. Are there any treatments for Marburg and/or Ebola infections in humans? Unlike disease caused by hepatitis C and B viruses (see Chapters 12 and 30), hepatitis A infection does not usually lead to chronic disease or liver cancer. These findings point to uncoating, rather than entry, as the level of the cell-specific block to replication of certain strains of minute virus of mice. • Viruses with very large double-stranded DNA genomes (400 to 1200 kb) are found in free-living unicellular algae and protozoa. A number of "glue" proteins (polypeptides IIIa, VI, VIII, and IX) mediate interactions between hexons and pentons, and with the viral DNA. Hemophilus type B is a close relative of Haemophilus influenzae, the bacterium that causes meningitis. Dementia can result from a reduced level of coagulation factor IX in the blood. Splicing generates up to six different mRNAs, which encode two four replication (Rep) proteins and two nonstructural (NS) proteins: DNA synthesis of cellular proteins. Interaction of IPS-1 with additional cellular proteins leads to the activation of IKK and TBK-1 and the nuclear translocation of NF-B, IRF-3, and IRF-7. Adenoviruses are the best-studied example of viruses that penetrate by acid-activated, virus-dependent endosome lysis (Figure 4.5c). CH034.indd 425 16/05/11 8:19 PM CH034.indd 426 16/05/11 8:19 PM SECTION X ANTIVIRAL AGENTS AND VIRUS VECTORS C H A P T E R 3 5 Antiviral Vaccines C H A P T E R 3 6 Antiviral Chemotherapy C H A P T E R 3 7 Eukaryotic Virus Vectors These three chapters deal with practical applications of virology that protect humans or animals against virus infection or that employ viruses as vectors that express specific genes for gene therapy or as antitumor agents. Following nicking of a concatemer, terminase separates the cohesive ends, remaining bound to the end of the DNA at cosB. The four distinct serotypes contain two pairs of mutually exclusive epitopes named "d" or "y" and "w" or "r". Proteasomes: large specialized complexes that degrade proteins in the cell and make peptides available to MHC class I proteins for presentation at the cell surface. In turn, viral DNA would then be threaded into the empty prohead to form a "provirion". If the helper is adenovirus (see Chapter 23), the adenovirus genes involved are E1A, E1B, E2, E4, and the VA RNA genes. Penicillin inhibits the formation of cross-links within peptidoglycan, a major component in bacterial cell walls. On the surface of the virion is a variable number of spherical projections about 16/05/11 8:00 PM Viruses of Archaea 105 15 nm in diameter, most likely involved in cell attachment. Gene E resides within gene D, and therefore E's nucleotides are translated in a different reading frame. Many bacteriophages have specialized tails that drill holes in the cell wall and membranes, and serve as a conduit for passage of the DNA genome through the hole into the cell. The early proteins of many DNA viruses induce the production of a number of cellular enzymes that are involved in the synthesis of DNA and of its building blocks, deoxyribonucleoside triphosphates. Some parvoviruses can only replicate if they infect a cell that is also infected with a larger "helper" virus. What is the role of a "reverse gyrase" for these thermophilic organisms? Activated PKR then phosphorylates a variety of cellular proteins. This protein is a transferase that catalyzes the formation of the first lipid-linked intermediate in cell wall biosynthesis. Its precursor, dimethyl sulfoniopropionate (DMSP) is thought to act as an osmolyte, regulating osmotic pressure within the cell. The helical nucleocapsids are visible as using a pyramidal structure (Figure 9.10) used by viruses from at least two different families. For example, a survey of naturally occurring baculoviruses on market cabbage demonstrated that over 110 million virus-containing polyhedra might be consumed in one serving of coleslaw. Gene 2, like gene 0.7, inhibits host RNA polymerase, ensuring that class I genes and host transcription are shut down. Many diseases were considered to be caused by mysterious elements in fluids termed virus ("poison" in Latin), but the distinction CH001.indd 6 between what are now called viruses and cellular microorganisms was not clear. Cytomegalovirus infections are usually inapparent in normal adults, but can be life-threatening or sight-threatening in the immunocompromised. Not all viruses that infect insect cells use clathrin-coated vesicles. Exocytosis: the process of delivery of material within vesicles derived from the endoplasmic reticulum to the outside of the cell by their passage through the Golgi membranes and fusion with the plasma membrane. Transcription factors: proteins that bind to specific DNA sequences in transcriptional enhancers or promoters and alter the transcriptional activity of the gene by interacting with other transcription factors and RNA polymerases. However, substantial information and stories about medical and historical aspects of virology are included, particularly in introductory sections of each chapter. The two colored circles show protein-coding regions on the two DNA strands. Genetic analysis has correlated this change in tropism to partial deletion of a structural protein gene and alterations in an accessory gene. Serum sickness: a hypersensitivity response to large amounts of antigen in the circulatory system. These cells then move to the subepithelial space and present bound virus to CD4-positive T cells under conditions that greatly enhance infection of the T cell. Rightward-reading genes are shown above each DNA segment, and leftward-reading genes are shown below. CH035.indd 441 441 The desire to establish herd immunity has led public health authorities either to enforce vaccination or to make annual vaccination difficult. Anti-apoptosis genes. This shows that amino acid sequence differences are not necessary to produce different strains. 10.6: Courtesy of Nicholas Acheson, with thanks to Cheng Kao; Fig. Together, these steps activate the major late promoter by a factor of 100 or more. It is not clear exactly how the Mx proteins block these steps, but a role of the GTPase activity of Mx proteins in their function is not understood. Virus species are grouped into genera by virtue of shared characteristics such as genome organization and size, virion structure, and replication strategies. On these altered messenger RNAs, protein synthesis continues beyond the normal termination codon until it encounters a downstream stop codon. Small DNA viruses could have evolved from escaped, self-replicating fragments of cellular genome DNA. λ DNA replication is directed by O and P, but carried out by host cell proteins Expression of the early genes O and P leads to DNA replication. Paramyxoviruses: 15–18 kb. A new pandemic strain of influenza A virus arose by genetic shift and spread worldwide in 2009 In April 2009, a new strain of human H1N1 influenza A virus was identified in the United States and Mexico, where it was initially associated with severe respiratory disease. Although as a DNA, extracted from virions of adeno-associated virus can self-anneal in vitro, producing a fully double-stranded DNA genome to be resistant to the antiviral effects of interferon. Several amino acids in the cucumber mosaic virus coat protein are needed for efficient aphid transmission have been identified. CJD: see Creutzfeldt-Jakob disease. DISTINCTIVE CHARACTERISTICS Multiple mRNAs arise by extensive alternative splicing. Common examples are squash, melons and cucumbers. 18.9: Reprinted by permission from Macmillan Publishers Ltd: Figures 1 a and c in Takeshi Noda, Hiroshi Sagara, Albert Yen, Ayato Takada, Hiroshi Kida, R. Alternatively, all RNA polymerase transcription could begin synthesis at a single promoter at the 3' end of the genome RNA, and progress from right to left through the genome, making the different mRNAs in sequence (Figure 15.7b). bcreidt.indd 487 Chapter 27 Fig. • Low risk human papillomaviruses such as types 1, 2, 6, and 11 cause benign warts • High risk human papillomaviruses such as types 16, 18, and 31 can cause cervical cancer and other malignancies. This protein accumulates in the lumen of the ER, Golgi, and endosome compartment of the cell. Thus it resembles the matrix proteins of other negative-strand RNA viruses. Packaging cell lines express retrovirus enzymatic and structural proteins Retrovirus vectors are produced by packaging cell lines that express all essential viral proteins: Gag (capsid proteins), Pol (reverse transcriptase and integrase), and Env (envelope proteins). Neuraminidase: enzyme that cleaves terminal sialic acid (N-acetyl neuraminic acid) from glycolipids or glycoproteins. In another strategy, the virus produces soluble receptors for tumor necrosis factor, interleukin-1, chemokines, and interferons, which bind to these cytokines and prevent them from interacting with their intended receptors. DISCLAIMER: The inclusion of certain drugs in this chapter should not be construed as a comment on their clinical merits relative to other treatments. Membrane attack complex: a collection of complement proteins that forms a pore in the membrane of pathogens or infected cells, killing the pathogen or cell. Although rarely fatal, the acute illness is severe, frequently involving seizures, and can lead to unpleasant aftereffects such as chronic seizures or epilepsy. Introduction to Virology 2 THE NATURE OF VIRUSES 3 Viruses consist of a nucleic acid genome packaged in a protein coat 3 Viruses are dependent on living cells for their replication 3 Virus particles break down and release their genomes inside the cell 3 Virus genomes are either RNA or DNA, but not both 4 WHY STUDY VIRUSES? However, other picornaviruses do not have an L proteinase. Such ambisense genomes are relatively rare in the RNA virus world, and are known only in the arenavirus (not covered in this book) also use this strategy to express their proteins. It was not known until 1951 that E. coli K-12 carries a prophage. Some of these are described in detail in Chapters 5 45 CH004.indd 45 16/05/11 7:52 PM 46 CHAPTER 4 Virus Entry Through 8. Some insect viruses spread as individual virions from cell to cell within the host organism, but wrap one or more virions in a specialized protein coat that is released when the insect dies. Four overlapping reading frames, seven viral proteins. mRNAs are synthesized and capped inside intact cores and extruded from the core into the cytoplasm. As a result, when virions are present in acidifying CH004.indd 54 endosomes, the interior of the particle is also exposed to a low pH, and this releases the interaction of nucleoproteins from their association with each other and with the matrix protein (M1). How does it accomplish this? Viroids and Hepatitis Delta Virus 378 Jean-Pierre Perreault, Université de Sherbrooke Martin Pelchat, University of Ottawa 32. As the DNA is packaged, the downstream cos site is bound by, and cut by, terminase, which can then package another genome into another prohead. These structural proteins are not translated directly from genome RNA, but from a subgenomic mRNA made during viral infection. A temperate phage: DNA can integrate into the host cell chromosome, creating a lysogen. A single gene encodes interferon-; it also lacks introns and is more distantly related to the interferon-₊ family. (a) Binding of the N-terminal domain of a picornavirus receptor to the "canyon" on the surface of the capsid. Diameter of nucleocapsid 30-60 nm, length 300 nm. 9.9a,b: Courtesy of Wolfram Zillig, reprinted by permission from Macmillan Publishers Ltd: figure 3a in David Prangishvili, Patrick Forterre and Roger A. C-

terminal arms of VP1 link adjacent pentamers together. Collectively, these findings point to a cellular response axis linking NF-B, IRF-3, and reovirus-induced apoptosis. Since alphaviruses also infect insects, particularly mosquitoes, vectors are being developed for use in insects. Binding of large T antigen to p300 can inhibit its transcriptional coactivation function and therefore reduce transcription of genes targeted by these factors. It has been shown to overcome host defense mechanisms and to enhance pathogenicity, apparently by inducing increased apoptosis in host immune cells responding to influenza virus infection. Directs and primes initiation of viral DNA replication. Natural resistance to HIV-1 infection is associated with a mutation in CCR5 that results in a loss of cell surface expression of the protein. What are IS elements? The two appendages (tails) at each pointed end have variable lengths; the maximum length of the virion including tails reaches about 1000 nm. What basic functions do bacteriophage T7 Class I–III genes perform? This DNA genome is transcribed in the cell nucleus to generate viral mRNAs as well as a pre-genome RNA transcript that is packaged in viral cores along with reverse transcriptase. They serve the cell as a sorting and recycling station for incoming fluid, ligands, receptors, and membrane proteins. The N-terminal part of HA2 forms a rigid alpha-helical structure, moving the fusion peptide out of the hydrophobic region of HA and toward the endosomal membrane, where it inserts itself into the membrane (see Figure 4.4 in Chapter 4 for details). Four partly overlapping open reading frames, designated C (for capsid protein), P (for polymerase), S (for surface protein), and X (for a regulatory protein), are translated to yield seven different viral proteins. Nonstructural proteins are multifunctional NS1 of the autonomous parvoviruses and its equivalent in adeno-associated virus, Rep78/68, are the principal viral regulatory proteins and play several roles in parvovirus replication. Hexagonal arrays of major capsomers form trisymmetrons (green, violet, and blue) and pentasymmetrons (yellow). barrier that stops ribosomes from reaching the authentic start site. The toll-like receptors bind to their adaptor proteins via TIR domains. Most viruses do not require special mechanisms to be released from the cell surface, but influenza virus does. 4.1, 4.2: Courtesy of Ari Helenius. Paramyxoviruses and Rhabdoviruses 175 Nicholas H. • During entry in endosomes, reoviruses lose their outer capsid shell, and the cores penetrate the endosomal membrane and enter into the cytoplasm. Messenger RNA is shown being translated by a ribosome. Such split AAV vectors have been used successfully, albeit at lower efficiency than standard AAV vectors, to deliver the 6.5-kb cDNA coding for dystrophin, in which mutations cause a severe type of muscular dystrophy. Forterre, O. As Table 33.1 shows, vectors have been developed from a large variety of viruses whose genomes can be positive- or negativestrand RNA, single- or double-stranded DNA, ranging 456 CH037.indd 456 16/05/11 8:22 PM Eukaryotic Virus Vectors from 5 to 200 kb in size. N-modified RNA polymerase simply transcribes through terminators tL1 and tR1 without taking any notice of them. The concentration of this repressor in the cell is limited. This discovery led to the replacement of the procaryote/eukaryote dichotomy by a trinity of All viruses have double-stranded DNA genomes except for one single-stranded DNA virus. Membrane fission: the process by which one membrane divides or "buds" into two. © 1994 Brooks/Cole, a part of Cengage Learning, Inc. Chapter 1 outlines the properties of viruses and how they replicate. • Herpesviruses enter the cell by fusion of their envelope with cell membranes; attachment and fusion are directed by several envelope glycoproteins. This ensures that newly made virions can leave the cell surface and proceed to infect other cells. Adeno-associated viruses were originally found contaminating preparations of adenovirus; they are fairly widespread since the majority of people have antibodies against them. EFFECTS OF INTERFERONS ON THE IMMUNE RESPONSE Interferons stimulate antigen processing via the proteasome and stimulate antigen presentation via MHC proteins. Splicing generates 10 or more different mRNAs. Early proteins E1 to E7 stimulate cell proliferation and enable viral DNA replication. Nef expression decreases the levels of CD4 and the major histocompatibility complex protein MHC 1 on the cell surface. These strain differences depend on variations in the SU protein (gp 120), particularly in a region that undergoes a high rate of evolution, designated variable domain 3. This makes sense, for the phage encapsidates only plus-strand RNA, and only this RNA can be translated to make more phage proteins. These vaccines carry no risk of infection but can induce antibody responses to the virus. Anaerobic methanogen: a microorganism that grows in the absence of oxygen and produces methane, often by reduction of carbon dioxide, to provide a source of energy. The nucleation point for this coating reaction is the replicase operator hairpin (Figure 5.5). In contrast, the transcription of 2 genes is totally dependent on viral DNA synthesis. Sindbis and Semliki Forest viruses are not highly pathogenic in humans, allowing their safe and easy use in the laboratory. For example, the [Het-s] prion state of the fungus Podospora anserina results in accumulation of amyloid fibrils made of the protein HET-s, and these fibrils appear to serve a functional role. Despite the present availability of vaccines and antiviral drugs, influenza viruses continue to kill hundreds of thousands of humans worldwide every year. Virions are assembled at the plasma membrane KEY TERMS Both paramyxoviruses and rhabdoviruses assemble their envelopes at the plasma membrane. Some archaea have histone-like proteins that associate with and condense genome DNA, as do eukaryotes but not bacteria. Antibodies: immunoglobulin proteins that recognize and bind to specific regions (epitopes) on a variety of types of molecules including proteins, carbohydrates, lipids, and nucleic acids. TATA Virus vectors are engineered to produce optimal levels of gene products The expression cassette (Figure 37.2) is one of the chief elements of a virus vector. Recent studies have demonstrated that the E protein of flaviviruses and the E1 glycoprotein of togaviruses have a similar structural fold and share similar functions in both fusion and icosahedral particle formation. W. (b) The 10 plasmid constructs are transfected into mouse L cells expressing T7 RNA polymerase from a replication-defective vaccinia virus strain. Once a gruesome and lethal disease • Late: virion structural proteins, virion transcription enzymes VIRUSES AND HOSTS Two subfamilies: Chordopoxvirinae: infect vertebrates: • • Humans: variola, vaccinia (vaccine strain), molluscum contagiosum Viruses that infect a variety of birds and mammals: monkeys, cattle, etc. Teng, Pennsylvania State University Loy Volkman, University of California, Berkeley Darlene Walro, Walsh University Jeannine Williams, College of Marin During the preparation of the first edition of this book, preliminary versions of a number of chapters were made available to students taking our virology course at McGill, and many of those students gave precious feedback that improved the book. The results of basic research with AcMNPV led to a renewed interest in using genetically modified baculoviruses for insect pest control, as well as the use of baculoviruses and insect cells as an efficient eukaryotic gene expression system. 24.4: Courtesy of Beate Sodeik, Hannover Medical School, reprinted from Fig. 4 (2006), 520-528; Fig. • Small, icosahedral, single-stranded circular DNA viruses may be evolutionarily related. Virions enclose the nucleocapsids within a lipid envelope derived from the membranes of the Golgi complex. To bypass this step, researchers have constructed vectors in which the single-stranded DNA molecule can fold back on itself in such a way that it carries a double-stranded DNA copy of the transgene of interest. C proteins also antagonize the antiviral effects of interferons by interfering with the function of Stat proteins (see Chapter 33). Kuru: prion disease of the Fore people in Papua New Guinea, caused by ingestion of prions during ritual cannibalism. Disseminated intravascular coagulation is a terminal event. The result of this clinical trial made news in 2000 and is regarded as the first example of a successful treatment of a human disease by gene therapy. Dendritic cells: cells that accumulate and present foreign antigen to lymphocytes in lymph nodes and elsewhere in the body. 2.6b: Reprinted from Liddington, R.C. et al., "Structure of Simian Virus 40 at 3.8 A Resolution," Nature 354 (1991): 278–284; Fig. Acheson, McGill University 2. This covalently bound dC residue is then used as a primer by the viral DNA polymerase for the further addition of nucleotides complementary to the template DNA strand (step 2, Figure 23.8). The virus life cycle initiates by binding of virions to a cellular receptor and internalization in an endosomal vesicle (upper left). The associated mortality was significant (30–50%) and many of those who recovered were left with permanent neurological sequelae. Another species of cucumovirus, peanut stunt virus, also harbors satellite RNAs. Peanut stunt virus satellite RNAs cannot be replicated by cucumber mosaic virus, and vice versa. Furthermore, its transcript contains signals that direct packaging of full-length plus-strand CH015.indd 180 copies of the viral genome with N protein to generate nucleocapsids. Most figures are designed to be simple and easily understood while one is reading the accompanying text, rather than comprehensive (and sometimes complicated!) descriptions of the entire replication cycle. Image created by Jean-Yves Sgro with software Qutemol. A newly emerged coronavirus caused a worldwide epidemic of severe acute respiratory syndrome (SARS) The field of coronavirus research took an abrupt turn when, in early 2003, an epidemic of severe and often fatal pneumonia broke out in southeast China, Hong Kong, and Vietnam, and subsequently spread to Toronto, Canada. A growing list of other key cellular proteins are also targeted for degradation by this complex. An example is one of the first retroviruses discovered, Rous sarcoma virus (Figure 28.8). Readthrough in phage Q arises when the UGA stop codon at nt 1742 that terminates the normal coat protein gene is occasionally read as a tryptophan codon (UGG). Table 35.3 Technological advances supporting antiviral vaccine development 1850 Germans add glycerin to "cow lymph" to stabilize and decontaminate smallpox vaccine. (b–d) The doublestranded template RNA is locally unwound, possibly by a helicase function of the 1 protein, and serves as a template for synthesis of full-length plus-strand mRNAs (light blue line). • Several different generations of adenovirus vectors have improved the capacity for incorporating foreign genes and reduced immune responses and toxicity of the vectors. See also Togaviruses nonstructural proteins, 151 packaging signal, 153 pathogenesis, 155 protein processing and modification, 154 receptors, 150 structural proteins, 152 vectors, 157, 457, 468 virion structure, 150 Single-cycle virus replication experiment, 11–12 Single-stranded DNA binding protein (ssDBP), 72, 73, 80, 280, 281, 291, 292, 307 Single-stranded DNA genomes, viruses with, 34–35 Single-stranded RNA bacteriophages, 61–69 binding to F-pilus, 60–61 discovery, 59 genera, 60 genome maps, 60 host ribosomal protein in replication, 65 maturation gene translation on nascent RNA, 65–66 MS2 bacteriophage, 62 plus strand synthesis, 65 Qβ bacteriophage, 62 replication proteins, 65 RNA secondary structure controls translation, 61–64 translation vs replication, 61 assembly and release of virions, 67 SiRNA, 121, 413 Site-specific integration, 468 Size variation of viral genomes, 35 Small hepatitis B surface protein (SHBs), 370 Small interfering RNAs (siRNAs), 121, 413 Small T antigen, 254–255 Smallpox, 312–313, 429, 430 global eradication program, 313 variolation, 313 Solfataric fields, 105 SOS genes, 89 'Spanish flu' pandemic, 210–211 Spleen focus forming virus (SSFV), 351 Spliceosome, 253 Spongiform encephalopathy, 388 Squamous cell carcinomas, 264 Structure, virus, 18–30 basic concepts, 18–21 budding, 26, 28 capsids with helical symmetry, 25 capsids with icosahedral symmetry, 21–24 core proteins, 28 dissassembly of virions, 29 genetic economy, 19 larger viruses in more complex packages, 23–24 packaging signals, 28 quasi-equivalence, 21–22 scaffolding proteins, 28 bindsub.indd 499 self-assembly, 19 triangulation number (T), 23 viral envelopes, 26 viral glycoproteins, 27 virion assembly, 28 Stuttering transcription, 216 Subacute sclerosing panencephalitis (SSPE), 176 Subgenomic mRNAs bunyaviruses, 204 coronaviruses, 161, 167–168 cucumber mosaic virus, 114, 117 togaviruses, 151, 153, 154, 156 Subunit vaccines, 431, 434 Sulfolobus islandicus filamentous virus (SIFV), 100, 102 Sulfolobus islandicus rod-shaped virus 1 (SIRV-1), 105 Sulfolobus islandicus rod-shaped virus 2 (SIRV-2), 106–107 Sulfolobus neozealandicus droplet-shaped virus (SNDV), 100, 103–104 Sulfolobus spindle-shaped virus 1 (SSV-1), 99–100 DNA transcription, 101–102 genome of, 105 Sulfolobus spindle-shaped viruses 6 (SSV-6), 99 Sulfolobus turreted icosahedral virus (STIV), 100 Supercoiled DNA, 249–250 Superoxide dismutase (SOD), 307 Swine flu, 211 Syncytia, 47, 164 Systemic mosaic symptoms, 116 T antigens, 254. Why are parvoviruses only able to productively infect cells going through the cell cycle? Other adenoviruses in cattle, mice, birds, etc. The DNA helicase-primase complex, along with DNA polymerase, fills in the complementary lagging-strand on ICP8 UL9 the extruded DNA to produce a double-stranded, linear concatemer. When RNA polymerase reads through the sib sequence during N-antiterminated transcription from PL, the mRNA forms a hairpin structure, SIB, that is a site for attack by double-stranded RNA-specific ribonuclease III (Figure 8.8; segments 1 and 2 pair with segments 3 and 4). 435 The role of the immune system in fighting viral infections 436 Adjuvants play an important role in vaccination with inactivated or subunit vaccines 436 Vaccines that stimulate cell-mediated immunity are being developed 436 13/05/11 2:11 PM xviii Contents NEW DEVELOPMENTS IN ANTIVIRAL VACCINES 437 New approaches to vaccine development show great promise 437 New adjuvants are being developed 437 New delivery systems for viral antigens 437 Vaccination with defined proteins 437 Use of live viruses with defined attenuation characteristics 438 Use of live vectors and chimeric viruses 439 Vaccines that can break tolerance 439 The changing vaccine paradigm 439 ADVERSE EVENTS AND ETHICAL ISSUES 439 Vaccine-associated adverse events 439 Ethical issues in the use of antiviral vaccines 441 36. Jaundice: yellowing of the skin due to the presence of bile pigments in the blood, often caused by poor liver function. The first surprise was the discovery that its genome consisted of single-stranded DNA; other known phage genomes were made of double-stranded DNA, as are all genomes of cellular organisms. The trigger for penetration is usually low pH, which induces a conformational change in the spike glycoproteins of enveloped viruses or in the capsids of non-enveloped viruses. Then the crossover occurs, inserting into the bacterial chromosome. Chapter 14 Fig. This creates a pore in the viral envelope that permits serum proteases and nucleases to enter the virus and destroy infectivity. The small envelope (E) protein is a 9–12 kDa protein that is present only in very limited numbers in the virion. By repressing the expression of these viral or host proteins at appropriate times in the viral life cycle, the virus may gain an advantage in establishing latency and persisting in humans. I then edited and sometimes rewrote these chapters into a common style, and in many cases I created or redesigned the illustrations. (b) Crystallographic averaging of a twodimensional crystal shows threefold symmetry. An interesting feature of the mimivirus genome is the presence of a range of DNA repair enzymes capable of correcting errors introduced by oxidation, ultraviolet light irradiation, and alkylating agents. Table 12.1 Members of the Flaviviridae family Genus Virus name Hosts Diseases Flavivirus Yellow fever Dengue (types 1–4) Japanese encephalitis West Nile Tick-borne encephalitis Monkeys, humans Monkeys, humans Birds, pigs, humans Birds, humans Rodents, birds, humans Hemorrhagic fever Fever, arthralgia, rash, hemorrhagic fever Encephalitis Fever, arthralgia, rash Encephalitis Pestivirus Bovine viral diarrhea Border disease Classical swine fever Ruminants Sheep, goats Pigs Persistent infection, mucosal disease Acute and chronic hemorrhagic symptoms Hepacivirus Hepatitis C Humans Acute and chronic hepatitis CH012.indd 138 16/05/11 8:03 PM Flaviviruses A live, attenuated yellow fever virus vaccine is available and widely used In the 1930s, a safe and effective vaccine (strain 17D) against yellow fever virus was developed by Max Theiler. P also serves as a chaperone that aids in the assembly of N protein onto RNA chains to form nucleocapsids. Since EBERs are abundantly expressed in latently infected cells, their presence is useful as a diagnostic tool for the identification of Epstein–Barr virus-infected cells in human biopsy material. Membrane penetration and apoptosis. This cellular response to virus infection can block translation of mRNA transcripts by induction of double-stranded RNA-dependent protein kinase R (PKR) and ribonuclease L (see Chapter 33). Exactly how the RNA subsequently passes from the vesicle into the cytoplasm is not known. A virus family with a penchant for sugar metabolism: hyaluronan and chitin The chloroviruses are unusual because they encode many enzymes involved in sugar metabolism. Tashjian, Jr., Ehrin A. E. However, an RNA primer at the very 5' end of a linear DNA molecule cannot be replaced, because there is no complementary DNA strand to copy beyond the end of the template. For example, the internal scaffolding proteins of the related phages ϕX174 and 3 have diverged 70% in amino acid sequence, yet they are cross-functional. The mechanism of switching from primed to unprimed RNA synthesis depends on the presence of CH018.indd 217 free NP protein, but is not fully understood. The replicase complex includes viral proteins 1a and 2a and host proteins. Their linear, 15-kb double-stranded DNA genomes (Figure 9.13) code for a DNA polymerase of the class that uses protein-primed DNA synthesis, similar to the mechanism used by adenoviruses (Chapter 23; see Figure 23.8). Inactivated virus and subunit vaccines do not replicate in the host, but are recognized by the immune system. These spikes are connected to proteins that are anchored in the underlying membrane, and that may be involved in capsid assembly and in delivery of the genome during infection. Immature flaviviruses at neutral pH also display distinct spikes on their surface (Figure 12.2a) and have a dramatically different appearance from the mature virus. In 1979, Robert Roeder used the adenovirus major late promoter to demonstrate specific initiation of transcription by RNA polymerase II in vitro, and he and others subsequently discovered many of the protein factors involved in transcription in mammalian cells by using this model system. This book is the result of an enjoyable and fruitful collaboration between myself and 49 other virologists flast.indd xxiv from around the world who contributed or revised chapters. Despite the small size of these algae and their genomes, twenty viruses have so far been Capsid isolated from both O. Formation of viral envelopes by budding is driven by interactions between viral proteins Viral envelope proteins inserted into a cell membrane tend to aggregate in local two-dimensional pools from which most of the cellular membrane proteins are excluded. Reassortment: the exchange of genome segments among different viral strains in viruses with fragmented genomes. NS3 623 Viral serine proteinase involved in polyprotein cleavage; RNA replicase component; nucleoside triphosphatase and helicase activities. Hantaviruses (Hantaan, Sin Nombre viruses) are transmitted by rodents and are not arthropod-borne. 10–12.3 kb. It is assembled from two major proteins, 1 and 2 (120 and 150 copies each, respectively), and minor core proteins 2 (probably 24 copies) and 3 (probably 12 copies), which constitute the viral RNA polymerase. messenger RNA appeared, and the genetic code was being cracked. Although both forms of HIV-1 may be present in an inoculum, only the R5 strain is sexually transmitted. Filoviruses 188 Heinz Feldmann, Division of Intramural Research, NIAID, NIH Hans-Dieter Klenk, University of Marburg Nicholas H. N F2 S S Fusion peptide C Extracellular medium F1 Plasma membrane or virus envelope C N F protein HN protein Cytoplasm or interior of virion Figure 15.4 Envelope proteins of paramyxoviruses. Early mRNAs are shown on left side, late mRNAs on right side (light blue lines). PB1 (light orange sphere with dotted outline) is probably also bound to the 5' end of genome RNA as it copies from the 3' end, and may block transcription at the oligo U region. Designation and naming of these steps is somewhat arbitrary but it is useful to help understand the important elements of the replication cycles of all viruses. Brügger, R. Following attachment to cell-surface carbohydrate (␣-linked sialic acid for type 3 reoviruses) and junctional adhesion molecule A (JAM-A), reovirus virions enter cells by clathrin-mediated endocytosis using a mechanism involving 1 integrin. Finally, transcription of the "late" genes (T1, 2, 3, 4, 7 and 8, and Tx) begins at about 6 hours after induction. The structures of virion termini are diverse: for Thermoproteus tenax virus 1 (TTV-1) (Figure 9.4), the termini are stubby tails; for Sulfolobus islandicus filamentous virus (SIFV), and Acidianus filamentous virus 3 (AFV-3), the termini taper and end in mop-like structures with six or three tail fibers, respectively (not shown); while for Acidianus filamentous virus 1 (AFV-1) (Figure 9.5), they have characteristic claw-like structures. Table 35.2 Milestones in antiviral vaccine development Pre-1700s Chinese doctors use powdered smallpox scabs to "immunize" intranasally. This distinguishes these viruses from the chloroviruses, which do not code for RNA polymerase, and therefore must depend on the cell's transcription machinery, which is normally located in the nucleus. NS1 352 RNA replicase component, possibly involved in replication complex formation. Insects are infected by ingesting occlusion bodies; infection spreads within the insect via budded virions In natural populations, insects become infected with baculoviruses by feeding on cadavers of infected insects or on leaves contaminated with occlusion bodies. The two subunits are held together by a cysteine bridge. 16/05/11 7:46 PM 10 CHAPTER 1 Introduction to Virology 1 mL transferred 1 mL 1 mL virus suspension 9 mL buffer dilution: 101 102 103 104 105 106 # plaques per Petri dish: 12 Average # plaques/mL: 12 PFU/mL of original virus suspension: 12 108 1.2 109 15 107 108 10 11 Figure 1.3 Plaque assay: an example. Entry Enfuvirtide (HIV) 3. Viral receptors have their own cellular functions, and are only fortuitously exploited by viruses. Low pH signals to the virus that it has reached an intracellular location and that the penetration reaction should now begin. One possible approach to treat Parkinson's disease would be to restore the production of dopamine by expressing the enzymes responsible for its synthesis. Chappell, Vanderbilt University v TOC.indd v 13/05/11 2:11 PM vi Brief Contents SECTION V: SMALL DNA VIRUSES OF EUKARYOTES 20. 16/05/11 8:01 PM 116 CHAPTER 10 Cucumber Mosaic Virus or triggering specific host responses. The prominent G protein spikes seen in the microviruses are not present in the gokushoviruses, nor is the external scaffolding protein. This opens up the possibility of disseminating DNAs that code for such RNAi in mosquito populations, perhaps by introducing them into transposons that will spread within natural populations of mosquitoes. This domain, located at the threefold axes of symmetry, forms protrusions called "mushrooms" on the surface of the virion, similar to those seen in some parvoviruses. Examples of drugs that were discovered this way are amantadine and acyclovir. Similarly, the E gene, which lies within the essential D gene, leads to host cell lysis; it can be considered a "luxury" gene, whose protein product is not required for replication, but increases the efficiency of release of newly replicated phage. RNA primers shown in orange. The protein pattern of new variant CJD is similar to that of BSE-infected animals, but quite different from that of sporadic or acquired CJD. There are two classes of oncogenic retroviruses: the acute transforming viruses, which transform cultured cells rapidly and induce cancer within a short time after inoculation into animals; and the nontransforming viruses, which induce cancer only after a long latency period. These protein-bound uridine residues hybridize to the poly(A) tail at the 3' end of viral RNA, and serve as a primer to initiate negativestrand RNA synthesis. Ten different class II promoters are used to initiate transcription over a 10-kb region of the T7 genome (Figure 7.1). 15.5a–b: Reprinted from Fig. Figure 36.4 shows three examples: acyclovir and ganciclovir, both active against herpesviruses, and azidothymidine (AZT), active against HIV-1. At this point no further virus is made, but release can take some time as cells slowly disintegrate after death. Examination of this structure revealed several unusual features for a viral envelope protein. The basic amino acids probably interact with the negatively charged phosphate groups of the genome RNA and may help to condense the RNA for encapsidation. Viruses with a single-stranded RNA genome that can directly code for viral proteins are called "positive-strand RNA viruses". Coxsackie B virus can infect heart muscle cells, causing severe and sometimes fatal damage in humans. Activated macrophages also release interleukin-12 (a mediator of T-cell differentiation), soluble protein lymphotoxins, and tumor necrosis factors, which induce apoptosis via specific cellular receptors (see Chapter 33, Figure 33.4). The process of integration of viral DNA into the host cell therefore has no obvious benefit for the virus, and can be considered as an "unplanned" byproduct of the infection process that has unfortunate consequences for the host. Because SARS-CoV can also infect certain cell types that lack ACE2, additional or alternate receptors are being sought. 10.9: Reproduced from DPV400 Figure 09 at ⬜g. ICP22 and UL13 activate the mitotic cyclin-dependent kinase cdc2, which in turn forms a complex with the viral DNA polymerase processivity UL5, 8, 52 UL30 UL42 Ori S RNA primer Ori S Ori L Figure 24.7 Establishment of bidirectional DNA replication. In principle, drugs can act by binding either to virions or to the cellular proteins to which virions attach (receptors). HIV-1 reverse transcriptase preferentially incorporates azidothymidine into DNA, leading to chain termination Azidothymidine (AZT; zidovudine) was the first drug approved for treatment of acquired immune deficiency syndrome (AIDS) caused by HIV-1, based on clinical studies showing that it could prolong life of patients with AIDS. However, 16/05/11 8:05 PM 190 CHAPTER 16 Filoviruses VP40 (matrix) VP24 GP (glycoprotein) VP30 NP (nucleoprotein) L, VP35 (RNA polymerase) (a) (b) (c) Figure 16.1 The structure of filoviruses. CH036.indd 454 16/05/11 8:21 PM Antiviral Chemotherapy KEY TERMS Acyclic Acyclovir Amantadine Azidothymidine Capsid-binding drugs Cell-based high throughput screen Enfuvirtide Ganciclovir Gangliosides Integrase Interferon Maraviroc Neuraminidase Nevirapine Non-nucleoside inhibitors Nucleoside diphosphate kinase Oseltamivir Peptidomimetic Pharmacokinetics Pleconaril Raltegravir Rational drug discovery Ritonavir Sialic acid Target-based high throughput screen Therapeutic index Thymidine kinase Thymidylate kinase Zanamivir FUNDAMENTAL CONCEPTS • Possible antiviral compounds are screened for activity by automated screening using infected cells or viral proteins as assays. In the vicinity of virus factories, the concentration of 3 may be high, so that any double-stranded RNA would be bound to 3 and PKR activation would be correspondingly low, allowing viral protein synthesis to continue. IB kinase complex (IKK): a serine-threonine protein kinase that phosphorylates IB, resulting in the dissociation and activation of NF-B. Viral protein VPg is covalently bound to 5' end. Early and abundant synthesis of the E protein would lead to premature cell lysis, before sufficient infectious progeny could be made. Occasionally, latently infected cells undergo a productive replication cycle as they traffic in the oral cavity and differentiate into plasma B cells. Cameron, Pennsylvania State University Howard Ceri, University of Calgary Jeffrey DeStefano, University of Maryland, College Park Rebecca Ferrell, Metropolitan State College of Denver Lori Frappier, University of Toronto Eric Gillock, Fort Hays State University Michael Graves, University of Massachusetts, Lowell Sidney Grossberg, Medical College of Wisconsin Tarek Hamouda, University of Michigan Medical Center Richard W. Adenovirus binding to cells is mediated by the fiber protein, which projects from the capsid. Depending on the threshold pH, conversion occurs 5–30 minutes after uptake into early and late endosomes, before the virus can be delivered to lysosomes. VIRUS STRATEGIES TO COUNTER HOST DEFENSES Viruses make proteins that mimic cytokines and cytokine receptors and interfere with host defenses Several large DNA viruses, including herpesviruses and poxviruses, possess genes coding for viral proteins that mimic cytokines or cytokine receptors. GENES AND PROTEINS Four nonstructural proteins (for viral RNA synthesis) translated directly from genome RNA. What is meant by the statement "the genome of hepatitis B virus is said to be an example of extreme efficiency"? In some cases, these invaginations are caveolae, which contain cellular proteins called caveolins and cavins. During translocation of the DNA into the prohead, the E shell subunits undergo rearrangement, causing the shell to expand and become more angular. The highly basic furin cleavage site of GP was mutated and an infectious virus clone was made by the multiplasmid system. Mimivirus has a unique mechanism for releasing its core Little is known of the precise molecular workings of the mimivirus infection cycle, but data are rapidly accumulating that allow a rough outline to be pieced together. This secondary structure is conserved among all hepadnaviruses despite many differences in the primary sequence. Can cause tumors in experimental animals but are not known to cause tumors in humans. Gajdusek was awarded the Nobel Prize in Medicine in 1976 for his work on spongiform encephalopathies. This fragment is missing N-terminal amino acids 23 to 89, but is otherwise identical in amino acid sequence and glycosylation pattern to PrPC. DNA replication can begin at either end of the linear DNA molecule (Figure 23.8). Baculovirus genomes carry repeated nucleotide sequences (hrs) that are transcriptional enhancers and may also function as initiators of DNA replication. Fusion by viral fusion proteins is therefore independent of metabolic energy in the form of ATP. Garrett, and David Prangishvili, "Viral Diversity in Hot Springs of Pozzuoli, Italy, and Characterization of a Unique Archaeal Virus, Acidianus Bottle-Shaped Virus, from a New Family, the Ampullaviridae," Journal of Virology 79 (2005), 9904–9911, with permission from American Society for Microbiology; Fig. These developments have led to constant surveillance of avian viruses circulating in poultry farms worldwide, coupled with attempts to produce a human vaccine that could protect against H5N1 and other highly pathogenic influenza virus strains. The L protein adds a methylated G cap at the 5' end of the growing mRNA chain, and elongates the chain until the end of the N gene. This assembled cDNA is transcribed in vitro by T7 RNA polymerase to produce a full-length genome RNA, which is introduced into permissive cells by electroporation. CH033.indd 405 Double-stranded RNA Secretion of interferon Transcriptional activation of antiviral genes Pro-Interleukin-1β 405 Figure 33.6 The interferon system. Thrips: a minute insect of the family Thripidae (order Thysanoptera) commonly found on plants, some of which are vectors for tospoviruses. the cytoplasm during genome replication, migrate to the plasma membrane where they interact with the matrix protein. Many chapters have text boxes that cover intriguing applications or recent developments in research. A bacterial cell is infected by a phage particle. The circularized DNA is localized near nuclear structures known as nuclear domain 10 (ND10), where early transcription takes place. • gene products direct viral DNA replication. 349 Frame 0 Figure 28.7 Ribosome frameshifting. Capsid: the protein coat that encloses a viral genome. These particles contain no core proteins, reverse transcriptase, or viral genome DNA. Not drawn to scale: the hairpin ends are 125 nt in length, and the entire genome is 5 kb long. Mouse L fibroblast cells were infected with reovirus strain T3D and examined 24 hours after infection using a confocal microscope. Electron micrograph of negatively stained vaccinia mature virus, showing surface ridges. These cellular genes (proto-oncogenes) code for growth factors, receptor proteins, protein kinases, or transcription factors involved in pathways that regulate cell growth and division. lipid bilayer that is at no time directly connected to a preexisting intracellular membrane. Human immunodeficiency virus and herpesviruses prevent these interactions by (1) blocking NK cell function, (2) preventing CH034.indd 424 the expression of cytokines that activate dendritic cells, (3) killing specific functional subsets of NK cells, and (4) changing the expression of specific NK cell receptors and coreceptors. The retinoblastoma protein (pRb), so-named because mutations in the Rb gene can lead to retinal tumors in humans, is one of the major cell-cycle control proteins. Since not all coronaviruses express HE, it is not considered an essential structural protein. Viruses of a particular genus tend to be transmitted by specific types of invertebrate vectors. Late proteins L1 and L2 form capsid. Apoptosis is a regulated form of cell suicide invoked when cells undergo extensive DNA damage, virus infection, or other insults. The best-understood mechanism involves an interaction between E1A proteins and the retinoblastoma (Rb) family of cellular tumor 16/05/11 8:11 PM 278 CHAPTER 23 Adenoviruses pRb p107 p130 p300 CBP CR1 N 289R TBP CR2 ATF CR3 poly(A) 13S mRNA N 243R CR1 CR2 poly(A) 12S mRNA 1000 500 1500 Nucleotides from left end of genome Figure 23.5 Major E1A proteins and interactions with cellular proteins. Small particles denoted "9S" and "6S" (named for their relative sedimentation rate in sucrose gradients) accumulate in cells infected with phage mutated in the internal scaffolding protein B. Nontranscribed sequences between genes are indicated by gray vertical bars; overlapping gene sequences are indicated by black vertical bars. Initial studies yielded promising results in adult monkeys; vaccinated animals displayed resistance to subsequent challenge with wildtype virus. KEY TERMS Aplastic crisis Erythema infectiosum Erythroid precursors Hemolytic anemia Nonpermissive cells Oncotropism Panleukopenia Permissive cells PKR Sickle cell disease Telomerase Tropism Uncoating FUNDAMENTAL CONCEPTS • Parvoviruses are among the smallest known viruses because their 5 kb DNA is single-stranded, and can therefore be packaged very compactly. Each protein subunit binds to 3 nucleotides of the single-stranded viral RNA. Compounds that prevent acidification of endosomes are often used as tools to study entry of acid-activated viruses. What is a "packaging signal" and what is its purpose during togavirus replication? HIV-1 strains that are most frequently transmitted between individuals can only infect cells that express CCR5. For example, CpG elements are short, unmethylated DNA motifs not commonly found in the human genome but often present in bacterial genes. Some viroids, and hepatitis delta virus, have enzymatic activity ("ribozymes": self-cleavage). Since Q replicase needs specific sequences at both ends of the RNA to replicate RNAs, half-molecules by themselves cannot be replicated. Under certain conditions, fragmented cellular DNA, resulting from cleavage during cell death, can also be packaged into capsids to form "pseudovirions". There are currently only four members in the avocado sunblotch viroid (ASBVd) group, also known as the Avsunviroidae Figure 31.1 Structure of viroid RNA. Which immuglobulin isotypes are these, and what is their structure? A short hydrophobic sequence, known as the fusion peptide, is found at one end of domain II, protected from the aqueous environment by the position of the neighboring E molecule within the dimer. In what is called Stage III replication, the viral C protein redirects plus-strand synthesis by binding to the replicative complex (shown in Figure 6.4) consisting of doublestranded DNA, the A protein, the rep protein, and DNA polymerase III. However, unscheduled DNA synthesis in fully differentiated cells is clearly not a normal cellular event; mechanisms have developed to eliminate such runaway cells from the body. Toll-like receptors signal through the adaptor proteins MyD88 and TRIF to activate kinases such as IKK and TBK-1. CH010.indd 124 16/05/11 8:01 PM C H A P T E R 1 1 Picornaviruses Bert L. The intergenic sequences between the transcriptional stop and start signals are always 3 nt long for Sendai 183 virus, but range from 1 to about 60 nt for other paramyxoviruses. Koster, A. PSV has a major virion structural protein of 33 kDa and two smaller structural proteins. For orthobunyaviruses, Gc is the major attachment protein for vertebrate cells, whereas Gn may contain the major determinants for attachment to invertebrate (mosquito) cells. • Structural proteins (capsid and envelope proteins) are synthesized by translation of an abundant subgenomic RNA. Other strategies rely on tumor-specific promoters to drive E1A gene expression or expression of cytokine or suicide genes to further improve the antitumor effects of replicative adenovirus vectors. Therefore, many complex diseases may obey a simple model of propagation in which the penetration of "seed proteins" into hosts determines spatial spread and disease progression. Graphics (1996), vol. Influenza virus transcription takes place in the cell nucleus, and uses newly synthesized cellular transcripts as a source of its capped primers; therefore, it is inhibited by agents that interrupt cellular RNA polymerase II transcription, such as actinomycin D or ␣-amanitin. The resulting accumulation of p53 could induce the early death of infected cells by apoptosis and severely limit virus production were it not reversed by other adenovirus gene products, particularly two E1B proteins. Parts (a–g) are electron micrographs of negatively stained virions, Part (h) is a computer-generated image derived from x-ray diffraction, Part (i) is a computer-generated image derived from cryoelectron microscopy, and part (j) is an electron micrograph of a thin section of infected cells. Interferon-␣ and - signal through the same receptor complex, which contains the interferon alpha receptor 1 (IFNAR1) and IFNAR2c proteins. Function in the mature virion is unknown. Because of their ability to interact directly with RNA, ribozymes are currently being developed as potential therapeutic agents for a wide range of diseases, including virus infections (human immunodeficiency virus, viral hepatitis, etc.), cancer, diabetes, cardiovascular disease, and osteoporosis. HIV-1 does not encode an enzyme that activates AZT by phosphorylation. A simpler and more quantitative application of cell lysis is to spread bacteria on the surface of nutrient agar in a Petri dish and to apply dilutions of a phage suspension. With this new drug delivery system, the possibilities are great: the delivery of genes encoding receptors for signaling molecules, membrane transport proteins, structural proteins, enzymes, transcription factors, and so on, can be used in a myriad of therapeutic applications. Two billion people worldwide have been exposed to hepatitis B virus. The virions carry two major structural proteins in nearly equal amounts. Genital herpes lesions are a common sexually transmitted disease, and chickenpox Over 100 known herpesviruses from many animal species. Olson, University of California, San Diego; Fig. • Retroviruses express three set of genes called gag (capsid proteins), pol (viral enzymes) and env (envelope proteins) on unspliced and singly spliced mRNAs. • Viral proteins are synthesized as polyproteins, which are subsequently cleaved by viral and cellular proteases to the individual viral proteins. Bunyavirus transcription is not sensitive to inhibition by actinomycin D but shares the same RNA capping strategy as influenza virus, which is sensitive to actinomycin D. The tail consists of two phage proteins, gp11 and 12, as well as six fibers made from trimers of gp17. Interaction is mediated by a carboxy-terminal domain of four amino acids (X-Ser/Thr-X-Leu/Val, where X any amino acid) on the E6 protein, and, most importantly, this is only found on those E6 proteins associated with the development of cervical cancer. One of the major target cellular genes activated is c-myc, which in turn activates many other cellular genes and can lead to unchecked cellular growth and division. Radially depthcued image created by Jean-Yves Sgro with software VMD with ambient occlusion. RNA-dependent RNA polymerase: an enzyme that synthesizes RNA using an RNA template. Can occasionally cause serious illness in certain individuals. Viroids in the Avsunviroidae family can cleave their multimeric linear RNAs in vitro in the absence of any proteins, yielding monomeric linear RNA molecules. A highresolution map showing a cross-section of the virion reveals concentric layers of density, from the outer layer of envelope proteins, through two layers of the lipid membrane, to the surface of the nucleocapsid. Hence, it is possible to generate AAV vectors with different cell tropis

has progressively expanded its presence into most of the United States and parts of Canada by virtue of its mosquito-to-bird transmission cycle. pRb and related proteins p107 and p130 repress transcription 16/05/11 8:09 PM 256 CHAPTER 21 Polyomaviruses from a large number of cellular genes when bound to members of the E2F family of transcriptional activator proteins. Cellular premessenger RNAs are rapidly spliced and exported to the cytoplasm, and therefore the pool of pre-messenger RNAs is quickly depleted when their synthesis is inhibited. 8. Second-generation adenovirus vectors. The three origins share extensive sequence homology and each contain a central palindromic sequence of 144 bp (oriL) or 45 bp (oriS) that can form a stem structure on single-stranded DNA. Naively, one would expect that most intact virus particles are infectious. Helical nucleocapsids. Endosome: intermediate membrane vesicles in the receptormediated endocytosis pathway. Tumor necrosis factor (TNF): a cytokine that activates the inflammatory response and can cause apoptosis. The cDNA was then transcribed in vitro and the RNA was introduced into cultured cells by transfection, leading to virus replication. This process triggers a rapid depolarization of the host cell membrane and the rapid release of potassium ions from the cell, probably directed by a virus-encoded potassium channel located in the virus internal membrane. The principle of genetic economy is an important factor that affects the evolution of designs for virus particles. These organisms can be genetically manipulated to express one or more viral proteins, and their intrinsic characteristics can be used to deliver vaccine antigens to desired locations (e.g., gut mucosa, dendritic cells). However, some are type II integral membrane proteins of the opposite polarity, with the C-termini facing outward and the anchor near the inward-facing N-terminus. Envelope is derived from host cell plasma membrane by budding. Many viruses cause chlorosis in plant leaves. A number of viruses (e.g., HIV-1 and adenovirus) require more than one type of receptor (Figure 4.2). The resistance elements are arrays of clustered, regularly interspaced short palindromic sequences, called CRISPR elements. Table 35.8 Some antiviral vaccines currently under development Virus family Virus species Most promising strategies Arenavirus Junin Live attenuated Lassa fever Recombinant vaccinia virus Calicivirus Norwalk Recombinant capsid "ghosts" Filovirus Ebola/Marburg DNA Recombinant vesicular stomatitis virus Flavivirus Hepatitis C Recombinant envelope glycoprotein, peptide, polypeptide Dengue Tetravalent attenuated, DNA Hepadnavirus Hepatitis B DNA, recombinant surface antigen including pre-S1/S2 regions; curative hepatitis B vaccine: DNA with CpG motifs Herpesvirus Epstein–Barr Whole inactivated, subunit (gp350) Herpes simplex 1, 2 Recombinant polypeptide, vectored Orthomyxovirus Influenza A Vectored, DNA, peptide Paramyxovirus Measles Vectored, DNA Parainfluenza Live attenuated Respiratory syncytial Subunit 6 and fusion protein, DNA, vectored, peptide Parvovirus B19 Empty capsid with VP1 protein Picornavirus Enterovirus 71 Whole inactivated Reovirus Rotavirus Live reassortants of simian or human origin Retrovirus HIV-1, 2 Recombinant polypeptide, DNA, vectored, chimeric UCtsadilised Hepatitis E Recombinant protein ORF2 Table 35.9 Severe adverse events associated with vaccination Vaccine implicated Event Smallpox ("lymph" vaccines) Sepsis due to bacterial contamination Yellow fever (1942) Vaccine lot contaminated with hepatitis B virus, leading to 28,000 cases of hepatitis B Inactivated polio (1955) Incomplete inactivation of virus, leading to 204 cases of paralytic disease (Cutter incident) Inactivated measles (1960s) Atypical (severe) disease upon exposure to natural measles infection Attenuated polio (Sabin) Vaccine-associated paralysis (~1 per 2.4 million persons vaccinated) due to reversion to more pathogenic strain Live vaccines (vaccinia, polio, measles) Dissemination and death in immunocompromised individuals Attenuated live measles Unexplained mortality in girls who received high titer formulations (relative risk of death doubled to age 5); more than 20 million doses of vaccine distributed Inactivated influenza A Apparent risk of Guillain Barré Syndrome (~1 per 1 million persons vaccinated), only in some years Rotavirus (1999) Intussusception —bowel folding and obstruction (vaccine withdrawn) CH035.indd 440 16/05/11 8:21 PM Adverse Events and Ethical Issues A great deal of energy is being expended internationally to refine the tools available for the surveillance of problems associated with vaccination. The various serotypes have different transduction efficiencies for different tissues. Activated PKR can also lead to activation of the important cellular transcription factor, NFκB. These viral proteins then direct replication of the viral genome. Some of the ribosomes translating ORF1a pause on a complex RNA structure known as a pseudoknot in the overlap between ORF1a and 1b, just upstream of the translation termination codon at the end of ORF1a. The sequence was used to design fragments that were commercially synthesized. Double-stranded RNA-dependent protein kinase (PKR). These contain both lipids and viral proteins, and are destined to become the mature virus envelope. Elliott, University of St. Andrews SECTION III: POSITIVE-STRAND RNA VIRUSES OF EUKARYOTES 18. AZT, like acyclovir and ganciclovir, is a nucleoside analogue with an altered sugar moiety. Sustained secretion of pro-inflammatory cytokines and their movement throughout the circulatory system lead to the characteristic hallmarks of a virus infection, including sleepiness, lethargy, fever, nausea, appetite suppression, and/or muscle pain. This suggests that the V protein normally interferes with some host mechanisms that reduce virus replication or spread. Amantadine blocks the M2 channel, inhibiting this process. He showed that warts on wild cottontail rabbits could be transmitted to domestic rabbits by inoculation with extracts of the warts. It is noteworthy that the transition from G1 to S phase is a crucial point in the cell cycle; once cellular DNA synthesis begins, the cell is committed to undergo mitosis. Several laboratories have generated transgenic mice that synthesize the hepatitis B virus X protein under the control of its own promoter. The immune response utilizes a variety of specialized immune cells in the circulatory system, and can be divided into two phases, the innate and the adaptive immune responses. A rare but fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML), is caused by JC virus, particularly in immunosuppressed individuals, including patients suffering from acquired immune deficiency syndrome (AIDS). Parvoviruses (Chapter 20) similarly contain one DNA replication gene and one capsid protein gene, although alternative initiation sites and splicing produce several proteins from each gene. Tonoplast: the membrane that surrounds the vacuole in plant cells. Satellite nucleic acids are presently classified based on the nature, length, and topology of their genomes. Many such products are currently under development or in field trials for a range of viral pathogens including measles, respiratory syncytial virus, influenza, HIV, dengue, and several herpesviruses. Work on this book began during a sabbatical year I spent in the laboratory of Steve Harrison at Harvard University. These human coronaviruses can cause repeated respiratory infections on an alternating 2- to 3-year basis in otherwise healthy hosts, and do not appear to induce long-lasting immunity. Twofold axis • Larger capsids with icosahedral symmetry usually are made from 60 T subunits, where T is the triangulation number; larger capsids may also have additional capsid proteins that strengthen the structure. Bottom: the end result, ritonavir. Within a section, smaller and simpler viruses are discussed first, then larger and more complex viruses. In some cases, these incidents can be explained by the biological properties of the product. Since virus binding is a prerequisite for infection, the presence or absence of an appropriate receptor is one of the most important factors determining which species and which cell types can be infected by a given virus. Numerous other acute transforming retroviruses contain a variety of oncogenes that are derived from cellular genes. M is involved in virus assembly and serves as a bridge between the nucleocapsid and the transmembrane envelope glycoproteins. The carbohydrate moieties (yellow-green) and glycosylated Asn and Ser residues (blue and gray, respectively) are shown as spacefilling atoms. They form dense, spatially and temporally extensive, monospecific blooms consisting of a mixture of colonial cells (within a gelatinous matrix) and unicellular cells that collapse suddenly in a virus-induced crash. This packaging reaction proceeds sequentially on each hexanucleotide sequence as the RNA is elongated. For example, mouse Mx1 in rodents blocks the replication of viruses such as orthomyxoviruses, which replicate within the nucleus, whereas cytoplasmic Mx2 in rodents blocks the replication of viruses such as rhabdoviruses and bunyaviruses, which replicate in the cytoplasm. The tail is attached at one vertex to a portal consisting of 12 molecules of gp8. Other hosts: cattle, dogs, deer, rabbits, monkeys, etc. T-cell receptor: a receptor on the surface of T lymphocytes that binds to specific antigens in association with major histocompatibility proteins, leading to activation of T cells and production of antibodies or cytotoxic T lymphocytes. Conserved regulatory sequences (Figure 15.4) that lie at the end of each gene signal termination of transcription of the gene, polyadenylation of the mRNA, and initiation of transcription of the next gene in line. For example, virus envelopes are fragile and can be disrupted, rendering the particle non-infectious. U A G-C CRNA tRNA G-C U-A U Pie Leu G-C G-C G-C G-C UUU UUG G-C 5'...CC AAA AAA CUC CAU AG-CCA...3' ----- process. The Rb protein is a major regulator of the cellcycle, and functions through its ability to bind to and repress the activity of a family of cellular transcription factors known as the E2F family (Figure 22.5). There is usually also a profound drop in white blood cell counts, and secondary bacterial infections can be found. However, horizontal gene transfer from the host has been predicted to contribute a minimal component of the mimivirus genome, although a lateral gene transfer has been suggested for at least one gene (see later). (a) Prototype reovirus gene segment cDNA in plasmid. DNA-dependent activator of interferon regulatory factors (DAI) is expressed in most cell types, and its levels dramatically increase following interferon signaling. For viruses that infect multicellular organisms, the problem becomes more complex. Viruses with double-stranded DNA or RNA genomes wind their own stiffer nucleic acid contents into regular coils. In addition to apoptosis, reovirus also causes cell-cycle arrest in infected cells. xxii flast.indd xxiii 13/05/11 2:23 PM xxiv Acknowledgements Michael Roner kindly agreed to write review questions that are placed at the end of each chapter in this edition. This kinase prevents apoptosis at both pre- and post-mitochondrial stages; its target is not known. Extracellular virus can be taken up by phagocytosis followed by rupture of the extracellular virus envelope due to a decrease in pH in the endosome, releasing a mature virus particle within the vesicle. Arrows inside circles show direction of rotation of the circular RNA through the RNA polymerase, which is shown here as stationary. CH037.indd 465 465 The major advantage of lentivirus vectors over other retrovirus vectors is their ability to transduce nondividing cells, which divide rarely or not at all in the body. However, some viruses can generate empty envelopes that bud in the absence of nucleocapsids; in this case, the envelope glycoproteins themselves drive budding. In extreme cases, virus particles can spread vast distances, and viruses in seawater and air may still have to travel extremely long distances inside the cell. Attachment of the adenovirus fibers to the primary CH004.indd 48 Many viruses enter the cell via receptor-mediated endocytosis 16/05/11 7:52 PM Virus Entry (a) (b) 49 vesicles, they move by microtubule-mediated transport into the perinuclear region of the cell, and they fuse with lysosomes. Contains unique regions, inverted repeat elements, and terminal direct repeat sequences. The preS1 domain does not contain any additional glycosylation sites, but it has a myristate residue at its N-terminus, a modification that serves to anchor the N-terminus in the membrane. Despite this, cytomegalovirus infection induces a roughly 30-fold increase in intracellular phosphorylated forms of ganciclovir. The new variant CJD, suspected to be transmitted to humans by consumption of BSE-contaminated beef, strikes mostly young people. Among the best examples of this approach are the cold-adapted influenza A vaccine (described above) and one of the chimeric rotavirus vaccines. This is translated into a single, long polyprotein that undergoes proteolytic processing to generate the individual proteins. This hypothesis formed the basis for studies seeking similar viruses in animals. However, downstream activation sequences, located beyond the transcription start site, are found in a number of 2 genes (Figure 24.6). The NS1 protein and other nonstructural proteins that follow E are not shown in their entirety. This twostage attachment strategy is similar to that proposed for several other viruses, including ϕX174, enteroviruses, adenoviruses, herpesviruses, and human immunodeficiency virus (Chapters 6, 11, 23, 24, and 29). Core shell protein 1 appears to possess RNA helicase activity and may locally unwind the double-stranded genome RNA to provide the polymerase access to the minus-strand template. VIRUSES AND HOSTS Three genera: • • Pestivirus, from Latin pestis (plague): infect pigs, cattle, other ruminants. Aquatic environments harbor large viruses The Phycodnaviridae (literally translated as "DNA viruses that infect algae") comprise a genetically diverse, yet morphologically similar, family of icosahedral viruses that infect marine or freshwater eukaryotic algae. The activation of macrophages by interferons, particularly interferon-, induces a strong respiratory burst resulting in the generation of toxic forms of oxygen that kill microbes and infected cells: these include nitric oxide (NO), superoxide (O-2), hydrogen peroxide (H2O2), singlet oxygen (O.), and hydroxyl radical (OH.). Classical activation pathway: an arm of the complement system that is triggered by antibodies bound to antigens on pathogens. Animal tests demonstrated that mice and guinea pigs were unaffected by exposure to baculoviruses; experimental or natural exposure of humans to baculoviruses caused no adverse health effects or signs of infection. Phosphorylated at Thr and Ser residues. After head maturation, the scaffolding protein dissociates, leaving T7 virions. CH027.indd 332 05/05/11 11:24 AM Coccolithoviruses 400 0 50 350 EhV-86 407,339 bp 300 100 150 250 200 Figure 27.9 Map of the genome of Emiliania huxleyi virus-86 (EhV-86). IE-0 is the only baculovirus protein known to be made as a result of RNA splicing; a small exon located about 4 kb upstream of the ie-1 start codon is spliced to the ie-1 open reading frame, producing a protein identical to IE-1 except for an additional 54 amino acids at its N-terminus. The RNAs of viroids in this family possess a highly conserved central region (C), and are not capable of self-cleavage. Rev. In such cases, the infected cells play an active role in transmitting the infection, with minimal exposure of the virus to the extracellular environment. Five different types of immunoglobulin molecules (IgM, IgD, IgG, IgE, and IgA) are synthesized by separate clones of B lymphocytes; each of these isotypes has its own individual functions in the adaptive immune response. CH030.indd 371 05/05/11 1:08 PM 372 CHAPTER 30 Hepadnaviruses only pregenome and preC RNAs have a second copy of the stem-loop near their 5ʹ ends. Despite incubation periods of months before symptoms appear, it was now possible to detect and to attempt to identify the infectious agent. Binding of virion structural proteins to cell surface receptors can initiate antiviral pathways. • Antibodies can protect against many virus infections and prevent reinfection, but in most cases the humoral response does not play an important role in recovery from virus infections. Ebolavirus species are named after their original site of discovery: Cote d'Ivoire, Reston, Sudan, Zaire, and tentatively Bundibugyo. They can serve as messenger RNAs for the C and P proteins, and can also serve as pregenome RNA (pgRNA), an intermediate in the synthesis of progeny viral genomes. Adenoviruses can be readily manipulated to allow expression of almost any gene by insertion of the coding sequences under control of a suitable promoter into the viral genome. These proteins also mediate cellular responses to many other cytokines and growth factors such as growth hormone, prolactin, and erythropoietin. In 1995, Michael Strauss reported the development of a baculovirus vector that could be used for efficient gene transfer into mammalian hepatocytes in vitro. The unusual symmetry-mismatched spikes of SH-1 seem to play a role in adsorption to the host cell. Five women and one girl, all victims of kuru, who were still ambulatory, assembled in 1957 in the South Fore village of Pa'iti. Production of viral proteins in bacteria, yeast, or eukaryotic cell expression systems. 3 Early viral proteins direct replication of the viral genome. However, these viruses may share immunogenic determinants with the target virus, resulting in an effective immune response against that virus. APOBEC3G is a member of a family of cellular proteins that deaminate cytidines (in RNA) or deoxycytidines (in DNA) to either uridine or deoxyuridine. Transcriptional activation occurs by binding of transcription factors to interferon gene enhancers Induction of interferon synthesis requires transcriptional activation of interferon genes, which are strongly ATF-2 c-JUN NF-B IRF-3 IRF-7 407 repressed in uninfected cells. Subsequently, envelope glycoprotein gD binds to at least two alternative receptors: nectin 1, a member of the nectin family of intercellular adhesion molecules; and herpesvirus entry mediator 16/05/11 8:12 PM Herpes Simplex Virus (HVEM), a member of the tumor necrosis factor (TNF) receptor family. Bound collectins are recognized by macrophages and other phagocytic cells and lead to uptake and destruction of the microorganism. Injection of similar virus 40 into newborn hamsters also produced tumors. The discovery and description of viruses of archaea (Chapter 9) is in its infancy. Despite vigorous worldwide efforts over the next 150 years to control smallpox with vaccination, in 1958 the disease was still endemic in numerous countries in South America, Africa, southeastern Asia, the Indian subcontinent, and China. Sanders, Purdue University Robert Sample, Mississippi State University Jeff Sands, Lehigh University Ann M. The mammalian reoviruses are the type species of the Orthoreovirus genus, which also contains viruses that infect birds and reptiles. Expression of proteins encoded by the 9-kb and 4-kb classes of mRNAs (Gag, Gag/pol, Env, Vif, Vpr, and Vpu) can then take place. These are driven by promoters (gray) that are recognized by cellular RNA polymerase II; mRNAs are exported to the cytoplasm where they are translated. Chapter 20). A variant form of this virus replicates preferentially in the respiratory tract. Epstein–Barr virus (a herpesvirus) encodes a small RNA that performs a similar function. Virus infection triggers synthesis of a large number of cytokines, several of which are important in coordinating the innate immune response, including interleukin-1, tumor necrosis factor (TNF), chemokines, and interferons, including interferon . al., "The structure and evolution of the major capsid protein of a large, lipid-containing DNA virus," Proc Natl Acad Sci USA, 99 (2002):14758-63, with permission from PNAS, Copyright (2002) National Academy of Sciences, U.S.A.; Fig. This sequence probably serves as a mimivirus-specific promoter similar to the familiar TATA box of higher eukaryotes. Arrowheads represent sites responsible for cleavage at specific sites on the polyprotein are denoted by arrows and symbols: see below for explanation of symbols. Explain why the best worldwide treatment for hepatitis B infections is an effective vaccination program. This expression system has the IPTG T7 RNA pol gene Host chromosome lac promoter T7 RNA polymerase T7 lysozyme T7 T7 rbs promoter Gene X pET vector Gene X mRNA Gene X protein Figure 7.4 Expression of foreign genes in T7-derived expression vectors. KEY TERMS 2', 5'-oligo(A) synthetase Apoptosis Caspases Cathepsins Cryoelectron microscopy Cyclin-dependent kinase (CDK) Double-stranded, RNAdependent protein kinase (PKR) Encephalitis Ependymal cells Hydrocephalus Inclusion bodies CH019.indd 235 Infectious subvirion particles (ISVPs) Interferons Jaundice Junctional adhesion molecule A (JAM-A) Myocarditis NF-B (nuclear factor-B) Ras Reassortant viruses Ribonuclease L Sialic acid Tight junction Trypsin 16/05/11 8:07 PM 236 CHAPTER 19 Reoviruses REVIEW QUESTIONS 1. First, different amounts of each protein are needed. Interferon mRNAs contain AU-rich elements that render them unstable, and the transcriptional activation of interferon genes is short-lived. • Adeno-associated virus vectors have recently shown some success in treating a congenital disease that results in blindness in children. • Retrovirus vectors can integrate viral DNA containing a foreign gene into target cells and can be adapted to infect specific cell types by expressing specific envelope glycoproteins. Because these viruses circulate between vertebrate and arthropod hosts, introduction of engineered viruses into mosquito populations could involve substantial risks to the vertebrate host. Virus infection has little effect on the invertebrate host even though the virus causes a systemic infection. receptor protein, CAR, triggers fiber dissociation. How they are formed is not well understood. Helical symmetry: symmetry defined by rotational and translational displacement about a single helix axis. This "rule of six" was discovered by making artificial "minigenomes" containing the two ends of the genome but lacking most internal coding sequences. This process can be visualized by electron microscopy of actively transcribing cores, which reveals multiple RNA transcripts emerging from core particles. State two problems faced by viruses with RNA genomes, and explain how the virus addresses these challenges. Below are shown functional domains of large T antigen; see text for details. 20.5: Reprinted from Fig. The group that developed ritonavir then made a correspondingly symmetrical inhibitor in which the proline was replaced with phenylalanine, and the peptide bond between the two phenylalanines was modified by converting the normal C:O to CHOH, creating a hydroxyethylamine (A-74702 in Figure 36.9). Similarly, why is only the right-hand LTR used for signaling cleavage and polyadenylation of the transcript? Class I gene products are needed for expression of the remainder of phage genes and to shut down host cell functions. p53 is a transcription factor that both activates and represses transcription, and regulates the progression of the cell cycle. Therefore, direct transmembrane protein interactions between the nucleocapsid and the glycoproteins may be involved in budding of bunyaviruses. Mature virions can be observed in host cells prior to their release. These nonnucleoside inhibitors bind to reverse transcriptase at a site close to the active site for DNA polymerase activity, and they drastically slow the rate of DNA polymerization. Adenovirus genomes can be seen as constructed from modular units similar to the early and late transcription units of the smaller and simpler polyomaviruses (Chapter 21). The first line of defense against virus infections is the interferon response (Chapter 33), triggered by the synthesis of double-stranded RNA (dsRNA) in infected cells. Coronavirus HE can cleave an acetyl group from sialic acid, resulting in the release of bound virus from the cell surface. This suggests that archaeal viruses may depend on the host cell replication machinery but can modify it to promote selective replication of viral DNA, as do some of the small DNA animal viruses (parvoviruses, polyomaviruses, and papillomaviruses, Chapters 20, 21, and 22). The broad end 16/05/11 8:00 PM 104 CHAPTER 9 Viruses of Archaea (a) (b) (a) (b) Fibers Core Packed circular genomic DNA (c) (c) Figure 9.6 Sulfolobus neozealandicus droplet-shaped virus (SNDV), family Guttaviridae. The MS protein contains an additional 55 N-terminal amino acids encoded by the preS2 domain (Figure 30.3). In other words, the ϕX174 internal scaffolding protein can productively interact with the coat, spike and external scaffolding proteins of phage G4 to build procapsids. Binding of large T antigen to these sites can interfere with the binding of RNA polymerase and general transcription factors such as TFIID, reducing the amount of early RNA synthesized. These compounds are toxic to DNA polymerase, so must be phosphorylated before they can be utilized. Apoptosis and the property of being positively supercoiled when isolated from virions, and were one of CH009.indd 101 Lytic Virion image Icosahedral head, flexible tail the first examples of such DNA found in nature. This is best illustrated by the conversion of the benign feline enteric coronavirus into lethal feline infectious peritonitis virus. Introduction of virus vectors into host cells or organisms can correct genetic diseases in which specific gene products are missing or defective. (b) 3D reconstruction of virion of Haloarcula hispanica virus SH-1. This strategy allows a relatively large genome to be packaged as distinct segments in small and simple virions. Furthermore, lysis of virus-infected cells can release viral double-stranded RNA into the extracellular medium. These data strongly suggest endemic foci for Marburg and Ebola virus in an area lying between the 10th parallels north and south of the equator in Central Africa. Unlike the Fuselloviridae, neither His-1 nor His-2 are believed to integrate their DNA into host cells, and their plaques are turbid. Viral mRNA synthesis is primed by stealing capped 5' ends of cellular pre-mRNAs in the nucleus. III extends the new 3' end created by cleavage of the plus-strand, and thereby synthesizes more plus-strand DNA. Vaccines and gene therapy. Receptor binding triggers fusion of the viral envelope with a cellular membrane. Envelope glycoproteins form stable trimers or tetramers in the plasma membrane. Their 28-nm diameter, non-enveloped T = 1 icosahedral capsid (Figure 20.1) resembles the capsid of the single-stranded DNA bacteriophage φ 174 (Chapter 6). The pregenome RNA starts just downstream of the beginning of the preC open reading frame and therefore this region is not translated in pg RNA (Figure 30.3). A number of small unicellular organisms including chlamydiae and rickettsiae, certain other bacterial species, and some protozoa can multiply only inside other host cells. EBNA-1 binds to multiple sites within oriP and directs replication of the viral genome, which usually replicates only once per cell division during latent infections. (Figure 24.1). RNA sequences in the 5' and 3' coding and noncoding regions of each genome segment are required to incorporate the segment into virions. In this model, capsid proteins bind to genome RNA in a complex that is not a structured nucleocapsid. Certain parvoviruses can integrate their DNA into a specific region of a human chromosome, in a manner reminiscent of site-specific integration of bacteriophage lambda (Chapter 8). DISEASES Hepatitis: incubation period 30–180 days. Crenarchaeota are hosts for seven distinct virus families, based on virion morphology and genome sequences. Therefore h 1 and k 1, and T 1 ≥ 12 ≥ 12 ≥ 3. KEY TERMS Adenoid Apoptosis Capsid Chromatin remodeling Cyclin-dependent protein kinase Hexon Histone acetyl transferases Histone deacetylases (HDACs) Immunoglobulin gene superfamily Integrins Interferon Inverted terminal repeat Oncogene Penton base PKR Retinoblastoma (Rb) Ribosome shunting Secondary receptor Terminal protein Tumor suppressor protein Ubiquitin FUNDAMENTAL CONCEPTS • Many human adenoviruses bind to both a primary receptor (CAR) and a secondary receptor (integrin), which leads to uptake in endocytic vesicles. Adenovirus transcription units are interspersed in such a way that virtually all of the viral genome is utilized for coding proteins on one or the other DNA strand. Weiner, Molecular Biology of the Gene, 4th ed., The Benjamin/Cummings Publishing Company, Inc. 26.4b: Copyright The Rockefeller University Press. Eleven proteins in virion. Self cleavage by the ribozyme generates authentic viral 3' ends. Once in the cytoplasm, miRNA precursors are further processed to mature miRNAs by Dicer, the enzyme involved in the generation of siRNAs. The processed miRNAs then associate with the RISC complex. Such syncytia can form both in cells cultured in vitro and in tissues of infected animals. The CI repressor is a mimic of LexA; both consist of two globular domains connected by a linker segment. 16/05/11 7:54 PM CHAPTER 5 Single-Stranded RNA Bacteriophages RNA secondary structures. They are needed, after DNA synthesis begins. At the same time, E2F activates both viral genes E2A and E2B by binding to a site near their common promoter. Among these newer approaches, chimeric live viruses share the strengths and weaknesses of the live attenuated vaccines. Liver and heart. Binds to viral DNA; interacts with cellular transcription factors. Live attenuated viruses, inactivated whole viruses, or purified viral proteins ("subunits") are inoculated into the host. A considerable majority of viral genes present in sequenced genomes of archaeal viruses have no detectable homologs other than in closely related virus species. A single molecule must survive during infection long enough to be translated and then replicated at least once. • Epstein–Barr virus encodes two small RNAs, transcribed by cellular RNA polymerase III, which inhibit PKR and enhance cell growth. CH018.indd 219 219 PB1-F2 may contribute in suppression of the host immune response A novel alternate reading frame that encodes the 87-90 amino acid PB1-F2 protein has recently been discovered in the PB1 polymerase gene segments of certain influenza A virus strains. By 1964 it became clear that cells cultured from the tumor biopsies contained herpesvirus-like particles; this new herpesvirus was named Epstein–Barr virus. These viruses were first classified as CH016.indd 189 189 rhabdoviruses, but differences in virion morphology and conserved protein sequences led to the establishment of a new family, the Filoviridae, a name based on the elongated shape of virions. A BRIEF HISTORY OF ANTIVIRAL VACCINES and occasionally reversed by waves of destruction wrought by viruses such as measles, yellow fever, and smallpox. These viruses have a double- or triple-layered capsid with icosahedral symmetry; the outer layer is removed during entry into the cell, activating transcription in the core. Host responses to cucumovirus infections reflect both a battle and adaptation between viruses and hosts Plants often exhibit different responses to infection with cucumber mosaic virus. Like 2', 5'-oligo(A) synthetase, PKR is activated by double-stranded RNA. Contains a number of virus proteins. To Gustav-Adolf Martini of the Department of Internal Medicine at the University of Marburg it was immediately clear that he was faced with an unusual infectious disease. Parvoviruses have repeatedly been shown to reduce the formation of tumors that arise spontaneously in animals or after treatment with chemicals or other viruses. Members of the immunoglobulin family have typical domains shown as green spheres. Bax and p21 are two examples of such genes. The target size for inactivation is several orders of magnitude lower than had ever previously been found for a virus, meaning that if there is a nucleic acid genome, it is unusually small. B cells that recognize and bind to antigens differentiate into plasma cells, which secrete antibodies. Both of these methods allow scientists to purify supercoiled DNA away from other forms. Subsequent images show channel formation and entry of the end of the DNA (black), followed by entry of the remainder of the DNA. Chapter 11 Fig. Becker Coronaviridae From Latin corona (crown), referring to spikes projecting from envelope VIRION Spherical enveloped particles studded with clubbed spikes. Abundant smaller spherical and filamentous forms lack nucleocapsid and are not infectious. RNA movement does not involve microtubules, although tobacco mosaic virus movement protein does bind to and colocalize with a microtubule-associating protein at a later stage of infection. All T7 genes are transcribed from left to right on the map as shown. Upon induction, these ends must be opened and joined to recreate the telRL site. and Fauci, A.S., "New concepts in the immunopathogenesis of HIV infection," Annual Review of Immunology (1995) 13: 487–512. (b) Cryoelectron microscopy image of virion of herpes simplex virus type 1. Transmembrane anchor: a domain of 20 hydrophobic amino acids that is embedded in a lipid bilayer and therefore anchors a protein in the membrane. But many viruses with RNA genomes smaller than 10 kb have non-enveloped capsids, while the capsids of many viruses with larger genomes (Flaviviridae, Togaviridae, Arteriviridae, Coronaviridae, and Roniviridae) are enveloped; envelopes may afford additional protection to the viral genome. As these RNAs developed and evolved, some of them may have acquired the property of binding covalently to a limited variety of amino acids, a role presently carried out by transfer RNAs and amino acid-activating enzymes. The secondary structure formed between the upstream complementary sequence (UCS) and the Shine–Dalgarno sequence (SD) does not allow ribosome binding. The initial human cases were widely reported in the press, leading the public to fear a widespread epidemic. Most genes produce a single mRNA and a single protein. VIRUSES AND HOSTS Over 100 known human papillomaviruses. Adjuvant systems Oil-in-water emulsions Elicit strong humoral response without major toxicity. Virus lacking Vif enters cells normally but generates a lower level of proviral DNA than wild-type virus. Flexible tube-shaped tail, 135 nm long, with terminal fiber. As a result, more single-stranded DNA becomes exposed on the complementary strand at each replication fork. Most retroviruses (with the exception of lentiviruses) have no means of entering an intact, interphase nucleus. These diseases include avian influenza; foot-and-mouth disease of cattle; infectious gastroenteritis and bronchitis in pigs, cattle, and chickens; sheep lung tumors caused by a retrovirus; canine distemper; and feline immunodeficiency virus. (a) Messenger RNA synthesis. The progeny virions, and more rarely the virus present in the initial inoculum, infect nerve endings and undergo retrograde transport along axons to the cell bodies of dorsal root neurons innervating the site of viral entry. Effects wild and domesticated ruminants (sheep and goats: scrapie; cattle: mad cow disease); mink, cats. (bacteriophages) could lead to understanding of the basic processes of life at a molecular level. Dermody, Vanderbilt University James D. Middle: location of the Autographa californica MNPV late expression factors and a set of overlapping cloned DNA fragments (A–M) used to transfect cells. Human bocavirus may be a cause of childhood pneumonia; however, more work is needed to establish the role of human bocavirus in respiratory illnesses. Regulation of mRNA transport by Rev requires two classes of sequences on viral RNA: (1) cis-acting (a) No Tat RNA Pol II CTD Cdk9 Cyclin T TAR RNA Tat (b) Tat PPP PPP PPP Figure 29.5 Mechanism of Tat function. When the particle can be crystallized, as is the case for many non-enveloped virions, a very detailed model can be obtained from x-ray diffraction data (e.g., simian virus 40; Figure 2.1b). There are at least three different mature virus membrane proteins that may facilitate attachment, and these each bind to ubiquitous cell surface glycosaminoglycans, consistent with the broad host range of the virus. Two mechanisms for entry of extracellular virus particles have been described. In cultured cells, reovirus-induced apoptosis does not require de novo synthesis of viral RNA and protein, indicating that an inducer of apoptosis is contained within infecting virions. CH036.indd 452 Cbz-Val-HN NH-Val-Cbz OH A-74704 Me S Me N Me O N N H Me Me H N O O N OH S N Ritonavir Figure 36.9 Steps in the development of ritonavir. The E1 polypeptide comprises most of the protein shell or "skirt" that covers the underlying lipid membrane (Figure 13.1b); its structure is similar to that of the flavivirus envelope protein (Figure 12.3). CH037.indd 469 16/05/11 8:22 PM CH037.indd 470 16/05/11 8:22 PM G L O S S A R Y -amanitin: a cyclic octapeptide produced by the fungus Amanita phalloides, which binds to and inhibits eukaryotic RNA polymerase II. Noble Research Institute Dalius J. MicroRNAs (miRNAs): short RNA molecules of approximately 22 nucleotides that bind to complementary sequences of target mRNAs, leading to degradation or translational repression. Detergent treatment of virions releases the nucleocapsids, which can be purified and visualized by electron microscopy (Figure 15.1b). The model in Figure 11.7 illustrates the steps postulated to lead to viral RNA synthesis. Nanoparticle vectors such as virus-like particles can elicit very powerful immune responses, particularly when viral antigens are combined with immunostimulatory molecules such as ligands for pathogen-recognition receptors (see Chapter 33). 7 in J.A. Lemm et al., "Polypeptide requirements for assembly of functional Sindbis virus replication complexes: a model for temporal regulation of minus and plus strand RNA synthesis" European Molecular Biology Organization Journal 13 (1994): 2925–2934, by permission of Oxford University Press; Fig. Oral Epithelium Resting B lymphocyte Lymphoid Tissue Bloodstream: Memory B Cells Plasma Cells Latency stage: Latency III Latency II Latency I Replication Viral gene expression EBNA1, 2, 3A, 3B, 3C, LP LMP1, 2A, 2B Small viral RNAs EBNA1 LMP1, 2A, 2B Small viral RNAs Lytic Genes Model cell types EBV-immortalized lymphocytes Nasopharyngeal Carcinoma Burkitt's Lymphoma Figure 24.11 Overview of events during infection by Epstein–Barr virus and stages of latency. These drugs inhibit attachment and/or entry at different steps depending on the drug-virus combination. Virus-induced release of cytokines may increase endothelial permeability and may be a major factor in the shock syndrome regularly observed in severe and fatal cases. This protein may play a role in targeting of movement protein to plasmodesmata. • Vpr retains infected cells in the G2 phase, increasing virus production. The resulting error rate during reverse transcription (1–10 mutations per proviral DNA molecule synthesized) renders HIV-1 infections a moving target for both the immune system and drug treatment strategies. Hepatocytes freshly explanted from duck liver support duck hepatitis B virus infection, but there are currently no in vitro cultivated cell lines that can be productively infected with hepatitis B virus. Illustrated are the two most common capsid morphologies: a roughly spherical shell (left) and a tubular rod (right). 159 CH014.indd 159 16/05/11 8:04 PM 160 CHAPTER 14 Coronaviruses Coronaviruses cause respiratory illnesses in humans and important veterinary diseases The search for viruses that cause upper respiratory infections in humans (the "common cold") was intense in the mid-1960s. However, because of the dramatic course of the disease, the high case/fatality rates, and the lack of effective vaccines and therapeutic measures, re-emergence of these viruses regularly causes high public concern. The discovery of small, self-replicating RNA pathogens changed this view. Cleavage at its N-terminus occurs in the lumen of the endoplasmic reticulum, but cleavage at its C-terminus is carried out in the cytoplasm by the NS2B/NS3 protease. Concatemer: a linear or circular oligomer of nucleic acid genome sequences produced by rolling circle replication and/or recombination. Emergence of the SARS coronavirus provided a dramatic demonstration of the adaptability of coronaviruses, and further showed that they can cross the species barrier with sometimes devastating results. These gene functions are therefore implicated in processing concatemers to unit-length molecules in preparation for packaging into phage heads. The cleavage of complement components C3 and C5 also generates small complement fragments C3a and C5a, which are called anaphylatoxins. KEY TERMS Antibody Apoptosis Chimera Chemokine Cytokine Helper virus Inverted terminal repeat Ligand Macrophages Packaging signal Polyadenylation signal Promoter occlusion Pseudotyping Ribozyme Silencing Transcriptional enhancer Transgene Tropism Virus vectors 469 FUNDAMENTAL CONCEPTS • Virus vectors are highly efficient means of expressing foreign genes within specific cell types. However, insensitivity of antigenome synthesis to -amanitin remains unexplained by this model. Many mRNAs contain signals within their 3' untranslated region that regulate translation or RNA stability. 4 also contain RNA 3. Spikes at fivefold symmetry axes are shown in blue. Only the plus strand is shown here. In today's terms, it is stunning that pre-exposure prophylaxis had been demonstrated in only 50 to 60 dogs before the first human, a preteen boy named Joseph Meister, received this vaccine in 1885. Virus entry, pathogenesis, virus release. Reporter gene: a gene coding for a protein that can be detected within cells easily and quantitatively. 16/05/11 8:09 PM Polyomaviruses regulatory role in several cellular signaling pathways by removing phosphate groups that stimulate the activities of proteins in those pathways. Simian immunodeficiency virus mutants with deletions of the Nef gene are viable, but have lower titers and fail to induce disease in infected monkeys. In contrast, many enveloped viruses form virions at the cell surface, where they acquire a lipid envelope, and therefore newly assembled virus particles are immediately released into the medium. T7 has a logical arrangement of genes that could have been designed by an engineer: three groups of genes are arranged in continuous blocks from left to right on the genome (Figure 7.1). This virus, subtype H2N2, had a new hemagglutinin gene and was the cause of the "Hong Kong" influenza pandemic, during which H2N2 viruses disappeared completely from people. Viruses need the following machinery provided by cells: • • • • Enzyme systems that synthesize amino acids, nucleotides, carbohydrates, and lipids Enzyme systems that generate useable chemical energy in the form of ATP Ribosomes, tRNAs, and enzymes used in protein synthesis Membranes that concentrate cellular macromolecules, small molecules, and ions WHY STUDY VIRUSES? Naïve lymphocytes enter from blood via capillaries. This timing reflects the rapidity of the activation of these emergency response genes. GENOME Linear double-stranded DNA, 40–60 kb. Binding of double-stranded RNA by TLR-3 only occurs at low pH, indicating that binding occurs within endosomes. When released from the host cell, it is lemon-shaped, with an overall dimension of approximately 120 300 nm (Figures 9.11b, c). In the future, it will be possible to synthesize therapeutic proteins directly in the cells of an individual. Phospholipids in membranes of archaea use ether linkages to join the glycerol phosphate polar ends and the non-polar lipid ends, which can contain cyclic moieties and branched hydrocarbon chains. However, infection with the lymph nodes persists throughout the course of the disease. Herein arrows are shown as interruptions connected by a V. A major global pandemic today (more than 33 million people infected). Envelopment and egress: three possible routes Most enveloped viruses bud from cytoplasmic membranes or the plasma membrane to form virions. • A recent anti-HIV drug, raltegravir, inhibits viral integrase. and Garman, E., "The origin and control of pandemic influenza," Science 293: 1776–1777, copyright 2001 AAAS. 05/05/11 2:45 PM Glossary Reverse transcriptase: an enzyme that synthesizes DNA using an RNA template: also an RNA-dependent DNA polymerase. Masaki Hayashi and colleagues defined the ϕX174 assembly pathway, and demonstrated that viral DNA can be packaged into capsids in vitro. This requires that they deliver their DNA genomes to the nucleus, where these enzyme systems are located. As a result, all three surface proteins contain a common S domain in their C-terminal portion (Figures 30.3 and 30.4). Future prospects for diagnosis and treatment of diseases caused by papillomaviruses The cervical smear test (Pap test, named for its inventor, George Papanicolaou) has proven to be a very effective means of reducing the incidence of death caused by cervical cancer in developed countries. Since the late 1970s, the AcMNPV-Sf virus-cell system has become the model for the study of baculoviruses. Pseudotype: see Pseudotyping. • Human prions are encoded by a host cell gene. on the surface of an infected cell (Figure 34.8). EF-Tu positions charged tRNAs on the ribosome during elongation of growing peptide chains. CH007.indd 84 What attracted scientists in the 1960s to the T7 phage as a prototype for research? Bcl-2 reverses this effect by binding to Bax, preventing oligomer formation. Before this step can occur, capsid proteins must reach the nuclear pore. The 2-kb and 1.45-kb RNAs are probably spliced from the 8.3-kb LAT transcript, but very little full-length transcript accumulates. Emerging avian influenza virus strains threaten domestic fowl and another possible human pandemic. The A gene overlaps with genes A*, B, and K. The two ends of genome RNAs are therefore maintained in close proximity. Some viral genomes are circular and others are linear. Other adjuvants that are being tested interact with toll-like receptors (TLRs), crucial signaling molecules that elicit strong innate responses to pathogens and also help to stimulate adaptive immune responses (see Chapter 33). n atio orm df Clou Cloud condensation nuclei Cloud Sulphate aerosol Climate negative feedback DMS Less sunlight to fuel phytoplankton growth AIR SEA Viruses E. Antiviral drugs are targeted to specific steps of virus infection As outlined in Chapter 1 and detailed in most chapters in this book, the infection of cells by viruses can be broken down into a common set of steps or stages (Figure 36.1). Viroid genomes are single-stranded, circular RNAs from 250 to 400 nucleotides long that do not code for any proteins and are not encapsidated. A surprising finding was that the 2-kb and 1.45-kb species are stable, unresolved lariat structures that accumulate in the nucleus. During the decade of active spread of H5N1 virus in animals and birds, there was increasing concern that mutation of these viruses could lead to human-to-human spread and a major influenza pandemic (widespread human epidemic). The nucleocapsid is formed from multiple copies of the nucleocapsid protein (N) bound to the viral RNA genome. Viral DNA introduced into cells during infection accumulates at nuclear bodies known as ND10 or PML structures, as they contain the promyelocytic leukemia protein, PML. Most proteins are packaged in the virion: • Nucleocapsid protein (N) RNA polymerase cofactor and accessory proteins (P/C/V) Matrix protein (M) Fusion protein (F) Hemagglutinin/neuraminidase, or envelope glycoprotein (H, HN, or G) RNA polymerase (L) • • • • • GENOME Single segment of linear ssRNA, negative sense. Tens of thousands of research papers have been published on the subject in the past 20 years. Viroceptor: a virus-coded protein that is a homologue of a cellular cytokine receptor. The host cell nucleus (and the chromosomes contained within), mitochondria, and the chloroplast remain intact throughout this period. Recently, antiviral agents active against rhinoviruses have been developed (see Chapter 36) and there is a large potential market for an effective and inexpensive agent against the common cold. • Disease outbreaks are self-limiting, because human-to-human transmission requires intimate contact with patients and is inefficient. (a) If a capsid contains certain multiples of 60 subunits, all these subunits cannot have their neighbors in nearly equivalent ways. Binding to a chemokine receptor triggers fusion between the HIV-1 envelope and a cellular membrane, leading to virus entry. These include the host species of origin (e.g., bovine papillomavirus, simian virus 40, porcine parvovirus), the locality where the virus was first isolated (e.g., Semliki Forest virus, Ebola virus, Norwalk virus), or the disease caused by the virus (e.g., measles virus, influenza A virus, hepatitis B virus). Highly successful vaccines help control the spread of measles, mumps, rabies, and influenza viruses. Collectively, 16/05/11 8:19 PM 424 CHAPTER 34 Innate and Adaptive Immune Responses to Virus Infection these proteins are known as virulence factors, as they are important for the virus to establish and maintain an infection within its host. Natural killer (NK) cells Recognize abnormal levels of self and signal antigen on cell surface MHC; kill by inducing apoptosis. In all cases to date, the bats from which the coronaviruses were isolated were not ill, and none of the viruses have been cultured from primary isolates in the laboratory. However, it may play a role similar to the M2 protein of influenza virus, and serve as an ion channel that allows entry of hydrogen ions into the particle to promote disassembly. CH027.indd 336 It was then shown to be a virus that replicates in an amoeba, and so was named after its host, Acanthamoeba polyphaga mimivirus. These genes have never been found in a virus; they are more commonly seen in animals and plants. Studies of these viruses led to the discovery of viral oncogenes, whose protein products (tumor antigens) interact with numerous cell signaling pathways to stimulate cell growth and division. Virus particles have a single mission: to mediate the transport of the viral genome from an infected cell to an uninfected cell, thus allowing the spread of infection from cell to cell and from organism to organism. Togavirus nucleocapsids are permeable to ribonuclease in vitro, suggesting that the RNA in nucleocapsids may be accessible to other proteins within the cytoplasm. The purified virus is then subjected to inactivation by treatment with formaldehyde, organic solvents, detergents, or high temperature. Cellular tRNALys3 molecules packaged in virions used as primers for reverse transcription. This exposed primer binding site (PBS) can hybridize with its complementary PBS' sequence at the other end of the growing minus-strand DNA. DNA packaging is complete when a sufficient length of the DNA is inserted and when the packaging machinery encounters the next a sequence in a manner similar to that of bacteriophage T4 and other phages. Sequence identity among satellite RNA variants is greater than 73%. 13.1: Courtesy Richard Kuhn, Purdue University; Fig. Green: unknown function; blue: DNA polymerase; red: viral capsid protein VP1. These vesicles are required for picornavirus RNA synthesis and may serve as a nucleation site for the formation of replication complexes. That is, a phage whose normal host is bacterial species A might recombine in intermediate species B with a phage whose usual host is species C, leading to the swapping of a block of genes between the phages. Nucleoside analogues will usually be toxic for host cells if they can be phosphorylated by host cell enzymes and if the phosphorylated form can inhibit host enzymes CH036.indd 448 such as DNA polymerases. Viruses of Algae and Mimivirus 325 SECTION VII: VIRUSES THAT USE A REVERSE TRANSCRIPTASE Aquatic environments harbor large viruses 325 Phycodnaviruses are diverse and probably ancient 326 Phycodnaviology: a field in its infancy 326 Conserved structure, diverse composition 327 28. These viruses then release the virion or its genome into the cytoplasm, and in some cases the genome is transported to the nucleus. Because reovirus replicates in humans but is largely nonpathogenic, this virus is now being tested in clinical trials as therapy for a wide array of human cancers. This involves copying of part of one template RNA, followed by dissociation of the replicase and the growing RNA chain, and association to a second template RNA to continue RNA synthesis then continues, generating a progeny RNA containing sequences from two different parental RNA molecules. Transcriptional promoters are shown at the left; proteins coded by each mRNA are shown at the right. VP35 (virion protein, 35 kDa) Phosphoprotein, associated with the nucleocapsid; required cofactor for viral RNA polymerase activity; interferon antagonist. Myocarditis: disease involving inflammation of the muscular walls of the heart. Some gene products are required for the subsequent transcription of early or genes, which are expressed between about 4 and 8 h after infection. Parvoviruses 238 Parvoviruses have very small virions and a linear, single-stranded DNA genome 238 Parvoviruses replicate in cells that are going through the cell cycle 239 Discovery of mammalian parvoviruses 239 Parvoviruses have one of the simplest-known virion structures 239 Single-stranded parvovirus DNAs have unusual terminal structures 240 Uncoating of parvovirus virions takes place in the nucleus and is cell-specific 240 DNA replication begins by extension of the 3' end of the terminal hairpin 241 The DNA "end replication" problem 241 Steps in DNA replication 243 Nonstructural proteins have multifunctional 243 Adenovirus functions that help replication of adeno-associated virus 244 In the absence of helper virus, adeno-associated virus DNA can integrate into the cell genome 244 Parvovirus pathogenesis: the example of B19 virus 244 21. Researchers who first extracted DNA of adenoassociated virus were puzzled by this, because the capsids of adeno-associated virus contain either plus or minus strands, and some particles contain double-stranded and therefore capsids of adeno-associated virus should contain twice as much DNA as capsids of minute virus of mice. When the particles are extracted, their experiments suggested that the DNA of adeno-associated virus were of the same size and density; however, their experiments suggested that the DNA of adeno-associated virus was double-stranded. • Associated with nucleocapsids; has a strong activating effect on synthesis of viral messenger RNAs for Ebola but not Marburg virus. Their surface is constructed from 72 capsomers visible by electron microscopy, each made of five molecules of the major capsid protein VP1 (Figures 21.1 and 21.2). 14.2a: Courtesy of Dr. Guy Schoehn, CNBS, France.; Fig. Its central role in controlling the cell cycle is emphasized by the fact that a majority of human tumors have mutations in the p53 gene. Diameter 80 nm, length 800 nm or more. The single binding site is constituted by the N-terminal variable regions of the heavy and light chains. Human cases of new variant Creutzfeldt-Jakob disease (nvCJD) appear to be linked to the BSE epidemic in the United Kingdom. Clone cDNA fragments 3. Binding of doublestranded RNA to TLR-3 and recruitment of the adaptor protein TRIF (see Figure 33.3) can lead to apoptosis 16/05/11 8:19 PM Interferons through the activation of caspase-8. Transcribed by cell RNA polymerase II. and E2. This is called "minus-strand strong-stop DNA" because it is a complementary copy of part of the positive-strand genome RNA, and DNA synthesis stops abruptly at the end of the RNA template during in vitro reactions. Each of the eight distinct genome RNAs of influenza virus is individually packaged into ribonucleoprotein complexes small enough to pass through the nuclear pores. MC DNA has been found in a large proportion of these rare but aggressive human skin tumors; the viral DNA is integrated in CH021.indd 248 Figure 21.1 Computer-generated image of mouse polyomavirus capsid. Orange sphere represents reverse transcriptase (P protein). The integration complex of these retroviruses is unable to pass through the nuclear pores, and therefore has access to the cellular chromosomes only after the nuclear membrane breaks down during mitosis. Examples: P22, L, LP-7. For example, the B19 virus was found during screening for an unrelated virus, hepatitis B virus, and only later was it shown to cause human disease. The total genome size ranges from just over 12 kb for orthobunyaviruses to nearly 19 kb for nairoviruses; CH017.indd 202 N Protein S Nucleocapsid L Protein M L Gc Protein Lipid envelope (b) Figure 17.1 Bunyavirus virions. This discussion would not be complete without mentioning the balancing responsibilities of the vaccine industry, governments, healthcare systems, and the healthcare providers to develop and administer the safest possible vaccines and to use these vaccine safety very seriously. If the infection begins to spread within the organism, the third level of host defense, the immune response, comes into play. Hydrolysis to ADP and release of the ADP molecule lead to a conformational change that rotates adjacent protein domains with respect to each other and CH021.indd 257 257 changes the size of the central opening of the hexamer that is in contact with the DNA. Enveloped viruses usually fuse their envelope with a cellular membrane, releasing the nucleocapsid 16/05/11 7:52 PM Virus Entry replication. macrophages are vital for development of both humoral antibody and cell-mediated immunity (see Chapter 34). Four different transcription factors are known to bind to the virus-inducible enhancer (VRE) within the interferon- promoter (Figure 33.7). Amantadine would block the entry of H⁺ ions, thereby preventing uncoating. Intracerebral inoculation of BSE-infected brain extract into macaque monkeys produced disease and pathology very similar to those of new variant CJD patients. In the case of retroviruses such as murine leukemia virus, integration of the proviral DNA occurs only in dividing cells. The double-stranded RNA templates remain within the core and can be repeatedly transcribed, giving rise to multiple copies of all mRNAs. Translation of reovirus mRNAs is regulated Newly synthesized viral proteins can be detected in infected cells as early as two hours after infection, and as their synthesis increases, there is a concomitant decrease in synthesis of cellular proteins. The image is formed by exclusion of the stain by the specimen, therefore the image is "negative." Nested set: a set of similar objects, each of which fits into the next larger object. • Immune attack by cytotoxic T cells and inflammatory cytokines (Fas, TNF) is responsible for liver damage. Feline panleukopenia virus, as the name suggests, affects blood formation in cats. (not SSB, as in normal replication) binds to the phage DNA being packaged and accompanies it into the new virion. The virus was introduced to animals from infected flocks. Furthermore, antibodies are not often of much use against viruses that have already established a chronic infection (human immunodeficiency virus, hepatitis B virus, papillomaviruses, herpesviruses). genetically engineered extraterrestrial organism! More recently, the atomic structure of the ϕX174 procapsid, an assembly intermediate containing a full complement of scaffolding proteins, was solved by a collaborative effort of the laboratories of Michael Rossmann, Bentley Fane, and Nino Incardona. Nevertheless, HE may play a role in binding and entry of coronaviruses into target cells, and could facilitate virus release from the cell surface. The two major E1A proteins are powerful transcriptional activators of a number of viral and cellular genes. Their linear, double-stranded DNA genomes also showed strong similarity to genomes of myoviruses and siphoviruses. (a1, a2) Uninfected cells; (b1, b2) 10 h after infection; (c1, c2) 26 h after infection. • Filovirus genomes have seven genes, and seven viral proteins are incorporated into virions. Autonomous parvoviruses grow better in transformed cells than in normal cells, partly because transformed cells are continually passing through the cell cycle. Heparan sulfate: a sulfated polysaccharide (glycosaminoglycan) made from repeating disaccharides. Alternative RNA splicing generates different mRNA species that together code for a grand total of about 50 distinct adenovirus proteins. Individual virus clones can be isolated from the agar overlay by picking, allowing isolation of genetically pure strains of viruses. and bound to the N terminus of VP1 via the uncoating. Virus particles are typically less or have no plasma membranes to the subsequent steps of the cell, and specific in the DNA. Viral proteins in this system are typically less than the genome (see later). a sequential synthesis medium by plaque formation in mammalian cells. Shown schematically is the pathway of virion assembly at membranes of the endoplasmic reticulum–Golgi intermediate compartment (below): the thick blue curved lines represent lipid bilayers. Glycoproteins and the orange knobbed stalks represent envelope proteins. Several vaccine strains are based on strain T3D. • Some viruses code for replication proteins not normally found in other organisms (RNA-dependent RNA polymerases, reverse transcriptase). This book is printed on acid free paper. Mutations or deletions in this late domain motif impair or block production of virus-like particles by VP40. The NS1 or Rep78/68 protein helps by unwinding the hairpin. • Coronaviruses have the largest known genomes of RNA viruses (up to 32 kb). The discontinuous transcription model can explain recombination between viral genomes Discontinuous RNA synthesis also can occur by the viral RNA polymerase switching between two different positive-strand RNA genome templates within the same cell. Helical structures extend along a single helix axis. Finally, several E3 proteins reduce the immune response, and could be used in immune suppression therapies. Toll-like receptor interactions can restore this dysregulated RNA regions and cleaves them at specific sites. CH001.indd 3 16/05/11 7:46 PM 4 CHAPTER 1 Introduction to Virology messenger RNAs, which in turn synthesize viral proteins using the enzyme systems, energy, ribosomes, and molecular building blocks that are present in the cell. It is most effective at agglutinating particulate or cellular antigens in the serum, activating complement for the early and inducing a specific immune response—a high mark for them for phagocytosis and phagosomal degradation. This analysis suggested that type 1 and type 3 reoviruses interact with different receptors expressed on ependymal cells and neurons. 9.5a,b: Modified from Figure 1 in: Bettstetter M., X. The idea is that the virus will replicate in and spread through the tumor tissue, killing the cancerous cells. At this point, the viral genome has already begun to replicate and may soon produce a simply become a new set of cellular genes, which can be transmitted indefinitely by the cell during cell division. It is noteworthy that, unlike most human cancers, cervical cancers caused by papillomaviruses do not usually have mutated p53 genes. The ends of the DNA are held together as a circle by hydrogen bonding between short repeated sequences called DR2, near the 5ʹ ends of both strands. Packaging of the brome mosaic virus genome is coupled to replication, a common feature of positive-strand RNA viruses. This T7 genome is called middle T antigen (MTAg). 3' GTAGTAGT... Myristoylation may also help LS fold properly at the membrane surface. Right: a cross-section along the black line shown in the picture on the left. Positive staining: staining of specimens for electron microscopy by use of heavy-metal salts that bind to the specimen and impart electron density. This ensures that M2 and NS2 are made in lesser amounts than the highly abundant M1 and NS1 proteins. However, the best-characterized antiviral effects are directed against viral mRNA synthesis and stability, and viral protein synthesis. The boundaries between these domains are defined by sharp changes in degree of sequence homology among different viroids. In some cases, Creutzfeldt–Jakob disease was shown to be transmitted from human to human by (1) corneal allografts, (2) reusable intracerebral electrodes used for electroencephalograms, (3) grafts of dura mater (covering of the brain) from cadavers, and (4) human growth hormone purified from pituitary glands of cadavers. The crystallization of tobacco mosaic virus challenged conventional notions about genes and the nature of living organisms Wendell Stanley found in the mid-1930s that highly purified tobacco mosaic virus could form crystals. To produce a retrovirus vector, a plasmid called the "transfer construct" is introduced into the packaging cell line (Figure 37.4b). Salient features of the eight structural and three replication proteins are provided in Table 19.2. Reovirus virions contain concentric layers of capsid proteins Virions of mammalian reoviruses are non-enveloped particles approximately 85 nm in diameter (Figure 19.1). The Host Factor is not needed because the 3' end of the minus-strand RNA is apparently not sequestered in a stem structure and is therefore available as a template. The cellular localization of Mx plays a large role in determining its antiviral activity. Fatal filovirus infections usually end with high levels of virus in the circulation and little evidence of an effective immune response. First, it allows encapsidation of RNA genomes of different lengths; several families have segmented genomes that vary in size. Once all viral mRNAs are made from newly synthesized DNA, replication and virion formation proceed. Transport of intact virions through the nuclear pore complex. RNA polymerase contains virus-coded RNA replicase plus three cellular proteins. However, elimination of V by mutating the editing site, or mutating the amino acid sequence of V in its specific C-terminal domain, reduces the virulence of Sendai virus in mice. In the case of togaviruses, uncoating of the virus takes place only after low pH-induced fusion of the viral envelope with an endosomal membrane. Colorado tick fever virus, can cause serious neurologic disease in humans. Interferons - and - play a more limited role in CH033.indd 411 modulating the adaptive immune response. The four-helix bundle structures of the two structural proteins are highly similar, and they also resemble the structure of the major capsid protein of the Rudiviridae. a-galactosidase: an enzyme that cleaves lactose to galactose plus glucose; often used as a reporter enzyme because artificial substrates such as Xgal produce colored cleaved products that are easy to detect and measure. These proteins were mutated to abolish the interaction with their natural receptors. Therefore, the DNA polymerase-preterminal protein complex can bind and initiate DNA replication (step 5). Two models for co-packaging of RNA 3 and RNA 4 have been proposed. Humphrey, W., Dalke, A. Internal scaffolding proteins must first assume a structure that directs the assembly of pentameric intermediates into a rigid capsid. Empty cores can be seen surrounded by a mass of DNA, which CH027.indd 338 The linear 1,181,404-bp mimivirus genome has no inverted terminal repeats, but does contain a nearly perfect 617-bp inverted repeat at positions 22,515 and 1,180,529. Other examples of virokines include homologs of interleukin-6, 8, 10, and 17, and secreted serine proteases. Two small VA RNAs transcribed by RNA polymerase III. The fourth mouse polyomavirus mRNA makes a protein with the same 79 N-terminal amino acids as the other T antigens. Aerosols, eye drops, and suppositories are being developed to direct vaccines to specific body locations. Genetics of prion diseases: mutations in the prion gene can increase occurrence of disease PrPC is encoded by a single exon of a unique gene (two copies per diploid genome). Note that repeats are not drawn to scale, as they are only 617 nt out of a total of 1,181,404 nt. (b) Late transcription, sponsored by Q-dependent antitermination of the PR' transcript (which continues beyond gene A, through all late genes). Vaccinia virus may be a variant of cowpox virus that has drifted significantly during two centuries of artificial propagation, or it may represent a species of poxvirus that has become extinct since its initial isolation. 1887 Petri describes overlapping glass plates to use for sterile cultures. A ganciclovir-resistance mutation that prevents cytomegalovirus from inducing phosphorylation of ganciclovir was mapped to a gene encoding a viral protein called UL97. number of cellular proteins that lead directly to cellular destruction. Positions of 5ʹ and 3ʹ splice sites are noted. IRFs and NFB induce synthesis of interferons, pro- and anti-inflammatory cytokines, and chemokines. In the case of cucumber mosaic virus and brome mosaic virus,

many encapsidated genome molecules lack the 3'-terminal A residue, and this can be added by cellular CCA-nucleotidyltransferase. An alternative method is to flood the extracellular space with truncated, soluble versions of the cellular receptor protein for a particular virus. The C open reading frame encodes two related, yet functionally distinct proteins: the core (C or HBc) protein and the E (or HBe) protein. (a) (b) Viruses in the family Rudiviridae (from the Latin rudis, "small rod") are non-enveloped, helical rods Envelope proteins Nucleoprotein encapsided genomic DNA (c) Figure 9.8 Pyrobaculum spherical virus (PSV), family Globuloviridae. 05/05/11 2:45 PM C R E D I T S Front Cover Enterobacteria Phage Phi X174: McKenna, R., Xia, D., Willingmann, P., Ilag, L.L., Krishnaswamy, S., Rossmann, M.G., Olson, N.H., Baker, T.S., Incardona, N.L. "Atomic structure of single-stranded DNA bacteriophage phi X174 and its functional implications," Nature (1992) 355: 137–143. If the replicase arrives first, there will be no new translation, allowing replicase to copy the RNA with no oncoming traffic. They are formed by budding of nucleocapsids through the plasma membrane, which is modified by incorporation of a virus-encoded fusion protein. One of the viral DNA fragments enabled transactivation of the reporter plasmid, and was found to contain an immediate early viral gene called ie-1. This has negative consequences for virus yield during vector production, which takes place at 37C, and during vector purification, which is done at room temperature or at 4C. As of March 7, 2010, WHO had counted more than 213 countries and overseas territories or communities that had reported laboratory-confirmed cases of pandemic influenza H1N1 2009, including at least 16,713 deaths. CH037.indd 459 16/05/11 8:22 PM 460 CHAPTER 37 Eukaryotic Virus Vectors Descriptions of vectors derived from bacteriophage T7, poxviruses, baculoviruses, flaviviruses, togaviruses, and bunyaviruses can be found in their respective chapters. Individual red blood cells settle to form a compact pellet in the bottom of the tube (right), but when agglutinated, form a thin shell on sides and bottom of tube (left). Expression of these viral oncoproteins in mice induces skin and cervical cancers. Of these, 127 are considered "early" genes (blue in Figure 27.2), because their RNA transcripts disappear before initiation of viral DNA synthesis, and 100 are considered "early/late" genes (orange), because their RNAs are detected at later times as well. A novel reverse genetic approach that has overcome the complexity, size, and toxicity of the coronavirus RNA genome is referred to as "in vitro assembly" (Figure 14.7). There are 10–50 million bacteriophages on average per mL of seawater, and even more in many soils. More NS1, which is toxic for cells, is produced in transformed cells. This allows production of nucleocapsids that can actively transcribe the genome, producing all viral messenger RNAs. In turn, newly synthesized viral proteins can replicate the genome, and viral envelope and matrix proteins incorporate nucleocapsids into infectious virus particles. These viruses have been termed "suicidal", as they are able to infect cells and produce a particular protein of interest within those cells, but do not produce new particles in the absence of helper genomes, and therefore cannot spread to neighboring cells. Only one in 300,000 measles victims are affected by SSPE, which 16/05/11 8:05 PM Paramyxoviruses and Rhabdoviruses 177 seems to result from a slow, persistent infection and strikes up to 10 years after the acute illness. However, attempts to confer active immunity using microbial products have been documented in many cultures extending back at least one to two centuries before Jenner's birth. Viral proteins inhibit activation of interferon-stimulated genes. See also Filoviruses Bundibugyo ebolavirus, 189 editing of GP mRNAs, 194 envelope glycoprotein, 193 genome map, 191 glycoproteins secreted, in virus pathogenesis, 195 proteins, 191 Reston ebolavirus, 189 Sudan ebolavirus, 189 viral mRNA synthesis in, VP30 activating, 195 Zaire ebolavirus, 189 Ectodomain, 27 Edmonston live virus vaccine, 176 Edmonston-B and Moraten measles vaccine strains, 433 Encapsidation, 233 Encephalitis, 4, 160, 176, 201–202, 234, 286 Encephalomyocarditis virus, 127 Endocytosis, 43, 46, 48, 49, 128, 141, 150, 178, 204, 229, 345, 401, 447, Endoplasmic reticulum–Golgi intermediate compartment (ERGIC), 169 Endosomes, 46–51 early endosomes, 48 late endosomes, 48–49 Enfuvirtide, 447 Enhanceosome, 407 Enteric infections, 365 Entomopoxvirinae, 314 Entry, virus, 45–54 enveloped viruses, 46 fusion proteins, 50 intracellular transport, 52 membrane fusion, 50–51 non-enveloped viruses, 46, 51–52 import into nucleus, 52 passage from endosomes to cytosol, triggered by low pH, 49–50 prevention of virus entry, 53 receptor-mediated endocytosis, 48–49 receptors interact with viral glycoproteins, 48 uncoating and release of viral genome, 54 vesicle transport, 46 virus receptors, 47–48 Envelopes, viral, 3, 19, 26–27 formation by budding, 28–29 glycoproteins inserted into lipid membrane to form, 27 made from lipid bilayer membranes, 26–27 penetration strategies, 46 Enzyme-linked immunosorbent assays (ELISA), 198 Ependymal cells, 234 Epidemics, 201, 210, 429 12/05/11 4:08 PM 494 Subject Index Epidermal growth factor (EGF), 423 Epitopes, 356, 367, 420 Epstein–Barr virus, 296–300 B-lymphocytes infected by, 297 discovery, 296–297 in latently infected B lymphocytes, 298 latent membrane proteins (LMPs), 298–299 linearized genome of, 297 microRNAs, 300 mucosal epithelial cells infected by, 297 nuclear antigens (EBNA), 299 small, untranslated RNAs (EBER), 300 Erythema infectiosum, 245 Erythroid precursors, 245 Ethidium bromide, 250 Eukaryotic elongation factor 1A (eEF1A), 115 Eukaryotic virus vectors, 456–469 AAV vectors, 465–469 adenovirus vectors, 460–462 antitumor therapy, 461 applications, 457 blood disorders, 464 chimeric viruses, 468 construction and production of, 458–459 engineered to produce optimal levels of gene products, 459–460 expression cassette, 459 gene replacement therapy, 458 lentivirus, 463–464 neurological disorders, 465 properties, 459 retargeting virus vectors, 468 retrovirus, 462–465 Euryarchaeota, 98 Evolutionary origin of viruses, 40–44 large DNA viruses, 43 retroviruses, 43 RNA world, 40–41 small- and medium-sized DNA viruses, 43 transition to the DNA-based world, 42–43 Exocytosis, 145, 207, 374 Exotic viral pathogens, 190 Exportin 1, 361 Extracellular regulated kinase (ERK), 403 Extracellular virus (EV), 316, 321 Ficolins, 417 Filopodia, 47 Filoviruses, 188–199. If, as seems likely, the prion hypothesis is correct, then this result means that other cellular factors may be required to help catalyze the conformational switch. The 16/05/11 7:57 PM Bacteriophage T7 83 Genetic engineering with T7 The unique properties of the RNA polymerases of the T7 family of phages make them very useful tools for biotechnological and genetic engineering applications. Second, replication and translation of the same RNA molecule can lead to problems. The cdk 9/cyclin T complex interacts with Tat but also binds to the loop sequences within TAR and stabilizes the interaction of Tat with TAR. • Some paramyxoviruses "edit" transcripts in the P/C/V gene by pausing and "stuttering", adding one or more nucleotides to the transcript and changing the reading frame downstream of the editing site. Subsequent cleavage of both DNA molecules could lead to ligation of the viral DNA to cellular sequences. Transmission is inefficient, as documented by secondary infection rates that rarely exceed 12%. Recombinant viruses are generated without a requirement for helper virus and free of any selection. The fragments were used to generate full-length cDNA and genome RNA for electroporation, allowing recovery of the replication competent bat/SARS virus. After mixing, 1 mL of that dilution was added to 9 mL of fresh dilution buffer and mixed; these steps were repeated a total of eight times. Thus, like acyclovir, it leads to chain termination when incorporated into a growing DNA molecule. Most people infected with poliovirus have few or no symptoms and the infection is limited to the enteric tract, but a small percentage suffer from muscle paralysis and brain stem involvement. Interferon- is encoded by a single gene containing three introns. First, they showed that the agent responsible for yellow fever could be passed to uninfected individuals using infected patient serum, and established that this agent was a virus by filtration methods. The 3D polymerase has been crystallized and its three-dimensional structure determined. 9.13: Part of Fig 6 reprinted from: Bath C, Cukalac T, Porter K, Dyall-Smith ML, "His1 and His2 are distantly related, spindle-shaped haloviruses belonging to the novel virus group, Salterprovirus," Virology 350 (2006), 228-239, with permission from Elsevier; Fig. Other viruses are also the subject of intense investigation as possible vectors for foreign genetic material. Synthesis of double-stranded genome RNAs occurs within core-like subvirion particles. We do know for certain that West Nile virus is now endemic in the lower 48 states and much of Canada, and is capable of causing occasional severe human disease and death. Other self-cleaving RNA structures have also been identified in viroid-like satellite RNAs (hairpin structure), (Figure 31.3b) and hepatitis delta virus RNA (delta structure) (Figure 31.3c). These coiled coils can adopt two distinct conformations, which correspond to the form present in virions and the post-fusion jackknife form. Passage of virions across the endoplasmic reticulum membrane appears to be mediated by VP2 molecules, which lie below the surface of each capsomere. 2.7: Reprinted from Burnett R.M., et al. Virions have an envelope containing virusencoded proteins and host-derived lipids. Arrowheads show 3' ends. For example, poxviruses produce proteins called CrmA, CrmB, and CrmC that bind to caspases, resulting in a delay in apoptosis and allowing the virus to replicate. The fivefold rotation axes (pentagons shown at the vertices of the icosahedron) pass from the center of the sphere through the positions where the tails of five commas converge. Perhaps for all these reasons, plus-strand synthesis is more efficient than minus-strand synthesis, and therefore more plus-strands are made. 455 • An anti-HIV drug, maraviroc, blocks attachment of the virus by binding to a cellular receptor. Papain-like cysteine proteinases: enzymes that have structural homology to the proteinase papain, and that use cysteine as the nucleophile for hydrolysis of peptide bonds. In the cleaved protein, this conserved sequence, the fusion peptide, often constitutes the N-terminus of the transmembrane subunit, and CH004.indd 50 plays a central role in the fusion reaction. X also promotes fibrogenesis by stimulating the production of fibronectin. 16/05/11 7:49 PM 26 CHAPTER 2 Virus Structure and Assembly Phage tails are specialized structures that allow binding to cellular receptors and help to introduce the viral genome in the bacterial cell by piercing the cell wall and membranes. Aquisition of these phage sequences confers host cell resistance to infection by the phage. Genome sizes in kb (thousands of nucleotides) are shown on left, and genome lengths in micrometers (m) or mm are shown above each line. The tegument includes at least one identified protein, GP41 (Ac80). Circular, single-stranded RNA, 240–500 nt. Antigenic drift: a slow change in the antigenic specificity of a protein due to the accumulation of point mutations in the gene coding for the protein. The ability of these viruses to transform normal cells into cancerous cells has led to intensive research efforts and significant understanding of the nature of tumorigenesis. Ribosome shunting: a mechanism by which ribosomes are able to locate initiation codons for translation of messenger RNAs without "scanning" the RNA from its capped 5⬘ end by the classical pathway using initiation factor eIF4F. For viruses of the Gammaretrovirus genus, suppression of translation termination is used. The remaining 120 commas have tail-to-tail interactions in clusters of six; for example, the clusters on the equator including the commas marked "B" and "C". In the absence of sufficient pre-messenger RNAs to serve as primers, influenza virus cannot make its own mRNAs. Thus virus replication is indirectly dependent on a healthy cellular RNA synthesizing system. Some primitive RNAs may have by chance contained nucleotide sequences that enabled them to bind to other ribonucleotides and form covalent bonds. The genome has inverted terminal repeat sequences of 580 bp. Prion proteins become infectious and pathogenic (PrPSc) as a result of protein conformational changes. E7 proteins of the highly oncogenic human papillomavirus types 16 and 18 bind more strongly to Rb than E7 proteins of non-oncogenic virus types 6 and 11. The best-characterized yeast prion state is associated with Sup35, a protein involved in translation termination. The virion binds to cell surface receptors. A termination signal releases the mRNA. Most 2 gene promoters contain few or no binding sequences for cellular transcription factors upstream of their transcription start sites. A second class of misfolding disease includes systemic amyloidoses, type II diabetes, and several common neurological diseases. E1A sequesters p300/CBP, a coactivator required for transcription of the interferon genes. Brügger, S. Papillomaviruses cause warts and other skin and mucosal lesions The first papillomavirus was discovered in 1933 by Richard Shope. While the viral and cellular determinants of this delivery system are not understood, the nucleocapsid protein and several replicase proteins of murine hepatitis virus have been detected at assembly sites in ERGIC. Reassortment between animal and human strains can create a virus that can replicate in humans but has acquired an HA or NA subtype derived from the animal virus. They appear to undergo a reversible structural transition when exposed to the cytoplasm, allowing ribosomes or initiation factors access to their RNA. Proteins with RNA-modifying activity have been demonstrated in vitro for nsp14 (exoribonuclease and cap N7 methyltransferase), nsp15 (endoribonuclease), and nsp16 (methyltransferase, which methylates the 5' cap structure). Proteins that bind to antibodies on the surface of immature B cells are taken up in the cell and digested; peptide fragments are presented on the surface of the B cell in association with MHC II protein. Virion proteins often undergo proteolytic cleavage during or after assembly, preparing the virion for uptake by target cells. However, the utility of some of these narrowly targeted vaccines may eventually prove to be limited, because 16/05/11 8:21 PM 438 CHAPTER 35 Antiviral Vaccines Table 35.7 New technologies for human antiviral vaccines A. Several groups have shown that hrs act as transcriptional enhancers and as initiators of DNA replication, at least in transient assays in cell cultures. The human disease was called "smallpox" in fifteenth-century France and England to distinguish it from "large pox", or syphilis, which caused larger lesions (pocks) and infected older (larger) people. Small T antigen inhibits protein phosphatase 2A and induces cell cycling Small T antigen forms a complex with a subunit of the trimeric cellular regulatory protein, protein phosphatase 2A (PP2A), inhibiting the phosphatase activity of this protein (Figure 21.8). Hundreds of thousands of cases of hemorrhagic fever with renal syndrome are reported throughout Europe and Asia every year, with case fatality rates ranging from less than 0.1% for Puumala-like viruses in Western Europe, to 15% for Hantaan-like viruses in China. Many other animal viruses are taken up into the cytoplasm in vesicles formed at the plasma membrane. Outbreaks of Ebola hemorrhagic fever caused by Bundibugyo, Sudan, and Zaire ebolaviruses have taken place in Gabon, the Republic of Congo, the Democratic Republic of Congo, Sudan, and Uganda. Monocytes and Macrophages Phagocytic cells in blood and tissues that take up and digest pathogens and cell debris; antigen presentation. Viruses with the largest RNA genomes (Coronaviridae, Roniviridae), as well as many plant viruses, have helical nucleocapsids. Genomic RNAs are represented by dark blue lines (length in nucleotides above each segment) and messenger RNAs as light blue arrows; host-derived sequences at the 5' end of mRNAs are shown as black rectangles. Release of particles is observed only in the stationary growth phase. This is crucial for understanding both the timing of particular events in the virus life cycle and which proteins are needed for each step. CREB-binding protein (CBP): a histone acetylase that binds to a cellular transcription factor called cyclic AMP response element-binding protein (CREB). They only rarely cause more severe respiratory diseases, gastroenteritis, and encephalitis. The ratio of physical virus particles to infectious particles can be much greater than 1 Measurement of the number of infectious virus particles by use of plaque assays, and of the number of physical virus particles in the same virus suspension by electron microscopy, allows calculation of the ratio of physical particles to infectious particles. PHAEOVIRUSES Seaweed viruses Phaeoviruses are latent viruses that infect the brown algae, more familiarly known as seaweeds. (Scale bars: a & b, 200 nm; c, 500 nm), (d) drawing of a typical fusellovirus. Selection for sexual reproduction as an antiviral mechanism will also maintain a high diversity, ensuring that there will always be a genetic variant that can adapt to a particular environmental condition. Prymnesioviruses infect phytoplankton from the algae class Prymnesiophyceae, in the division Haptophyta (generally referred to as haptophytes). Since then, other flavivirus cDNA clones capable of producing infectious virus have been constructed. Like intrinsic cellular defenses, innate immune responses can be rapid, and they usually act locally at the site of infection. However, there is evidence that mature virions of some coronaviruses, including porcine transmissible gastroenteritis virus, possess a stable spherical core structure. These immature virions are transported within vesicles through the Golgi apparatus, where the envelope proteins are glycosylated. et al., "Microtubulemediated transport of incoming herpes simplex virus 1 capsids to the nucleus," The Journal of Cell Biology 136 (1997): 1007– 1021, with permission from Rockefeller University Press; Fig. Intrinsic cellular defenses: rapid and usually local host responses by an organism to infection by a pathogen. Although certain flaviviruses have been brought under control through vaccination and vector management, a large number remain significant threats to human health. Strong splice donor (SD) and splice acceptor (SA) sequences as well as a strong polyadenylation signal (pA) ensure efficient processing and export of mRNA. In a multicellular organism, the loss of abnormal cells through apoptosis can be considered as a defense mechanism against disease, including virus infection and cancer. Thus viral messenger RNAs are both capped and polyadenylated, like cellular mRNAs. After releasing the mRNA, the RNA polymerase has two choices: it can again scan the genome RNA for a start site and begin synthesis of the next mRNA, or it can dissociate from the genome. 5 in N. Based on electron microscopic studies of phleboviruses, entry into cells is by endocytosis. Acheson.—2nd ed. Vif is required to overcome the action of a host cell protein, APOBEC3G. Once viral structural proteins are synthesized, assembly of nucleocapsids occurs in the nucleus. The slower adaptive immune response is mediated by populations of specifically activated B lymphocytes, which synthesize virus-specific antibodies, and T lymphocytes, which differentiate into cytotoxic T lymphocytes that kill virus-infected cells. Many viral genes counter host defenses against virus infection. They kill continent-sized blooms of their host organism E. Replication starts at the 3' end of the RNA and proceeds toward the 5' end. Adenoviruses that do not express E1B genes CH037.indd 461 461 but are otherwise intact replicate poorly in normal cells but well in many tumor cells. For example, upon entering a peripheral neuron, herpes simplex virus type 1 or rabies virus must move distances measured in centimeters or meters along the nerve axon to reach the cell body, where the nucleus is located. Adenovirus infection of human cells usually results in cell death, and therefore no transformed cells can survive. Rodent cells also possess a closely related CAR protein that allows their infection by human adenoviruses. The 5'-proximal two-thirds codes for the nonstructural proteins required for replication and transcription of the RNA (Table 13.2), and the 3'-proximal one-third codes for the capsid and envelope proteins (Table 13.3). Cleavage of DMSP by the membrane-bound enzyme DMSP lyase during virus infection produces DMS, which is thought to attract certain protozoanlike grazers that engulf the algae. • Cell transformation and tumor formation results from integration of viral DNA into the genome of nonpermissive cells and expression of viral early genes, turning on cell cycling and increasing cellular growth and multiplication. Infection by herpesviruses can lead to the activation of a signal transduction pathway that results in the activation of four cellular kinases. The same arguments for genetic economy, simplicity, and strength apply to these structures: they must be built from identical subunits and are therefore symmetric. Class Class II DNA metabolism 1.3 2 3 4 Class III Virion structure and assembly 5 6 10 7 8 9 10 11 12 13 14 15 20 16 30 17 18 19 kb TE I Tϕ 1.1A 1.1B II III Figure 7.1 Genetic and transcriptional map of the T7 genome. The survival strategy used by adenoassociated virus, and its ability to integrate at a specific site into the human genome, are unique among animal viruses and resemble the strategy used by bacteriophage lambda (Chapter 8). E6 reverses p53 increase by binding to E6AP and p53, leading to degradation of p53. Early accounts of silkworm diseases are found in both ancient Chinese and Western literature. Surprisingly, the viral transcriptional machinery cannot make mRNAs on its own, but uses cellular RNAs as primers for initiating viral mRNA synthesis (Figure 18.7). These proteins, together with nsP4, form complexes that efficiently initiate synthesis of positive-strand genome RNA (replication), and larger amounts of subgenomic mRNA (transcription), from promoters (gray squares with directional arrows) on the antigenome RNA template. These particles can extend for several micrometers and may be an efficient means for cell-to-cell transmission. 16/05/11 7:59 PM 94 CHAPTER 8 Bacteriophage Lambda The scaffolding protein Nu3 helps E, the major head protein, to form a closed icosahedral shell containing many (approx. 19; Fig. There are no signs of brain inflammation, suggesting that there is no infectious organism present. The extracellular space is on the left, the cytoplasm is in the center, and the nucleus is on the right. Natl. • Picornaviruses encode their viral proteins as large precursor polyproteins that are subsequently cleaved by viral-specific proteinases. CH035.indd 438 Use of live viruses with defined attenuation characteristics The first intentional exposure of healthy subjects to a live vaccine-strain virus can make both vaccinator and vaccinee quite nervous, particularly when the vaccine has been produced by random passage. CH005.indd 64 Replicase binds to the RNA at two internal positions, one of which is the start of the coat gene. Vif acts by binding to APOBEC3G and inducing ubiquitination and degradation of this protein by proteasomes. Can induce immune responses that reduce their utility. akashiwo host strains and HaV clones suggest that this strain-dependent infection plays an important role in determining clonal composition and maintaining intraspecies diversity in natural H. be used to infect another species to protect against a closely related pathogen. Knowledge of the cellular factors involved in initiation of translation of viral RNA and of RNA synthesis, as well as the roles of viral proteins such as the RNA-dependent RNA polymerase and the 3C and 2A proteinases, may help in the development of novel antiviral agents that can target these critical steps in virus replication and reduce or eliminate the most serious disease symptoms. • Poxvirus cores contain a full complement of viral enzymes that direct transcription of early genes and processing of viral transcripts, which are extruded into the cytoplasm. It was then realized that this virus was associated with a serious illness—aplastic crisis in anemia patients. Nevirapine: non-nucleoside reverse transcriptase inhibitor. Early endosomes are responsible for dissociation of incoming ligandreceptor complexes, for recycling of empty receptors to the cell surface, and for dispatching non-recycled material to late endosomes and lysosomes to be degraded. Unlike single-stranded RNA, double-stranded RNA is stable and has been detected within the lung fluid of individuals infected with viruses such as influenza virus. 16/05/11 7:49 PM 28 CHAPTER 2 Virus Structure and Assembly PACKAGING OF GENOMES AND VIRION ASSEMBLY Multiple modes of capsid assembly Capsid assembly mechanisms depend on the nature of the final structure and on the characteristics of the viral genome. These recombinant genes are incorporated into progeny virions. • Coronaviruses have helical nucleocapsids, unusual for positive-strand RNA viruses. Some of the immediate early genes including ie-0, ie-1, ie-2, and pe38 are clustered within a 7-kbp region, but there do not appear to be other significant groupings of genes by function or time of expression. Lipid rafts: microdomains in plasma membranes of eukaryotic cells that are enriched in cholesterol, glycolipids, and sphingolipids and in certain membrane proteins. Nearly 100% of human cells exposed to baculoviruses can express the desired gene (provided high multiplicities of infection are CH025.indd 309 used), and expression of transduced genes under the control of mammalian promoters is high. Dermody Reoviridae From respiratory enteric orphan viruses VIRION Naked icosahedral capsid (T 13), Diameter 60–85 nm. VIRAL ENVELOPES Many viruses enclose the nucleocapsid in a lipid membrane, called an envelope. They carry long inverted terminal repeats. Amantadine inhibits uncoating of most influenza virus strains. Vaccines that can break tolerance Until very recently, vaccine development efforts have focused almost exclusively on products capable of preventing or modifying acute disease. These mutant viruses nevertheless replicate well in cells that have a defective interferon response. This coiled-coil structure brings the transmembrane domain, which is anchored in the viral envelope, close to the fusion peptide, which is inserted into the cell membrane, and therefore brings the two membranes in close proximity (see Chapter 4 for a discussion of fusion by influenza HA protein). The identity of this second factor is unknown. Many virus isolates that infect other crops, and were therefore given different names, were later found to be identical to cucumber mosaic virus. This gene was designated src, for sarcoma. 36.10b: Courtesy of Elspeth Garman, reprinted with permission from Graeme, L. The L proteinase cleaves itself from the VP4 protein and also cleaves cellular initiation factor eIF-4G. Recombinant baculoviruses as gene therapy vectors In 1985, Lois Miller's group demonstrated that foreign gene expression from recombinant baculoviruses is cell typedependent. With the advent of gene transfer technologies, new horizons are opening for the development of CH037.indd 458 biopharmaceuticals based on recombinant DNA technology. stiff neck, or swelling of lymph nodes) that requires hospitalization in a minority of individuals. MutS is part of a DNA mismatch repair enzyme system that binds to regions on DNA where bases are mismatched as a result of DNA damage; it is highly conserved from bacteria to humans. The template for both mRNA synthesis and genome replication is the negative-strand genome assembled into an intact nucleocapsid. • Misfolded proteins of yeast and other fungi can form self-propagating states that resemble prions. All of these viruses are phycodnaviruses, assigned to the family Prasinoviridae. It is a eukaryotic cell that has an elaborate armor of calcareous (chalk) plates called coccoliths. Viral envelopes contain viral glycoproteins inserted into the lipid membrane by a transmembrane anchor. RNA 4A is not packaged in many strains of cucumber mosaic virus, and RNA 5 is only synthesized in some strains. This complex can be made in vitro and is infectious for F cells; thus it can bind to pili in the absence of the capsid, and direct the phage RNA into the cytoplasm. Other flaviviruses, including pestiviruses and hepatitis C virus, share with picornaviruses the use of an internal ribosome entry site (IRES) to direct initiation of viral protein synthesis. Oseltamivir: neuraminidase inhibitor used for treatment of influenza A and B virus infections. For example, nitric oxide is generated in macrophages as a result of the interferon-dependent induction of the inducible form of nitric oxide synthase (iNOS). Its single hydrophobic membranespanning anchor is usually near the amino terminus. Diameter 45 nm. The hemagglutinin-esterase (HE) is only present in some coronaviruses and is not shown here. In summary, viruses in their simplest form contain a nucleic acid genome, packaged in a protein coat. This is reminiscent of the way in which bacteriophage constructs and fills its capsid (Chapter 8). How does the filovirus GP gene make both a secreted and membrane-bound protein from the same gene? Hosts have evolved multiple mechanisms to suppress their expression; these include DNA methylation to block transcription, interfering RNA to silence translation of mRNAs, and APOBEC proteins (see Chapter 29) that sequester RNA into particles within the cytoplasm. Recombinant viruses or expression vectors can be used to make virus-like particles (right) containing an immunogenic viral protein: these virus-like particles are inoculated into the host where they induce an immune response against the targeted virus. The mechanisms by which herpesviruses establish latent infections and reactivate are presently under intense study. The reovirus outer capsid protein 3 appears to influence this selective inhibition of cellular protein synthesis via its effects on PKR activity. Some examples of virions with icosahedral symmetry Parvoviruses are among the smallest viruses, with a 5 kb single-stranded DNA genome. CH020.indd 242 16/05/11 8:08 PM Parvoviruses RNA primers 5' 3' 3⬘ 5' 3' 5' DNA template strand DNA polymerase extends primers New DNA strands 3' 5' Remove RNA primers DNA polymerase fills in gaps Figure 20.6 RNA primers and the DNA "end replication" problem. In the case of the so-called "gene guns," this can be a quick and efficient system for localized delivery of DNA vaccines or other antigens. Topoisomerases: enzymes that relieve torsional stress in supercoiled DNA molecules by cleavage and resealing of phosphodiester bonds. This requirement may mean that interactions of PrPSc that lead to its stabilization or aggregation are stronger between molecules of the same amino acid sequence and therefore the same threedimensional conformation. These unusual stuctures enable DNA replication by serving as primers for DNA polymerase. Tegument proteins interact with cellular machinery to activate viral gene expression and to degrade cellular messenger RNAs The precise functions of only a few of the tegument proteins are known. Nonstructural proteins are made as a polyprotein that is cleaved by a viral proteinase Once in the cytoplasm, nucleocapsids release the viral genome RNA so that it can be translated by cellular ribosomes. The onset of the disease is sudden, with fever, chills, headache, muscle pain, and loss of appetite. Tat binds to the TAR sequence on nascent (growing) RNAs, but not to the same sequence on proviral DNA. The five edges of the capsid that radiate from this vertex are decorated with a starfish-like feature that opens gaps between the five adjacent faces of the capsid. The distinct location for E alters its folding and antigenicity when compared to the nearly identical C protein. Male and female (haploid) gametophytes produce swimming gametes. NS4A and NS4B. Transcription by cellular RNA polymerase II begins in the left-hand LTR, using the enhancer and promoter sequences located in the U3 (a) CMV pA gag pol CMV pA env (b) ⌿ U3 R U5 Transgene U3 R U5 Transfer construct U3 R RNA carried by retrovirus vector Transcription ⌿ R U5 Transgene Reverse transcription and integration ⌿ U3 R U5 (c) U3 R U5 Promoter ⌿ Transgene Transgene U3 R U5 ⌬ R U5 DNA in target cell Transfer construct Transcription R U5 ⌿ Transgene ⌬ R Transgene ⌬ R RNA carried by retrovirus vector Reverse transcription and integration ⌬ R U5 ⌿ Transgene ⌬ R U5 DNA in target cell Figure 37.4 Production of retrovirus vectors. Chitin is an insoluble linear homopolymer of D-N-acetylglucosamine linked by -1,4 glycosidic bonds. The promoters for replication of both plus- and minus-strand 123 satellite RNAs have not been identified and molecular mechanisms of replication of satellite RNAs remain unclear. Inactivated viruses. Direct infection of cells lining the gut or vagina has not been detected, indicating that the virus must overcome this physical barrier to establish the infection. This is about 10-fold greater than the estimated number of bacteria. The size exclusion limits of plasmodesmata connecting epidermal and mesophyll cells are similar. Within the virion the two RNAs exist as a dimer, held together in a head-to-head configuration by interaction of sequences known as a "kissing loop" located in the U5 region. This disease can occur when a patient previously infected with one dengue virus serotype is subsequently infected with a second serotype within a period of one to two years. 2, reprinted with permission from Badger, J. Messenger RNA capping. A minority of polymerases reads through this terminator to form longer RNAs. The use of multiple promoters leads to the production of a nested set of overlapping primary transcripts of the class II region with a common 3' end. They produce subgenomic RNAs, which express the foreign protein. Most of the commonly used adenovirus vectors are missing E1A and E1B genes (and often many more) and therefore cannot replicate in normal cells. Unsurprisingly, viruses infecting the same host are most similar, and overall approximately 80% of the genes are found in all six sequenced genomes. Virus-like particles Viral proteins, alone or in combination, that can either spontaneously self assemble or be coaxed into assembling with lipids to form nanoparticles resembling viruses. Rossmann, "Cryo-EM Study of the Pseudomonas Bacteriophage PhiKZ," Structure 15 (2007), 1099-1104, with permission from Elsevier and Michael G. Most viruses adjust the timing of their penetration by carefully titrating the activation threshold to a pH in the 6.5–5.3 range. Integration (downward arrow) and excision (upward arrow) are catalyzed by the proteins shown next to the arrows. Can be caused by absence of retinoblastoma protein (pRb), a tumor suppressor protein. Many have only been recently discovered, and their importance in controlling algal blooms and in influencing global levels of oxygen and carbon dioxide has led to a lot of recent research on these viruses. Mutations in the genes that code for ICP0 and ICP4 strongly reduce expression of and genes. These are included because bacteriophages are among the best-known viruses, and because much of our knowledge of molecular biology and virology began with their study. Genes are shown on the circularized 152-kb map of herpes simplex virus type 1 DNA. NS2 121 Nonstructural protein that directs nuclear export of viral nucleocapsids; interacts with M1 8 CH018.indd 213 Proteins encoded 890 16/05/11 8:07 PM 214 CHAPTER 18 Influenza Viruses matrix protein (M1) and a third envelope protein (M2). In brome mosaic virus, it remains unknown how RNA 3 and RNA 4 are packaged together into a single virion. Not all fungal prion states, however, are deleterious. Is worldwide eradication of poliovirus possible? In addition, the head shells of all tailed dsDNA viruses and herpes simplex virus contain a special vertex, the portal vertex, formed by the portal protein, a dodecamer of radially disposed subunits. Within an endocytic vesicle, the viral outer capsid undergoes cathepsin-mediated proteolysis, generating infectious subvirion particles (ISVPs). It was quickly found that prion infectivity is unusually resistant to inactivation by ionizing or ultraviolet irradiation, which damage nucleic acids. A function for E has not been established. Within the core is a tubular structure folded into three sections. Replication of AAV requires the presence of a helper virus, either an adenovirus (thus the name) or a herpesvirus. At the same time, the part of HA2 that is inserted into the virus envelope is brought closer to the extended N-terminal region. Baculoviruses are widely used to express foreign proteins In the early 1980s, several lines of evidence indicated that the very late gene product, polyhedrin, is not essential for virus replication in cell culture. Diameter 33 nm. Virus particles are transported toward the nucleus using interactions between the hexon protein and microtubules. In fact, herpesvirus mutants lacking the 134.5 gene are highly attenuated. Immature T cells whose T-cell receptor recognizes these peptides are activated and differentiate into Th1, Th2, or Tc cells, expressing CD4 or CD8 surface proteins. 2 in Jackson, G.S. and Clarke, A.R., "Mammalian Prion Proteins," in Current Opinion in Structural Biology 10 (2000), 69–74, with permission from Elsevier. Start and stop codons are shaded in green. Matrix protein associates with the cytoplasmic face of the plasma membrane, probably by interacting with the cytoplasmic tails of the viral envelope glycoproteins. Slow progressors (70–80%) develop late stage symptoms in 8 to 10 years. The capsid effectively wraps itself in the membrane, and progressive formation of contacts between the surface of the capsid and the cytoplasmic tails of the viral glycoproteins drives pinching-off of the bilayer by membrane fusion. What are virus-like particles (VLPs) and how were they used to produce the human papillomavirus vaccines? Proteins are shown in shaded boxes. Temperate phage: a bacterial virus that can either replicate to produce progeny virions and cell lysis, or enter a repressed prophage state that can be harbored in a cell for multiple generations. Models include myosin-like contractions of terminase subunits, or rotation of the portal protein to winch or screw the DNA into the head shell. The picornaviruses have solved this problem by linking all of their protein-coding regions into a single unit, and synthesizing a single, large polyprotein (Figure 11.4). Encapsidation of the RNA initiates the budding of particles into the endoplasmic reticulum. Vesicles containing virions are then transported to the cell surface via exocytosis, eventually releasing their contents to the exterior. Negative contrast electron micrographs of (a) virions being extruded from S. The number of the hexameric V disks, 32, is dictated by the length of H, which Isao Katsura and Roger Hendrix have shown acts as a tape measure by extending up the central hole of V hexamers. The vesicular stomatitis virus G protein has been widely used to engineer recombinant enveloped viruses, particularly retroviruses, as it allows them to infect many cell types. The eclipse phase of the replication cycle follows, resulting in the appearance of a ring-like virogenic stroma in the nucleus, and production of viral nucleocapsids. Diameter 100 nm. Gene D lacks a promoter but retains the transcription terminator. Envelopes are used by many viruses as an external layer in which their nucleocapsids are wrapped. An example is the influenza virus hemagglutinin, HA, which is cleaved into a peripheral N-terminal fragment called HA1 and a C-terminal transmembrane fragment called HA2 (Figure 18.4; see also Figure 2.12). The genome usually integrates in a specific region of human chromosome 19, at position 19q13.3. The exact mechanism of integration is not known. Upon further incubation at temperatures above 75C, appendages protrude from both pointed ends of the virion, and the lemon-shaped virion body shrinks to approximately 85 150 nm (Figures 9.11a, d–h). Hemagglutination: the agglutination or clumping of red blood cells. "WIN compounds" (named for the company that originally synthesized them) inhibit human rhinoviruses by binding to a pocket under the receptor-binding canyon. Numerous different rhabdoviruses infect insects, plants, and vertebrates; this is one of the few virus families whose members infect such a wide variety of hosts. The expression of viral proteins on the surface of infected B lymphocytes leads the infected host to produce cytotoxic T lymphocytes (see Chapter 34) that attack and kill most of the infected B-lymphocytes. At the peak of virus formation, about 10 times more subgenomic RNA than genome RNA has accumulated. The use of molecular clones of the virus genome greatly facilitated the study of hepatitis C virus, because up until 2005, virus replication could not be carried out in cells cultured in vitro or in non-primate animals. All segments have a 5' cap structure and 3' tRNA-like structure. Finally, the high mobility group protein HMG-1, a small histone-like protein associated with chromatin, binds to the minor groove of DNA within positive regulatory domains IV and II, promoting the binding of ATF-2/c-Jun and NF-B. Pasteur's most potent ally in convincing Parisians to try vaccines was fear. Hepatitis B virus also uses reverse transcriptase during its replication, but it packages a DNA genome within its virions. T7 DNA polymerase uses the RNA as a primer for synthesis of DNA (gray arrow). This raises the possibility that the vector might integrate near an oncogene and cause cancer by disturbing the function or expression of this oncogene. All RNA viruses (except retroviruses) must synthesize an RNA-dependent RNA polymerase to replicate their genomes, as this enzyme is not present in host cells. A hairpin at 16/05/11 7:54 PM 66 CHAPTER 5 Single-Stranded RNA Bacteriophages 3' U U U C C G C 10 G CC G G G p p p 5' G AG G U 20 G U C 30 C • G U U A GCUC A C U C C G A GC U G U C 120 U UCS 40 str U U U C C G C 10 G CC G G AG 5' hairpin U 20 C GG C • U U • G G C • GG p p p 5' U C UGACCUGUG A U 30 A U 130 C G U • G 120 UCS U A SD C G 40 100 50 C G U U U A C C G U A A U C G A G C U G U A UC C U U A C C U GGA AU U U A A U G U C U U U A G C G A G A C G CC 100 C G 60 70 U A A U C G C G "Cloverleaf" A 80 A U • U G G• U G C 90 C G U C AU uc tur e Maturation start U G A C C U GUG U U G G SD A G G A G G 3' Ribosome unknown (b) Nascent RNA (a) Fully folded RNA Figure 5.8 Translation of maturation gene on nascent RNA. These include proteosomes, virosomes, liposomes, and virus-like particles (VLPs). When nsP2 was expressed in bacteria in the absence of other viral proteins, it was shown to have an RNA helicase activity that may be needed for optimal RNA replication/transcription. In 1957, a new influenza A virus suddenly appeared in Southeast China. Simian virus 40 and Epstein–Barr virus both encode miRNAs that target early viral messenger RNAs. These miRNAs accumulate during the late phase of infection, when early viral proteins are no longer needed for virus replication but can become targets of the host adaptive immune response. Signaling pathways shown in Figure 33.3 lead to activation of cellular transcription factors. The loss of cell surface MHC 1 means that the cell cannot present viral antigens to circulating cytotoxic T lymphocytes, thus masking the infection from the immune system. The viral N protein forms the helical nucleocapsid along with genome RNA (Figures 15.1b, 15.2b, and 15.5). Gene 9 protein functions as a scaffolding protein for assembly of the prohead. Many of the mutations found within viral oncogenes are similar to those found in altered genes associated with tumor formation in animals or humans, suggesting common tumor-inducing mechanisms. But how and when did viruses first appear? Within 1 to 2 months, the primary infection resolves but there is little recovery of the CH029.indd 356 1. Requiring vaccination when no vaccine is 100% safe raises ethical issues. Making new and mutant coronaviruses: reverse genetics and synthetic biology The ability to introduce specific mutations into a viral genome is a powerful tool to define functions of viral proteins in replication, pathogenesis, and virulence. Johnson, Charles L. Cucurbit: a plant in the family Cucurbitaceae. Coen DISCOVERY OF ANTIVIRAL DRUGS "Serendipity"—trying out compounds used for other purposes: • Amantadine (inhibits influenza virus uncoating) Acyclovir (inhibits herpesvirus DNA replication) • VP1 Chemical modification of known active compounds: • Ganciclovir (inhibits herpesvirus DNA replication) Azidothymidine (inhibits HIV-1 reverse transcription) • High throughput screening assays of many compounds: • CF3 N Nevirapine (inhibits HIV-1 reverse transcription) Raltegravir (inhibits HIV-1 integrase) • Ritonavir (inhibits HIV-1 protease) Zanamivir (inhibits influenza neuraminidase) • Picornavirus capsid proteins HIV-1 envelope proteins and host chemokine receptors. Whereas the cells responsible for nonspecific innate immunity are broadly distributed in the blood, tissues, and organs throughout the body, pathogen-specific immune B and T lymphocytes are mostly localized in specialized organs of the lymphatic system. The spacer segments frequently contain sequences that match sequences found in bacteriophage genomes. The capsid gene is translated first, and the carboxyterminal half of this polypeptide folds to form a serine protease catalytic site that self-cleaves the capsid protein from the nascent polypeptide chain while translation continues downstream (Figure 13.4). Viruses are known to infect members in the genera Ectocarpus, Feldmannia, Hincksia, Myriotrichia, and Pilayella. Why would this be true? DNA J is a cochaperone protein, first described in E. When phosphorylated at tyrosine 527, c-Src is inactive (not shown). Gene therapy is an expanding application of virus vectors Vaccination and gene therapy are the most widely publicized uses of virus vectors. Up to the present, influenza viruses that cause widespread human disease have been limited to strains that contain HA types 1, 2, or 3. Signal transduction mediated by CD150/SLAM may regulate T-cell proliferation and interferon-γ production. This is a result of the complex interplay between adenovirus early gene products and cellular signaling pathways leading to apoptosis (see Chapter 23). The mechanism ensuring that only one copy of (b) Figure 19.7 Formation of inclusions in reovirus-infected cells. In addition to Q RNA itself, a number of small (100–200 nt) RNAs can be efficiently replicated in vitro by this RNA polymerase. These attenuated viruses replicate in humans and therefore can often induce protective immune responses as effectively as the parent viruses, while causing little or no disease. Fiber-less particles lie close to the edge of the virus factory (blue, purple, and red arrowheads); fiber-covered particles lie farther away (yellow arrowhead). The major capsid protein, VP1, decorates the outside of the virion, and a small basic protein, VP3, binds to the DNA genome inside the particle. However, the danger of these highly pathogenic strains adapting to humans and causing a serious pandemic is present. Although human transmission and epidemic disease appears to have been eradicated, additional cases in 2003 and 2004 arose in laboratory settings and from animal-to-human transmission. Virions of a number of bacteriophages contain both elements with icosahedral symmetry (the capsid) and elements with helical symmetry (the tail) (Figure 2.10). Genomic analysis of bunyaviruses isolated from mosquitoes caught in the field shows evidence for naturally occurring reassortment. Uncoating: the removal of the protein coat, or capsid, from a viral genome within a cell, in preparation for its expression and replication. Poxviruses also produce soluble forms of cytokine receptors ("viroceptors") that perform the dual functions of blocking the production of antiviral cytokines and inhibiting B cell activation, thus delaying the onset of the humoral immune response. Most viral genes are present in only one copy per genome; the UL region contains 65 protein coding sequences, and the US region contains 14. Large replicase polyprotein is cleaved by viral proteinases to form numerous functional proteins. Five of these protomers then assemble into a 14S pentamer. The pH-dependence of envelope-membrane fusion was shown directly by lowering the pH of medium bathing cells to which large numbers of virions were bound; virus envelopes fused directly with the plasma membrane when the pH was reduced. Serious adverse effects following immunization are rare but sometimes important. The matrix (M) protein lies on the inner surface of the envelope. In contrast to paramyxoviruses, rhabdoviruses have an unusual bullet shape (Figure 15.2), due to wrapping of the helical nucleocapsid in a supercoil or "skeleton" 50 nm in diameter, about 2.5 times the diameter of the nucleocapsid. Transcription from this promoter generates mRNAs encoding the NS1 and NS2 proteins. Species: in virology, a taxonomic grouping of virus isolates that share a high degree of nucleic acid and amino acid sequence homology in addition to having a common genome structure and size, common virion structure and replication strategy, and (often but not always) a limited host range. As noted above, some picornaviruses encode a proteinase (protein 2A for the enteroviruses and rhinoviruses or the L protein for aphthoviruses) that cleaves eIF-4G, one of the essential protein components required for recognition of the 5' end of capped cellular mRNAs. Infected cells are therefore unable to translate most cellular mRNAs, allowing the cap-independent translation of viral RNAs to take over the cellular protein synthesis machinery. DISEASES Respiratory syndromes including pneumonia, but not common colds. Because most virology research has been carried out in the fields of bacterial, plant, and vertebrate viruses, the majority of viruses that have been discovered infect organisms in one of these groups. This mechanism of transmission is widespread among plant pathogens. As a result, the endosome membrane ruptures, and the virion together with other contents of the endosome is released into the cytosol. Anti-inflammatory cytokines. Pseudovirion: virions of polyomaviruses that encapsidate fragments of cellular chromatin instead of the viral genome. These viruses depend on cellular enzymes to transcribe and to replicate their genes. Examples: bacteriophages T3, ϕII, H, SP6, and gh-1. In spite of a high degree of self-complementarity, however, they are not believed to be hydrogen bonded to each other. What aspects of coronavirus replication most likely contribute to their ability to rapidly change host range, possible even in vitro? GENOME Circular double-stranded DNA, 8 kb. Following infection by nearly all picornaviruses (Figure 11.5b), cap-dependent translation in the host cell is abolished by the proteolytic cleavage of eIF-4G or by sequestration of the cap-binding protein eIF-4E. Therefore, insertion of a T7 late promoter sequence upstream of any DNA sequence allows in vitro synthesis of substantial quantities of homogeneous RNA from the desired DNA sequence. One advantage of this system is that it draws our attention to the distinct kinds of RNA polymerases needed by viruses in each group, and indicates whether these enzymes are available in the cell or must be provided by the virus. CH018.indd 214 HA2 Fusion Peptide S S N C Transmembrane domain N Plasma membrane or virus envelope C Figure 18.4 Influenza A hemagglutinin protein (HA). Images created by Jean-Yves Sgro, University of Wisconsin, Madison, with software Qutemol and VMD. The core is surrounded by a bilayer "core wall" structure of unknown composition. Reovirus liver and bile duct disease and myocarditis have become particularly well-established experimental models of viral injury at these sites. Some viroids have a region complementary to certain cellular RNAs. This suggests that disease could result from inhibition of the function of these RNAs, or their cleavage directed by viroid RNA (e.g., trans-acting hammerhead cleavage). The cleavage of the prM protein by Mature virus release Virus attachment and entry Virus maturation Furin cleavage of prM Virus fusion and disassembly 145 pr TGN RNA Golgi CAP CA AP A P RNA RN R NA Protein translation, polyprotein processing on membranes CAP C A AP P CAP CAP CA C A AP P Immature virus assembly and budding ER Nucleus Viral RNA replication on membranes Figure 12.6 Flavivirus replication pathway. HE and S are incorporated into the membrane through interactions with the M protein. • Viral mRNAs are capped by viral capping enzymes during transcription, and are extruded from the core into the cytoplasm. 05/05/11 1:08 PM Hepadnaviruses 369 (a) S preS1 X pg preC DR1 DR2 (b) RNA polymerase II E preC Poly(A) DR1 DR1 DR2 P C pg Poly(A) LS MS SS preS1 Poly(A) MS SS S Poly(A) X X Poly(A) Figure 30.4 Transcription of hepatitis B virus DNA. The unusual structure of adeno-associated virus DNA, which is mostly single-stranded with terminal hairpins, makes it appear like damaged DNA to the infected cell. Careful quantitation of the effect of UV irradiation on synthesis of different viral mRNAs showed that the single-entry model is correct. Their interactions with cellular macromolecules and organelles can be analyzed, and their size, number per cell, and rates of synthesis and turnover can be studied. The fusion peptides—present as polypeptide loops—are hidden in the dimer interphase. There are four D proteins for every viral capsid protein. DISTINCTIVE CHARACTERISTICS Associated with aquatic environments. The majority of the capsid subunits bind to RNA non-specifically, perhaps just through neutralization of the negative charge on the phosphate groups. Under some stress conditions, virus infection may benefit plants. An adenovirus/retrovirus chimera utilizes the ease of production, high titer, and wide tropism of adenoviruses in conjunction with the ability of retroviruses to integrate their proviral DNA. Eight transcriptional promoters are used by cellular RNA polymerase II to initiate transcription, and twelve polyadenylation signals define the 3⬘ ends of mRNAs. Transcription units can be divided into three classes, depending on the timing of their use during the infectious cycle. The circular minus-strand RNAs then serve in the same fashion as templates for the synthesis of multimeric, linear plus-strand RNAs, which are cleaved and circularized (steps 5–8). Of the 365 protein-coding genes of PBCV-1, 227 are expressed before viral DNA synthesis begins. Unlike siRNAs, which are produced from long and perfectly complementary viral double-stranded RNA molecules, miRNAs are typically made from imperfect RNA hairpins in transcripts synthesized by cellular RNA polymerase II (Figure 33.11, right side). Biochemical experiments failed to uncover the correct answer, but, as was true for amantadine, molecular genetics succeeded in defining the drug target. • Research on the replication of bacteriophage and animal virus DNAs laid the foundations for understanding the enzymes involved in cellular DNA replication. Below are sequences corresponding to the end of the upstream gene, the intergenic sequences, and the start of the downstream gene. KEY TERMS Helicase Lipopolysaccharide Primosome Procapsid Rolling circle Scaffolding proteins FUNDAMENTAL CONCEPTS • Overlapping reading frames in genomes of microviruses maximize genetic coding capacity. Section VIII covers small infectious entities that are not viruses: viroids, which are virus-like nucleic acids that replicate but code for no proteins; and prions, which are infectious proteins that contain no detectable nucleic acid. Furthermore, their study has helped uncover basic mechanisms of gene expression in mammalian cells. These complement fragments trigger tissue mast cells to release histamine and other mediators that augment blood flow and vascular leakage, and attract leukocytes to the site of infection. • Poxvirus envelopes are assembled de novo within virus factories in the cytoplasm; they are not formed by budding from pre-existing cellular membranes as are most animal virus envelopes. These are usually cleaved into unit-length molecules. It is also known that Rep78 is required to promote integration. In addition to viral RNAs and proteins, the yeast chaperone protein YDJ1 (Yeast DnaJ homolog) and the levels of unsaturated fatty acids in the infected cell are also important for the initiation of minus-strand RNA synthesis. In contrast to retroviruses, integration of AAV is site-specific. The general answer is: by changing 5'– 3' 3'–5' 5' 3' Ribosome 5' RNA replicase 3' Figure 5.4 Collision between ribosome and RNA polymerase. ITR: inverted terminal repeat; ⌿: packaging signal; Cre: recombinase. Cellular p53, like Rb, is a tumor suppressor protein. Infection of cells by coronaviruses induces dramatic rearrangement of the subcellular architecture, most notably of cytoplasmic membranes. How do virions get into cells? et. Furthermore, the translation termination signals for large T antigen on the early mRNA (UGA) and for VP1 on the late mRNA (UAA) are separated by only 8 nucleotides. PCNA binds to DNA polymerase and ensures that it does not dissociate from the DNA template. Current vaccines are highly purified, and the only common side effect is soreness at the site of vaccination. Left: a model of Sendai virus nucleocapsid, showing N protein subunits bound to genome RNA (black) in a left-handed helix 20 nm in diameter, with 13 protein subunits per turn and a pitch of 5.3 nm. The third CII-activated int xis exo N cI cro cII O P Q PInt PRE PAQ CII CII CII Figure 8.6 CII-activated promoters in lysogenization. The infection causes an acute but relatively short-lived reduction in red cell production in the bone marrow. Top: genome RNA is shown with sequence elements believed to participate in formation of replication complex (not drawn to scale). There is also cross-talk between these two defense responses, such as the involvement of salicylic acid in both RNA silencing and the hypersensitive response. What molecular criteria are used to classify viruses? Louis Pasteur and Robert Koch firmly established the science of bacteriology in the latter part of that century by isolating and growing a variety of bacteria, some of which were shown to cause disease (e.g., tuberculosis). GENES AND PROTEINS Two transcription units: early and late. pH-independent penetration 16/05/11 7:52 PM 50 CHAPTER 4 Virus Entry Table 4.1 Acid-activated and pH-independent viruses Acid-activated penetration pH-independent penetration Orthomyxoviruses (influenza A and B viruses) Most retroviruses (HIV-1) Togaviruses (Semliki Forest and Sindbis viruses) Herpesviruses (HSV-1) Flaviruses (tick-borne encephalitis virus) Paramyxoviruses (Sendai, measles) Rhabdoviruses (rabies, vesicular stomatitis virus) Some coronaviruses Filoviruses (Ebola virus) Some picornaviruses (poliovirus) Bunyaviruses (Uukuniemi virus) Reoviruses Adenoviruses (types 2 and 5) Some picornaviruses (rhinovirus) Some retroviruses is triggered by interactions between the virus and its specific receptor. Vaccinia virus genes required for DNA replication are expressed immediately following infection, and are therefore called early genes. A vaccine based on live bacteria would be inexpensive to produce and could be administered orally. This allows replication, expression, and packaging of the genome of vesicular stomatitis virus. These promoters could have different strengths, allowing control of the amount of each mRNA produced. When the template is antigenome RNA, there are no further transcription initiation signals downstream of the promoter within the trailer sequence, and RNA polymerase therefore dissociates from the template. After these Stat dimers are released, they bind to cytoplasmic IRF-9 and the trimeric complex translocates to the nucleus, where it binds to sequences within the promoter regions of a variety of interferon-stimulated genes to activate their transcription. From top to bottom: circoviruses have genomes of less than 2 kb; many positive-strand RNA viruses have genomes of approximately 12 kb; medium-size bacteriophages such as lambda have genomes of 50 kb; the largest known viral genome, that of mimivirus, is 1.2 Mb long. Both the minus and plus DNA strands are then extended by reverse transcriptase to the ends of their respective template strands. Unfortunately, the human Trim 5␣ homolog fails to recognize HIV-1. Finally, the virus could have been passed on to humans in a form that was virulent and transmissible, causing the pandemic. The result is the synthesis of a progeny viral plus-strand DNA circle and regeneration of the double-stranded replicative form DNA each time the replicative machinery makes a full turn on the template DNA. Pregenome RNA is both an mRNA and a template for synthesis of genome DNA. The three ␣-helical regions in the C-terminal region are at the left, and at the right is the flexible N-terminal region containing the octapeptide repeats (blue half-circles) with bound copper ions (green spheres). Two of the most important killing mechanisms involve the production of reactive oxygen and reactive nitrogen intermediates. This new disease was named severe acute respiratory syndrome (SARS), and the causative agent was quickly identified as a previously uncharacterized coronavirus. Rudiviruses escape from the cell by means of unique pyramidal structures Unlike many other viruses of crenarchaeota, infection by rudiviruses kills host cells. Gene expression is controlled by the strength of promoters and transcriptional terminators Unlike most DNA viruses, microviruses do not produce regulatory proteins to ensure temporal control of gene expression. Baculoviruses also code for proteins that inhibit apoptosis (programmed cell death). The simplest form of this plasmid contains the transgene flanked by the two LTRs of HIV in addition to the other essential cis-acting elements, which are the packaging signal, the RRE, and the cPPT. (a) Transmission electron micrograph of a thin section of Vero E6 cells from which Ebola virus particles are budding. However, very little research has been conducted on them. and Cassinotti, P., "Parvoviruses," 261–279, Topley & Wilson's Microbiology and Microbial Infections, Vol. Serotypes are defined by antibody recognition sites on the small surface protein. It also uses scaffolding proteins to assemble virions, cleaving genome-length fragments from oligomers made during DNA replication, and stuffing them into procapsids, which are subsequently joined to a helical tail structure. Influenza virus (Figure 2.1g), which contains helical nucleocapsids, can adopt a variety of shapes and sizes from roughly spherical to elongated and filamentous (see Figure 18.2). • Smallpox is the only human infectious disease that has been totally eradicated. Transcription of the provirus DNA may also be affected by external stimuli, which can change the mix of active transcription factors present. This completes the synthesis of the subgenomic RNA. These three proteins accumulate in regions of the membrane enriched in cholesterol, named lipid rafts, which are believed to be the site of virion formation by budding. It is not clear why the virus has three origins, since any two can be inactivated and the virus can still replicate in cultured cells. Chapter 2 examines the unique features of the intricately constructed symmetrical capsids that most viruses use to package their genomes, and discusses how lipid envelopes are formed. • Mimivirus appears to replicate exclusively in the cytoplasm of infected cells. CR3 contains two regions involved in transactivation: a zinc finger that interacts with the TATA-binding protein (TBP) of the basal transcription machinery, and a promoter-targeting region that activates cellular transcription factors such as ATF (Figure 23.5). Joining these two domains is the central domain I, which contains the amino terminus of the protein. However, AAV DNA has been found in the form of stable, unintegrated double-stranded monomers and concatamers in the nucleus. Prangishvili, A unique virus release bcreidt.indd 485 485 mechanism in the Archaea, Proc. Since viruses displace the normal reproductive organs, the algae are rendered sterile; however, this does not affect their vegetative growth. When associated with middle T antigen, c-Src loses a phosphate group at tyrosine 527 and phosporylates itself at tyrosine 416 (Figure 21.9). Each large protrusion found at the 12 icosahedral vertices is composed of five copies of the major spike protein G (total 60 copies) and one copy of the DNA pilot protein H. Proteolytic processing by cellular signal peptidase (arrows with white squares) produces PE2, 6K, and E1. (a) and (b) show virus bound to cell surface; (c) and (d) show fusion of the viral envelope with the plasma membrane; (e) and (f) show nucleocapsids (arrows) detaching from the tegument (arrowhead) at the plasma membrane; (g) and (h) show empty capsids (arrows) at the nuclear pores (fibers shown by arrowheads) after release of DNA into the nucleus. This approach was demonstrated to be effective for retargeting lentivirus vectors to B lymphocytes by using an antibody against CD20, a protein specifically expressed at the surface of these cells. Future research will likely follow two major lines: (1) exploration of the diversity of archaeal viruses in different environments, and isolation and characterization of new virus–host systems; and (2) in-depth analysis of the biology of selected archaeal viruses. Thus T7 DNA does not have a circularly permuted sequence, as does the DNA of the unrelated phage T4. Maraviroc: an antiviral agent that binds to the cellular CCR5 chemokine receptor membrane protein, which serves as a co-receptor for HIV-1; binding of maraviroc can therefore block HIV-1 entry. DISC activates caspase 8, which in turn activates caspases 3 and 7. (Singular: microvillus.) Mitotic arrest-defective (MAD): proteins that control entry into anaphase, preventing separation of chromosomes on the mitotic spindle in dividing cells. 4 in Berns, K.I. and Linden, R.M., "The Cryptic Lifestyle of Adeno-Associated Virus," Bioessays 17 (1995): 237–245, with permission from John Wiley and Sons; Fig. The cleaved, 55 kDa terminal protein (TP) is represented by a black square. For most viruses, packaging the viral genome is under tight control. The GroE gene was so named because certain mutations in the E gene allowed growth on these E. However, this has not yet happened, and it is very difficult to predict whether such a virus will appear, particularly since determinants of pathogenicity and transmission are still incompletely understood. This primed state has been likened to a "Jack-in-the-box", awaiting activation by some triggering step. Compact helical nucleocapsids. The many ways in which viral genomes are uncoated and released The ultimate goal of virus entry is to release the viral genome within the host cell in a form able to be translated, transcribed, or replicated. Recent observations suggest that apoptosis is a primary line of host defense and that herpes simplex virus, like many other viruses, has evolved a number of mechanisms designed to block this host response to infection, allowing infected cells to survive long enough to produce progeny virus. The two intermediate transcription units are named IX and IVa2. Picornaviruses 125 Picornaviruses cause a variety of human and animal diseases including poliomyelitis and the common cold 125 Poliovirus: a model picornavirus for vaccine development and studies of replication 126 TOC.indd x Picornavirus virions bind to cellular receptors via depressions or loop regions on their surface 127 Genome RNA may pass through pores formed in cell membranes by capsid proteins 128 Translation initiates on picornavirus RNAs by a novel internal ribosome entry mechanism 128 Essential features of picornavirus IRES elements 130 Interaction of picornavirus IRES elements with host cell proteins 131 Picornavirus proteins are made as a single precursor polyprotein that is autocatalytically cleaved by viral proteinases 131 Picornaviruses make a variety of proteinases that cleave the polyprotein and some cellular proteins 131 Replication of picornavirus RNAs is initiated in a multiprotein complex bound to proliferated cellular vesicles 131 RNA synthesis is primed by VPg covalently bound to uridine residues 133 Virion assembly involves cleavage of VP0 to VP2 plus VP4 133 Inhibition of host cell macromolecular functions 134 12. Similarly, computer viruses transfer information from computer to computer, propagating themselves, although their "program" is carried by electronic bytes rather than by a nucleic acid. Virus vectors are produced by transfection of cells with plasmids containing deleted genomes For gene replacement, an ideal vector should be capable of delivering a transgene specifically to the appropriate target cells and expressing that gene sufficiently strongly to obtain a therapeutic effect. Epstein–Barr virus infects mucosal epithelial cells and B-lymphocytes The genome of Epstein–Barr virus is approximately 180 kb in length, similar to that of herpes simplex virus. However, sequence analysis of the genome of HIV-1 revealed it to be considerably more complex than many other retroviruses. These homotrimers are extended perpendicular to the membrane, exposing the fusion peptides, which insert into the target membrane. Acanthamoeba polyphaga mimivirus: 911 genes. Viruses are the only known forms of life that can have genomes made of RNA. Anti-prion protein antibodies and antibody fragments can block prion formation in cultured cells in a dose-dependent fashion. Other lentiviruses block effects of high dUTP levels by encoding and packaging a dUTPase, which hydrolyzes dUTP to dUDP, rendering it unavailable for the synthesis of viral DNA. In 1963, Alfred Hershey and coworkers, and Hans Ris, showed that the ends of chromosomes could bind to each other, cyclizing the molecule. (b) Viral DNA entering the host cell. 1947 to differentiate between infectious (enteric) and serum hepatitis. CH035.indd 442 To everyone's relief (including Joseph's), the vaccine appeared to work and its popularity spread. The first model proposes that preformed nucleocapsids migrate to the plasma membrane, where they bind to sections of the membrane that are densely populated with virus envelope glycoproteins, and the enveloped particle is formed by budding from the surface of the infected cell (Figure 13.4; see also Figure 2.11a). VP4, the N-termini of VP1, and the genome RNA bound at its 5' end to VPg are all located in the interior of the capsid (above). However, plus-strand DNA synthesis is usually not completed by the time the virion is released from the host cell, and stalls when the dNTP pools within the virion are exhausted. The transgene carried by lentivirus vectors can be expressed from a strong promoter such as the CMV promoter, or from an inducible promoter that allows regulation of protein levels. Shown at left is c-Src, a protein tyrosine kinase inserted into the plasma membrane via a N-terminal myristate residue. Scrapie infectivity was found to be resistant to treatment with agents that were usually effective inactivators of nucleic acids (psoralen cross-linking agents, nucleases, high concentrations of zinc ions, ammonium hydroxide) while sensitive to agents that were usually effective inactivators of proteins (strong alkali, phenol, sodium dodecyl sulfate). Chloroplast RNA polymerase resembles bacterial RNA polymerase in its resistance to ␣-amanitin. Two genera of RNA phages have subtle differences RNA phages have been placed into two genera on the basis of genetic maps, serological cross-reactions, and RNA size. Sialic acid: a 9-carbon acidic sugar derived from mannose, found on many glycolipids and glycoproteins. al., "Structural studies of the giant mimivirus," PLoS Biol. (Scale bar: 30 nm). system, and the fetus. Such a strategy was successfully employed to treat children suffering from severe combined immunodeficiency disease (SCID-XI). Icosahedral togavirus nucleocapsids assembled in the cytoplasm approach the plasma membrane and bind to the cytoplasmic tails of viral envelope proteins (Figure 2.11a). Such "generosity" to the host is thought to provide a durable intracellular refuge for the virus population in harsh conditions of natural habitats (often with temperatures above 80°C and pH values below 3). Analysis of the region sequence predicts a series of inverted repeats that fold into a three-dimensional stem-loop structure (Figure 30.5). (a) Cryoelectron microscopic image of purified virions of La Crosse virus (Scale bar: 100 nm). All other families in this group have

fragmented genomes (as many as eight genome segments). Two sequenced species of Ostreococcus, O. et al. • Many viral genomes contain specific packaging sequences that are recognized by capsid proteins, ensuring that only intact genomes are encapsidated. Type III interferons are produced in a similar fashion to type I interferons. A-74702 is a very weak inhibitor, but adding symmetrical valine-carboxybenzyl groups at both ends (A-74704 in Figure 36.9) resulted in a 40,000-fold increase in potency. Wp EBNA-LP EBNA-2 EBNA-3A EBNA-3B EBNA-3C EBNA-1 LMP-2A LMP-2B TR OriP IR1 IR2/OriLyt LMP-1 IR3 IR4/OriLyt TR Figure 24.10 Linearized genome of Epstein-Barr virus and transcripts expressed during latent infections. Because of the link with cancer, the focus of research on human papillomaviruses is now largely centered on the oncogenic human papillomavirus types 16, 18, and 31. Viruses with single-stranded RNA genomes fall into two categories. Virus groups that are covered in detail in this book are highlighted in green. The U3 region contains binding sites for other cellular transcription factors such as Sp1, LEF, Ets-1, and USF, which also play a role in modulating HIV-1 transcription. Procapsids are assembled with the help of scaffolding proteins, and single-stranded genome DNA is inserted into procapsids during Stage III DNA replication. (a) Seventy-two pentamers of VP1 are centered on lattice points of a T 7 icosahedral surface lattice. Some of these early proteins dissolve the core and release viral DNA into the cytoplasm, where further transcription and DNA replication take place. Unlike most DNA viruses of vertebrates, poxviruses replicate exclusively in the cytoplasm, importing all the transcription machinery they need within the virion core. Without exception, all of these viruses have unfragmented genomes; that is, all viral 2 Kb 12 Kb 50 Kb 0.7 m 4 m 16 m 0.4 mm 35 genes are contained in a single DNA molecule. However, these clinical trials were disappointing because the level of factor IX produced was lower than predicted from preclinical studies using animal models. Currently, treatment of Marburg and Ebola hemorrhagic fever is symptomatic and supportive. As already mentioned, binding of HIV-1 to the CD4 protein triggers a change in the HIV-1 glycoprotein trimers that allows them to bind to coreceptors in the cell membrane. This lifestyle resembles that of temperate viruses such as bacteriophage lambda, which integrates its DNA into the host cell genome and only replicates under certain conditions. Major (CTL) factor in limiting spread of viral infections. The arrangement of the operators at PR is shown in Figure 8.5a and b; PL has a similar arrangement of three operators (Figure 8.5c). Genomes are usually less than 2 kb long and in some cases code for no proteins. The viral gene segments may be maintained in circular form as shown here. AAV virions are stable; they are resistant to heat inactivation as well as large pH variations. Prophage: a repressed bacteriophage chromosome that is integrated into the bacterial chromosome or replicates as a free plasmid. Mice with a transgene containing the P102L mutation spontaneously develop a lethal scrapie-like disease. Several other diseases, including nasopharyngeal carcinoma and Hodgkin's lymphoma, have been linked to infection with Epstein–Barr virus. Pyroptosis: a form of programmed cell death that occurs during inflammation and requires activated caspase 1. CH036.indd 451 CH3 N H N O N N Nevirapine Figure 36.8 Nevirapine, a non-nucleoside inhibitor of HIV-1 reverse transcriptase. Capped 5' ends of cellular premessenger RNAs are used as primers for synthesis of viral mRNAs The PB2 protein in nucleocapsids contains a domain that recognizes the 5' cap structure found on all eukaryotic mRNAs. Capped cellular premessenger RNAs are synthesized in the nucleus by cellular RNA polymerase II, and undergo polyadenylation and splicing before being transported as mature mRNAs to the cytoplasm. In principle, the best targets are essential viral proteins that are sufficiently different from their cellular counterparts that the activity of the viral protein can be inhibited without affecting the function of similar cellular proteins. These fusion peptides insert into the cell membrane (step 2), and a cluster of such trimers forms (step 3). Internalization within an endosomic vesicle occurs quickly (within 10 minutes), after which the virion escapes to the cytosol in a process dependent on the acidic pH of the endosome. It might transmit hepatitis B virus to you! The first hepatitis B virus vaccine was prepared from the blood of paid chronic carriers. When phosphorylated, pRb dissociates from its E2F partner, allowing transcription of these cellular genes, entry into the cell cycle, and expression of proteins required for both cellular and viral DNA replication (see Figure 23.6). By convention, this is labeled the positive (or plus) strand. The herpesvirus cytomegalovirus causes infected cells to express increased CD55 and CD46 on their surface. Given the role of the GALT in regulating intestinal flora, it is has been suggested that its depletion may result in release of bacterial products such as lipopolysaccharide into the circulation, inducing a state of chronic immune activation known to persist in the chronic phase of HIV disease. These proteins have been termed class II fusion proteins, as they differ in several physical and functional aspects from the class I fusion proteins represented by influenza virus HA protein (see Chapters 4 and 18). This seems to be a rare event, as it has occurred only a few times in the past century, but the results can be disastrous. DISEASES Chickenpox, mononucleosis, pneumonia, hepatitis, encephalitis. Phage transcripts are cleaved by host RNAse III to small, stable mRNAs. DNA synthesis is initiated by phage RNA polymerase. • Bunyavirus transcription is coupled to translation and is primed by a cap-snatching process. The LexA repressor has a weak autoprotease activity that is enhanced by binding to the activated RecA protein, with the result that the LexA repressor cleaves itself into two domains and loses repressor activity, allowing induction of SOS gene transcription. Virus particles are very small: between 20 and 500 nanometers (nm) in diameter. As part of the "inflammosome," AIM2 and NLRP3 signal through the adaptor ASC to activate caspase-1, which cleaves pro-interleukin-1 to mature interleukin-1. Similar phages infect a variety of bacterial hosts, including marine bacteria. The core promoter sequences for minus-strand RNA synthesis include a stem loop within the 3'-terminal tRNAlike structure of the three viral genome RNAs. The 5' noncoding region and the noncoding region between the two reading frames of RNA 3 contain important sequences regulating the accumulation levels of genome and subgenomic RNAs. The minimum promoter sequence for synthesis of subgenomic RNA 4 contains a stem-loop structure and lies in the noncoding region upstream of the coat protein reading frame on RNA 3. • Poxviruses have evolved elaborate mechanisms for evading the host immune system. Baculoviruslike particles have been found in shrimp (Decapoda), but there are no equivalent baculoviruses that infect vertebrates, plants, bacteria, or archaea. Since most of the protein machinery used to replicate the genomes of these viruses is host-derived, substitution of the small part of the viral DNA that directs replication by bacterial host cell proteins (the replication origin) with a eukaryotic replication origin could potentially allow replication of a prokaryotic virus in a eukaryotic cell. This has been dubbed the "Cheshire cat" escape strategy, after the disappearing antics of another famous character in Lewis Carroll's book. Consequently, initial steps of virus entry are poorly understood. Attachment factor: a cell surface component involved solely in binding of virions to cells, but not in virus uptake. Having a second copy may be a backup mechanism in case one of the RNA molecules is damaged. The use of second- and third-generation adenovirus vectors greatly reduces problems caused by host immune responses. These are polypeptide hormones that communicate with other cells in the organism, inducing an antiviral state or stimulating the innate and adaptive immune systems to fight the infection. The parvovirus capsid protein therefore uses up about one-third of the coding capacity of the viral genome. RNA polymerase, RNA helicase, and RNA-modifying enzymes are encoded by the replicase gene The coronavirus replicase gene encodes multiple proteins that are known or predicted to direct viral RNA CH014.indd 165 165 synthesis or to interact with or modify RNAs (Table 14.2). The chloroviruses may follow the same pathway. Another tegument protein known as virion host shutoff (Vhs) protein, a product of the UL41 gene, interacts with several cellular proteins and mediates the Viral gene Class ICP4 α (IE) selective degradation of both cellular and viral mRNAs during the first few hours after infection. This forces bending of the membrane around the icosahedral nucleocapsid, culminating in fusion of the highly curved membrane and release of the virion from the cell. Following several days of incubation, transfected cells are lysed by repeated freezing and thawing, and viable virus is isolated by a plaque assay using L cells. Effects of mutations in viral proteins on cytopathic effects and on pathogenesis Infection of vertebrate cells with alphaviruses is usually cytopathic. The negative strands have an additional guanosine at their 3'-termini; this protrudes at the end of the replicative form double-stranded RNA, is required for efficient synthesis of plus-strand satellite RNAs, and may be required for the formation of multimers. The aggregate that forms retains gp160 inside the cell and therefore reduces gp120 incorporation into the virions released (Figure 29.7a). This stem recreates the replication origin found at both ends of the parental double-stranded DNA molecule. 2 in Longworth, M.S. and Laimins, L.A., "Pathogenisis of human papilomaviruses in differentiating epithelia," Microbiology and Molecular Biology Reviews 68 (2004): 362–372, with permission from the American Society for Microbiology. A map of the mouse polyomavirus genome is shown in Figure 21.4; the genome of simian virus 40 is organized in a very similar fashion. Sequencing of genomes of a many animal species has revealed that a significant fraction of the genome (8% in humans) is composed of retrovirus DNAs; these are called "endogenous retroviruses". Viruses can also induce apoptosis by binding to the cell surface, altering the organization of cellular membranes, modifying the cytoskeleton, disrupting the cell cycle, or activating cellular signal transduction pathways (see Chapters 21 and 23). These vaccines exploit the capacity of purified viral proteins to self-assemble into viruslike particles (VLP). It is activated by cellular nucleoside kinases and, in its triphosphate form, selectively inhibits viral reverse transcriptase. For example, p300 is associated with a number of cellular transcription factors, including p53 and AP-1. As a result, it makes a small and large T antigen, but no middle T antigen. Viral mRNA synthesis is primed by stealing capped 5' ends of cellular mRNAs. Bunyaviridae family. DNA entry at this stage progresses at about 250 bp/second, reflecting the high rate at which the phage RNA polymerase transcribes DNA. The DNA J domain of large T antigen is believed to act by helping to separate the Rb protein from a complex containing E2F and other proteins, thus stimulating transcription of cell-cycle and S-phase genes. CH028.indd 348 the right-hand LTR as they pass through that region on the proviral DNA, inactivating its ability to initiate transcription. Rather, reovirus-induced cytopathic effects directly cause injury of heart muscle cells. However, spread from plant to plant is often carried out by insects that feed on plant juices, ingesting virions and reinjecting them into other plants. A polyadenylation signal in the right-hand LTR directs cleavage of the transcript precisely at the R/U5 boundary, and the RNA 3⬘ end is polyadenylated by host cell enzymes. Extracellular virions are extruded through the plasma membrane by actin tails A small fraction of mature virus undergoes further maturation to become extracellular virus (EV), which is specifically designed for export and infection of neighboring cells. Furthermore, sizable regions of subterminal sequence are conserved among homologous genes of different virus strains. Similar folds are shared by capsid proteins of other animal and plant viruses, suggesting a common functional motif required for subunit packing and assembly of icosahedral virus particles. Because of the increasing interest in global climatic changes that are currently being observed, it is likely that research into algae (and their viruses) will become increasingly fashionable and essential in the future. These include mutants that (1) lack glycoprotein D, an envelope protein; (2) have a temperature-sensitive UL36 tegument protein that does not properly assemble virions; (3) lack ICP4 or ICP27, important viral regulatory proteins, and (4) lack the major subunit of ribonucleotide reductase, encoded by UL39. HSV: herpes simplex virus; VZV: varicella zoster virus. Replication complexes are associated with cytoplasmic membranes Membrane association of viral RNA synthesis is a common pattern among positive-strand RNA viruses of eukaryotes (see Chapters 10 through 13). • Baculoviruses are widely used as efficient eukaryotic expression vector systems, taking advantage of the highly active polyhedrin gene promoter to drive foreign gene expression. These functions may have been added on to the original functions of the P gene as the virus evolved, by the addition of new translation initiation sites and the RNA editing mechanism. huxleyi can grow in huge numbers to form what is known as a "bloom". Nucleocapsids also contain a trimer of RNA polymerase proteins PA, PB1, and PB2. Promoter occlusion may occur by dislodging of transcription factors from the downstream promoter by transcribing RNA polymerases that initiate upstream. When all of these proteins are in place, transcription of early genes begins. The integrase removes the two 3'-terminal nucleotides of each strand of the linear viral DNA. These viruses contain seven genes, arranged in an order similar to those of paramyxoviruses and rhabdoviruses. Binding to cell receptor 2. IgA is involved in providing immune defenses in the mucous membranes of the gut, respiratory and urogenital tracts. Molecular hybridization with oligonucleotide probes, or the polymerase chain reaction (PCR), can be used to detect specific viral DNA or RNA molecules. Furthermore, vaccinated individuals were protected against development of pre-malignant cervical lesions. By mimicking cytokines, virokines can augment virus replication or suppress host defenses. IRES sequences have also been found in other virus mRNAs and in a number of cellular mRNAs. (a) Type I IRES GNRA IV A/C-rich V VI III II AUG 568 I 5' pyrimidine-rich Polyprotein ORF 3' AUG 743 (b) Type II IRES I A/C-rich GNRA A Essential features of picornavirus IRES elements Secondary structure elements in RNA (stem loops) can be detected both by computer prediction from the primary sequence and by treating RNAs in vitro with structure-specific chemical and enzymatic probes. 419 marrow contains multipotent stem cell precursors for both the red and white blood cell populations, including lymphocytes. However, the RNA primer at the very 5 end of a linear DNA (orange "X") cannot be replaced because there is no DNA upstream. All 10 reovirus double-stranded RNAs are simultaneously transcribed, (b) λ2 σ2 λ1 λ3 RNA pol μ2 (c) (d) () mRNA 5 cap Figure 19.6 Synthesis of reovirus messenger RNAs. (a) Diagram of the viral core transected through one of the 12 spikes. This can lead to enhanced antigen presentation, which may result in clearance of chronic viral infections. For use in vaccines, poxviruses can be engineered to express foreign proteins under control of viral transcriptional promoters (see below). These non-infectious particles contain little or no LS protein, since its presence favors the retention of surface proteins within the endoplasmic reticulum. Sialic acid is a common target bound by surface proteins of many animal viruses. Replicating DNA in infected cells is found as high molecular weight, branched head-to-tail multimers called concatemers, suggesting that DNA is made by a rolling circle mechanism, which can produce linear multimers from a circular template. coli RNA polymerase 2.5 Single-stranded DNA binding protein 3 Endonuclease, degrades host cell DNA and cleaves T7 DNA concatemers 3.5 Lysozyme, inhibits T7 RNA polymerase 4 RNA primase/helicase, initiates lagging-strand DNA synthesis 5 DNA polymerase 6 Exonuclease, degrades host cell DNA T7 RNAs are cleaved by host cell ribonuclease III to smaller, stable mRNAs The primary RNA transcripts of T7 phage undergo post-transcriptional processing by the host enzyme, ribonuclease III. In more recent constructs, the U3 region of the left-hand LTR, which contains the promoter and enhancer elements, has been replaced with a strong constitutive promoter such as CMV, or with the U3 region of another retrovirus such as Rous sarcoma virus (RSV). Furthermore, a number of viruses have double-stranded RNA genomes. This finding demonstrates that the 9S and 6S particles are the first intermediates in the assembly pathway and that the internal scaffolding protein B is required for capsid assembly to proceed. in 1974 from three-year-old Japanese boy (family name Oka) • Initial growth in human lung fibroblast cells • Twelve passages in primary guinea pig fibroblasts • Two passages in WI-38 cells (human diploid fibroblasts) • Three to six passages in MRC-5 cells (human diploid lung cells) • Licensed for use in Japan and Korea in late 1980s, and in USA in 1995 CH035.indd 433 16/05/11 8:21 PM 434 CHAPTER 35 Antiviral Vaccines therefore virus infectivity, while retaining the antigenicity of viral proteins. • Most plant viruses have positive-strand RNA genomes. Circular satellite RNAs, like viroids, have a highly base-paired structure. The chaperone protein may be involved in the assembly of the replicase complex, and fatty acid levels may affect the assembly of membranes within this complex. The translation products include a viral RNA polymerase, which can then replicate the viral genome and synthesize more viral messenger RNAs. These viruses therefore do not package an RNA polymerase in their virions. Type I membrane proteins have a signal sequence that is cleaved from their N-terminus by a peptidase as they are inserted in the endoplasmic reticulum during synthesis. The earliest known written reference to measles is by an Arab physician, Rhazes of Baghdad, in the tenth century. Other viruses have class II fusion proteins with a somewhat different design. For mouse polyomavirus, the 3' ends of the mRNAs overlap by some 40 nt, and the AAUAAA signals encoded by the two DNA strands are separated by a single nucleotide (shown by dashed lines). Figure 34.1 Cells involved in the innate and adaptive immune responses. The original virus strain could have been passed from bats to an animal that served as an intermediate, or amplifying host. Herpesviruses 285 Bernard Roizman, University of Chicago Gabriella Campadelli-Fiume, University of Bologna Richard Longnecker, Northwestern University 25. The RNA polymerase then resumes elongation. During the early phase (Figure 28.3) the virus enters the cell, makes a DNA copy of its RNA genome, and inserts this copy into the host cell genome. molecular mechanism of this apparently translational inhibition is not yet clear. Protrusion of tails does not require the presence of host cells, an exogenous energy source, or any cofactors. However, there are several ways to minimize the likelihood of contracting the virus. If viral DNA replication is inhibited, expression of both intermediate and late genes is prevented. . Chlorella infected with these viruses produce either chitin or a chitin/hyaluronan on the surface of infected cells. Figure 14.1 shows a map of the genome of murine hepatitis virus. (Figure 10.4). Plus-strands are light blue, minus-strands are black. Innate and Adaptive Immune Responses to Virus Infection 415 Malcolm G. One such case is the host resistance (R) gene, whose phenotype is a hypersensitive response (HR) to infection. However, much work has been done with cloned filovirus genes or with nonpathogenic virus-like particles made with filovirus proteins. Since phage infections involve introduction of foreign DNA into the host cell, phages are often the target of this sort of restriction. The disease was imported from the Caribbean in July of that year and quickly established itself in the city due to the presence of an unusually high number of mosquitoes. CH001.indd 17 16/05/11 7:46 PM C H A P T E R 2 Virus Structure and Assembly Stephen C. (Scale bars: (a) to (c), 500 nm; (d) to (h), 100 nm). 16.1a–c: Courtesy of Dr. Yoshihiro Kawaoka and Dr. Takeshi Noda. Name three ways viruses adapt to the host organisms in which they replicate. The reaction proceeds as follows (Figure 28.5): U5 C GTAGG C CC G HO AA TG C GA H G O CG CC U3 TG GOH U3 R U5 CCCGG AC U3 R U5 R U5 CA GGCCC GT HOG AA CH028.indd 347 R AA Integration sites appear to be distributed randomly over the host genome. For example, an occasional serious complication with a rotavirus vaccine might theoretically be tolerable in the developing world, where rotaviruses kill more than a million children every year. Normally p53 is rapidly degraded, is present in very low concentrations in the cell, and is inactive as a transcription factor. Two-dimensional crystals of PrPSc have been analyzed by electron microscopy (Figure 32.4b) and a model of the structure of this protein has been proposed (Figure 32.6). TYPES OF ANTIVIRAL VACCINES Live wild-type viruses that infect other animals but are attenuated in humans. The explosion of research on RNA phages laid the foundations for the careers of a number of modern day molecular biologists. PrPC is found in neuronal synapses, binds copper, has unknown function. Bunyavirus mRNAs are not polyadenylated at their 3' ends. (c) Vaccinia virus. Polyadenylation signal: in eukaryotes, a consensus AAUAAA sequence in messenger RNAs that is recognized by proteins that bind to the RNA, cleave it downstream of the AAUAAA to create a new 3⬘ end, and add a poly (A) tail to the 3⬘ end. Condit, University of Florida 27. Each chapter begins with an outline. Simian virus 40 late transcription is initially blocked by binding of a cellular protein to viral DNA near the late promoter. As discussed previously, these E1B proteins blunt the pro-apoptotic effects of E1A, and inhibit p53-induced growth arrest and apoptosis. These results suggest that, at least in some cell types, growing bunyavirus mRNAs can be translated before their synthesis is completed, and that translation is required for the proper elongation of mRNAs. Ribosomes translating or scanning the mRNA in the 5' to 3' direction could dislodge interactions between the growing mRNA chain and the template genome RNA that might otherwise slow down or block elongation by the viral RNA polymerase. CH015.indd 185 185 The V protein, which shares common N-terminal sequences with P but has a distinct and highly conserved C-terminal region, is not normally required for replication of paramyxoviruses in cells cultured in vitro. Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030-5774, (201)748-6011, fax (201)748-6008, website .com/go/permissions. Disseminated intravascular coagulation: systemic clotting of the blood; can be caused by activated neutrophils. Direct consumption of antigen-containing food can sometimes result in acquisition of immunity to the targeted viruses. The 1918 pandemic influenza A virus was probably not a reassortant virus In an experimental tour de force, scientists recently recovered genome fragments of the influenza A virus implicated in the 1918 pandemic from preserved human tissue, and determined the complete genome sequence of this virus. Protein 3a (30 kDa), also known as the movement protein (MP), is a nonstructural protein essential for virus movement in host plants. The fact that Marburg and Ebola virus species are pathogenic for monkeys and apes argues against the idea that these animals may be a reservoir for the virus; they are more likely secondary hosts, as are humans. Genome RNAs are capped at their 5' termini and polyadenylated at their 3' termini, as are eukaryotic mRNAs. A 150- to 200-nt repeated sequence (R) is present at both the 5' and 3' ends of the genome RNA. In most instances, the viral DNA cannot produce infectious virus because of mutations that disrupt or delete reading frames or gene expression control sequences. All picornaviruses initiate protein synthesis via an internal ribosome entry site (IRES) that lies within the highly structured 5' noncoding region. PrPC (Figure 32.3) is a 207-amino acid glycoprotein normally expressed on the surface of neurons, lymphocytes, follicular dendritic cells, and other tissues. Phagocytosis by leukocytes is greatly augmented by binding of complement fragments or antibody to virus particles; phagocytic cells have receptors that recognize these components on the surface of pathogens. CEACAM1 is a member of the immunoglobulin family and is widely distributed in all mammalian species. Like other positive-strand RNA viruses, coronavirus genomes are infectious; introduction of purified genome RNA into a cell will initiate infection and result in production of new virus particles. Satellite viruses and satellite nucleic acids require a helper virus to replicate A number of very small virus-like genomes replicate only in association with other viruses. The single-stranded, positivesense RNA genome contains a 5' methylguanosine cap and a 3' poly(A) tail. They would serve as a reservoir for the virus, from which it occasionally emerges to infect primates, probably via direct contact or contact of secretions/excretions. Currently being investigated as gene therapy vectors. In reading frame –1, the protein is extended to a size of 421 amino acids before a termination codon is encountered. Transcribed by cellular RNA polymerase II. The right half, from about 50 to 90 map units, codes for the viral capsid (Cap) proteins. Single-Stranded RNA Bacteriophages 59 Jan van Duin, University of Leiden 6. The most familiar unit of evolving information is the gene, but evolution can occur based on any mode of information transfer. SARS ORF6: interferon antagonism. The only known human viroid-like agent, hepatitis delta virus, has an RNA genome but does not encode its own RNA polymerase. Pseudoknot: an RNA secondary structure in which bases in the loop at the end of a stem form hydrogen bonds with bases at a distant site on the same RNA molecule. But his proposal does explain exactly the principle by which parvoviruses duplicate the ends of their linear DNA. Viruses are obligatory intracellular parasites. Reovirus infection of mice causes pathology in a wide range of other organs and tissues, including the liver and bile ducts, heart muscle, lungs, exocrine pancreas, and endocrine tissues. A number of cellular proteins known to be involved in trafficking and sorting of intracellular vesicles have been shown to bind to proteins that contain such late domains. Additional stop-transfer and signal sequences in the PE2, 6K, and E1 regions of the polyprotein lead to insertion of the 6K protein and translocation of the E1 portion into the endoplasmic reticulum membrane. It causes fetal abnormalities and birth defects when women are infected in the first trimester of pregnancy. occlusion-derived virus, which is responsible for spreading of the virus from one host to another (vertical transmission). Hepatitis C virus blocks interactions between NK cells and dendritic cells by inhibiting the production of dendritic cell proteins that are required for binding to NK cells. As budding begins, each of the 240 capsid protein subunits of the core binds to an E2 protein molecule in the membrane. Pharmacokinetics: study of the fate of a drug when administered to an organism, including its uptake, its distribution, its degradation, and its clearance. The practice was replaced by vaccination in the nineteenth century. Synthetic and natural double-stranded RNAs are also potent inducers of interferon synthesis, and double-stranded RNA may represent the natural signaling molecule by which virus infection leads to interferon production (Figures 33.1, 33.2, and 33.3). The 116-amino acid Rev protein contains both a nuclear localization signal and a nuclear export signal, indicating that this protein can shuttle continuously between the nucleus and cytoplasm. The viral E1 protein is a DNA helicase that binds to the origin of replication and locally unwinds the viral DNA, allowing cellular DNA polymerases and other cellular proteins to carry out viral DNA replication. 5'-terminal cap, 3' poly(A) tail. Hundreds to tens of thousands of copies of the viral genome can be made in each cell. Furthermore, recent developments allow recovery of fully infectious virus from cloned DNA copies of the genomes of negative-strand viruses. Antiviral drugs, in conjunction with viral mutations that confer drug resistance, can help to identify the roles of viral proteins and dissect the details of how these proteins do their jobs. Some movement proteins remain in the plasmodesmata. Most of these late promoter sequences are not associated with a consensus TATA box. It is tempting to speculate that the gokushoviruses, like the ancient coelacanth fish, have persisted by occupying a niche free of competition from larger phage species. Extension of transcription beyond the polyadenylation site could also produce RNA antisense to ICP4 mRNA. In contrast, the B-free variant (see text box on page 75) requires 20 minutes and a much higher critical concentration of the external scaffolding protein D. Although relatively efficient, this method has the disadvantage that vector preparations are contaminated by adenovirus virions. The 3.5-kb pg and preC transcripts are exceptional in that they extend more than once around the circular DNA genome. Includes chapters on important human pathogens such as herpes viruses, hepatitis B and C viruses, human immunodeficiency virus, influenza viruses, measles virus, poliovirus, smallpox virus, West Nile virus, and others.New and improved comprehensive, illustrative art program. Below, replication: when sufficient N protein is made, the L protein initiates RNA synthesis directly at the 3' end of genome RNA and replicates the genome via an antigenome copy, which is encapsidated. The RNA primers are subsequently removed and DNA polymerase extends upstream DNAs, filling in the gaps. Such plasmids can be effective mimics of viral infection and can elicit broader immune responses than soluble proteins, because the viral proteins are synthesized in cells within the host and thus are more efficiently recognized by the immune system. • Another anti-HIV drug, enfuvirtide, blocks a conformational change in viral envelope protein gp41 that is required for fusion and viral entry. Tumor formation by acute transforming retroviruses is directed by proteins that are not required for virus replication. How the RNA then gets inside the cell is not known. Differences in the 5' untranslated region of the genome have been correlated with changes in survival of infected animals. Transcription of class II and III genes requires a novel T7-coded RNA polymerase The initial steps of infection described above can take place in the presence of an inhibitor of protein synthesis, but further transcription and entry of the remainder of the genome is blocked unless phage class I proteins are synthesized. However, the live vaccine is still preferred in resourcepoor settings with lower rates of vaccine coverage. For a detailed discussion of virion structure and assembly, see Chapter 2. This gene arrangement closely resembles that used by picornaviruses, a family of non-enveloped positive-strand RNA viruses (Chapter 11). Mutations that destabilize the lysis hairpin by introducing base mismatches allow lysis gene translation independent of the coat gene. Seed transmission occurs when the virus infects seed tissues after fertilization, and enters the newly developing plant during embryogenesis. D. Ekefjärd, T. In response to appropriate signals, the integrated retrovirus genome can be transcribed by cellular RNA polymerase II, giving rise to full-length viral genome RNA and to spliced versions that serve as messenger RNAs for viral structural and enzymatic proteins. In the case of HA and some other viral spike glycoproteins, the activating cleavage occurs just to the N-terminal side of a hydrophobic sequence of about 10–12 amino acid residues. Interestingly, the distinction between helperdependent and autonomous parvoviruses may not be as rigid as first thought. 16/05/11 8:21 PM New Developments in Antiviral Vaccines However, protection by other live attenuated vaccines such as measles and mumps may be lost over time in the absence of periodic boosting by either revaccination or exposure to natural disease. At a late stage in the maturation process of the virion, a cellular furin protease cleaves prM, releasing the "pr" peptide into the extracellular medium when the virion is released from the cell, and leaving the small M protein associated with the virus particle. 05/05/11 2:45 PM Glossary Jelly-roll β-barrel: folding of a polypeptide chain into a number of parallel strands, usually as two sheets, found in many virion capsid proteins. Interferons are secreted into the extracellular medium. The transfer construct provides the transgene expression cassette, which is replicated and packaged in AAV capsids because of the inverted terminal repeats (ITR). Diagnostic tests are now available based on the detection of viral DNA and are often used in conjunction with the Pap test. Internal envelope is not formed by budding but is assembled de novo. • In the absence of sufficient concentrations of CI repressor, transcription begins at promoters PL and PR on the lambda chromosome, but terminates shortly downstream at terminators tL1 and tR1. Borelli, Sangita Venkataraman, Gabriel Lander, Padmaja Natarajan, John E. (c) Assembly of viral capsids in the cytoplasm. The seven viral genes are shown above the genome map along with the leader and trailer sequences. In that year, the World Health Organization launched the Global Smallpox Eradication Program, a massive, internationally coordinated program of surveillance and vaccination, which ultimately lead to the eradication of smallpox worldwide, certified in 1979. In the process, late endosomes are formed that have the characteristics of multivesicular bodies; they contain intralumenal Binding of viruses to their receptors occurs via specific binding sites exposed on the surface of the virion. Brooks, III and Vijay S. 17.1a: Courtesy of B. It also interacts with the gene 4 helicase to stabilize the replication complex. For this reason RNA phages are used as index organisms to reveal the potential presence of pathogenic enteroviruses in drinking water. How they enter and exit their host cells is largely unknown. SS has four ␣-helical hydrophobic regions. This is called promoter occlusion. PKR (double-stranded RNA-dependent protein kinase): a cellular serine-threonine protein kinase that inactivates protein synthesis by phosphorylating eukaryotic initiation factor 2B (eIF-2B), in response to virus infection or the presence of double-stranded RNA. It would seem that the virus-infected cells make a more appealing snack for grazers! High concentrations of DMS can also prevent further infection of E. Orthomyxovirus virions must contain at least one copy of each genome segment in order to be able to initiate an infection. RNase H digestion of the 3' end of the tRNA, now part of an RNA-DNA hybrid, removes the tRNA from the minus-strand DNA copy and exposes the primer binding site on the plus-strand strong-stop DNA. However, release of such a virus into nature may be difficult to justify unless extensive testing shows its safety for humans and other animals. Target-based high throughput screen: method for screening large numbers of drugs for their ability to affect biological activity of a purified protein by use of automated in vitro assays. A viral tegument protein known as -transinducing factor (-TIF) or VP16 forms a complex with at least two key cellular proteins, Oct-1 Figure 24.4 Entry and uncoating of herpes simplex virus. For example, compared with herpes simplex virus DNA polymerase, cellular DNA polymerase alpha uses acyclovir triphosphate less efficiently as a substrate and it is less potently inhibited by the next deoxynucleoside triphosphate. CH006.indd 76 What early discovery about bacteriophage ϕX174 challenged the concept of the gene? By 1879, Victor Galtier had succeeded in passaging the virus in rabbits and had demonstrated limited efficacy with a rabbit tissue vaccine in herbivores. These mRNAs are translated and produce all eleven reovirus proteins, which can then direct a productive infectious cycle. These nucleocapsids subsequently reacquire an envelope by budding into Golgi membranes. Injection is fascinating and more than a little mysterious. The viral genome, an 8-kbp molecule of double-stranded circular DNA, is associated with cellular nucleosomal histones. A typical recombinant DNA used for gene expression in mammalian cells includes a strong transcriptional enhancer and promoter, appropriate sequences at the 5⬘ end of the mRNA for efficient initiation of translation, RNA splicing signals, and finally a strong polyadenylation signal. Members of the Levivirus genus (also called supergroup A) have RNAs approximately 3500 nt in length, and code for four proteins: maturation (or A) protein, coat protein, lysis protein, and the RNA replicase, encoded in that order proceeding from the 5' end of the genome (Figure 5.1). Early gene expression Analysis of viral macromolecules reveals the detailed pathways of virus replication This analysis of the virus growth cycle is very elementary and gives only an overview of the beginning and the end of steps in virus replication. In vitro experiments have shown that on short messenger RNAs containing only 5 codons, the ribosome shuffles back and forth between the start and stop codons, making multiple copies of a pentapeptide without ever leaving the template. They have capped 5'-termini and contain a 3'-terminal CCC sequence. Parvoviruses replicate in cells that are going through the cell cycle The small size and limited genetic coding capacity of parvoviruses make them exceptionally dependent on host cell functions. Ribavirin, a drug that is effective against other agents that cause viral hemorrhagic fever, has no effect on filoviruses. In professions where the virus might be expected to be in the environment, care when handling potentially contaminated surfaces, fluids, and sharp objects will minimize accidental exposure. Crimean–Congo hemorrhagic fever, a tick-borne disease found in Eastern Europe, Asia, and Africa, results in severe illness and high case fatality. An origin-binding protein (UL9) binds to specific 10 bp sequences in oriS or oriL and unwinds the two DNA strands with the help of the single-stranded DNA binding protein ICP8 (Figure 24.7). In this model, the N-terminal region of PrPSc is folded into a highly stable, parallel -helix. The decrease in the rate of synthesis of gene products at 4 hours postinfection involves several proteins in addition to the tegument protein Vhs. Interferon- alone is able to stimulate macrophage activation. In May 1790, a severe influenza epidemic struck Philadelphia, then the capital of the fledgling United States of America, and George Washington, the newly elected President, was close to death but eventually recovered. Many paramyxoviruses have a strict requirement that the length of their genomes must be an exact multiple of six nucleotides. • Paramyxoviruses and rhabdoviruses have six to ten genes arranged in a highly conserved order on the viral genome, beginning with nucleocapsid protein and ending with the viral RNA polymerase. This may be another means of reducing the likelihood of inactivation by genome degradation, by reducing the size of each genome fragment; however, these viruses can only infect their hosts Table 3.3 Viruses with positive-strand RNA genomes Genome size (kb) and topology Capsid symmetry Virus group Hosts Example Leviviridae Bacteria Bacteriophage Q 1 3–4 Linear Icosahedral No Marnaviridae Algae Heterosigma akawisho RNA virus 1 9 Linear Icosahedral No Barnaviridae Fungi Mushroom bacilliform virus 1 4 Linear Icosahedral No Bromoviridae Plants Cucumber mosaic virus 3 8 Linear Icosahedral No 2 other families Plants and 8 genera Closteroviridae Plants 5 other families Plants and 5 genera CH003.indd 37 Genome segments Envelope Tomato bushy stunt virus 1–3 4–15 Linear Icosahedral No Beet yellows virus 1-2 15–19 Linear Helical No Tobacco mosaic virus 1–5 6–19 Linear Helical No 1–2 6–10 Linear Icosahedral No 1 26 Linear Helical Yes 2 families and 1 genus Invertebrates Cricket paralysis virus Roniviridae Invertebrates Gill-associated virus Nodaviridae Vertebrates & Flock house virus invertebrates 2 4–5 Linear Icosahedral No Astroviridae Vertebrates Human astrovirus 1 6–7 Linear Icosahedral No Picornaviridae Vertebrates Poliovirus 1 7–9 Linear Icosahedral No Caliciviridae Vertebrates Norwalk virus 1 7–8 Linear Icosahedral No Flaviviridae Yellow fever virus Vertebrates & invertebrates 1 10–12 Linear Icosahedral Yes Togaviridae Vertebrates & Semliki Forest virus invertebrates 1 10–12 Linear Icosahedral Yes Arteriviridae Vertebrates Equine arteritis virus 1 13–16 Linear Icosahedral Yes Coronaviridae Vertebrates SARS coronavirus 1 28–31 Linear Helical Yes 16/05/11 7:52 PM 38 CHAPTER 3 Virus Classification: The World of Viruses if at least one virus particle carrying each of the genome fragments gets into the same cell. Some of these DNA genomes are circular; others are linear. The nuclear localization signal of simian virus 40 large T antigen was one of the first such signals identified. Interestingly, none of these early genes have homologs in protein sequence databases, and most of the reading frames are modest in length. The structure of the ␣-helical part (ribbon helices) was derived from the known structure of the C-terminal region of PrPC. More importantly, 55K and a protein coded by the adenovirus E4 transcription unit, called E4orf6 (for "open reading frame 6"), form an E3 ubiquitin ligase complex, involving members of the cellular Cullin protein family and Elongins B and C, which targets bound proteins for degradation in the proteasome. The operator affinities for Cro are OR3 OR2 OR1. Mieke Mommaas, Eric J. Lentiviruses are able to replicate in resting cells, but other retroviruses cannot. Any partial hybridization of the insert to an RNA molecule other than the target would be very unlikely to align both ends of the MDV-1 RNA half-molecules in such a way that ligation could occur. Steps in DNA replication 1. NS5. All share the same 5' leader sequence and all contain overlapping sequences at their 3' ends. Furthermore, bacteriophages are the source of many tools commonly used in modern molecular and cell biology laboratories. VIRUSES AND HOSTS Six genera of Phycodnaviridae: Chlorovirus, Coccolithovirus, Phaeovirus, Prasinovirus, Prymnesiovirus, and Raphidovirus. Expressed during fetal development, Syncytin-1 can induce membrane fusion, and is active in the formation of the fused trophoblast layer of cells surrounding the fetus. All Ebola virus species tested make sGP as well as GP, but Marburg virus makes only GP. Muzyczka and K.I. Berns, "Parvoviruses," Chapter 32 in Fundamental Virology, D.M. Knipe and P.M. Howley (eds), Lippincott Williams & Wilkins (Baltimore) (2001); Fig. PB1-F2 is localized in mitochondria. PDZ domains are protein–protein recognition modules that organize diverse cell-signaling assemblies. 26.1c: Reprinted from Baroudy BM, Venkatesan S, Moss B, "Incompletely base-paired flip-flop terminal loops link the two DNA strands of the vaccinia virus genome into one uninterrupted polynucleotide chain," Cell 28 (1982), 315–324, with permission from Elsevier; Fig. First, the full-length RNA produced during genome replication is encapsidated by binding to N protein molecules, while mRNAs are not encapsidated. The translocated RNA fragment serves as a primer for subsequent synthesis of plus-strand DNA. However, their complex interactions with cellular signaling pathways that facilitate high levels of viral DNA replication and progeny virus production can occasionally transform a normal cell into a tumor cell. Particle reconstructions from cryoelectron micgrographs of mimivirus particles, using fivefold symmetry averaging. These genome segments are encapsidated by the nucleocapsid (N) protein to form ribonucleoprotein complexes with helical symmetry. CH027.indd 340 05/05/11 11:24 AM SECTION VII VIRUSES THAT USE A REVERSE TRANSCRIPTASE C H A P T E R 2 8 Retroviruses C H A P T E R 2 9 Human Immunodeficiency Virus C H A P T E R 3 0 Hepadnaviruses The viruses covered in this section use a reverse transcriptase, an enzyme that produces a DNA copy of an RNA molecule, during their life cycle. Many plant viruses are also transmitted by insects. Vpr also facilitates packaging within the virion of the cellular enzyme uracil DNA glycoslase. Matrix protein VP40 directs budding and formation of filamentous particles VP40, the most abundant protein in filovirus particles, is associated with the viral envelope. viral minichromosomes. The lipid bilayer of the envelope ensures impermeability to ions or nucleases that might damage the viral genome located inside the envelope. Indeed, one of the most widely used human cell lines, HeLa, was derived from a cervical cancer many years ago and has been shown to contain papillomavirus DNA and continues to express the E6 and E7 proteins. (b) View of a hexamer of large T antigen molecules showing the central channel through which DNA passes and the -hairpins that contact the DNA and pull it through the channel. As a result, the structures of larger and more complex virions depart in one way or another from the simple designs described above. These vectors tend to be less stable than replicons, particularly when the inserted foreign genes are large (greater than 2 kb). Nucleocapsids are exported from the nucleus in a complex with matrix protein and NS2 The NS1 protein interferes with polyadenylation of cellular mRNAs Remember that during the intial stages of infection, nucleocapsids are dissociated from the matrix protein as a result of low pH within the virion, brought about by the activity of the M2 ion channel. Because of this, vaccine developers are focusing on highly conserved, essential viral proteins and are considering vaccination with mixtures or "cocktails" of defined proteins. Thus, one of the advantages of the two-scaffolding protein system is speed. NS1 binds to and inhibits signaling by RIG-1, a cytoplasmic sensor that responds to RNA virus infections by the induction of interferon synthesis via interferon response factor-3. californica multiple nucleopolyhedrovirus 1 80–180 Circular Polyomaviridae Virus group Hosts Example Corticoviridae Bacteria Bacteriophage PM2 1 10 Plasmaviridae Mycoplasma Bacteriophage L2 1 Tectiviridae Bacteria Bacteriophage PRD1 1 Podoviridae Bacteria Bacteriophage T7 1 16–70 Siphoviridae Bacteria & archaea Bacteriophage lambda Myoviridae Bacteria & archaea Fuselloviridae 32–35 63 100–560 Linear or Icosahedral circular Linear Internal &/or external Icosahedral Internal Helical External Vertebrates Mouse polyomavirus 1 5 Circular Icosahedral No Papillomaviridae Vertebrates Human papillomavirus 16 1 8 Circular Icosahedral No Adenoviridae Vertebrates Human adenovirus 5 1 25–45 Linear Icosahedral No Asfarviridae Vertebrates African swine fever virus 1 170–190 Linear Icosahedral Internal & external Herpesviridae Vertebrates Herpes simplex virus 1 1 125–240 Linear Icosahedral External Iridoviridae Vertebrates & invertebrates Frog virus 3 1 135–303 Linear Icosahedral Internal & external Poxviridae Vertebrates & invertebrates Variola virus (smallpox) 1 130–375 Linear Complex External CH003.indd 36 16/05/11 7:52 PM Major Virus Groups The great disparity of viral genome size and complexity Viral genomes (Figure 3.2) range from slightly less than 2000 nucleotides (coding for 2–3 proteins) up to nearly 1.2 million nucleotides (coding for over 1200 proteins). Viruses are important disease-causing agents As living organisms arose and evolved during the past 4 billion years on Earth, they were probably always accompanied by viruses that could replicate within cells and pass from cell to cell. In principle, any step of viral infection can be targeted for inhibition. 2005. Current efforts in this area are directed at enhancing baculovirus stability in the human body and extending the life span of the viral genome in non-permissive cells. Virions subsequently enter the cell by endocytosis. When the layer of bound water molecules that normally prevents fusion is eliminated in a small local spot in the middle of the HA cluster, the two bilayers proceed to fuse (step 5). Procapsids are assembled by the use of scaffolding proteins The assembly pathway of ϕX174 (Figure 6.5) was elucidated by analyzing the intermediate capsid structures that accumulate in cells infected with virus mutants. The domains involved in binding by histone acetyl transferases (p300/CBP), Rb family members, and cellular transcription factors TATA-binding protein (TBP) and ATF are indicated. 378 CH031.indd 378 16/05/11 8:16 PM Viroids and Hepatitis Delta Virus Viroids are small, circular RNAs that do not encode proteins Until the early 1970s, viruses were believed to be the smallest agents of infectious diseases. Filovirus transcription, replication, and assembly As with paramyxoviruses and rhabdoviruses, transcription and replication of filovirus genomes take place in the cytoplasm of infected cells. MV membrane lateral body core MV DNA replication uncoating I uncoating II intermediate mRNA early proteins (VITF) intermediate proteins (VLTF) early mRNA nucleus late mRNA late proteins (VETF) WV MV EV EV IV EV membrane Figure 26.5 The vaccinia virus life cycle. Entry by fusion is mediated by envelope glycoproteins and may occur at the plasma membrane or in endosomes Herpes simplex virus initially binds to heparan sulfate proteoglycans at the host cell surface via viral envelope glycoproteins gB and gC. The rule of six (see text box) is less strictly observed, and the difference in strength between the promoters on the genome and the antigenome is reduced. T7 DNA polymerase can also act as a "proofreading" 3' to 5' exonuclease to remove misincorporated nucleotides. In contrast, both Vpr and IN interact directly with components of the nuclear pore. caused the deaths of 20 million people—more than all the battles of World War I. Up to that time, all known RNA polymerases were multi-subunit enzymes, so the discovery of this single-subunit RNA polymerase, which does not depend on any host proteins for function, galvanized the transcription field. Briedis, McGill University Terence S. Different members of a genus may infect different organisms or different tissues in the same organism, and therefore may not usually come in contact with each other. Two to six weeks following exposure to the virus, individuals can develop a mononucleosis or influenza-like syndrome (fever, malaise, lethargy, nausea, diarrhea, headaches, 16/05/11 8:15 PM 356 (a) CHAPTER 29 Human Immunodeficiency Virus Acute Infection AIDS Viral RNA (CD4⩽ T Cells ( )) Clinical Latency GALT. In its absence, the RNA polymerase appears less stringent in the recognition of the RNA sequences that direct its activity. In shorthand, the linear genome sequence can be written anb-UL-banc-US-ca, where the subscript n designates one or more copies of the a sequence and the prime designates complementary sequences. For this reason an elegant scheme has been designed to inactivate the transcriptional activity of the LTR after integration. Flavivirus: infect humans, monkeys, bats, birds, etc. Cucumber mosaic virus has the largest known host range among all viruses, infecting more than 1200 species of plants including monocots and dicots, herbaceous plants, shrubs, and trees. These viruses are able to infect cultured cells from a wide variety of animal species and replicate rapidly, producing high titers of progeny (about 10,000 virions per infected cell in 12 to 16 hours). P, the pitch of the helix, is the distance along the helix axis that corresponds to exactly one turn of the helix; P u p. Several courses of disease following acute infection have been documented in the absence of treatment: Virus load setpoint 0 6 Weeks 12 1 3 5 7 Years 9 11 (b) Entry of virus into the host Localized replication at site of infection Release of virus into the circulation Seeding of lymph nodes throughout body Immune clearance of virus from peripheral circulation; continued replication in lymph nodes Replication and destruction of lymph node architecture Release of virus into the circulation Loss of immune response; susceptibility to opportunistic infections. Production of AAV vectors usually requires a helper virus The essential genes that must be provided by helper adenoviruses include E1A and E1B, open reading frame 6 of early region 4 (E4orf6), E2A, and the small VA RNAs. One of the first strategies employed to produce AAV vectors was to cotransfect 293 cells, which express adenovirus E1A and E1B genes, with a transfer plasmid and a packaging plasmid (Figure 37.6). By convention, genes are numbered sequentially within individual Hind III restriction endonuclease fragments, and designated as being transcribed rightward or leftward (Figure 26.2). They begin with molecular detection systems such as toll-like receptors, which recognize a variety of molecules that are specific to viruses or other pathogens. The host cell is shown as a rounded box. Expression of this gene in vivo can lead to potent induction of both humoral and cellular responses. Hardy, Indiana University Hans Heidner, University of Texas at San Antonio Richard Kuhn, Purdue University Alexander C. The Orthomyxoviridae family includes influenza virus types A, B, and C, as well as two distinct tickborne mammalian viruses in the Thogotovirus genus and a virus that infects Atlantic salmon in the Isarvirus genus. VP30 expression activates Ebola virus transcription by counteracting the inhibitory effect of this stem-loop structure on the initiation of transcription of the NP gene by viral RNA polymerase. Severe acute respiratory syndrome (SARS): pneumonia in humans with 10% fatality rate. The viral genome is a circular, partly singlestranded DNA with overlapping reading frames Hepatitis B virus has one of the smallest viral genomes known: the circular DNA is 3.2 kb long. Makes large amounts of non-infectious spherical and filamentous particles. Therefore the conformational change from PrPC to PrPSc could involve formation of this -helix structure. Its symptoms resemble those of Lassa fever and Ebola (see Chapter 16) viruses. They are used to study the evolutionary connection between RNA and DNA genomes. There is evidence that one strand may be nicked 5 nt from the termini; this could be used as a primer during DNA replication, as is thought to occur with a number of linear doublestranded DNA viruses such as poxviruses. Hippocampus: a section of the brain that is located inside the temporal lobe and is part of the limbic system. Ends have complementary single-stranded extensions (cohesive ends). At this point the bloom was effectively "dead", and up to 1 million E. Until recently, diagnosis of prion disease in individuals with suspicious clinical presentations was made by histological examination of brain biopsies or, more commonly, autopsy specimens. BS, bundle sheath cells; C, companion cells; M, mesophyll cells (these surround the vascular bundle, but are not part of it); S, sieve elements; VP, vascular parenchyma cells; X, xylem cells. Top: Hind III map of the entire genome. Macromolecules are often separated from each other by electrophoresis on polyacrylamide or agarose gels. A virus that infects one animal species can sometimes Table 35.4 Classification of currently available human antiviral vaccines Live wild-type viruses Vaccinia (cowpox)a Live attenuated viruses Adenovirusb Influenza A (cold adapted) Production of vaccines against avian influenza strains has been problematic Nevertheless, the following story illustrates how technological limitations persist to modern times. Consequently, the presence of Tat is essential only if the wild-type LTR is used to control expression of the transgene or the RNA of the vector genome. New delivery systems for viral antigens Chimeric molecules that contain cytokines, lectins, or monoclonal antibodies fused to viral antigens can help to direct the antigen to an appropriate target cell or CH035.indd 437 437 enhance immune responses by interacting with cells in the immune system. Which two papillomavirus proteins help to immortalize HeLa cells? The resulting virus genome RNAs are replicated by the viral RNA polymerase, which is usually provided by co-infection of the cells with an expression plasmid. The viral capsid associates with cellular microfilaments and reverse transcription of the viral genome begins. It is theoretically possible that the doublestranded genome RNA could be denatured within the cell, allowing the positive-strand RNA to be translated; however, this is energetically unlikely and does not occur. CH011.indd 129 16/05/11 8:02 PM 130 CHAPTER 11 Picornaviruses 5' end of an mRNA. • Hepatitis delta virus infects hepatocytes in humans and other animals; its replication requires coinfection with hepatitis B virus. 16/05/11 8:09 PM Polyomaviruses Paradoxically, polyomaviruses cause few if any tumors in their natural hosts. Like most viruses, reoviruses demonstrate an extraordinary degree of genetic economy; nontranslated regions on different gene segments range from 12 to 83 Spike 2 Attachment protein 1 Outer capsid 3 and 1 S1 S2 S3 S4 M1 M2 M3 L1 L2 L3 Inner capsid 2 and 1 RNA polymerase 3 and 2 Figure 19.1 The reovirus virion. Poxviruses encode and package in virions a full complement of enzymes required for viral mRNA synthesis and modification, and thus provide an ideal model system for studying the basics of mRNA metabolism. Golan, Armen H. Nucleocapsids are composed of multiple copies of the capsid (C) protein, and each virion also contains 180 copies of two envelope glycoproteins, M and E. The diversity of the epitopes recognized by the immune system also appears to play a role; recognition of a limited number of epitopes is associated with a poor prognosis. Such an assay has been used to discover novel inhibitors of herpesvirus and HIV replication. But the 1918 HA protein was present in viruses that circulated in swine, and it had varied little over the past 90 years, perhaps because commercial swine live short lives and therefore herds develop little immunity to the virus. Exportins: proteins that mediate the export from the nucleus to the cytoplasm of proteins or RNAs. Family: in virology, a taxonomic grouping of genera whose members share common virion structure, genome organization and size, and replication strategy, and for which there is evidence of a common evolutionary origin by comparison of nucleotide and amino acid sequences. Genome RNA is encapsidated by N protein (green ovals). This condenses the viral DNA into a "minichromosome" containing about 25 such nucleosomes (Figure 21.3), giving a total of 200 histone molecules per viral DNA molecule. Coronavirus spike proteins bind to a variety of cellular receptors The S proteins of several alphacoronaviruses are known to bind to aminopeptidase-N, a family of zinc-binding metalloproteinases found in cellular plasma membranes. In a few cases, infection can spread from one cell to the next without formation of a complete virus particle. What is the role of the J protein in ϕX174? 1A reprinted from Jantti, J. Nucleic acid genomes are shown as black curved lines, capsid proteins as green spheres, and envelope proteins as orange knobbed spikes. See also Paramyxoviruses gene order, 180 plus-strand RNA synthesis, promoter for, 181–182 structure of, 178 virions of, 177 Rhinoviruses, 125–128 Ribonuclease H domain, 370 Ribosomal frameshifting, 349 Ribosome shunting, 282 Ribozymes, 192, 381, 459 Rickettsiae, 3 Rift Valley fever infection, 201 Ritonavir, 445, 452–454 RNA bacteriophages, see Single-stranded RNA bacteriophages RNA helicase, 116, 117, 142, 143, 144, 151, 153, 165, 166, 228, 230–231, 318, 401 RNA interference (RNAi), 379, 412–414 in plants and invertebrates, 412–413 microRNAs in vertebrate gene expression control, 413–414 RNA world, 40–41 RNA-dependent protein kinase (PKR), see Protein kinase R (PKR) RNA-induced silencing complex (RISC), 121, 413 RNA silencing, 116, 121, 122, 382, 413 Rolling circle replication mechanism, 75, 93, 291, 380 Ross River virus, 149 Rotaviruses, 5, 226, 431, 434, 439, 440 Rous sarcoma virus, 6, 255, 342–343, 350–351 Rubella virus, 149 Rudiviridae, 100, 105–106 Rule of six, 185 Salterprovirus genus, 108 SARS coronavirus, 160–161, 164, 165, 167, Satellite nucleic acids, 40, 41 Satellite RNAs, 122–123, 379, 383 Satellite viruses, 40, 41, 383 Scaffolding proteins, 16, 28, 72, 73–75, 76, 82, 93–94, 281, 293–294 Scrapie, 388, 389–390, 393–394 Secondary bacterial infections, 211 Secondary receptors, 13, 48, 276 Self-assembly, 19, 310 Self-cleaving RNA structures, 381 Selfish Gene, The, 394 Self-replicating alphavirus vector, 156 Sendai virus, 178 Serpin, 322, 392 Serum sickness, 374 Severe acute respiratory syndrome (SARS), 4, 5, 160–161 Severe combined immunodefiency disease (SCID-XI), 464 Shine-Dalgarno sequence (SD), 64, 68 Sialic acid, 10, 47, 48, 164, 178, 211, 214, 220, 221, 228, 229, 234, 251, 453, 454 Sickle cell disease, 245 12/05/11 4:08 PM Subject Index Sieve elements, 119 Signal peptidase, 142, 154 Signal sequence, 27 Simian immunodeficiency virus (SIV), 355 Simian virus 40 (SV40), 247–248 capsid, 20, 24 entry via caveolae, 49, 251 large T antigen, 256, 258 late transcription, control, 260 miRNAs, 414 vector, 457 Sin nombre virus, 201, 202 Sindbis virus, 149. Confocal microscope: a microscope that uses focused lasers and computer imaging to visualize thin segments in living or fixed cells. Segments are color-coded to denote their origins, shown at the left, in birds, humans or swine, and the approximate year of transmission from one animal host to another is also shown. Protein S provides a route through the cytoplasmic membrane for R. Cytotoxic T lymphocytes also cause the rejection of organ transplants, can kill cancer cells, and can participate in autoimmune reactions that cause diseases such as thyroiditis, diabetes, systemic lupus erythematosus, and perhaps multiple sclerosis. The fundamental properties of cellular life as we know it would have then been established. Formation of the octamer results in the formation of a large (2.4 kb) DNA loop. However, both type 1 and type 3 reovirus strains use JAM-A as a receptor, which is not required for reovirus infection in the brain. The long control region contains the viral origin of DNA replication as well as enhancer sequences that control viral gene expression. The original methods for the study of such viruses relied on inoculation of animals or plants with filtered extracts from infected individuals, and their observation for the effects of virus infection. The occlusion bodies dissolve in the alkaline conditions of the insect gut (the acid pH of the human gut does not dissolve occlusion bodies). Shown at left is a cross-section of one center of 5-fold symmetry on a virion docked at the cell surface by binding to the poliovirus receptor. Sequences of the terminal regions of the AFV-1 genome are unusual; the short inverted terminal repeat CGGGGGGGG is followed at either end by a 350-bp region containing numerous short direct repeats of the pentanucleotide TTGTT and close variants thereof. Linear, double-stranded adenovirus type 5 DNA showing the first 8 nt of the inverted terminal repeats. The viral genome encodes proteins that enable it to replicate and to be transmitted from one cell to another, and from one organism to another. This would resemble the coordinated transcription and translation known to take place in bacteria, which (unlike eukaryotic cells) do not physically segregate their genomes from the protein synthetic machinery. 13.4: Adapted from Figs. Indeed, the ancestors of phycodnaviruses could be between 2 and 2.7 billion years old and could have witnessed the divergence of prokaryotes and eukaryotes. Maestra and Emilio Cornalia independently observed polyhedral bodies in silkworm larvae and made the connection between the presence of polyhedra and the symptoms in the diseased silkworms. When cells growing at suboptimal temperatures (75⬚C) are infected with ATV, instead of lysogeny, the result is a productive infection and lysis of the cells. The linear DNA genomes of rudiviruses range in size from 24 to 35 kb, and carry long inverted terminal repeats, comprising up to 7% of the total genome length. Cavins: proteins that bind to caveolins and regulate function of caveolae. These subheadings (collected in the Table of Contents) can also be read separately to provide an overview of the material presented in the chapter, and to follow the steps of the virus replication cycle. Virions are assembled at this membrane system (Figure 14.6). coli Yes No Binding to plus-strand RNA; inhibition of ribosome binding to coat start site Host Factor E. The stability of CII is enhanced by CIII, which inhibits the host proteases. Furthermore, tumor viruses such as polyomaviruses and retroviruses (Chapters 21 and 28) were shown in the late 1960s to integrate copies of the virus genome into the host cell during transformation. A recombinant vaccine is available CH030.indd 375 In 1986, Pablo Valenzuela and William Rutter of Chiron Corporation produced the first recombinant hepatitis B vaccine in yeast (Recombivax), and this is the basis of the vaccines still used today. 27.4: Courtesy of Michael Rossman, reprinted from Figure 2 in Nandhagopal, N., et. Probably all different forms of life can be infected by viruses. The HK97 shell is remarkable in that neighboring subunits of gp5, its major head protein, become covalently joined in pentameric and hexameric rings. In both cases, a reverse transcriptase initiates DNA synthesis near the 5⬘ end of the template RNA, then jumps to a repeated sequence at the 3⬘ end, and copies the remainder of the RNA, degrading the template as it progresses. Throughout the 1920s and 1930s, many different bacterial viruses were identified and classified, including the Microviridae (Table 6.1). The accumulated p53 either induces cell-cycle arrest in G1, allowing sufficient time to repair damaged DNA, or induces apoptosis, which eliminates these abnormal cells. My own teaching experience and conversations with numerous colleagues convinced me that there is a real need for a concise, up-to-date textbook organized around the concept of virus families and designed specifically for teaching university students. Quasi-spherical virions are much smaller and may be more readily incorporated 16/05/11 8:07 PM 212 CHAPTER 18 Influenza Viruses Figure 18.2 Electron micrographs of spherical and filamentous influenza virus A particles. 05/05/11 1:08 PM Hepadnaviruses The pregenome RNA is packaged by interaction with polymerase and core proteins The pregenome RNA (pgRNA), in addition to serving as mRNA for C and P proteins, is used as a template to produce the DNA genome of hepatitis B virus. Coiled helical nucleocapsid. Nef has been implicated in alterations of signaling in T cells, resulting in general activation of the cell and promoting virus replication. Can arise spontaneously or by ingestion of infected tissue. These two strains reproducibly transmit different behavioral changes, with different neuropathology and incubation periods. Other strategies have been developed to minimize contamination with nonrecombinant virus and to reduce the time and effort required for vector production. Baculoviruses 302 Eric B. Burkitt became fascinated with the tumor and he eventually embarked on what he called his "long safari", documenting the incidence of the lymphoma in sub-Saharan Africa. Second strand transfer. Plasma cells Synthesize and secrete antibodies T lymphocytes Activated by binding via T-cell receptor to antigens presented by MHC I or II on surface of infected or antigen-presenting cells; differentiate into T helper cells and cytotoxic T lymphocytes. These are encoded within regions of the Epstein–Barr virus genome defined by restriction endonuclease fragments BamHI A and H, and have therefore been named BART (BamHI A Rightward Transcript) and BHRF1 (BamHI H Reading Frame 1) miRNAs. Interestingly the BART miRNAs are most robustly expressed in cells derived from nasopharyngeal carcinomas, whereas the BHRF1 miRNAs are not detected in nasopharyngeal cells but are expressed in B lymphocytes. The stargate can be seen on some particles (red arrowheads). One pathway has 12 of the pentamers coming together to form an 80S empty "procapsid" (lower part of Figure 11.8). Influenza type A has eight distinct genome segments each assembled in a helical nucleocapsid. How would you construct a subunit chimeric vaccine against a human virus? In many cases, the assembled virion is in an energetically metastable state, primed to dissociate and release its cargo upon binding to or entering the host cell. Mimivirus early transcription appears to be driven by a highly conserved AAAATTGA sequence in the region immediately upstream of the messenger RNA start site of about one-half of mimivirus genes. • The resulting virus cores can either continue to synthesize viral mRNAs, or can be further encapsidated to make mature reovirus virions. Discuss some of the ethical issues surrounding "herd immunity." 4. Glycosylated; retained in virions of Semliki Forest but not Sindbis virus. Fibrils formed in vitro from recombinant yeast prion proteins and then introduced into yeast cells have been shown to result in inheritable prion states, providing final evidence that proteins alone can act as selfpropagating infectious agents. 12 spikes project from surface. Both viral and cellular proteinases are required to process the viral polyprotein, which is inserted into the membrane of the endoplasmic reticulum. nsP2 807 Cysteine proteinase, RNA helicase. Rubella virus, the cause of German measles, is the only member of the togavirus family that is not 149 transmitted via arthropod vectors. Capsomers are linked by the flexible carboxy-terminal arms of VP1, which extend across the gap and embrace VP1 molecules in neighboring capsomers, stabilizing the capsid structure (see Figure 2.6b). Viruses must be able to enter and replicate in their host organism, and must then be released and transmitted to another individual. Plaque assays were subsequently developed for animal, plant, and insect viruses using cells cultured in vitro. This nascent DNA chain then folds into a new hairpin (step 3) that can prime further DNA synthesis (step 4). Detection of an oncogenic papillomavirus type in patients with an abnormal Pap smear is valuable in patient management, especially when an infection appears to be persistent. Each gene contains its own transcriptional promoter, and genes are closely packed; intergenic regions larger than 100 bp are rare, and coding sequences sometimes overlap. Each subunit contains the same 8-stranded antiparallel -barrel shape that is found in capsid proteins of many other icosahedral viruses (see Chapter 2). Subsequently, Rev binds to importin , which docks Rev to the cytoplasmic face of the nuclear pore (Figure 29.6). Viruses were first distinguished from other microorganisms by filtration In the last decade of the nineteenth century, Russian scientist Dimitrii Ivanovski and Dutch scientist Martinus Beijerinck independently showed that the agent that causes tobacco mosaic disease could pass through fine earth or porcelain filters, which retain bacteria. Treatment of papillomavirus-induced precancerous lesions involves the removal of the infected lesions. capsids. 1 Cap poly(A) 1 ε 2 poly(A) 1 3. Actinomycin D: a tricyclic unsaturated hydrocarbon linked to two cyclic pentapeptides, made by a streptomyces species, that binds tightly to dG residues in DNA and blocks transcription by DNA-dependent RNA polymerases. What cellular enzymes accomplish this genome replication? Indeed, glycosylation of Vp54 probably occurs independently of the host endoplasmic reticulum–Golgi system. These defense responses are known to be activated through analysis of plant transcriptomes, but it is not clear that they are involved in any resistance to cucumber mosaic virus. (-) LAT ORF P/O OriS c OriS RNA 134.5 134.5 0 Us11 47 4 UL22 UL24 UL25 UL27.5 UL32 UL31 UL29 UL30 UL38 UL36 UL33 UL34 Figure 24.2 Map of herpes simplex virus genes. Additionally, parts of the chapter on Interferons were incorporated into the new chapter on Cellular Defenses. However, more than 50% of human tumors

lack p53; in such cells, inactivation of p53 by the 55K protein is not required for virus replication, and therefore the ONYX015 virus replicates actively in tumor cells and kills them selectively. Reassortment can occur at high frequency with viruses that have fragmented genomes. 444 CH036.indd 444 16/05/11 8:21 PM Antiviral Chemotherapy The AIDS epidemic led to a pressing need for drugs active against human immunodeficiency virus (HIV) and opportunistic pathogens, and coincided with the development of expanding knowledge about virus genetics, molecular biology, enzymology, and protein structure. Thus interferon directs the immune system to generate a cytotoxic response, which is most effective in suppressing viral infections, as infected cells are killed and eliminated. It should be noted that the "blue-green algae" are prokaryotic cyanobacteria (the evolutionary source of algal chloroplasts) and their viruses are not considered here. Protein tyrosine kinase: an enzyme that phosphorylates proteins at specific tyrosine residues. The final result is a second double-stranded DNA molecule (step 7, bottom of Figure 23.8). Twofold symmetry axis: an axis around which an object rotated 180° appears identical to the original object. by Nicholas H. Reducing initiation of RNA synthesis could allow the initial translation of RNAs 1 and 2 immediately after entry into the cell, providing sufficient levels of viral RNA polymerase to subsequently carry out viral RNA replication. The vast majority of viral genes are late genes, designated 1 and 2. In fact, not only does E7 induce cell-cycle progression, but this also results in an increase in p53 levels. This is followed by the synthesis of plus-strands that are then either translated, making more viral proteins; replicated, making more RNA; or packaged, making virions. Shortly after infection, host chromosomal DNA begins to be degraded, presumably to aid in inhibiting host transcription and/or to provide a readily available source of nucleotides for viral DNA replication. VIRUSES OF ARCHAEA HAVE UNUSUAL MORPHOLOGIES Lemon, droplet, rod, or bottle shapes with surface fibers. Scientists who classify viruses that infect distinct groups of organisms tend to use different criteria. Like other viral glycoproteins, fusion proteins are synthesized, folded, and assembled into oligomers in the endoplasmic reticulum of infected cells. It had been appreciated for some time that infectious diseases were transmitted by air, water, food, or close contact with sick individuals. The a sequence varies from 200 to 500 bp in length in virus isolates, and contains no open reading frames. • Cells have antiviral defense mechanisms. Antibodies come in a variety of forms The basic "Y" structure of antibodies (Figure 34.7), also called immunoglobulins (Ig), consists of two linked dimers, each containing a light chain and a heavy chain. Adenoviruses have a prominent trimeric fiber attached to each penton base, with a receptorbinding site located in a knob at the tip (see Chapter 23). As obligate intracellular pathogens, viruses can remain almost invisible to most of the cells and molecules of the host immune systems; this chapter will explain how the problem of "seeing" inside the cell is dealt with by the immune system. The level of seroconversion (an indicator of exposure to the virus) in the human population has remained relatively constant and low (~3%), but an increase in infections of the host (bird) or vector (mosquito) population could have a significant impact on the number and perhaps severity of human infections. The viral E2 protein also acts as a transcriptional regulator of viral promoters through its ability to bind to multiple specific sequences within the long control region. For most other mammalian and avian retroviruses ("C-type" viruses), as well as lentiviruses, assembly of the core occurs directly at the plasma membrane during budding. In everyday life, there can be packages with different structural 18 CH002.indd 18 16/05/11 7:49 PM Basic Concepts of Virus Structure characteristics. CH029.indd 361 361 Together, the Tat and Rev proteins strongly upregulate viral protein expression Tat and Rev are essential for virus replication because they regulate HIV-1 transcription and transport of mRNAs that code for viral structural proteins. This coldsensitive strain was developed and tested over a 30-year period. Given that one of Chikungunya's mosquito vectors, Aedes albopictus, is widespread, the threat of an endemic alphavirus in Europe and elsewhere is a major public health concern. Production of massive amounts of non-infectious particles may provide a "stealth" mechanism for evading the immune response; they can divert the immune attack by binding to antibodies and complement, allowing the less abundant virions to traverse the bloodstream undetected. By about 24 hours, only viral proteins are made, indicating that baculovirus infection results in the shutoff of host cell protein synthesis. It is not presently known whether these genes are transcribed by a virus-coded RNA polymerase, which could be packaged within the virion, or cellular RNA polymerases, which reside in the nucleus. Intrinsic defenses can protect the infected cell or nearby cells from damage by inducing a variety of protective systems within minutes to hours of the initial infection. Plasmids carrying either AcMNPV hrs or early promoter regions have been shown to replicate by this assay when transfected into insect cells that are subsequently infected with AcMNPV. Fusion can occur among numerous nearby cells, leading to the production of giant multinucleated cells, called syncytia. CH032.indd 394 The nature of the prion infectious agent Prions challenge our traditional concepts of infectious agents. This foreshadowed discoveries that numerous other animal viruses also make polyproteins that are cleaved by viral proteinases, now prime targets for antiviral drugs. Regions irrelevant for translational controls are shown as dashed lines; numbers in dashed hairpins show how many nucleotides have been omitted. This implies that there has been selective pressure for maintenance of heterozygosity in humans, suggesting that prion diseases similar to kuru may have been commonplace over the past 500,000 years of human evolution. One-mL aliquots from this 108-fold dilution were applied to four different Petri dishes of susceptible cells and plaques (bottom) were allowed to develop. The genome RNA of a negative-strand virus must be packaged in a nucleocapsid to be transcriptionally active, and it needs the virus-coded RNA polymerase and in some cases appropriate viruscoded cofactors to be expressed. Because the RNA polymerases from the T7 group (T7, T3, and SP6 phages) differ in promoter recognition, it has been possible to make plasmids with several different promoters, so that transcription patterns can be controlled by the choice of which RNA polymerase is used. These Key Terms are collected at the end of each chapter as a review aid, and definitions are given in a combined glossary at the end of the book. Special features of the T7 family of phages T7 and related phages have a number of properties that make them objects of special interest to biologists as well as CH007.indd 82 to biotechnologists: (1) These phages have mechanisms for modulating and overcoming the classic host restriction-modification system; (2) T7 and many of its relatives are able to grow efficiently only in E. 97) in Claverie, J.M., Abergel, C., Ogata, H., "Mimivirus," Curr Top Microbiol Immunol. 16/05/11 8:15 PM Human Immunodeficiency Virus 363 (a) Cytoplasm Lumen Endoplasmic reticulum CD4 gp160 Vpu β-TrCP Degradation by proteasome (b) CD4 Cytoplasm nef Plasma membrane AP–2 Clathrin Figure 29.7 Down-regulation of CD4 expression. Ribosomes initiate translation on all four early mRNAs at the first AUG codon they encounter, at nt 175 on the polyomavirus genome. Associated with membranes in cytoplasm; may translocate to nucleus. Small- and medium-sized DNA viruses could have arisen as independently replicating genetic elements in cells A number of small DNA viruses replicate their genomes and express their messenger RNAs by using host cell DNA and RNA polymerases. No single cellular receptor has been unambiguously identified CH013.indd 150 for alphaviruses; different proteins have been identified as receptors for Sindbis virus in different host cells. • Class II ("middle") genes are transcribed by the phage T7 RNA polymerase, and include genes coding for DNA replication functions. It has the familiar "hand" structure of RNA and DNA polymerases, with a finger region, a palm region, and a thumb region; the palm region includes the enzyme's active site. Even though effective vaccines against smallpox (Edward Jenner, 1798) and rabies (Louis Pasteur, 1885) were developed, there was no understanding of the nature of these disease agents, which we now know to be viruses. Although PrPC is highly conserved among mammalian species, attempts to identify its normal function by genetic means have proven frustrating. In similar fashion, the 1968 Hong Kong virus contained an HA gene and a PB1 gene derived from an avian virus but other genes derived from the previously circulating human H2N2 virus. In the case of AFV-1, adsorption proceeds via interaction of the terminal claws of the virion with pili of the host cell, resulting in folding of the claws. The genome can be condensed into a compact sphere like a ball of string, about 18 nm in diameter. There are two proposed pathways leading to the production of infectious picornavirus particles. • Plasma cells produce antibodies, or immunoglobulins, which bind to viral proteins and either prevent the virus from infecting cells or target the virus for degradation or phagocytosis. Other DNA viruses such as adenoviruses and herpesviruses code for their own histone-like core proteins. (a) T7 RNA polymerase (green sphere) initiates an RNA chain (light blue arrow) at promoter 1.1. (b) T7 RNA polymerase is displaced by T7 DNA polymerase (dark blue oval) in an AT-rich region shortly downstream of the promoter. The Flavivirus genus includes at least 73 distinct viruses, including many human pathogens that are transmitted by bloodsucking arthropods (Table 12.1). 57 Internal External External Envelope 37 74 34 Genes Temperate, integrates genome; productive infection kills cells Lytic Productive infection does not kill cells Productive infection does not kill cells Productive infection does not kill cells Lytic Productive infection does not kill cells Temperate, integrates genome; Productive infection does not kill cells Life cycle Lemon shape with two tailed ends Rod, 23 600–900 nm, terminal tail fibers Spherical Bottle with fibers at blunt end Droplet shape, pointed end with fibers Icosahedral capsid with 12 turrets Filament 24–40 400–2200 nm; terminal fibers or claws Lemon or elongated shape Virion shape Virion image 100 CHAPTER 9 Viruses of Archaea 16/05/11 8:00 PM Viruses of Archaea 101 Table 9.2 Viruses of Euryarchaeota Family or genus name Genome, size Example of virus species, abbreviation unassigned Circular ss DNA 7 kb Halorubrum pleomorphic virus1 (HRPV-1) unassigned Circular ds DNA 8 kb Salterprovirus Genes (ORFs) Envelope Life cycle Virion shape 9 External Productive infection does not kill cells Roughly spherical Haloarcula hispanica pleomorphic virus 1 (HHPV-1) 8 External Productive infection does not kill cells Roughly spherical Linear ds DNA 14–16 kb Haloarcula hispanica virus 1 (His-1) 35 External Lytic Lemon shape unassigned Linear ds DNA 31 kb SH-1 56 Internal Lytic Icosahedral capsid with 12 spikes Myoviridae Linear ds DNA 76 kb Halovirus HF-1 117 None Some are Icosahedral temperate, head, some lytic contractile tail Siphoviridae Linear ds DNA 30.4 kb Halovirus psiM-1 37 None in adsorption to the surface of host cells. Because some essential lentivirus proteins are toxic for cells, it is difficult to establish good producer cell lines for lentivirus vectors without tight regulation of expression of the packaging proteins. Adenoviruses rely on a variety of host proteins for optimal DNA replication and production of adequate levels of precursor deoxynucleotides. At the end of the virus life cycle, lipid membranes reform around the progeny DNA and associated proteins to make the virus core, just as nuclei are reformed by enclosure of chromatin within a lipid membrane after mitosis. DNA polymerase then extends the DNA chains. Hairpin 1, 2/3, 4 at the 3' end of int mRNA made from PL is long enough to be cleaved by RNAse III, resulting in instability, but hairpin 2/3 at the 3' end of int mRNA made from Pint escapes RNAse III cleavage and this mRNA is more stable. Virions packaged with mutant J proteins have altered dimensions and lower infectivity, showing that the J tether is important in virion structure. Minor nucleocapsid protein VP30 activates viral mRNA synthesis in Ebola virus Filovirus nucleocapsids contain four proteins, three of which (NP, VP35, and L) correspond to their counterparts (N, P, and L) in nucleocapsids of paramyxoviruses and rhabdoviruses. Chapters are designed to accompany a 50-minute lecture on the subject, or in some cases, two or three such lectures. Coat protein is also required for virus movement within plants, and is involved in aphid transmission of cucumber mosaic virus between plants. Transit of HIV-1 across the mucosa can be facilitated by local abrasions, inflammation, ulcerations or interaction with dendritic cells. They can be replicated by the nonstructural proteins made by the replicon RNA. 260 Only non-permissive cells can be transformed 261 Transformed cells integrate viral DNA into the cell chromosome 261 22. Natural killer (NK) cells recognize virus-infected cells lacking major histocompatibility complex (MHC) molecules and target them for death by apoptosis. Adenovirus vectors have been extensively used for gene transfer into mammalian cells since the mid-1980s. Growth under various applied selections, selections starting with deliberately constructed ancestors, and tests of host/phage evolution have been employed to study predictive models, concepts of optimality, and traditional evolutionary arguments. The virus genome remains latent in the vegetative filamentous cells until the formation of algal reproductive organs in the mature host. Several genes lie within these repeats and are therefore present in two copies per genome. Indeed, the two algal virus species Pyramimonas orientalis V01 and Chrysochromulina ericina V01 (infecting P. Viruses evade adaptive immune responses via antigenic drift and shift. Genetic alterations in a virus can also result in changes in tissue tropism. At the opposite extreme, uncoating of the DNA genomes of hepatitis B virus, parvoviruses, and polyomaviruses takes place only after the capsids have been transported into the nucleus through the nuclear pores. In this fashion, interferons modulate the immune response and increase host defenses against certain intracellular bacteria and viruses. Scientists had begun to use light microscopes to discover and describe fungi and bacteria in the first half of the nineteenth century. • Baculoviruses infect only invertebrates, notably insects, with a very high degree of host species specificity. ICP0 also activates transcription of genes by a number of other pathways, not fully understood, involving interactions with a variety of cellular proteins. Control of transcription in coronaviruses generates a series of subgenomic mRNAs. How are these generated? Because DNA molecules are more stable than RNAs, they would have a tendency to accumulate within primitive RNA-based cells even before they could be replicated. Replication is shown beginning at one of the three possible origins. Protease inhibitors were developed rationally rather than by random screening, high throughput, or otherwise. Plaque assay: a method for enumerating infectious virus particles by generation and counting of plaques, or focal BGLOSS.indd 479 479 areas of cell death, on a monolayer of cells. Intasome: an assembly of the integrase and the host protein IHF on the phage lambda attp site; the intasome captures the bacterial attb site and carries out recombination between the two att sites. SARS coronavirus and murine hepatitis virus have analogous replicase proteins that will be referred to as nsp1 through nsp16 (Figure 14.1 and Table 14.2). coli was collected in 1922 from a patient with an intestinal disorder. Src was one of the first oncogenes identified, and its presence in a virus lent credence to the theory that certain cancers were caused by oncogenes of viral origin. Both 1 and 2 possess an enzymatic activity that can remove the phosphate from the 5'-terminal nucleotide to yield a 5'-diphosphate end; this prepares the RNA for cap addition. • Interferons-␣, -, and - are expressed by most cell types, whereas interferon- is expressed by activated NK cells and T lymphocytes. These viral mimics bind and sequester, down-regulate or increase the decay of critical host complement activators such as C3b, C4b, and C5b. (a) Electron micrograph of negatively stained virions of simian virus 5, showing fringe of envelope proteins projecting from the surface. The achievements of this era include improved vaccines for smallpox and rabies as well as poliovirus, measles, mumps, and rubella vaccines. The flower-like envelope projections, which consist of trimers of E1-E2 heterodimers, reorganize to form trimers of E1, exposing a hydrophobic fusion domain in that protein. Both of these vaccines contain L1 of papillomavirus types 16 and 18, responsible for most cervical carcinomas; one of the vaccines also contains L1 of types 6 and 11, responsible for most genital warts or condylomas. The structural proteins are needed in large amounts for virion assembly (240 molecules of each protein per virus particle), while only small amounts of the nonstructural proteins are required since they act catalytically. Precapsids package viral DNA under the direction of packaging proteins that recognize the terminal a repeats and cleave off genome-length, linear DNA fragments. Removal of tRNA and ppt primer. Unlike most enveloped viruses, there is a large amorphous mass between the capsid and the envelope, called the tegument, which contains about 14 virus-coded proteins. The E1A proteins activate E2F by a similar mechanism: they bind to the Rb pocket, releasing E2F from the embrace of Rb. E2F is therefore free to activate transcription of its target genes (Figure 23.6). During replication CH025.indd 310 Scientists have developed the baculovirus/insect cell expression system to make VLPs corresponding to a variety of human pathogenic viruses. New variant CJD is distinct from sporadic CJD; it has a distinct pathology, has a far younger age of onset than sporadic CJD, and presents with very different initial symptoms. If the defective helper genomes are mutated so that they lack signals for RNA packaging, only replicon genomes will be packaged into virions. A further conformational change is needed to close the gap. Chapters are designed to tell stories about specific virus families, presenting information on well-studied viruses. Some of the most feared, widespread, and devastating human diseases are caused by viruses (see Table 1.1). B-cell receptors: transmembrane proteins on the surface of B lymphocytes that consist of a ligand binding domain (membrane-bound antibody) and an intracellular signaling domain. The increased cloud cover reflects heat and sunlight back out to space. in length. Viruses with fragmented genomes face the problem of ensuring that at least one copy of each genome segment is packaged into the virion. In most cases, each negative-strand genome segment codes for a single messenger RNA and one or two viral proteins. N3 AZT triphosphate Preferentially blocks HIV reverse transcriptase Figure 36.7 Phosphorylation of azidothymidine (AZT). The 240 petals are heterodimers composed of one molecule each of the glycoproteins E1 Table 13.1 Representative togaviruses Genus and virus name Vertebrate host Arthropod host Sindbis virus Birds Mosquitoes Semliki Forest virus Birds, rodents, primates Mosquitoes Ross River virus Marsupials, rodents Mosquitoes Chikungunya virus Primates Mosquitoes Venezuelan equine encephalitis virus Rodents, horses, birds Mosquitoes Western and Eastern equine encephalitis viruses Birds Mosquitoes Humans None Alphavirus Rubivirus Rubella virus CH013.indd 149 16/05/11 8:04 PM 150 CHAPTER 13 Togaviruses Envelope protein Transmembrane and helix Viral RNA Capsid protein Lipid bilayer membrane Figure 13.1 Cryoelectron microscopic image reconstruction of a togavirus virion. Ambisense coding strategies are also employed by tospovirus M and S segments, and by both the L and S segments of viruses in the Arenaviridae family. The conservation of gene order is notable given the high frequency of recombination observed in coronaviruses and the experimental demonstration that genes can be artificially reordered without affecting virus replication. The other one-third of the genome contains genes coding for virion structural proteins and group-specific nonstructural proteins. CH017.indd 209 16/05/11 8:06 PM C H A P T E R 1 8 Influenza Viruses Dalius J. It is a small 97 aa protein with an N-terminal external domain (residues 1–23), a single transmembrane domain (residues 24–44), and a larger internal domain (residues 45–97). Binding sites are boxed and the factor is noted above or below the box. Release of the virus occurs upon fusion of the vacuoles with the plasma membrane. Marcellus Adenoviridae From Greek adenos (gland), for site of first isolation, adenoids VIRION Naked icosahedral capsid (T 25) with 20 triangular faces. E1A,B L1 L2 L3 E2B Adenovirus vectors are widely used in studies of gene transfer and antitumor therapy Adenoviruses (Chapter 23) have a double-stranded linear DNA genome of 25 to 45 kbp, depending on the serotype. Marco Vida of Cremona, an Italian bishop of the sixteenth century, described in poetic form an infection of silkworm that is reminiscent of baculovirus pathogenesis. The ribonuclease H activity degrades the pregenome RNA during the process of genome replication. Prions are transmissible, replicable, and variable disease-causing agents that are distinct from viruses. The polyprotein CH011.indd 131 131 is first cleaved into three fragments, called P1, P2, and P3 (Figure 11.4). About 200 bp of the cosQcontaining end of the DNA protrude down into the tail of a virion from within the head (Figures 8.3 and 8.9). CH012.indd 147 16/05/11 8:03 PM C H A P T E R 1 3 Togaviruses Milton Schlesinger Sondra Schlesinger Revised by: Richard Kuhn Togaviridae From Latin toga (gown or cloak), referring to virus envelope VIRION Spherical enveloped particle with a fringe of projections. An m.o.i. of 10 to 100 plaque-forming units per cell is often used in studies of bacterial or animal viruses. Figure 5.5 shows that three regions of secondary structure near the replicase start site could contribute to impeding the ribosome from binding to that site. RNA-dependent RNA polymerases, to help replicate RNAs in the RNA world 2. Consequently, it was erroneously inferred that archaeal viruses constituted a variety of the ubiquitous head-tail bacteriophages. Each protein subunit binds to a fixed number of nucleotides in the RNA: 3 in the case of tobacco mosaic virus; 6 in the RNA 25 case of paramyxoviruses. Library of Congress Cataloging-in-Publication Data Acheson, N. Viral DNA is inserted into host DNA by a concerted cleavage and ligation reaction. Viruses of algae are among the largest viruses known, with genomes ranging up to 500 kb. This chapter will mostly use examples from the paramyxovirus family. susceptible rodents and other mammals at high concentrations, mouse polyomavirus can cause multiple tumors in parotid glands, skin, kidneys, bone, lungs, liver, and many other tissues. 32.2: Nobel Lecture, December 13, 1976 by D.C. Gajdusek. Further support for this model comes from the finding that introduction of translational stop codons into the coat protein gene upstream of nt 1427 inactivates translation of the replicase gene (the ribosome never gets to stem MJ). However, viruses that lack the ability to express MS protein still retain the ability to produce infectious virions. It is known that parts of the F1 polypeptide chain form elongated, triple-stranded, coiled-coil complexes, similar to those formed by fusion proteins of Ebola virus, influenza virus, and human immunodeficiency virus (see Chapters 16, 18, and 29). These inhibitors are divided into two major groups called P35 and inhibitors of apoptosis (IAP). Viral capsid proteins are labeled. Sequence elements in the intergenic region and the polyadenylation region are shown in Figures 21.5 and 21.6. or several nearby sites that serve as promoters (PE and PL) where transcription begins. Among the genes dispensable for replication in vitro are a number that block host responses to infection. Initiation factors required for transcription of early genes (virus early transcription factors, VETF) are packaged in infecting virions and act within the core upon its release into the cytoplasm. Hepatitis delta virus can replicate only in cells infected with hepatitis B virus, since it borrows the hepatitis B surface proteins to package and form an envelope around its own capsid proteins and genome RNA. Seaweeds predominantly inhabit temperate nearshore marine environments around the world. One of these templates is called midi variant-1 (MDV1). This protein likely helps to condense the genome DNA during packaging into capsids. West Nile virus was originally isolated in Uganda in 1937 and is found in Israel and throughout the Middle East, as well as in parts of Europe and Asia. Physical and chemical barriers such as the skin, mucous membranes, tears, and acidic or basic environments are the first line of defense. 16/05/11 8:00 PM 110 CHAPTER 9 Viruses of Archaea The failure to recognize genes involved in DNA synthesis in the genomes of most archaeal viruses is puzzling, especially considering that many genomes have exceptional features such as covalently closed ends and telomere-like terminal structures that must be involved in genome replication. This led the way to biochemical and genetic analyses allowing detailed understanding of picornavirus gene expression. The ppt primer is also removed. Two distinct human coronaviruses, called 229E and OC43, were isolated and studied. After several such cycles all the cells in a circular area surrounding the original infected cell are lysed. This restricted Interferon signal transduction is carried out via the Jak–Stat pathway Cytokines and interferons are intercellular messengers that induce many important biological responses in target cells. Viruses with filamentous or rod-shaped capsids have diameters between 7 and 30 nm but can be as long as several thousand nm (several micrometers, mm), which approaches the diameter of many mammalian cells. See also Bat coronaviruses adaptability of, 169–171 spike proteins, binding, 164 discontinuous transcription, 167–168 envelope fusion, 164 evolution of, 169–171 genome replication, 166–167 genome sequences, 161–162 hemagglutinin-esterase, 163 bindsub.indd 493 messenger RNAs, nested set, 167 viral proteinases, 165 replicase gene translation, 164–165 replication complexes in cytoplasmic membranes, 165–166 respiratory illness by, 160 SARS caused by, 160 subgenomic RNAs, 167–168 veterinary diseases by, 160 virion assembly and release, 169 in vitro assembly of genome cDNA, 170 Coxsackievirus and adenovirus receptor (CAR), 125, 229, 276 Crenarchaeota, 98 viruses of, 100 Creutzfeldt–Jakob disease (CJD), 388–389, 393 Crimean–Congo hemorrhagic fever, 202 CRISPR elements, 87 Cryoelectron microscopy, 19, 150, 229 Cucumber mosaic virus (CMV), 112–123 cell-to-cell movement, 119 coat protein for spreading, 118–119 evolution of, 120 genome, 113–114 host responses to infections from, 120–121 icosahedral symmetry, 113 infection symptoms, 118 long-distance movement, 119 movement proteins, 118–120 packaging of viral genomes, 114, 117 plants response to, 121–122 RNA replication associated with membranes, 117 satellite RNAs, 122–123 3a protein for spreading, 118–119 3' terminal regions of viral RNAs, 114–115 transmission in nature by aphids, 115 viral proteins, 116–117 Cyclin-dependent kinase (CDK), 234, 278, 292, 360 Cytokine signaling, 322 Cytokines, 198, 211, 321, 360, 399, 403, 436, 458 anti-inflammatory cytokines, 403 chemokines, 403 interferons, 403 pro-inflammatory cytokines, 403 Cytopathic infection, 149 Cytoplasmic membranes, replication complexes in, 117–118, 132, 144, 165–166 Cytotoxic T lymphocytes, 356, 399, 416, 422, 436 Dane particles, 366–367 DC-SIGN (dendritic cell-specific intercellular adhesion molecule-grabbing nonintegrin), 193 Delta self-cleaving structure, 381, 383, 384 Dendritic cells, 179, 355, 390, 399 Dengue virus, 137, 13 Deoxyguanosine, 449 Detection and titration of viruses, 9–11 Dicots, 113 Dimethylsulfide (DMS), 331, 332 Disassembly of virions, 29 Discontinuous transcription, 167–168 Divergent transcription, 250–251 DNA 'end replication' problem, 241–243 DNA factories, 320 DNA gyrases, 250 DNA helicase, 72, 73, 80, 81, 93, 243, 256, 257, 259, 266, 267, 291, 292, 307, 318 DNA packaging, J protein role in, 77 493 DNA vaccines, 435 Domains of life, 97 Domestic ruminants, prion diseases in, 388 Dorsal root neurons, 296 Double subgenomic alphavirus RNA vector, 156 Double-stranded DNA genomes, viruses with, 35 Double-stranded RNA-dependent protein kinase (PKR), 382, 410 Droplet-shaped virus, 103 Drosophila melanogaster, 399 Drowsy (DY) encephalopathy, 394 Dynactin, 52 Dynein, 52 Early endosomes, 48 Ebola hemorrhagic fevers, 4 Ebola viruses, 188–190. Since replicons lack structural protein genes, production of virus particles requires cotransfection with 16/05/11 8:04 PM 156 CHAPTER 13 Togaviruses (a) Self-replicating vector foreign gene replicase genes 5' 3' (⩽) genome 3' 5' (⩾) antigenome 3' (⩽) subgenomic RNA 5' (b) Double subgenomic RNA vector structural protein genes foreign gene replicase genes 5' 3' (⩽) genome 3' 5' (⩾) antigenome 5' 3' 5' (⩽) subgenomic RNAs 3' Figure 13.5 Alphavirus expression vectors. Diameter 9 0–100 nm. Cellular RNA polymerases normally transcribe only double-stranded DNA templates into RNA. The consensus is that girls 11 to 12 years old should FUNDAMENTAL CONCEPTS KEY TERMS Apoptosis Basal cells Condyloma Heparan sulfate Integrin Keratinocytes Mucosal epithelium Pap test be the principal target for vaccination since it is important to vaccinate people before most sexual activity begins and before the infection occurs. Even though retrovirus vectors do not encode any viral genes, possible recombination events between sequences of the transfer construct and viral genes integrated in the packaging cells may generate a replication-competent retrovirus. Progeny virus particles are assembled in the nucleus, and are released upon cell death and shedding at the surface of lesions. In cell-to-cell movement, coat protein may protect and stabilize the protein 3a-viral RNA complex or alter the conformation of protein 3a to facilitate the movement of viral RNAs, but no direct interaction between coat protein and 3a has been detected. Synthesis of progeny double-stranded genomes occurs within subviral particles In addition to being used for protein synthesis, reovirus mRNAs have a second function: they serve as templates for the synthesis of progeny double-stranded genome RNAs. Production of genome RNAs (see below) is a two-step process. Diameter of enveloped virion 200 nm. Coccolithoviruses may have circumvented this defense by acquiring the ability to control the timing of the apoptotic cascade during the infection process, essentially short-circuiting host-controlled apoptosis. After cleavage by furin, the pr peptide is not released from the virion until it is exposed to a neutral pH upon secretion from the host cell. Inoculation of cynomolgus monkeys with these vectors resulted in complete protection against disease from an otherwise lethal challenge dose. Total genome length 18–24 kb. (a) 103 Viral genomes are linear double-standed DNAs, varying between 16 and 56 kb in length. Single-Stranded RNA Bacteriophages 59 The discovery of RNA phages stimulated research into messenger RNA function and RNA replication 59 RNA phages are among the simplest known organisms 59 Two genera of RNA phages have subtle differences 60 RNA phages bind to the F-pilus and use it to insert their RNA into the cell 60 Phage RNA is translated and replicated in a regulated fashion 61 RNA secondary structure controls translation of lysis and replicase genes 61 Ribosomes translating the coat gene disrupt secondary structure, allowing replicase translation 62 Ribosomes terminating coat translation can reinitiate at the lysis gene start site 63 Replication versus translation: competition for the same RNA template 64 Genome replication requires four host cell proteins plus the replicase 64 A host ribosomal protein directs polymerase to the coat start site 65 Polymerase skips the first A residue but adds a terminal A to the minus-strand copy 65 Synthesis of plus-strands is less complex and more efficient than that of minus-strands 65 The start site for synthesis of maturation protein is normally inaccessible to ribosomes 65 Synthesis of maturation protein is controlled by delayed RNA folding 66 Assembly and release of virions 67 13/05/11 2:11 PM Contents 6. nsp8 Primase, polymerase. This makes sense, as infected cells can be recognized and killed before they produce large amounts of virus, which could then spread throughout the body. Interestingly, UL97 does not phosphorylate natural nucleosides and it is not homologous to nucleoside kinases. Table 30.1 and Figure 30.6 summarize in schematic form the similarities and differences between the reverse transcription mechanisms and the replication cycles of retroviruses and hepadnaviruses. Also, a weak or non-neutralizing antibody response generated by a vaccine can sometimes lead to immunopathology (respiratory syncytial virus, atypical measles) or even facilitate virus entry into target cells (dengue virus). Aramayo, T. Host Factor is a poly(A) binding protein that, in the uninfected cell, stimulates translation of the mRNA of a transcriptional sigma factor. Envelope proteins derived from viruses such as Ebola, Molokai, rabies, influenza, and respiratory syncytial virus have been successfully employed to alter the tropism of retrovirus vectors. The DNA polymerase will then use this nascent RNA chain as a primer, and begin synthesizing DNA in a rightward direction. Genetic bottleneck: an event during which only a limited number of individuals of a species survive, resulting in a narrow amount of genetic diversity in a population. Papillomavirus genotypes are considered distinct if the nucleotide sequences of specific regions of the viral genome (L1, E6, and E7 genes) differ by more than 10%. Two gene products govern peptidoglycan hydrolysis. Induction of the SOS genes occurs as follows. See also Ebola viruses; Marburg viruses assembly, 191–192 budding of, VP40 in, 195–196 causing hemorrhagic fever, 190 cloned cDNA copies, 192 diagnosis, 198 Ebola virus proteins, 191 filamentous particle formation, VP40 in, 195–196 filovirus infections, pathogenesis of, 197–198 genome map, 191 glycoprotein mediates entry by fusion, 192–194 in Africa, outbreaks, 196–197 infection, clinical features of, 198 multiplasmid transfection systems, 192 pathogenesis, 197–198 RNA editing of GP mRNA, 194 RNA replication, 191–192 virion structure, 189, 190 secreted glycoproteins, 195 transcription, 191–192 transmission to humans, 197 treatment options, 198 vaccine candidates, 198 virus-like particles (VLPs), 198 bindsub.indd 494 Filterable viruses, 6 Fission, 46 Fivefold rotation axes, 21 Flaviviruses 137–146 cell entrance, by pH-dependent fusion, 141 diseases caused by, 137–138 E protein, 140–141 RNA synthesis, 144–145 genome organization, 141–142 hepatitis C virus, 139 live, attenuated yellow fever virus vaccine, 139 membrane topology of flavivirus structural proteins, 143 polyprotein processing, 142–143 replication pathway, 145 virion structure, 139–140 virus assembly at intracellular membranes, 145–146 West Nile virus, 137–138, 146 yellow fever, 138 'Flu', 211 Asian flu, 211 Hong Kong flu, 211 Spanish flu, 210–211 swine flu, 211 Folate receptor-α, 193 Foot-and-mouth disease virus, 127 Formalin-inactivated vaccine, 126 Four-helix bundle structures, 102 F-pili, RNA bacteriophage binding to, 62–63 F-restriction, 85 Fulminant hepatitis, 374, 383 Furin protease, 154 Furin, 142–143, 145, 146 Fuselloviridae, 99–102 Fusion peptide, 50–51, 140–141, 178–179, 193–194, 214 Fusion proteins, 50–51, 141, 164 Gag/Pol polyprotein, 348–350 β-Galactosidases, 155 Ganciclovir, 445, 449 Gangliosides, 178, 251, 454 Gastroenteritis, 4, 160 Gene guns, 437 Gene replacement therapy, 457 Genetic bottlenecks, 120 Genetic drift, 207 Genetic economy, 19 Genetic shift, 207 Genital herpes, 5, 285, 286,448 Genital tract cancers, oncogenic HPV in, 264 Genome packaging, 28–29 Genomes, length of viral and cellular, 39 Geodesic dome, 24 Gerstmann–Sträussler–Scheinker syndrome, 388 Global smallpox eradication program, 313 Globuloviridae 2, 100 Glycolipids, 13, 47 Glycosaminoglycans, 127, 316 Gokushoviruses, 77–78 Golgi membranes, virus assembly at, 206–207 Granulins, 304 Granzyme B, 418 Green fluorescent protein, 192 Guanylyltransferase, 318, 320, 330 Gut-associated lymphoid tissue (GALT), 356 Guttaviridae, 100, 103 Hairpin structures in viroids, 381 Haloarcula hispanica pleomorphic virus 1 (HHPV-1), 109 Halorubrum pleomorphic virus 1 (HRPV-1), 108 Hammerhead structures in viroids, 381 Hantavirus pulmonary syndrome, 202 Helical symmetry, 25–26 Helicase, see DNA helicase, RNA helicase Helper virus, 40, 122, 185, 241, 244, 351, 383, 459, 461, 465, 466 Hemagglutination, 10, 177, 204 assay, 10, 214 Hemagglutinin (HA) protein, 48, 50–51, 212–214, 219–222, 448 Hemagglutinin-esterase (HE) protein, 161, 163, 164, 166 Hematopoiesis, 407 Hemifusion, 50 Hemocoel, 306 Hemolytic anemia, 245 Hemorrhagic fevers, 5 dengue virus, 138, 141 filovirus, 189, 197, 198 bunyavirus, 201–202 Hepadnaviruses, 365–375. Adenoviruses 274 Philip Branton, McGill University Richard C. This explains why terminally differentiated (non-dividing) cells in the body are not usually targets for retrovirus infection. Cells lacking PML appear to be unable to respond to exogenous interferon. The mimivirus exhibits the genome compaction typical of DNA viruses, as a full 90.5% of its DNA is occupied by protein-coding sequences, with an average distance between open reading frames of only 157 nucleotides. The adaptive immune response takes days to weeks to deploy its maximum effects. Prangishvili, "Four newly isolated fuselloviruses from extreme geothermal environments reveal unusual morphologies and a possible interviral recombination mechanism," Environmental Microbiology 11 (2009), 2849–2862, with permission from John Wiley and Sons Inc.; Fig. Transmitted by direct contact. These soluble receptors can bind to virions, thus preventing their interaction with the natural receptor on the cell surface. • Scaffolding proteins help to assemble capsids but are subsequently ejected and do not form part of the mature capsid. However, chimpanzees living in the wild and infected with SIVcpz appear to suffer from significant disease, loss of fertility, and early death, the higher pathogenicity perhaps reflecting a relatively recent acquisition of this virus. The x-ray diffraction structure shows that some of the viral DNA is distributed with the same icosahedral symmetry as the capsid proteins. Poxviruses replicate in the cytoplasm, but splicing of cellular RNAs takes place in the nucleus. These include TATA-box-like elements and initiator motifs related to the sequence ATCA(G/T)T(C/T). This suggests that these genes have been acquired during evolution by the virus from the host cell genome. The unedited mRNA has an open reading frame (frame 0) that stops at a UAA codon and produces the 364-amino acid precursor of sGP. ISSN:1077-2626. As a result, host organisms have acquired a variety of means to protect themselves against infection and the pathological effects of infection. Parvoviruses have very small virions and a linear, single-stranded DNA genome Parvoviruses, as their name implies, are among the smallest of all viruses. However, T7 uses its own replication proteins. As a result, editing during mRNA synthesis did not take place, and only mRNA coding for GP was made, because the extra A residue in the mRNA transcript changes the reading frame. Smallpox plagued humans for at least 3000 years. The NP protein and polymerase proteins contain nuclear localization signals that mediate interactions with cellular importin-␣. 25.1, 25.2, 25.3: Courtesy Dr. Eric B. Nevertheless, satellite RNA variants differ in the ways they modify symptoms of cucumber mosaic virus infection. All preparations were negatively stained with 3% uranyl acetate, except for (b), which was platinium shadowed. In mouse polyomavirus, these 12 binding sequences are grouped in four sites denoted 1/2, A, B, and C (Figure 21.5). If the phosphodiester bond between two nucleotides of one of the DNA strands is cleaved ("nicked"), this supercoiled molecule will unwind and become a relaxed circular DNA (Figure 21.3, top right). T lymphocytes: white blood cells that mature in the thymus and are involved in the adaptive immune response, regulating development of B lymphocytes for antibody production, and generating cytotoxic T lymphocytes, leading to killing of target cells. Promoter occlusion: silencing the use of a transcriptional promoter, usually as a result of the presence of an active upstream promoter. Dependoviruses require a helper virus to replicate. Viruses can also infect and kill bacteria, archaea, algae, fungi, and protozoa. As DNA synthesis progresses, cellular histones present in the S-phase nucleus assemble on the growing DNA chains to form nucleosomes. The genomes of six of the seven families are circular, and those of the seventh family, Parvoviridae, have hairpin ends. Chapter 20 Fig. Human Immunodeficiency Virus 354 334 Human immunodeficiency virus type 1 (HIV-1) and acquired immunodeficiency syndrome (AIDS) 355 HIV-1 was probably transmitted to humans from chimpanzees infected with SIVcpz 355 HIV-1 infection leads to a progressive loss of cellular immunity and increased susceptibility to opportunistic infections 355 13/05/11 2:11 PM xvi Contents Antiviral drugs can control HIV-1 infection and prevent disease progression, but an effective vaccine has yet to be developed 356 HIV-1 is a complex retrovirus 357 HIV-1 targets cells of the immune system by recognizing CD4 antigen and chemokine receptors 357 Virus mutants arise rapidly because of errors generated during reverse transcription 358 Unlike other retroviruses, HIV-1 directs transport of proviral DNA into the cell nucleus 359 Latent infection complicates the elimination of HIV-1 359 The Tat protein increases HIV-1 transcription by stimulating elongation by RNA polymerase II 360 The Rev protein mediates cytoplasmic transport of viral mRNAs that code for HIV-1 structural proteins 360 Together, the Tat and Rev proteins strongly upregulate viral protein expression 361 The Vif protein increases virion infectivity by counteracting a cellular deoxycitidine deaminase 361 The Vpr protein enhances HIV-1 replication at multiple levels 362 The Vpu protein enhances release of progeny virions from infected cells 362 The Nef protein is an important mediator of pathogenesis 362 30. These two viral genes are expressed in most cervical cancers. A compound identified in such a screen was then modified systematically, and eventually maraviroc was selected for clinical trials. CPSF, as its name implies, is responsible for the cleavage of cellular pre-mRNAs shortly downstream of the consensus AAUAAA polyadenylation signal; PABII CH018.indd 218 16/05/11 8:07 PM Influenza Viruses facilitates production of full-length poly(A) chains on the 3' terminus created by cleavage. For example, when non-enveloped viruses such as adenoviruses bind to cell surface receptors like integrins, the cell responds with the production of several cytokines, including interleukin-1␣. Consequently, infection is only possible in cells that undergo mitosis. No course would be expected to use all the chapters in the book, nor necessarily in same order in which they appear. Furthermore, reverse genetics has allowed rapid generation of viral vaccines and propelled the use of viruses as gene delivery vehicles. TR terminal repeats; IR internal repeats; OriP and OriLyt are replication origins used during latent and lytic infection, respectively. See also Human papillomaviruses (HPV) cervical cancer, 264, 269–270, differentiating epithelial cells, infection by, 265–266 E1 protein, function, 267 E2 protein, function, 267 E7 protein, function, 267–268, 269, 270 E6 protein, function, 268–269, 270 genes and functions, 266 genetic and transcriptional map, 265 12/05/11 4:08 PM Subject Index genomes, 264 growth in cell culture, difficulty of, 264 human papillomaviruses (HPV), 263 mucosal lesions, 263–264 sites of infection, 264 skin lesions, 263–264 vaccines, 271 viral mRNA synthesis, 266–267 warts caused by, 263–264 Paramecium bursaria chlorella virus-1 (PCBV-1), 327–330 genome, 327–328 capsid protein, 329 capsid structure, 328 carbohydrates on capsid protein, 328 chitin synthesis, 330–331 hyaluronan synthesis, 330 pentasymmetrons, 328 transcription in cell nucleus, 329 trisymmetrons, 328 virus entry by degradation of cell wall, 329, 330 virion assembly in cytoplasm, 329 Paramyxoviruses, 175–186. Upon fusion of the viral envelope with the endosomal membrane, the individual genome segments are released freely into the cytosol. However, M1 is required for the production of infectious VLPs. The function of M1 may predominantly be to package the genome in virions. Cre elements, along with stem-loop I, may also serve to distinguish viral RNA from the many molecules of polyadenylated cellular mRNA present in the cytoplasm, allowing the viral RNA polymerase to concentrate on copying only viral RNA. Genome RNA may pass through pores formed in cell membranes by capsid proteins Rhinoviruses and foot-and-mouth disease virus have been shown to enter the cell by receptor-mediated endocytosis (see Chapter 4). Replication is initiated by a site-specific nick in one of the terminal hairpins, revealing a 3' end that primes DNA synthesis (step 1). Human Rhinovirus 3: Zhao, R., Pevear, D.C., Kremer, M.J., Giranda, V.L., Kofron, J.A., Kuhn, R.J., Rossmann, M.G., "Human rhinovirus 3 at 3.0 A resolution," Structure (1996) 4: 1205–1220. To ensure simultaneous infection, cell cultures are infected with a sufficient number of virus particles such that each cell receives at least one infectious particle. Within the nucleus, Rev binds to the Rev-responsive element (RRE) in viral RNAs, and recruits exportin 1 and Ran GTP to generate a complex capable of exporting the RNA to the cytoplasm. Only two other genes have homologs in databases: a glycosyl transferase and a thymidylate kinase. Structural proteins are synthesized as a polyprotein that undergoes cleavage and membrane association. Syncytium: a group of cells that share a common cytoplasm with more than one nucleus. 2.1i: Courtesy of Richard Kuhn, Purdue University; Fig. • Bunyaviruses assemble and mature intracellularly at the Golgi apparatus. A small number of people in Britain have succumbed to a neurological disease believed to have arisen from ingestion of tissues of prion-infected cattle. Togavirus replicons expressing Marburg virus proteins protected cynomolgus monkeys from infection with the virus. • Togaviruses can cause serious disease such as encephalitis in humans and horses. It is proposed that the CRISPR element expresses RNAs containing the phage sequences, which act as antisense RNAs, preventing translation of phage mRNAs. Much remains to be understood about the details of this intriguing phage resistance mechanism. GENOME Linear single-stranded RNA, positive sense. Covalently closed hairpin ends: no free 3' and 5' ends. Viruses in Tospovirus genus infect plants, are transmitted by thrips. It was discovered by virtue of disease symptoms in cash crops, but it can also establish non-symptomatic infections. X-rays cannot be focused, however, and direct x-ray imaging of the virion is not possible; CH002.indd 19 19 instead, diffraction patterns are captured and analyzed. Bunyaviruses were among the earliest animal viruses discovered: Nairobi sheep disease (in 1910) and Rift Valley fever virus (in 1930). 100 nt repeat 100 nt repeat 35 kbp 5' CACATCA... 1 CH001.indd 4 1 nanometer 109 meter or 106 millimeter. Signaling by CD40 turns on a cascade of events leading to activation of the important cellular transcription factor, NF B. The second step in virion construction is the assembly of the coat protein around the RNA to form an icosahedral shell. The Nef protein is an important mediator of pathogenesis Nef (negative effector) is a 210-amino acid protein that is localized at the inner face of the plasma membrane via a residue of myristate added to its N-terminal amino acid. (b) The coated vesicles detach from the plasma membrane and the clathrin coat dissociates. RIG-I plays an essential role in the recognition of single-stranded RNA viruses, including paramyxoviruses, influenza viruses, vesicular stomatitis virus, and Japanese encephalitis virus, while MDA5 is required for the recognition of picornaviruses. CD40L is typically expressed on activated T lymphocytes and provides a key costimulatory signal for B-lymphocyte maturation when combined with signals through the B-cell receptor (BCR). Nonstructural proteins organize protein processing, viral RNA replication, and capping The nonstructural proteins (NS1 through NS5) are involved in the replication of the genome RNA. Epizootic: an epidemic in one or more animal species. Enveloped virions are usually more flexible than non-enveloped virions, and they can have a variety of shapes. Virions form by budding into the Golgi cisternae. CH030.indd 376 05/05/11 1:08 PM SECTION VIII VIROIDS AND PRIONS C H A P T E R 3 1 Viroids and Hepatitis Delta Virus C H A P T E R 3 2 Prions This section covers two categories of very small infectious agents that are not viruses. The single open reading frame is translated into a large polyprotein that is cleaved by viral proteinases into intermediates P1–P3 and finally into the capsid and replication proteins shown. This shows that ribosomes that have recently terminated can search for nearby start codons in both the 5' and 3' directions. Fortunately, about 90% of infected CH030.indd 374 adults resolve their infection within six months and show no further symptoms. Initially these were based upon incidental observations of virus-like particles in electron micrographs. The proof that these drugs act as expected is that drugresistance mutations are located in the HIV-1 gene encoding the protease. Expression of Nef in mouse T cells and macrophages causes a disease that resembles the late stages of AIDS. These viruses package a circular DNA 15 genome that is partly double-stranded, with a singlestranded gap of variable length on one strand. However, a rare doubly spliced CH021.indd 254 simian virus 40 mRNA codes for a protein similar to mouse polyomavirus tiny T antigen. VIRUSES AND HOSTS Viruses in four genera (Orthobunyavirus, Phlebovirus, Nairovirus, and Hantavirus) infect vertebrates. Arthropod-borne: transmitted (usually to mammals and birds) by arthropod vectors (e.g., mites, ticks, fleas, mosquitoes). coli cell is called a lysogen, because the expression of the prophage can 85 CH008.indd 85 16/05/11 7:59 PM CHAPTER 8 Bacteriophage Lambda cro cII O P Ea47 int xis exo bet gam cIII N cI Ly sis re gu la tio Nul A W B C Nu3 D E FI FII Z U V G T H M L K I J lom stf tfa La te ibe il f Ta il Ta He ad n Infection begins with binding to the cell surface and injection of the DNA into the cell. Like cellular messenger RNAs, the viral RNA has a methylated cap at its 5' end, but it is not polyadenylated at its 3' end. Phylogenetic analyses using the sequences of the coat protein gene and the 5' noncoding sequences of genome RNA 3 indicate a common ancestor for each subgroup. In poliovirus-infected cells, PKR is proteolytically cleaved. RNA made from a DNA template Retroviruses DNA viruses DNA-dependent RNA polymerase THE DNA WORLD Figure 3.3 Possible steps in the evolution of life and the emergence of viruses. Therefore, it is not surprising that hepatitis C infection is one of the primary indicators for a liver transplant. The book is organized into ten sections and 37 chapters. Not all reovirus proteins are produced in the same amount. Viruses can undergo reassortment ("antigenic shift") by exchanging genome segments between related strains. This system, using a single transcription unit and producing multiple mRNAs by different RNA processing reactions, may be a simpler and more economical means than having 16/05/11 8:11 PM Adenoviruses distinct transcriptional enhancers and promoters for each of the many late mRNAs needed. Boxes indicate TATA boxes or sequence elements that interact with cellular (SP1, CCAAT) or viral (TIF, ICP4) proteins. Recombinant baculoviruses and VLPs for vaccine production Many viruses construct their capsids and envelopes by spontaneous self-assembly of multiple copies of a small number of distinct protein subunits. This is potentially a clever antiviral strategy and will create a reservoir of resistant haploid cells that can have sex and possibly create a huge variety of new E. With one exception, each gene codes for a single protein, and seven proteins are incorporated into virions (Figure 16.2). The diagrams on the second page show virions at a scale of 200 nm per inch, in order to be able to accommodate all the larger virions on a single page. A polyadenylation signal, 5'-AAUAAA-3', specifies the 3' ends of each transcript (Figure 21.6). Genera are in turn grouped into virus families. Each HA trimer then folds back on itself like a jackknife, forcing the two membranes closer together in the middle of the cluster (step 4). Inter- and intramolecular recombination also occurs in vivo, both in experimental plants and in natural populations. van den Worm, Jessika C. Diseases Long growth cycle, slow spread, restricted host range. Uncoating of parvovirus virions takes place in the nucleus and is cell-specific Parvoviruses are particularly dependent on host cell functions, a result of their small genomes and limited coding capacity. However, several vaccines produced with alternate technologies are either available or rapidly approaching the market. As a result there is competition between replicase and ribosomes for this site. These results suggest that a ribosome that has reached the coat stop codon and released the coat protein does not immediately leave the mRNA, but makes a short random walk along the RNA in either direction. This increases processivity of the polymerase and generates increased levels of full-length transcripts. Hyperthermophile: an organism that grows at temperatures above 80°C. When the next nucleoside triphosphate binds, viral DNA polymerase is "frozen." Figure 36.6 Mechanism of inhibition of herpes simplex virus DNA polymerase by acyclovir triphosphate. The major function of MHC proteins is to bind to peptides produced by degradation of antigens and to display them on the cell surface, leading to the activation of specific antiviral immune responses (see below). Some have likened the poxvirus immune defense to "star wars". To a large extent this is made possible because these are localized surface lesions, and papillomaviruses do not give rise to systemic infections. This translation or replication defect may therefore be a major cause of the inability of the Sabin vaccine strains to cause paralytic disease, while replicating well in intestinal cells and therefore inducing a strong immune response. Tat increases phosphorylation of the carboxyterminal domain by recruitment of cyclin-dependent kinase (cdk)-9/cyclin T to the transcription complex (Figure 29.5). Individuals progressing toward end-stage disease have high levels of virus in the blood, indicative of the failure of the immune system to contain the infection. Total genome length 10–15 kb. From 1994 to 2005, several outbreaks of Zaire ebolavirus disease occurred in the border region of Gabon and the Republic of Congo. Four of these are in the same reading frame, and ribosomes that initiate protein synthesis at these sites produce a nested set of proteins, denoted C', C, Y1, and Y2, with different N-termini but with a common C-terminus. Some live virus vaccines can be taken orally or nasally, which simplifies their delivery. These protease inhibitors are now used along with other drugs in some of the very successful triple therapy drug combinations that efficiently suppress HIV-1 replication and virus levels, and lead to healthier and longer lives for people infected with HIV-1. These trimers then close like a jackknife, bringing the two membranes in close proximity, resulting in fusion (steps 4 to 6). Virus-coded proteins on the surface of the virion bind to specific proteins, carbohydrates, or lipids on the cell surface. Right: viral proteins produced from the different classes of viral mRNA. A* Not essential for virus growth. • The reovirus outer capsid protein 3 binds to double-stranded RNA, reducing activation of PKR and therefore allowing viral protein synthesis to continue. 9.6c: Courtesy of Philippe Lemercier; Fig. Cilia are thin, elongated extensions of the plasma membrane containing microtubules; cilia move rhythmically to sweep up foreign particles and mucus. They had also made the key discovery that viral virulence disappeared over a 15- to 20-day period of drying spinal cord from infected rabbits. The cellular tRNA molecule that is bound to the primer-binding site (PBS) on the viral RNA serves as a primer for the initiation of synthesis of DNA complementary to the genome RNA. Because VP16 is a tegument protein that enters neurons with the infecting virus, little VP16 actually makes it to the nucleus of the neuron, which is a long distance along the nerve axon from the site of infection. There siRNAs are unwound and a single strand is hybridized to viral genome RNA or mRNAs, which are subsequently cleaved. Smaller particles are also seen; these may contain fewer than three nucleocapsids, and therefore would be non-infectious. Plasmid-based DNA vaccination may be particularly well suited for the delivery of either single or small groups of viral proteins to the immune system. Circulating leukocytes and monocytes migrate to regions producing cytokines and phagocytose pathogens. If these mechanisms used by parvoviruses can be exploited in the future for treatment of cancer, then infection with certain parvoviruses could turn out to be of benefit for the host. The precise pairing of envelope glycoproteins to capsid proteins in the core precludes incorporation of host cell surface proteins into the released virus particles. Shortly thereafter, R. Computer analysis of baculovirus genome sequences revealed a common set of about 29 homologous core genes that are present in all species. The vector DNA integrated in the target cell is identical to the DNA in the transfer construct. These enzymes, including p300 and the cyclic AMP-response elementbinding protein, CBP (Figure 23.5), play an important role in the transcriptional activation of large blocks of cellular genes. Three repressor-binding sites, called operators, overlap the PL and PR promoters. These strategies take advantage of the characteristics of each component vaccine to generate specific patterns of response (more antibody, more cell-mediated response, or balanced humoral and cellular responses). Marco Tarini, Paolo Cignoni, Claudio Montani, "Ambient Occlusion and Edge Cueing for Enhancing Real Time Molecular Visualization," IEEE Transactions on Visualization and Computer Graphics (2006), Volume 12, Issue 5, pp. New accumulations of endoplasmic reticulum membranes appear early in the infectious cycle, and numerous smooth 16/05/11 8:03 PM Flaviviruses vesicles can be found later in infection. This intricate mechanism explains why the replication of influenza virus is blocked by treatment of infected cells with inhibitors of cellular RNA synthesis such as actinomycin D or ␣-amanitin. Mol. What hantavirus disease appeared in the Four Corners region of the United States in 1993? Small envelope protein. These researchers observed that the transgenic mice were predisposed to developing liver tumors within a year of their birth, and they are more sensitive to exposure to liver carcinogens. A similar stuttering mechanism, repeated many times, accounts for the production of long poly(A) tails at the end of each viral mRNA (see above). Viral envelope proteins assemble in the plasma membrane and direct budding of virions Viral hemagglutinin, neuraminidase, and M2 ion channel proteins are synthesized and inserted into membranes of the endoplasmic reticulum. Rev is transported into the nucleus via nuclear pores by direct interaction with importin beta. Larger and more complex viruses may have numerous capsid proteins, and some viruses make scaffolding proteins that are involved in virion assembly but are subsequently discarded and do not form part of the mature virion. Contaminated food in restaurants can lead to a local outbreak of many infections in a short time. • Vaccines are generally held to a very high safety standard because they are administered to healthy subjects to prevent disease. CAPSIDS WITH ICOSAHEDRAL SYMMETRY Geometric principles originally discovered by mathematicians in ancient Greece demonstrate that closed shells made of identical subunits can have one of only three kinds of symmetry—tetrahedral (a tetrahedron is a polygon with 4 identical triangular faces), cubic (a cube has 6 identical square faces), or icosahedral (a regular icosahedron has 20 triangular faces; from Greek eikosi 20). HIV Nef mutants make virus particles that have reduced capacity to infect cells. Shah, F. The production and secretion of cytokines can be induced by cellular recognition of virus binding, entry, uncoating, or replication; by the accumulation of viral nucleic acids; and by virus-induced stress responses. One such adjuvant contains an emulsion of squalene (a fat-soluble hydrocarbon isolated from fish oil) vitamin E (also mostly hydrocarbon) in water. Prions 387 Prions are proteins that cause fatal brain diseases 387 Prion diseases were first detected in domestic ruminants 388 Bovine spongiform encephalopathy ("mad cow disease") developed in Britain and apparently spread to humans 388 Human prion diseases can be either inherited or transmitted 388 The infectious agent of prion diseases contains protein but no detectable nucleic acid 389 PrPSc is encoded by a host cell gene 390 Differences between PrPC and PrPSc 390 The prion hypothesis: formation of infectious and pathogenic prions from normal PrPC 391 Is the prion hypothesis correct? Although aluminum-based adjuvants help the immune system to respond to many viral antigens, they do not stimulate type 1 T helper lymphocytes (Th-1 cells), involved in the cellular immune response. In the coming years, altered forms of these versatile molecules will surely emerge as a new class of drugs. 4.5: Courtesy of Ari Helenius, modified from a figure from Whittaker, G.R. et al., "Viral entry into the nucleus," Annual Review of Cell and Developmental Biology 16 (2000): 627–651. Tospoviruses also encode an NSm protein, but they use an ambisense strategy. Used as biological insecticides. These repeated, short TRS (5'-UCUAAAC-3' for murine hepatitis virus) are located at the 5' ends of each of genes 2 through 7; there is an additional TRS at the 3' end of the leader sequence in genome RNA. It is a dimer of the basic immunoglobulin "Y" structure, with the addition of a joining J chain and a secretory protein (molecular weight 60,000) added during its secretion through the mucosal epithelium. Therefore, in contrast to research on animal 303 viruses, where the goals are to cure or prevent infection, research on baculoviruses eventually focused on enhancing viral pathogenesis and virulence. Roizman, "Herpesviruses", Ch. 33 in Fundamental Virology, D.M. Knipe and P.M. Howley (eds), Lippincott Williams & Wilkins (Baltimore) (2001); Fig. Thus an infected cell responds both by setting up its own intracellular defensive measures and by sending signals to nearby cells that in turn activate their defenses against infection. Because viruses leave no fossil record, we can only speculate on their first appearance based on what we know about their genomes, their proteins, and their replication strategies. However, even production of infectious filoviruses by plasmid transfection is a hazardous procedure because of the deadly consequences of infection, and must be done under biocontainment conditions. Members of these families, all with double-stranded DNA genomes, infect exclusively hyperthermophilic crenarchaeota, the optimal growth temperature of which exceeds 80□C. 1a 111 kDa 5' Cap OH 3' RNA 1 3357 nt OH 3' RNA 2 3050 nt OH 3' RNA 4A 689 nt OH 3' RNA 3 2216 nt OH 3' RNA 4 1031 nt OH 3' Satellite RNA ~335 nt 2a 97 kDa 5' Cap 2b 11 kDa 5⬘ CAP 3a 30 kDa CP 24 kDa 5' Cap CP 24 kDa 5' Cap 5' Figure 10.4 Cucumber mosaic virus genome organization. Describe a "DNA factory". 2910 3' 2920 AAAUAACUUAUUUGUAAU HOOC 2930 L mRNA VP1 Figure 21.6 Polyadenylation signals and overlapping 3' ends of viral mRNAs. mRNAs are shown in light blue; C-terminal parts of large T antigen and VP1 are shown in orange. Because the DNA is circular, this leads to the production of very long late RNAs that can contain multiple, tandem repeats of the DNA sequence, similar to the production of long DNAs by rolling circle DNA replication. The small genomes of these viruses have limited coding capacity, and instead of coding for their internal structural proteins, they simply pick them up in the cell nucleus, where viral DNA replicates. Is there a common theme to transcriptional control within the parvoviruses? All but live attenuated vaccines are administered with an adjuvant to augment the immune response. This and their cell-type dependence largely account for the biology and pathology of parvovirus infections. However, measles remains a major problem in the developing countries of Africa and South America. As they evolved, these independently replicating DNAs could have acquired genes coding for proteins that bind to the DNA, providing a package for its extracellular protection and delivery. (a) Region coding for CI and Cro. MDA5, on the other hand, preferentially recognizes long double-stranded RNA molecules and the synthetic double-stranded RNA mimic polyinosinic-polycytidylic acid (polyI:C). 2–3 reprinted from S.C. Harrison, "Principles of Virus Structure", Chapter 3 in Fundamental Virology 4th ed. The helper adenovirus is engineered to contain loxP sequences flanking its packaging signal. Given the enormous volume of the oceans, scientists have calculated that there may be as many as 1031 bacteriophages in the world. The Mx proteins. Table 18.1 Influenza A virus strain A/PR/8/34 genome RNAs and proteins RNA segment Length (nucleotides) 1 2341 PB2 759 Binds to cap structures on cellular pre-mRNAs; part of transcriptase complex 2 2341 PB1 757 Cleaves cellular pre-mRNAs to create primer; RNA polymerase activity for both transcription and replication PB1-F2 87-90 A protein that is localized in mitochondria and enhances apoptosis in immune cells Protein size (aa) Protein function 3 2233 PA 716 Part of transcription and replication complexes, exact role unknown 4 1778 HA 566 Hemagglutinin; major surface glycoprotein; receptor-binding; mediates membrane fusion at low pH; antigenic determinant 5 1565 NP 498 Nucleocapsid protein; binds to and encapsidates viral RNA; control functions in RNA synthesis 6 1413 NA 454 Neuraminidase; major surface glycoprotein; receptor destruction; dissociation of virus aggregates; antigenic determinant 7 1027 M1 252 Matrix protein; interacts with envelope, nucleocapsids, and NS2 M2 97 Integral membrane protein; ion channel activity essential for virus uncoating and maturation NS1 230 Nonstructural protein that down-regulates host cell mRNA processing; suppresses RIG-1 signaling and interferon induction; sequesters dsRNA and reduces interferon response; contains a PDZ domain for protein–protein recognition that can interact with diverse cell-signaling assemblies and reduce apoptosis of infected cells. Holin: a small, bacteriophage-coded protein that self-associates in the bacterial membrane under certain conditions of pH and ionic concentrations to open holes in the membrane, allowing phage endolysin to enter the periplasmic space and attack the bacterial cell wall. The 9S particles are pentamers of the major capsid protein F, and the 6S particles are pentamers of the major spike protein G. DNA is enclosed within an internal lipid membrane. While this may allow double-stranded DNA phages to produce high numbers of progeny particles, it delays their production. Collectively, these effects on host cell functions ensure that the infected cell devotes its metabolic activities primarily to the expression and replication of picornavirus RNAs. It is notable that hepatitis A virus, a picornavirus with a very slow replication cycle, does not inhibit host cell protein or RNA synthesis and, for the most part, does not produce observable cytopathic effects during infection of cells in culture. The envelope construct encodes the gene for the envelope protein, in this example VSV-G. Only a selection of these interactions will be discussed here. Another kinase that is activated is Tank-binding kinase 1 (TBK-1), which phosphorylates two interferon regulatory factors, IRF-3 and IRF-7 (Figure 33.3). However, the measured rate of movement during retrograde transport of herpesvirus capsids in neurons is 400 times faster than that. Open reading frames are shown as colored rectangles, and the names of encoded proteins and their molecular weights are shown above. • Many archaea grow and survive in extreme environments of temperature, acidity, or salinity. These three proteins combine to form the capsid shell of picornaviruses, and VP4 remains buried beneath the shell. For these reasons, use of these two tissues in humans as a platform for systemic delivery of therapeutic proteins was considered a promising application of AAV vectors. Others use cellular machinery to assist assembly or to facilitate budding. Unlike the membranebound toll-like receptors, these are soluble, cytosolic proteins, and can therefore bind to nucleic acids localized in the cytosol. CHO: carbohydrate residues added to two asparagine residues; GPI: glycophosphatidyl inositol membrane anchor; S–S: putative disulfide bond; A, B, C: ␣-helical regions in PrPC. These changes greatly stimulate its protein kinase activity. Transcription begins precisely at the junction of the U3 and R regions within the left-hand LTR and proceeds through the entire genome and the right-hand LTR. infection. The E protein may serve to facilitate budding of the virion into the lumen of the endoplasmic reticulum and Golgi membranes during particle maturation (see below). This subtype H2N2 virus caused the "Asian flu" pandemic. (a) The geodesic dome, designed by Buckminster Fuller, erected for Expo '67 in Montreal. differ substantially from host proteins. p53 activity is regulated in part by at least two cellular proteins that control its stability in opposite ways. Clinical trials using adeno-associated virus vectors Transduction of the liver and skeletal muscle of laboratory animals with AAV vectors is nontoxic, relatively efficient, and provides long-term transgene expression. cells, host cells have evolved mechanisms of recognizing double-stranded DNA in the cytoplasm (Figure 33.2). Prion proteins in yeast and other fungi do not share sequence similarity with mammalian prion proteins. Dynactin: an adaptor protein complex that allows dynein to bind to its cargo. Co-chaperone: a protein that associates with chaperones and stimulates their protein-folding and unfolding activities. The use of a different envelope protein also improves the safety of the vector by reducing the likelihood of producing replication-competent HIV-1. (a) Hammerhead self-cleaving structure of peach latent mosaic viroid. The fourth nucleocapsid protein, VP30, is required for optimal levels of viral messenger RNA synthesis from Ebola virus genomes. However, like inactivated virus vaccines, multiple doses of subunit vaccines are normally required for protection, and periodic boosting may be necessary to ensure long-term immunity. birds. However, occasionally a glutamine-tRNA misreads the UAG codon as CAG and inserts glutamine at that site. 16/05/11 8:00 PM Viruses of Archaea CH009.indd 99 99 Viruses of Archaea have diverse and unusual morphologies Fuselloviridae are temperate viruses that produce virions without killing the host cell The first archaeal viruses that were isolated in the 1970s and early 1980s resembled bacteriophages from the family Myoviridae and Siphoviridae, with icosahedral heads and contractile helical tails. Tat increases viral RNA abundance not by increasing the rate of initiation of transcription by RNA polymerase, but rather by increasing the elongation

efficiency of RNA polymerase molecules (Figure 29.5). However, like retroviruses and unlike other DNA viruses, hepadnaviruses code for a reverse transcriptase. Consequently, cells expressing these mutated viral forms of the protein behave as if they are receiving a growth stimulus independent of src pol LTR env gag LTR Rous sarcoma virus pol erb B gag Avian erythroblastosis virus gag myc Avian myelocytomatosis virus Figure 28.8 Genomes of acute transforming retroviruses. Double-stranded RNA activates 2', 5'-oligo(A) synthetase, which produces oligomers of ATP joined by 2', 5' linkages. Related phages: 434, ϕ80, 21, HK022, N15. Typically, transmissible gastroenteritis virus causes digestive tract disease in young swine. Lentiviruses: human, feline, bovine, and simian immunodeficiency viruses Spumaviruses: simian foamy virus. Cancers mediated by oncogenic papillomaviruses occur in only a small percentage of people who become infected with papillomaviruses. DISEASES Acquired immune deficiency syndrome (AIDS) was first described in 1981. In 1994, the third Ebola virus species, Cote d'Ivoire ebolavirus (CIEBOV), was isolated from an ecologist who was infected after having examined a dead chimpanzee. It is not known whether these viruses also have a nuclear phase in their life cycle, as the chloroviruses and some other members of the NCLDV group apparently have. This mutant is no longer dependent on Host Factor for its replication. Several of the new approaches outlined in Table 35.7 have the proven capacity to break tolerance in animal models. Omission of certain clones (B, C, D, E, F, and I) had no effect on reporter gene activity (shown by presence of a band of acetylated chloramphenicol, labeled AC). Topoisomerase I is required to help release the tension created in the circular, double-stranded DNA molecule as the DNA polymerase and helicase proceed along the DNA molecule, twisting the double helix in front as it is unwound behind. These have undergone deletion of the E1A and E1B genes. 13.3: Modified from Fig. These viruses are probably transmitted to primates from fruit bats. These oligomers bind to and activate ribonuclease L, which degrades viral and cellular mRNAs. PKR bind to the same double-stranded RNA molecule, they phosphorylate each other, inducing a conformational change that greatly increases their serine-threonine kinase activity (Figure 33.10). 27.10: Courtesy of Karen Weynberg, republished from 16/05/11 2:35 PM 488 Credits Figure 4.6 d in Ph.D. thesis of Karen Weynberg; Fig. After allowing the cells to settle, the tubes or plates are examined; the highest dilution that will agglutinate the aliquot of cells is considered to have one hemagglutinating unit (HAU) of virus. Introns are shown as interruptions in mRNAs. Names of proteins coded by mRNAs are shown adjacent to the corresponding mRNAs. Adenovirus genomes also contain one or two copies of VA genes, transcribed at high levels by cellular RNA polymerase III, the enzyme used by the cell to transcribe tRNA and other small RNA genes. Viral gene expression Acyclovir (Herpesviruses) Ganciclovir (Cytomegalovirus) Nevirapine (HIV) Raltegravir (HIV) 5. Although rodent cells can be readily infected by human adenoviruses, they are non-permissive for viral replication and therefore survive infection. Among the families that infect vertebrates, those with smaller genomes (Polyomaviridae, Papillomaviridae, and Adenoviridae) do not have enveloped virions, but those with larger genomes all have enveloped virions. Complement is a set of serum proteins that recognize foreign macromolecules on pathogens or cells and mark them for destruction. Are expensive to manufacture and may require the handling of highly pathogenic viruses Are less sensitive to storage conditions than live attenuated viruses. Nonpermissive cells may block virus infection at many possible stages, including virion binding, uptake, gene expression, genome replication, and virion assembly. Q is also a transcriptional antiterminator. Replication and transcription: synthesis of genome and subgenomic RNAs Structural proteins are cleaved during translation and directed to different cellular locations The antigenome RNA serves as a template for the synthesis of both full-length and subgenomic positivestrand RNAs. Genome RNA synthesis (replication) initiates at the 3' end of the antigenome RNA and proceeds to its 5' end, to make a complete complementary copy of the negative-strand RNA (Figure 13.3). When cells growing in a monolayer are infected, the progeny virus is released into the medium and can travel to distant sites, infecting other cells. Also, since a single replicase protein can make multiple copies of RNA, fewer replicase proteins are needed than coat proteins. This is one of the steps that lead to B-lymphocyte proliferation during an immune response. The viral genome is converted within the nucleus into a covalently closed, circular form (Figure 30.2). Despite the intriguing success of these vaccines, preexisting immunity to adenovirus in the human population may eventually limit the utility of this approach. The capsid or a subviral particle remains Table 3.4 Viruses with negative-strand RNA genomes Capsid symmetry 3–4 11–12 Linear Helical No 4–6 17–18 Linear Helical No Borna disease virus 1 9 Linear Helical Yes Lassa fever virus 2 11 Linear Helical Yes Influenza A virus 6–8 10–15 Linear Helical Yes Hosts Example Ophiovirus Plants Citrus psorosis virus Tenuivirus Plants & invertebrates Rice stripe virus Bornaviridae Vertebrates Arenaviridae Vertebrates Orthomyxoviridae Vertebrates & invertebrates CH003.indd 38 Genome size (kb) and topology Virus group Genome segments Envelope Rhabdoviridae Vertebrates, invertebrates, & plants Rabies virus 1 11–15 Linear Helical Yes Paramyxoviridae Vertebrates Measles virus 1 13–18 Linear Helical Yes Filoviridae Vertebrates Ebola virus 1 19 Linear Helical Yes Bunyaviridae Vertebrates, invertebrates, & plants California encephalitis virus 3 11–19 Linear Helical Yes 16/05/11 7:52 PM Major Virus Groups 39 Table 3.5 Viruses with double-stranded RNA genomes Virus group Hosts Example Genome size (kb) Capsid and topology symmetry Envelope Cystoviridae Bacteria Bacteriophage phi6 3 13 Linear Icosahedral Yes Totiviridae Fungi & protozoa Saccharomyces cerevisiae virus L-A 1 5–7 Linear Icosahedral No Penicillium chrysogenum virus 4 13 Linear Icosahedral No Chrysoviridae Fungi Partitiviridae Fungi & plants Penicillium stoloniferum virus S 2 3–4 Linear Icosahedral No Birnaviridae Invertebrates & vertebrates Infectious bursal disease virus 2 5–6 Linear Icosahedral No Reoviridae Fungi, plants, invertebrates, & vertebrates Rotavirus A 10–12 19–31 Linear Icosahedral No intact in the cell while messenger RNAs are transcribed from each genome segment by viral RNA polymerase molecules packaged with the genome RNAs; the resulting messenger RNA is extruded into the cytoplasm. Viruses in this group have genomes with a limited size range of 9–19 kb. So far no toxicity has been associated with the vector injection, and there has been improvement of motor function and quality of life of the treated patients. The ribozyme (right) is then ready to process a new molecule of substrate RNA. These viruses have been isolated from extreme geothermal environments in regions of active volcanism and tectonics in Europe, North America, and Asia. Divergent transcription from a central control region containing origin of DNA replication. On June 11, 2009, the World Health Organization raised the warning level for this virus to the highest available—Phase Six—indicating that influenza had entered a pandemic stage of worldwide spread. How virions are released has not been investigated. Each phage measures approximately 200 nm (0.2 m) in length from top of head to base of tail. The polyprotein is processed by both viral and cellular proteinases After virus uptake and release of the genome RNA in the cytosol, the RNA is bound by ribosomes and translated, giving rise to the polyprotein. Arrows indicate direction of transcription of genes. The activity of NK cells is augmented by interferon-␣, which is one of the first cytokines produced by host cells in response to viral infection. (b) Schematic diagram showing the envelope and three nucleocapsids (L, M, and S) of a bunyavirus virion, with the four structural proteins labeled. The eighth tube therefore is diluted by a factor of 108 compared with the original virus suspension. At some point, the mimivirus cores apparently release their viral DNA into the cytoplasm. The antitumor effects of parvoviruses could be exerted in several ways. T7 DNA became one of the first well-studied phage genomes (along with that of phage ϕ X 174) and its molecular weight was established as 25 106 daltons, or 40,000 base pairs, both by classical physical 77 CH007.indd 77 16/05/11 7:57 PM 78 CHAPTER 7 Bacteriophage T7 Searches for phages with properties similar to T7 have yielded many isolates, some of which are nearly identical to T7 and some of which are more distantly related. Thus killing of infected cells by activated cytotoxic lymphocytes appears to be the major mechanism by which the immune system clears viral infections from the body. The Gag/Pol polyprotein is made by suppression of termination and use of alternative reading frames The unspliced, full-length RNA is used to synthesize Gag and Gag/Pol polyproteins, the precursors of the MA, CA, NC, PR, RT, and IN proteins (Figure 28.2). Once inside the cell, nucleocapsids are transported to nuclear pores. What is the key trigger for virus penetration into endosomes? Chapter 13 Fig. Live vaccine-strain viruses are also shed in the stool of vaccinated children and this can immunize close contacts (e.g., family members) or boost their immunity. Alphaviruses: 11 kb; rubella virus: 9 kb. INTERFERONS: STRUCTURE AND SYNTHESIS Interferons-␣, , and have antiviral activity and are produced by most cell types. Virions enter the cell either in specialized vesicles or by direct penetration through the plasma membrane. 16/05/11 8:00 PM Viruses of Archaea (a) VP1 and VP2 covering genomic DNA VP3 VP4? If a start codon is encountered, the ribosome may reinitiate translation (Figure 5.7); if not, it will eventually detach from the mRNA. Furthermore, when the NP stem-loop was placed at the beginning of the next downstream gene, VP35, transcription of that gene also became dependent on expression of VP30. One capsid protein (C). The recombinant retrovirus vectors can infect nearby cells and integrate and express the transgene they carry. Many viruses need to recruit cellular proteins to complete the final pinching-off of the lipid bilayer and release a completed particle. Two additional cellular proteins recognize viral double-stranded DNA within the cytoplasm, namely absent in melanoma 2 (AIM2) and (hold your breath for this name!) nucleotide-binding domain, leucine-rich repeat-containing (NLR) family, pyrin domain-containing 3 (NLRP3) protein. The remainder of this section describes four families of animal viruses, many of which cause a variety of diseases in humans or animals. However, only group A adenoviruses yield tumors by direct injection of virus into animals. These sequence elements necessarily overlap with one or more coding regions (see inset in Figure 30.3). Some of the larger DNA viruses could have evolved from these small viruses through recombination and gene duplication events. The HA1 subunits (not shown in Figure 4.4) remain attached but no longer form a trimeric domain at the top of the oligomer. This RNA is in turn used as a template to produce more plusstrand RNAs. Although they are fully complementary, plus- and minus-strands do not anneal to form a doublestranded RNA. Unlike transcription, genome replication does not use a primer but begins at the 3' end of genome RNA and continues to the 5' end, making a complete copy. Viral mRNAs are made from two promoters and two polyadenylation signals Numerous viral messenger RNAs have been described. about the intracellular signals that regulate the switch from plasmid to vegetative viral DNA replication during differentiation in keratinocytes. 275 constructed from two basic building blocks, the hexon and the penton base (Figure 23.2). hepatitis B, 375 effects on immune system, 411, 422–423 effects on reovirus replication, 231, 233, 234 in cellular defense, 405–412 inhibition by adenovirus VA RNA, 277 inhibition by bunyavirus NSs protein, 208 inhibition by coronavirus proteins, 166 inhibition by Ebola virus proteins, 191 inhibition by herpesvirus proteins, 295, 300 inhibition by influenza virus NS1 protein, 213, 219 inhibition by paramyxovirus C and V proteins, 184–185 inhibition by poxvirus proteins, 321–322 interferon-α effects on NK cells, 418 interferon-γ effects on cytotoxic C lymphocytes, 422 interferon-γ effects on macrophages, 418 interferon alpha 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virus, 245 Panzootic influenza, 431 Pap test, 270 Papain-like proteinases (PLPs), 165 PLP1, 165 PLP2, 165 Papillomaviruses, 23, 263–271. • To date, only live attenuated vaccines reliably elicit both humoral and cellular immune responses • Among the most promising new vaccine technologies include DNA vaccines, chimeric vaccines, nanoparticle vaccines, and virus-like particles. • Cytokines produced by infected cells work in concert with leukocytes, macrophages, and natural killer cells to recognize and kill virus-infected cells. The virion is subjected to a series of changes prior to its activation in the endosome. Fatal cases develop clinical signs early during infection, and death commonly occurs between days 6 and 16 after the development of hemorrhage and shock due to low blood volume. The distal end of the T5 transcript codes for the integrase protein, named ORF D-335. Toll-like receptors (TLRs), located either on the cell surface or in endosomal membranes, are specialized proteins that recognize a variety of conserved molecular structures typically found in pathogens ("pathogen associated molecular patterns") (Table 33.1). Furthermore, three out of the six genes contain introns, but only one of the 78 and genes contains an intron. In contrast to inactivated or attenuated virus preparations, VLPs contain no viral genetic information, so they are not infectious and cannot replicate. Specific packaging signals direct incorporation of viral genomes into virions A variety of mechanisms have evolved to ensure specific genome packaging. However, a class of intriguing cellular targets of E6 is proteins that possess PDZ domains, named after the prototype proteins of this class (PSD95, Dlg, and ZO-1). (b) eIf-4F eIF-4A IRES eIF-4G (a) eIF-4F eIF-4E Met eIF-4AGTP eIF-4G Cap AUG Cleavage of eIF-4G by viral proteinase eIF-2 AUG 40S eIF-4E host cell factors Preinitiation complex Met Met GTP 4G GTP eIF-2 eIF-2 Cap AUG 40S AUG 40S 40S ribosome docking at IRES by host cell factors 40S scanning Met 60S Cap 40S Met 60S AUG Initiation complex 40S AUG Initiation complex Figure 11.5 Initiation of protein synthesis. Because NP is bound to growing RNA chains and incorporated into nucleocapsids, its concentration in the nucleus will drop during genome replication, favoring mRNA synthesis until more NP is imported into the nucleus. Reproduced by permission. Unlike viroids, they are encapsidated and can therefore be transmitted more efficiently from plant to plant. CH026.indd 316 16/05/11 8:13 PM Poxviruses out these processes. Late messenger RNAs are made from newly replicated genomes Many viruses synthesize a distinct set of "late" messenger RNAs after genome replication has begun. and analyzed for infectious virus, viral mRNA, viral proteins, or viral DNA. The best-studied chlorovirus is Paramecium bursaria chlorella virus-1 (PBCV-1), which infects Chlorella NC64A, a strain isolated from P. Retention of cis-acting replication and packaging sequences in the vector genome. The responses vary with specific combinations of virus strains and plant species or cultivars, and environments, indicating a constantly evolving symbiosis between virus and host. The unique gene content of the majority of archaeal viruses may reflect exceptional features of virus–host relationships. Table 29.2 HIV-1 structural proteins HIV-1 is a complex retrovirus Table 29.3 HIV-1 nonstructural proteins The basic replication pattern of lentiviruses is identical to that of other retroviruses. • The existence of prions suggests that information coded in the three-dimensional structure of a protein can be transferred from organism to organism and propagate itself without the intervention of a nucleic acid. This book was set in 10/12 Janson Text Roman by MPS Limited, a Macmillan Company, and printed and bound by Markono Print Media Pte Ltd. ISVPs may in fact be the form of virus particle that infects intestinal cells. This protein contains the same 295 N-terminal amino acids as GP, including the N-terminal signal sequence, but has a different C-terminal region that does not include the transmembrane domain of GP (Figure 16.6). Diameter 55 nm. However, since 1980 many effective antiviral agents have been developed. Journal of General Virology 73, 3065–3077. This chapter focuses on the final stage of the journey for viruses of vertebrates: entry of the virion into a host cell, its transport to the proper intracellular location, and disassembly of the virion to release the viral genome. The initial cleavages are rapid, so that little if any full-length polyprotein can be found in an infected cell, unless amino acid analogues that inhibit proper protein folding are incorporated during synthesis. Material from tumors was used to infect cell cultures or laboratory rodents and the viruses that emerged were considered as possible candidate oncogenic viruses. Virions of Sulfolobus neozealandicus droplet-shaped virus (SNDV) resemble elongated droplets with varying dimensions, 110–185 nm in length and 95–70 nm in width (Figure 9.6). It may prove useful to modify the concept of an "infectious agent" to include any transfer of information between cells or organisms that results in repetitive selfpropagation. A virus that infects amoebae, called mimivirus, has by far the largest known viral genome; recently sequenced, it contains nearly 1.2 million base pairs, larger than the genomes of many bacteria and archaea. Recent studies indicate that L-SIGN, a cell surface lectin, may also serve as a receptor for the SARS coronavirus. Simian Virus 40: Stehle, T., Gamblin, S.J., Yan, Y., Harrison, S.C., "The structure of simian virus 40 refined at 3.1 A resolution," Structure (1996) 4: 165–182. All viruses use nucleic acid as a repository for the information necessary for their replication. Togavirus virions contain a nucleocapsid with icosahedral symmetry wrapped in an envelope of the same symmetry Togavirus virions are enveloped spherical particles with a diameter of 70–80 nm. Can be packaged with the G envelope protein of vesicular stomatitis virus to allow infection of many cell types. Plasmodesmata are found as two types, primary and secondary, based on the developmental stage. (eds) Biological Macromolecules and Assemblies, John Wiley and Sons (1984); Fig. These chapters are each designed to tell a story about the viruses being considered, and to portray the "personality" of those viruses, with the idea that this will help students to learn about and remember each virus group. Unfortunately some of the children that were treated with this retrovirus developed T-cell leukemia, a cancer resulting from the unrestricted proliferation of T lymphocytes. 3' end has a 70-nt poly(A) tail. Paralogous: homologous gene sequences that presumably arose by a gene duplication event are called "paralogous" genes. In 1977, the H1N1 virus reappeared in humans in China and then spread around the world. Subunit vaccines. 237 SEC-V.indd 237 12/05/11 5:03 PM C H A P T E R 2 0 Parvoviruses Peter Beard Parvoviridae From Latin parvus (small), referring to size of virion VIRION Naked icosahedral capsid (T 1). Their underlying hope, largely achieved, was to use viruses as a tool to help understand how cells work. The molecular chaperone Hsp70, a cellular protein, has been implicated in P1 processing; it is possible that Hsp70 assists in the interaction between 3CD and P1. Binding of small T antigen to protein phosphatase 2A inhibits its activity, allowing phosphorylated MAP kinase to accumulate and to activate transcription factor AP-1, which enhances cyclin D1 transcription. Although production of AAV by transfection of three plasmids can be scaled up by using suspension cultures of 293 cells, this production method is cumbersome. 16/05/11 8:06 PM Bunyaviruses 209 KEY TERMS ␣-amanitin Actinomycin D Ambisense Encephalitis Epidemic Exocytosis Genetic drift Genetic shift Hantavirus pulmonary syndrome Hemagglutination Hemorrhagic fever Hemorrhagic fever with renal syndrome Integrins Ionophore Ligand Lumen Monensin Reassortant viruses Ribozyme Thrips Transovarial transmission Tropism Type I transmembrane protein FUNDAMENTAL CONCEPTS • • • • Most bunyaviruses are transmitted by arthropod vectors. In most cases, both the 5' and the 3' ends of the genome RNA of the vector must be precisely reproduced by transcription from the plasmid DNA to enable replication of the transcripts by viral RNA polymerases. DISEASES Humans: AIDS, leukemia. (a) The icosahedral nucleocapsid of Semliki Forest virus interacts directly with the cytoplasmic tail of virus envelope glycoproteins during budding. The DNA-binding domain of large T antigen (Figure 21.11) recognizes G(A/G)GGC pentanucleotide sequences on double-stranded DNA. These unrecognized infections can result in spread of virus to uninfected individuals. 16/05/11 8:21 PM 432 CHAPTER 35 Antiviral Vaccines Figure 35.1 Major types of antiviral vaccines. Retargeting was rendered possible by inserting into the envelope protein the binding sequence of an antibody targeted against a specific cell receptor. • The smallest viruses are almost totally dependent on their host cells for the enzymatic machinery to express and replicate their genomes, while the largest viruses require only substrates, energy, and protein synthesis machinery. This can happen if the left-hand LTR is inactivated by mutation or deletion, allowing the right-hand LTR to initiate transcription (Figure 28.9a). DISEASES Chronic, progressive, invariably fatal central nervous system degeneration. Circulating antibodies can bind to proteins on the surface of virus particles within the body and neutralize their infectivity. 161 Coronaviruses have large, single-stranded, positive-sense RNA genomes 161 Coronaviruses fall into three groups based on genome sequences 161 Coronaviruses have enveloped virions containing helical nucleocapsids 162 Coronavirus virions contain multiple envelope proteins 163 Coronavirus spike proteins bind to a variety of cellular receptors 164 The virus envelope fuses with the plasma membrane or an endosomal membrane 164 The replicase gene is translated from genome RNA into a polyprotein that is processed by viral proteinases 164 RNA polymerase, RNA helicase, and RNA-modifying enzymes are encoded by the replicase gene 165 Replication complexes are associated with cytoplasmic membranes 165 Genome replication proceeds via a full-length, negative-strand intermediate 166 Transcription produces a nested set of subgenomic mRNAs 167 Subgenomic mRNAs are transcribed from subgenomic negative-sense RNA templates made by discontinuous transcription 167 The discontinuous transcription model can explain recombination between viral genomes 168 Assembly of virions takes place at intracellular membrane structures 169 Adaptability and evolution of coronaviruses 169 TOC.indd xi xi SECTION IV: NEGATIVE-STRAND AND DOUBLE-STRANDED RNA VIRUSES OF EUKARYOTES 15. An added domain of the goshukovirus capsid protein performs some of the functions of 16/05/11 7:56 PM 76 CHAPTER 6 Microviruses the missing external scaffolding protein. RNA 3 encodes the 3a or movement protein, and the coat protein (translated from subgenomic RNA 4). Recently, it was shown that infectious prions could be generated from purified recombinant prion protein. The new nucleoside analogues are more easily tolerated. According to one theory (Model 1 in Figure 24.9), virions retain their envelopes and are transported to the Golgi membranes within vesicles that bud from the outer nuclear membrane. CH029.indd 364 16/05/11 8:15 PM C H A P T E R 3 0 Hepadnaviruses Christopher Richardson Hepadnaviridae From hepatitis and DNA VIRION Spherical enveloped particle. Cyclin D1 is an important activator of the cell cycle. It was the first bunyavirus whose complete genome sequence was determined, and it was the first segmented negative-strand virus from which infectious virus was recovered by transfecting cells with cloned cDNAs. Rift Valley fever virus, a member of the Phlebovirus genus, uses an ambisense coding strategy on its S genome segment (see below). Conversion of RNA to DNA is a complex process that proceeds as follows (Figure 28.4): 1. For N to function, it binds to specific mRNA sequences, called NUT (N-utilization) sequences (Figure 8.4a). Intrinsic cellular defense systems are deployed in three stages: (1) pathogen recognition, (2) signal amplification, and (3) pathogen control. The capsids of many small, icosahedral, plus-strand RNA viruses grow around the viral RNA, condensing it into a compact "ball of string" as the assembly proceeds. In contrast, phospholipids in membranes of bacteria and eukaryotes use ester linkages, and their non-polar parts are usually made from unbranched fatty acids. Viruses with more limited capacity to do harm, such as agents of mild childhood diarrhea and the common cold, are currently being targeted. The host cell RNA polymerase transcribes the class I genes, simultaneously spooling the DNA out of the capsid into the cytoplasm at a rate of 40 bp per second. 3' 1 5' 7 2 3' 5' 3' 5' 6 5' 3 3' 3' 5 5' 4 Figure 31.2 Rolling circle replication of circular RNAs. Steps 1 to 8 show symmetric replication, in which both plus- and minus-strand circles are formed and used as templates for RNA synthesis. This DNA is made by reverse transcribing a genome-length RNA molecule that is one of the transcription products of the fully double-stranded DNA. In the NS3 C-terminal domain there are amino acid sequence motifs found in RNA helicases as well as in nucleoside triphosphatases. nsP4 610 RNA polymerase. • In contrast to transcription, replication of viral RNA begins when sufficient quantities of nucleocapsid protein are available to encapsidate nascent transcripts; in this mode, the RNA polymerase makes a full-length copy of the genome RNA and does not terminate transcription before the 5' end of the genome RNA. Reovirus infection also activates caspases, which are cellular proteases that mediate apoptosis. Some of the mutations are located in the external scaffolding and coat protein genes, and allow these proteins to interact in the absence of protein B. All other viroids identified to date fall into the potato spindle tuber viroid (PSTVd) group, also known as the Pospiviroidae family or group B. A protein shell 3 nm thick surrounding such a sphere will have about 30,000 amino acid residues. Togaviruses 148 Most togaviruses are arthropod borne, transmitted between vertebrate hosts by mosquitoes 148 Togavirus virions contain a nucleocapsid with icosahedral symmetry wrapped in an envelope of the same symmetry 149 Togaviruses enter cells by low pH-induced fusion inside endosome vesicles 150 13/05/11 2:11 PM Contents Nonstructural proteins are made as a polyprotein that is cleaved by a viral proteinase 151 Partly cleaved nonstructural proteins catalyze synthesis of full-length antigenome RNA 151 Replication and transcription: synthesis of genome and subgenomic RNAs 153 Structural proteins are cleaved during translation and directed to different cellular locations 153 Assembly of virions and egress at the plasma membrane 154 Effects of mutations in viral proteins on cytopathic effects and on pathogenesis 155 Alphaviruses have been modified to serve as vectors for the expression of heterologous proteins 155 Alphavirus vectors have multiple potential uses 156 14. This leader is then spliced to an mRNA "body"; multiple alternative 3' splice sites give rise to several different mRNAs for each of the five late gene families. Old world monkeys experimentally infected with HIV-1 express Trim5␣, a protein that promotes rapid degradation of the capsid before reverse transcription can occur. [DNLM: 1. Icosahedral or conical capsid. Postreplicative RNA synthesis, capping, and polyadenylation are carried out by the same enzymes used for early mRNA synthesis (see Table 26.2), freshly synthesized from early mRNAs. Initiation of transcription of intermediate genes requires two virus-coded factors (VITF) synthesized from early viral mRNA, and one cellular factor. It is believed to be a distant cousin of hepadnaviruses (see Table 3.6). It initially attaches to cells by interactions between the virion hemagglutinin protein and cellular sialic acid (Figure 36.10a; also known as N-acetyl neuraminic acid), which is present on many membrane glycoproteins and gangliosides. Murine hepatitis virus binds to carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1 or CD66a). Examples of such heptamer sequences are A AAA AAC (HTLV-1), A AAU UUA (Rous sarcoma virus), and U UUU UUA (HIV-1). This allows the viral genome to be replicated to a high copy number by the cellular DNA replication machinery. These are in turn copied by RNA polymerase to make more full-length genome RNA (not shown) or the six subgenomic mRNAs. CH014.indd 168 16/05/11 8:04 PM Coronaviruses "template switching" may be a normal feature of coronavirus replication and may be important for genome repair and generation of new strains of coronaviruses. Seroconversion: the appearance in serum of antibodies specific to a pathogen, indicating exposure to the pathogen but not necessarily overt disease. Four types of illness are caused by bunyaviruses in humans: fever, encephalitis, hemorrhagic fever, and a fatal respiratory syndrome. Nucleocapsids enter the nucleus, where mRNA synthesis and RNA replication occur Influenza virus nucleocapsids contain multiple copies of the NP protein that wrap the single-stranded genome RNAs in an unusual twin helical conformation with a central loop (Figure 18.5). Interferon- primarily stimulates components of the adaptive immune response VIRAL DEFENSES AGAINST INTERFERON RESPONSES Homologues of interferon-regulatory factors block transcription of interferon genes. Reovirus strains are distinguishable by unique electrophoretic profiles of their double-stranded RNA gene segments. The virus capsid is formed from 1680 trimeric capsomers of Vp54 (Figure 27.4b, c), which are arranged in groups as 20 curved triangular facets (trisymmetrons; 66 capsomers each) and 12 curved pentagonal facets (pentasymmetrons; 30 capsomers each) on the surface of the virion (Figure 27.3). The interconnective portions form a protein shell that covers most of the lipid bilayer except for small holes. The presence of coat protein increases the accumulation of plus-strand viral RNAs in infected cells, probably because it stabilizes viral RNAs by packaging them in capsids. Shown are examples of the smallest and largest known capsids with icosahedral symmetry, and two intermediate-size capsids. Three enzymes: protease (PR), reverse transcriptase (RT), integrase (IN). One has also to bear in mind that the plus-strand needs extra protection because it is the infectious agent. I. In conditions where there is little or no free N protein (such as at the beginning of infection), the RNA polymerase transcribes for a short distance (40–60 nt), then terminates and releases an uncapped "leader" RNA. Instead of walking, the tailless, spherical ϕX174 may "roll" along the cell surface in a similar fashion. For RNA viruses with multiple genome segments, this is usually due to reassortment of genome segments between two virus strains that infect the same cell. The activity of nsp5 is thought to be regulated by cleavage site specificity as well as by the flanking nsp4 and nsp6 proteins, both of which are hydrophobic membrane-spanning proteins that are likely membrane-associated. Anaphylatoxins: fragments of complement proteins that trigger inflammation. The S RNA of most orthobunyaviruses (Figure 17.2) encodes two proteins, the nucleocapsid protein (N) and a nonstructural protein (NSs). Chapter 15 Fig. Perhaps the most interesting aspect is not their presence but their number; at least ten proteins have accessory roles in protein synthesis. The 2002–2003 SARS epidemic sparked a flurry of investigation in the coronavirus field, including examination of archived samples of patients admitted to hospitals with respiratory symptoms of unknown etiology. 16/05/11 8:21 PM 442 CHAPTER 35 Antiviral Vaccines The story of the rabies vaccine Human rabies is a devastating disease with periods of relative lucidity and apparent madness alternating over days to weeks in a downward spiral to inevitable death. The viral envelope contains host-derived lipids and encases a tightly packed superhelical nucleoprotein (Figure 9.8b). ...TGATGATG ...ACTACTAC 3' 5' Figure 23.3 Terminal inverted repeats on adenovirus DNA. This step usually involves interaction with a specific cellular receptor on the cell surface or in the cytoplasm. • Alterations in secondary structures within phage RNA control translation of the four phage genes. It has been postulated that rolling circle replication produces DNA knots that need to be resolved for efficient transcription. Once in the cytoplasm, these RNA molecules are further processed by Dicer to short miRNAs. MiRNAs are incorporated into the RISC complex where they are hybridized to cellular or viral mRNAs. Perfect matches lead to mRNA degradation, whereas imperfect matches block protein synthesis. VP24 a'S a L L a'S a Sa L a'S a'S a L 293 L a a'S a L a'S a' a'S a L a L a'S a L a'S a a S L a'S a L L a'S a L a' L a'S a L a'S a a a S L a' Figure 24.8 Packaging of herpesvirus DNA. They are still used for such purposes, and a number of vectors are available commercially as kits, along with selection systems for recombinant viruses and susceptible cell lines for propagation. The viral genome (dark blue) is shown as a helical nucleocapsid covered with N protein (green ovals) and containing RNA polymerase components L (orange spheres) and P (thin gray ovals). • Small T antigen interacts with protein phosphatase 2A, stimulating the MAP kinase pathway and bringing the cell into the S (DNA synthesis) phase of the cell cycle. CH005.indd 61 How is the access of ribosomes to the start sites of the lysis and replicase genes made dependent on translation of the coat gene? Most survivors were scarred for life, many were blinded, and some suffered debilitating limb deformities. Alternatively, the extracellular virus envelope may rupture upon initial contact of the particle with the cell surface, releasing a mature virus particle, which enters as described above. Cross-protection is known to occur between strains of the same viroid, and between different viroids that are very similar in sequence. Their capsids contain 60 copies of a protein of about 520 amino acid residues. Thus, recombination and reassortment have likely played important roles in the evolution of cucumber mosaic virus strains. Amyloid-like formations formed by other chromosomally encoded yeast and fungal proteins, such as Sup35, Rnq1, HET-s, and Swi1 have been shown to result in adoption of distinct and inheritable "prion states." Prion states in these simple organisms have been studied extensively and have been shown to result from aggregation of cellular proteins into self-propagating amyloid fibrils whose transmission occurs naturally through cell division. HA forms a trimer in the virus envelope, and is responsible not only for binding of virions to the cell surface but also for entry of the virus genome into the cell via the fusion pathway. 27.12a-d: Reprinted from Fig 5 A, B, D and E in Xiao, C. The fusion-activating changes involve major alterations in the interactions between folded domains and between protein subunits in oligomers. orange, whereas single-stranded RNA fluoresces orange. Genome RNA is contained within helical nucleocapsids (b) Figure 15.2 Structure of rhabdoviruses. The complex structures of the tail and the internal core provide the machinery for packaging the viral genome and for inserting it into cells during infection. CH001.indd 8 One outcome of the phage group's work was the demonstration, by Alfred Hershey and Martha Chase in 1952, that the DNA of a bacteriophage is injected into the host cell and the protein coat remains outside the cell. A highly diverse family of viruses, reflected in a wide variation of encoded proteins. CH010.indd 121 121 symptoms including stunting, mosaic lesions, chlorosis, necrosis, distorted morphology of various organs, and interrupted vegetative and reproductive development. This strategy is known as pseudotyping. The L protein then adds nucleotides to this primer RNA, using the genome RNA as a template. However, this required mixture CH032.indd 392 of the recombinant protein with cellular extracts and prolonged and repeated cycles of incubation under conditions that favor protein misfolding. 16/05/11 8:22 PM Eukaryotic Virus Vectors with cassettes controlling the expression of the foreign genes. Further spread throughout the world is occurring, probably via infected migrating waterfowl. Vertical lines on mRNAs (light blue) show transcription start sites. This can be conveniently done by the use of the Cre recombinase, derived from bacteriophage P1. Are less immunogenic than other vectors. Unfortunately, VSV-G is toxic to cells in which it is made. Oncogenic human papillomaviruses are a major cause of genital tract cancers Since 1976, it has been recognized that human papillomavirus infections of the genital tract frequently lead to benign genital warts, neoplasias, and invasive squamous cell carcinomas. The goal in designing chimeric viruses (also known as hybrid vectors) is to combine into one vector the advantageous properties of different viruses. Thus VP40 appears to be the major viral protein that directs budding and virion formation. RNA phages do not have the specialized tail assemblies that are used by many DNA phages to inject their genomes. However, it has the disadvantage that productive infection by such viruses requires the delivery of at least one of each of the particles containing different genome segments into the same cell. The 1 protein has the most sequence diversity of any reovirus protein and is the serotype-specific antigen. These researchers demonstrated that a replication-defective lentivirus could be used to stably express a reporter gene in mouse brain neurons, which are nondividing cells. A segment of concatemeric DNA is shown cleaved at a cos site by terminase, which then binds to a prohead. Cold chain: a system to maintain the storage temperature for a temperature-sensitive product such as live vaccines all the way from the manufacturer to the site of administration. The major capsid protein of bacteriophage ϕKZ is colored blue, the vertices are colored gray, the tail helices are in various colors, and the baseplate is colored gray. Applications of retrovirus vectors: treatment of neurological disorders Interestingly, a recent gene therapy trial using a selfinactivating lentivirus vector for the transfer of the ABCD1 transporter gene in autologous hematopoietic stem cells resulted in the successful treatment of adrenoleukodystrophy (ALD). 16/05/11 8:17 PM CH032.indd 396 16/05/11 8:17 PM SECTION IX HOST DEFENSES AGAINST VIRUS INFECTION C H A P T E R 3 3 Intrinsic Cellular Defenses Against Virus Infection C H A P T E R 3 4 Innate and Adaptive Immune Responses to Virus Infection Organisms have coexisted with viruses for most of evolutionary time. infected leaf, moves along the phloem following the translocation stream, and eventually enters young plant tissues, following the same route as photoassimilates (Figure 10.8). To distinguish his procedure from variolation, Jenner named it vaccination (vacca means "cow" in Latin), and within 10 years vaccination against smallpox had been adopted worldwide. BGLOSS.indd 476 Integrase: a retroviral enzyme that catalyzes the integration into cellular DNA of the double-stranded viral DNA produced by reverse transcription. Some coronavirus S proteins can induce cell-cell fusion at neutral or alkaline pH values, suggesting that these virions fuse directly with the plasma membrane. These enzymes are mostly involved in the regulation of cytokine maturation. The diagrams on the first page show virions at a scale of 50 nanometers (nm) per inch. Sophisticated virus purification and detection methods. NK cells appear to constitute a very important early defense mechanism for limiting the severity of viral disease. Viroid: a small (250–400 nt), circular RNA that can replicate in the absence of a helper virus by reprogramming cellular DNA-dependent RNA polymerases to copy its genome RNA. Viruses with positive-strand RNA genomes bring their messenger RNA directly into the cell in the form of the genome. Depending on the virus, the genome can be either RNA or DNA, and it can be either single-stranded or double-stranded. Many viruses damage or kill their host cells and therefore cause disease and death. The lesions become necrotic, and virus does not spread beyond the lesions. Antibodies (immunoglobulins) are formed from dimers of heavy chains and light chains. For example: • Viruses with single-stranded DNA genomes tend to be very small. Both host nucleic acids and proteins have been proposed to be the initial viroid target. Paramyxoviruses enter the cell by fusion with the plasma membrane at neutral pH Paramyxoviruses recognize a variety of different cellular receptors. It may be possible in the future to exploit site-specific integration for gene transfer applications using adeno-associated, virus-based vectors (see Chapter 37). 16/05/11 8:05 PM 180 CHAPTER 15 Paramyxoviruses and Rhabdoviruses ss56 13 C U A CU UC G G U C U G G AA C C U GC 4 G U UUU 2 1 G UU CUCUUG UCU H 3 AC O A A OH UG 5 GG G U U GU A UU UA U A 2 14 6 AC 1 15 11 12 UC A A AAUCC UUA 15 14 16 7 10 GG A U C CU A A 16 CC 9 8 ACU G UU AC A UC 5.3 nm GU Leader and genome promoter Figure 15.5 Structure of paramyxovirus helical nucleocapsid. Dynein: motor complex that typically carries cargo in the minus end direction (towards the center of the cell) along microtubules. Viral inclusions do not contain membranes but are intimately associated with the cytoskeleton. Interferon was discovered in 1957 by Alick Isaacs and Jean Lindenmann. Pathogenesis of filovirus infections Marburg and Ebola viruses cause similar pathological changes in humans. Antiviral Vaccines 428 A BRIEF HISTORY OF ANTIVIRAL VACCINES 429 Early vaccine technology was crude but effective 430 Embryonated chicken eggs and cell culture played major roles in vaccine development in the twentieth century 431 Production of vaccines against avian influenza strains has been problematic 431 TYPES OF ANTIVIRAL VACCINES 431 Advantages and drawbacks of vaccine types 434 New categories of antiviral vaccines 434 HOW DO ANTIVIRAL VACCINES WORK? Not much room for maneuver As the smallest free-living eukaryote, Ostreococcus represents a significant challenge for the replication of a large virus. More often, the same glycoproteins mediate both attachment and membrane fusion. A CRISPR element consists of several protein encoding genes and an array of direct repeats, 25–50 bp long and in some cases containing short palindromes. The target RNA substrate (left) anneals to the ribozyme (step 1) and is cleaved (scissors), producing two products that are released (step 2). Other parvoviruses were discovered by chance. Since their invention, recombinant baculoviruses have become one of the most widely used vehicles for the high-level expression of foreign proteins. Effective, safe drugs have a high therapeutic index. Protein 2C is also believed to bind to the RNA and bring it to the vesicles where RNA synthesis takes place. (a) Branched structure proposed for peach latent mosaic viroid, a member of group A. • Viral capsid proteins are imported into the nucleus where they condense around progeny viral minichromosomes and assemble into mature virions. Immediately after exposure to low pH in late endosomes (step 1, Figure 4.4), the fusion peptides in the HA2 subunits (red filaments shown at top of the extended polypeptide chains) are exposed and insert into the outer bilayer leaflet of the target membrane (step 2). • Ribosomes translating the coat protein gene disrupt secondary structure nearby the replicase gene start site, allowing ribosomes to bind to and translate that gene. In early 2010, seasonal strains of influenza A H3N1 and H1N1 as well as influenza B virus began to cocirculate with pandemic H1N1 virus. The late genes code for viral proteins involved in assembly of budded virions, while the very late genes are involved in the formation of occluded virions. The first AAV vectors constructed were derived from serotype 2 (AAV2). 27.11: Courtesy of Nathan Zauberman; Fig. 3⬘ ends of growing DNA chains (black) are shown as arrows. Three additional proteins (gp14, gp15, and gp16) form an internal core around which the DNA is wrapped. The three internal core proteins are then inserted via the tail into the cell, forming a channel through the outer membrane, the peptidoglycan cell wall, and the cytoplasmic membrane. Discuss the composition of this particle. A base-paired RNA stem induces stalling upstream by the translating ribosome (green oval) at the heptamer sequence, shown in orange. 2.8c: Reproduced from Eight_Allotropes_of_ Carbon.png, Wikipedia Commons; Fig. ADVERSE EVENTS AND ETHICAL ISSUES Vaccine-associated adverse events Although the currently available antiviral vaccines are among the safest pharmaceutical products ever introduced, none is 100% safe. Multimerization Figure 20.7 Functional domains in adeno-associated virus Rep78 protein. In comparison, the smallest cellular genomes contain approximately 400,000 nucleotides, and the genomes of mammals contain approximately 3 billion nucleotides. (b) DNA polymerase extends the plus-strand, filling in the ss gap (orange). These cellular signal transduction events lead to many changes in the transcriptional profile of an infected cell, inducing synthesis of a wide array of cytokines, chemokines, and stress-related molecules. The viral genome is then integrated into the host cell genome, and is transmitted during cell division to all the cells in the developing host thallus. Should the virus succeed in infecting host cells, the second level of defense against infection comes into play: these responses are called intrinsic cellular defenses and are discussed in detail in Chapter 33. The mosaic structure of phage chromosomes challenges the classification of phages based on tail structure, because phages with related tail structures may be unrelated across much or all of their chromosomes, and conversely, phages that are genetically highly related may differ in tail structure. Its 4.7-kb, singlestranded DNA genome contains only two genes: rep, which encodes replication and integration enzymes, and cap, which produces the capsid structural proteins (Chapter 20). In support of this hypothesis, woodchuck hepatitis virus variants that lack E are incapable of establishing persistent viral infections. Attachment Pleconaril (Picornaviruses) Maraviroc (HIV) 2. 05/05/11 2:45 PM Glossary Panzootic: increase in infectious disease incidence in animal species over time and large geographic range (e.g., panzootic of rabies in raccoons extending up eastern North America through the 1970s to the present). Capsid proteins oligomerize and bind to genome RNA in the cytoplasm to form nucleocapsids, which migrate to the plasma membrane, as shown here, or alternatively form during association with viral glycoproteins at the plasma membrane. These include cloning a full-length cDNA in a bacterial artificial chromosome or in a recombinant vaccinia virus. One to three proteinases. It soon became apparent that this protein was related to type B hepatitis, and the Australia antigen is now known as the hepatitis B surface antigen. The HA protein of the 1918 virus acquired the ability to bind to the ␣-2,6 sialic acid present in the human respiratory tract by virtue of a single amino acid change from an HA that binds to the avian ␣-2,3 sialic acid. PBCV-1 encodes two chitinases, a chitosanase, a glycanase, gluconase, and glucouronic lyase. Most single-stranded RNA animal viruses form a double-stranded RNA replication intermediate when replicating their genome. These peculiar features arise from the mechanism of generation of the genome DNA via reverse transcription (see below). RNA replication requires the synthesis of a complementary (minus-strand) copy of the plus-strand RNA. Translation, on the other hand, moves in the opposite direction on the RNA. Such issues are obviously controversial and highly charged. For these reasons, their study is important, and development of effective vaccines and chemotherapeutic measures is a priority. These RNAs replicate and are encapsidated together with genome and subgenomic RNAs in plants infected by certain viral strains. This virus was subsequently found in cynomolgus macaques in the export facility in the Philippines, and more recently it was discovered in pigs in the Philippines, raising the possibility of an amplifying host with potential implications for public health, agriculture and food safety. Clues are beginning to emerge which suggest that self-duplication is the main mechanism by which the genome size has expanded. The three Sabin attenuated poliovirus vaccine strains very occasionally revert to neurovirulence, and we now know the sites on the viral genome of many of the CH035.indd 435 435 mutations that affect virulence (Table 35.6). This partly accounts for the variety of baculovirus genome sizes. This led to the realization that viruses could be engineered to bring foreign genes (transgenes) into cells, and that those genes could in some cases be stably integrated into the cellular genome, replacing defective host cell genes. The molecular pathways that lead to synthesis of early viral proteins from viral messenger RNAs depend on the chemical nature and strandedness of the genome. This occurs by cleavage of the DNA, removal of the region coding for the constant region of the heavy chain, and joining of the upstream segment coding for the variable region to another downstream constant region. DISTINCTIVE CHARACTERISTICS A subgenomic mRNA codes for virion structural proteins. These genes include cyclins, DNA polymerase alpha, thymidine kinase, DNA ligase, histones, and others. Genes 2–7 are expressed from subgenomic messenger RNAs. Where known, the names or function of gene products are shown above the genome map. KEY TERMS Antigenome Apoptosis Arthropod-borne -galactosidase Chloramphenicol acetyltransferase Cryoelectron microscopy Cytopathic Encephalitis Furin protease Green fluorescent protein Heparan sulfate Hippocampus Interferon Laminin receptor Methyltransferase Packaging signal Palmitoylation Persistent infection Polyprotein Receptor-mediated endocytosis Replicon Reporter gene RNA helicase RNAi Signal peptidase Subgenomic RNAs FUNDAMENTAL CONCEPTS • Most togaviruses are transmitted among warm-blooded hosts by mosquitoes. How do parvoviruses replicate the ends of their linear DNA genomes? Acyclovir triphosphate is an inhibitor of herpesvirus DNA polymerases. It remains unknown how RNA 3 and RNA 4 are packaged together into a single virion. The IgD molecule appears on the surface of immature and naïve B-lymphocytes simultaneously with IgM, and IgD may act as an antigen receptor. Reverse genetics of bunyaviruses For many years detailed molecular analysis of the replication of negative-strand RNA viruses was hampered because of our inability to manipulate their genome RNAs directly. ATP hydrolysis by terminase powers translocation of the DNA into the prohead. Its structure is shown in Figure CH036.indd 450 36.4. Like acyclovir, ganciclovir contains a guanine base linked to an acyclic sugar-like molecule, and lacks a 2' CH2 moiety. Cells derived from Burkitt's lymphomas also show the latency I gene expression pattern. Both reovirus 3 capsid protein and vaccinia virus SK-1 protein block PKR activation by binding strongly to double-stranded RNA in the infected cell. These findings demonstrate that reovirus-induced apoptosis requires functional NF-B, which presumably enhances the expression of genes that induce this cellular response to reovirus infection. How would blocking an ion channel lead to inhibition of uncoating? 157 Alphavirus vectors may be useful for treatment of neurological diseases Virologists who study alphaviruses have an interest in understanding the ability of these viruses to infect neurons. The entry of enveloped viruses into cells also triggers an antiviral response. Person-to-person transmission by physical contact with case patients or their secretions/excretions is the main route of infection in human outbreaks. From the trans-Golgi network, mature virions are packaged into vesicles that are targeted to the plasma membrane for release of progeny virus. Viruses evade complement by down-regulating, sequestering, or degrading critical complement components. The insertion of DNA requires energy derived from ATP hydrolysis by the packaging motor. This double-stranded DNA, called replicative form (RF) DNA, is synthesized during Stage I DNA replication (Figure 6.3), using only host cell proteins. Do not encode proteins. Ribosomes translate the first 191 codons and continue translation of this mRNA in the same reading frame downstream of the splice junction. cores also contain a spectacular array of virus-coded enzymes required for early mRNA synthesis and modification. STEPS IN THE VIRUS REPLICATION CYCLE 1. Polymerase-DNA transfers to 3' DR1 site ε Cap ε 2 1 1 poly(A) 4. 19/05/11 12:19 PM Baculoviruses The virion envelope has a trilaminar structure typical of lipoprotein unit membranes. There are still about 40 million cases worldwide per year resulting in nearly 1 million deaths. Re co mb ina tio n 86 Q S R Rz bor cos sib attP ori cos Figure 8.2 The genetic map of DNA. Hematopoiesis: the formation of the cellular components of the blood from stem cells found within the bone marrow. CH024.indd 290 16/05/11 8:12 PM Herpes Simplex Virus Immediate early () genes regulate expression of other herpesvirus genes A major function of the genes is to set up a regulatory cascade that leads to properly timed expression of other viral genes. The recruited immune cells limit virus replication and stimulate the adaptive immune response, which helps to clear virus and protects against a future infection by the same virus. Mx (for "myxovirus") proteins are interferon-inducible, 70- to 80-kDa proteins with the ability to hydrolyze GTP. These toroidal coils distribute themselves around the DNA molecule after removal of histones (Figure 21.3). A quick review The events of the lytic/lysogenic decision may be summarized as follows (see Tables 8.2 and 8.3). Anaphase: phase of mitosis during which the chromosomes begin to migrate to opposite ends of the cell. Core proteins readily form dimers, and interactions between dimer pairs produce the icosahedral structure of the nucleocapsid. Interferon has an important effect on Th1 cell development and plays a dual role in this process. Adapted from Fig. Aligned head to tail, these phages would therefore cover the following distance: 1031 0.2 m 0.2 1025 meters 2 1024 meters ꞡ 2 ∂ 1021 kilometers. This step is reminiscent of the transfer of the retrovirus "minus strong-stop" DNA to the repeat region at the 3⬘ end of the retrovirus genome RNA. This movement is long-distance movement, which transports the virus from the initially infected tissue to remote parts of the plant. However, adenovirus vectors lacking E4 are retained less stably in transduced cells and produce lower amounts of transgene protein. In order to stimulate the immune response, these vaccines are often mixed with adjuvants such as CH035.indd 436 aluminum hydroxide or aluminum phosphate salts. Robert Sinsheimer began detailed investigations in the late 1950s. Some viruses can form capsids in the absence of the viral genome. This progressive neurodegenerative disorder is caused by the death of neurons in the substantia nigra region of the brain. DISTINCTIVE CHARACTERISTICS Reovirus family has members that infect a broad range of hosts from fungi to humans. Rb is considered a tumor suppressor protein since loss of the Rb genes is associated with the development of different forms of cancers, most notably retinoblastoma, a childhood tumor of the eye. Rev interacts with a sequence on viral RNA that is named the Revresponsive element, or RRE. C-terminal cytoplasmic domain binds to capsid during assembly. CH023.indd 275 16/05/11 8:11 PM 276 CHAPTER 23 Adenoviruses of the T 25 icosahedral capsid. A schematic diagram showing the HA1 (blue) and HA2 (green) fragments created by proteolytic cleavage of the full-length polypeptide (HA0) shown inserted in the plasma membrane or the virus envelope at neutral pH. It is known that RNA polymerase I, used by the cell to transcribe ribosomal RNA genes, does not interact with the cellular factors used for cleavage and polyadenylation of RNA polymerase II transcripts. No typical symmetry elements. Rachel, and R. Viral genes are organized as two families, called early and late genes, and each gene family is expressed from a single transcription unit. Subsequently a smaller outbreak of Ebola disease occurred in Sudan in 1979. These stimulate the activated B cell to divide and differentiate into plasma cells, which are large cells with enhanced protein-synthesizing and processing machinery that produce prodigious amounts of antibodies with the same specificity as the original B-cell receptor. These characteristics may reflect the fragility of single-stranded DNA compared with double-stranded DNA; small size and circularity or hairpin ends ensure that the genome DNA cannot be easily attacked by nucleases. Th cells express an MHC II recognition protein called CD4 on their surface, while Tc cells express an MHC I recognition protein called CD8. This structural information implies that one copy of each of the eight different nucleocapsids is incorporated into a bundle either before or during virus budding. This has raised fears of wider epidemics due to these emerging agents. The virus ravaged native populations in North and South America when introduced by European explorers and colonists. Increased levels of algae will fix increased amounts of CO2 and have a buffering effect on the concentrations of this "greenhouse gas". Extension from this 3' end (step 2) copies the sequence of the melted hairpin to its 5' end, approximately 100 nt. Measles virus infection can lead to strong immunosuppression of the host; infection CH015.indd 179 of cells in the immune system by the virus may play an important role in measles pathogenesis. For example, many antibiotics are directed against bacterial cell wall synthesis or bacterial protein synthesis, which are clearly distinct from their human counterparts. Expression of coccolithovirus genes is temporally regulated Microarray analysis of transcribed genes in infected cells showed that a set of genes located between kb 200 and 300 on the genome are specifically transcribed at very early times (within 1 h) after infection. One is CD46, a complement-binding protein found on most human cell types. A variety of stimuli induce the degradation of IB, resulting 16/05/11 8:15 PM 360 CHAPTER 29 Human Immunodeficiency Virus in the transport of NFB into the nucleus where it can activate transcription. For this reason, these retroviruses can only productively infect cells that undergo mitosis. The second is a transfer plasmid that contains the left-hand end of the genome, the transgene cassette, and a homologous sequence to the right of the transgene cassette that allows for recombination to occur. CH005.indd 62 16/05/11 7:54 PM Single-Stranded RNA Bacteriophages 63 Coat termination 57 U C A U C U A U A U•G C G C G G U A U A AA AC C A U A UC U G C G G C UU C G C A GC A C G C C G AU GCGUC U G C C U A G C G A G A 70 A A 1727 U A G A C G C C A U GA A A AC G C U U G CC A A C UA GG G UA C A U G U C U elongating ribosome AUG Replicase start starting ribosome 43 AUG 1335 Coat start Figure 5.6 Activation of replicase translation. The infectious agent is transmitted following contact between a wounded uninfected plant and an infected plant, a contaminated agricultural tool, or a carrier insect. The presense of satellite RNAs can affect the replication of the corresponding helper virus and modulate disease symptoms caused by the virus. Binding to integrins leads to the migration of virus-receptor complexes to clathrin-coated pits, where they are taken up into endosomes (see Chapter 4). Further transcription of the cDNAs provides antigenome RNAs that are encapsidated during transcription by newly synthesized N protein, and these RNAs can then be replicated by the bunyavirus L polymerase. • Herpes simplex viruses establish latent infections in neurons, where they are transcriptionally silent except for some RNAs that represent unusually stable introns. (a) Distribution of 60 protein subunits in the shell of a parvovirus capsid. Richard Mulligan and Paul Berg expressed rabbit -globin in monkey cells in 1979 using the SV40 genome as a vector. The mRNA sequences at the editing site, and their products of translation, are shown below. Arrows indicate direction of transcription and location of polyadenylation sites. Some large DNA viruses could have evolved from degenerate intracellular parasites. Diameter 120–160 nm. The procedure excited a limited cowpox disease on Phipps's arm, but otherwise "the indisposition attending it was barely perceptible". This implies that there are DNA recognition sites on the inside of the parvovirus capsid, and these are probably involved in packaging the viral DNA. Genome segment 7 codes for the 16/05/11 8:07 PM Influenza Viruses 213 Hemagglutinin (HA) Neuraminidase (NA) Nucleocapsid protein (NP) Ion channel (M2) RNA polymerase complex (PA, PB1, PB2) Matrix protein (M1) NS2 protein Figure 18.3 Schematic diagram of influenza virus virion. The cellular proteinases responsible for processing include signal peptidase and furin. Explain the concept of "plasmid replication". Military use only. Shown is a typical transgene with two exons. Reverse genetics: The process of engineering specific genetic changes in the genome of an organism and then looking for resulting phenotypic changes. When they are transcribing concatemers, RNA polymerases therefore pass through the concatemer junction. Caveolae: small flask-shaped invaginations of the plasma membrane that are enriched in cholesterol, glycosphingolipids, and the protein caveolin. The cellular control protein p53 was first discovered as a 53-KDa protein present in immunoprecipitates of simian virus 40 large T antigen. Late: shuttles between nucleus and cytoplasm to transport viral mRNAs. ICP47 CH024.indd 291 Binds to TAP1/TAP2 transporter, blocking presentation of antigenic peptides to immune system via MHC class I protein. N- and C-termini are shown. which viral glycoproteins have been inserted. In some cases, the termini are masked by hydrophobic ligands, precluding exonucleolytic degradation from either 3' or 5' ends. Three virus-encoded proteins in the preintegration complex facilitate this transport: MA, Vpr, and IN. Derepression of late transcription by dilution of a repressor. There have been several reports of joggers contracting hepatitis B from contaminated brambles or thorns on their running paths. Complex lysogenic and lytic regulatory circuits. Reovirus serotype 3 virions are likely to bind first to sialic acid residues on cell-surface glycoproteins and glycolipids. A number of veterinary diseases of economic importance are caused by coronaviruses. This chapter presents an overview of the different virus groups that have been recognized and named. Antigenic shift results from reassortment of influenza virus genes during mixed infections with two or more virus subtypes. Editing introduces an additional A residue (shown in orange) that shifts the reading frame to that of GP. Herpesvirus capsids and adenovirus capsids have been shown to interact with dynein in the cytoplasm, and use it for retrograde transport. It has been estimated that 20% of the microbes in the Earth's oceans are destroyed each day by virus infections. These replication complexes are often associated with cellular membranes. Since cellular mRNAs are capped in the nucleus, cytoplasmic RNA viruses must produce their own capping enzymes (for details, see Chapters 19 and 26). Proteoglycan: extracellular macromolecule composed of large negatively charged polysaccharide chains and protein. KEY TERMS RNA world Satellite nucleic acid Satellite virus Species Taxonomy Viroid FUNDAMENTAL CONCEPTS • Viruses are classified into families, genera, and species based on characteristics of their genomes, capsids, and replication strategies. The amino acids known to be involved in aphid transmission are found in the E-F, F-G, and H-I loops. The hosts of gokushoviruses are obligate intracellular parasitic bacteria, some of which (chlamydia) multiply within eukaryotic cells. How does phage MS2 ensure that only a limited number of copies of the maturation protein are produced? The information that determines their three-dimensional structures is built into the way in which their protein subunits bind to each other and to the packaged RNA or DNA. The NF-B heterodimer is bound in the cytoplasm to an inhibitor, IB␣; when this inhibitor is phosphorylated by IKK, it releases NF-B, which then translocates to the nucleus. Beneath the canyon floor is the drug-binding site—a "pocket" in the VP1 capsid protein. In the endoplasmic reticulum, PE2 spans the membrane twice at its carboxy terminus; the 6K protein spans the membrane at both its amino and carboxy termini; and E1 spans the membrane once at its carboxy terminus (Figure 13.4). 15.2b: Courtesy of Professor Rob Ruigrok, Université Joseph Fournier,

Grenoble, France; Fig. At the cellular level, studies show that lysogenization requires that a cell be infected by more than one phage, and that the likelihood of lysogenization decreases with increasing cell size. GENOME Linear, double-stranded DNA, 125–250 kb. • In contrast, the Cro repressor, made from the initial transcript starting at P R, suppresses synthesis of the CI transcript when it has reached high concentrations, allowing lytic growth. Most viruses are too small to be detected by light microscopy, but can be readily seen with the electron microscope. (a) (b) Figure 2.3 A virus particle with icosahedral symmetry. 05/05/11 2:45 PM Glossary Furin: a host cell endopeptidase, localized in the trans-Golgi compartment of the cell's secretory pathway, that cleaves proteins at paired arginyl residues. Our company is built on a foundation of principles that include responsibility to the communities we serve and where we live and work. Alphavirus vectors have also been engineered to express proteins of unrelated viruses, with the prospect of developing them as vaccines. Ligation. In this model, only one of the leading DNA strands is extended, as the DNA polymerase copies the remaining intact circular template strand. 273 SEC-VI.indd 273 12/05/11 5:04 PM C H A P T E R 2 3 Adenoviruses Philip Branton Richard C. Subsequently, viral cDNAs were put under the control of a eukaryotic promoter, enabling production of viral RNA by transcription of DNA introduced into the nucleus of a eukaryotic cell by transfection. Phagosomes can deliver viral components to endosomes, where they are recognized by toll-like receptors. • Some baculoviruses are used as effective biological pest control agents, both in agriculture and forestry. The Asian (H2N2) and the Hong Kong (H3N2) viruses are reassortant viruses. Multicellular organisms encode hundreds of different transcription factors. COOH UGAAUAA B mRNA 2900 minichromosome. Study of bacterial viruses (bacteriophages) by the "phage group" led to understanding of the nature of genes and helped found molecular biology. These were the first double-stranded RNA viruses to be described. As late as the 1880s, pioneering microbiologists were restricted to growing "germs" either in whole animals or on potato slices and in jars containing broth. This long-distance interaction effectively prevents ribosome binding. In the first phase of intensive molecular sophistication, it is sometimes hard to believe that products developed half a century ago are still in use. By making reassortant viruses between virulent and nonvirulent bunyaviruses, it was shown that the M segment (and hence the proteins coded by M) controls virulence. In a second ancient lineage, the "double barrel trimer" fold is found in the major capsid protein of viruses infecting hosts from all three kingdoms: bacteria (phage PRD1), eukaryotes (adenovirus), and archaea (Sulfolobus phage STIV). These recognition elements are located at the 5′-termini of RNAs 1, 2, and 3 and in the intergenic region of RNA 3, which also interacts with the 5′-terminal region of RNA 3. A narrow aqueous channel is formed between 16/05/11 7:52 PM Virus Entry 1 2 3 51 4 H⊕ 5 6 Figure 4.4 A model for influenza virus hemagglutinin-mediated membrane fusion. (a) (b) Putative circularized Q-like form obtained by pairing the two inverted repeats. Because ribosomes translate the (positive-strand) genome RNA in the 5′-3′ direction, and RNA polymerases proceed in the opposite direction while synthesizing progeny negative strands, initiation of RNA synthesis during translation of the same RNA molecule could lead to collisions between translating ribosomes and transcribing RNA polymerases (see Figure 5.4). The 3′-terminal regions of cucumber mosaic virus genome segments can fold to form a transfer RNA-like structure All genome and subgenomic RNAs of cucumber mosaic virus and its relatives contain a conserved noncoding region at their 3′-termini, which is about 300 nucleotides in length and can fold to form a tRNA-like "cloverleaf " or pseudoknot structure (Figure 10.5). Studying the interactions of viral and cellular proteins has helped scientists understand a lot about the cell cycle and signaling pathways. Gene segments are classified as large (L), medium (M), or small (S) based on their relative electrophoretic mobilities. In contrast, infection of B-lymphocytes leads to a long-term, latent infection, with expression of a limited class of latency genes, and little or no production of progeny virus. Among the many agents that cause hepatitis are a number of unrelated viruses. A striking finding was made when knockout mice lacking the PrP gene were generated: these mice are resistant to prion disease caused by inoculation with PrPSc from infected mice, and cannot propagate infectious prions. The N-terminal region of large T antigen, called the DNA J domain, is also required for gene activation via the Rb pathway. What remains is an RNA-free particle, the "B-particle". lucimarinus, have genomes of 12.6 and 13.2 Mb, respectively. Ribosome shunting has parallels to initiation of protein synthesis at "internal ribosome entry sites" (IRES elements) of picornaviruses and flaviviruses (see Chapters 11 and 12). ⩽ ssRNA Viral RNA-dependent DNA polymerase (reverse transcriptase) Figure 1.7 Baltimore classification of viruses. Translated codon triplets are shown in capitals in bold type; nucleotides that lie beyond a translation termination signal are shown in small, unbolded type. The machinery that spools the DNA of herpesviruses and most double-stranded DNA bacteriophages into the procapsid recognizes specific packaging sequences on viral DNA, and often involves cleavage of the DNA genome from a larger replicative intermediate (see Chapters 6, 8, and 24). Because of this, they are presumed to be evolutionarily related. Transformed cells integrate viral DNA into the cell chromosome Only 1 to 10 out of every 100,000 infected nonpermissive cells end up being transformed; these can be detected as dense, rapidly growing colonies on a monolayer of normal fibroblasts growing on the surface of a Petri dish. Interferons are major players in host defense against most viruses, and are discussed in detail below. GENOME Linear, single-stranded RNA, positive sense. Cleavages carried out by cellular signal peptidase are shown as orange arrows. Coinfection of cells with different viral strains produces a collection of progeny reassortant viruses containing various combinations of gene segments from each parent. The host GroES and GroEL chaperone complex assists in the formation of the portal vertex. However, their use as antibacterial agents in humans is not accepted in most countries; there are significant unsolved problems, not the least of which is the induction of immune reactions to the bacteriophage. The carboxyterminal signal sequence of the precursor to the C protein anchors that protein to the membrane (see Figure 12.5); this sequence penetrates into the lumen of the endoplasmic reticulum or smooth vesicles. Cholera toxin B subunit Elicits strong humoral response without toxicity. Roesch, B. This protein acts as a competitive inhibitor of bacterial type I restriction enzymes by mimicking their DNA substrate. Nucleotide numbers for the A2 strain of mouse polyomavirus are shown inside the circular map. Activation or induction of new transcription factors: Clinical data show that treatment with Gendicine can both extend and improve the quality of the patient's life. Once in the nucleus, NF-B, IRF-3, and IRF-7 act as transcription factors and induce the expression of interferons, other cytokines, and interferon-stimulated genes (ISGs). Vaccinia virus, a large, enveloped DNA virus of the poxvirus family, triggers a complex signaling cascade that induces transient ruffling of the plasma membrane followed by the internalization of fluid and virus particles in large vacuoles called macropinosomes. These two viruses are serologically and morphologically identical. These particles resemble authentic filovirus particles and can enter susceptible cells, but will replicate only if filovirus nucleocapsid and envelope proteins are expressed by plasmid transfection in the same cells. The remaining portions of the polyproteins are processed by the nsp5 proteinase, producing nsp4 through nsp16 in all coronaviruses. 2.1b: Courtesy of Jean-Yves Sgro, University of Wisconsin, Madison; Fig. After internalization, the virus particle is still separated from the cytosol by the barrier of the endosomal membrane, which it can overcome by the same strategies that other viruses use at the plasma membrane (see below). This handsome capsid contains a total of 11 viral proteins, but is CORE Figure 23.1 Adenovirus virion. These correspond to the eight viral genome RNAs assembled in nucleocapsids. All except one have double-stranded DNA genomes, and most archaeal viral genes have no counterparts in the database of sequenced genes from any of the three domains of life. Evidence for this model comes from studies with capsid protein mutants in which preformed nucleocapsids are not detected in the cytoplasm of infected cells, but intact enveloped virions are still able to bud from the plasma membrane. 16/05/11 8:07 PM 224 CHAPTER 18 Influenza Viruses REVIEW QUESTIONS 1. However, cells have a variety of defense mechanisms that can interfere with many steps in virus infection. et al., Journal of Virology 7 (1997): 1162–1172. The uridylylated vpg primer is then translocated to the 3′ end of negative-strand templates for initiation of positive-strand RNA synthesis. At the tip of the tail (Figure 8.3) is a single fiber, the J protein. The precise nature of the systemic signal is unknown. Ras: a cellular signaling protein that is activated by receptor tyrosine kinases and in turn activates the MAP kinase BGLOSS.indd 480 pathway, which itself induces transcription of cell-cycle and other genes. A recent problem (2004–2005) with the campaign for the eradication of polio is a great example of an unexpected "adverse event." What was the adverse event? When the complex between envelope proteins, matrix protein, and nucleocapsids is formed, virions are made by budding from the plasma membrane (for a diagram showing budding, see Figure 2.11). Material between the capsid and the envelope is called "tegument". Immunoglobulin family: a family of membrane proteins that have characteristic repeated domains usually stabilized by disulfide bonds, first described in immunoglobulins. Virus vectors have been designed for four major objectives: (1) the production of specific proteins in cultured cells; (2) vaccination of animals or humans against specific diseases; (3) gene therapy in animals or humans, usually to replace deficient genes; and (4) anticancer agents that specifically kill tumor cells. These additional amino acids are primarily hydrophilic and are believed to reside on the external face of hepatitis B virions. Filoviruses belong to the so-called "exotic viral pathogens" and are prime examples of (re) emerging infectious agents. 05/05/11 2:45 PM Glossary Transcriptional enhancer: a region that contains consensus binding sequences for transcription factors and that enhances the transcription of specific genes. Vaccination of domestic animals and control of infections in wild animals have reduced the incidence of human rabies infections in developed countries; only about one case per year now occurs in North America. Normally, single gravious virions are occluded within small occlusion bodies called granules. In animal models of myocarditis, RNA sequences in the 5′ noncoding regions of coxsackie B virus genomes have been shown to be involved in virulence, again implicating specific cellular factors in translation or replication of viral RNA. All coronavirus replicase proteins tested can be found in membrane-associated complexes in the cytoplasm. Deletion of the domain of the tobacco mosaic virus movement protein that interacts with pectin methylesterase results in a loss of cell-to-cell movement. Viruses with negative-strand RNA genomes have helical nucleocapsids; some have fragmented genomes Some of the most widespread and/or deadly human diseases (measles, influenza, rabies, Ebola hemorrhagic fever, Lassa fever) are caused by viruses with negativestrand RNA genomes (Table 3.4). A number of viruses have been adapted for use as vectors. This poses a problem for positive-strand RNA viruses such as the picornaviruses. It is released from Sulfolobus host cells with the help of virus-induced pyramidal structures, as described for the rudivirus SIRV-2 (Figure 9.10). CH017.indd 207 This suggests that only some genetic variants of the virus are virulent for humans. The binding of 3CD to this cloverleaf stem-loop structure is facilitated by the binding of a cellular protein, poly r(C) binding protein 2 (PCBP2). About 1% of those infected suffer from a severe disease known as fulminant hepatitis, which results in massive levels of liver damage and often is fatal. Simple polypeptides could have been assembled by such adaptor RNAs on templates of self-replicating RNAs, and those polypeptides that helped to protect the RNAs and increase their rate of replication would have enhanced selection and further multiplication of their template RNAs. Thus a self-replicating RNA linked to a primitive proteinsynthesizing system developed. The type member of the flavivirus genus, yellow fever virus, will be used as an example throughout this chapter due to its historical importance and the wealth of information available on its biology. Comparison of the nucleotide sequences of the virulent Asibi strain and the 17D vaccine strain derived from it provided insight into the determinants of attenuation. Additional protein modifications carried out during vesicle transport to the cell surface include cleavage by furin protease (arrow with white circle) in a trans-Golgi compartment to form E2 and E3, as well as glycosylation. The repressor is the CI protein (pronounced "see-one"), product of gene cI. However, both strands of this relaxed molecule are covalently closed 16/05/11 8:09 PM 250 CHAPTER 21 Polyomaviruses and Stra vage a cle Remove histones Top DNA ligase oiso Add histones mer DN Ag ase I yras e Minichromosome Supercoiled DNA Relaxed DNA Figure 21.3 Gymnastics of circular DNA molecules. DISTINCTIVE CHARACTERISTICS Genomes contain extensive overlapping reading frames. The major transcripts are illustrated below. RNA synthesis is primed by VPg covalently bound to uridine residues The proteins that actually direct viral RNA synthesis are derived from P3. A circular, double-stranded DNA molecule is shown as a minichromosome, bound to nucleosomes (left). Another way of saying this is that viruses are obligatory intracellular parasites. VPg (protein 3B) linked to U residues acts as a primer for the 3D RNA polymerase, and is rapidly cleaved from protein 3A (scissors) or some other 3B-containing precursor polypeptide. Following host cell wall degradation, the internal membrane of the virion fuses with the host cell membrane, resulting in entry of the viral DNA and virion-associated proteins into the cytoplasm. Each transcription unit has one 16/05/11 8:09 PM Polyomaviruses 251 Intergenic region PE PL ST Ag VP 2 TAg T 1/5292 5000 MTA g VP 3 1000 4000 1 3000 VP 2000 LT Ag Polyadenylation signals Figure 21.4 Map of mouse polyomavirus genome. Paramyxoviruses are discussed in Chapter 15. Genetic relatedness is used to classify viruses. coli. Receptors also bind to virions, but they play a more active role by inducing conformational changes in the particles, triggering cell signaling pathways, or mediating fusion. Prohead: term used frequently to designate procapsids of bacteriophages. Finally, recent fears that smallpox might be used as a biological weapon have spawned renewed interest in development of both improved vaccines and antipoxvirus drugs. Many vaccines are susceptible to both aggregation and degradation at high temperatures. When expressed alone, E4orf4 kills a wide range of cancer cells, but it has little effect on normal human cells. PVR: poliovirus receptor; CAR: Coxsackie virus and adenovirus receptor; LDL: low density lipoprotein. Hairpin structures, which result from the presence of inverted repeat sequences in viral RNAs, can also form. What is Dicer and how does it contribute to antiviral defense? Overlapping reading frames allow efficient use of a small genome Seven of the eleven ϕX174 genes (genes A through E and gene K) lie in a region of overlapping reading frames. CH006.indd 70 reading frames that covers nearly one-half the genome (Figure 6.1). The amplified RNA can be detected by gel electrophoresis and ethidium bromide staining. These results are consistent with the prion hypothesis: pre-existing PrPC is required to propagate infectious prions and to cause prion disease. Model of poliovirus type I based on x-ray crystallography. Hepatitis delta virus is a blood-borne pathogen that replicates in the liver. Nicholas H. The Gag and Gag/Pol proteins interact with each other to initiate assembly of the virus core. The class II and class III primary transcripts are also cleaved at specific sites by ribonuclease III to form smaller functional mRNAs. The stem structures that remain at the 3′ ends of the mature T7 mRNAs after cleavage render them relatively resistant to degradation by other host nucleases, making the phage mRNAs substantially more stable and long-lived than most host cell mRNAs. Class III gene expression is regulated by the promoter strength As the phage DNA enters the cell via transcriptional winching, the five class III promoters become available for transcription by T7 RNA polymerase. Influenza vaccine production depended on a single technology: the creation of reassortant vaccine-strain virus (see Chapter 18), and CH035.indd 431 431 Measles Mumps Polio (Sabin) Rotavirus (human-recombinant) Rubella (german measles) Varicella (chickenpox) Yellow fever Whole inactivated viruses Hepatitis A Influenza A Influenza B Polio (Salk) Rabies Tick-borne encephalitis Subunit vaccine Hepatitis B surface antigen Virus-like particles Human papillomavirus Chimeric virus Rotavirus (human-bovine) a b Smallpox eradicated (supply of vaccine tenuous). However, the live yellow fever vaccine is expensive to manufacture and are relatively unstable, requiring careful attention to refrigeration via the cold chain, a problem in developing countries. This is because the chance of encountering a block to transcription should increase as a function of the distance between the promoter and the end of the mRNA. Scarred Egyptian mummies provide evidence for the existence of smallpox as early as the second millennium B.C. Written records suggest that smallpox was endemic in regions of Egypt and India by the first century, from where it spread throughout China, southwestern Asia, Europe, and northern Africa by the 312 CH026.indd 312 16/05/11 8:13 PM Poxviruses tenth century A.D. European exploration and colonization in the sixteenth, seventeenth, and eighteenth centuries brought the disease to North and South America, southern Africa, and Australia, so that by the twentieth century, smallpox was common in virtually all densely populated areas worldwide. Like small and middle T antigens, large T antigen interacts with a number of cellular proteins that control cell metabolism and the cell cycle. When this stem-loop structure was disrupted by mutation, transcription of Ebola virus minigenomes was no longer dependent on VP30 expression. In 1982, Fred Sanger and coworkers published the sequence of the 48,502 bp DNA molecule. LamB is a maltoseinducible porin for transport of maltose and maltose oligosaccharides. Most herpesviruses can establish latent infections One of the most intriguing characteristics of herpesviruses is their ability to establish latent infections. The 7 kb genome codes for 9 proteins. Outbreaks of virus diseases in domesticated animals can lead to destruction of thousands or millions of animals to avoid even more widespread epidemics. The overlap of control sequences and coding sequences in a 400-nt stretch of the genome is shown in the inset. The rash is extremely useful in differential diagnosis. This concept is illustrated in Figure 2.5a (compare with Figure 2.2c). Nonstructural proteins are translated from genome RNA. Virions enter cells in caveolae and are transported to the nucleus Both mouse polyomavirus and simian virus 40 bind to sialic acid-containing glycoproteins and gangliosides on the cell surface and are subsequently internalized in caveolae, which are specialized vesicles formed at the plasma membrane. Human cervical cancers caused by papillomaviruses do not have mutated p53 like most human cancers; how do papillomaviruses target p53? Images are colored red at smaller diameters through yellow and green to blue at greater diameters. Elongation by RNA polymerase II is stimulated by increasing the extent of phosphorylation of the carboxy-terminal domain (CTD) of RNA polymerase II. 26.5: Courtesy of Richard Condit. Important veterinary diseases: severe endemic and epidemic respiratory, hepatic, gastrointestinal, and neurological diseases in mammalian and avian species. Continuous instead of batch processing led to a greater risk of cross-contamination between feeds from different species. There are nine known immunoglobulin isotypes, each presumably with a different function in the immune response. into tiny droplets in respiratory aerosols; therefore these may be responsible for person-to-person transmission. The host immune response is mediated by circulating specialized cell types Innate immune responses are rapid but non-specific; adaptive immune responses are slower but long-lasting and highly specific All complex multicellular organisms have evolved a broad spectrum of defenses against infection by viruses and other microorganisms. Another endocytic mechanism used by viruses is called macropinocytosis. However, in resting T cells the preintegration complex remains in the cytoplasm. Insertion of a provirus DNA into this region of a gene can result in the use of the polyadenylation signal within the lefthand LTR, generating an mRNA missing its normal 3′ untranslated region. Taken together, this information suggests that heterozygosity at position 129 protects against both inherited and infectious prion disease. Based on the genome sequence of SARS coronavirus and what is known about other human coronaviruses, it seemed likely that SARS coronavirus originated as an animal virus. This sequence motif (positions 17–21) is the only conserved region shared with the similarly functioning M2 protein of influenza B virus; it is also found in the p7 proteins of some genotypes of hepatitis C virus (a flavivirus). Surprisingly, many of the most serious cases of illness, and many deaths, occurred in young, healthy people, unlike the "seasonal" flu, which mostly affects older people. Phage-coded DNA polymerases. Cervical warts and precancerous tumors are routinely removed by surgery or physically destroyed by freezing with liquid nitrogen or laser vaporization. Vesicle transport: the transfer of vesicular membranes and their contents between two membrane compartments by coupled membrane fission and fusion. Production of viruses, virus-like particles, or viral proteins in plants. RETROVIRUS VECTORS Retrovirus vectors incorporate transgenes into the cell chromosome Retroviruses (Chapter 28) use reverse transcriptase to generate a double-stranded DNA copy of their RNA genome and subsequently integrate this DNA into the cellular chromosome. The AAV transcriptional promoters were modified to function optimally in insect cells. Therefore these viroids probably replicate in chloroplasts using the chloroplast RNA polymerase. This theory is not easy to prove or disprove. This vector is now being evaluated in patients with Parkinson's disease in a Phase I/II clinical trial. (a) In the absence of Tat, RNA polymerase II molecules that initiate transcription at the HIV-1 promoter lack processivity and most are released from the template before reaching the polyadenylation site. Some of the sequences within the LTR responsible for promoter silencing have been characterized and can be deleted to correct the problem. 1985 Hepatitis B surface antigen gene cloned and expressed. for activation of interleukin-1. Likewise, the adenovirus hexon is a defined trimer subassembly. Carboxylic ionophores, such as monensin and nigericin, are also effective in breaking down proton gradients, as are inhibitors of the vacuolar proton ATPase such as bafilomycin A1. There is no polyadenylation signal on the antigenome RNA, allowing multigenome-length transcripts to accumulate by rolling-circle replication. Parvoviruses (diameter of 20 nm) are also thought to enter the nucleus in this fashion (Figure 4.5e–g). Viroid RNAs could, by virtue of their sequence or structure, act in the place of certain cellular RNAs. Homologies have been observed between potato spindle tuber viroid and a group I intron, as well as U3B RNA (involved in RNA splicing). In addition, coronaviruses infecting many animals such as giraffes, cheetahs, and Beluga whales have also been identified. However, like other negative-strand RNA viruses, influenza virus genomes remain associated with both sequence elements, aligned on one side of the nucleocapsid, to initiate transcription. 22.1a–b: Courtesy of Norman H. Interestingly, lentivirus vectors are also less prone to silencing. The specific interaction often involves an RNA sequence element containing a stem-loop structure (the folded packaging sequence). This is reminiscent of the conformational changes that take place in the fusion proteins of enveloped viruses like influenza and HIV-1 upon receptor binding (Chapter 4). More effective vaccines and antiviral agents are currently being developed by a number of laboratories and pharmaceutical companies. Pseudotyping: production of a recombinant virus (pseudotype) that carries a foreign receptor-binding protein, allowing the virus to bind to and infect different cell types or species. In an experimental system, the level of variation in evolved populations of cucumber mosaic virus is higher than that found with tobacco mosaic virus or cowpea chlorotic mottle virus, a related bromovirus, but varies with different virus–host combinations. Humans or domestic animals can be infected by coming in contact with members of this population, and they can suffer from disease, but do not necessarily transmit the agent efficiently to other members of their species. Most of the vertebrate poxviruses cause blistering skin diseases, while the insect poxviruses cause a generalized necrosis of infected larvae. DISEASES Humans: poliomyelitis (paralysis), myocarditis, hepatitis, meningitis, common cold. • Poxviruses replicate exclusively in the cytoplasm although they have DNA genomes. Other members of the genus Cucumovirus are peanut stunt virus and tomato aspermy virus. Most human and animal papillomavirus genomes contain 8 to 10 identifiable open reading frames. Other proteins (not shown) link together hexons and pentons. When the cell is exposed to stressful conditions such as virus infection or DNA damage, p53 becomes stabilized by phosphorylation, and its level increases. Translation of all four early mRNAs starts at an AUG at nt 175 (to the left). protein to at least two distinct cell surface proteins. People whose immune systems are impaired can suffer reactivation of cytomegalovirus or Kaposi's sarcoma virus with dire results. • Viral envelopes usually incorporate viral glycoproteins with a transmembrane anchor. Coinfection of cells with the two viruses results in synthesis of T7 RNA polymerase from the first virus, which can then transcribe the gene of choice from the T7 promoter in the second virus. ICP0 associates histone deacetylases from chromatin in ND10 structures, therefore relieving transcriptional silencing that occurs as a result of strong DNA binding by deacetylated histones. Other plant virus RNAs with a 3′-terminal tRNA-like structure are recognized by tRNA synthetases that covalently link them to histidine or valine residues. • Prion diseases are not usually transmitted among different species, but bovine spongiform encephalopathy ("mad cow disease") developed in Britain and apparently spread to humans. GENES AND PROTEINS 150–250 genes (vaccinia virus: 200). This results in a linear, double-stranded DNA with long terminal repeats at both ends. Introduction to Virology 2 Nicholas H. Consequently, T cells that would support an immune response against HIV-1 are exposed to virus while interacting with dendritic cells; these conditions enhance the ability of the virus to replicate by facilitating entry of proviral DNA into the nucleus, and eventually lead to productive infection and death of the T cells. Members of a virus family share overall genome 16/05/11 7:52 PM Major Virus Groups organization, virion structure, and replication mechanisms. • Nucleocapsids are packaged in an envelope derived from the plasma membrane of the cell. Virological synapse: a small region of contact established between neighboring cells that allows passage of enveloped viruses from one cell to the next without exposure to the extracellular milieu. The ligated MDV-1 RNA is then released and amplified by Q polymerase. ABV has the only genome besides those of the salterproviruses His-1 and His-2 that carries an identifiable DNA polymerase gene. Table 30.1 Comparison of reverse transcription by retroviruses and hepadnaviruses Step Retroviruses Hepadnaviruses Template for DNA synthesis Plus-strand virion RNA Plus-strand pregenome RNA Site of DNA synthesis Residual capsid after virus entry (cytoplasm) Newly assembled capsid before virion formation (cytoplasm) Primer for initiation of minus-strand DNA synthesis Cellular tRNA bound near 5⊕ end of genome RNA Polymerase protein bound near 5⊕ end of pregenome RNA First switch: to 3⊕ end of RNA template Via terminal repeat (R) sequence Via subterminal DR1 sequence RNA template degradation RNAse H activity in reverse transcriptase RNAse H activity in Polymerase Initiation of plus-strand-DNA synthesis RNAse H-resistant polypyrimidine tract in genome RNA RNAse H-resistant capped 5⊕ RNA fragment bound to DR2 sequence Second switch: to 3⊕ end of minus-strand DNA Via primer-binding site Via DR1 sequence Final product ds linear DNA with long terminal repeats Partly ss/ds circular DNA Fate of DNA Integrated into cellular chromosomal DNA Incorporated into virions CH030.indd 372 05/05/11 1:08 PM Hepadnaviruses Steps 4 and 5. Different strains of cucumber mosaic virus have been found worldwide and have caused several severe epidemic crop diseases in many regions of the world. The infection was horrific in its presentation. For many negative-strand RNA viruses, packaging occurs as the RNA chain is being synthesized. However, while phylogenetic analyses using sequence variations in the genes coding for other viral proteins, as well as noncoding regions in viral genomes, maintain the subgroup designations, they indicate CH010.indd 120 different evolutionary histories for each genome RNA and each gene. Penetration requires the viral DNA pilot protein, also called the DNA pilot protein. • Polyomavirus DNA becomes supercoiled upon removal of the histones, but remains in an open circular form within the cell as it is always associated with histones. Antibodies and memory lymphocytes leave via the efferent lymphatic vessel. Woese showed that there are three different classes of cellular organisms, each with a distinct set of ribosomal RNAs and ribosomes. This is unique to retroviruses, and it may be important in the reverse transcription step that makes a full-length DNA copy of the genome within the capsid once it has entered the cell (see Chapter 28). Many of the proteins produced are involved in protecting the cell from virus infection or activating the immune system. Infection with exogenous PrPSc would introduce a "seed" that initiates aggregation. Although hantaviruses can be present in large amounts in lungs and kidneys of infected rodents, they do not cause obvious CH017.indd 201 disease in the animal host. Each primary transcript from the major late promoter is cleaved at only one of these sites, and the resulting polyadenylated RNA is spliced in a complex fashion to generate one of the 18 possible late mRNAs. Three short exons in the first 4 kb of these transcripts are spliced together to form the noncoding 200-nt "tripartite leader". The first steps in the development of life on Earth: the RNA World Within perhaps 300 million years after the formation of the Earth, once the oceans had formed and cooled, macromolecules may have arisen by spontaneous 16/05/11 7:52 PM The Evolutionary Origin of Viruses 41 Table 3.7 Satellite viruses, satellite nucleic acids, and viroids Example Function of encoded protein Linear Capsid protein Virus group Hosts Satellite viruses Plants & Tobacco necrosis invertebrates satellite virus ss RNA 1–3 1–3 Single-stranded RNA satellites Plants Tomato leaf curl virus satellite DNA ss DNA 1 0.7–1.3 Circular Unknown function Double-stranded RNA satellites Fungi & protozoa M Satellites of saccharomyces cerevisiae L-A virus ds RNA 1 0.5–1.8 Linear Toxin Circular singlestranded RNA satellites Plants Tobacco ringspot virus satellite RNA ss RNA 1 0.35 Circular No encoded proteins Small linear single- Plants stranded RNA satellites Cucumber mosaic virus satellite RNA ss RNA 1 0.7 Linear No encoded proteins Linear Replication Circular Replication and packaging Large singlestranded RNA satellites Plants Bamboo mosaic virus satellite RNA ss RNA 1 0.8–1.5 Deltavirus Humans Hepatitis delta virus ss RNA 1 1.7 Viroids Plants Avocado sunblotch viroid ss RNA 1 polymerization of simple organic molecules such as ribonucleotides and amino acids. Surface shaded views of dengue virus type 2 as determined by cryoelectron microscopy and image reconstruction. It would be hard to imagine people lining up today to be inoculated with crudely purified pus from cowpox lesions or dried spinal cord from rabbits infected with rabies. Papillomaviruses are not easily grown in cell culture A long-standing problem in studying papillomaviruses has been the difficulty in propagating them in cultured cells in vitro. Repeated sequence elements are shown in small letters (a, b, c) and unique sequence elements are shown in capital letters (UL, US). This regulation occurs by the production of microRNAs, also known as miRNAs. These are similar in length to small interfering RNAs, but are produced by a different mechanism. The complex encounters the cytoplasmic tails of viral glycoproteins anchored in the plasma membrane, and the capsid proteins in the complex bind on a 1:1 basis to these tails. Over a 6-month period this CH014.indd 160 epidemic led to more than 8000 illnesses and nearly 800 deaths worldwide. In AcMNPV, these homologous regions (hrs, see Figure 25.3) consist of multiple copies of a 60 to 70 bp repeat carrying an imperfect inverted repeat sequence of 30 bp CH025.indd 307 307 Table 25.2 Early Autographa californica MNPV proteins involved in DNA replication Protein Mol. Enfuvirtide: a peptide that blocks a conformational change in HIV-1 gp41 necessary for fusion of the viral envelope with the cell membrane. Both simian virus 40 DNA and mouse polyomavirus DNA have 12 such sequences that lie within a 160-nt span in the intergenic region. 9.4a: Courtesy of Wolfram Zillig; Fig. CH006.indd 73 73 Summary of viral DNA replication mechanisms Figures 6.3 and 6.4 illustrate the contrasting mechanisms used for plus- and minus-strand synthesis. For positive-strand RNA viruses, mutagenesis requires a reverse genetic approach, in which the RNA genome is cloned as a complementary DNA (cDNA) in a plasmid vector. Phycodnavirology: a field in its infancy Reports of probable viruses infecting at least 44 different species of eukaryotic algae have appeared since the early 1970s. This has been experimentally demonstrated for murine hepatitis virus. In the "double subgenomic" alphavirus gene expression vector (Figure 13.5b), coding regions for the structural proteins are retained and a second subgenomic RNA promoter is inserted into the genome, allowing the synthesis of both the viral structural proteins and CH013.indd 156 foreign protein, each on different subgenomic RNAs. This type of vector produces a viable virus that can spread from cell to cell or from host to host. For phagocytic cells to "see" a foreign organism they must recognize specific molecules. It is 50 nm in diameter compared with 70 nm for togavirus particles, and togaviruses have a T 4 icosahedral structure, implying 240 subunits. Three human type III interferon genes are clustered on chromosome 19: interferon-1, interferon-2, and interferon-3, also known as interleukin-29, interleukin-28A, and interleukin-28B, respectively. Two forms of vaccinia virions have different roles in spreading infection Vaccinia virions are large (310 240 140 nm), enveloped, ovoid or brick-shaped particles (Figure 26.2a) that CH026.indd 315 containing as many as 100 proteins. The most consistent nomenclature for the replicase protein domains is "nonstructural protein" (nsp), although possible roles these proteins might play in virion structure have not been ruled out. • Other inhibitors of HIV reverse transcriptase are not nucleoside analogues, but bind near the active site of the enzyme. It has 05/05/11 1:08 PM 370 CHAPTER 30 Hepadnaviruses are implicated in the development of hepatocellular carcinoma (liver cancer). Because this activity is missing, between 1 and 10 nucleotide errors are introduced during synthesis of each proviral DNA molecule. W., 303 Charny, Patrick, 366 Chase, Martha, 7–8 Condamine, Conte de la, 313 Cornalia, Emilio, 302 Crick, Francis, 8 d'Herelle, Felix, 7, 8, 69 Darwin, Charles, 9, 357 Daubenton, Baron, 302 Davidson, Louis, 302, Dawkins, Richard, 394 Delbruck, Max, 7, 77 Doolittle, Sears, 113 Dulbecco, Renato, 7 Eddy, Bernice, 247 Edmonston, David, 176 Ellis, Emory, 7 Enders, John, 7, 126, 176, 430, 431 Epstein, Anthony, 297 Fane, Bentley, 70 Fields, Bernard, 226 Finlay, Carlos, 7 Franklin, Richard, 127 Frosch, Paul, 7, 430 Fuller, Buckminster, 24 Hausmann, Rudolf, 78 Hayashi, Masaki, 71 Hendrix, Roger, 94 Henle, Gertrude, 297 Henle, Werner, 297 Hershey, Alfred, 7, 8, 86 Hilleman, Maurice, 274 Human, Mary, 85 Incardona, Nino, 70 Isaacs, Alick, 7, 405 Ivanovski, Dimitrii, 7, 71 Jagger, Ivan, 113 Jenner, Edward, 6, 7, 313, 429, 430 Johnson, James, 113 Joklik, Wolfgang, 226 Kaiser, Dale, 86 Kaplan, Paul, 366 Katsura, Isao, 94 Klug, Aaron, 23 Koch, Robert, 6, 431 Kornberg, Arthur, 69 Lederberg, Esther, 86 Lindenmann, Jean, 7, 405 Loeb, Tim, 59 Loeffler, Friedrich 7, 430 Lot, Herve, 113 Luria, Salvador, 7, 85 Lwoff, Andre, 7 MacCallum, F.O., 365 MacLeod, Colin, 8 Maestra, A., 302 Maizel, Jacob, 127 Martini, Gustav-Adolf, 188 McCarty, Maclyn, 8 Meister, Joseph, 442 Metchnikoff, Elie, 430 Miller, Lois, 309 Montagnier, Luc, 7, 355 Montagu, Lady M.W., 430 Mulligan, Richard, 457 Nelmes, Sarah, 313 Northrup, John, 7 Gajdusek, D. is amplification in embryonated eggs. coli outer membrane protein, LamB, named because it confers susceptibility to infection. Autophagy: a cellular process responsible for the degradation of cytoplasmic components by ingestion into vesicles called autophagosomes, which fuse with lysosomes containing digestive enzymes. Unlike the other picornaviruses discussed here, important disease determinants for this virus appear to reside in the viral capsid proteins. Innate host defense responses (for example, induction of interferon; see Chapter 33) can play an important role as does the adaptive immune response. The prolonged transgene expression observed with AAV vectors is most likely a consequence of the transcription of this episomal DNA. Immunity after even a single dose of these vaccines is typically long-lived (at least 10 years in most cases). Yellow fever is a devastating human disease transmitted by mosquitoes Yellow fever was first recognized in 1648 in the Yucatan. Several alphaviruses, including eastern and western equine encephalitis viruses and Ross River virus, cause disease in animals and humans. The concept of quasi-equivalence A more economical way to build a capsid with 180 subunits is to use 180 identical protein subunits, rather than 60 copies each of three different proteins. Virus particles can be seen forming by budding into the lumen of these membranes. Thus, unlike acyclovir and ganciclovir, activation of this drug is not selective; it occurs in uninfected as well as in infected cells. In this case, exogenous PrPSc would catalyze the rearrangement of a molecule of PrPC, or of a partially destabilized intermediate, to the more stable PrPSc conformation. The next year (2000) proved pivotal for the presence of West Nile virus in North America. C-Src phosphorylates middle T antigen tyrosines 250, 315, and 322, which serve as binding sites for cellular proteins involved in cell-cycle signaling: Shc, phosphatidyl inositol 3 kinase (PI3K), and phospholipase C (PLC). Such annealing is inhibited by the high degree of internal secondary structure found in each single-stranded RNA. huxleyi-specific coccolithoviruses per mL of water were found in the middle of the high reflectance water. 3 binds to double-stranded RNA, competing with PKR for binding. But it was not until 1990 that the equivalent experiments were carried out in humans, with limited success. 1972 First DNA cloning performed. Transport Cytoplasm Nucleus 6. These oligomers self-cleave and then ligate to reform circular viroid RNAs. Viroids are therefore "ribozymes," or RNAs with catalytic activity. The resulting wrapped virus (WV) therefore consists of a mature virus particle encased in two additional bilayer membranes. This thin finger of cytoplasm called the infection thread is pulled towards the host cell through the spike. The virus moves out of the phloem to invade new tissue in the reverse of this process. 1,180,529 1,181,145 22,515–23,131 (A+C) excess reversal (400,00) R1: OBP 193-2580 Leading → ←Leading 616/617 Identical nucleotides ← (in) ing ad Le ding → L ea 1,158,273 bp (A+C) excess reversal (400,000) R1: OBP 617 bp 22.514 bp 259 bp (b) Figure 27.15 Schematic structure of the mimivirus genome. CH031.indd 386 16/05/11 8:17 PM C H A P T E R 3 2 Prions Dalius J. This association is correlated with degradation of movement protein by the proteasome pathway. Transcription of interferon genes is activated by virus infection or double-stranded RNA Virus infection is the best natural inducer of interferon production, although other infectious agents can also induce interferon production. A number of different RNA-containing plant viruses support the replication of satellite RNAs, presumably by using their own RNA-dependent RNA polymerases to replicate the satellite RNA, and their capsid proteins to encapsidate it. If two transgenes must be expressed coordinately, they can be cloned as two independent transcription units with their own enhancers, promoters, splice sites, and polyadenylation signals. Lentiviruses make a complex set of singly and doubly spliced mRNAs. Six regulatory proteins control virus production and pathogenesis. A variable number of accessory or groupspecific genes are interspersed between these structural protein genes. Cellular proteins such as those in the complement pathway, or cytotoxic cells such as natural killer cells, bind to infected cells and mark them for destruction. Viroids in the Pospiviroidae family are found in the cell nucleus, whereas viroids in the Avsunviroidae family are found within chloroplasts in the cytoplasm of plant cells. The organization of the book gives wide latitude to course coordinators to make their own choices of which virus groups will be covered. Tat is a powerful transcription factor that stimulates transcription from the LTR. This protein remains covalently attached to the 5′ end of the DNA it has cut and is incorporated into new virions along with the progeny DNA (and therefore it is not strictly a nonstructural protein). These viruses belong to the family Herpesviridae, one of the 3 families of herpesviruses that comprise the order Herpesvirales. 1930–45 Introduction of vaccines for Japanese B encephalitis (1930), yellow fever (1935), and influenza (1936). coli lacking the fertility plasmid F; that is, they are femalespecific or "F-restricted" phages; (3) finally, because T7 is so well-studied and replicates rapidly, it can be used to study microbial evolution, al., "Microarray analysis of Paramecium bursaria chlorella virus 1 transcription," Journal of Virology 84 (2010):532-42, PMID: 19828609, with permission from American Society for Microbiology; Fig. Fluid distribution problems and platelet abnormalities in disease victims indicate dysfunction of capillary endothelial cells and platelets. The outer fibril layer becomes partially digested within the phagosome, allowing interaction of the capsid surface with the phagosomal membrane. These short DNAs are extended by DNA polymerase up to the RNA primers of the adjacent DNA chains, and the primers are removed by ribonuclease H. The hypersensitive response is normally elicited by a single viral protein of a specific strain; for example, in cowpea it is elicited by the viral capsid protein. Genes are kept to a minimum. Coronaviruses 159 Coronaviruses cause respiratory illnesses in humans and important veterinary diseases 160 A newly emerged coronavirus caused a worldwide epidemic of severe acute respiratory syndrome (SARS) 160 SARS coronavirus may have originated from related bat coronaviruses 160 How did a bat coronavirus mutate and enter humans to become SARS coronavirus? This process ensures the synthesis of Gag and Gag/Pol in a particular ratio, so that the required quantities of each protein necessary for proper virus assembly are present. There appears to have been a segmental 200-kb duplication of the telomeric regions at both ends of the linear genome; furthermore, tandem duplications are common throughout the genome. Transmission is mainly by aphids, but the virus can also be transmitted by parasitic plants, in seeds, and mechanically. nsp3 One or two papain-like proteinase domains (PLP1, PLP2) responsible for cleavage of nsp1, nsp2, and nsp3; zinc ribbon motifs with predicted transcription factor-like function; transmembrane sequences with membrane integration; PLP2 and homologs with ubiquitinase activity; interferon antagonist. Prions are normal cellular proteins that under certain circumstances can undergo structural changes and become pathological, causing neurological diseases in humans and other animals. However, p53-mediated apoptosis of virusinfected cells could limit the ability of viruses to complete their life cycle and thus restrict the spread of the virus. tauri, and O. Canine parvovirus infects puppies and causes disease and death. The specificity of canine parvovirus for dog cells rather than cat cells maps to the capsid protein gene. Lipopolysaccharide: A complex molecule containing fatty acids and oligosaccharides, found in the outer membrane of Gram-negative bacteria. Interacts with M protein; may form icosahedral shell. Transcription by T7 RNA polymerase x x 5. A related approach, called a target-based high through screen, starts with the identification of a viral gene product (almost always a protein) that would make a good drug target. Reovirus serotype 3 particles interact with which two cellular molecules to initiate infection? Hepadnaviruses 365 At least seven distinct viruses cause human hepatitis 365 The discovery of hepatitis B virus 366 Dane particles are infectious virions; abundant non-infectious particles lack nucleocapsids 366 The viral genome is a circular, partly single-stranded DNA with overlapping reading frames 367 Nucleocapsids enter the cytoplasm via fusion and are transported to the nucleus 367 Transcription of viral DNA gives rise to several mRNAs and a pregenome RNA 368 The roles of hepatitis B virus 369 The pregenome RNA is packaged by interaction with polymerase and core proteins 371 Genome replication occurs via reverse transcription of pregenome RNA 372 Virions are formed by budding in the endoplasmic reticulum 373 Hepatitis B virus can cause chronic or acute hepatitis, cirrhosis, and liver cancer 374 Hepatitis B virus is transmitted by blood transfusions, contaminated needles, and unprotected sex 374 A recombinant vaccine is available 375 Antiviral drug treatment has real success 375 SECTION VIII: VIROIDS AND PRIONS 31. Unlike smallpox and measles, there are two good vaccines for poliovirus: both the inactivated (Salk) and live attenuated (Sabin) vaccines are highly effective in preventing polio. HIV-1 infection leads to a progressive loss of cellular immunity and increased susceptibility to opportunistic infections In the majority of cases, HIV-1 is transmitted upon exposure to mucous membranes, usually during sex or ingestion of breast milk. The single 8-containing portal is located at one of the 12 vertices of the icosahedral shell, called a prohead. Most bunyaviruses are transmitted by arthropod vectors, including mosquitoes and ticks The Bunyaviridae family includes over 300 named viruses in five genera (Table 17.1). mucoproteins (myxo mucus). Cis-acting replication elements (cre): sequence elements located in viral RNA that are required for initiation of viral RNA replication. The 30- to 36-kb linear, double-stranded viral DNA genome is organized into coiled domains by the histonelike polypeptide VII. Such inactivated viruses can retain their ability to induce an immune response but are incapable of replication and therefore do not cause disease. Amantadine: an antiviral agent that inhibits the activity of the M2 ion channel of influenza A viruses and blocks uncoating. Full-length RNA serves as mRNA for Gag and Gag/Pol and is packaged in virions. Viroplasm: name given to localized regions in the cytoplasm of infected cells where poxvirus DNA accumulates and where virion assembly takes place. CH021.indd 252 16/05/11 8:09 PM Viruses are excellent tools for studying multiple aspects of cell biology, including embryogenesis and cellular differentiation. This allows activation of the late promoter and increased levels of late transcription. Core protein 2 stabilizes microtubules within viral inclusions and associates with nonstructural protein NS, forming an organizing center for formation of inclusions. VIRUSES AND HOSTS Two genera, found in a variety of gram-negative bacteria: • GENOME Linear single-stranded RNA, positive sense, 4 kb. The idea of gene therapy arose in the late 1950s, when it was realized that viruses are able to transport their own genetic material into cells. The advantages of using baculoviruses in human gene therapy include (1) the known capacity of baculoviruses to tolerate large inserts of foreign DNA, (2) the inability of baculoviruses to replicate in mammalian cells, including a lack of toxic or adverse immune responses, (4) ease of manipulation in insect cell cultures, and (5) production of high virus titers. VIRUS REPLICATION CYCLE 1. G-C G-C tRNA tRNA A-U Phe Leu G-C G-C G-C G-C G-C UUU UUG G-C 5′...CCA.AAA.AAC.UCC.AUA.G-CCA...3′ - - - - - - C U Frame 1 Why have these viruses evolved such a complex scheme for the generation of the Gag/Pol polyprotein? 487 New York: A. It was therefore expected that virus-receptor interactions would be the factor that determines the specificity of infection. NL63, a recently discovered alphacoronavirus, also binds to ACE2. T antigens are oncogenes that can transform nonpermissive cells in vitro. Again, this is drawn as a linear molecule in Figure 28.4 but could involve a circular intermediate, with the plus-strand strong-stop DNA acting as a bridge between the two ends of the minus-strand DNA. Transfect packaging cell line Viral replication and structural proteins 4. nsp13 RNA helicase, nucleoside triphosphatase activity in vitro. DISTINCTIVE CHARACTERISTICS DNA circularizes upon entry via cohesive ends. How does a virus with a dsRNA genome inhibit cellular DNA synthesis? The precise differences between herpesvirus and cellular DNA polymerases that contribute to selectivity of acyclovir remain poorly understood. Filoviruses 188 Marburg and Ebola viruses: sporadically emerging viruses that cause severe, often fatal disease 188 Filoviruses are related to paramyxoviruses and rhabdoviruses 189 Filoviruses cause hemorrhagic fever 189 Filovirus genomes contain seven genes in a conserved order 189 Filovirus transcription, replication, and assembly 190 Cloned cDNA copies of viral mRNAs and viral genome RNA are used to study filoviruses 192 Multiplasmid transfection systems allow recovery of infectious filoviruses 192 Filovirus glycoprotein mediates both receptor binding and entry by fusion 192 13/05/11 2:11 PM xii Contents Ebola virus uses RNA editing to make two glycoproteins from the same gene 194 Do the secreted glycoproteins play a role in virus pathogenesis? Flaviviruses 137 Richard Kuhn, Purdue University 13. This superinfection form of DNA results from the toroidal coiling of the doublehelical DNA around the nucleosomes in the intact minichromosome. The mature virus particle contains an outer envelope composed of lipid and viral proteins. The expression of these genes is placed under the control of a strong promoter such as the cytomegalovirus (CMV) immediate early promoter. Often located immediately upstream of a gene, but can be found within the transcribed sequence or thousands of base pairs from the transcription start site. These surveillance activities are essential not only to ensure the safety of new vaccines but also to protect the enormous societal investment already made in national and international vaccination programs. Future research and development may well reveal situations in which their use will be able to control otherwise runaway infections by bacteria that have developed resistance to many commonly used antibiotics. Live virus strains can occasionally cause serious illness and even death in subjects with abnormal immune systems. A brief description of the function of each viral gene is presented in Table 22.2. The infectious cycle follows differentiation of epithelial cells During infection of the mucosal epithelium (Figure 22.3), virions must first reach the non-differentiated basal cells of the epithelium where they bind to components of the extracellular matrix and then to the major viral receptors: heparan sulfate proteoglycans and the cellular surface protein, 6-integrin. The dynamics of this movement are not understood, but the ultimate outcome is the assembly of 805 ribosomes at the initiator AUG and the start of polypeptide chain synthesis. These viruses may be relics of the transition between the RNA world and the DNA world; reverse transcriptase was probably responsible for generating DNA copies of the RNA molecules postulated to have been the genomes of primitive cells. This "scanning" model was further tested by introducing an additional start codon a short distance downstream from the authentic lysis start. CH037.indd 464 16/05/11 8:22 PM Adeno-Associated Virus Vectors Two strategies could be used to reduce the risk of cancer in patients being treated with this vector. Three distinct theories account for the mechanism by which virus particles exit the cell. This capsid is called "symmetric" replication, since both plus- and minus-strand coronaviruses in the same fashion. Coronaviruses have the largest known viral RNA genomes; besides causing important veterinary diseases, some cause upper respiratory infections in humans. Morphologically these viruses are highly similar to members of the family Fuselloviridae; however, genome sequences reveal no relationships to viruses of that family. An early strategy would be effective only if a significant proportion of mosquitoes received the gene that expresses the antisense RNA; infection by the Sindbis vector itself would not likely achieve this result. The loss of red cell production in this disease causes such a reduction in hemoglobin concentration that B19 infection can be fatal if not quickly treated by transfusion. Electroporate mouse cells x x 6. The so-called "Spanish flu" pandemic (worldwide epidemic) of 1918–1920 DISEASES Symptoms include high fever, sore throat, cough, headache, muscular pain. Togaviruses, particularly Semliki Forest virus, served as models to establish this means of entry, which is used by many enveloped RNAcontaining viruses (see Chapter 4). Transcribed by cellular RNA polymerase. The expression of TLR-7, TLR-8, and TLR-9 is largely restricted to plasmacytoid 16/05/11 8:19 PM Detection of Virus Infection by Host Cells dendritic cells, which are major interferon-producing cells within the blood and B lymphocytes. Organisms from flies to humans have a variety of primary "sensors" on their cells that can detect foreign molecules. Self-inactivating retrovirus vectors are much less likely to induce tumors in cells following integration of the proviral DNA into the cellular genome. A number of viruses interact with nuclear targeting receptors such as importins, and (also called karyopherins), and their genomes enter the nucleus through the nuclear pore complexes. Why were RNA phages so interesting to researchers during the 1960s through the 1980s? Phosphorylated IRF-3 and IRF-7 form dimers that are translocated to the nucleus. His group and that of S. In the second step, acyclovir triphosphate serves as a substrate and is incorporated by the viral DNA polymerase into the growing DNA chain opposite a dC residue on the template strand. Capsid proteins are then assembled into capsids that package viral minichromosomes. America Akabane virus Cattle: abortion and congenital defects Midge Africa, Asia, Australia Human: severe hemorrhagic fever with renal syndrome (HFRS); 5–15% fatality Field mouse Eastern Europe, Asia Puumala virus Human: mild HFRS, nephropathica epidemica; 0.1% fatality Bank vole Western Europe Sin Nombre virus Human: hantavirus pulmonary syndrome; 40% fatality Deer mouse N. These tumors grow rapidly and can eventually kill the host animal. The reassortant viruses produced were checked for their ability to replicate in the lung at core body temperature, but grow well in the cooler nasal mucosa, where they elicit an excellent immune response without any danger to the vaccinated individual. However, virions contain numerous copies of receptor-binding proteins on their surface, so the loss or denaturation of a few protein molecules should not lead to a loss of infectivity. CTD: carboxy-terminal domain of the large subunit of RNA polymerase II. Required for synthesis of subgenomic RNA. Explain how rubivirus escape from the cell. All 180 contains on the surface of this 16/05/11 7:49 PM 22 CHAPTER 2 Virus Structure and Assembly (a) (b) (c) (d) Figure 2.2 Elements of icosahedral symmetry. Linear vaccinia virus genomes have covalently sealed hairpin ends and lack introns The genome of vaccinia virus is a 200-kb doublestranded DNA molecule that contains 10-kb inverted repeats at each end (Figure 26.1a). Prangishvili, "Independent virus development outside a host," Nature 436 (2005), 1101-1102; Fig. So far, these host factors have not been identified. Most mammals have between 10 and 15 toll-like receptors, with humans and mice having 10 and 13, respectively. The protein shell must therefore be made of a large number of identical protein subunits, in order not to exhaust the coding capacity of the genome. They represented a superb source of pure messenger RNA that could be produced in large amounts: up to 1013 phage particles per mL can be made within a few hours after infection of bacterial cultures, and the phages can be easily purified. It is known that small RNAs such as tRNA (~75 nts) fold up correctly within milliseconds after denaturation. The first coronaviruses that infect humans were identified in the 1960s, and some 60 to 80 genera of human coronaviruses are thought to infect primarily bats or other mammals in the near future. The left half, from 5 to 50 map units, codes for the nonstructural (NS) or replication (Rep) proteins (a map unit is 1% of the length of the viral genome, or approximately 50 nucleotides). Thus, a large range of protein functions important for replication have been assembled and incorporated as the result of the plasticity and adaptability of the coronavirus genome. Filovirus infections are transmitted to humans from an unknown animal origin It is believed that filoviruses naturally infect and circulate in one or more animal species in Central Africa and perhaps in South Asia. PrPC has been implicated in copper regulation, copper buffering, and copper-dependent signaling. The acyclic sugar-like molecule accounts for the name of the compound and for aspects of acyclovir action. Prangishvili, 2006. BVDV usually causes a fatal disease in cattle. Cells infected with the virus produce far fewer progeny virus particles than when no defective interfering particles are present. This does not mean, however, that the virus is no longer ethically acceptable when used in research. Their small sizes and the finding that many virus-encoded proteins are "user-friendly" have resulted in the biochemical and structural characterization of several PBCV-1 enzymes. Class II ("middle") genes occupy the next one-third of the genome. 18.10: Figure 1 from Rebecca J. If effective, such a vaccine could eventually replace the more expensive injected vaccines and contribute to the prevention of a major cancer worldwide. AAV capsid components were apparently displayed as foreign antigens at the surface of the transduced liver cells, which were eliminated by the host immune system. Following attachment of virions to cell surface receptors, entry into the cell is achieved by endocytosis within clathrin-coated vesicles. To overcome this problem, influenza virus encodes another envelope protein, the enzyme neuraminidase. Mimivirus has a capsid diameter of 450 nm and a total diameter including fibers of 700 nm. Unclear are also the mechanisms that allow stable transmission of the viral genome during division of the host cell, especially in the case of viruses with linear DNA genomes that do not integrate into the host chromosome. In normal cells, the protein p53 is present at very low levels. Large doses lead to arrest of the cell cycle by a mechanism dependent on the cellular p53 protein, and cell-cycle arrest stops virus replication. Many clinically useful antiviral drugs are nucleoside analogues that target viral DNA polymerases. Oncolytic: able to kill or lyse tumor cells. Protein 1a acts as a chaperone to bring protein 2a, the RNA polymerase, to the membrane where it first copies the positive-strand RNA (light blue) into a complementary negative-strand RNA (dark blue), and then synthesizes progeny positive-strand RNAs. The negative-strand RNA remains within the membrane complex. We also set out to improve the graphic qualities of the book, by introducing full-color figures and by incorporating the impressive computer-generated figures of virus structures created by Philippe Lemercier, of the Swiss Institute of Bioinformatics, Swiss-Prot Group, University of Geneva. However, infection of young animals with virus lacking Nef induced disease, and adults experienced disease progression over a prolonged period of time. Suggested functions of these conserved sequences are recognition by viral RNA polymerase, RNA packaging into capsids, and translational control, but the role of these sequence elements has not been precisely defined. These are DNA sequences that are transposable elements and code for a transposase that catalyzes the transposition of the DNA element. Silencing: reduction or elimination of the activity of a transcriptional promoter, often because sequences in the promoter region are methylated. Within the nucleus, the ribonuclease III enzyme Drosha cleaves the primary miRNA into a smaller miRNA precursor, which is then exported to the cytoplasm. K. A hydrophobic sequence near the C terminus serves to anchor the protein in the plasma membrane. 16/05/11 8:05 PM Filoviruses 193 T7 Terminator Ribozyme T7 Promoter Ebola virus genome cDNA Cytoplasm Nucleus T7 RNA Pol 5′ 3′ T7 POL Ribozyme cleavage NP, VP30, VP35, L VP35 NP L Ebola virus nucleocapsid VP30 Viral mRNAs Figure 16.4 Production of infectious filoviruses by transfection of cells with multiple plasmids. Diameter 50 nm. Some coronaviruses have a spherical core. This is expressed only EBNA-1, the three LMPs, and the small viral RNAs. Cells derived from nasopharyngeal carcinomas also show the latency II gene expression pattern. Major symptoms are fever, hemorrhages, liver dysfunction, intravascular coagulation, and shock. What are the two reports that Epstein–Barr virus uses to infect B cells? Discuss the role of nsP2 in determining the fate of virus-infected cells. In all cases, treatment resulted in a general improvement of vision and reduced light sensitivity in patients. ϕX174 binds to glucose residues in lipopolysaccharide on the cell surface The icosahedral capsid (Figure 6.2) contains 60 copies of the viral capsid protein F. As a virus envelope by budding through the inner nuclear membrane, become de-enveloped at the outer nuclear membrane, and are subsequently re-enveloped at Golgi membranes. Viral proteins required for packaging of the genome, transcription and replication are expressed on a set of plasmids transfected along with the genome-expression plasmid. As a result, a current hypothesis is that SARS coronavirus, and perhaps all human coronaviruses, may have originated from bat coronaviruses. from Priola, S.A., Chesebro, B., and Caughey, B., "A view from the top: Prion diseases from 10,000 feet," Science 300: 917–919, copyright 2003, AAAS. 3′ U A C U C A AA (a) A U GAGACG A A G U C U C UGA G U AG 5′ A U G A G U C C U U UGUGC 5′ A A A C A GA G C U C G U U A A C G C C C G G G C U A G C G C A C G G C U A C U A G C G G C C C C U G U G C C G G G A A G U 5′ U 3′ G C C G deita C G G C A A U A G A G A G C G C U A C U C G C C A C C U A (b) C UGUGC U A C A CG A A (b) C Cleavage. They then demonstrated by chemical and physical studies that the viral genome consists of double-stranded RNA. Most transcriptional units encode only one protein. The interaction between receptor and virus particle often results in a loss of infectivity. Second, all of the early genes encode proteins that are needed to redirect cellular functions to serve the virus and the cell. Reprinted with permission from Sauder, P., Ph.D. Thesis, University of Lausanne (2000). Many millions of people around the globe are already infected with viruses such as hepatitis B and C, and human immunodeficiency virus. Nanoparticle vaccines delivering one or more viral proteins in virus-like particles, virosomes, proteosomes, etc LIVE ATTENUATED VIRUS VACCINES Naturally stimulate both cell-mediated and humoral immunity. The rule of six may have evolved as a mechanism to prevent replication of these edited genomes, which would not produce viable virus if packaged. Ambisense coding strategy in some viruses. Some retroviruses have been shown to generate a special region where viruses are preferentially assembled and transmitted—a virological synapse—in the contact area between the virus-producing cell and its neighbor. Cucumber Mosaic Virus 112 19. In other words, if the capsid surrounding a genome was made of a set of quasi-equivalent designs, the protein subunits do not need to evolve three alternative binding sites for interactions with each other, as in the case of poliovirus; instead, they need to have built-in flexibility. Although they also carry reverse transcriptase, pararetroviruses package an RNA genome that is reverse transcribed into DNA once it enters the host cell, whereas hepadnaviruses package a DNA genome that is reverse transcribed from a pregenome RNA in the cell during virus assembly (see below). Immediately downstream of the leader is the N gene, followed by the P/C/V, M, F, HN, and L genes. Some of these transcripts are polyadenylated at the early polyadenylation site, giving rise to abundant mRNAs that express the E1 and E2 proteins. However, there are indications that the virus enters clathrin-coated pits and undergoes low pH-dependent fusion within vesicles in the cytoplasm. These proteins are best known for recognizing microbial components such as peptidoglycans and toxins, and endogenous stress-related molecules such as uric acid and ATP. The viruses targeted by most vaccines developed up to the present time either cause acute infections by one prevalent serotype (measles, rubella, smallpox) or can be neutralized by antibodies before reaching their target tissues (poliovirus, hepatitis B, rabies, human papillomaviruses). C3b also has an enzymatic activity that triggers the activation of the remaining cascade of complement proteins (C5–C9), resulting in the formation of a membrane attack complex. Encephalitis: disease involving inflammation of the brain. Poliomyelitis: disease caused by poliovirus, involving central nervous system lesions that can lead to paralysis. There are advantages to very early or late steps such as attachment, entry, and 16/05/11 8:21 PM 446 CHAPTER 36 Antiviral Chemotherapy STAGE OF VIRUS LIFE CYCLE EXAMPLE OF INHIBITOR 1. VIRAL RNA polymerase as well as a packaging signal for assembly of nucleocapsids. GENES AND PROTEINS Four capsid proteins: matrix (MA), capsid (CA), nucleocapsid (NC), p6. Th1 cells synthesize interferon- and interleukin-2, and promote cellmediated immunity and the cytotoxic response (Figure 34.8). synthesis) and replication (genome synthesis). The poliovirus capsid has an internal cavity about 19 nm in diameter, which contains the single-stranded RNA genome of about 8 kb. • Reverse transcription is initiated on a primer provided by a cellular tRNA bound to virion RNA. Members of these professions are encouraged to be vaccinated. Extending this metaphor, the virus carries a MIRV (multiple independently-targeted reentry vehicle) warhead, and launches this missile in a preemptive strike on the host's first lines of defense. The best-characterized members of the family are brome

mosaic virus and cucumber mosaic virus. Conditions within the endoplasmic reticulum lead to extrusion of the N-terminus of VP2 through the capsid surface, allowing the N-terminal myristate to interact with lipid membranes and leading to release of the virions into the cytosol. In plants infected with cucumber mosaic virus, both virus-induced defense responses and viral doublestranded RNA-triggered RNA silencing are part of the host immune response to virus infection. On the other hand, in conjunction with chemotherapy, the use of Onyx-015 resulted in complete or partial regression of tumors and to a significant extension of the life of the patients. Coronaviruses tend to move from host species into new species. Eight different helical nucleocapsids containing NP and the RNA polymerase trimer of PA, PB1, and PB2 are enclosed within the envelope. Viral RNA polymerase initiates transcription exclusively at the 3' end of the viral genome There are two general models by which viral RNA polymerases could initiate synthesis of viral messenger RNAs on the genomes of mononegaviruses. Following amplification of the virus in the local lymph node, virus is released into the circulation, seeding the infection into all the lymphatic tissues of the body. release, because inhibitors of these steps do not have to enter cells to exert activity. Nevertheless, extreme care should be taken with blood, secretions, and excretions of infected patients. 9.2a,c: Courtesy of Wolfram Zillig; Fig. What is the current theory on the origin of SARS and perhaps all human coronaviruses? How then is the maturation protein made? PrPC contains three ␣-helices in the C-terminal half of the molecule, while the N-terminal region, containing octapeptide repeats that bind to copper ions, is relatively unstructured. (Figure 9.3). Human Immunodeficiency Virus 354 Alan Cochrane, University of Toronto 30. When carefully controlled, these treatments preferentially inactivate the virus genome and 16/05/11 8:21 PM Types of Antiviral Vaccines 433 Figure 35.2 Recombinant antiviral vaccines. In addition to evading the antiviral effects of complement, viruses inhibit the activity of cells involved in innate resistance, such as natural killer (NK) cells and dendritic cells. Mature virions contain 60 copies of each of four proteins: VP1, VP2, VP3, and VP4. The virus was subsequently adapted to monkey kidney cells and chick embryo fibroblasts, and served as the basis of the Edmonston live virus vaccine, introduced in 1961 (see Chapter 35). However, some very small satellite viruses and viroids (see below) have circular RNAs. Most of these viruses have genomes in the size range of 5 to 19 kb; an exception is the Coronaviridae, whose genomes are as long as 31 kb (the largest known RNA virus genomes). Furthermore, there is extensive amino acid identity in the translocation ATPases of the tailed dsDNA phages and the herpesviruses. CH037.indd 460 ≁ transgene ≁ E4 Complementing cell lines ITR (E1/E3-deleted) Second generation ITR ≁ ITR E2A First generation ITR ADENOVIRUS VECTORS L4 E3 L5 E1 ITR transgene (E1/E3/E4-deleted) transgene (E1/E3/E2-deleted) E1, E4 ITR Third generation ITR stuffer transgene E1, E2 ITR E1, Cre (helper adenovirus) stuffer ≁ ITR ≁ Conditional replicative (onyx-015) (E1B-deleted) ITR E1 Figure 37.3 Production of adenovirus vectors. These progeny genomes can be used as templates for synthesis of more viral messenger RNAs or for further genome replication. This virus showed extremely high pathogenicity for mice and for embryonated chicken eggs, and these properties required the 1918 versions of HA, NA, and the three polymerase proteins. (a) Equilibrium folding of the 5' untranslated region of MS2 RNA. Erythema infectiosum: Latin for "infectious rash." A childhood viral disease with typical rash and fever, also known as "fifth disease." Erythroid precursors: cells on the pathway of differentiation from hemopoietic stem cells to mature red blood cells. Promoters (P) and transcription terminators (T) are indicated on a linearized map of ϕX174 DNA. Both genomes and antigenomes are always found complexed with N protein. Molluscum contagiosum: the only existing natural poxvirus infection of humans; relatively rare but more common in immunocompromised patients. Therefore uncoating is incomplete, and viral double-stranded RNA is never released to the cytosol. Cleavage of fusion proteins such as influenza virus hemagglutinin or human immunodeficiency virus gp160 primes them for a dramatic, fusioninducing conformational rearrangement triggered by low pH (influenza hemagglutinin, see Figure 4.4) or by receptor and coreceptor binding (HIV-1 gp160; Chapter 29). Anthony Epstein attended a lecture by Burkitt at Middlesex Hospital in London in 1961. INTERFERONS Virus-infected cells secrete interferons, which protect nearby cells against virus infection Of the virus-induced cytokines, interferons possess the greatest antiviral activity. Roossinck Bromoviridae From Latin Bromus, a genus of grasses in the family Gramineae Refers to the hosts of the type genus, Bromovirus VIRION Non-enveloped icosahedral capsid (T 3). EPSTEIN–BARR VIRUS Epstein–Barr virus was discovered in lymphomas in African children Epstein–Barr virus is a very successful pathogen, present in over 95% of the adult population. Regions rich in S proteins bud into the lumen of the endoplasmic reticulum in the absence of cores to produce the abundantly secreted 22-nm diameter spheres and filaments (Figure 30.7). A single amino acid change in the E2 glycoprotein of Sindbis virus has a profound effect on the neurovirulence of the virus. Only a minority of infections with oncogenic human papillomaviruses result in cervical cancer, and many of the lesions regress spontaneously without treatment. The large genome size of some of these viruses is probably a result of the superior qualities of double-stranded DNA as a gene storage facility compared with other forms of nucleic acids: increased resistance to degradation, a low error rate during DNA replication, and the ability to recognize and repair mutations. Many viruses have been associated with their host organisms during millions to billions of years of evolution. The blunt end of the virion is formed by sealing of the envelope behind the coiled nuclocapsid as the virion is released from the cell membrane. In each case, differential splicing then generates the specific mRNAs that the virus needs. Because occluded and budded virions are enveloped at distinct sites in the cell, there are differences in the protein composition of the two kinds of virions. • Influenza virus neuraminidase (NA) protein cleaves sialic acid, the cellular receptor that binds to HA, facilitating efficient virus release. One such mechanism employs restriction endonucleases that recognize specific DNA sequences as sites for degradation. Two envelope proteins (M and E). This RNA cannot form the complete SIB hairpin; therefore these transcripts are not cleaved by RNAse III, and are more stable. Luria was one of the founding members of the "phage group", a coalition of physicists, biologists and chemists who chose during the 1940s to study bacteriophages in order to understand the molecular basis of life, and who invented the field of molecular biology. Again, the alternative use of several splice sites leads to the production of a number of late mRNAs. Only mRNAs that start at the late 16/05/11 8:10 PM Papillomaviruses promoter and end at the late polyadenylation site code for L1 and L2 capsid proteins. Mature virus particles are wrapped in Golgi-derived cisternae containing specific viral extracellular virus proteins (center left of Figure 26.5). This virus is more commonly encountered in countries with poorly developed sewage systems and low hygiene. GENOME Linear, double-stranded DNA, 30–36 kb. In addition, there are 60 copies of the DNA binding protein J inside the virion. Viruses that replicate in the nucleus transport their genomes into the nucleus via the nuclear pore complex. Among other colleagues who contributed significantly to this course during recent years are Alan Cochrane, Matthias Gotte, John Hiscott, Arnim Pause, and Mark Wainberg. The 2b protein of cucumber mosaic virus is also an RNA silencing suppressor. These lines further ensure safety by reducing the possibility of generating replication-competent virus by recombination. • Both mouse polyomavirus and simian virus 40 can cause tumors in experimental animals and transform cells in vitro, but they rarely if ever cause cancer in nature. These oligomers create holes in the membrane, resulting in the leakage of cytochrome c and other mitochondrial proteins into the cytosol. The inflammatory response is triggered by cytokine production by infected cells. 9.11a-h: Reprinted by permission from Macmillan Publishers Ltd: Figure 1 in Häring M., G. Cellular DNA-dependent RNA polymerase II appears to be involved in the replication of Pospiviroidae, since their replication can be inhibited by low concentrations of ␣-amanitin, a known inhibitor of this enzyme. BK and JC viruses were first isolated in the early 1970s. Initiation of early transcription requires two additional proteins: a specificity factor that binds to RNA polymerase, and a 2-subunit initiation factor (VETF) that binds to the promoter. However, capsid formation occurs at a much lower concentration of coat protein in the presence of phage RNA, assuring that empty capsids will not form and all phage RNA becomes packaged. Methods have been developed to target retrovirus vectors to a specific cell type by modifying the gene encoding the viral envelope protein. However, polyomaviruses were first discovered as agents able to induce formation of tumors in mice and other rodents. These antibodies bind to epitopes on intact proteins or other antigens with which B cells come in contact in the circulation. In contrast, the F protein is a type I integral membrane protein, with a long N-terminal domain facing outside and a short C-terminal internal domain. The viral genome is associated with nucleosomes formed from two molecules each of the four cellular histones H2A, H2B, H3, and H4. To become fusion-active, they generally must undergo several maturation steps. Mucosal epithelium: epithelial cells of the mucosal membrane. Boxes labeled 1 and 2 represent DR1 and DR2 sequence elements. S has been dubbed a holin, as it multimerizes in the membrane, forming a pore. These changed factors specifically activate the late promoters. To date, the only described chloroviruses infect symbiotic chlorella, often referred to as zoochlorellae, such as those associated with the protozoan Paramecium bursaria (Figure 27.1), the freshwater coelenterate Hydra viridis, and the heliozoon Acanthocystis turfacea. This phase has been called the "latent phase" of infection because the infecting virus has disappeared and no new progeny virus has yet been made. Brome mosaic virus RNA replication has been analyzed in yeast cells Brome mosaic virus has been extensively studied as a model system for translation and RNA synthesis by positive-strand RNA viruses. See also individual virus genera or species Chlorovirus, 327–331 Coccolithovirus, 331–334 Host algal species, 326 Paramecium bursaria chlorella virus-1 (PCBV-1), 327–331 Phaeovirus, 335 Prasinovirus, 334–335 Prymnesiovirus, 335–336 Raphidovirus, 336 Picornaviruses, 125–135 cell receptors and coreceptors, 127 cis-acting replication elements (cre), 133 diseases caused by, 125–126. These viruses enter host cells in a variety of ways. Virus Physiological Phenomena. Hepatitis B virus can cause chronic or acute hepatitis, cirrhosis, and liver cancer Hepatitis B virus does not immediately kill infected cells but instead leads to chronic, persistent infections. Two elements derived from the herpesvirus Epstein–Barr virus (see Chapter 24) have been useful for allowing long-term maintenance of vector DNA in dividing cells. These are among the simplest of viruses, and they control the translation of their genome RNA by modulating RNA secondary structure. The images were obtained using cryoelectron microscopy and three-dimensional image reconstruction. A variety of specialized cellular proteins can detect invading pathogens; the best-characterized pathogen-detecting proteins are the toll-like receptors. The genome coding strategies of two representative bunyaviruses are shown in Figure 17.2. Most bunyavirus proteins are coded by messenger RNAs copied from the genome, and therefore the genomes are considered negative-strand RNAs. However, some bunyaviruses have "sense" and "antisense" protein coding regions on the same RNA genome; for an example, see the phlebovirus S RNA segment in Figure 17.2. In this case, the messenger RNA for the N protein is copied from the genome RNA, but the messenger RNA for the NSs protein is of opposite polarity, and must be copied from the antigenome RNA (see below). Fortunately, adenovirus can be partly removed by heat denaturation and by density gradient centrifugation. The matrix and Vpr proteins within these complexes presumably interact with importins (Figure 4.5a). (a) Left LTR 351 Retroviruses lacking oncogenes can transform cells by insertion of proviral DNA near a proto-oncogene In contrast to acute transforming viruses, nontransforming retroviruses lack oncogenes. A variety of naming conventions are used for viruses of vertebrates. The simplest virus capsids consist of one protein that forms either a closed shell or a helical tube within which the viral genome is packaged (Figure 1.1). • Amantadine inhibits uncoating of influenza virus by blocking an ion channel protein that allows H⩽ to enter virions within endosomes. More than 1000 such genes have been identified to date; a partial list is presented in Table 33.4. Antiviral activities induced by interferons The ability of interferons to confer an antiviral state to uninfected cells is their defining activity. • Oncogenic papillomaviruses cause cancer through the combined action of two early viral proteins: E6 and E7. Therefore, herpesvirus-infected cells contain a much higher concentration of phosphorylated acyclovir than do uninfected cells. 57 SEC-II.indd 57 12/05/11 5:02 PM 58 SECTION II Viruses of Bacteria and Archaea Archaea were only recently distinguished from bacteria; although they are small, unicellular organisms with circular DNA genomes and no nucleus, they are as different from bacteria as they are from eukaryotes. nucleocapsid into a vesicle that forms a 42-nm enveloped Dane particle within the lumen of the Golgi vesicle (Figure 30.7). Ribonucleoproteins that make up the spliceosome bind to both ends of the intron as well as to an internal branch site near the 3⬘ splice site. Projecting from the penton bases like antennae on a space satellite are fibers made of trimers of polypeptide IV. Marburgvirus and Ebolavirus are two distinct genera within the filovirus family. Unexpectedly, the ability of parvoviruses to infect certain cell types or species seems to be determined at the stage of virus uncoating. and HCF-1. Viruses with single-stranded DNA genomes must first convert their DNA into a double-stranded form, as there are no known enzymes that will transcribe single-stranded DNA directly into RNA. These subunits will not all be in identical positions on the surface of the capsid, and therefore they will have distinct binding interactions with their nearest neighbors, corresponding to their distinct environments. CH035.indd 430 16/05/11 8:21 PM Types of Antiviral Vaccines Embryonated chicken eggs and cell culture played major roles in vaccine development in the twentieth century The technological leaps that had the greatest impact on antiviral vaccine development in the twentieth century were (1) serial passage of human viruses in animals; (2) the adaptation of viruses for growth in embryonated chicken eggs; and (3) the growth of viruses in vertebrate cells cultured in vitro. A fraction of RNA polymerase molecules dissociate from the genome at the end of each gene, and therefore cannot reinitiate transcription at the next downstream gene. • Latent herpesviruses may be reactivated regularly, as in cold sores, or only after many years, as in shingles. (a) Structure of amantadine. Ritonavir: a successful protease inhibitor of HIV-1 that was developed by rational methods One example of these drugs is ritonavir (structure in Figure 36.9), a peptidomimetic drug (it mimics a peptide). When N levels are high (central part of figure), the RNA leader is encapsidated and RNA polymerase does not terminate but copies the entire genome, to form full-length antigenome nucleocapsids. Progressive multifocal leukoencephalopathy (PML) is a rare demyelinating disease caused by human polyomavirus JC. Some members of the T7 family of phages supplement this strategy by destroying the methyl group donor substrate (S-adenosyl methionine) present in the host cell. Eventually, the resident viral genes, localized within an internal membrane system, could have taken over the functions of the prokaryotic genome, which would have subsequently been lost. Red blood cells (RBC; small orange circles in central tube) are mixed with virus (small green spheres), or with buffer, and are allowed to settle. the doubly spliced 2-kb class of RNAs, which encode Tat, Rev, or Nef. Many genes looking for a function With almost 500 genes and a double-stranded DNA genome of 408 kb, the coccolithovirus E. The first step in the generation of a viral vector from a positivestrand RNA virus is to convert the RNA genome to a DNA copy (cDNA) that can be cloned into a bacterial plasmid, modified in various ways, and then transcribed back into an infectious RNA. This organization of gene expression assures that sufficient newly replicated virus genomes will accumulate before synthesis of structural proteins begins. Bacteriophage T7 77 T7: a model phage for DNA replication, transcription, and RNA processing 77 T7 genes are organized into three groups based on transcription and gene function 78 Entry of T7 DNA into the cytoplasm is powered by transcription 79 Transcription of class II and III genes requires a novel T7-coded RNA polymerase 79 Class II genes code for enzymes involved in T7 DNA replication 80 T7 RNAs are cleaved by host cell ribonuclease III to smaller, stable mRNAs 80 Class III gene expression is regulated by delayed entry and by promoter strength 80 DNA replication starts at a unique internal origin and is primed by T7 RNA polymerase 80 Large DNA concatemers are formed during replication 81 Concatemer processing depends on transcription by T7 RNA polymerase and occurs during DNA packaging into preformed proheads 82 Special features of the T7 family of phages 82 8. The emphasis of this textbook is on virus replication strategies; it is directed towards university students studying microbiology, cell and molecular biology, and the biomedical sciences. The hexon is actually constructed as a trimer of the adenovirus hexon protein, but each protein subunit contains two very similar subdomains, so that the assembled hexon has pseudo-sixfold symmetry. However, a number of other viruses of euryarchaeota are morphologically similar to viruses of crenarchaeota. A cutaway model of the mature virus (MV) particle, showing internal structures. An interesting twist on this hypothesis has been proposed. If this hypothesis is correct, an inherent plasticity would allow other proteins to compensate for reduced B protein function or for its absence. By means of rational drug design, zanamivir and oseltamivir were discovered as inhibitors of influenza virus. During the late phase of infection, polyadenylation signals are frequently misread, resulting in extended downstream transcription and the accumulation of large amounts of complementary RNAs that can anneal to each other. Immunoprophylaxis: treatment of an infectious disease by injection of antibodies directed against the disease agent. However, the capacity of these transposable elements to alter genome structure and gene function also makes them a strong driving force in evolution. the unspliced 9-kb full-length RNA, used to produce Gag and Gag-Pol proteins 2. • Cucumber mosaic virus is often transmitted by aphids, but it also can be transmitted mechanically, by parasitic plants and by seeds. Today several serotypes (AAV1 to 12) that can infect primate cells have been described. BGLOSS.indd 477 477 Macrophages: cells present in a variety of tissues and organs that engulf and digest extracellular debris, cell fragments, and pathogens, and are involved in antigen presentation to cells of the adaptive immune system. The nature of the immune response is also crucial. Insert transgene(s) Ori ≁ Transgenes Vector genome 3. coli) and take part in ATP-dependent protein folding and unfolding reactions that are vital in assembling or disassembling multiprotein complexes. After 1 hour, fresh medium is added to each culture and the cells are incubated at 37C in a humidified chamber with a 5% CO2 atmosphere. Packaging of brome mosaic virus RNAs involves specific sequence- and/or structure-dependent RNARNA interactions and coat protein-RNA interactions. This enzyme is usually required for transcription and replication of RNA. It is likely that the mimivirus tricks the amoeba into internalizing it by having the appearance of an innocuous bacterial food source. Ligates linear plus-strand DNA to form full-length, single-stranded circles. Viral envelope protein gp350/220 binds to CD21, leading to uptake of the virus into endocytic vesicles. The role of the immune system in fighting viral infections Cellular immune responses (see Chapter 34) mediate recovery from the vast majority of viral illnesses. The most notable feature of E6 proteins made by oncogenic papillomaviruses such CH022.indd 268 as types 16 and 18 is their ability to mediate the destruction of p53 through the ubiquitin proteolysis pathway (Figure 22.6). Parts of the overlapping reading frames at the stop site for translation of sGP are shown at right. All bunyaviruses contain three genome segments, named L, M, and S for large, medium, and small. • The prion hypothesis proposes that misfolded forms of a cellular protein can catalyze the refolding of properly folded native protein molecules into similarly misfolded conformations. Reverse transcription 5. A number of baculoviruscoded proteins such as the viral proliferating cell nuclear antigen (PCNA), ubiquitin (vUBI), and superoxide dismutase (SOD) share a high degree of amino acid 19/05/11 12:19 PM 308 CHAPTER 25 Baculoviruses sequence similarity with homologous cellular genes. RNAs can be produced by in vitro transcription of plasmids containing cloned copies of the modified MDV-1 RNA, using bacteriophage T7 RNA polymerase. • Cellular immunity is critical for recovery from most viral infections. • Viral DNA synthesis begins at either end of the linear genome with a protein primer covalently bound to a nucleotide. PrPSc is encoded by a host cell gene Soon after the purification of PrPSc, its N-terminal amino acid sequence was determined. The maintenance of satellite RNAs is largely determined by RNA 1, indicating that this element of the viral replicase interacts with satellite RNA. Brackets indicate promoters PRM and PR, with their 10 and 35 boxes shown. 16/05/11 7:56 PM C H A P T E R 7 Bacteriophage T7 William C. Both E1 and E2 are anchored in the lipid membrane with carboxy-terminal portions of their polypeptide chains protruding into the interior of the particle, as can be seen in Figure 13.1b. There are presently nine known members of the IRF family of transcription factors, of which five are thought to contribute to type I and/or type III interferon production. A viral gene was identified by mapping mutations that inactivated tumor formation by Rous sarcoma virus in chickens, but that did not affect virus replication. Some enveloped animal viruses fuse their lipid envelopes directly with the plasma membrane of the cell, releasing the viral capsid and genome into the cell. Kevin also launched the present second edition, and both Associate Editor Michael Palumbo and Senior Production Editor Joyce Poh have been of constant and unwavering help throughout this process. Editing, the addition of one or more nucleotides to a growing mRNA molecule at an internal editing site, can also take place during genome replication. Palmitoylated. The plus-strand genome is first copied into a complementary minus-strand RNA. Return instructions and a free of charge return shipping label are available at www.wiley.com/go/returnlabel. Terminal protein: an adenovirus-coded protein that is covalently bound to the 5⬘ ends of linear viral DNA. 3b, Smith, T., et al., "Neutralizing antibody to human rhinovirus 14 penetrates the receptor-binding canyon," Nature 383 (1996): 350–354; Fig. Given their similairities, it is likely that viroids and viroid-like satellite RNAs have evolved from a common ancestor. Virus spreads between hosts by direct contact, aerosol and fecal–oral transmission, and contact with contaminated surfaces. In order for one protein to carry out such a wide variety of interactions, it must assume several different conformations. Because the hairpin end can fold back on itself, it can serve as a primer for DNA polymerase, eliminating the need for an RNA primer. The fate of the infected cell depends partly on the outcome of a battle between cellular mechanisms that lead to apoptosis and viral mechanisms that suppress it. Some viruses have internal lipid membranes that lie underneath an external capsid. Attachment proteins of the two other reovirus serotypes also bind to JAM-A but not to sialic acid. When a specific lymphocyte clone is activated with the help of Th2 cells (Figure 34.6), it first produces IgM, and later the other immunoglobulin isotypes. Gastroenteritis: inflammation of the gastrointestinal tract resulting in diarrhea, vomiting, and/or cramps. It was found that minigenomes whose total length is an exact multiple of six nucleotides replicate much more efficiently than minigenomes of other lengths. CH002.indd 24 (c) Figure 2.8 Structures that share symmetry elements with icosahedral viruses. The phage group helped to found the field of molecular biology, which developed rapidly during the 1950s and 1960s. Virus structure is studied by electron microscopy and X-ray diffraction Most virus particles are too small to be visible by light microscopy. VP1 VP3 VP2 "canyon" Figure 2.4 Picornavirus capsids. Many viruses first bind to a relatively non-specific primary receptor such as a carbohydrate, and subsequently bind to a specific cell surface protein that serves as a secondary receptor. The receptor-binding sites in non-enveloped viruses are formed either by protrusions or by cavities in the capsid surface. A tumor virus, Rous Sarcoma virus, was isolated from sarcomas of chickens in 1911, and was only many years later recognized as a representative of the important retrovirus family, of which human immunodeficiency virus (HIV) is a member. It appears that E4 is required for efficient expression of transgenes under the control of several promoters used in these vectors, particularly the cytomegalovirus and Rous sarcoma virus promoters. VP3 is formed from VP2 by proteolytic cleavage of about 20 N-terminal amino acids, after its incorporation into the capsid. Upon entering the cell, retroviruses use reverse transcriptase packaged within the virion core to make a fully double-stranded DNA copy of their single-stranded RNA genome. There is only one late transcription unit, with a single promoter but five different polyadenylation sites. But how do they do this? Kaposi's sarcoma, Burkitt's lymphoma, nasopharyngeal carcinoma. Vpr may do this by targeting for degradation cellular proteins that are needed to pass from the G2 phase to mitosis. This model states that the conformational change leading to conversion of PrPC into PrPSc is a reversible reaction, CH032.indd 391 ... Also, a virus hypothesis would not easily explain genetically inherited prion diseases. Most viruses preferentially package only one of the two DNA strands, but some viruses package both strands, making two kinds of virions that are identical except for the polarity of their single-stranded DNA genomes. (c) Scanning electron micrograph of Ebola virus particles budding from the surface of Vero E6 cells (bar: 500 nm). Therefore, these clones must code for factors essential for transcription of late genes. The (full-length) minus DNA strand is joined at its 5⬘ end to a tyrosine residue in the P protein, a virus-coded DNA polymerase with reverse transcriptase activity. Part of the excitement is that we are taking the knowledge we have gained over many years about viruses and are putting it to work to treat viral diseases. Conclusion The discovery and description of unique and varied species of archaeal viruses has significantly influenced the field of prokaryotic virology. In 1973, David Dane found virus-like particles in the serum of patients with type B hepatitis; these particles subsequently became known as Dane particles and were shown to be virions of hepatitis B virus (HBV) (Figure 30.1). The upper arm is PL; the lower arm is the PRM–PR region (also shown in b). Filovirus Outbreaks in Africa Ebola 2001/02/03/05 Ebola 1976/79/2004 Sudan Ebola 1976/77 Ebola 1994/96/97 2001/02/03 Rep. Entry of animal viruses into cells is discussed in detail in Chapter 4. This leads to a shift in the reading frame, allowing continued translation through ORF1b and production of a larger ORF1ab polyprotein, which is cleaved into as 16/05/11 8:04 PM Coronaviruses many as 16 mature proteins. CH008.indd 90 promoter, PAQ, is located within gene Q. When serving as a template for mRNA synthesis and RNA replication (see below), the RNA genomes of paramyxoviruses and rhabdoviruses always remain associated with N protein as nucleocapsids; naked genome-length RNA is never found within the cell. The b sequence contains four open reading frames. NS1 can bind to both single- and double-stranded RNA and to two host cell proteins: (1) cleavage and polyadenylation specificity factor (CPSF) and (2) poly(A)-binding protein II (PABII). Table 37.1 Some eukaryotic virus vectors and their applications Application Virus family Virus species Protein production Adenovirus Adenovirus type 5 Retrovirus Human immunodeficiency virus type 1 Baculovirus Autographa californica nucleopolyhedrovirus Togavirus Semliki Forest virus, Sindbis virus Adenovirus Adenovirus types 2 and 5 Coronavirus Transmissible gastroenteritis virus, mouse hepatitis virus, infectious bronchitis virus Flavivirus Japanese encephalitis virus, yellow fever virus Herpesvirus Bovine herpesvirus Orthomyxovirus Influenza A and B viruses Paramyxovirus Sendai virus, simian virus 5 Parvovirus Adeno-associated virus Picornavirus Poliovirus Poxvirus Vaccinia virus, canarypox virus, fowlpox virus Rhabdovirus Rabies virus, vesicular stomatitis virus Togavirus Semliki Forest virus, Sindbis virus, western equine encephalitis virus, Venezuelan equine encephalitis virus Adenovirus Adenovirus types 2 and 5 Baculovirus Autographa californica nucleopolyhedrosis virus Flavivirus Kunjin virus, tick-borne encephalitis virus Herpesvirus Cytomegalovirus Retrovirus Human immunodeficiency virus type 1, simian immunodeficiency virus type 1, murine leukemia virus Paramyxovirus Sendai virus, human parainfluenza virus Parvovirus Adeno-associated virus, B19 virus Polyomavirus Simian virus 40 Togavirus Semliki Forest virus, Sindbis virus Adenovirus Adenovirus type 5 Herpesvirus Herpes simplex virus Paramyxovirus Newcastle disease virus, simian virus 5 Parvovirus Minute virus of mice, H-1 parvovirus, LuIII virus Poxvirus Vaccinia virus, avian poxviruses Reovirus Reovirus Rhabdovirus Vesicular stomatitis virus Recombinant vaccines Gene replacement therapy Antitumor agents CH037.indd 457 16/05/11 8:22 PM 458 CHAPTER 37 Eukaryotic Virus Vectors Both adenoviruses (35-kb genome) and baculoviruses (135-kb genome) proved to be more effective vectors for protein production because of their larger genome sizes and the ease of producing recombinant virions. It is presently unclear whether this disease crossed the species barrier from sheep and goats to cattle, or whether it is a rare, preexisting disease of cattle that was propagated by feeding remains of diseased cattle to livestock. Single-stranded DNA binding protein (SSB) first coats the intracellular phage DNA except for a specific short, highly base-paired "hairpin" region. In summary, even a partial list of the many poxvirus immune defense functions reveals an ingenious multifaceted attack on the host defense system. For example, the envelope glycoproteins of HIV-1 and other lentiviruses first bind to CD4, a glycoprotein of the immunoglobulin family. Jak–Stat pathway: signaling by interferons, other cytokines, and growth factors is mediated by a pathway that includes the Janus tyrosine kinases (Jak kinases) and the signal transducers and activators of transcription (Stat proteins), via a cascade of tyrosine phosphorylation events leading to the activation of transcription of a variety of target genes. Pathology and diagnosis of prion diseases A number of human diseases are caused by production of misfolded proteins. These phages have no distinct lysis protein; lysis is carried out by the maturation (A2) protein. Like PRM (activated by low levels of CI; see Table 8.1), PRE is a weak promoter that, in this case, is activated by CII. Cleaved to produce mature M protein, releasing pr. Shown in orange is the 708-amino acid large T polypeptide. How is plus-strand DNA synthesis carried out? Transcription is influenced by two enhancer elements (Enh I and Enh II) (Figure 30.3), which are binding sites for several cellular transcription factors. Eukarya: one of the three domains of life; all eukarya, also called eukaryotes, have a nucleus, as well as other membranebounded organelles such as mitochondria or chloroplasts. In the case of adenovirus, a viral cysteine protease is activated by entry into the reducing cytosolic environment. The order of genes is highly conserved among all coronaviruses. Long-term maintenance of virus genomes. Papillomaviruses 263 Papillomaviruses cause warts and other skin and mucosal lesions 263 Oncogenic human papillomaviruses are a major cause of genital tract cancers 264 Papillomaviruses are not easily grown in cell culture 264 Papillomavirus genomes are circular, double-stranded DNA 264 The infectious cycle follows differentiation of epithelial cells 265 Viral mRNAs are made from two promoters and two polyadenylation signals 266 Viral E1 and E2 proteins bind to the replication origin and direct initiation of DNA replication 267 Viral E7 protein interacts with cell-cycle regulatory proteins, particularly Rb 267 Viral E6 protein controls the level of cellular p53 protein 268 Synergism between E6 and E7 and the predisposition to cancer 269 Cells transformed by papillomaviruses express E6 and E7 gene products from integrated viral DNA 270 Future prospects for diagnosis and treatment of diseases caused by papillomaviruses 270 SECTION VI: LARGER DNA VIRUSES OF EUKARYOTES 23. However, the single-cycle approach does simplify the study of virus replication and is nearly universally used. For example, annual vaccination of healthcare providers against seasonal influenza virus strains can reduce the impact of influenza on hospitalized and institutionalized individuals, although the danger of influenza to the (younger and healthier) vaccinated individuals is not as great. PRE is located downstream of the cro gene, so that RNA polymerase initiating at PRE transcribes cro backward, prior to transcribing cI (Figure 8.6). The 5.3-kb circular, double-stranded DNA SV40 genome was one of the first eukaryotic virus genomes to be cloned in a bacterial plasmid, and its complete nucleotide sequence was determined in the late 1970s. In transit from the endoplasmic reticulum through the Golgi complex to the plasma membrane, fusion proteins may be cleaved by cellular proteases. Viral RNA is synthesized in association with membrane vesicles that are induced during virus infection. No mammalian enzyme phosphorylates acyclovir as efficiently as do the thymidine kinases of these two herpesviruses, although some phophorylation does occur in uninfected cells (1–3% of that found in cells infected with herpes simplex virus). Eukaryotic cells cultured in vitro have been adapted for plaque assays In vitro culture of human, animal, insect, and plant cells was achieved in the mid-twentieth century, and allowed more convenient and cheaper growth and titration of many viruses. The 3D protein is the RNA-dependent RNA polymerase that adds nucleotides to the primer, copying the template RNA strand. (Scale bar: 100 nm). Poxviruses also encode dozens of proteins that have evolved to confound the 16/05/11 8:13 PM 314 CHAPTER 26 Poxviruses host inflammatory and immune responses. CH014.indd 161 16/05/11 8:04 PM 162 CHAPTER 14 Coronaviruses programs to determine evolutionary relatedness, or phylogeny. Cucumber mosaic virus requires protein 3a (movement protein) and coat protein for cell-to-cell movement and for long-distance spread within infected plants via the vasculature Systemic infection of plants by cucumber mosaic virus depends on movement of viral genomes from initially CH010.indd 118 infected cells to neighboring cells, vascular cells, and other distant tissues. America La Crosse virus Human: encephalitis Mosquito N. An alternative to introduction of viral antigens is the insertion of their genes into expression plasmids, which are then introduced into cells in the body. These nucleocapsids exit the nucleus and travel to the basal cell plasma membrane, where they form virions by budding. 2.3a: from VIPERdb2: an enhanced and web API enabled relational database for structural virology, Mauricio Carrillo-Tripp, Craig M. The core contains the virus DNA, presumably within the tubular structure. Three late proteins—VP21, pre-VP22a, and VP24—form the scaffold. They have been valuable models for investigating togavirus uptake, structure, and replication, and have also been used as important tools for studying cellular pathways such as cleavage and modification of glycoproteins and their transport to the plasma membrane. CH028.indd 343 16/05/11 8:15 PM 344 CHAPTER 28 Retroviruses The virus genome (Figure 28.2) is a positive-strand RNA 7 to 10 kb long, complexed with the nucleocapsid protein (NC). It is believed that they cause disease by direct interaction between the viroid RNA, or other viroid-specific RNAs generated in the course of the infection, and cellular targets. From the middle of the thirteenth century until recent times, human population growth has been slowed Table 35.1 Impact of antiviral vaccines on disease frequency: pre-vaccination era vs. Self-complementary hairpin structures at genome termini. In addition, structures not usually found in uninfected cells, such as convoluted membranes, membrane whorls and multivesicular bodies, have been observed in coronavirus-infected cells. Before viruses were recognized as infectious agents, scientific efforts were focused on establishing a cause for a particular disease. EBNA-2 carries out this activation by binding to the promoters of viral or cellular genes via a cellular DNA-binding protein called RBP-Jk. EBNA-LP binds to EBNA-2 and enhances its activation activity, as do a number of general cell transcription factors, leading to recruitment of RNA polymerase II to these genes and their transcription. It undergoes N- and O-glycosylation and proteolytic processing by a furin-like endoprotease, and is released into the medium of infected cells as two distinct molecules: mature sGP (N-glycosylated) and delta-peptide (O-glycosylated), the approximately 40 amino acid C-terminal portion of the protein. Retroviruses are found only in vertebrates, but a variety of retroviruslike elements, some of which make virus-like particles, have been found in plants, invertebrates, and fungi. Other examples are discussed below. Nuclear factor B (NFB) is activated upon phosphorylation and degradation of its inhibitor. After reverse transcription and integration, the U3 regions of both the left and right LTRs will be deleted and both LTRs will therefore be transcriptionally inactive (see the mechanism of reverse transcription in Chapter 28). Many enveloped viruses are assembled by budding at the plasma membrane, and can be released with little or no effect on the host cell. 398 CH033.indd 398 16/05/11 8:19 PM Detection of Virus Infection by Host Cells RNA INTERFERENCE Plants, invertebrates and fungi do not produce interferon, but use RNA interference as a host defense mechanism. "For strange effects and extraordinary combinations we must go to life itself, which is always far more daring than any effort of the imagination." Sherlock Holmes Roots . Lines are drawn in proportion to genome lengths. For example, a number of viruses of vertebrates are transmitted from one animal to another by bloodsucking invertebrate arthropods (mosquitoes, ticks, sandflies), in which they can also replicate. Infection of Escherichia coli by phage can have two possible outcomes. Hepatitis delta virus shares some characteristics of viroids: it has a small, single-stranded circular RNA genome with self-cleavage activity. For gene therapy. Tc cells are primed to respond when their T-cell receptor binds to a peptide displayed in association with a MCH I protein CH034.indd 422 Interferons stimulate antigen processing and presentation Interferon promotes protective immune responses by its ability to increase the expression of major histocompatibility complex proteins. Infected cells that were grown for several months continually released virus particles, showing that the virus exists in a "carrier state" in these cells. However, both CD4 and gp160, the precursor of gp120, are made in the endoplasmic reticulum, and they can bind to each other at that intracellular site. Antigenic shift: a rapid change in the antigenic specificity of a protein. Attachment of adenovirus to the ␣v3 integrins also activates a signaling cascade inside the cell that temporarily increases endocytic activity and prepares the cell for the incoming virus. The phage then "walks" along the surface of the cell, binding to lipopolysaccharide residues one after the other, until it finds a second receptor, which leads to delivery of the viral DNA. CH028.indd 346 16/05/11 8:15 PM Retroviruses The reverse transcriptase of retroviruses lacks the 3'-to-5' exonuclease activity that cellular DNA polymerases use for proofreading. Late viral genes are expressed from newly replicated viral genomes. Hopkins, Jeffrey W. William Studier and Rudolf Hausmann independently obtained large collections of conditional-lethal T7 mutants, which they mapped and classified in terms of phenotypes. CH011.indd 132 16/05/11 8:02 PM Picornaviruses cytoplasm is a common theme encountered with a variety of positive-strand RNA viruses (see also Chapters 10, 12–14); these vesicles help to localize all the elements required for efficient viral RNA replication. Individual genes are represented as rectangles (direction of transcription: R: rightward, L: leftward). GENOME Linear single-stranded RNA, negative sense. Starting from the 3' end at this nick, the cellular DNA polymerase then elongates the DNA chain in the other direction, to fully copy the left end hairpin. Peter Panum, a Danish physician, was the first researcher to realize (in 1846) that a measles infection conveyed lifelong immunity. CH015.indd 183 16/05/11 8:05 PM 184 CHAPTER 15 Paramyxoviruses and Rhabdoviruses P V W C' C Y1 CAACAAAAAAGGGC P CAACAAAAAAGGGGC V CAACAAAAAAGGGGGC W Y2 81 GCCACGGCU C' 104 114 UUACCGCAUGGAUCAAGAUGCCU P/V/W C 183 201 CGGAUGUUA UCGAUGCUG Y1 Y2 Figure 15.9 Sendai virus P/C/V gene: open reading frames, translational start sites, and "stuttering" site. Subgenomic mRNAs are made by an unusual discontinuous transcription mechanism. Because of their unusual function and their central role in infection, many fusion proteins have been characterized in great detail. 2.10: Reprinted from Fig. How do polyomaviruses transform cells in vitro and cause tumors in vivo? Although these mutations are spread across the genome, the envelope protein has a high number with 12 amino acid substitutions. Shown is an encounter between a ribosome translating an RNA in the 5' to 3' direction, and an RNA polymerase making a copy of the RNA in the 3' to 5' direction. The polyomaviruses (Figure 2.6a) and papillomaviruses have capsids that assemble from 72 pentamers of the major capsid protein, VP1. This shortlived intermediate undergoes one final proteolytic cleavage event that produces VP4 and VP2 from the VP0 precursor, thus producing the fully infectious virion. The myelin component of the early vaccine was eventually implicated. A number of viruses that infect archaea have been recently described; all but one of these contain double-stranded DNA genomes, and in many cases their virions have distinct and unusual morphologies. Aquareoviruses infect fish and mollusks. Therefore, there were fewer obvious targets for chemotherapy against viruses than against bacteria. 11.3: Adapted from J.M. Hogle and V.R. Racaniello, "Poliovirus receptors and cell entry," Molecular Biology of Picornaviruses, Semler, B.L. and Wimmer, E. It was surprising to discover that the N-terminal region of large T antigen can, by itself, complement DNA J-minus mutants of E. Wt. (kDa) Sequence features Functions 1a RNA 1 110 Methyltransferase domain; RNA helicase domain containing NTP-binding and RNA-binding motifs 5' capping of viral RNAs; RNA synthesis; support of satellite RNAs; virus movement and seed transmission in specific hosts 2a RNA 2 97 RNA-dependent RNA polymerase RNA synthesis; virus movement 2b RNA 4A 15 Basic domains in C- and N-terminal regions; nuclear localization signal; binding of single-stranded RNA and siRNA Long-distance movement; suppression of RNA silencing; virus accumulation 3a RNA 3 30 Binding of single-stranded RNA Cell-to-cell and longdistance movement; virus accumulation Coat protein RNA 4 24 Arginine-rich domain at N-terminal region; RNA binding Encapsidation; aphid transmission; cell-to-cell and longdistance movement CH010.indd 116 16/05/11 8:01 PM Cucumber Mosaic Virus Replication of viral RNA is associated with intracellular membranes, and requires coordinated interaction of viral RNAs, proteins, and host proteins When viral genome RNAs are released from capsids within plant cells, they serve as messenger RNAs that direct synthesis of proteins 1a, 2a, and 3a. To understand the time course of events taking place during the replication cycle, scientists usually study cultures containing thousands to millions of infected cells, because only then can sufficient viral nucleic acid or proteins be isolated and analyzed. A number of viruses have internal lipid membranes that lie beneath an external capsid with icosahedral symmetry. These microvilli can contact neighboring cells, infecting them with extracellular virus. coli, Shigella, Salmonella ϕX174, G4, 3 Bdellomicrovirus Bdellovibrio (obligate intracellular bacteria) ϕMH2K Chlamydiamicrovirusa Chlamydia (obligate intracellular bacteria) CHP1 and CHP2 The Mollicute bacteria, Spiroplasma SpV4 Microvirus a a Spiromicrovirus a These genera have been grouped into a distinct subfamily, called the Gokushoviruses. (b) Single-entry model: RNA polymerases initiate transcription only at the 3' end of the genome, and transcribe each gene in sequence. However, should the CI concentration become very high, OR3 is also occupied and transcription from PRM is turned off until the CI concentration returns to a normal level. In two clinical trials, patients were treated with AAV vectors carrying factor IX either by direct injection into muscle or by infusion in the liver via the hepatic artery. The 19K protein is a functional homologue of the cellular protein Bcl-2, which is a suppressor of apoptosis. Small amounts in virions; enhances virus assembly and budding, possible role in hydrogen ion transport. For example, many viruses of higher plants spread from cell to cell throughout a plant by passage through intracellular channels (plasmodesmata), using specific viral proteins to enhance passage of unencapsidated viral genomes. PB1 then uses this capped fragment as a primer for copying genome RNA (dark blue line) into viral mRNA (light blue line). Such DNA fragments must have contained a nucleotide sequence that was recognized by the cellular DNA replication machinery as a replication origin. • Retrovirus vectors have been used successfully to treat children suffering from severe combined immunodeficiency disease, but several of these children developed leukemia. Retroviruses have a unique replication cycle based on reverse transcription and integration of their genomes The first members of the retrovirus family were described at the beginning of the twentieth century, when a virus responsible for equine infectious anemia • • • Simple retroviruses: Rous sarcoma virus (chickens), mouse mammary tumor virus (mice), Jaagsietke sheep retrovirus. Fusion can take INFECTED CELL Budding Exocytosis place either at the plasma membrane or at the membranes of early or late endosomes. T7 RNA polymerase is easily purified, is a very efficient and rapid enzyme, and is highly specific for its own late promoter sequences. CH024.indd 299 16/05/11 8:12 PM 300 CHAPTER 24 Herpesviruses kinases are activated when the B-cell receptor binds to an antigen, leading to a cascade of events that eventually generates plasma cells, which produce specific antibodies (see Chapter 34). For viruses with a type II IRES element, the conserved AUG is used for initiation of translation. Although it is nominally an early protein, the 11.6-kDa protein is expressed at high levels during the late phase when its mRNA is made using the major late promoter and an E3 polyadenylation site. Bacteria Archaea Lower eukaryotes (fungi, protozoa, algae) Plants Invertebrates Vertebrates (including humans) Note that these categories do not correspond to those in the current "six-kingdom" model of classification CH003.indd 32 of organisms; instead, this is a pragmatic grouping of life forms that is useful for the present discussion of virus classification. The expression of these genes inhibits the normal process of apoptosis, allowing for greater production of budded virus and development of the very late stage of virus infection. Alternative splicing of late transcripts, in contrast, generates distinct proteins by removing AUG start codons, leading to initiation of translation at different start sites, in the same or different reading frame. Viroids are small, single-stranded RNAs that do not code for any proteins, yet can replicate and cause disease in plants. For viruses that infect unicellular organisms, this usually involves death and lysis of the host cell, and many viruses code for specialized late proteins that lead to cell death. This outstanding display of biodiversity is mirrored in genome structure, size and, crucially, life style, and creates a myriad of problems for authors aiming to produce a chapter summarizing the general properties of such a diverse family! This is further exacerbated by an extensive lack of knowledge of the fundamental workings of these interesting, but mysterious, viruses. Similar tRNA-like structures are found at the 3'-termini of the genomes of a number of other positive-strand RNA plant viruses, including other members of the Bromoviridae family, as well as turnip yellow mosaic virus and tobacco mosaic virus. Donald Summers and Jacob Maizel found that picornavirus proteins are synthesized as large polyproteins, which are subsequently cleaved into the various structural and enzymatic viral proteins. CH011.indd 135 16/05/11 8:02 PM 136 CHAPTER 11 Picornaviruses 4. The virus is efficiently transmitted in aerosol droplets derived from coughs and sneezes. Antigenic change occurs in two ways: (1) slowly but continuously ("antigenic drift"), and (2) suddenly but episodically ("antigenic shift"). Readthrough RNAs hybridize with early transcripts and lead to their degradation. In the case of the hepadnaviruses, this reverse transcriptase is used to synthesize the partially single-stranded DNA genome that is packaged in virions. Cro binds to the same operators as CI repressor, but with different affinities, and without cooperativity. CH016.indd 194 16/05/11 8:05 PM Filoviruses changes the reading frame downstream of that site by one nucleotide (frame –1). For example, interferon- can switch production from IgM to IgG2a and IgG3 in stimulated B cells. • Rolling circle replication makes long concatemers of lambda DNA that are cleaved and packaged into proheads; scaffolding proteins are shed during packaging. Toroidal coil: a spring-like coil that consists of a cylindrical form bent into a circle. In cases where the capsid is released into the cell, a variety of pathways lead to disintegration of the capsid and release of the genome, called uncoating. Viruses are grouped into species, genera, and families This chapter outlines information about the major virus groups presently identified, with particular emphasis on viruses that infect bacteria and vertebrates, the subject of most of the remainder of this book. Virus infectivity can be inhibited by monoclonal antibodies specific for the orthobunyavirus Gc protein, and an orthobunyavirus Gc mutant is defective in the fusion process, showing that Gc is involved in entry into the cell. The homologous genes mainly code for proteins involved in virion assembly, capsid and tail formation and DNA modification. In 1957, the new Asain human virus contained an HA gene, an NA gene, and a PB1 gene that were all apparently derived from an avian influenza virus, while the other genes were the same as those in the original H1N1 human virus. The [Het-s] state has been shown to mediate heterokaryon compatibility and prevents exchange of nuclei between incompatible yeast genotypes. An example of the former: the side tail fibers of phage T4 and The interacting Rz and Rz1 protein pair, called a spanin, is proposed to span the periplasm, linking and subsequently fusing the inner and outer cell membranes: Rz is an integral cytoplasmic membrane protein, and Rz1 is an outer membrane lipoprotein. These particles contain reduced levels of both core proteins and outer capsid proteins compared with mature virions. These are shown in an extended, uncoiled form. In 1983, a retrovirus isolated from the blood of individuals with AIDS was characterized by groups led by Luc Montagnier in Paris and Robert Gallo in Maryland. Coccolithoviruses infect the cosmopolitan and ecologically important coccolithophore Emiliania huxleyi, a tiny (5 m diameter) marine alga (Figure 27.6) that floats freely in the ocean. • The phycodnaviruses and mimivirus are full of novel genes of unknown function. Usually require multiple doses to achieve protection. Hepatitis B virus, in contrast, initiates DNA synthesis on the pregenome RNA template by covalently linking the first nucleotide of the DNA chain to the hydroxyl group of a tyrosine residue in the polymerase protein itself. The antigenome RNAs are then replicated and assembled into virions. This core is associated with the virus membrane protein (M). Although all parvoviruses employ the same principle, the scheme is simpler with adeno-associated virus since the two ends of the viral DNA are identical. ICP34.5 binds to protein phosphatase 1 and redirects it to efficiently dephosphorylate eIF-2, thereby reversing the effect of the activated PKR. CH001.indd 12 16/05/11 7:46 PM Steps in the Virus Replication Cycle There is often a long period during which progeny virus is being produced, but at some point virus replication stops, often because the host cell dies. What were the two accomplishments of the Yellow Fever Commission that brought yellow fever under control in the United States? (c) Sixty commas distributed with icosahedral symmetry on the surface of a sphere. This virus appears sporadically in the eastern half of the United States, with a number of notable outbreaks in Florida near Orlando. P53 regulates apoptosis in normal cells. 32.1: Peter Morrison/AP/Wide World Photos; Fig. Avian retroviruses and human immunodeficiency virus type 1 (HIV-1) employ a different strategy. 19/05/11 12:19 PM 310 CHAPTER 25 Baculoviruses Polyhedrin promoter Inserted foreign gene Multiple cloning site Viral flanking sequences Cleave; ligate foreign gene sequence Recombinant transfer vector Bacterial plasmid sequences (a) Construction of transfer vector mRNA expressing foreign gene ⩽ Flanking sequences Infect and transfect cells; recombination Viral genome Recombinant viral genome (b) Production of recombinant virus Figure 25.5 Preparation of a recombinant baculovirus designed to express a foreign gene product. What is this material and what is its composition? The genetic map (Figure 8.2) shows that genes are arranged in functional blocks. Schematic views of several known virus receptors, shown protruding from the plasma membrane at the cell surface. 33.11: Reprinted by permission from Macmillan Publishers Ltd: from Figure 2 in Gunter Meister & Thomas Tuschl, "Mechanisms of gene silencing by double-stranded RNA," Nature 431 (2004), 343-349. Importin (also called karyopherin): receptor protein that interacts with nuclear localization signals in proteins. Its envelope contains hepatitis B surface (S) proteins and lipids; however, electron microscopy does not reveal a typical unit membrane structure, and the envelope contains a lower proportion of lipids than most membranes and viral envelopes. Infection at 85⬚C, the optimal growth temperature for host cells, results in integration of the viral genome into the host cell chromosome, leading to lysogenization of the host cell and suppression of lytic functions of the virus. In contrast, the S RNAs of most hantaviruses and nairoviruses contain only a single open reading frame, encoding the nucleocapsid protein. Schematic diagram showing relative positions of structural proteins and RNAs in the virion. F-pili: filamentous protrusions from the surface of E. However, penetration may follow a pathway similar to that of the large, tailed bacteriophage T4. tauri. These multistranded structures result from highly efficient reinitiation of synthesis of positive strands on PCBP2 5' 3CD UU the template RNA, before the synthesis of previously initiated positive strands is completed. Viral proteins are transported back into the nucleus (4 and 6), where they mediate genome replication (5) and transport of progeny nucleocapsids back to the cytoplasm (7). Replication protein C helps to dissociate DNA polymerase ␣/primase and load DNA polymerase onto the 3' ends of the growing DNA chains. Consider a 5000-nucleotide single-stranded RNA or DNA genome, which can code for proteins totaling about 1500 amino acid residues (1 amino acid per 3 nucleotides). Progeny virions are released from the host cell 16 2. Although very different in primary amino acid sequence, VP1, VP2, and VP3 all fold into a common structure that resembles a wedge shape, or a jelly-roll, composed of an eight-stranded -barrel 127 Figure 11.2 Picornavirus virion structure. Most experiments on virus binding and entry have been done with 16/05/11 8:05 PM 194 CHAPTER 16 Filoviruses replication-defective pseudotypes, using filovirus GP incorporated into the envelopes of unrelated recombinant viruses; these systems may not reflect the authentic entry pathways of filoviruses. Retroviruses 342 Alan Cochrane, University of Toronto TOC.indd vi 37. Virus Entry 45 Ari Helenius, Swiss Federal Institute of Technology, Zurich SECTION II: VIRUSES OF BACTERIA AND ARCHAEA 5. CH014.indd 167 16/05/11 8:04 PM 168 CHAPTER 14 Coronaviruses Figure 14.5. Briefly, the viral RNA polymerase initiates transcription at the 3' end of (positive-strand) genome RNA and makes a (negative-strand) RNA copy that stops at the end of a transcription-regulating sequence (TRS). It is notable that different members of the Reoviridae family contain between 10 and 12 gene segments, which allows each segment to be transcribed at one of the 12 spike bases. Viral RNA is converted into a double-stranded DNA copy by reverse transcription In the cytoplasm, deoxynucleoside triphosphates penetrate into the core, and the process of conversion of viral RNA to double-stranded DNA begins. nsp2 Unknown, dispensable for replication in cultured cells of murine hepatitis virus and SARS coronavirus. More recently, CH016.indd 197 197 viral RNA and antiviral antibodies were detected in fruit bat species from known outbreak sites, and Marburg virus has been isolated from wild-caught Rousettus aegypticus, a cave-dwelling fruit bat species. Furthermore, the presence of many non-optimal codons in both the E and H genes most likely reduces the level of synthesis of these proteins. Complement. Reverse transcription progresses for only a few nucleotides beyond the 5⬘ stem-loop, after which the enzyme and the newly synthesized DNA are translocated onto an identical sequence in the direct repeat region 1 (DR1) near the 3⬘ end of the pregenome RNA. Adenoviruses types 2 and 5 also utilize certain cell surface integrin proteins, including integrins ␣V3 and ␣V5, as secondary receptors. 4. For more information, please visit our website: www.wiley.com/go/citizenship. CH031.indd 381 16/05/11 8:16 PM 382 CHAPTER 31 Viroids and Hepatitis Delta Virus It is unclear how the multimeric plus-strands of Pospiviroidae are cleaved to give monomers, because a self-cleavage activity has not been found. T7 and its relative, T3, are the smallest of the T phages. High levels of late transcripts are made after DNA replication begins Initiation of late transcription on both mouse polyomavirus and simian virus 40 DNA occurs at multiple promoter sites within a 100-nt region that overlaps with the transcriptional enhancer. However, the monophosphate species of AZT blocks the cellular enzyme, thymidylate kinase, contributing to toxicity. (c) Production of a self-inactivating retrovirus vector. Dependoviruses, like adeno-associated virus rely on coinfection by a helper virus to push the cell into the S phase and for other functions. • A terminator of transcription, T, releases the T7 polymerase at the end of the class II region. However, the bulk of X protein resides in the cytoplasm, and its major function may be to regulate signal transduction pathways. Nucleocapsid has icosahedral symmetry (T 4). Interferons provide an early line of defense against viral infections, and are part of the host's intrinsic cellular and innate immune responses to infection. Therefore, even though a cell takes up an intact and potentially infectious virion, it may not produce any progeny virus. Thymidine kinase (UL23), ribonucleotide reductase (UL39/UL40), and deoxyuridine triphosphatase (UL50) are involved in producing nucleoside triphosphates used as building blocks for DNA replication, while uracil N-glycosylase (UL2) is involved in DNA repair. Viroids and RNA viruses may have originated in the RNA world Viroids and small single-stranded RNA satellites share some unique characteristics that indicate that they may be relicts of the RNA world that survive to this day. Campbell proposed in 1961 that the prophage inserts into the bacterial chromosome by site-specific recombination between a circular form of the chromosome and the bacterial chromosome. How To Use This Book This textbook is designed to be used in a modular fashion. The finding that mutations in E2 can alter receptor binding confuses attempts to identify specific cellular receptors. A final question is why the Q plus-strand conceals its 3' end even though replication needs unpaired nucleotides to initiate. Retroviruses are the best-known viruses that use reverse transcriptase; the enzyme was first discovered in these viruses. CH023.indd 276 16/05/11 8:11 PM Adenoviruses x y pVIII 33K 100K E1B 55R 171R 217R 243R 289R E1A 0 VA RNA 2 V pVII penton (III) I II Tripartite leader 1 L5 L4 23K (protease) hexon (II) L3 pVI IX 131R/168R 19K/93R 19K/156R 19K/84R 19K/55K fiber (IV) z 277 3 20 IVa2 IIIa 52/55K 40 80K pTP DNA pol L2 14.7K 14.5K 11.6K 10.4K/14.5K 6.7K/gp19K 12.5K E3 L1 60 72K DBP 80 100 Orf 1/2/3/4 E2A Orf 3/4 Orf 6 Orf 6/7 E2B E4 Figure 23.4 Transcriptional map and gene products of adenovirus type 5. Means "shivering" or "trembling" in the Fore language. These caspases cleave a CH033.indd 404 EXTRINSIC TNFα TNF receptor PATHWAY Adaptor Procaspase-8 DISC MDA-5 IPS-1 Caspase-8 Bax Cytochrome c p53 Apoptosome Procaspase-9 DNA damage; Viral DNA replication INTRINSIC PATHWAY Caspase-9 Procaspases-3/7 Caspases-3/7 APOPTOSIS Figure 33.4 Intrinsic and extrinsic pathways of apoptosis. • To make vectors, the transfected cells must either be engineered to express essential viral genes, or be infected with a helper virus that provides these functions. Interferons and the development of CD4-positive helper T cells The distinct helper T-cell populations are defined by the cytokines they produce after stimulation. This protein was isolated and found to be a monomeric, 100 kDa RNA polymerase that is highly specific for class II and class III T7 phage promoters. The nearest start site on Sendai virus RNA is for the N messenger RNA, 56 nt from the 3' end of the genome RNA (Figure 15.5). In cases where homologs are found, they are nearly exclusively from other viruses or plasmids of archaea, such as with the bicaudavirus ATV (Figure 9.12). Formation of stable transformants requires coexpression of E1A and either E1B 19K or E1B 55K proteins, preferably both. What is meant by the term "moving template" model of transcription used by reovirus cores to synthesize mRNA? Innate immune response: the part of the immune response that acts by rapid and relatively nonspecific detection of pathogens. Each monomer consists of three domains. To make room for these chapters, a chapter on human T-cell leukemia virus was removed, but it is available for book users on the text's companion website (www.wiley.com/college/acheson). Most importantly, advances in molecular biology have given us the capacity to modify these organisms to carry foreign genetic material. Reoviruses also have been used to study RNA synthesis. A site named sib, located just beyond the attP site downstream of the int gene (Figure 8.2), contains a transcription termination sequence. Marine field surveys and cross-reactivity tests between H. 16/05/11 7:52 PM 54 CHAPTER 4 Virus Entry shows that hepatitis B virus capsids (diameter of 34 nm) and simian virus 40 capsids (diameter of 48 nm) can pass intact through the nuclear pores. 16/05/11 8:05 PM Paramyxoviruses and Rhabdoviruses The "rule of six" Mutational analysis of paramyxovirus genomes revealed an intriguing length restriction for genome replication. 16/05/11 8:21 PM 452 CHAPTER 36 Antiviral Chemotherapy Due to the ability of the virus to rapidly develop resistance to these compounds, they are used in combination with other drugs. Elongation of minus-strand DNA; RNA template degradation ε 1' Cap 2' 1 5. Viruses have developed many ways to evade complement actions. RNA phage genomes contain extensive regions of secondary structure. The intrinsic pathway can be induced in response to DNA damage or viral DNA replication, which activate the cellular protein p53. What role does the viral E7 protein play in replication? Although interferon- possesses antiviral activity, it is primarily an immune modulator rather than an antiviral agent. Likewise, a Type IV IRES structure has been proposed for the porcine teschovirus genome RNA. Each of these components contains a stretch of unique sequences, UL, and US, flanked by inverted repeats. Eventually the amoeba undergoes lysis at approximately 14 hours post infection. Virions have dimensions of approximately 60 100 nm. Modification of domains on the fiber protein involved in receptor binding can lead to the production of differently targeted vectors. The number of algal species (mostly singlecelled microalgae) has been estimated to be as high as several million. Segmented genome. The NSm coding region is contained in the 5'-terminal part of the tospovirus genomic RNA, but is translated from a subgenomic mRNA. Adaptive mutations in this gene can simultaneously create mutations in the overlapping genes and change their functions. From 95 to 98% of the biomass in the oceans is microbial (the CH001.indd 5 remaining 2–5% being made up of all other forms of life, including fish, marine invertebrates, marine mammals, birds, and plants), and roughly half of the oxygen in the Earth's atmosphere is generated by photosynthetic activity of marine microbes. Subunit vaccines generally stimulate only humoral immunity. Th1 cells contribute to the maturation of Tc cells via their production of interleukin-2 and interferon-. The normal cellular protein, called PrPC, is a copper-binding protein of uncertain function. Like the leader sequence on genome RNA, the trailer region, located at the 3' end of the antigenome RNA, contains the promoter for genome replication, and its negative-strand transcript contains signals for genome packaging. • Phycodnavirus genomes harbor genes that encode the smallest known versions of many proteins. The inflammatory response is mediated by cytokines and migrating leukocytes The accumulation of leukocytes at sites of infection, the local activation of their antimicrobial functions, and the production of cytokines are collectively called the inflammatory response, because these activities lead to local inflammation of tissues. Table 4.1 lists viruses known to enter through an endosomal, acid-triggered pathway, and viruses that use pH-independent entry. KEY TERMS 2', 5' oligoadenylate synthetase Complement Concatemer Cytokine Endemic Glycosaminoglycan Guanylyltransferase Interferons Interleukin-1 Micelle Microvilli (singular: microvillus) Phagocytosis PKR Pock Resolvase Ribose methyltransferase Serpin Tumor necrosis factor (TNF) Vaccination Variolation Viroplasm FUNDAMENTAL CONCEPTS • Immunization against smallpox (1796) was the first successful vaccination attempt. Individual viral membrane proteins such as the influenza viruses hemagglutinin and neuraminidase can be purified and crystallized, allowing determination of their molecular structures. However, virus isolates obtained from the brains of children who died from infection by La Crosse virus in 1960, 1978, and 1993 revealed remarkable genetic stability: only 51 nucleotide changes were detected in the 4526 nt M genome segment over this 33-year period. The proteins are named based on their function, glycosylation status, or molecular weight, and are listed in Table 16.1 in the order of their genes from the 3' end of the genome. cleavage N Leu-Asn-HN Ile O O Leu-Asn-Phe-Pro-Ile HN NH OH A-74702 Protease inhibitors can interfere with virus assembly and maturation Many viruses encode proteases that are essential for viral replication, often at the stage of assembly and maturation. RNA polymerases that initiate transcription within the lefthand LTR can dislodge transcription factors bound to U3 R U5 U3 R U5 Proviral DNA Transcription Capping Cleavage & polyadenylation Cap R U5 Gag Pol U3 R Poly (A) Full-length RNA 5' ss 3' ss Splicing Cap R U5 Env U3 R Poly (A) Spliced RNA Figure 28.6 Production of retrovirus RNAs. Transcription initiates at junction of U3 and R, and RNA is cleaved and polyadenylated at junction of R and U5. The girl shows the spastic strabismus, often transitory, which most children with kuru developed early in the course of the disease. • Togaviruses synthesize RNA polymerase, capping enzyme, and proteinases by translation of full-length genome RNA. It is important that the immune response not be prolonged beyond what is necessary to fight the present infection, and these anti-inflammatory cytokines do just that. Knockout mice lacking PrPC remain healthy throughout development, suffering only minor electrophysiological defects. Plants and simpler eukaryotes use RNA interference as a defense mechanism against virus infections. Influenza virus enters cells via receptor-mediated endocytosis, yet replication of the virus is inhibited by drugs that block ion channels. The genomes of other herpesviruses show variations on the theme of interspersed unique and repeated sequences. In severe cases, the sheep's wool is rubbed off their sides and back. The cII gene, located just downstream from cro (Figure 8.6), is transcribed from the PR promoter and sponsored by N antitermination. Roberts, Joan Argetsinger Steitz, and Alan M. Vpu binds to the cytoplasmic domain of CD4 and through interaction with -TrCP directs CD4 to the proteasome degradation pathway. 2.11a: Adapted from Fig. Many do not have an identifiable DNA polymerase gene. Phosphorylation results in the formation of an inactive complex that involves eIF-2 bound to guanosine diphosphate 16/05/11 8:19 PM Interferons 411 Met GTP Met eIF-2 GTP eIF-2 GTP 40S eIF-2 GDP Preinitiation complex GTP GDP Met GDP eIF-2 eIF-2B eIF-2 60S P P 40S Initiation complex Activated PKR GDP eIF-2 eIF-2B P ds RNA Inactive PKR Figure 33.10 Mechanism of antiviral activity directed by PKR. Although some parvoviruses were originally isolated from tumor-bearing animals or tumor cells, they are not oncogenic. Steps 2 and 3. This allows the cell to survive long enough to produce high levels of progeny adenovirus virions before eventually succumbing to the effects of virus infection. Moore et al., "Ataxia in prion protein (PrP)-deficient mice is associated with upregulation of the novel PrP-like protein doppel," Journal of Molecular Biology 292 (1999), 797–817, with permission from Elsevier; Fig.

Certain enveloped viruses of bacteria, archaea, and algae have a rigid capsid surrounding an internal lipid bilayer, which is stuffed full of genome DNA or RNA. This poses a problem: if the infecting viral DNA is first transported to the nucleus, where it is transcribed to generate 05/05/11 11:24 AM 330 CHAPTER 27 Viruses of Algae and Mimivirus (a) (b) (c) (d) Figure 27.5 Infection of Chlorella strain NC64A by PBCV-1. All cellular organisms store the information required to sustain life, to grow, and to reproduce exclusively in DNA molecules, and all RNA molecules in these organisms are transcribed from DNA sequences. Infected children have different disease outcomes; only about 50% manage to clear the virus and 40% become chronic carriers, perhaps due to the underdeveloped state of a child’s immune system. The primary receptor is usually sufficient for attachment and inducing conformational changes, the secondary receptor (or “coreceptor”) may be needed to trigger endocytosis or penetration. Raphidoviruses infect algae from the class Raphidophyceae, which are often associated with toxic red tides and subsequent fish kills, particularly in the aquaculture industry. Thioredoxin interacts electrostatically 3⬘ 3⬘ 3⬘ 5⬘ (c) Figure 7.3 Initiation of DNA replication at the leftward T7 promoter. Inserted within a turret-like opening of the spike pentamer is the trimeric attachment protein, 1 (36 copies). It contains a transmembrane domain and initially associates with the endoplasmic reticulum in infected cells. On the other hand, phage RNA replicases incorporate approximately 35 nucleotides per second into a growing chain. RNA helicase: an enzyme that unwinds helical structures (stems) in single-stranded RNA molecules, or unwinds the strands of double-stranded RNA molecules. Surprisingly, these CH017.indd 208 antigenome RNAs can act as messenger RNAs even though they are neither capped nor polyadenylated, and small amounts of viral proteins are made in the transfected cells. Advantages and limitations of AAV vectors Because AAV does not cause disease, AAV vectors are considered to be very safe for administration to humans. A final development would have been the generation of a polypeptide with DNA polymerase activity, enabling the replication of the DNA genomes. host plant (Figure 10.11). Some 95% of the known bacteriophages are tailed phages with double-stranded DNA genomes. • Plants and invertebrates do not express interferon, but instead use RNA interference to fight against viral infections. The linear dsDNA genome is shown in a two segments for convenience. Tropism: the ability of a virus (or other pathogen) to infect and replicate in a particular tissue, organ, or species. The C-terminus of the polymerase protein interacts with the core protein to begin encapsidation only when the polymerase is bound to the stem-loop. Efforts to develop antiviral drugs have focused mainly on those viruses for which effective vaccines or public health measures are not available, and that cause widespread and easily diagnosed diseases whose treatment would earn substantial profits. ACV is incorporated into growing DNA chain, blocking further chain growth pppdC ACV dC dG 3. Viruses have developed a number of strategies to counter both intrinsic cellular defenses and adaptive immune responses. Viruses similar to SARS coronavirus were found in caged civet cats and raccoon dogs, carnivores that are captured or farmed and sold in markets for food in Guangdong province, in southern China. For example, the 10 head assembly genes are followed by 11 tail assembly genes, and so on. PRE, the promoter for repression establishment, is used to make the initial dose of CI repressor that in turn activates PRM. Glycosylation of the ectodomain ensures that the external surface of virus particles is hydrated, reducing protein/protein interactions that could aggregate the virions. In the case of polyomavirus, Figure 1.5 shows that small amounts of “early” viral messenger RNAs are made beginning about 8–10 hours after infection, and early viral proteins, known as T antigens, begin to appear shortly afterward. (b) Outline of an icosahedron, showing fivefold (pentagons), threefold (triangles), and twofold (oval) symmetry axes. This allows researchers to study host responses to viral infections, and may lead to development of useful immunotherapeutic and anti-inflammatory reagents. It remains to be determined if these are distinct compartments with a required process of delivery, or whether sites of replication and assembly are part of an interconnected and continuous replication/ assembly network. Patients infected with filoviruses must be isolated, and protection of medical and nursing staff is required. Given this track record, the study of viruses will undoubtedly continue to shed light on many important aspects of cell and molecular biology. Azidothymidine: nucleoside analogue used for treatment of human immunodeficiency virus infections. Electron micrographs of thin sections of infected cells reveal virions with a doughnutlike core structure that represents the helical nucleocapsid underlying the viral envelope; the helical nucleocapsid can also be seen in negatively stained disrupted virions. RNA genomes are shown as curved light blue lines; they are converted to double-stranded DNA (provirus), shown as parallel straight dark blue lines, by reverse transcription. All known RNA viruses have linear genomes. Some examples of these adaptations are: • Specific mechanisms for transmission between individual cells or organisms (via direct contact, diffusion through liquid media, dispersion via the circulatory system, respiratory droplets, stool or urine contamination of food or water, insect vectors, etc.) • Entry pathways into cells (binding to specific cell surface receptors, passage through cell walls, entry by membrane fusion, uptake via vesicles, etc.) • Interaction with cellular RNA or DNA polymerases, RNA processing enzymes, protein synthesizing systems, membrane systems, cellular signaling proteins, and other cellular proteins used by the virus to express and replicate its genes As a result of these adaptations, viruses that are genetically and evolutionarily related tend to infect related organisms. was isolated. Notably, this is the first T7 gene transcribed as the DNA enters the cell, so the protection is established rapidly. KEY TERMS Adjuvant Autoimmune demyelination Cellular immune responses Chimera (adj: chimeric) Cold chain Cytokines Cytotoxic T lymphocytes Dendritic cells DNA vaccines Epidemic Epizootic Gene gun Herd immunity Humoral immune response Immunogenic Lectins Monoclonal antibodies Neutralizing antibodies Pandemic Panzootic Primary cell cultures Proteosomes Tolerance Toll-like receptors Type 1 and type 2 T helper lymphocytes Zoonotic FUNDAMENTAL CONCEPTS • No vaccine can ever be 100% safe, but vaccines are among the safest medical interventions ever introduced. Which of the hepatitis B virus proteins is associated with the ability of the virus to cause cancer? Copyright The Nobel Foundation, 1976; Fig. The idea worked and highly potent inhibitors were derived. A crucial discovery came from studies of people who had been exposed repeatedly to HIV-1, yet did not develop AIDS. Several factors contribute to the lack of expression of viral genes in neurons. Viruses that use reverse transcriptase package this enzyme in the virion, as viruses with negative-strand or CH003.indd 39 Genome segments The length of viral and cellular genomes One turn of the DNA double helix contains 10 base pairs and is 3.4 nanometers (nm) in length. Early viral proteins direct replication of viral genomes Once early viral proteins are made, they act to promote replication of the viral genome. • In response to viral infection, cytokine signaling and sensing of intracellular stress can lead to apoptosis, or programmed cell death. Viruses of Archaea 97 Archaea, the third domain of life 97 Viruses of Archaea have diverse and unusual morphologies 99 Fuselloviridae are temperate viruses that produce virions without killing the host cell 99 Genomes of fuselloviruses are positively supercoiled 101 Transcription of SSV-1 DNA is temporally controlled 101 Filamentous enveloped viruses of the Lipothrixviridae come in many lengths 102 A droplet-shaped virus is the only known member of the Guttaviridae (from the Latin gutta, “droplet”) 103 Acidianus bottle-shaped virus (ABV): its name says it all! 103 The genome of Pyrobaculum spherical virus has nearly all open reading frames encoded on one DNA strand 104 Viruses in the family Rudiviridae (from the Latin rudis, “small rod”) are non-enveloped, helical rods 105 Rudiviruses escape from the cell by means of unique pyramidal structures 106 Acidianus two-tailed virus (ATV) has a virion with tails that spontaneously elongate 106 Infection with ATV at high temperatures leads to lysogeny 106 Two related viruses of hyperhalophiles resemble fuselloviruses by morphology but not by genetics 108 Two unusual viruses with icosahedral capsids and prominent spikes 108 A virus with a single-stranded DNA genome is closely related to a virus with a double-stranded DNA genome 108 Comparative genomics of archaeal viruses 109 Conclusion 110 13/05/11 2:11 PM x Contents SECTION III: POSITIVE-STRAND RNA VIRUSES OF EUKARYOTES 10. 9.7d: Courtesy of Philippe Lemercier; Fig. Deletions in E2A (single-stranded DNA binding protein), E2B (viral DNA polymerase/preterminal protein), and E4 were engineered. Lipids are delivered to crescents from the endoplasmic reticulum in a novel form, perhaps as small vesicles or micelles containing viral envelope proteins. It is well known that human immunodeficiency virus uses this strategy. Antiviral drugs are useful for discoveries in basic research on viruses Aside from their importance in treating viral diseases, antiviral drugs make excellent laboratory tools. Their major advantage is that they present no danger of inadvertent infection with live pathogenic virus, which can be a problem with the other vaccine categories. Preintegration complex: a complex of retrovirus capsid proteins and newly synthesized proviral DNA in a state ready to be integrated into the cellular genome. P has four characteristic domains: an N-terminal domain for priming minus-strand DNA synthesis; a spacer domain; a reverse transcriptase domain, which occupies roughly 40% of the protein; and a ribonuclease H domain. 05/05/11 11:24 AM 328 CHAPTER 27 Viruses of Algae and Mimivirus Figure 27.2 Map of the genome of Paramecium bursaria chlorella virus-1 (PBCV-1). 18.1: Courtesy of Robert Dourmashkin, London Metropolitan University; Fig. Components of the virion are labeled; see Table 29.2. For the sake of clarity, only one of the two genome RNA molecules is shown covered by NC protein. The molecular motors used include the microtubule-dependent motor dynein and an adaptor complex called dynactin. 16/05/11 8:02 PM Picornaviruses Interaction of picornavirus IRES elements with host cell proteins Because no viral proteins other than VPg accompany picornavirus RNA upon entry into a cell, the factors that direct translation at an IRES must be preexisting cellular proteins. Central nervous system tissue from such animals reveals large vacuoles in the cortex and cerebellum, giving the brain the appearance of a sponge. 20.4: Courtesy of N. The 2A proteinase of enteroviruses and rhinoviruses cleaves at the P1/P2 junction, and it is also responsible for cleavage of eIF-4G. Interaction of these regions would lead to the creation of a “Q”-like circular form of DNA containing short and long tail extensions, 259 bp and 22,515 bp, respectively (Figure 27.15). Why is only the left-hand LTR used for signaling initiation of transcription? The nucleocapsid of Sendai virus is wound as a 20-nm-diameter, left-handed helix, with 13 subunits of N protein per turn of the helix, a pitch (distance between adjacent turns) of 5.3 nm, and a central hole 5.5 nm in diameter. Non-enveloped viruses interact with cellular membranes, leading to the penetration of either the capsid or the genome into the cell, beginning the replication cycle. The mature virions are then secreted into the extracellular environment via exocytosis. As much as 50% of the population of Europe is estimated to have been killed by these agents between 1300 and 1700 A.D. The indigenous populations of the Americas were decimated at least in part by epidemics of viral diseases in the 100 years following the arrival of Europeans in the mid-sixteenth century. The lipid bilayer provides a permeability barrier that protects the viral genome from the environment, so that enveloped viruses do not need to assemble their proteins as perfectly as do viruses that lack envelopes. This approach is called a cell-based high throughput screen, because it involves applying chemicals to cells infected with the virus and assaying the effect on virus replication. In a novel approach currently being tested in clinical trials, a replication-competent adenovirus mutant is injected into human tumors. CH023.indd 279 279 signal to the cell that something is wrong, and activated p53 induces growth arrest and cell death by apoptosis. The recent discovery in bats of many new coronaviruses belonging to two groups of mammalian coronaviruses (alpha and beta) has redefined the spectrum of coronaviruses and raises the intriguing possibility of bats as evolutionary pools for new coronaviruses in other animals. This compound acts by inhibiting a viral enzyme, integrase. An anti-HIV drug, enfuvirtide, which binds to HIV-1 virions and prevents virus entry, was approved by the FDA in 2003. 1885 Pasteur and collaborators introduce air-dried rabbit spinal cord as rabies vaccine. Infection with hepatitis B virus nevertheless remains a problem for certain occupational groups such as morticians, doctors, dentists, dialysis workers, hemophiliacs, mental care facility workers, tattoo-parlor workers, firefighters, police officers, daycare workers, and barbers. Their high academic standards and constant effort ensured its success. It is the most successful test invented for cervical cancer prevention. The development of in vitro transcription assays led to the identification of viral proteins required for transcription from late and very late promoters (Table 25.3). This argument suggests that a large, enveloped DNA-containing virus that had evolved to code for most of the functions required for its replication could have established itself persistently in a prokaryotic cell. Similarly, the N-antiterminated transcript from PR extends through cII and the replication genes O and P all the way to gene Q, which encodes the late gene activator. Other applications such as gene replacement therapy require long-term expression, ideally for the lifetime of the individual. ICP0. 16/05/11 8:07 PM Influenza Viruses 215 PB2 PA 5' 3' PB1 Figure 18.5 Helical nucleocapsid of influenza virus. In vivo studies demonstrated a rather simple transcriptional pattern for both SIRV-1 and SIRV-2, with very few cases of temporal regulation. In mosquitoes infected with two virus strains, reassortment has been documented in salivary glands and ovaries, and reassortant viruses can be transmitted both transovarially and by feeding. Thus, this new technique provides a means to directly and precisely engineer the viral genome in the context of infectious virus. Another outbreak of Marburg virus disease began in Angola in 2004–2005, and is the biggest outbreak so far encountered. Several of these strategies are already in advanced-stage clinical trials. (“Thrips” is both singular and plural.) Thymidine kinase: an enzyme that phosphorylates thymidine to thymidine monophosphate. In contrast, genomes of complex plants and animals contain mostly noncoding sequences; for example, the human genome codes for about 30,000 proteins, or one protein per 100,000 nucleotides. This allows the mRNA transport and translation machinery to concentrate on these RNAs. The Cullin-based E3 ubiquitin ligase complex discussed above, containing the E1B 55K protein and the E4orf6 protein, is responsible for selective transport of late mRNAs to the cytoplasm (Table 23.1). NS1 is translocated into the lumen of the endoplasmic reticulum, where it is cleaved from the polyprotein at its N-terminus by signal peptidase (Figure 12.5) and at its C-terminus by an unknown protease. (b) Extension of DNA chains beyond the initiation region by DNA polymerase associated with replication protein C and proliferating cell nuclear antigen (PCNA). Short DNA fragments joined to RNA primers are found in replicating T7 DNA, so it is clear that T7 DNA replication resembles that of the E. Retinoblastoma: a tumor of the retina. Mature virions or capsids are shown as green hexagons, genomes as diagonal lines, viral mRNAs as blue lines, and viral proteins as orange ovals. • Influenza viruses are negative-strand RNA viruses whose eight genome segments code for a total of 11 different viral proteins. GENOME Circular, double-stranded DNA, 80–180 kb. 9.11i: Courtesy of Philippe Lemercier; Fig. 16/05/11 8:21 PM 450 CHAPTER 36 Antiviral Chemotherapy pppdG pppACV dC dG ACV dC dG 1. VIRUSES AND HOSTS Paramyxoviruses: • • Humans: measles, mumps, respiratory syncytial, parainfluenza viruses Animals: Newcastle disease virus (chickens), canine distemper virus, rinderpest virus (cattle) viruses infecting birds, pigs, dogs, cats, seals, whales, etc. Cell-to-cell movement: movement of a plant virus from one cell to another. Carboxylic ionophors: compounds that bind monovalent cations (including protons) and increase their membrane permeability. During translation of subgenomic mRNA, the capsid protein is released from the growing polypeptide chain by self-cleavage (arrow with black square). 16/05/11 8:12 PM 294 CHAPTER 24 Herpesviruses Viral DNA in the form of head-to-tail concatemers is cleaved and stuffed into the preformed capsids by the interaction of several viral late proteins with highly conserved packaging sites (pac1 and pac2) located in the a sequence near the termini of the DNA. Cyclin-dependent kinases (CDKs): protein kinases that, in combination with cyclins, regulate the cell cycle by phosphorylating other proteins. (a) (c) (b) (d) 337 Why such a large genome? Viruses in these two groups differ fundamentally in how their replication cycle begins. There are four families of icosahedral single-stranded DNA viruses: Microviridae (bacteriophages), Geminiviridae (plant viruses), and Circoviridae and Parvoviridae (animal viruses). Viroids replicate via linear multimeric RNA intermediates By convention, the plus polarity is assigned to the most prominent viroid RNA species, and the minus polarity to its complementary strand; since viroids do not code for proteins, these designations are arbitrary. As a consequence, they suffered from a number of opportunistic infections (the most prevalent being pneumonia) that were often fatal. Inside the procapsid, B proteins interact with each other across the twofold symmetry axes. Much of the impetus for this effort came from the successes and the lessons learned from the development of anti-HIV-1 protease inhibitors. Genes are expressed in three temporal classes: • • • (immediate early): early gene activation (six genes) (early): DNA replication, late gene activation (late): virion proteins, regulatory proteins VIRUSES AND HOSTS Three subfamilies: alpha, beta, and gammaherpesvirus. Integration takes place at random sites within the cellular genome. Chronic hepatitis can be controlled by the nucleoside inhibitors to a large extent, but treatments are expensive and only available in developed nations. It also provided the basis for the generation of poliovirus vaccines during the 1950s. The flavivirus virion contains a lipid bilayer and envelope proteins arranged with icosahedral symmetry Flaviviruses have a small (25–30 nm) nucleocapsid enclosed within a lipid-containing envelope. The first Ebola virus vaccine to protect nonhuman primates was based on injection of DNA plasmids coding for both GP and NP, followed by boosting with an adenovirus recombinant vector expressing GP. An example is shown in Figure 2.5b. However, under conditions of reduced immune response found in newborns, transplant recipients, and AIDS victims, 285 CH024.indd 285 16/05/11 8:11 PM 286 CHAPTER 24 Herpesviruses herpesviruses can cause serious and often fatal disease, including encephalitis, pneumonia, and hepatitis. These are viruses in which some or all viral genes are removed by genetic engineering and are replaced by foreign genes. Key Features of This Book • A concise, up-to-date textbook designed for universitylevel virology courses for students in biomedical sciences and microbiology • Written in a simple and clear style for students with a background in cell and molecular biology 16/05/11 7:20 PM Preface • Explains replication mechanisms of viruses representing many of the major virus families • Many full-color figures complement the text and illustrate virus structure, genome organization and individual steps in virus replication • Each chapter is designed to tell a story about a specific virus family and to portray the “personality” of the virus covered • Chapter introductions give historical background and information about viral diseases • Includes study aids such as thumbnail sketches of each virus group, informative chapter subheadings, text boxes outlining recent research and applications, a list of fundamental concepts after each chapter, sample test questions, and a comprehensive glossary with definitions of numerous terms • An introductory section provides basic information about the history of virology, virus replication, virus structure, classification of viruses, and virus entry into cells • A section on viruses of bacteria and archaea covers four of the best-known bacteriophages: single-stranded fpref.indd xxi • • • • • • xxi RNA phages, ϕX174, T7 and lambda; as well as a survey of the known viruses of archaea Five sections containing 21 chapters cover a wide variety of viruses that infect animals, plants, algae and insects, with emphasis on viruses that cause human disease Includes chapters that cover important human pathogens such as Ebola virus, hepatitis B and C viruses, herpes viruses, human immunodeficiency virus, influenza viruses, measles virus, poliovirus, SARS coronavirus, smallpox virus, West Nile virus and others A chapter on viroids: small infectious nucleic acids that do not code for proteins but cause important plant diseases A chapter on prions: infectious proteins that cause mad cow disease and Creutzfeld–Jacob disease in humans A section on host defenses, with discussion of intrinsic cellular responses, innate and adaptive immune responses to virus infections A concluding section with chapters on antiviral vaccines, antiviral chemotherapy, and virus vectors 16/05/11 7:20 PM fpref.indd xxii 16/05/11 7:20 PM ACKNOWLEDGMENTS This textbook is the outgrowth of an undergraduate science course in virology taught by myself and colleagues in the Department of Microbiology and Immunology at McGill University for 25 years. Condensed with a small basic virus-coded protein. E1A proteins are the kingpins of the adenovirus growth cycle E1A is the first viral gene to be transcribed. The two receptors are differently distributed in human cells and tissues. Cold-adapted influenza vaccines are produced by combining the H and N genes of the targeted virus strain with six other gene segments from mutant viruses known to have restricted growth at 37⬚C. Table 25.2 lists some early viral proteins known to be involved in viral DNA replication. In this procedure, anti-hepatitis B immunoglobulins are injected into the exposed individual to neutralize circulating virus and prevent it from entering target cells. Virus was transmitted by direct contact with patients, but also via aerosol droplets and contaminated stool. These additional genes express such functions as restriction systems, antibiotic resistance, or toxins. Other cytokines that are rapidly made following virus infection include Table 33.2 Cytokines and their functions Type Examples Function Interferons Interferon-␣/ Antiviral defense; dendritic cell maturation; natural killer cell activation Interferon- Antiviral defense Interferon- Macrophage activation; inhibition of interleukin-6; antiviral defense Interleukin-1 Costimulator of T cells; induces fever, acute-phase proteins (liver) and metabolic wasting Interleukin-6 Stimulates B cell growth; costimulator of T cells; induces acute-phase proteins (liver) Interleukin-12 Stimulates growth of natural killer cells and T cells; induces production of interferon- and tumor necrosis factor-␣ Tumor necrosis factor-␣ Activates neutrophils, induces fever, acute-phase proteins (liver), and metabolic wasting; lyses infected cells Interleukin-8 Recruits neutrophils; promotes angiogenesis RANTES Recruits T cells, eosinophils, and basophils; activates natural killer cells CXCL10 (IP-10) Recruits monocytes, macrophages, T cells, natural killer cells, and dendritic cells Pro-inflammatory cytokines Chemokines Anti-inflammatory cytokines Interleukin-4 CH033.indd 403 Inhibits macrophage function; promotes Th2 cells; stimulates B cells Interleukin-10 Inhibits macrophage function; stimulates B cells Transforming growth factor- Inhibits T cells and macrophages; induces production of immunoglobulin A 16/05/11 8:19 PM 404 CHAPTER 33 Intrinsic Cellular Defenses Against Virus Infection tumor necrosis factor alpha (TNF␣), interleukin 6 (IL-6), and interleukin 12 (IL-12). Infected cells often undergo fusion with neighboring cells as a result of the presence of fusion-competent envelope proteins in their membranes. There are no data on the possible role of the delta-peptide. One of these genes codes for the same ribose-methyltransferase that participates both in mRNA 5' cap formation and in 3' end polyadenylation. O. University of Helsinki, Finland. Have been widely used in human trials. A computer-generated image of adenovirus type 2 virion, viewed along a threefold symmetry axis. A copy of the RNA template for reverse transcriptase could have been packaged along with reverse transcriptase, allowing transmission of this gene from cell to cell by a virus. Explain how the transcriptional promoter of Sendai virus can contain two elements separated by more than 60 nucleotides. This so-called oncotropism of parvoviruses may well turn out to be useful because it could be a basis for targeting a lytic virus to cancer cells. This process involves extension of the plus-strand DNA across the single-stranded gap region to form a fully double-stranded DNA, removal of the terminal primers (the P protein on the minus-strand, and the short, capped RNA primer on the plus-strand), and covalent CH030.indd 368 ligation of the resulting 3⬘ and 5⬘ ends of each DNA strand (Figure 30.2b, c, and d). It is not clear if an outer envelope (calyx) is present on granules. Spike (S) Virion structural protein; receptor binding, fusion, virus entry, host range, tropism, immune response, syncytia formation. See also Human adenoviruses cell killing induced by, 283 cell transformation/oncogenesis, 283 cellular receptors for, 47, 48 DNA replication, 280–281 E1A proteins, 277–279 E1B proteins, 279–280 enteric infections in humans, 274 gene expression, controlled at transcription level, 276–277 gene products of type 5, 277 inverted terminal repeats, 276, 280–281 late promoter, 281 multiple late mRNAs, 281 oncogenic nature, 274–275 respiratory infections in humans, 274 transcription of viral genome, 276–277, 280–282 tripartite leader, 281–282 uptake in endosomes, 46 vectors, 460–462 virions, 275 Adjuvants, 434, 436, 437 Adrenoleukodystrophy (ALD), 465 Aedes albopictus, 149 AIDS, see Acquired immunodeficiency syndrome Algae, viruses of: see Phycodnaviruses Algal blooms, 326, 331, 332, 333 Alphaviruses, 149–151 expression vectors, 155–156 New World, 155 Old World, 155 uses, 156–157 ‘Altruistic’ vaccination, 441 Amantadine, 53, 215, 445, 447–448 α-Amanitin, 205, 216, 306, 381–384 Ambisense coding strategies, 200, 203–206 Ampullaviridae, 99, 100, 103, 104 Amyloid, 392 Anaerobic methanogens, 98 Anaphylatoxins, 417 Anti-apoptosis genes, 295 Antibodies, 10, 13, 53, 195, 198, 204, 300, 356, 366, 399, 416–422 424, 436, 458 Antibody specificities, diversity of, 421–422 Antibody-dependent cellular cytotoxicity, 418 Antibody-dependent enhancement, 141 Antigenic drift, 220, 424 Antigenic shift, 220–221, 424 Antigenome RNA, 132, 133, 151–153, 168, 181–186, 203–206, 208, 216–218, 383–384 Antigens, 419, 434–435, 437–438 Anti-inflammatory cytokines, 403–404 Antitumor effects of parvoviruses, 245 Antiviral chemotherapy, 444–454 acyclovir, 448–449 amantadine, 447–448 azidothymidine (AZT), 450–451 discovery, 444–445 drugs preventing attachment and entry of virions, 446–447 ganciclovir, 450 maraviroc development, 447 neuraminidase inhibitors, 453 non-nucleoside inhibitors targeting viral replication enzymes, 451–452 nucleoside analogues target viral DNA polymerases, 447–448 peptidomimetic drugs, 452 protease inhibitors, 452–453 ritonavir, 452–454 targeted to specific steps of virus infection, 445–446 Antiviral drugs, 53, 444–448, 453 against hepatitis B virus, 375 against HIV-1, 356–357, 359 Antiviral vaccines, 428–442 adjuvants role in, 436 adverse events, 439–442 for avian influenza strains, 431 cell culture in development of, 431 changing vaccine paradigm, 439 chimeric viruses, 431, 439 with defined proteins, 437–438 early vaccine technology, 430 Edmonston-B and Moraten measles vaccine strains, 433 embryonated chicken eggs in development of, 431 ethical issues in, 441–442 inactivated viruses, 432 live attenuated viruses, 431–432, 438–439 live vectors, 439 live wild-type viruses, 431–432 milestones in development, 430 multivalent peptide vaccines, 435 new categories of, 434–435 new developments in, 437–439 Oka-strain varicella virus, 433 pre-vaccination era vs. Dark green: known function; green: predicted function; light green: unknown function. Virus assembly also takes place at intracellular membranes Unlike many other enveloped viruses, which derive their envelope from the plasma membrane of the cell, flaviviruses assemble their virions inside the cell by using intracellular membranes for envelope formation (Figure 12.6). Therefore, hemagglutination assays are much less sensitive than plaque assays, but they are rapid and cheap. The mechanism of lysis mediated by E protein is similar to that of antibiotics like penicillin, which inhibits cell wall biosynthesis. • Autonomous parvoviruses replicate in cells that normally cycle and therefore frequently enter S phase. Try to say that before brushing your teeth in the morning! AcMNPV replicates well in cells from the cabbage looper (Trichoplusia ni) and the fall armyworm (Spodoptera frugiperda, Sf) cultured in vitro. Membrane fusion: the process by which two membranes combine to form one. However, it was subsequently shown that the infectious agent affecting both the human and crow populations was West Nile virus, a virus not previously seen in North America. (a) 3D reconstruction of virion of Sulfolobus turreted icosahedral virus (STIV). One strategy would be to decrease the vector dose in order to reduce the odds of an integration event near a cellular proto-oncogene. The production of the recombinant virus occurs in two stages (Figure 25.5). This gives virus vectors an enormous advantage over other methods for Are less immunogenic than first-generation adenovirus vectors. Poxviruses produce large concatemeric DNA molecules that are resolved into monomers The presently accepted model describing poxvirus DNA replication (Figure 26.6) is fashioned after the “rolling hairpin” model of DNA replication in parvoviruses (see 16/05/11 8:13 PM Poxviruses 319 Table 26.3 Enzymes of DNA metabolism encoded by vaccinia virus No. of subunits Mol. Many organisms, including bacteria such as E. Mosquitoes and midges transmit orthobunyaviruses, ticks transmit nairoviruses, and sandflies or ticks transmit phleboviruses. BGLOSS.indd 475 475 Hantavirus pulmonary syndrome: an often-fatal lung disease resulting from infection by hantaviruses such as sin nombre virus, transmitted by rodents. The range of viruses against which vaccines are currently under development is impressive (Table 35.8). Granzyme B is a protease that can activate both caspase-3, the “executioner caspase,” as well as the pathway leading to release of mitochondrial proteins and the formation of the apoptosome (see Figure 33.4). For example, polyomaviruses release their circular DNA as a minichromosome, covered with nucleosomes made from cellular histones. Eukaryotic Virus Vectors 456 Many viruses can be engineered to deliver and express specific genes 456 TOC.indd xviii Virus vectors are used to produce high levels of specific proteins in cultured cells 457 Gene therapy is an expanding application of virus vectors 458 Virus vectors are produced by transfection of cells with plasmids containing deleted genomes 458 Virus vectors are engineered to produce optimal levels of gene products 459 ADENOVIRUS VECTORS 460 Adenovirus vectors are widely used in studies of gene transfer and antitumor therapy 460 Replication-defective adenovirus vectors are propagated in complementing cell lines 460 Replication-competent adenovirus vectors are useful tools in antitumor therapy 461 Advantages and limitations of adenovirus vectors 461 RETROVIRUS VECTORS 462 Retrovirus vectors incorporate transgenes into the cell chromosome 462 Packaging cell lines express retrovirus enzymatic and structural proteins 462 Strategies for controlling transgene transcription 463 Lentivirus vectors are used for gene delivery to non-dividing cells 463 Production of lentivirus vectors requires additional cis-acting sequences 463 Applications of retrovirus vectors: treatment of blood disorders 464 Applications of retrovirus vectors: treatment of neurological disorders 465 Advantages and limitations of retrovirus vectors 465 ADENO-ASSOCIATED VIRUS VECTORS 465 Adeno-associated virus vectors can insert transgenes into a specific chromosomal locus 465 Production of AAV vectors usually requires a helper virus 466 Clinical trials using adeno-associated virus vectors 467 Advantages and limitations of AAV vectors 467 GLOSSARY 471 CREDITS 484 NAME INDEX 489 SUBJECT INDEX 491 13/05/11 2:11 PM PREFACE This book is written for students who are learning about viruses for the first time in a university course at the undergraduate or graduate level. Could new viruses still be arising by some of these mechanisms? How DNA traverses the outer and inner lipid membranes and the periplasm of the gramnegative E. Electron micrograph of a male E. Gp16 is also responsible for transporting the first 850 base pairs of the left end of T7 DNA through this channel into the cytoplasm, exposing three closely spaced promoters recognized by the host Escherichia coli RNA polymerase. All picornavirus IRES elements contain a pyrimidine-rich tract 20 to 25 nucleotides from a conserved AUG triplet. At that time, most scientists believed that genes were made of proteins, not nucleic acids, because only proteins were believed sufficiently complex to encode genetic information. The copy of the TRS at the 3' end of the nascent RNA chain forms an RNA-RNA hybrid with the complementary TRS at the end of the leader sequence on the genome RNA. A complex between Rb and E2F binds to cellular and viral genes, and bound histone deacetylase (HDAC) represses transcription by altering chromatin structure. The polymerase plays a critical role in hepadnavirus genome replication and pregenome RNA encapsidation. Multiplasmid transfection systems allow recovery of infectious filoviruses Both of these complications have been solved by the use of multiple-plasmid systems (Figure 16.4). These genomes can replicate only if present in a cell that is simultaneously infected with a helper virus; therefore they have been called satellite viruses and satellite nucleic acids (Table 3.7). Reoviruses 225 Ping Xu, J. Many such conversion genes appear to have hopped into the phage genome during integration/excision events as a cassette composed of a promoter, the open reading frame, and a transcription terminator. The bat “SARS-like” coronavirus sequence was available from direct sequencing of bat secretions, but virus could not be grown in culture, and thus no virus or RNA was available. Retroviruses are presently grouped into seven genera (Table 28.1), based on differences in morphology and genome organization. Presumably, this plethora of proteins is required immediately upon release of the virion core into the cytoplasm. Research beginning in the 1940s by the “Phage Group”, an informal grouping of scientists, established many of the basic principles of molecular biology. However, vectors that lack the E4 region usually express lower levels of transgene proteins expressed from viral promoters. The cDNA fragments are excised from plasmids by cleavage with restriction endonucleases, and ligated in vitro into a full-length cDNA. When studies on the molecular biology of arthropod-borne viruses were undertaken, it became apparent that not all such viruses are closely related. A genome fragment (green) encoding an immunogenic viral protein is engineered into the genome (red) of a nonpathogenic virus or into a plasmid DNA. 16/05/11 8:19 PM 402 CHAPTER 33 Intrinsic Cellular Defenses Against Virus Infection with both adaptor molecules. (b) The Vp54 trimer viewed from the inside of the virus, with each monomer in a different color. However, when free-ranging or farmed animals of these species were examined, no viruses similar to SARS coronavirus were found. Initiation of RT activity ε 5' end of newly synthesized DNA is covalently attached to polymerase protein. The IgG molecule is the most abundant immunoglobulin. High concentrations of pppdG can reverse inhibition at this stage. Capsids are represented as green icosahedra, genomes as black circles, messenger RNAs as blue lines with translating ribosomes, viral replication proteins as orange ovals, and viral structural proteins as green squares. Viral E7 protein interacts with cell-cycle regulatory proteins, particularly Rb Papillomaviruses do not code for their own DNA replication enzymes. It is also cleaved by a cellular furin protease into two subunits: a large amino-terminal ectodomain, GP1, and a shorter carboxy-terminal transmembrane protein, GP2. Bacteriophage chromosomes are mosaics of DNA segments that have been swapped due to rampant lateral exchange with other bacteriophage genomes. In 293 cells expressing Cre recombinase, the packaging signal of the helper virus is deleted, resulting in DNA that still can produce viral proteins, but cannot be packaged into virus particles. Envelopes are shown as light blue membrane bilayers, and envelope proteins are shown as yellow spikes inserted in the membrane. The second protein component of the virion, of about 124 kDa, is associated with tail fibers. The 40S subunit scans the mRNA, searching for an AUG codon in an appropriate sequence context. As with single-stranded DNA genomes, single-stranded RNA genomes are susceptible to degradation by nucleases or by divalent cation attack, and this may be a factor that limits their size. Hepatitis C virus, unknown prior to 1989, has infected 4 million people in the United States alone and is responsible not only for acute and chronic hepatitis, but also for hepatocellular carcinoma. In humans, some of these proteins are also called human leukocyte antigens, or HLA. The N-terminal tails of 1 trimers are inserted into the 2 spikes, whereas the C-terminal globular heads project away from the virion surface. • One Ebola virus gene makes two distinct proteins by RNA editing; one of these proteins, sGP, is secreted by infected cells and is not present in virions, but has unknown functions. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning or otherwise, except as permitted under Sections 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate percopy fee to the Copyright Clearance Center, Inc. Viral miRNAs target both viral and cellular messenger RNA species. 1960 Production of reassortant influenza A viruses in eggs. Five different promoter elements (preC, pregenome, preS1, S, and X) direct initiation of transcription by RNA polymerase II (Figure 30.4). Leser, Northwestern University; Fig. Cells transformed by papillomaviruses express E6 and E7 gene products from integrated viral DNA The most important and interesting disease aspect of human papillomaviruses such as types 16 and 18 is their involvement in cervical cancer. The polyprotein is cleaved during translation and transport into the lumen of the endoplasmic reticulum to yield the two proteins, which are notably rich in cysteine residues. More than a decade of frustrating efforts with DNA vaccines that work beautifully in animal models but less well in humans has led to the concept of sequential, multiple-formulation vaccination strategies referred to as “prime-boost” (beginning with a DNA vaccine “prime”, followed by a live attenuated vaccine “boost”). The replication complex, which retains proteins A and C, rep, and DNA polymerase III, can presumably bind to another procapsid to synthesize and package another phage genome. Expression of Mx affects virus replication by interfering with the transcription of influenza virus and other RNA viruses. The outer circle shows groups of genes expressed in different temporal classes, and the direction of their transcription. More effective drugs with shorter treatment durations are under development. Kuru was characterized by cerebellar movement disorders and a shivering-like tremor progressing to total muscular incapacity, dementia, and death within one year of the onset of symptoms (Figure 32.2). The modifications also vastly improved the uptake and stability of the drug in the body (pharmacokinetics). One is pegylated interferon (see Chapter 33), an immune system modulator that works by stimulating cytokine synthesis and by enhancing host antiviral responses. Advantages and drawbacks of vaccine types The immune response elicited by live wild-type or attenuated vaccines comes closest to that observed in natural infection, because these viruses multiply within the vaccinated individual. 7.2: Redrawn from Fig. 16/05/11 8:04 PM 154 CHAPTER 13 Togaviruses Continued translation of the subgenomic mRNA beyond the capsid gene produces an N-terminal signal sequence that translocates the PE2 polypeptide to the rough endoplasmic reticulum (Figure 13.4). The major capsid protein of polyomaviruses forms stable pentamers, and mechanisms have evolved for specific interactions with either five or six neighboring pentamers. The A protein is simultaneously transferred to the newly formed 5' end resulting from this cleavage, recreating the original replication complex. NS1 also further stimulates transcription from the p4 promoter, leading to increased production of its own mRNA. • Hepadnaviruses package the RNA pre-genome template in the cytoplasm and use viral reverse transcriptase to generate the partially double-stranded viral DNA genome. Hepatitis B virus can also be passed on to babies at birth. Late messenger RNAs are made from newly replicated genomes 15 6. Many bacteriophages lyse their host cell, and this lysis causes a loss in light diffraction leading to clearing of the bacterial culture; “clear lysis” serves as an indicator of phage replication. A viral genome containing 1.2 million base pairs is 600 times longer, or 420 m (0.4 mm). Another capsid protein, VP26, is located at the tips of the capsomers. Sixty tetramers of the external scaffolding protein D then organize twelve 12S* particles into “procapsids”. The products of genes 1.2 and 1.7 also contribute to the cessation of host transcription by complex effects on nucleotide pools. Second, insect cells are cotransfected with infectious, wild-type viral DNA and the transfer vector carrying the foreign gene (step b). The mechanics of the DNA packaging motor are not fully understood. • Introns removed by splicing of some polyomavirus early RNAs are unusually small, and their small size reduces the efficiency of splicing. Compare and contrast the two types of genome replication used by the two families of viroids, referred to as symmetric and asymmetric replication. In so doing, Nef acts to kill cells that could otherwise help to clear the infection, prolonging the lifetime of the infected cell and maximizing production of new virus. Viral integrase cleaves 2 nucleotides from the 3' ends of the viral DNA and then joins these 3' ends to host DNA in a concerted cleavage-ligation reaction at sites 4–6 nt apart on the host DNA (dashed arrows). Their discovery has helped provide a detailed understanding of the process of tumorigenesis. These are in turn phosphorylated by c-Src and activated, leading to increase in cell metabolism and cell cycling. 2', 5'-oligo(A) synthetase and ribonuclease L. • Polyomaviruses have two divergent transcription units that lie on either side of a central control region, which includes the origin of DNA replication and transcriptional enhancer elements. These interactions result in formation of a CI octamer (Figure 8.5c). • Many viruses have genomes not found within any other extant life form (single-stranded DNA, positiveand negative-strand RNA, double-stranded RNA). In 2007, the FDA approved an anti-HIV drug named raltegravir. Upon contact with the pilus, the maturation protein is cleaved into two fragments. A number of animal viruses have also been adapted to grow in embryonated chicken eggs, which are readily available from farms; this reduced both the expense and the time of virus assays. As virions are assembled and extruded from the cell, the viral protease becomes activated. • Tubular capsids have helical symmetry, and their length depends on the length of the enclosed nucleic acid genome. Cryoelectron microscopy reveals a spherical particle with a smooth, golf ball-like appearance (Figures 12.1, 12.2c), in contrast to the fringed envelope of togaviruses and many other enveloped viruses. Viral double-stranded DNAs in the cytoplasm are recognized by at least three different cellular proteins In addition to recognizing double-stranded RNA, a form of nucleic acid not routinely produced in uninfected CH033.indd 401 401 Cytoplasm 5' ppp ssRNA ppp Short dsRNA Long dsRNA RIG-I MDA-5 dsDNA dsDNA DAI NLRP3 dsDNA AIM2 Nucleus Figure 33.2 Cytosolic sensors that recognize viral nucleic acids. Autophagy has been shown to be required for TLR7-mediated recognition of vesicular stomatitis virus particles in the cytoplasm, by delivering viral RNA to vesicles containing TLR-7 (Figure 33.1). Adaptive immunity, provided by humoral antibodies and specific B- and T-lymphocyte responses, is much slower to respond (days to weeks). (a) Virus factories (VF) surrounded with virus particles at various stages of assembly. West Nile virus, originally discovered in Uganda and prevalent in Africa, southern Europe, and the Middle East, suddenly appeared in North America in 1999. A short RNA fragment at the capped 5⬘ end, including the DR1 region, remains resistant to ribonuclease H degradation. The ribosomal translocation process then drives uncoating. Scales are different for each image. Neither mice nor monkeys naturally infected with mouse polyomavirus or simian virus 40 develop tumors. • Ribosomes that terminate translation of the coat protein gene can scan forward or backward on viral RNA and initiate translation at the nearby lysis gene start site. Sixty of them have tail-to-tail interactions in clusters of five; for example, the five commas whose tails meet at a point immediately above the equator, near the tail of the comma marked “A”. Lee, and J. Depending on where the RNA polymerase pauses, the product is either full-length antigenome RNA (no pause; not shown) or one of the six subgenomic negative-strand RNAs shown at bottom left. While current therapies delay or prevent disease progression, they do not eradicate the infection. Some effort has been spent in searching for a plant cell enzyme that can catalyze cleavage of viroid RNA multimers, but none has yet been found. In fact, the beginning of the lysis and replicase genes lie within RNA secondary structures that are too stable to allow ribosome binding (Figure 5.5), and this is the reason that they are not translated on their own. In severe cases, the rash became confluent and/or hemorrhagic. However, the integration site contains a copy of the sequence in adeno-associated virus DNA where Rep78 binds and cleaves. In addition to the worldwide AIDS epidemic that started in the early 1980s, there have been localized outbreaks in Africa of the highly fatal Marburg and Ebola hemorrhagic fevers during the past 30 years, a short-lived epidemic in southern Asia and Canada of severe acute respiratory syndrome (SARS) in 2003, and spread of acute and chronic hepatitis via both hepatitis B and C viruses. In the absence of protein synthesis, many prematurely terminated mRNAs are made. Toll-like receptors located within endosomes recognize both singleand double-stranded RNA and DNA (Figure 33.1). These capsids bind to fibers that extend from the cytoplasmic face of the pore, and the capsids are subsequently disassembled, allowing the viral DNA and associated proteins to enter through the nuclear CH004.indd 53 53 pore (Figure 4.5c, d). Pro-inflammatory cytokines: cytokines that function to enhance inflammation. AZT monophosphate is then converted to AZT diphosphate by cellular thymidylate kinase. The capacity of attachment protein 1 to bind to cell-surface sialic acid CH019.indd 234 is a determinant of viral tropism for bile duct epithelium and inflammatory damage to bile ducts. Ribozyme: a ribonucleic acid that acts as an enzyme. H. Rightward reading transcripts shown at top, leftward reading transcripts shown at bottom. nsp4–10 Intermediate product, unknown function. Virokines can alter the host immune response and protect infected cells from detection. This technique facilitated the use of T7 DNA in many experiments requiring intact, complementary DNA strands. Those that recognize host proteins are destroyed while those that recognize foreign antigen are activated and produce many clones of themselves. CH027.indd 339 susceptible to UV damage, whereas exposure to oxidizing conditions during envelopment in the phagocytic vesicle may trigger significant oxidative damage. Hervé Lot et al. As recombinant vaccines. We have simultaneously gained considerable experience with live bacteria used as vaccines, such as Bacille Calmette–Guerin (BCG, against tuberculosis) and attenuated Salmonella typhi (against typhoid fever). There is a long history of their medical use, particularly in the Republic of Georgia, in an institute cofounded by one of the discoverers of bacteriophages, Felix d’Herelle. Classification of organisms that are genetically and evolutionarily related to each other is useful because detailed knowledge about one organism can often be generalized and applied to related organisms. Virus vectors: viruses that are genetically modified to express foreign genes and/or to infect cell types or species not originally targeted by the virus. As with other RNA viruses, cucumber mosaic virus has an error-prone RNA polymerase that contributes to rapid generation of mutations, giving rise to much genetic variation. Protein kinases: enzymes that modify proteins by the covalent addition of phosphate groups. Dynein moves CH004.indd 52 RT Microtubule Endosome IN MA Vpr (b) Influenza virus NUCLEUS IN MA Vpr Pre-integration complex vRNPs M1 Clathrin Dynein (c) Adenovirus Microtubule Endosome DNA and associated proteins Microtubule Dynein (d) Herpesvirus Endosome Tegument proteins VP16 Incoming capsid (e) Hepatitis B virus DNA and associated proteins (f) Parvovirus DNA Recycling endosome Microtubule (g) Simian Virus 40 DNA and associated proteins Intact virus Caveosome Smooth ER Caveolin Minichromosome Figure 4.5 Entry and intracellular transport of viruses that replicate in the nucleus. Binds to genome RNA packaging signal via positively charged amino terminus. Electron microscopic studies revealed that viral nucleocapsids condense on the cytoplasmic side of areas of the Golgi vesicles at sites where viral glycoproteins are present. They are found in anoxic environments such as marshes, the guts of animals, undersea sediments, volcanic springs, and deep underground. These viruses have been isolated and their nucleotide sequences compared. A rate-limiting step during AAV infection is the conversion of the single-stranded DNA into doublestranded DNA. These open reading frames are shown on a linearized genome map in Figure 22.2. Unlike E2 E7 Genes L1 E5 E6 E1 L2 E4 LCR Control elements Genome 0 P early 1 Poly(A)early P late 2 3 4 5 Poly(A)late 6 7 8 Kb Transcripts Figure 22.2 Genetic and transcriptional map of human papillomaviruses. 16/05/11 8:21 PM Antiviral Chemotherapy Hydroxyethylamine compounds not only mimic the original peptide, they also mimic the transition state of protease catalysis (i.e., the catalytic intermediate that binds most tightly to the enzyme). NS1 has also been shown to prevent the maturation of human primary dendritic cells, thereby limiting host T-cell activation. The best-studied paramyxoviruses are Sendai virus (a virus isolated in Sendai, Japan), Newcastle disease virus (a virus that infects chickens), simian virus 5 (in reality, a canine parainfluenza virus), and measles virus. Furthermore, there is concern that filoviruses could be intentionally spread as a bioterrorist measure. GP forms trimers in the plasma membrane and in the virion envelope. The negative-strand subgenomic RNA templates are themselves generated by discontinuous transcription of full-length positive-strand genome RNA. These scientists were studying the phenomenon called viral interference, in which infection of cultured mammalian cells by one virus can interfere with a subsequent infection of the cells by the same or another virus. Processivity: the ability of a polymerase to extend a growing chain without detaching from the template. Negative (or minus) strand: for RNA viruses, the strand complementary to the RNA that contains protein-coding regions. RRE AAAAA Importin Beta RRE AAAAA Cytoplasm Nucleus Rev RRE AAAAA Exportin 1 Ran GTP Figure 29.6 Mechanism of Rev function. Members of a species usually infect a limited number of organisms or specific target cells and tissues. 66 (2006):31-124. Mekambo of Gabon Congo Nzara Watsa Yambuku Ebola 2000/01 Maridi Juba Gulu Uganda DR Congo Marburg Mt. Elgon 1980/87 Kenya Lake Victoria Mweka Kikwit Ebola 2007/08 Uige Marburg 1998-2000 Marburg 2004/05 Angola Ebola 2007 Ebola 1995 Ebola 1994 Marburg 1975 Figure 16.8 Locations of filovirus outbreaks in Africa. Under these conditions, not only is the mRNA for gene X the major RNA in the cell, but it also is able to compete effectively for most of the protein synthetic apparatus, so that gene X product becomes the major protein in the cell. The elucidation of the mechanism of action of amantadine represents a triumph of molecular genetic approaches using drug-resistant viruses. Some plant viruses also have a double-stranded DNA genome that is made from an RNA by reverse transcriptase. This allows the phage to concentrate its efforts on production of large amounts of virion structural and maturation proteins (Table 7.3) during the final stages of infection. However, many viruses do encode miRNAs. Of particular interest, viruses that have been found to make miRNAs are all nuclear DNA viruses, including members of the herpesvirus, adenovirus, and polyomavirus families. Transcription produces a nested set of subgenomic mRNAs The expression of all coronavirus genes downstream of the replicase gene occurs from a series of subgenomic mRNAs (Figure 14.4). The incidence of this cancer could be dramatically reduced with appropriate healthcare intervention such as a routine Pap smear and followup treatment, although this is rendered difficult in many countries because of the lack of appropriately trained pathologists and because of cultural inhibitions. 26.7: Courtesy Mariano Esteban, National Center of Biotechnology, Madrid, Spain. High-titer stocks can be produced easily, and high levels of transgene expression can be achieved with these vectors. 5 R -1 hr1 a eg da t d 26 da a16 41 I B hr o t ro -p v en lef lef-2 hr1 0 -ex pp 3 gp1 4 p246 gp6 7 vcat h chitin ase lef-7 pk2 The baculovirus genome is a covalently closed circular, double-stranded DNA molecule. Other characteristics such as the order of genes on viral genomes, the mechanism of viral messenger RNA synthesis, the presence of viral genes coding for DNA or RNA polymerases, and the use of reverse transcriptase during replication, are also useful criteria for virus classification. The nuclear localization signal on VP1 directs this complex to the nucleus via the nuclear pores. Some of these pathways have been outlined above. : alk. The mimivirus is currently the virus with the largest fully sequenced genome: linear, double-stranded DNA of 1,181,404 base pairs and a staggering 911 predicted protein-encoding genes plus six tRNA genes. The persistence and shedding of live attenuated viruses in some hosts, the appearance of virulent revertants, and their capacity to spread through contaminated water represent major challenges for the eradication effort. GENES AND PROTEINS Seven genes, transcribed in series from 3' end of genome by viral RNA polymerase Most genes produce a single mRNA and a single protein. • E1B proteins reverse E1A-induced apoptosis by reducing p53 levels. These are individually wrapped within helical nucleocapsids, which are packaged in a lipid envelope derived from the plasma membrane to form the virion. DETECTION AND MEASUREMENT OF VIRUSES The plaque assay is widely used to measure virus infectivity. First, if virus-coded nucleoside kinases can phosphorylate nucleoside analogues more efficiently than their cellular counterparts, these drugs should be more active in infected than in uninfected cells. Several autonomous parvoviruses can cause disease in animals; canine parvovirus, feline panleukopenia virus, and Aleutian mink disease virus are well-known examples. Moreover, satellite RNAs are not essential to helper virus replication, and most do not encode any protein. (a) Stargate structure (blue) seen down the unique fivefold vertex. DISTINCTIVE CHARACTERISTICS Proteinaceous infectious agent that contains no nucleic acid and consists only of a single species of protein called PrP. • A rolling circle mechanism produces multimeric concatemers of viral DNA that are cleaved into genome-length fragments during packaging into preformed capsids. Proteasome Proteoglycan Raft culture Retinoblastoma (Rb) Squamous cell carcinoma Tumor suppressor protein Ubiquitin Ubiquitination • Papillomaviruses cause warts on skin and in oral or genital mucosa. Vectors containing the fiber protein of adenovirus 35 transduce tumor cells expressing CD46 more efficiently than vectors made with the fiber protein of serotype 5, the most common serotype used. Enzymes representing each of these steps can be purified from virions, so that the enzymology of each process can be dissected in detail (Table 26.2). Synthesis of brome mosaic virus protein 2a in yeast requires the host protein DED1, an RNA helicase. Mature progeny virions begin to appear by 18–20 hours after infection, and virus titers increase slowly for the next 24 hours, by which time most of the infected mouse cells are dead. CH022.indd 265 16/05/11 8:10 PM 266 CHAPTER 22 Papillomaviruses Table 22.2 Functions of human papillomavirus genes Gene Function E1 DNA helicase, binds to replication origin, initiates DNA replication E2 Binds to specific sites on viral DNA, enhances binding of E1 to replication origin, activates or represses transcription of viral genes, binds to mitotic chromosomes and aids in segregation of viral genomes between two daughter cells E4 Function largely unknown; most abundantly expressed viral protein, binds keratins and may help virus maturation and release E5 Small, membrane-associated protein, enhances growth factor responses in infected cell, stimulates cell proliferation E6 Directs ubiquitin-mediated degradation of cellular p53 and other target proteins, inhibits cell-cycle block and apoptosis (oncogenic papillomaviruses only) E7 Binds to and degrades Rb protein, releases E2F, activates cell cycling and DNA replication genes, stimulates cell and viral DNA replication L1 Major capsid protein L2 Minor capsid protein, helps encapsidate viral DNA Note: Other human and animal papillomaviruses have additional genes designated E3 and E8. The helper virus activates transcription of the integrated adenoassociated virus genome, which leads to synthesis of large amounts of the Rep proteins, excision of adenoassociated virus DNA from the cellular DNA, and finally production of progeny adeno-associated virus particles. Africa Human: encephalitis, hemorrhagic fever, retinitis; 1–10% fatality Mosquito Africa Human: fever Sandfly Europe, Africa, Asia More than 650 plant species; various symptoms Thrips Worldwide Phlebovirus Rift Valley fever virus Domestic ruminants: necrotic hepatitis, hemorrhage, abortion Sandfly fever virus Tospovirus Tomato spotted wilt virus (Chapters 12 and 13). The mutants isolated so far all contain a single amino acid change in the C-terminal half of nsP2 and have a decreased level of viral RNA synthesis. Copyright 1965, 1970, 1976, 1987, reprinted by permission of Pearson Education, Inc (Atlanta); Fig. It is still uncertain whether direct shuttling, targeted protein degradation, or both are responsible for the selective transport and stabilization of viral mRNAs. Nevertheless, the effect is that late adenovirus mRNAs predominate in the cytoplasm during the late phase of infection. The P protein recruits the E. Phylogenetic analysis shows that the mimivirus MutS protein is most closely related to MutS homologues in the bacterium Nitratiruptor (a nitrate-reducing bacterium found in deep-sea hydrothermal vents) and the eukaryote Octocorallia (“soft corals”, marine organisms that form colonial polyps). These viruses, called NL63 (isolated from the Netherlands) and HKU1 (isolated in Hong Kong), have since been determined to circulate in human populations worldwide. Viruses with a reverse transcription step in their replication cycle can have either RNA or DNA genomes Three virus families are grouped separately from other viruses because they have a reverse transcriptase that makes a DNA copy of an RNA during their replication cycle (Table 3.6). Figure 36.5 shows what happens to acyclovir in cells infected with herpes simplex virus or varicella zoster virus. Thus the competition between ribosomes and replicase for the coat protein start site is in fact mediated by the same (cellular) protein! Once the polymerase complex binds, assuring absence of ribosomes on the RNA it is about to copy, it looks for the 3' end of the RNA. VP4 is not visible as it is in the interior of the virion. A number of octapeptide amino acid repeats in the N-terminal region bind copper ions via histidine residues. Delivery technologies Aerosol A variety of ingenious mechanical devices are being studied for aerosolization of antiviral vaccine preparations for delivery either to the nasal or the lower respiratory mucosa. The smallest open reading frame encodes the 154–amino acid X protein. Virus particles can be seen and counted by electron microscopy. Therefore, DpnI can be used to distinguish between input plasmid DNA (methylated) and progeny unmethylated DNA. The prM protein associates with the E protein in the endoplasmic reticulum to form a heterodimer. Recent studies have shown that APOBEC3G also directly impairs the reverse transcription reaction, significantly reducing the yield of proviral DNA. The same host cell proteins used in Stage I DNA replication (Figure 6.3) mediate this reaction. E1A proteins indirectly induce apoptosis by activation of cellular p53 protein Expression of E1A proteins leads indirectly to stabilization and activation of the cellular tumor suppressor protein, p53 (Figure 23.7; see also Figure 22.6). NK cells recognize activating and inhibitory receptors on the surface of other cells. KEY TERMS Budding Capsid Confocal microscope DNA-dependent RNA polymerase Encephalitis Envelope Filterable virus Gastroenteritis Glycolipid Glycoprotein Hemagglutination assay Hemorrhagic fever Hepatitis Latent infection Lysis Multiplicity of infection (m.o.i.) Negative (or minus) strand Obligatory intracellular parasite Oncogenes Plaque assay Plaque-forming units (PFU) Plasmodesmata Poliomyelitis Positive (or plus) strand Reverse transcriptase RNA-dependent DNA polymerase RNA-dependent RNA polymerase Scaffolding protein Sialic acid Tropism Uncoating Virion FUNDAMENTAL CONCEPTS • A virus particle, or virion, consists of a nucleic acid genome enclosed in a protein coat and in some cases a lipid-containing envelope • Viruses depend on cells for molecular building blocks, energy, and the machinery for protein synthesis CH001.indd 16 16/05/11 7:46 PM Steps in the Virus Replication Cycle 17 • Viruses can have genomes made of either RNA or DNA • Viruses replicate by uncoating and expressing the viral genome inside the cell, directing synthesis of viral proteins, replicating the genome, and encapsidating it to form new progeny virus particles • Viruses can be detected and enumerated by plaque assays, hemagglutination assays, and electron microscopy • The sequence of steps during virus replication can be studied by infecting all cells in a culture at a high multiplicity of infection • Seven different classes of virus can be distinguished based on the pathway to synthesis of early mRNAs; this is known as the Baltimore classification system. Zoonotic disease: a disease that can be transmitted to human beings from animals. Fusion proteins undergo major conformational changes that lead to membrane fusion When activated by low pH or by receptor binding, fusion proteins undergo a series of changes resulting in a dramatic alteration in protein architecture, properties, and membrane association. My approach was to ask a number of prominent virology researchers and teachers to write chapters on viruses that they knew well, using a set of criteria that I provided. Alternative transcription start sequences called initiators are present at the beginning of many viral and cellular genes that lack TATA boxes, but play a similar role. X-ray crystallography has revealed that E2 contains three immunoglobulin-like domains matching the three domains of E1. (b) Rod-like structure proposed for group B viroids, showing the five domains (TL: terminal left; P: pathogenic; C: central; V: variable; TR: terminal right). This strategy has been used to prepare specific RNA substrates for study of RNA conformation, ribozymes, and RNA processing. Cloned cDNAs representing the 10 reovirus dsRNA gene segments are flanked by the bacteriophage T7 RNA polymerase promoter and hepatitis delta virus ribozyme sequences. • Viruses with negative-strand RNA genomes are among the major infectious disease agents of humans, but are not found in prokaryotes. Energy derived from hydrolysis of ATP to ADP powers the movement and denaturation of the DNA strands. Although relatively few viral proteins can self-assemble like the hepatitis B virus sAg and the human papillomavirus L1 protein, recombinant proteins that combine a multimerization domain from one protein with an antigenic viral protein can create a family of novel VLPs. Other nanoparticle vaccines can be generated by mixing viral antigens with either lipids (e.g., liposomes, nano-emulsions) or proteins that tend to aggregate (e.g., proteosomes). Host cell elongation factors that affect translation of viral proteins can be indirectly involved in resistance. Reactivation of latent virus can lead to recurrent disease, such as repeated outbreaks of labial or genital herpes, or herpes zoster (“shingles”), a localized rash that can occur years after the initial chickenpox infection. Pathogenic viruses were repeatedly passaged through eggs or tissue culture cells under varying conditions until they acquired an attenuated phenotype. CH013.indd 152 16/05/11 8:04 PM Togaviruses At this stage, little or no positive-strand RNA is produced. Encodes two proteins from one open reading frame by RNA editing. Data adapted from Passarelli and Miller, J. Secondary structure in RNA consists of regions of self-complementary base sequence that form stemloop structures. Imperfect RNA hairpins in cellular transcripts are recognized and cleaved by the enzyme Drosha and are exported from the nucleus. Polymerase skips the first A residue but adds a terminal A to the minus-strand copy Surprisingly, polymerase initiates RNA synthesis not at the 3'-terminal A, but at the second nucleotide, a C. This protein, active as a hexamer, does not associate with the replicase but acts directly on the phage RNA and is thought to expose the 3'-terminal residues (CCAOH), which are base-paired to nearby G residues. These amino acids are distributed either on the external surface or the internal surface of the virion. Bernard Fields and his CH019.indd 226 coworkers used genetic approaches to study the pathogenesis of reoviruses in rodent models of infection and disease, especially viral neurologic illness. Immune stimulating complexes (ISCOM) More complex liposomal preparations that retain the capacity to elicit strong immune responses, but are more stable. These compounds had usually been synthesized for some other purpose, and discovery of their antiviral activities was often serendipitous. The carbohydrate portion of Vp54 (Figure 27.4a), which is oriented to the outside of the particle, contains seven sugars: glucose, fucose, rhamnose, galactose, mannose, xylose, and arabinose. Herpesviruses directly evade the antiviral function of dendritic cells by interfering with antigen presentation and processing. This is unusual; most viruses bind to cellular receptors via proteins that protrude from the surface of the virion. All interferon family members share the ability to enhance the expression of MHC 16/05/11 8:19 PM Virus Strategies to Counter Host Defenses class I proteins and promote the development of CD8positive cytotoxic T cells. (b) The arrangement of VP1 subunits in a 6-coordinated group. Viruses—genetics. domains of life, the Archaea, Bacteria, and Eukarya (eukaryotes) (Figure 9.1). Rhabdovirus genomes are somewhat smaller (11–12 kb). nsp15 Endoribonuclease. • Adeno-associated virus vectors can also integrate foreign genes into the cell chromosome; they are considered safer because these viruses cause no known human disease. All victims of new variant Creutzfeldt–Jakob disease in the United Kingdom up to the present have been found to be homozygous for methionine at position 129. CH013.indd 154 16/05/11 8:04 PM Togaviruses Initiation of the budding event is thought to occur when a nucleocapsid in the cytoplasm recognizes the cytoplasmic tails of arrays of the E1/E2 glycoprotein heterodimers localized in the plasma membrane. Replication complexes are often observed on double-membrane vesicles by electron microscopy (Figure 14.3). However, these deadly zoonotic (animalborne) virus infections have had relatively little impact on human civilization due to their local distribution and their low capacity to spread from person to person. Adjacent to the repeated sequences at either end are unique regions designated U5 (80–200 nt) and U3 (240–1200 nt). Cheshire Cat dynamics: sex to avoid virus infection The Red Queen’s race in Lewis Carroll’s book, “Alice’s Adventures in Wonderland”, is a common metaphor for an evolutionary arms race. A virus suspension was subjected to 10-fold serial dilutions by adding 1 mL of the original suspension to 9 mL of a dilution buffer. Gene 1 PLP2 PLP1 Genes 2–7 3CLpro MP1 2 3 4 Hel 12 13 ExoN OMT EndoN MP2 5' Cap 1 Pol 5 6 HE 14 8 10 7 9 11 S E M N Poly(A) 3' 15 16 2a 4 5b ORF1a ORF1b Figure 14.1 Organization of the coronavirus genome. Measured aliquots of dilutions of virus suspensions can be applied to grids, and the number of virus particles in a given area can be counted. B lymphocytes: white blood cells that originate in bone marrow and are involved in the adaptive immune response, especially in steps leading to the production of humoral antibodies. Part of the interferon response, this enzyme is activated by double-stranded RNA. Replication pattern follows differentiation of epithelial cells in skin or mucosa. Epidemics of influenza occur every winter, usually between December and March in the Northern Hemisphere. To carry out these functions, macrophages must first become activated, a process that enhances cytocidal activities. (b) Activation of PRM by CI prophage repressor. It was soon found that interferon induced by one kind of virus can inhibit replication of many different virus types. The role of interferon in macrophage activation and cellular immunity Macrophages function as key effector cells in innate and adaptive immune responses. (c) C60, known as buckminsterfullerene. Replicative intermediate: for RNA viruses, an RNA molecule on which one or several growing complementary RNA strands are being synthesized. Another variation is seen for reoviruses, which are non-enveloped double-stranded RNA viruses. Carstens, Queen’s University 26. Phages that can either

grow lytically or exist as a repressed prophage are called temperate phages. Many of our ancestors may have succumbed to prion disease as a result of consumption of human tissues. A rare complication of measles virus infection is a progressive, fatal neurological disease called subacute sclerosing panencephalitis (SSPE). They also interfere with the transport of influenza virus ribonucleoprotein complexes from the cytoplasm to the nucleus, where they are transcribed and replicated. CH019.indd 228 The attachment protein of type 3 reovirus contains two receptor-binding domains: one in the tail, which binds to sialic acid, and another in the head, which binds to junctional adhesion molecule A (JAM-A) (Figure 19.4). Finally, an alternate pathway that spontaneously activates complement and normally serves to eliminate senescent cells also can amplify complement activation by the classical and lectin pathways. Although many different gene therapy vectors have been developed, this chapter will focus on three of the most widely used vectors: those derived from adenoviruses, retroviruses, and parvoviruses (Table 37.2). GENES AND PROTEINS Five to nine genes, transcribed in series from the 3' end of the genome by viral RNA polymerase. Secondary structure of MS2 RNA between nts 1427 and 1807. At this point no more DNA enters the cell unless transcription is allowed to proceed. Antigenome RNA serves as a template for synthesis of progeny genomes (not shown) and in some cases ambisense mRNAs (bottom). Transcripts initiated at the S promoter encode both the small (SS) and medium (MS) surface proteins, while those initiated at the preS1 promoter encode the large surface protein (LS). Therefore, these viruses must bring along with the viral genome an RNA-dependent RNA polymerase that enables them to produce viral messenger RNAs. This enzyme is packaged within the virion. (i) Drawing of ATV. 1946–75 Introduction of vaccines for polioviruses types 1–3 (Sabin attenuated strains and Salk inactivated virus); measles, mumps, and rubella viruses; tick-borne encephalitis virus; mouse brain, duck embryo, and tissue culture vaccines for rabies virus; inactivated influenza A and B viruses; and adenoviruses. Signal peptidase: a host protease that cleaves the signal sequence from membrane or secretory proteins during or shortly after their transport into the lumen of the endoplasmic reticulum. The 11-kb single-stranded alphavirus RNA genome (Figure 13.2) contains two separately translated modules. Once the double-stranded DNA is made (and integrated into the cellular genome: see Chapter 28 for details), it is transcribed into viral messenger RNAs by host cell RNA polymerase II, as for most DNA viruses. Histone deacetylases (HDACs) bound to Rb are targeted to E2F-regulated genes and induce transcriptional repression by compressing chromatin structure, reducing access of proteins that activate transcription. This is called "asymmetric" replication. The x-ray structures of these three polypeptides have been determined and are shown in Figure 12.4. NS1. To date, the only viruses reported in this genus infect the red tide-forming species Heterosigma akashiwo; these viruses are referred to as Heterosigma akashiwo virus (HaV). Cleaved in trans-Golgi vesicles by a furin-like cellular protease; rarely incorporated into virions. Despite the overall similarity of coding regions among genomes of different parvoviruses, they use 16/05/11 8:08 PM 240 CHAPTER 20 Parvoviruses map units 0 20 40 60 80 100 MVM NS1 p4 NS2 VP1 p38 VP2 AAV Rep78 p5 Rep68 Rep52 p19 Rep40 VP1 p40 VP2, VP3 Figure 20.2 Genes and transcripts: Minute virus of mice and adeno-associated virus. The orientation of the prM-E heterodimer is parallel to the lipid bilayer, similar to the orientation found in mature virions. When protein B is present, five copies will bind to the underside of a 9S particle, yielding the 9S* particle. However, by using genetic engineering methods, vaccine developers now are able to create molecularly defined, attenuated vaccine-strain viruses. First, the foreign gene is cloned adjacent to the polyhedrin promoter in a plasmid 19/05/11 12:19 PM Baculoviruses hr1 hr2 hr3 hr4L hr4R 309 hr5 EcoRI 0 100 mu lef-2 lef-1 lef-6 lef-10 lef-11 lef-12 lef-8 dnapol lef-9 p143 lef-3 lef-4 lef-7 p35 ie-1 lef-5 pe38 ie-2 pp31 p47 A M E I B G C J M F K H D L AC Library AcMNPV C A B C D E F G H I J K L M Clones omitted from library Polyhedrin promoter activity Figure 25.4 Identification of baculovirus late expression factors (LEFs) by a transient expression assay. This would enable such RNAs to extend themselves, and by using base-pairing, replicate themselves in the absence of any protein-based enzymes. Hydrocephalus: swelling of the cerebral ventricles. This sexually transmitted virus caused an ongoing worldwide epidemic of acquired immune deficiency syndrome (AIDS) that has killed millions of people and remains unchecked despite the availability of effective anti-HIV chemotherapy. Since one molecule 16/05/11 7:54 PM Single-Stranded RNA Bacteriophages of maturation protein is packaged per plus-strand RNA molecule, this mechanism assures the appropriate level of its synthesis. This gene is organized as two partially overlapping open reading frames, ORF1a and ORF1b (Figure 14.1). Viruses with negative-strand RNA genomes must first synthesize complementary positive-strand copies of the genome, which serve as viral messenger RNAs. However, the cell does not produce an enzyme able to carry out this step, so the virus itself must supply this enzyme. In this approach, a large cDNA (more than 4.5 kb) is split into two AAV vectors. Innate immunity is exercised by a variety of cells and serum proteins that non-specifically recognize foreign pathogens or infected cells. An N-terminal signal sequence is cleaved from the precursor during insertion into the membrane. While most chlorella species are free-living, many species have symbiotic relationships with aquatic organisms CH027.indd 327 Figure 27.1 Paramecium bursaria filled with the symbiotic alga Chlorella NC64A, which is a host for chloroviruses. KEY TERMS Abortive Apoptosis Caveolae Cochaperone Cyclins Divergent transcription DNA gyrase Ethidium bromide Gangliosides Helicase Intergenic region Myristate Nonpermissive Nucleosomes Oncogene Polyadenylation signal Progressive multifocal leukoencephalopathy (PML) Protein tyrosine kinase Sialic acid Sodium dodecyl sulfate Spliceosome Supercoiled TATA box Topoisomerase I Toroidal coil Transcription factors Transcriptional enhancer FUNDAMENTAL CONCEPTS • Polyomaviruses are among the smallest double-stranded DNA viruses, with a circular genome of 5.3 kb. Bunyavirus reassortment has been studied experimentally both in cells cultured in vitro and in mosquitoes. Their precise position and spacing is vital for optimal transcription. (d) Section through the center of a particle in the same orientation as in (c) shows a depression in the core (white) below the stargate vertex. Instead of an RNA primer (used by cells and by most DNA viruses) or a DNA primer (used by parvoviruses), adenoviruses use the preterminal protein as a primer for replication. Osmolyte: an organic compound that can affect osmosis, affecting cell volume and fluid balance. Copying of the remainder of the repeated sequence allows formation of a second repeat and release of a full-length DNA molecule containing repeats at both ends. This problem also applies to linear cellular chromosomes. Uptake into phagocytic vesicles is followed by fusion with lysosomes and activation of lysosomal proteases and nucleases that degrade virus particles. The unusually small polyomavirus introns have apparently evolved to reduce splicing efficiency between the 5' splice site at nt 748 and either of the two 3' splice sites. Capsids are formed by the assembly of 12 such pentamers into a closed shell by one of two pathways; they may assemble directly around viral RNA (upper pathway) or they may form an empty 80S procapsid, which then incorporates viral RNA (lower pathway). Virus replication cycles vary greatly, depending on the host cell, genome type, and complexity of the virus. Rolling circle replication subsequently produces multimeric concatemers of viral DNA At some point one of the strands in the parental DNA is nicked, resulting in rolling circle replication (Figure 24.8). It is therefore not surprising that most clinically useful antiviral drugs are enzyme inhibitors that target genome replication, maturation, and release. Aphids: small insects that feed on plants using sucking mouthparts called stylets. COCCOLITHOVIRUSES Viruses that control the weather There are not many viruses that have a clear link to controlling the weather, yet the coccolithoviruses ("cocco", derived from Greek kokkis, meaning berry or grain, referring to their shape; and "lith", from Greek lithos, meaning stone) are able to make this bold claim. 1 1' Figure 30.5 Reverse transcription of pregenome RNA to form hepatitis B virus genome DNA. As these DNA viruses replicated and evolved, virus-coded proteins that specifically bind to the viral origin of replication and attract the cellular DNA replication machinery would ensure preferential replication of viral DNA. Arthropod-borne viruses ("arboviruses") 471 BGLOSS.indd 471 05/05/11 2:45 PM 472 Glossary generally replicate both in the arthropod vector and in the vertebrate host. The structure showed that sucrose molecules (a dimer of glucose and fructose) were bound 16/05/11 7:56 PM Microviruses 71 Table 6.2 Gene products of X174 Protein Function A DNA replication. Viruses within the Bromovirus and Cucumovirus genera have similar packaging strategies, producing three different virions, each containing one or two viral genome segments. Like many other plant viruses, cucumber mosaic virus moves through plasmodesmata from the epidermis to mesophyll tissues, where the majority of replication occurs. In addition to the N protein, about 300 copies of P protein and 50 copies of L protein are packaged with the nucleocapsid; both are required for transcription once the virus enters the cell. How picornavirus genome RNAs engineer their translation and how the virus eventually takes over the cell's translation machinery is a fascinating story. Strategies for controlling transgene transcription Transgene expression in target cells can be controlled by the enhancer/promoter elements of the LTR or by using a second promoter that directly controls the transgene (Figure 37.4c). These include amantadine, a small molecular weight molecule that specifically blocks the M2 proton channel of influenza A virus and thus prevents acidification of the virus interior. A number of these proteins contain predicted membrane-spanning sequences. Genome RNA x x 2. The mechanisms and strategies by which incoming viruses enter the nucleus differ from one family to the next. Therefore, there is a strong link between induction of early viral transcription and initiation of viral DNA replication. Stressed cells degrade much of their contents by forming specialized membrane compartments called phagosomes, which are similar to lysosomes. cell. America Tick Eastern Europe, Africa, Asia Hantavirus Hantaan virus Nairovirus Crimean-Congo hemorrhagic fever virus Human: hemorrhagic fever; 20–80% fatality Nairobi sheep disease virus Sheep, goats: fever, hemorrhagic gastroenteritis, abortion Tick E. In both of these trials no adverse event following AAV treatment was detected, demonstrating the safety of the vector. Capsid contains six proteins, envelope contains at least ten glycoproteins. 05/05/11 11:24 AM Chloroviruses was isolated; that virus infects the marine unicellular alga Micromonas pusilla. Lectins: plant proteins that bind to specific oligosaccharides in cell membranes. Circular DNA becomes supercoiled upon removal of histones When viral genomes are gently released from the capsid without denaturing the bound nucleosomes, they can be visualized as circular molecules by electron microscopy, and the minichromosome resembles a necklace of "beads on a string" (Figure 21.3). Expression of many coccolithovirus transcripts may therefore occur in the cytoplasm rather than the host nucleus. Figure 36.2 is a model showing how picornaviruses bind to their cellular receptor via the so-called "canyon" in the surface of the capsid. In 1989, a full-length cDNA clone of yellow fever virus was constructed that could be used to generate infectious viral RNA in vitro. Negative contrast electron micrographs of (a) two intact virions, (b) two disrupted virions releasing the helical nucleoprotein core (Scale bars: 100 nm), (c) drawing of a globulovirus. It terminates with a narrow channel, 2 nm in width, and an anchor-like structure formed by two furled filaments, each 4 nm wide. Gramineae: "true grasses," including cereals, bamboo, and many marsh and grassland plants. The name of the best-known family member, ϕX174, reflects its history. Despite the high degree of similarity among members of the aminopeptidase-N family, the utilization of these receptors by coronaviruses is for the most part species-specific. T cells that recognize and bind to peptides on MHC molecules differentiate into helper and cytotoxic T cells. PB1 (orange sphere) copies genome RNA into a plus-strand complementary RNA that is coated with NP protein as a viral nucleocapsid. GENES AND PROTEINS ϕX174: 3 transcriptional promoters and 4 terminators control gene expression. Interferon-2 and interferon-3 share 96% amino acid sequence identity but they share only 81% amino acid sequence identity with interferon-1. Ancestors of mimivirus may have transferred genes from bacteria to eukaryotes MutS is an interesting DNA repair gene that provides a telling insight into the checkered history of the genome of the mimivirus. Thus, effective repression of PRM depends on OR1, OR2, OL1, and OL2 for CI octamerization, and on OL3 and OR3 for CI tetramerization. These figures complement the text and draw the attention of students to important concepts.Text boxes throughout the book feature exciting and current developments in molecular virology or practical applications of viruses in science and medicine. Protomers: subunits used to build a larger structure. Basal cells: cells in the epidermis of mammalian skin that divide and differentiate into the outermost squamous cells of the epithelium. Autophagy is an intrinsic cellular process that increases cell survival during periods of stress, such as nutrient starvation and infection. The folded structure of the parvovirus capsid protein is based on a domain known as a jelly-roll β-barrel (Figure 2.3b). Given the nature of the infecting agent and its transmission route, preventive steps could be taken to reduce the spread of the virus. Antigens: molecules or parts of a molecule that can induce antibody formation by binding to a specific antibody or T-cell receptor. Nef also interacts via its proline-rich domain with members of the Src family of tyrosine protein kinases and alters their activity. Introduction of a hamster or human prion transgene into such mice restored susceptibililty to infection with hamster or human prions, respectively. The six early transcription units are named E1A, E1B, E2A, E2B, E3, and E4. FUNDAMENTAL CONCEPTS • Coronaviruses are responsible for common colds, a number of important veterinary diseases, and an outbreak of severe acute respiratory syndrome (SARS). 16/05/11 7:49 PM Capsids with Helical Symmetry CAPSIDS WITH HELICAL SYMMETRY An alternative to a closed shell for packaging viral genomes is an elongated tube. However, the clinical use of drugs to treat viral diseases in humans lagged behind, and the first antiviral drugs were introduced only in the 1960s. 5' end is capped and methylated. Replicase complexes containing this nsP2 mutation regain the ability to carry out negative-strand RNA synthesis when combined with mutations in P123 that destroy the sites of cleavage between the individual nonstructural proteins nsP1, nsP2, and nsP3. To help understand the role of sGP, an infectious virus with a mutation in the editing site of the GP gene was made by the multiplasmid system. Diameter 80–120 nm. The 20 non-polar amino acids of the transmembrane anchor form an alpha helix that neatly spans the 3-nm-thick hydrophobic part of a lipid bilayer membrane. Many bacteriophages are constructed from a head with icosahedral symmetry and a tail with helical symmetry. This protein forms a pore in the plasma membrane of a target cell, leading to apoptosis, often with the help of cytolytic proteins such as granzyme B. Genes are functionally grouped. Extensive internal base pairing. The space between the envelope and the nucleocapsid is denoted the tegument. As a result, ribosomes translating the edited mRNA make GP, a protein of 676 amino acids. This is the basis of vaccination (Chapter 35), still by far the most successful antiviral strategy available. Furthermore, recombination can occur between the DNA of the helper virus and the third-generation adenovirus vector, resulting in a hybrid population containing elements belonging to both vectors. Cells respond to infection by synthesizing and secreting interferons and other cytokines. Herpes simplex virus establishes latent infection in neurons Latency is a hallmark of all known herpesviruses in their natural hosts. A major 210 CH018.indd 210 16/05/11 8:07 PM Influenza Viruses reason is the ability of influenza viruses to escape immune surveillance by changing the amino acid sequences of their surface glycoproteins. narrow size range. 1373, in Mavis Agbandje-Mckenna, Antonio L. They consist of a set of nucleic acid genes enclosed in a protein coat, called a capsid, which in some cases is surrounded by or encloses a lipid membrane, called an envelope (Figure 1.1). Early messenger RNAs are extruded from cores into the cytoplasm, where they are translated into early virus proteins. coli strains and screened for phages that would produce plaques on male (F) but not female (F) bacteria. Stat proteins bind to interferon-stimulated genes in the nucleus and activate transcription. At this point the herpesvirus DNA polymerase, product of the UL30 gene, binds to the RNA primers and extends them as DNA chains. Synthesis continues through the entire length of the genome (200,000 nt) using one DNA strand as template, then through the distal hairpin, and back through the entire length of the genome using the complementary strand as template (step 5), to generate a head-to-head dimer. The insertion of a reporter gene into a virus genome allows for the detection of virus infection and replication by assaying for the appearance of the reporter protein. Therefore, the humoral response is vital for protection against infection by viral pathogens, while cellular immunity is important for recovery from viral infections. CH010.indd 122 Satellite RNAs can either attenuate or increase severity of symptoms in infected plants Cucumber mosaic virus satellite RNAs are highly structured, with more than 50% internal base pairing. Dried pus from a smallpox or cowpox lesion would likewise seem quite benign in the setting of a smallpox epidemic with 20 to 25% mortality. Viruses can infect all forms of life Viruses also infect animals, plants, and insects of importance to humans. Programmed cell death, or apoptosis, results from a complex cascade of events in which a cell can self-destruct (Figure 33.4). Int synthesis is controlled by retroregulation After excision of a prophage induced to lytic growth, and also during a lytic infection, high levels of Int are no longer necessary. What was discovered about T7 RNA polymerase that galvanized the field of transcription? A likely possibility is that the pilus with the attached RNA retracts into the cell, dragging the RNA and the cleaved maturation protein along with it. CH031.indd 382 Interaction of viroid RNA with cellular RNAs or proteins may disrupt cell metabolism The molecular mechanisms by which viroids cause disease in plants remain mysterious. Binding of the cellular receptor molecule to the canyon is thought to release the lipid molecule from the pocket. On the other hand, it is commonplace for many double-stranded DNA viruses to have coding regions for mRNAs and L RNA codes for viral RNA polymerase The L genome segments of all bunyaviruses encode a single large protein, the L protein, which is translated from an mRNA complementary to the genome RNA. Polyprotein synthesis and processing is a common expression strategy among other positive-strand RNA viruses including picornaviruses, flaviviruses, and togaviruses (Chapters 11 through 13). Figure 6.2 The atomic structure of ϕX174. For instance, Ngari bunyavirus, isolated from humans suffering hemorrhagic disease, was shown to be a reassortant between Bunyamwera and Batai viruses, each of which causes relatively mild febrile illness. This complicates the machinery required for virus replication (Figure 18.6). However, it is difficult to explain how changing a few nucleotides can convert a severe variant into a mild one, since these changes generally are not located in the regions proposed to base pair. Wilson, Bigelow Laboratory for Ocean Sciences SECTION X: ANTIVIRAL AGENTS AND VIRUS VECTORS 35. The 185-amino acid core protein is the major component of the nucleocapsid, which packages the viral genome in the mature virion. ICP0 activates transcription of and genes by relieving the silencing of these genes, whereas ICP4 recruits cellular transcription factors to their promoters (Figure 24.6). Gene replacement therapy for genetic diseases has seen some recent success, and a variety of virus vectors directed against human cancers are being developed. How does T7 solve the problem of RNA primer-directed replication of linear DNAs? These particles are composed primarily of lipids and hepatitis B surface proteins. The transfer construct derived from AAV2 can be efficiently packaged into virions made of capsid proteins of different serotypes. The HA2 domain of the protein serves independently as an acid-activated membrane fusion factor. Thus the presence of double-stranded RNA in a cell is perhaps the most reliable indicator that a replicating virus is present, and it makes sense that the cell recognizes and responds to this molecule. Polyhedrin: protein that forms the polyhedral occlusion bodies of nucleopolyhedroviruses. The HN and F proteins are shown projecting from the viral envelope. How and when did viruses first appear during evolution? E1B proteins suppress E1A-induced apoptosis and target key proteins for degradation, allowing virus replication to proceed The E1B region also produces multiple mRNAs by alternative splicing. Cloned cDNA copies of viral mRNAs and viral genome RNA are used to study filoviruses Because of the severity of human filovirus disease and the lack of vaccines and therapeutic agents, experimental study of live infectious Marburg and Ebola viruses is restricted to a very small number of laboratories that have high-level biological containment (Biosafety Level 4 [BSL-4]). akashiwo to HaV differs among clonal strains in the laboratory. Synergism between E6 and E7 and the predisposition to cancer Although p53 and Rb are multifunctional tumor suppressor proteins, they intersect in a common regulatory pathway. Eight different genotypes can be represented in progeny virus: the two parental genotypes and six possible reassortant genotypes, each with two segments from one parent and one segment from the other parent. An effective rotavirus vaccine has recently been developed. Assembled nucleocapsids are retained in the nucleus of infected cells where they become associated with membranes, eventually becoming enveloped either singly or as groups of multiple nucleocapsids. Full-length genome or antigenome RNA is always encoated in nucleocapsid proteins. Proteins encoded by coccolithovirus genomes are remarkably different from those of other viruses, as 80% of their protein-coding sequences have no matches in current protein databases. It is likely that viruses infect all of these species, although not all of these viruses will be assigned to the Phycodnaviridae family. The first step in DNA replication is unusual. Interferon- stimulates macrophage activity. the stop and start sites (but not coding regions) of several Ebola virus genes and one Marburg virus gene actually overlap by a few nucleotides, so that the 3' end of one mRNA lies downstream from the 5' end of the next mRNA (Figure 16.3). NP interacts either with the RNA polymerase complex or with the 5' and 3' ends of the genome RNAs in nucleocapsids, and the RNA polymerase begins copying the genome RNA directly from its 3' terminus, with no intervening primer. CH004.indd 51 For example, in the case of dengue virus, at neutral pH the fusion proteins are homodimers that lie parallel to the surface of the viral envelope (see Figure 12.3). GENES AND PROTEINS All genes are translated as single polyprotein followed by proteolytic cleavage. Transcription within the cells generates the ten different viral mRNAs, each with its authentic 5' end. The transfer process begins with the assembly of virions in the infected cell, followed by their release, transmission within the organism or in the environment to uninfected cells, and finally, entry into a new host cell. So carries a site-specific recombination system for integration into and excision from the bacterial chromosome (Figure 8.7). Other viruses bind to cell surface proteins that are found only on specific cell types, and are often species-specific, thus limiting the tropism of the virus to a particular tissue and organism. Engineering of DNA has generated a variety of powerful cloning vectors for genetic and genomic studies. Immunogenic: able to induce an immune response. Required for initiation of transcripion of minus-strand RNA and modulation of nsP2 protease activity. RNA transcripts with this 5' end are not efficiently exported to the cytoplasm, perhaps because they are missing a critical RNA export sequence. Most small viruses with icosahedral symmetry are roughly spherical, but many CH002.indd 21 21 larger viruses (adenoviruses, phycodnaviruses, many tailed bacteriophages) have capsids with the shape of a regular icosahedron. Eye drops, troches, suppositories Formulations that deliver vaccine antigens to conjunctival, vaginal, or gastrointestinal mucosae. However, it is striking that for such a large virus, so far few proteins with truly novel metabolic functions have been identified on the mimivirus genome. The complete P1234 polyprotein is formed only when there is readthrough of this codon, which occurs 10 to 20% of the time, depending on the host cell. • Small, linear satellite RNAs that replicate and are encapsidated along with cucumber mosaic virus RNAs can either worsen or attenuate symptoms of infection by the virus. Picornaviruses have an unusually long (600–1200 nt) stretch of 5' noncoding sequences upstream of the AUG codon at which protein synthesis begins. Virus vectors are used to produce high levels of specific proteins in cultured cells The first virus used as a vector for protein production in mammalian cells was simian virus 40 (SV40), a 457 polyomavirus. These helper viruses provide functions needed for efficient replication and transcription of AAV genomes and abundant synthesis of capsid 16/05/11 8:22 PM 466 CHAPTER 37 Eukaryotic Virus Vectors proteins. Vestergaard, K. to major histocompatibility complex (MHC) molecules, which are normally expressed on all cell types. This is followed by the beginning of viral DNA replication at 12–15 hours after infection. All five of these viruses appear to be widespread in the human population and are aquired during early childhood, but usually cause no apparent disease. A self-cleaving ribozyme (see Chapter 31) is engineered into the plasmid so that it is transcribed along with the genome RNA, and cleaves itself to generate the exact 3' end of the genome. The genomes of all known satellite viruses are less than 2 kb in length, and those that contain no protein-coding regions are less than 700 nucleotides in length. Insertion of gene(s) of interest into the vector genome. The activity assay is then used to screen large libraries of chemicals. Host range mutations change amino CH006.indd 71 acids primarily in the spike proteins G and H, indicating that the spikes are also involved in attachment to host cells, and arguing that a second host cell receptor may be required for DNA penetration. It is the role of the viral E7 protein to induce cellular DNA synthesis. These membranes are the sites of viral RNA synthesis, and are thus referred to as replication complexes. Once sufficient P123 has been made, trans-cleavage generates nsP1, P23, and finally nsP2 and nsP3. The discovery of human immunodeficiency virus (HIV), a lentivirus, in the mid-1980s (see Chapter 29) 343 led to an explosion of work on this newly emerged human pathogen, responsible for a worldwide epidemic of acquired immunodeficiency syndrome (AIDS). Experiments have shown that if the start codon of the coat gene is deleted, preventing ribosomes from translating this gene, there is no synthesis of either the lysis or the replicase protein. Despite their extreme simplicity, viroids cause plant diseases as varied as those caused by plant viruses. Abortive: an infection that does not lead to a complete virus replication cycle. Two envelope proteins: surface (SU) and transmembrane (TM). Viral genomes contain multiple genes for capsid proteins Among the genes of novel function found are Nmyristoyl-transferase, 3-dehydroquinate synthase, multiple glycosyl- and methyl-transferases, prolyl 4-hydroxylase, and 6-phosphofructokinase. Rachel, R. Interestingly, cleavage between nsp1–nsp2 and nsp2–nsp3 is not required for viral replication in cultured cells, as these cleavage sites can be deleted without drastic effects on virus replication. The important role played by baculoviruses in insect diseases suggested that they might be harnessed to control insect pests. The 3D RNA polymerase absolutely requires a primer to copy RNA molecules, similar to most DNA polymerases but unlike many other RNA polymerases. There is overwhelming evidence that some eukaryotic viruses share common ancestors with bacteriophages. Interleukin-2: a cytokine that stimulates T-cell growth and proliferation. Wilson Phycodnaviridae From Greek phykos, referring to seaweed, and DNA, referring to the nature of the genome Mimiviridae From "microbe mimicking", because it stains gram-positive, like some bacteria VIRION Regular icosahedral capsids, diameter 100–500 nm. One theory is that high levels of E protein may suppress the host immune system and prevent it from eliminating cells that contain hepatitis B virus. Experimentally transferred to mice, hamsters, chimpanzees. Occlusion–derived virus (a) (b) Polyhedron (NPVs) Enveloped virions Baculoviruses produce two kinds of particles: "budded" and "occlusion-derived" virions The baculovirus infectious cycle is unique because two types of virions are produced: budded virions and occlusion-derived virions. Binding leads to a major rearrangement (shown at right) during which VP4 is externalized and the hydrophobic N-termini of the five VP1 molecules (only two shown here) insert into the membrane to form a channel through which the viral RNA can pass into the cytoplasm. This means that lentivirus vectors can transduce stationary cells as well as dividing cells. It has been estimated that there are over 170 million hepatitis C seropositive individuals in the world. Induction occurs in cells that have suffered DNA damage caused by ultraviolet Table 8.1 Control of PRM and PR by CI and Cro repressors Occupancy at operator Protein CI Cro CH008.indd 89 Concentration OR3 OR2 Activity of promoter OR1 PRM PR None Weak On Low Activated Off High Off Off None Weak On Low Off On High Off Partially off 16/05/11 7:59 PM 90 CHAPTER 8 Bacteriophage Lambda to OR3 and blocks PRM. After transport through early and late endosomes, simian virus 40 and other polyomaviruses are brought to the endoplasmic reticulum, where penetration to the cytosol takes place. A wheat germ RNA ligase known to participate in splicing of tRNA introns can circularize linear unitlength potato spindle tuber viroid RNAs. This enzyme, which produces 3'-5' phosphodiester bonds, is localized in the nucleus, and therefore appears a logical candidate for the activity that ligates Pospiviroidae RNAs. How do viroids cause disease? The best-characterized model is reovirus pathogenesis in the mouse central nervous system. However, many details of the process are different, as described below (Figure 30.5 and Table 30.1). Type II interferon, called interferon- or "immune interferon," is only distantly related to the type I interferons. degree of sequence and organizational homology with the genome of HRPV-1. The coupling between translation of the coat and replicase genes works like this. Lineage: a collection of individual organisms linked by close genetic relationships. Some bunyaviruses cause severe hemorrhagic fever, respiratory disease, or encephalitis Although most bunyaviruses have little impact on humans, some are important pathogens, either directly by causing disease in humans, or indirectly by affecting domestic animals or food crops (Table 17.1). Finally, a set of Review Questions is included as a further review tool and to alert the student to the kinds of knowledge that might be expected in test questions. Attempts to define a receptor for hepatitis B virus have yielded various candidates, including apolipoprotein-H, fibronectin, interleukin-6, annexin V, and an unknown 80-kDa protein. This approach was used to discover non-nucleoside reverse transcriptase inhibitors such as nevirapine. Known genes are labeled. Table 11.2 lists some cell surface receptor molecules used by various picornaviruses to bind to target cells. DISTINCTIVE CHARACTERISTICS Tegument proteins function in early steps of virus replication: activation of transcription and host shutoff. 16/05/11 7:49 PM 30 CHAPTER 2 Virus Structure and Assembly KEY TERMS Buckminsterfullerene Budding Capsid Core proteins Cryoelectron microscopy Ectodomain Envelope Genetic economy Glycoprotein Helical symmetry Hexon Icosahedral symmetry Jelly-roll -barrel Matrix protein Myristate Negative staining Nucleocapsid Packaging sequence FUNDAMENTAL CONCEPTS Palmitoylation Penton Positive staining Pseudovirion Quasi-equivalent Scaffolding proteins Self-assembly Signal sequence Synchrotron Transmembrane anchor Triangulation number • Viruses enclose their nucleic acid genomes in protein capsids; some viruses also have an internal or external lipid-containing envelope. • Phage RNA polymerase binds to the start of the coat gene at the same site where ribosomes bind; therefore an RNA can be either replicated or translated, but not both at the same time. CAPSID V II VII III X TP DNA IIIa IV VI VIII IX Figure 23.2 Structure of adenovirus virion. Algae are a diverse group of photosynthetic eukaryotic organisms that are ubiquitous in marine, freshwater and terrestrial habitats. Over 500 cases were reported, with lethality rates of 88% in Zaire and 53% in Sudan. Messenger RNAs whose genes lie near the (proximal) 3' end are less affected by a given UV dose than mRNAs whose genes lie near the (distal) 5' end of the genome. Viroids trick cellular RNA polymerases that normally transcribe DNA genomes into replicating the viroid RNA by making long RNA oligomers. Their protrusion presumably results from a conformational change in a virion structural protein. The envelope construct. Within each of these families there is a wide range of genome sizes, gene organization, and replication mechanisms. When this occurs in response to the presence of damaged DNA, cells will not replicate until the damaged DNA has been repaired. Examples of crises of public confidence in vaccines include the pertussis vaccine in Japan in the mid-1990s (a real association with fever and hyporesponsiveness), measles vaccine in the United Kingdom (a spurious association with autism), and poliovirus vaccine in northern Nigeria in 2004–2005 (a politically motivated claim of attempted genocide). Coat protein (CP; 24 kDa) is the only protein in the virus capsid. The genome of Pyrobaculum spherical virus has nearly all open reading frames encoded on one DNA strand The virions of Pyrobaculum spherical virus (PSV) and Thermoproteus tenax spherical virus 1 (TTSV-1) are enveloped, spherical particles, approximately 100 nm in diameter (Figure 9.8a, c). Subsequent modification of the immunization protocol accelerated protection. The polyprotein open reading frame is shown at reduced scale. Similarly, there are more mRNAs that encode the J, F, and G proteins than mRNAs that encode the H protein, because transcription is successively reduced at a series of terminators at the ends of each of these four genes. The branching could result from initiation of replication at several origins on the concatemers, as well as intermolecular recombination between replicating DNAs. However, Table 24.3 Herpes simplex virus proteins that direct viral DNA replication Protein Activity UL9 Binds to replication origins UL29 (ICP8) Binds to single-stranded DNA UL5 UL8 UL52 Helicase-primase complex UL30 DNA polymerase UL42 Processivity factor 16/05/11 8:12 PM 292 CHAPTER 24 Herpesviruses herpesvirus initially sets up bidirectional replication on the circular viral DNA, and only later shifts to rolling circle replication. f1 turned out to be a filamentous phage with a single-stranded DNA genome, and f2 was the first RNA-containing phage to be discovered. (a) Virus particles bound to the cell surface via their spike glycoproteins are trapped in clathrin-coated pits. The complementary plus DNA strand is joined to a short, capped RNA at its 5⬘ end. Monomeric circular antigenome and genome RNAs are formed by self-cleavage and ligation of multimeric linear RNAs (steps 3, 4 and 7, 8). This cycle can generate several new circular plus-strand RNA daughter molecules from each plus-strand template, because transcription continues for multiple rounds at both steps 1 and 5. Fission: see Membrane fission. Note that the sphere shown in Figure 2.2c has icosahedral symmetry, but it does not have the shape of an icosahedron (Figure 2.2b). Scrapie is a naturally occuring prion disease of sheep; kuru was a human disease transmitted among members of a New Guinea tribe by handling prion-infected human tissue. These defenses can be conveniently separated into two categories: intrinsic cellular defenses and host immune responses. Tropical spastic paraparesis (TSP): a neurological disease, caused by HTLV-1 infection, that leads to demyelination of motor neurons. Retrotransposons may have been the precursor of retroviruses. This was the first human virus shown to be oncogenic. The primosome enables primase, one of its components, to generate short complementary RNA primers at different locations around the single-stranded phage DNA. the final 32 kb are pulled into the cell during transcription by the phage T7 RNA polymerase • Class I ("early") genes are transcribed by cellular RNA polymerase and include the gene coding for phage T7 RNA polymerase. Most of these vaccines continue to be used effectively even though we remain ignorant of the precise mechanisms of attenuation and protection against infection. The cell therefore grants free passage for its lethal cargo through a maze of obstacles within the cytoplasm. If present in germ line cells, the viral genome (and therefore virus) can be inherited, and will persist by "vertical transmission". There appears to be a correlation between the decrease in cytopathogenicity and the decrease in viral RNA synthesis, but it is not known which factors are most critical in producing cytopathic effects nor why mutations in the nsP2 gene allow cell survival. However, filamentous particles are also made. The replication origins, including the first 50 nt of each inverted terminal repeat, contain binding sites for a complex between the adenovirus DNA polymerase and preterminal protein. If the nick is sealed with DNA ligase (lower right), this becomes a relaxed, closed circular DNA molecule. Sequence analysis indicates that RNAs of Pospiviroidae family members are characterized by five domains: TL (terminal left), P (pathogenic), C (central), V (variable), and TR (terminal right) (Figure 31.1b). KEY TERMS Apoptosis Ascites Cirrhosis Complement Enteric Epitope Exocytosis Fulminant hepatitis Hepatitis Hepatocellular carcinoma Immunoprophylaxis Interferons Jak–Stat pathway Jaundice Myristate Myristoylation NF-B (nuclear factor-B) Ras Ribonuclease H Serum sickness Src kinases Tumor necrosis factor (TNF) Tumor suppressor protein 05/05/11 1:08 PM 376 CHAPTER 30 Hepadnaviruses FUNDAMENTAL CONCEPTS • Hepatitis B virus, like retroviruses, encodes a DNA polymerase with reverse transcriptase activity. Subgenomic mRNAs are transcribed from subgenomic negative-sense RNA templates made by discontinuous transcription Experimental evidence supports a model in which each subgenomic mRNA is transcribed from a corresponding subgenomic negative-strand RNA. Proteosomes: vesicles made from the outer membrane of Neisseria bacterial species, into which exogenous antigens are introduced. Minute virus of mice (MVM), an autonomous parvovirus, was first discovered contaminating a preparation of a mouse adenovirus, suggesting that these viruses, though competent for replication, are not above using some help from a larger virus if it is available. Nef binds to CD4 and to the adaptor protein AP2 on the plasma membrane, and this complex is recruited into clathrin-coated pits and endosomes (Figure 29.7b). Viruses can transfer genes between organisms. However, 05/05/11 1:08 PM 368 CHAPTER 30 Hepadnaviruses preS2 preS1 S P 1/3219 HBV genome (adw subtype) 3219 bp Enh 1 Some regions of interest Enh 2 C Enh 1 DR2 Enh 2 preC RNA start pg RNA start DR1 -Stem Loop Poly (A) Signal X preC preC RNA pg RNA C preC 1961 Poly(A) signal 1060-1260 1590-1600 1635-1714 1785-1791 1818,1819 1824-1835 1847-1907 1916-1921 X P Enh 2 DR1 DR2 1561 Figure 30.3 Coding and signaling regions on the hepatitis B genome. This coiling is mediated by the rhabdovirus matrix (M) protein. Some members of the Reoviridae are important pathogens Other recognized genera of Reoviridae that infect animal hosts are Rotavirus, Orbivirus, Coltivirus, and Aquareovirus. RIG-I and MDA5 recruit the adaptor protein IPS-1 upon detection of viral RNAs (Figure 33.3). As do VA RNAs, EBERs may be involved in modulating the cellular interferon response by inhibiting activity of PKR, a protein kinase that can interrupt protein synthesis. During granulovirus infection, the cytoplasm merges with the nucleus and a distinct nuclear membrane structure is no longer present. • E1A proteins bind to cellular Rb protein, dissociating it from cellular E2F protein and stimulating transcription of other viral genes and numerous cellular genes that bring non-dividing cells into the cell cycle. VA RNA is a short, double-stranded RNA that binds to and inhibits RNA-dependent protein kinase (PKR). The e antigen was named for its "early" appearance in serum during an acute hepatitis B virus infection. Although such vectors are more efficient at transducing cells, the disadvantage of double-stranded AAV vectors is a smaller capacity for foreign DNA sequences. To avoid adenovirus contamination of AAV vector preparations, it is now current practice to produce AAV by transfecting the cells with a helper plasmid that codes for all essential adenovirus functions, eliminating the adenovirus vector (Figure 37.6). DNA-dependent DNA polymerases, to replicate the DNA genomes Retroviruses could have originated during the transition to DNA-based cells At this stage of evolution, retroviruses and other viruses that use a reverse transcriptase could have emerged. It has been identified as the polymerase responsible for both genome replication and subgenomic mRNA synthesis. has been a valued source of knowledge and understanding for more than 200 years, helping people around the world meet their needs and fulfill their aspirations. Cleavage of concatemers and copying of the terminal repeat is carried out during packaging of the DNA into the prohead, with the help of gene products 18 and 19. Live viruses replicate in host cells and produce progeny virus. The N-terminal region of protein 2a interacts with protein 1a, and phosphorylation in this region abolishes this interaction and virus replication. Partial repression of PR and PL by Cro reduces synthesis of early proteins that are already present in sufficient amounts to allow the lytic phase to proceed. Transcription of DNA is controlled by: • • • CI, cro: transcription repressors N, Q: transcription antiterminators CII: transcription activator VIRUSES AND HOSTS A genus within the family Siphoviridae. Some of these compounds exhibit less or different toxicity than AZT and some can be administered less frequently. In general, higher levels of messenger RNAs are made for proteins that are required in greater abundance. Ligation of peach latent mosaic viroid (an Avsunviroidae family member found in the chloroplast) can occur without the participation of host proteins. Filovirus genomes contain seven genes in a conserved order The complete nucleotide sequences of both Marburg and Ebola virus strains have been determined, allowing mapping of their coding regions. No projections, "golf ball" appearance. Two other human polyomaviruses, named WI and KU, were recently discovered in the respiratory tracts of children, but their association with human disease is not clear. The principle is outlined below (Figure 5.9). The functions required for complementation of third-generation adenovirus vectors are provided by coinfection with a helper virus, usually a first-generation adenovirus vector. Acidianus two-tailed virus (ATV) has a virion with tails that spontaneously elongate The virion of Acidianus two-tailed virus (ATV), the only known member of the family Bicaudaviridae (from Latin bi, "two", and cauda, "tail"), exists in two conformations. CH002.indd 22 sphere have head-to-head, neck-to-neck, and tail-to-tail interactions with their neighbors. This chapter focuses on intrinsic cellular defenses and the signaling responses by infected cells, particularly CH033.indd 399 399 DETECTION OF VIRUS INFECTION BY HOST CELLS Host cells sense virus infection with toll-like receptors and a variety of other molecular detection systems Organisms have evolved multiple ways of recognizing different aspects of virus infection, including virus attachment and entry, virus replication, and release of virus components from infected cells. After resolution of the acute infection, varying levels of viral RNA genomes ("virus load setpoint", Figure 29.1) can be detected in the blood of different individuals. PMID: 19216436, with kind permission of Springer Science+ Business Media. Viruses can spread from one cell to another within an organism, or they can move from one organism to another; virions that follow these two pathways are not necessarily identical. Influenza type B is limited to humans, and type C can infect both pigs and humans. RNA is capable of self-cleavage (hepatitis delta virus ribozyme). • Rabies virus is a rhabdovirus that infects bats, small mammals, dogs, and humans; the ensuing encephalitis is nearly always fatal. The late phase involves expression of viral RNA, synthesis of viral proteins, and assembly of virions. Various insults to the cell, including infection by polyomaviruses, can lead to increases in the concentration and the activity of p53. Shunting is directed by complementary regions between the tripartite leader and the 18S RNA, which is part of the 40S ribosomal subunit. The virion is 50 nm in diameter and resembles a golf ball. The total number of protein subunits in a capsid is equal to 60 T. The last patient was admitted in November of the same year. CH001.indd 14 The seven groups in the Baltimore classification system 1. Prasinovirus MpV-SP1 Micromonas pusilla Dominant single-celled green algae found in both warm and cold marine waters. Genes for virokines and viroceptors are almost certainly derived from host cell genes that were incorporated into viral genomes and evolved by mutation and selection to confer an advantage in virus replication, spread or survival. Multinucleated giant cells: see Syncytium. A major determinant of neurovirulence for all three poliovirus serotypes is found in the stem-loop V region of the IRES (Figure 11.6). Following the capsid protein is the precursor to the membrane protein, prM (Figure 12.5). In fact, the closer the coat gene stop codon is to the lysis start codon, the more lysis protein is made. Some viruses form circular intermediates 16/05/11 7:57 PM 82 CHAPTER 7 Bacteriophage T7 that overcome this problem, but no circular forms of T7 DNA have been found. This would allow DNAs to take over the function of storing the information for protein synthesis, while the RNA copies could continue to serve as messengers and amino-acid-binding adaptors for templating protein synthesis. Dendritic cells Bind to antigens and virus particles in tissues; antigen presentation to T lymphocytes. Most cells in an organism are not dividing, but are differentiated and in a resting (G0) state. (b) Folding intermediate of the same region on nascent RNA, allowing translation of the maturation gene. Enzymes that direct DNA replication are encoded by early mRNAs Table 26.3 lists viral gene products with known or presumed functions in DNA metabolism. Transmission of viruses that infect genital tract mucosa is largely through sexual contact with an infected individual. Although the exact mechanism by which these proteins interact with VP40 to form budding virions is not clear, it may involve ubiquitination of VP40 or associated proteins, and association with endosomes before transport to the plasma membrane. Interestingly, splicing of adenovirus early and late transcripts (see Chapter 23) follows the same pattern to generate distinct proteins. Perhaps as a result of this delay of cell death, coccolithoviruses have burst sizes as large as 1000 progeny virions per algal cell. Left: a view of a Sindbis virion at 2.0 nm resolution. It appears that the mimivirus has a heavy requirement for additional translation factors, which is perhaps no surprise given the potential number of proteins it encodes, yet these genes still contribute a mere ten proteins among the potential predicted total of 911 mimivirus proteins. More recently, strains of live attenuated recombinant vesicular stomatitis virus and human parainfluenza virus that express either Marburg or Ebola virus GPs were constructed. The 5'-termini of the genome and subgenomic RNAs are capped with 7-methylguanosine, as are cellular messenger RNAs. The 3'-terminal regions of all viral genome RNA segments can fold into a transfer RNA (tRNA)-like structure (see below). Different viral proteins are synthesized during these specific time frames, although some viral proteins expressed early continue to be synthesized late. Small amounts of this protein are incorporated into the virion envelope. Parvovirus genomes are linear, not circular Figure 20.1 Virion of minute virus of mice. Supercoiled DNA can be separated from relaxed or linear DNA molecules The properties of circular DNA molecules were first understood by studying the genomes of polyomaviruses. Alternatively, viruses can establish a latent state in the nucleus, or integrate their genes into host chromosomes. Injection of proteosomes into animals can induce immunity to these antigens. Tax-responsive elements (TRE): 21 bp repeated sequences in HTLV-1 proviral DNA that help form a complex with Tax and other proteins, recruiting histone acetylases to the DNA and increasing viral gene transcription. Top: a natural substrate of HIV-1 protease. Microviruses, represented by bacteriophage ϕX174 (Chapter 6), first revealed that genes can overlap each other in a viral genome. Acute transforming retroviruses express mutated forms of cellular growth signaling proteins Study of the mechanisms by which retroviruses transform cells in culture and induce tumors in animals has provided important insights into the causes of cancer. It turns out that the RNA polymerase provides the first primer for leading-strand synthesis. The low pH within a late endosome promotes the fusion of viral and cellular membranes by the E protein and results in the release of genome RNA into the cytoplasm. The major internal repeat element contains six to twelve tandem repeats of 3 kb, depending on Cp 297 the virus strain. Unlike most other RNA-containing viruses, orthomyxoviruses replicate in the nucleus. Most viral envelope proteins are type I integral membrane proteins, with the N-terminus facing outward and the transmembrane anchor nearest the C-terminus of the polypeptide. Normally, eIF-2B catalyzes the exchange of GDP for GTP, and the regenerated eIF-2-GTP complex binds to initiator methionine tRNA and forms a translation initiation complex with mRNA and a 40S ribosomal subunit. Once cells have been infected locally, viruses can then spread from cell to cell in the plant through intercellular channels called plasmodesmata, which normally allow communication and passage of metabolites between cells (see Chapter 10). In 1977, the laboratories of Philip Sharp and Richard 274 CH023.indd 274 16/05/11 8:11 PM Adenoviruses Roberts discovered RNA splicing by studying adenovirus late mRNAs, and were jointly awarded the Nobel prize for this discovery. However, their production is relatively expensive and requires the manipulation of virulent pathogens. These peptides are complexed with specialized presenting molecules called major histocompatibility complex (MHC) proteins, which are located on the surface of all cell types in the body (except red blood cells). After binding to the cell membrane (above) via its HA1 subunit (not shown in this figure) and being brought into an endosome, the trimeric HA molecule, shown as colored cylinders, rearranges upon exposure to low pH (step 1), exposing the fusion peptides (red filaments) at its extremity. The Hepacivirus genus contains a single member, hepatitis C virus. IRES contain extensive secondary and tertiary structures that interact with a variety of cellular proteins. This crash leads to a rapid shift in the composition of the bacterial community due to the massive flux of released organic nutrients. Genes required for virion formation are expressed after DNA replication has begun, and comprise two classes, intermediate and late. Removal of the large intron generates VP1 mRNA. Measles virus has a hemagglutinin (H) and a fusion (F) protein. In humans, infection results in an encephalitis that is invariably fatal once the first symptoms have appeared, although there may be a long latent period between exposure and disease. For examples of virion assembly using scaffolding proteins, see Chapters 6 (bacteriophage ϕX174) and 8 (bacteriophage lambda). Many DNA viruses transcribe both strands of certain regions of their DNA genomes, giving rise to self-complementary RNAs that can anneal to form double-stranded RNA. • Many viruses of archaea have envelopes and unusual morphologies with no obvious symmetry. Chapter 12 Chapter 18 Fig. Furthermore, a number of chapters were read and reviewed in detail by the following McGill undergraduate students, who contributed insightful comments and suggestions: Jonathan Bertram, Yasmin D'Souza, Eric Fox, Caroline Lambert, Kathryn Leccese, Edward Lee, Alex Singer, Brian Smilovici, and Claire Trottier. Tospoviruses are plant viruses that are transmitted by thrips and are able to replicate both in vector and in plant cells. Because these vaccines are inactivated, they cannot cause influenza. The extreme terminal 100 bp exist in two related forms (Figure 26.1c). Based on both these approaches, coronaviruses were traditionally clustered into three groups. In the early 1950s, John Enders, Thomas Weller, and Frederick Robbins began the first systematic study of cell culture, using tissues from children who had died at birth. Polypeptides coded by these RNA CH003.indd 41 Genome Genome size (kb) Genome segments and topology 0.25–0.4 Circular No encoded proteins genomes could be inserted into the lipid membranes and control the passage of small molecules and ions, forming the first primitive cells. GENES AND PROTEINS Six to nine genes code for more than 20 proteins. Enzymes make good drug targets because they are usually at low concentrations inside cells (due to being catalysts), they are relatively well-understood mechanistically, and they normally interact with smallmolecule substrates. Initially, these structural domains were presumed to have specific functional roles; for example, the P domain was associated with pathogenicity. Clinical use of other adenovirus functions. It controls gene expression by making a variety of repressors that bind to viral DNA, and by controlling termination of RNA polymerase at specific sites on viral DNA. Monomers can be arranged as direct repeats (head to tail) or inverted repeats (head to head; tail to tail) depending on how they are formed. Synthesis is initiated by the ppt RNA primer and extends through the U3-R-U5 long terminal repeat just formed, and on through the 18 nucleotides of the tRNA that were initially hybridized to the primer binding site on genome RNA. Indeed, it was the discovery of this host-controlled modification in the early 1950s by Salvador Luria and Mary Human and by Giuseppe Bertani and Jean Weigle that led to the identification and characterization of site-specific restriction endonucleases. No diseases have been associated with infection by dependoviruses. The World Health Organization estimated in 2009 that 25 million people have died of AIDS and that more than 33 million people worldwide were living with HIV-1 infections, most of these being inhabitants of Africa and South-East Asia. What is the scanning model? Following virion attachment, the viral genome and associated protein is released into the cytoplasm and moves to the nucleus within 5 minutes. Liss (1983), pp. The natural target cells of adenoviruses are terminally differentiated, non-dividing epithelial cells. This turns out to be a highly regulated process, for two reasons. Late viral proteins package viral genomes and assemble virions 16 7. Lectin receptor-activated pathway: an arm of the complement system that begins with recognition of mannose residues on microbial carbohydrates. In many ways, viruses work like Trojan horses; they can only infect a cell that provides extensive assistance during entry. The use of reassortant viruses showed that these distinct patterns of central nervous system disease depend on the source of the 1-encoding S1 gene. The changing vaccine paradigm Use of live vectors and chimeric viruses Because of many years of experience with hundreds of millions of vaccinated people, the advantages and dangers of live virus vaccines such as vaccinia and measles are now well known. The U3-R-U5 sequence is called a "long terminal repeat" (LTR). It is known that the first step in this process must be the binding of the maturation protein to the RNA. MODERN VACCINE PRODUCTION Virus production in cultured cells or embryonated eggs. Single-stranded genomes are packaged into procapsids as they are synthesized Genome biosynthesis and packaging occur concurrently. Four viral proteins are associated with genome RNA in nucleocapsids: NP (nucleoprotein), VP35 (RNA polymerase cofactor), VP30 (transcription activator; only shown for Ebola virus), and L (viral RNA polymerase). Multiple subtypes are distinguished within each influenza virus type by means of variation in the structure of their two surface glycoproteins. However, differences in genome size and organization and in virus replication strategies led taxonomists to reclassify these viruses into two distinct families, the Polyomaviridae and Papillomaviridae. In either case, the envelope proteins would be glycosylated within the Golgi, and further transport of mature virions to the extracellular space is via secretory vesicles. However, interference between the LTR promoter and the second promoter (promoter occlusion) often occurs. As discussed above, several late expression factors are also required for replication of transfected reporter plasmids, supporting the hypothesis that DNA replication is required for efficient late gene expression. 16/05/11 8:07 PM Reoviruses T1L 227 T1L x T3D progeny T3D L segments M segments S segments Figure 19.2 Generation of reassortant viruses. Cell lysis caused by E protein leads to release of phage The E protein is responsible for cell lysis. It has the canonical Gly-Arg-Arg motif found in other RNA virus RNA polymerases, and it also contains sequences that have homology with methyltransferases. Steps 6 and 7. However, mutants of APOBEC3G that lack deoxycytidine deaminase activity retain some antiviral activity. The spike protein has three distinct active sites for the three enzymatic activities required for capping: (1) formation of the cap itself by adding a GMP residue (derived from GTP by pyrophosphate release) to the 5'-diphosphate end of the RNA; (2) methylation of the cap guanine at the N7 position; and (3) methylation of the ribose sugar of the first template-coded G residue. Polyadenylation signals (AAUAAA) are bracketed; they are separated by only one nucleotide pair, GC, shown by a vertical dashed line. The active polymerase complex contains one copy of each of the four proteins (we will refer to the active enzyme as polymerase while reserving the term replicase for the phage-coded subunit). The virion contains six major proteins in the size range between 15 to 80 kDa. The 24-kb doublestranded linear DNA genome encodes 57 proteins, including a DNA polymerase. Standard suspensions of tiny latex spheres or other small uniform objects are often added to the virus suspension to help establish absolute numbers of virus particles per unit volume. DNA coding for the SARS receptor-binding domain was inserted in the bat virus spike sequence. Reverse transcriptases do not have efficient proofreading mechanisms to remove incorrectly incorporated nucleotides in proviral DNA. The result is cytotoxic T lymphocytes (right), which recognize and kill infected cells displaying the peptide in question. NS1 also activates the phosphatidylinositol 3-kinase (PI3K)/ Akt pathway, leading to inhibition of caspase-9 activity and suppression of apoptosis. This may help in the release and dispersal of virions from some cell types. By the late eighteenth century variolation became well established in Europe and North America, reducing significantly the impact of smallpox where practiced, and setting the stage for the discovery of vaccination at the turn of the nineteenth century. To solve this problem, we come back to the inverted terminal repeat sequences on adenovirus DNA. Activation of IRF-3 by pathways shown in Figure 33.3 can also induce apoptosis, in part by transcriptional induction of proteins such as the death domain-associated protein Daxx, and in part by causing the translocation of proteins to the mitochondria and induction of the mitochondrial apoptosis pathway. Prevention: • • vaccination with recombinant hepatitis B surface antigens injection with anti-hepatitis B immunoglobulin after exposure to virus. Microvilli: finger-like projections of the plasma membrane, containing a core of actin filaments, on the surface of various cell types such as those of the intestinal epithelium. 1–3 reprinted from Choppin, P.W., "On the emergence of influenza virus filaments from host cells." Virology 21 (1963): 278–281; Fig. Brome mosaic virus RNA synthesis takes place on cytoplasmic membranes The assembly of the brome mosaic virus RNA synthesis complex in yeast occurs in the membranes of the endoplasmic reticulum. Host cell protein synthesis is inhibited and in many cell types there appears to be induction of programmed cell death, or apoptosis. Because these protein variants are not as well recognized by the immune system as those of the original infecting virus strain, these viruses can selectively replicate and be propagated. Vpu has two known activities within the cell. Reading frames for the 289-amino acid and 243-amino acid protein products are shown (boxes) with the critical amino terminal region (N) and conserved regions (CR1, 2, and 3). These oligomers can be dimers or ring-shaped hexamers or octamers. How cells recognize entry of these enveloped viruses is not yet understood. This protease cleaves some capsid proteins, releasing the DNA from its attachment to the inside surface of the capsid shell. Thanks to the following McGill University students who worked with me on chapter summaries, permissions and editing in the final phases of preparation of the book: Meoin Hagege, Jennifer LeHuquet, Melany Piette, Pooja Raut, and Emilie Mony. Virus-coded enzymes for transcription and RNA processing are packaged in the virion core. Contains reverse transcriptase. The virus codes for an integrase protein. Gene Segments, Hosts, and Years of Introduction PB2, PA ~1998 PB2, PA ~1968 PB1 ~1998 PB1 Triple Reassortant PB1 ~1918 HA, NP, NS Classical Swine ~1979 NA, M Eurasian Swine HA, NP, NS NA, M PB2 PB1 PA HA NP NA M NS 2009 A(H1N1) Figure 18.10 Origin of genome segments of the 2009 H1N1 pandemic influenza A strain. Pro-inflammatory cytokines. Prior to the development of these tools, phage genetics was limited to plaque-morphology and host-range mutants. Plasma cells: terminally differentiated B cells specialized in producing and secreting antibodies. Therefore N-antiterminated transcription from PL on prophage DNA is not subject to retroregulation, and sufficient amounts of Int and Xis are produced for prophage excision. Due to its segmented genome, influenza virus can evade humoral immunity via antigenic shift. Densoviruses: infect insects. The first level of defense comprises physical barriers, including the skin and mucosal secretions in the gut and respiratory tracts that non-specifically prevent access of viruses to cells they can infect. Component of virion envelope projections; type 1 transmembrane glycoprotein with two positively charged C-terminal amino acids in the cytoplasm; palmitoylated and glycosylated; functions in low-pH activated membrane fusion. An unintended result is the ability of adenoviruses to transform non-permissive cells and to induce tumor formation in experimental animals, thus making E1A an oncogene. Extension of both DNA strands. High titers of preformed neutralizing antibodies can confer immunity against most viral pathogens. Secretory vesicles transport virions to the cell surface, where the vesicles fuse with the plasma membrane and release virions. This induces the ubiquitination of CD4 and its subsequent degradation by proteasomes, thus releasing gp160 and increasing surface expression of its cleavage products, gp 41 and gp 120. The structures of the low pH post-fusion form of the E protein have been determined for dengue and tickborne encephalitis viruses using x-ray crystallography. Microorganisms such as these algae will probably be the first organisms to react and adapt to a changing ocean. Unless the immediately prior host cell has "marked" the phage DNA with the host-specific modification, allowing it to escape the restriction endonucleolytic attack, the phage DNA may be cleaved and the infection aborted. Virions or nucleocapsids are transported within the cell to sites of transcription and replication (cytoplasm or nucleus). Viruses are important disease-causing agents. What the general population calls "flu" is usually a mild upper respiratory illness (the "common cold") caused not by influenza virus but by a number of upper respiratory viruses including rhinoviruses and coronaviruses. DAI binds to bacterial, viral, and mammalian DNA, and has been shown to mediate cellular responses to herpesviruses and poxviruses. As multicellular, filamentous macroalgae, the brown algae differ from the unicellular, microalgal hosts of the other Phycodnaviridae. DNA packaged by cleavage from linear concatemers. The spike (S) glycoprotein is the major surface transmembrane protein protruding from the CH014.indd 163 163 viral envelope (Figure 14.2b). A mutated and highly active form of c-Src is encoded by the acutely transforming retrovirus Rous sarcoma virus, which produces cancer in chickens (see Chapter 28). Togaviruses 148 Milton Schlesinger, Washington University in St. Louis Sondra Schlesinger, Washington University in St. Louis Revised by: Richard Kuhn, Purdue University 14. The slightly enlarged "head" ends of filamentous particles (see Figure 18.2) also contain a single bundle of eight nucleocapsids, and their thinner tails appear to be empty extensions of the envelope. Cyclins: cellular proteins that regulate the cell cycle by associating with and activating cyclin-dependent kinases. • Viral mRNAs are selectively incorporated into replicase particles, where the viral RNA polymerase copies them, making full-length, double-stranded RNA genomes. New cancer therapies. Their genomes are among the smallest of all viral genomes, ranging from less than 2 kb up to 9 kb. • The flavivirus particle is covered with a flat layer of envelope proteins arranged with icosahedral symmetry, giving the virus an appearance of a golf ball. The CI repressor blocks expression of the lytic program by regulating three nearby promoters: PL, PR, and PRM When infects active cells in logarithmic growth, the phage grows lytically in most of the cells. Since the level of CI produced from PRE depends on the level of CII, the lysis/lysogeny decision depends finally on the intracellular concentration of CII protein. Under such circumstances previously healthy animals would develop scrapie, albeit with an incubation period of from 2 to 5 years. Plaque-forming units (PFU) per mL are calculated as shown. The genome of PSV is a 28-kb linear, doublestanded DNA molecule. Coronaviruses cause devastating outbreaks of gastroenteritis and respiratory infections in cattle (bovine coronavirus), pigs (transmissible gastroenteritis virus), and chickens (infectious bronchitis virus), with mortality rates as high as 100% in newborn animals. The majority of gene therapy strategies using virus vectors target specific tissues or organs within the body. DNA polymerase I subsequently removes the RNA primers and fills in the gaps, and DNA ligase joins the DNA chains to form a covalently closed, circular, double-stranded RF DNA. For minute virus of mice, VP2 is the major capsid protein; only a few copies of VP1 are incorporated into the capsid. Both viruses and computer viruses can cause disease or damage to their host cell or computer. The final result is the synthesis of two circular, double-stranded 16/05/11 8:09 PM Polyomaviruses 259 RNA primer DNA polymerase α-primase Replication protein A Large T antigen hexamer 5' 3' 5' 3' Topoisomerase I (a) RNAse H Replication fork DNA polymerase δ (b) Replication protein C PCNA DNA ligase Lagging strand Leading strand (c) Figure 21.12 Mechanism of bidirectional replication of polyomavirus DNA. In recent clinical trials it was observed that injections of Onyx-015 directly into solid tumors, or its delivery through the intra-arterial or intravenous routes of administration, was safe but was ineffective at killing cancer cells. Replication of viral genome 6. HA2 is anchored in the virus envelope and contains a hydrophobic fusion peptide near the amino terminus created by protease cleavage. This means that the virus can enter the cell and express its early genes, but can neither replicate its DNA nor express late proteins. nuclear pores and are uncoated, releasing the singlestranded viral DNA. This is the first successful gene therapy trial using a HIV-based vector and so far no side effects have been reported. Some types cause cervical carcinoma, a sexually transmitted disease and major cause of cancer in women. The development of prophylactic vaccines has also been an important issue in research on filoviruses in recent years. Reassortment generates new viruses that can cause pandemics because of changed surface antigens (HA and NA). RNA interference (RNAi) may be also be involved in viroid pathogenesis. In type II proteins, the transmembrane anchor itself serves as the signal for membrane insertion. This leads to a cascade of signaling events too complex to detail here. 20 nm The exact nature of the conformational change that leads to fusion has not been determined for paramyxoviruses. 16/05/11 8:19 PM 406 CHAPTER 33 Intrinsic Cellular Defenses Against Virus Infection be proteins that could be purified and used to protect cells against virus infection. 22.3: Modified from Fig. Every base pair in the genome of hepatitis B virus is involved CH030.indd 367 (a) (b) 3' DNA polymerase Polymerase 5' DR1 5' Cap 5' 3' 3' DR2 DR1 3' DR2 Cap 5' Nuclease (d) (c) DNA ligase 3' 5' DR1 DR2 DR1 5' 3' DR2 Figure 30.2 Structure of hepatitis B virus genome, and its conversion to a covalently closed, circular DNA. These genes encode virokines, which mimic cytokines, and viroceptors, which mimic cytokine receptors. All cells must be infected simultaneously, and with some luck the events of the virus replication cycle will be synchronized so that similar steps will be taking place at the same time in all cells. The motile, flagellated, haploid cells lose their chalk armor and are instead covered by organic scales, which may act as a physical barrier to infection. 5 Table 1.1 Some human diseases caused by viruses Disease Virus Family Acquired immunodeficiency syndrome

(AIDS) Cervical carcinoma Chickenpox “Cold sores” Common cold HIV-1 Retrovirus Human papillomavirus types 16, 18, 31 Varicella virus Herpes simplex virus type 1 Adenoviruses Coronaviruses Rhinoviruses Papillomavirus Herpesvirus Herpesvirus Adenovirus Coronavirus Picornavirus Diarrhea Norwalk virus Rotaviruses Herpes simplex virus type 2 Dengue virus Ebola and Marburg viruses Lassa fever virus Hepatitis A virus Hepatitis B virus Hepatitis C virus Calicivirus Reovirus Herpesvirus Flavivirus Filovirus Arenavirus Picornavirus Hepadnavirus Flavivirus Influenza A and B virus Measles virus Epstein–Barr virus Cytomegalovirus Mumps virus Poliovirus types 1, 2, and 3 Othomyxovirus Paramyxovirus Herpesvirus Herpesvirus Paramyxovirus Picornavirus Rabies virus SARS coronavirus Rhabdovirus Coronavirus Variola virus Human papillomavirus types 1, 2, 4 Yellow fever virus Poxvirus Papillomavirus Flavivirus Genital herpes Hemorrhagic fevers Hepatitis Influenza Measles Mononucleosis Mumps Poliomyelitis Rabies encephalitis Severe acute respiratory syndrome (SARS) Smallpox Warts Yellow fever Viruses are the most abundant form of life on Earth Recent studies of soil and seawater have revealed that bacterial viruses, also called bacteriophages, are much more numerous than previously imagined. and Digard, P., “The influenza virus nucleoprotein: a multifunctional RNA-binding protein pivotal to virus replication,” Journal of General Virology 83 (2002): 723–734, with permission from Society for General Microbiology; adapted from Compans, R.W., Content, J., and Duesberg, P.H., “Structure of the ribonucleoprotein of influenza virus,” Journal of Virology 10 (1972): 795–800, with permission from American Society for Microbiology; Fig. Influenza A viruses encode a protein (M2) that forms membrane channels allowing free passage of H⩽ ions at low pH (see Figure 36.3). Curiously, the HA protein of this virus strongly resembles the HA protein of the original 1918 pandemic virus. OM: outer membrane; PG: peptidoglycan layer; CM: cytoplasmic membrane. The envelope contains two viral glycoproteins, named Gn and Gc. Thus bunyavirus particles contain only four viral proteins: N, L, Gn, and Gc. Virions examined by cryoelectron microscopy are spherical, with a diameter of about 100 nm (Figure 17.1a). The energy needed to induce fusion is derived from the conformational change in the fusion protein. The infectious agent of prion diseases contains protein but no detectable nucleic acid Initial efforts to identify a conventional infectious agent in affected nervous tissue were fruitless. Although these genes may not be essential for virus replication, they likely enhance some stage of virus multiplication in their natural hosts. Penton base: structural subunit of adenovirus virions, made from pentamers of the penton protein and located at the 12 vertices of the icosahedral virion. and Schulten, K., "VMD - Visual Molecular Dynamics", J. (ed.). Occlusion-derived virions initiate infection in the insect gut epithelium (see below). dsDNA DNA polymerase (or reverse transcriptase) 7. One of the most convincing arguments for the importance of intact P123 in the synthesis of negative strands is based on a mutation in the nsP2 proteinase that leads to unusually rapid proteolytic processing of P123. In the cervix, the cells that become infected are non-differentiated basal cells of the epidermis within a region known as the “transformation zone”. However, strains resistant to the drug were reported within months, leading to controversy about its use in patients with mild disease. Poliovirus (Figure 2.4) illustrates one way in which viruses have evolved to produce larger icosahedral shells and thus to package larger genomes. A BRIEF HISTORY OF VIROLOGY Viruses were first distinguished from other microorganisms by their small size and their ability to pass through fine filters that retain bacteria. PR OR3 cI ⩾10 cI mRNA OR2 ⩾35 OR1 cro mRNA ⩾10 cro ⩾35 PRM (a) RNA polymerase OR2 PRM (b) (c) PR OR1 OL3 OL2 OL1 OR3 OR2 OR1 RecA* (d) Figure 8.5 Transcription and its repression at PRM and PR. (c) The close proximity of the viral and cellular membranes results in their fusion, releasing the virion core into the cytoplasm. Thus, large T antigen acts as a DNA helicase. Capsomers in neighboring trisymmetrons are related by a 60° rotation, giving rise to the boundary between trisymmetrons. Virions are assembled and released from the cell, which eventually shuts down most viral transcription and returns to the original lysogenic state and normal growth rate. It has been most useful in certain settings, such as nursing homes, for prevention of infection among contacts of flu victims. The close association between capsid proteins and the single-stranded RNA in helical nucleocapsids may afford additional protection against degradation, particularly inside the cell. Because of their lack of toxicity, their weak antigenicity, their demonstrated long-term transgene expression, and their very large insert capacity, thirdgeneration adenovirus vectors are very promising for gene therapy applications. Like herpes simplex virus, Epstein–Barr virus DNA circularizes upon entering the cell via a terminal repeat (TR) sequence. MS2 phage will be used to discuss translational control in this chapter. For example, self-cleavage of the capsid protein VP0 in the picornaviruses (Chapter 11) creates a small internal peptide, VP4, which emerges and helps mediate penetration when receptor binding forces the virus particle to expand or dissociate (see Figure 11.3). For many viruses these include the carbohydrate moities of proteoglycans or glycoproteins. A chimeric vaccinia virus expressing the major glycoprotein of rabies virus is currently in use in the northeastern part of North America to control a major rabies epizootic in raccoons. The nsps 3, 4, and 6 are integral membrane proteins and are proposed to nucleate the formation of replication complexes and possibly induce membrane modifications. Infectious subvirion particles (ISVPs): in reoviruses, particles that have lost part of the external capsid as a result of protease digestion in endosomal vesicles or the intestine. Moreover, the eye tends to tolerate the presence of foreign antigens such as the AAV capsid. Since the development of AZT, several other nucleoside analogues have have been developed and approved for use against HIV. To circumvent 16/05/11 8:22 PM 464 CHAPTER 37 Eukaryotic Virus Vectors this problem, vectors are often produced by simultaneously transfecting susceptible cells with three different plasmid constructs, referred to as packaging, envelope, and transfer constructs (Figure 37.5). It is not known whether these early transcripts are prematurely terminated or degraded between 2 and 5 hours after induction. Further replication by the same process yields higherorder concatemers (not shown). The numbers of these proteins per virion are 60:60:60:12, respectively. Recently, a new mechanism came to light. Much work has therefore been accomplished by this route. The capsid protein is inserted into the endoplasmic reticulum via a 20-amino acid signal sequence at its carboxy terminus (Figure 12.5). Nucleoside diphosphate kinase: an enzyme that phosphorylates nucleoside diphosphates (e.g., deoxyguanosine diphosphate) to nucleoside triphosphates. Children affected with SCID-XI die at an early age because of the absence of T-cellmediated and humoral immune responses. 2a,b in Watanabe, S., et al., “Production of novel Ebola virus-like particles from cDNAs: an alternative to Ebola virus generation by reverse genetics.” Journal of Virology 78 (2004): 999–1005. Flaviviruses 137 Flaviviruses cause several important human diseases 137 Yellow fever is a devastating human disease transmitted by mosquitoes 138 A live, attenuated yellow fever virus vaccine is available and widely used 139 Hepatitis C virus: a recently discovered member of the Flaviviridae 139 The flavivirus virion contains a lipid bilayer and envelope proteins arranged with icosahedral symmetry 139 Flavivirus E protein directs both binding to receptors and membrane fusion 140 Flaviviruses enter the cell by pH-dependent fusion 141 Flavivirus genome organization resembles that of picornaviruses 141 The polyprotein is processed by both viral and cellular proteinases 142 Nonstructural proteins organize protein processing, viral RNA replication, and capping 144 Flavivirus RNA synthesis is carried out on membranes in the cytoplasm 144 Virus assembly also takes place at intracellular membranes 145 13. Reverse genetics allows direct engineering of the viral genome without a need to devise complicated selection strategies for isolation of viral mutants. Cleavage of HA by cellular proteases into two subunits activates its ability to carry out membrane fusion. KEY TERMS Concatemer Intasome Lysogen Operator Prophage Rolling circle Scaffolding proteins SOS genes Telomerase Temperate phage FUNDAMENTAL CONCEPTS • Bacteriophage lambda is a temperate phage that can integrate its DNA into the host cell chromosome and repress all phage gene expression, creating a lambda lysogen. Cucumber mosaic virus supports the replication of parasitic satellite RNAs that can dramatically alter the virus symptoms. A., “Structural and genomic properties of the hyperthermophilic archaeal virus ATV with an extracellular stage of the reproductive cycle,” J. coli hosts. The study of viruses has led to numerous discoveries in molecular and cell biology Because viruses replicate within cells but express a limited number of viral genes, they are ideal tools for understanding the biology of cellular processes. This structural design is reminiscent of the telomeric ends of linear chromosomes of eukaryotes. John Enders and his team at Harvard University made a major breakthrough in 1954, when human embryonic kidney cells cultured in vitro were used to propagate measles virus from a young patient named David Edmonston. To restrict diffusion of the progeny virus, the infected cells are overlaid with nutrient medium in melted agar, which solidifies on cooling. Adenoviruses 274 Adenoviruses cause respiratory and enteric infections in humans 274 13/05/11 2:11 PM xiv Contents Adenoviruses can be oncogenic, but do not cause cancer in humans 274 Virions have icosahedral symmetry and are studded with knobbed fibers 275 Fibers make contact with cellular receptor proteins to initiate infection 276 Expression of adenovirus genes is controlled at the level of transcription 276 E1A proteins are the kingpins of the adenovirus growth cycle 277 E1A proteins bind to the retinoblastoma protein and activate E2F, a cellular transcription factor 277 E1A proteins also activate other cellular transcription factors 278 E1A proteins indirectly induce apoptosis by activation of cellular p53 protein 279 E1B proteins suppress E1A-induced apoptosis and target key proteins for degradation, allowing virus replication to proceed 279 The preterminal protein primes DNA synthesis carried out by viral DNA polymerase 280 Single-stranded DNA is circularized via the inverted terminal repeat 280 The major late promoter is activated after DNA replication begins 281 Five different poly(A) sites and alternative splicing generate multiple late mRNAs 281 The tripartite leader ensures efficient transport of late mRNAs to the cytoplasm 281 The tripartite leader directs efficient translation of late adenovirus proteins 282 Adenovirus-induced cell killing 283 Cell transformation and oncogenesis by human adenoviruses 283 24. 445 Antiviral drugs are targeted to specific steps of virus infection 445 Drugs preventing attachment and entry of virions 446 Amantadine blocks ion channels and inhibits uncoating of influenza virions 447 Nucleoside analogues target viral DNA polymerases 447 Acyclovir is selectively phosphorylated by herpesvirus thymidine kinases 448 Acyclovir is preferentially incorporated by herpesvirus DNA polymerases 449 Cytomegalovirus encodes a protein kinase that phosphorylates ganciclovir 450 HIV-1 reverse transcriptase preferentially incorporates azidothymidine into DNA, leading to chain termination 450 Non-nucleoside inhibitors selectively target viral replication enzymes 451 Protease inhibitors can interfere with virus assembly and maturation 452 Ritonavir: a successful protease inhibitor of HIV-1 that was developed by rational methods 452 Neuraminidase inhibitors inhibit release and spread of influenza virus 453 Antiviral chemotherapy shows promise for the future 453 37. If an invading pathogen is able to breach these barriers, cells exposed to the pathogen respond with intrinsic cellular defense systems. Progeny plus-strand DNA molecules are cleaved from the growing strands on the rolling circle by the A protein, and are ligated to form single-stranded circles. Interferons also play an important role in antigen processing by regulating the expression of proteins required to generate antigenic peptides. Their ability to infect a wide range of cells and to express genes at high levels make them ideal tools to fight disease in humans and other mammals. For wild-type ϕX174, the time required to reach these critical concentrations is exceptionally short. In a new approach, Charles Arntzen, formerly of the Boyce Thompson Institute at Cornell University and now at Arizona State University, is currently testing edible hepatitis B vaccines engineered into bananas! Another method of prevention is through immunoprophylaxis, used primarily on newborns of mothers who are carriers of hepatitis B virus. E2F was first described as a transcription factor regulating the adenovirus E2 promoter (hence its name), but it also regulates promoters of many genes coding for cellular proteins involved in DNA synthesis and cell-cycle progression factors. Each turn of the helix is separated by 2.3 nm. Mutational analysis has indicated that the kinetic trap involves the small hairpin between nts 25 and 43 shown in Figure 5.8b. The diagnostic window for IgM antibodies lies within 2 days and about 5 months after onset of disease, and IgG-specific antibodies can be detected for many years starting as early as 6 days after onset of disease. More than 1000 cellular genes are activated by the Jak–Stat pathway. Antiviral treatments can reduce the viral load to base levels in 48 weeks and are generally administered indefinitely to prevent reemergence of the virus. When the hepatitis B vaccine was introduced into the United States, children were one of the first groups targeted for vaccination; why? Wt. in KDa) Name Abbreviation Matrix MA p17 Capsid CA p24 Nucleocapsid NC p7 Protease PR p14 Reverse transcriptase RT p66/51 Integrase IN p32 Surface protein SU gp120 Transmembrane protein TM gp41 Virion protein R Vpr p15 Name Abbreviation 357 Alternative name (M. Mutational analysis has shown that sequences extending up to 30 nucleotides from the 3' end are important for transcription and replication. Which hepatitis virus is a member of the Flaviviridae? T7 makes large plaques on susceptible host cells and has a linear genome of double-stranded DNA. Sheep are susceptible to infection with bovine spongiform encephalitis (BSE) at the same frequency and incubation time as scrapie, although sheep PrP differs from bovine PrP at amino acids 98, 100, 143, 146, 155, 168, 198, and 218. A number of other early proteins, as well as a variety of cellular proteins, help form RNA replication complexes in the cell. Ependymal cells: epithelial cells that line the brain and spinal cord. DISTINCTIVE CHARACTERISTICS RNA-dependent RNA polymerase in virion transcribes genome into mRNAs. A single transcriptional promoter at the 3' end of RNA; mRNAs are made by a start–stop mechanism. Virus taxonomy takes into account the ability of viruses to infect different categories of organisms. NS2B 130 Part of viral proteinase that cleaves viral polyprotein. CH021.indd 256 16/05/11 8:09 PM Polyomaviruses and function, see the chapters on papillomaviruses and adenoviruses (Figures 22.6 and 23.7). Once synthesis has begun, it can continue in the absence of S1, EF-Tu, and EF-Ts, since the replicase protein has RNA polymerase activity by itself (but the complex usually stays intact). (b) Nef binds to the cytoplasmic tail of CD4 at the inner surface of the plasma membrane, and via interaction with AP-2 increases uptake of CD4 into clathrin-coated pits, lowering its concentration on the cell surface. Short, genome-encoded poly(A) tail at 3' end. Parvoviruses have one of the simplest-known virion structures The structures of the spherical capsids of several parvoviruses have been solved at atomic resolution by x-ray crystallography (Figure 20.1). Shortly afterward, in 1911, Peyton Rous showed that the Rous sarcoma virus induces tumor formation in chickens. The immobilized target RNA is then hybridized to the modified MDV-1 RNA (Figure 5.9a). Cell death is usually necessary to release virus particles trapped in the nucleus or the cytoplasm. Whether or not infection will produce disease is dependent both on the virus and the host. The high levels of recombinant proteins synthesized by firstgeneration adenovirus vectors in mammalian cells have 16/05/11 8:22 PM 462 CHAPTER 37 Eukaryotic Virus Vectors only recently been surpassed by the lentivirus vector system. These proteases are normally present as inactive “procaspases.” Two main apoptosis pathways are known: the extrinsic pathway, triggered by a cell surface receptor, and the intrinsic, or mitochondrial, pathway, triggered by a variety of stress responses within the cell. Infection with ATV at high temperatures leads to lysogeny The circular, 62.8-kb double-stranded DNA genome of ATV (Figure 9.12) is the largest genome among known viruses of crenarchaeota. Matrix proteins located at the cytoplasmic face of the membrane bind to the nucleocapsids and cooperate with the glycoprotein layer to drive the budding process. Th1 cells help activate cell-mediated immunity, and Th2 cells help activate antibody production (see below). Like , N15 virion DNA has cohesive ends that cyclize the molecule upon injection. Insect cells were cotransfected with plasmids containing a variety of specific viral DNA fragments and a reporter plasmid driven by an early promoter. Viruses make homologues of MHC proteins that prevent NK cell recognition of infected cells. How RNAs 4A and 5 and the satellite RNAs are packaged remains unknown. The epidemic eventually ends because transmission of the infection is transient and the spread of the virus is inefficient. Most of the information in this chapter comes from studies with influenza type A virus. Acqua (in silkworms) independently established the viral etiology of polyhedrosis. Helicase: an enzyme that unwinds DNA or RNA helices. Interferons modulate production of cytokines that favor development of Th1 cells and a cytotoxic T-cell response. This specificity of killing suggests that E4orf4 could be of use therapeutically in the treatment of human malignancies (see below). Circular viral DNA is bound to nucleosomes (orange rounded squares) formed from cellular histones. President Washington and many other prominent government leaders and staff left the city for fear of infection. Hepatitis B virus is distinct in the world of viruses with its very compact 3.2- kb circular DNA genome, extensive use of overlapping reading frames, production of a pregenome RNA, and dependence on a reverse transcription step for replication. These papillomavirus oncoproteins are therefore able to act at two distinct points in the same pathway by abrogating both p53 and Rb activity. Capped at 5' end but no poly(A) tail at 3' end. MT7 is rapidly secreted in high levels from virus-infected cells. 16/05/11 8:08 PM 244 CHAPTER 20 Parvoviruses tio n Helicase loc a Zn -fi ng er ar Nu cle NT Pbin din g Co ile dco il liz a DNA-binding (E1, E2a, E4orf6, and VA RNA) that help adenoassociated virus replication. Marburg and Ebola viruses: sporadically emerging viruses that cause severe, often fatal disease In August 1967, three laboratory workers of the Behringwerke in Marburg, Germany, became ill with a hemorrhagic disease after processing organs from African Green monkeys (Cercopithecus aethiops). Patients who develop a predominantly cytotoxic T-cell– based immune response have a better chance of longterm survival. ericina, respectively) actually are closer phylogenetically to the largest known virus, Acanthamoeba polyphaga mimivirus. Other viruses package the “antisense” strand, which cannot be translated because it does not contain meaningful coding regions; only its complementary copy codes for viral proteins. Electron microscopy of samples collected from archaea-rich extreme geothermal and hypersaline environments revealed that head-tail phage are rare among archaeal viruses. In contrast, the genome RNA of negative-strand viruses is not infectious by itself because it requires the viral RNA polymerase to function. These are the simplest vaccines to make and are among the most effective. A fragment of the E protein of tick-borne encephalitis virus (a close relative of yellow fever virus) has been crystallized and its structure was determined by x-ray diffraction analysis. 16/05/11 8:19 PM Virus Strategies to Counter Host Defenses A cowpox virus protein, V203, has an endoplasmic reticulum-retention signal and is able to retain MHC I within the endoplasmic reticulum. The altered picornavirus virions may participate in the formation of a pore within the plasma membrane, allowing the genome RNA to pass directly from the virion through the membrane into the cytoplasm (Figure 11.3). 15.1b: Courtesy of Professor Rob Ruigrok, Universite Joseph Fournier, Grenoble, France; Fig. These multimers are subsequently cleaved and circularized by host enzymes into plus-strand progeny circles. 5' 3' G C G C G C U A C G C G C U A G C G C A G C G A C U A C G C C CUC U UG C GC G A U U S G C C G G C G U CU 3' 2 G G C U G G G ɩ ɩ 5' 5' 3' G C G C G C U A C G C G C U A G C G C A G C G A C U A C G C C CUC U UG C GC G A U U S G C C G G C G U CU Figure 31.5 Proposed cleavage of an RNA substrate by a trans-acting hepatitis delta virus ribozyme. Translation of the C and S open reading frames starts at two or three alternative sites (preC or C and preS1, preS2, or S). Some viruses devote a glycoprotein exclusively to attachment. These include the TATA-binding protein (TBP), the general transcription factor TFIIB, and the transcriptional co-activator p300, which has histone acetylase activity (Figure 21.10). • The RNA polymerase begins transcribing at the 3' end of the genome RNA and sequentially transcribes five to ten genes, terminating and releasing each mRNA before starting the next one. Crenarchaeota: one of the major phyla of Archaea, including most known hyperthermophiles, such as Sulfolobus solfataricus, but also many marine organisms that are not thermophiles. Surprisingly, cleavage of GP to produce GP1 and GP2 is not required either for production of infectious virions or for pathogenicity in animals. This is defined as the number of infectious virus particles added per susceptible cell. The distance between the pyrimidine-rich tract and the conserved AUG appears to be critical; if it is much smaller or larger than 20–25 nt, initiation of translation is reduced. Why infections with the same virus can lead to these two very different outcomes is not known. Mature virus then fuses its envelope with the vesicle membrane to release the virion core into the cytoplasm. Retroviruses pseudotyped with VSV-G can infect many different cell types and are stable. Many viruses can be engineered to deliver and express specific genes When viruses are genetically modified to deliver and express either their own or foreign genes, they are called virus vectors. In some flaviviruses, binding of antibody to the virus particle can result in enhanced uptake into cells expressing immunoglobulin Fc receptors. 16/05/11 8:11 PM 284 CHAPTER 23 Adenoviruses In human adenoviruses, E1A is the oncogene that stimulates uncontrolled cell-cycle progression. Second, the association of E1A with p300/ CBP prevents p53 from activating transcription of the Mdm-2 gene, further reducing the level of Mdm-2. In diseases such as cystic fibrosis and ␣1-antitrypsin deficiency, degradation and/or mistrafficking of misfolded proteins causes loss of protein function. These are then extended by cellular DNA polymerase III to make a complete complementary copy of the phage DNA. Genes were originally given sequential numbers from 1 to 19; additional genes that were discovered subsequently were given decimal numbers such as 0.3 or 1.2 to indicate their position in the genetic map (Figure 7.1). Neuraminidase cleaves sialic acid from the membrane glycoproteins, permitting release of the virus; without this enzyme, the virus remains stuck and cannot spread to other cells. Additional conserved features in picornavirus IRES elements include a 4-nt GNRA sequence (where N any nucleotide and R A or G), and (A ⩽ C)-rich sequences in specific loop regions of the predicted stemloop structures. Empty capsids remain at the cytoplasmic side of nuclear pores for several hours until they disintegrate. OtV-1, OtV-2, and OtV-5 contain 11, 14, and 6 genes respectively with close homology to genes of the host, O. the first 850 bp enter spontaneously via the phage tail upon binding of the phage to the cell 2. The linear genomes of chloroviruses contain hundreds of genes, and each virus species encodes some unique proteins The PBCV-1 genome (Figure 27.2) is a linear, 330-kb, double-stranded DNA molecule. Scaffolding proteins help promote formation of a precursor particle (“procapsid”) by coassembly with capsid proteins, but are not retained in the fully completed virion. Cells differentiate as they pass from the basal layer (bottom) to the surface (top). “Red Queen dynamics” can also help explain the bloom–bust cycles of E. The Th2 cell responds by producing cytokines, among which are interleukins-4, -6 and -10. • Most of the genes of viruses of archaea have no homology to known genes. Here u is 16.33 (there are 3 16.33 49 subunits in three turns of the helix), p is 0.14 nm, and P is 2.3 nm. Five different reasons for the increase in the ratio of late to early transcription have been suggested. Such a jump could have occurred during evolution. Ligand: a specific molecule that is bound by a protein. These arguments about the origin of viruses are only speculations Family Genus (plural: genera) Helper virus Lineage Order Ribozyme Because there are very few hard data on which to base theories of the origin of viruses (and the origin of life itself!), the above ideas are really only informed speculation. The family Coronavirinae contains three genera: Alpha-, Beta-, and Gammacoronaviruses. There are presently more bat coronavirus sequences in databases than coronaviruses from all other animals combined, including humans. The A protein remains on the replicative form DNA to help synthesize further progeny plus-strand DNA. In addition to these regular epidemics, more severe pandemics occur at irregular 10- to 50-year intervals. However, with further studies this view has changed radically. In one model, formation of PrPSc is a nucleationdependent oligomerization process (Figure 32.7). The extreme 5'-terminal four nucleotides (5'-GCUA-3') and 3'-terminal five nucleotides (5'-UCAUC-3') are conserved among all 10 reovirus gene segments. These elements, along with binding sequences for other cellular transcription factors, are found upstream of the promoters of all genes (Figure 24.6). Surprisingly, another sequence element beginning 79 nt from the 3' end of the genome is also required for proper transcription and replication. In addition to its involvement in aplastic crisis, B19 is also known to cause a common childhood disease called erythema infectiosum (“infectious rash”). However, for humans RNA phages are harmless creatures. How are the numerous viral proteins, some of which are encoded in different reading frames, made from this RNA? This is true for some bacteriophages and for a small number of animal viruses. The identification of Marburg virus (MARV) within two months of its first appearance is even by present-day standards a remarkable accomplishment of medical virology. The end result of virus infection is death and liquefaction of the insect from two to nine days after infection, depending on virus dosage and insect host. Plaque-forming units (PFU): infectious virus particles as detected by a plaque assay. A system fully driven by T7 RNA polymerase is also available; in that case, all plasmids are transcribed in the cytoplasm. Virol. (Scale bars: 100 nm); (d) drawing of ABV. Increased numbers of T cells from HIV-infected individuals undergo spontaneous apoptosis even though they are not directly infected. However, at least six other viral proteins are known to be associated with the envelope of occlusion-derived virions. These “virus factories” grow larger and coalesce as the virus replication cycle progresses. In between the early and late promoters is a noncoding region of approximately 400 nucleotides that contains several important sequence elements involved in both transcription and genome replication; this is called the intergenic region or the control region (Figure 21.5). A number of elegant studies using transgenic mice have been done to assess the relative contribution of E6 and E7 to cancers caused by human papillomaviruses. The influenza A virus M2 protein contains a HisXXX-Trp motif (where X can be any amino acid) that appears to constitute the main functional element of the M2 channels. • Induction of lytic gene expression in a lysogen involves inactivation of the lambda CI repressor by self-cleavage stimulated by cellular RecA protein. It turns out that Cavalier-Smith’s proposal is not actually used by eukaryotic cells; instead cells use the enzyme telomerase to extend and maintain the ends of their linear chromosomes. This mode produces multimeric doublestranded DNAs, called concatemers, that are the DNA packaging substrate. Many nonstructural viral proteins are expressed in infected cells, and these can also be recognized by the immune system. This cutaneous route of variolation resulted in primary and satellite lesions at the site of inoculation, but a much milder generalized CH026.indd 313 313 infection, and an overall mortality rate of approximately 1%, significantly lower than the natural infection. Depending upon the amount of E2 protein expressed, it can act as either a transcriptional activator or repressor of viral gene expression. Protein 3CD is believed to bind to stem loop I at the 5' end of the positive-strand RNA, bringing the RNA into the replication complex. In the intasome, some of the Int proteins have free binding domains; these free motifs enable the intasome to capture an attB site on a bacterial chromosome by binding to its core sequence. Although the hepatitis B surface antigen has been found in other primates, humans remain the primary reservoir for this virus. The S1 subunit, which forms the clubbed end of the spike, recognizes specific cellular receptors and initiates attachment. RNA primers (blue) initiate DNA replication by copying a short sequence on the DNA template strand, and primers are then extended (orange) by DNA polymerase. Alternatively, genes can be transferred directly into cells in the body by injection of virus vectors. The ability to switch the major structural component of the virus capsid could have profound implications for future potential host interactions, although this is an avenue of research yet to be explored. How does the immune system provide for antibody diversity? Plants can also be engineered to produce virus-like particles that can be purified and used as vaccines. EBERs may also provide a survival function during latent infection by acting on cellular proteins involved in protein synthesis such as L22 and La, thereby enhancing cell growth and division. Such DNAs would therefore have become primitive viruses, able to jump from one cell to the next. Recent work has found that infections by bacteria or viruses can lead to a new pathway toward cell death called pyroptosis. DNA vaccines: express immunogenic viral proteins but cannot generate virus. ICP4 can repress transcription of its own gene by direct binding to the ICP4 promoter region (Figure 24.6). This residual RNA serves as a primer for the subsequent synthesis of plus-strand DNA by reverse transcriptase. As a result of this technological limitation, it was initially quite difficult to make candidate vaccines against these dangerous viruses. In the late 1980s, independent work from the laboratories of Nahum Sonenberg and Eckard Wimmer showed that initiation of protein synthesis is capindependent and occurs at internal ribosome entry sites on picornavirus RNAs. This is in contrast to most cellular mRNAs, on which ribosomes recognize the capped 5' end of the RNA and begin protein synthesis at the first AUG codon encountered. The E7 proteins of all papillomaviruses are small, multifunctional proteins approximately 100 amino acids CH022.indd 267 Ad E1B 55K p53 HPV E6 Figure 22.4 Common pathways used by viral oncogenes. Many viruses carry genes whose expression can suppress apoptosis in infected cells, thereby prolonging the virus replication cycle. In humans and experimental animal systems, herpes simplex virus replicates in skin or mucosal cells at the portal of entry, producing infectious virus. The lipid bilayer and transmembrane alpha helices crossing the membrane are clearly seen. Members of the Pospiviroidae family fold into a rod-like secondary structure that includes short, double-stranded regions separated by small, single-stranded loops (Figure 31.1b). Cilia cut tangentially in this electron micrograph of a thin section are shown projecting from an epithelial cell. This is not trivial, because biological membranes provide effective barriers against movement of proteins and nucleic acids, let alone large macromolecular assemblies such as virus particles. A heavy fringe of fibers surrounds the capsid, within which several internal layers and the core can be seen. The presence of so many major capsid proteins in each of these viruses is, so far, unique among the phycodnaviruses. The best guess is that any protruding nucleotides will be a target for host exonucleases (tRNA, which has a protruding CCAOH 3' end, is continuously repaired by tRNA nucleotidyl transferase). • Members of most but not all virus families infect only organisms within one of the six major divisions of life. Positive (or plus) strand: the RNA strand that contains coding regions for proteins. These enzymes may well have been inherited from the RNA world. Most large T antigen is localized in the nucleus of infected cells, although some is found in the cytoplasm and at the plasma membrane. Capsids are shown as hexagons, and the virus envelope is shown as a gray circle. regions of the world, to discover other viruses transmitted by mosquitoes. Most people who are acutely infected with hepatitis B virus do not even realize they have it. Protein J packaged with DNA Figure 6.5 The ϕX174 morphogenetic pathway. Transcription of viral genes is temporally controlled and probably occurs in the cell nucleus Circumstantial evidence indicates that the viral DNA and probably DNA-associated proteins quickly move to the nucleus, where early transcription is detected CH027.indd 329 within 5 to 10 minutes post infection. What are they? The polymerization step for Avsunviroidae is clearly different since it is unaffected by high levels of ␣-amanitin. Negative contrast electron micrographs of (a) virions of SSV-1, (b) virions of SSV-6, (c) virions of SSV-1 being extruded from Sulfolobus shibatae. In contrast, human influenza A viruses code for HA proteins that bind preferentially to ␣-2,6 sialic acid, the major form found in the human respiratory tract. Flavivirus RNA synthesis is carried out on membranes in the cytoplasm The synthesis of the nonstructural proteins results in the establishment of active RNA replicase complexes. Dendritic cells, which present antigens to the immune system, can be isolated from an individual, exposed to a viral antigen, and reintroduced into the body. This suggests that the barrier for coronaviruses to “jump” from one species to another might be much lower than previously predicted. DNA containing these motifs binds to toll-like receptor 9 (TLR-9). Although not usually associated with human illness, blooms of H. Consistent with these findings, strain-specific differences in the capacity of reovirus to induce apoptosis segregate genetically with the viral S1 and M2 gene segments, which encode components of the outer capsid: attachment protein 1 and membrane-penetration protein 1, respectively. Cervical cancer in women is associated with infection primarily with the human papillomavirus types 16, 18, and 31, but a number of other types are also implicated (see Table 22.1). Lymphotoxin: a cytokine, also known as tumor necrosis factor β (TNF β), that kills infected cells by inducing holes in the plasma membrane. The predicted RNA-dependent RNA polymerase (nsp12) is a 100-kDa protein encoded in ORF1b, and a second, non-canonical RNA polymerase has been identified in nsp8. All of the proteins involved in these diseases undergo a conformational alteration to a common structure in the amyloid fibril, a “cross ” repeat structure in which strands are aligned perpendicular to the axis of the amyloid fiber. A circular map of the herpes simplex virus genome is shown in Figure 24.2. Envelope glycoproteins Tegument Capsid DNA (a) (b) Figure 24.3 Structure of the herpesvirus virion. These are proteins, lipids, or carbohydrates that have their own cellular functions, and are only fortuitously exploited by viruses; therefore they tend to be highly conserved, and are unlikely to undergo mutations that could impede virus uptake. For some viruses, the plasmodesmata are too narrow to allow passage; virusencoded “movement proteins” are expressed that cause expansion of plasmodesmata into wider channels. The viral RNA must also contain the central polypurine tract (cPPT). Thus small T antigen indirectly stimulates the MAP kinase pathway and helps bring the cell into the S phase. Receptor tyrosine kinases: cell surface receptor proteins that respond to binding of signaling molecules by phosphorylating specific tyrosine residues on intracellular proteins, activating a variety of signaling pathways leading to activation or inactivation of numerous cellular genes. Nonessential viral proteins can also be targeted if they activate a drug, transforming it into an inhibitor of virus replication. Endosomes are membranous organelles in which the virion is enclosed after undergoing receptor-mediated endocytosis (see below). Furthermore, they can be isolated easily from the blood of a variety of animals, are sturdy during manipulation, and can be stored for days or weeks. Integrins: a family of cell surface proteins (usually heterodimers) that interact with extracellular adhesion proteins and initiate intracellular signaling pathways. These neurons synthesize the neurotransmitter dopamine, which controls motor function and movement. nucleocapsids with icosahedral symmetry; coronaviruses thus resemble negative-strand RNA viruses in having helical nucleocapsids. For this purpose, it is convenient to divide all living organisms into the following six broad categories: 1. coli Yes Yes Initiation of RNA synthesis by bound GTP EF-Ts E. One general conclusion of this new field appears to be that wild type T7 is both a robust and highly optimized bacteriophage. Note that initiation of DNA replication at the leftmost T7 RNA polymerase promoter avoids head-on collisions between DNA polymerases and RNA polymerases, because they all travel in the same direction; transcription from class I genes, which could affect replication moving leftward, has ceased. Scrapie: prion disease in sheep, characterized by rubbing or scraping of the skin. Localizes to nuclear domain 10 structures. The text contains a number of new four-color figures which focus on the individual steps in virus replication and helps draw students’ attention to important concepts and details.Additional coverage on viruses of Archaea, plant viruses, viruses of algae and the giant mimivirus PDF 82.7 MB Password: vetbooks.ir Help Download 2ND EDITION FUNDAMENTALS OF MOLECULAR VIROLOGY NICHOLAS H. The two subfamilies of poxviruses are based on host range: Chordopoxvirinae infect vertebrates and Entomopoxvirinae infect insects. coli replication proteins. Lariat structures are generated from introns during RNA splicing; these two LATs are therefore stable introns. Shown are the major landmarks on the positive-strand RNA genome of picornaviruses. However, community spread of live vaccine strains also explains the fact that two-thirds of those who suffer from vaccine-associated paralytic polio are close contacts of vaccinated children. Conclusion: viroids may be a link to the ancient RNA world The various small self-replicating RNA pathogens share a considerable number of characteristics and may well have evolved from a common ancestor. Chapman (in gypsy moths) and C. All rights reserved. Met refers to methionine, used for initiation of most proteins, bound to methionine transfer RNA, which is bound to the 40S submit. M: mitochondrion; N: nucleus. To prevent membrane fusion more directly, peptides have been designed that bind to conformational intermediates of viral fusion proteins and inhibit their function. This could explain two differences between transcription (mRNA synthesis) and replication of genome RNA. The sialic acid–binding domain is shown folded at the top. In the early 1980s a group of viruses that infect freshwater unicellular, chlorella-like green algae were characterized; they were called “chloroviruses”. must make their own RNA polymerase and package at least one copy of this enzyme with the nucleocapsid in the virion. Gene gun: apparatus that projects tiny particles (gold or other inert material) coated with DNA into cells at high speeds, allowing penetration of cell membrane and intracellular expression of proteins encoded by the DNA. Some cellular genes have been shown to contain IRES. The vital role of interferons in antiviral defense has been demonstrated by studies in which animals that cannot mount an interferon response succumb to viral infections more rapidly and at a lower infectious dose than normal animals. Once this critical size has been acquired, then monomeric precursors of PrPSc can add irreversibly to allow growth of the PrPSc particle (c). It remains possible that a small virus might transmit prion disease by infecting and replicating in brain neurons, leading by some change in the infected cell to the conformational change in PrPC that causes disease. Neutralizing antibodies: antibodies that inactivate or “neutralize” virus infectivity when they come in contact with the virus particle. In many cases, sequences in viral RNA or viral proteins are associated with specific phenotypic changes in specific hosts under certain conditions. Herpes simplex virus blocks the presentation of antigenic peptides to the immune system (see Chapter 34). Genome replication Figure 27.13 Core membrane of mimivirus fuses with phagosome membrane. By interaction between their transmembrane regions, S proteins aggregate in specific regions of endoplasmic reticulum membranes and exclude host membrane proteins. The displaced single-stranded DNA can begin a new replication cycle, or can be packaged if capsid proteins are present. Replicon: a nucleic acid that, when transfected into cells, is capable of replication directed by proteins encoded in the nucleic acid. Interspecies recombination also occurs during mixed infections. • Single-stranded RNA bacteriophages have four genes on a linear RNA of 3500–4200 nucleotides. After an initial infection, viral DNA is harbored in a latent state in neurons, B or T lymphocytes, or other cell types. Moreover, parvovirus infection triggers an interferon-induced innate antiviral response (see Chapter 33) in normal cells but not in cancer cells. CH028.indd 350 16/05/11 8:15 PM Retroviruses the external environment of the cell, and show uncontrolled growth and division. Recombination between both input and progeny T7 genomes occurs and allows standard genetic mapping by recombination frequency measurements. Fusion 3. Semler, University of California, Irvine 12. The genome has 190-bp inverted terminal repeats that contain multiple copies of 5-bp direct repeats and a 16-bp direct repeat. To spread within a plant, they produce “movement proteins” that alter plasmodesmata, channels that connect the cytoplasm of neighboring cells. Since the level of CII determines the lysis/lysogeny decision, an important question becomes: What controls CII? The first practical application of an insect virus is credited to Edward Steinhaus, for the control of the alfalfa caterpillar in California in the 1940s. Top: rolling circle replication mediated by herpesvirus DNA polymerase (UL30) and processivity factor (UL42) generates head-to-tail concatemers of herpesvirus DNA. When tested in non-human primates, the vector significantly reduced disease severity. Likewise, adeno-associated virus Rep52 may be involved in encapsidation and interactions with cellular regulatory pathways, but again little is known. White: unknown function; blue: homologues in archaeal viruses; green: homologues in archaeal plasmids; red: transposases; black: virion proteins. Filterable virus: the name given to viruses when they were first distinguished from other microorganisms on the basis of their small size and their ability to pass through fine-pore filters. Most of these newly synthesized cellular mRNAs, as well as pre-existing cellular mRNAs, are degraded by Vhs, an endoribonuclease with a substrate specificity similar to that of ribonuclease A. Endemic: natural to a specific animal or human population or place; indigenous; native. Instead, the interactions between neighboring protein subunits must be well defined and specific. Cutting requires the introduction of nicks, staggered 12 bp apart, so that the single-stranded cohesive ends are made. All possess catalytic activity (hairpin or hammerhead self-cleaving structure). Therefore, with appropriate screening and effective treatment options, the development of malignant cervical cancer is relatively rare in developed countries. See also individual entry ligation, 382 symmetric replication, 380 ribozymes, 381, 385 RNA interaction with cellular RNAs, 382 RNA interference, 382 RNA structure, 379 Virokines, 423 Virological synapse, 47 Viroplasm, 321 Virulence factors, 424 Virus-like particles (VLPs), 198, 219, 310, 431, 435, 437 Virus vectors, 456. Karyopherin: see Importin. At least three viral genes that influence the length at the 3' end of postreplicative mRNAs have been identified. Viroids are circular, single-stranded RNAs 250 to 400 nt long that are not encapsidated. Viroid RNAs could therefore interfere with splicing by replacing functional RNAs in splicing complexes. GENES AND PROTEINS 56 genes code for 59 proteins. Recombination is a rare event and incompletely digested adenovirus genomes may give rise to nonrecombinant virus. The RNA polymerase complexes are located at the base of each spike in the core. The attachment protein binds to one or two cellular receptors The reovirus attachment protein 1 is a long, fiberlike molecule with head-and-tail morphology. RNA primers are synthesized by copying B C 1 A 3' 5' A' A' A C B 2 B DNA polymerase extends 3' end A' B' C' A A A B C A' A' C B 3 Rep protein binds and nicks 4 Rep protein unwinds hairpin; DNA polymerase extends 3' end 5 Hairpins refold; DNA polymerase extends 3' end 6 ss DNA displaced; new ds DNA formed A A' nick site C 7 A' C B A A C' B' A' B A C A' C⬘ A A' B' B' B A A' A' A C C' ɩ C⬘ A A' B' C' A A' A B C A' B' Figure 20.4 Replication strategy of adeno-associated virus DNA. Initiation of transcription of late genes requires at least three viral protein factors (VLTF) produced from intermediate mRNAs, and may require in addition an early gene product and a cellular factor. P binds to L, probably as a tetramer, and directs the binding of L to the promoter at the 3' end of the genome or antigenome RNA in nucleocapsids. Human immunodeficiency virus takes a different approach; it prevents the maturation of dendritic cells and induces the expression of interleukin-10, a cytokine with immune suppressive function. An RNA polymerase complex, composed of one molecule of 3 and one or two molecules of 2, is located at the base of each of the 2 spikes. The black images above and underneath the bilayer are envelope and capsid protein molecules. Bacteriophages specific to a variety of pathogenic bacteria have been isolated and characterized. Cytotoxic T lymphocytes have the capacity to kill cells with which they come into contact if those cells express surface MHC type I complexed with the specific peptide that their T-cell receptor recognizes. [Note: in biology, the name “prion” also refers to a group of 6 or 7 different species of small petrels (oceanic birds) restricted to the Southern Ocean surrounding Antarctica.] Prion disease: neurodegenerative disease caused by the accumulation of prions in the brain, leading to spongiform encephalopathy, and characterized by accumulation of amyloid fibrils and plaques. CH016.indd 198 Filovirus diagnosis, treatment options, and vaccine candidates Diagnosis of Marburg and Ebola virus infections is generally achieved in two ways: measurement of host-specific immune responses and detection of viral particles or particle components. (Plural: syncytia.) Systemic mosaic: mosaic disease symptoms found throughout the infected plant. Coronaviruses 159 Mark Denison, Vanderbilt University Michelle M. Which coronavirus protein is associated with syncytia formation? This stuttering mechanism recalls a similar mechanism used by paramyxoviruses and rhabdoviruses (Chapter 15) for polyadenylation of their mRNAs. Stuttering and termination of transcription on influenza genome RNAs is believed to result from blockage of further movement of the RNA polymerase by PB1 itself, because it remains bound to the terminal 12 to 13 nt at the 5' end of the same genome RNA it is copying. Often depends on the initial interaction of the pathogen with cell surface receptors. Other transcripts from the late promoter extend through most of the viral genome to a second major polyadenylation site. Chapter 33 Fig. These channels traverse the cell wall and provide a conduit for transport of viral genomes and other macromolecules. (a) Type I IRES, found in genome RNAs of enteroviruses and rhinoviruses. The best-known substrate is the alpha subunit of translation initiation factor eIF-2, which is phosphorylated by PKR at serine-51. ICP47. Chapter 23 Fig. nsp14 3' to 5' exoribonuclease; ExoN mutants of murine hepatitis virus and SARS coronavirus with mutator phenotype; possible RNA proofreading; N7 cap methyltransferase. Drugs preventing attachment and entry of virions Inhibition of attachment and entry prevents all subsequent steps in virus infection and permits clearance of virus particles by immune and other mechanisms. These genes are probably the minimum set required for virus replication and form a pool that are the essence of what constitutes the baculovirus family. A “delayed early” transcript from the T9 gene is detected starting 5 hours after induction, at about the time that viral DNA replication begins. NS2A. Drug companies performed target-based screens for compounds that could prevent binding of chemokines to CCR5. These have been classified in seven recently created virus families: spindle-shaped Fuselloviridae, filamentous Lipothrixviridae, dropletshaped Guttaviridae, bottle-shaped Ampullaviridae, spherical Globuloviridae, rod-shaped Rudiviridae, and two-tailed Bicaudaviridae. A. Differ from organisms in the other two domains by virtue of their cell surface proteins, the chemical nature of their lipids, and their translation, transcription, and replication machinery. Satellite RNAs can be either linear or circular. This “flip-flop” sequence inversion, resulting from the nicking, unfolding, and copying of the hairpin, can be detected in adeno-associated virus DNA, and was instrumental in testing the validity of the replication model. CH036.indd 455 16/05/11 8:21 PM C H A P T E R 3 7 Eukaryotic Virus Vectors Rénald Gilbert Bernard Massie USES OF VIRUS VECTORS For recombinant protein production. Receptor-mediated endocytosis σ1 229 and becomes elongated. Most cellular mRNAs employ cap-dependent mechanisms to initiate translation near their 5' end. In contrast, a number of cytoplasmic RNA viruses such as togaviruses, paramyxoviruses, and reoviruses cap their transcripts with virus-coded enzymes. al., “Antigenic and Genetic Characteristics of Swine-Origin 2009 A(H1N1) Influenza Viruses Circulating in Humans,” Science 325 (2009), 197–201, reprinted with permission from AAAS. Instead, infection spreads directly through special channels, called plasmodesmata, which connect the cytosolic compartments of neighboring cells. A decision is made during infection to progress to lytic growth (virus production and lysis) or lysogeny (integration of viral DNA and continued growth of the cells). Coen, Harvard University SECTION VII: VIRUSES THAT USE A REVERSE TRANSCRIPTASE 28. Plasmodesmata: membrane-lined connections between plant cells that allow small molecules to pass between cells. CH007.indd 83 dual advantages of strict regulation of expression and high yields upon induction. CH014.indd 169 Secretory vesicle Golgi membranes ER/Golgi intermediate compartment Figure 14.6 Assembly, maturation, and release of coronavirus virions. As a result of this change, 5 to 6 bp of dsDNA are pulled through the hexamer by the motion of a -hairpin that is in contact with the DNA. A' represents sequences complementary to A, B' to B, and C' to C. Once uncoated, the viral genome can be used as a template for synthesis of Nucleic acid genome Protein capsid Lipid envelope Envelope proteins Figure 1.1 Schematic diagram of virus particles. The same RNA polymerase therefore participates in two very different reactions: (1) synthesis of single-stranded mRNAs in viral cores, using a double-stranded RNA template that remains intact and can be used multiple times, and (2) synthesis of double-stranded genome RNAs within replicase particles, using a single-stranded mRNA template to which the negative-strand copy remains hydrogenbonded after synthesis. 9.12: Figure 4 reprinted from: Prangishvili, D., G. A DNA helicase-primase complex made from three other proteins (UL5, UL8, and UL52) then binds to the single-stranded DNA and synthesizes RNA primers, the first step in the initiation of DNA replication. Intergenic region: for polyomaviruses, a 400-nt region located on the viral genome between the early and late genes. For Sendai virus and related paramyxoviruses, each N subunit binds to exactly six nucleotides in the genome; thus a 15-kb genome contains about 2500 copies of the N protein. Unlike Sendai virus, measles virus H protein has no neuraminidase activity. Noncoding regions at each end of the genome are indicated by a solid line. These proteinases must act autocatalytically at least initially since they are part of the polyprotein, and therefore they must be enzymatically active before they are released by self-cleavage. In addition, the falling price of tallow (animal fat) led to a decrease in the amount of expensive processing using organic solvents. Two related viruses of hyperhalophiles resemble fusellovíruses by morphology but not by genetics The genus Salterprovirus has been introduced for classification of two spindle-shaped, double-stranded DNA viruses that infect hyperhalophilic euryarchaea: Haloarcula hispanica viruses 1 and 2 (His-1 and His-2) (Table 9.2). No homologs have been identified in related lentiviruses such as HIV-2 and simian immunodeficiency virus. Small black rectangles with directional arrows represent promoters for RNA synthesis. A specific role for nsP3 has not yet been identified. This lateral association is possible because membrane proteins can move in the plane of the fluid membrane. A defined structure for the nucleocapsid has not been obtained. Until very recently, the process of attenuation was essentially “black-box” science. ICP0 is best described as a promiscuous activator protein, because its expression activates transcription of many genes introduced into cells by either infection or transfection. The outermost bilayer leaflets fuse first (this is called hemifusion), followed by the inner bilayer halves. Coccolithophore: single-celled haptophyte algae that produce calcium carbonate plates called coccoliths. Nevertheless, late adenovirus mRNAs are efficiently translated; by what mechanism? Palmitoylated and membrane-associated. Euryarchaeota: one of the major phyla of Archaea, including hyperhalophiles, methanogens, thermophiles and some hyperthermophiles. These fibers can be detected by their characteristic binding of the dye, Congo red. Binding sites for transcription factors NFB and NFAT, whose activities are sensitive to external stimuli, are present in the U3 region of the LTR. Bats have been discussed before as potential reservoirs for the Ebola virus outbreaks in Sudan and the Democratic Republic of Congo, the Kenyan Marburg virus disease cases, and the more recent Marburg virus disease outbreak in eastern Democratic Republic of Congo. RNA virus genomes are generally either plus or minus sense. • Although not yet proven, it is likely that fruit bats serve as a reservoir for filoviruses in nature. Although shown in the figure as free, single-stranded RNA, the negative-strand antigenome RNA most likely exists in a double-stranded form, hybridized to positive-strand viral RNA. For example, the transforming potential of the mouse retrovirus spleen focus forming virus (SSFV) is due to mutations in the viral env gene that allow it to mimic the activity of erythropoietin and to stimulate growth of cells that respond to this hematopoietic growth factor. Because hepatitis delta virus needs a viral protein for packaging, it is considered an RNA satellite of hepatitis B virus. This disease caused a total loss of regional cucurbit crops. Of these seven, three are known to have specific enzymatic activities: NS5 is an RNA-dependent RNA polymerase; NS3 is a multifunctional, multidomain protein that contains both a proteinase activity and an RNA helicase acitivity; NS2B has been shown to complex with NS3 and is a required cofactor for proteinase activity. Surprisingly, mRNA synthesis is stimulated by addition of methylated cap analogues such as mGpppAm, or naturally capped mRNAs such as alfalfa mosaic virus RNA 4. Most RNA polymerases that transcribe the late genes of mouse polyomavirus do not terminate transcription after passing through the late polyadenylation signal, but continue transcription along the DNA template. • Many, but not all, viral envelopes are derived by budding from preformed cellular membranes. The immune response is mediated by a variety of specialized cell types that originate primarily in bone marrow, and circulate through the body in the blood and lymphatic systems as well as in most tissues. ϕX174 delivers its genome into the cell through spikes on the capsid surface When ϕX174 virions are placed in solutions of high ionic strength, the DNA genome is extruded from the capsid via the spikes, located at the axes of fivefold symmetry. When the stack of V disks reaches to the end of H, U binds, terminating tail polymerization. Because it contains most of the amino acid sequences of small C N Exterior Cytoplasm Tyr 250 ~P 315 ~P 322 ~P Tyr 416 SHC ~P PI3K ~P Activated MAP kinase Phosphatidyl inositol 3 phosphate PLC ~P Inositol triphosphate ~P 527 C N C-Src Middle T (active) antigen Figure 21.9 Middle T antigen binds to c-Src and activates pathways that stimulate cell metabolism and the cell cycle. 4.4: Reproduced from the Ph.D. thesis of Jennifer Gruenke, University of Virginia, with permission from Jennifer Greunke and Judith M. A subpopulation of T lymphocytes within lymph nodes differentiates into cytotoxic cells that kill virus-infected cells. The reaction is broken into four stages. The fusion peptide is predicted to play a major role in the fusion of the viral and cellular membranes that leads to the release of the genome RNA into cells. To prevent integration of the vector by means of its natural integration mechanism, the lentivirus integrase was mutated to abolish its function. This can be achieved by strict barrier nursing techniques and addition of HEPA-filtered respirators for aerosol protection when feasible. The most striking lesions are found in the liver, spleen, lymph nodes, adrenal glands, and kidney. These discoveries were all awarded Nobel prizes, although Peyton Rous had to wait 55 years between his discovery and his prize! Retroviruses have been isolated from numerous animal species, including chickens, mice, cattle, horses, cats, monkeys, and humans. In some cases (NS3 and NS5), domain structures have been determined. We study them in a course on virology because they are small, noncellular infectious agents. Fusion involves rearrangement of multimers of protein subunits but only minor conformational changes in protein tertiary structure. IPS-1 dimerizes and binds to the outer mitochondrial membrane, and its recruitment leads to activation of caspase-3, as shown in Figure 33.4, triggering apoptosis. The viral strucural proteins are often identified by their glycosylation status and their molecular weights; for example, the human immunodeficiency virus SU protein is called gp120, TM is gp41, and CA is p24. Phospholipase C cleaves phosphatidylinositol, generating inositol triphosphate, which releases calcium ions from the endoplasmic reticulum. CH026.indd 323 16/05/11 8:13 PM 324 CHAPTER 26 Poxviruses • Poxvirus DNA can replicate only after the core has been dissolved and the DNA genome is released into the cytoplasm. The functions of other nsps in replication are less well studied, but there are data suggesting roles for these proteins in virulence (nsp1), cell-cycle control (nsp1), cell membrane modification, and replication complex formation (nsp4, nsp6). Viral mRNA accumulates faster than it is degraded, but cellular mRNA synthesis is inhibited by other herpesvirus proteins; as a consequence, viral protein synthesis predominates. Viral DNA in the nucleus is circularized, either by direct ligation of the ends or by recombination between the a sequences at the ends. Furthermore, these viruses can transform normal rat or hamster fibroblasts grown in vitro into cell lines that are immortal, form dense and rapidly growing colonies, have reduced requirements for growth factors, and in some cases can cause tumors when injected into animals. Negatively stained preparations of PrP 27–30 were observed by electron microscopy. HIV is transmitted through sexual contact and blood exchange. (b) (a) (c) (h) (d) (e) (g) (f) (i) Figure 9.11 Acidianus two-tailed virus (ATV), family Bicaudaviridae. They found that the infectious agent could pass through a Berkefeld filter, and was therefore a “filterable virus”. The number of virus-specific proteins synthesized dramatically increases beginning at about 6 hours post-infection, and the synthesis of host cell proteins gradually decreases from that time onward. B molecules at the portal vertex are simultaneously cleaved to remove 20 amino acids from their amino termini. Thus transcription-dependent DNA entry is one factor responsible for the delayed transcription of class III genes compared with class II genes. However, some chloroviruses 05/05/11 11:24 AM Coccolithoviruses have a chitin synthase instead of, or in addition to, the hyaluronan synthase. CH016.indd 199 16/05/11 8:05 PM C H A P T E R 1 7 Bunyaviruses Richard M. The viral E2 protein is also involved in viral DNA replication. Most known satellite viruses and nucleic acids replicate in plants, but an insect satellite virus (chronic bee paralysis virus), several double-stranded RNA satellite nucleic acids of fungi and protozoa, and a human satellite virus (hepatitis delta virus) are known. Also called N-acetyl neuraminic acid. Reovirus is an acronym for respiratory enteric orphan virus; infections of humans by reoviruses types 1 to 3 usually involve the respiratory and intestinal tracts with minimal or no associated disease symptoms (therefore the virus is an “orphan”). Delta structure: structure of a ribozyme derived from hepatitis delta virus. Hemorrhagic fevers are caused by some nairoviruses and hantaviruses. Subacute sclerosing panencephalitis (SSPE): a progressive, fatal neurological disease that is a rare complication of measles virus infection. These virions incorporate the RNA genomes of either the helper virus itself or the defective transforming virus. Oligomers of another Bcl-2 family member, called Bax, stimulate the caspase cascade and lead to apoptotic cell death (see Figure 33.4). The wide distribution of related sequences is proposed to occur by serial genetic exchanges between phages sharing common host species. In some strains of influenza A, a second reading frame on RNA 2 codes for an additional short protein named PB1-F2. (c) Primers are extended by T7 DNA polymerase, and replication forks move toward opposite ends of DNA (horizontal arrows). This reading frame does not terminate until very near the 3' end of the mRNA (Figure 21.6). Although cells can be infected by transfection with naked singlestranded DNA, transfection efficiency is enhanced if H protein is added to the DNA. V. The fiber forms a triple helical structure anchored to the penton base at one end and has a terminal knob at the other end. • In vertebrates, virions are made by budding of nucleocapsids through the cytoplasmic membrane. At this point, two outcomes are possible, depending on the level of CII in the cell. Especially remarkable in their uniqueness and diversity are double-stranded DNA viruses that infect hyperthermophilic crenarchaeaota in extreme geothermal environments. This concept is supported by the observation that mutation or deletion of the left-hand LTR can lead to initiation of transcription in the right-hand LTR, resulting in transcription of cellular genes downstream of the proviral DNA (see below). Mutational analysis has defined optimum consensus DNA sequences that distinguish early, intermediate, and late promoters. Carstens, Queen’s University, Kingston, Ontario; Fig. Us10 Us9 U s7 Us6 Us3 Us1/Us1.5 4 Us4 U s2 OriS RNA Us8/ Us5 Us8.5 UL2 U L5 U L4 a b UL8/UL8.5 US UL9/UL9.5 UL11 a UL10 LAT 140 b 150 0 UL13 UL14 10 13 20 UL54 UL17 UL18 40 HSV-1 110 UL53 UL52 UL16 UL15 30 120 UL55 UL12 Kbp 0 UL56 UL19 0 50 10 UL50 UL49.5 90 UL48 80 UL20.5 70 UL23 UL45 UL43.5 UL44 OriL UL43 UL42 UL41 UL26/UL26.5 UL27 UL28 UL UL40 UL37 UL39 UL35 UL20 UL21 60 UL47 UL46 U L7 UL6 c ORF P/O UL49 UL3 OriS 0 UL51 UL1 ? A CI octamer formed through long-range cooperative interactions is shown in gray. Some of these viruses interfere with normal cellular processes and cause disease (although many other viruses infect their host organisms without causing overt disease). In this example, cells are infected with vaccinia virus expressing T7 RNA polymerase, then are transfected simultaneously with four plasmid DNAs. One DNA contains the vesicular stomatitis virus genome sequence downstream from a T7 promoter. • One product of the initial transcription is the lambda N-antiterminator protein, which allows transcription to proceed beyond terminators tL1 and tR1, making “early” mRNAs. • Early gene products include proteins that excise and circularize lambda DNA, DNA replication proteins, and another antiterminator protein, Q. RNA polymerase is shown as an orange oval. A small (22 to 24 amino acids) virus-encoded protein called VPg is covalently attached to the 5' end of genome RNA, and a poly(A) tract of 30 to 100 nucleotides (depending on the virus strain) is present at the 3' end. Some of these accessory genes have been shown to serve functions in pathogenesis, such as inhibition of interferon-regulated gene expression. Later during lytic growth, sufficient Cro accumulates to partially fill OR2 and OR1, along with OL2 and OL1. Fortunately, Reston ebolavirus, the first Ebola virus species with an origin outside of Africa, does not appear to be pathogenic to humans despite several serologically documented human exposures. What two models for co-packaging have been proposed? The transmembrane helices (cylinders) span the membrane, and a short internal domain interacts with the matrix protein underneath the viral envelope. The answer is that ribosomes only bind to the maturation start site on RNA chains that are in the process of being synthesized, that is, “nascent” chains. DISTINGUISHING CHARACTERISTICS Cucumber mosaic virus has the broadest known host range of any known virus and is distributed worldwide. Viruses are responsible for many cases of human encephalitis, meningitis, pneumonia, hepatitis, and cervical cancer, as well as warts and the common cold. David Baltimore and Richard Franklin were the first to demonstrate the presence of an RNA-dependent RNA polymerase in virus-infected cells in the early 1960s. NEW DEVELOPMENTS IN ANTIVIRAL VACCINES New approaches to vaccine development show great promise In the last 50 years, spectacular advances in molecular biology, immunology, biotechnology, and protein chemistry have been the driving forces behind antiviral vaccine development. Capsids of polyomaviruses and papillomaviruses assemble around double-stranded DNA genomes pre-wound into nucleosomes by capture of cellular histones. Below: exploded map of the 16-kb Hind III D fragment. RNA is shown in light blue, newly synthesized DNA in gray. These four receptor components are transmembrane proteins with a binding domain on the extracellular side and an intracellular domain that binds to one of the Jak kinases, either Jak1 or Tyk2 (Figure 33.8, top left). For example, the genome RNAs of bacteriophages such as Qβ and MS2 express their four viral proteins from three or four different initiation sites, and control of RNA phage gene expression is largely carried out at the level of initiation of protein synthesis (see Chapter 5). • Viruses with double-stranded DNA genomes are widespread among all organisms except plants, and they can have genomes ranging from 5000 to more than a million base pairs. Most negative-strand RNAs are associated with one or more growing positive-strand molecules in a replicative intermediate. Small RNAs identical to viroid sequences have been detected in infected plants, and it has been shown that these siRNAs were produced by the cellular enzymatic machinery following viroid infection. Site-specific integration. • The Q protein allows transcription to proceed rightward on circular lambda DNA, transcribing all “late” genes in a single, long transcript. Acheson VIRUS CLASSIFICATION Viruses are classified into related groups based on: • Genome composition (DNA or RNA, single- or double-stranded) Genome topology (linear or circular; single or multiple segments) Capsid symmetry Presence or absence of an envelope Genetic relatedness (nucleotide and amino acid sequence similarity) Mechanisms for expressing messenger RNAs and replication of genomes Host organisms • • • • • • THE MAJOR VIRUS GROUPS Viruses are classified into species, genera, families, and (in some cases) orders, within the following categories: • • • • • • • Viruses with single-stranded DNA genomes Viruses with double-stranded DNA genomes Viruses with positive-strand RNA genomes Viruses with negative-strand RNA genomes Viruses with double-stranded RNA genomes Viruses that use a reverse transcriptase Satellite viruses, satellite nucleic acids, and viroids THE EVOLUTIONARY ORIGIN OF VIRUSES RNA viruses may be relicts of the prebiotic RNA world. Transition from the original folded state to the more stable state is a highly improbable event, because a large energy barrier separates the two states. Despite its profound impact on susceptibility to HIV-1, the deletion has little negative impact on human health, making it an attractive target for intervention with a small molecule. Transcription from PR antiterminated by N also proceeds beyond the Q gene into the late genes; this transcription gives a level of late gene expression equal to about 20% of the PR', Q-sponsored level. The RNA polymerase repeatedly transcribes this U run by “stuttering” (see below), giving rise to a poly(A) tract some 200 nt long, and then releases the mRNA. This binding triggers conformational changes on the upper surface of the particle. See Procapsid. These two proteins are identical except for a 46-amino acid region in the 289R protein that is missing in the 243R protein because of removal of a longer intron by CH023.indd 277 alternative splicing. CH028.indd 351 16/05/11 8:15 PM 352 CHAPTER 28 Retroviruses Memories of infections past and the enemy within: endogenous retroviruses and retrotransposons The stable insertion of proviral DNA into the host chromosome means that, even in the absence of expression, the viral genome will be replicated each time the infected cell divides. Although retrovirus vectors can remain viable for several months at –80□C in the presence of cryoprotectants (glycerol, sucrose, or serum), the virion loses its infectivity within a few hours at 37□C or within a few days at 4□C. Vaccination campaigns against diseases that are highly transmissible and are therefore public health risks can be unpopular, and the ethics of requiring vaccination with products that are never 100% safe are discussed.

Enzymatic machinery within the host cell is able to recognize foreign double-stranded or structured single-stranded RNAs and process them into 21 to 26 nucleotide small interfering RNAs (siRNAs). Live wild-type viruses. Ascites: fluid that accumulates in the abdominal cavity as a result of impaired liver function or other pathologic conditions. A number of 16/05/11 8:19 PM The Innate Immune Response B lymphocytes Activated by binding to antigens via B-cell receptor (surface antibodies); differentiate into plasma cells by interacting with T-helper cells. Other cellular proteins may also function in the replication complex. (a) Immediate early transcription (N) and early, N-antiterminated transcription (N) initiated at PL and PR. The subsequent course of the infection can be roughly divided into three phases: (1) acute infection; (2) a subsequent clinically latent phase of variable duration; and finally (3) AIDS (Figure 29.1). Transmission electron micrographs of a late phagosome in a cell infected with mimivirus, showing three particles at different stages of uncoating. • Some, however, generate pyramidal structures that appear to pierce the cell wall, releasing virions and killing the host cell. The virus codes for an integrase that carries out this reaction. Gene replacement therapy was initiated in the mid-1980s with the successful transfer of the adenosine deaminase gene to mouse bone marrow stem cells to correct a severe immune deficiency, using a retrovirus vector. 23.9: Fig. Paul Kaplan detected endogenous RNA/DNA-dependent DNA polymerase (reverse transcriptase) within the core of hepatitis B virions. • • Two large open reading frames are joined by a frameshift These genes are translated as polyproteins, then cleaved to 14–16 individual proteins Other genes are translated from multiple 3'-coterminal, subgenomic mRNAs. • • • Three to four envelope proteins (HE, S, E, M) One nucleocapsid protein (N) Four to six nonstructural or accessory proteins VIRUSES AND HOSTS Subfamily Coronavirinae: three genera based on genome homologies. Prion diseases were first detected in domestic ruminants Sheep and goat herders in Britain have for hundreds of years observed a disease in their animals that they termed scrapie. And where does viral DNA replication occur? PKR is believed to play an important role in regulation of cellular protein synthesis in the absence of virus infection. Although virions of hepatitis B virus contain DNA, the viral genome is transcribed into a full-length RNA copy within the infected cell, and that RNA is subsequently reverse-transcribed into DNA. Satellite nucleic acid: a nucleic acid genome that is able to replicate only in the presence of a helper virus, and which is packaged as a virion by capsid proteins coded by the helper virus. Viral RNA isolated from purified virions was found to be infectious by mechanical inoculation of plants. This is true of many non-enveloped viruses that replicate and assemble in the nucleus or in the cytoplasm of eukaryotic cells, and of most bacteriophages. Synthesis of mRNAs that lie more distant from the promoter, toward the 5' end of the genome (HN and L), should be more strongly inhibited, because the polymerase would have to travel through most of the genome before it began to make those mRNAs, and therefore would be more likely to encounter a block. Polyomavirus capsids are constructed from pentamers of the major capsid protein The virions of polyomaviruses are naked capsids approximately 45 nm in diameter. While ONYX-015 does seem to kill cancer cells selectively, the basis for this effect now seems more complex than simply the presence or absence of p53. Semen or other body fluids may be infectious for some time during convalescence. (b) Cells were stained with fluorescent antibodies that detect reovirus proteins, which are revealed as bright patches within cells. These diagrams, and the figures illustrating the opening pages of each chapter in this book, were drawn by Philippe Lemercier, Swiss Institute of Bioinformatics, Swiss-Prot Group, University of Geneva. Glycoprotein: protein that has covalently bound carbohydrate residues. Baculoviruses 302 Insect viruses were first discovered as pathogens of silkworms 302 Baculoviruses are used for pest control and to express eukaryotic proteins 303 Baculovirus virions contain an elongated nucleocapsid 303 Baculoviruses produce two kinds of particles: “budded” and “occlusion-derived” virions 304 Baculoviruses have large, circular DNA genomes and encode many proteins 305 Insects are infected by ingesting occlusion bodies; infection spreads within the insect via budded virions 306 Viral proteins are expressed in a timed cascade regulated at the transcription level 306 Immediate early gene products control expression of early genes 307 Early gene products regulate DNA replication, late transcription, and apoptosis 307 Late genes are transcribed by a novel virus-coded RNA polymerase 308 Baculoviruses are widely used to express foreign proteins 308 26. Retrovirus virions are much less stable than virions of other vectors such as adenoviruses and adeno-associated viruses. The receptor-binding domain has been mapped for well-studied coronaviruses such as murine hepatitis virus and SARS coronavirus, but the exact location of this domain within the S proteins of other coronaviruses has not yet been determined. The outer capsid protein 3 is degraded and lost from virions, and the exposed 1 protein is cleaved to form two fragments, and ϕ, that remain bound to the ISVP. The T-cell receptors bind not to intact proteins but to small peptide epitopes produced by intracellular digestion of proteins. Instead, it prevents formation of the peptideloading complex within the endoplasmic reticulum. This is reminiscent of viral interference, in which the presence of one virus inhibits replication by a superinfecting virus. Four chapters in Section II cover wellstudied bacteriophages. Gene order and expression are similar to those of paramyxoviruses and rhabdoviruses. These defenses are organized in a multilevel strategy that helps the organism resist infection and combat infectious diseases. Some known ribozymes can cleave or ligate RNAs. Ritonavir: protease inhibitor used for treatment of human immunodeficiency virus infections. A recently emerged coronavirus caused the unexpected SARS epidemic in the early part of this century. Evolution of peptide synthesis amino acids activated amino acids templated peptide synthesis oligopeptides 3. Ten years later, James Johnson reported that this virus also infects tobacco plants, in which it causes mosaic disease. 16/05/11 7:57 PM C H A P T E R 8 Bacteriophage Lambda Michael Feiss λ-like bacteriophages λ is the Greek letter “lambda” VIRION Head is a naked icosahedral capsid (T 7), diameter 63 nm. Alternate origins are known to exist because T7 mutants that have lost the primary origin are able to replicate well. The 11 kb positive-strand RNA contains a methylated cap at its 5' end and is polyadenylated at its 3' end. Following packaging of the DNA the capsid changes conformation, sealing the DNA inside the capsid. Regulates transcription both positively and negatively. Hepatitis C virus: a recently discovered member of the Flaviviridae Hepatitis C virus was first identified, not by the traditional method of isolation and growth of virus in culture, but rather by direct cDNA cloning of the viral genome. The envelope surrounds the virus nucleocapsid, which contains a single molecule of viral genome RNA. Proviral DNA: a DNA copy of a retrovirus genome that is inserted into the chromosome of an infected host cell. Capsid-binding drugs block attachment and/or entry of picornaviruses, especially rhinoviruses, a major cause of the common cold. The 118-nt 5' noncoding region is followed by a single, long open reading frame of 3411 codons. These two types of structure have therefore been named type I and type II internal ribosome entry sites, respectively. Viroids are transmitted among plants by sucking insects or by mechanical means, like many plant viruses. Immature virions are shown as circles. They showed that the multiples of 60 that lead to quasi-equivalent structures are given by the triangulation number series, T h2 ⩽ hk ⩽ k2, where h and k are any two integers, including zero. At this point the RNA polymerase pauses, dissociates the nascent RNA chain from the TRS, and brings the nascent chain to the TRS located at the end of the leader sequence in the template RNA. Several factors help predict clinical outcome. Hepatitis B virus can linger on drying surfaces like door handles for over a week and still remain infectious. • HIV-1 codes for multiple auxiliary proteins that overcome barriers to virus replication. A ribosome translating the coat gene arrives at its stop codon, releases coat protein, and carries out a short random walk during which it may encounter the lysis or replicase start sites. Subsequent cleavage of the membrane anchor of the C protein by the viral proteinase (see above) releases the capsid from direct contact with the membrane. New adjuvants are being developed Until very recently, only aluminum salts were used as adjuvants in human vaccines. For example, Sulfolobus islandicus rod-shaped virus 2 (SIRV-2), causes massive degradation of the host cell chromosome and lyses the cell. Influenza Viruses 210 Influenza viruses cause serious acute disease in humans, and occasional pandemics 210 Influenza virus infections of the respiratory tract can lead to secondary bacterial infections 211 Orthomyxoviruses are negative-strand RNA viruses with segmented genomes 211 Eight influenza virus genome segments code for a total of 11 different viral proteins 212 Hemagglutinin protein binds to cell receptors and mediates fusion of the envelope with the endosomal membrane 214 M2 is an ion channel that facilitates release of nucleocapsids from the virion 214 TOC.indd xii Nucleocapsids enter the nucleus, where mRNA synthesis and RNA replication occur 215 Capped 5' ends of cellular premessenger RNAs are used as primers for synthesis of viral mRNAs 215 Viral mRNAs terminate in poly(A) tails generated by “stuttering” transcription 216 Two influenza A mRNAs undergo alternative splicing in the nucleus 216 Genome replication begins when newly synthesized NP protein enters the nucleus 217 Nucleocapsids are exported from the nucleus in a complex with matrix protein and NS2 218 The NS1 protein interferes with polyadenylation of cellular mRNAs 218 The NS1 protein also suppresses a variety of host cell antiviral response pathways 219 PB1-F2 may contribute to suppression of the host immune response 219 Viral envelope proteins assemble in the plasma membrane and direct budding of virions 219 Neuraminidase cleaves sialic acid, the cellular receptor that binds to HA 220 Influenza virus strains vary in both transmissibility and pathogenicity 220 Genetic variability generates new virus strains that can cause pandemics 220 The 1918 pandemic influenza A virus was probably not a reassortant virus 221 Genome sequences from some previous influenza A virus strains confirm the antigenic shift hypothesis 221 Highly pathogenic avian influenza A H5N1 strains in poultry farms are a potential threat but are poorly transmitted among humans 221 A new pandemic strain of influenza A virus arose by genetic shift and spread worldwide in 2009 222 19. huxleyi, which seem to be controlled by the coccolithovirus. These are the same histones that package and condense cellular chromosomal DNA. Nevertheless, there are antiviral agents that act at nearly every step of viral infection. Garrett, and D. Sequences of the terminal 12 to 13 nucleotides at both the 5' and 3' ends of viral RNAs are highly conserved among all genome segments of a given virus type, and are highly self-complementary. The recruitment of viral RNAs into the RNA synthesis complex involves cis-acting recognition elements. If recombinant DNA technologies can be used in their preparation, subunit vaccines are potentially the simplest and least expensive vaccines to make. Influenza received its name in eighteenth-century Italy as a reference to the imagined cause of epidemics—the influence of the stars. They are capped at their 5' ends, and cleaved and polyadenylated at their 3' ends, by protein machinery used by the cell for generating its own 253 messenger RNAs. Early transcripts then undergo alternative splicing within the nucleus to generate four different messenger RNA species. DNA synthesis using the newly formed hairpin generates a new double-stranded DNA molecule with a hairpin end, and also results in displacement CH020.indd 243 243 of progeny single-stranded DNA. VA I RNA binds to PKR as do other double-stranded RNAs, but fails to activate the protein kinase, perhaps because it is too small to bind to two PKR molecules at the same time (see above). Secretion of interferon- is induced by mitogenic stimuli, antigens, Interferons are a first line of host defense against viruses; however, therapeutic use has been limited A gene coding for interferon-␣ was cloned by Charles Weissmann and collaborators in 1980, allowing the protein to be produced in large amounts and opening up the possibility of therapeutic use. Ancient ancestors. Replication-competent adenovirus vectors are useful tools in antitumor therapy The disadvantages of some adenovirus vectors, namely cytotoxicity and induction of an immune response, are assets in applications such as vaccination or cancer gene therapy. DNA polymerase ␣/RNA primase reinitiates synthesis on single-stranded DNA generated by continued helicase action of large T antigen as the two replication forks travel in opposite directions on the DNA template. It contains, besides the preS2 and S domains, an additional 119 N-terminal amino acids in the preS1 domain. This reaction can be measured by separating the acetylated from unacetylated chloramphenicol, and is used as a reporter assay to measure activity of genes. Monkeypox, cowpox, tanapox are animal poxviruses that occasionally infect humans. Filoviruses are related to paramyxoviruses and rhabdoviruses Marburg and Ebola viruses have enveloped virions 80 nm in diameter and of varying lengths, including filamentous particles between 800 and 1200 nm long (Figures 16.1 and 16.2). These oligomers contain up to 10 adenylate residues. 2.4: from VIPERdb2;, op. (Scale bar, 100 nm). Consequently, many of these viruses can penetrate directly through the plasma membrane, where they first encounter their receptors. Their action results in the expression of viral proteins in two stages. Using capped cellular RNAs as primers for transcription allows these viruses to make their own capped mRNAs even though they do not code for a capping enzyme. More recently, a new species, Bundibugyo ebolavirus (BEBOV), was described as the causative agent of a disease outbreak in Uganda with 149 cases (25% case fatality rate). • The infectious agent of prion diseases contains protein but no detectable nucleic acid. Two types of virion are made: budded and occlusionderived virions. Middle: viral messenger RNA, shown in light blue, with the open reading frame shown above. (f) Adenovirus. More recently, these compounds have shown effectiveness in reducing severe disease symptoms and deaths due to the emerging influenza H5N1 (“bird flu”) and pandemic H1N1 (“swine flu”) strains, which has led to stockpiling and more widespread use of these drugs. ICP27 inhibits splicing of mRNAs during the early stages of infection, also contributing to blocking host responses. Adenovirus-induced cell killing Two viral proteins have been implicated in cell killing and virus release. Without electron microscopes, Sertic and Bulgakov were able to distinguish different groups, or “races”, of bacteriophage, primarily by plaque morphology and ultrafiltration. GP (glycoprotein) Heavily N- and O-glycosylated type I transmembrane protein; single envelope glycoprotein of filoviruses; responsible for both receptor binding and fusion with cellular membranes; is cleaved into two subunits: GP1, N-terminal portion, and GP2, C-terminal portion with membrane anchor and fusion peptide. Seven different polypeptides are derived from this region. Measles and some other enveloped viruses induce fusion of infected cells with neighboring uninfected cells. Many of the complexities involved in national and international vaccine program decisions are exemplified by the ongoing global effort targeting poliovirus. CH009.indd 102 Filamentous enveloped viruses of the Lipothrixviridae come in many lengths The family Lipothrixviridae (from the Greek lipos, “fat” and thrix, “hair”) presently includes nine different species of rod-shaped or filamentous viruses of variable flexibility and lengths, depending in part on genome length. CH031.indd 380 16/05/11 8:16 PM Viroids and Hepatitis Delta Virus Polymerization. This test is based on the recognition of morphologically abnormal cervical cells indicating the presence of premalignant or malignant lesions. In plants, the cell wall prevents diffusion of virus particles between cells. Recombinant interferons ␣ and are presently used to treat chronic infections by hepatitis B and C viruses, cancers such as melanoma, multiple myeloma, renal cell carcinoma and Kaposi’s sarcoma, and the neurodegenerative disease, multiple sclerosis. L (large protein) Viral RNA-dependent RNA polymerase. Serial passage of pathogenic viruses in animals, eggs, or cells grown in vitro often results in the acquisition of a variety of mutations that reduce the pathogenic properties of the virus in its natural host. Strictly speaking, adeno-associated viruses, which are single-stranded DNA viruses whose replication depends on coinfection with adenoviruses or herpesviruses, should be included in this category, but those viruses are closely related to numerous other parvoviruses that replicate independently of helper viruses. One copy of maturation protein bound to RNA genome. At the early uncoating stage (1), a partial opening of the inner protein shell at the stargate assembly is initiated; the stargate is denoted by two red arrows. Ubiquitination: covalent linkage of ubiquitin to a protein, marking it for degradation by proteasomes. However, when infects metabolically sluggish, stationary phase cells, most of the infected cells are lysogenized. Bacteriophage T7 77 William C. Filovirus genes are believed to be transcribed in sequence starting at the 3' end of the genome to yield seven different subgenomic messenger RNA species by a start–stop mechanism described in Chapter 15 (see Figure 15.7). Both Tat and Rev are then transported to the nucleus where they act to augment transcription of provirus DNA (Tat) and the transport of viral mRNAs to the cytoplasm (Rev). The size limitation may reflect the packaging preference of alphavirus capsids for RNAs the size of genome RNA. The extracellular form of NS1 is capable of eliciting a protective humoral immune response, and has been shown to help the virus evade the immune surveillance of the host complement system (see Chapter 34). Bunyaviruses can cause encephalitis, hemorrhagic fevers, or severe respiratory syndrome in humans. The clathrin coat is seen in these thin-section electron micrographs as a densely stained layer on the cytosolic side of the plasma membrane. DNA is shown as a gray coil within the capsid. Early viral genes are expressed: the Baltimore classification of viruses 14 The seven groups in the Baltimore classification system 14 4. In children with suboptimal immunity, virus shedding can persist for months or years. Real influenza is a severe acute disease in all age groups with symptoms of sore throat, cough, high fever, headache, and muscular aches and pains. The terminal 8 to 11 nucleotides of genome RNAs are highly conserved among all three segments for a given virus, and among all viruses within a genus (Table 17.2). However, other genes are unique to particular species within the family; these genes likely determine individual species adaptation including host range or tissue tropism, virulence factors, and differences in virus morphology. More recently, high-level protein production has been achieved with other vector systems, particularly those derived from togaviruses such as Semliki Forest virus and retroviruses such as human immunodeficiency virus type 1. In the next half-century, scientists isolated a large number of viruses that infect mammals and birds and are transmitted by a variety of arthropod vectors, including mosquitoes, ticks, and sandflies. CH019.indd 232 16/05/11 8:07 PM Reoviruses 233 sufficient capsid proteins have been synthesized, assembly is completed by the addition of a layer of outer capsid proteins to replicase particles, resulting in the formation of infectious double-shelled virions (Figure 19.8). The core contains at least one molecule of reverse transcriptase (P), as well as the viral genome. Peng, R. DISTINCTIVE CHARACTERISTICS Occlusion bodies contain virions in a form that allows insect-to-insect transmission. The VLPs have a safety advantage over live attenuated vaccines but still need booster immunization to protect nonhuman primates against lethal challenge. In the upper atmosphere, oceanic DMS is oxidised into acidic particles (methanesulfonate, sulfur dioxide, and sulfuric acid) that eventually form cloud condensation Figure 27.7 True color satellite image of a milky E. Virions must be assembled, exit the cell in which they are made, withstand the extracellular environment, attach to and enter another cell, and release the viral genome. Lentiviruses like HIV-1, in contrast to other retroviruses, direct their preintegration complexes to enter the nucleus via the nuclear pores. Short, inverted terminal repeat sequence. As we saw above, E2F binds to the strong p4 promoter and helps activate gene expression. As is the case with acyclovir, the triphosphate species exerts antiviral activity. These examples show that defined subassemblies of protein subunits confer significant advantage. Autocrine loop: self-stimulation of growth by a cell population that produces a growth-promoting molecule and responds to the same molecule. GENOME Linear or circular double-stranded DNA, 160–1200 kb. Virions are then transported from the lumen to the outside of the cell. The debates surrounding some childhood vaccination programs are only slightly less vigorous today. (GDP) and the recycling factor eIF-2B. Viruses of Archaea 97 David Prangishvili, Institut Pasteur 11. In addition to inhibiting prophage expression, the repressor turns off the lytic genes of any incoming chromosome. Helical nucleocapsid. • Upon entry into the cell, the RNA genome is reverse transcribed to double-stranded proviral DNA within the viral nucleocapsid. They are released from the host cell by a novel mechanism involving the formation of large pyramidal protrusions that transect the cell envelope, rupturing the S-layer. Recombinant viruses can be easily identified by screening for their polyhedra-negative phenotype. Study of the structure and activity of the early gene products (T antigens) led to the discovery of a number of important cellular regulatory pathways that are disrupted or modified during transformation and tumor formation. 428 CH035.indd 428 16/05/11 8:21 PM A Brief History of Antiviral Vaccines 429 Usually require multiple doses to achieve protection. • Herpesvirus nucleocapsids appear to bud through the inner nuclear membrane to acquire an envelope, but the precise mechanism by which they acquire their final envelope and exit the cell is uncertain. Antibody-dependent cellular cytotoxicity: a mechanism by which macrophages or NK cells kill cells that express a foreign antigen on their surface by recognition of surfacebound antibodies. Thus, one role of VP30 is to activate Ebola virus transcription. The T7 DNA polymerase (gp5) has structural similarity to mammalian DNA polymerase and HIV reverse transcriptase. Genes grouped by function. The transition to the DNA-based world At some point during the evolution of the RNA world, polypeptides produced by self-replicating RNAs could have acquired the abilities to (1) catalyze the production of deoxyribonucleotides from ribonucleotides and (2) use these deoxyribonucleotides to make DNA copies of the RNA genomes (i.e., the first reverse transcriptase). The binding of antigenic epitopes with the specific cell surface receptors activates the lymphocytes to perform their immune function. The presence of complementary early and late RNAs in the nucleus leads to the formation of double-stranded RNAs, which are degraded by cellular enzymes. T7 primase/helicase (orange hexamers) loads onto the replication fork and generates short RNA primers (light blue arrows) for DNA synthesis on the opposite strand. Internal amino acids that affect transmission may be involved in virion stability; external amino acids may be recognition sites for interactions with aphids that affect uptake or release of virions from mouthparts during probing (Figure 10.3). 932 from Molecular Biology of the Gene, 4th ed. It can replicate in the (cooler) nasal passages but not in the bronchi or lungs, and therefore causes few or no disease symptoms. By use of this somewhat more rational procedure, effective antiviral drugs such as the nucleoside analogues ganciclovir and azidothymidine were discovered. In the latter, the surface glycoprotein genes of human rotaviruses have been inserted into a non-pathogenic bovine rotavirus. VIRION ASSEMBLY Many capsids self-assemble. Seven nonstructural proteins. 325 CH027.indd 325 05/05/11 11:23 AM 326 CHAPTER 27 Viruses of Algae and Mimivirus Given the number of potential hosts, it is surprising that so few phycodnaviruses have been isolated to date (see Table 27.1 for some examples). The first contains a copy of the bacteriophage T7 RNA polymerase gene (see Chapter 7) under transcriptional control of a vaccinia virus promoter, and the second contains the coding sequence for the gene of choice to be expressed, cloned downstream of a T7 RNA polymerase promoter. However, these proteins are encoded by information in the viral nucleic acid genome, and their activities serve to express and replicate the genome. Full-length negative-strand RNA (not shown in Figure 14.5) can serve as a template for production of genome RNA by the viral RNA polymerase. Lentiviruses and human T-cell leukemia viruses have additional regulatory genes expressed from multiply spliced mRNAs. VIRUSES AND HOSTS Seven genera are recognized: Alpha- to Epsilonretrovirus, Lentivirus, Spumavirus. In South America, Oropouche virus can cause epidemics involving tens or hundreds of thousands of people. ⩾ ssRNA 3. Advantages and limitations of adenovirus vectors Several features make first-generation adenovirus vectors attractive vehicles for gene transfer and protein production: (1) they can be easily produced at high titers; (2) they can transduce many cell types; (3) they do not require cell division for transgene expression; (4) their genome remains episomal, and therefore the target cell does not undergo permanent mutation. However, because adenoviruses do not integrate their genome into cellular chromosomes, cell division leads to dilution of the transgene and eventually to loss of transgene expression. What is it called? This situation presents a problem for the immune system. RNAs 1 and 2 are translated, and protein 1a subsequently binds to the B box near the 5' end of viral RNAs and targets it to cytoplasmic membranes. That is, variation among these algae appears to correlate with depth in the ocean and not with “horizontal” geographic location. The Rev protein promotes the efficient transport of unspliced or singly spliced viral RNA from the nucleus to the cytoplasm. Unfortunately, the H5N1 viruses were highly pathogenic for avian cells and rapidly killed the embryos before the required reassortant viruses could be produced. It is the blue whale of viruses. The envelope of Sulfolobus spindle-shaped virus 1 (SSV-1) contains lipids with a composition similar to that of host cell membranes. In extreme cases, 90% or more of a virus preparation consists of particles with defective genomes. Antiviral Vaccines 428 Brian Ward, McGill University 36. Overlapping rounded boxes enclose molecules that would have been part of the RNA world and the present DNA world. Retroviruses package a virus-coded reverse transcriptase (an RNAdependent DNA polymerase) within the virion, and use this enzyme to produce a double-stranded DNA copy of their RNA genome after they enter the cell. C-Src then phosphorylates several tyrosine residues on middle T antigen itself. Acyclic: a version of a compound in which a group that is usually cyclic is broken or removed. • Reoviridae have double- or triple-layered capsids with icosahedral symmetry, and from 10 to 12 different double-stranded RNA genome segments. As the DNA polymerase extends the growing DNA chain, it displaces the other strand of parental DNA, CH023.indd 280 which is then coated with the single-stranded DNA binding protein. Agard, and Stanley B. The combination of these approaches will allow testing of mechanisms of coronavirus host species movement and adaptation. The sites and years of filovirus episodes and outbreaks in African countries are shown. These motifs can be formed by annealing two different RNA fragments in vitro, such that one RNA fragment acts as the ribozyme and the other as the substrate (Figure 31.5). As a result, polio can be targeted for eradication. All of the oral poliovirus vaccine strains also have the capacity to persist in sewage for weeks, potentially infecting and therefore immunizing or boosting immunity in community members exposed to contaminated water. • Epstein–Barr virus causes mononucleosis and, in certain regions of the world, malignant lymphomas. The drug consists of a guanine base attached to what is essentially a broken sugar ring (Figure 36.4). The ind gene product apparently controls the virus productive life cycle; beginning approximately 1 hour later, transcription from two “early” genes, T5 and T6, can be detected. In the reoviruses, a protector protein called 3 is cleaved by acid-activated proteases within the intestine or in lysosomes to expose another protein, 1, that is responsible for penetration of the virus core into the cell (see Figures 19.4 and 19.5). Viral proteins VPg (black circle), 3AB (orange line plus black circle), 3CD, 3D, and 2C are shown bound to RNA or cytoplasmic vesicles. They are the best-characterized vectors, and the most common vectors used in preclinical and clinical trials. In an intriguing variation, the prophage DNA of the -like phage N15 is linear, and is not integrated into the cell chromosome; such linear plasmids are rare in E. Furthermore, first-generation adenovirus vectors are strongly immunogenic, causing the loss of transgene expression in immunocompetent hosts. A molecule in the shape of a stable closed shell composed of 60 carbon atoms was discovered in 1985 and named buckminsterfullerene. Two unusual viruses with icosahedral capsids and prominent spikes A number of archaeal viruses still remain unclassified. The nonstructural proteins nsp1–16 (shown below the map) are generated by cleavage from the 1a/1b fusion protein by viral proteinases. Rather, AZT is a substrate for cellular thymidine kinase. attP is also designated POP', to symbolize its three parts. Because of their capacity to integrate their genome into the chromosomes of nondividing cells such as neurons, it should be possible to use lentivirus vectors to treat other neurological disorders such as Parkinson’s disease. 14, pp. Keratinocytes in these cultures can be transfected with cloned viral DNA and are able to produce infectious viral particles. The Vp54 protein that makes up the capsid of the PBCV-1 virion contains a carbohydrate portion unexpectedly containing what? Hemagglutinin-esterase protein. Other immediate early baculovirus genes, including ie-2, pe38, and ie-0, were identified based either on their ability to transactivate other viral genes or on the time of their expression in infected cells. This is achieved by using the terminal hydroxyl group of the 3' hairpin as a primer CH020.indd 241 for host cell DNA polymerase. The C-terminal part of 3AB, protein 3B, is identical to VPg, a protein that is covalently attached to the 5' end of genome and antigenome viral RNAs. VPg becomes covalently attached to uridine residues via a tyrosine side chain near its N-terminus, a process known as uridylylation. This pattern indicates that there are selection pressures on reovirus gene segments to maintain certain nucleotide sequences independent of their protein-coding function. This approach required several months for immunity to develop. However, it contains the 3' CHOH group that is missing in acyclovir. The nucleocapsid has a diameter of 40 nm and is composed of 240 copies of the capsid protein, also arranged in a T 4 icosahedral lattice. Dendritic cells collect virus by the binding of the HIV-1 SU protein (gp120) to DC-SIGN, a cell-surface protein. 2 reprinted from Cornell, C.T. and Semler, B.L., “Gene expression and replication of 16/05/11 2:35 PM 486 Credits the picornaviruses,” Encyclopedia of Molecular Cell Biology & Molecular Medicine, 2nd ed. Zanamivir and oseltamivir were designed based both on this structure and that of neuraminidase bound to a transition state inhibitor. The conserved sequences at the 5' termini are complementary to the sequences at the 3' termini, which permits the ends of the RNA to base pair and form panhandle structures. Transcription of late genes is activated after the onset of viral DNA replication, and late transcripts become the major RNAs made in the infected cell. One of the most promising applications of retrovirus vectors is the treatment of blood disorders. They use cellular RNA polymerases to replicate RNA. Acheson THE NATURE OF VIRUSES Virus particles contain: • A nucleic acid genome (either DNA or RNA) A protein coat (capsid) that encloses the genome In some cases, a lipid membrane (envelope) • • The infectious virus particle is called a virion. In spite of their small size, parvoviruses have a number of unique properties that set them apart from other viruses. In the process the scaffolding is dismantled and the scaffolding proteins are ejected from the capsid. 16/05/11 7:52 PM CH004.indd 56 16/05/11 7:52 PM SECTION II VIRUSES OF BACTERIA AND ARCHAEA C H A P T E R 5 Single-Stranded RNA Bacteriophages C H A P T E R 6 Microviruses C H A P T E R 7 Bacteriophage T7 C H A P T E R 8 Bacteriophage Lambda C H A P T E R 9 Viruses of Archaea Viruses that infect bacteria are called “bacteriophages”, or “phages” for short. Dependovirus infection has not been associated with disease. The text provides a thorough presentation of basic and contemporary concepts in virology for a student’s first exposure to the field.Chapter stories. In this application they function primarily as vectors to deliver genes that correct genetic deficiencies associated with human diseases. 9.10a1-c2: Adapted From Figure 4 in: Bize A, E. • Hepatitis delta virus RNA and certain viroids possess a self-cleaving structure essential for their replication. Arrows above and below the black line show genes transcribed, respectively, from left to right or from right to left. Comparison of viral oncogenes with their cellular counterparts revealed that the viral forms have mutations or deletions that alter the activity of the proteins. of transcription (Stat proteins). X-ray crystallographic studies of the 3 protein complexed with oligoribonucleotides suggest that the growing messenger RNA chain passes up into the spike turret and the minus-strand template of the genome RNA passes down into the core interior via independent channels within the RNA polymerase. Reovirus isolates that are most capable of myocardial injury induce little interferon and are resistant to its antiviral effects. Why is maturation of bunyaviruses inhibited by monensin? wt. Several water-based community outbreaks of flaccid paralysis caused by vaccine-derived revertant polioviruses (including Type 1) have been described. Transgene: a foreign gene that is introduced artificially into a cell and expressed, sometimes as part of a virus vector. Newly made ICP0 also localizes in ND10 structures and performs multiple functions. Vpu acts by binding CH029.indd 362 to CD4 and to the cellular protein ß-TrCP. Prion proteins can be produced by recombinant DNA technology in bacteria; these proteins can be folded into states that resemble the native PrPc and the altered PrPsc conformations. On the other hand, the unbridled use of vaccines for everything from tooth decay to cancer has the potential to tarnish the relatively good name that vaccines have enjoyed for almost half a century. What is the primer for initiating adenovirus DNA replication? Cell fusion causes tissue destruction and may be responsible for pathogenic effects of some paramyxoviruses. RNA-containing viruses are therefore unique, and they face two related problems as a result of their RNA genomes: (1) they must synthesize messenger RNAs from an RNA template, and (2) they must replicate their genome RNA. Enteric: intestinal. The rings, in addition, loop through rings formed by neighboring pentamers and hexamers, generating protein chainmail! DNA is inserted into preformed proheads by an ATP-dependent mechanism Concatemeric DNA generated by rolling circle replication is cut to generate unit-length virion DNA molecules during packaging of the DNA into the prohead shell (Figure 8.9). In the course of the epidemic, 17 more persons engaged in this kind of work were admitted to hospital, and two medical staff members became infected while attending to the patients. This is at least partly mediated by large T antigen interacting with pRb, which stimulates transcription of genes that raise levels of p53. During capsid assembly, one or a few capsid protein subunits bind to this packaging sequence, initiating both genome packaging and capsid assembly. Interferon-, also called fibroblast interferon, is a glycosylated protein produced mainly by fibroblasts and epithelial cells. Concatemer processing depends on transcription by T7 RNA polymerase and occurs during DNA packaging into preformed proheads Surprisingly, transcription by the T7 RNA polymerase is also required for cleavage of concatemers. For example, cowpox virus actually uses rodents as a reservoir host, cattle being only an occasional and accidental host for the virus. It should be possible to take advantage of these viral proteins to develop new therapies. Harrison CHARACTERISTICS OF CAPSIDS Virus capsids are assembled from many copies of a small number of viral proteins: principle of genetic economy. 1885 Pasteur and colleagues fix biological characteristics of rabies by passage in rabbit brains and discover that virulence in spinal cord is lost with drying. The Tc cell differentiates and develops into an active cytotoxic T lymphocyte with specificity for the antigen first encountered. Group A arboviruses were reclassified into the Alphavirus genus of the Togaviridae family (Table 13.1), so named in reference to their envelope (“toga” means “cloak” in Latin). Arrowheads denote 3' ends of RNAs. Low level of readthrough of Tϕ is indicated on one class III transcript but can occur on all transcripts. Its surface displays an array of acidic residues that mimics the phosphate backbone of DNA, similar to a 24 base-pair segment of bent DNA. Prior to DNA sequence analysis, different strains of hepatitis B virus were classified based on the antigenic epitopes contained within SS (see above). However, E. It is probable that uncoating occurs when endosomes become acidified, initiating fusion of the viral membrane and endosomal membrane, followed by release of the nucleocapsids into the cytoplasm. Human ideas, for example, can be transmitted by human communication from generation to generation. Below: the pathway by which the primary transcript is converted into more than 25 different mRNAs in three size classes by alternative splicing. The K3L gene product, a homologue of eukaryotic protein synthesis initiation factor eIF-2␣, competitively inhibits the dsRNA-activated protein kinase PKR, relieving the inhibition of protein synthesis induced by interferon. This argument makes most sense for the giant mimivirus (Chapter 27) and the poxviruses (Chapter 26). In endocytic vesicles, virions undergo partial degradation by cellular proteases, resulting in the generation of infectious subvirion particles (ISVPs). Therefore these viral proteins make good drug targets. HEPATITIS DELTA VIRUS (HDV) The only viroid-like satellite RNA known to infect humans and animals. The roles of these eleven viral proteins in the viral replication cycle are described in detail below. Signaling by interferons is mediated by a pathway that includes the Janus tyrosine kinases (Jak kinases) and the signal transducers and activators Interferon Interferon receptors Jak 1 Tyk 2 Jak 1 P P P P P P Tyk 2 Jak 1 Stat2 P P P P P P P P Stat1 Stat2 P P P P Tyk2 Stat2 Plasma membrane Stat1 Stat1-Stat2 heterodimer Stat1 ⩽ IRF-9 P P Cytoplasm Nuclear membrane Nucleus ISG mRNA P P Interferon-stimulated genes Figure 33.8 Interferon signal transduction. 16/05/11 8:10 PM Papillomaviruses E7 E6 DNA damage stabilizes/ activates E6AP binds to p53 induces P53 p53 Degradation in proteasomes activates transcription of APOPTOSIS es uc d in p21 / Bax CELL CYCLE ARREST Figure 22.6 Interaction of papillomavirus E7 and E6 proteins with p53. This complex process involves several steps and exploits a variety of cellular activities ranging from endocytosis to nuclear import. Such an E. Large quantities of virions were purified by sucrose gradient sedimentation for crystallization and subsequent x-ray structure determination. For example, supercoiled DNA migrates more rapidly during electrophoresis in agarose gels than does the same DNA when it is a relaxed circle or a linear molecule, because the supercoiled form is more compact. See also Icosahedral symmetry Capsomers, 249 Cap-stealing bunyaviruses, 205 influenza viruses, 217 Carboxylic ionophors, 53 Carboxy-terminal domain (CTD) of RNA polymerase II, 293, 360 Cardiovirus, 126 Caspase recruitment domains (CARDs), 401, 403 Caspases, 219, 233, 279, 333, 363, 404–406, 418 Cathepsins, 194, 229 Caveolae, 49, 251 Caveolins, 49 Cavins, 49 CD4 protein, 47–48, 345, 357–359, 362–363, 420, 464 CD4 expression, down-regulation, 363 CD4-positive T cells, 355, 356, 357, 363, 423, 424 Cell killing, adenovirus-induced, 283 Cell lysis, 9, 407, 418, 421 by bacteriophage φX174, 72, 75 by bacteriophage T7, 82 by bacteriophage λ, 85, 94–95 by Acidianus two-tailed virus (ATV), 106 by Haloarcula hispanica virus 1 (His-1), 108 by phycodnaviruses, 330, 335, 338 by single-stranded RNA bacteriophages, 60, 61, 63, 67 Cell response to virus infection, 402–405 Cell transformation by human adenoviruses, 283–284 by papillomaviruses, 270 by polyomaviruses, 261 by retroviruses, 351 Cell-based high throughput screen, 445 Cell cycle, 239, 243, 254–256, 265, 267–269, 277–279, 298 Cell-cycle arrest, 166, 228, 234, 245, 266, 268–269, 362 Cell-mediated immunity, vaccines that stimulate, 436–437 Cell-to-cell movement, 116, 118–120 Cellular immune responses, 436 12/05/11 4:08 PM Subject Index Cellular mRNAs polyadenylation of, 218–219 viral mRNA synthesis primed by, 204–205 Central nervous system, 234 Central or primary lymphoid organs, 419 Chaperones, 73, 93, 117, 118, 131, 184, 185, 256 Chemokine receptors, 47, 48, 345, 357–358, 359, 447, 464 Chemokines, 322, 358, 364, 403–404, 409, 423, 447 Chikungunya virus, 149 Chimeras, 382, 468 Chimeric vaccines, 434 Chimeric virus, 431, 468 Chlamydiae, 3 Chloramphenicol acetyltransferase, 155, 192 Chordopoxvirinae, 314 Chromatin remodeling, 279 Chronic wasting disease, 389 Ciliated epithelium, 211 Circoviridae, 34 Circular DNA genome, 70, 92, 249–250, 260, 264, 305, 366, 367, 369 becoming supercoiled, 249–250 gymnastics of, 250 Cirrhosis, 139, 374 cis-acting replication elements (cre), 133 Class I fusion proteins, 51, 141, 164 Class II fusion proteins, 51, 141, 164 Classical complement activation pathway, 416 Classification, virus, 31–44 based on molecular criteria, 31–32 major virus groups, 33–40 naming conventions, 33 virus families, 32–33 virus genera, 32 virus species, 32 Clathrin-coated pits, 48, 194 Cleavage and polyadenylation specificity factor (CPSF), 218 Clonal selection theory, 420 Coccolithophore, 331 Coccolithoviruses, 331–333 caspase activation, 333–334 control of algal blooms, 331 effects on climate, 332 genome, 333 release of dimethylsulfide, 331 sphingolipid biosynthesis, 333 Cochaperone, 256 Coiled coils, 51, 163, 180, 244 Cold chain, 434 Cold sores, 5, 285, Collectins, 417 Common cold, 5, 125–126, 160, 211, 274, 439, 446 Complement system, 297, 322–323, 366, 399, 416–418, 424 Concatemers, 84–85, 93, 291, 319 processing, 85 Condylomas, 264 Confocal microscopes, 13 Core proteins, 28, 79, 81, 227, 229, 232, 321,366, 370, 371, 373 Coronaviruses, 159–171. All retroviruses package two identical copies of the viral genome. Their hosts, Chlorella, are small, unicellular, nonmotile, asexual green algae with a global distribution. Myristoylation: covalent binding of the 14-carbon fatty acid myristate to N-terminal glycine residues on proteins, leading to their association with lipid membranes. What two common defense responses are induced in infected hosts? p53 responds to a variety of signals by binding to and activating transcription of genes that can lead either to blockage of the cell cycle or apoptosis (programmed cell death). The viral envelope is the transport vessel in the passage from one cell to the next, and the nucleocapsid is its cargo (Figure 4.1). Orthomyxoviruses share a common structure and mode of replication. How do the coccolithoviruses control the weather? These plus-strand antigenome RNAs are then themselves copied in a similar fashion by the RNA polymerase complex, in the presence of NP, to generate nucleocapsids containing full-length, minus-strand genomes (Figure 18.8). The T7 RNA polymerase supplied by vaccinia virus transcribes all four genes simultaneously, synthesizing the RNA genome of vesicular stomatitis virus and messenger RNAs for all three replication proteins. The S1 protein is instrumental in binding messenger RNA sequences to the ribosome during initiation of translation in bacteria. CH014.indd 171 16/05/11 8:04 PM CH014.indd 172 16/05/11 8:04 PM SECTION IV NEGATIVE-STRAND AND DOUBLESTRANDED RNA VIRUSES OF EUKARYOTES C H A P T E R 1 5 Paramyxoviruses and Rhabdoviruses C H A P T E R 1 6 Filoviruses C H A P T E R 1 7 Bunyaviruses C H A P T E R 1 8 Influenza viruses C H A P T E R 1 9 Reoviruses The single-stranded RNA genomes of negative-strand RNA viruses cannot be translated, because they are complementary to the positive-strand mRNAs that encode viral proteins. Minute virus of mice uses one promoter (p4, located 4 map units from the left end) to synthesize the mRNA encoding the nonstructural proteins and a second promoter (p38) for the mRNA encoding the capsid proteins. The split junction of the cDNA is flanked by one half of an intron carrying a splice donor or acceptor sequence. The different forms of the poxvirus virion probably serve different roles in nature. Small, flask-shaped invaginations in the plasma membrane are enriched in cholesterol and sphingolipids. Reoviruses are excellent models for studies of viral replication and pathogenesis. Both papillomaviruses and adenoviruses make analogous proteins that interact with p53. Membrane fusion is mediated by specific viral “fusion proteins” Penetration of enveloped viruses involves a membrane fusion event mediated by viral glycoproteins. Like many plant viruses with divided genomes, its genome RNAs are packaged in three separate but otherwise identical capsids. These changes are triggered by: • interaction of viral surface proteins with cellular receptors in membranes, or exposure to low pH inside endosomes • Enveloped viruses penetrate by fusion of the virus envelope with either: • the plasma membrane, or membranes of intracellular vesicles • Non-enveloped viruses enter the cytosol by either: • • forming transmembrane channels through which the viral nucleocapsid or genome can pass, or inducing lysis of endocytic vesicles INTRACELLULAR TRANSPORT Virions and virus nucleocapsids are transported within the cell along microtubules using the cellular transport motor dynein. Presumably these transcripts are extruded from the intact core into the cytoplasm, where they are translated. Interferon response. Fortunately, these strains have not yet shown signs of spreading efficiently among humans. Virions of cucumber mosaic virus are compact (29 nm) spherical particles with icosahedral symmetry (Figure 10.2). The hydrophobic pocket lies just below the canyon floor. Retrovirus reverse transcriptases use a cellular tRNA bound to their genome RNA as a primer to initiate synthesis of a DNA copy (Chapter 28). Because of the strand transfer, this DNA molecule contains a copy of the R sequence sandwiched in between the U3 sequence, originally at the right end of the viral RNA, and the U5 sequence, originally at the left end. These genera roughly correspond to the previous coronavirus groups I (alpha), II (beta), and III (gamma), but with some differences based on the criteria of relatedness of the most conserved coding sequences. Some milestones in vaccine development are presented in Table 35.2. Early vaccine technology was crude but effective Like all medical interventions, the development of new vaccines has been limited by the availability of appropriate technologies (Table 35.3). For mouse mammary tumor virus and Mason– Pfizer monkey virus (“B-” and “D-type” viruses), the core is assembled within the cytoplasm, generating spherical structures designated “A-type particles” visible by electron microscopy. In 2008, we launched a Corporate Citizenship Initiative, a global effort to address the environmental, social, economic, and ethical challenges we face in our business. The 511-nt 3' noncoding region contains an 87-nt sequence that is predicted to form a stem-loop structure. The membranes in these structures most likely originate from the endoplasmic reticulum, although other membranes have been implicated. How might the terminal hairpin structure of AAV single-stranded DNA lead to death of tumor cells infected by the virus? Enhancement of virus release from the plasma membrane. All cultured representatives of the phylum Crenarchaeota are hyperthermophiles, which grow optimally above 80⬚C. These strategies utilize a virus genome that is carried in bacteria as a plasmid or cosmid. 16/05/11 8:03 PM 142 CHAPTER 12 Flaviviruses 5'UTR 3'UTR CAP Structural Non-structural pr M C Capsid prM NS1 E 2A 2B NS3 prM 4A 4B NS5 Methyltransferase-polymerase Protease-helicase Envelope NS2B/NS3 (cytoplasm) Signalase (ER) Furin (ER lumen) Unknown (ER lumen) Figure 12.4 Organization of the flavivirus genome. Since the genome contains two identical copies of the b sequence, these four viral genes are therefore present in two copies per genome, albeit in inverted orientation. The exception is a 1788-nucleotide CHLOROVIRUSES Known chloroviruses replicate in Chlorella isolated from symbiotic hosts The chloroviruses are the best studied of all phycodnaviruses. NP does not bind to influenza virus mRNAs, presumably because they are capped and contain cellular mRNA sequences, not viral sequences, at their 5' ends. The threedimensional structure of fragments of the glycoproteins of these viruses reveals a trimeric spike standing perpendicular to the viral envelope and dominated by long central alpha-helical coiled coils. The polymerase protein is believed to recognize and interact directly with the stem-loop, initiating both encapsidation and reverse transcription of the pregenome RNA. Double-stranded RNA-dependent protein kinase: see PKR. Consequently, the majority of all cancers have undergone either a mutation in the p53 gene or alterations in genes encoding p53 regulatory molecules. Telomerase: an enzyme that maintains and extends ends of linear chromosomes in eukaryotic cells by using a small template RNA to make repeated DNA sequences. The orthobunyavirus and phlebovirus NSs proteins are nonessential virulence factors that are involved in inhibition of host cell transcription and in antagonizing the host interferon response. As anticancer agents. Both RIG-I and MDA5 contain a C-terminal RNA helicase domain and two N-terminal caspase recruitment domains (CARDs). The RNA phages also attracted attention because scientists were intrigued about how their RNA genomes were replicated, since cells normally make RNA only from a DNA template. Molec. Can be caused by inherited mutations in PrP gene or transmitted from infected animals, usually of same species. Transcription ends by stuttering and termination at an oligo U region near the 5' end of genome RNA. By 5 to 6 h, the cytoplasm becomes filled with infectious progeny virus particles and by 6 to 8 h, localized lysis of the host cell releases progeny virions (Figure 27.5d). When N levels are low (lower part of figure), RNA polymerase terminates and reinitiates transcription at multiple sites, producing mRNAs as shown. However, plasmodesmata connecting mesophyll cells and vascular-related cells (bundle sheath cells, adjacent phloem parenchyma cells, sieve elements, and companion cells; see Figure 10.8) have different morphological and transport features. Therefore, p53-activated p21 expression inhibits progression of the cell cycle from G1 to S phase, inhibiting cell proliferation. In contrast, unicellular organisms that replicate inside other cells invariably remain intact and retain their genomes within their own cellular membranes. Many of these cells are memory T cells that have a very long lifetime in the body. See also Bacteriophage Lambda (λ); Bacteriophage T7; Microviruses; Single-stranded RNA Bacteriophages abundance, 5, 6 against bacterial diseases, 8 cell entry mechanism, 14 classification, 33, 35 discovery, 7 entry of DNA into virion, 28, 29 “phage group”, 7–8 plaque assay, 9 scaffolding proteins, 28 Bacteriophage Lambda (λ), 85–95 chaperone proteins, 93 CI repressor, 88–89 Cro, 89–90 DNA entry, 86–87 DNA packaging/virion assembly, 93–94 DNA replication, 93 excision of DNA, 92 genetic map, 86 Int synthesis control, 96 integration, 92 lytic transcription program, 87–88 outline of λ biology, 86 phage adsorption, 86–87 promoters, 91 prophage state, 85 regulatory gene products, 92 retroregulation, 93 scaffolding proteins, 93–94 telomerase, 93 temperate phage, 85 lytic transcription program, 87–88 Bacteriophage T7, 77–84 bindsub.indd 492 Class I gene products, 80 Class II gene products, 80 Class III gene products, 81 DNA concatemers, 81–82 DNA entry into cytoplasm, 79 DNA replication, 80–82 expression of foreign genes, 83 gene organization, 78–79 genetic engineering with, 83 genetic and transcriptional map, 78 ribonuclease III cleavage of mRNAs, 80 viral RNA polymerase, 79 special features, 82–83 Baculoviruses, 302–311 budded virions, 304 discovery, 302–303 early gene products, 307–308 expression of foreign proteins, 303, 308–311 gene expression regulated at transcriptional level, 306–307 genomes, 305–306 immediate early gene products, 307 infecting insects, 306 late gene products, 308 occlusion-derived virions, 304–305 in pest control, 303 recombinant baculoviruses as gene therapy vectors, 309–310 representative baculoviruses, 303 virions, 303–304 Bafilomycin A1, 53 Baltimore classification system, 14–15 Basal cells, 265 Bat coronaviruses, SARS origin from, 160–162, 171 Bax, 269, 279–280, 404 B-cell receptors, 420 Bcl-2, 279–280 Bicaudaviridae, 1, 100 BK virus, 248 Blood disorders, treatment, 464–465 Bovine spongiform encephalopathy (BSE), 388, 393, 394 Brome mosaic virus (BMV), 113, 117, 118 Bronchitis, 160 Buckminsterfullerene, 24 Budded baculovirus virions, 304 Budding, 16, 26, 28–29, 46, 145, 154–155, 164, 169, 177, 186, 190, 195, 196, 206–207, 212, 219, 220, 294–295, 304, 306, 349, 362, 373, 374 Bunyaviruses, 200–209 arthropod vectors, 200–201 cell entrance by endocytosis, 204 encephalitis, 201–202 evolutionary potential of, via genome reassortment, 207–208 genome replication, 206 genome RNAs, 202–203 hemorrhagic fever, 201–202 infectious bunyavirus rescue from cloned cDNAs, 208 L protein, 202, 203, 204–206 mRNA synthesis primed by cellular mRNAs, 204–205 pathogens, 201 premature termination of mRNAs prevention, 206 protein coding strategies, 203 respiratory disease, 201–202 reverse genetics, 208 transmission virions of, 202 virus assembly at Golgi membranes, 206–207 c-Jun N-terminal kinase (JNK), 403 c-src, 255, 350 c-Src kinase, 370 Cancer cervical, 264, 269–270 nature of, study, 8–9 liver, 370, 374, 383 lymphoma, 296 retroviruses and, 350, 464–465 virus vectors in treatment of, 9, 232, 239, 245, 283, 322, 461 Capping viral mRNAs cucumber mosaic virus, 116 flaviviruses, 144 togaviruses, 151, 153 reoviruses, 228, 231 baculoviruses, 308 poxviruses, 318 phycodnaviruses, 330 Capsids, 3, 19 capsid-binding drugs, 446 with helical symmetry, 25–26. Infectious fractions of scrapie-infected brain contain no demonstrable nucleic acid even when analyzed by highly sophisticated techniques. The ability to assume alternate structures challenges the widely held assumption that folded proteins are rigid and assume only one conformation. Mouse polyomavirus was discovered as a tumor-producing infectious agent While studying the transmission of leukemia in mice in the early 1950s, Ludwig Gross unexpectedly discovered that extracts of mouse tissues caused salivary gland tumors when injected into baby mice. The “Jack-in-a-box” metaphor is particularly apt in the case of herpesviruses and certain bacteriophages, in which DNA is coiled in a spring-like fashion into the virion by the action of an energy-requiring protein spooling machine during virion assembly. What Is New in the Second Edition The first edition of this book was well-received and was adopted as a text by over 100 university-based virology courses in North America and overseas. Antiviral Chemotherapy 444 The discovery and widespread use of antiviral compounds began relatively recently 444 Antiviral drugs are useful for discoveries in basic research on viruses 445 How are antiviral drugs obtained? It is possible that they began as independently replicating cells, which at some point parasitized other cell types and became obligatory intracellular parasites. An example of such an assay is the use of a dye that measures viability and proliferation of cells following virus infection. The vector retains the cis-acting sequence elements of the virus genome required for replication (ori) and packaging (⊬), and the deleted viral genes are replaced Ori ⊬ Replication Structural proteins proteins Virus genome 1. The viroid-like domain necessary for rolling circle replication is at the right. Acquired cellular genes. The smallest spherical virions have a diameter of approximately 20 nanometers (nm) (Figure 3.1); 50,000 such virions lined up in a row would cover a length of 1 millimeter. Mitotic spindle checkpoint: a control mechanism that prevents the onset of anaphase until chromosomes are aligned properly on a bipolar spindle; controlled by mitotic arrestdefective (MAD) proteins. Gene products 1.3, 2.5, 4, and 5 act at the replication fork to synthesize progeny DNA molecules. A major factor accounting for this delay is that viruses replicate within host cells, using many elements of the host cell machinery. Only a few molecules of the enzymes reverse transcriptase, protease, and integrase are needed, but many molecules of the structural proteins encoded by Gag are required to form a single virion. It forms a stable 1:1 complex with a normal host cell protein, E. Discontinuous transcription: mechanism that copies and joins together nonadjacent sequences on an RNA or DNA template by displacement of the RNA polymerase along with its nascent RNA chain from one site to another. Retrograde transport: transport of macromolecules within nerve axons in the direction from the nerve periphery to the nucleus, or cell body. Virus particles attached to the plasma membrane can be artificially activated to penetrate prematurely simply by lowering the pH in the medium bathing the cells, showing that low pH is all that is needed as a cue for penetration. Approximately 2 109 virus particles are made and destroyed every day in an untreated infected individual. Chapter 6 Fig. The form of RNA polymerase II that participates in initiation contains few phosphate groups within its carboxy-terminal domain. Once transcription of interferon genes is activated, interferon mRNAs are transported to the cytoplasm and interferons are synthesized and secreted into the extracellular medium (Figure 33.6). Replicase particles. Eventually, some of the fitter genotypes will succeed in generating new blooms when the environmental conditions are favorable. Such degree of novelty provides a tantalizing glimpse of the potential benefits locked within, but many of the gene secrets still remain a mystery. Capsid subunits are shown in green; capsids with icosahedral symmetry are shown as circles or polygons, and capsids with helical symmetry are shown as chains or coils. Coronavirus polyproteins are processed by virusencoded proteinases at cleavage sites flanking each mature protein domain. The exact mechanism of baculovirus DNA replication is not well understood, although recent data suggest that homologous recombination may be involved. Tolerance: a state in which the immune system accepts as “self” organisms or cells of foreign origin. (a) Intact virion; (b, c) partially disrupted virions revealing an inner cone-shaped core composed of a toroidally supercoiled nucleoprotein filament. Which papillomavirus protein has DNA helicase activity? Although avocado sunblotch viroid is also believed to adopt a rod-like secondary structure, two other members of the Avsunviroidae family, peach latent mosaic viroid and chrysanthemum chlorotic mottle viroid, have complex secondary structures (Figure 31.1a), in which several stem-loops (hairpins) emerge from a central core. Figure 18.7 does not attempt to show this spatial relationship between the polymerase complex and the ends of the genome RNA. Viruses with the capacity to establish latent infections (herpesviruses), viruses with multiple serotypes and limited cross-neutralization (respiratory syncytial virus, dengue virus), and those that rapidly mutate their structural proteins (influenza A virus, human immunodeficiency virus, hepatitis C virus) present much more difficult targets for vaccines that generate predominantly antibody responses. The capsid is constructed from 60 protein subunits arranged with T 1 icosahedral symmetry. The conserved nucleotides essential for catalytic activity are boxed. 16/05/11 7:56 PM 72 CHAPTER 6 Microviruses Stage I DNA replication generates double-stranded replicative form DNA Microviruses depend on the host cell RNA polymerase to synthesize their mRNAs. Because cellular RNA polymerases require a double-stranded DNA template, microviruses must transform their single-stranded DNA genome into double-stranded DNA before viral genes can be transcribed. It is spread by direct contact between individuals and by contamination of food or water. Each of these viruses encodes a thymidine kinase that is able to phosphorylate not only thymidine and certain other nucleosides, but also a variety of nucleoside analogues, including some like acyclovir, which contain a purine instead of a pyrimidine base. The foot-and-mouth disease virus 3C proteinase also cleaves histone H3, a cellular protein associated with transcriptionally active chromatin in the cell nucleus. When scientists isolated the RNA polymerase activity from infected cells, they found to their surprise that it is a complex made of four proteins, only one of which (the replicase protein) is coded by the phage genome. Some poxviruses are transmitted among different animal species, resulting in sometimes misleading nomenclature. CI produced from PRM maintains prophage repression. Because of its tendency to aggregate, PrPSc has not been crystallized, but physical measurements show that it contains less ␣-helical content and substantial amounts of strands, and therefore has undergone a significant conformational change. Painful, isolated, pus-filled blisters covered the entire body as well as the mouth and airway, making breathing difficult and eating and drinking virtually impossible. 9.14a: Reprinted by permission from Macmillan Publishers Ltd: Fig 3 in Ortmann A.C., B. • Viruses have developed many different strategies to counteract innate and adaptive immune responses. The related virus, brome mosaic virus (BMV), has a much narrower host range, largely limited to the members of the Gramineae (grasses). These organisms apparently profit from the photosynthetic capacities of the chlorella, which they harbor, and like chlorella themselves, they become green! Surprisingly, these viruses have not been observed to replicate in chlorella living within their symbiotic hosts, but they replicate well (and can form plaques) in these algae when cultured in vitro. The decision to go lytic or lysogenic rests on whether sufficient repressor is produced to shut down transcription of the lytic genes. defective helper genomes. RNA primers are laid down on the separated DNA strands, and DNA polymerase extends these primers to begin copying the DNA. This recombinant virus can be used to vaccinate an animal, which will become immune both to the poxvirus and to rabies virus. DISTINCTIVE CHARACTERISTICS An unusually large and complex virus. Their genomes are 180- to 190-kb linear, double-stranded DNAs, and code for between 232 and 268 different proteins and four to five tRNAs. OtV-2 contains 42 unique protein coding sequences not found in OtV-1 or OtV-5. Most transforming retroviruses that have picked up cellular genes have also lost large segments of viral genes by deletion, rendering them unable to replicate on their own; they depend on coinfection with a replication-competent retrovirus. Raltegravir: an antiviral agent that acts by inhibiting HIV-1 integrase. F. Virus-like particles that contain a mini-genome, coding only for green fluorescent protein or chloramphenicol acetyltransferase, convenient markers of infected cells, have been made by modifications of the above multiplasmid procedure. The capsid is formed from 180 identical subunits, shown as 60 groups of three subunits labeled A, B, and C. These include Nedd4, a ubiquitin ligase, and Tsg101, which is part of a multiprotein complex that directs vesicles to endosomes, where they bud into the lumen and form multivesicular bodies. These defects have been traced to the proteolytic cleavage of specific subunits of the different RNA polymerases by the 3C viral proteinase. huxleyi, Phaeocystis species play important roles in CO2 and sulfur cycling, which ultimately have major implications on global climate; infection by viruses is known to affect these processes. These are systems that act rapidly, within minutes of the initial infection of a cell, and locally, within the infected cell or in nearby cells. The reovirus core is a highly structured “mRNA factory” (Figure 19.6). A schematic diagram showing functional domains of simian virus 40 large T antigen is shown in Figure 21.10. These related sequences are found not only in phages infecting the same host, but also in phages infecting distantly related bacteria, including gram-negative, gram-positive, and mycobacteria. Infections of humans by this virus are mild or without symptoms. A new bloodborne hepatitis virus, distinct from A and B, was named C, and is a member of the Flaviviridae (Chapter 12). Acheson, McGill University 17. Transcription from PAQ produces an antisense transcript of Q that lowers Q expression, delaying late gene expression. Envelope proteins are synthesized on ribosomes bound to the endoplasmic reticulum and are inserted into the cell membrane by the standard route for cellsurface integral membrane proteins. 16/05/11 8:21 PM 454 CHAPTER 36 Antiviral Chemotherapy HO HO HO HO O H N OH COOH OH H N OH O O H N NH H2N H2N O O HN O HO COOH O O Zanamivir Sialic acid Oseltamivir (a) ARG 371 TYR 406 ARG 118 ARG 292 GLU 119 GLU 277 ARG 156 ASN 294 GLU 276 ASP 151 ALA 246 ARG 152 ARG 224 TRP 178 ILE 222 (b) Figure 36.10 Rational design of neuraminidase inhibitors. The Epstein–Barr encoded-RNAs (EBERs) are two small, untranslated RNAs that are transcribed by RNA polymerase III and resemble adenovirus VA RNAs (Chapter 23). Polyomaviruses are therefore not “professional” tumor viruses. The virus was likely accidentally brought into the United States via an infected bird or mosquito, perhaps from an airplane that landed at a New York airport. Over the course of clinical latency, virus replication persists in the lymph nodes, resulting in a gradual depletion in the level of circulating CD4-positive T cells and destruction of the lymph node architecture. EBNA-1 binding within oriP also enhances expression of the latency genes. This may be related to differences in the target cells of the virus, since both men and women appear equally susceptible to the development of HPV-induced anal cancer. The development of symptoms associated with viroid 16/05/11 8:17 PM Viroids and Hepatitis Delta Virus infections could result from the activation or downregulation of targeted genes by siRNAs, but no specific targets have yet been identified. However, 50% of the planet’s oxygen is produced by marine microalgae, and algae play a pivotal role in global carbon fixation from atmospheric CO2. CH010.indd 119 16/05/11 8:01 PM 120 CHAPTER 10 Cucumber Mosaic Virus movement protein, even though they share no detectable sequence similarity. For example, HA proteins of most avian influenza A viruses bind preferentially to ␣-2,3 sialic acid, the major form of sialic acid found in the avian enteric tract. Second, polypeptide IVa2, which is encoded by an intermediate gene, binds to viral DNA at a site shortly downstream of the position where late transcription begins. It has been estimated that virtually the entire population of the United States aged 20 and below by 1961 received such contaminated vaccine at some point. It was originally called the “promiscuous transactivator” since it increases the synthesis of many viral and cellular gene products. Intrinsic Cellular Defenses Against Virus Infection 398 INTRODUCTION 399 DETECTION OF VIRUS INFECTION BY HOST CELLS 399 Host cells sense virus infection with toll-like receptors and a variety of other molecular detection systems 399 Several toll-like receptors recognize viral nucleic acids 400 13/05/11 2:11 PM Contents Other cellular proteins are also involved in recognition of viral RNAs 401 Viral double-stranded DNAs in the cytoplasm are recognized by at least three different cellular proteins 401 RESPONSE OF THE CELL TO VIRUS INFECTION 402 Virus-mediated signal transduction leads to activation of cellular transcription factors 402 Cellular recognition of virus infection leads to production of cytokines 403 Recognition of virus infection can trigger death of infected cells 404 Other antiviral signal transduction pathways involve “inflammasomes” 405 INTERFERONS 405 Virus-infected cells secrete interferons, which protect nearby cells against virus infection 405 Interferons are a first line of host defense against viruses; however, therapeutic use has been limited 406 Interferons ␣, , , and are made by different cells, bind to different receptors, and have distinct functions 406 Transcription of interferon genes is activated by virus infection or double-stranded RNA 407 Transcriptional activation occurs by binding of transcription factors to interferon gene enhancers 407 Interferon signal transduction is carried out via the Jak–Stat pathway 408 Antiviral activities induced by interferons 409 Interferons have diverse effects on the immune system 411 Viruses have developed numerous strategies to evade the interferon response 411 RNA INTERFERENCE 412 Small interfering RNAs are involved in combating virus infections in plants and invertebrates 412 MicroRNAs are used to control gene expression in vertebrates 413 34. CH036.indd 447 447 Amantadine blocks ion channels and inhibits uncoating of influenza virions Amantadine (Figure 36.3) was discovered by random screening in the 1960s. Capsid, shown as an icosahedron, is constructed from core protein. The innate (“alternative”) pathway involves a nonspecific interaction of the complement system with the virion surface, while the adaptive (“classical”) pathway involves a reaction of the complement system with antibody-coated virus particles. Aluminum salts preferentially stimulate type 2 T helper lymphocytes (Th-2 cells), which secrete cytokines that favor the production of antibodies (see Chapter 34). Transcription of viral DNA gives rise to several mRNAs and a pregenome RNA In contrast to retroviruses, hepadnavirus DNA is not immediately integrated into the host cell genome, but remains in a free, circular form. Therefore, as RNA viruses replicate and spread from cell to cell or animal to animal, variants in antigenic domains of the envelope glycoproteins appear. Attachment 2. Above are shown regions that bind to cellular proteins involved in cell signaling or DNA replication: Pol ␣ DNA polymerase ␣/RNA primase; Rb retinoblastoma protein; TBP TATA-binding protein. PRASINOVIRUSES Small host, big virus We now will

discuss viruses that infect the world’s smallest free-living eukaryote: the green alga Ostreococcus (Figure 27.10). Viruses within the Bromovirus and Cucumovirus genera have similar packaging strategies. Figure 12.1 Dengue virus type 2 particles visualized by cryoelectron microscopy. During packaging, B proteins are extruded from the procapsid, probably through the same pores. What is the prion hypothesis, and how does it explain prion diseases? Scaffolding proteins may increase rate of assembly and help small phages to compete against larger phages The assembly pathways of all viruses include a rate-limiting step. Ovocylindrical occlusion bodies 0.3 0.5 μm and containing only one virion are made by granuloviruses. The presence of the target RNA is therefore signaled by the appearance of a band of MDV-1 RNA in the gel. During an infection, immediate early transcription from PL and PR produces N and Cro. Dengue virus and Japanese encephalitis virus, also transmitted by mosquitoes, cause serious human disease throughout tropical regions of the world. The integrase binds to the two ends of linear viral DNA and brings them together and in close proximity to cellular DNA. Occlusion-derived virions: virions embedded in occlusion bodies, formed in the nucleus. This protein plays a double role in entry: it binds to cell surface receptors and directs fusion of the viral envelope with host cell membranes. Inactivated viruses: retain immunogenicity but are no longer infective. 90 A quick review 90 Breaking and entering: the insertion of prophage DNA into the bacterial chromosome 90 Excision of DNA from the bacterial chromosome 92 Int synthesis is controlled by retroregulation 93 DNA replication is directed by O and P, but carried out by host cell proteins 93 Assembly of heads involves chaperones and scaffolding proteins 93 DNA is inserted into preformed proheads by an ATP-dependent mechanism 94 Host cell lysis 94 9. The O protein binds to multiple sites in the replication origin, ori (actually located within the O gene; Figure 8.2), forming a nucleoprotein structure, the “O-some”. Table 11.1 shows the current genera in the Picornaviridae family and some representative members in each genus. This small protein contains a transmembrane domain at its C-terminus followed by a signal sequence that is required to insert the E protein in the correct orientation in the membrane. The molecular mechanisms of symptom modification caused by satellite RNAs are not yet understood (for more discussion of this topic, see Chapter 31). 9.8a,b: Modified from Fig 1 b and c in: Häring M., X. Are incapable of replication and therefore do not cause disease if properly made. Large T antigen interacts directly with a number of cellular proteins involved in transcription. CH023.indd 278 Rb and its family members p107 and p130 contain a pocket domain that binds to both the E2F and E1A proteins (Figure 23.6). The infective particle can then “reproduce” by breakage to smaller, stable particles (d). Unlike retroviruses (Chapter 28), polyomaviruses do not normally integrate their DNA into the host cell chromosome during replication; this rare event is carried out by cellular enzymes that pick up degraded fragments of viral DNA and insert them into the host genome. Epidemics were documented during the Saracen invasions of the eighth century and continued into the Middle Ages. Using this approach, it has been possible to develop lentivirus vectors that are able to integrate a gene expression cassette at a specific gene locus. The distinct roles of the CH020.indd 240 minor capsid proteins are not well understood, although their absence can reduce infectivity of virions. Calyx: a carbohydrate-rich layer on the surface of occlusion bodies, sometimes called the outer or occlusion body envelope. E Host cell lysis. Necrogenic: refers to a pathogen that causes necrosis, or death. In addition, at late stages of infection transcription of cellular genes is also grossly reduced, possibly as a result of changes in the chromatin structure of cellular DNA. There are at least 20 CH032.indd 393 393 known mutations in the PrPC gene that are associated with prion disease, presumably because of a high rate of spontaneous PrPSc formation. RNA interference could determine viroid pathogenicity and cross-protection RNA interference may also be implicated in viroid pathogenesis. Left: formation of a simple enveloped virus particle by budding at the plasma membrane (above) or at an internal cellular membrane (below), followed by release via exocytosis. Which of these proteins are functional cellular receptors remains to be established. Chimeric vaccines are based on live organisms that are either nonpathogenic in humans (e.g., strains of the bacterium Lactobacillus, and a variety of attenuated viruses) or known and “trustworthy” (e.g., attenuated Salmonella Ty21a, and vaccine-strain measles virus). With population growth, the great trading cities of the Middle Ages became large enough to maintain foci of these diseases year round. Subunits of host translation initiation factor eIF3 are associated with the replicase complex purified from infected cells. Bats are probable reservoir hosts. Laminins are a family of proteins, each containing three polypeptides of about 820 kD, that are a component of the basal lamina, which lies underneath epithelial cells. 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(c) and (d) Diagrams showing a polyhedron (nucleopolyhedrovirus) and a granule (granulovirus) with occluded virions. Viruses have a variety of distinct morphologies, genome and particle sizes, and mechanisms of replication. Each particle is viewed along a threefold axis. DNA J and its eukaryotic analogues interact with a cellular chaperone called Hsp70 (known as DNA K in E. Lipid envelope is derived from the plasma membrane (vertebrate cells) or internal membranes (insect cells). 24.11, 24.12: Courtesy of Richard Longnecker. It is clear from the recorded history of filovirus outbreaks that all of them have so far been self-limited and that the total number of human infections hitherto recorded does not exceed 3000 cases. al., “An icosahedral algal virus has a complex unique vertex decorated by a spike,” Proc Natl Acad Sci USA 106 (2009):11085-9, with permission from PNAS; Fig. Primers are formed and elongated on both DNA strands, and as a result, bidirectional DNA synthesis is initiated. Supercoiled: double-stranded (usually circular) DNA molecules that are overwound and as a result are twisted into a coiled structure. Retroviruses 342 CHLOROVIRUSES 327 Known chloroviruses replicate in Chlorella isolated from symbiotic hosts 327 The linear genomes of chloroviruses contain hundreds of genes, and each virus species encodes some unique proteins 327 Chlorovirus capsids are constructed from many capsomers and have a unique spike 328 Virus entry begins by binding to and degradation of the host cell wall 329 Transcription of viral genes is temporally controlled and probably occurs in the cell nucleus 329 Progeny virions are assembled in the cytoplasm 329 Small and efficient proteins 330 A virus family with a penchant for sugar metabolism: hyaluronan and chitin 330 COCCOLITHOVIRUSES 331 Viruses that control the weather 331 Many genes looking for a function 332 Expression of coccolithovirus genes is temporally regulated 332 Cheshire Cat dynamics: sex to avoid virus infection 333 Survival of the fattest: the giant coccolithovirus genome encodes sphingolipid biosynthesis 333 PRASINOVIRUSES 334 Small host, big virus 334 Viral genomes contain multiple genes for capsid proteins It works both ways 334 Not much room for maneuver 335 TOC.indd xv xv PRYMNESIOVIRUSES AND RAPHIDOVIRUSES 335 The lesser-known Phycodnaviridae 335 MIMIVIRUS 336 The world’s largest known virus 336 Mimivirus is unquestionably a virus 336 Why such a large genome? GENOME Linear single-strnded DNA, 5 kb. Smallpox strains varied in mortality between 1–40%, but the average case fatality rate in endemic countries was approximately 25%. Because picornavirus RNAs are not capped, a novel mechanism is used to direct ribosomes to the initiator AUG codon. 16/05/11 8:06 PM 208 CHAPTER 17 Bunyaviruses Parent 1 Parent 2 L L đ M T7 Terminator T7 Promoter Ribozyme M S L S M S Coinfection T7 RNA polymerase L Positive-sense M RNAs S Progeny Translation Parental viruses L L M M S S Transcription Replication Bunyavirus proteins: L, NSm, Gn Gc, N, NSs L Negative-sense M nucleocapsids S Reassortant viruses L L L M M M S S S L L L M M M S S S Figure 17.5 Reassortment of bunyavirus genome segments. Although the precise mechanism of membrane penetration is not well understood, ISVPs are thought to breach the endosomal membrane by a process that requires 1-mediated formation of small pores. Virus Classification: The World of Viruses 31 Nicholas H. This gene order is highly conserved, both in paramyxoviruses and in rhabdoviruses (in which the G gene replaces the F and HN genes). Because members of a virus family may have evolved separately over considerable periods of time in distinct host organisms, they often share only limited nucleotide or amino acid sequence homology in a small number of genes. This activity involves binding of GTP and its hydrolysis to GDP. Hyaluronan: an anionic, non-sulfated, usually large molecular weight glycosaminoglycan formed in the plasma membrane. New categories of antiviral vaccines Several new types of vaccine (Figure 35.2) targeting various viruses are newly licensed or under late-stage development. Prymnesiovirus CbV-PW1 Chrysochromulina brevifilum Single-celled marine algae. This could be due to a requirement for replication in specific vascular-related cells for movement to occur, or could imply a more direct role for the 1a protein in virus movement. Proteinase K treatment of the PrPSc species corresponding to HY and DY gives rise to digested proteins with distinct amino termini, although the parent PrPC proteins have the same amino acid sequence. These are caused by toxicity resulting from the aggregation of specific misfolded proteins into highly ordered structures made up of fibers known as amyloid. The looping that accompanies octamerization also brings OR3 and OL3 near to each other, enabling cooperative interactions between CI dimers at OR3 and OL3 (Figure 8.5c), which leads to repression of PRM. Cattle: foot-and-mouth disease. 05/05/11 1:08 PM 367 Hepadnaviruses Eight major strains with different genome sequence variations (genotypes) and four major antigenic strains (serotypes) of hepatitis B virus are found worldwide. 6; Fig. This success is even more impressive considering that the treated individuals were affected by recurrent cancer of the head and neck, or by gastrointestinal cancer that had spread to the liver, two types of cancer with particularly poor prognosis. A schematic diagram showing a simplified virus replication cycle in seven steps, as described in text. Gene segments can be reassorted during coinfection of cells by different strains. Thus gene D8L is the eighth gene from the left end of the Hind III D DNA fragment, and is transcribed in a leftward direction. Endosymbiotic: relationship in which a unicellular organism is taken up into the cells of another organism and continues to reproduce, retaining at least minimal genetic information, eventually becoming dependent on the engulfing organism. This releases the RNA from the virion, and it becomes sensitive to RNAse degradation. Binding by cellular transcription factors called nuclear factor I and nuclear factor III (also known as Oct I) enhances the efficiency of initiation of DNA replication, perhaps by increasing the affinity of the preterminal protein/DNA polymerase complex for the replication origin. • Middle T antigen binds to protein kinases such as c-Src and activates them, phosphorylating middle T antigen itself, and leading to activation of cellular signaling proteins such as SHC, PLC, and PI3K. This molecule is an exact complementary copy of the genome, and it does not contain the 5’ primer sequences used to make mRNAs. Therefore the L protein initiates genome replication at the first nucleotide at the 3’ end of the genome. Despite considerable effort, therapeutic and/or preventive vaccines remain a distant hope, as the most straightforward strategies have yielded very limited success. Among the largest viruses currently known. While there are currently no recombinant poxvirus vaccines licensed for use in humans, some are being evaluated in clinical trials, in particular for immunization against HIV. The core is covered with a beehive-like structure, the surface of which appears to be either helical or stacked. These neuraminidase inhibitors provide one of the best examples of rational drug design starting from the structure of a protein and a natural ligand. As the particle is assembled, the core is extruded outward, resulting in a C-shaped bulge on the surface of the plasma membrane, hence the designation “C-type”. At present, it is unclear which of the multiple activities of Nef are directly involved in pathogenesis in infected animals. Negative-strand genome RNA must be encapsidated to be transcribed and replicated, and therefore synthesis of the genome RNA alone does not lead to virus production. coli as a bacterial protein required for replication of bacteriophage lambda DNA (Chapter 8). Most of these “early” genes are preceded by a repeated GTTCCC(T/C)AA sequence that may be part of a promoter for RNA polymerase. In mRNA transcribed from an edited genome, the reading frame of the protein that is made normally from the unedited mRNA would no longer be accessible by ribosomes. One of the small RNA strands is used as a “guide” that hybridizes to complementary sequences within the viral genome RNA or messenger RNAs. A ribonuclease within the silencing complex then cleaves the hybridized viral RNA molecule. HDAC Rb HDAC represses S phase genes ycle lc Cel Rb E2F P P P Cdk X E2F activates S phase and E2 genes E1A Ade n infec ovirus tion Rb E1A Figure 23.6 S-phase gene activation by E1A proteins. In experimental systems, significant genetic bottlenecks are encountered by viruses during systemic spread in plants, and during transmission by aphids. These virion figures and many others can be found at Viralzone (. and S. CH011.indd 126 16/05/11 8:02 PM Picornaviruses Because poliovirus had been so intensively studied for vaccine development, it and several related picornaviruses became model systems during the 1960s for understanding how RNA viruses replicate in eukaryotic cells. NS2B is a cofactor for the proteinase activity found within NS3. Flaviviruses and togaviruses have lipid envelopes, and most are transmitted between host animals by mosquitoes or ticks. Contains important transcription and replication control sequences. However, there is no 3'-hydroxyl group on acyclovir on which to add the next deoxynucleoside triphosphate. LMP-2B is transcribed from a promoter just upstream of the LMP2A initiation site and contains an additional exon near its 5' end. One hypothesis is that a limiting host factor is required for viroid replication, transport, or accumulation. Cytokine signaling. Where in the cell do polyomaviruses assemble progeny virions? This has obviously limited work on these viruses. Finally, the E1A amino terminus and part of CR1 engage in a complex with a distinct set of cellular proteins, the histone acetyl transferases. (b) Binding of pleconaril, a capsid-binding drug, to the hydrophobic pocket displaces the bound lipid and interferes with attachment of virion to receptor and/or entry of viral genome into the cytoplasm. Orange lines: newly synthesized DNA strands. Polyomaviruses 247 Nicholas H. Linear monomers resulting from hammerhead-mediated self-cleavage have been shown to self-ligate in vitro, producing 2'-5' phosphodiester bonds. What is the “single-entry model” of transcription of viral genomes, and how does it control the levels of individual viral mRNAs? The membrane (M) glycoprotein is the most abundant structural protein in the coronavirus virion. During the first hour or two after adding virus, most of the infecting virions are taken up into the cells or are subsequently washed away by changing the medium. pppACV acyclovir triphosphate; pppdG deoxyguanosine triphosphate; pppdC deoxycytidine triphosphate; dC deoxycytidine in the template strand; dG deoxyguanosine in the template strand. This complex is disrupted either during the cell cycle, by phosphorylation of Rb by cyclin-dependent kinase (Cdk; above), or during infection, by binding of adenovirus E1A proteins (below), allowing E2F to transcriptionally activate cellular and viral genes. Nonfatal cases show fever for several days. Cryoelectron microscopy data indicate that E2 has a shape similar to that of E1, but E2 forms the external part of the petals that project outward. Lipid rafts are believed to be targets for binding of certain viruses during virus entry, as well as for assembly of certain enveloped viruses. Monoclonal antibodies: antibodies produced by cloned antibody-producing cells. Following virus uptake, genome replication begins by 2 hours post infection, and virions are assembled in the cytoplasm from 6 hours until 20 hours post infection, after which cellular lysis occurs. In most cases, the natural vertebrate hosts of these viruses are wild mammals and birds; humans (and horses) are usually dead-end hosts as far as the virus is concerned, because they do not transmit disease to other individuals. Surprisingly, transcription by T7 RNA polymerase was found to be necessary for the initiation of T7 DNA replication. DNA genome replicates in the cytoplasm using exclusively virus-coded enzymes. This virus infects liver cells and can cause a lifelong infection, which can give rise to liver cancer or degeneration; like HIV, it is sexually transmitted. Below the baseplate, the device that punctures the cells is colored red. At this sequence, which contains a stretch of 6 A’s followed by 3 G’s, the RNA polymerase occasionally “stutters”, and adds one or more extra G residues within the stretch of 3 G’s (Figure 15.9). worldwide, smallpox has now been eradicated from the planet in a magnificent and unprecedented international collaboration of science and society. The 2009 H1N1 virus presumably arose as a recombinant between viruses of Eurasian and North American swine. Therefore, the infectious cycle is abortive and does not result in the production of progeny virus or kill the infected cell. In tobacco mosaic virus, as well as in the nucleocapsids of negative-strand RNA viruses like measles or influenza, the RNA winds along a groove that follows the helical path of the protein subunits. RNA interference is initiated by the double-stranded RNA-specific ribonuclease III enzyme called Dicer (Figure 33.11). Bacteriophage Lambda 85 Michael Feiss, University of Iowa 9. Therefore the translating ribosome occasionally shifts the reading frame back one nucleotide, and resumes translation of the sequence (Figure 28.7). CH027.indd 334 In common with the genetic promiscuity of their relatives, there is evidence of horizontal gene transfers between host and virus in all three sequenced OtV genomes. Instead, initiation of translation at the lysis gene depends on termination of translation at the end of the coat gene, 50 nt downstream (Figure 5.5). Individuals who lack the ability to make soluble antibodies via the humoral immune response recover from most viral infections in spite of their defect, showing that antibody formation is not required for recovery. CH008.indd 94 At the end of lytic growth, the host cell is lysed to release progeny phages. Presently archaea are recognized as a major constituent of the Earth’s biosphere, playing an important role in both the carbon cycle and the nitrogen cycle. DISTINCTIVE CHARACTERISTICS Can replicate in both vertebrate and invertebrate hosts. Table 37.2 Properties of selected virus vectors Transduced cells Vector Transgene capacity Dividing Nondividing First-generation adenovirus 7 kb Yes Yes No Strong Third-generation adenovirus 35 kb Yes Yes No Weak Oncoretrovirus 8 kb Yes No Yes Weak Lentivirus 8 kb Yes Yes Weak Adeno-associated virus 4.5 kb Yes Yes Integrates transgene 1 Yes /No Antigenicity Weak 1 Efficient integration requires the presence of the Rep protein. This strongly backed up the chemical and enzymatic data published eight years earlier by Oswald Avery, Maclyn McCarty, and Colin MacLeod showing that a bacterial gene was made of DNA. 6 and 7 in Schlesinger, S. Particles still associated with the surface of the Extracellular space TARGET CELL Fusion Endocytosis Figure 4.1 Exit and entry of enveloped viruses occurs by vesicle transport. Using vaccinia virus recombinants it has been shown that the targeting of bunyavirus glycoproteins to the Golgi complex is a property of the glycoproteins alone, and does not require other viral proteins or virus assembly. • Paramyxoviruses are spherical or elongated enveloped viruses with a negative-strand RNA genome, and share many other characteristics with rhabdoviruses and filoviruses. Occupancy of the OR operators by CI is summarized in Table 8.1. light or some other agent. An endonuclease activity that specifically cleaves capped mRNAs was detected in vitro, and this activity has been mapped to the L protein by expression studies using a recombinant vaccinia virus. Pandemic: increase in infectious disease incidence over time and a large geographic range spanning many countries and continents (e.g., the human influenza pandemic that spread worldwide in 1918–1920). They are divided into four major groups: viroids, satellite RNAs, viroid-like plant satellite RNAs, and hepatitis delta virus. There are actually four subclasses of IgG. Similar stem-loop structures are present in other flavivirus genomes, and biochemical and genetic experiments suggest a role for this region in viral RNA replication. The preC and pregenome promoters are closely spaced, only 10 to 15 nucleotides from each other (see insert, Figure 30.3). By the end of October of that year, Werner Slenczka and Rudolf Siegert of the Institute of Hygiene had identified the causative agent as a new virus that soon received the name of the town where 188 CH016.indd 188 16/05/11 8:05 PM Filoviruses it was discovered. In addition, some cell types cultivated in vitro can be transformed by transfection with viral DNA. Some genes of endogenous retroviruses have even been adapted by the host organism for specialized functions. In 1957, Burkitt saw a young boy with swelling of both sides of his jaws that proved to be a lymphoma. The web of interactions between CII, CIII, and the proteases is not completely defined. The reduction in pH results in a rearrangement of the E dimer into the fusion-active trimeric state. Primary infection of B lymphocytes results in latency III; the six EBNAs, three LMPs, and several small RNAs coded by the virus genome are expressed in these cells. Polyomavirus genes are organized in two divergent transcription units As a result of their small genome size, most polyomaviruses code for only 6 to 8 proteins. Alignment of E1A proteins from different adenovirus serotypes reveals three regions with highly conserved amino acid sequences, termed CR1 (for “conserved region 1”), CR2, and CR3 (Figure 23.5); the 243R protein is missing CR3. Bacteria: one of the threee domains of life, distinct from Archaea and Eukarya. Protein 3a binds to viral RNAs and forms a ribonucleoprotein complex that transports viral RNAs through plasmodesmata, possibly by interacting with 119 unidentified host proteins. HIV-1 is characteristic of a subfamily of retroviruses named the lentiviruses (Table 29.1), so named because of the slow progression of diseases caused by lentiviruses. Divergent transcription: transcription of adjacent genes that progresses in opposite and diverging directions from a central point. Nucleocapsid (N) Structural protein, forms helical nucleocapsid with genome RNA. Clathrin-coated pits: invaginations of the plasma membrane involved in endocytosis, and covered on their cytosolic surface by a clathrin protein coat. CH004.indd 46 16/05/11 7:52 PM Virus Entry infected cell may reach neighboring cells through contacts established via filopodia and other actin-based extensions. CONSTRUCTION OF VECTORS Deletion of some or all virus genes. Papillomaviruses share many of these properties with the polyomavirus family (Chapter 21), with which they were previously grouped. Coding regions for viral proteins, with their molecular weights and cleavage sites, are shown below mRNAs. CH017.indd 203 Bunyavirus protein coding strategies: negative-strand and ambisense RNAs proteins on both DNA strands, in different regions of their genomes. A number of central nervous system disorders in humans were shown to have similar symptoms and progression, and to share the spongiform pathology of the brain; these include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome, and fatal familial insomnia (Chapter 32.1). E1 is therefore the papillomavirus homologue of the polyomavirus large T antigens (Chapter 21). Cucumber mosaic virus infects monocots, dicots, herbaceous plants, shrubs, and trees, including more than 1200 species from 85 plant families. Liamas-Saiz, Feng Wang, Peter Tattersall, and Michael G. The cytoplasmic receptors RIG-I and MDA-5 detect single-standed or double-stranded viral RNA; DAI, NLRP3, and AIM2 bind to cytoplasmic, double-stranded DNA. What are these cellular receptors? Some possess catalytic activity (hammerhead self-cleaving structure). Experiments have shown that infection of mosquitoes with such a recombinant vector will effectively inhibit replication of yellow fever virus or dengue virus in the mosquito. The previous assumption that src was an oncogene of viral origin was proved incorrect. The mimivirus was one of the first viruses to blur these boundaries. 2 CH001.indd 2 16/05/11 7:46 PM The Nature of Viruses 4. Seven categories of viruses are distinguished based on the nature of the viral genome and the pathway leading to synthesis of early messenger RNAs. Numbering is arbitrary. RNA evolution Viroids nucleotides oligonucleotides self-replicating RNAs 2. Viroids do not code for proteins and are not packaged into virions, but are probably transferred as naked RNAs from one host to another via insects or mechanical abrasion. Interferons play a major role in the “rapid reaction” first line of defense against viral infections. Nucleotides 1-41 (A) and 85-125 (A') are base-paired, as are nucleotides 42-50/54-62 (B) and nucleotides 64-72/76-84 (C). The sequence repeat at the left end of UL is designated ab, and the internal inverted repeat is designated ba. The classic definition of a virus is an obligate intracellular parasite that lacks the core protein synthesizing apparatus (ribosomes and associated factors) and does not have systems to supply chemical energy or metabolic needs. Qutemol: . The DNA may interact with the protein Z of the tail, because the tails of Z-less virions do not have inserted DNA. 34.3: From STARR, Biology: Concepts and Applications, 2E. As infection proceeds, these bodies grow and become highly structured. The integrated DNA, flanked by attL and attR, is shown in orange at bottom. Viral DNA replication occurs in fully differentiated keratinocytes, which do not normally divide, and therefore DNA synthesis does not normally occur in these cells. How does this reverse transport occur? Viral proteins have evolved to modulate many aspects of cell growth, apoptosis, and the immune system. Genome replication occurs via reverse transcription of pregenome RNA The generation of the hepatitis B virus DNA genome from pregenome RNA is formally similar to the synthesis of retrovirus proviral DNA from genome RNA (Chapter 28). The 100 known adenoviruses include 50 human virus serotypes, distinguished by their sensitivity to neutralizing antibodies. NS3. Since all of these additional reading frames are conserved among various clinical isolates of the virus, they must play important roles in the virus life cycle. Macropinocytosis: uptake at the cell surface of relatively large amounts of extracellular fluid and particles within vacuoles 0.5 to 5 μm in diameter. stem-loops are shown at both ends of pregenome RNA (pg RNA). Vesicular stomatitis virus can replicate in cells cultured in vitro from many different species, and it has been extensively studied. In terms of mass, this many phages would weigh about 100 million tons, or the equivalent of 1 million blue whales (the largest animal on Earth). This can be a major cause of the high ratio of physical particles to infectious particles of some viruses. The basic life cycle of rhabdoviruses is very similar to that of paramyxoviruses, justifying their inclusion together in this chapter. RNA-dependent DNA polymerase: an enzyme that synthesizes DNA using an RNA template; reverse transcriptase. This strategy has been termed the “moving template” model of transcription, because it postulates that the template RNA moves through the fixed RNA polymerase. Many satellite RNAs attenuate symptoms of virus infection. This domain can therefore be considered as a candidate marker of oncogenic potential. Vp54 folds into two jelly-roll -barrels (Figure 27.4a), structures commonly found in capsid proteins of many other viruses, including bacterial and animal viruses (e.g., adenovirus). Formation of prions in fungi results in phenotypic changes that vary with the aggregating protein. Some proteins were first identified as virion proteins (e.g., VP5) or glycoproteins (e.g., gp), or were designated as infected cell proteins found in cell extracts (e.g., ICP25). Briedis Prions From proteinaceous infectious particles PROPERTIES Small, filterable infectious particles that contain protein but no detectable nucleic acid. Recombinant retroviruses are produced by coinfecting the cells with a second adenovirus carrying the retrovirus env, gag, and pol genes. These siRNA molecules are then bound by a collection of proteins known as the RNA-induced silencing complex (RISC). Initiation of plus-strand DNA synthesis 1 1 1' 2' Cap 1' 7. DISTINCTIVE CHARACTERISTICS Two identical copies of the genome per virion. 16/05/11 7:52 PM 44 CHAPTER 3 Virus Classification: The World of Viruses This resembles the breakdown of the nuclear membrane during mitosis. Isotype: in immunology, the type of constant region in the heavy chain of an immunoglobulin. Six more cases, including two people VIRUSES AND HOSTS Two genera: Marburgvirus and Ebolavirus. This is a rarely used mechanism of initiation of protein synthesis. The H and N genes code for the envelope glycoproteins hemagglutinin and neuraminidase, which are the most immunogenic influenza virus proteins. Because of the hairpin, this 3 end is hydrogen-bonded to a complementary sequence further along on the same DNA strand (Figure 20.3), allowing the same DNA molecule to serve both as primer and template for DNA replication. Even though 16/05/11 8:19 PM 414 CHAPTER 33 Intrinsic Cellular Defenses Against Virus Infection mammals encode the RNAi machinery, to date siRNAs complementary to viral RNAs have not been found within virus-infected cells. Chemokine PVR receptors (CC and CXC) CAR Integrin ␣v3/5 Cationic Aminoamino acid peptidase N transporter Figure 4.2 Cell surface glycoproteins used as virus receptors. Phylogenetic studies based on comparison of ribosomal RNA sequences— molecular phylogeny, pioneered by Carl Woese starting in the 1970s—radically changed this view. However, the RNA phages are much easier to detect by a rapid plaque assay on male E. This emphasizes an important difference in replication strategy between positive-strand and negativestrand RNA viruses. Chimera: an organism or genome resulting from the artificial combination (usually by genetic engineering) of genomes of two or more distinct species. However, this is an inefficient process, and the contribution of integrated AAV DNA to the production of the transgene protein is unlikely to be important. 05/05/11 2:45 PM Glossary Cirrhosis: severe damage to the liver, involving loss of normal structure, caused by environmental factors or infection by pathogens. This ultimately led Stanley Table 32.1 Species affected by prion diseases Species Disease Abbreviation Sheep Scrapie Elk, mule deer Chronic wasting disease CWD Mink Transmissible mink encephalopathy TME Cattle Bovine spongiform encephalopathy BSE Cats Feline spongiform encephalopathy (probable cross-species spread from BSE) FSE Zoo ruminants Transmissible spongiform encephalopathy of zoo ruminants TSE Humans Creutzfeldt-Jakob disease CJD (New) variant Creutzfeldt–Jakob disease vCJD Gerstmann-Sträussler-Scheinker syndrome GSS Fatal familial insomnia FFI Kuru CH032.indd 389 16/05/11 8:17 PM 390 CHAPTER 32 Prions Signal sequence Octapeptide repeats Alpha-helices s-s A N 1 23 58 89 142 B CHO CHO GPI C C 231 254 Figure 32.3 Domains and modifications of the prion protein. This has similarities to the mechanism by which adenoviruses initiate replication of their linear, double-stranded DNA genomes (see Chapter 23), but in the case of hepadnaviruses the polymerase plays the roles of both the adenovirus terminal protein and the DNA polymerase. The packaged particle is called the provirion because it still contains the external scaffolding protein D. The virion (or the viral genome) enters the cell Once they have located an appropriate cell, bacteriophages and plant viruses are presented with the problem of passing through a rigid cell wall, as well as the outer cell membrane(s). Both prM and E have two C-terminal domains that span the lipid bilayer. The nucleocapsids enclosed in this way are sometimes flexible, helical rods and sometimes more rigid, roughly spherical, closed shells. Although the genes can be transcribed at a low level by basal cell transcription factors, expression is not optimal. In countries that can achieve and sustain high rates of vaccine coverage, the inactivated vaccine can provide excellent protection without any risk of disease from revertant viruses. Thermophile: an organism that grows at temperatures above 45 °C but below 80 °C. Thus massive conversion of PrPC to PrPSc would require an initial nucleation process. These movement proteins also accompany the viral genomes as they pass from cell to cell. These responses are carried out by proteins expressed from the interferon-stimulated genes (ISGs). Each isomer accounts for one-quarter of the DNA packaged into virions. Virus genomes code for proteins that enable their replication and transmission, and thus viruses are self-propagating. Viroceptors can bind to host cytokines and inhibit host responses to infection. Infection with AAV vectors does not trigger a strong cellular immune response, which allows for long-term transgene expression. They are stable and relatively resistant to ribonucleases. Denis Burkitt was an Irish physician pursuing a medical career in Kampala, Uganda. Genomes of fuselloviruses are positively supercoiled Sulfolobus spindle-shaped virus 1 (SSV-1) is perhaps the best-studied archaeal virus, having been isolated by the laboratory of Wolfram Zillig in the mid-1980s and intensively studied by that laboratory. Of these proteins, only Tat and Rev are essential for virus production in many cell lines. T lymphocytes respond to peptides on the surface of antigen-presenting cells Antigen-presenting cells such as macrophages, dendritic cells, or B cells take up antigens and degrade them to short (12–18 amino acid) peptides, which are then presented on the cell surface complexed with MHC class II protein (Figure 34.5). Every patient required support from the others in order to stand without the aid of the sticks they had been asked to discard for the photograph. Is the prion hypothesis correct? For an invading virus, the ability to control 05/05/11 11:24 AM 334 CHAPTER 27 Viruses of Algae and Mimivirus when your host will die and to ensure your own survival is an effective propagation strategy. Caspases linked to both the intrinsic (mitochondrial) and extrinsic (death receptor) pathways of apoptosis induction are activated by reovirus. The M and E proteins play important roles in the formation of virus envelopes by budding. Type I integral membrane protein Type II intregal membrane protein Virion X-ray diffraction • The smallest capsids with icosahedral symmetry are constructed from 60 identical subunits. Three genome segments: S, 1–3 kb; M, 3–5 kb; L, 6–12 kb. This is shown in Figure 2.11b for influenza virus. 1. Such transformed cells invariably contain most or all of the early region of the viral genome, integrated into the cellular genome at apparently random positions. • E1A proteins indirectly induce apoptosis by increasing levels of cellular p53 protein. Greve, Q. Electron micrographs show that only a very small section of the sacculus (the cell wall network) has dissolved, usually at the equatorial growth zone. Even transient losses of public confidence in vaccination programs can have devastating effects. It appears that only the 5⬘ stem-loop is functional in packaging, and only in the pregenome RNA. Surface proteins are inserted into membranes of the endoplasmic reticulum and Golgi apparatus. The E3 region is not required for virus production in cultured cells in vitro and its deletion increases the space available (total 7 kbp) for insertion of exogenous DNA. Minute virus of mice NS2 protein is needed for efficient viral replication only in certain cell types, but the reasons and mechanism are not clear. E1 deleted adenoviruses can replicate only in cell lines such as human embryonic kidney 293 cells, which express integrated copies of the E1 region and therefore complement these functions. • Some capsids are assembled around the nucleic acid genome; others are pre-assembled and the genome is stuffed into the capsid. Franklin D. Because they do not express viral proteins, these cells cannot be readily distinguished from uninfected cells by the immune system. Like NF-B, IRF-3 also is required for reovirus to induce maximal levels of apoptosis. By contrast, the major capsid protein of adeno-associated virus is VP3; the larger VP2 is made by occasional initiation of protein synthesis at an ACG initiation codon upstream of the VP3 AUG start codon but in the same reading frame. The progeny DNAs can be used as a template for further transcription, or can be replicated. The genetic makeup of an individual’s immune system can be an important determinant in the development and progression of AIDS. The envelope contains the external surface protein (SU), bound by noncovalent interactions to the transmembrane protein (TM), which traverses the lipid bilayer. Studies with rat hippocampus slice cultures show that neurons are selectively infected by Sindbis and Semliki Forest virus replicons. Other cellular proteins are also involved in recognition of viral RNAs Although toll-like receptors were the first pattern recognition receptors identified, alternative mechanisms for sensing virus infection were subsequently discovered. This helps with decisions on directing the patient toward further tests and treatments, and more stringent subsequent monitoring. It should be possible to read each chapter in 30–60 minutes, including examination of figures and tables. Once sufficient genome RNAs are made, picornavirus virions can be assembled. This form could be involved in replication of the viral genome. Interestingly, gp42 is underrepresented in virions made from B lymphocytes, probably because it binds to HLA class II protein on the surface of virus-producing cells and therefore is less likely to be incorporated into the viral envelope. Experiments confirmed that GP remained uncleaved at the furin cleavage site in this mutant virus, but the virus was able to infect and replicate in monkey cells in culture and in infected cynomolgus macaques, leading to a lethal outcome indistinguishable from infection with wild-type Ebola virus. La Crosse virus is a significant cause of childhood encephalitis in the Midwestern United States, averaging about 100 cases per year. For some viruses (e.g., HIV), maturation takes place after release of virions from the cell. 1' 1 6. GENES AND PROTEINS Four virus-coded proteins: • • • • Coat protein Maturation protein Lysis protein RNA replicase The discovery of RNA phages stimulated research into messenger RNA function and RNA replication The RNA coliphages were discovered by Tim Loeb and Norton Zinder in 1961 as the result of a search for phages whose replication depends on E. CH023.indd 284 16/05/11 8:11 PM C H A P T E R 2 4 Herpesviruses Bernard Roizman Gabriella Campadelli-Fiume Richard Longnecker Herpesviridae From Greek herpein (creep), referring to spreading of lesions VIRION Enveloped icosahedral capsid (T 16). Distinct entry and transport pathways of seven viruses are shown schematically. ZFNs are custom-made artificial nucleases that bind to a specific nucleotide sequence. There does not appear to be a universal termination signal for bunyavirus transcription; however, two sequences that have been suggested as termination sites are 3'-G/CUUUUU-5' and 3'-GGUGGGGGGUGGGG-5'. Second, these mRNAs have heterogeneous 3' ends, resulting from a transcription termination mechanism that is fundamentally different from the process used during early transcription. Another approach is to have Sindbis virus express a protein toxic to the insect, serving essentially as an insecticide. DED1 also regulates the synthesis of brome mosaic virus protein 1a by interacting with sequences near the 5' end of viral RNA 1. Developments in the past 20 years have led to more than 1500 clinical trials of gene therapy, most using virus vectors. One of the simplest methods is to mix a virus suspension with an electron-dense stain, usually phosphotungstate or uranyl acetate, and to spread the mixture on a grid for 16/05/11 7:46 PM The Virus Replication Cycle: An Overview Virus Buffer RBC Figure 1.4 Hemagglutination assay. In contrast, members of the Allolevivirus genus (supergroup B) have a longer genome of approximately 4200 nucleotides. Order: in virology, a rarely used taxonomic grouping of virus families whose members share distinctive characteristics of replication strategy, but which may diverge widely in host organism and genome size, and which may share little or no nucleotide sequence homology. They: • bind in a sequence-specific manner to viral DNA origins and promoters • have ATP-binding, ATPase, and helicase activities • are sequence-specific endonucleases • are transcriptional activators • prevent infected cells from progressing through the cell cycle The biological importance of the last activity may be to keep infected cells in S phase and so increase the time available for viral replication. See also Epstein–Barr virus anti-apoptosis genes, 295–296 β proteins, 291 blocking host responses to infection, 295–296 DNA replication, 291–292 envelopment and egress, 294 entry by fusion, 288–289 functions of early (α) proteins, 291 genome, 287 latent infection in neurons, 296 nomenclature of genes and proteins, 288 promoter sequences, 290 rolling circle DNA replication, 292 12/05/11 4:08 PM Subject Index scaffold during assembly, 293 tegument proteins, 288, 289–290 viral DNA packaging, 293 viral nucleocapsids assembled in nucleus, 293–294 virion host shutoff (Vhs) protein, 290 virions of, 294 Herpesvirus entry mediator (HVEM), 288 Herpesviruses, 285–300. RNA secondary structure controls translation of lysis and replicase genes Figure 5.3 Binding of RNA phage to F-pili. Historically, the options for mechanical delivery of vaccines into the human body have been remarkably limited, based for the most part on injection with needles, contributing to many people’s fear of vaccines. However, VP1 forms stable pentamers, not hexamers, and these pentameric capsomers fit into “sixfold holes” on the surface of the capsid. The actual structure of viroids inside the cell remains undetermined, and it is possible that these RNAs adopt several alternative conformations during the different steps of their life cycles. • Reoviruses have been used to study aspects of viral pathogenesis in mice. Virions are non-enveloped rod-shaped particles (Figure 9.9), with widths of 23 nm and different lengths in the range of 610 nm to 900 nm, proportional to the length of their linear doublestranded DNA genomes. Addition of nucleosomal histones to a supercoiled DNA molecule can regenerate a circular minichromosome (left). Class I Early 0.3 0.7 1 T7 genes are organized into three groups based on transcription and gene function The sequence of T7 DNA predicts that it can encode 56 proteins, defined by open reading frames with recognizable ribosome binding sites and protein synthesis initiation sequences. It is mediated by nonstructural protein 1s and occurs independently of the apoptotic response to infection. A similar phenomenon has been observed for the scaffolding proteins of various herpesviruses. CH012.indd 141 141 This form of the protein is arranged as a trimer with the distal end of domain II, containing the fusion peptide, exposed and oriented toward the target membrane. This circular viral DNA uses cellular RNA polymerase II to direct the synthesis of a set of five messenger RNAs, one of which also serves as a pregenome RNA (pg RNA). They recognized that phages are sets of genes wrapped in a protein coat, and therefore were ideal objects for studying how genes (RNA or DNA) are replicated and expressed. 9. Because Rep78 both binds to and cleaves DNA at a specific site, it can be imagined that adeno-associated virus DNA is brought to its target sequence in the host cell chromosome via interaction between Rep78 molecules bound to the viral genome and cellular DNA. Like E. Beginning with Zinke’s observation in 1804 that rabies could be transmitted by the injection of saliva from a rabid animal, several investigators worked through the nineteenth century to develop a successful rabies vaccine. Rossmann, "Functional implications of the structure of the murine parvovirus, minute virus of mice," Structure (1998) Nov 15, vol 6 (11), 1369–1381, with permission from Elsevier. In plants infected with RNA viruses, doublestranded replicative intermediate viral RNAs can be generated. Shepherd, Ian A. TATA box: a consensus sequence, often TATAAA, to which the TATA-binding protein (TBP), a part of the general transcription factor TFIID, binds. 16/05/11 7:49 PM 24 CHAPTER 2 Virus Structure and Assembly Other structures, large and small, display icosahedral symmetry The principles of construction of closed shells using identical subunits are universal. L. Copyright 1965, 1970, 1976, 1987 by The Benjamin/Cummings Publishing Company, Inc. This RNA can be amplified in vitro some 1011 times in about 30 minutes by Q replicase. Viruses that could be neutralized by the same antisera were considered to be related and were classified in the same group. Specific marker sequences can be incorporated into the viral RNAs and/or proteins that will allow distinction of vaccinated subjects. Release of a latch that seals the particle leads to release of the genome. Although no evidence has linked adenoviruses to human cancer, these viruses have been used as models for understanding the molecular biology of cancer. Early vaccine scientists showed that the levels of neutralizing antibodies correlate well with protection against many virus infections, and therefore this was one of the primary goals of vaccine development. Reovirus infection of mice with a defect in NF-B activation due to absence of the p50 subunit results in massively increased levels of myocardial apoptosis and marked cardiac dysfunction compared to wild-type animals. It codes for 72 proteins, most of which have no matches in databases. Furthermore, knowledge of the three-dimensional structure of the protein can enable visualization of its active site and understanding of its mechanism of action. 22 Uracil DNA glycosylase* 1 25 dUTPase 1 16 dsDNA break repair 1 50 Thymidine kinase 1 19 Thymidylate kinase 1 23 Ribonucleotide reductase 2 121 Function Protein DNA replication, repair, recombination Nucleotide metabolism * Genes coding for these proteins have been shown to be required for carrying out viral DNA replication. These genomes have a high degree of internal base pairing and therefore resemble double-stranded RNAs over much of their length. A third theory is based on the observation that nuclear pores become enlarged and the Golgi membranes are fragmented during viral replication. At this point, the virus must begin making messenger RNAs (“transcription”) by copying its negativesense RNA genome segments (step 2). However, other cases were labeled “sporadic” and have no obvious genetic component. Adjuvants play an important role in vaccination with inactivated or subunit vaccines Because they are able to replicate in the body, live attenuated virus vaccines induce both cellular and humoral immunity, as do most natural viral infections. The cellular proteintrafficking machinery translocates these proteins via the Golgi network to the cell surface, where virus assembly takes place. (a) ... Two major promoters are used by human papillomavirus types 16 and 31: an early promoter located upstream of the E6 gene, and a late promoter located upstream of the E1 gene (Figure 22.2). Viruses with negative-strand or double-stranded RNA genomes import their RNA polymerases into the host cell, and the capsids serve as tiny intracellular machines for synthesizing viral messenger RNAs. Because of this lifestyle, and because they make their own RNA replication machinery, these viruses may be less dependent on the specific cellular environment where they replicate, giving them freer range to adapt to a wide variety of organisms. 19.5a–c: Courtesy Max L. Burkitt found that the geographic distribution of patients with this lymphoma followed closely the incidence of malaria, suggesting that a transmissible infectious agent was involved. Mutations, including deletions in viral genes, occur frequently during genome replication, and such defective genomes are often incorporated into virus particles. For example, plantar warts of the feet are associated with human papillomavirus type 1, and common hand warts are associated with human papillomavirus types 2, 4, and 7. It is subsequently transported through the Golgi apparatus and the endosome compartment and finally arrives at the plasma membrane. • Inhibitors of uncoating of viral genome: • Influenza M2 ion channel The discovery and widespread use of antiviral compounds began relatively recently Both bacteria and viruses (filterable infectious agents) were first shown to cause disease in the late nineteenth century. In addition to its protease activity, nsP2 contains nucleoside triphosphate-binding motifs in its amino terminal region that are homologous to those found in bacterial helicases. Vertical bars show 5' ends of RNAs, corresponding to transcriptional promoters; arrowheads represent 3' ends of mRNAs, corresponding to polyadenylation sites. (b) Model of part of the active site of influenza neuraminidase bound to zanamivir, shown as ball-and-stick model in the center (small orange spheres O; larger orange spheres N). In the absence of Rev, these mRNAs are retained in the nucleus and therefore are not translated, because translation is confined to the cytoplasm. The M protein is a smaller type I transmembrane protein of approximately 80 amino acids. The World Health Organization estimates that over 2 billion people worldwide have been exposed to hepatitis B virus, and around 350 million of these individuals are chronic carriers. Penetration into the cytosol occurs from these vacuoles. It is reminiscent of similar recombination events between retrovirus genomes that can occur during reverse transcription (Chapter 28). Plant viruses often penetrate as a result of damage to the cell wall caused by abrasion or a wound caused by an insect. Each phage was designated by T (for “type”) and arbitrarily assigned a number. Boxes indicate operator sites for binding by CI repressor. nsp5 Chymotrypsin-like proteinase (3CLpro or Mpro) responsible for cleavage of nsp4 through nsp16 in all coronaviruses. This results in the symptoms that define the “flu” syndrome: fever, headache, chills, malaise, and muscle aches. The only exceptions are archaeal head-tail viruses, which carry genes with homologs in genomes of bacterial head-tail viruses from the families Myoviridae and Siphoviridae. Reservoir: a population of organisms in which a pathogenic agent circulates in nature, not necessarily causing overt disease. As CII decays, PRE, PInt, and PAQ return to their unactivated states. Coat protein plays different roles in cell-to-cell movement and long-distance movement. Virions can be visualized by negative staining, which reveals the structure of the virion by the exclusion of electron-absorbing molecules such as uranyl acetate (Figures 2.1a–g). Strong transcriptional enhancer and TATA box ensure high-level transcription from a defined site. • After fusion, herpesvirus nucleocapsids are transported to the cell nucleus where they release their viral DNA into the nucleus via the nuclear pores. After “flickering” open and shut briefly, the fusion channel remains permanently open and starts to expand. ADENO-ASSOCIATED VIRUS VECTORS Are considered safe because the parent virus is nonpathogenic for humans. Thirteen different interferon-␣ genes have been identified and are clustered on the short arm of human chromosome 9. A great deal of effort has been made to discover drugs active against these enzymes. The HIV protein Nef is able to induce a loss of MHC 1 from the surface of virus-infected cells. Epstein and his colleague Yvonne Barr immediately began collaboration with Burkitt by studying biopsies sent from Uganda. Herpesviruses and poxviruses make a number of proteins that are similar to complement control proteins. A fraction of nsP2 is found in the nucleus of infected cells. Tumor virus oncogenes 267 Cell tumor suppressor genes HPV E7 Ad E1A pRB SV40 LTAg Viral E1 and E2 proteins bind to the replication origin and direct initiation of DNA replication Regulatory DNA sequences known as the long control region (LCR) of human papillomaviruses extend from the end of the L1 gene to the beginning of the E6 gene (Figure 22.2). • Study of gene expression in small DNA viruses led to the identification of promoters for eukaryotic RNA polymerases. The resulting double-stranded RNA activates a cellular protein, double-stranded RNA-dependent protein kinase (PKR). E4orf6 targets the 55K protein to the nucleus, but both 55K and E4orf6 also contain nuclear export signals that are involved in shuttling these complexes out of the nucleus. In 1971, Wu and Kaiser showed that the cohesive ends were complementary, single-stranded, 12 base-long extensions at the 5' ends of each DNA strand. Reasoning that the apparently protective effects of cowpox could be artificially induced using the procedures of variolation, Jenner inoculated eight-year-old James Phipps with cowpox from the hand of a milkmaid, Sarah Nelmes, on May 14, 1796. Other chronically-infected individuals progress to cirrhosis, which is characterized by fibrin deposition in the liver, the production of ascites fluid, infiltration of lymphocytes, flu-like symptoms, high serum ALT levels, and hemorrhaging. • Ganciclovir is phosphorylated by a cytomegalovirus protein kinase and preferentially inhibits activity of the viral DNA polymerase. Viruses are dependent on living cells for their replication Viruses can replicate only within living cells. The core contains a small, highly basic protein, P6.9 (also known as Ac100), which is bound to the DNA. However, unlike the original peptide and the actual transition state, they cannot be cleaved. Translation initiates on picornavirus RNAs by a novel internal ribosome entry mechanism Once in the cytoplasm, the genome RNA must be translated by cellular ribosomes to produce viral CH011.indd 128 proteins. The small, highly base-paired VA (for “virus-associated”) RNAs are involved in translational control by inhibiting activity of the cellular, double-stranded, RNA-dependent protein kinase (PKR), one of the pathways by which interferon acts (see Chapter 33). A number of university and college teachers of virology reviewed the concept of the book, or parts of the manuscript at various stages, and offered helpful suggestions and comments. Drug resistance mutations can provide selectable markers for engineering interesting mutant viruses and performing viral genetics. Virions can be transported inside endocytic vesicles (prior to penetration), or as free nucleocapsids within the cytoplasm (after penetration) (Figure 4.5). Reassortment is restricted to closely related bunyaviruses, and even then certain combinations of viruses appear genetically incompatible. However, RNA in virions made with defective or missing maturation protein is sensitive to RNAse degradation. Three activities have been attributed to Nef, but it is unclear which of these activities is important for disease. A protein subunit must take on the appropriate configuration when it enters a growing icosahedral capsid, and the more choices it has available, the more likely it is to make a mistake—which could result in a fatal flaw in the CH002.indd 23 particle. Note that several protein coding regions overlap, allowing the efficient use of a small genome. These mRNAs undergo splicing that removes most of the 3- to 5-kb region that lies between the late promoter and the beginning of the L1 and L2 open reading frames. CH021.indd 261 16/05/11 8:09 PM 262 CHAPTER 21 Polyomaviruses • Two to four early messenger RNAs and two to four late messenger RNAs are made by splicing primary transcripts of the early and late regions. T7 phage binds to lipopolysaccharide molecules in the outer cell membrane via its tail fibers (Figure 7.2). Genes coding for translation factors and DNA repair enzymes With such a large genome size, it is no surprise that the mimivirus encodes a wide variety of proteins with a diverse array of potential functions. Baines, McGill University Karen Mossman, McMaster University 312 Richard C. Roosinck, J. This system allows researchers to introduce directed mutations into viral genes and to study their effects on virus production, infectivity, and pathogenicity in infected animals. In the most widely used “self-replicating” alphavirus vectors (Figure 13.5a), the structural protein genes are replaced by a heterologous gene—initially, reporter genes such as the gene producing -galactosidase or the gene encoding chloramphenicol acetyltransferase, and subsequently any foreign gene of interest. Many of the early genes carry promoters and transcription start sites that mimic normal host insect gene promoters. What were the first two applications of baculoviruses as insecticides? No coding sequences are found on the short tail; however, there are 12 protein-coding regions on the longer tail, 7 of which have predicted functions involved in DNA replication or binding. A new live influenza vaccine has recently been approved for use in humans between 6 and 55 years old. • These viral oncoproteins inactivate critical cellular tumor suppressor pathways mediated by p53 and Rb proteins. Hemagglutination is a cheap and rapid method for detection of virus particles. Fusion of the endosome membrane with the viral envelope leads to the release of the nucleocapsid into the cytoplasm. Because numerous transcription factors are involved, and because the same enhancer region powers both early and late transcription, the exact mix of transcription factors that activate late transcription is not fully understood. C has also been shown to modulate the activity of the RNA polymerase. devastating to the silk industry. They have a 60-nm icosahedral capsid and a short, stubby tail attached to one of the icosahedral vertices. Budding is driven primarily by the interactions between envelope glycoproteins and matrix or nucleocapsid proteins. Most alphaviruses are transmitted by mosquitoes. These exons are randomly selected and combined by a process of somatic recombination durding lymphocyte differentiation and development. These sequence elements are approximately 20 nucleotides in length and do not appear to affect transcript stability. It is long-lived, with a half-life of about 25 days. From the humblest of origins (it was originally isolated from a water cooling tower in Bradford, England), it has since taken the world by storm, and currently holds the world record for being the largest known virus. F-pili (singular: pilus) to maneuver their single-stranded RNA genomes into the cell. In this mutant genome, two U residues in the stretch of 7 were exchanged for C residues, and an additional U residue was added (Figure 16.6). Intense Phaeocystis blooms can lead to anoxia and impressive foam 05/05/11 11:24 AM 336 CHAPTER 27 Viruses of Algae and Mimivirus formation on beaches during their decline, hence their label as harmful algal blooms. Seven nucleocapsids are arranged parallel to each other as a cylindrical barrel and an eighth nucleocapsid lies in the center of the bundle. Core proteins condense viral DNA by neutralizing the negative charges of the phosphate groups on DNA. These enzymes are prevented from acting on the cell’s own DNA by modification of the host cell DNA, frequently by methylation of one of the nucleotides in the restriction enzyme recognition sequence. Picornaviruses make a variety of proteinases that cleave the polyprotein and some cellular proteins Picornaviruses encode three distinct proteinases: the L, the 2A, and the 3C proteinases. Phages in the T7-like group infect many different host bacteria, including marine organisms, the plague bacillus (Yersina pestis), Serratia, Klebsiella, and Pseudomonas. Experiments in several species have identified host proteins (Ref1, Lv1) that can block this stage of the infection. A variety of auto-assembling vesicles or nanoparticles, within which viral antigens can be trapped, are being tested as delivery systems for subunit vaccines. Phylogenetic analysis of sequenced representative genomes places the Phycodnaviridae within the Nucleo-Cytoplasmic Large DNA Viruses (NCLDVs), a “superfamily” of diverse viruses that also includes the Poxviridae, Iridoviridae, Asfarviridae, and Mimiviridae. CH008.indd 88 16/05/11 7:59 PM Bacteriophage Lambda 89 because the cooperating repressors must lean toward each other, so CI at OR2 leans toward CI at OR1(Figure 8.5b), and cannot interact with a third repressor at OR3. Release of the viral nucleocapsids from the virion is facilitated by the M2 H⩽ ion channel. Compare antigenic shift and antigenic drift. Outside of the United States, please contact your local representative. The 3' end of this mRNA is specified by the classical polyadenylation signal, AAUAAA, which directs RNA cleavage by cellular enzymes and the addition of poly A to the cleaved 3' end of the RNA. These three pro-inflammatory cytokines activate immune cells within the circulatory system. interferons stimulate the expression of antiviral genes, and the mechanisms by which antiviral proteins inhibit virus replication. The division into sections is based on the nature of the virus genome and virus replication strategies: positive-strand RNA viruses (Section III), negative-strand and double-stranded RNA viruses (Section IV), DNA viruses (Sections V and VI), and viruses that use a reverse transcriptase (Section VII). Kurochkina, Paul R. Trypsin expression is limited to the upper respiratory tract in humans, and therefore these viruses are less likely to infect lung and other tissues in the body, leading to milder and more self-limiting infections. PI 3-kinase synthesizes phosphatidylinositol 3-phosphate, a phospholipid that controls such cellular functions as proliferation, vesicle trafficking, and organization of the cytoskeleton. The ability of ligands for these receptors (Mip1␣, Mip1ß, and Rantes for CCR5; SDF-1 for CXCR4) to block virus entry demonstrates their significant role in the infection process. The Octocorallia homologue of MutS, known as Msh1, is encoded on the mitochondrial genome of all octocorals, but is not present in mitochondria of even the closely related hexacorals. Negatively stained preparations of infected cells. Some genes are separated by short untranscribed intergenic sequences. DNA polymerase ␣/RNA primase binds to a region of single-stranded DNA unwound by the DNA helicase activity of large T antigen hexamers and bound by replication protein A. How were viruses first distinguished from other microorganisms, and what did this ultimately reveal about viruses? During the Ebola virus disease outbreaks in 1976 and 1995, transmission within hospital settings via contaminated syringes and needles was a major problem. Alphavirus gene expression vectors, described in the next section, are also cytopathic. The three capsid proteins, VP1, 2, and 3, have overlapping amino acid sequences, because they are translated in the same reading frame (Figure 20.2). completion, only a fraction of these two viral mRNAs are spliced, and both unspliced (M1 and NS1) mRNAs and spliced (M2 and NS2) mRNAs are exported from the nucleus. The scale is numbered clockwise in kbp. A similar mechanism probably occurs with several other enveloped viruses that have class I fusion proteins, including retroviruses, paramyxoviruses, and filoviruses. How do the two forms of poxvirus virions differ, and are they both equally infectious? The ecology and epidemiology of West Nile virus is not yet understood well enough to predict its future effects on either birds or humans in North America. Therefore, their capsids (“preintegration complexes”), which contain proviral DNA made by reverse transcription as well as several viral proteins, must wait in the cytoplasm until the cell divides. The ratio of physical particles to infectious particles is greater than 1 for many viruses. This conversion is carried out by a cellular DNA polymerase. Like AZT, these compounds are phosphorylated by cellular kinases and their triphosphates inhibit HIV-1 reverse transcriptase. Neuraminidase cleaves sialic acid, the cellular receptor that binds to HA The roles of HA and M2 in virus entry were discussed above. T7 virion is shown attached to outer cell membrane via tail fibers (left image). Many viruses enclose their nucleocapsids in lipidbilayer membranes called envelopes. Mutational studies of viral genes determined that vif, vpr, vpu, and nef are not essential for production of infectious HIV-1 in many cell culture systems in vitro. Large T antigen hexamers bind to the origin of DNA replication and locally unwind the two DNA strands Once the cell has entered the S phase, viral DNA replication can begin. • Acyclovir is a nucleoside analogue that is preferentially phosphorylated by herpes simplex virus thymidine kinase and is incorporated into DNA by the viral DNA polymerase, blocking further DNA replication. You might say, “This is all well and good, but when a chromosome is injected into a cell, there is no repressor in the cell! If CI activates its own gene, whence comes the repressor that initially activates PRM?” The answer is that has a system, involving a second cI promoter, to provide the CI repressor needed to activate PRM. Phaeoviruses only infect these free-swimming gametes. Examples include the smallest eukaryotic ATPdependent DNA ligase; the smallest type II DNA topoisomerase (capable of cleaving double-stranded DNA 3 to 50 times faster than the human homologue); CH027.indd 330 the first RNA guanylyltransferase (RNA capping enzyme) whose atomic structure was resolved; a small prolyl-4-hydroxylase that converts proline incorporated into polypeptide chains into hydroxyproline in a sequence-specific fashion; a dCMP deaminase that is also capable of deaminating dCTP (usually enzymes from two different protein families are used for these reactions); and a small (94 amino acid) potassium ion channel protein; ATCV-1 has an even smaller version with only 83 amino acids. • E1A proteins also engage cellular histone acetyl

transferases, resulting in relaxed chromatin and increased transcription of many cellular genes. Their limited number of viral genes code for (1) virion structural proteins, (2) proteins that stimulate production of cellular enzymes involved in DNA replication, and (3) proteins that CH003.indd 43 43 recognize and bind to the viral DNA and assemble the cellular DNA replication machinery (for examples, see bacteriophage ϕX174, parvoviruses, polyomaviruses, and papillomaviruses, in Chapters 6, 20–22). Other paramyxoviruses cause several important human diseases Mumps virus, human parainfluenza viruses, and respiratory syncytial virus are paramyxoviruses that cause serious systemic and respiratory infections, predominantly in children. 21.11: Reprinted from Fig. LATs have been shown to protect cells against apoptosis, perhaps contributing to the survival of neurons that express low levels of viral proteins. A region corresponding to the fusion peptide of other enveloped viruses lies near the N-terminus of GP2 and is believed to mediate fusion by insertion into cellular membranes, followed by conformational changes in GP that bring the virus envelope into close contact with the membranes (see Chapter 4). Breaking and entering: the insertion of λ prophage DNA into the bacterial chromosome To be stably inherited as a prophage, the circularized chromosome must integrate into the bacterial chromosome; otherwise, the non-replicating circles are simply diluted out as the cells divide. SUBUNIT VACCINES Usually stimulate only humoral immunity if administered without adjuvants. The terminal filaments are involved (a) (c) (b) Genomic DNA (d) Putative capsid Integrase Tail Figure 9.2 Sulfolobus spindle-shaped viruses 1 (SSV-1) and 6 (SSV-6), family Fuselloviridae. (a) Diagram of herpesvirus particle with major elements denoted. Sci. However, there are upward of 30,000 human fatalities per year worldwide, mostly in less developed countries. Recognizing its unique properties, • Gokushoviruses, from Japanese gokusho (very small), infect obligate intracellular parasitic bacteria. Human diseases: Creutzfeldt–Jakob disease (CJD), kuru. Furthermore, ribonuclease L cleavage of cellular RNAs can result in the accumulation of small RNA species that stimulate RIG-Ilike receptor pathways, leading to amplification of the cellular interferon response. The other 80% of the GP mRNA codes for the precursor of sGP, which is therefore made in larger amounts than GP. This results in an extracellular virus (EV) particle that is attached to the cell membrane. The terms “hepatitis A” and “hepatitis B” were first introduced by MacCallum in Chronic or associated diseases: cirrhosis, liver cancer, serum sickness. Negative contrast electron micrographs of (a) full-length virion (Scale bar: 200 nm), (b) terminal structure at higher magnification, (c) drawing of a rudivirus. Cucumber mosaic virus replicase can generate recombinant RNAs in vitro by base pairing-independent copy-choice recombination. CH021.indd 255 255 T antigen, middle T antigen can also bind to and inactivate PP2A. A final chapter in Section II covers exciting new knowledge about the sometimes bizarre viruses that infect archaea, members of the third domain of life alongside bacteria and eukaryotes. Adenovirus E1B 55K protein and E4orf6 protein combine to form a complex that transports adenoassociated virus mRNAs from the nucleus, where they are made, to the cytoplasm. A major problem with integrative vectors derived from retroviruses is the fact that integration into the cell genome occurs at random locations, which can result in insertional mutagenesis with dire consequences. Virions are assembled within the host cell and “slip” out of the cell without creating holes in the cell membrane or S layer (Figure 9.2c). Unlike positive-stand RNA viruses, the genomes of negative-strand RNA viruses always remain in the form of a ribonucleoprotein complex, which is usually constructed with a single major capsid protein that forms a helical nucleocapsid enclosing the RNA. Chemokines: secreted proteins that attract leukocytes and are active in inflammatory reactions. Staining of macromolecules can be observed in fixed or living cells by microscopy, especially by using computerized methods that allow visualization of horizontal layers of thickness 1 micrometer (m) or less within individual cells. Subsequent epidemiological studies suggested that the syndrome was due to a transmissible agent that was acquired through sexual contact or blood exchange; hemophiliacs, recipients of blood transfusions, and intravenous drug users were also affected. The 54-kDa major capsid protein (Vp54) is a glycoprotein that comprises approximately 40% of the total protein in virions, the remainder composed from at least 110 different virus-encoded proteins. The ratio of unspliced to spliced segment 7 and 8 viral mRNAs in the cytoplasm is approximately 9:1. Orthomyxoviruses are negative-strand RNA viruses with segmented genomes Myxoviruses were originally named because virions of influenza and several other enveloped, negativestrand RNA viruses bind to sialic acid residues in CH018.indd 211 211 Figure 18.1 Epithelial cells of the upper respiratory tract infected with influenza virus. Rubella virus has not been studied in as much detail, but is likely to have similar morphology and symmetry. KEY TERMS Concatemer Holin Lipopolysaccharide Nested set Processivity Prohead Scaffolding protein 16/05/11 7:57 PM 84 CHAPTER 7 Bacteriophage T7 FUNDAMENTAL CONCEPTS • The linear, double-stranded genome of bacteriophage T7 enters the cell in three phases: 1. Certain early genes encode distinct intermediate transcription factors (VITF) that trigger intermediate gene expression. Repression of early transcription by large T antigen. (b) The surface of the immature virus at the low pH found in the trans-Golgi network. The F protein directs fusion with the plasma membrane. These changes facilitate interactions of the ISVP with endosomal membranes, leading to delivery of the core particle into the cytoplasm and activation of the viral transcriptase. For example, cloning vectors with both T7 and T3 promoters arranged to promote convergent transcription from opposite DNA strands have been useful in applications of sequence analysis to both DNA strands of the same fragment and in preparation of antisense RNAs. The T7 RNA polymerase/late phage gene promoter system has been exploited to generate a family of highly efficient gene expression vectors, called pET vectors (for plasmid, expression, T7 [Figure 7.4]). Bacteriophage T7 (Chapter 7) is a double-stranded DNA virus with a stubby tail structure. Activation of the kinase Hck results in increased expression of interleukin-6, tumor necrosis factor-␣, and interleukin-1, all activators of HIV-1 16/05/11 8:15 PM 364 CHAPTER 29 Human Immunodeficiency Virus replication. However, this report was largely ignored until the early 1990s, when high concentrations of viruses in aquatic environments were being described. Proteins coded by these genes include many of those shown in Tables 25.2 and 25.3. A library of plasmids, each carrying one of these genes, is sufficient to support expression of a transfected reporter gene under the control of an AcMNPV late or very late promoter. Yet, despite the conserved structure, the phycodnaviruses are probably the most genetically diverse of any virus family. PRM is a weak promoter, but in a prophage, PRM is activated to produce sufficient CI repressor to shut down PR and PL, as follows. There undoubtedly remain many viruses yet to be CH003.indd 33 33 discovered, particularly in poorly studied organisms such as archaea, protists, small invertebrates, and marine organisms. Because late transcripts are in excess, most of the early transcripts but only a fraction of late transcripts are degraded. EIF4F is dephosphorylated by an adenovirus host shutoff protein during the late phase of infection, and it can no longer direct scanning of mRNAs, resulting in the inhibition of translation of most cellular mRNAs. However, viral mRNAs containing the tripartite leader continue to be translated efficiently, because the leader allows ribosome shunting that avoids the scanning mechanism. Reprinted by permission of Pearson Education, Inc. Regulation is also achieved on the translational level. It involves recognition of foreign antigens by B and T lymphocytes, leading to the production of antibodies and the generation of cytotoxic T lymphocytes that kill infected cells. Study of virus replication cycles by these and other methods has led to a general understanding of the various steps in virus replication. Also, faster sailing vessels ensured periodic delivery of unwanted viral passengers to all parts of the known world. First, there are multiple translational start sites between nt 81 and 203 of the Sendai virus P/C/V mRNA. It injects its DNA part way into the host cell, and uses transcription by cellular RNA polymerase to complete entry of its DNA. The virion or viral genome enters the cell; the viral genome is uncoated. PMID: 18479185; Fig. coli) and P22 (a member of the Podoviridae that grows in Salmonella). CH002.indd 30 In simple terms, how do virions transmit the genome of a virus from one cell to another? Transcriptional enhancer, origin of DNA replication (ORI), and large T antigen binding sites are shown above and below map. Eye and gastrointestinal infections. Rather, they grow productively in tumor cells, killing these cells. Baines Karen Mossman THE INNATE IMMUNE RESPONSE Innate immune responses are rapid but non-specific responses of the host to infection. First, progeny plus-strand DNA is made by a rolling circle mechanism using replicative form DNA as a template. It turns out that effective repression of PRM requires long-range interactions of the CI tetramers at OR1 and OR2 with CI tetramers at OL1 and OL2. Packaging of individual segments of viral genomes into separate particles is commonly found in plant viruses but is rare or absent in viruses infecting other organisms. However, when the histones are removed by treating with the strong protein denaturant sodium dodecyl sulfate and extraction of the proteins with the organic solvent phenol, the circular DNA now becomes coiled about itself like a rubber band held between the thumbs and forefingers of both hands and twisted. Chapters contributed by prominent virologists were edited to conform to a clear and accessible style. These two proteins are crucial players in the formation of polyadenylated cellular messenger RNAs in the cell nucleus. Some genes have compound promoters, allowing for their expression throughout infection. In some of these viruses, the U3 region contains sequences that enhance recognition of the polyadenylation signal near the 3' end of viral RNAs, ensuring that cleavage and polyadenylation take place only at that end. • Antiviral vaccines have had enormous positive impacts on global health: for example, eradication of smallpox, control of measles and polio. Activation of a PKR plant homologue may be the triggering event in viroid pathogenesis, as PKR is induced by interferons and activated by double-stranded RNAs in mammalian cells (see Chapter 33 and Figure 33.10). ss single-stranded; ds double-stranded;   positivestrand RNA;   negative-strand RNA. The eight segments shown within the circle at the right are labeled to denote major proteins coded by each segment. DNA polymerase ␣/primase lays down new RNA primers on 16/05/11 8:09 PM 258 CHAPTER 21 Polyomaviruses (a) (b) D1 hairpin hairpin D2 D3 ATP (c) Channel D1 17A -hairpin 1 Figure 21.11 Structure of simian virus 40 large T antigen. Initiation of the reverse transcription process begins when P binds to the 5' stem-loop. The gene of interest, say gene X, is transcriptionally silent until expression is activated, at which time a massive takeover of the cellular transcription and translation capacities occurs. Among the most promising include DNA vaccination, pulsed dendritic cell vaccination, and the use of potent Th1-biasing adjuvants such as ligands for toll-like receptors. It is tempting to speculate that most viruses with single-stranded DNA genomes may have evolved from a common ancestor (see Chapter 6). Why would you predict that the phycodnaviruses may play a role in understanding global warming? These include tumor necrosis factor receptor-associated factors (TRAFs), tumor necrosis factor receptor-associated death domain protein (TRADD), inhibitory B kinase (IKK), and NF B inducing kinase (NIK), leading to activation of NF- B. Removal of patients from multiple antiviral drug therapy invariably results in reemergence of the infection. It also makes its own RNA polymerase, which transcribes late genes and has been used in many applications in molecular biology and genetic engineering. However, the morphologically altered Golgi complex retains its ability to glycosylate and transport glycoproteins destined for the plasma membrane. The “single cytoplasmic envelopment” model (3) predicts that nuclear pores become enlarged and allow translocation of capsids to the cytoplasm, where they become enveloped at Golgi membranes. If this UAA stop codon and the UAG stop codon that immediately follows it are mutated so that termination does not take place until the next UGA codon (25 nt farther along the RNA), there is no expression of the lysis gene. Enveloped virus-like particles can be formed in the intermediate compartment when only M and E are expressed together in cells, indicating that these proteins are sufficient for assembly and budding. N-terminal parts of the coding regions for T antigens and VP2 are shown in orange. Perforin makes holes in the cell surface membrane as well as intracellular membranes. Students who understand what diseases are caused by particular viruses, and the importance of these diseases in human history, may be motivated to learn more about those viruses. Diameter 26 nm. The same outcome can be achieved more simply by infecting cells with the virus that expresses T7 RNA polymerase, followed by transfection with a DNA molecule containing any coding sequence cloned downstream from a T7 promoter. CH016.indd 195 5' NP mRNA A A U U A G A A G G A G 5' 195 poly(A) 3' U A A U U U U C C U C UC 3' Figure 16.7 Stem-loop at 5' end of NP mRNA that controls transcription of Ebola virus genome. As the shell is filled, the next cos site of the concatemer approaches the terminase located at the portal. The incoming capsids apparently contain proteins that bind to the motors. CH002.indd 28 In helical capsids, such as tobacco mosaic virus (Figure 2.9) or the nucleocapsids of many negative-strand RNA viruses, the RNA extends along a groove in the helical array of protein subunits. Interestingly, all genes in the right half of the genome of His-2 are read from left to right, and all genes in the left half are read right to left, implying that transcription diverges from the center of the genome toward the genome ends. Often characterized by the nature of their photosynthetic apparatus, the products of multiple and complex endosymbiotic events, the algae do not represent a single evolutionary group. However, when injected into DISEASES Usually persistent, nonsymptomatic infections. Members of the Gokushovirus subfamily appear to recognize proteins rather than sugars on host cell surfaces. Like inactivated vaccines, subunit vaccines also typically include an adjuvant to enhance immunogenicity. GP1 Fusion peptide SS Extracellular medium N GP2 Plasma membrane or virus envelope C Cytoplasm or interior of virion Figure 16.5 Ebola virus envelope glycoprotein. Twelve spikes project from the core through the outer capsid. Subsequently, Stat1 and Stat2 proteins are recruited by binding to the phosphorylated receptor, and they in turn are tyrosine-phosphorylated by the activated Jak kinases, leading to the dimerization of the Stat proteins (Figure 33.8, top right). During lytic growth, there is a shift to rolling circle replication (see Chapters 6 and 24). • Within infected cells, viral proteins are processed into small peptides, which are presented on the surface of infected cells by MHC proteins for recognition by B and T lymphocytes. To reduce such reactions, MDV-1 RNA can be added as two half-molecules (Figure 5.9b), which are joined when properly aligned on the target RNA by adding RNA ligase to the reaction. Reovirus strains that do not recognize sialic acid may 16/05/11 8:07 PM Reoviruses bind directly to JAM-A, although these strains may also use carbohydrate receptors other than sialic acid. Spread of filovirus infections among humans is limited to close contacts The usual pattern seen in large outbreaks of filovirus hemorrhagic fever begins with a single case, who disseminates infection to several other people. Additional sequences downstream of the late initiator motif appear to be responsible for the high transcriptional activity of very late genes such as polyhedrin and P10. For viruses with a type I element, translation is initiated at an AUG located 30–150 nucleotides downstream from the conserved AUG. Cells provide molecular building blocks such as nucleotides and amino acids, a source of chemical energy, the cellular protein-synthesizing machinery, and the controlled intracellular environment needed to carry out life processes. Proteins W and FII bind to the portal vertex, stabilizing the filled head and creating a binding site for tail attachment. Treatment of p50-negative mice with interferon protects heart cells from apoptosis following reovirus infection. As a result, the two strands of an adjacent AT-rich segment of the DNA become unpaired. Expression of this protein dramatically increases the amount of viral RNA produced in infected cells, thus its name, abbreviated from “transactivator of transcription”. Therefore, in order to replicate their double-stranded DNA genomes, they must use the machinery of the host cell. This does not require either S1 or the Host Factor. (2001), D.M. Knipe and P.M. Howley (eds), Lippincott Williams & Wilkins (Baltimore); Fig. No clear excess levels of cancer or of specific tumors have been detected in people during the 45 years since they received contaminated poliovirus vaccine. First, there is evidence that transcription and replication initiate at different sites, implying the use of different polymerases. As a general rule, if it can vary, it inevitably does vary within the Phycodnaviridae. However, due to incomplete knowledge about many immunologically mediated reactions, most adverse events following immunization are poorly understood and therefore are difficult to foresee. Furthermore, it keeps most viral double-stranded RNA segregated (a) within subviral particles and away from the cytosol, reducing activation of cellular signaling pathways that suppress virus replication. RNA synthesis directed by most positive-strand RNA viruses, including bromoviruses, picornaviruses, togaviruses, and coronaviruses (Chapters 10, 11, 13, and 14), is also associated with membranes in the cytoplasm of infected cells, and in some of these cases is understood in more detail. This action enhances the immunogenicity of viral proteins by increasing the quantity and repertoire of peptides displayed in association with MHC class I proteins. BSE: bovine spongiform encephalopathy; prion disease in cattle. A pattern of interlocking arms from the C-termini of the capsid subunits holds the structure together (Figure 2.6b). The 240 capsid protein molecules are each in direct contact with one of the overlying 240 envelope protein heterodimers, leading to identical symmetry arrangements in both nucleocapsid and envelope, unusual for enveloped viruses. This “giant” virus replicates in the amoeba for which it is named, and therefore is not a virus of algae. A typical burst size is usually greater than 300 viruses per infected cell. Transcriptional stuttering occurs within the P reading frame at sequence shown in the middle, adding one or two G residues that change the reading frame, producing proteins V or W. (b) A larger view of a nucleosome, around which DNA is wrapped as a toroidal coil. To enter plant tissues and access cells, plant viruses rely on insect bites, grafting, and other forms of mechanical damage. These families have remarkably similar features. A virus with a single-stranded DNA genome is closely related to a virus with a double-stranded DNA genome The only known archaeal virus with a single-stranded DNA genome, Halorubrum pleomorphic virus 1 (HRPV1), infects a hyperhalophile and has poorly shaped roundish virions with surface extensions. 1.2c: Courtesy of The John Curtin School of Medical Research, ANU. • Many of these viruses do not kill the host cell during productive infection; their method of exit from the cell is unknown. Extreme examples of the unique genetic content of archaeal viruses are provided by the globuloviruses PSV and TTSV-1, the genomes of which are almost terra incognita, containing no genes with homologs in extant databases. Synthesis of the subgenomic RNA (transcription) initiates at a promoter that lies at the junction between the genes for nonstructural proteins and those for structural proteins. CH009.indd 106 The only requirement is a temperature in the range of host cell growth, above 75⬚C. However, subsequent studies evaluating this strategy for an Ebola virus vaccine were less encouraging. Air travel by infected individuals quickly spread the disease to 32 countries, resulting in the first pandemic of the twenty-first century. Shells with icosahedral symmetry can accommodate exactly 60 structural subunits on their surface (Figure 2.2b, c). 18.5: Redrawn from Portela, A. A number of plant viruses have fragmented positivestrand RNA genomes that are packaged separately into distinct particles. The early promoters of both simian virus 40 and mouse polyomavirus overlap with one or more of the G(A/G)GGC binding sites for large T antigen. This feature allows the determination of which viral proteins are involved in particular biologic properties of the virus. The newly synthesized minus-strand DNA remains associated with its template plus-strand in a double-stranded replicative form. In this form, ingestion or introduction into the central nervous system can lead to propagation of the misfolded form and neurological disease, dementia, and death. Ribonuclease L then degrades both host and viral mRNAs, leading to inhibition of virus replication by suppression of viral protein synthesis. Two identical genome RNAs in each virion. It is administered by a nose spray, in contrast to inactivated vaccines, which require injection. Viruses of Algae and Mimivirus 325 Michael J. One strategy is to prevent complement binding to antibody-antigen complexes. The use of overlapping reading frames can be understood as a way of increasing the amount of genetic information encoded in a small viral genome. Neurons were transduced by the vector and an increase of dopamine production during several months was seen. viruses can undergo mutation of the targeted gene and therefore escape the immune response directed against a single antigen. A great variety of specific B- and T-cell receptors are created by somatic recombination. Influenza Viruses 210 10. Transcriptase activity can be detected in detergent-disrupted bunyavirus preparations. C proteins are required for formation of infectious paramyxovirus virions. A complex of proteins P123 and nsP4 initiates minus-strand RNA synthesis (replication) from a promoter (black with directional arrow) at the 3' end of genome RNA; 5'-terminal sequences are also required. Reverse transcriptase (polymerase protein) generates genome DNA from pregenome RNA within capsid during virus assembly. Viruses can be crystallized: they lie on the edge between chemical compounds and life. Recombinant poxvirus vaccines are now used widely in heterogeneous 3' ends, made on opposite DNA strands from convergent viral promoters. Shortly thereafter the laboratory of Michael Bishop and Howard Varmus showed that the ability of Rous sarcoma virus to induce tumors results from the acquisition of an altered cellular gene (an oncogene) by the virus. Virions and non-infectious particles are formed by budding into the lumen of endoplasmic reticulum and Golgi membranes, and are released upon fusion of vesicles with the plasma membrane. 3CD also plays a role in RNA replication (see below). Early vaccines made possible by growth in embryonated eggs include the yellow fever virus strain 17D (1932) and influenza virus (1936). However, export and translation of the 2-kb (doubly spliced) class of viral mRNAs is not dependent on Rev. When the space required for the nucleus, the chloroplast, ribosomes, and other essential intracellular structures is taken into consideration, this makes for a very crowded infection process! Experiments reveal a typical burst size of 6 to 15 virus particles per cell. CH015.indd 187 16/05/11 8:05 PM C H A P T E R 1 6 Filoviruses Heinz Feldmann Hans-Dieter Klenk Nicholas H. Persistent infection: an infection (in this case, by a virus) of cultured cells or of an animal during which the virus is not eliminated, but continues to replicate for a prolonged time at a low level, without killing the host. Only cells that bind to a specific antigen will be recruited to respond. 6.2, 6.5, 6.6: Courtesy of Bentley Fane. This severe respiratory disease, which is a classic example of an emerging viral disease, has an overall fatality rate of 40%. Experimental laboratory animals such as suckling mice, in which many animal viruses are able to replicate, were adopted for use because they are relatively easy and inexpensive to raise. However, the 2A protein in other picornaviruses has no proteinase activity. This can be achieved by treatment with agents that interfere with the formation of clathrin-coated vesicles or block transport via the nuclear pore complexes. They replicate via rolling circle forms, RNA cleavage, and ligation. Because of these difficulties, pharmaceutical companies have not always been enthusiastic about investing resources in the development of antiviral drugs. The gene products are mostly involved in viral DNA replication. Gangliosides: sialic acid–containing glycosphingolipids found in cellular membranes. This enables interactions between the C protein and the envelope proteins, which are also anchored to the membrane but reside within the lumen. Loeb and Zinder plated filtered samples of raw New York City sewage on E. Opportunistic infections: infections that flourish in an immune-deficient individual but that are usually absent from healthy individuals. Thus the DNA replication cycle is complete. On the simplest (T 1) capsids, there are only clusters of 5 subunits, and therefore only one jump is required between such clusters: h 1, k 0. A cardboard box is rigid, symmetrical, and fixed in volume and shape; a plastic bag is flexible, variable in volume and shape, and roughly spherical when stuffed full. These reports were followed in the early 1990s by research into viruses of marine filamentous brown algae. GENOME Linear double-stranded RNA, 10–12 segments. These coronaviruses may be unique among known viruses in having both a spherical core shell (probably with icosahedral symmetry) and an internal helical nucleocapsid. These proteins are inserted into the endoplasmic reticulum as they are translated (Figure 30.7). Figure 32.2 Fore victims of kuru. Since then four other effective nucleoside analogues have been approved. The 31.5-kb genome of murine hepatitis virus is shown. Macrophages/ monoctes and dendritic cells seem to be the first and preferred site of filovirus replication. Surprising results, however, have been achieved in nonhuman primates with post-exposure vaccination using live attenuated recombinant strains of vesicular stomatitis virus expressing Marburg or Ebola virus GP (50–100% survival if given early after infection). Binds to F-pili projecting from surface of F1 (male) Escherichia coli. Nine of the 10 reovirus gene segments code for a single protein; the exception is the S1 gene, which codes for both the 1 attachment protein and the 1s nonstructural protein in overlapping reading frames. The viral DNA contains inverted terminal repeat sequences of approximately 100 bp (Figure 23.3). Many of these pathways activate cell metabolism and the cell cycle. Early viral genes are expressed (Baltimore classification scheme). As is described below, 3-terminal hairpins function as primers for the initiation of viral DNA replication. CH019.indd 229 16/05/11 8:07 PM 230 CHAPTER 19 Reoviruses Virion ISVP Core Figure 19.5 Structure of reovirus virions and subvirion particles. Enveloped viruses code for glycoproteins that are inserted into lipid membranes and that direct formation of the viral envelope by a process called budding. However, their original promise as major antiviral drugs has not been fulfilled. CH030.indd 373 05/05/11 1:08 PM 374 CHAPTER 30 Hepadnaviruses Exocytosis ER/GOLGI Budding NUCLEUS Surface proteins Core proteins, polymerase, RNA Reverse transcription Figure 30.7 Assembly of nucleocapsids and virions of hepatitis B virus. Infect humans, mammals, birds, fish, etc. CH006.indd 74 Threefold axes Procapsid Virion Figure 6.6 The ϕX174 procapsid and virion. It was soon recognized that mouse polyomavirus and simian virus 40 are closely related, and they were initially grouped together with the papillomaviruses (Chapter 22), in a family called 247 CH021.indd 247 16/05/11 8:09 PM 248 CHAPTER 21 Polyomaviruses the Papovaviridae. Some genes lying within the b and c segments have names related to their time of expression (0, 4, and 134.5) or other properties (LAT latency-associated transcript; ORF P/O open reading frames P and O). Phagocytosis: the uptake of particulate material from the extracellular medium by vesicles in phagocytic cells. huxleyi to produce a massive flux of dimethylsulfide (DMS) into the atmosphere, whose oxidation products subsequently seed cloud formation, blocking the vital fuel of phytoplankton growth, sunlight. Virus entry into host cells is recognized by an unknown mechanism, independent of nucleic acid. These consist of purified viral proteins that are known to be immunogenic. Finally, late genes encode early transcription factors, which are packaged into virions in preparation for a subsequent round of infection. Virus infection leads to production of double-stranded RNA, which activates transcription of interferon genes. The p53 protein is normally present at low levels in the cell in a latent, inactive form. Apparently, this is easier to accomplish than forming pentamers for the fivefold positions and hexamers for the sixfold positions. Following injection, DNA circularizes via the cos (“cohesive”) sequences at its ends, and the circle is sealed by host DNA ligase. 05/05/11 2:45 PM 474 Glossary Dicots: flowering plants that have two cotyledons, or seed leaves, which emerge from the germinating seed. Since parvoviruses nevertheless need the cellular DNA replication machinery for their own replication, they are able to productively infect only cells that are going through the cell cycle. DNA vaccines are based on DNA plasmids that encode one or more viral proteins in a form that can be expressed in host cells. Shock: inability of circulatory system to maintain adequate blood supply to vital organs; most often caused by low blood volume due to loss of blood. Rotavirus is an important cause of gastroenteritis worldwide and infant mortality in the developing world. Many viral proteins interact with other viral or cellular proteins in functional complexes, and these interactions can be targeted by drugs. The stalled ribosome is able to equilibrate between these two states because of the similar strength of base pairing interactions. This virus replicated and produced infectious virions, but induced cytopathic effects in cultured cells more rapidly than wild-type virus, and gave smaller plaques and lower titers of infectious virus. Unlike viroids, are encapsidated by helper virus proteins. AAV can infect dividing as well as nondividing cells, and its life cycle involves integration of its genome into the host chromosomes. 16/05/11 8:15 PM Human Immunodeficiency Virus However, treatment is costly and requires a high degree of compliance (¬95% adherence to medication schedule) to contain the infection. Furthermore, neurons contain two transcription factors named Luman and Zhangfei, which bind to HCF-1 and prevent formation of the active transcription factor complexes. Two additional genera: Thogotovirus (transmitted by ticks) and Isavirus (infects fish, particularly salmon). Smallpox influenced the course of history, killing rulers, disabling attacking armies, and decimating native populations infected by advancing conquerors. Most modern cultivars of cucumber are either resistant to cucumber mosaic virus, meaning that they cannot be infected, or they are tolerant of cucumber mosaic virus, meaning they become infected but do not show any symptoms. As the leading strands are extended toward each replication fork, large T antigen continues to act as a helicase by unwinding the template DNA. 16/05/11 8:06 PM Bunyaviruses 207 Figure 17.4 Electron micrograph of cells infected with Uukuniemi virus (a phlebovirus) showing virus particles budding into Golgi vacuoles (center) and small vesicles (arrows) budding from Golgi cisternae (Scale bar: 200 nm). The carboxyterminal domain of ICP34.5 is homologous to a corresponding domain of a cellular protein, GADD34. The timing and levels of baculovirus gene expression are transcriptionally controlled. Replication starts with the synthesis of a full-length negative-strand RNA, using the initial positive-strand RNA as a template. Some of the surviving infected B-lymphocytes have a latency II expression pattern. Increased efficiency of processing and export of late RNAs. Before the onset of DNA replication, most mouse polyomavirus late transcripts initiate at a single promoter sequence. Adenovirus E1A protein inhibits the production and action of interferon at the level of transcription. After primary replication in the intestine, type 1 reovirus strains spread through the bloodstream to the brain where they infect ependymal cells, leading to nonlethal hydrocephalus. The viral RNA polymerase presumably synthesizes the initial AAA sequence, then backs up while still gripping the nascent RNA, adds another AAA sequence, then backs up and repeats the process several more times before clearing the promoter and continuing downstream. Because of their structural variability, the virions of most enveloped viruses cannot form crystals, and therefore high-resolution structures of complete virions cannot be obtained by x-ray diffraction. Sixty of the 72 pentamers have six nearest neighbors rather than five! Therefore, the pentamers at these positions have to fit into a “sixfold hole” on the capsid surface, even though they have fivefold symmetry. This leads to an initial loss in the titer of virus detected in the medium. For viruses, the targeted molecules include glycoproteins and nucleic acids, but in particular double-stranded RNA, which is a byproduct of the expression of many viral genomes. Several commercial vaccines now contain oil-in-water-based adjuvants that can both enhance and broaden the antibody response. Both the terminal protein and the inverted terminal repeats are involved in viral DNA replication (see below). Inactivated whole virus vaccines, like live virus, can generate immune responses to a wide range of viral antigens. Nomenclature of herpes simplex virus genes and proteins Partly because of the size and complexity of the viral genome, a number of different naming conventions have been used to identify herpesvirus genes and proteins. Receptors interact with viral glycoproteins, surface protrusions, or “canyons” on the surface of the virion After binding to cell receptors, some viruses are able to penetrate directly into the cytosol by membrane fusion and other mechanisms. Where do flaviviruses acquire their envelopes? Progeny virions are assembled in the cytoplasm Assembly of virus particles takes place in localized regions in the cytoplasm, called virus assembly centers (Figure 27.5c), starting at 2 to 3 h after infection. 10.3: Courtesy of Keith Perry, from VIPERdb2, op. The combined action of these gene products ensures that the conditions in the cell are right for adeno-associated virus replication. The twofold axes (ovals at the center of each edge of the icosahedron) pass through the midpoints of the edges of those triangles, where the heads of two commas lie opposite each other. These viruses contain hundreds of genes that supply many functions usually provided by cellular genomes. Although their genome (5 kb) is actually larger than that of hepatitis B virus (3 kb) and is approximately the same size as the genomes of the polyomaviruses, parvoviruses have small virions because their DNA is single-stranded, and can therefore be packaged very compactly. Many temperate phages carry genes encoding toxins and other proteins that alter the host phenotype. One advantage of helical nucleocapsids is that they can accommodate a variety of genome lengths; the longer the genome, the longer the helical nucleocapsid that assembles on the genome. Envelope derived from plasma membrane. Second, the viral RNA polymerase changes its mode of synthesis, and no longer recognizes the intergenic termination-polyadenylation-reinitiation sequences. Furthermore, pharmaceutical companies have a great deal of experience in developing enzyme inhibitors as drugs. This pocket is lined with hydrophobic amino acid side chains, and is ordinarily occupied by a lipid molecule. A new type of hantavirus disease called hantavirus pulmonary syndrome was described in the southwestern United States in 1993, and was linked to a virus called Sin Nombre (“no name”) virus. However, reporter genes can be expressed in mammalian cells if they are driven by mammalian promoters engineered into the baculovirus genome. Virus particles have multiple copies of receptorbinding proteins on their surface, and red blood cells contain many copies of surface receptors. 2.2: Figs. Toll-like receptor ligands Elicit strong humoral and cellular immune responses. The 3'-terminal 135–200 nts of viral genome RNAs function as promoters for the synthesis of minusstrand RNAs, and the 3'-terminal region of minus-strand RNAs are promoters for synthesis of plus-strand RNAs. The intergenic region of the minus-strand of RNA 3 contains the promoter for synthesis of subgenomic RNA 4. The viral A protein cleaves the plus-strand on replicative form DNA at a specific site (step 1) and is covalently linked to the resulting 5' end of the DNA via a tyrosine residue in the protein. DNA replication initially proceeds in a bidirectional fashion from a replication origin The mechanism of herpesvirus DNA replication can be reconstructed only from knowledge of the proteins involved and in comparison with better-studied systems, because it has so far been impossible to reconstitute origin-dependent herpesvirus DNA replication in vitro. Several topical creams are also available; these have poorly understood antiviral activity or work by stimulating a local cellular immune response against the virus. This would have to occur immediately upon infection before the virus genome has had a chance to replicate (i.e., when only one copy of the genome is present, ensuring that one haploid progeny cell must be virus-free and therefore can survive). For example, patients infected with a strain of virus that lacks the viral nef gene progress to AIDS very slowly if ever. These dimers bind to the operator hairpin, precluding further translational starts of the replicase gene by excluding ribosome binding. Prangishvili, “Morphology and genome organisation of the virus PSV of the hyperthermophilic archaeal genera Pyrobaculum and Thermoproteus: a novel virus family, the Globuloviridae,” Virology 323 (2004), 233242, with permission from Elsevier; Fig. Phagosome: a vacuole formed around a particle taken into a cell by phagocytosis. Upon completion of the review period, please return the evaluation copy to Wiley. However, this reduced expression of MHC proteins can be recognized by NK cells, which are no longer inhibited from attacking the cell. Entry of reovirus into cells using a pathway that bypasses 1 attachment to its receptors, JAM-A and sialic acid, leads to apoptosis, indicating that signaling pathways triggered by interactions between 1 and these receptors are dispensable for this process. The lysis area can be seen as a clear “plaque” against the cloudy background of the uninfected cells, which grow in multiple layers on the surface of the agar in the Petri dish. For togaviruses, each viral glycoprotein molecule in the envelope contacts a capsid protein molecule lying underneath the membrane, with the result that the viral glycoproteins are distributed on the envelope with the same symmetry as the proteins in the underlying capsid. Interferons have diverse effects on the immune system Interferon-, or immune interferon, is produced by a restricted set of immune cells (T lymphocytes, macrophages, and natural killer cells) in response to immune and/or inflammatory stimuli, and its major function is to stimulate the development and actions of immune effector cells. Reverse transcriptase is shown as orange sphere. Conversion of this protein into amyloid fibrils depletes active Sup35 monomer from the cell, and the resultant phenotype is defective in protein synthesis. Families of early (E, in blue), intermediate (in black), and late (L, in orange) mRNAs are shown in relation to the viral genome, divided into 100 map units (⌀350 nt per map unit). The Vpu protein enhances release of progeny virions from infected cells Vpu (virion protein unique to HIV-1) is an 81-amino acid protein that is inserted into membranes via its amino-terminal domain. CH022.indd 271 16/05/11 8:10 PM CH022.indd 272 16/05/11 8:10 PM SECTION VI LARGER DNA VIRUSES OF EUKARYOTES C H A P T E R 2 3 Adenoviruses C H A P T E R 2 4 Herpesviruses C H A P T E R 2 5 Baculoviruses C H A P T E R 2 6 Poxviruses C H A P T E R 2 7 Viruses of Algae and Mimivirus This section covers DNA viruses with genomes ranging from 25 kb to over 1000 kb, larger than the genomes of some cellular organisms! This is a very diverse group of viruses, and our understanding of the largest DNA viruses is still at a very primitive level. Most importantly, six weeks later Jenner challenged Phipps with smallpox by variolation, and found that he was resistant. Envelope assembled at plasma membrane. Resolution of dimers or concatemers into unit-length molecules (step 6) requires a virus-coded resolvase. It happened that the vector integrated itself near the T-cell proto-oncogene LMO2, whose transcription was then activated by the enhancer located within the LTR of the retrovirus vector. Most have very big icosahedral capsids, with internal lipid envelopes and a core containing the genome. In this proposal, the family Coronaviridae has two subfamilies: Coronavirinae and Torovirinae. (eds.) (2002), with permission of ASM Press, Washington, D.C.; Fig. 3. Viral movement proteins are often associated with secondary plasmodesmata, which normally originate from primary plasmodesmata. Depends on hepatitis B virus for its replication. Circularization of DNA joins the late structural genes, originally on the left half of the genome (Figure 8.2), to the right 16/05/11 7:59 PM 88 CHAPTER 8 Bacteriophage Lambda As a first step in understanding how the level of repressor is controlled, let’s look at repressor synthesis in a prophage. Transcription efficiency may be influenced by gene order, formation of RNA secondary structures at the 3' ends of the genes or within the intergenic sequences, overlapping genes, and presence of duplicated termination sites. However, few of these viruses have been studied in detail. These developments could lead to both favorable and unfavorable outcomes. The trapezoids represent a jelly-roll -barrel domain, and the ovals represent loops that form contacts about the threefold axes. Topoisomerase I can produce the same relaxed molecule by cleaving a phosphodiester bond on one strand of supercoiled DNA, allowing the strands to rotate around each other, and resealing the cleaved bond. However, when a termination codon for coat gene translation was engineered upstream of the lysis start codon, the authentic lysis initiation site was again used, because it is closer to the termination site. Therapeutic index: the ratio of the dose of a drug that exerts a half-maximal toxic effect, to the dose that exerts a halfmaximal therapeutic effect. CH021.indd 262 16/05/11 8:09 PM C H A P T E R 2 2 Papillomaviruses Greg Matlashewski Revised by Lawrence Banks Papillomaviridae From Latin papilla (nipple) and Greek –oma (tumor) referring to warts (papillomas) VIRION Naked icosahedral capsid (T 7). Prions are proteins that cause fatal brain diseases The concept that a protein can transmit an infectious disease from one individual to another without the intervention of a nucleic acid genome has caused considerable surprise and controversy within the scientific community, but has now become widely (if not universally) accepted. Binding 2. Above, transcription: viral L protein (orange sphere) cleaves capped cellular mRNAs and uses the 5' end fragment (black line) as a primer for transcription of genome RNA. 283 Cell transformation and oncogenesis by human adenoviruses Adenoviruses (and other DNA tumor viruses) can oncogenically transform cells as a result of their ability to induce active cycling in cells that are normally in a resting state. The word virion is used to denote a complete, infectious virus particle. Acute infection with hepatitis C virus is similar to other forms of acute viral hepatitis. Examples include green fluorescent protein, beta-galactosidase, and chloramphenicol acetyltransferase. A schematic cross-sectional view of a human adenovirus type 5 virion, with components shown as indicated. The organization of the genome clearly established the flaviviruses as a group of enveloped, positive-strand RNA viruses different from the togaviruses, with which they had been previously grouped because of similar virion morphology, genome size, and transmission by arthropod vectors. The majority of cancers that do retain wild-type p53 genes suffer mutations in other genes whose products are required to activate p53 function. A good example can be seen in predator–prey dynamics, particularly virus–host interactions in which hosts must rapidly evolve immune mechanisms to allow survival of the species: “It takes all the running you can do to stay in the same place”. This can also help to avoid recognition of the virus by the host immune system. CH016.indd 193 only four to eight C-terminal amino acids project into the cytosol. Recent evidence suggests that most influenza A virus particles contain exactly one copy of each genome segment in an ordered bundle that can be seen in high-resolution electron micrographs (Figure 18.9). When the polymerase reaches the end of the plus-strand template, it completes the minus-strand by copying the G’s at the 5' end of the plus-strand into C’s. They can also be used to detect antibodies that bind to viral surface antigens, because addition of such antibodies will inhibit hemagglutination. • • Some use viral scaffolding proteins to assemble virions and package genomes. The fibers dissociate, the penton base proteins bind to arginine-glycine-aspartate sequences in the ␣v3/5 integrin (the coreceptor), and when the virion is exposed to low pH, it converts to the lytic form. This means that the first 100 nt at the 5' and 3' ends of each DNA strand are complementary and can base-pair, allowing single DNA strands to circularize, forming a short panhandle end. Simultaneously, host cell DNA polymerase ss plus-strand DNA DNA pol III DNA pol I RNA primer DNA ligase ds RF DNA Figure 6.3 Stage I DNA replication. Transcription of filovirus genomes begins at the NP gene start site, shortly downstream from the 3' end of the genome (Figure 16.3). However, omission of other clones (A, G, H, J–M) resulted in the absence of chloramphenicol acetyl transferase activity (band representing unmodified chloramphenical, labeled C). 1897 Loeffler and Frosch describe “filterable viruses” (foot-and-mouth disease virus). Middle T antigen stimulates protein tyrosine kinases that signal cell proliferation and division Middle T antigen is localized at the plasma membrane of infected cells, where it is inserted via a hydrophobic region near its C-terminus (Figure 21.9). The antigen-binding site is capable of binding to an antigenic determinant with a molecular weight of between 500 and 1500 Da. Therefore the binding site can accommodate an epitope or antigenic determinant of about six sugar molecules or thirteen amino acid residues. Icosahedral symmetry: symmetry of a regular icosahedron, with 12 axes of fivefold symmetry, 20 axes of threefold symmetry, and 30 axes of twofold symmetry. Nucleocapsids enter the cytoplasm via fusion and are transported to the nucleus Replication of hepatitis B virus is most efficient in liver cells (hepatocytes), although other cell types, including monocytes, epithelial cells, and endothelial cells, have been found to support minimal viral replication. QW 160] QR389.A24 2011 616.9'101—dc22 2011002024 Printed in Asia 10 9 8 7 6 5 4 3 2 1 ffirs.indd ii 19/05/11 10:33 AM I dedicate this book to four mentors whose enthusiasm for virology stimulated my interest when I was a student, and who encouraged me to follow my own path. The SOS genes of undamaged cells are repressed by the cellular LexA repressor. Following DNA damage or virus infection, p53 becomes stabilized and activated, and its concentration increases. It contains signals for packaging viral DNA in capsids, and enhances recombination between genomes. In HPV-infected cells (bottom half), E7 binds to Rb, activating E2F and leading to cell DNA replication and cell-cycle progression. GENES AND PROTEINS Each genome segment codes for 1–3 proteins: • • • L segment: RNA polymerase (L) M segment: Two viral envelope glycoproteins (Gn, Gc), cleaved from a precursor during translation S segment: Nucleocapsid protein (N) Zero to three nonstructural proteins are also made, depending on virus strain. This began with the success of acyclovir, a drug directed against certain herpesviruses. These genes are clustered both by function and by the timing of their expression during the life cycle of the phage. CH021.indd 260 CHAPTER 21 Polyomaviruses when large T antigen concentration increases, early transcription decreases. • Viruses with different genome types and replication strategies may have originated independently at different times during the evolution of life. Unlike the togaviruses, which encode their structural proteins on a subgenomic RNA, the flaviviruses have only one messenger RNA, identical to genome RNA. Finally, immune therapy, although not being convincingly successful, should not be dismissed at this time. T antigens bring resting cells into the DNA synthesis (S) phase of the cell cycle The principal function of the early proteins is to enable viral DNA replication. The spectrum of drugs available is changing rapidly, and the drugs described here are used only as examples of the mechanisms of a much larger group of compounds. Dane particles are infectious virions; abundant non-infectious particles lack nucleocapsids The hepatitis B virion, or Dane particle (Figure 30.1), has a diameter of 42 nm. They have no known function. Supercoiled DNA has different physical properties than relaxed circular DNA and linear DNA of the same nucleotide sequence and molecular weight. CH036.indd 446 Capsid-binding drugs act by direct binding to virions. Retroviruses have a single-stranded, positive-sense RNA genome, but they follow a pathway different from other positive-strand RNA viruses. On the one hand, more humans throughout the world will likely enjoy protection against more viral diseases than ever before. Non-enveloped viruses must use other mechanisms to penetrate membrane barriers because they have no lipid membrane. Hemagglutinin: a protein that binds to receptor molecules on the surface of red blood cells, leading to their agglutination. Vaccines against the important human pathogens responsible for influenza and yellow fever were developed during the 1930s. Oncotropism: the ability of a virus to replicate more efficiently in cancer cells than in normal cells. Although productive infection does not lyse the cells, it does slow cellular growth, and therefore plaque assays can be carried out by examining lawns of Sulfolobus cells for lower densities of cells, the result of slow growth of virus-infected cells. Viruses evade innate immune responses Viruses have evolved many strategies to block innate immune proteins, pathways, and cells. 2.8b: PhotoDisc, Inc.; Fig. The pr peptide can be seen as the blue extended oval on the gray E protein surface. TAR forms a stem-loop structure in viral RNA, and two regions are essential for its function: (1) a bulge in the stem, which forms the recognition element for Tat binding; and (2) the nucleotide sequence within the loop. This process of expressing a new receptor-binding protein in a recombinant virus is called pseudotyping. It became clear that Rous sarcoma virus had acquired a mutated copy of the c-Src gene, probably by integrating its proviral DNA nearby the c-Src gene and incorporating that gene into the viral genome during subsequent transcription from the LTR. Highly pathogenic avian influenza A H5N1 strains in poultry farms are a potential threat but are poorly transmitted among humans Starting in 1997, highly pathogenic avian H5N1 strains spread through commercial poultry farms in Hong Kong, and a small number of humans were infected with this virus, causing six deaths. This cleavage renders HA conformationally metastable, like a loaded spring; it responds to low pH with a major conformational change. The virus can also be passed from mother to newborn babies during birth. This DNA has the unusual property of being partly double-stranded, with a single-stranded region of variable length (Figure 30.2a). 1 transcription start site. A 55-kDa virus-coded terminal protein is covalently linked via a serine residue to each of the 5' ends of the viral DNA (Figure 23.3). These are “chemo-attractant” cytokines that serve to recruit immune cells such as lymphocytes and antigen-presenting cells to the site of infection. With no effective vaccines and limited access to drug treatments, the number of infected people continues to grow as the pandemic expands into other developing countries. HIV replicates in and kills lymphocytes and macrophages. The abortive phage infection progresses more or less normally in the early phase, but the class II and class III mRNAs fail to express proteins. Cucumber mosaic virus has a positive-strand RNA genome enclosed in a compact capsid with icosahedral symmetry In 1959, John Tomlinson reported that cucumber mosaic virus has an RNA genome, and he observed virus particles by electron microscopy. Polyomaviruses are tumorigenic in animals when administered at high concentrations, but rarely cause tumors in nature. Two examples of such deleted viral genomes are shown in Figure 28.8. The nontransforming “helper virus” provides the structural and enzymatic proteins necessary for producing virions. Binding of the drug to the pocket displaces the lipid molecule. It is clearly impossible for the viral genome to code for a single structural protein of that size. Bacteriophages have evolved complex mechanisms for puncturing this barrier and penetrating the membranes of bacteria. When the nsp2 protein was deleted from the polyproteins of both murine hepatitis virus and SARS coronavirus, viable mutants were recovered, demonstrating that nsp2 is not required for virus replication. At some point these macromolecules would have been confined within lipid membranes to form cells. Replication of viral genome 5. Evaluation copies are provided to qualified academics and professionals for review purposes only, for use in their courses during the next academic year. 27.7: Courtesy of The Remote Sensing Group, Plymouth Marine Laboratory, Plymouth PL1 3DH, United Kingdom; Fig 27.8: Courtesy of Willie Wilson; Fig. The products of five of the six genes (Table 24.2) are involved in the regulation of viral and cellular gene expression. More than 90% of infected stationary phase cells become lysogenized. For a discussion of linear satellite RNAs of cucumber mosaic virus, see Chapter 10. Viroids were first discovered as the causative agent of potato spindle tuber disease. Highly base-paired structure like viroids. While the external fibril layer remains within the phagosome, small spherical compartments can be seen in the cytoplasm 2 hours post infection; these precisely match the size of the intact virion core containing the viral genome. Polyprotein: a precursor protein that is cleaved by proteolysis during or after synthesis to generate several mature functional proteins. Adenovirus E1A protein acts as a transcriptional activator both of adeno-associated virus genes and of cellular genes involved in DNA replication, by binding to the retinoblastoma (Rb) protein and releasing it from the cellular transcription factor E2F (see Figure 23.6). DISEASES Many are associated with human disease: yellow fever, dengue hemorrhagic fever, tick-borne encephalitis, West Nile virus, hepatitis C virus. Observations made with both kinds of transformed cells have led to a detailed understanding of the molecular mechanisms by which viral gene products induce cell transformation. These primitive cells would have contained small RNA genomes that coded for only a few polypeptides, whose main functions were to replicate the genome RNA and to provide for the entry of substrate nucleotides and amino acids. They also make proteins that inhibit apoptosis, which is induced when the cell undergoes unscheduled DNA replication; otherwise, the infected cell would die before producing progeny virus. Replication of the genome occurs via the synthesis of a full-length complementary antigenome (positive sense) RNA, which then serves as the template for the synthesis of progeny negative-stranded RNA molecules. Cytosolic receptors such as MDA5 (upper left) recruit IPS-1 upon activation, also leading to release of mitochondrial proteins and apoptosis. Study of viruses reveals basic mechanisms of gene expression, cell physiology, and intracellular signaling pathways. Folate receptor-`: a cellular membrane protein that binds to folic acid, a soluble B vitamin. Hypoxemia: low oxygen level in the blood, leading to lack of oxygen supply to tissues and organs. RNA viruses are said by some to be the simplest viruses. • Recognition of viral nucleic acids, particularly double-stranded RNA, leads to the activation of transcription factors, including interferon regulatory factors (IRFs) and NFB. Viral or bacterial vectors Genes that encode viral antigens of interest can be inserted into non-pathogenic viral vectors (avian poxviruses, Semliki Forest virus) or bacterial vectors (attenuated Salmonella typhi) that retain some useful characteristics of the vector organisms (binding and entry, tissue tropism). Smith and Max Summers came up with the ingenious idea that the very strong polyhedrin promoter could be used to drive the high-level synthesis of a foreign protein by infecting cells with recombinant viruses. Their survival strategy appears to be speed, producing progeny at a time when most double-stranded DNA phages are just beginning to make the transition to middle or late gene expression (see Chapters 7 and 8). In these surveys, two additional coronaviruses were identified and classified. DED1 is an essential gene for translation of yeast messenger RNAs in yeast. It has been speculated that this may be the result of widespread cannibalism among humans in the past. A spike, formed by pentamers of the 2 protein (60 copies), projects from the inner capsid through the outer capsid at the 12 axes of fivefold symmetry. Nucleoprotein structures 16/05/11 7:59 PM Bacteriophage Lambda assembled at attL (IHF, Int) and attR (Int, IHF, Xis) combine, and excision occurs. Natural killer cells recognize virus-infected cells and kill them via apoptosis pathways Natural killer (NK) lymphocytes are capable of selectively and rapidly killing virus-infected cells and cancer cells either directly or by recognition of “cell-surface bound antibody”. As will be illustrated in detail below, drugs can block the functions of specific viral proteins that direct specific steps in virus infection. KEY TERMS Apoptosis B lymphocyte Complement Concatemer Cyclin-dependent kinase Dorsal root neurons Encephalitis Heparan sulfate Hepatitis Interferons Latency Latent infection Lumen Major histocompatibility complex (MHC) microRNA Nectin NF B PKR Processivity Proteasomes Proteoglycans Retrograde transport Rolling circle mechanism T lymphocyte Tegument Topoisomerase Tropism Tumor necrosis factor Ubiquitin ligase FUNDAMENTAL CONCEPTS • Most herpesviruses establish latent infections in their hosts after an initial primary infection. The activated B cell usually requires help from a Th2 cell, whose T-cell receptor can recognize and bind to the surface-displayed peptide. Rubella infection (German measles) can lead to fetal abnormalities. The outgrowth of these cells renders them susceptible to subsequent random mutations that result in their transformation. These phages came on the scene just as the first evidence for the existence of • Levivirus (Examples: f2, MS2, R17) Allolevivirus (Example: Q) DISTINCTIVE CHARACTERISTICS One of the smallest and simplest viruses known. However, both of these methods can distort or blur structural details of virions because of staining artifacts and sample damage by the electron beam. However, the consequences for human biology of vaccinating against these types of viral agents are currently unknown. As a result, retrovirus populations cannot be considered uniform but rather are a collection of variants or quasispecies. Plasmid-based Reverse Genetics for Reovirus “Reverse genetics” describes the process by which plasmid copies of viral genomes are manipulated to introduce specific mutations to test hypotheses about functional domains in viral proteins. Viruses follow the molecular rules and regulations that control events inside the cell, but manipulate these pathways to serve their own ends. Herpesviruses 285 Herpesviruses are important human pathogens 285 Most herpesviruses can establish latent infections 286 HERPES SIMPLEX VIRUS 286 Herpes simplex virus genomes contain both unique and repeated sequence elements 286 Nomenclature of herpes simplex virus genes and proteins 288 The icosahedral capsid is enclosed in an envelope along with tegument proteins 288 Entry by fusion is mediated by envelope glycoproteins and may occur at the plasma membrane or in endosomes 288 Viral genes are sequentially expressed during the replication cycle 289 Tegument proteins interact with cellular machinery to activate viral gene expression and to degrade cellular messenger RNAs 289 Immediate early (␣) genes regulate expression of other herpesvirus genes 291 gene products enable viral DNA replication 291 TOC.indd xiv DNA replication initially proceeds in a bidirectional fashion from a replication origin 291 Rolling circle replication subsequently produces multimeric concatemers of viral DNA 292 DNA replication leads to activation of 1 and 2 genes 292 Viral nucleocapsids are assembled on a scaffold in the nucleus 293 Envelopment and egress: three possible routes 294 Many viral genes are involved in blocking host responses to infection 295 Herpes simplex virus establishes latent infection in neurons 296 Latency-associated transcripts include stable introns 296 EPSTEIN–BARR VIRUS 296 Epstein–Barr virus was discovered in lymphomas in African children 296 Epstein–Barr virus infects mucosal epithelial cells and B-lymphocytes 297 Epstein–Barr virus expresses a limited set of proteins in latently infected B lymphocytes 298 Epstein–Barr virus nuclear antigens direct limited replication of the viral genome and activate viral and cellular genes 299 Latent membrane proteins mimic receptors on B lymphocytes 299 Small, untranslated viral RNAs expressed during latent infections target host defense mechanisms 300 25. But there is just one parvovirus known to cause disease in humans, and that is the B19 virus. Eleven of the virus-coded proteins are believed to be virus structural proteins. (a) Differential interference contrast image. Members of the newly recognized domain, the archaea, had previously been considered to be bacteria, as they are unicellular microorganisms that have circular chromosomes and no nucleus. 7–8.5 kb. In contrast to CD4⩽ T cells, HIV-1 infection of macrophages results in a low level of virus replication, with the bulk of the virus accumulating in intracellular vacuoles. This can be blocked by activated p53 via p21. Phycodnaviruses are diverse and probably ancient Members of the Phycodnaviridae are currently grouped into six genera (named after the hosts they infect): Chlorovirus,Coccolithovirus,Phaeovirus,Prasinovirus,Prymnesiovirus, and Raphidovirus (Table 27.1). An enlargement at left shows red blood cells bound together by virus particles. The promoter for plus-strand RNA synthesis consists of two sequence elements separated by one turn of the ribonucleoprotein helix All paramyxoviruses and rhabdoviruses have conserved sequences at the 3' ends of their negative-strand genome RNAs as well as their positive-strand antigenome RNAs. These conserved sequence elements are required for initiation of transcription (mRNA 16/05/11 8:05 PM 182 CHAPTER 15 Paramyxoviruses and Rhabdoviruses (a) Multiple-promoter model 3⬘ 5⬘ X N P/C/V M F L HN (b) Single-entry model 5⬘ 3⬘ N X N P/C/V M N P/C/V M L F HN Figure 15.7 Alternative models for transcription of mononegavirus genome RNA. Parvoviruses may therefore replicate better in cancer cells in part because these cells lack an effective antiviral defense. This leads to removal of the phosphate residue at tyrosine 527, and autophosphorylation of c-Src at tyrosine 416 (~P), activating its protein tyrosine kinase activity. • Rev directs export of most HIV-1 mRNAs from the nucleus to the cytoplasm. A remarkable feature of this system is that newly induced Stat DNA binding activity can be detected in the nucleus within minutes of cytokine binding. VP40 (virus protein, 40 kDa) Associates with membranes and resembles matrix (M) protein; directs budding of virus envelopes at the plasma membrane to form virions. Large T antigen assembles the cellular DNA synthesis machinery to initiate viral DNA replication This process generates a localized region of singlestranded DNA that can now serve as a template for DNA replication. CH009.indd 107 16/05/11 8:00 PM 108 CHAPTER 9 Viruses of Archaea 54 241 192 383b 104a 273 79 70 409 59a 209 82 48 119 117 1334 326b 545 618 362 170 61 58 175 127 80 211 45 98a 240 115 167 86 111 100 59b 189 60 161 155 387 131 187 326a 330 220 98b 84 81 137 ATV 62730bp 892 710 457 193 145 104b 213 653 277 286 34 161b 147 383a 800 529 227 301 38 134 567 1940 Figure 9.12 Genome of Acidianus two-tailed virus (ATV). 16/05/11 8:05 PM Paramyxoviruses and Rhabdoviruses mRNAs are synthesized sequentially from the 3' to the 5' end of the genome RNA The viral RNA polymerase (L protein) begins transcription at the 3' terminal nucleotide of the viral genome (Figure 15.8). The most closely related cellular proteases are not symmetrical dimers. Th2 virokines can alter the host Th1 immune response by preventing the synthesis or release of Th1 cytokines from infected cells, competing for host cytokine receptors, interfering with cellular signal transduction pathways, or inducing host responses that are advantageous to virus replication. Approximately 97% of the known phages of free-living bacteria are large, double-stranded DNA viruses. Karlsson, K. 3TC interferes with the DNA polymerase activity of the P protein. This changes the reading frame; in Sendai virus, edited mRNA with one extra G gives rise to a protein called V, and mRNA with two extra G’s gives rise to a protein called W. processivity factor 1 49 DNA primase* 1 90 Topoisomerase I 1 36 ssDNA binding protein 1 34 DNA ligase* 1 63 Holliday junction resolvase 2 42 Protein kinase (BAF antagonist)* 1 34 Multifunctional “scaffold” protein* ? This also gives rise to two progeny genomes, as the DNA polymerase molecules pass each other on the complementary DNA strands like trains on separate tracks. The RNA catalyzes a specific biochemical reaction in a manner that can transform several substrate molecules per ribozyme molecule. After a slight improvement, the characteristic smallpox skin rash developed and fever resumed, lasting approximately 12 days. Erythroviruses: B19 (humans). Genes boxed above the central line are transcribed from left to right, those below are transcribed from right to left. These differences in envelope composition reflect the different target cells that budded and occlusion-derived virions infect in the insect. In clinical trials, oral pleconaril was found to reduce cold symptoms and virus shedding, but it was not approved by the U.S. Food and Drug Administration (FDA) due to safety concerns. Jak kinases phosphorylate Stat proteins, which dimerize and transit to the nucleus. Sensen, “Elucidating the transcription cycle of the UV-inducible hyperthermophilic archaeal virus SSV1 by DNA microarrays,” Virology 365 (2007), 48-59, with permission from Elsevier; Fig. This means that most progeny virus can be detected only by lysing the cells after harvesting them. Present no danger of inadvertent infection with live virus. Early RNAs are made in the intact core and extruded into the cytoplasm. Poxviruses make several proteins that target host defenses against invading pathogens As obligate intracellular parasites, viruses have no choice but to confront host defenses (see Chapter 33 and 34). Efforts are continuing to provide definite proof of the prion hypothesis. This disease is caused by mutations in a cellular receptor (the c cytokine receptor subunit) whose function is required for differentiation of T lymphocytes. Acheson Polyomaviridae From Greek poly (many) and -oma (tumor) VIRION Naked icosahedral capsid (T 7). • In some cases, virions subsequently bind to a second receptor, which mediates entry into the cell. The PR' promoter (Figure 8.4b) constitutively expresses a short transcript that ends at tR'. From Morbidity and Mortality Weekly Report 1999, 48, 243–248. 24.3b: Courtesy of Wah Chiu, Baylor College of Medicine, from Zhou, A.H., et al., “Visualization of tegument/ capsid interactions and DNA in intact herpes simplex virus type 1 virions,” Journal of Virology 73 (1999): 3210–3218; Fig. It was shown recently that orthobunyavirus mRNAs, unlike cellular mRNAs, are efficiently translated in a process independent of polyA-binding protein (PABP). These are alphavirus RNAs that have undergone large deletions in the nonstructural protein genes, but retain the promoter for the subgenomic RNA and the structural protein genes. However, at the end of the virus replication cycle, binding of virion hemagglutinin proteins to sialic acid CH036.indd 453 453 on the cell surface inhibits release of newly formed virus particles. Translation of this protein starts in the same reading frame as VP2 but at an AUG that codes for an internal methionine in VP2; thus VP3 is identical to the C-terminal two-thirds of VP2. O N3 Azidothymidine Figure 36.4 Structures of selected nucleosides and nucleoside analogues. This enzyme has the unique ability to produce oligomers of ATP via a 2', 5' linkage, in contrast to the normal 3', 5' linkage found in natural RNAs (Figure 33.9). Cells from embryos or newborn animals are often used because of their ease of growth. (e) Human papillomavirus. Genetic engineering to modulate virus pathogenicity, infectivity, or immunogenicity. The alternative template-assisted, catalyzed conversion model is similar to what is seen with a number of proteases (subtilisin and ␣-lytic protease) and the serpin family of protease inhibitors. coli and other bacteria that enable F bacteria to transfer a partial (single-stranded) copy of their chromosome to F cells, which do not possess pili. The function of the NS1 protein in replication remains obscure, but several lines of experimental evidence suggest that it functions in an early step in RNA synthesis. Transcription of the proto-oncogene (exons shown as filled boxes) initiates within the viral LTR, either as a result of inactivation of the left-hand LTR (a) or deletion of the right-hand LTR (b). Others incorporate cellular DNA or RNA instead of the viral genome into the capsid. In undifferentiated basal epithelial cells, the early promoter directs transcription of RNAs that code for all six early gene products. This 90 kDa protein functions as an RNA/DNA-dependent DNA polymerase (reverse transcriptase). Viruses that are evolutionarily and genetically related tend to infect organisms within only one of the above six categories. Type II transmembrane protein: an integral membrane protein oriented with its carboxy terminus facing the lumen of the endoplasmic reticulum or the extracellular space and its amino terminus facing the cytosol. Most other arthropod-borne viruses are members of the Flaviviridae family (Chapter 12; named after yellow fever virus) or the Bunyaviridae family (Chapter 17); these viruses have distinct genome organization and replication strategies. In most cases, the cell is not lysed and continuously releases virions by budding into

the extracellular medium. Via successive rounds of binding and cleavage, a single ribozyme molecule can therefore cleave many substrate molecules, establishing a classic enzyme/ substrate relationship. Their small number of genes and their strong dependence on cellular macromolecular synthesis also make them useful model systems for studying mammalian RNA synthesis and processing, DNA replication, and signal transduction. Figure 29.1 Events associated with progression to AIDS. Hepadnaviruses can be seen as a special case of the category of viruses that have single-stranded DNA genomes (#5 above), because like those viruses, their genomes are made fully double-stranded by cellular DNA polymerases after entering the cell. About four out of 10 ribosomes successfully navigate the pseudoknot structure and therefore terminate at the end of ORF1a, resulting in a lower abundance of proteins in the ORF1b region, most of which are involved in viral RNA replication and modification. Coding regions are shown in orange. Copyright © 2011, 2007 John Wiley & Sons, Inc. A site on the surface of the capsid, near the threefold axes of symmetry, binds reversibly to glucose. This resource has basic information on many viruses and facilitates entry into protein and nucleic acid databases relevant to each virus family or species. Theiler’s murine encephalomyelitis virus causes a neurologic disease in mice that ranges from paralytic polio-like symptoms to a persistent infection accompanied by chronic demyelination, depending on the strain of virus. As noted above, both human–human and human–bovine chimeric rotavirus vaccines have recently been licensed, and many other conceptually similar vaccines are currently in clinical trials. (b) A diagram showing occluded virions. However, the dependoviruses require coinfection with an adenovirus or a herpesvirus to undergo productive replication; these viruses are therefore named adeno-associated viruses (AAV). (a) Bunyaviruses encapsidate a segmented RNA genome in a simple enveloped particle Like the orthomyxoviruses and the reoviruses (Chapters 18 and 19), the genome of bunyaviruses consists of multiple distinct RNA molecules. Transmission efficiency varies with different hosts, aphids, and virus strains. The capped RNA transcripts are extruded through channels within the spikes to the exterior, where they are released into the cytoplasm (Figure 19.6). Natural killer cells can recognize and kill infected cells that are lacking certain cell surface proteins. Symptoms range from rashes and high fevers to joint pains and encephalitis, and the outcome can be fatal in both humans and domestic animals such as horses. As in the case of Onyx015, Gendicine works better in combination with conventional treatments such as radiotherapy. Interferons-␣, , and primarily induce genes involved in blocking virus replication. Acyclovir is selectively phosphorylated by herpesvirus thymidine kinases Acyclovir exhibits a very high therapeutic index (TI dose that exerts a 50% toxic effect divided by dose that exerts a 50% antiviral effect). If such a reassortant virus can replicate well in the animal host, it is propagated. Open reading frames of the six early (E) genes and two late (L) genes are shown in boxes. 3' hammerhead 3' 381 U GA C AGU G U C AA A C GA C A G A hairpin G A A A C A C A C C UA G G U C C A U U A U A U G (d) O O CH 3' O BASE1 O OH 2' O P CH 3' O O Mg O BASE1 O 2' P O O O 5' CH O BASE2 5' OH CH O O BASE2 OH O OH G U G U U Figure 31.3 Self-cleaving RNA structures and the cleavage reaction. The product of the vlf-1 gene, very late factor, is involved in the high-level expression of very late genes. How does the replicase copy the genome RNA starting at its 3' end while ribosomes are translating the same RNA in the opposite direction (Figure 5.4)? A proteinaceous infectious agent called a prion (“pree-on” or “pry-on”) appears to contain no nucleic acid, and only a single species of protein called PrP (prion-associated protein). Most plant viruses are named in a similar manner, after the host where they were first discovered and the disease symptoms caused in that host. Study of tumor viruses led to discoveries in molecular biology and understanding of the nature of cancer Virus research underwent an explosive development in the second half of the twentieth century that led to the discovery of many new viruses and basic concepts in cell and molecular virology. Rabies is a fatal human encephalitis caused by a rhabdovirus Rhabdoviruses were named because of the unusual rodor bullet-shaped morphology of virions. Uncoating Amantadine (Influenza A) 4. Virus titers drop as the immune system responds with both cytotoxic T lymphocytes and antibodies (see Chapter 34). Once initiated, nucleocapsid formation continues by adding N protein subunits every six nucleotides without a further requirement for specific binding sites on the RNA. The Sendai virus attachment protein (HN) has neuraminidase activity, which, like the influenza virus neuraminidase (Chapter 18), can release progeny virions that bind to the surface of the cell from which they are extruded. Hemolytic anemia: a disease caused by shortage of red blood cells due to their fragility. White; Fig. huxleyi killed by viruses over geological time. (a) Bacteriophage fd. CH012.indd 146 16/05/11 8:03 PM Flaviviruses 147 • Like other positive-strand viruses, flavivirus RNA synthesis takes place in association with cytoplasmic membranes. Herpesvirus nucleocapsids are assembled in the nucleus, and need to exit this compartment and acquire an envelope. and Howard Scott et al. Viral DNA enters the nucleus of the neuron, is circularized, and remains dormant; transcription is repressed and there is no DNA replication. Finally, three other cellular proteins, replication protein C (RPC), proliferating cell nuclear antigen (PCNA), and DNA polymerase (delta), are recruited to the complex (Figure 21.12b). These factors cannot bind efficiently if the ends of the intron are too close to each other, as they mutually interfere with each other’s binding (“steric hindrance”). This virus has a 20-kb circular, double-stranded DNA genome that, unlike genomes of most viruses of archaea, is extensively methylated. The second edition includes five new chapters: two survey chapters, “Viruses of Archaea” and “Viruses of Algae and Mimivirus”; a chapter on a well-studied plant virus, “Cucumber Mosaic Virus”; and two chapters on the host response, “Cellular Defenses Against Virus Infection” and “Innate and Adaptive Immune Responses to Virus Infection”. The role of the 6K protein, which is found in reduced levels relative to E1, E2, and capsid in the virion, is obscure. coli lives, for example, in the intestinal tract of humans and other animals. The functions of the small, hydrophobic NS4A and NS4B proteins are not known, although NS4A has been implicated in the formation of virus-induced membrane structures that support RNA synthesis. • Retroviruses lacking oncogenes can also transform cells if their proviral DNA is inserted nearby a gene encoding a cellular signaling protein whose transcription is stimulated by the retrovirus promoter/enhancer. Nicking of one strand (upper right) allows the supercoiled form to unwind to its lowest-energy relaxed circular state; a portion of the double-helix containing a nick is shown enlarged above. A long-standing problem in studies of viruses in the Reoviridae family was recently solved with the development of a fully plasmid-based reverse genetics technology for mammalian reoviruses (Figure 19.9). Bunyaviruses that use an ambisense strategy transcribe some of their mRNAs from antigenome templates (Figures 17.2 and 17.3). No one had imagined that a virus might encode an ion channel. All genes are flanked by highly conserved sequences that signal transcription termination, polyadenylation, and transcription reinitiation. CH023.indd 283 It was hypothesized that in normal cells infected with this virus p53 will remain active, leading to poor replication of the virus, little spread, and only limited pathological consequences. This mutant over produces GP and makes no sGP. Studies on experimentally infected wild animals showed that fruit-eating and insect-eating bats support replication and circulation of high titers of Ebola virus without necessarily becoming ill. What role does small T antigen play in bringing resting cells into the DNA synthesis phase of the cell cycle? If, however, the miRNA is perfectly complementary to its target, the mRNA is degraded, as occurs with siRNAs. So far, more than 500 different human miRNAs have been discovered, and many are expressed in a developmental or tissue-specific manner. Vaccination with defined proteins With the exception of the vaccines for hepatitis B virus and human papillomavirus, which contain single viral proteins, all antiviral vaccines presently in use are based on either live attenuated, live chimeric, or inactivated whole viruses. However, the vast majority of new coronavirus sequences that have been submitted to databases are from bat viruses; roughly half of the coronaviruses now known are bat coronaviruses. These vaccines are generally less sensitive CH035.indd 434 to storage conditions and are safer for immunocompromised individuals. The hairpins and immediate flanking sequences have important functions in DNA replication. Virus mutants arise rapidly because of errors generated during reverse transcription Once released into the cytoplasm, the viral nucleocapsid partially breaks down to permit access to nucleotide CH029.indd 358 pools within the cell. Each particle contains about the same amount of RNA, ranging from 3000 to 3360 nt. On the other hand, if the ribosome binds first to the coat protein gene, replicase will not be able to bind to its template, and therefore the ribosome is free to complete its voyage unhindered. Coronaviruses have enveloped virions containing helical nucleocapsids The coronavirus virion is a spherical enveloped particle, ranging in size from 75 to 160 nm, with a characteristic crown of spike proteins that gives the virus family its name (Figure 14.2). It is interesting to note that all paramyxoviruses for which the rule of six strictly applies use RNA editing for expressing one or more viral proteins. Removal of template RNA. 12.1–12.6: Courtesy Richard Kuhn, Purdue University. (b) The structure of PrPC has been determined by nuclear magnetic resonance and x-ray crystallography (Figure 32.5). Advantages and limitations of retrovirus vectors The main advantage of retrovirus vectors is their capacity to integrate their genome efficiently into the cell chromosome, which ensures the permanent presence of the transgene in transduced cells and their progeny. In China in the tenth century A.D., practitioners pulverized pock scabs from individuals undergoing mild smallpox infections, and blew the powder through a tube into the nostrils of the recipient, resulting in a generalized but nevertheless relatively mild infection. C K N M F E I G L J H O,P DNA glycosylase capping enzyme subunit MV core protein D1R D3R D4R D A early transcription initiation factor RNA polymerase subunit DNA primase D5R D6R D7R mRNA decapping enzymes D9R D10R D2L D8L IMV core protein MV membrane protein early B D11L D12L early transcription termination ATPase D13L Cresent scaffold proteins capping enzyme subunit late early + late Figure 26.2 Functional organization of the vaccinia virus genome. RNA replication is associated with vesicles in cytoplasm. S assembly is regulated so that the pore forms at the proper time. The erythrovirus B19, named for its specificity for red blood cell precusors, is noteworthy as a parvovirus that is pathogenic for humans (see below). coli thioredoxin. Similarities between the atomic structure of parvovirus and microvirus capsid proteins are striking, as are the mechanisms employed for DNA packaging (however, parvovirus genomes are linear, not circular). Both the small and large forms of this protein contain nuclear localization signals and RNA-binding regions. 389 Similarities between symptoms and brain pathology in these diseases and kuru and scrapie led to extensive efforts to identify a causative infectious agent. 1975–present Introduction of vaccines for hepatitis B virus, hepatitis A virus, varicella zoster virus (chickenpox), live, coldadapted influenza virus, rotavirus, and human papillomavirus. Finally, VLPs that mimic the enveloped viruses can be generated in a number of prokaryotic and eukaryotic expression systems, including plants. 382 Interaction of viroid RNA with cellular RNAs or proteins may disrupt cell metabolism 382 RNA interference could determine viroid pathogenicity and cross-protection 382 Circular plant satellite RNAs resemble viroids but are encapsidated 383 Hepatitis delta virus is a human viroid-like satellite virus 383 Hepatitis delta virus may use two different cellular RNA polymerases to replicate 383 RNA editing generates two forms of hepatitis delta antigen 384 Conclusion: viroids may be a link to the ancient RNA world 384 32. Vaccination has led to a decrease from 4 million cases per year in the United States to less than 500 cases per year. 16/05/11 7:59 PM C H A P T E R 9 Viruses of Archaea David Prangishvili Archaea (singular: archaeon) From Greek archaios, “ancient” THE THREE DOMAINS OF LIFE: ARCHAEA, BACTERIA, EUKARYA Archaea are unicellular organisms lacking a nucleus that are phylogenetically distinct from bacteria and eukaryotes • • • • Cell walls made of S layer of protein Ether linkages in phospholipids Proteins initiated with methionine, not N-formyl methionine RNA polymerase and promoters resemble eukaryotic transcriptional machinery Many archaea thrive in extreme environments • • • • Thermophiles (¬45C) and hyperthermophiles (¬80C): volcanic hot springs Acidophiles (pH 0–3): volcanic hot springs Halophiles (high salt concentrations): salt lakes Anaerobic environments (methanogens) Two major phyla of archaea: Crenarchaeota and Euryarchaeota. Capsid proteins that recognize and bind to receptors on the surface of susceptible cells would have been selected. Although they contain substantial open reading frames, there is no evidence that LAT RNAs express any proteins, and their accumulation as introns in the nucleus argues against a role as mRNAs. The role of LATs remains controversial. Data from images of many different virions are combined by computer programs to build an overall averaged image of the structure at a higher resolution than is possible by direct imaging of individual particles (e.g., Semliki Forest virus, Figure 2.1i). budding Virion Plasma membrane cytosol Trans-Golgi vesicle Cleavage Glycosylation Palmitoylation Nucleocapsid lumen E2 E1 E3 RNA C N Capsid pE2 Endoplasmic reticulum cytosol 6K Figure 13.4 Processing and modification of Sindbis virus structural proteins. Many enveloped viruses with helical nucleocapsids have an internal matrix protein that mediates the association between the viral glycoproteins inserted into the plasma membrane and the nucleocapsids in the cytoplasm. The amino terminus of M is exposed on the outer surface of the envelope and is in contact with the spike protein; the carboxy terminus extends within the virion underneath the envelope, and is in contact with the viral nucleocapsid. C DNA packaging. Six to eight different segments. The middle circle (green) shows location of individual genes. • Viroids and hepatitis delta virus RNA are replicated by host cell RNA polymerases that normally transcribe DNA. Parvoviruses have very few genes Table 20.1 Some representative parvoviruses Subfamily Example Host Autonomous parvoviruses Minute virus of mice (MVM) Canine parvovirus Mouse Dog Dependoviruses Adeno-associated virus (AAV) Human CH020.indd 239 Erythroviruses B19 virus Human Densoviruses Culex pipiens densovirus Mosquito The coding region of parvovirus DNA (Figure 20.2) can be divided into two approximately equal sections. Shown are the enzymatic steps that result in the phosphorylation of acyclovir to the mono-, di-, and triphosphate forms, the last of which blocks viral replication. Several viral gene products interfere with cellular transcription or translation and inactivate cellular defenses, therefore increasing the chance that progeny viruses are made in large numbers. As a combined result of interference with proper 3' end processing and cleavage near their 5' cap structures, most cellular pre-mRNAs are degraded in the nucleus, and cellular mRNA production is therefore suppressed. In Arabidopsis (thale cress), elongation factors that specifically reduce the synthesis of movement protein result in resistance. 32.6a, b: Fig. Furthermore, they cannot use the right-hand LTR to drive transcription of adjacent cellular genes, which could be problematic if the proviral DNA integrated CH037.indd 463 463 next to an oncogene (see Figure 28.9). Chapter 25 Fig. Phosphorylated Shc leads, through an activated Ras protein, to the activation of the MAP kinase pathway referred to above. Fusion releases the nucleocapsid and tegument proteins into the cytoplasm. This results in the formation of multinucleated cells called syncytia. Steps along the entry pathway are shown in Figure 24.4. Viral genes are sequentially expressed during the replication cycle Like the smaller DNA viruses of eukaryotes discussed in this book, transcription of herpesvirus genes is carried out in the nucleus by cellular RNA polymerase II. Research on animal, insect, and plant viruses has shed light on the functioning of these organisms, their diseases, and molecular mechanisms of replication, cell division, and signaling pathways. These include entecavir, telbivudine, tenefovir, and adefovir. The released virions pass through the peritrophic membrane and invade the columnar epithelial and regenerative cells of the midgut by attachment and fusion of the viral envelope with the cell microvilli. Integration of viral DNA into the cellular genome only occurs during the development of the cancer; viral DNA in productively infected cells is present as a free plasmid. Hosts are members of the genera Thermoproteus, Sulfolobus and Acidianus, isolated from terrestrial hot springs of Iceland, USA, Russia, and Italy. I am grateful to Professors Dal Briedis, Mike DuBow, and John Hassell, with whom I collaborated in designing and offering this course. Also important is the nature of the virus itself. However, vaccines based on virus-like particles are expensive to manufacture and require injection of several doses, and poorer countries may have difficulties in adopting their widespread use. The time course of appearance of infectious virus particles, viral mRNA, DNA, and proteins during a typical replication cycle of mouse polyomavirus in baby mouse kidney epithelial cells. Palmitoylation: covalent attachment of palmitic acid to cysteine sulfhydryl groups in a protein; increases affinity of the protein for lipid membranes. Carstens Baculoviridae From Latin baculum (stick), referring to shape of virion VIRION Enveloped rod-shaped nucleocapsid. They replicate not by disintegration and reassembly, but by growth and division into daughter cells. As a result of these studies, it has been determined that HIV-1 is a zoonotic infection likely transmitted in the early 1900s from butchered chimpanzees infected with the chimpanzee strain of SIV (SIVcpz) to humans in West-Central Africa. Interestingly, synthesis of full-length mRNAs of some bunyaviruses has been shown to depend on ongoing protein synthesis in certain cell types. Usually used for observation of thin sections of cells. (a) Fiber Penton base Peripentonal hexon (b) Hexon Group of nine hexons Figure 2.7 Structure of the adenovirus capsid. The membrane fusion pathway has the advantage that viral macromolecules do not need to pass directly across the hydrophobic interior of any cellular membrane. Many cell surface proteins that are used as virus receptors have immunoglobulin-like folds, and several viral structural proteins are known to have such folds. integrated prophage. This enzyme complex leads into the NF-B signal transduction cascade. Similarly, enzymes involved in DNA replication in archaea are more like those used by eukaryotes than bacteria. Development of maraviroc Maraviroc is an inhibitor of HIV-1 attachment and entry that was approved by the FDA in 2007. The target RNA in the clinical sample is first hybridized to a complementary DNA oligonucleotide that carries a magnetic bead at one end, which allows binding to a solid support (the magnet). M2 forms tetramers by lining up four parallel transmembrane ␣-helices, thus creating a small pore in the viral envelope. The most dramatic success with gene therapy employing recombinant AAV occurred in three trials for Leber congenital amaurosis, a genetic disorder progressively leading to blindness by age 10. (a) Viral genome with inverted repeats (orange and black arrows), and a likely origin of replication (green diamond). The difference comes mostly from a region on the genome used to code for a protein called readthrough protein or protein A1. Human adenoviruses have recently become of great importance as gene therapy vectors and are one of several promising new oncolytic virus anticancer agents. However, the species barrier is not absolute. P123 does not undergo autoproteolysis and it is not cleaved during the early phase, suggesting that the protease domain in nsP2 is only able to cleave other polyprotein molecules than the one in which it lies. Poliovirus: a model picornavirus for vaccine development and studies of replication John Enders and coworkers showed in the late 1940s that poliovirus and other human viruses could be grown in cells cultured in vitro. Macrophages localized in tissues are activated by infection and kill viruses or infected cells using toxic oxygen compounds Specific antiviral antibodies bound to virus particles and virus-infected cells can also lead to engulfment or attack by macrophages (Figure 34.1), leading to cell lysis, often referred to as antibody-dependent cellular cytotoxicity. This could contribute to the buildup of vesicles in infected cells. If viral DNA is inserted adjacent to or within a cellular proto-oncogene, the altered pattern of expression of the gene can lead to tumor formation if the infected cell is not killed by the resident retrovirus. Chappell Terence S. Viral replication is one stimulus that can induce apoptosis. Douglas, and M.J. Young, “Hot crenarchaeal viruses reveal deep evolutionary connections,” Nat. Secondary transcription Early replication complexes Replicase particles Virion assembly Figure 19.8 Assortment of gene segments and virion assembly. Hosts of these viruses are members of the genera Sulfolobus and Acidianus, and experiments are usually carried out at approximately 80°C and in medium of pH 3. The number of positive strands synthesized in infected cells exceeds the number of negative strands by a ratio of 40 to 1. APOBEC3G therefore functions to defend the cell against infection by mutating the DNA copy of the 16/05/11 8:15 PM 362 CHAPTER 29 Human Immunodeficiency Virus HIV-1 genome. Normally, only one or a few phages bind and initiate infection. It is even less stable in metabolically active cells than in inactive cells. Without these cleavages, the progeny virions are unable to infect cells and therefore the infection does not spread. Overcoming host restriction/modification. Ethical issues in the use of antiviral vaccines Many of the viruses targeted by vaccine programs are among the most highly transmissible infectious agents known. However, it is not understood how the large viral genome is transported through the cytoplasm, nor how it enters the nucleus. 61 Phage RNA is translated and replicated in a regulated fashion Once inside the cell, the phage RNA begins to function as a messenger RNA for the synthesis of phage proteins. 3 in F.W. Studier and J.J. Dunn, Cold Spring Harbor Symposia on Quantitative Biology 47 (1983): 999, with permission from Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY); Fig. 111 SEC-III.indd 111 16/03/11 7:07 PM C H A P T E R 1 0 Cucumber Mosaic Virus Ping Xu Marilyn J. As a result, the virions find themselves in endosomes whose pH is lowered. The virus was demonstrated to be subtype H1N1. • “Empty” capsids that contain no viral genome can be made in large numbers. Hyaluronan is a polymer of disaccharides, themselves composed of D-glucuronic acid and D-N-acetylglucosamine, linked together via alternating -1,4 and -1,3 glycosidic bonds. Afterward, these proteins must be extruded through holes in the capsid, presumably by assuming an alternate, flexible structure. RSV: Rous sarcoma virus U3 region (promoter); RRE: Rev-responsive element; cPPT: central polypyrimidine tract. VIRUSES AND HOSTS 12 genera, including Orthoreovirus, Rotavirus, Orbivirus. This started in the United Kingdom as early as 1926, but the volume of recycled animal protein increased rapidly over the twentieth century. As a result, this glycoprotein is secreted into the endoplasmic reticulum but is not anchored in the membrane. (a) Structure of the Vp54 monomer with strategic amino acids labeled. Alternate pathway: an arm of the complement system that induces formation of the membrane attack complex in the absence of pathogen-specific antibodies. (a) In the absence of Vpu, CD4 interacts with gp160 in the endoplasmic reticulum, resulting in formation of a complex that blocks transport to the cell surface. The primary lymphoid organs are the bone marrow and thymus; the secondary lymphoid organs are the spleen, lymph nodes, Peyer’s patches, adenoids, and tonsils. When particular compounds were known to have antiviral activity, a variety of other compounds with similar structures were tested for activity. The remainder of the US region, containing genes, is not shown. This implies that the template RNA is actually threaded through the RNA polymerase complex as elongation proceeds, while its 5' end remains bound to the complex. Cauliflower mosaic virus, a plant virus, also has circular DNA, has overlapping reading frames, and uses a reverse transcriptase during its replication. 27.1: Courtesy of James Van Etten; Fig. Furthermore, most of the genomes of the Paramyxovirinae subgroup (measles, mumps, Sendai, etc.) sequenced to date have lengths that are exact multiples of six nucleotides. Its precursor interacts with E during virus assembly (see below and Figure 12.2a, b). Newly synthesized nucleocapsids containing minusstrand genomes can be transcribed to produce more viral mRNAs or can generate more full-length, plus-strand RNAs for genome replication (Figure 18.6); the balance between these activities depends on the amount of NP 16/05/11 8:07 PM 218 CHAPTER 18 Influenza Viruses 5' PB1 AG 3' UC ppp GA ppp 5' Genome RNA Free NP protein pppAG PB1 UC GA ppp pppAG PB1 CU GApp UC 5' pppAG CU 3' Antigenome RNA GA ppp 5' 3' UC Genome RNA Figure 18.8 Replication of influenza virus genome RNA. Therefore, the synthesis of these mRNAs must await the production of sufficient N protein for genome replication to begin. The importance of NS1 in facilitating virus replication was shown by poor replication of influenza viruses that have a mutated NS1 gene. • Baculoviruses undergo a temporally regulated replication cycle in insect cell cultures, producing both budded and occlusion-derived virus. See also Eukaryotic virus vectors Warts, 263–264 West Nile virus, 137 entry into North America, 146 Wrapped virus (WV), 321 X protein, 369 X-ray diffraction, 19 Yellow fever, 138 1793 epidemic in Philadelphia, 138 proteins of yellow fever virus, 143 vaccination, 139 Yellow Fever Commission, 138 Zanamivir, 222, 445, 454 Zoonotic disease, 197 Zoonotic infections, 429 12/05/11 4:08 PM Animals including mice, dogs, cats, pigs, cattle, turkeys, bats: murine hepatitis virus (MHV), transmissible gastroenteritis virus (TGEV), avian infectious bronchitis virus (IBV) DISEASES Coronaviruses are responsible for up to 30% of common colds in humans. Viruses using CXCR4 are designated X4 (or T celltropic) viruses, whereas those using CCR5 are termed R5 (or macrophage-tropic) viruses. Early genes are expressed within virus cores, which are released into the cytoplasm after fusion of the virion envelope with cellular membranes during virus entry (Figure 26.5). The smallest mRNA encodes the X protein. Infection of monkeys with simian immunodeficiency virus containing point mutations in the Nef gene resulted in the rapid generation of revertant viruses with a functional nef gene, showing that there is considerable selection pressure for an active Nef protein. It cannot be excluded that archaeal viruses may encode unusual DNA polymerases, which cannot be identified by comparative sequence analysis. Viruses. The increased level of these proteins contributes to the shift to vegetative DNA replication, during which many thousands of viral DNA molecules per cell are made. Reconstructed from cryoelectron microscopy images. This cleavage is not carried out by the known viral proteinases (L, 2A, 3C, or 3CD) and, because the cleavage site is found on the inside of procapsids and provirions and would not therefore be accessible to proteolytic enzymes, there has been speculation that viral RNA or the capsid proteins themselves participate in the cleavage reaction. Because each virus particle carries many such sites, there are usually multiple receptor-virus contacts. Bunyaviruses use a similar cap-stealing mechanism to prime viral mRNA synthesis, but they replicate in the cytoplasm and use mature cellular mRNAs as a source of caps (Chapter 17). The stain tends to form electron-dense pools around virus particles; virus particles exclude the stain and therefore show up as light images against a dark background, and much fine surface detail can be seen (Figure 1.2). Has a satellite virus: hepatitis delta virus (see Chapter 31, Viroids). ss56 is the start site for synthesis of N mRNA. Because they do not replicate, these vaccines are usually less efficacious, are often administered with adjuvants, and may require booster doses. Nucleocapsids protect the enclosed viral RNA from ribonuclease digestion. Chloramphenicol acetyltransferase: an enzyme that transfers an acetyl group from acetyl-coenzyme A to chloramphenicol. If the multiple-promoter model were correct, the level of inhibition of mRNA synthesis by UV irradiation of the viral genome should depend only on the length of the mRNA being made. The CH013.indd 153 153 In addition to being produced in large amounts, the subgenomic mRNA, which encodes viral structural proteins, is also very efficiently translated. It plays a role in the long-distance movement of cucumber mosaic virus in infected plants, and helps to counteract the host defense system of RNA silencing (see below). Scientific understanding of steps in viral replication and the availability of automated tests to detect antiviral activity of thousands of compounds have led to significant breakthroughs. orientalis and C. The RNA polymerase eventually terminates transcription at that position. Approximately 50 “housekeeping” genes, involved in processes common to all poxviruses such as transcription, DNA replication, and morphogenesis, are conserved among the poxviruses and are generally clustered in the center of the genome (Figure 26.2). TLR-3 is essential for the recognition of infection by multiple viruses, including encephalomyocarditis virus, respiratory syncytial virus, herpes simplex virus, influenza viruses, and West Nile virus. Unlike paramyxoviruses, rhabdoviruses enter the cell by endocytosis, and fusion with the endocytic membrane occurs at low pH, releasing the nucleocapsid into the cytoplasm. Budded virions: for baculoviruses, virions that are produced by budding from the plasma membrane (see Occluded virions). Cleavage by proteinases PLP1 and PLP2 (black, cleavage sites shown as vertical arrows) yields nsp1–3, and cleavage by 3CLpro (black) yields nsp4–16. For high throughput screening, it is important that the target protein can be expressed and purified easily, and assayed rapidly (i.e., within hours or even minutes) using robots. It is believed that the large hepatitis B surface protein undergoes proteolytic cleavage as it interacts with the cellular membrane, exposing a fusion peptide. Key to the function of Tat is the presence of a sequence element located just after the HIV-1 transcription start site designated the Tat-responsive element (TAR) (Figure 29.5). The gokushoviruses, on the other hand, infect obligate intracellular bacteria, a niche where very few double-stranded DNA phages have been isolated. Human hepatitis B virus became the archetype of the Hepadnaviridae family. Assembly of the icosahedral capsid is initiated on a donut-shaped dodecamer of B, the “portal protein”. CD4 and CD8 proteins direct these different T-cell populations to interact with specific target cells displaying either MHC type II or type I proteins on their surface. The two ends of the nucleocapsid are capped asymmetrically, giving polarity to the virion. Coincident with the onset of synthesis of late proteins, ICP27 begins to shuttle between the nucleus and cytoplasm, exporting viral mRNAs. CH024.indd 293 Viral nucleocapsids are assembled on a scaffold in the nucleus An immature capsid lacking DNA is assembled by accumulation of the major capsid protein VP5 and several other proteins around a scaffold (Figure 24.8). CH011.indd 136 16/05/11 8:02 PM C H A P T E R 1 2 Flaviviruses Richard Kuhn Flaviviridae From Latin flavus (yellow), referring to jaundice caused by yellow fever virus VIRION Spherical enveloped particle. siRNAs become part of a RISC complex that cleaves viral RNAs with homologous sequences. Mature virus and extracellular virus use distinct viral structural proteins for binding to cell receptors. Immunization against smallpox by inoculation with cowpox was introduced by Edward Jenner, an English physician, in 1796. The infectious cycle ends when the accumulation of the lysis protein in the cytoplasmic membrane has caused the collapse of the cell wall. The outer surface of the virion consists of 80 flower-like structures each made up of three subunits, projecting outward in the form of petals (Figure 13.1a). During reverse transcription, the growing DNA chain is transposed (“jumps”) from one end of the RNA template to the other end; chain transfer can alternatively occur to the second packaged copy of the RNA genome. When this occurs in vivo, such cells can eventually evolve to form tumors. These cleavages may be within the same polyprotein molecule, or one 3C region can cleave within another polyprotein molecule. Concurrently, the local population of crows in the greater New York City area suffered from disease, and many were found dead. All known viruses in this group have helical nucleocapsids. The genome, although linear, contains no free 5' and 3' ends; each terminus is sealed in a hairpin structure. It is generally thought that herpesviruses use the nuclear membrane to form an envelope as a means of escape from the nucleus. Cytokines that are produced within a virus-infected cell are secreted and can act either on nearby cells or at distant sites. When N levels drop (top), trailer RNA is not encapsidated; RNA polymerase terminates and does not complete replication of the antigenome. Ciliated epithelium: epithelial cells that line body surfaces such as the respiratory tract and which are covered with cilia. The 5⬘ ends (transcription initiation sites) are shown as vertical lines and 3⬘ ends (polyadenylation sites) are shown as arrowheads on mRNAs. PE and PL: early and late promoters. Translation termination signals for large T antigen (UGA) and VP1 (UAA) are underlined. Hundreds of known virus species. CH018.indd 224 16/05/11 8:07 PM C H A P T E R 1 9 Reoviruses James D. 239 Discovery of mammalian parvoviruses Several autonomous parvoviruses were discovered during the hunt for tumor viruses. A lentivirus vector was constructed to express a glycoprotein whose sole function is to mediate fusion. A nasal spray formulation of pleconaril is currently under investigation. Virus assembly takes place at Golgi membranes Maturation of bunyaviruses occurs at the smooth membranes in the Golgi apparatus (Figure 17.4), and is inhibited by the drug monensin, a monovalent ionophore that disrupts protein transport through Golgi membranes. The main difference between the mimivirus and all other viruses is sheer size. Their role in the viral genome is not understood. The tripartite leader directs efficient translation of late adenovirus proteins The cellular translation initiation factor eIF4F normally binds to the 5' cap structure on mRNAs and enables CH023.indd 282 the 40S ribosomal subunit to scan mRNAs for an appropriate AUG initiation codon (see Figure 11.5a). Most modern cultivars of cucumber are infected asymptomatically. The successful adaptation of rabies virus to the human diploid fibroblast cell line WI-38 in the early 1960s finally permitted the development of a rabies vaccine that is both effective and safe. Before DNA replication begins, low but detectable CH021.indd 259 levels of late transcripts are made. These giant viruses are as large as some of the smallest free-living cellular organisms, including mycoplasma and some archaea, and unlike most viruses, are retained by small-pore filters. Replicase genes are translated directly from genome RNA. Granzyme B: a cytolytic protein produced by cytotoxic T lymphocytes and natural killer cells; induces apoptosis in infected target cells. 9.5c: Courtesy of Philippe Lemercier; Fig. The hydrophobic region may be involved in binding the capsid to the viral envelope. However, most tumor cells lack the cellular p53 protein, or other proteins that it activates. In viral nucleocapsids, PB2 (small green sphere) binds to the capped 5' end of a cellular premessenger RNA (orange line) and PB1 (orange sphere) cleaves the RNA 10–13 nt from its 5' end, preferentially after an A or G residue. Furthermore, AAV vectors poorly infect professional antigen-presenting cells, whose function is to induce the cascade of events leading to the activation of the immune system (see Chapter 34). This model proposes that the polyadenylation signal is not always recognized by the cellular cleavage factors, allowing production 16/05/11 8:17 PM 384 CHAPTER 31 Viroids and Hepatitis Delta Virus large delta antigen (214 aa) N N small delta antigen (195 aa) C C Translation 5' Cap poly(A) 3' cleavage/ poly(A) site Transcription by RNA polymerase II editing site 1014 956 1630 1600 1682/1 300 903/902 819 687/688 771 delta selfcleaving motifs Figure 31.4 Hepatitis delta virus genome, mRNA, and proteins. The glycoprotein projections can be readily seen. Image reconstruction from cryoelectron microscopy. Their amino termini are rich in positively charged lysine and arginine residues, but their carboxy termini CH006.indd 75 75 contain aromatic and hydrophobic amino acids. 11.4: Adapted from Leong, L.E., Cornell, C.T., and Semler, B.L., “Processing determinants and functions of the cleavage products of picornavirus polyproteins,” Molecular Biology of Picornaviruses, Semler, B.L. and Wimmer, E. • All herpesvirus mRNAs are made by cellular RNA polymerase II; transcription factors regulate the transcriptional program into several temporal classes called (immediate early), (early), 1 and 2 (late). These particles elegantly illustrate the above principles of construction of molecular packages from a limited number of proteins. PP2A plays an important MAP kinase Protein phosphatase 2A Small T antigen binds to and inactivates PP2A PP2A (inactive) MAP kinase kinase +ATP MAP kinase ~P (activated) Phosphorylates and activates PP2A STAg AP-1 (Fos/Jun) Binds to cyclin D1 enhancer, stimulates transcription Cyclin D1 Figure 21.8 Small T antigen stimulates MAP kinase pathway. The ability of Epstein–Barr virus to infect B lymphocytes is determined by its binding to two key proteins found on the surface of B-lymphocytes: CD21, a complement receptor, and HLA class II (also known as MHC class II), which is involved in immune recognition (see chapter 34). For viruses such as influenza virus and some rhinoviruses, which require transport to late endosomes before penetration can occur, proper maturation of the endosomal vacuoles is essential. This elicits a DNA damage response, even though the cellular DNA is not actually damaged. Use of this plaque assay (Figure 1.3) allows scientists to count the number of infectious virus particles in a suspension with a high degree of precision and reproducibility. Large T antigen hexamers bind to the origin of DNA replication and locally unwind the two DNA strands. Hexons consist of trimers of the hexon protein (polypeptide II); each hexon has six nearest neighbors, hence the name. Actin tails are assembled within the cell beneath the extracellular virus particles, resulting in the formation of microvilli extending from the cell into the extracellular space, with extracellular virus particles perched at their tips. Capping of mRNAs also requires two enzymes. The epidemic that struck Philadelphia in 1793 is of particular note due to its impact on the fledgling government of the United States located in that city. This approach allows the testing of “mix and match” libraries of mutations in different genes to determine phenotypic effects. The specificity of editing is likely determined by the primary and secondary structural characteristics of the hepatitis delta virus RNA. DC-SIGN (dendritic cell-specific intercellular adhesion molecule-grabbing nonintegrin): a type II transmembrane mannose-binding protein found on dendritic cells, involved in their interaction with T cells during antigen presentation. They are the only known present-day biological entities with RNA genomes. 377 SEC-VIII.indd 377 12/05/11 5:04 PM C H A P T E R 3 1 Viroids and Hepatitis Delta Virus Jean-Pierre Perreault Martin Pelchat Viroids Name means “virus-like” particles VIROIDS Autonomously replicating pathogens. Typically parvoviruses will infect cells of the intestine, the hematopoietic 16/05/11 8:08 PM Parvoviruses Antitumor effects of parvoviruses Parvoviruses have a long-standing relation with cancer. The atomic structure of the ϕX174 procapsid (Figure 6.6) demonstrates that the external scaffolding protein can assume at least four different conformations. 417 Cytotoxic T lymphocytes Bind to and kill cells expressing specific antigens. Ribonuclease H (RNAse H) has removed initial RNA primers. 741, with permission from Elsevier. Evolutionary potential of bunyaviruses via genome reassortment Like other viruses with segmented genomes, bunyaviruses evolve by two major pathways: genetic drift, or the slow accumulation of point mutations, insertions, or deletions; and genetic shift, caused by the exchange of genome segments via reassortment. This is best illustrated by experiments showing that p53 knockout mice, genetically engineered to contain no p53 genes, develop normally in every respect except that they succumb to a variety of tumors at a young age (a few months old). Nowadays we may recoil at the idea of spraying powdered scabs up the nose, but the inevitability and destructiveness of smallpox went a long way toward easing any squeamishness about the purity or the origins of early vaccines. • Once a promising candidate is discovered, chemists synthesize analogues that may have higher antiviral activity. Recognition of virus infection can trigger death of infected cells Many cells also respond to a virus infection by inducing a self-destruction process called apoptosis, or programmed cell death. These protrusions eventually break apart the membrane, creating large apertures through which virions escape from the cell (Figure 9.10). It is possible that microviruses evolved from a gokushovirus ancestor. Interferons released from virusinfected cells bind to receptors on nearby uninfected cells and stimulate transcription of a set of genes encoding proteins with antiviral activities, resulting in protection of those cells against viral infection (Figure 33.6). The plasmids used to produce these cell lines contain neither the long terminal CH037.indd 462 repeats (LTR) nor viral packaging signals (⌿), ensuring that no virus genomes are made or packaged. • A tegument protein called Vhs degrades host cell as well as viral messenger RNAs. • gene products regulate viral and cellular gene expression. The threat of spread to developed countries was highlighted by a brief but notable outbreak in Italy in the summer of 2007. Virion: a complete, infectious virus particle. Can give long-lived immunity after even a single dose. It contains 14 cross-linked cysteine residues, providing for high stability, and it can be glycosylated at asparagine-146. Again, about one-third of the genome is used to encode the virion structural proteins. Adenoviruses make different amounts of the various late mRNAs (and therefore of their protein products) by controlling which polyadenylation sites and 3' splice sites are recognized most efficiently. Molecular virology. How can they express their numerous genes from a single RNA molecule that serves both as a genome and as a messenger RNA, in a cellular environment that favors mRNAs that code for only one protein? The prion hypothesis proposes that prions transform a pre-existing normal cellular protein into an infectious, pathogenic agent. Caveolin: major membrane protein component of caveolae. The extracellular virus particle is required for cell-to-cell and tissue-to-tissue spread of virus in an infected animal. • In some cases, fusion occurs directly at the cell surface, with the plasma membrane. Figure 32.6 -helical model of PrP 27–30. Some of these proteins are secreted and act on nearby cells, inducing synthesis of antiviral proteins that reduce or inhibit virus replication. Other localization methods use the higher-resolution electron microscope coupled with specific staining: for example, using colloidal gold particles linked to antibodies. P remains bound to L during RNA synthesis. Nearly all cultivated archaeal species have been isolated from environments with extreme features, such as volcanic hot acid springs, as well as from undersea hydrothermal vents (“black smokers”), which can have temperatures well over 100C at the high pressures found thousands of meters under the ocean. The various adenovirus vectors engineered up to the present are shown in Figure 37.3. First-generation adenovirus vectors. These cells are stimulated to differentiate into antibody-producing plasma cells when they come in contact with activated Th2 helper cells whose T-cell receptor shares specificity for the displayed peptide. The F protein is cleaved into two subunits (F1 and F2) joined by a disulfide bond on mature virions; this cleavage reveals the hydrophobic fusion peptide responsible for virus entry (see below). • The existence of phycodnaviruses has been known for a little over three decades. Recombinant adenoviruses can be produced by either: (1) subcloning the transgene directly into the plasmid containing the virus genome; (2) using recombination within bacteria to insert the transgene into the virus genome; or (3) using specific recombinases such as Cre recombinase (see below) to insert the transgene. Genetically inherited prion diseases would result from an increased stability of mutated PrPSc or an increased tendency of the protein to undergo aggregation and thus stabilize the altered conformation. Fields Virology has become the classic reference book for knowledge about human and animal viruses during the past 25 years; that book is also organized in chapters that cover specific virus families. The discovery of human immunodeficiency virus (HIV) brought to the fore public awareness of retroviruses. Some virions contain a layer of matrix protein between the nucleocapsid and the envelope. Immediate early and early genes are transcribed by the host cell RNA polymerase II, but late and very late genes are transcribed by a virus-coded RNA polymerase. In the case of animal viruses, cells of many different tissues (especially from embryos or newborn animals) can be induced to grow in a monolayer on a glass or plastic surface underneath liquid media. B Internal scaffolding protein, required for procapsid morphogenesis; 60 copies in procapsid. The major symptoms are fever, internal bleeding, liver damage and dysfunction, disseminated intravascular coagulation, and shock. Only a small fraction of these particles successfully integrates proviral DNA into infected cells, but this still translates into approximately 104 to 105 virus variants being generated every day. A trimer consisting of RNA polymerase proteins PA, PB1, and PB2 is bound via PB1 to the 5' and 3' ends of the RNA. This mechanism allows large amounts of late viral mRNAs to be made and translated even though Vhs is being made at the same time. Virus present in individual plaques can be isolated by sampling with a needle or Pasteur pipette, allowing the “cloning” of progeny virus derived from a single virion that initiated the infection leading to the plaque. This raises the question of whether BSE was acquired during the epidemic in cattle by ingestion of remains not of scrapie-infected sheep, but of cattle that had been infected by a rare, spontaneous case of BSE sometime in the past. Five structural proteins translated from a subgenomic mRNA: • • • one capsid protein three envelope proteins a small hydrophobic protein Most togaviruses are arthropod borne, transmitted between vertebrate hosts by mosquitoes Walter Reed, working in Cuba in 1900, demonstrated that yellow fever virus (see Chapter 12) was transmitted to humans by mosquitoes. B lymphocytes respond to antigens and are stimulated to differentiate to plasma cells by interaction with Th2 cells When a particular B lymphocyte comes in contact with an antigen that its receptor antibody can recognize, the B cell is activated, the antigen is internalized and partially degraded, and individual peptides are displayed on the cell surface in association with MCH II proteins (Figure 34.6). 337 Mimivirus has a unique mechanism for releasing its core 337 Virus replication occurs exclusively in the cytoplasm 337 Genome replication 338 Genes coding for translation factors and DNA repair enzymes 338 Ancestors of mimivirus may have transferred genes from bacteria to eukaryotes 339 Conclusion 340 Retroviruses have a unique replication cycle based on reverse transcription and integration of their genomes 342 Viral proteins derived from the gag, pol, and env genes are incorporated in virions 343 Retroviruses enter cells by the fusion pathway 344 Viral RNA is converted into a double-stranded DNA copy by reverse transcription 345 A copy of proviral DNA is integrated into the cellular genome at a random site 347 Sequence elements in the long terminal repeats direct transcription and polyadenylation by host cell enzymes 348 Differential splicing generates multiple mRNAs 348 The Gag/Pol polyprotein is made by suppression of termination and use of alternative reading frames 348 Virions mature into infectious particles after budding from the plasma membrane 349 Acute transforming retroviruses express mutated forms of cellular growth signaling proteins 350 Retroviruses lacking oncogenes can transform cells by insertion of proviral DNA near a proto-oncogene 351 29. • Woodchucks, ground squirrels, ducks, and transgenic mice containing the viral genome have been used to study the molecular virology and immunology of hepatitis B. Th2-cell dependent isotype switching in B cells requires recombination of the assembled specific variable gene sequence of the heavy chain from one constant gene sequence to the next (e.g., IgM to IgG to IgE to IgA). Top line: distance in nucleotides from left end. Many of these cases were Figure 32.1 Incineration of British cattle infected with mad cow disease. Nucleocapsids, formed in B lymphocytes Coiled-coil Dendritic cells Encephalitis Fusion peptide Gangliosides Hemagglutination Hemagglutinin Interferon Nested set Neuraminidase Pseudotyping Sialic acid Subacute sclerosing panencephalitis (SSPE) Synctia (singular: syncytium) T lymphocytes Type I integral membrane protein Type II integral membrane protein FUNDAMENTAL CONCEPTS • Rhabdoviruses are enveloped, bullet-shaped viruses with a negative-strand RNA genome. Some viruses such as Epstein–Barr virus directly interfere with complement-dependent functions. • Like other positive-strand RNA viruses, coronaviruses induce intracellular membrane proliferation and viral RNA replication takes place in association with these membranes. CH024.indd 298 16/05/11 8:12 PM Epstein–Barr Virus 299 Epstein–Barr virus nuclear antigens direct limited replication of the viral genome and activate viral and cellular genes expression is strongly reduced, leading to loss of expression of the viral LMP genes and the cellular genes that promote cell growth and division. The adenoviruses build their capsids with two kinds of structural subunits. These homologs show either that these bacterial and archaeal viruses have a common ancestor, or that there was genetic exchange between bacterial and archaeal viruses some time during evolution. First, newly replicated viral DNA interacts with a different set of DNA binding proteins, thus altering DNA structure to make the major late promoter more accessible to cellular transcription factors. The live vaccine can be administered orally, increasing compliance and avoiding needle-related injuries. Mutations in C reduce virulence in experimental animals, probably because interferon-induced pathways restrict virus replication. Small, untranslated viral RNAs expressed during latent infections target host defense mechanisms Two additional classes of viral genes are expressed during latency, but their products are RNAs that do not code for proteins. Circular plant satellite RNAs resemble viroids but are encapsidated Plant satellite RNAs are small, single-stranded RNAs that depend on a helper virus for replication and encapsidation, but show little or no sequence similarity with their helper virus. This figure shows a hypothetical series of steps that could have led from pools of complex organic molecules such as nucleotides and amino acids to the generation of self-replicating RNAs, which over time could have evolved to organize templated protein synthesis and eventually self-sustaining life forms. Messenger RNAs that contain cis-acting repressive sequences cannot leave the nucleus unless they contain an RRE, in which case Rev supplies a pathway for their export. On the outer surface of the procapsid, D proteins selfassociate across both the twofold and threefold axes of symmetry. Subunit vaccines: contain one or more viral proteins but no viral genome. Intact bacteria diffract visible light and CH001.indd 9 9 therefore dense liquid cultures appear cloudy. There are several different patterns of budding. This is a significant factor in the accumulation of mutations leading to genetic heterogeneity (“drift”) in viral RNA populations. These phosphotyrosines act as part of binding sites for a set of cellular proteins including Srchomology 2 domain protein (Shc), phosphatidylinositol 3-kinase (PI3K), and phospholipase C (PLC). Cytopathic: causes cell injury and death. At the molecular level, the outer shell of a virion can be a rigid, symmetrical container called a capsid (derived from the Latin capsa box). Viral nucleocapsids are transported through the nuclear pores into the nucleus (1), where viral mRNAs are synthesized (2). The origin of the virus was most certainly West Africa, with multiple reintroductions of the virus in the New World due to the extensive slave trade. Experiments with other plant virus RNAs have shown that eukaryotic elongation CH010.indd 115 115 factor 1A (eEF1A) binds more efficiently to aminoacylated RNAs than to RNAs lacking a 3'-terminal bound amino acid residue, and that binding of this factor is correlated with increased translation of the RNA. How is minus-strand DNA synthesis carried out? To generate an immune response, T cells are activated by presentation of antigen fragments during contact with dendritic cells within lymph nodes. She, C. Both intermediate and late CH026.indd 320 promoters contain the sequence TAAA at the RNA start site, usually located just upstream of the translation initiation ATG for a given gene. Single-stranded parvovirus DNAs have unusual terminal structures One of the most distinctive features of parvoviruses is the structure of the linear, single-stranded viral DNA. Another alternative is to use the fiber protein of adenovirus serotypes that are known to infect target cells more efficiently. Zanamivir: neuraminidase inhibitor used for treatment of influenza A and B virus infections. Affected areas contain microscopic insoluble amyloid fibrils and macrocrystalline arrays known as amyloid plaques. Virion assembly is studied by electron microscopic examination of cells infected with wild-type or mutant viruses (Figure 26.7); viral structural and assembly proteins are localized using labeled antibodies. In many of these respects, the filovirus GP resembles surface glycoproteins of paramyxoviruses, rhabdoviruses, and orthomyxoviruses. Lymphatic system: a network of vessels and lymphoid tissues within the body that functions to transport extracellular fluids containing pathogens, macromolecules, and immune cells to lymphoid organs. Thus during each round of replication, a short sequence would be lost at each end of the DNA. GENES AND PROTEINS Codes for approximately 60 proteins. To regulate translation, the access of ribosomes to the AUG start codons of phage genes is strongly restricted. 16/05/11 8:21 PM Adverse Events and Ethical Issues 443 REVIEW QUESTIONS 1. Numerous details in the replication cycles are omitted. Diameter 125 nm. If linear DNAs are provided with hairpin ends, they effectively contain two tandem inverted copies of the sequences at the ends of their DNA. CH028.indd 352 retrotransposons. Schleper, P. How do polyomavirus proteins interact with Rb to induce the cell to enter the S (DNA synthesis) phase of the cell cycle? CH015.indd 177 Figure 15.1 Structure of paramyxoviruses. For example, HIV-1 reverse transcriptase can be expressed and purified, and its DNA polymerase activity can be easily assayed in vitro. Viral DNA: blue; transgene DNA: green; stuffer DNA: orange. No virus replication takes place in transformed cells. Thus, NF-B appears to play organ-specific roles in reovirus pathogenesis. Proteins 2B and 3A both mediate the inhibition of cellular protein secretion, perhaps by blocking membrane trafficking from the endoplasmic reticulum through the Golgi apparatus to the plasma membrane. One individual recently survived rabies encephalitis after drug treatment and induced coma. Cytokine secretion is a hallmark of both the intrinsic cellular defense system and the innate immune response, and cytokines mediate communication between the innate and adaptive immune systems (see Chapter 34). There are fears that a new deadly pandemic of human influenza could occur if a recently emerged, highly pathogenic strain of avian influenza virus mutates to a form that is easily transmitted among humans. These virus groups can replicate in both warm-blooded mammals and cold-blooded arthropods. Right: Mammalian cells use similar enzymatic machinery to generate microRNAs (miRNAs). The transcribed antigenome (positive-strand) RNAs are directly translated to give bunyaviral replication and structural proteins. The gene 17.5 protein is a small membranespecific protein called a holin. 3, p. The Tat protein increases HIV-1 transcription by stimulating elongation by RNA polymerase II Tat is a highly basic 86-amino acid protein produced by doubly spliced HIV-1 mRNA; it is localized in the cell nucleus. The ability to study cell transformation in vitro by these viruses allowed scientists to study the biochemical and signaling pathways that are altered upon infection by polyomaviruses. Even some viral messenger RNAs are packaged in the tegument and can be translated in newly infected cells before most viral genes have been transcribed. Marburg virus was first isolated from patients in Europe, but the origin of the European cases could be traced back to foci in Uganda from where vervet monkeys held in Entebbe were imported into Germany and former Yugoslavia. This protein contains a serine residue whose hydroxyl group forms a phosphodiester bond with deoxycytidine, in a reaction that cleaves dCTP to dCMP. Hyperhalophile: an organism that grows in hypersaline waters with concentrations of sodium chloride above 1.5 molar. Virus entry begins by binding to and degradation of the host cell wall PBCV-1 initiates infection by attaching to the chlorella cell wall by its spike; attachment is followed by degradation of the host wall at the point of contact by virus-packaged enzymes (Figure 27.5). Note also that in the prophage state, sib is separated from int by site-specific recombination at sib 2 3 1 4 att P 3' Pint xis PL RNAse III 5' 3' 2 int int mRNA from PL 34 5' int mRNA from Pint Figure 8.8 Retroregulation of int synthesis. Alphaviruses have the unusual ability to replicate in both invertebrate and vertebrate hosts. In humans infected with Epstein–Barr virus, approximately one to ten memory B lymphocytes per million B lymphocytes in the blood harbor viral DNA. • Transcription of late genes is activated by a number of transcription factors that are induced by the action of T antigens. • Scaffolding proteins mediate capsid assembly by lowering thermodynamic barriers and ensuring the temporal nature and fidelity of the assembly pathway. The combined effects of mutations, recombination, reassortment, selection, and genetic bottlenecks all contribute to the dynamic longterm evolution of cucumber mosaic virus in nature. More recently, another anti-HIV drug, maraviroc, which blocks attachment by binding to a cellular receptor for HIV-1, was approved by the FDA (see text box). Comparing bacteriophage genome sequences shows that blocks of genes, often having related functions, have been swapped from phage to phage by genetic recombination. In practice, these official names are often shortened to “bacteriophage” or “phage” followed by the number. Their task upon infecting a cell is very straightforward: make their proteins, replicate their RNA, assemble progeny phage particles, and leave the cell. However, RNAs generated from genome segments 7 and 8 (those encoding the M1 and NS1 proteins) contain splicing consensus sequences that are recognized by the cellular splicing machinery in the nucleus. RNA replication is associated with cytoplasmic membranes. LS is believed to interact with the core protein at the cytoplasmic face of the Golgi membranes, pulling the Hepadnavirus Retrovirus Exit Entry Entry Assembly, exit Reverse transcription, assembly Reverse transcription Packaging Transport, integration RNA export Transport, repair Transcription CYTOPLASM NUCLEUS Figure 30.6 Schematic comparison of replication cycles of retroviruses and hepadnaviruses. Procapsids often change in 05/05/11 2:45 PM 480 Glossary structure or protein composition when they incorporate the viral genome to form a mature capsid. During the subsequent 20 years, millions of cows in the United Kingdom and parts of CH032.indd 388 continental Europe were slaughtered in attempts to control this disease (Figure 32.1). Examples of T 1, 3, 4, 7, 13, 16, 25 and greater are known. Inner circle: G C content. Will it be possible to isolate replicon expression vectors that are not cytopathic to neurons? The NS1 protein also suppresses a variety of host cell antiviral response pathways Perhaps more important to the virus is inhibition of the innate cellular antiviral responses (see Chapter 33) by NS1. However, cytomegalovirus does not encode a homologue of herpes simplex virus thymidine kinase (which phosphorylates ganciclovir very efficiently). • Vaccine science has evolved over time with advances in understanding of the immune response and developments in technology such as egg culture, tissue culture, and recombinant DNA technologies. First analyses of the infectious agent suggested that these diseases might be caused by a flavivirus known as St. Louis encephalitis virus. • Linear parvovirus DNA circumvents the “end replication problem” by having terminal hairpin sequences that can prime DNA synthesis. The large genome size is clearly not due to the accumulation of noncoding or junk DNA, as in many eukaryotic organisms. 2.1j: Courtesy of Jan Orenstein, George Washington University Medical Center; Fig. In the presence of amantadine, release of viral nucleocapsids is incomplete, blocking infection (see Chapter 36). Examples of such chimeric products include genetically modified vaccine-strain measles virus carrying the structural proteins of respiratory syncytial virus, and attenuated Salmonella typhi expressing rotavirus glycoproteins. Also complicating HIV treatment is the rapid emergence of drug-resistant strains, which currently represent approximately 10% of new infections in developed countries. It is no surprise that “rogue” phycodnavirus members lying on their own distinct branches of the NCLDV tree are beginning to appear. 24.9: Copyright Bernard Roizman. Harrison, Harvard University 3. However, these antibodies can neutralize virus samples harvested several months previously. Each virus has its own array of specific proteins required for its replication, and therefore different antiviral drugs may have to be developed for each virus. Three heterodimers converge to form the spike-like projection, with the pr peptide forming the small knob at the distal end of the spike. Cell surface proteins exploited by viruses as receptors include members of the immunoglobulin family (CD4, PVR, CAR, CD46); the low-density lipoprotein (LDL) receptor family; a variety of multimembrane-spanning transport proteins; integrins; and others. Thus, it is likely that these proteins remain associated with the endoplasmic reticulum and play a role in RNA replication. CH006.indd 72 16/05/11 7:56 PM Microviruses 3' 5' A protein (1) ds RF DNA DNA pol III (2) rep SSB (3) Elongation (5) Cleavage Cleavage, ligation (4) ss plus-strand DNA Figure 6.4 Stage II DNA replication. Meanwhile, Allan Campbell isolated a large set of mutants with conditional lethal mutations. Replication versus translation: competition for the same RNA template Once the replicase is made, it can begin to generate new copies of phage RNA. Lamivudine (3TC), a nucleoside analogue that was originally developed for the treatment of HIV (see Chapter 36) was also shown to be active against hepatitis B virus by Dr. Lorne Tyrrell (University of Alberta) and Biochem Pharma (Montreal) in 1994. The origin of replication itself is predicted to be located 400,000 nt from the left end of the genome. Negative staining: staining of specimens for electron microscopy by use of heavy-metal salts that absorb electrons but do not bind to the specimen. However, hepatitis delta virus resembles viroids and is quite different from the hepadnaviruses. Both E1B 19K and 55K proteins are potent inhibitors of apoptosis, and could be of value in diseases characterized by excess or unwanted apoptotic cell death, such as certain neurological disorders (e.g., Alzheimer’s disease) or stroke. Thus, only a few-fold purification is needed to obtain homogeneous protein X for further study. This capacity to spread within families and communities is a significant advantage in regions with low rates of vaccine coverage. Second, all late mRNAs contain the same leader sequence at their 5' ends. Viral DNA is replicated by three viral proteins: preterminal protein (pTP, blue oval), DNA polymerase (DNA pol, orange circle), and single-stranded DNA binding protein (ssDBP, small green circles). Some produce unique pyramidal structures that lead to exit of virions and cell death. Despite the high similarity in genome content, each of the six sequenced chloroviruses contains genes that encode unique proteins and functions. The movement protein subsequently accumulates in membrane-associated virus replication complexes (VRCs). This IRES bears structural similarity to that of hepatitis C virus, a flavivirus. The x-ray structure for the dengue virus NS3A proteinase domain confirmed sequence analyses and genetic studies that suggested that NS3 is a serine proteinase having a catalytic triad consisting of His, Asp, and Ser residues. Adenovirus uses a similar mechanism to enter cells by first binding to the coxsackievirus and adenovirus receptor (CAR) and subsequently binding to ␣V3 and ␣V5 integrins. GENES AND PROTEINS Paramecium bursaria chlorella virus-1 (PBCV-1): 366 genes. What key advantage does the overlapping of reading frames impart? (b) Transcriptional map. The minus-strand, which does have protruding C’s, may occasionally perish. C G Five different poly(A) sites and alternative splicing generate multiple late mRNAs 5 C C G 6 C G C 7 C G G C Figure 23.8 Adenovirus DNA replication. This problem is solved in part by splicing of the primary transcript (Figure 28.6). Interferons are synthesized and secreted, then bind to receptors on nearby cells, inducing expression of genes that make antiviral proteins. The scourge of yellow fever outbreaks in the United States continued through the nineteenth century and was not brought under control until the Yellow Fever Commission headed by Walter Reed produced two significant results. The positive-sense genome RNA alone, in the absence of any viral proteins, is infectious when introduced artificially into susceptible cells. The crescents ultimately develop into spheres called “immature virions”, and in the process enclose viroplasm, a substance of intermediate electron density that is known to contain several viral core proteins. Terminase undocks from the filled head, remaining bound to the end of the concatemer to form a new complex that is ready to bind to another prohead. Late transcripts are made from a single strong promoter and five alternative polyadenylation signals. Secondary transcriptase particles, like replicase particles, lack the full complement of virion proteins, but the two particle forms are physicochemically distinct. These are grouped into three families based on the tail structure: Myoviridae have a long contractile tail, Siphoviridae have a long non-contractile tail, and Podoviridae have a short non-contractile tail. Current evidence indicates that the matrix protein (M1) is imported into the nucleus and binds to Although the NS1 protein is the most abundant viral protein in virus-infected cells, its roles have only recently begun to be appreciated. Host cell proteins are probably also involved in these reactions. Some retroviruses position this signal within the U3 region so that only the copy present in the right-hand LTR is transcribed. First, all late mRNAs are made by turning on a single, highly active promoter, allowing coordinate expression of late proteins when 16/05/11 8:11 PM 282 CHAPTER 23 Adenoviruses 0 16.5 50 100 map units Adenovirus DNA Major late promoter 1 2 Primary transcript for late genes Transcription 3 5' Cap L1 L2 L3 Poly A addition at one of 5 possible sites (L4 3' L5) For example Cap An L1 L1 L2 L3 3 possible 3' splice sites RNA splicing Cap Cap Cap L1 4 possible 3' splice sites RNA splicing to assemble tripartite leader and join it to different 3' splice sites in poly A proximal exon L1 mRNAs An An An An Tripartite leader L3 mRNAs L3 Cap An Cap Cap An An An Figure 23.9 Production of late adenovirus mRNAs. Transcription initiates at the major late promoter, and the RNA is cleaved and polyadenylated at one of 5 possible sites. In the case of Sendai virus, the only conserved nucleotides are C residues at positions 79, 85, and 91 (Figure 15.5). This recombination depends on the products of genes 3, 4, 5, and 6 as shown both in vivo and in vitro. Second, the machinery responsible for genome replication does not recognize the cleavage/polyadenylation signal that leads to mRNA 3' end formation. O is a 15-bp-long “core” sequence found in both attP and attB, where the DNA crossing-over reaction takes place. the 5' end is followed by a “cloverleaf” (three “leaves”, one stem) structure in which the Shine–Dalgarno sequence (SD) of the maturation gene is base-paired to an upstream complementary sequence (UCS). Virions follow the exocytosis pathway and are released to the extracellular space by fusion of vesicles containing virions with the plasma membrane (Figure 12.6). Packaged by hepatitis B virus S protein. On the other hand, if stop codons in the coat gene are introduced by mutagenesis between nts 1678 and 1725 or even somewhat upstream of the lysis protein start codon, lysis protein is made. The modified genome RNAs (referred to as replicons) serve as mRNAs for the translation of the nonstructural proteins, and their replication is carried out by these proteins. Genes encoding proteins are drawn above the double line, and promoters and terminators are below the double line. Resulting secondary bacterial pneumonias cause many of the deaths attributed to influenza virus infection. Often, a very efficient class III T7 ribosome binding site is included in the plasmid construction. These genes flank the ind gene and are transcribed in divergent directions, away from the ind gene. Many tumor cells have mutations in the gene for p53 that inhibit its capacity to induce apoptosis. Another safety issue concerns the fact that the integration of proviral DNAs of retroviruses can happen at numerous sites in the cell genome. Linear DNAs need another way to solve this problem. Two different strains of influenza virus replicating within the same cell can recombine genome segments, in a process called reassortment, to form a new strain that contains a mixture of the genome segments from the

two original strains. How a protein kinase can phosphorylate a nucleoside analogue is an interesting problem in enzymology. At the molecular level, how is HIV latency controlled? Messenger RNAs synthesized within the core are extruded into the cytoplasm of the infected cell, where they are translated and direct viral replication. Discuss the three size classes of mRNAs generated by HIV-1, in contrast to the two size classes generated by simpler retroviruses. Scaffolding proteins: proteins that are involved in the assembly of complex biological structures such as viral capsids, but are not retained in the final structure. Start sites for transcription are shown by arrows. Are viruses living or inanimate? Then growing points for DNA replication are set up using E. It is believed that NS2 therefore piggybacks onto nucleocapsid/matrix protein complexes and signals the cellular protein export system to transport the complexes through the nuclear pores to the cytoplasm (step 7, Figure 18.6). (a) Cap-dependent initiation directed by EIF-4F and other initiation factors (not all shown). These include shutoff of cap-dependent translation, shutoff of host cell RNA synthesis, induction of cytoplasmic vesicles, and alteration of intracellular transport pathways between the endoplasmic reticulum and Golgi apparatus. This protein has not been carefully studied. Virus replication occurs exclusively in the cytoplasm The remaining replication cycle appears to take place entirely in the cytoplasm in virus “factories” that remain 05/05/11 11:24 AM 338 CHAPTER 27 Viruses of Algae and Mimivirus increases in size as the infection progresses and viral DNA is replicated. Therefore, EF-Tu would be acting as a kind of primer for the initiation of minus-strand RNA synthesis. Taken together, these data indicate that bunyaviruses, like influenza viruses (see Chapter 18), use a “cap-snatch” mechanism to prime messenger RNA synthesis. However, transformed cells do not generally produce virus, and express a limited range of viral proteins. DNA replication initiating on a single-stranded DNA template requires a primer. Many victims suffer from acute hepatitis, which gives the skin a yellow tinge, hence the name. The spread of SARS coronavirus infections usually took place among family members of victims or in hospital care settings, and occurred principally during symptomatic illness. Ribosomes translating the coat gene disrupt secondary structure, allowing replicase translation Ribosomes contact the messenger RNA over a stretch of about 20 nt upstream and 15 nt downstream from the initiator AUG. There may be other roles that sGP plays, particularly in the (unknown) natural host of this virus. CH003.indd 35 Members of nine different virus families with positive-strand RNA genomes infect vertebrates, and positive-strand RNA viruses classified into twelve different families and seven unassigned genera infect plants (Table 3.3). The hypersensitive response involves tightly controlled cell death and induced resistance of neighboring cells. Bacteriophage T4 interacts reversibly with lipopolysaccharide via its long tail fibers. 6a–c in Gai, D., et al., “Mechanisms of conformational change for a replicative hexameric helicase of SV40 large tumor antigen,” Cell 119 (2004), 47–60, with permission from Elsevier. Effective antiviral responses also require interactions between NK cells and dendritic cells. Hepatitis delta virus has also been associated with the development of liver cancer. Using several different drugs simultaneously reduces the likelihood that a virus will become resistant to all of the drugs, and certain mutations that provide resistance against one drug may make the same protein more sensitive to a second drug or lessen the ability of the virus to replicate. As these proteins are transported to the Golgi stacks, they are modified by palmitoylation, the addition and trimming of oligosaccharides, and a reorientation of the carboxy terminus of PE2 from a membrane-embedded to a cytosolic location. Since then, more than 100 individuals have been affected by this disease, called new variant Creutzfeldt-Jakob disease (nvCJD), with an incidence in the United Kingdom of 10 to 15 cases per year. This domain is a virulence determinant that can interact with PDZ-binding protein(s) and modulate pathogenicity through additional mechanisms; mutations in this small domain have strong effects on virus pathogenicity. The phlebovirus NSm protein is not essential for virus viability but plays some role in virulence, while the orthobunyavirus NSm protein has a function in virion assembly and morphogenesis. This RNA is packaged along with the genome RNA (RNA 3) from which it is derived. Furthermore, treatment with the nematode-derived anticoagulation protein rNAPc2 resulted in partial survival (33%) of Ebola virus-infected nonhuman primates but failed against Marburg virus infection. A reduction of dopamine in the brain results in tremors, aberrant movements and rigidity, the main symptoms of Parkinson’s disease. Latency: a state of inactivity in which the viral genome is present in the cell in a dormant state; little or no viral transcription or replication occurs. Beyond the end of the L gene is a short (50–150 nt) noncoding “trailer” sequence. They are therefore ideal vectors for gene replacement therapy, as transgenes introduced into target cells remain stably integrated. Variolation led to vaccination, which has eradicated smallpox worldwide Early attempts at smallpox prevention, called variolation, took advantage of the fact that infection with variola virus by an unnatural route resulted in a disease of reduced severity and decreased mortality. However, the P/C/V gene has a more complex coding strategy (Figure 15.9). CH005.indd 66 Synthesis of maturation protein is controlled by delayed RNA folding However, the folding of the 5' untranslated region of MS2 RNA to its final equilibrium configuration is unusually slow; in vitro experiments show that it takes minutes to refold the heat-denatured cloverleaf structure. (Scale bar: 250 nm). Steps (1) to (5) are described in the text. E1A proteins bind to the retinoblastoma protein and activate E2F, a cellular transcription factor How do E1A proteins induce cells to enter the S phase of the cell cycle? Cellular immune responses: adaptive immune responses mediated by T lymphocytes. At the nuclear pore the DNA is released and enters the nucleus. RNA primers are blue; newly synthesized DNAs are orange. Although the adenovirus protein E3-19K binds to TAP, it does not prevent peptide transport. The early primary transcript is polyadenylated at a site downstream of the E5 gene, and these transcripts are CH022.indd 266 spliced in a variety of ways to generate numerous early mRNAs. Many of these RNAs contain multiple open reading frames, but it is not clear how these multiple coding regions are translated by ribosomes. The complete, infectious virus particle is called a virion. As with prion diseases, these amyloidoses can be initiated by inherited mutations in the gene coding for the responsible protein. Now, nucleocapsids must be transported back out of the nucleus to the cytoplasm, so that they can be incorporated into progeny virions. NS2 contains a nuclear export signal, and it can also bind to the matrix protein. In the last decade, it has become clear that the cellular immunity generated by some live attenuated vaccines can be very long-lived (¬50 years for smallpox vaccine). The immune response generates specific antibodies that recognize and bind to viral antigens. Dorsal root neurons: sensory neurons whose ends are in peripheral organs and whose cell bodies are grouped in dorsal root ganglia near the spinal cord. Early investigators were able to explain the high frequency of transmission of the disease by ritual funeral preparation of victims, including ritual cannibalism of their brains. The obvious conclusions are that the mimivirus is either a serial acquirer of genetic information (by horizontal gene transfer) or it has a propensity for duplication of its own genes. Host resistance to cucumber mosaic virus often involves multiple genetic loci; however, in some cases resistance is conferred by a single gene. (a) Structures of sialic acid, zanamivir, and oseltamivir. NS2A 224 RNA replicase component, either direct role in replication or targeting replicase complex to membranes. Both AIM2 and NLRP3 contain a pyrin domain that mediates protein-protein interactions. Indeed, it has been suggested that more than one-third of mimivirus genes have at least one homologue on the genome. Johns Hopkins III James D. Six to eight replication proteins. Furthermore, because inactivated viruses cannot multiply within the host, they tend to be less successful in inducing a robust immune response. Three patterns of gene expression have been detected in infected B-lymphocytes. Lumen: the inner cavity of cellular vesicles, such as the endoplasmic reticulum. It has been speculated that filamentous virions, which can grow as long as the diameter of an epithelial cell, are responsible for cell-to-cell transmission of virus; such virions could infect a neighboring cell while still being extruded. Algae also have cell walls; how does the chlorovirus PBCV-1 enter a cell? In 1994, the first West African species of Ebola virus (Cote d’Ivoire ebolavirus) was isolated during an outbreak of hemorrhagic fever among wild chimpanzees in a rain forest of Ivory Coast. However, the 125-nt, self-complementary sequences at the two ends of adenoassociated virus DNA are identical, except for the orientation of the internal 42 nucleotides labeled B and C in Figure 20.3 (see below). These sequences allow the two ends of a fulllength, single-stranded DNA molecule to anneal over the first 100 nt, forming a panhandle structure with a short, double-stranded stem (step 4). The genomes of many different viruses of eukaryotes have been cloned and can be manipulated in bacterial plasmids and subsequently reintroduced into their natural host cells to produce recombinant virions. The product of the class I phage gene 0.3 (OCR, overcoming classical restriction) is a protein with an unusual form of molecular mimicry. Causality is hard to prove; some human tumors were shown to contain simian virus 40 DNA and express viral early proteins. Late genes are transcribed by a novel virus-coded RNA polymerase Once DNA replication begins, another set of baculovirus genes is expressed. • RNA phages bind to F-pili and enter the cell by release of RNA from the virion and probably by retraction of the F-pilus bound to viral RNA into the cell. This results from the ease with which antigenic domains in the virus envelope glycoproteins, HA and NA, can change. With greater than 15 million people affected worldwide, hepatitis caused by hepatitis delta virus infection is a serious public health problem. To test this hypothesis, a lentivirus vector expressing the three enzymes involved in the synthesis of dopamine was constructed and injected into the brains of animal models for Parkinson’s disease. Therefore, it must be able to coevolve with its host. The palindromic sequences at one end then reform hairpins to prime further DNA synthesis. One of its major effects is to permit the infection of non-dividing cells by serving as a signal for the active transport of the preintegration complex into the cell nucleus (see above). Rhabdoviruses: rabies (fatal encephalitis in humans), transmitted by bites of infected animals; vesicular stomatitis virus in cattle. In some cases (but not in T7) this gene product also exhibits S-adenosylmethionine hydrolase activity (SAM-ase), which depletes the source for methyl groups for DNA modifications. This can reduce class II promoter usage by lowering the overall availability of active T7 RNA polymerases, with the result that the stronger class III promoters are favored over the weaker class II promoters. CH034.indd 416 Immune responses can be classified as innate or adaptive. It could be argued that if we lived in a world without the well-studied human viruses, we would all live longer; yet if we lived in a world without the poorly studied algal viruses, we would all certainly be dead! With only approximately 150 formally identified viruses, and 100 or so others mentioned in the literature, it is clear that most phycodnaviruses, containing an enormous reservoir of genetic diversity, remain to be discovered. At the same time, late genes L1 and L2 are expressed, producing the viral capsid proteins. Certain of these people have a deletion in the gene encoding CCR5 and therefore lack this chemokine receptor. Cytokines: secreted proteins that act on cells of the innate and adaptive immune systems to stimulate or inhibit various immune functions. 27.9: Courtesy of Mike Allen; Fig. The cover was printed by Markono Print Media Pte Ltd. • Live attenuated virus vaccines, inactivated virus vaccines, and subunit vaccines have all been successful. What are the two major receptors utilized during infection of human mucosal epithelium by papillomaviruses? The encapsidated RNA is resistant to ribonuclease treatment. With time, some of the progeny of these intracellular parasites would lose genes that were no longer needed, and other genes that can package the viral genome and transmit it to susceptible cells could have evolved. Peripheral or secondary lymphoid organs: lymphoid tissue (e.g., spleen, lymph nodes, tonsils) where foreign or altered native antigens interact with lymphocytes. Internal ribosome entry sites (IRES): regions on viral RNAs (particularly picornaviruses and flaviviruses) that bind to ribosomes and lead to cap-independent initiation of protein synthesis at internal sites distant from the 5⬘ end of the RNA. LMP-2A binds to these two kinases and to E3 ubiquitin ligases (E3), all of which are important in modulating LMP-2A signaling. Poxviruses remain a subject of intense research interest Although smallpox has been eradicated, interest in poxviruses persists, and with good reason. Wherever a phage binds to a bacterial cell and replicates, that cell releases the progeny phage particles, which are then taken up by neighboring cells and further replicated. Many DNA viruses organize their gene expression in this fashion. Leucine zipper domain: in proteins, an alpha-helical region with leucine residues at each 7th position, which enables dimerization between proteins. Virus replication complexes containing movement proteins are transported rapidly within the cell and halt briefly at plasmodesmata. The former strategy has the advantage that the cell provides all the systems and signals needed to move the incoming vesicle in the direction of the nucleus. Circular single-stranded RNA, ~1680 nt. However, the replication of a retrovirus can result in mutagenesis of the host cell because it involves integration of proviral DNA into the host genome. Third-generation adenovirus vectors. Large-scale transfection of mammalian cells with plasmids has begun to supplant the use of recombinant viruses for the purposes of protein production. Some have termed these proteins “prionoids,” since they seem to infect neighboring cells (and possible other hosts) just as do prions. Host cell proteins bind to the IRES and help to dock the 40S ribosomal subunit onto the RNA. the termini vary among poxviruses, and they encode functions that are distinct for each virus such as host range and pathogenesis. Gene order is conserved among different paramyxoviruses and rhabdoviruses Paramyxovirus and rhabdovirus genomes contain between five and ten genes, each coding for a specific messenger RNA. Poxviruses have evolved a number of genes that cleverly combat host defenses against infection (Table 26.4). Furthermore, production of full-length mRNAs in vitro is stimulated by addition of the protein synthetic machinery and is reduced by inhibitors of protein synthesis. • One small, double-stranded DNA virus is closely related to a single-stranded DNA virus; both infect hyperhalophiles. 9.8c: Courtesy of Philippe Lemercier; Fig. SARS coronavirus uses angiotensin-converting enzyme 2 (ACE2), another metalloproteinase, as a receptor. In the case of Sendai virus, the first 12 nucleotides at the 3' end of the genome are identical to the first 12 nucleotides at the 3' end of the antigenome. Alternatively, analogues can be incorporated into the growing DNA chain and prematurely terminate its elongation. • Reverse transcriptase contains a ribonuclease H activity that degrades RNA in RNA/DNA hybrids. Nine human herpesviruses, including herpes simplex virus, varicella-zoster virus, Epstein–Barr virus, cytomegalovirus. Coding regions for virion structural proteins are in the 5'-proximal region of the viral RNA and are therefore present in the N-terminal part of this polyprotein; the viral RNA polymerase is coded at the most distal 3' region of the viral RNA (Figure 12.4). In comparison, the total length of the genome contained within the 23 pairs of chromosomes in a single human cell, 3 billion base pairs, would be approximately 1 meter if stretched out end-to-end. The intrinsic pathway (lower left) responds to developmental signals and internal stress such as virus infection by activating cellular p53, which leads to the binding of oligomerized proteins such as Bax to the mitochondrial membrane, release of cytochrome C, and activation of caspase-9 by the “apoptosome.” Both caspases 8 and 9 activate caspases-3 and -7, which are the “executioner” caspases that lead to cell death. The Pap smear is still the most widely used and effective test to detect cellular changes indicative of disease such as cervical neoplasia. Certain of these transcription factors recognize sequences in the viral enhancer and bind, displacing a small number of nucleosomes from that region. 2.9: Reprinted from S.C. Harrison, “Principles of Virus Structure”, op. This process, termed primary transcription, can begin as soon as the viral nucleocapsids are released into the cytoplasm. Protein 2b has two conserved basic domains at its N- and C-terminal regions, including a nuclear localization sequence at the N-terminal region. Some members of the Reoviridae and Rhabdoviridae are also arthropod-borne. The start site for synthesis of maturation protein is normally inaccessible to ribosomes So far we have ignored translation of the maturation protein, whose coding region lies near the 5' end of the phage RNA. The viral envelope proteins may have regular side-by-side interactions or may be clustered by virtue of interacting through their cytosolic “tails” with a multivalent internal viral protein or protein assembly. A domain within the amino-terminal half of the capsid protein binds to a specific region of the positivestrand genome RNA called the packaging signal. Endosymbiosis is believed to be at the origin of a number of organelles such as mitochondria and chloroplasts, and is BGLOSS.indd 474 therefore considered to be mutually advantageous to both the invading and the receiving organism. After thorough washing to remove all unbound RNAs, the hybridized MDV-1 RNA is released by denaturation and amplified by Q polymerase. However, certain strains of satellite RNA cause more severe symptoms in infected plants such as systemic necrosis, severe chlorosis, and white leaves (Figure 10.10). Much of what is known about papillomavirus replication is derived from studies on warts, or on cells transformed by papillomaviruses and propagated in vitro. Thus, just three molecules of the DNA genome of mimivirus, the largest known virus, stretched out end-to-end, would cover a length of more than 1 millimeter. It is possible that these small DNA viruses originated from fragments of cellular DNA that broke away from the cell genome and were able to replicate independently within the cell as plasmids. • The X protein is a multifunctional oncogenic protein that can initiate hepatocarcinogenesis. The steps postulated to have taken place in the evolution from simple self-replicating RNAs to DNAbased cellular life forms imply the existence of nucleic acid polymerases of four types: 1. As a result, a nested set of negative-strand RNAs with common 5' ends but different lengths is produced. RNA 5, when present, is packaged together with one of the three genome RNAs. Occasionally, three RNA 4 molecules are packaged in a single particle in the absence of RNA 3. (eds.), Virus Taxonomy, VIIIth Report of the International Committee on Taxonomy of Viruses, (2004) p. Deletion of the vif gene reduces infectivity of HIV-1 in cell cultures and in animal models used to test pathogenicity. DNA or RNA genomes are shown as coils or double helices. This DNA copy is then transported to the nucleus where another packaged viral enzyme, integrase, directs the insertion of the viral DNA into the cellular chromosome at random sites. These different functions determine the structural characteristics of virions. Recently, nsp14-exonuclease mutants of murine hepatitis virus were shown to have a mutator phenotype and profoundly increased mutation rates, consistent with an RNA proofreading function in nsp14. The DNA copy of the R sequence hybridizes with the R sequence at the 3⬘ end of the genome RNA. These improvements were facilitated by an iterative process of synthesizing an inhibitor and solving the structure of the protease bound to the inhibitor by x-ray crystallography. 345 The actual geometry of how this transfer takes place within the virus core during reverse transcription is not clear. Reprinted from Moyer, R.W. and Condit, R.C., “Poxviruses,” Topley & Wilson’s Microbiology and Microbial Infections, London: Edward Arnold Publishers (2005), reproduced by permission of Edward Arnold. How is it replicated? The enormous diversity of antibody specificities Antibody specificity is a property of the N-terminal variable amino acid sequences of the immunoglobulin proteins. VA RNA therefore suppresses inhibition of viral protein synthesis by PKR that occurs when it is activated by longer, viral double-stranded RNA (see Chapter 33). Following phosphorylation by cellular kinases, certain nucleoside analogue triphosphates are selective inhibitors of the viral enzyme responsible for this reverse transcription step. 16/05/11 7:59 PM 92 CHAPTER 8 Bacteriophage Lambda Table 8.3 Roles of regulatory gene products Gene product Action Result CI Represses PR and P L Shuts off early transcription, excludes exogenous phages (“immunity”) Activates PRM Maintains CI expression during lysogeny At high concentration, represses PRM Controls CI levels during lysogeny Represses PRM Shuts off CI expression during lytic growth Turns down PR and PL Reduces early transcription during late phase Activates PRE Turns on CI expression, directs cell to lysogeny Activates Pint Makes Int protein, leads to prophage integration Activates PAQ Reduces Q expression, lowers late transcription CIII Blocks proteolysis of CII Directs cell to lysogeny N Antiterminates at tR1, tL1, and downstream terminators Activates early transcription from PR and PL Q Antiterminates at tR' Activates late transcription from PR' Cro CII A cos Prophage/host cell interactions R cI N J attP int POP' gal BOB' attB Int, IHF, Xis gal attL int N cl R bio Int, IHF cos A J attR bio Figure 8.7 Integration and excision of DNA. NS2A binds to the NS3 and NS5 proteins, as well as to sequences within the 3' noncoding region of the genome RNA. Retroviruses are the only RNA viruses whose genome is replicated by the host cell enzymatic machinery. Basic polypeptides V and X are also bound to the viral DNA. Therefore it appears that a host RNA polymerase must be responsible for transcribing the viral genome, and host RNA polymerases are localized in the nucleus. In some experimental situations, coat protein is not necessary for cell-to-cell movement of cucumber mosaic virus. Several parvoviruses of animals are responsible for serious diseases. Indeed, even before the discovery of interferon, it was appreciated that double-stranded RNA acts as a “viral toxin” and leads to many of the symptoms associated with a virus infection. Infection of mosquito cells is often not cytopathic but instead causes a persistent infection. The three-dimensional structures of the virions of many different picornaviruses have been determined by x-ray crystallography (Figure 11.2). Laminin receptor: a cell surface protein that binds to laminin. Therapeutic applications of human adenoviruses In the near future we will almost certainly see adenoviruses used in a variety of clinical applications. The genotypes, denoted Group A through Group H, are distributed in specific regions of the world. Later (2), the opening of the stargate allows for the extrusion of the viral membrane toward the phagosome membrane. Another possibility is that a bat coronavirus might have directly infected a person in a form that was not pathogenic, and was then passed to an animal that again served as an intermediate host. 16/05/11 8:21 PM 436 CHAPTER 35 Antiviral Vaccines Table 35.6 Attenuated oral polioviruses: reversion to neurovirulence Virus strain Number of mutations Number of amino acid changes Number of mutations required for return to neurovirulence Sabin strain 1 57 23 ¬10 Sabin strain 2 23 5 5–6 Sabin strain 3 ~6 3 1–2 From Minor, P. Rb protein binds to E2F, blocking activation of cell-cycle genes. The attentive reader will note that some interesting observations can be made about viruses grouped in these different tables. with the DNA polymerase to tether the polymerase to its template DNA strand. 36.2b: Fig. Viral structural proteins are often the most effective antigens, because they are displayed on the surface of the virion and are easily accessible to the protective elements of the immune system that prevent disease. During its gestation, I was ably helped by a succession of editors at Wiley: Joe Hefta, Keri Witman, Patrick Fitzgerald, and finally Kevin Witt, under whose tutelage the book first saw the light of day. Some viruses such as herpes simplex virus type 1 and cytomegalovirus can interfere with MHC-I expression and peptide presentation, effectively blocking viral antigen expression on infected cells and preventing recognition by Tc cells. Env proteins are made from singly spliced mRNA. Viral double-stranded RNA is cleaved by the enzyme Dicer (DCR-1 and DCR-2) to make small interfering RNAs (siRNAs). Three different in-frame start codons are utilized within the S open reading frame. This is regulated by the strength of the respective promoters: the promoter at the 3' end of the antigenome is 10-fold stronger than the promoter at the 3' end of the genome. These enzymatic activities link viroids and circular RNA satellites to the ancient RNA world (see below), in which it is postulated that RNAs were able to replicate themselves in the absence of proteins by serving as enzymes (ribozymes). Transcripts initiated at the nearby preC promoter encode the E protein. In contrast, the mature virus particle is stable, and is likely the form responsible for transmission of infections between individuals. Share other advantages of subunit vaccines. As with parvoviruses, the three poliovirus capsid proteins are all folded as jelly-roll -barrels. SH may form pentamers and create an ion channel in membranes. Myristate: a C14 linear, saturated fatty acid that can be joined via its carboxyl group to N-terminal glycine in a number of proteins; the fatty acid becomes embedded in a cellular membrane, linking the protein to the membrane. p. These proteins in turn form a complex, the “apoptosome,” which leads to activation of caspase 9; caspase 9 in turn activates caspases 3 and 7, as above. Bacteriophages can be used as targeted antibiotics against bacterial diseases Bacteriophages may be called upon to play an important role in the fight against bacterial diseases in humans and animals. Thymidylate kinase: an enzyme that phosphorylates thymidine monophosphate (thymidylate) to thymidine diphosphate. nsp12 RNA-dependent RNA polymerase. huxleyi by coccolithoviruses, although the mechanisms by which this antiviral property works are not understood. Before the viruses causing hepatitis were identified, these pathogens were classified by modes of transmission and epidemiology. Genes near the ends of the genome are usually transcribed toward the nearest end, while genes in the center of the genome can be transcribed in either direction. Nef may enhance infectivity by modification of virion structure; however, no difference in the structure of virions produced in the presence or absence of Nef has been detected. The locations of genes essential for viral DNA replication are shown in orange, and immediate early genes are shown in green, in the same circle. The hosts of ATV are Acidianus species isolated from a hot spring on a solfataric field near Naples, Italy. The capsid is formed of 60 trimers (shown to the right) of VP1 (blue), VP2 (green), and VP3 (red), grouped in 12 pentamers, with 5-fold symmetry axes in the centers of the blue regions. Proteins that are involved in inflammation and apoptosis often contain pyrin domains. One of the first cytokines produced by virtually all virus-infected cells is interferon. In fact, heterozygosity in the PrP gene, particularly at position 129, is present at unexpectedly high levels in humans worldwide. THE VIRUS REPLICATION CYCLE: AN OVERVIEW The single-cycle virus replication experiment Use of the plaque assay enables the quantitative study of the kinetics of virus replication. Nicked circular or linear DNA can bind more ethidium bromide than covalently closed circular DNA, because the strands can freely rotate about each other CH021.indd 250 and therefore unwinding is less constrained. Lysis: breaking open of a virus-infected cell, leading to its death and the release of virus particles within the cell. Becker, Vanderbilt University SECTION IV: NEGATIVE-STRAND AND DOUBLE-STRANDED RNA VIRUSES OF EUKARYOTES 15. Mesyanzhinov, and Michael G. Genetic drift: see Antigenic drift. Late gene expression 6. By far the largest of the flavivirus nonstructural proteins is the RNA-dependent RNA polymerase, NS5. Lethality can be high (up to 90%). Considerably more research has been done on the cell biological and molecular mechanisms involved in movement of tobacco mosaic virus within infected plants. Membrane protein. SS has at least two transmembrane regions. The penton base binds to these integrins via a domain similar to that used by extracellular adhesion molecules that interact with integrins. C and N: carboxy and amino termini. SD SA enhancer 459 pA le transgene Transcription, splicing Promoter region Cap mRNA AAAAA Figure 37.2 Typical mammalian expression cassette. The precise roles of these proteins in IRES function are not known. However, stop codons placed beyond nt 1434 allow replicase synthesis to proceed at wild type levels. This is in contrast to a number of paramyxoviruses and orthomyxoviruses (Chapters 15 and 18), in which infectivity, and therefore pathogenicity in animals, depends on cleavage of the envelope glycoprotein. 1a–h in Sodeik, B. It is not known whether proteins required for viral DNA replication also play some role in regulating late or very late gene transcription. Gene X is placed under the control of a late T7 phage promoter and thus cannot be expressed in a normal bacterial cell. Low levels of viral transcripts are made in sensory neurons, leading to formation of small amounts of progeny virions. Unlike their helper virus, the 3'-termini of these satellite RNAs do not fold into tRNA like structures, and they cannot be aminoacylated. However, this release mechanism is not universal for viruses of archaea; in many cases productive infection does not lead to cell lysis or death. Viruses with RNA genomes must code for such an enzyme, which is not available in the host cell. These seven genes code for nonstructural proteins (Table 6.2). B19 was discovered by accident during screening of blood donations. While several cellular transcription factors are involved in producing cytokines, NF-B is a key factor in this response. At this stage, the cell has become a factory for the expression and replication of viral genomes. Continued research on baculovirus gene regulation and gene function, and the basis of host range specificity, will provide important information for future applications of baculoviruses in biotechnology. An object with icosahedral symmetry has fivefold, threefold, and twofold rotation axes. Thus RNA polymerase serves as a winch to transfer the DNA from the capsid into the cell while making messenger RNAs (Figure 7.2). Binding of NS1 to these proteins blocks their functions, and therefore inhibits the normal 3' end cleavage and polyadenylation of cellular mRNAs. Because the polyadenylation of viral mRNAs occurs by a distinct mechanism and therefore does not depend on these cellular enzymes, the synthesis of viral mRNAs is not affected. Hemagglutinin-esterase (HE) Envelope protein expressed by some but not all coronaviruses. Herpesviruses can also serve as helper viruses, and exposure of the cell to certain agents that affect cell gene expression (ultraviolet light, chemical carcinogens, etc.) can allow virus replication to proceed in the absence of a helper virus. Each of these cell types carries out specific functions that are listed in Figure 34.1, and they will be encountered as we discuss the different elements of the mammalian immune response. Cucumber mosaic virus supports replication of satellite RNAs and also of defective RNAs derived from RNA 3. Thus the vaccinia virion is a “mini-nucleus”, a simple and easy-to-purify mRNA synthesis machine that has proved to be of extraordinary value for dissecting the biochemical details of transcription, mRNA 5' end capping, and mRNA 3' end polyadenylation. These viruses usually have multiple genome fragments (up to 12). A common problem that arises when the transgene is controlled by the LTR is reduction or loss of transgene expression. 1.2 Mb Most plant viruses and many viruses of vertebrates have positive-strand RNA genomes Figure 3.2 Size variation of viral genomes. Chemokines. E3 64 Amino-terminal part of PE2 containing the signal sequence. Once microviruses acquired an external scaffolding protein, which appears to be the more critical of the two, the internal scaffolding protein may have evolved into an “efficiency protein” that aids capsid assembly but is not strictly required for any one reaction. Reverse transcriptase-polymerase chain reaction (RT-PCR) and antigen detection enzyme-linked immunosorbent assays (ELISA) are most frequently used for acute laboratory diagnosis. The majority of known plant viruses have positive-strand RNA genomes. Nu1 binds to an anchoring site cosB, and protein A cleaves the DNA at an adjacent nicking site, cosN. Latent in secretory glands and lymphoreticular cells. CH029.indd 360 16/05/11 8:15 PM Human Immunodeficiency Virus repressive sequences (CRS) present in the gag, pol, and env regions inhibit the transport of RNAs to the cytoplasm; (2) a 240-nt sequence within env, termed the Rev response element (RRE), is a target for Rev binding and required for Rev action (Figure 29.6). 261 The low frequency of cell transformation in vitro (and tumor formation in vivo) is probably due to the low probability of integration of the early region of viral DNA into the cellular genome. However, it is also reasonable that many jurisdictions require those who refuse measles vaccine to keep their children out of school in the event of a measles outbreak. Nucleocapsids are assembled in the nucleus. These cancers are rare or localized to specific populations, and other factors probably contribute to their incidence. Released virions Plasma membrane Secretory vesicle CYTOPLASM Golgi membranes Model 3 Model 2 Model 1 Enlarged nuclear pore Normal nuclear pore Nuclear membrane NUCLEUS Figure 24.9 Envelopment and egress of herpesvirus virions. Transcription by host RNA polymerase then begins at two promoters, PL and PR (initiating transcription toward the left and right, respectively), located on either side of the repressor gene, cI (Figure 8.4). (Atlanta). 16/05/11 7:57 PM 80 CHAPTER 7 Bacteriophage T7 Table 7.1 Selected class I gene products Gene Function 0.3 Inhibits host type I restriction endonucleases 0.7 Inhibits E. Coding regions in the genome of hepatitis B virus are organized in a highly efficient fashion (Figure 30.3). Moreover, if the next deoxynucleoside triphosphate (pppdC in the figure) is present and binds to the enzyme, this induces the polymerase to freeze in a “dead-end complex,” leading to apparent inactivation of the enzyme. CMV: cytomegalovirus promoter; U3, U5, and R, unique 3⬘, unique 5⬘ and repeat regions of the retrovirus long terminal repeat; C: packaging signal; pA: polyadenylation signal. Thus, as is the case for acyclovir and herpes simplex virus, ganciclovir has selective activity against cytomegalovirus at two steps—activation by phosphorylation and inhibition of DNA polymerase. Large green oval is viral DNA polymerase, small gray oval is dNTP binding site, horizontal lines are template and growing daughter DNA strands. 1798 Jenner publishes Variolae Vaccinae, the use of cowpox inoculation to protect against smallpox. 15.2a: Courtesy of Guy Schoehn, CNBS, France; Fig. Segmented genome is composed of RNA 1 (~3.4 kb), RNA 2 (~3.1 kb), and RNA 3 (~2.2 kb). 523–540. MDV-1 RNA is first modified to contain a short sequence complementary to the target RNA; this sequence does not interfere with the replication of the RNA by Q polymerase. (a) An early doublemembrane vesicle showing a connection (arrow) to a reticular membrane. Most of the class II gene products are concerned with phage DNA replication. Bernander, and D. This leads to trimerization of the receptor, recruitment of adaptor molecules, and assembly of a protein complex called the “death-inducing signaling complex” or DISC. P1 is subsequently cleaved into capsid proteins VP0, VP1, and VP3; VP0 is cleaved into VP2 and VP4 during virion maturation. A British cell biologist, Thomas Cavalier-Smith, suggested how cells could solve the end replication problem for their linear chromosomes. Through this sequence E6 can target several PDZ domain-containing cellular substrates, including Dlg and Scribble. Transmission by droplets and small-particle aerosols has been observed among experimentally infected and quarantined imported monkeys. Peng, K. Can block cleavage of cytokines and therefore block their normal biological functions. Besides their unique morphologies, these viruses have exceptional genomes in which the majority of genes have no detectable homologs in other viruses or cellular life forms. Because type 3 reovirus also binds to sialic acid, the distribution or density of cell-surface carbohydrates may determine the types of cells infected by a given reovirus strain. Below: four isomeric forms of viral DNA with different orientations of the long (L) and short (S) components due to frequent recombination at the repeated sequence elements. 4.3a–c: Courtesy of Jurgen Kartenbeck, German Cancer Research Center; Fig. RNA oligonucleotide fingerprinting studies on field isolates of La Crosse virus revealed that no two isolates were identical. CH024.indd 294 16/05/11 8:12 PM Herpes Simplex Virus According to another theory (Model 2 in Figure 24.9), virions lose their envelope by fusion with the outer nuclear membrane, releasing nucleocapsids into the cytoplasm. As the RNA polymerase traverses the genome from right to left (Figure 14.5), it can pause and dissociate at any one of the TRSs located at the beginning of genes Leaders 2 through 7, or it can transcribe through all six TRSs and continue to the end of the genome, making a fulllength, negative-strand antigenome RNA. Foot-andmouth disease virus has an L proteinase, located at the very N-terminus of the polyprotein (Figure 11.4). The M2 protein allows hydrogen ions to enter the virion, releasing the RNP from the matrix protein. Why? An 800-nt polyadenylated messenger RNA is synthesized by cellular RNA polymerase II and is translated into proteins called hepatitis delta antigens. In all cases the singlestranded DNA is converted into a double-stranded form before being transcribed, so the polarity of the packaged strand is of little importance. For example, herpesviruses rapidly activate c-J un N-terminal kinase (JNK), extracellular regulated kinase (ERK), phosphoinositide 3-kinase (PI3 kinase), and mitogen-activated protein kinase (MAPK). The full significance of these interactions and how they affect viral replication and tumourigenesis are unknown. Archaea: one of the three domains of life, distinct from Bacteria and Eukarya by virtue of phylogenetic relatedness (based on ribosomal RNA sequences). However, complementary DNA copies of mRNA sequences for each of the viral proteins have been cloned in bacterial plasmids, and the proteins can be expressed either in bacterial or mammalian cells. Spontaneous mutations in feline enteric coronavirus accessory proteins alter the virulence, resulting in severe immunopathology that is almost always lethal. During the initial stages of synthesis of each mRNA molecule, the 2 spike protein catalyzes cap addition to the RNA 5' end. (eds.). Complete genome sequences are also available for five other chloroviruses. This 75-amino acid hydrophobic protein short-circuits the membrane potential, eventually leading to degradation of peptidoglycan. The complement of the single open reading frame encoding both forms of delta antigen is shown as a black rectangle. Ribosomes terminating coat translation can reinitiate at the lysis gene start site Independent access of ribosomes to the lysis gene start site (nt 1678) is precluded by the “lysis hairpin” (Figure 5.5). The integrase brings the two 3'-OH ends of viral DNA close to two phosphodiester linkages 4 to 6 nt apart on the host DNA, and joins viral to cellular DNA strands. At first, amantadine, which is a weak base, was thought to inhibit the acidification of endosomes, the cellular compartment in which influenza uncoating occurs. How do filoviruses ensure the production of high levels of their nucleocapsid protein? Therefore, RNAs whose length is an exact multiple of six nucleotides will have the last six nucleotides at their 3' end fully bound to an N protein subunit in a precise manner. Material between capsid and envelope, called the “tegument”, contains at least 14 viral proteins. One result of these interactions is that infected cells can grow and divide indefinitely, and if the cell is not killed by the virus it can eventually evolve into a tumor cell. • Viruses with double-stranded RNA genomes can have up to 12 different genome fragments; they are transcribed by a packaged RNA polymerase. Terminal direct repeat sequence of 150 bp. (a) Transmission electron micrograph of frozen-hydrated bovine papillomavirus type 1. GENES AND PROTEINS One to three transcriptional promoters on same DNA strand. In the 1970s and early 1980s, a number of changes were made in the rendering process for transforming diseased and otherwise unusable animal carcasses into meat, bone meal, and animal feed. Each chapter finishes with a list of Fundamental Concepts, statements outlining the most important facts or conclusions that the reader should have learned. MAJOR VIRUS GROUPS Study of the major groups of viruses leads to understanding of shared characteristics and replication pathways For convenience and simplicity, many of the major known virus groups (families and unassigned genera) are listed below in seven separate tables, based on the nature of their genomes and their replication strategy. In this way, a nucleus would be established, producing the first eukaryotic cell. PKR is a serine-threonine protein kinase that is present in normal cells at low levels; its expression is increased by interferon treatment. Many genital infections with human papillomaviruses are transient and result in no disease. Zaire ebolavirus re-emerged twice in the Democratic Republic of Congo: in Kikwit in 1995, with 315 cases (81% case fatality rate); and in Kasai Occidental Province in 2007, with 264 cases (71% case fatality rate). They can act as a competitive inhibitor, blocking binding of the natural nucleoside triphosphate to the active site of the enzyme. However, the concentrations of drug required to inhibit endosomal acidification are much higher than those needed for a therapeutic effect. Viral pathogens have been serious threats to health and society throughout the evolution of Homo sapiens. Some of these phages, including T7, were isolated from a commercially available mixture of phages being sold for treatment of intestinal infections. The structures of virions have evolved to carry out the task of transmitting the RNA or DNA genome of a virus from one cell to another and from one host organism to another. Blue lines correspond to RNA, black lines correspond to DNA. 9.7a-c: Reprinted from Fig 4 A, Fig 5 A and G in: Monika Haring, Reinhard Rachel, Xu Peng, Roger A. F-pili are filamentous structures of E. Infection of vertebrate cells quickly inhibits host cell protein synthesis and leads to cell death. INDUCTION OF ANTIVIRAL ACTIVITY Interferons interact with specific receptors on the surface of target cells. Thank you, all. High concentrations of non-infectious particles can be found in the serum together with Dane particles during the acute phase of infection. 16/05/11 8:10 PM 268 CHAPTER 22 Papillomaviruses Activates transcription of P21 P53 Blocks CDK Rb Cyclin tes la ory ~P Rb h osp Ph Rb E2F E2F E7 Blocks transcription of cell-cycle genes Bin ds Activates transcription of cell-cycle genes CELL-CYCLE PROGRESSION to R b Rb E7 CELL-CYCLE ARREST Figure 22.5 Papillomavirus E7 protein activates cell-cycle progression. In 1881, Robert Koch introduced gelatin and agar-based culture systems and, in 1887, R. By virtue of selfcomplementary sequences, it has a structure similar to double-stranded RNA. • The viral matrix protein, VP40, can stimulate budding at membranes and produces virus-like particles when expressed in mammalian cells. It would take only 2000 such virions lined up in a row to cover a length of 1 millimeter. Fifty-nine phage proteins have been identified by genetic or biochemical evidence; some genes give rise to more than one protein by initiation of translation at internal AUG codons. This chapter will concentrate on two well-studied human herpesviruses: herpes simplex virus type 1 and Epstein–Barr virus. What kind of genome does it have? Alloleviviruses have in addition about 12 copies of the readthrough protein in their capsid. Hepatitis B virus is transmitted by blood transfusions, contaminated needles, and unprotected sex Transmission of hepatitis B virus is primarily through blood or sexual contact. In contrast, HIV-1 is able to transport the preintegration complex into the nucleus CH029.indd 359 After integration of the HIV-1 proviral DNA into the host cell genome, there are two possible outcomes: latent infection, in which viral RNA is not expressed, or active infection, leading to production of progeny virus. But when cells are transfected with naked viral DNAs instead of being infected with virions, the cell-specific block is overcome and normally nonpermissive cells produce virus. There was less replication in vascular endothelial cells, hepatocytes, and tubular epithelium of the kidney. For example, many enterohemorrhagic E. Fusion peptide: a short hydrophobic region within a viral envelope protein that is inserted into the target cellular membrane during virus-induced membrane fusion. The ability of a retrovirus to infect specific cell types (tropism) is determined by the envelope protein, which mediates attachment of the virion to the cell surface as well as entry by fusion or endocytosis. Two kinds of occlusion bodies have been described: polyhedra 0.15 to 15 μm in diameter, which can contain many virions, are made by nucleopolyhedroviruses. Presently, most herpes simplex viral genes are numbered sequentially from one end of the unique sequence in which they are found. 347 Host repair enzymes GGGCCTG U3 R U5 CCCGGAC Cell DNA U3 R U5 CAGGCCC GTCCGGG Proviral DNA Cell DNA Figure 28.5 Integration of proviral DNA into host cell DNA. There is still no cure for individuals infected with hepatitis B virus, and infection remains a major worldwide health problem, particularly because it can be a persistent, chronic infection that can eventually lead to serious liver disease. NS4A 149 RNA replicase component, may recruit NS1 and other proteins into replicase complex. Viruses can replicate only within living cells. Adenoid: growth of lymphoid tissue in the upper part of the throat, behind the nose. What effects does picornavirus infection have on host cells? At a specific 8-nt sequence in the concatemer junction, RNA polymerases pause, forming an unusual DNA structure that recruits the DNA processing machinery, which cleaves the DNA at this 8-nt sequence. Viroids differ from viruses not only by their small size and lack of encapsidation of their RNA; viroids do not code for any proteins and therefore must rely on host enzymes for their replication. Alan Hay and collaborators at the National Institute for Medical Research in London (UK) isolated a set of influenza virus mutants resistant to amantadine. However, lentiviruses code for proteins that mediate the active transport of the viral DNA complex through the nuclear pore (shown in Figure 28.3; see also Chapter 29). Because there are approximately 1013 cells in a single human being, the aggregate length of all of the DNA in a single individual is 1013 meters (1010 kilometers), or 50 times the distance between the Earth and the sun! double-stranded RNA genomes package their respective RNA polymerases in the virion. Plasmid DNA produced in bacteria is methylated, but when it is introduced into eukaryotic cells and undergoes replication, the DNA is no longer methylated. P designates regions that are phosphorylated, NLS designates the nuclear localization signal, and Zn designates a zinc-binding site. A potentially important new human parvovirus was recently discovered, the human bocavirus (HBoV), which derives its name from the fact that it is closely related to bovine parvovirus and to the minute virus of canines. ALD is a fatal brain disease, manifested by a malfunction in myelin turnover caused by mutations in the ABCD1 transporter gene. Genome segment 8 codes for two nonstructural proteins (NS1 and NS2), although NS2 is also found in small amounts in virions. In contrast, the deep “canyons” present around the pentons in picornaviruses constitute indented receptorbinding sites (see Chapter 11). A number of DNA viruses make early proteins that interact with pRb and stimulate the cell cycle. Adaptability and evolution of coronaviruses The spectrum of diseases observed in coronavirus infections reflects the capacity of these viruses to adapt to changing environments and to new hosts. Of the genes that do have database matches, most are similar to core genes of viruses in the NCDLV group. Current therapy calls for the simultaneous use of multiple drugs affecting several steps of the virus life cycle in an attempt to generate a higher barrier for the virus to overcome. 1237–1244. One of the pentasymmetrons has at its center a unique spike that projects 25 nm outward from the virion, and this is likely the organelle with which virions contact the cell wall of host cells and initiate its degradation (see below). Viroids and Hepatitis Delta Virus 378 Viroids are small, circular RNAs that do not encode proteins 379 The two families of viroids have distinct properties 379 TOC.indd xvi Viroids replicate via linear multimeric RNA intermediates 380 Three enzymatic activities are needed for viroid replication 380 How do viroids cause disease? Virus is transported along the axon to mucosal sites where the virus can replicate. Note the large inclusion structures in the cytoplasm that contain reovirus proteins and the absence of reovirus proteins in the nucleus (Scale bar: 25 m). Examination of genome sequences of many coronaviruses isolated from animals and humans at different stages in the pandemic, and from bats, suggest that the second pathway, although seemingly more complicated, might be correct. PKR, a latent protein kinase activated by double-stranded RNA, interferes with translation of mRNAs by phosphorylation (and consequent inactivation) of eukaryotic initiation factor 2␣ (eIF-2␣). Upon infection the genome circularizes; a linear genome is shown for convenience. CH008.indd 96 Lambda phage infection of E. For example, bacteriophages are currently classified into families based on the nature of their genomes and on their capsid and tail morphology. An additional improvement is the deletion of the enhancer/ CMV gag rev pro pA Packaging construct pol CMV pA Envelope construct VSV-G RSV R U5 ≁ RRE Promoter Transgene ◎ R U5 Transfer construct cPPT Figure 37.5 Production of lentivirus vectors. This exonuclease activity is used by cellular DNA polymerases to remove regions that contain mispaired bases, allowing their repair. Most importantly, no noticeable toxicity or immune response was observed. Approximately equal amounts of the four mRNAs are made. All known DNA polymerases need a primer, usually RNA, to initiate DNA synthesis, which proceeds in the 5' to 3' direction. Yeast prions also exhibit other properties of their mammalian counterparts, exhibiting both strain variability and barriers to interspecies transmission. However, fulllength transcripts from these genes are only detected by 5–6 hours after induction. The genomes of these viruses are smaller than the microvirus genomes. Figure 27.6 Electron micrograph of cells of the coccolithophore Emiliania huxleyi and a bound virion of EhV-86. There are two major differences between genome replication and transcription. The enveloped virion is then released into the extracellular space directly, or after transport through the secretory pathway. The membrane fusion is expected to generate huge gaps in the cell envelopes, completing cell lysis. It is likely that a complex global program that evolves from use of the live vaccine to the inactivated vaccine and continues vaccination long after cases of polio disappear, will be needed before eradication can be achieved. Packaging of viral genomes Brome mosaic virus has been used as a model for the study of packaging of segmented viral genomes. Beginning at the 3' end, the polymerase copies the single-stranded genome DNA up to its 5' end, generating a double-stranded DNA molecule with an intact hairpin at one end (step 2 in Figure 20.4). Activities in different cell layers are shown in the middle. Early proteins E6 and E7 can transform cells in vitro, are expressed in cervical carcinomas. Signal sequences: gray rectangles; transmembrane segments: orange rectangles; processed viral envelope proteins: orange spikes; viral RNA: blue curved line. These genes are essential for virus replication in non-dividing cells (e.g., neurons) but not in dividing cells, which can make cellular analogues of these enzymes. N-terminus is at left, C-terminus at right; the mature protein begins at amino acid 23 and ends at amino acid 230. 7.1: Redrawn from Fig. These include four proteins that may bind to the origin of replication, a chromosomesegregation ATPase, and two DNA-binding proteins. Raphidovirus HaV01 Heterosigma akashiwo CH027.indd 326 Single-celled, free-swimming marine algae that tend to produce toxic surface aggregations known as harmful algal blooms. The three-domain system proposed by Carl Woese, based on sequence analysis of ribosomal RNAs. • • • • • bacteria; instead, they are usually made of an impermeable “S layer” of proteins that in many cases are directly associated with the cytoplasmic membrane. The capsid is enclosed in an envelope that contains at least 10 different glycoproteins (gB through gM) and several other non-glycosylated membrane proteins. All three origins are flanked by genes whose promoters direct divergent transcription away from the origin, reminiscent of the replication origins of polyomaviruses. Measles is a serious childhood disease caused by a paramyxovirus Measles virus was probably a newly emerging virus some 6000 years ago during the urbanization stage of human civilization. Parvoviruses replicate in rapidly growing cells that are in specific states of differentiation, but not in resting cells that make up most of the body tissues. This extra 5' noncoding region may aid in recognition of the AUG by ribosomes. CH011.indd 134 16/05/11 8:02 PM Picornaviruses 135 Picornavirus pathogenesis and disease Picornaviruses cause a number of important and well-studied diseases in humans and animals. The genome of retroviruses contains a sequence known as the “kissing loop”. Nucleosomes: complexes assembled from two copies each of the four cellular histones H2A, H2B, H3, and H4. This enzyme opens the dsDNA at telRL and seals the resulting ends, creating covalently closed hairpins at both ends of the duplex. The positions of conserved sequence elements are shown in orange. These highlights encourage students to discover new information about virology from other sources.Key terms, fundamental concepts, and review questions at the end of each chapter help students review and organize information they have learned.Extended coverage. These objects range in size from tens of meters (geodesic domes) to 0.7 nm in diameter (C60), and all share unusual strength, stability, and simplicity of construction based on their use of identical subunits joined in a symmetrical pattern. The T 1 inner capsid encloses the 10 double-stranded RNA gene segments and is covered by a T 13 outer capsid. Some coronaviruses, notably betacoronaviruses, possess a hemagglutininesterase (HE) protein on the surface of the virion. These views, revealed by confocal microscopes using focused laser light sources, show the localization of viral macromolecules within the nucleus, or in association with organelles such as the endoplasmic reticulum, lysosomes, or the plasma membrane. 16/05/11 8:21 PM Antiviral Chemotherapy The intracellular metabolism of AZT is very similar to that of thymidine (Figure 36.7). When CH019.indd 233 Reoviruses induce apoptosis via activation of innate immune response transcription factors NF-B and IRF-3 Reoviruses can induce apoptosis (programmed cell death) in cultured cells and in host animals. See Chapter 28 for a complete description of how this occurs. Elements shown with a prime are inverted. In humans, integration occurs preferentially on chromosome 19 at a site called AAVS1. 1953 Description of first immortalized cell line (HeLa). 3C then cleaves at its own N-terminus within P3 to generate 3AB and 3CD. Although hepatitis C virus will not be discussed in detail in this chapter, it is perhaps the most interesting member of the family from a clinical perspective. The B-cell receptors are membrane-bound antibodies. It turns out that all hyperthermophilic microorganisms, both archaea and bacteria, code for a “reverse gyrase” that introduces positive supercoils into DNA. Most adenovirus serotypes, as well as Coxsackievirus B3 (a picornavirus; see Chapter 11) utilize the same receptor on human cells, termed Coxsackievirus and adenovirus receptor (CAR). Early and late genes are indicated by shading, and a function for each gene, where known, is indicated. WHY STUDY VIRUSES? Of historical interest, one of the earliest reports of a phycodnavirus was that of a putative prymnesiovirus observed in thin sections of a Chysochromulina species in 1974; this was well before the importance of algal viruses had been appreciated. Based on these results, both vaccines have been licensed for use in the general population in many different countries. By this mechanism, viruses with many combinations of the different HA and NA subtypes, as well as other viral genes, are generated in multiply infected animals in the wild. It has been suggested that they inhibit ICP0 and ICP34.5 expression by an antisense mechanism involving microRNAs, therefore inhibiting viral gene expression. The production of these second-generation vectors required the development of producer cell lines expressing the corresponding deleted genes, which are required for virus replication. Acidification within the endosomal vesicle causes a conformational change in the capsid, resulting in the dissociation of capsid subunits and release of the viral RNA. Viral mRNAs terminate in poly(A) tails generated by “stuttering” transcription Each genome RNA segment contains a short poly(U) stretch 15 to 22 nt from the 5' end of the RNA. Co-expression of VP40 with GP and NP increases production of such particles, which resemble mature filovirus virions. In animals infected with Ebola virus, replication was extensive in macrophages, monocytes, dendritic 16/05/11 8:05 PM 198 CHAPTER 16 Filoviruses cells, and fibroblasts of many organs. In 1918, an influenza pandemic killed 20 million people worldwide. RNA polymerase II holoenzyme: a complex consisting of eukaryotic RNA polymerase II plus accessory proteins, which is active in transcription. Several groups are trying to exploit this amplification capacity to detect minute amounts of DNA or RNA molecules in clinical samples, for instance HIV-RNA. This prevents its incorporation into virions, and therefore counteracts this cellular antiviral defense mechanism. 16/05/11 8:04 PM 152 CHAPTER 13 Togaviruses TABLE 13.3 Sindbis virus structural proteins Protein Length (aa) Properties and functions Capsid 264 Forms nucleocapsid; contains serine-like protease that autocatalytically cleaves itself from the polyprotein. Because it is based primarily on virion morphology, this classification system is somewhat arbitrary. It may be that one virus (HHPV-1) packages the double-stranded replicative intermediate form of the DNA genome, and the other (HRPV-1) packages the single-stranded DNA form made during rolling circle replication (see replication of ϕX174 DNA in Chapter 6, Figure 6.4). akashiwo as a ubiquitous and problematic bloom-former in coastal regions. Negative contrast electron micrographs of virions. In this chapter, emphasis in different sections is placed on results found with either mouse polyomavirus or simian virus 40, but these two viruses have a common overall replication strategy and they share many details of structure, genome organization, and gene function. Proteins of yeast and other fungi can form self-propagating states resembling prions Reed Wickner proposed that an inheritable yeast phenotype [URE3] is the result of a prion-like, self-propagating change in the conformation of the nitrogen metabolism protein Ure2. 29.2: Courtesy Liwie Rong, McGill University. Thus RNA editing in Ebola viruses limits the amount of GP made, allowing the infected cell to produce higher levels of virus. Foods Genetic manipulation of plants has made it possible to consider the delivery of viral and other antigens in foods such as bananas. E6 proteins from high-risk human papillomaviruses have also been shown to interact with several CH022.indd 269 269 other cellular proteins involved in signal transduction, apoptosis, DNA replication, and calcium metabolism. Receptors (viral): cell surface proteins, lipids, or carbohydrates to which specific viruses attach and begin the process of entry into the cells. However, sheep and goat herders have long recognized that scrapie behaves like an infectious disease. A circular template can be made from an adenovirus vector by inserting two recombination sites, such as loxP sites, in the vector backbone. Retroviruses enter cells by the fusion pathway The retrovirus life cycle takes place in two phases. Injection of CpG DNA can elicit innate cellular Th-1-like responses and therefore increase the host response to antigens. This fascinating mechanism, termed “induced substrate inhibition,” involves three steps (Figure 36.6). These innovations set the stage (in 1931) for the first use of eggs to culture viruses; an egg can be thought of as a sterile tissue culture flask with a calcium shell. The Gag/Pol polyprotein is incorporated into the assembling virus core by interaction between its CA region and the corresponding region of Gag. The slightly longer preC RNA is not packaged, apparently because translation of the preC open reading frame (which overlaps with the stem-loop) interferes with packaging. Humans become infected by inhaling aerosolized excretions from infected rodents. Mosaic disease: a disease that causes symptoms on plant leaves that result in a mosaic pattern of light and dark green, or green and yellow. Baculoviruses can attach to and penetrate nonpermissive cells from a wide variety of tissues and species, and the viral genome is transported to the nucleus, but viral promoters are not recognized in these non-permissive cells, so no viral gene expression occurs. As the antigenome RNA serves only as a template for viral genome synthesis, it is made in about 10-fold lower amounts than the genome. There is evidence that the earliest stages (virus entry and uncoating) and the final stages (assembly and release) of virus infection can be inhibited by interferon treatment of cells. A number of influenza type A virions (small spherical particles) are shown attached to the cilia. An overview of the variety of known viruses is given in Chapter 3. Foreign genes can be spliced into poxviruses and expressed during virus infection, providing research vectors for eukaryotic gene expression as well as promising recombinant vaccines. Up to 1% of the cells within the lymph nodes and circulation of recently infected individuals contain integrated HIV-1 provirus, but only about 1% of those infected cells actively express viral RNA. This section of the book introduces the student to the study of viruses, their structure and classification, and how they enter cells and begin their replication cycles. It is a target for neutralizing antibody and cytotoxic T-lymphocyte responses to virus infection. Reovirus RNA replication and virion assembly take place in intracytoplasmic inclusions (Figure 19.7), detectable as early as 8 hours postinfection. See also Measles virus cell entrance by fusion, 178–180 envelope proteins, 178–179 gene map, 181 gene order, 180 genome, within helical nucleocapsids, 178 helical nucleocapsid, 180 human diseases, 177 mRNA synthesis, 183–184 N protein controls transcription/replication switch, 185–186 P/C/V gene coding/functions, 184–185 promoter, 181–182 replication, 183 RNA editing, 184 RNA polymerase, 180, 181 rule of six, 185 structure, 177 transcription, 183 virion assembly, 186 virions, 177, 178 Parvoviruses, 21, 238–245 adeno-associated viruses (AAV), adenovirus functions as helper virus, 244 antitumor effects, 245 B19 virus, 244–245 discovery, 239 DNA ‘end replication’ problem, 241–243 DNA replication, 241–243 genes and transcripts, 239–240 hairpin ends on DNA, 241 integration of viral DNA, 244 minute virus of mice, nonstructural proteins, 243–244 replication, 239 representative parvoviruses, 239 single-stranded parvovirus DNAs, 240 uncoating, 240–241 virions, 238, 239 Peptidomimetic drug, 452 Perforin, 418 Peripheral or secondary lymphoid organs, 419 Peritonitis, 160 Peritrophic membrane, 306 Permissive cells, 241, 261 Persistent infection, 149 pET vectors, Phaeovirus, 335 brown algae, 335 integration of genome in host DNA, 335 bindsub.indd 497 temperate life cycle, 335 Phagocytosis, 322, 417 Phagosomes, 401 ‘Phage group’ stimulated studies of bacteriophages, 7–8 pH-dependent fusion, 48, 49, 50–51, 54, 141, 150, 164, 178, 194, 204, 214, 345 pH-independent viruses, 49–50, 52, 54 Phosphatase 2A (PP2A), Phosphoinositide 3-kinase (PI3 kinase), 403 Physical virus particles to infectious particles ratio, 11 Phycodnaviruses, 325–336. This mechanism of transmission therefore often results in the delivery of many virus particles simultaneously into the same cell. Trisymmetrons: triangular capsid substructures composed of multiple trimeric or hexameric capsomers. VP24 (virus protein, 24 kDa) Associated with membranes; interferon signaling antagonist; involvement in host adaptation. Thus there is a remarkable economy of function, with two enzymes, the cap guanylyltransferase and the cap ribose-methyltransferase, performing dual functions essential for both 5' end and 3' end maturation of the early mRNA. These structures are believed to serve a number of important functions in virus replication. • The host immune response is composed of a rapid, non-specific innate response, and a slower, highly specific adaptive immune response. What is induction? In an alternative pathway (not shown), replication can begin at both ends of a double-stranded DNA molecule, as both ends are identical. How do influenza viruses manage to cap their own mRNAs and greatly reduce cellular protein synthesis in one fell swoop? The packaged DNA is cleaved from the concatemer at a specific site within a 20-nt direct repeat flanking the terminal a sequence. Lecturers might want to supplement material given in the text with experimental methods or results, which are not covered because of lack of space. For example, the cellular receptor for adenovirus 35 is CD46, a protein located at the surface of many tumor cells. Semler Picornaviridae From Spanish pico (point; 10⩾12), referring to small size of virions, and RNA VIRION Naked icosahedral capsid (T = 1). (a) Plasmid constructs used to establish a packaging cell line expressing gag, pol, and env gene products. Rolling-circle replication: synthesis of a linear RNA or DNA from a circular template by an RNA or DNA polymerase that cycles around the template numerous times without detaching, resulting in an extended linear molecule that contains tandem repeats of the complement of the template sequence. Electron micrographs of elongated influenza virions budding from infected cells cultured in vitro, and sectioned in a horizontal plane (left) or a vertical plane (right), show a specific arrangement of eight internal rod-like structures of different lengths (seen in serial sections through particles, not shown). 1950 Enders, Weller, and Robbins launch systematic study of cell culture. These hairpin structures are typically found within the introns of capped and polyadenylated transcripts (“primary miRNAs”). Replication of linear parvovirus genomes involves self-priming and specific cleavage of hairpin sequences by viral nucleases; this is a model that may apply to linear DNA genomes of much larger viruses. Nucleocapsids are assembled in the cytosol and reverse transcription generates the DNA genome. Membrane associated; palmitoylated; C-terminus is signal sequence for E1. Second, if viral DNA polymerases are more sensitive to inhibition by the phosphorylated analogues than are cellular DNA polymerases (or other cellular enzymes), replication of the viral genome in infected cells should be inhibited at drug concentrations low enough to avoid toxic effects on uninfected cells. Induction is a way for to bail out of a damaged cell and move on to more fertile hunting grounds. The DNA “end replication” problem Besides acting as transcriptional activators, the NS proteins also directly control the replication of viral DNA. Distinguished from the adaptive immune response, which develops more slowly but is highly specific for particular antigens and usually results in development of both specific antibodies and cytotoxic cells. In humans, local or systemic stimuli—such as physical or emotional stress, hyperthermia, exposure to ultraviolet light, menstruation, and so on—induce reactivation of herpes simplex virus from latency. • Human prion diseases can be transmitted or inherited. Ac acetyl; Me methyl. At the time of writing, the major influenza vaccine manufacturers are still heavily dependent on embryonated eggs. Binding of reoviruses to JAM-A on endothelial cells that line blood vessels is required for spread of the virus via the circulatory system. These are spatially linked to sites of assembly and budding of immature virus on membranes of the endoplasmic reticulum (ER) (lower right). particles along microtubules from the cell periphery in the direction of the microtubule-organizing center, located close to the nucleus. These genomes can be transcribed by cellular RNA polymerase II immediately upon arrival in the cell nucleus. Variolation: artificial inoculation with variola (smallpox) virus, designed to produce immunity to natural smallpox infection. (c) Nucleases remove the 5⬘ ends of both strands. The two copies of oriS are shown flanked by genes. Whether the viral RNA is threaded into an empty capsid or is bound by 14S pentamers prior to particle assembly, only positive-strand viral RNAs with a VPg protein linked to the 5' end are packaged. (b) Electron micrograph of negatively stained nucleocapsids purified from vesicular stomatitis virus. huxleyi are non-motile, CH027.indd 333 333 coccolith-bearing cells, perhaps ironic given some early thinking that coccoliths actually played an antiviral role. Bacteriophage T7 and related phages have evolved a strategy to avoid these methylation-dependent restriction systems by inactivating the restriction endonuclease. C-Src can modify a number of other cellular proteins by phosphorylating specific tyrosine residues on those proteins. If this were the case, it is not clear why the virus should purify along with the prion protein, nor why the virus should remain resistant to treatments that normally destroy nucleic acids. Parvovirus capsid protein subunits are arranged on the surface of the capsid with icosahedral symmetry (Figure 2.3a; compare with Figures 2.2b and c). Like the NS2A and NS2B proteins, they contain several transmembrane domains, and NS4A also contains a signal sequence. It binds to the cellular epidermal growth factor receptor, and plays an important role in stimulating the growth of cells, resulting in increased virus replication. In a healthy person this is not serious. The genome of yellow fever virus is an RNA molecule 10,862 nucleotides in length. Further characterization

of the replication cycles of archaeal viruses and their interactions with the host cell will surely lead to interesting discoveries. SOS genes: a collection of LexA-regulated genes that function to repair damaged DNA. The genomes of the pestiviruses and hepatitis C virus are similar in layout but differ in the number and type of proteins. Also in the 1960s, it became possible to study the genome of a phage by physicochemical techniques. Genome segments 1 to 6 each code for a single protein: three RNA polymerase subunits CH018.indd 212 (PB2, PB1, and PA), the envelope glycoprotein hemagglutinin (HA), the nucleocapsid protein (NP), and a second envelope glycoprotein, neuraminidase (NA). CH020.indd 245 16/05/11 8:08 PM 246 CHAPTER 20 Parvoviruses • Dependent parvoviruses replicate in cells infected with a helper virus, which induces entry of the cell into S phase. This finding so challenged the concept of the gene, reported The New York Times, that some scientists were searching for hidden messages in the DNA sequence, hypothesizing that ϕX174 was a 69 CH006.indd 69 16/05/11 7:56 PM 70 CHAPTER 6 Microviruses Table 6.1 Phages in the Microviridae family Genus Hosts Examples E. Maraviroc blocks infection of HIV-1 strains that use CCR5 for attachment and entry, but not HIV-1 strains that use CXCR4. This condition is therefore termed spongiform encephalopathy. An infectious influenza virus with the sequence of the 1918 strain was generated in a high-level biosecurity laboratory by reverse genetic engineering and its properties were tested. Transformed cells are, in addition, more sensitive to the toxic effects of NS1. When the genome-expression plasmid is transfected into such a cell, authentic viral genome RNA is synthesized. Proviral DNA is blue; host DNA is black. This model is suggested by the presence in the cytoplasm of free, fully formed nucleocapsids, and by electron microscopic images showing budding virus particles that contain such nucleocapsids. Furthermore, the class II gp3.5, which has lysozyme activity, binds specifically to T7 RNA polymerase and inhibits its activity. Because viruses can efficiently target specific cell types and express their genes at high levels, they can be used as vectors for expression of a variety of genes. Multiple copies of the C protein condense on the genome RNA to form the nucleocapsid. is a common childhood disease that can be serious particularly when people are first exposed as adults. Eight influenza virus genome segments code for a total of 11 different viral proteins Of the eleven viral proteins produced by influenza type A viruses, nine are packaged in virions (Figure 18.3 and Table 18.1). BGLOSS.indd 472 Capsid-binding drugs: a class of drugs that bind to hydrophobic pockets on picornavirus capsids and inhibit viral attachment and/or entry. This common protein modification enables interaction of proteins with lipid membranes (see below). Differential splicing produces 3 to 4 mRNAs from each transcription unit. each of the 10 unique RNA segments is packaged into each particle is not understood. Although generally accepted, the prion hypothesis has not convinced all scientists in the field. They enter the cytosol by a pH-independent mechanism through the plasma membrane or an endosomal membrane, during which an outer layer of capsid proteins is removed, but the inner capsid remains intact. Only one in 300,000 measles victims are affected by SSPE, which seems to result from a slow, persistent infection and strikes up to 10 years after the acute illness. Sequences called cis-acting replication elements (cre), located in various regions of the genome RNA depending on the virus (Figure 11.7), are required to initiate picornavirus RNA synthesis by serving as templates for uridylylation of the VPg primer. DISTINCTIVE CHARACTERISTICS Viroids are the only known infectious, pathogenic RNAs that are not packaged and do not code for any protein product. Lysogen: a bacterial cell containing a prophage. Guanylyltransferase: an enzyme that transfers a guanosine residue to the phosphorylated 5⬘ end of an RNA, creating an RNA “cap” structure. Each hexagon is believed to be formed from trimers of PrP 27–30 dimers. With this mechanism of picornavirus attachment and entry in mind, how capsid-binding drugs act can be understood. sequence near the middle of the genome, which encodes 11 tRNA genes. Risky activities to avoid include intravenous drug use and unprotected sexual intercourse with multiple partners. The coat protein will form capsids even in the absence of phage RNA. Low amounts of Rep78 are then made, even in the absence of helper virus. Polymerase protein. For the examples shown in Figures 2.5a and b, it takes two single jumps in two different directions to get from the fivefold axis above the equator to the fivefold axis below the equator. Apoptosis: programmed cell death, involving a number of signaling pathways that lead to activation of caspases (proteases), fragmentation of chromosomal DNA, condensation of the nucleus, and blebbing of the cytoplasm. A small number of copies of the readthrough protein are incorporated into the capsid; their function is unknown. Viruses that contain mutations in E1A genes make strongly reduced amounts of other early gene products, and therefore little DNA or progeny virions. CH010.indd 114 RNA 4 is a subgenomic RNA equivalent to the 3' half of RNA 3; it encodes the coat protein. This complex binds to gene promoters and leads to their transcription. Purified genome RNA can be used to infect cells, showing that none of the structural proteins present in the virion is needed to initiate the replication cycle once the RNA has entered the cell. 3' and 5' ends of linear RNAs are labeled. This proteinRNA interaction is believed to lead to capsid formation by serving as a nucleation site for the further addition of coat dimers. Unlike acyclovir (Figure 36.5), AZT is not selectively phosphorylated by a viral kinase, but follows the phosphorylation pathway of thymidine in the cell. Fig. They use distinct transcriptional promoters and initiation factors, but are otherwise similar and can be considered together. After attachment via virion glycoproteins, bunyaviruses enter the cell by endocytosis No cellular receptor has yet been identified for most members of the Bunyaviridae family. Mutations of the RPE65 gene, involved in the retinoid cycle, are responsible for 15% of cases of Leber congenital amaurosis. CH010.indd 123 16/05/11 8:01 PM 124 CHAPTER 10 Cucumber Mosaic Virus 2. Pore formation has not been directly demonstrated, but this is an attractive hypothesis to explain entry of viral RNA from altered virions at the cell surface. Reovirus infection of mice with experimentally induced tumors leads to tumor regression. Virus diseases affecting domesticated plants such as potatoes, tomatoes, tobacco, coconut trees, and citrus trees are common and widespread. Furthermore, antiviral drugs provide a terrific avenue for learning more about viruses. This modification permits transcription of viral RNA without the need of the Tat protein. Production of virus can be induced by subjecting the host cell to stress conditions, including irradiation with ultraviolet light, treatment with mytomycin C, or freezing-thawing. To make sense out of this large collection of viruses, scientists have attempted to classify viruses into related groups. CH024.indd 295 295 ICP34.5. Herpesvirus genes are tightly packed and are transcribed from both DNA strands. The envelope protein most often used is that from vesicular stomatitis virus (VSV-G), as this enables the vector to infect many different cell types. Recent epidemics in Southeast Asia and Australia with a number of human fatalities were caused by two newly discovered paramyxoviruses named Nipah and Hendra. In recent years, T7 phage has emerged as a useful model system for the study of microbial evolution because of its rapid reproduction, relatively simple structure, well-understood biology, and simple regulatory strategies. Gray, University of Texas, Dallas: reprinted from Gray, C.W., R.S. Brown, and D.A. Marvin, “Adsorption complex of filamentous fd virus,” Journal of Molecular Biology 146 (1981): 621–627; Fig. In vitro ligate cDNA fragments T7 x genome 4. HA is first synthesized in the cell as a fusion-incompetent protein that is inserted into the membrane of the endoplasmic reticulum and transported via the Golgi apparatus to the plasma membrane, where it is incorporated into virions (see below). (b) Tat recruits cyclin T and cyclin-dependent kinase-9 (Cdk9) to the transcription complex shortly after initiation, resulting in hyperphosphorylation (P) of the carboxy-terminal domain of RNA polymerase II. Experimental evolutionary studies. The TMV movement protein can complement the function of missing or mutated cucumber mosaic virus protein 3a. Virions are assembled by budding at endoplasmic reticulum and are released by exocytosis. In newborn mice, reoviruses cause patterns of disease that vary with the virus serotype. The genome of cucumber mosaic virus encodes five multifunctional proteins The three genome segments of cucumber mosaic virus encode a total of five different proteins, denoted 1a, 2a, 2b, 3a, and CP (Figure 10.4 and Table 10.1). Fusion: see Membrane fusion. This requires three conserved glycoproteins: gB, gH, and gL. Most have internal or external lipid envelopes. Genes are distributed on both DNA strands. A variety of proteins, including interleukin-6 and annexin V, have been reported to interact with the preS1 region of LS; these may be cellular receptors for the virus. Raltegravir came out of a drug discovery program that utilized a target-based assay of integrase. A 399-nt intron (133 triplet codons) keeps the ribosome in reading frame 0, as with small T antigen mRNA. Second, it blocks the development of Th2 cells by inhibiting interleukin-4 production, which is required for Th2 cell formation. SINEs are dependent on the reverse transcriptase provided by LINEs for their replication. HA is a 549-amino acid type I transmembrane protein, with its N-terminus exposed to the outside of the virion, a hydrophobic transmembrane domain near the C-terminus, and a short C-terminal tail projecting inward (Figure 18.4). Each plaque usually arises from a single infectious virus particle. Supercoiling of relaxed DNA molecules can be catalyzed by specialized enzymes found in bacteria, called DNA gyrases, which carry out the reverse reaction of topoisomerase I, using ATP as an energy source. Virions of various lengths have been observed by electron microscopic examination of AcMNPV mutants that produce genomes of different sizes. Modifications such as glycosylation of the ectodomain and palmitoylation of cysteines near the inner margin of the lipid bilayer occur in the endoplasmic reticulum and in the Golgi complex. Transovarial transmission is an important mechanism for the maintenance of these viruses in nature, as the virus can survive in the egg until conditions are favorable for the arthropod to hatch. The P protein is a cofactor required for viral RNA synthesis carried out by the L protein. The K-12 () cells grew to form a cell lawn, but there were plaques in the lawn caused by virions in the supernatant of the K-12 () culture. An antigen-presenting cell ingests proteins and displays peptide fragments on the cell surface in association with MHC II protein. Entry of viral DNA into the capsids is carried out by a multimer of UL6 protein located at one vertex of the capsid, and involves several other nonstructural proteins. 311 KEY TERMS α-amanitin Apoptosis Budded virions Calyx Granulin Hemocoel Microvilli Occlusion body Occlusion-derived virions Peritrophic membrane Polyhedrin Tegument Ubiquitin Virogenic stroma FUNDAMENTAL CONCEPTS • The family Baculoviridae consists of a large number of different species of rod-shaped enveloped viruses with double-stranded circular DNA genomes varying from 80 to 180 kb pairs. • The polypeptide chain of many capsid proteins is folded as a jelly-roll -barrel. Novel 100 kDa phage-coded RNA polymerase transcribes class II and III genes. This feature makes these two proteins ideal targets for potential therapeutic intervention in malignancies induced by human papillomaviruses. The intensive study of bacteriophages led to discovery of some of the fundamental principles of molecular biology and genetics. (2004), 93–117, Meyers, R.A. (ed.), Weinheim: Wiley-VCH. Therefore, most normal cells are protected against NK cell attack if they are expressing MHC proteins. Mdm-2 binds to p53 and targets it for ubiquitin-mediated degradation, whereas p14Arf binds to Mdm-2 and blocks its binding to p53, thus stabilizing p53. The second phase of Stage II DNA replication is the formation of more replicative form DNA from the progeny plusstrand viral DNA (step 5). However, an RNA sequence just to the left of U3, consisting of a polypurine tract (ppt), is resistant to digestion, and remains hybridized to the newly synthesized minus-strand DNA. cit., Fig. • An alternative assembly model proposes that a complex between genome RNA and capsid proteins forms a nucleocapsid only upon interaction with viral envelope proteins at the cytoplasmic membrane. The cytoplasmic tails of HA (10 to 11 amino acids, Figure 18.4), NA (6 amino acids), and M2 (54 amino acids) are crucial to virion formation, probably because they interact with matrix protein (M1) bound to virus nucleocapsids and transported from the nucleus to the cytoplasm. Genome replication begins once sufficient N protein is made Viral genome replication requires synthesis of a fulllength complementary positive-sense RNA, called the antigenome or replicative intermediate RNA. Note that these genes use distinct promoters but share polyadenylation sites in common with E1B and E2B. INACTIVATED VIRUS VACCINES Often stimulate only humoral immunity if administered without adjuvants. DNA in any one of these orientations is infectious, and genomes frozen into only one orientation by removing the inverted repeats are also able to generate infectious virus, but exhibit reduced virulence in experimental animal systems. How might such a drug target influenza virus replication? Cleavage of CI repressor in cells with damaged DNA leads to prophage induction As CI is the repressor used to establish and maintain a lysogenic state, the dimeric Cro protein is the repressor that establishes and controls a lytic infection by turning off the synthesis of CI. Baculoviruses contain multiple copies of highly homologous DNA sequences located in several different specific regions of the genome. This causes the appearance of local lesions, whose spread is usually limited by host inflammatory and immune responses. Used to detect and quantitate nucleic acids. 85 Phage adsorption and DNA entry depend on cellular proteins involved in sugar transport 86 TOC.indd ix ix The lytic transcription program is controlled by termination and antitermination of RNA synthesis at specific sites on the genome 87 The CI repressor blocks expression of the lytic program by regulating three nearby promoters: PL, PR, and PRM 88 Cleavage of CI repressor in cells with damaged DNA leads to prophage induction 89 The Cro repressor suppresses CI synthesis and regulates early gene transcription 89 Making the decision: go lytic or lysogenize? 24.2, p. Messenger RNAs transcribed from both genome segments 7 and 8 can be spliced, each giving rise to two distinct mRNA species that code for different viral proteins. The new start codon is used in place of the authentic start codon, as it is closest to the termination codon and thus forms a 16/05/11 7:54 PM 64 CHAPTER 5 Single-Stranded RNA Bacteriophages A Lysis start U G to lysis U U• G U U UA 57 A G C A C A U GC A G A UGCG GCU C A C G C C G AU G C GU C U G C C U A G C G A G A 70 U A G G A G U A C A A to replicase UAA Ribosome Coat termination CC A UU C A U A C C C A U Replicase start G U C GAAG A CU A GG U AA G U G C 1427 U C U 43 1335 G U Coat start A Figure 5.7 Termination of translation followed by reinitiation. Every time a ribosome reads the coat gene, it disrupts base pairing at stem MJ as it passes through that part of the coat gene open reading frame (Figure 5.6). Obligatory intracellular parasite: an infectious element or organism that can replicate only within other cells. This disease is now called postinfectious encephalomyelitis, and is known to be mediated by autoreactive T cells. Permissive: a cell type is considered permissive for infection by a particular virus if it can be productively infected and give rise to progeny virus. CH025.indd 305 19/05/11 12:19 PM 306 CHAPTER 25 Baculoviruses The sequences of 51 other baculovirus genomes, ranging from 81 to 179 kbp, are also known. These could slow down the spread of influenza A virus during a pandemic, or reduce symptoms in infected people. Widely used for high-level expression of foreign proteins including vaccine production. The reassortant virus can therefore express a new surface antigen that is not recognized by antibody in its natural host (see Chapter 18). Note that between nt 797 and 811, two different reading frames are used to make parts of STAg and LTAg, and between nt 811 and 831, all three reading frames are used to make parts of MTAg, LTAg, and TTAg. Simian virus 40 has two 5' splice sites analogous to those of mouse polyomavirus, but only one 3' splice site. The DNA of other bacteriophages (e.g., bacteriophage T7) is not spring-loaded sufficiently to drive complete injection into the cell; rather, after partial entry by injection, the remainder of the genome is actively spooled into the cell by the transcriptional machinery (Figure 7.2). The herpesviruses are also distant cousins to this group. Ebolavirus species are named after their original site of discovery: Bundibugyo (tentative), Cote d’Ivoire, Reston, Sudan, and Zaire. Removal of histones generates a supercoiled molecule (center) due to redistribution of the toroidal coils. An effective vaccine is available. Infection of tumor cells with an adenovirus encoding p53 restores its function and triggers cell death. This mechanism provides for the synthesis of large amounts of mRNAs for proteins needed in high concentration, such as N and M, Trailer RNA Abortive replication (low N levels) 5' 3' Antigenome RNA Replication (high N levels) RNA polymerase (L/P) 3' N 5' Genome RNA Transcription (low N levels) Leader RNA AAAA N P/C/V AAAA AAAA M AAAA F AAAA HN L AAAA Figure 15.8 Paramyxovirus transcription and replication. This design has two advantages. These vaccines do not contain other components of the virus, in particular the nucleic acid genome. et al., “Distinct DNA exit and packaging portals in the virus Acanthamoeba polyphaga mimivirus,” PLoS Biol. Top right: quasi-spherical particles budding from the cell surface. Includes bibliographical references and index. The addition of eIF3 to replicase complexes isolated from transfected yeast cells stimulates RNA synthesis in vitro. In infected cucumber and tobacco plants, the cucumber mosaic virus replicase complex is associated with the tonoplast, the membrane surrounding the large central vacuole typically found in many plant cells. However, viruses that use reverse transcriptases to replicate would have continued to exist and replicate in these DNA-based cells, using their DNA-dependent RNA polymerases to transcribe the DNA form of the retrovirus genome. Progressive multifocal leukoencephalopathy (PML): a rare, fatal demyelinating disease of humans caused by the polyomavirus JC. • Activated T lymphocytes can secrete cytokines that stimulate B lymphocytes to develop into plasma cells. coli chromosome with the attP and attB sites aligned. This is the simplest type of capsid that can be constructed with icosahedral symmetry. The viral RNA polymerase, 3, and the polymerase cofactor protein, 2, are thought to reside at the base of the turret formed by pentamers of the 2 protein. Excision repair excises single-stranded DNA oligonucleotides, which in turn bind to and activate the cell’s RecA recombination protein such that it acquires a “coprotease” activity. Upon sequencing, all of the mutations were found to lie within the M2 gene (the other gene in the M segment is the M1 gene). They localize at nuclear pores where they are disassembled in a sequential manner, and the DNA is released into the nucleus. factor and topoisomerase II. 32.7: Reprinted from Fig. Human herpesvirus 8 produces viral homologues of the interferon regulatory factors, called v-IRFs, which block interferon gene activation by inhibiting, rather than stimulating, transcription when bound to interferon genes. RNAs 1, 2, and 3 are genome RNAs, and simultaneous infection with all three RNAs is required to establish a productive infection. in cellular mRNA metabolism. Human bocavirus is frequently found in children suffering from acute respiratory and intestinal infections. How are antiviral drugs obtained? the internal cavities of the virus and the cytosol (step 6). Apparently, the remaining operator and R32 hairpin structures together are not strong enough to block the entry of ribosomes. Noble Research Institute Marilyn J. The virions are large, layered structures between 100 and 500 nm in diameter, with a double-stranded DNA-protein core surrounded by a lipid bilayer, which is itself enclosed in a capsid with icosahedral symmetry. Ankyrin: a family of adaptor proteins that mediate the attachment of integral membrane proteins to the spectrinactin based membrane skeleton. Type A hepatitis was predominantly transmitted via the fecal–oral route while type B hepatitis was transmitted primarily through blood contact. The chalk of the White Cliffs of Dover, epitomized in Dame Vera Lynn’s famous war-time song, is formed from the coccoliths of algae like E. Explain why only 25% of the genomes of herpes simplex virions are identical. division; the generation time of the host cells increases from about 24 hours to about 48 hours. It is striking that all but 4 of the 48 open reading frames lie on the same DNA strand. Capsid subunits of some viruses require genome RNA to assemble; however, others can form empty shells in the absence of RNA. Mucociliary flow: flow of mucus and debris along the epithelial lining of the respiratory tract modulated by the movement of cilia on the epithelial cells. RNA 4A is also a subgenomic RNA, equivalent to the 3'-terminal one-quarter of RNA 2; it encodes the 2b protein, and is packaged by some strains but not by others. CH029.indd 357 The CD4 antigen, found on the surface of both helper T lymphocytes and macrophages, is the primary receptor for the virus. Binding of E1A proteins to histone acetyl transferases increases histone acetylation and thus promotes entry into the cell cycle by enhancing expression of cell-cycle genes. Agriculture may be thought of as an idea that first arose in the ancient Middle East and then spread and evolved among humans for thousands of years. First, by binding to Rb (Figure 23.6) they activate expression of p14Arf, which is encoded by an E2F-inducible gene. Acheson, McGill University 4. Synthesis of plus-strands is less complex and more efficient than that of minus-strands The next step is to copy the minus-strand RNA into plus-strands. Typically one protein is encoded per genome segment. In spite of the availability of a vaccine, millions of individuals still are chronically infected with the virus. (b) Bacteriophage lambda. The prion hypothesis proposes that information coded in the three-dimensional structure of a protein, and therefore ultimately in its amino acid sequence, can be transferred from organism to organism and propagate itself, without the intervention of a nucleic acid. Early in the history of vaccine development there were occasional setbacks due to contaminated vaccine lots (e.g., infections from bacterial contamination of vaccinia-induced pustules) and serious vaccine-associated side effects (e.g., encephalomyelitis following rabies vaccine). The selection of specific immunocompetent B and T cells is accomplished by virtue of the antigen-specific receptors expressed on their surface. Site-specific integration into host genome (adeno-associated virus). CH014.indd 166 Genome replication proceeds via a full-length, negative-strand intermediate The proteins in replication complexes direct both genome replication and transcription, the production of viral mRNAs. Genome replication requires the synthesis of a full-length, negative-strand copy of the viral genome, which in turn is used to direct synthesis of full-length, positive-strand genome RNAs. Negative-strand templates account for only 1 to 2% of total viral RNA produced in an infected 16/05/11 8:04 PM Coronaviruses (a) 167 (b) Figure 14.3 Membrane structures induced by SARS-coronavirus infection. However, it is now recognized that there are many important differences in pathogenesis between bovine papillomavirus and human papillomaviruses (HPV). • The nucleocapsids of many DNA viruses are transported to the nucleus by dyneins on microtubules, and either pass directly through the nuclear pores or are disassembled at the nuclear pore. Immunodetection using western blots or direct fluorescent detection before and after proteinase digestion have allowed detection of as little as 10⩾8 g (106 molecules) of abnormal PrP. How do non-enveloped viruses penetrate the cellular membrane? IV: immature virus; MV: mature virus; WV: wrapped virus; EV: enveloped virus; VETF: viral early transcription factors; VITF: viral intermediate transcription factors; VLTF: viral late transcription factors. Knobbed fibers protruding from each of 12 vertices. The small delta antigen and several other cellular factors are also involved in genome replication. Viruses evade adaptive immune responses Viruses evade both the humoral and cell-mediated arms of the adaptive immune response. Polymorphonuclear leukocytes and monocytes (Figure 34.1) can spontaneously phagocytose free virus particles or debris from virus-infected 16/05/11 8:19 PM 418 CHAPTER 34 Innate and Adaptive Immune Responses to Virus Infection cells. Six regulatory proteins: Vif, Vpu, Vpr, Tat, Rev, Nef. Hepatitis delta virus is only detected in the presence of hepatitis B virus. The capsid therefore provides a structure that positions each RNA polymerase molecule and the various genome segments, and this allows reiterated transcription of each segment and directional transfer of the mRNAs to the cytoplasm. Stanislaus Von Prowzek showed in the early 1900s that jaundice of the silkworm is caused by a filterable 302 CH025.indd 302 19/05/11 12:19 PM Baculoviruses agent, but he believed the agent to be a bacterium and the occlusion bodies to be by-products of the disease. Indeed, many advances in rational drug discovery have come from efforts to develop antiviral drugs. RNAs containing this mutation, when transfected into cultured cells, are unable to produce new virus progeny, suggesting that intact P123 is essential for negative-strand RNA synthesis. RNA-induced silencing complex (RISC): a multi-protein complex that uses one strand of an siRNA or miRNA as a template for recognizing complementary mRNA sequences to mediate RNA interference. The first intracellular step in their replication cycle is therefore the synthesis of positive-sense viral mRNAs by this viral RNA-dependent RNA polymerase, using the negative-sense genome as a template. These membranes, located in the perinuclear region of the cell, lie in between the endoplasmic reticulum and the Golgi apparatus, and are part of an interconnecting system of membranes involved in modification and secretion of cellular proteins. (a) Quasi-equivalent arrangement of 180 commas in a T 3 icosahedral surface lattice on a sphere. The higher the basal level of viral RNA, the more rapidly patients progress to full-blown AIDS. In contrast, plus-strand DNA is made from the double-stranded replicative form template by a continuous rolling circle mechanism utilizing the viral A protein, DNA polymerase III, the single-stranded DNA binding protein SSB, and rep helicase (Figure 6.4). National public health officials are also stockpiling chemotherapeutic agents active against influenza A, such as the neuraminidase inhibitors zanamivir and oseltamivir (see Figure 36.10a). Viruses can be acquired by aphids and transmitted within seconds to minutes. Although not a major public health threat, infection of humans with this mosquito-borne virus can lead 137 CH012.indd 137 16/05/11 8:03 PM 138 CHAPTER 12 Flaviviruses to neurological effects and is occasionally fatal. Lysosomotropic agent: weakly basic compound that elevates the pH in acidic organelles of the cell. The term reovirus in its common usage refers to the mammalian reoviruses and will be used as such in this 225 CH019.indd 225 16/05/11 8:07 PM 226 CHAPTER 19 Reoviruses Table 19.1 Genera within the Reoviridae Genus Number of gene segments Hosts Orthoreovirus 10 Mammals, birds, reptiles Rotavirus 11 Mammals, birds Orbivirus 10 Mammals, birds, arthropods Coltivirus 12 Mammals, arthropods Aquareovirus 11 Fish, mollusks Cypovirus 10 Insects Idnoreovirus 10 Insects Fijivirus 10 Plants, insects Oryzavirus 10 Plants, insects Phytoreovirus 12 Plants, insects Seadornavirus 12 Insects, humans Mycoreovirus 11–12 Fungi chapter. the Salk and Sabin vaccines, and the World Health Organization is using these vaccines in a far-reaching plan to eradicate poliovirus worldwide. It is now clear that a growing number of intracellular proteins are prone not only to pathological aggregation but can also be released and “infect” neighboring cells. Clinical features of infection The incubation period of Marburg and Ebola virus infections varies between 4 and 21 days. transfer vector, replacing the polyhedrin-coding region (step a). The product of the M2 gene is a small protein that spans the virion envelope. 327 from different classes in the animal kingdom including Rhizopoda, Ciliata, Hydrozoa, and Turbellaria. Capsids contain at least eight other proteins of unknown functions. All viruses in the family infect plants, but host ranges vary dramatically. Packaging signal: sequence on a DNA or RNA genome that is recognized by viral proteins involved in packaging the genome in the capsid. The translation of cellular mRNAs is strongly inhibited in reovirus-infected cells; by approximately 10 hours after reovirus infection, most proteins synthesized in the cell are viral. One such organism, Methanopyrus kandleri, can grow at temperatures as high as 122□C. Parvovirus pathogenesis: the example of B19 virus In a remarkable way the biological properties of parvoviruses help to determine the diseases they cause. This means that a double-stranded viral DNA genome of 2000 nucleotides measures approximately 700 nm (0.7 micrometers, m) from end-to-end (Figure 3.2). 1884 Metchnikoff descibes role of phagocytes in immunity. Little is presently known (a) (b) Figure 22.1 Papillomavirus virions. At intervals, samples of the infected cells or of the medium are harvested Late Early 1000 100 T antigens Viral DNA 80 60 Infectious virus 10 Viral capsid proteins Viral mRNA 40 Viral RNA, DNA, or proteins % of maximum value Virus, PFU per cell 100 20 Infectious virus 1 0 0 10 20 30 40 Hours after infection Figure 1.5 Replication cycle of mouse polyomavirus. The hemagglutinin of some other paramyxoviruses also has a neuraminidase activity, and is therefore called HN. Using filtration as a diagnostic tool, numerous viruses infecting humans, other vertebrate animals, plants, insects, and bacteria were described during the first half of the twentieth century. Marcellus, McGill University 24. In contrast, bunyavirus transcription takes place in the cytoplasm, and uses preformed cellular mRNAs as primers; thus it is not sensitive to these drugs, because cellular messenger RNAs are abundant and stable. The mechanism of crossprotection in viroids is not understood. If so, then such vectors could be considered as a means to treat neurological diseases by replacement gene therapy. Binding to interferon brings two or more receptor molecules together, and their associated Jak kinases phosphorylate tyrosine residues on each other as well as on the cytoplasmic domains of the receptor molecules (Figure 33.8, top center). H2N2 strains persisted until 1968, when another new virus suddenly appeared in China. Three virus-coded enzymes—a protease (PR), an integrase (IN), and a reverse transcriptase (RT)—are associated with the virus core. However, some mutants of these vectors are able to persist in mammalian cells without causing cytopathic effects. A gene encoding a possible origin of replication DNA-binding protein (R1: OBP) is located at the extreme left end. Electron microscopy and x-ray crystallographic studies have revealed the detailed structure of the hexamer form of large T antigen (Figure 21.11). Recombination and other studies indicate that S is possibly a modular protein and that domains might be exchanged with relative ease during mixed infections with distinct coronaviruses. Flavivirus genome organization resembles that of picornaviruses The modern era of flavivirus research began with the determination of the nucleotide sequence of the 17D strain yellow fever virus RNA genome in 1985. All known coronaviruses have a similar genome organization, in which the 20–22 kb replicase gene (gene 1) comprises the 5' twothirds of the RNA genome. Electron microscopy reveals roughly spherical virions of size varying from 150 to 350 nm in diameter, with a visible fringe of glycoproteins projecting from the envelope (Figure 15.1a). 16/05/11 8:07 PM 222 CHAPTER 18 Influenza Viruses Because of increased travel and rapid transportation, the next pandemic could be particularly hard to deal with. This leads to production of three early viral proteins that direct adenovirus DNA replication. In the case of rhabdoviruses, the matrix protein is responsible for condensing the helical nucleocapsid into a coil or “skeleton” that defines the unusual bullet shape of the virion. Interferons can influence immunoglobulin isotype switching. This results from interruption of synthesis of the second DNA strand by the viral reverse transcriptase (DNA polymerase) during virion maturation (see Chapter 30). Envelope: a lipid bilayer membrane, usually derived from a cellular membrane, into which viral glycoproteins are inserted. At this point the NS2 protein comes into play. A common epitope present on the surface protein of all known hepatitis B strains is called determinant “a”. Other parvoviruses that are not 245 covered here, the densoviruses, infect invertebrate species. The terminal loops are incompletely base paired, as are similar terminal loops in the genomes of poxviruses (see Figure 26.1). 11.6: Reprinted from Stewart, S.R. and Semler, B.L., “RNA determinants of picornavirus and cap-dependent translation initiation,” Seminars in Virology 8 (1997), 242–255, with permission from Elsevier; Fig. In this way, the presently known DNA world could CH003.indd 42 have emerged. 2.5a: Reprinted from S.C. Harrison, “Principles of Virus Structure,” op. Expression of Rev was found to induce accumulation of the 9-kb and 4-kb viral RNAs in the cytoplasm, demonstrating that Rev is required for transport of the unspliced and singly spliced viral RNAs from the nucleus to the cytoplasm. Compare with Figure 2.2c, a T 1 arrangement. 1900 Walter Reed demonstrates that yellow fever is caused by a filterable virus. Chapter 1 Fig. In addition to the standard gag, pol, and env genes, six additional reading frames were identified: vif, vpr, vpu, tat, rev, and nef (Figures 29.2 and 29.3, Tables 29.2 and 29.3). Interferon regulatory factors (IRFs) are activated by phosphorylation. This is due in part to the high degree of variation in the virus, both within one individual and within the population. It is presently unclear whether the pointed end or the filaments on the broader end are involved in adsorption to the cell surface and channeling of viral DNA into host cells. Acts as ubiquitin ligase (E3). This RNA polymerase can specifically recognize the doublestranded RNA genome and transcribe its negativestrand into positive-strand messenger RNAs. Viruses with double-stranded DNA genomes must first transcribe their genomes into messenger RNAs, using a DNA-dependent RNA polymerase. Protein 1a induces the formation of CH010.indd 117 117 spherical invaginations within the endoplasmic reticulum membrane, recruits viral RNA to these invaginations, and directs the 2a protein to the membrane (Figure 10.6). • Vif inactivates the host protein APOBEC-3G, which inhibits reverse transcription and increases mutations in proviral DNA. Genus: in virology, a grouping of closely related virus species. Thus hepatitis delta virus makes two forms of the delta antigen: small (195 amino acids) and large (214 amino acids) (Figure 31.4). 16/05/11 8:15 PM 346 CHAPTER 28 Retroviruses R U5 PBS Gag 1 tRNA PBS Pol ppt U3 R Synthesis of minus-strand strong-stop DNA Pol Gag Env Env ppt U3 R R' U5' 2 PBS Gag RNAse H digestion Pol Env ppt U3 R R' U5' 3 PBS Gag Pol First strand transfer Env ppt U3 R R' U5' 4 PBS Synthesis of full-length genome copy Pol Gag Env ppt U3 R U3' R' U5' PBS' 5 RNAse H digestion ppt U3' R' U5' PBS' 6 Synthesis of plus-strand strong-stop DNA ppt PBS' U3' R' U5' 7 RNAse H digestion U3 R U5 PBS PBS' U3' R' U5' 8 Second strand transfer U3 R U5 PBS PBS' U3' R' U5' 9 Extension of both DNA strands U3 R U5 U3 R U5 U3' R' U5' U3' R' U5' LTR LTR Figure 28.4 Reverse transcription. This DNA-protein complex docks at the portal vertex of a prohead, and DNA packaging proceeds. The main hurdle limiting their use is the production of clinical-grade vectors due to the complexity of the production process and to the low levels of helper virus contaminating vector stocks. The newly synthesized, double-stranded DNA can be transcribed to provide more messenger RNA, or can again be nicked and extended, to generate further progeny DNAs. The attentive reader will notice that the sequence at the 3' (left) end of the original adeno-associated virus DNA molecule shown in Figure 20.4 reads ABCA' in the 3' to 5' direction, and the sequence at the 3' (right) end of the progeny single-stranded DNA shown at the bottom of Figure 20.4 reads AC'B'A', so that the order and orientation of B and C are inversed. • Many fungal viruses have double-stranded RNA genomes. Recombinant viruses engineered to incorporate one or more immunogenic proteins Chimeric vaccines: benign or attenuated microbes (viruses or bacteria) that express specific immunogenic viral proteins. Many togaviruses were isolated from mosquitoes or infected animals in tropical regions of the world, and their names often recall the localities where they were first found. Creutzfeldt-Jakob disease: prion disease in humans. C 101 Encapsidation of genome RNA. Genes are color-coded by putative function: red, information transfer; sky blue, degradation of large molecules; yellow, central or intermediary metabolism; light blue, kinases; pink, virus-specific; light green, no known function; dark green, no known function but contain transmembrane helices; gray, miscellaneous. Both 5' ends covalently bound to a virus-coded terminal protein. Filovirus glycoprotein mediates both receptor binding and entry by fusion The glycoprotein (GP) (Figure 16.5) is synthesized as a 676- (Zaire ebolavirus) or 681- (Marburg virus) amino acid precursor protein that is inserted into the lumen of the endoplasmic reticulum. This approach has been used to discover anti-HIV protease inhibitors such as ritonavir and anti-influenza neuraminidase inhibitors such as zanamivir. How do enveloped viruses penetrate the cellular membrane? Subgenomic mRNA: a messenger RNA that includes only part of the sequence of the genome of an RNA virus. Many viruses come in simple, symmetrical packages The simplest virus particles assemble spontaneously from their protein subunits and nucleic acid genomes; this process is called self-assembly. Alphavirus cDNAs were initially placed under the control of a promoter for bacteriophage T7 or SP6 DNA-dependent RNA polymerase. An example of a virus replication cycle: mouse polyomavirus An idealized example of a single-cycle growth curve for mouse polyomavirus (see Chapter 21), a small DNA virus that replicates in cultured baby mouse kidney cells, is shown in Figure 1.5. Each 6-cm diameter Petri dish contains approximately 5 million cells, and 0.5 mL of a suspension containing 100 million (108) PFU/mL of polyomavirus is used to infect the cells (m.o.i. 10 PFU/cell). Over 125 CH011.indd 125 16/05/11 8:02 PM 126 CHAPTER 11 Picornaviruses 100 different types of human rhinoviruses have been described, making it unlikely that an effective vaccine against these cold viruses would ever be developed. Gene D mRNAs are more abundant than any other transcript, and more of this protein is needed than any other: 240 copies of the D scaffolding protein are required to build one virion. CH013.indd 157 16/05/11 8:04 PM 158 CHAPTER 13 Togaviruses • Like other positive-strand RNA viruses, togavirus RNA synthesis is carried out in the cytoplasm in association with membranes. A number of cellular proteins, including APOBEC3G, are incorporated into HIV virions. DNA VACCINES Can be engineered to stimulate both cell-mediated and humoral immunity. The inner circle shows conservation of genes among the four sequenced SSV genomes: dark gray, conserved; gray, conserved in three out of four genomes; light gray, not conserved or conserved only in two of the four genomes. 45 Enveloped and non-enveloped viruses have distinct penetration strategies 46 Some viruses can pass directly from cell to cell 46 TOC.indd viii A variety of cell surface proteins can serve as specific virus receptors 47 Receptors interact with viral glycoproteins, surface protrusions, or “canyons” on the surface of the virion 48 Many viruses enter the cell via receptor-mediated endocytosis 48 Passage from endosomes to the cytosol is often triggered by low pH 49 Membrane fusion is mediated by specific viral “fusion proteins” 50 Fusion proteins undergo major conformational changes that lead to membrane fusion 50 Non-enveloped viruses penetrate by membrane lysis or pore formation 51 Virions and capsids are transported within the cell in vesicles or on microtubules 52 Import of viral genomes into the nucleus 52 The many ways in which viral genomes are uncoated and released 54 SECTION II: VIRUSES OF BACTERIA AND ARCHAEA 5. All other chapters have been revised and updated.Information on human pathogens. Receptor-mediated endocytosis: endocytic uptake of molecules from the extracellular space involving their specific binding to plasma membrane receptors. Associated with the nucleocapsids is the RNA polymerase (L) protein. Top: viral proteins (N amino terminus, C carboxy terminus). Once the critical concentrations are achieved, subsequent steps occur quickly, ensuring that the assembly of each capsid goes to completion. Researchers therefore looked for possible animal carriers in open-air markets in areas where SARS was first observed. CH031.indd 384 As a result of editing, the UAG termination codon that normally ends the open reading frame at amino acid 195 is changed to a UGG codon, which codes for tryptophan. 1721 Lady Montagu brings concept of variolation (inoculation with pus from recovering smallpox victim) from Turkey to England. Green fluorescent protein: a jellyfish protein that emits green light when exposed to ultraviolet light. coli, mitochondria, plasmids, and a number of DNA viruses, solve this problem by circularizing their DNA—thereby eliminating the ends. Prion diseases are not usually transmitted among different species To a large extent, prion diseases respect species barriers; prions from one animal cannot usually infect animals of a different species. Bovine spongiform encephalopathy (“mad cow disease”) developed in Britain and apparently spread to humans Cattle have increasingly been fed meat and bone meal prepared from animal sources as the beef industry has evolved. The most highly purified preparations of PrPSc contain 100,000 protein molecules per infectious unit. Therefore the transcriptional promoter consists of two distinct sequence elements, brought into proximity by the structure of the helical nucleocapsid. coli cell showing numerous MS2 phages attached to F-pili (enlarged in inset). Polyomaviruses and papillomaviruses use cellular histones to condense their DNA genomes; the resulting DNA-protein complex, which resembles cellular chromatin, is called a “minichromosome” (see Figure 21.2). Bleeding into the gastrointestinal tract is most prominent, and localized skin hemorrhages and hemorrhages from puncture wounds and mucous membranes are common. ACHESON John Wiley & Sons, Inc. CD4-positive T lymphocytes: T lymphocytes that carry CD4 antigen on their surface. The non-enveloped picornaviruses release their genome RNA directly into the cytosol, leaving the viral capsid within an endosomal vesicle. determinations as well as by measurement of its contour length (12 m) in electron microscope images. The terrible reality of these viruses and the impact of vaccines are readily apparent from the data shown in Table 35.1. Edward Jenner and his European counterparts are widely credited with discovering vaccination in the late 1700s (see Chapter 26). Topoisomerase I: enzyme that cleaves a phosphodiester bond in a double-stranded DNA, forming a temporary covalent bond with the DNA, and allows one strand to rotate around the complementary strand, subsequently resealing the phosphodiester bond and leading to relaxation of overwound (supercoiled) DNA. All the treated patients had already suffered significant visual loss. Unscheduled DNA synthesis results in activation of apoptosis, leading to cell death, and this process is dependent on the activity of the cellular p53 protein. 16/05/11 8:15 PM Retroviruses 353 REVIEW QUESTIONS 1. DNA polymerase ⎵/primase binds to the single-stranded DNA and lays down a short (6–9 nt) RNA primer that remains hydrogen-bonded to the DNA template. Activation of late transcription leads to initiation at multiple sites upstream of this promoter sequence, and RNAs with these 5' ends are more efficiently processed into mRNAs and exported to the cytoplasm (Figure 21.5). Chlorovirus capsids are constructed from many capsomers and have a unique spike The PBCV-1 virion (Figure 27.3) has a diameter of 190 nm and consists of an icosahedral outer capsid (T169) surrounding an internal lipid bilayer, within which is enclosed the viral DNA and many viral proteins. Following cleavage of P1, five protomers, each containing one molecule of VP0, VP1, and VP3, self-assemble to form a 14S pentamer structure. Isotype switching is also essential for increasing the affinity of antibodies (via further somatic mutations in the variable regions) and for production of long-term immune memory cells. Blue arrows denote the PRM (CI) and PR (Cro) transcripts. This phage was used as a model system for studying DNA replication and virion assembly using scaffolding proteins. The use of cellular histones by polyomaviruses (as well as papillomaviruses) for DNA condensation and packaging is a striking example of genetic economy. There remains a maverick category of viruses in the Baltimore classification system, of which the best known representatives are the hepadnaviruses (see Chapter 30). The standard K-12 laboratory strain of E. 1.2a: Courtesy of Robley Williams, University of California at Berkeley and Harold Fisher, University of Rhode Island; Fig. The leader region contains signals for initiation of RNA synthesis by the viral RNA polymerase. The low pH within virions weakens the interaction of the matrix protein M1 with viral nucleocapsids, facilitating their release into the cytoplasm upon membrane fusion. “Mimivirus” was chosen as a shortened version of microbe mimicking virus. This suggests the location of the second receptor and indicates that the genome is delivered directly into the cytoplasm, where replication occurs, and not into the periplasmic space. For example: 16/05/11 7:46 PM 6 CHAPTER 1 Introduction to Virology Phages lined up through the universe Scientists estimate that there are approximately 1031 tailed bacteriophages on Earth. Among these proteins are the TATA-binding protein (TBP), present in a complex called TFIID, which binds to a consensus TATA box sequence element on the viral DNA (Figure 21.5). A single copy of the maturation protein is bound to the encapsidated RNA at sites near both its 5' and 3' ends. When these S proteins are incorporated into the plasma membrane of an infected cell, cell fusion can occur, resulting in the formation of large multinucleated giant cells, or syncytia, which are observed in infected cells in vitro and in some infections in vivo. Poxviruses are notoriously stable in the environment; the smallpox vaccine can be freeze-dried, and dried pox scabs or virus-contaminated surfaces can retain infectivity for prolonged periods of time. CH033.indd 408 16/05/11 8:19 PM Interferons expression of the type III interferon receptor complex may allow the host to rapidly eliminate viruses at the major sites of virus entry before the virus can establish an infection, without activating additional immune responses. Moreover, vaccination has been a very successful approach to controlling viral diseases, further dampening the interest of pharmaceutical companies to develop drugs against these diseases. Satellite RNAs spread epidemically as parasites in natural populations of cucumber mosaic virus. 2.3b: Courtesy of Stephen C. Parvoviruses bind to cells via receptors that have in some cases been identified: globoside (blood group P antigen) for B19 virus; transferrin receptor for canine parvovirus; and heparin sulfate proteoglycan, fibroblast growth factor receptor 1, and integrin V5 for adeno-associated virus type 2. Degradation of CD4. Two other class I gene products play important roles in the infection (Table 7.1). Virus Entry 45 How do virions get into cells? Furthermore, these nascent RNAs must not yet have reached their equilibrium folding configuration. It has an overall length of 230 nm and a width varying from 75 nm at the broad end to 4 nm at the pointed end (Figure 9.7). Individual virus diseases often represent smaller potential markets than the wide variety of diseases caused by bacteria. Systematic examination of infections by phage with mutations in class I genes revealed that the protein product of gene 1.0 is essential for class II and class III gene transcription. Some viruses can pass directly from cell to cell The release of virions from an infected cell is not always necessary for transmission of infection to a neighboring cell. T, the triangulation number, is equal to h2 ⩽ hk ⩽ k2, where h and k are integers. Based on analysis of open reading frames and transcriptional promoters, it has been estimated that AcMNPV encodes about 152 proteins. VIRUSES AND HOSTS Autonomous parvoviruses: minute virus of mice, canine and feline parvoviruses. Above: transcripts of the six genes (ICP0, 4, 22, 27, 47, and US1.5) and three other genes (ICP34.5, ORF P/O) are shown as blue arrows. Furthermore, some HA proteins are readily cleaved by intracellular furin proteases, allowing them to infect many cell types because their fusion mechanism is activated upon cleavage. DNA-dependent RNA polymerases, to express the information in the DNA via messenger RNAs 4. Because some variants of prion proteins seem to protect against prion disease, vaccination or gene therapy are other possible solutions to curing or preventing prion diseases. Adenoviruses cause respiratory and enteric infections in humans nomenclature were adopted in 1956, acknowledging the original source of the virus from adenoid tissue. Interferon-f : a cytokine produced by activated T lymphocytes and natural killer cells that functions in the innate and adaptive arms of the immune response by activating cells such as macrophages. There the viral DNA associates with the nuclear matrix via the terminal protein, and transcription of viral genes commences. What are two likely origins for the smallpox vaccine strain, vaccinia virus? a short stretch of the template DNA strand, and are then extended by DNA polymerase to make a full complementary DNA strand (Figure 20.6). Replication-defective adenovirus vectors are propagated in complementing cell lines The development of adenovirus vectors has involved the following genetic modifications: (1) eliminating the capacity of the vector to replicate on its own; (2) increasing its capacity to package exogenous genes; (3) reducing the host immune response to viral proteins; and (4) retargeting the vector to specific cell receptors. Nevertheless, computer viruses are analogous to real viruses, because their sequence of bytes (analogous to nucleic acid) codes for programs (analogous to enzymes that direct nucleic acid replication) that enable them to be replicated, de novo, using elements provided by a computer (analogous to the cell). HOW DO ANTIVIRAL VACCINES WORK? Viral envelope protein gp42 binds to HLA class II and helps direct virus entry. Electron micrographs show that the vast majority of adsorbed ϕX174 particles are imbedded at points of adhesion between the cell wall and the inner membrane. Long-lived memory cells can provide life-long protection against re-exposure to a specific pathogen. Thus their lifestyle is very similar to those of the positive-strand RNA flaviviruses and togaviruses 200 CH017.indd 200 16/05/11 8:06 PM Bunyaviruses 201 Table 17.1 Some pathogens in the family Bunyaviridae Genus/Virus Host: disease Vector Distribution Orthobunyavirus Oropouche virus Human: fever Midge S. PE2 487 Precursor to E2; palmitoylated and glycosylated. • These small, circular, replicating RNAs may be a relict from the ancient RNA world. The extremely large and complex coronavirus RNA genome, and the presence of genome regions resistant to stable cloning, have been formidable obstacles to cDNA cloning and mutagenesis. Virus-like particle (VLP) vaccines include “nanoparticles” bearing viral antigens that can be produced using a wide range of technologies. Tissue macrophages are activated by interferon- to kill microbes and infected cells by producing toxic oxygen and nitrogen compounds. In one model, prior packaging of RNA 3 alters the conformation of the coat protein and promotes its interaction with RNA 4, leading to packaging of both RNAs in the same capsid. Ganciclovir triphosphate is a more potent inhibitor of cytomegalovirus DNA polymerase than of cellular DNA polymerases. 3395 3569 replicase coat 3' lysis 1678 1902 MS2 1 61 5' 1320 1344 maturation (A2) 1742 2352 coat 4118 4217 replicase 3' readthrough (A1) 1344 2330 Q Figure 5.1 Genetic maps of phage MS2 and Q RNAs. First and last nucleotides are shown for all open reading frames (boxes). The transfer plasmid contains the transgene expression cassette flanked by the AAV inverted terminal repeats (ITRs), which include the viral origin of DNA replication and the DNA packaging signal. Neonatal infections, birth defects. Another ethical problem arises from the concept of “altruistic” vaccination. EF-Ts recycles the resulting EF-Tu/GDP to EF-Tu/GTP; an intermediate in this process is a complex of EF-Tu and EF-Ts. 16/05/11 7:54 PM Single-Stranded RNA Bacteriophages 65 Table 5.1 Proteins needed for Q RNA replicase activity Protein Coded by genome of Required for synthesis of: Minus-strand Plus-strand Replicase Q Yes Yes RNA-dependent RNA polymerase EF-Tu E. The disease spectrum of B19 therefore well illustrates the basic principle of parvovirus pathogenesis resulting from infection of and damage to dividing cells. The book approaches learning about virology by presenting a set of chapters each of which covers a specific virus family, using one or two well-studied viruses as examples. Scrapie got its name from the habit of affected animals to itch, rub, and scrape their skin. Such black-box vaccines continue to be introduced. Although DNA can be delivered to cells using a variety of physical and chemical methods, virus vectors are capable of much higher gene transfer efficiency. In a process called antigenic drift, a number of viruses can change epitopes on viral structural proteins with mutations in regions normally recognized by antibodies. It binds to distinct sites near the replication origin, and forms a complex with the E1 protein. S forms trimers whose stalk-and-club morphology gives the virion a crownlike fringe of proteins. Importantly, while all these cytokines influence primarily the innate response, interferon also activates critical components of the adaptive immune response. Polyomaviruses and papillomaviruses have circular, double-stranded DNA genomes. A plasmid containing the gene X sequence under the control of the T7 promoter is introduced into a bacterium in which the T7 RNA polymerase is made from an inducible lac operon promoter. Practiced in various forms from the tenth through the eighteenth centuries in several countries worldwide, variolation produced a disease of diminished severity while conferring lifelong immunity to smallpox. CH009.indd 109 The most prominent feature of our understanding of genomes of known archaeal viruses is the extremely low number of genes with recognizable functions. The T 1 inner capsid surrounds the 10 tightly packed, double-stranded RNA gene segments, forming the viral core. This protein is aptly named, because its function remains elusive; many contradictory findings have been made over the years. Although this effectiveness is diminished in the elderly and chronically ill, vaccination still reduces illness and death in these populations. Virions containing RNA 4 alone can be assembled efficiently in vitro, but 94% of virions assembled in vivo that contain RNA 16/05/11 8:01 PM 118 CHAPTER 10 Cucumber Mosaic Virus 5' B box 3' Translation Protein 1a tRNA-like structure Protein 2a (RNA polymerase) Binding to B box Membrane targeting Formation of replication complex RNA replication Figure 10.6 Formation of brome mosaic virus replication complexes. Picornaviruses, on the other hand, undergo conformational changes during binding to surface receptors or after entering endosomes, so that viral proteins form membrane channels that allow the genome direct access to the cytosol. This plasmid encodes all viral enzymes and structural proteins necessary for the production of lentivirus virions except for the envelope protein. DNAs are shown as black lines and RNAs as light blue (positive-strand) or dark blue (negative-strand) lines. OR1 and OR2 overlap the 10 and 35 segments of PR, so that CI bound to these operators blocks transcription from PR. factors may contribute to the preference of this virus for liver cells. Thus free of repression, E2F can activate expression of S-phase genes, allowing progression into S phase. Are among the most successful antiviral vaccines. Filopodia: small, actin-containing projections from the cell surface. Many important events of the virus replication cycle take place during the latent period. Pyramidal structures are seen in b1 and b2; c1 and c2 show lysed cells with holes in the cell wall. Capsid consists of two or three concentric protein shells. An important target for many viruses is the double-stranded, RNA-activated protein kinase (PKR). Diameter 42 nm. Protein 3a targets the plasmodesmata between various cell types including sieve elements and companion cells, and alters its size exclusion limits. Figures 2.2b and c show side-by-side drawings of an icosahedron and a sphere onto which 60 commas are distributed with icosahedral symmetry. It was a surprise to discover that these viruses bind to and enter permissive and nonpermissive cells equally well. The GP gene makes both GP and sGP by producing two messenger RNAs that differ by a single A residue, added at an editing site within the gene (Figure 16.6) in a manner similar to RNA editing of the P/C/V gene of paramyxoviruses (Chapter 15). 26.4a: Reprinted from Fig 4C in Condit, R.C., Moussatche, N., Traktman, P., “In a Nutshell: Structure and Assembly of the Vaccinia Virion,” Adv Virus Res. Furthermore, virus infections can be transmitted from parent to offspring if an egg or sperm cell becomes infected and contains integrated proviral DNA. ATPase and RNA helicase activities of nsp13 may be critical in replication and packaging of genome RNA. Five alternative start sites (ACG or AUG) in a 120-nt region are shown at bottom. If, on the other hand, there were a single entry site for RNA polymerase at the 3' end of the genome, synthesis of mRNAs that lie closest to the 3' end (N and P/C/V) should be least inhibited by UV irradiation. Some phycodnaviruses have an additional external envelope. The X-ray crystal structure of the nsP2 proteinase revealed two domains; the N-terminal domain is consistent with a cysteine proteinase and the C-terminal domain resembles a methyltransferase, but its function is not understood. Late viral proteins package viral genomes and assemble virions Structural proteins used to package viral genomes and to assemble the capsid are usually the most abundant viral proteins made in an infected cell. In contrast, most eukaryotic messenger RNAs code for a single protein. It contains five unique sequence elements (U1 to U5) interspersed with one major repeated sequence element (IR1) and three smaller internal repeats (IR2 to IR4) (Figure 24.10). 16/05/11 7:49 PM 20 CHAPTER 2 Virus Structure and Assembly (a) (b) (c) (f) (e) (h) (d) (i) (g) (j) Figure 2.1 Structures of some typical virus particles. Founded in 1807, John Wiley & Sons, Inc. Diameter 70–90 nm. In some retroviruses, more complex splicing patterns generate additional mRNAs. For example, Rous sarcoma virus (see Figure 28.8) has an alternative 3' splice site downstream of the env gene that permits the expression of the Src protein. Insect viruses that kill silkworms, used for centuries in Asia and Europe to produce silk, have plagued that industry over the ages. (c) Surface of the Vp54 trimer also viewed from the inside of the virus, colored according to charge distribution (positive is blue, negative is red). Once N is made in sufficient quantity, “early” transcription can take place. To be sure, the amino acid sequence of prions is encoded in nucleic acid genes of the host organism, but only the protein, not the prion gene, is transmitted and infectious. This leads to the formation of oligomers of Bax and similar proteins at the outer mitochondrial membrane. In the initial stages of infection, about one-half of patients are asymptomatic, while others suffer from flu-like symptoms, jaundice, elevated liver enzymes (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) in the blood, and serum sickness, a hypersensitivity response to large amounts of viral antigen in the serum. These vesicles fuse with endosomes, which subsequently undergo acidification. gene transfer. This is called the “second strand transfer” since, like the first strand transfer, the plus-strand strong-stop DNA is transferred from one end of the template to the other end. • Epstein–Barr virus infects B lymphocytes and establishes a variety of types of latent infections. Both progeny virus and viral proteins made within infected cells are recognized by the immune system, which generates specific antibodies and cytotoxic T lymphocytes (below). The architect Buckminster Fuller exploited these principles to construct “geodesic domes” of various sizes from steel bars clustered in groups of 5 or 6 on their surface; a good example is the dome built for Expo ’67 in Montreal (Figure 2.8a). Presently the ICTV recognizes 87 virus families, and 13 genera that are not yet assigned to families. The enveloped virion contains three small structural proteins. • Activated T lymphocytes can develop into cytotoxic T cells that recognize and kill virus-infected cells. Many insect viruses are named using the Latin name of the insect species from which the virus was isolated, followed by the virus genus name (e.g., Aedes aegypti densovirus, Autographica californica multiple nucleopolyhedrovirus, Chironomus luridus entomopoxvirus). This is similar to the mechanism by which polyomaviruses and eukaryotic cells initiate their DNA replication, but polyomaviruses use the cellular DNA polymerase /RNA primase. For example, the HA1 domain of the trimeric influenza virus hemagglutinin (HA) protein contains the sialic acid binding sites with which the virus binds to the cell surface. OtV-1 Ostreococcus tauri Single-celled green algae famous for being the smallest free-living eukaryote. This was the first demonstration that transcription by RNA polymerase II could be modulated by an RNA element on the nascent transcript. CH001.indd 13 4. DISTINCTIVE CHARACTERISTICS Entry of DNA into cell is powered by transcription. They then used influenza genetics to map the mutations to the M segment of the virus. Jenner and his contemporaries were nevertheless the first to recognize the full potential of active immunization and to systematically study vaccines and vaccination. In the absence of coat gene translation, the lysis gene is not expressed. Three LMPs (LMP-1, -2A, and -2B) are made from coding regions extending in either direction from the terminal repeat element of the circularized genome. This allows the phage to make lower levels of gene products coded at the left end, and higher levels of gene products coded at the right end of this part of the genome. The development of compounds active against a broad spectrum of viruses is unlikely, because different viruses do not share common features N O CH3 Pleconaril CH3 Inhibitors of viral genome replication: • Herpesvirus nucleoside/nucleotide kinases Virus DNA and RNA polymerases HIV-1 integrase TARGETS OF ANTIVIRAL DRUGS Inhibitors of virus attachment and entry: • O O N Rational design, often with the aid of three-dimensional structures of viral proteins: • CH3 • • Inhibitors of virion maturation: • HIV-1 protease Inhibitors of virus release from the cell: • Influenza neuraminidase that distinguish them from their host cells. The ratios of the volumes of these capsids is 1:64:1000:15,000. The cortical actin region just beneath the plasma membrane is highly structured and constitutes a difficult obstacle. Ectodomain: the part of a membrane protein that projects into the extracellular space. Some viruses cause a self-limiting febrile illness, often with rash and rarely fatal. HA binds to sialic acid-containing receptors, which are found on red blood cells as well as most other cell types. However, this process most likely involves specific recognition of nucleotide sequences and secondary structures at the termini of each mRNA (Figure 19.3) by viral proteins (for further discussion of this topic, see Chapter 18). ϕX174: a tiny virus with a big impact Although viruses were first discovered and distinguished from other microorganisms by Martinus Beijerinck and Dimitrii Ivanowski in the late 1890s (tobacco mosaic virus), the first bacteriophage was not identified until 1917 by Felix d’Herelle. Injection of these plasmids into a human or animal results in transient expression of the viral proteins in host cells (e.g., myocytes, immune cells). The spheres contain only small and middle hepatitis B surface proteins (SS and MS), whereas the filaments also contain large hepatitis B surface proteins (LS). At an entry rate of 250 bp/second, it takes about 1 minute of transcription starting at the first class II promoter (5.9 kb from the left end) until the first class III promoter (19 kb from the left end) enters the cell, and about 2 minutes until the most distal class III promoter (34 kb from the left end) enters (Figure 7.1). Three Table 33.4 Selected cellular proteins induced by interferons CH033.indd 409 Protein Function Induced by interferon type dsRNA-dependent ser/thr protein kinase Phosphorylates eIF-2⎵, inhibits protein synthesis ⎵/ 2', 5'-oligo(A) synthetase Activates ribonuclease L ⎵// Ribonuclease L mRNA degradation ⎵/ Myxovirus-inhibiting protein GTPase; inhibits replication of negative-strand RNA viruses ⎵// Interferon regulatory factor-1 Transcription factor ⎵/, Interferon regulatory factor-7 Transcription factor ⎵// Interferon-stimulated gene protein-15 Cytokine immunomodulator ⎵// Interferon-stimulated gene protein-56 Inhibition of translation ⎵// Cytokine-responsive gene protein-2 Chemokine ⎵/, Class I major histocompatibility complex antigen Antigen presentation ⎵/, Class II major histocompatibility complex antigen Antigen presentation Rev-binding protein-27 Inhibits Rev-dependent HIV activation ⎵/, Immunoglobulin G-Fc receptor Binds to IgG Fc fragment Nitric oxide synthase Macrophage activation Mn-dependent superoxide dismutase Superoxide scavenger RING-12, proteasome component Protein degradation 16/05/11 8:19 PM 410 CHAPTER 33 Intrinsic Cellular Defenses Against Virus Infection interferon-induced systems that are understood in some detail are (1) the Mx proteins; (2) 2⬘, 5⬘-oligo(A) synthetase and ribonuclease L; and (3) PKR (doublestranded RNA-dependent protein kinase). 175 CH015.indd 175 16/05/11 8:05 PM 176 CHAPTER 15 Paramyxoviruses and Rhabdoviruses The mononegaviruses: a group of related negative-strand RNA viruses Among the negative-strand RNA viruses, four families (Paramyxoviridae, Rhabdoviridae, Filoviridae, and Bornaviridae) share a number of fundamental characteristics: • Their genome is a single, linear RNA molecule, packaged in a helical nucleocapsid. The prion protein accumulates selectively and abnormally in neurons in the central nervous system during the course of the disease. Interferon- is an immune modulator that is produced by lymphocytes and macrophages. Except for the NS1 protein, all other nonstructural flavivirus proteins are believed to reside within the membrane or on the cytoplasmic side of the membrane. After uptake of virus into endosomal vesicles, protein pumps in the vesicle membrane generate a drop in intravesicular pH, leading to conformational changes in the viral envelope glycoproteins. The nucleotide sequence at each end of parvovirus DNAs contains self-complementary regions. It has been possible to separate these two functions for retargeting purposes. Sequences surrounding the initiator AUG codons and differences in the length and secondary structure of 5' nontranslated regions also likely account for different levels of synthesis of viral proteins. Epidemic: increase in infectious disease incidence over time and/or geographic range. Alternatively, the invaginations are formed by the induction of membrane curvature caused by the tight, multivalent interaction of capsid proteins on the virion with receptor gangliosides on the cell surface. This vector is introduced into a host cell that contains the T7 RNA polymerase gene under the control of the lac operon regulatory region (lac promoter). These species may or may not show overt signs of infection. Endocytosis occurs frequently via clathrin-coated pits and vesicles (Figure 4.3). Thereafter, use of genetic markers such as virus-encoded DNA polymerases, a core gene present in all NCLDVs, revealed that phycodnaviruses are a diverse and ubiquitous component of aquatic environments. DISTINCTIVE CHARACTERISTICS Strong dependency on cell cycle. Virus latency is regulated by transcriptional control elements present in the HIV-1 LTR. During the early phase of the infectious cycle, full-length negative-strand antigenome RNAs are synthesized by a replicase complex formed between P123 and nsP4 (Figure 13.3). Ionophores: small lipid-soluble molecules that bind specifically to certain ions and transport them across membranes. NK cells carry several activating receptors that bind to cell-surface proteins expressed by proliferating cells (Figure 34.2). PKR regulation and cancer. These elements are related to the retroviruses by their mechanism of replication; they express an RNA that is converted into DNA by reverse transcriptase and is subsequently integrated into the genome. Amino acid numbers are shown below the map. As reverse transcription proceeds, the RNA template is degraded by the ribonuclease H activity of P. in coding for at least one viral protein! The genome also contains two enhancer elements (Enh 1 and 2) that regulate levels of transcription, a polyadenylation signal, a packaging signal (), and direct repeats (DR1 and DR2) that are involved in reverse transcription. Activated Th1 cells (top) help to activate Tc cells by production of interleukin-2 and interferon-. These vectors, however, can already provide valuable tools for expressing proteins and for identifying and studying neuronal functions. Much less is known about the functions of the minor nonstructural proteins. (b) Drawing of TTV-1. In 1947, Gernot von Bergold identified virus-like, rod-shaped particles in polyhedral bodies by electron microscopy, and subsequently found that they contained nucleic acids and proteins. (b) LMP-2A (left) mimics the functions of an activated B-cell receptor (BCR; right). 16/05/11 8:04 PM Togaviruses close to the end of the nsP3 coding region, generating a P123 polyprotein (Figure 13.3). Rous sarcoma virus is replication-competent because it contains all structural genes; however, most transducing retroviruses have deletions of structural genes and often express their oncogenes as fusion proteins. Hematopoietic stem cells isolated from the affected individual are infected with a retrovirus containing a normal version of the faulty gene causing the disease. Reassortant viruses: viruses that have packaged different genome segments from two (or more) parent viruses and therefore express some viral proteins from one strain and some viral

proteins from the other strain. For a more complete description of pRb interactions, see chapters on papillomaviruses and adenoviruses (Figures 22.4, 22.5, and 23.6). Binds to Stage II DNA replication complex, directing concomitant Stage III replication and entry of plus-strand viral DNA into the procapsid. (c) The CI repression loop. Within each section, virus groups are listed in order of increasing genome length and therefore complexity. Acidianus bottle-shaped virus (ABV): its name says it all! (c) Figure 9.5 Acidianus filamentous virus 1 (AFV-1), genus Gammalipothrixvirus. The early proteins of DNA viruses induce entry of the cell into the DNA synthesis (S) phase of the cell cycle. Signal sequence: amino acid sequence that directs insertion of the N-terminus of proteins into the endoplasmic reticulum during protein synthesis, and is cleaved from the protein in the process. coli Yes Yes Unknown S1 E. Reoviruses were the first double-stranded RNA viruses discovered Reoviruses were first isolated from stool specimens of children during the 1950s by Albert Sabin, Leon Rosen, and their colleagues. These RNAs may have represented the first self-replicating macromolecules in the RNA world (see Figure 3.3). • Polyomavirus early proteins, called T antigens, have multiple interactions with cellular signaling proteins. The pointed end of the virion is covered by a beard of dense filaments. For example, the tegument of occluded virions contains a 41-kDa glycoprotein, GP41, not present in budded virions; however, GP41 may be involved in the egress of nucleocapsids from the nucleus prior to the formation of budded virions. Its single hydrophobic membranespanning anchor is usually near the carboxy terminus. Type 1 and type 2 T helper lymphocytes: T lymphocytes that secrete cytokines that contribute to the development of cellular immune responses (Th1 cells) or humoral immune responses (Th2 cells) Type I transmembrane protein: an integral membrane protein oriented with its amino terminus facing the lumen of the endoplasmic reticulum or the extracellular space and its carboxy terminus facing the cytosol. Specific packaging signals on virus genomes are recognized and bound by viral proteins. Unraveling the mechanisms by which these processes are controlled has been a fascinating puzzle. Nucleocapsids, along with some of the tegument proteins, are transported to the nuclear pores by dynein motors along the microtubular network of the cell. Armstrong, April W. Screening of the blood supply has led to dramatic drop in spread of transfusion-related hepatitis C infections. These virions would therefore appear four times larger if they were shown at the same scale as the first page. All six of these subgenomic RNAs extend to the 3' end of the genome RNA and, like genome RNA, have poly(A) tails. As the number of viral DNA molecules increases due to DNA replication, a smaller proportion of viral DNA molecules contain bound repressor. This drift mechanism is common to a number of RNA viruses, including human immunodeficiency virus, and is a major complicating factor in production of vaccines. In this case, the symmetry is helical. Clinical studies have shown that mutations in the human p53 gene are present in over 50% of all human tumors. This cell damage is due to decreased production of type I interferon in response to viral infection. Interestingly, most Burkitt's lymphomas have permanently activated c-myc genes due to translocation of the c-myc gene to actively transcribed regions. In turn, chimpanzees likely acquired SIVcpz from red-capped mangabeys and greater spot-nosed monkeys, as SIVcpz appears to be a recombinant between the viruses that infect these two monkey species. In much of what follows, we will use minute virus of mice and adeno-associated virus type 2 (AAV2) as examples, these being the two parvoviruses whose replication is best understood at the molecular level. Viroids are composed only of a circular, single-stranded RNA molecule 250–400 nucleotides in length, a size significantly smaller than the genomes of the smallest viruses. a terminal a repeats; L and S long and short components; a internal a repeats (b and c regions are not shown). If a strong secondary structure in the messenger RNA prevents interaction with one of the above components, ribosomes will not efficiently bind and therefore protein synthesis will not begin at that site. The future is bright for the phycodnaviruses; the future is green! Ankyrin Apoptosis Caspase Ceramide Chitin Coccolithophore Coccoliths Endosymbiotic Guanylyltransferase Hyaluronan Inverted repeat Jelly-roll -barrel Osmolyte Paralogous Pentasymmetrons Topoisomerase Trisymmetrons Ubiquitin FUNDAMENTAL CONCEPTS • Phycodnaviruses are large, double-stranded DNA viruses infecting algae. A closer view of the envelope proteins (Figure 15.4) shows that H or HN is a type II integral membrane protein, with a transmembrane domain located near the N-terminus, a long external C-terminal projection, and a short N-terminal sequence facing inside the virion. A copy of proviral DNA is integrated into the cellular genome at a random site gene. Plant viruses are presently grouped into 24 different families, but a large number of genera (most of which contain viruses with positive-strand RNA genomes) have not yet been assigned to families. Certain poxviruses can interfere with two major players of host antiviral defenses. Several structural proteins are found in all coronaviruses, in the following order: hemagglutinin-esterase (HE), spike (S), small envelope protein (E), membrane protein (M), and nucleocapsid protein (N). These cells are particularly susceptible to CH022.indd 264 oncogenic transformation by the high-risk human papillomavirus types such as 16 and 18. A negatively charged arginine-rich domain in the N-terminal region of the coat protein is localized on the internal surface of the virion and interacts with encapsidated RNA (Figure 10.3). This process has been observed in vitro and is known as antibody-dependent enhancement. Unlike most cellular mRNAs, reovirus mRNAs do not contain 3' poly(A) tails. The 3 protein modulates PKR activation. The P1 polypeptide is cleaved by the 3C/3CD proteinase to generate VP0, VP3, and VP1, which assemble into a 5S protomer. The N-antiterminated transcript from PL extends leftward through the general recombination genes gam, bet, and exo, the site-specific recombination genes xis and int, across the locus for site-specific recombination, attP, all the way to Ea47 (Figures 8.2 and 8.4a). Upon binding of the virion to a cell surface receptor, the F1/F2 (fusion) protein undergoes a conformational change at neutral pH that allows insertion of the fusion peptide into the plasma membrane of the cell. There are two identical LTRs, one at each end of the proviral DNA. Availability of the vaccine worldwide, especially in poorer countries, is still a major problem. Can be expensive to make and store. Moreover, production of factor IX was transient, and antibodies against AAV capsids were detected, which could prevent reuse of the same vector. examples include the lom and bor genes, which encode prophage-expressed outer membrane proteins. Once viral DNA replication has begun, much larger amounts of viral messenger RNAs can be detected, and these "late" messenger RNAs are copied from a different set of viral genes. The prM-E heterodimers are oriented perpendicular to the surface of the lipid bilayer. The plus-strand is incomplete. An example is the env gene of human endogenous retrovirus W, called syncytin-1. 16/05/11 8:19 PM The Adaptive Immune Response THE ADAPTIVE IMMUNE RESPONSE Viruses and other infectious agents produce a variety of proteins and other macromolecules that are recognized by the host as foreign; these molecules can give rise to specific immune responses and are termed antigens. This second edition has new chapters on viruses of plants, algae, and archaea, host responses to virus infection, and the host immune system. Scientists are working on the possibility of discovering drugs against other viruses that encode similar proteins. This is mainly due to the cell types that they target for establishment of long-term latency: neurons for herpesvirus type 1 and B lymphocytes for Epstein–Barr virus. Assembly of virions 7. Like other negative-strand RNA viruses, the template for both transcription and replication of bunyaviruses is the helical nucleocapsid, not naked RNA. In 1950, the Insect Pathology Research Institute in Sault Ste-Marie, Ontario, successfully controlled sawfly populations with applications of baculovirus. How do they combat influenza virus infections? DISEASES Symptoms include fever, rash, arthritis, and encephalitis; can be fatal. It binds to and sequesters interferon-, and therefore inhibits binding of interferon- to host cell receptors, thus reducing the Th1 immune response to viral infection. Cells that have integrated a functional early region can continue to express T antigens, and all daughter cells inherit this ability. Because of this property of self-assembly, expression of viral structural proteins in the absence of the viral genome often leads to the production of artificial virus-like particles (VLPs). • DNA concatemers are cleaved within the terminal repeats in a reaction dependent on transcription by T7 RNA polymerase and are simultaneously packaged in phage proheads, which mature by shedding scaffolding proteins. Mad cow disease: popular name for bovine spongiform encephalopathy (BSE). Primosome: a protein complex that synthesizes RNA primers on which DNA replication can be initiated. Describe two types of adenovirus vectors that have been used as antitumor agents. (b) The matrix (M) protein of influenza virus interacts both with the cytoplasmic tail of surface glycoproteins (HA and NA) and with helical nucleocapsids (RNP) during budding. The "single nuclear envelopment" model (1) predicts that after budding through the inner nuclear membrane, virions are enclosed in vesicles derived from the outer nuclear membrane, and are transported to the plasma membrane without further envelopment. Protein 1a (110 kDa) contains two domains: a methyltransferase domain in the N-terminal region, involved in capping the 5' ends of viral RNAs, and an RNA helicase domain in the C-terminal region. p53 blocks the cell cycle and induces apoptosis in response to virus infection. The inability of rat and hamster cells to replicate mouse polyomavirus or simian virus 40 DNA has been traced to a lack of interaction between some elements of the cellular DNA replication machinery and the viral large T antigen. (b) Figure 9.4 Thermoproteus tenax virus 1 (TTV-1), genus Alphalipothrixvirus. Thickness of lines indicates approximate transcript abundance. self-priming mechanism of replication, similar to that proposed for large eukaryotic DNA viruses including poxviruses. Each of the negative-strand subgenomic RNAs is copied by viral RNA polymerase to generate a nested set of positive-strand subgenomic mRNAs (bottom right of Figure 14.5). To accomplish this, most viruses piggyback on the machinery that cells utilize for protein and nucleic acid trafficking. How is the cellular gene c-myc targeted by Epstein–Barr virus? 10-kb inverted terminal repeats. Core proteins: proteins associated with the viral genome inside the capsid. Early viral genes are expressed: the Baltimore classification of viruses Once in the appropriate compartment of the cell, the viral genome must direct expression of "early" proteins that will enable genome replication. Once the bound ADP residues dissociate from the large T antigen molecules, another set of ATP molecules binds, and the cycle continues. All are uniformly fatal. What affects the phage's decision to go lytic or lysogenic? Considering the niche in which the microviruses must compete, rapid replication may be the key to their survival. Van Etten, "Assembly site of the virus PBCV-1 in a chlorella-like green alga: ultrastructural studies," Virology 154 (1986): 240–245, with permission from Elsevier; Fig. Release of animal viruses can occur by a number of pathways. The design of the two neuraminidase inhibitors presently available, zanamivir and oseltamivir (Figure 36.10a), began when the three-dimensional atomic structure of the neuraminidase was determined by x-ray crystallography in 1983. AIDS is caused by loss of cellular immunity due to depletion of T lymphocytes; illness and death is a result of infection by a variety of pathogens that can no longer be repulsed by the weakened immune system. In summary, the expression of E7 leads to induction of unscheduled DNA synthesis in fully differentiated keratinocytes. • Rotaviruses, members of a genus in the Reoviridae family, cause severe gastroenteritis and are a major cause of disease and death in children in the developing world. • Hepacivirus, from Greek hepatos (liver): hepatitis C virus, in humans only. Furthermore, since maturation protein cannot be translated from the full-length genome, replicase needs only to compete with ribosomes that bind at the coat start site to create a ribosome-free template, which is needed for unimpeded synthesis of minus-strand RNAs. Assembly and release of virions The assembly of virions has not been studied in great detail. It is selectively phosphorylated by viral thymidine kinase and, in its triphosphate form, selectively inhibits viral DNA polymerase. Monocots: flowering plants that have a single cotyledon, or seed leaf, which emerges from the germinating seed. Furthermore, there is no evidence that mammalian viruses encode suppressors of RNA silencing, which might be expected if this were a major antiviral mechanism available to mammalian cells. • Another approach is to design drugs based on knowledge of the active sites of the enzymes to be targeted. The lysis of the infected cell to liberate approximately 100 progeny phage particles is dependent on two T7 functions. Proviral DNA can remain unexpressed in the host cell for long periods of time, like any other cellular gene, if the cell does not contain the appropriate set of transcription factors. Alternatively, proteins, nucleic acids, and lipids are revealed by positive staining in plasticized thin sections of infected cells (e.g., human immunodeficiency virus; Figure 2.1j). The disease progresses in three stages: (1) an initial stage marked by high fever and accompanied by muscle pain, headaches, and severe vomiting; (2) a quiescent stage in which the fever dissipates and the patient appears to be in remission; and (3) a final stage marked by a repeat of the original symptoms in a more severe form that results in multi-organ failure with a mortality rate of between 20 and 50%. The DNA J domain acts as a cochaperone to dissociate pRb from E2F. Alternatively, the infecting DNA can enter a prophage state, in which the lytic genes are repressed Some phages in the Myoviridae and Podoviridae families share segments of genome homology with λ phage. CI dimers show pairwise cooperativity, so that when a CI dimer binds to OR1, it facilitates the binding of a second CI dimer to OR2 by protein–protein interactions between the two repressor dimers (Figure 8.5b). What modifications or additions to these vaccines could enable the stimulation of both humoral and cellular immunity in a vaccinated individual? The virus replicates predominantly in the mesophyll cells, and then moves in the direction of the arrows, from the mesophyll into the vascular bundle to the sieve elements for transport along with the photoassimilate. CH035.indd 429 16/05/11 8:21 PM 430 CHAPTER 35 Antiviral Vaccines The fruits of their labors have fundamentally changed human history. Re pli Cell Division Induction ion Lysis Lysogenization Re gu lat Lytic Growth In the late 1950s, Dale Kaiser began studying genes involved in the establishment of lysogeny. and the viral DNA integrates into and is passively replicated with the host chromosome (Figure 8.1). The protein is glycosylated, forms dimers, and is found both secreted and in association with membranes in infected cells. Single segment, 19 kb. Budded virions (Figure 25.1) consist of single nucleocapsids surrounded by an envelope. Matrix proteins often serve as a bridge between the envelope glycoproteins incorporated into the lipid membrane and the nucleocapsids enclosed within the envelope. An unspliced late mRNA is translated to produce viral capsid protein VP2. Arrows show direction of transcription. Interestingly, p50-negative mice exhibit greatly reduced levels of apoptosis in the central nervous system in comparison to normal animals, in keeping with the proapoptotic effects of NF-B in cell culture. The virus envelope contains two or three glycoproteins. Transcription of SSV-1 DNA is temporally controlled Within 1 hour after exposing cells carrying an integrated genome of SSV-1 to ultraviolet light, a short (200–300 nt) "immediate early" viral RNA named T-ind is produced 16/05/11 8:00 PM 102 CHAPTER 9 Viruses of Archaea Early A-153 B-277 T9 Late A-92 C-102a B-251 C-84 T1/2 Late extended D-335 A-82 VP2 VP3 VP1 Early E-54 F-92 D-244 B-115 C-166 E-178 SSV1 B-78 F-93 E-51 E-96 D-63 15.5 kb F-112 C-792 T5 B-49 Late C-124 A-291 B-129 A-100 A-132 A-79 T4/7/8 C-80 T-ind T6 Immediate early C-102b A-45 Tx T3 Early Late Figure 9.3 Genome of Sulfolobus spindle-shaped virus 1 (SSV-1). This permits the synthesis of Tat, Rev, and Nef. DNA replication leads to activation of 1 and 2 genes Although 1 genes can be transcribed in the absence of viral DNA replication, their optimal expression requires viral DNA synthesis, presumably because of the increase in the number of template DNAs available for transcription. Alternatively, there may be extremely low levels of haploid cells present naturally at all times, which have the opportunity to proliferate when the more vigorous diploid community becomes decimated by viral attack. Complexes that contain reovirus mRNAs bound to nonstructural viral proteins NS and NS and outer capsid protein 3 are postulated to represent the first step in reovirus assembly (Figure 19.8). The P protein may take part in the extension of the plus-strand, but inhibitor studies have shown that all of these steps (strand extension, primer removal, and ligation) can be carried out by host cell enzymes in the nucleus. Several other viruses of crenarchaeaota have not yet been assigned to families. For example, maternal antibodies can inactivate measles vaccine-strain virus in some children younger than 12 months of age. Electron micrographs of (a) two-tailed virions; (b) virions being extruded from a cell of Acidianus convivator; (c) virions in culture medium after release from infected cells, 2 days after infection; (d) as for (c), but purified by CsCl density gradient; (e–h) as for (d), but incubated at 75□C for 2, 5, 6, and 7 days, respectively. The attP–attB exchange during integration shuffles the att sequences, creating attL (BOP') and attR (POB') sites at the left and right junctions between the prophage DNA and the bacterial chromosome (Figure 8.7). This pathway is activated by a cascade of phosphorylation events on tyrosines located in the receptor molecules, in the intermediary Jak kinases, and in the Stat proteins that activate transcription of a variety of target genes (Figure 33.8). 6. Because this restricted tropism severely limits cell types that can be transduced, the HIV-1 env gene is not used to generate lentivirus vectors. According to this hypothesis, nucleocapsids exit through the enlarged nuclear pores without an envelope (Model 3 in Figure 24.9), and become enveloped at multivesicular bodies derived from fragmented Golgi membranes. Distinct sites within the PB1 protein bind to the conserved sequences at the 5' and 3' ends of the genome RNA (Figure 18.5). In the G1 phase of normally cycling cells, Rb becomes phosphorylated by cyclin-dependent protein kinases (Cdks), and the phosphorylated Rb no longer binds to E2F. Sialic acid– binding domain HA1 Hemagglutinin protein binds to cell receptors and mediates fusion of the envelope with the endosomal membrane The influenza hemagglutinin protein (HA) was so named because influenza viruses can agglutinate (clump) red blood cells. This complex binds to specific response elements in viral DNA that have a central consensus sequence TAATGARTT. In contrast, occlusion-derived virions (Figure 25.2) are formed in the nucleus where they become enveloped CH025.indd 304 Granule (GVs) Occlusion body protein Occlusion body envelope (c) (d) Figure 25.2 Occluded baculoviruses. Infection in adolescence and adulthood can cause infectious mononucleosis, a disease whose symptoms are caused by proliferation of infected B lymphocytes and their subsequent destruction by activated T lymphocytes. Latently infected individuals can remain without symptoms for months or years, or even their entire life. The RNA genome is in a helical nucleocapsid associated with 4 viral proteins: the viral polymerase (L), the nucleoprotein (NP), and proteins VP35 and VP30. The two DNA strands are covalently linked at their ends. T7 solves this problem by forming large linear DNA oligomers, called concatemers, via a terminal repeat sequence of about 150 bp at each end of its DNA. Virus factories. 10.2: Courtesy of Robert Milne; Fig. Below: latency-associated transcripts (LATs). bcreidt.indd 488 16/05/11 2:35 PM N A M E I N D E X Acqua, C., 303 Ahlquist, Paul, 117 Arntzen, Charles, 375 Avery, Oswald, 8 Baltimore, David, 7, 14, 127, 342 Barr, Yvonne, 297 Barré-Sinoussi, Françoise, 7 Beijerinck, Martinus, 6–7, 71 Berg, Paul, 7, 457 Bertani, Giuseppe, 85 Bishop, Michael, 7, 343 Blumberg, Baruch, 7, 366 Bulgakov, N., 69 Burkitt, Denis, 296 Campbell, Allan, 86 Carroll, Lewis, 333 Caspar, Donald, 23 Cavalier-Smith, Thomas, 242 Chanock, Robert, 160 Chapman, J. No individual can possibly write knowledgeably about the large spectrum of viruses that a virology course should cover, so a collaborative approach was necessary. Humans are infected by virus-containing saliva from bites of infected animals, often domestic dogs or bats. Fundamentals of molecular virology / Nicholas H. The coordinated activities of nsps in RNA synthesis and modification remain to be shown during virus infection, but mutagenesis studies have confirmed the importance of these proteins for replication. The start site for translation of the P protein is in a favorable context for recognition by the ribosome (AUG followed by a G at position 4) and is therefore most often used. CH032.indd 395 Describe how prion diseases in humans are both infectious and inheritable, and discuss how they are transmitted. This shows that ribosomes look for the lysis start codon from the 3' direction. CH034.indd 421 The large IgM macroglobulin is the first antibody produced in an immune response. To exit from the infected cell, non-enveloped viruses typically rely on rupture of cellular membranes. This section will briefly describe how the adaptive immune system recognizes and responds to viral infections. CH004.indd 55 What are the four basic steps of virus entry into a host cell? T7 RNA polymerase not only takes over transcription of the phage genome, but it also continues the work begun by the host RNA polymerase of spooling the phage DNA into the cell. These proteins are involved in envelope formation, as well as the formation of non-infectious particles found in serum. However, some viruses of bacteria and archaea are released by extrusion from the cell membrane and do not kill their host cells. Mutations leading to amino acid changes in the viral E2 glycoprotein rapidly accumulate during passage in cultured cells; these mutations allow the virus to bind to heparan sulfate, which is found on many cell surface proteins. Vaccination: a term coined by Edward Jenner and initially used to describe immunization against smallpox using BGLOSS.indd 483 483 cowpox. This exposes specific amino acid sequences in the penton base proteins that are recognized by binding sites in integrins. Structure determined by x-ray crystallography. Mice are resistant to infection with hamster prions, which have 16 differences in amino acid sequence from mouse PrP. Can cause cancer by expressing mutated cellular regulatory genes or by integrating into or nearby cellular regulatory genes. These "naïve" B and T cells are subsequently selected and activated upon contact with antigens in the lymph nodes or spleen to generate the immune response. The same issues apply to vaccine design. Fully deleted vectors have been given several names: third generation, helper dependent, high capacity, or "gutless." Those vectors offer two main advantages: (1) no viral genes are present, eliminating problems of immune response to viral proteins; (2) up to 35 kb are available for insertion of transgenes for expression. 5. Pock: a localized blistering lesion on skin or mucosae caused by poxviruses or other agents. These cores bud through the plasma membrane in regions where the SU and TM proteins have accumulated, acquiring an envelope. Several hundred cases of hantavirus pulmonary syndrome have since occurred in North and South America. N protein levels control the switch from transcription to genome replication Once sufficient N protein is made, genome replication can begin (Figure 15.8). The ASC adaptor then recruits procaspase-1 through its caspase activation domain. Thus, it more closely resembles the natural compound, deoxyguanosine, which may account for its greater toxicity. The primer for elongation by DNA polymerase III is the 3' end of plus-strand DNA cleaved by the viral A protein. William, 78 Summers, Donald, 127 Summers, Max, 308 Tamm, Igor, 225 Temin, Howard, 7, 342 Theiler, Max, 7, 139 Tiollais, Pierre, 366 Tomlinson, John, 113 Tyrrell, David, 160 Tyrrell, Lorne, 375 Valenzuela, Pablo, 366, 375 Varmus, Harold, 7, 343 Verma, Inder, 463 Vida, Marco, 302 Von Bergold, Gernot, 303 Von Prowzek, Stanislaus, 302 Washington, George, 138 Watson, James, 8 Weigle, Jean, 85 Weissmann, Charles, 406 Weller, Thomas, 7, 430, 431 Wickner, Reed, 393 Wimmer, Eckard, 127 Woese, Carl, 97 Zillig, Wolfram, 101 Zinder, Norton, 59 489 bindauth.indd 489 12/05/11 4:08 PM bindauth.indd 490 12/05/11 4:08 PM S U B J E C T I N D E X Abortive infection, 82, 261 Acid-activated viruses, 50, 51, 53 Acidianus bottle-shaped virus (ABV), 100, 103–104 Acidianus filamentous virus 1 (AFV-1), 102–103 Acidianus filamentous virus 3 (AFV-3), 102 Acidianus two-tailed virus (ATV), 106–108 Acquired immunodeficiency syndrome (AIDS), 4, 5, 7, 248, 285, 343, 355, 356, 362, 445, 447, 450 Actin, 52,120, 321,363 Actinomycin D, 205, 216 Acute transforming retroviruses, 350 Acyclovir, 444–445, 448–449 acyclovir monophoshate, 449 incorporated by herpesvirus DNA polymerases, 449–450 Adaptive immune responses to virus infection, 155, 321, 332, 399, 404, 411, 419–422 antibodies, forms of, 420–421 antibody specificities, diversity of, 421–422 B-cell receptors, 420 B lymphocytes, 420 cells involved in, 417 cytotoxic T cells, 422 immunoglobulin G molecule, 421 primary and secondary lymphoid organs, 419 T-cell receptors, 420 T lymphocytes, 420 viruses evading, 424–425 Adeno-associated virus (AAV), 239–244 AAV vectors, 465–469 DNA replication, 241–243 integration into cell genome, 244 replication, adenovirus functions in, 244 Adenosine deaminase (ADAR1), 384 Adenoviruses, 274–284. An alternative interpretation of experimental results suggests that RNA polymerase II is used for both transcription and replication. Virus-like particles tend to be much more effective immunogens than proteins that are not assembled into such a structure (see below). Cucumber mosaic virus has caused several crop disease epidemics worldwide and catastrophic crop losses in other plants, most notably tomato. huxleyi has adopted a novel sex strategy to avoid coccolithovirus infection. Likewise, the tropism of different MVM strains for fibroblasts or lymphocytes is determined by the capsid protein. The vascular parenchyma, companion cells, and sieve elements make up the phloem. As shown in Figure 21.7, excision of the smallest intron (48 nt) removes exactly 16 triplet codons. Forterre, R. The coupling of protein synthesis, RNA synthesis, and virion assembly on membranous structures assures that newly synthesized genome RNA can associate with C protein and initiate the assembly process. The P protein remains linked to DNA throughout the process, so presumably the active site extends the 3⬘ end of the growing DNA molecule, while the 5⬘ end remains attached to another part of the same molecule of P, forming a circular complex. The short gaps that remain are filled in by DNA polymerase , and adjacent DNA molecules CH021.indd 258 are then ligated, forming a continuous lagging strand (Figure 21.12c). Int binds to the CH008.indd 91 arm sequences strongly; the arms also have binding sites for the E. Foot-andmouth disease virus, caused by a picornavirus, was the first animal virus discovered (1898). • Cucumber mosaic virus produces two subgenomic RNAs, one of which (RNA 4) codes for the coat protein. The molecular mechanisms underlying genetic variation and adaptation of cucumber mosaic virus are being uncovered through research in experimental evolution. In cells infected with oncogenic papillomaviruses, this pathway is not under normal control since p53 levels are suppressed by E6, and the pRb protein is bound by E7, resulting in activation of E2F-mediated genes (Figures 22.5 and 22.6). From 1989 to 1996, several Ebola virus infections occurred in cynomolgus monkeys imported from the Philippines to different facilities within the United States including Reston, Virginia, and a facility in Siena, Italy. Ribonuclease L: an enzyme that, when activated by binding of 2⬘, 5⬘-oligoadenylate, degrades cellular and viral mRNAs. Part of the interferon response. Some NF-B-responsive genes function in host innate immune defense mechanisms; these include interferon-stimulated genes and the gene encoding interferon- (see Chapter 33). Given the large number of vaccine types available, discuss why a live attenuated virus vaccine may be the best choice. The linear double-stranded viral DNA resulting from reverse transcription remains associated with components of the virus core in a preintegration complex. Likely involved in genome unwinding, separation, and packaging; may be virulence factor. Exit Figure 1.6 Steps in virus replication cycle. The bone marrow and thymus are central or primary lymphoid organs responsible for the production of naïve B lymphocytes (B for bone marrow) and T lymphocytes (T for thymus), respectively. via the glucose moiety to a distinct site on the major capsid protein F. The major envelope protein of flaviviruses lies parallel to the lipid membrane. Virions contain a negative-sense, single-stranded RNA genome 19 kb long, wrapped in a helical nucleocapsid. Chapter 16 Fig. Small DNA viruses usually use host cell DNA polymerases to replicate their genomes; larger DNA viruses often code for their own DNA polymerases as well as other enzymes involved in DNA replication. • Recombivax vaccine is recombinant surface antigen expressed in and purified from yeast. This system has provided valuable tools to understand the more general roles of the Rb and p300/CBP families, cell-cycle progression genes, and p53. As one of the first lines of defense against incoming viruses, the complement system is a centerpiece of innate immunity. The prion domains of certain yeast proteins have an exceptionally high content (approximately 40%) of two "amyloidogenic" amino acids, glutamine and asparagine. • Viruses that do not have envelopes can penetrate membranes by lysis of the endosomal membrane, or by insertion of viral proteins into the membrane, allowing passage of the viral genome directly into the cytosol. This commitment requires the support of adequate surveillance systems and a program to fairly compensate the rare individual who suffers a serious adverse event as a result of vaccination. Lai, Oklahoma State University Lorie LaPierre, Ohio University Maria MacWilliams, University of Wisconsin, Parkside Phillip Marcus, University of Connecticut Nancy McQueen, California State University, Los Angeles Joseph Mester, Northern Kentucky University Thomas Jack Morris, University of Nebraska Brian Olson, Saint Cloud State University Arnim Pause, McGill University Marie Pizzorno, Bucknell University Sharon Roberts, Auburn University Michael Roner, University of Texas at Arlington Miroslav Sarac, Our Lady of the Lake College David A. The viral proteins that are inserted into this membrane do not need to form a perfectly tight barrier as in a capsid, and therefore are not usually organized in a symmetrical fashion. In India at about the same time, variolation was accomplished by seeding freshly scratched skin of a recipient with pus from an active smallpox infection. Therefore the recent discovery of a viral DNA primase that is required for DNA replication implies that the actual mechanism of vaccinia virus DNA replication may deviate significantly from this model. Cytokines bind to specific cell surface receptors and initiate cellular signal transduction pathways, leading to activation of transcription of numerous cellular genes. As soon as the first six nucleotides at the 5' end of the growing viral RNA chain have been made, they bind to a molecule of N protein. CD4 Polio Adenovirus 2 and 5 Murine Leukemia virus Human Corona virus Measles virus Some rhinoviruses CD46 LDL-receptor 2. Of the 10,862 nucleotides in the yellow fever genome, 68 nucleotide differences resulting in 32 amino acid substitutions were noted between the two strains. Ribosomal frameshifting is induced by the presence of two sequence elements within the RNA: a heptamer sequence, where the ribosome stalls, and a secondary structure downstream of the heptamer that induces ribosome stalling (Figure 28.7). CH024.indd 292 16/05/11 8:12 PM Herpes Simplex Virus 3' a UL42 UL30 a'S a L 5' 3' L a'S a a'S a L a'S a L L 5' 3' 5' VP5 VP21 VP26 VP22a etc. Antiviral antibodies are also important for limiting reinfection with the same virus and can sometimes provide partial or complete protection against closely related viruses. To date, slightly more than three dozen viruses infecting organisms from the archaeal phyla Crenarchaeota and Euryarchaeota have been described (Tables 9.1 and 9.2). Once these proteins detect the presence of a pathogen, they set off a highly coordinated response that involves receptor-mediated signaling, activation of gene transcription, secretion of proteins, and cell-to-cell communication. First, the tRNA-like structure may protect viral RNAs from degradation by cellular nucleases, as RNAs covalently bound to an amino acid are rendered resistant to ribonucleases that recognize 3' ends of RNAs. Second, the tRNA-like structure may enhance translation of the RNA by interacting with cellular proteins involved in translation elongation. In addition, many viral proteins cause the down-regulation or degradation of cell-surface proteins such as MHC class I and II, CD4, and CD8. Cytomegalovirus Human herpesvirus 6A, 6B Human herpesvirus 7 Mononucleosis, hepatitis, fetal abnormalities Roseola Roseola Epstein-Barr virus Mononucleosis, Burkitt's lymphoma, nasopharyngeal carcinoma Kaposi's sarcoma Growth primarily in epithelial cells and B or T lymphocytes. In a research field often dominated by the claim "my virus is bigger than yours!" the mimivirus stands head and shoulders above its peers as the largest. These are broadly distributed in epithelial and fibroblast cells in the small intestine, renal epithelium, and central nervous system, and on monocyte and granulocyte lineages. Early endosomes are complex organelles composed of tubular and bulbous membrane elements, found in the peripheral cytoplasm. (d) The cleavage of CI stimulated by activated RecA protein. Originally published in The Journal of Cell Biology 169:269-83. Transport to the cytoplasm involves binding of the nuclear export signal on Rev to the cellular protein exportin 1, which mediates the docking of Rev to the nuclear pore. Each mRNA sequence could be preceded by its own promoter region, which would allow RNA polymerases to bind to each promoter region independently and begin synthesis of the different mRNAs (Figure 15.7a). Four nucleotides at the 5' end and five at the 3' end are invariant among all 10 viral gene segments. The synthesis of RNA is asymmetric, generating more than 10 times as many plus-strands as minus-strands. It has been proposed that host cell enzymes carry out cleavage of these RNA molecules. Inner capsid (T 1), or core, contains RNA polymerase and capping enzymes. Raft culture: in vitro culture system used to differentiate epithelial cells by growth at an air–liquid medium interface. MDV-1 RNA 1st-generation detection probe INSERT Target RNA Magnet Magnetic bead (a) DNA oligonucleotide Ligate, then amplify 2nd-generation detection probe IN MDV-1 RNA Detection of minute amounts of RNAs by using the power of Q replicase Among all the single-stranded RNA phages, Q is famous for its replicase. Virions of larger enveloped viruses are often somewhat irregular in shape, and their envelopes have no obvious symmetry. coli RNA polymerase encounters a strong termination signal (TE) about 8 kb into the genome from the left end (Figure 7.1), where it releases the mRNA and detaches from the DNA. 1998 (USA) Annual reported cases: Disease Dates Smallpox 1900–04 Pre-vaccination 48,164 Post-vaccination (1998) 0 Decrease (%) 100 Poliomyelitis (paralysis) 1951–54 1,314 1* 100 Measles 1958–62 503,282 89 100 Mumps 1968 152,209 606 99.6 Rubella 1966–68 47,745 345 99.3 Congenital rubella syndrome 1958–62 823 5 99.4 *Single case of vaccine-associated paralytic poliomyelitis. Subheadings within each chapter are explanatory phrases, telling the reader what will be discussed in the next several paragraphs. As DNA-based cells evolved, they would no longer need to maintain genes for reverse transcriptase activities, and they therefore could have lost genes that code for these enzymes. Ubiquitin ligase: an enzyme that catalyzes covalent binding of ubiquitin to proteins, marking them for degradation in proteasomes. All IRFs share a high degree of homology in their N-terminal DNA-binding domains, and they bind to the consensus DNA sequence GAAANNGAAANN (where N can be any RNA POL II TFIID TATA HMG1 IV HMG1 III I II PRD Figure 33.7 Transcriptional activation of the interferon- gene. (b) A cluster of double-membrane vesicles, showing a connection between the outer membranes and reticular membrane structures (arrow). SSB: single-strand DNA binding protein; priA–C and dnaB–C: primosome proteins. Vaccine technology has progressed rapidly, and there are now dozens of effective antiviral vaccines. Immunoglobulin diversity is 16/05/11 8:19 PM 422 CHAPTER 34 Innate and Adaptive Immune Responses to Virus Infection CD4 Th1 cell IL-2 IFN-γ Cytotoxic T lymphocyte Tc cell CD8 T-cell receptor MHC I peptides viral proteins Infected cell Infected cell Lysed cell Figure 34.8 Generation of cytotoxic T lymphocytes and killing of virus-infected cells. Genome replication requires four host cell proteins plus the replicase How the replicase actually binds to the coat protein start site is fascinating. Reprinted from Fig. Adenoviruses do not integrate their genome during productive infection, but the adenovirus genome can persist as a stable episome in some cell types for months or even years. Development of self-sampling tests based on DNA detection (see below) may offer some solutions to these problems. Virtually all human adenoviruses are capable of transforming rodent cells in culture, and such cells usually form tumors when injected into newborn or immunologically compromised rodents. What is known about the site-specific integration of adeno-associated virus DNA in the host cell DNA? DISTINCTIVE CHARACTERISTICS Many flaviviruses (but not hepatitis C virus) are transmitted from host to host by mosquitoes or ticks. CH005.indd 68 16/05/11 7:54 PM C H A P T E R 6 Microviruses Bentley Fane Microviridae From Greek micro (small), referring to size of phage VIRION Naked icosahedral capsid (T 1). Genotypes vary in the severity of disease symptoms, in the ability to induce chronic liver disease, and in their response to interferon or chemotherapy. A single polyadenylation signal specifies the 3⬘ ends of all viral RNAs. This gives rise to five classes of RNA transcripts, ranging in size from 900 to 3500 nucleotides (Figure 30.4). Prusiner, "Structural studies of the scrapie prion protein by electron crystallography," Proceedings of the National Academy of Sciences, USA 99 (2001): 3563–3568; Fig. LMP-2A also mimics a cellular receptor, but in this case it is the B-cell receptor (BCR), which when paired with LMP-1 can drive resting B lymphocytes to proliferate and differentiate into memory B cells. Because the CI dimers cooperate only in pairs, binding to OR3 is not facilitated by repressors cooperatively bound at OR1 and OR2. The NC portion of Gag binds to a packaging signal designated psi (), located near the 5' end of viral genome RNA (Figure 28.2). 16/05/11 8:22 PM Retrovirus Vectors region, and ends in the right-hand LTR where the polyadenylation signal defines the RNA 3⬘ end. See also Common cold; Poliomyelitis genome organization, 128, 129 inhibition of host cell macromolecular functions, 134–135 internal ribosome entry sites (IRES), 128–131 membrane vesicles in replication, 131–132 model for cell entry, 128 pathogenesis, 135 poliovirus, 126–127, 435 protein synthesis initiation, 129 proteinases, 131, 133 precursor polyprotein, 131 replication of RNA, 132–133 rhinoviruses, 125–126 virion assembly, 134 virion structure, 22, 127 VPg and initiation of RNA synthesis, 133 Plant satellite RNAs, 41, 122–123, 379, 381, 383, 385 Plaque assay, 9–10 Plaque-forming units (PFU), 10 Plasma cells, 419 Plasmodesmata, 46, 116, 118 Pleconaril, 447 Plus-strand strong-stop DNA, 345 Poliomyelitis, 4, 125–126 Poliovirus, 21, 126–127 in vaccine development, 126–127 Sabin attenuated strains, 435, 436 Salk inactivated vaccine, 430, 431, 435 worldwide eradication possibility, 435, 441 Poly r(C) binding protein 2 (PCBP2), 133 PolyA-binding protein (PABP), 206, 218 Polyadenylation of cellular mRNAs, 218–219 Polyadenylation signal, 251, 459 Polyhedrin gene, 308 Polymerase chain reaction (PCR), 13 Polymerase protein, 370 Polyomaviruses, 23, 247–261 alternative splicing of viral RNAs, 253, 260 binding to sialic acid, 251 capsids, construction, 248–249 control regions, 252–253 circular DNA genome, 249 discovery, 247–248 divergent transcription, 250–251 DNA replication mechanism, 259 497 early mRNAs, 253 entry in caveolae, 251 genes, organization, 250–251 human polyomaviruses, 248 in vitro transformation of cells by, 260–261 large T antigen, 255–259 late transcription, 259–260 middle T antigen, 255 model for tumorigenesis studies, 248 mouse polyomavirus, discovery, 247 simian virus 40 (SV40), 247–248 small T antigen, 254–255 supercoiled DNA, 249–250 tumors in vivo, 260–261 viral DNA integration into cell chromosome, 261 virions, 248–249 Polyproteins bunyavirus, 203, 204 coronavirus, 161, 164, 165 flavivirus, 141, 142, 143, 144 picornavirus, 127, 129, 130, 131 retrovirus, 348–350 togavirus, 151, 152, 153, 154 Positive regulatory domains (PRD), 407 Positive staining, 19 Positive-strand RNA genomes, viruses with, 35–38 Pospiviroidae (group B), 379 Poxviruses, 312–323. The system has found utility in two areas: over-expression of foreign proteins for purification and biochemical analysis, and expression of foreign proteins for assay of their intracellular function. Measles virus infection can lead to immunosuppression due to its choice of cellular receptors. It is likely that when the RNA polymerase comes across a 3' end that is not properly packaged, it cannot begin transcription or genome replication efficiently, and that RNA molecule is therefore lost to the replication system. HERPES SIMPLEX VIRUS Herpes simplex virus genomes contain both unique and repeated sequence elements All herpesviruses contain a double-stranded linear DNA genome, which is between 120 and 230 kb in length depending on the virus species. Their "early" genes, transcribed immediately upon entry of the genome into the nucleus, carry out many of the same functions as the early genes of the small DNA viruses, with the same result: induction of the cellular machinery for DNA replication. RNA 2 encodes the 2a protein: the RNA polymerase component of the replicase, and the 2b protein: a silencing suppressor (translated from subgenomic RNA 4A). (b) Diagram showing the polypeptide chain of the canine parvovirus capsid protein in the same orientation as the red trapezoid in (a), with a jelly-roll -barrel in red (upper left) and loop structures in blue (lower right). Viruses play a fundamental role in cycling nutrients in the global ecosystem, as they kill or lyse up to 20% of the microbial biomass in the earth's oceans each day. Two promoters (Pearly and Plate) and two polyadenylation signals (Poly Aearly and Poly Alate) control production of transcripts, shown at bottom. This means that such viruses can evolve and spread in both vertebrates (or plants) and invertebrates. A host cell signal peptidase cleaves the polyprotein at two sites that define the carboxy termini of the PE2 and 6K proteins. How does herpes simplex virus ensure packaging of full-length genomes? However, virus infection slows host cell CH009.indd 104 (d) Figure 9.7 Acidianus bottle-shaped virus (ABV), family Ampullaviridae. VP1 has additional N-terminal sequences and is translated from an alternatively spliced mRNA. 16/05/11 8:01 PM Cucumber Mosaic Virus A U GCU AG U A CUUUGGAUCCUC U U CAGG C |||| G GUCC G |||||| ||||| GAAACC AGGAGACCA-3' A A U G-C G CC A C G A U-A G A C U C A-U C G G 5' C-G U C G C U-G C G C C C G U-G A U C G U C-G U C G C U A-U A G G-C U C C-G U U U A-U C A A CUG Figure 10.5 The pseudoknot configuration of the tRNA-like 3' structure of cucumber mosaic virus genome RNAs. RNA 2 of the Fny strain is shown. They contain unusual inverted repeat sequences in their promoter regions. Figure 5.8a shows the secondary structure of the 5' untranslated region of MS2 RNA, just upstream of the maturation start codon. Matrix protein: in virology, virus-coded proteins that lie just underneath the viral envelope and form a connecting bridge between the envelope and the viral nucleocapsid. To simplify AAV vector production, a method has been developed that is based on infection of suspension cultures of insect cells by recombinant baculoviruses. For every 180 copies of the coat protein, the phage needs only 1 copy of the maturation protein to construct virions, and a few copies of the lysis protein to leave the cell. Members of this family are divided into five subfamilies (a) TL P C V TR (b) Viroids can be classified into two families based on comparative sequence analyses and whether they possess a central conserved region (Table 31.1). Infection of cells with mature virus may occur by direct fusion of the mature virus membrane with the plasma membrane, or alternatively by uptake into vesicles followed by fusion with the membrane of the endosomal vesicle. The gene to be expressed (X) is inserted in a pET vector downstream of a T7 late promoter and a T7 gene 10 ribosome-binding site (rbs). Viruses missing the polyhedrin gene could be easily distinguished from polyhedrin-positive virus by microscopic examination of plaques in cell monolayers. This untranslated "leader" sequence is joined to an mRNA "body" sequence that includes one or more of the open reading frames in genes 2 through 7 (for murine hepatitis virus). ICP0 also acts as an E3 ubiquitin ligase to degrade several components of ND10, including the cellular proteins PML and SP100, and it causes a dispersal of the components of ND10. Since then, over 30 different viroids have been identified as the causative agents of a number of diseases that affect plants of economic importance. This peptide drug blocks a conformational change in the viral envelope protein gp41, which is required for fusion between the viral envelope and the cell membrane. Binding to CD4 induces a conformational change in gp120 that exposes regions that interact with the chemokine receptor. As a result other cellular genes (IL-2 and IL-2 receptor) are also induced, resulting in cell proliferation due to an autocrine feedback that stimulates growth of infected cells. They stabilize the virus capsid and prevent uncoating. Penetration 4. HIV-1 protease is an excellent target for antiviral chemotherapy because it is an enzyme that is essential for HIV replication; it cleaves the Gag precursor of viral capsid proteins within the assembled virion. THE EVOLUTIONARY ORIGIN OF VIRUSES Because viruses need living cells for their replication, they must have evolved hand-in-hand with their hosts as these organisms evolved. Further support for this notion was provided by sequencing the 5' ends of bunyavirus mRNAs. They were found to contain an additional 10 to 18 nucleotides at their 5' ends whose sequence is unrelated to the viral genome sequence. What other characteristics are used for virus classification? Extracellular virus is a mature virus particle wrapped in an additional lipid bilayer containing a distinct set of embedded viruscoded proteins. Poxvirus genes do not contain introns and therefore viral mRNAs are not spliced. During the transition from ISVP to core (70 nm in diameter), the attachment protein and the 1 protein fragments are lost, leaving the 2 spike protein and the viral core proteins. (c) Hepatitis A virus (a picornavirus). Edmonston) • Twenty-four passages (35□–36□C) in primary human kidney cells • Twenty-eight passages in primary human amnion cells • Six passages in chicken embryos • Marketed as Edmonston B vaccine in 1963 • Seven passages (36□C) in chicken embro fibroblasts • Forty passages (32□C) in chicken embryo fibroblasts • Marketed as "further attenuated" measles vaccine (Moraten strain) in 1968 Oka-strain varicella virus • Isolated by Takahashi et al. The structure of this protein closely resembles that of the adenovirus fiber protein, also a trimer (see Chapter 23). Diameter 70 nm. Similarly, the ability of a bunyavirus to cause a disseminated infection in its mosquito vector maps to the M segment and is therefore controlled by viral glycoproteins. This mechanism works as follows (Figure 15.8, top). At the top is a cutaway view to reveal the RNA, protected within a groove, in the interior of the capsid. However, dendritic cells collect HIV-1 virions on their surface. Nucleic Acids Research 37, D436-D442 (2009); doi: 10.1093/nar/ gkn840; Fig. VIRUSES AND HOSTS Fifty human serotypes in six subgroups (A through F). Blooms can only be seen from space when the cells are dying and the chalky shell is released into the surrounding sea. A host RNA polymerase copies plus-strand circular RNA (top of Figure 31.2) into single-stranded linear, complementary minus-strands (step 1). Certain viruses do not always undergo a productive growth cycle, but instead lodge one or more "silent" copies of their genome in the host cell until conditions are appropriate for a lytic cycle (lysogeny or latent infection; see Chapters 8, 9, 24, and 28). Many of the vectors used either have too broad a tissue tropism or are not able to infect the targeted tissue. Genetic variation can be reduced by selection or by genetic bottlenecks. Following integration of proviral DNA and its transcription at a basal level, only the doubly spliced 2-kb RNAs (Figure 29.3) are transported to the cytoplasm. Metalloproteinases: enzymes that degrade proteins by cleaving peptide bonds, and that use metal ions such as zinc in the catalytic mechanism. Secondary and subsequent infections occur in close family members or among medical staff. • HIV-1 infects and kills primarily CD4-positive T cells, resulting in severe immune deficiency for ¬90% of people infected. Watson Igor Tamm Purnell Choppin ffirs.indd iii 19/05/11 10:33 AM ffirs.indd iv 19/05/11 10:33 AM B R I E F C O N T E N T S SECTION I: INTRODUCTION TO VIROLOGY 1. Analysis of molecular chimeras between viroids of the Pospiviroidae family of different pathogenicity levels has revealed that the severity of disease symptoms is the result of complex interactions among three out of the five viroid domains. Very little is known about how the protein 3a-viral RNA complex moves from the sites where viral RNA and protein synthesis take place to the plasmodesmata. The discovery of single-stranded RNA phages (Chapter 5) allowed scientists for the first time to study a purified messenger RNA. 05/05/11 11:24 AM Chloroviruses (a) (b) 329 (c) Figure 27.4 Structure of PBCV-1 capsid protein Vp54. What is the one member of the togavirus family that is not transmitted by an arthropod vector, and what diseases does it cause in humans? This explains the region of single-stranded DNA found within the genomes of 373 purified virions. The ability of some vaccines to induce "herd immunity" has long been thought of as a positive property. Filamentous particles are seen in longitudinal, transverse, or crosssections (bar: 500 nm). Papillomaviruses 263 Greg Matlashewski, McGill University Revised by: Lawrence Banks, International Centre for Genetic Engineering and Biotechnology, Trieste 29. PrPSc can catalyze its own formation from PrPC in animals. CAR is a cell surface protein of unknown function, whose amino acid sequence shows that it is a member of the immunoglobulin family (Figure 4.2). In contrast to many highly symmetrical nonenveloped virions, many enveloped viruses such as influenza virus (Figure 2.1g) have virions of variable shape and size. This allows one part of the terminal sequence to base pair with the other part, enabling the molecule to fold back on itself to form hairpin-shaped or T-shaped structures (Figure 20.3). However, binding of large T antigen to p53 suppresses its transcriptional activation function, allowing the cell to enter S phase and avoiding p53mediated apoptosis. Delete some or all virus genes 2. Alternatively, the drug (a) Picornavirus receptor Canyon Hydrophobic pocket VP1 (b) CF3 N O N CH3 O CH3 Pleconaril N O CH3 Figure 36.2 Receptor and drug binding to the picornavirus capsid. S-S: cysteine bridge joining the N-terminal region of F1 to F2 after cleavage. The virus-coded RNA polymerase is responsible for transcription of late and very late genes. Phage adsorption and DNA entry depend on cellular proteins involved in sugar transport ca tio n Figure 8.1 Outline of biology. Finally, mimivirus is a real giant among viruses, with a genome of 1.2 Mb. It replicates in an amoeba, and its huge size originally fooled scientists into believing it was a small bacterium. No viral RNA polymerase has been detected, either by examining the known virus-coded protein sequences or by enzymological methods. Poliovirus containing a single point mutation in stem-loop V of genome RNA, a lesion present in attenuated Sabin vaccine strains, shows pronounced growth defects in neuroblastoma cells compared to the wild-type virus. Antiviral drugs can control HIV-1 infection and prevent disease progression, but an effective vaccine has yet to be developed At present, recommended treatment involves use of a combination of (usually three) drugs targeted at the viral enzymes reverse transcriptase and protease, although drugs targeting other aspects of the viral life cycle (virus entry and integration of proviral DNA) are also available. Many of the technologically crude vaccines made from these "natural products" actually worked well, and their popularity spread. Insufficient CI results in the lytic program. Whether a sufficient amount of CI is produced from PRE transcripts is the crux of the choice to go lytic or lysogenic. Prior to their appearance in the plasma membrane, a host cell furin protease converts PE2 to E2 and E3. The variable parts of heavy and light chains form antigen-binding sites Antigen-presenting cells such as dendritic cells and macrophages digest antigens and present digested fragments in association with MHC molecules to T cells. This results in liberation of the viral nucleocapsids from within the envelope, so that they can find their way to the nucleus for transcription. Various cytoplasmic receptors recognize viral nucleic acids. What enzyme is responsible for this? The mechanisms that control the switch between early and late messenger RNA synthesis have been extensively studied as paradigms for understanding how gene expression is controlled in the host cell. The resulting double-stranded DNA is then transcribed by a cellular RNA polymerase to generate viral mRNAs. Note that either of the complementary DNA strands can be packaged in virions. The agent was isolated from patients in both countries and was named after the Ebola River in northwestern Zaire. When several of these viruses were understood to have a very different lifestyle from the influenza viruses, the myxoviruses were split into two families, orthomyxoviruses and paramyxoviruses (ortho correct; para alternative). neozealandicus; (b) virions at higher magnification (Scale bars: 200 nm); (c) drawing of SNDV. The combined signaling of LMP-1 and LMP-2A drives B-cell proliferation and maturation, allowing Epstein–Barr virus to establish a long-lived latent infection in memory B lymphocytes. Picornaviruses cause a variety of human and animal diseases including poliomyelitis and the common cold Picornaviruses (pronounced "pic-or-na-viruses") were so named because they are small (pico, implying "small") RNA-containing viruses. Informational DNA Reverse transcriptase DNA DNA polymerase 5. Figs. • The replicase genes are translated to produce two overlapping polyproteins, which are cleaved to generate up to 16 distinct viral proteins. • The influenza virus NS1 and PB1-F2 proteins suppress a variety of host cell antiviral responses. This is a process that believers of the "Gaia hypothesis" (which postulates that planet Earth is a self-regulating "organism") can extol, since something as small as a virus can control the weather. As it progresses, the DNA polymerase encounters the other end of the same DNA strand and displaces it from the template as it continues around the circular DNA. Measles virus can bind via the H 16/05/11 8:05 PM Paramyxoviruses and Rhabdoviruses 179 HN: Hemagglutinin-neuraminidase F: Fusion N: Nucleocapsid L: Polymerase P: Phosphoprotein SH: Small hydrophobic M: Matrix Figure 15.3 Schematic diagram of paramyxovirus virion. Oncogenic papillomaviruses, simian virus 40, and adenoviruses have all evolved to express proteins that target and inhibit the activity of p53, albeit through different mechanisms (Figure 22.4). Therefore, nucleocapsid, RNA polymerase, and cofactor proteins must be expressed in a cell independently of genome RNA in order to recover virus from transfected plasmids. Tumor cells or cells of the immune system are considered polyclonal if they have numerous different origins or antigenic specificities. p53 bound to this complex is ubiquinated, transferred to the proteasome, and degraded (Table 23.1). Copying of full-length genome. In Sindbis virus, translation terminates most of the time at a UGA codon positioned Partly cleaved nonstructural proteins catalyze synthesis of full-length antigenome RNA The nonstructural proteins carry out synthesis of the different viral RNAs, a highly regulated event in infected cells. Pectin methylesterase can also bind to RNA. Diameter 30 nm. But in patients with a hemolytic anemia, for example sickle cell disease, the red cells have a much shorter lifespan in the blood. (a) Electron micrograph of a thin section through a polyhedron in which baculovirus particles are occluded. Latent infection complicates the elimination of HIV-1 Figure 29.4 Model of HIV-1 entry. These phages instead subvert the bacterium's 1308 1335 1724 1761 maturation (A) 5' replicase proteins. Cells transformed by papillomaviruses are in fact unable to produce new virus particles, because only a part of their genome is present 16/05/11 8:10 PM 270 CHAPTER 22 Papillomaviruses in the transformed cell, and furthermore it is frequently integrated into the cellular genome and can no longer replicate independently. The successes with influenza virus, HIV-1, and herpesviruses lead to optimism that other viral diseases will be susceptible to antiviral therapies in the future. Consequently, the mRNA is no longer regulated in the appropriate fashion and expression of the protein can be altered. The innate immune response also acts quickly, and involves the action of sentinel cells, including macrophages, dendritic cells, and natural killer (NK) cells, as well as complement. This involves binding of the capped 5' end of a cellular mRNA by the L protein, cleavage at a site CH017.indd 205 10 to 18 nt from the 5' end, and alignment of the 3' end of the cleaved RNA fragment with the 3' end of the genome RNA. Reverse transcriptase, the enzyme responsible for this conversion, exists as a dimer that has two distinct activities: an RNA/DNAdependent DNA polymerase activity, and a ribonuclease H activity. Cell surface components involved solely in binding of virions to cells are called attachment factors. Given the variety and number of these viruses, it is striking that virtually no known double-stranded DNA viruses (for the exception, see reverse transcriptase viruses below) infect higher plants. The resolution of structures determined by x-ray diffraction is much greater than the resolution obtained by electron microscopy, allowing precise determination of the position of every atom in the virion. This could be one explanation for the potentially low barrier to species jumping by coronaviruses; if the receptor-binding domain is capable of being exchanged easily during recombination, the receptor specificity of the recombinant virus and therefore its target cell type could quickly adapt to a new species. 1998 (USA), 429 rabies vaccine, 441 recombinant, 433 subunit vaccine, 431, 434 technological advances supporting, 430 types of, 431–435 under development, 440 vaccines that break tolerance, 439 vaccines that stimulate cell-mediated immunity, 436–437 vaccine-strain viruses, passage histories for, 433 virus-like particle (VLP) vaccines, 435 virus-like particles, 431 whole inactivated viruses, 431 Aphthovirus, 126, 130, 134 Aplastic crisis, 245 Apoptosis, 155, 401, 404–405, 412, 418 adenovirus, 279–280, 283, 284 baculovirus, 307 coccolithovirus, 333 Epstein-Barr virus, 300 extrinsic pathway, 404 granzyme B, 418 herpes simplex virus, 295–296 human immunodeficiency virus, 356, 363 influenza virus, 213, 219 intrinsic pathway, 404 NK cells, 418 p53, 256 papillomavirus, 268–269 PKR, 411 polyomavirus, 256 reovirus, 228, 233–234 Archaea, 97–98 Archaeal viruses, 99–110 Acidianus two-tailed virus (ATV), 106–108 Ampullaviridae, 103–104 Bicaudaviridae, 106–108 comparative genomics, 109–110 Crenarchaeota, 98 differences between Archaea, Bacteria, and Eukarya Euryarchaeota, 98 Fuselloviridae, 99–102 Globuloviridae, 104–105 Guttaviridae, 103 Lipothrixviridae, 102–103 491 bindsub.indd 491 12/05/11 4:08 PM 492 Subject Index Archaeal viruses (Continued) lysogeny, 106 morphologies, 99 positively-supercoiled DNA genomes, 101 pyramidal structures for cell lysis, 107 Rudiviridae, 105–106 Salterprovirus, 108 Sulfolobus spindle-shaped virus 1 (SSV-1), 101–102 temperate viruses, 99 Arthropod-borne viruses, 148–149 Ascites, 374 Asialoglycoprotein receptor, 193 Asian flu, 211, 221 Assembly, virion, 28–29 core proteins, 28 multiple modes of, 28 packaging signals, 28 scaffolding proteins, 28 viral envelope formation 28–29 Attachment factors, 47 Attachment proteins, reovirus, binding cellular receptors, 228–229 Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV), 303 genetic map of, 305 Autophagy, 401 Avian influenza virus strains, 431 Avsunviroidae (group A), 379 Azidothymidine (AZT), 445, 449–451 B lymphocytes, 179, 286, 399, 416, 420 B19 virus, 244–245 Bacteriophage MS2: see Single-Stranded RNA Bacteriophages Bacteriophage ϕKZ virion, 25 Bacteriophage ϕX174: see Microviruses Bacteriophage Qβ: see Single-Stranded RNA Bacteriophages Bacteriophages. There is no proof of any successful strategy in specific pre- and post-exposure treatment of human filovirus infections. Multiplicity of infection (m.o.i.): the number of infectious virus particles inoculated per cell. Early genes are transcribed, permitting an initial replication phase that results in 50 to 100 copies of DNA per cell. However, many viruses have specific packaging signals that ensure incorporation only of viral nucleic acid into virions. Cytomegalovirus encodes a protein kinase that phosphorylates ganciclovir Ganciclovir was the first drug approved for use against human cytomegalovirus, another herpesvirus. Injection of spinal cord material from a rabid rabbit would certainly be less intimidating to someone who had just been bitten by a rabid wolf. Are cheaper to manufacture and test, and can be very stable. Thus studies of a bacteriophage were important in proving the chemical nature of genes. Genes within these fragments can be mutated independently, creating libraries of mutated fragments. What transcription process do these viruses share and what is different? Experimental observations suggest that when a population of CRISPR-containing cells is infected by a bacteriophage, short DNA sequences derived from the phage chromosome are processed and inserted as spacer sequences in the CRISPR element. Reoviruses replicate more efficiently in transformed cells cultured in vitro than in their normal counterparts. X is not found in mature virions or nucleocapsids, but it stimulates viral gene transcription during the early phases of infection. RNA viruses tend to mutate rapidly because their RNA polymerases do not have proofreading mechanisms to eliminate mutations that occur during genome replication. Rapid progressors (10–15% of infected individuals) develop late stage symptoms in 2 to 3 years. Are safer for immunocompromised individuals. Subsequently, virions can diffuse laterally ("walk") on the cell surface until they encounter and bind to JAM-A within a tight junction. and heavy chains, which are responsible for the millions of distinct binding specificities of antibodies. Differential affinity of the viroid RNA for this factor could determine which of two coinoculated viroids prevails, and the interaction of viroid RNA with the host factor could determine pathogenicity. Its termini consist of 35-nt-long covalently closed hairpin loops flanked by identical 2221-bp inverted repeats. Collectins: proteins that contain C-type lectin domains, which bind to complex carbohydrates on microorganisms. 169 Released virions Plasma membrane Assembly of virions takes place at intracellular membrane structures Once genome replication and transcription have taken place, the action shifts to a different set of intracellular membranes, the endoplasmic reticulum–Golgi intermediate compartment (ERGIC). Virions are then transported inside these vesicles through the cytosol to the lumen of the endoplasmic reticulum, near the nuclear membrane (see Figure 4.5). Since the 3D polymerase begins copying the positive-strand RNA at its 3' end, it is possible that CH011.indd 133 133 the 5' and 3' ends of the genome RNA are brought together at this point. We have learned a great deal about the molecular characteristics of some of these products in recent years. Virions attach to the side of the pilus via their maturation protein (Figure 5.3). Bacteriophages can mutate rapidly because of their rapid growth cycles, and they can readily exchange genes with each other by recombination or by integration into (and excision from) the genomes of their host bacteria. Viroids replicate by a rolling circle replication mechanism that generates linear multimers from a circular RNA template (Figure 31.2). The signal sequence functions to insert the NS1 protein into the endoplasmic reticulum, where it functions in the early steps of RNA synthesis. CI bound to OR2 is physically near PRM and forms favorable contacts with RNA polymerase (Figure 8.5b); these contacts make PRM a much better promoter. A well-studied example is fusion carried out by the HA protein of influenza virus. 112 CH010.indd 112 16/05/11 8:01 PM Cucumber Mosaic Virus 113 Mosaic disease in cucumber plants led to the discovery of cucumber mosaic virus (CMV) The symptoms caused by infection with cucumber mosaic virus and the transmission of this virus among plants were first described separately by Sears Doolittle and Ivan Jagger in 1916. • The mimivirus is the world's largest sequenced virus, with a genome of 1.2 Mb. • Mimivirus extrudes its core into the cytosol via a "stargate" structure in its capsid that opens up within phagosomes in infected amoebae. The genetic potential for reassortment of the vast number of known bunyaviruses should not be underestimated when considering the possibility of new and emerging pathogenic viruses. The D gene, which encodes a head shell stabilization protein, is also thought to have been acquired by a ancestor phage by lateral genetic transfer. Therefore the various signaling pathways that turn on cell cycling, reduce dependence on growth factors, and increase cell growth rate are permanently altered. One such cytotoxic protein is perforin, a poreforming protein with some similarity to complement component 9. After binding to cell receptors, virions enter the cell within an endosomal vesicle. When the concentration of coat protein becomes sufficiently high in the cell, dimers are formed. However, the gene organization and replication strategies of flaviviruses and togaviruses are quite distinct. • Large T antigen binds to Rb protein, releasing it from the transcription factor E2F and allowing transcription of cellular genes that direct DNA synthesis and move the cell into the S phase. Ribosomes translating this mRNA make a protein that shares the same first 79 amino acids with small and middle T antigens, because the 5' splice site lies exactly 79 triplet codons beyond the initiator AUG. Two common defense responses are induced in infected hosts. In fact, these host chaperone proteins were first discovered by isolating E. The double nuclear membrane is shown traversed by nuclear pores. Plant cells are surrounded by a substantial physical barrier, the cell wall, which impedes movement of macromolecules between cells. This makes red blood cells an ideal substrate for assaying viruses. Strain variation and crossing of the species barrier Distinct strains of prions can produce distinct and reproducible patterns of disease, incubation time, and glycosylation and protein cleavage patterns of PrPSc. In some cases, these strains are attributed to different conformational structures of PrPSc. For example, two strains of transmissible mink encephalopathy exist: hyper (HY) and drowsy (DY). Newly synthesized DNA is shown in orange. This tumor appears mainly in immunosuppressed individuals or in people over 65. Thus one would expect the cloverleaf structure to be formed long before the ribosome binding site is made, as addition of a further 20 nucleotides beyond nt 123 could take as long as half a second. Many viral genes are involved in blocking host responses to infection Virus replication in cells cultured in vitro requires a relatively small number of genes—at most 37 out of the 84 identified to date. This activity of Nef requires its interaction with another adaptor protein, AP1. NFB is held in the cytoplasm in an inactive state by association with the inhibitor IB. The full text and figures of the chapter on Human T-cell Leukemia Virus Type I that appeared in the first edition but was not included in the second edition are also available at that site. Modification of cell signaling. In a normal infection the vast majority of progeny virions remain intracellular mature virus. The current adenovirus name and Adenoviruses can be oncogenic, but do not cause cancer in humans Human adenovirus type 12 can induce malignant tumors in hamsters. My job has been to craft the original chapters that I received into what I hope are readable and easily understood units. The infection progressed though an asymptomatic phase lasting approximately 12 days, followed by sudden onset of high fever, splitting headache, and vomiting lasting four days. The best-studied orbivirus is bluetongue virus, an economically significant pathogen of sheep and cows named for a symptom affecting sick animals. 1974 First recombinant proteins expressed in bacteria. Strikingly, when expressed by itself in mammalian cells, VP40 can direct the formation of viruslike particles that bud from the plasma membrane and are released into the medium. Eukaryotic Virus Vectors 456 Rénald Gilbert, NRC Biotechnology Research Institute, Montreal Bernard Massie, NRC Biotechnology Research Institute, Montreal 13/05/11 2:11 PM C O N T E N T S SECTION I: INTRODUCTION TO VIROLOGY 1. Each gene has its own transcriptional promoter. The symmetry of a helical assembly of subunits is defined by two parameters: u, the number of subunits per turn, and p, the displacement along the helix axis between one subunit and the next. This model is based on the structure of the virus genome and on two additional observations: (1) replication appears to initiate within or near the hairpin termini; and (2) head-to-head and tail-to-tail concatemers (oligomers of genome-length units) accumulate during infection. CH013.indd 158 16/05/11 8:04 PM C H A P T E R 1 4 Coronaviruses Mark Denison Michelle M. A structure is set up in which two replication forks progress in opposite directions from the origin (Figure 24.7). Genetic variability generates new virus strains that can cause pandemics

Unlike what is seen with a number of other respiratory viruses, protective immunity after infection with influenza A virus does not last for a lifetime. Thus genes UL1 to UL56, and US1 to US12 (47), are located respectively in the L and S unique segments. A fraction of newly synthesized viral proteins are normally degraded to peptides in proteasomes, especially as a consequence of misfolding. RNA editing generates two forms of hepatitis delta antigen A fraction of hepatitis delta virus antigenome RNAs are edited during replication, resulting in the deamination of a specific adenosine residue (at nucleotide 1014) to an inosine. Are pandemic strains of the always the result of reassortment, or have some been the result of antigenic drift? Which cell–cell interaction is required for generation of antigen-specific T cells, and how does this interaction proceed? Dismantling of the scaffold generally leads to rearrangement of the capsid subunits into a "mature" form of the shell. Because these viruses share the same basic genome structure and replication mechanism, they have been grouped in the order Mononegavirales (for "single [RNA fragment], negative-strand virus"). New rotavirus vaccines show promise in reducing the morbidity and mortality associated with rotavirus infection. Given the simplicity and elegance of this strategy, it is not surprising that the entry of enveloped viruses such as parainfluenzaviruses or togaviruses is often very efficient. Microviruses 69 Bentley Fane, University of Arizona 7. Activation of B cells leads to formation of a germinal center and the production of plasma cells, which secrete antibodies. HPV infection leads to increased cell division and retention of cell nuclei in outer layers. Each cell type in an organism may harbor a different combination of transcription factors, each of which recognizes specific binding sites on DNA. CH005.indd 63 However, activation of the lysis gene is not directly coupled to opening of this hairpin by translation of the overlapping coat gene, as with the replicase gene. Cell-free translation of hepatitis A virus RNA is stimulated by the addition of mouse liver extract but not by other tissue extracts, indicating that tissue-specific factors may regulate viral cell translation KEY TERMS Cis-acting replication elements (cre) Hepatitis Internal ribosome entry site (IRES) Jelly-roll -barrel Myocarditis Poliomyelitis Promoters Receptor-mediated endocytosis FUNDAMENTAL CONCEPTS • Picornaviruses display wide ranges of species and cell susceptibilities for viral infection. The movement protein of tobacco mosaic virus binds to single-stranded RNAs, interacts with host cytoskeleton elements, and targets and dilates plasmodesmata. Acad. Multivesicular bodies: cytoplasmic vacuoles containing many small membrane vesicles and lysosomal degradative enzymes. Other insects, including mosquitoes (Diptera), sawflies (Hymenoptera), and caddis flies (Trichoptera), can be infected by baculoviruses (Table 25.1). Since non-infectious particles contain the viral surface antigens, they can induce an immune response in the infected host. The functional protein can then be produced after transcription and RNA splicing. What are some more examples of these molecules, and how are they recognized? Some three-quarters of the viral genome encodes the viral RNA polymerase. Some DNA viruses have circular DNA genomes; some of these viruses that code for early transcripts, and they are made in large amounts for reasons explained above. Protein evolution RNA viruses oligopeptides RNA-binding proteins RNA polymerase 4. However, the development of chronic hepatitis, which occurs in up to 85% of infected individuals, results in an increased risk of liver cirrhosis and hepatocellular carcinoma. Archaea, the third domain of life By the mid-twentieth century, a widely accepted view of evolution postulated a single evolutionary line leading from prokaryotes to unicellular eukaryotes and then to higher, multicellular eukaryotes. These plasmids are introduced into cells where they are transcribed either by endogenous cellular RNA polymerases or by bacteriophage T7 RNA polymerase, which is provided by specialized cell lines or by a vaccinia virus vector (see Chapters 7 and 26). Snijder, "SARSCoronavirus Replication Is Supported by a Reticulovesicular Network of Modified Endoplasmic Reticulum," PLoS Biology (doi:10.1371/journal.pbio.0060226), September 2008, Volume 6, Issue 9,e226, pp 1957–1974. Such proteins could be expressed in target cells using viral vectors. Structural surface proteins of many viruses bind to carbohydrate residues found in surface glycoproteins and glycolipids that are widely distributed on many cell types. There has been recent success in propagating several human papillomaviruses in specialized raft culture systems, which are capable of maintaining fully differentiated epithelial cells (keratinocytes) over long periods of time. There are twelve known caspases in humans. RNA polymerases that initiate transcription at class III promoters do not encounter strong termination signals before the righthand end of the viral DNA. Although the use of AAV as a gene transfer vehicle was first documented in 1984, its utility as vector for gene therapy applications has only been exploited only since the mid-1990s. The rule of six is explained as follows. Hence, efficient production of lentivirus vectors requires the presence of both Rev and the RRE. Subsequent generations of rabies vaccines produced in suckling mouse brain and duck embryos had progressively lower but dramatically lower incidences of such complications (1/8000 and 1/32,000 respectively). Expression of Vpu results in enhanced release of virus from the cell surface. The positive-sense genome RNAs of picornaviruses range from 7 to 8.5 kb in length (Figure 11.4). (2008) May 13;6(5):e114. 11.1: Courtesy of Ny Carlsberg Glyptotek, Copenhagen; Fig. Initially, protein H and the incoming DNA are associated with the outer cell membrane at the site of cell wall adhesion regions. The adenovirus 19K protein mimics Bcl-2 by binding to Bax and preventing oligomer formation, thereby blocking apoptosis (Table 23.1). Children were intentionally inoculated with fully virulent variola virus at inconspicuous places on the body (e.g., lower back, buttocks) to prevent subsequent, and less controlled, naturally occurring leishmania infections in more visible locations. The direct repeats alternate with non-repetitive spacers, 25–75 bp long. Neuraminidase is the target for currently available anti-influenza chemotherapy. Transcripts initiated at the pregenome promoter have a dual role. Only non-permissive cells can be transformed Transformation of cells by polyomaviruses can take place only if the cells are nonpermissive for viral replication. Whenever necessary, these "receptor-destroying enzymes" release the virions by cleaving the sialic acid residues to which they are bound. Not surprisingly, a variety of viruses make use of cellular transport systems that are normally used for moving membrane vesicles and other large objects within the cell. TATA boxes that direct initiation of transcription at selected promoters are shown. Examples: orthomyxoviruses, paramyxoviruses, coronaviruses, filoviruses, retroviruses. Determined by x-ray crystallography. 328 (2009):89–121. Neuraminidase inhibitors inhibit release and spread of influenza virus The inhibitors of influenza virus neuraminidase are perhaps the best examples of the success of a purely rational approach to drug design. Discuss some of the reasons why broad-spectrum antibiotics have been produced in large numbers, but broad-spectrum antivirals are unlikely. One approach is to CH036.indd 445 445 find a way to assay virus replication that can be automated, using robots, and performed relatively rapidly (i.e., within one to a few days). These MHC II-peptide complexes are recognized by T lymphocytes. Consequently, activation of the E2F transcription factors results in progression of the cell cycle from the G1 phase to the S phase, during which DNA synthesis takes place. For a more complete description of p53 control 400 500 p53 p53 P NLS Transactivation 700 Pol ␣ TBP P J domain 600 C Zn binding DNA binding ATPase DNA helicase Figure 21.10 Functional and protein-binding domains of simian virus 40 large T antigen. Class III promoters have a higher affinity for T7 RNA polymerase than class II promoters due to slight sequence differences, and therefore higher levels of class III transcripts are made. RELATED VIRUSES AND HOSTS The family Bromoviridae consists of five genera: Alfamovirus, Bromovirus, Cucumovirus, Ilarvirus, and Oleaevirus. This reaction is similar to that used to repair gaps in double-stranded cellular DNA. When sufficient levels of class II proteins have been made, the T7 DNA polymerase can displace the RNA polymerase in the AT-rich region shortly after it has begun transcription at one of these promoters. The phage MS2 RNA polymerase initiates RNA synthesis at the second nucleotide from the 3'-terminus. Naïve B cells express two immunoglobulin isotypes on their surface that may serve as antigen receptors. • For the related brome mosaic virus, it has been shown that proteins 1a (capping enzyme) and 2a (RNA polymerase) act together to assemble viral replication complexes bound to cytoplasmic membranes. Both human and mouse interferon-␣ genes lack introns and share a high degree of homology at the nucleotide sequence level, suggesting that the gene cluster was derived from a common ancestor gene by successive duplications. but PrPSc monomers are less stable than PrPC, resulting in little or no accumulation of PrPSc. Aggregation of PrPSc would promote the conversion of PrPC by binding to and stabilizing the otherwise unfavored PrPSc conformation. Interestingly, sequences at both the 3' and 5'-termini of genome RNA are required for synthesis of negative-strand antigenome RNA, implying that the replication complex may be bound to both ends of the RNA. 05/05/11 2:45 PM 476 Glossary Humoral immune response: all aspects of the response to foreign antigens by the adaptive immune system that result in production of antibodies. Most satellite RNAs cause cucumber mosaic virus infection of tomato to be asymptomatic, but the WL2 satellite RNA (a) induces a white chlorosis, and the D satellite RNA (b) induces a systemic necrosis. (b) Capsid of Norwalk virus, a human pathogen with a T 3 structure, with a superimposed surface lattice in black, corresponding to the triangles on (a). 47 A variety of cell surface proteins can serve as specific virus receptors The first step in infection of a target cell is binding of the virion to the cell surface. Demyelination: loss of the myelin sheath covering axons in the central nervous system, leading to dysfunction and loss of muscle control. • Protein 2b, made from subgenomic RNA 4A, suppresses salicylic acid-mediated virus resistance and counteracts host antiviral responses by blocking the systemic signal of host RNA silencing. 67, 3481–3488 (1993). The glucosebinding site was identified by accident. This presumably occurs because the PB1 subunit has been displaced and is no longer bound to the 5' end consensus sequence on the RNA genome. Paramyxoviruses and Rhabdoviruses 175 The mononegaviruses: a group of related negative-strand RNA viruses 176 Rabies is a fatal human encephalitis caused by a rhabdovirus 176 Measles is a serious childhood disease caused by a paramyxovirus 176 Other paramyxoviruses cause several important human diseases 177 Paramyxovirus and rhabdovirus virions have distinct morphologies 177 Viral envelope proteins are responsible for receptor binding and fusion with cellular membranes 177 Genome RNA is contained within helical nucleocapsids 178 Paramyxoviruses enter the cell by fusion with the plasma membrane at neutral pH 178 Gene order is conserved among different paramyxoviruses and rhabdoviruses 180 Viral messenger RNAs are synthesized by an RNA polymerase that initiates transcription exclusively at the 3' end of the viral genome 181 The promoter for plus-strand RNA synthesis consists of two sequence elements separated by one turn of the ribonucleoprotein helix 181 mRNAs are synthesized sequentially from the 3' to the 5' end of the genome RNA 183 The P/C/V gene codes for several proteins by using alternative translational starts and by mRNA "editing" 184 Functions of P, C, and V proteins 184 N protein levels control the switch from transcription to genome replication 185 Virions are assembled at the plasma membrane 186 16. To reduce immune rejection, adenovirus vectors were further crippled by deletion of genes in the E2 or E4 regions (Figure 37.3). The ultimate "objective" of the virus is not to induce malignant transformation, but to replicate by completing a productive growth cycle. However, its particles exhibit greater heterogeneity than those of the alphaviruses. Furthermore, like all viruses, it uncoats and replicates its genome within a host cell, then packages it within a virion. The best-characterized action of interferons on B-cell activation is their ability to influence immunoglobulin (Ig) isotype switching. These viruses may have spread as a result of clearing forests for lumbering and farming; they apparently were transmitted from infected wild fruit bats to domestic livestock and then to humans. Selective encapsidation of full-length viral RNA is achieved by positioning the psi sequence downstream of the 5' splice site, so that only unspliced viral RNA contains the packaging signal. They have been marketed aggressively to patients, using, for example, actors from sitcoms to play the role of influenza virus. The strong p4 promoter contains binding sites for cellular transcription factors E2F, Ets, Sp1, and TATA-binding protein TBP (Figure 20.5). However, when used alone its benefits were rather limited, and, like all anti-HIV drugs, it is now used in combination with other drugs. Ribonuclease L cleaves mRNAs and is activated by oligonucleotides generated by another protein induced by interferon, 2', 5'-oligo(A) synthetase, which is also activated by double-stranded RNA. Viruses that express HE can also bind to cells by interacting with sialic acid, found on a variety of cell membrane glycoproteins and glycolipids. 24.23, p. Interestingly, human MxA, which is found in the cytoplasm, has antiviral activity against a range of viruses, regardless of the compartment where the virus replicates. Hypersensitive necrosis: a localized area of tissue death on a plant leaf that is induced in response to a pathogen, and is associated with limited spread. Joining of the adjacent 5' CH026.indd 319 and 3' ends by DNA ligase would then produce mature poxvirus DNA molecules. Besides hairpin sequences at the ends of the DNA, the mechanism requires a site-specific endonuclease that cuts DNA within the hairpin—and both are present in parvoviruses. The proviral DNA is therefore able to integrate into the cellular genome in the referred nucleus. Activation of cdc2 leads to increased expression of a subset of 2 genes, but the exact mechanism is not understood. Acheson November 2011 Designed for students learning about viruses for the first time at the undergraduate or graduate level, Fundamentals of Molecular Virology is presented in a style which relates to today's students and professors. What are these sensors called and what types of molecules do they "see"? When damaged nucleotides such as pyrimidine dimers are removed by the cell's excision repair system, a set of bacterial genes is induced whose products are involved in helping the cell to survive irradiation; these genes are called the SOS genes. U C A. The stable integration of the viral DNA also renders "curing" of a retrovirus infection difficult, requiring the destruction of all cells containing proviral DNA, whether or not it is actively expressed. • Most viruses that infect archaea have double-stranded DNA genomes, and some are positively supercoiled, probably an adaptation to extreme environments. The role of many envelope proteins is twofold. Polymerase binds to 5' ε stem-loop on pg RNA ε ε Cap 1 2 2. Cucumber mosaic virus supports replication of defective and satellite RNAs Satellite RNAs are small, parasitic RNAs that are completely dependent on a helper virus for replication, encapsidation and transmission (see Chapter 31). 2.6a: VMD image courtesy of Dr. Jean-Yves Sgro, University of WisconsinMadison; Fig. Members of the Pestivirus genus cause economically important disease in cattle, pigs, and sheep. The modified residue forms a Watson–Crick base pair with cytosine rather than uridine during subsequent replication, changing the original adenosine to a guanosine. For example, see Figure 1.3. Hemagglutination is a convenient and rapid assay for many viruses A number of animal viruses bind to sialic acid residues or other carbohydrates on cell surface proteins and lipids. Using ϕX174, Arthur Kornberg and coworkers elucidated many of the fundamental mechanisms of DNA replication. In the context of viral illness, tolerance can occur when infection occurs at a very young age or can be induced as a part of the biology of the infecting virus (HIV or human papillomaviruses). The same viral proteins that serve as fusion agents in the virion envelope are responsible for cell– cell fusion when they are incorporated into the plasma membrane of the infected cell. MA encodes a classical nuclear import signal that interacts with the importin family of proteins. DISASSEMBLY OF VIRIONS: THE DELIVERY OF VIRAL GENOMES TO THE HOST CELL Virions are primed to enter cells and release their genome Virus particles are more than passive packages that simply enclose the viral genome. Binding leads to dimerization of receptors, phosphorylation, and activation of Jak kinases. However, in this book, virus family names are usually referred to in their English version for sake of simplicity (e.g., "picornaviruses" instead of "Picornaviridae"). A typical virus vector is unable to replicate because of deletion of some or all essential viral genes (Figure 37.1). Picornavirus virions bind to cellular receptors via depressions or loop regions on their surface Picornaviruses have a non-enveloped, icosahedral capsid that encloses a single-stranded, positive-sense genome RNA. Carrascosa, Mariano Esteban, and Dolores Rodriguez, "Vaccinia virus 15-kilodalton (A14L) protein is essential for assembly and attachment of viral crescents to virosomes," Journal of Virology (1998), vol 72 (2):1287–96. Six of these mRNAs are exported directly to the cytoplasm where they are translated by ribosomes (step 3, Figure 18.6). Most proteins are packaged in virion: • Nucleocapsid protein (NP) • RNA polymerase cofactor (VP35) • Matrix protein (VP40) • Envelope glycoprotein (GP, cleaved into GP¹ and GP²) • Minor nucleocapsid protein (VP30) • Membrane protein (VP24) • RNA polymerase (L) Ebola makes additional secreted glycoproteins (sGP, ␣-peptide). Large-scale trials have shown strong antibody production in vaccinated individuals and nearly 100% protection against infection by targeted viruses. The observation of head-to-head and tail-to-tail linked replicative intermediates suggests a 16/05/11 8:01 PM 106 CHAPTER 9 Viruses of Archaea (b) (a) Tail fiber CP Genomic DNA (c) Figure 9.9 Sulfolobus islandicus rod-shaped virus 1 (SIRV-1), family Rudiviridae. Early during lytic growth, either during an infection or induction of a lysogen, Cro binds The Cro repressor suppresses CI synthesis and regulates early gene transcription Prophage DNA within a lysogen can be induced, or derepressed, so that lytic growth ensues. Self-assembly: ability of a structure such as a viral capsid to assemble spontaneously from its protein subunits, without the aid of other machinery. Although usually not severe, this disease may lead to serious birth defects if a fetus is infected during pregnancy. Cellular RNA polymerase II then transcribes this template starting from the leftmost promoter (p4 in minute virus of mice, or p5 in adeno-associated virus, see Figure 20.2). number of the possible variants of the virus, something that the virus can easily overcome. In contrast, infection of the genital tract with non-oncogenic human papillomavirus types 6 and 11 can produce cervical warts (also known as condylomas), which are benign and only rarely progress to cancer. E7, E1A, and large T antigens have been shown to interact with many of the same cellular targets, and these interactions result in deregulation of the normal cell cycle, resulting in the induction of cellular DNA synthesis (Figure 22.4). The enzymes necessary for reverse transcription and integration are produced by the packaging cell line and are incorporated along with the RNA into virions. The packaging signal initiates encapsidation and ensures that genome RNA is efficiently packaged, excluding subgenomic RNA, antigenome RNA, and unrelated cellular RNAs from incorporation into nucleocapsids. The crystal structure of these virions was resolved for AFV-1. coli DNA bending protein, IHF, which bends the arms to allow int to make bridging contacts with the core sequences. However, expression persists for only a few weeks in immunocompetent animals, because the host immune system destroys transduced cells expressing viral proteins. The tripartite leader is formed by splicing 3 small exons; it is spliced to the mRNA body at one of several alternative 3' splice sites. Chaperones have since been shown to play a major role in the proper folding of many proteins and in the assembly of multi-subunit protein complexes. Bound transcription factors facilitate the association with viral DNA of the multiple cellular proteins required for transcription. PCBP2 was previously mentioned as taking part in recognition of the IRES by 40S ribosomes, and therefore it has a role in both translation and replication of viral RNA. DISTINCTIVE CHARACTERISTICS Unusual partly single-stranded, partly double-stranded circular genome results from incomplete genome replication in cell. M1 seems to interact directly with the cytoplasmic tail of M2 to promote recruitment of internal viral proteins and viral nucleocapsids to the plasma membrane, thus facilitating efficient assembly and budding of infectious virions. Studies of translational control have identified sequences in the 3' nontranslated region of the S4 mRNA that interact with cellular proteins and affect translation efficiency. High concentrations of noninfectious hepatitis B virus particles can be found in the serum of patients; these were once used as a source of material for preparation of a vaccine. As a result, the viral HA2 protein is now attached both to the viral membrane (via the transmembrane sequence) and to the target membrane (via the fusion peptide). Viral proteins that bind to dsRNA reduce activation of PKR. The use of such primary cell cultures, and the subsequent establishment of immortalized cell lines such as HeLa, dramatically accelerated the development of antiviral vaccines in the late 1950s and early 1960s. Hemagglutinin-esterase is not present in all coronaviruses. Release can happen by disintegration of the vesicle membrane or by fusion of the viral envelope with the vesicle membrane. Transformed cells derived from cervical carcinomas can be propagated in vitro. There proviruses profit from the presence of the cellular machinery for transcription, RNA processing, RNA export, and DNA replication. cit.; Fig. Assembly of the nucleocapsid takes place at the cytoplasmic face of membranes with which prM and E transmembrane proteins are associated. Innate and Adaptive Immune Responses to Virus Infection 415 The host immune response is mediated by circulating specialized cell types 416 Innate immune responses are rapid but non-specific; adaptive immune responses are slower but long-lasting and highly specific 416 THE INNATE IMMUNE RESPONSE 416 Complement proteins mark invading pathogens for destruction 416 The inflammatory response is mediated by cytokines and migrating leukocytes 417 Macrophages localized in tissues are activated by infection and kill viruses or infected cells using toxic oxygen compounds 418 Natural killer cells recognize virus-infected cells and kill them via apoptosis pathways 418 TOC.indd xvii THE ADAPTIVE IMMUNE RESPONSE 419 Primary and secondary organs of the immune system harbor B and T lymphocytes 419 B and T cells have specific surface receptors that recognize antigens 420 T lymphocytes respond to peptides on the surface of antigen-presenting cells 420 B lymphocytes respond to antigens and are stimulated to differentiate to plasma cells by interaction with Th2 cells 420 Antibodies come in a variety of forms 420 The enormous diversity of antibody specificities 421 Cytotoxic T cells are generated upon interaction of Tc cells with MCH I-bound peptides 422 EFFECTS OF INTERFERONS ON THE IMMUNE RESPONSE 422 Interferons and the development of CD4-positive helper T cells 423 The role of interferon in macrophage activation and cellular immunity 423 Effects of interferons on antibody production 423 VIRUS STRATEGIES TO COUNTER HOST DEFENSES 423 Viruses make proteins that mimic cytokines and cytokine receptors and interfere with host defenses 423 Viruses evade innate immune responses 424 Viruses evade adaptive immune responses 424 SECTION X: ANTIVIRAL AGENTS AND VIRUS VECTORS 35. Seen in this light, external capsids are simply a more elaborate and structured version of the envelope glycoproteins that coat the membrane of many viruses. This assay is then used to screen large libraries of chemicals (hundreds of thousands of compounds) for their ability to inhibit virus replication in cultured cells. The scheme has several advantages as an expression system: (1) recombinant vaccinia viruses are relatively easy to prepare by homologous recombination between an infecting vaccinia virus genome and a transfected DNA that contains the foreign gene embedded within a nonessential virus gene; (2) the entire process takes place in the cytoplasm, obviating the need for RNA splicing or nuclear-cytoplasmic inactivates two components of the complement system, C3B and C4B, which are critical to both the innate and adaptive complement pathways. 16/05/11 7:49 PM Capsids with Icosahedral Symmetry 23 A C B (a) (b) Figure 2.5 More complex capsid protein arrangements. The study of viral and cellular oncogenes has led to major advances in the detection and treatment of cancer. • Because they are dangerous pathogens, research on infectious filoviruses is restricted to laboratories with high biological containment. • The protein subunits in both the nucleocapsid and the envelope of togaviruses are arranged with icosahedral symmetry. With further development, use of such viruses as anticancer agents in humans can be envisioned. The E protein has a very different fate and function. (Plural: genera.) Glycolipid: lipid molecule with a polar head group that contains carbohydrate. This negative-strand RNA is used as a template for the synthesis of additional positive-strand RNAs via multistranded replicative intermediate structures (diagrammed in Figure 11.7). Polyomavirus particles are assembled in the cell nucleus and are not efficiently released from the cell until after cell lysis. Genes are expressed in four temporal classes: • • • • Immediate early: activation of early genes Early: DNA replication, activation of late genes (viral RNA polymerase) Late: assembly of budded virions (capsid and envelope proteins) Very late: assembly of occluded virions (polyhedrin) VIRUSES AND HOSTS Four genera: • Alphabaculovirus: infect lepidopterans (moths and butterflies), enclose virions in polyhedra Insect viruses were first discovered as pathogens of silkworms Throughout the ages, economic pressures have helped to establish the direction of basic research. Virions can be either roughly spherical or filamentous (Figure 18.2). Reoviruses 225 Reoviruses were the first double-stranded RNA viruses discovered 225 Some members of the Reoviridae are important pathogens 226 Reoviridae have segmented genomes made of double-stranded RNA 226 Reovirus virions contain concentric layers of capsid proteins 227 The attachment protein binds to one or two cellular receptors 228 During entry, the outer capsid is stripped from virions and the core is released into the cytoplasm 229 Enzymes in the viral core synthesize and cap messenger RNAs 230 Translation of reovirus mRNAs is regulated 231 Interferon and PKR: effects on viral and cellular protein synthesis 231 Synthesis of progeny double-stranded genomes occurs within subviral particles 232 13/05/11 2:11 PM Contents Reoviruses induce apoptosis via activation of innate immune response transcription factors NF-κB and IRF-3 233 Studies of reovirus pathogenesis in mice 234 SECTION V: SMALL DNA VIRUSES OF EUKARYOTES 20. 1 in: Daniel Kolakofsky, Laurent Roux, Dominique Garcin, and Rob W. Two major E1A mRNAs, denoted 13S and 12S (for their slightly different sedimentation rates in sucrose gradients), encode proteins of 289 and 243 amino acid residues, respectively (289R and 243R; Figure 23.5). The virion (or the viral genome) enters the cell 14 3. The second most abundant membrane protein produced by the middle hepatitis B surface protein (MS, or MHBs). Have limited capacity for insertion of large foreign genes. Naming of plant viruses usually makes reference to the plant species in which the virus was first isolated and to the disease symptoms caused by the virus (e.g., tobacco mosaic virus, tomato bushy stunt virus, maize streak virus). Cooperative interactions of CI dimers at OL3 and OR3 are required for PRM repression; these dimers are shown in light green. Cryoelectron microscopy: a method for visualizing biological structures, including viruses, at high resolution (to about 1 nm) by suspending unstained objects in a thin sheet of noncrystalline ice at liquid nitrogen temperatures. The four partially overlapping open reading frames (C, P, S, and X) are shown with arrowheads pointing in the direction of transcription and translation. Small and efficient proteins Many enzymes encoded by PBCV-1 are either the smallest or among the smallest known proteins encoded by phycodnaviruses. UL42, a viral protein that is bound to the DNA polymerase, increases the processivity of the polymerase, allowing it to extend growing DNA strands over considerable distances without dissociating. This disease was called kuru ("shivering" or "trembling" in the Fore language). The most direct means of preventing virus entry is to intercept the virus with neutralizing antibodies before it reaches the cell. These complexes include not only the RNA-dependent RNA polymerase NS5 and other viral proteins, but presumably also host proteins recruited for the purpose of genome replication. It has been calculated that free diffusion of particles the size of herpesvirus capsids within the cytoplasm over a distance of 10 micrometers would take 2 hours. These features make them good inhibitors. Primary Multiple Mechanisms Viral Proteins Cytoplasm miRNA precursor exportin-5 Other sources of dsRNA 413 Dicer cleaves double-stranded RNAs resulting from virus infection into small interfering RNAs (siRNAs) that are 21 to 23 nucleotides in length, and have 2- to 3-nt 3ʹ overhangs at both ends. Disassembly (cysteine proteases) 4. The virion contains at least eleven proteins with molecular masses in the range of 12–90 kDa, and modified host lipids. The protease then cleaves the Gag and Gag/Pol polyproteins into the individual structural (MA, CA, and NC) and enzymatic (PR, RT, and IN) proteins, and they rearrange to form the electron-dense core characteristic of mature virions. Hepadnaviruses 365 Christopher Richardson, Dalhousie University SECTION VIII: VIROIDS AND PRIONS 31. The 3' leader contains the promoter for the 16/05/11 8:05 PM Filoviruses Leader 191 Trailer NP VP35 VP40 sGP/GP VP30 VP24 L 3' 5' end CUACUCUAAUUCUUUU start GP mRNA 5' Cap ppl(A) 3' VP30 m RNA Figure 16.3 Map of Ebola virus genome. This strongly resembles the mechanisms by which cellular RNAs are expressed, modified, and transported from the cell nucleus to the cytoplasm. (2009) Apr 28;7(4):e92.PMID: 19402750; Fig. Genome sequences from some current Ebola virus strains confirm the antigenic shift hypothesis The H1N1 subtype that emerged in 1918 was still present in 1933, when the first human influenza virus was isolated in the laboratory, and continued to circulate CH018.indd 221 221 among humans into the 1950s. Epidemics are larger than "outbreaks" (which are more geographically limited) and smaller than "pandemics." Epitopes: short regions on antigens such as proteins (usually 10 amino acids or less) or carbohydrates, to which specific antibodies bind. The hepatitis B virus DNA genome was eventually cloned and sequenced by Pierre Tiollais, Patrick Charny, Pablo Valenzuela, and William Rutter in 1979. When a MAP kinase is phosphorylated at specific serine and threonine residues (by MAP kinase kinase), it can in turn activate the nuclear transcription factor AP-1, which activates transcription of the cyclin D1 gene among others (Figure 21.8). Large DNA viruses could have evolved from cellular forms that became obligatory intracellular parasites An alternative origin has been suggested for some of the largest DNA viruses. Hemorrhagic manifestations develop during the peak of the illness, and can predict the likely progress of the disease. These cell systems have helped to elucidate much of the hepadnavirus life cycle. CH033.indd 412 Some viruses evade apoptosis or the inflammatory response by synthesizing caspase inhibitors Many viruses make caspase inhibitors. Once this happens, other ribosomes can initiate protein synthesis at the start codon that is placed adjacent to the 3' end of the coat and initiate Lysis hairpin 1308 1335 MS2 1724 1761 1678 AA G A 1678 A A Replicase maturation (A) coat U U Lysis C G lysis start C G U A C U A G C G C (part shown) A C A C G G Coat termination Operator U A A U hairpin A U U A C C UU C A 1727 G C A A A U G C G C U A A U G C C G A C A U G C 1761 A C U A • U A U G C G Replicase A U G C G C start G C U A C G U A MJ U A U AA AC C G A U C A UC GC C A U U C A G A G U C G C GC GAAGA CAA 57 A C G C C G AU GCGUC U G C C U A G C G A G A 70 1902 62 GGUA AGU C UU C U GUU AC U A A C G 1427 C 1807 U 3' C U 43 1335 G U A 24 R32 Coat start Figure 5.5 Translational control by secondary structure. CH015.indd 186 16/05/11 8:05 PM Paramyxoviruses and Rhabdoviruses 187 • Both virus families transcribe their genomes in a sequential manner; the viral RNA polymerase initiates transcription exclusively at the 3' end of the genome and travels along the genome, terminating and releasing individual mRNAs and then reinitiating transcription of the subsequent mRNA, until it reaches the 5' end of the genome. 449–461; Fig. The λ lytic transcription program is controlled by termination and antitermination of RNA synthesis at specific sites on the genome Transcripts from PL and PR express the N and cro genes; these "immediate early" transcripts end at transcription terminators tL1 and tR1 (Figure 8.4a). After the production of poxvirus early mRNAs, the poxvirus core membrane is broken down, allowing viral DNA replication to be directed by early viral proteins. The RNA helicase domain includes two conserved motifs, one for binding of ribonucleoside triphosphates and one for binding to RNA, both of which are essential for viral replication. Archaeal initiation factors for translation are more complex than in bacteria, and archaea initiate protein synthesis with methionine, as do eukaryotes, not N-formyl methionine, used by bacteria. Tail Host cell lysis Virion Figure 8.9 DNA packaging and virion assembly. Virions bind to receptors on the cell surface Viruses must first recognize and bind to cells that they infect. Objects with icosahedral symmetry have 30 twofold rotation axes. J binds to an E. Viral functions that take place in different layers are shown at right. (b) Beginning at the left end, features are: methylated cap; repeat region (R); untranslated 5' sequence (U5); primer binding sequence (PBS); 5' splice site (5' ss); psi (ψ) packaging sequence; gag, pol, and env reading frames for viral structural genes; 3' splice site (3' ss); polypurine tract (ppt) used during reverse transcription; untranslated 3' sequence (U3); repeat region (R), poly(A) tail. Inhibitors of influenza virus neuraminidase are being used as drugs against flu infections. Most RNA viruses encode their own RNA-dependent RNA polymerases to carry out both these functions. The three host cell proteins are crucial factors in the cellular translational apparatus. In normal cell cycle (top half), CDK/cyclin complex phosphorylates Rb, releasing it from E2F and leading to expression of cell-cycle genes. While interferon- can be induced without IRF-7 by the constitutively expressed IRF-3 (along with NF-B and ATF-2/ cJun), interferon-␣ induction requires IRF-7. These algae have a global distribution and they are often associated with large-scale blooms. Various genetic engineering tricks are used to produce RNAs with precise 3' ends, including the use of T7 transcriptional terminator signals and self-cleaving ribozymes (see Chapter 31). ISVPs interact with endosomal membranes, leading to delivery of transcriptionally active core particles into the cytoplasm. For chapters that cover virus families, these outlines are "thumbnail sketches" that contain some basic information about virion structure, genome organization, replication strategies, diseases caused, and distinctive characteristics shared by viruses in that family. Blue arrows in the periphery show the location and orientation of viral coding regions. Precursor to capsid protein; signal sequence inserted in membrane is cleaved by viral protease. But the proteins the host cell machinery that cannot function. Rb was first identified as a gene that is deleted or mutated in human retinoblastomas and other cancers. Both of these pathways have many components, and only a simplified outline is shown in Figure 33.4. The extrinsic pathway can begin with the cytokine tumor necrosis factor ␣ (TNF␣), which binds to its receptor on the cell surface. A rare event can lead to its misfolding into a beta-helix configuration, resistant to protease digestion. 9.6a,b: Courtesy of Wolfram Zillig; Fig. 16/05/11 8:11 PM Adenoviruses TP 5' 3' C G G C 3' 5' G C 5' 1 ssDBP C C G pTP DNA pol 2 C C G G C 3' G C 3' 5' 5' 3 C G ȴ G C 4 281 The major late promoter is activated after DNA replication begins More than half of the adenovirus genome is dedicated to coding for at least 18 different late mRNAs. These mRNAs encode structural proteins, scaffolding proteins, a protease, and other products required for packaging of progeny virus. However, the small genome size was a handicap, because it limits the size of foreign DNA that can be packaged in the virion. 16/05/11 8:00 PM Viruses of Archaea (A1) (B1) 107 (C1) (C2) (A2) (B2) Figure 9.10 Changes in Sulfolobus host cells following infection with SIRV-2. Large T antigen molecules bind cooperatively to these sequences and assemble at a specific site denoted the DNA replication origin (Ori) to form two circular hexamers that enclose the DNA like a wheel on an axle. The most efficient strategy to eliminate the helper virus is to remove the packaging signal on the helper virus genome made during propagation of the vector. CH026.indd 321 Wrapped virus is transported to the cytoplasmic side of the plasma membrane, where the outermost membrane of the wrapped virus fuses with the plasma membrane. A vaccine based on the L2 protein will soon be tested. Baculoviruses are insect viruses that are used for insect control, expression of useful eukaryotic proteins, and production of vaccines. A protective vaccine (see text box) would be extremely valuable for at-risk medical personnel in Africa and researchers working with infectious filoviruses. Although VP30 is present in nucleocapsids, it is not known if VP30 directly binds to this stem-loop, either in the genome RNA or in the growing mRNA chain. Although this approach has met with great success, the emergence of viruses resistant to combinations of three drugs suggests that even this barrier may not be sufficient to control the infection over the long term. Replicase particles can synthesize full-length, double-stranded RNAs by copying the packaged plusstrand mRNAs This type of viral RNA replication is referred to as "plasmid replication". What is structurally unusual about the E protein that contributes to the smooth surface of tick-borne encephalitis virus? Hepatitis A virus is now known to be a member of the Picornaviridae (Chapter 11). As they evolved, they would have undergone selection for the production of a stronger capsid that could better package and protect the genome DNA. Virus particles can be seen and counted by electron microscopy A variety of staining or shadowing methods can be used to detect virus particles by electron microscopy. The viral replicase complex plays a role in selective packaging of viral RNAs and maintaining uniform-sized viral particles. Genome replication begins when newly synthesized NP protein enters the nucleus Viral proteins that contain nuclear localization signals, including NP and the RNA polymerase subunits, are transported from their site of synthesis in the cytoplasm back into the nucleus (step 4, Figure 18.6). P35 has only been found in AcMNPV and its close relatives, whereas iap genes are apparently present in most baculoviruses and they are often present as multiple copies. Another possibility is that the presence of a rigid cell wall would seem to rule out budding through the cytoplasmic membrane as a mechanism of formation of these internal envelopes. Thus, it could potentially serve as a target for antiviral drug intervention for the alphaviruses. CH007.indd 79 79 these proteins, gp16, is able to enzymatically degrade the peptidoglycan layer as it bores through, creating a hole for the passage of the DNA. pg RNA also serves as a template for virus replication. DNA replication starts at a unique internal origin and is primed by T7 RNA polymerase T7 DNA is replicated bidirectionally starting at an origin about 5.9 kb from the left end of the DNA. The RNA replicase of Q can be easily purified, while the replicases of leviviruses are much more difficult to work with, and so most of the work on RNA replication has been done with Q. Mature virions are formed within virus "factories" Assembly of vaccinia virions is a unique and complex process that provides fascinating insights into intracellular protein trafficking and organelle biogenesis. Sequence changes in the pathogenicity (P) domain can affect infectivity and the severity of symptoms. If all protein subunits on the surface of a capsid are identical, then the subunits will be identical (or nearly so), generating a symmetrical object. It does not appear to be a transmembrane protein, but is located on the cytoplasmic face of the plasma membrane, or the inside of the virus envelope. Once HIV-1 mRNAs are in the cytoplasm, Rev can remain bound and can enhance their translation by a mechanism that is not yet understood. These RNA polymerases can be distinguished based on their differential susceptibility to α-amanitin, a specific inhibitor of cellular RNA polymerase II; α-amanitin inhibits early transcription but not late or very late transcription. (j) Human immunodeficiency virus (a retrovirus). Emerging paramyxoviruses Hendra and Nipah cause respiratory and neurological disease in pigs, humans, horses, and bats. BSE has since apparently itself crossed the species barrier and has infected humans. pe38 ie-2 ie- K alk ie-1 6 94 Baculoviruses have large, circular DNA genomes and encode many proteins ptp bro ctx polh orf 7 orf16 29 pk1 56 odv-e 27 -ec 8 odv-ec 4 ko d 49 0 ie- 0 1 3 cg1 twn fgf 69 odv- n p 80 p pr gp1 6 le-f5 ptp ode v-e 56 p3 tp 7 n vv1 i e-1 -ec m r t l f 3 a 10 p9 0 p 40 p6 p48 p1 ape 25 p4 7 7 v lef 16 ie-14 m t g i l 0 pe38 ptp o ike -ph 11 le f 12 orf 10 orf 11 o p3 nd s 13 o 2 1 gta H hr2 hr4c1 J E AcMNPV (133,894 bp) hr4b5 hr4b4 hr3 hr4b21 L hr4b 2 hr4aa1 O hr4 5 he6 0 vp8 5 p4 2 p140 p ,9 p6 f-5 le 8k 3 305 lef-11 K T M odv-e6 6 pcna lef8 N D G lef -10 C 2 le 5K f-9 hr3 3 lef- l po a dn pl 4 3 F p95 lef vp3 -4 cg9 9 0 p15 pnk/ pnl od ve 25 slp 6 6 orf 1 hcfiap2 vlf-1 gp41 Figure 25.3 Genetic map of Autographa californica MNPV. The viruses thus have a life cycle similar to that of temperate bacteriophages such as lambda (Chapter 8), with the important difference that they do not kill the host cell upon induction, but rather produce virions and then revert to a non-productive state. DNA is inserted into preassembled heads (λ involves chaperones and scaffolding proteins A large number of proteins are involved in the construction of virions; some are incorporated into the head or tail structures in virions, some are cleaved during incorporation, and some are used as scaffolding proteins but are not incorporated into the mature virus particle. These are termed latency I, II, and III (Figure 24.11). 16/05/11 8:05 PM Filoviruses KEY TERMS Antibody Asialoglycoprotein receptor Chloramphenicol acetyltransferase Clathrin-coated pits Cytokines DC-SIGN Disseminated intravascular coagulation Ectodomain Endemic Endosome Folate receptor-␣ Furin 199 FUNDAMENTAL CONCEPTS Fusion peptide Green fluorescent protein Hemorrhagic Inclusion bodies Integrins Interferon Macrophage Multivesicular bodies Pseudotype Reservoir Ribozyme Shock Ubiquitin ligase Ubiquitination Zoonotic disease • Marburg and Ebola viruses are members of the family Filoviridae, and are related to paramyxoviruses and rhabdoviruses. Although both these viruses infect humans via the oral mucosa, they have very different lifestyles and cause distinct diseases. Two dimers of a heavy chain and a light chain, joined by a disulfide bond, are themselves joined by disulfide bonds to form an IgG molecule. What are these proteins and how do they induce apoptosis? They are vehicles CH002.indd 29 29 evolved to deliver their cargo (the viral genome) to a host cell according to a programmed set of instructions (see Chapter 4, Virus Entry). Caspases: cysteine-aspartic proteases that are activated during the events leading to apoptosis. However, the situation is more complex, and expression of disease symptoms is now thought to be controlled by discrete determinants located within different domains. Three tandem stop codons are used to ensure termination of translation. Mixed infection with different virus strains produces a collection of progeny reassortant viruses in which the inheritance of specific parental gene segments can be deduced (Figure 19.2). The endolysin, once in the periplasmic space, degrades the cell wall, leading to cell lysis. Some paramyxoviruses "edit" the P mRNA region at a specific site, generating mRNAs with distinct protein-coding capacities. Non structural proteins 5' Cap nsP1 nsP2 nsP3 151 nsP4 Structural proteins c PE2 6K E1 An 3' Figure 13.2 Alphavirus genome RNA. Stated another way, CI activates its own gene to ensure that sufficient repressor is produced to maintain prophage repression. 26.1a,b: Reprinted from Moss, B., et al., in Mechanics of DNA Replication and Recombination, Cozzarelli, N. 16/05/11 8:04 PM 170 CHAPTER 14 Coronaviruses The capacity of coronaviruses to undergo RNARNA recombination, coupled with the high theoretical error rate of the viral RNA polymerase (1 mutation in every 10,000 nucleotides), generates coronavirus genomes with changes that may be selectively advantageous in new environments or hosts. Neurological symptoms correlate with virus replication in the central nervous system. 27.15: Courtesy of Jean-Michel Claverie, reprinted from Figure 4 (p. Summers, Yale University 8. This suggests that the H protein may stimulate stage I DNA synthesis (see page 72) by piloting the penetrating DNA to the proper site of replication. This last situation resulted in the reintroduction of polio into many countries and has likely set back polio eradication efforts by many years. Six early and two delayed early transcription units: E1A, E1B, E2A, E2B, E3, E4; IVa2, IX. • Filoviruses cause sporadic outbreaks of severe hemorrhagic fever with high case fatality rates in Central Africa. Virus then enters minor veins in the 16/05/11 8:01 PM Cucumber Mosaic Virus Cell wall C C V Vacuole Vacuole V Plasmodesma Figure 10.7 Diagram of a plasmodesma. Bunyaviruses 200 Richard M. The two DNA strands are locally separated, and the separated strands are extruded through channels within the hexamer. Glycosaminoglycan: polysaccharides formed from repeating units made up of two different 6-carbon sugars, one of which is an amino sugar, usually N-acetyl glucosamine or N-acetyl galactosamine. Compounds that block virus attachment, uptake, uncoating, DNA or RNA replication, assembly, and release are now available for use against specific viruses. These changes are subtle, yet their effects are significant, and virologists are trying to understand what steps in infection or in the response to infection are altered by these mutations. Typically containing just a single mitochondrion, chloroplast, and Golgi body, this tiny marine alga has a global distribution and is found at a wide range of ocean depths. Three enzymatic activities are needed for viroid replication Rolling circle replication as depicted in Figure 31.2 requires three distinct enzymatic activities for completion: (1) an RNA-dependent RNA polymerase, to generate the multimeric linear strands; (2) an RNA-cleaving activity (endoribonuclease), to process them to unit-length linear molecules; and (3) an RNA ligase, to circularize the linear monomers. Much of the information in this chapter is derived from the ICTV's 8th Report (2005) and recent updates (see index.asp). Toll-like receptors: a set of cell-surface and endosomal proteins that recognize molecules specific to invading pathogens (e.g., lipopolysaccharide, CpG-containing DNA sequences, double-stranded RNA) and activate innate and adaptive immune responses via a protein kinase cascade. Although most bunyaviruses mature by this pathway, Rift Valley fever phlebovirus has been observed to bud directly at the surface of infected rat hepatocytes. Although this turned out to be one of the best viral vaccines known, yellow fever continues to inflict misery and death in unvaccinated populations in South America and Africa. The polypeptide chain is folded into a series of strands that interact with each other to form a rigid blocklike structure. The copying of ideas from one person to another is imperfect, just as the copying of genes from parent to child is sometimes inaccurate. Note that the loss of viral sequences at the ends of viral DNA has no effect on progeny virus because these ends are not used in the synthesis of viral RNA (see below). Most capsids are constructed as either: • Closed spherical shells with icosahedral symmetry or Elongated tubes with helical symmetry. Viruses are often adapted to replicate well only in their natural host; they can infect other species, but replicate poorly and have limited or no pathogenic effects. Apoptosis: the process of programmed cell death. Hepatitis delta virus may use two different cellular RNA polymerases to replicate The genome of hepatitis delta virus (Figure 31.4) is a 1682-nt single-stranded circular RNA molecule that can be folded into a rod-like structure by virtue of extensive internal base pairing. (a) Three (asymmetrical) commas, arranged with threefold symmetry. NTP nucleoside triphosphate. Thus the mature virus particle may provide for stability in the environment while extracellular virus allows efficient spread in the body. IgG is involved in agglutination of cellular antigens, neutralization of toxins and viruses, and binding to bacteria and virus particles, leading to their uptake and destruction by phagocytosis. The reovirus disassembly intermediates can be visualized using the technique of cryoelectron microscopy and three-dimensional image reconstruction (Figure 19.5). Viral fusion proteins are generally type I transmembrane proteins most of whose mass lies external to the viral membrane, and they are usually organized as oligomers. 9.3: Figure 9 in Sabrina Fröls, Paul M.K. Gordon, Mayi Arcellana Panlilio, Christa Schleper, Christoph W. The steps are shown in Figure 20.4, using adeno-associated virus as the example. Inverted terminal repeat: a nucleotide sequence present at both ends of a nucleic acid molecule but in the opposite orientation; the two ends of a single-stranded DNA with such a sequence are complementary and therefore can anneal to each other. As a result of these host–virus protein interactions, cervical cancer is among the most common forms of malignancy in the world. Table 25.1 Some representative baculoviruses CH025.indd 303 Genus Virus Host Alphabaculovirus Autographa californica nucleopolyhedrovirus (AcMNPV) Bombyx mori nucleopolyhedrovirus (BmNPV) Lymantria dispar multinucleocapsid nucleopolyhedrovirus (LdMNPV) Alfalfa looper Silkworm Gypsy moth Betabaculovirus Cydia pomonella granulovirus (CpGV) Codling moth Gammabaculovirus Neodiprion lecontei nucleopolyhedrovirus (NeleNPV) Redheaded pine sawfly Deltabaculovirus Culex nigripalpus nucleopolyhedrovirus (CuniNPV) Mosquito 19/05/11 12:19 PM 304 CHAPTER 25 Baculoviruses Envelope glycoproteins Tegument Cap Envelope Capsid DNA Base Figure 25.1 Budded baculovirus virion. CH030.indd 366 366 a number of animals (see chapter opener), and some of these viruses have served as models for the study of hepadnavirus replication and pathogenesis. Translation efficiency in eukaryotic cells is usually strongly dependent on the poly(A) tail, which is bound by cellular proteins and interacts with the translational machinery. 27.5a,b: courtesy of James Van Etten, reprinted from Figure 2c and f in Meints, R.H. et al., "Infection of a Chlorella-like alga with the virus, PBCV-1: ultrastructural studies," Virology 138 (1984):341-6, with permission from Elsevier; Fig. However, despite the controversy, the mimivirus is a bona fide virus. To date, only three mammalian reovirus serotypes have been identified despite isolation from a broad range of hosts. The "swine flu" pandemic of 2009–2010, which caused upward of 16,000 deaths worldwide, did not lead to widespread serious illness as was feared. 15.1a: Courtesy of George P. Human papillomaviruses tend to infect either mucosa (genital tract, oral and nasal cavity) or skin. • Influenza virus nucleocapsids travel to the nucleus, where three viral RNA polymerase proteins (PA, PB1, and PB2) direct mRNA synthesis, using capped RNAs from the cell as primers, and primer-independent RNA replication. During assembly of virions the 80-kDa preterminal protein is cleaved to generate the 55-kDa terminal protein, which remains covalently attached to the genome after packaging. Four early mRNAs are made by differential splicing of a common transcript The 2.7-kb early transcripts of mouse polyomavirus extend about half way around the circular viral DNA (Figure 21.4). Affected areas contain microscopic insoluble amyloid fibrils (Figure 32.4a) and macrocrystalline arrays known as amyloid plaques. This association apparently protects the E protein from CH012.indd 143 a premature conformational change that would lead to exposure of the fusion peptide in the low pH environment in Golgi vesicles. (b) Production of a simple retrovirus vector by transfection of a transfer construct into the packaging cell line. How are the virions of some of the simplest viruses assembled? These mRNAs code for early proteins that were first detected in virus-induced tumors, and therefore are called tumor antigens, or T antigens. It is now recognized that papillomaviruses are widespread in animals and humans. The clonal selection theory states that antigenspecific cells of the immune system with a great variety of binding specificities are created in an antigen-independent manner in the bone marrow. This may involve an intracellular receptor involved in uncoating. Membrane (M) Virion structural protein that contacts both spike and nucleocapsid protein. Antigens and antigen-presenting cells enter via the afferent lymphatic vessels. Top: organization of unique and repeated sequence elements. However, the members of individual genera within each family share significant biological characteristics. This is also a sign of infection, because most DNA is usually restricted to the nucleus in healthy, uninfected eukaryotic cells. Five of these capsid proteins have closest similarity to capsid proteins of Pyramimonas orientalis virus-1 (PoV-1), two have closest similarity to capsid proteins of Heterosigma akashiwo virus-1 (HaV-1), and one has closest similarity to a capsid protein encoded by Paramecium bursaria chlorella virus NY-2A, all viruses in different genera of the phycodnavirus family. Activation of T cells by Nef is caused by CH029.indd 363 modulation of the activity of a number of cellular protein kinases involved in signaling pathways. Positions of major gene classes I, II, and III are indicated by heavy lines. This antigenome nucleocapsid can in turn be replicated by the viral RNA polymerase, producing full-length encapsidated genomes. GENOME Linear ss RNA, negative sense. Other functions encoded include enzymes for DNA replication, recombination, and repair; nucleotide metabolism and transport; transcription; protein and lipid synthesis, modification and degradation; signaling; sugar metabolism; and eight major capsid proteins. This process forms a "concatemer junction" that contains the two complementary hairpin ends in a perfectly base-paired duplex (see Figure 26.1d). Prions are infectious proteins; they do not contain any detectable informational nucleic acid. Discuss the process of membrane fusion carried out by the influenza hemagglutinin. Had a virulent and transmissible H5N1 virus "emerged" while the manufacturers were struggling to overcome this problem, the results could have been devastating. While some mutations may result in attenuated disease, others may increase virulence and disease. Production of lentivirus vectors requires additional cis-acting sequences Lentiviruses are complex retroviruses that encode six accessory proteins (Vif, Nef, Tat, Rev, Vpr, and Vpu) in addition to the proteins coded by the gag, pol, and env genes (Chapter 29). Carleton Gajdusek Baruch Blumberg Howard Temin and David Baltimore Paul Berg 1975 1976 1976 1975 1980 Cellular oncogene is part of a retrovirus genome RNA splicing in adenovirus Prions: infectious proteins Human papillomaviruses cause cervical cancer Discovery of AIDS virus (HIV-1) 1976 Michael Bishop and Harold Varmus 1989 1977 1975–1990 1972–1984 Phillip Sharp and Richard Roberts Stanley Prusiner Harald zur Hausen 1993 1997 2008 1983 Luc Montagnier and Françoise Barré-Sinoussi 2008 Foot-and-mouth disease (cattle) Yellow fever (humans: transmitted by mosquitoes) packaged in virions, but they share many attributes of life, including the ability to mutate, evolve, and reproduce themselves, when they enter cells that can support their replication. Retroviridae package the RNA copy of the genome, while Hepadnaviridae and Caulimoviridae package the DNA copy of the genome. The Phaeovirus EsV-1 Ectocarpus siliculosus Multicellular filamentous brown algae (seaweed). It also can inhibit apoptosis mediated by Fas, tumor necrosis factor (TNF), transforming growth factor , or p53. cIII N t L1 cI PL cro PR NUTL cII O P Q t R1 NUTR ≥N ≥N ⩽N ⩽N (a) Q PR' tR' S R Rz Nu1 A cos ≥Q ⩽Q (b) Figure 8.4 The lytic transcription program. r be induced, causing prophage excision, genome replication, and cell lysis (lysogen means "able to generate lysis"). The roles of hepatitis B virus proteins X protein. However, papillomaviruses have a significantly larger genome, their gene map is organized in a different fashion, and they share a distinct lifestyle, all justifying their classification in their own family. This may be related to their mechanism of replication; the genome RNA must first be transcribed within the cell to generate complementary, positive-strand messenger RNAs, and the helical nucleocapsid may provide stability and structure to the genome during this process. Subsequently, the structure of the neuraminidase complexed with sialic acid was solved, showing that sialic acid occupies two of three well-formed pockets on the enzyme (Figure 36.10b). Since EF-Tu has a GTP binding site, it could bring GTP to the replicase to initiate RNA synthesis. If serum and virus are taken from an HIV-infected individual, the antibodies in the serum cannot neutralize the virus that is currently present. Vectors can also be used as vaccines that generate immune responses against a variety of unrelated pathogens. Spliceosome: a complex made up of a number of small ribonucleoproteins bound to pre-messenger RNAs. Carries out cleavage and joining reactions leading to production of spliced messenger RNAs. Spongiform encephalopathy: disease accompanied by large vacuoles in the cortex and cerebellum, giving the brain the appearance of a sponge. Vaccines are now more than 200 years old, as the first successful antiviral vaccine was shown by Jenner in 1796 to prevent smallpox. Viral genomes are released into the cell by passage through the vesicle membrane or by disintegration of capsids within the cell (uncoating). Cathepsins: a family of intracellular proteases with a variety of specificities. These genes code for viral stuctural proteins, proteins involved in the assembly of capsids and packaging of viral DNA, and many other proteins whose functions are only beginning to be discovered. TYPES OF ANTIVIRAL VACCINES Antiviral vaccines can be grouped into several broad categories (Table 35.4 and Figures 35.1 and 35.2): 1. Regulation of gene expression is accomplished primarily at the level of transcription. Recombinant poxviruses can be constructed that express antigens of another pathogen, for example, the rabies virus surface glycoprotein. Most primary infections occur in childhood, resulting in largely asymptomatic or unrecognized infections. Blue arrows: early genes; orange arrows: early/late genes; green arrows: late genes. Although it has not been proven to be involved in the death of adenovirus-infected cells, E4orf4 could play an important role. Because of this, S has been a focus of much research. What is the "kissing loop"? The cleaved glycoprotein fragments GP1 and GP2 are shown linked by a cysteine bridge (SS). CH024.indd 300 16/05/11 8:12 PM Epstein–Barr Virus 301 • Viral DNA is circularized once it enters the nucleus. This rearrangement results in folding down the proteins onto the surface of the virion and exposes the furin cleavage site. The absence of Vif within infecting virions cannot be compensated by expressing it in the cells being infected. In addition, the generation of insertions and deletions in viral RNAs during infection in plants was shown to be dependent on both viral RNA structure and the plant host. AZT triphosphate is a more potent inhibitor of HIV reverse transcriptase than of human DNA polymerases; this accounts for the selectivity of AZT. LMP-2A also allows activated B lymphocytes to survive (antiapoptosis) by activating the Ras/PI3-Kinase/Akt pathway (Figure 24.12b). Quax, M. Internal core and lateral bodies. Quasispecies: virus variants that differ by a small number of mutations. This RNA can bind to ribosomes and is translated into viral proteins as the first step in virus replication. CH008.indd 93 93 attP; sib is only downstream of int after the prophage has been excised. 16/05/11 8:07 PM 234 CHAPTER 19 Reoviruses Mutants of reovirus with alterations in the 1 protein are diminished in the capacity to activate NF-B and induce apoptosis, pointing to a key role for 1 in the reovirus cell-death mechanism. Before simian virus 40 was identified, several batches of the Salk inactivated poliovirus vaccine unknowingly contaminated with live simian virus 40 were administered by injection to millions of people. The transfer construct contains the transgene expression cassette and all the necessary cis-acting DNA sequences. Applications of retrovirus vectors: treatment of blood disorders The packaging construct. In contrast, RNA phages rarely if ever produce double-stranded RNA. This prompted substantial discussion about how the virus was introduced and whether it would establish itself in the United States and become an endemic disease. CH021.indd 249 249 The circular DNA genome is packaged with cellular histones Packaged within the capsid is the viral genome, a 5.3-kb circular, double-stranded DNA molecule (Figure 21.2a). Describe two properties of the parvovirus genome that are not common among DNA viruses. 18.2: Courtesy of P.W. Choppin. The 150-kb herpes simplex virus genome has an unusual structure; it consists of two covalently linked components, long (L) and short (S) (Figures 24.1 and 24.2). Ethidium bromide: a fluorescent polycyclic aromatic molecule that intercalates between bases in DNA or RNA. • To economize on restricted genome size, viruses often encode multifunctional proteins. High-intensity x-rays are produced in giant synchrotrons, and the diffraction data are analyzed by sophisticated computer programs to yield the three-dimensional structure of the virion. ORF1b is one of the most highly conserved regions in the coronavirus genome, underscoring the importance of the proteins coded in this region. It is the balance between activation and inhibition that decides whether NK cell will attack and kill a particular cell within the body. The c sequence contains a single open reading frame, also present in two copies per genome. • Satellite viruses and viroids have very small genomes of from 250 to 1800 nucleotides, and are highly dependent on host cells or other viruses for packaging as well as replication. Transfer of plus-strand DNA to 3' DR1 site on minus-strand DNA; elongation of plus-strand DNA 1 2' 1' Cap Polymerase extends plus-strand DNA along minus-strand DNA template, forming circular ss/ds DNA. The P1234 polyprotein is cleaved in stages to yield initially a complex of P123 and nsP4, followed by a complex consisting of nsP1, nsP23, and nsP4 and finally by a complex consisting of the individual polypeptides nsP1, nsP2, nsP3, and nsP4. This drug, referred to as Gendicine, is a first-generation adenovirus vector encoding the tumor suppressor p53, and was approved for use in China in 2003. Interaction with ribosomes within the cytoplasm may therefore initiate nucleocapsid breakdown and release the genome RNA, allowing its translation. Holland Cheng & Yoshihiro Kawaoka, "Architecture of ribonucleoprotein complexes in influenza A virus particles," Nature 439 (2006), 490–492; Fig. During the early phase this promoter has a low activity, and the rare transcripts terminate prematurely, so that only low levels of L1 mRNAs are made. Survival of the fattest: the giant coccolithovirus genome encodes sphingolipid biosynthesis There are several surprises in the coccolithovirus genome, including a group of genes involved in sphingolipid biosynthesis that is thought to be responsible for the production of the sphingolipid, ceramide. CH014.indd 162 16/05/11 8:04 PM Coronaviruses (a) Spike protein (S) Membrane protein (M) Small envelope protein (E) Nucleocapsid protein (N) (b) Figure 14.2 Coronavirus virion. 16/05/11 8:06 PM 206 CHAPTER 17 Bunyaviruses Coupled translation and transcription may prevent premature termination of mRNAs The presence of a capped primer at the 5' end of elongating transcripts (Figure 17.3) could allow binding by ribosomes and initation of protein synthesis before synthesis of the mRNA chains is completed, because transcription takes place in the cytoplasm. Accordingly, more antiviral drugs target genome replication than any other stage of virus infection. What are the host ranges of influenza virus types A, B and C? Studies with duck hepatitis B virus have suggested that release of the viral genome into the nucleus occurs by interaction of the core with the nuclear pore complex. This also shuts off cellular mRNA synthesis, freeing up energy and nucleotides for phage transcription. CH009.indd 110 16/05/11 8:00 PM SECTION III POSITIVE-STRAND RNA VIRUSES OF EUKARYOTES C H A P T E R 1 0 Cucumber Mosaic Virus C H A P T E R 1 1 Picornaviruses C H A P T E R 1 2 Flaviviruses C H A P T E R 1 3 Togaviruses C H A P T E R 1 4 Coronaviruses Viruses are the only known organisms on earth that can encode their genetic information in RNA molecules; these may be relics of the ancient RNA world. Additional endemic areas in Africa or on other continents may also exist. A growing list of diseases in which fibrillar accumulation causes cellular damage in the central nervous system includes Alzheimer's disease, in which neurodegeneration is associated with deposition of the amyloid protein; Parkinson's disease, which is associated with deposition of ␣-synuclein; and Huntington's disease, in which an unusually long tract of polyglutamine in a protein leads to brain lesions. Some viruses specifically package the negative RNA strand in virions. Most retrovirus and lentivirus vectors are pseudotyped with the vesicular stomatitis virus G glycoprotein, a protein conferring a very wide tropism. The largest known virions (mimivirus) have a diameter of approximately 500 nm, and they can be barely seen with a light microscope. Subsequently, several pharmaceutical companies developed target-based high throughput screens against HIV-1 reverse transcriptase, leading to the identification of a number of promising compounds that are not nucleoside analogues. Because the versions of HA and NA in this strain had not previously been circulating among humans, a large part of the population was susceptible to virus infection. CI monomers are shown as dumbbells, and RNA polymerase is shown as a large orange asymmetric arrow. Soluble homologues of cytokine receptors block cytokine production and inhibit B-cell activation and antibody production. This explains the smooth, golf ball-like surface of the virion. Balancing the negative and positive effects of retrotransposition is key in maintaining the viability of the host. Distinct genotypes can be distinguished within the algae in the Ostreococcus genus; these genotypes correlate not with geographic location but with their ability to grow in environments with high or low ambient light. 16/05/11 7:52 PM 52 CHAPTER 4 Virus Entry Other non-enveloped viruses pass through cellular membrane barriers without apparent lysis or major membrane perturbations. Viruses with double-stranded RNA genomes have fragmented genomes and capsids with icosahedral symmetry Most viruses with double-stranded RNA genomes (Table 3.5) contain multiple genome segments, each of which codes for a single messenger RNA and usually a single viral protein. What impact does this trigger have? Like C, V can inhibit the host interferon response, and it is known to stimulate degradation of Stat proteins. The provirion may represent the end of the intracellular assembly pathway. The history of vaccine development is troubled by several unfortunate incidents in which vaccinated individuals have suffered severe 16/05/11 8:21 PM 440 CHAPTER 35 Antiviral Vaccines "adverse events" (Table 35.9). Therefore scientists widened the net and started to sample other animal species. M 75 Small envelope glycoprotein. This virus was distinguished from retroviruses that cause mouse leukemia by its smaller size and greater stability. The capsids of many viruses are constructed as shells with icosahedral symmetry; tetrahedral and cubic symmetries have not been observed. Diameter 6 0 nm. Some bunyaviruses are maintained in nature by transovarial transmission within the arthropod host. huxleyi DMS ⩽ DMSP Figure 27.8 Interaction of algae and their viruses with climate. 2.1a: Electron micrograph courtesy of Dr. Carla W. Viruses with double-stranded RNA genomes are faced with the same problem as those with negativestrand RNA genomes: the cell does not produce an enzyme that can generate an mRNA by transcribing 16/05/11 7:46 PM 5 Steps in the Virus Replication Cycle 4. Vaccinia virus, a poxvirus, was the world's first known antiviral vaccine, and was used against the deadly smallpox virus. Some Figure 27.11 Transmission electron micrograph of a mimivirus particle in a thin section. They contain viral proteins and double-stranded RNA, virions at various stages of maturation, and at late times crystalline arrays of mature virions. Eukaryotic initiation factors eIF-2␣, and eIF-4B are known to interact with picornavirus IRES elements, and eIF-2, eIF-4E, and eIF-4F all stimulate cap-independent translation. ICP27 Early: colocalizes with spliceosomes and blocks RNA splicing. The rules of symmetry would suggest that the remaining 60 capsomers should be made from three or six molecules of VP1, because each such capsomer has six nearest neighbors. Incoming molecules sorted for degradation are exposed to a successively decreasing level of pH as they follow the maturation program from early to late endosomes, and ultimately to lysosomes. Formation of double-stranded replicative form DNA from single-stranded genome DNA, carried out entirely by host cell proteins. Poxviruses 312 Smallpox was a debilitating and fatal worldwide disease 312 Variolation led to vaccination, which has eradicated smallpox worldwide 313 Poxviruses remain a subject of intense research interest 313 13/05/11 2:21 PM Contents Linear vaccinia virus genomes have covalently sealed hairpin ends and lack introns 314 Two forms of vaccinia virions have different roles in spreading infection 315 Poxviruses replicate in the cytoplasm 316 Poxvirus genes are expressed in a regulated transcriptional cascade controlled by viral

transcription factors 317 Virus-coded enzymes packaged in the core carry out early RNA synthesis and processing 318 Enzymes that direct DNA replication are encoded by early mRNAs 318 Poxviruses produce large concatemeric DNA molecules that are resolved into monomers 318 Postreplicative mRNAs have 5' end poly(A) extensions and 3' end heterogeneity 319 Mature virions are formed within virus "factories" 320 Extracellular virions are extruded through the plasma membrane by actin tails 321 Poxviruses make several proteins that target host defenses against invading pathogens 321 PHAEOVIRUSES 335 Seaweed viruses 335 Phaeoviruses have a temperate life cycle and integrate their genomes into the host 335 27. Acheson Daniel Kolakofsky Christopher Richardson Revised by: Laurent Roux Paramyxoviridae From Greek para (alternate) and myxa (mucus): virions bind to mucoproteins Rhabdoviridae From Greek rhabdo (rod), referring to shape of virion VIRION Paramyxoviruses: spherical, diameter 150–300 nm, also found as filaments. The IE-1 protein is responsible for transactivating other early genes and is considered the major regulator of early baculovirus gene expression. In contrast to Env, both Gag and Gag/Pol proteins are released into the cytosol upon translation. A third hypothesis to explain the large size of the mimivirus genome is that it has evolved from a free-living cellular organism that became an intracellular parasite and lost many of the requirements for cellular existence. From the top down are shown: (a) the double-stranded DNA genome, showing terminal loops; (b) an enlargement of the inverted terminal repeat, emphasizing the tandem repeats within the terminal repeat; (c) the two hairpin loops (nonitalic font) as they are joined to the terminal repeats (italic font); (d) the duplex structure formed at a concatemer junction, consisting of the unfolded hairpins base-paired to each other. Like the leader sequence at the 3' end of the genome RNA, there is a "trailer" sequence at the 3' end of the antigenome RNA. The symmetry of the object is defined by the collection of all such operations that apply to it. Latent infection: an infection in which a pathogenic agent remains present in the organism but replicates slowly or not at all and produces few or no symptoms. Since the doubly spliced 2-kb RNAs (and most cellular mRNAs) do not contain a cis-acting repressive sequence, their export does not require Rev. Interferon-` : a family of type I interferons that are rapidly produced by many cell types, particularly blood cells, that have antiviral and anti-proliferative activities, and that are an important component of the intrinsic cellular response and the innate immune response to viral infections. • Baculoviruses engineered to express caspid proteins of other viruses can result in the synthesis of large amounts of virus-like particles (VLPs) in infected insect cells. The same kind of protein fold is found in icosahedral capsids of many other viruses. Lentiviruses such as human immunodeficiency virus are capable of transporting their integration complex into intact nuclei, and vectors based on lentiviruses are therefore useful for expressing transgenes in nondividing cells. Why is it a good model? GENOME Linear double-stranded DNA, 40 kbp. There is still an urgent need for vaccines that can protect against viruses that cause significant illness and death, such as respiratory syncytial virus, HIV, dengue virus, and hepatitis C virus. The first infection is believed to expand the population of cells that can be infected by a second serotype via antibody-dependent enhancement of virus entry. One of the first coronavirus diseases to be recognized, in 1912, was feline infectious peritonitis, although the virus was isolated much later. Nectins: a family of cell surface proteins, with an immunoglobulinlike structure, that function in cellular adhesion. When an inducer of the lac promoter is added, T7 RNA polymerase is made and gene X is transcribed. nucleotides in length (Figure 19.3). Variations around the core structural theme include an outer lipid layer in coccolithoviruses (a consequence of the budding exit mechanism through the cell membrane), a star-shaped surface structure on mimivirus (associated with entry of the viral genome into the cell), and the short filaments on the capsid of some phycodnaviruses as well as the long "hairy" appendages of mimivirus. An inducer of the lac operon (IPTG) turns on T7 RNA polymerase expression and leads to expression of gene X in the pET vector. Most of the RNAs initiated at these promoters end at a single, stong terminator (Tϕ) located 24 kb from the left end of the genome. (a) LMP-1 (left) resembles a constitutively active CD40 receptor (right) by stably interacting with proteins normally found bound to an activated CD40 receptor. The nucleocapsid contains a single molecule of circular, doublestranded DNA arranged as a cylindrical core. How does new variant Creutzfeldt–Jakob disease differ from sporadic Creutzfeldt–Jakob disease? The RNA polymerase then scans the genome for a nearby mRNA start site, and begins mRNA synthesis. As discussed above, a similar strategy has been employed to modify the tropism of AAV vectors by means of capsids derived from different serotypes. The remainder of the structural polyprotein, including PE2, 6K, and E1, is translocated as a unit to the endoplasmic reticulum by means of a signal sequence at its N-terminus. Figure 2.9 shows a diagram of the tobacco mosaic virus particle, in which many copies of a single protein subunit bind to the viral RNA and form a tube with helical symmetry. Between 1997 and 2009, many parts of the world experienced a major panzootic (widespread epidemic in animals) of H5N1 avian influenza strains. The crystal structure of nsp5 for several coronaviruses has recently been solved, and it is a target for development of antiviral agents specific for SARS coronavirus. The binding of transcription factors to the interferon- gene enhancer has been carefully studied and serves as a paradigm for the interaction between transcription factors and the general transcriptional machinery. One answer may be that many people, particularly older individuals, had partial immunity against this virus because of previous exposure to strains of H1N1 virus that have circulated over the last 50 years in the human population. • Adenovirus genes are organized in six early, two intermediate, and five late families. More than 20,000 deaths per year in North America continue to be caused by influenza virus, most among elderly or otherwise debilitated or immunocompromised individuals. DNA replication begins by extension of the 3' end of the terminal hairpin Incoming parvovirus single-stranded DNA must first be converted to a double-stranded form to be able to function as a template for transcription. Numerous cellular oncogenes were discovered incorporated into retrovirus genomes. So far little is known about how these viral proteins or RNAs or the virions reprogram cell activities in the infected plants except for the 2b protein (see below). Recover and analyze mutant viruses Figure 14.7 In vitro assembly of genome cDNA and production of infectious genome RNA. Rift Valley fever infection results in a range of disease syndromes, from severe febrile illness to retinal 16/05/11 8:06 PM 202 CHAPTER 17 Bunyaviruses disease to a fatal hemorrhagic disease. Finally, Section X finishes the book by reviewing some important applications in virology: antiviral vaccines, antiviral chemotherapy, and virus vectors. Instead of being assembled in the cytoplasm and migrating to the membrane, the nucleocapsid is assembled from the Gag precursor protein directly at the membrane during budding (Figure 2.1j; see Chapter 28). Since a persistently infected host is the most likely reservoir for a zoonotic disease, chronic infection in bats or other small mammalian species may be considered as a possible mechanism that regulates survival of filoviruses in nature. Because they are difficult to grow in vitro, most of these viruses were identified by direct cloning of their DNA in bacterial plasmids, followed by nucleotide sequence analysis. In this system, the transgene expression cassette flanked by retrovirus LTRs is delivered to cells using a first-generation adenovirus vector. Granulin: protein that forms ovoid occlusion bodies of granuloviruses. Briedis, McGill University SECTION IX: HOST DEFENSES AGAINST VIRUS INFECTION 33. Rigid constraints on structure are likely essential for the functional requirements of M in virus assembly. The production of different amounts of viral proteins is entirely dependent on transcriptional promoters, transcription terminators, and ribosome binding sites on mRNAs (Figure 6.1b). Three major types of interferon, designated types I (interferons ␣ and), II (interferon-) and III (interferons 1, 2, and 3) have been described. Then the replicase adds on CH005.indd 65 Function a single, untemplated A residue—so the 3' end of the minus-strand looks like the initial 3' end of the plusstrand, C's with a terminal A. serum proteins, including ficolins, which bind to N-acetyl glucosamine, and collectins, which bind to mannose residues expressed on microbial membrane carbohydrates and lipopolysaccharides, activate complement by the lectin receptor-activated pathway. Efimov, Lidia P. Since viruses are pathogens and cause major medical and veterinary problems, every step in the replication cycle must be viewed as a potential target for inhibition by antiviral drugs. These molecules are folded in a stable state as originally synthesized, even though a distinct, more stable folding state exists. CH020.indd 246 16/05/11 8:08 PM C H A P T E R 2 1 Polyomaviruses Nicholas H. RNA synthesis is associated with these structures (Figure 12.6), and most of the nonstructural proteins are localized there. For example, plants infected with cucumber mosaic virus are more tolerant to drought and to cold temperatures, compared to uninfected plants. Experiments using deletion mutants have shown 16/05/11 8:07 PM 220 CHAPTER 18 Influenza Viruses Figure 18.9 Selective packaging of influenza virus nucleocapsids. On the one hand, if the host cell is metabolically active, instability keeps the CII concentration low, resulting in insufficient CI production from PRE to repress PL and PR. The death protein is a membrane glycoprotein found in the Golgi membranes, endoplasmic reticulum, and most predominantly in the nuclear membrane; how it kills cells is not known. DISEASES Infections with Marburg and Ebola viruses cause severe hemorrhagic fever. The proteinase responsible for these cleavages is encoded in a domain at the C-terminal end of nsP2. Examples include canarypox viruses that express HIV sequences, and alphaviruses such as Semliki Forest virus that express paramyxovirus proteins. Others bud at internal cell membranes or form their envelopes de novo; many of these viruses use cellular transport vesicles to reach the cell membrane and to exit the cell. Each protein has a number of functions and biochemical activities (Figure 20.7). A cellular tRNA is used as a primer to initiate reverse transcription. Conserved structure, diverse composition Despite the wide host range displayed by the Phycodnaviridae, all family members, as well as mimivirus, have similar structure and morphology. Basta, R. The alternative assembly pathway proposes that the 14S pentamers first associate with viral genome RNA and subsequently combine with other 14S pentamers to form the provirion directly. Monensin: monovalent ionophore that disrupts the passage of proteins through the Golgi complex, and has multiple effects on glycosylation. The first antiviral drugs were discovered by testing chemical compounds for their ability to inhibit virus replication in labor- and time-intensive assays of viral replication, such as assays of cytopathic effect or plaque assays. Coronaviruses, like many viruses, were initially categorized by antigenic relationships. In 2002, it was shown that West Nile virus could also be transmitted by blood transfusions and tissue transplantation. Polyclonal: derived from a number of individual clones. The env gene of HIV-1 produces an envelope glycoprotein that targets the CD4 antigen and chemokine receptors, present on lymphocytes, macrophages, and certain other cell types. The AAV genome is a single-stranded DNA molecule. This replaces a confusing terminology based on relative sizes of these two proteins. Some bacteriophages complete their replication cycles in as little as 20 minutes; some animal viruses can take several days to complete one replication cycle. BGLOSS.indd 473 473 Condyloma: growth resembling a wart on the skin or a mucous membrane, usually of the genitals or anus, caused by infection with a human papillomavirus. It was later demonstrated that there was a causative link between the leukemia and the gene therapy treatment. Recurrent eye, mouth, and genital lesions. Interferon-␣, previously called leukocyte interferon, is produced by peripheral blood leukocytes and many other cell types in response to viral infection or treatment with double-stranded RNA. Positivestrand RNA is shown in light blue, negative-strand RNA in dark blue. Poxviruses evade activating receptors of NK cells by secreting soluble competitive inhibitors of these receptors. Plasmid DNA An expression plasmid containing the gene that encodes a viral antigen is introduced into the body. Under these conditions, the enzymes and precursors needed to support cellular or viral DNA synthesis are not normally present in the cell nucleus. Public health isolation measures appear to have interrupted the chain of person-to-person infection, but not without serious economic loss for the cities involved, a significant drop in travel to Southeast Asia and China, and the illness and death of hospital personnel caring for victims. Alternatively, Hsp70 could help fold P1 so that its cleavage sites are recognized by 3CD. Acute transforming retroviruses: retroviruses that transform cells rapidly and efficiently by means of cellular oncogenes incorporated into the viral genome. The high-resolution crystal structures of polyomavirus capsid proteins and of the adenovirus hexon protein reveal that these proteins, like the parvovirus and picornavirus capsid proteins, contain jelly-roll -barrels. These VLPs exhibit excellent antigenic properties and often stimulate strong protective immune responses when used as vaccines. The 16/05/11 8:10 PM Papillomaviruses 265 the polyomaviruses, all papillomavirus mRNAs are transcribed from only one of the DNA strands. Initiation of synthesis of the MS2 lysis protein is explained by the "scanning" model. Most mRNAs are unspliced. The viral proteinase resides within the protein NS3; however, an accessory protein, NS2B, is also required to carry out CH012.indd 142 proteolytic processing with high efficiency. For orthobunyaviruses and some phleboviruses a small nonstructural protein (NSm) is encoded as part of the polyprotein and is cleaved during translation (Figure 17.2). At some point during this pandemic, H1N1 strains disappeared completely from the human population. Sheep scrapie can be used to infect mice and hamsters, a discovery that eventually led to the purification and identification of prions. Circular forms of both the naked RNAs and the helical ribonucleoprotein nucleocapsids (Figure 17.1b) have been observed by electron microscopy. H DNA pilot protein, required for DNA delivery; also called the minor spike protein; 12 copies in virion. Wt. in KDa) Viral infectivity factor Vif p23 Virion protein unique to HIV-1 Vpu p16 Transactivator of transcription Tat p15 Regulator of expression of virion proteins Rev p19 Negative effector Nef p27 splicing of the HIV-1 primary transcript generates more than 25 mRNAs that fall into three size classes (Figure 29.3): 1. Irradiation of viral genome RNA with CH015.indd 181 ultraviolet (UV) light inhibits transcription by forming dimers between adjacent uridine residues on the template RNA. Because 1 light year (the distance traveled by light in one year) 1013 kilometers, 2 1021 kilometers 2 1021/1013 light years ᵹ 2 ȡ 108 light years (200 million light years). The nucleocapsid protein (NP, small green spheres) binds to negativestrand viral genome RNA (blue line) to form a nucleocapsid with a twin helical conformation. Allen William H. Multimers of both positive and negative strands of satellite RNAs, probably replicative intermediates, can be detected in extracts from infected plants. In the case of dengue virus, such a mechanism has been proposed to explain the disease known as dengue hemorrhagic fever or dengue shock syndrome. Rev is returned to the nucleus, where the cycle is repeated. This channel allows protons to enter the interior of the virion during acidification within the endosome (see Figure 36.3 in Chapter 36 on antiviral chemotherapy). When examined by electron microscopy, the enveloped virion appeared to be surrounded by a crown or corona of studded projections, and was therefore named a coronavirus. The characteristics and disease implications of these latent infections will be described in detail after a discussion of the strategy of lytic infection employed by the best-studied model, herpes simplex virus. New variant Creutzfeldt-Jakob disease (nvCJD or vCJD): prion disease in humans believed to be caused by transmission of prions from diseased cattle. Once in the vascular system, the virus moves in a passive manner along with photoassimilates. Caveolae and the caveolar pathway are thought to play a role in signaling and in the uptake of cholesterol and selected ligands. Virus release begins only in the early stationary growth phase of host cells and does not result in any detectable cell lysis. 40S and 60S refer to the small and large ribosomal subunits. Hepatitis delta virus is a human viroid-like satellite virus Hepatitis delta virus (HDV) is unique among human pathogens in that it shares several characteristics with both plant viroids and viroid-like plant satellite RNAs. It has been proposed that hepatitis delta virus evolved from a primitive viroid-like RNA through the capture of a cellular transcript. In the 1990s, most developed countries replaced the live Sabin (oral) polio vaccines with inactivated vaccines in their national programs to avoid the small but measurable risk of reversion (approximately 1 per 2 million doses). The presence of satellite RNAs generally reduces the accumulation of cucumber mosaic virus particles in infected plants, but surprisingly, the same satellite RNAs do not affect the accumulation of tomato aspermy virus. In this way, concepts that are learned about simpler viruses can be applied when more complex viruses are encountered. This could have also have hastened the transformation of cells from RNA-based to DNAbased genes. One major late transcription unit: five subclasses of late mRNAs, L1 through L5. Surface proteins. If these small introns are artificially lengthened by insertion of as little as 40 nt into the viral genome, almost all splicing events target the nearest 5' splice site at nt 748, and no large T antigen mRNA is made. Recently, a combination of reverse genetics, commercial cDNA synthesis and rational design was used to recover the first bat coronavirus to replicate in culture. Cells infected simultaneously 16/05/11 8:07 PM Influenza Viruses with two virus strains can produce reassortant virions that contain some genome segments from one strain and some genome segments from the other strain (see Figure 17.5). They are also ideal biological molecules with which to study RNA structure/function relationships. These viruses are the only two known members of the family Globuloviridae (from the Latin globulus, "small ball"). Inr initiator element; DAS downstream sequence activator element. There are presently no drugs 05/05/11 1:08 PM Hepadnaviruses or treatments that ensure clearance of hepatitis B virus from infected individuals. 484 bcreidt.indd 484 16/05/11 2:35 PM Credits Chapter 7 Fig. Immune response: the set of responses to foreign organisms or macromolecules that are used by an organism to protect against disease by mobilizing a variety of proteins and specialized cells in the circulation and in tissues that recognize, respond to, and destroy foreign pathogens and tumor cells. Artificial virus-like particles (VLPs) containing influenza surface proteins can be assembled in the absence of M1. For example, changes in two amino acids in or close to the region of the putative catalytic center of the RNA polymerase can change the host response from hypersensitive necrosis (localized plant tissue lesions) to systemic mosaic symptoms. In order for the virus to keep the cell alive long enough to complete its replication, it has developed a means to counteract p53-mediated apoptosis. The host cell provides the virus with all of the other biological molecules required for its reproduction. 238 CH020.indd 238 16/05/11 8:08 PM Parvoviruses like those of the single-stranded DNA bacteriophages. These three domains share a common ancestor and are as distinct from each other as any two of them are from the third. The second complexity arises at a specific editing site in the middle of the P reading frame. ATP then powers translocation of the DNA into the prohead, changing the prohead's shape. Vhs is also responsible for the transition from to and protein synthesis by degradation of gene mRNAs. Interestingly, the RNA degradation activity of Vhs made after the onset of viral DNA synthesis is suppressed by binding to newly synthesized VP16 and VP22, and this inactive complex is incorporated into progeny virions. Briedis Orthomyxoviridae From Greek ortho (correct or normal) and myxa (mucus); virions bind to mucoproteins VIRION Enveloped particles, quasi-spherical or filamentous. Induction of interferon-1 and interferon-2/3 resembles induction of interferon- and interferon-␣, respectively. There are three replication origins; two of these (Ori-lyt) are used during productive infection and one (Ori-P) is used during latent infection. EBNA-1 also acts to segregate the daughter genomes into each of the two progeny cells during cell division, presumably by binding viral DNA to cellular chromosomes. Surface lattice: a set of lines drawn on the surface of an object that connect points defining axes of symmetry. Junctional adhesion molecule A (JAM-A): a cellular membrane protein found in tight junctions; member of the immunoglobulin superfamily of cell-adhesion molecules (CAMs). Therefore, virus particles bud from the infected cells, but they are immature and do not successfully infect other cells. Differences between PrPC and PrPSc Proteinase K treatment of PrPC present in brains of normal animals results in complete digestion of the protein, while similar treatment of PrPSc from diseased animals gives a proteinase-resistant 27- to 30-KDa core CH032.indd 390 (a) (b) Figure 32.4 Prion fibrils and two-dimensional crystals. They in turn bind to another interferon-induced protein, ribonuclease L, and activate its ribonuclease activity. Table 31.1 Classification of viroids Family Characteristics Subfamily Examples Avsunviroidae (group A) Self-cleaving RNA; self-ligating; replicate in chloroplasts Avsunviroid Avocado sunblotch viroid (ASBVd) Pelamoviroid Peach latent mosaic viroid (PLMVd) and chrysanthemum chlorotic mottle viroid (CChMVd) Unassigned Eggplant latent viroid (ELVd) Pospiviroid Potato spindle tuber viroid (PSTVd) and 8 others Cocadviroid Coconut cadang-cadang viroid (CCCVd) and 3 others Hostuviroid Hop stunt viroid (HpSVd) Apscaviroid Apple scar skin viroid (ASSVd) and 11 others Coleviroid Coleus blumei-1 viroid (CbVd) and 4 others Pospiviroidae (group B) CH031.indd 379 RNAs do not self-cleave; replicate in nucleus 16/05/11 8:16 PM 380 CHAPTER 31 Viroids and Hepatitis Delta Virus (Table 31.1) based mainly on the presence of highly conserved sequences within the C domain, and in part on the presence of sequence homologies in the other domains. Non-enveloped viruses penetrate by membrane lysis or pore formation The proteins of non-enveloped viruses can also undergo major conformational changes in response to receptor binding or low pH in endosomes. Enveloped viruses circumvent this problem by using vesicle transport and coupled fusion ("coming together") and fission ("breaking apart") of membranes. Hexons lie at the 240 sixfold positions of a T 25 icosahedral capsid, and pentons lie at the 12 fivefold positions (Figure 2.7). Primary cell cultures: cells grown in vitro that are derived directly from an animal tissue. Late proteins (VP1, 2, 3) make virus capsid. The S2 subunit, which forms the stalk, has a short carboxy-terminal tail, a hydrophobic transmembrane domain, and an exterior domain with two long alphahelical regions. This enzyme is believed to play an important role in reducing local unwinding of DNA at high temperatures. The structures of the E proteins from multiple flaviviruses have been solved by x-ray crystallography; these proteins have a very similar architecture to the tick-borne encephalitis E protein. The Vpr protein enhances HIV-1 replication at multiple levels Vpr (virion protein R) is a 100-amino acid protein that is recruited into virions (10–100 molecules per virion) by virtue of its interaction with the carboxy-terminal region of Gag. PKR is a key factor in determining host susceptibility to infection. There are indications that injury to bile ducts leading to jaundice in newborn humans may also be caused by reovirus infection. Most have a majority of genes with unknown functions and no homology to any gene in databases. The arrows and scissors indicate the cleavage sites in (a)–(c). Activated macrophages use a variety of interferon-induced mechanisms to kill microbial targets. Despite intensive information programs and the availability of treatment, the number of infected people also continues to increase in many developed countries, people between the ages of 16 and 40 being most at risk. There are four main types of cytokines: interferons, pro-inflammatory cytokines, chemokines, and anti-inflammatory cytokines (Table 33.2). The higher resolution of the electron microscope reveals the general outlines of a virus particle, and shows the bumps and projections formed by oligomers of viral structural proteins on the surface of the virion (Figure 2.1). This activity is dependent upon the transmembrane portion of Vpu. coli (EHEC) strains carry -like prophages that express the gene for a shiga-like toxin, which substantially enhances the pathogenicity of the host. CH011.indd 130 J H D F C E K G A-rich loop L 5' B pyrimidine-rich AUG Polyprotein ORF 3' 834 Figure 11.6 Secondary structures of internal ribosome entry sites. For example, the original chapter on herpes simplex virus now is entitled "Herpesviruses", and includes a substantial section on Epstein–Barr virus. 32.3: Adapted from Fig. Diameter 28 nm. Electron micrographs of infected cells show densely staining material, probably helical nucleocapsids, lying on the cytosolic face of membranes in the intermediate compartment. Monocytes: white blood cells that phagocytose foreign particles or pathogens. The prion hypothesis: formation of infectious and pathogenic prions from normal PrPC Prusiner's original statement of the prion hypothesis proposed that a misfolded form of a protein (PrPSc) can catalyze the refolding of properly folded native protein molecules (PrPC) into a similar misfolded conformation. (b) Stages of HIV-1 infection. Viruses with double-stranded DNA genomes include the largest known viruses Viruses with double-stranded DNA genomes (Table 3.2) show a wide variation in genome size (between 5 and 1180 kb; Figure 3.2). Translation of the RNA leads to the establishment of membrane-bound RNA replication factories (lower left). CH024.indd 289 16/05/11 8:12 PM 290 CHAPTER 24 Herpesviruses ICP34.5 US1.5 oriS ICP27 ICP0 orfP/O b' ICP4 a'n oriS ICP22 ICP47 ICP4 c' c UL US 8.3 Kb 2.0 Kb 1.45 Kb LATs Figure 24.5 Transcriptional map of the b and c regions of herpes simplex virus. The idea was to maximize energetically favorable interactions with specific amino acid residues of the enzyme in the three pockets. THE NATURE OF VIRUSES Viruses consist of a nucleic acid genome packaged in a protein coat Viruses are the smallest and simplest forms of life on Earth. Mediterranean-area doctors use directed leishmania-infected sandfly bites to induce long-term protection from reinfection. The G glycoprotein of vesicular stomatitis virus (VSV-G) is often used for pseudotyping retroviruses. All tissue abnormalities of prion diseases are confined to the central nervous system. Circular DNA is shown above the E. C K B D A* E A J H F G (a) A A* C D B K TJ PA PB (b) J F G H E TF TG TH PD Figure 6.1 The ϕX174 genome. Garten et. Did all icosahedral ssDNA virus families evolve from a common ancestor? It may be possible to increase stability of retrovirus vectors by introducing more stable versions of envelope proteins and reverse transcriptases into these vectors. Protein 1a can also affect seed transmission, host resistance, and symptom severity. Such viruses were originally classified as arthropod-borne viruses ("arboviruses"). Construction of first-generation vectors was routinely performed by transfecting 293 cells with two DNA fragments. In all cases, the end result is the release of the virus core into the cytoplasm. Scaffolding proteins are used for virion assembly. All of these structures align the target cleavage site by base pairing with other regions of the RNA molecule. Several herpesvirus glycoproteins cause the shedding or internalization of viral protein-antibody complexes, while the spike protein of some coronaviruses displays receptor activity for the Fc (constant) portion of immunoglobulins. 16/05/11 2:35 PM Credits Chapter 21 Fig. Unencapsidated, circular single-stranded RNA, 250–400 nt. From Greek siphon (tube), referring to long, non-contractile tail. This involves deleting certain viral genes and inserting into the viral genome an expression cassette that contains control sequences and foreign genes to be expressed. 27.5d: Reprinted from Figure 1d in Yanai-Balser, G.M., et. From Greek podos (foot), refering to short tail. Objects with icosahedral symmetry have 20 threefold rotation axes, the number of triangular faces on an icosahedron. Mad cow disease is a recently discovered prion disease, transmitted by feeding cattle with meal made from discarded carcasses of prion-infected cattle and other animals. Virus-like particles can be formed by expressing only the E and M proteins together in cells, suggesting an important role for E in virion formation and budding. The empty capsid of the infecting OtV-1 virion is still attached to the host cell membrane. MAP kinase kinase activates MAP kinase by phosphorylation; protein phosphatase 2A inactivates MAP kinase by removing phosphates. The autonomous parvoviruses can replicate in cells that spontaneously enter the DNA replication phase of the cell cycle. Arresting or delaying cells in G2 may be beneficial for virus replication since transcription directed by the HIV-1 LTR is the most active at this stage of the cell cycle. Cohen, David A. Each transcription unit gives rise to multiple mRNAs by RNA splicing. Intrinsic cellular defense systems respond rapidly (within minutes) to an infection, induce a set of intracellular antiviral defense mechanisms within infected cells and their immediate neighbors, and direct the production and secretion of cytokines, including interferons, that signal the presence of an infection within the organism. Unlike apoptosis, pyroptosis is dependent on caspase-1 and is characterized by the release of pro-inflammatory cytokines. • A signal on the genome RNA ("packaging sequence") binds to a sequence in the capsid protein to ensure efficient packaging of the viral genome RNA into the nucleocapsid. Cap-independent translation also utilizes some of the same eukaryotic initiation factors that are involved in cap-dependent translation. It is located at tight junctions of epithelial and endothelial cells and mediates cell-to-cell interactions. For example, influenza viruses and paramyxoviruses bind to sialic acid residues present on glycoproteins and glycolipids. Peptidomimetic: drug that mimics a peptide, but is not itself a peptide. This host specificity resides in CH018.indd 220 certain viral proteins that have adapted to function optimally in a particular species. • Vpu enhances release of HIV-1 virions from the cell surface. The result is a complex nucleoprotein structure called an intasome. 2.1b, 2.1e–g: Courtesy of Robley Williams, University of California at Berkeley and Harold Fisher, University of Rhode Island; Fig. 05/05/11 11:24 AM 340 CHAPTER 27 Viruses of Algae and Mimivirus Conclusion KEY TERMS The phycodnaviruses and mimivirus represent an incredibly diverse group of viruses with very large genomes but a common virion architecture. In fact, filoviruses induce immunosuppression in the infected host, which appears to be a major factor for the rapid generalization and the severity of the disease. Within the lymph nodes, B lymphocytes respond to foreign antigens by differentiating into plasma cells, which produce specific antibodies. Onyx-015 is a conditionally replicative adenovirus that lacks the E1B region and preferentially replicates in certain cancer cells. Many of these phages encode proteins that participate in host cell takeover and that exclude the replication of other phages. RNA and DNA polymerases that carry out different steps are shown. The hammerhead, hairpin, and delta self-cleaving RNA motifs (Figure 31.3a–c) have been studied extensively. Numerous genes have been inserted into adenovirus vectors for experimental vaccination, including genes encoding hepatitis B surface and core antigens, herpes simplex glycoprotein, rabies virus glycoprotein, respiratory syncytial virus F protein, and several HIV-1 proteins. On the other hand, transcripts initiated at the PInt promoter, which are not subject to N-antitermination, terminate upstream of segment 1 in sib. Both of these pathways are activated by double-stranded RNAs in virus-infected cells. These CD40 LMP2A BCR TRAFS TRAFS Lyn P TRAFS NIK TRADD Lyn E3 P Syk P P Syk P E3 IKK IKK TRAFS NIK NF-B (a) (b) Ras/PI3-Kinase/Akt Figure 24.12 LMP-1 and LMP-2A mimic functions of normal B-lymphocyte proteins. The growing strands are usually base-paired to the template RNA only near their growing 3⬘ ends. Without these, a virus is just a package of genes; once inside a cell, a virus organizes a "factory" that produces progeny virus particles and sends them on their way to infect other cells. Chapter 37 discusses vectors made from adenoviruses, retroviruses, and adeno-associated viruses. The antigen-binding sites lie at the tips of the upper arms of the Y; these are the variable regions of both light 16/05/11 8:19 PM The Adaptive Immune Response 421 T-cell receptor Th2 cell B-cell receptor (surface antibody) CD4 peptides B cell antigen IL-4 IL-6 IL-10 antigen MHC II Activated B cell Plasma cell secreted antibodies Figure 34.6 Antibody production by B cells. Polymorphonuclear leukocytes: a collection of white blood cells, including neutrophils, eosinophils and basophils, that have granules within their cytoplasm and lobed nuclei. A second defense response is RNA silencing, a common and widespread antiviral defense response of plants. The bone Antigens, antigen-presenting cells Afferent lymphatic vessels Capsule Lymphoid follicle (B cells) Adenoids tonsils Thymus Thoracic duct Spleen Lymphatic vessels Peyer's patches (in intestinal wall) Appendix Lymph nodes Bone marrow Figure 34.3 Anatomy of the human immune system. Single-stranded DNA is circularized via the inverted terminal repeat At this point the double-stranded progeny DNA can serve as a template for further replication, but in each round only one double-stranded DNA molecule is formed, and there would therefore be no net accumulation of viral DNA. Anti-inflammatory cytokines: cytokines that function to reduce inflammation. • Coronavirus virions are assembled within the endoplasmic reticulum/Golgi intermediate compartment and secreted via vesicles that fuse with the cell membrane. GENES AND PROTEINS mRNAs are transcribed by cellular RNA polymerase II from five promoters. Stabilization of p53 in response to DNA damage or viral oncogenes such as E7 results in transcriptional activation of the p21 gene. Shown in the nucleus are plasmids used to make bacteriophage T7 RNA polymerase and four Ebola virus proteins that form nucleocapsids. IRFs function in a number of biological processes such as response to pathogens, cytokine signaling, regulation of cell growth, and hematopoiesis. 10.1: Courtesy of Tom Zitter; Fig. Transgenic animals that express one or more of the early genes of polyomaviruses in specific tissues are being studied as model systems for tumor formation. This double-stranded DNA is subsequently transcribed into messenger RNAs by cellular RNA polymerase, as for other DNA viruses. Why is x-ray diffraction unsuitable for the study of the structure of enveloped viruses? Viruses therefore adapt to the host organisms in which they replicate, and evolve as these organisms evolve. Chapter 4 Fig. This depolarization is likely to serve two purposes: it may prevent infection by a second virus, and it lowers the turgor pressure within the cell, which aids entry of viral DNA. Eventually, many of the virus particles taken up into the cells are uncoated, rendering intracellular virus non-infectious, and thus the titer of infectious intracellular virus also drops. Top: locations of hrs superimposed on the EcoRI fragment map. Over the next few years, the virus expanded its presence into all of the contiguous 48 states and the adjacent Canadian provinces. PRODUCTION OF VECTORS Transfection of genome into cell lines engineered to express virus genes deleted in the vector, or Transfection of genome into cells that are co-infected with helper viruses that express missing genes. Although PrPC is a soluble protein, PrPSc tends to form oligomers and aggregates that can be detected as fibrils in purified preparations or in infected brains (Figure 32.4a). Expression of FasL on the surface of the infected cell is also increased by Nef, inducing apoptosis of surrounding uninfected CD8-positive and CD4-positive T cells by interaction between FasL and Fas. Different virus strains bind different cellular tRNAs. Viral proteins are generated from three genes designated gag (for "group-specific antigen"), pol (for polymerase), and env (for envelope proteins). Virus infection causes loss of the ciliated epithelium, leading to loss of the ability of the respiratory tract to clear viruses or bacteria by mucociliary flow that normally traps these agents in mucus and disposes of them. The Jak–Stat pathway represents an extremely rapid signaling system between the plasma membrane and the cell nucleus, and explains at least part of the basis for the specificity of signals that are induced by different cytokines. Pina, M. IgM is short-lived, with a half-life of about 3.5 days. 14.3: Reprinted from Fig 2A and B in: Kevin Knoops, Marjolein Kikkert, Sjoerd H. However, research progress was hindered by the repeated failure to amplify ribosomal RNA sequences. Signaling begins by the binding of the cytokine to a cell-surface receptor. This attaches the membranes to each other but does not cause them to fuse because the distance between them is still too great. DNA synthesis initially extends from the tRNA primer up to the 5⬘ end of the viral RNA, resulting in a short (100–400 nt) DNA molecule that is a copy of the U5 and R regions. More recently, a new classification system for coronaviruses (Table 14.1) has been accepted by the International Committee on Taxonomy of Viruses. This editing event is carried out by a cellular enzyme known as adenosine deaminase (ADAR1) acting on RNA. (a) A schematic diagram of a virion in cross-section, showing pentamers of VP1 (green spheres), with single molecules of VP2 or VP3 (gray ovals) underneath the capsid surface. Roosevelt, President of the United States of America from 1933 to 1945, used a wheelchair because of paralysis caused by poliomyelitis, although many people were unaware of the extent of his disability. The formation of these unusual structures likely involves both stimulation of cellular processes to synthesize new membranes and modification of existing membranes by viral replicase proteins. • Protease inhibitors active against HIV inhibit maturation of virus particles. A mammalian cell line engineered to express T7 RNA polymerase enables transcription of these three cDNAs into positive-strand antigenome RNAs with the authentic 5′- and 3′-terminal sequences. ensemble of four host proteins, modifies RNA polymerase so that transcripts initiated at PL and PR are no longer terminated. Preferential replication of reovirus in transformed cells also might result from greater efficiency of disassembly of reovirus virions. • Capsids of small viruses are constructed from one or a small number of identical subunits; they are therefore highly symmetrical. Virion 16/05/11 8:13 PM 316 CHAPTER 26 Poxviruses Figure 26.3 Vaccinia virus virion. Virus produced by B lymphocytes has reduced infectivity for B lymphocytes but enhanced infectivity for epithelial cells, allowing productive infection and virus transmission. Secondary transcription and encapsidation. This effect cannot be reversed by expression of Nef in cells infected with Nef-minus virus. This system permits selective introduction of desired mutations into cloned cDNAs encoding each of the 10 viral gene segments, followed by isolation of mutant viruses from cells transfected with the plasmid constructs. Edited antigenome RNAs give rise to genome RNAs by replication; these genome RNAs are then transcribed by RNA polymerase II to form modified messenger RNAs. Up to 30% of hepatitis delta virus messenger RNA molecules contain this modified termination codon. Bunyaviruses 200 Most bunyaviruses are transmitted by arthropod vectors, including mosquitoes and ticks 200 Some bunyaviruses cause severe hemorrhagic fever, respiratory disease, or encephalitis 201 Bunyaviruses encapsidate a segmented RNA genome in a simple enveloped particle 202 Bunyavirus protein coding strategies: negative-strand and ambisense RNAs 203 L RNA codes for viral RNA polymerase 203 M RNA codes for virion envelope glycoproteins 203 S RNA codes for nucleocapsid protein and a nonstructural protein 204 After attachment via virion glycoproteins, bunyaviruses enter the cell by endocytosis 204 Bunyavirus mRNA synthesis is primed by the capped 5' ends of cellular mRNAs 204 Coupled translation and transcription may prevent premature termination of mRNAs 206 Genome replication begins once sufficient N protein is made 206 Virus assembly takes place at Golgi membranes 206 Evolutionary potential of bunyaviruses via genome reassortment 207 18. Because vaccinia virus is the vaccine strain, it is relatively safe to work with and has been studied in clinical and laboratory situations for over 200 years. The ϕ (Greek letter phi) in ϕX174 refers to "phage"; the X is the Roman numeral 10; "race X" bacteriophages were unusually small, as determined by ultrafiltration, and the first isolate was placed in a test tube labeled 174. TLR-9 recognizes both single-stranded and doublestranded viral DNA from various members of the herpesvirus family as well as unmethylated CpG dinucleotides in bacterial DNA. Uncoating of herpesvirus and adenovirus genomes takes place at the nuclear pore, as the capsids are dissassembled there. The single-stranded DNA molecule is tightly compacted within the capsid. HE has functional similarities to two influenza virus proteins: the receptor-binding hemagglutinin protein (HA) and the receptor-destroying enzyme neuraminidase (NA) (see Chapter 18). (b) gp120 undergoes a conformational change that exposes regions that bind to chemokine receptors (CCR5 or CXCR4). PrPC is presumed to function in transmembrane transport or signaling. Study of tumor viruses led to the discovery of viral and cellular oncogenes. Phycodnaviruses have particle diameters from 100 to 220 nm, and contain double-stranded DNA genomes ranging from 160 to 560 kb. When E2F is freed from bound Rb, it can induce the expression of a number of genes whose products initiate and carry out cellular DNA synthesis. The remaining 9% of infected adults become chronic carriers, almost half of whom may eventually develop liver cancer. Most filovirus outbreaks have occurred in equatorial Africa Marburg virus and the Ebola virus species Bundibugyo, Cote d'Ivoire, Sudan, and Zaire ebolavirus are indigenous to the African continent (Figure 16.8). Equine, bovine, feline immunodeficiency viruses. A number of families that suffer from genetic Gerstmann–Sträussler–Scheinker syndrome have a common double mutation in PrPC at amino acids 178 and 200 (D178N; E200K), while other such families have a mutation of leucine to proline at amino acid 102 (P102L). 3 in Condit, R.C. and Niles, E.G., "Orthopoxvirus genetics," Current Topics in Microbiology and Immunology 163 (1990): 1–39, with kind permission of Springer Science and Business Media (Heidelberg); Fig. However, aluminum-based adjuvants do not efficiently induce cellular immune responses. Proto-oncogene: a cellular regulatory gene that, when mutated or overexpressed, can cause cellular transformation and oncogenesis. (a) Tobacco mosaic virus. Electron micrograph of a thin section of vaccinia mature virus, showing the envelope, lateral bodies, the core wall and the internal tube. The preterminal protein primes DNA synthesis carried out by viral DNA polymerase Once E1A has induced the cell to enter S phase and the early viral gene products have accumulated, viral DNA replication begins. Other advantages of these simpler vaccines include ease of manufacture and the possibility of combining several antigens in multivalent formulations to protect against a range of pathogens. In contrast, CH001.indd 10 individual red blood cells slide to the bottom of the tube and form a compact, dark red pellet (Figure 1.4). Togaviruses enter cells by low pH-induced fusion inside endosome vesicles The E2 glycoprotein binds to cellular receptors. Such cellular parasites always contain their own ribosomes and protein synthetic machinery, and their genes code for enzymes that direct many of the basic metabolic pathways. E2 423 6K 55 E1 439 Carboxy-terminal part of PE2, a component of virus envelope projections. Besides their antiviral effects, interferons inhibit cell growth and division and stimulate the immune system. Virus infection induces both extrinsic and intrinsic pathways of apoptosis, leading to the activation of cysteine proteases called caspases. This site contains a stretch of seven U residues, which are transcribed into A residues in positive-sense messenger RNA. Instead of releasing free progeny plusstrands, this complex now associates with preformed procapsids (Figure 6.5) and inserts the plus-strand DNA into the procapsid as this DNA strand is displaced from the double-stranded template. These noncoding sequences contain extensive secondary and tertiary structure (see pg 130). Harvest recombinant virus Figure 37.1 Construction and production of a typical virus vector. The travel distance can be a million times the capsid diameter! Diffusion of macromolecular assemblies in the cytosol is severely limited due to molecular crowding by organelles, the cytoskeleton, and macromolecules. First, it facilitates Th1 cell production by enhancing the synthesis of interleukin-12 in antigen-presenting cells (Figure 34.5). Four IS elements are present in the viral DNA. These can be bound to proteins on the cell surface. A delicate control mechanism senses the amount of N protein present in the cell, allowing full-length genome RNA to be made only if there is sufficient N protein to encapsidate it. Over the next 20 years, study of the RNA phages became a growth industry. Temperature-sensitive mutants defective in DNA replication have been isolated in six of these genes (noted in Table 26.3), proving that each of those enzymes is required for viral DNA replication. An alternative start site lies in a distinct and overlapping reading frame that extends through most of the mRNA to give rise to a longer protein denoted P (the polymerase cofactor). It can be thought of as deoxyguanosine missing the CHOH moiety at the 3' position and the CH2 moiety at the 2' position of ribose. Their promoters lie upstream of CH030.indd 369 the polyadenylation signal, but RNAs initiated at these promoters are not cleaved on first passage of the polyadenylation signal, which lies very close to the promoter region. Upon binding to its target DNA sequence, the ZFN creates a double-strand break that is quickly repaired by the cellular machinery by homologous recombination with the sister chromatid. The polymerase is not competing with ribosomes for binding to the minus-strand RNA, explaining the lack of requirement for S1. G Major spike protein; 60 copies in virion. Reddy. A related process may mediate uncoating of some positive-strand RNA animal viruses. However, the four glyscolyated asparagine residues are not located in typical eukaryotic consensus sequences, suggesting that PBCV-1 encodes most, if not all, of the machinery Figure 27.3 Structure of PBCV-1 virion. The retrovirus proviral DNA is larger than the genome RNA. Nevertheless, these viruses are widespread and, in those individuals where there is long-term persistent viral infection and lack of immune clearance, oncogenic papillomaviruses are potent human carcinogens. It was quickly discovered that polyomavirus early genes act as oncogenes both in cells cultured in vitro and in intact animals. 409 Multiple cellular responses affecting cell growth and differentiation, resistance to a wide range of pathogens, and modulation of immune functions are induced by interferon via the Jak–Stat pathway. The major functions are involved in memory and navigation. Vaccination for smallpox has now been discontinued, since the small chance of serious complications from vaccination exceeds the negligible risk of contracting smallpox. Three promoters (PA, PB, and PD) and four terminators (TJ, TF, TG, and TH) have been detected. The original member of this family, APOBEC1, was so named because it deaminates a specific cytidine residue in the mRNA coding for Apolipoprotein B, allowing two proteins to be made from the same gene using either the native or edited messenger RNA. However, these vectors are more difficult to produce; it is almost impossible to generate a stable cell line that complements all of the essential adenovirus genes, due to the toxicity associated with the over-expression of most of these proteins. IVa2 cooperates with factors bound to an upstream enhancer to activate transcription. Proteolytic processing of the rubella virus nonstructural polyprotein (p200) also appears to regulate replication and transcription of the viral RNA. This rearrangement creates openings in the shell lattice for D trimers that fill the sites and greatly stabilize the shell. The presence of the RNA polymerase holoenzyme (6 subunits of RNA polymerase), together with a family of novel promoter sequences, indicates that coccolithoviruses have their own transcription machinery. As this plus-strand copy is synthesized it is complexed with NP protein, eventually forming nucleocapsids containing plus-strand antigenome RNAs. In this mode, the RNA polymerase does not stutter and terminate at the poly(U) stretch, but continues to the 5' end of the genome RNA, generating a full-length plusstrand copy. The gene 4 primase/helicase protein subsequently binds to this replication fork, laying down a short RNA primer on the opposite ("lagging") DNA strand and simultaneously unwinding the DNA to allow the T7 DNA polymerase to continue forward on the leading-strand. However, the antiviral activity of interferon is generally limited to the animal species in which the interferon is produced. This proteinase cleaves at specific dipeptides of the form glutamine-X, where X can be glycine, serine, or a number of other amino acids with hydrophobic side chains. Many different strains of this virus have been found all over the world. The fully double-stranded DNA is nicked at a specific site, just opposite the original 3' end, by Rep78/68 of adeno-associated virus (or its equivalent NS1 in the case of minute virus of mice). Following a mixed infection of a cell with two parental viruses, progeny containing segments derived from the different parents may be produced (Figure 17.5). F-pili are made from a single protein polymerized into a long (1–2 m), ribbon-like structure that protrudes from the cell. circles, as in the original supercoiled molecule but unlike "nicked" relaxed circular DNA. Noncoding regions that include replication promoters and the internal promoter for subgenomic RNA synthesis from antigenome RNA are shown in black. IgM is a pentamer of the basic immunoglobulin structure, and contains a 15-kDa J chain that joins the ringed structure. The first isolate, named f1, made turbid plaques but the second isolate, named f2, made clear plaques; Loeb and Zinder decided to concentrate their work on phage f2. For example, the U3 sequence of mouse mammary tumor virus contains elements that confer responsiveness to glucocorticoids. Moreover, it inhibits viral DNA polymerases more potently than cellular DNA polymerases. One of these viruses has a circular single-stranded DNA genome, but all others have either linear or circular double-stranded DNA genomes. Antigenome RNA is in turn replicated to form genome RNA. Because the infected neurons eventually die, the current alphavirus vectors would not be appropriate for any type of treatments in the human nervous system. Regulatory sequences in genome and subgenomic RNAs that coordinate the levels of translation, and the switch from translation to replication, have been identified. First, one copy of each of the 10 reovirus mRNA molecules is assembled into a subviral particle; second, each plus-strand mRNA within this particle is copied by viral RNA polymerase to form the 10 different double-stranded genome RNAs. This mechanism ensures that one molecule of each gene segment is incorporated into every virion. This protein counteracts host antiviral responses by blocking the systemic signal of RNA silencing, and therefore facilitates local and systemic infection. A TATA box just upstream of the U3/R junction directs the initiation of transcription by cellular RNA polymerase II (Figure 28.6). Functions of P, C, and V proteins The functions of the different proteins made by the P/C/V gene are still being worked out. Autonomous parvoviruses replicate only in S-phase cells. Acheson, McGill University Daniel Kolakofsky, University of Geneva Christopher Richardson, Dalhousie University Revised by: Laurent Roux, University of Geneva 16. TFIID recruits RNA polymerase II and a number of general transcription factors, named TFII A to H, to a site approximately 30 nt beyond the TATA box. One origin (oriL) is in the middle of the UL region, and the other two (oriS) are in the c inverted repeats flanking the US region (Figure 24.2). Unlike most other viruses, retroviruses package two identical copies of the genome in each virion. Immature virions then develop the distinguishing internal core and lateral body structures by rearrangement and assembly of the material captured inside spheres, and in the process acquire the final brick shape to become mature virus (MV). Cellular recognition of virus infection leads to production of cytokines One of the first responses of an organism to virus infection is the secretion of cytokines (Table 33.2), which are proteins that stimulate protective reactions in the 403 infected host. • In other cases, enveloped virions are first taken up in endosomes, caveosomes, or pinocytic vesicles before fusion occurs. Integrase catalyzes a crucial step in HIV genome replication: integration of the DNA copy generated by reverse transcriptase into host cell DNA. A map of the Zaire ebolavirus genome is shown in Figure 16.3. As with paramyxoviruses and rhabdoviruses, the gene coding for the nucleocapsid protein (NP) is located closest to the 3' end of the genome, and the gene coding for the RNA polymerase (L) is located closest to the 5' end. Virions are formed by budding in the endoplasmic reticulum Because more S mRNA is made than preS1 mRNA, larger amounts of the SS and MS proteins are made than LS protein. The largest known viruses are some 500 nm in diameter; their genomes are as large as 1.2 million nucleotides, and they code for over 1200 proteins. This implies that they are in genetic contact with each other and are therefore members of a common genetic lineage. This family includes hundreds of viruses of mammals, birds, and reptiles. Scientists working in England and France discovered in 1915–1917 that bacterial cultures could be lysed by filterable agents, the first known bacterial viruses. 20.2: Reproduced by permission of Edward Arnold from Fig. However, unlike T cells activated by antigens, T cells activated by Nef cannot effectively mount an immune response. Alternatively, the enzyme topoisomerase I can bind to the DNA and unwind the supercoils by breaking a phosphodiester bond, allowing one of the broken ends to rotate around the opposite DNA strand, and then resealing the phosphodiester bond. • Hepatitis B virus can cause cirrhosis and is the leading cause of hepatocellular carcinoma (liver cancer). Thus, the biological functions of these satellite RNAs depend on their direct interactions with viral or host cell proteins, nucleic acids, or other cellular components. Movement of the polymerase away from the point of initiation is facilitated by phosphorylation of the carboxy-terminal domain. Both the genome RNA and the subgenomic mRNA have methylated caps at their 5' ends and are polyadenylated at their 3' ends, resembling cellular messenger RNAs. Translation of genome RNA begins at an initiator AUG near its 5' end. Prior to the sudden emergence of the porcine H1N1 pandemic influenza virus in Mexico in mid-2009, the principal focus of the world's influenza experts had been avian influenza. Chapter 26 Fig. These viral IRFs block the interferon response, allowing more efficient replication of human herpesvirus 8. It is not known how these elongation factors inhibit synthesis of movement protein. These findings suggest that internal scaffolding proteins may be inherently flexible and may undergo folding directed by their contacts with structural proteins. different strategies for controlling the expression of viral genes. The mimivirus virion is highly complex and has been shown to contain at least 130 different virus-coded proteins, including a set of proteins homologous to the subunits of eukaryotic RNA polymerase. show sequence identity. 05/05/11 2:45 PM 482 Glossary Spasticity: continuous contraction of certain muscles, usually resulting from damage to brain centers that control voluntary movement. Polyhedrins and granulins from different baculoviruses have highly conserved amino acid sequences. The S protein is synthesized as a single polypeptide chain that in some coronaviruses is cleaved by a cellular proteinase to yield an amino-terminal S1 domain and a carboxy-terminal S2 domain. CH035.indd 443 16/05/11 8:21 PM C H A P T E R 3 6 Antiviral Chemotherapy Donald M. In 1856, A. Virions produced in the absence of Nef appear to have a reduced ability to complete proviral DNA synthesis upon infection of target cells. The same enzyme then extends the primer by adding a short (20–30 nt) DNA molecule. In 1978, Fred Sanger and colleagues were the first to determine the nucleotide sequence of the entire genome of an organism, and that organism was ϕX174. A number of related polyomaviruses have been discovered in rodents, birds, cattle, monkeys, and humans. During asymmetric replication, the multimeric minus-strand linears resulting from step 2 are directly copied into mutimeric plus-strand linears (dashed horizontal line with vertical arrow), thus skipping steps 3–6. CI's weak autoprotease activity is also stimulated by activated RecA, leading to linker cleavage that inactivates CI (Figure 8.5d). Homozygosity at this locus was also present among most victims of kuru. The genome of SSV-1 is a circular double-stranded DNA molecule of 15.5 kb and carries 34 open reading frames, most of them of unknown function (Figure 9.3). These viruses usually cause tumors only under very specific conditions: injection of high titers of virus into particularly susceptible animals shortly after birth. Lagging-strand DNAs are extended up to the 5⬘ end of the leading-strand DNA and the strands are linked by DNA ligase to produce a single, covalently joined daughter strand on each template strand. Which elements of the host antiviral responses does the influenza NS1 protein inhibit? These are covalently attached to core proteins to form proteoglycans, found on the cell surface. Capped RNA fragment remains at 5' end of template 1' Cap 2' Capped RNA fragment translocates to DR2 and acts as primer for plus-strand DNA synthesis. An example of a capsid- binding drug is pleconaril, which is active against many picornaviruses, including rhinoviruses. Simian immunodeficiency virus. Because the genome RNA is infectious, viable mutant viruses are produced. HOST CELL RESPONSE TO VIRUS INFECTION Double-stranded RNA is a potent stimulator of signal transduction cascades. At this point, the A protein cleaves off a full-length plus-strand and ligates it to form a single-stranded circle (step 4). This could have eventually led to the selection of RNAs that coded for a polypeptide with RNA polymerase activity, increasing the rate and efficiency of RNA replication beyond that of a purely RNA-based enzymatic activity. Cytoplasm: the portion of a euykaryotic cell enclosed by the plasma membrane, excluding the nucleus. JAM-A is a transmembrane protein with two immunoglobulin-type domains in the extracellular region. The preS2 domain also contains a glycosylation site at asparagine-4 that is always glycosylated in the MS protein. This allows the capsid to undergo a conformational change that results in the eventual entry of the genome RNA into the cytoplasm. Tenaillon, R. It is important to understand that the safety demanded of a vaccine can change in different situations. This allowed scientists to introduce mutations into the viral genome and therefore to study the functions of each gene. Its amino terminus and most of its mass reside on the outside of the envelope. ϕX174 attaches to host cells via sugar residues, most likely glucose, in the lipopolysaccharide that is a part of the outer membrane of gram-negative bacteria. Domain III Fusion peptide Domain I Domain II Figure 12.3 The atomic structure of the tick-borne encephalitis virus E protein dimer. Surprisingly, analysis of its genome sequence suggests that this pandemic virus strain arose from an avian virus by mutation ("drift"), rather than by reassortment. Because many (approximately 105) red blood cells are used in each tube, one hemagglutinating unit represents 105 or more virus particles. The separation of the complementary strands of T7 DNA was particularly easy by equilibrium sedimentation in CsCl in the presence of excess poly riboG. The results were astounding: many of the genes overlapped! One region of DNA could even encode part of four different genes (Figure 6.1). However, the 3' splice site at nt 811 lies just downstream of the UAG that terminates small T antigen translation. Viroids use cellular DNA-dependent RNA polymerases to replicate their RNA genomes. Inactivation of CI leads to transcription from PR (see Table 8.1) and PL, so that the early genes cro and N are transcribed. Cbz carboxybenzyl; Me methyl. The expansion of the B-lymphocyte population and their killing by cytotoxic T lymphocytes can lead to the symptoms of infectious mononucleosis. These reactions probably occur in sequence as the RNA progresses through the 2 spike during transcription. J Small DNA-binding protein, needed for DNA packaging; 60 copies in virion. Astonishingly by today's ethical standards, the appearance of the attenuated phenotype was typically demonstrated by inoculation of children! Several of our most successful vaccines, including yellow fever, measles, mumps, rubella, and Sabin polio, were generated in this way. A cDNA CH016.indd 192 copy of the viral genome is placed under the control of a bacteriophage T7 promoter such that transcription directed by T7 RNA polymerase (see Chapter 7) initiates at the exact position of the 5' end of the genome. Like all anti-HIV drugs, enfuvirtide is administered as part of a multi-drug cocktail, which reduces the chances of the development of drug resistance. Autoreactive T lymphocytes: T lymphocytes that recognize antigens on normal cells in the body, and destroy them. Mutations are then introduced in the cDNA, the mutant RNA genome is transcribed from the mutated cDNA, and the infectious RNA is introduced into cells to generate infectious mutant viruses. These have included not only the "Spanish flu" pandemic in 1918–1920, but also the "Asian flu" in 1956 (60,000 deaths in North America) and the "Hong Kong flu" in 1967. GENES AND PROTEINS mRNAs are full-length copies of each genome segment. associated with the original virion core structure (Figure 27.14), similar to the life cycle of poxviruses (see Chapter 26, Figure 26.5 and 26.7). Ubiquitous in the marine environment. The molecular biology of poliovaccines. Our understanding of these drugs comes largely from x-ray crystallographic studies of complexes between picornaviruses and such drugs. Viral glycoproteins are inserted into the lipid membrane to form the envelope Most of the proteins found in viral envelopes have a relatively large glycosylated external domain (ectodomain), a hydrophobic transmembrane anchor of about 20 amino acid residues, and a short internal tail. The transfer construct. Messenger RNA export. (b) Section through the edge of a particle in the same orientation after removing the stargate structure, showing a depression in the core (in white). Virus production does not result in cell lysis, nor does it slow growth of cells, which double every 24 hours. Note the pore at the 3-fold axis on the procapsid, absent in the virion. One factor affecting protein abundance is the length of the mRNA; the longer the transcript, the longer the time required for its synthesis, and therefore long mRNAs CH019.indd 231 231 are less abundant than shorter transcripts. The identification of Reston ebolavirus in 1989 suggested for the first time the presence of an Asian filovirus that may be associated with wild nonhuman primates. Jenner's bold experiment represented a synthesis of three concepts, all of which were fairly common knowledge at the time: (1) cutaneous variolation produced resistance to smallpox, (2) milkmaids were rarely pockmarked and often resistant to variolation, and (3) milkmaids often acquired pock-like lesions on their hands, called cowpox disease, presumably from milking cows bearing similar lesions on their teats. This is the subject of the present chapter; most of the information presented here refers to the mammalian immune system, particularly that of man. Once doublestranded RNA is made, the replicase particles are able to synthesize more plus-strand viral mRNAs. Transcription by these "secondary transcriptase particles" accounts for the bulk of the mRNA made in reovirus-infected cells. The budded virions produced by these infections are released into the hemocoel and spread to infect other tissues including hemocytes, fat body, and epidermis. After exposure to low pH, the dimer dissociates and homotrimers are formed. This not only inhibits expression of cellular genes, most of which make spliced mRNAs, but also inhibits the expression of the three genes (ICP0, ICP22, and ICP47) that contain introns. There is a conserved motif (Tyr-X-Leu) in the carboxy-terminal tail of the E2 glycoprotein that interacts with a hydrophobic pocket on the surface of the capsid protein. The resulting molecule, shown in step 7 of Figure 30.5, is identical to the molecule shown in Figure 30.2a. attP is ~250 bp long, with multiple binding sites for Int; in contrast, attB is about 25 bp long. Sindbis virus (named for an Egyptian village) and Semliki Forest virus (from Eastern Uganda) are two togaviruses that have been studied in great detail in the laboratory. These dimers block progression of the RNA polymerase along the template, and therefore inhibit all RNA synthesis downstream of the lesion. Which of the two hantavirus glycoproteins is involved in the interaction so far remains unknown. This is also called local movement. Sickle cell disease: a hereditary form of anemia caused by an abnormal type of hemoglobin and characterized by unusual crescent-shaped red blood cells—"sickle cells." Sieve elements: a cell type in the phloem, sieve elements combine and form tubes that are used to transport nutrients. Phaeoviruses have a temperate life cycle and integrate their genomes into the host The life cycle of the filamentous brown alga, Ectocarpus, is perhaps the best studied, and it serves as a model organism for studying the molecular genetics of brown CH027.indd 335 335 algae. These structures have been shown to be responsible for the production of monomers both in vivo and in vitro. Table 24.2 Functions of herpes simplex virus ␣ proteins Protein Activities ICP0 Promiscuous activator of gene transcription (viral and cellular). The virons of these two viruses also resemble each other, and they are clearly related. CH031.indd 385 KEY TERMS ␣-amanitin Chimera Delta structure Fulminant hepatitis Hairpin structure Hammerhead structure Helper virus Interferon PKR (double-stranded, RNA-dependent protein kinase) Ribozyme RNA interference Rolling circle replication 16/05/11 8:17 PM 386 CHAPTER 31 Viroids and Hepatitis Delta Virus FUNDAMENTAL CONCEPTS • Viroids, plant satellite RNAs, and hepatitis delta virus are small, circular, single-stranded infectious RNAs. • Viroids and plant satellite RNAs do not encode any proteins. This blood-borne pathogen replicates in the liver and frequently causes fulminant CH031.indd 383 383 hepatitis in both primate and nonprimate hosts. Viral genomes are very compact and economical; most nucleotide sequences are used for protein coding, and a good yardstick is one protein coded per 1000 nucleotides. THE INNATE IMMUNE RESPONSE Complement proteins mark invading pathogens or infected cells for destruction The term complement refers to a self-regulating cascade of over a dozen proteins that were originally shown to "complement" the specific antibacterial activities of antibodies by a classical activation pathway. CH025.indd 308 Table 25.3 Autographa californica MNPV proteins that regulate late transcription Protein Mol. paper) 1. However, HIV-1 does not enter cells unless its envelope glycoprotein gp120 subsequently binds to one of two host cell chemokine receptors, CXCR4 or CCR5. ADENOVIRUS VECTORS Are easy to produce at high titers. The functional relevance of these energetically costly biosynthesis pathways remains to be elucidated; however, it is interesting to note that the chloroviruses also encode genes for enzymes involved in sugar degradation, which are essential for viral infection. The lytic growth cycle produces up to 200 progeny viruses per infected cell and causes cell lysis. At the junction between each gene is a highly conserved set of "intergenic sequences" that control transcription termination, polyadenylation, and reinitiation. The horror of watching an individual die from rabies virus encephalitis or Ebola virus hemorrhagic fever is surely the same today as it was for our hunter-gatherer ancestors. Such recombination is particularly frequent in 3' noncoding regions, probably because they have substantial secondary structure and share common sequences. (b) Electron micrograph of negatively stained nucleocapsids purified from Sendai virus. Envelope glycoproteins are also arranged with icosahedral symmetry, T 4. This is an alternative folding configuration that is stable enough to temporarily prevent the RNA from reaching the equilibrium structure. • Herpes simplex virus genomes have inverted repeat sequences flanking unique sequence elements, as well as direct terminal repeated sequences. Virions have a lipid-containing envelope and two major structural proteins. Receptor binding, fusion of virus envelope with cell membrane. Morrighan, Science. RNA 1 has a single open reading frame (protein 1a), RNA 2 has two overlapping reading frames (proteins 2a and 2b), and RNA 3 has two non-overlapping reading frames (protein 3a and coat protein [CP]). Virion formation begins by budding of nucleocapsids through the inner nuclear membrane. The remaining parts of both light and heavy chains have invariant or constant amino acid sequences. If the phage had not made the proper arrangements, the replicase and the translating ribosome would meet somewhere on the RNA and sit facing each other forever (Figure 5.4)! Thus a molecule of phage RNA that begins to be translated must not be allowed to begin replication, and vice versa. Häring, R. Because these are important mediators of immune responses (see Chapter 34), their absence can be important in disease progression. Further modifications increased potency by more than 100-fold, resulting in a compound that can inhibit the protease when present at picomolar (10-12 M) concentrations. The virion adsorbs to a receptor on the host cell with its bearded end. However, different members of a virus family may vary significantly in virion size and genome

length, and may have a variety of unique genes not shared by other family members. This activity, along with the nucleoside triphosphatase in the C-terminus of NS3, is involved in the formation of the methylated cap found at the 5' end of flavivirus genomes. E 493 Major envelope glycoprotein. • Some virus particles may contain defective genomes. These include resistant and 16/05/11 8:01 PM Cucumber Mosaic Virus susceptible responses, symptomatic and asymptomatic infections, disease and tolerant responses. DISTINCTIVE CHARACTERISTICS The largest known RNA virus genomes. Activities such as nursing and preparing bodies for burial are associated with an increased risk. The predicted 366 PBCV-1 protein-encoding genes are evenly distributed on both strands and, with one exception, intergenic space is minimal. Finally, many of the scientific advances outlined above have to some extent democratized the process of vaccine development, which was once the exclusive purview of the major pharmaceutical companies. Injection of these vectors into animals showed them to be effective in inducing an immune response and, in the case of influenza and herpes viruses, protecting the animal from subsequent infection. (a) Changes in viral RNA and CD4-positive T cell levels in the peripheral circulation from acute infection to end-stage disease. This has been especially true in the Americas, which suffered dramatic outbreaks of yellow fever, transmitted by mosquitoes, in the 1700s and 1800s. CH025.indd 311 19/05/11 12:19 PM C H A P T E R 2 6 Poxviruses Richard C. 29.1: Reprinted from Panteleo, G. Immunization with one or a small group of viral antigens will generate an immune response to only a limited 16/05/11 8:15 PM Human Immunodeficiency Virus (a) Virion gp41 gp120 CD4 Plasma membrane Cytoplasm CCR5 (b) gp41 (c) 359 via the nuclear pores, and can therefore infect cells that are not actively dividing. These virions subsequently infect the midgut connective tissue, tracheal epidermal cells and tracheoblasts, initiating a second round of replication. RT IN HIV-1 targets cells of the immune system by recognizing CD4 antigen and chemokine receptors Figure 29.2 Diagram of structure of HIV-1 virion. • Prion diseases of humans may have much in common with more familiar ailments such as Alzheimer's and Parkinson's diseases. At a later stage, viral DNA enters the spheres to form a discrete electron dense nucleoid, perhaps a precursor to the tubular structure in the interior of the core in mature virions. coli cell is by no means clear. Recent advances have been made in concentrating and detecting abnormal PrP in blood and lymphoid tissues 16/05/11 8:17 PM Prions such as tonsils. Phyla (singular, phylum): a taxonomic rank, or grouping of related organisms, between "kingdom" and "class." Picornavirus 3C-like proteinases: enzymes that have structural homology to the picornavirus 3C proteinase, which is involved in cleavage of picornavirus polyproteins into mature proteins. These mutants, however, become fully virulent in mice lacking components of the interferon pathway that control PKR. They may also be significantly cheaper to manufacture, because they do not require expensive virus growth in cell culture systems and testing of the virus produced. Each of these mRNAs is translated into a unique protein by use of reading frames beginning at different AUG initiation codons within the mRNA body. Their presence enables the replication machinery to begin to produce full-length, plus-strand copies of the eight viral genome segments (step 5, Figure 18.6; Figure 18.8). The related toll-like receptors TLR-7 and TLR-8 recognize guanosine- and uridine-rich single-stranded CH033.indd 400 CpG CpG DNA TLR-7/8 TLR-3 dsRNA ssRNA Phagosome Endosome Cytoplasm Nucleus Figure 33.1 Toll-like receptors that recognize virus components. This is a common route for production of vaccines when a live virus vaccine is not available. SERT (b) Figure 5.9 Detection of minute amounts of target RNAs using Q replicase. Recent studies CH013.indd 155 155 suggest that in some viruses, the so called New World alphaviruses, the sequences controlling cytopathogenicity reside in the capsid protein and affect the survival of vertebrate cells through a different mechanism than nsP2 of the Old World alphaviruses. That region serves as a signal for the assembly of a primosome by seven different host cell proteins. PM plasma membrane; NE nuclear envelope. These are involved in adsorption to the host cell. Conical capsid with probable icosahedral symmetry. Transcription termination occurs in response to a sequence element, U5NU, in the growing RNA chain, CH026.indd 318 and requires two protein factors: a DNA-dependent ATPase and the same cap guanylyltransferase that modifies the mRNA 5' end. D External scaffolding protein, required for procapsid morphogenesis; 240 copies in procapsid. For an idea of how this is carried out, see Figure 20.4. Assembly of progeny virions begins by the formation of an empty prohead structure that includes the gene 9 product as well as the proteins of the mature capsid. Cytomegalovirus produces a non-functional MHC class I decoy that binds to the inhibitory receptors on NK cells, preventing NK-cell activation. Recombinant AAV vectors are deleted of all viral genes and consequently do not express the Rep proteins. Flaviviruses enter the cell by pH-dependent fusion Although possible cellular receptor molecules have been suggested for several flaviviruses, none has been clearly identified. Onyx-015, also known as H101, has been commercialized in China for the treatment of head and neck cancer since 2005. • T7 DNA replication begins at the first transcription initiation sites for T7 RNA polymerase and is mediated by RNA primers made by T7 RNA polymerase. GENES AND PROTEINS Each genome segment codes for one or two proteins • • • • • • • • Envelope glycoproteins: hemagglutinin (HA) and neuraminidase (NA) Integral membrane protein (M2) with ion channel activity Matrix protein (M1) Nucleocapsid protein (NP) Three RNA polymerase proteins (PA, PB1, and PB2) Nonstructural protein (NS1) Minor structural protein (NS2) Additional nonstructural protein (PB1-F2) VIRUSES AND HOSTS Influenza types A, B, and C: hosts include birds, various mammals and humans. These differences in growth are not observed in non-neuronal cells, indicating that a host cell factor in neurons may have a defective interaction with the mutated RNA. TLR-2 TLR-4 TLR-9 Several toll-like receptors recognize viral nucleic acids Viral nucleic acids are the most potent inducers of intrinsic and innate antiviral responses. The sequences at the 3' and 5' ends of minute virus of mice are different from each other. Hairy-cell leukemia: a malignant blood disease caused by proliferation of a type of B cell. RNAs are shown in blue and DNAs in black. The terminal A or G residue is hydrogen-bonded to the 3'-terminal U residue on genome RNA, and PB1 acts as an RNA polymerase by adding ribonucleotides complementary to the genome RNA sequence, proceeding toward its 5' end. There is evidence that one copy of each of the eight genome segments is incorporated in a bundled form into each virion. Thus bats would be the main animal 16/05/11 8:04 PM Coronaviruses 161 It is still not clear which pathway was taken by viruses to become SARS coronavirus, but several possibilities exist. Recently the first crystal structure of the head shell of a tailed dsDNA bacteriophage was determined for the -like phage HK97. Conversely, proteins or antibodies that bind to the receptor on the cell surface can be used. Two identical molecules, 7–10 kb, packaged in virions. Infected animals suffer from neurological symptoms that can cause tameness or unusually aggressive behavior, leading to direct contact with other animals or humans and thus helping to spread virus infection. Interestingly, the first commercial gene therapy product to be approved was also an adenovirus used against cancer. Because viroids do not code for any proteins, their effects on plants must be a consequence of direct interactions between the viroid RNA and host constituents. NS2B. 23.1, 23.2: Reprinted from Fig 1a in Rux, J.J, and Burnett, R.M., "Review: Adenovirus Structure," Human Gene Therapy (2004) 15: 1167–1176, with permission from Mary Ann Liebert Publishers. (a) Negative contrast electron micrographs of virions (Scale bar: 50 nm), (b) terminal structures at higher magnification, (c) drawing of a typical gammalipothrixvirus. There has been a suggestion that the presence of Nef might facilitate the passage of the nucleocapsid from virions into the cytoplasm by altering the structure of the actin matrix that lines the inner surface of the plasma membrane, where nucleocapsids are released after fusion. The newly introduced rotavirus vaccines based on recombinant human and bovine chimeric viruses are good examples. The 3' and 5' ends of the DNAs of some parvoviruses such as minute virus of mice have different sequences, which leads to a modified and more complex replication scheme, notably involving a dimer-length replicative intermediate. The 55K protein binds directly to p53 and blocks transcription from p53-dependent promoters, therefore inhibiting p53-dependent apoptosis. These proteins are called virokines and viroceptors, and they combat host immune responses against these viruses. Two of the four possible introns (62 nt and 48 nt in length) removed during splicing of mouse polyomavirus early transcripts are unusually small. LEF-6 20 mRNA transporter? Recognition of cytosolic DNA by DAI leads mainly to the production of interferon. Since the GDP-GTP exchange reaction is blocked, insufficient met-tRNA-eIF-2GTP complex is formed, and translation initiation can no longer take place. The carbohydrate groups (glycans) are shown as blue ovals at top left. 05/05/11 11:24 AM Mimivirus newly isolated phycodnaviruses have shown even closer phylogenetic relationships with the mimivirus lineage, further justifying its inclusion in this chapter. DISEASES Viroids are transmitted between plants via mechanical breaks and insects. Sheehy, College of the Holy Cross Kenneth Stedman, Portland State University Carol St. Angelo, Hofstra University Suresh Subramani, University of California, San Diego William Tapprich, University of Nebraska, Omaha Milton Taylor, Indiana University Michael N. At least four 365 CH030.indd 365 05/05/11 1:08 PM 366 CHAPTER 30 Hepadnaviruses other hepatitis viruses, named D (or delta) to G, are presently known. Humans: human coronaviruses 229E, OC43, NL63, HKU-1; SARS coronavirus. Prostration, lethargy, wasting, and diarrhea seem to be more severe in filovirus hemorrhagic fever than in patients suffering from hemorrhagic fevers caused by other viruses. When we considered creating a second edition, my editor and I solicited reviews and suggestions for improvements from a number of university teachers. The helper plasmid provides adenovirus helper functions (listed on right). However, the A* and K proteins are not essential for virus replication, and the B protein has a flexible structure that is highly tolerant of amino acid substitutions (up to 70% of its sequences can be altered with minimal effects on its functions). (b) Open reading frames and translation products are shown above each RNA. Large T antigen has a conserved binding site for the Rb protein (Figure 21.10). This 371 occurs in a "packaged" RNA-protein complex including both the C and P proteins. CH034.indd 420 Figure 34.5 Generation of helper (Th) and cytotoxic (Tc) T lymphocytes. Release of the virions into the extracellular milieu results in release of the pr peptide and a fully infectious mature virion. Poxviruses have at least two different strategies for interfering with cytokine function. Since one turn of the helical nucleocapsid contains 78 nucleotides wrapped in 13 molecules of N protein (each N molecule binds to six nucleotides), these three conserved nucleotides lie exactly one turn of the helix from the 3' end of the RNA (Figure 15.5). Prevost, P. Antibiotics active against bacterial diseases began to be widely used in the 1940s. Some examples of such experiments are described below. Finally, a variety of devices are being tested to deliver tiny particles coated with either antigens or plasmid DNA at high velocity into superficial tissues and cells. More modern and rational approaches have been used to discover some newer drugs. (kD) RNA synthesis Transcription initiation RNA polymerase 8 410 Early gene specificity factor 1 94 Early gene initiation factor 2 166 Cap guanylyltransferase 2 130 Ribose-methyltransferase 1 39 Cap guanylyltransferase 2 130 DNA-dependent ATPase 1 72 mRNA 3' end polyadenylation Poly(A) polymerase 2 94 Nucleic acid topology RNA helicase 1 77 DNA helicase 1 56 DNA topoisomerase I 1 37 Protein kinase I 1 34 Protein kinase II 1 52 Protein phosphatase 1 20 mRNA 5' end capping Transcription termination Protein modification Virus-coded enzymes packaged in the core carry out early RNA synthesis and processing When purified vaccinia virions are permeabilized in vitro with a detergent and incubated with ribonucleoside triphosphates and S-adenosylmethionine, early genes are transcribed, and the resulting mRNAs are capped, methylated, polyadenylated, and extruded from the virion core. These vaccines typically require the addition of an adjuvant (a non-antigenic additive that enhances immunogenicity) to elicit a strong immune response. The four C-terminal residues of the NS1 protein constitute a PDZ ligand domain of the consensus sequence X-Ser/Thr-X-Val (where X is any amino acid). The roles of these conserved elements have not been precisely defined, but they may provide important RNA structure and sequence information required for binding of cellular proteins and the 40S ribosomal subunit. 37 yet been discovered. To understand this it is useful to consider the forces that drive the binding of ribosomes to translational start regions on prokaryotic messenger RNAs. There are at least three contributors to this binding energy: (1) base complementarity between 16S ribosomal RNA and the Shine-Dalgarno sequence just upstream of the start codon on the messenger RNA, (2) interaction of the anticodon on the initiator fmet-tRNA with the AUG start codon on the messenger RNA, and (3) binding of ribosomal protein S1 to pyrimidine-rich sequences frequently found upstream of the Shine–Dalgarno sequence. New virions can be seen assembling at the edges of these virus factories (Figure 27.14a). DNA replicates by a rolling circle mechanism. Class II fusion proteins: envelope glycoproteins that achieve fusion by a mechanism distinct from that of class I fusion proteins. Problems with virulent revertants led most wealthy countries to switch from the Sabin live vaccine to the Salk inactivated vaccine in the 1990s. Furthermore, unlike other circular DNA viral genomes, neither DNA strand forms a covalently closed circle. Apoptosis. (kDa) LEF-1 31 Primase for DNA replication LEF-2 24 Primase accessory factor, associates with LEF-1 LEF-3 45 Single-stranded DNA binding protein/ helicase transporter P143 143 Helicase, host range factor, transcription factor IE-1 67 May bind to replication origins DNAPOL 114 Function DNA polymerase with an EcoRI restriction site at its center. There is a single Marburgvirus species. These mRNAs are transported back to the cytoplasm (3) where they are translated. Propagation of the resulting vector usually requires coinfection with a wild-type virus or expression of missing viral proteins by the infected cell. • The polyomavirus genome is packaged with cellular histones to form a "minichromosome". The late promoter is active in differentiated keratinocytes (Figure 22.3). ss/dsDNA DNA polymerase (or reverse transcriptase) 5. Each large T antigen monomer in a hexamer binds a molecule of ATP. It is not known how this occurs. Biol. ISBN 978-0-470-90059-8 (pbk. Comparative genomics of archaeal viruses (a) (b) Figure 9.14 Archaeal viruses with icosahedral symmetry. The details of the inhibition of herpes simplex virus DNA polymerase by acyclovir triphosphate have been worked out with the purified enzyme in vitro. Infection by positive-strand RNA viruses delivers a messenger-sense RNA directly to the cytoplasm, where it can be translated by cellular ribosomes. Polyadenylation of freshly terminated mRNA 3' ends is catalyzed by a 2-subunit poly(A) polymerase, one subunit of which is the same cap ribose-methyltransferase that participates in mRNA 5' cap formation. This is carried out via the acetylation of nucleosomal histones and other proteins, resulting in a more relaxed chromatin structure ("chromatin remodeling") that is more accessible to specific transcription factors and is more easily transcribed. CD4 antigen: a protein on the surface of helper T lymphocytes that is involved in interactions with other cells in the immune system. Both Gn and Gc are type I transmembrane proteins, with hydrophobic membrane anchor regions near their C-termini. At this point, autonomous parvoviruses like minute virus of mice must wait until the cell enters the S phase to begin their replication cycle. • Loss of public confidence in vaccines can have devastating effects both for individuals who refuse vaccination and for populations. Some individuals at each end of the spectrum are quite prepared to label those at the opposite extremes as "abusers" of children. The small delta antigen is required for viral RNA replication, while the large delta antigen is required for genome packaging and is an inhibitor of viral RNA replication. The sequence overlap between the GP and VP30 genes, and the positions of the 3' and 5' ends of their mRNAs, are shown below. This is the basis of hemagglutination assays for viruses. During a cycle of productive infection with most of the known species, neither formation of any significant amounts of cell debris nor a decrease in cell density was observed. transport of any of the component RNAs or proteins; (3) the system is very specific and efficient, owing to the remarkable activity and promoter specificity of the T7 RNA polymerase and to the amplification of expression by synthesis of numerous T7 RNA polymerase molecules, which efficiently and repeatedly transcribe the gene of choice. Reovirus infection leads to activation of nuclear factor kappa B (NF-B), a family of transcription factors known to play important roles in regulating cellular stress responses, including apoptosis, and interferon regulatory factor-3 (IRF-3), which is required for expression of type I (or antiviral) interferons in many types of cells. Smallpox and rabies vaccines were groundbreaking developments in medical history. The packaging construct provides AAV Rep and Cap proteins. VP40 is a matrix protein, the spikes are formed by trimeric GP1/GP2 complexes, and VP24 is associated with the envelope. Viral envelope proteins are responsible for receptor binding and fusion with cellular membranes Figure 15.3 shows a schematic diagram of a typical paramyxovirus virion, emphasizing localization of proteins (not drawn to scale). How do the adenovirus E1A proteins induce apoptosis via p53? Human prion diseases can be either inherited or transmitted During the 1950s, an apparently transmissible spongiform encephalopathy of humans was identified among the Fore people in the mountainous interior of Papua New Guinea. Cucumber mosaic virus is transmitted in nature by aphids Cucumber mosaic virus is transmitted to other plants by more than 80 species of aphids, small insects that feed by sucking sap from plants. Influenza virus infections of the respiratory tract can lead to secondary bacterial infections The virus initially infects epithelial cells in the upper respiratory tract (Figure 18.1). The nature of the ends of the linear genome is presently unclear. This tether prevents the single-stranded genome from forming secondary structure by base pairing with itself. The two best characterized proteins, denoted 19K and 55K (for their molecular weights), are produced from a single 2.2-kb mRNA by initiation of translation in two different reading frames. These may be sugar residues on glycolipids or glycoproteins, or a variety of cellular membrane proteins. DISTINCTIVE CHARACTERISTICS Filoviruses are prime examples of (re)emerging infectious agents. Infectious subvirion particles can be formed extracellularly in the intestine or in the laboratory by in vitro treatment of reovirus virions with proteases. The proteinase activity of NS3 maps to the N-terminal one-third of the protein. K Not essential for virus growth. The smallest members of this group are the adenoviruses, whose linear DNA genomes code for 30 to 50 proteins. Tc cells are generated by the encounter between an antigen-presenting cell and a naïve CD8-positive T cell (Figure 34.5). Chemical analysis indicated that the ends of T7 DNA have unique sequences; this was a major achievement prior to the development of DNA sequencing methods. Another termination codon, UGA, is present just 7 codons beyond the splice junction, making an 85-amino acid protein called tiny T antigen (TTAg). Not surprisingly, plant and invertebrate viruses have evolved strategies to block RNA interference, including the production of proteins known as suppressors of RNA silencing (see Chapter 10). 5' cap 3' (ꞁ) genome RNA Translation/readthrough P1234 P123 Cis-cleavage at 3/4 site P123 P1 nsP4 23 nsP4 Trans-cleavage at 1/2 and 2/3 sites Replication nsP2 nsP4 nsP1 nsP3 nsP2 nsP3 nsP1 nsP4 3' 5' (ꞁ) antigenome RNA Replication 5' cap Transcription 3' 3' 5' cap (ꞁ) genome RNA (ꞁ) subgenomic RNA Figure 13.3 A model for the temporal regulation of minus- and plus-strand RNA synthesis. A proteinase K-resistant diglycosylated species is particularly prominent among the new variant CJD cases. However, in most cases, the H or HN protein is also required for fusion, implying that F interacts with H or HN to produce the fusion-active state. Phylogenetic analysis based on conserved genes in the NCLDV superfamily places mimivirus as a member of this group of viruses, closely related to the Phycodnaviridae family. 21.1: Courtesy of Jean-Yves Sgro, Institute for Molecular Virology, University of Wisconsin-Madison. Examples of how some adenovirus gene products help adeno-associated virus replication are: 1. The following discussion of the replication of HIV-1 will emphasize the unique features of this virus, including the roles of these additional viral proteins. Sections III through VII cover viruses of eukaryotes, with some emphasis on viruses that infect humans, although included are chapters on viruses that infect plants, insects, and algae. When bound to middle T antigen in the presence of activated c-Src, these proteins are in turn phosphorylated by c-Src and therefore activated. The recent discovery that coronaviruses may regulate replication fidelity suggests they may have novel mechanisms for adaptation to new hosts. Introns smaller than approximately 70 nt are not normally found in mammalian cells. Their discovery stimulated an enormous amount of research whose underlying hope was that interferons could be used to prevent and cure virus infections in humans. As a result, opportunistic infections by other pathogens are often fatal. About this time, phage genetics blossomed because of the discovery of conditional-lethal nonsense and temperature-sensitive mutants. and Schlesinger, M.J., "Togaviridae," Fundamental Virology, 3rd ed., Fields, B.N., Knipe, D.M., and Howley, P.M., (eds.). Latently infected B lymphocytes eventually exit the cell cycle and differentiate into long-lived memory B lymphocytes with a latency I gene expression pattern. During latency II, EBNA-2 Latent membrane proteins mimic receptors on B lymphocytes LMP1 LMP-1 is an integral membrane protein that mimics the cellular protein CD40 (Figure 24.12a), which normally responds to the presence of the CD40 ligand (CD40L). 27.5c: Reprinted from Figure 2d in Meints, R. The three envelope proteins spike (S), membrane (M), and small envelope (E) are shown. It is selectively phosphorylated by the viral UL97 protein kinase and, in its triphosphate form, selectively inhibits viral DNA polymerase. Despite the limited number of isolates, known species of archaeal viruses encompass an exceptionally broad range of unique morphological and genomic features. The protein is shown with the N-terminus at the left and the C-terminus at the right. These DNA polymerases are most similar in sequence to the type B DNA polymerases found in certain viruses of bacteria and eukaryotes, like the tectiviruses or adenoviruses, and are unusual in using proteins attached to the 5'-termini of linear dsDNA genomes to prime replication. However, there is logic to the progression of steps in virus replication. Development of viral resistance against 3TC was initially a problem but a combination drug approach alleviated this problem substantially. Viruses that bud outward from the cell surface, like influenza virus, contain phospholipids and cholesterol in the proportions characteristic of the plasma membrane. Inside the tube resides a filament 2 nm in width. Middle: two early symmetric inhibitors in which the peptide bond is replaced by a hydroxyethylamine. Stuttering most likely happens when the RNA polymerase pauses, and the growing RNA chain CH015.indd 184 slips backward on the RNA template by one or more nucleotides. The amino acid coded by each triplet is shown directly below. Two influenza A mRNAs undergo alternative splicing in the nucleus Transcription generates a complete set of eight viral mRNAs, one from each genome segment. The smallest known viruses are 20 nanometers1 (nm) in diameter; their genomes contain fewer than 2000 nucleotides, and they code for as few as 2 proteins. Ribbon diagram showing the amino acid chains of the hemagglutinin trimer, with each monomer in a distinct color. In contrast, type 3 reovirus strains spread primarily by neural routes to the central nervous system and infect neurons, causing fatal encephalitis. Hepatitis C virus is transmitted by blood transfusion, by contaminated needles of intravenous drug users, and by sexual contact; unlike most flaviviruses, it is not known to be transmitted by an arthropod host. The nucleotide sequence at this start site can form a stem-loop structure in either the genome RNA or the complementary NP mRNA (Figure 16.7). Excision of λ DNA from the bacterial chromosome The same two proteins, Int and IHF, plus an excision protein named Xis, are involved in the reverse reaction that generates a circular DNA molecule from the CH008.indd 92 "Lysogenic conversion" is the term for a common phenomenon in which an integrated prophage expresses one or more genes, in addition to the prophage repressor, that affect the host cell's phenotype. Cre probably interact with specific viral proteins. The matrix and nucleocapsid proteins are generated by cleavage of the Gag precursor during and after virion formation. • The simplest icosahedral capsids contain 60 T protein subunits. However, gene therapy is still largely an experimental procedure, and much work remains to be done to make it available for general use in humans. Virions are then taken up by endocytosis and viral DNA enters the nucleus, where it is maintained as a free, circular minichromosome in the basal cells. These are stem MJ, the "operator hairpin" containing the AUG itself, and stem R32. As they mature, virions become smaller and the doughnut-shaped core becomes more compact and dense. Genome codes for 11 proteins including: • 2 DNA replication/packaging proteins 2 scaffolding proteins 4 virion structural proteins 1 lysis protein • • • VIRUSES AND HOSTS Two subfamilies of Microviridae: • Microviruses infect free-living bacteria. On the other hand, if the host cell is metabolically inactive, resulting in fairly stable CII, sufficient CI is produced from PRE to repress PL and PR. This mechanism avoids the production of genome RNAs when insufficient N protein is available to encapsidate them; these RNAs could form double-stranded hybrids with viral mRNAs, leading to induction of antiviral proteins (see Chapter 33). Genes for four aminoacyl-tRNA synthetases, an mRNA cap-binding protein, translation initiation factors, tRNA methyltransferases, and peptide release factor are clearly of interest since they are all involved in protein synthesis. Type I interferons include interferon-␣ and interferon-. Typically, these blooms collapse over a period of 2 to 3 days, releasing a biogenic sulfur gas called dimethyl sulfide (DMS) into the atmosphere, a bit like an oceanic sneeze! DMS is the smell commonly associated with the sea, but has a multitude of functions in algae. Do the secreted glycoproteins play a role in virus pathogenesis? Condit Poxviridae From English pocks (pox), referring to blistering skin lesions VIRION Complex, ovoid- or brick-shaped particles, 310 x 240 x 140 nm Surface ridges or "tubules". These enzymatic activities play important roles in viroid replication, during which RNA oligomers are synthesized. In some cases, only the viral genome is transported in plasmodesmata; in other cases, assembled virions or nucleocapsids are transported. Most other positive-strand RNA viruses have Table 14.1 Representative human and animal coronaviruses [Order Nidovirales, Family Coronaviridae, Subfamily Coronavirinae] Genus Species Disease Alphacoronavirus (Group I1) Human coronavirus 229E Colds, pneumonia Human coronavirus NL63 Bronchiolitis, colds, pneumonia Porcine transmissible gastroenteritis virus Pneumonia, gastroenteritis Canine coronavirus Gastroenteritis Feline infectious peritonitis virus Peritonitis, enteritis Bat coronaviruses (HKU2, HKU8, 512) Asymptomatic (enteric shedding) Human coronavirus OC43E Colds, pneumonia Human coronavirus HKU1 Colds, Pneumonia Murine (mouse) hepatitis virus Hepatitis, encephalitis Bovine coronavirus Pneumonia, gastroenteritis SARS coronavirus Pneumonia, gastroenteritis Bat coronaviruses (HKU4, 5, 9) Asymptomatic Avian infectious bronchitis virus Tracheitis, kidney infection Beluga whale coronavirus SW1 Hepatitis 1 Betacoronavirus (Group II) 1 Gammacoronavirus (Group III) 1 Previous taxonomy of virus groups within previous genus Coronavirus (Groups I, II, and III). The carboxy-terminal cytoplasmic tail of M is thought to interact with packaging signals in N to ensure specific incorporation of only full-length viral genome RNA into virions. These include virion-associated RNA or DNA polymerases (retroviruses, negative-strand viruses, reoviruses), and tegument proteins of herpesviruses. VMD-Visual Molecular Dynamics: http:// www.ks.uiuc.edu/Research/vmd/. Small interfering RNAs (siRNAs): short, double-stranded RNA molecules of 20 to 25 base pairs that stimulate RNA interference and antiviral signaling pathways. At the moment, no proven effective treatments exist for the prevention, cure, or even delay of progression of prion diseases. It is notable that no negative-strand RNA viruses of prokaryotes (bacteria and archaea) have yet been discovered. Peritrophic membrane: chitinous protective sheath secreted around food in insect gut. (a) (b) Paramyxovirus and rhabdovirus virions have distinct morphologies Virions of paramyxoviruses consist of a loose-fitting envelope surrounding a helical nucleocapsid. GP2 is inserted into a lipid bilayer (initially the endoplasmic reticulum, later the plasma membrane and the virion envelope) via its C-terminal transmembrane domain (shown in gray). However, the AAUAAA is located within the R region of other retroviruses, and therefore it is transcribed and present at both the 5' and 3' ends of viral RNA. This relaxation occurs because one of the DNA strands can now rotate freely about the other strand, allowing the DNA to come to the lowest-energy topological state. The results are expressed as PFU/cell for virus (logarithmic scale on left) or as a percent of the maximum amount of the viral macromolecule (linear scale on right). 9.1: Reproduced from File:Phylogenetic_tree.svg, Wikipedia Commons; Fig. structure (see Figures 2.3b and 2.4). Table 3.1 Viruses with single-stranded DNA genomes CH003.indd 34 Genome segments Genome size (kb) and topology Capsid symmetry Envelope Circular Icosahedral No 5–9 Circular Helical No 1–2 3–6 Circular Icosahedral No 6–8 6–9 Circular Icosahedral No Beak and feather disease virus 1 2–4 Circular Icosahedral No Vertebrates & invertebrates Adeno-associated virus 1 4–6 Linear Icosahedral No Vertebrates Torque teno virus 1 1 4 Circular Icosahedral No Virus group Hosts Example Microviridae Bacteria Bacteriophage ϕX174 1 4–6 Inoviridae Bacteria Bacteriophage M13 1 Geminiviridae Plants Maize streak virus Nanoviridae Plants Banana bunchy top virus Circoviridae Vertebrates Parvoviridae Anelloviridae 16/05/11 7:52 PM Major Virus Groups How small are viruses? Genetic economy: principle that organisms with genomes of limited size use their protein-coding capacity economically, for example, by making capsids with multiple copies of a few different proteins. (b) Enlargement of a single particle in cross section. Table 33.5 gives some examples of a growing list of viral gene products that interfere with the interferon response. 75 7. Such vectors, referred to as self-inactivating retrovirus vectors, are safer because they cannot produce viral RNA after integration into their target cells. Experiments performed with extracts from poliovirus-infected HeLa cells showed that RNA synthesis by all three classes of mammalian RNA polymerases (pol I, II, and III) is inhibited. ICP4 Major regulatory protein, required for all and virus gene expression. Flaviviruses were previously grouped with the togaviruses (Chapter 13), as they are both small, enveloped viruses, and many are transmitted by arthopod vectors, within which they also replicate. Here again large T antigen has a vital function. Members of four families (Bornaviridae, Paramyxoviridae, Rhabdoviridae, and Filoviridae) share several distinctive characteristics: a single genome segment with a common gene order, transcribed in series from a single promoter at the 3⬘ end of the genome. Viruses are important players in the regulation of the Earth's ecology. Thus HIV-1 attacks at the very heart of the immune system, as both CD4-positive T cells and 16/05/11 8:15 PM 358 CHAPTER 29 Human Immunodeficiency Virus gag vif pol vpr tat rev env vpu nef Transcription Full length 9 Kb An Gag Pol Splicing 4 Kb Class 2 Kb Class Env An Vpr Vif Vpu An Tat Rev Nef Structural proteins Regulatory proteins Figure 29.3 Genome structure and RNA splicing pattern of HIV-1. They can cause plant diseases, but the basis of their pathogenicity is not well understood. of the virion is decorated with about 20 thin rigid filaments, each 20 nm long and 3 nm thick, which appear to be inserted into a disc and are interconnected at their bases. VP24 is actually a viral protease that cleaves itself from a precursor protein, producing VP21 as another cleavage product. One approach is to use a Sindbis virus strain that is not pathogenic for insects to express an interfering RNA (RNAi, see Chapter 33) directed against human pathogens such as yellow fever virus or dengue virus (both flaviviruses). They cause a variety of diseases, including hepatitis (hepatitis A virus), myocarditis (Coxsackievirus), the common cold (rhinoviruses), and paralytic poliomyelitis (poliovirus). To date, prymnesioviruses have been isolated from members of the genera Chrysochromulina and Phaeocystis. Int is a phage protein that catalyzes crossing-over between the 16/05/11 7:59 PM Bacteriophage Lambda 91 Table 8.2 Activities of promoters Promoter Phase of growth cycle Genes transcribed Control mechanism PR Prophage None Repressed by CI Immediate early cro Terminated at tR1 Early cro, cII, O, P, Q Antiterminated by N Late cro, cII, O, P, Q (reduced levels) Repressed by Cro Prophage None Repressed by CI Immediate early N Terminated at tL1 Early N, cIII, recombination genes Antiterminated by N Late N, cIII, recombination genes (reduced levels) Repressed by Cro Prophage None Terminated at tR' Immediate early None Terminated at tR' Early Lysis and structural protein genes (low) Antiterminated by N Late Lysis and structural protein genes (high) Antiterminated by Q Prophage cI, anti-cro (very low) Weak constitutive activity Immediate early cI, anti-cro (very low) Weak constitutive activity Early cI, anti-cro (high) Activated by CII Late cI, anti-cro (very low) Weak constitutive activity Prophage cI (moderate) Activated by CI Immediate early cI (very low) Weak constitutive activity Early None Repressed by Cro Late None Repressed by Cro Prophage int (low) Weak constitutive activity Immediate early int (low) Weak constitutive activity Early int (high) Activated by CII Late int (low) Weak constitutive activity Prophage Antisense Q (low) Weak constitutive activity Immediate early Antisense Q (low) Weak constitutive activity PL PR' PRE PRM Pint PAQ Early Antisense Q (high) Activated by CII Late Antisense Q (low) Weak constitutive activity phage attP site, near the int gene, and the bacterial attB site, located between the galactose (gal) and biotin (bio) operons. Vaccination in healthy adults is 60 to 90% effective in preventing clinical illness caused by virus strains similar to those used to manufacture the vaccine. Like smallpox and measles, humans are the only known host for the polioviruses. Michelitsch, Vincent Guénebaut, Surachai Supattapone, Ana Serban, Fred E. Although relatively few cases of H5N1 disease occurred in humans, human mortality rates were very high (33–70%). It is taken up within the host cell CH027.indd 337 Figure 27.12 Stargate structure on the mimivirus capsid. This results in diminishing quantities of mRNAs as their genes lie farther from the 3' end of the genome. By substitution of individual clones with smaller fragments, a total of 19 specific genes (late expression factors or lefs) were identified that were capable of transactivating the expression of late but not early promoters. This protein contains an N-terminal transmembrane helix that most likely mediates penetration. CH028.indd 349 16/05/11 8:15 PM 350 CHAPTER 28 Retroviruses The Gag protein is targeted to the plasma membrane by the fatty acid myristate, linked post-translationally to its N-terminal amino acid. This fusion peptide is hidden within a hydrophobic region of HA, near the lipid membrane of the virus envelope. Activated PKR phosphorylates the a subunit of eukaryotic initiation factor 2 (eIF-2), which in turn shuts off protein synthesis (see Chapter 33). Importantly, AAV vectors produced in insect cells are indistinguishable from those produced in mammalian cells. However, their exact role in virion formation is not yet understood, and little or no C is incorporated into virions. LIFE CYCLES OF VIRUSES OF ARCHAEA Many are temperate viruses that integrate their genome into host cell DNA and replicate without killing the host cell. Many of these viruses have lipid membranes and bizarre shapes. 439 these viruses persist in the body because of the establishment of some degree of tolerance by the human immune system. Prangishvili "AFV1, a novel virus of the hyperthermophilic crenarchaeon Acidianus," Virology 315 (2003), 68-79, with permission from Elsevier; Fig. Cell-cycle arrest may be a result of hyperphosphorylation and consequent inactivation of the cyclindependent kinase (CDK), p34cdc2/cdk1, by 1s. A number of clinical trials using baculovirus/insect cell-derived VLPs corresponding to such diverse viruses as human immunodeficiency virus and influenza virus are currently under way. Alternative splicing can produce a truncated form of E2 that lacks the transactivating function, and this further contributes to the regulation of viral gene expression. suppressor proteins (Figures 23.5 and 23.6). What is the role of CII? Therefore some viral proteins are made from messenger RNAs copied from the genome RNA, while other proteins are made from messenger RNAs copied from the antigenome RNA. Alternatively, there may be host proteins that recognize and interact with different viroid structures. Pestiviruses and hepatitis C virus are not transmitted by arthopod vectors; a major transmission pathway for hepatitis C virus was via blood transfusions due to contamination of the blood supply. It is this binding that leads to the formation of the inner shell (made of capsid proteins) and outer shell (made of envelope glycoproteins), and membrane bending and budding produces virions. Stetter, R. Twenty-nine class II gene products are made from the resulting mRNAs. Most of the class II proteins participate in T7 DNA replication and recombination (Table 7.2). The absence of inhibitory receptors, for example MHC proteins, triggers release of NK cell factors which induce apoptosis in the target cell. Fusion releases the virion core into the cytoplasm. Later it was discovered that they represented two distinct Ebola virus (EBOV) species, Sudan ebolavirus (SEBOV) and Zaire ebolavirus (ZEBOV). Prion proteins (PrPC) are encoded by the host genome. M2 is an ion channel that facilitates release of nucleocapsids from the virion The M2 protein is crucial in the next phase. 16/05/11 7:52 PM 42 CHAPTER 3 Virus Classification: The World of Viruses THE RNA WORLD 1. However, none of these agents has yet been shown to have significant efficacy in reducing or preventing prion disease in animals. A highly antigenic nineamino acid loop that is conserved among cucumoviruses is located on the outside of the virion, and mutations in this region affect efficiency of transmission by aphids. In many cases, CH035.indd 439 Just as there can be no one-size-fits-all for the development of antiviral vaccines, there is no single optimal vaccination strategy for all viruses. • Synthesis of single-stranded microvirus genome DNA takes place concurrently with DNA packaging. GENOME Linear single-stranded RNA, negative or ambisense. Pentasymmetrons: pentagonal capsid substructures composed of multiple trimeric or hexameric capsomers surrounding a central pentameric capsomer. Virulence factors: pathogen-encoded proteins that enable the pathogen to counteract aspects of the host immune response and therefore increase its own replication, usually to the detriment of the host organism. Coiled-coil: a protein structural element consisting of several alpha-helical chains coiled about each other, forming a rodshaped domain. • The influenza virus M2 protein is an ion channel that facilitates release of nucleocapsids from the virion. Comparison of image reconstructions of the virion (85 nm in diameter) and ISVP (80 nm in diameter) reveals the loss of 600 finger-like subunits, which correspond to the 600 copies of 3 in the virion. HIV-1 virions initially attach to cells by binding to the host cell membrane protein CD4. Human herpesvirus 8 CH024.indd 286 16/05/11 8:12 PM Herpes Simplex Virus an b UL b' an' c' US L 287 c a S Figure 24.1 The genome of herpes simplex virus. • Cucumber mosaic virus has a tripartite plus-stranded RNA genome. As a result, all late mRNAs have the same tripartite leader at their 5' ends. This also results in increased release of chemokines from infected cells, recruiting uninfected T cells to the sites of virus infection and activating them, thus making them susceptible to productive infection by HIV-1. In the absence of gene transcription, the rest of the genome remains silent, with the exception of a class of RNAs called latencyassociated transcripts (LATs). Pathogenicity could also be the result of molecular mimicry. Three essential proteins made by E2 genes carry out viral DNA replication. This property is the basis for the rapid and sensitive hemagglutination assay that allows detection of many viruses (see Figure 1.4). These include togaviruses, flaviviruses, rhabdoviruses, and hepadnaviruses. Newly synthesized DNA is shown in blue. The matrix protein is shown in black just beneath the envelope, and the helical nucleocapsid is shown in green. Picornaviruses 125 Bert L. When the viral RNA polymerase reaches this poly(U) sequence, it pauses and stutters, reading through the CH018.indd 216 poly(U) sequence a number of times and repeatedly adding complementary A residues to the transcript to generate a poly(A) tail (Figure 18.7). • Synthesis of picornavirus proteins occurs via an internal ribosome entry site (IRES) found in the 5' noncoding regions of viral genomic RNAs. • Picornaviruses carry out membrane-bound RNA replication in the cytoplasm of infected cells, yielding proteinlinked progeny RNAs. • Many picornaviruses inhibit cellular macromolecular functions during infection, including host protein synthesis, RNA synthesis, DNA synthesis, or vesicular trafficking. Binding of eIF-4F to an IRES is specified by the central portion of its eIF-4G subunit, replacing the function of eIF-4E, which normally recognizes the cap and directs binding of this complex to capped mRNAs. Picornavirus proteins are made as a single precursor polyprotein that is autocatalytically cleaved by viral proteinases Many bacterial messenger RNAs contain several different protein coding regions and can initiate protein synthesis at various internal sites. In this way the target can be separated from the bulk of the other RNAs in the sample. Coronavirus virions contain multiple envelope proteins Spike protein. RNase H digests the RNA part of the newly made DNA-RNA hybrid (but not the RNA-RNA hybrid at the primer binding site), removing the RNA template and exposing the DNA copy of the U5 and R sequences. Most regions of both DNA strands are transcribed by cellular RNA polymerase II. Chapters 5 through 8 discuss a selection of well-studied bacteriophages that have served as models for understanding cell processes and the replication cycles of other viruses. This enzyme can remove deoxyuridine residues incorporated into viral DNA during reverse transcription because of high levels of dUTP in the cell. However, this vaccine must be delivered by needle, with attendant risks for the vaccinee, the vaccinator and the community (e.g., inadequate disposal of used needles). these capsomers are surrounded by five nearest neighbors, and define the fivefold symmetry axes typical of capsids with icosahedral symmetry. Either the viral DNA is released directly into the nucleus, or the nucleocapsids first enter through the nuclear pore and then are uncoated in the nucleus. Virus replication eventually leads to cell lysis. See also Epstein–Barr virus, Herpes simplex virus diseases, 285–286 latent infections by, 286 Hexons, 23, 275 Histamine, 417 Histone acetyl transferases, 279 Histone deacetylases (HDACs), 278 Holin, 85 Hong Kong flu, 211 Host cell antiviral response pathways, suppression, 219 Human adenoviruses cell transformation by, 283–284 oncogenesis by, 283–284 therapeutic applications of, 283 Human diseases caused by viruses, 5 Human herpesviruses, 286 Human immunodeficiency virus (HIV), 354–364 acute infection phase, 355 AIDS, 355 antiviral drugs control infection, 356–357 as complex retrovirus, 357 clinically latent phase, 355 genome map, 358 latent infection complicates elimination of, 359–360 long-term non-progressors, 356 Nef protein as pathogenesis mediator, 362–364 nonstructural proteins, 357 proviral DNA transport into cell nucleus, 359 rapid progressors, 356 Rev protein mediates viral mRNA transport, 360–361 RNA splicing pattern, 358 slow progressors, 356 structural proteins, 357 targeting cells of immune system, 357–358 Tat protein increases transcription, 360 transmitted to humans from chimpanzees, 355 Vif protein counters APOBEC3G, 361–362 virus mutants arise rapidly, 358–359 Vpr protein enhances virus replication, 362 Vpu protein enhances release of progeny virions, 362 Human papillomaviruses (HPV), 263 functions of, 266 Genetic map of, 265 infection sites, 264 oncogenic HPV, 264 transcriptional map of, 265 Human polyomaviruses, 248 Human T-cell leukemia virus type 1 (HTLV-1), 351 Humoral immune response, 436 Hydrocephalus, 234 Hyper (HY) encephalopathy, 394 Hyperhalophiles, viruses of, 98, 108 Hypersensitive necrosis, 116 Hypersensitive response (HR), 121 Hyperthermophiles, 98 Hypoxemia, 160 bindsub.indd 495 IκB, 359, 402 Icosahedral ssDNA virus families, 77–78 Icosahedral symmetry, 21–24 elements of, 22 fivefold axes, 21 quasi-equivalence concept, 21–23 threefold axes, 21 twofold axes, 21 virions with, 21 Immune system, effects of interferons on, 411 Immunoglobulin family, 47, 276 Immunoglobulin G molecule, 421 Immunoprophylaxis, 375 Importins, 53, 215, 359, 361 Inclusion bodies, 192, 225 Infectious subvirion particles (ISVPs), 229–230 Inflammasome, 405 Inflammatory response, 417 Influenza viruses, 210–223. Similar processes probably lead to uncoating and release of the viral genome. The prasinoviruses are morphologically similar to other phycodnaviruses, with icosahedral capsids of diameter approximately 120 nm. Cucumber mosaic virus can also be transmitted through seeds to progeny plants. Presently, administration of therapeutic proteins is restricted to secreted and circulating proteins such as antibodies, serum proteins, cytokines, and hormones. One to three capsid (Cap) proteins: formation of virion. It was recently found that NLRP3 also mediates innate immunity to influenza virus via recognition of viral RNA. Negative-strand RNA viruses have not been found in bacteria, archaea, fungi, or algae, and only a few are known to infect vascular plants. GENES AND PROTEINS All genes translated as single polyprotein followed by proteolytic cleavage. Viruses multiply inside cells by expressing and replicating their genomes. The 3 nucleotides associated with necrosis are shown in orange. The inverted repeats flanking US are designated ac and ca. The effect of these overlaps on mRNA synthesis is not known, but they may control the level of initiation of synthesis of mRNA of the downstream genes. Children treated by retrovirus vector-mediated therapy demonstrated a complete recovery of their immune system; they were able to leave their protective isolation in the hospital and return home with their parents to enjoy a normal life. Many virus preparations consist of fully intact virions that contain infectious genomes. Although there is often little knowledge of the mechanisms of attenuation in these "black box" viruses, these are among the most successful vaccines currently used. Virions of three additional families of doublestranded DNA phages contain lipid membranes either outside or inside their protein capsids. However, this viral silencing suppressor sometimes triggers a hypersensitive response-associated resistance in specific hosts, indicating that complex molecular strategies are employed by viruses and their hosts for defense, counter defense, and perhaps eventual adaptation. Binding of E2 to E1 increases the affinity of E1 binding to the replication origin. The second stage of replication, formation of genome RNA from antigenome templates (steps 5 and 6 in Figure 31.2), is sensitive to ␣-amanitin, and therefore presumably uses RNA polymerase II. When Tc cells come in contact with infected cells, they are activated if their T-cell receptor recognizes these peptides. Widely used in cell biology for intracellular localization of specific cellular proteins fused to green fluorescent protein by genetic engineering. A second promoter, called PRM for repressor maintenance, is located near PR but initiates transcription in the opposite direction (Figure 8.5a); this promoter directs transcription of cI mRNA. These steps are studied by extracting from the infected cell the macromolecules that constitute viral genomes, messenger RNAs, and proteins. Viral genes can be classified in at least four groups based 289 on the time of their expression during the replication cycle. All viruses contain genomes made of one and only one type of nucleic acid. Assembly and disassembly are not simply reverse processes. The different interferon-␣ subtypes all have the same number of amino acids and share a high degree of amino acid sequence homology; differences in molecular weights are due only to different levels of glycosylation. Twelve of Human polyomaviruses are widespread but cause disease only rarely Five distinct human polyomaviruses are presently known. The Rev protein mediates cytoplasmic transport of viral mRNAs that code for HIV-1 structural proteins Mutations in the rev gene of HIV-1 result in the loss of synthesis of all proteins encoded by the 9-kb (unspliced) and 4-kb (singly spliced) classes of viral RNA; therefore this gene was named regulator of expression of virion proteins. It may be involved in bringing genome RNAs in contact with the vesicles. (a) Attachment of PBCV-1 to the algal cell wall and digestion of the wall at the point of attachment. Analysis of gene function by site-directed mutagenesis was not possible, because infectious RNA cannot be expressed from a cloned DNA copy. The delta ribozyme is therefore extremely interesting, because it can function in the environment of human and animal cells. A short 5' non-translated leader region is followed by gene 1, which consists of two overlapping reading frames (ORF1a and 1b) that are translated as polyproteins from genome RNA. Viral antigens trapped on or in these particles can promote both humoral and cellular immune responses. A chloramphenicol acetyl transferase reporter gene under the control of a baculovirus late promoter was transactivated by a combination of viral genes expressed on different transfected plasmids. It is not yet clear why this pandemic virus was so mild in its impact. Instead of being derived from a degenerate intracellular parasite, a large DNA virus could have been the precursor to the nucleus in the evolution of the first eukaryotic cells. Once the DNA polymerase has copied the first 100 nt of the double-stranded DNA stem, it proceeds along the single-stranded DNA, displacing the single-stranded, DNA-binding protein as it goes (step 6). CREB binds to cyclic AMP response elements on DNA and activates transcription. Only after CD4 binding do these viruses engage the chemokine receptors CCR4 or CXCR5, which allow penetration by membrane fusion (see Figure 4.2 and Chapter 29). nsp4, nsp6 Membrane-spanning proteins, may localize replication complexes to membranes; glycosylation of nsp4 important for replication complex formation. Long-term non-progressors (5%) show no decline in CD4-positive T-cell levels. 16/05/11 8:02 PM 134 CHAPTER 11 Picornaviruses Inhibition of host cell macromolecular functions Infection by nearly all picornaviruses leads to a number of dramatic effects on host cell macromolecular functions. The virus factors are the cap guanylyltransferase referred to above, and one of the smaller RNA polymerase subunits. These observations suggest a potential use of reoviruses as a treatment for cancer. TLR-3 recognizes double-stranded RNAs. The expression of TLR-3 is widespread and includes dendritic cells, macrophages, fibroblasts, and epithelial cells. In 1943, Glaser and Wendell Stanley concluded, based on biochemical analysis, that polyhedra contain nucleoproteins. These receptor molecules are produced by genes that rearrange during lymphocyte development to generate millions of different binding specificities; each individual B- or T-cell expresses many copies of a surface receptor that has a single binding specificity. coli replication helicase DnaB to the O-some. In those viruses, cleavage reveals the fusion peptide and allows it to insert into cellular membranes and direct virus fusion. Short tail with short tail fibers. In this model, immature lymphocytes have many different antigen receptors. This occurs in two distinct phases. For example, Epstein–Barr virus encodes miRNAs that target PUMA, a pro-apoptotic protein, and CXCL-11, a potent T-cell chemokine. Mouse polyomavirus is commonly found in wild mice and their inbred laboratory cousins but causes little or no overt disease. Protein subunit 2.3 nm Figure 2.9 The helical nucleocapsid of tobacco mosaic virus. 33–38. This approach has been replaced by comparison of nucleotide sequences of viral genome RNAs using computer reservoir for coronaviruses, as has been proposed for other "emerging" viruses such as Ebola, Hendra, and Nipah viruses. The locations of homologous repeat sequences (hrs) usually associated with multiple EcoRI sites are labeled on the outer circle and shown in black. Patient isolation and contact tracing are key elements for the public health response. Solfataric: refers to sulfurous fumaroles or springs of volcanic origin. Based on analysis of antigenicity of the coat protein and of RNA sequences, cucumber mosaic virus strains can be divided into three subgroups: IA, IB, and II. These include genes encoding dTDP-D-glucose 4,6 dehydratase, ribonucleotide-triphosphate reductase, mucin-desulfatating sulfatase, an aquaglyceroporin, an alkyl sulfatase, a potassium ion transporter, ubiquitin, a calcium-transporting ATPase, and a Cu/Zn superoxide dismutase. 1936 Stanley isolates nucleic acids from tobacco mosaic virus. Three major genes, each translated as a polyprotein and cleaved into mature proteins: • • • gag: nucleocapsid proteins pol: reverse transcriptase, protease, and integrase env: envelope proteins Gag and Pol proteins are made from unspliced mRNA. Below: an engineered virus mutant that does not undergo editing because the run of U residues in the editing site (A's in the complementary mRNA) is interrupted by two C residues (underlined G's in the mRNA). and smaller amounts of mRNAs for envelope proteins and the RNA polymerase (L). However, at least four different viral 16/05/11 8:12 PM 296 CHAPTER 24 Herpesviruses mutants have been shown to allow apoptosis to proceed. translation there, even though this site is some 340 nt downstream from the position where the ribosome is translating the coat protein gene. • Most bacteriophages are tailed and have doublestranded DNA genomes. (a) Initiation of DNA synthesis at viral replication origin. In other areas of the cytoplasm, lower concentrations of 3 would allow PKR activation, leading to selective inhibition of cellular protein synthesis. A significant quantity of poxvirus dsRNA is produced during the late phase of infection as a result of synthesis of transcripts with 16/05/11 8:13 PM 322 CHAPTER 26 Poxviruses Table 26.4 Poxvirus genes that interfere with host defense systems System Virus gene Activity Protection against host defense Interferon K3L Homologue of eukaryotic initiation factor 2␣ (eIF-2␣) Pseudosubstrate for interferon-induced protein kinase (PKR) E3L Double-stranded RNA binding protein Sequesters dsRNA, an activator of interferoninduced PKR and 2',5'-oligo A synthetase B8R Secreted, soluble interferon- receptor Binds to and inhibits interferon- B14R Serine protease inhibitor (serpin) Inhibits proteolytic activation of interleukin-1, decreasing inflammatory response B16R Soluble interleukin-1 receptor Binds to and inhibits interleukin-1 Tumor necrosis factor (TNF) B28R, A53R Soluble TNF receptor Binds to and inhibits TNF-␣ and Complement C3L C4B and C3B binding protein Inhibits classical and alternative complement pathways Chemokines B29R/C23L Chemokine binding protein Interferes with leukocyte migration Interleukin-1 Medical uses of poxviruses Poxviruses hold potential for medical use in two areas, recombinant vaccines and treatment of cancer. Simian virus 40 was found as a contaminant of Salk poliovirus vaccine Simian virus 40 (SV40) was discovered some 10 years later as a contaminant of rhesus monkey kidney cell cultures that were being used for production of human poliovirus vaccines. The fivefold averaged cryo-EM structure of PBCV-1 viewed down a quasi-twofold axis. Claire Trottier helped organize these student reviews. Black parts of lines show introns; open reading frames are shown in orange. The 3C proteinase actually exists in three different forms, and each form has a different role in polyprotein cleavage. The use of protease inhibitors in combination with reverse transcriptase inhibitors led to strong reductions in the amount of HIV-1 circulating in patients and to dramatic improvements in the health and longevity of the patients. Blooms typically contain around 10,000 to 100,000 algae/mL of seawater over areas up to 100,000 km2, which would give a total of 1020 to 1021 organisms in the top 10 cm of seawater! Light reflected by coccoliths in these massive and impressive blooms can even be seen from space (Figure 27.7); blooms can range from the size of a small country to a whole continent. 16/05/11 8:21 PM How do Antiviral Vaccines Work? Rhabdoviruses: 11–12 kb. CH028.indd 344 16/05/11 8:15 PM Retroviruses • CD4 and the chemokine receptors CCR5 and CXCR4, for HIV-1 (see Chapter 29) • the amino acid transporter EcoR/Rec-1, for murine leukemia virus • the phosphate transporters Pit-1/Glvr-1 and Pit-2/ Glvr-2, for the gibbon ape leukemia virus • Tva, a homologue of the low-density lipoprotein receptor, for avian sarcoma leukosis virus type A Following interaction of SU with its receptor, the viral envelope either fuses directly with the plasma membrane, or the virus undergoes receptor-mediated endocytosis and fusion is initiated by a decrease in pH within the endosome. Most DNA viruses use a host cell RNA polymerase for this purpose, but some of the larger DNA viruses bring their own RNA polymerase into the cell along with their genome, or make an additional RNA polymerase later in the cycle to transcribe a subset of their genes. The virus strain, ONYX-015, is a mutant that does not express the E1B 55K protein, which plays a vital role in inactivating and degrading p53. Retrovirus populations exist as quasispecies in their hosts because of frequent errors made by reverse transcriptase. USA 106 (2009), 11306-11311, with permission from PNAS; Fig. While the interferon receptor proteins IFNAR1 and IFNAR2c are expressed in virtually all cell types throughout the body, IL28R␣ is only expressed in a few cell types, most notably epithelial cells. The virus that appears is not drug-resistant, and is probably generated from the latently infected cells. Certain retroviruses have acquired copies of genes that encode cellular signaling proteins, and in some cases these viruses can transform normal cells into cancer cells; their study has led to great advances in the understanding of oncogenesis. This protein is required for infection of cell cultures via fusion of the viral envelope and the cell membrane. It can be drawn in a shape that resembles a hammerhead. Virions exist in two infectious forms: • Mature virus (MV): one lipid membrane Extracellular virus (EV): two lipid membranes • GENOME Linear, double-stranded DNA, 150–250 kb (vaccinia virus: 200 kb). The viral proteinases include one or two papainlike cysteine proteinases (PLP1 and PLP2) and a chymotrypsin-like cysteine proteinase that resembles the picornavirus 3C proteinases (nsp5, also known as 3CLpro or Mpro) (Figure 14.1). None of these viruses has a lipid envelope, and viruses in six out of the seven families have icosahedral Viruses with single-stranded DNA genomes are small and have few genes Given the great variations in size and shape of known viruses (Figure 3.1), it is striking that viruses with singlestranded DNA genomes (Table 3.1) lie within a very 500 nm 200 nm 80 nm 20 nm 12 nm 300 nm 2000 nm Figure 3.1 Size variation of viral capsids. Nucleocapsids are shown as circular; they are joined by complementary RNA sequences at the 3' and 5' ends. Early gene products regulate DNA replication, late transcription, and apoptosis A large number of early baculovirus proteins are made. The activation of cellular signaling pathways is an essential process for the entry or replication of many viruses. Among the targets of Vhs are proteins associated with activation of the interferon pathway, a major host response to infection. Squamous cell carcinoma: a type of skin cancer arising from the upper layers of skin epidermis. Poly C binding protein 2 (PCBP2) is one of several cellular proteins believed to be involved in viral RNA replication. Note that the RNA is highly structured. (kDa) Function P47 47 Viral late RNA polymerase subunit LEF-4 54 Viral late RNA polymerase subunit, RNA capping LEF-8 102 Viral late RNA polymerase subunit LEF-9 59 Viral late RNA polymerase subunit LEF-7 27 Stimulatory for transient DNA replication LEF-5 31 Transcription initiation factor? Six distinct EBNAs (EBNA-1, -2, -3A, -3B, -3C, and LP) are made from mRNAs differentially spliced from extraordinarily long primary transcripts that initiate near Ori-P and can extend halfway around the 180-kb genome of Epstein–Barr virus (Figure 24.10). It therefore seems likely that members of the Avsunviroidae family need only a host cell RNA polymerase activity for their replication, since they provide their own self-cleavage and self-ligation activities. After initial translation of viral proteins, there is a switch from translation to the synthesis of minus-strand RNAs and then plus-strand RNAs. Replication of viral RNAs requires the assembly of a replicase complex, which involves viral RNA and proteins, host proteins, and cell membranes. Inhibiting the expression of these proteins using siRNA technology inhibits the growth of HeLa cells and results in apoptosis, demonstrating that E6 and E7 continue to drive cell proliferation many years after the initial transforming events. Seven of these proteins work together to carry out essential steps in DNA replication (Table 24.3). Proteins Rz and Rz1 are required for lysis of cells in the presence of high concentrations of divalent cations. NK cells can detect abnormalities in the expression of cell-surface proteins that may be altered by viral infection. Influenza neuraminidase differs sufficiently from cellular enzymes to make it an excellent drug target. CH016.indd 190 Figure 16.2 Schematic view of a filovirus virion. It is very unusual that introns accumulate in cells, because they are usually rapidly degraded after splicing occurs. Serpin: serine protease inhibitor. A variety of cancers in monkeys, mice, cats, sheep, birds, etc. Nevertheless, once the process begins, it is autocatalytic, as the product catalyzes formation of more of itself from the originally folded protein. The RNA polymerase that transcribes genes of archaea has a number of subunits with counterparts in eukaryotic RNA polymerase II, including the TATA-binding protein TBP. Presently, a major focus of vaccine designers is the harnessing of cell-mediated immunity to create vaccines for the above-mentioned elusive and complicated viral targets, which remain serious threats to human health. The incidence of human disease in North America is low except for occasional local outbreaks. The immune response also produces clones of cytotoxic lymphocytes that recognize specific viral antigens expressed in infected cells and kill those cells, limiting and controlling the spread of the infection. In this way, large T antigen controls its own levels of synthesis. Asialoglycoprotein receptor: a liver-specific protein that binds to and internalizes glycoproteins that lack terminal sialic acid. Diameter 29 nm. Hepatitis: disease involving damage to and inflammation of the liver. By stimulating PI-3 kinase, Nef also inactivates Bad, a pro-apoptotic factor. The problem was to make such a book accessible for beginning students but not to over-simplify the material. Altogether, the fusion process is rapid and efficient. 405) E subunits. However, studies of their biochemistry and cell biology revealed numerous differences between archaea and bacteria: • Cell walls of archaea do not contain muramic acid and D-amino acid peptides as do cell walls of most 97 CH009.indd 97 16/05/11 7:59 PM 98 CHAPTER 9 Viruses of Archaea Figure 9.1 Phylogenetic tree of life. Such a virus can rapidly spread throughout the human population if antibodies against that subtype are not present, and can cause a pandemic. Among other differences, the basic C-terminal region is removed from E by proteolytic cleavage. CH027.indd 331 331 When conditions are right, E. Because the 5'- and 3'terminal 8 to 11 nucleotides of bunyavirus genome RNA are exactly complementary, the 3' end of the antigenome is identical to the 3' end of the genome, and this is probably the signal for initiation of genome replication by the RNA polymerase. Mimivirus is unquestionably a virus Easily visualised by light microscopy, it is clear to see why this hairy 750-nm monster was mistaken for a bacterium at first glance (Figure 27.11). Variants of this adjuvant have been used successfully for inactivated human influenza vaccines (including pandemic vaccines) in Europe and Canada. Packaging most likely occurs through one of the pores that are found at the threefold axes of symmetry in the procapsid but not in the virion (Figure 6.6). RNAs are therefore transcribed from complementary genome strands. The particles containing different genome RNAs are physically and morphologically indistinguishable. (a) Circular DNA is transcribed by host RNA polymerase II, which recognizes five promoters (preC, pg, preS1, S, and X) to generate five classes of capped mRNAs that share a common 3⬘ polyadenylated end. Integration of proviral DNA must therefore await the disintegration of the nuclear membrane when the cell divides. 16/05/11 7:52 PM 40 CHAPTER 3 Virus Classification: The World of Viruses Table 3.6 Viruses with a reverse transcriptase Virus group Hosts Example Capsid symmetry Envelope Caulimoviridae Plants Cauliflower mosaic virus 1 7–8 Circular ds DNA Icosahedral No Hepadnaviridae Vertebrates Hepatitis B virus 1 3–4 Circular ss/ds DNA Icosahedral Yes 7–13 Linear ⩽ RNA Yes Retroviridae Vertebrates Human immuno1 (dimer) deficiency virus type 1 Hepadnaviruses have an unusual DNA genome: it is circular and mostly double-stranded, with a singlestranded region of variable length. These viral glycoproteins have been implicated in many biological attributes of the virus including hemagglutination, virulence, tissue tropism, neutralization by antibodies, and cell fusion. Large T antigen activates or suppresses transcription of cellular genes by binding to a number of important cellular regulatory proteins Large T antigen is a complex protein with a surprisingly large number of different functions. Ethidium bromide molecules insert themselves between adjacent nucleotide base pairs, unwinding the double helix by a small amount. 395 KEY TERMS Amyloid BSE Creutzfeldt–Jakob disease (CJD) Dendritic cells Dura mater Kuru Mad cow disease New variant Creutzfeldt–Jakob disease (nvCJD) Prion Prion disease Scrapie Serpin Spongiform encephalopathy Transgene FUNDAMENTAL CONCEPTS • Prions are proteins that can cause fatal brain diseases. Interferon modifies the activity of several components of proteasomes, which digest viral proteins and make peptides available to MHC class I proteins. CH036.indd 449 449 N HO N ON N O HSV or VZV thymidine O kinase NH2 HO PO N ON N N NH2 OH Acyclovir Acyclovir monophoshate Preferentially phosphorylated in cells infected with HSV or VZV Cellular kinase O HO N O O PO PO OH OH N ON N NH2 Acyclovir diphosphate Cellular kinase O O HO O O PO POPO N ON N N NH2 OH OH OH Acyclovir triphosphate Preferentially blocks HSV and VZV DNA polymerase Figure 36.5 Phosphorylation of acyclovir. The H protein is responsible for binding to receptors on the cell surface, and can also bind to receptors on red blood cells and agglutinate them (hemagglutination). Major histocompatibility complex (MHC) proteins: cell surface proteins that bind to antigens or antigen fragments and present them to cells involved in the immune response. Transcripts initiated at PA are unstable, keeping the levels of the A and A* proteins low; the terminator for PA transcripts is unknown. For this reason, retroviruses pseudotyped with VSV-G are produced either by transient transfection of the producer cells with a plasmid encoding the VSV-G gene, or by using a producer cell line containing the VSV-G gene tightly regulated by an inducible promoter. Pap test: a diagnostic test for cervical cancer at an early stage, by examination of exfoliated cells stained with Papanicolaou stain. Procapsid: a precursor to a viral capsid that usually does not contain the viral genome. Thus optimum gene expression depends on the presence of a tegument protein that is introduced into the cell at the time of infection. Viruses often escape humoral immunity by changing the proteins recognized by antibodies. MicroRNAs are used to control gene expression in vertebrates DCR-2 R2D2 DCR-1 DCR-2 DCR-1 P Viral suppressors, ERI-1 Ago2 P P P Armitage R2D2 Argonaute Ago2 P Helicase Argonaute Ago RISC P 7mG AAAA miRNA P 7mG AAAA Ago mRNA cleavage Translational repression Figure 33.11 RNA interference and microRNA. Many immune cells produce anti-inflammatory cytokines, which suppress the activity of the pro-inflammatory cytokines and help to return the immune system to a resting state. 902, in James D. • Two phenotypes of virions are produced in infected insects: 1. Ribosomal 60S subunits serve as cellular uncoating factors. 291 gene products enable viral DNA replication The function of most genes is to replicate the viral DNA. A new kind of infectious agent that can transmit a disease and replicate itself without the intervention of informational nucleic acids. Nibert, Harvard Medical School. Therefore, it has been suggested that an ancestral mimivirus was involved in transfer of this gene from marine bacteria to the ancestors of the Octocorallia lineage, perhaps 450 million years ago. Such agents work either by occupying the receptor sites, or by inducing receptor internalization and down-regulation. The NSs protein is encoded in an

mRNA corresponding to the 5' half of the S RNA; this mRNA is copied from antigenome RNA (see below). It has been shown that large ribosomal subunits can bind to capsid proteins and disrupt nucleocapsids, liberating genome RNA. RNAse III cleavage at the SIB hairpin leads to further degradation of the mRNA into the adjacent int coding sequence, limiting expression of the Int protein. A much lower virus titer is detected in the medium surrounding the cells (not shown). IgE mediates allergies by virtue of its ability to attach to tissue basophils known as mast cells. The conserved tRNA-like structures at the 3'-termini of genome and subgenomic RNAs are required for in vitro assembly of brome mosaic virus particles, but these structures are not required for packaging of another bromovirus, cowpea chlorotic mottle virus. Cell death induced by mutants lacking the gD gene is blocked by expressing either glycoprotein D or glycoprotein J in infected cells. Binding of large T antigen to these proteins can lead to both stimulation and repression of gene expression. A total of 240 hexons cover the 20 faces Virions have icosahedral symmetry and are studded with knobbed fibers Adenovirus virions have a remarkable resemblance to a regular icosahedron, with 20 equilateral triangular faces and 12 vertices (Figures 23.1 and 23.2). 16/05/11 8:06 PM Bunyaviruses 203 Table 17.2 Consensus 3' and 5' terminal nucleotide sequences of Bunyaviridae genome RNAs Orthobunyavirus 3' UCAUCACAUGA……………….UCGUGUGAUGA 5' Hantavirus 3' AUCAUCAUCUG………………………AUGAUGAU 5' Nairovirus 3' AGAGUUUCU……………………….AGAAACUCU 5' Phlebovirus 3' UGUGUUUC…………………………..GAAACACA 5' Tospovirus 3' UCUCGUUAG……………………..CUAACGAGA 5' Bunyavirus L RNA 3' 6875 nt Phlebovirus 5' 3' 6404 nt L 237 kDa L 259 kDa Gn 32 kDa S RNA 3884 nt 4458 nt M RNA NSm 18 kDa Gc 110kDa 961 nt 5' NSm 14kDa Gn 55kDa Gc 62 kDa 1690 nt N 26 kDa NSs 11kDa N 28 KDa NSs 30 KDa Figure 17.2 Coding strategies of genome segments of orthobunyaviruses and phleboviruses. They could be descended from self-replicating RNA molecules that arose in the RNA world. The presence of the product PrPSc would then catalyze more transformation of PrPC to PrPSc. If this model is correct, genetically inherited prion diseases would result from mutations in PrPC that increase the population of unstable intermediates, or the rate of spontaneous conversion of PrPC to PrPSc. The nucleation-polymerization model (Figure 32.7) resembles the well-studied processes of crystal growth, tubulin polymerization, viral capsid assembly, and bacterial flagellar polymerization. The dominance of alum is now being challenged by a rapidly expanding repertoire of adjuvants that manipulate the immune response in increasingly sophisticated ways. The switch from synthesis of negative to positive strands is correlated with proteolytic processing of P123 (Figure 13.3). Changes in gp120 conformation expose the fusion domain of gp41, resulting in its insertion into the cell membrane. What do we mean by the term structure? The word is derived from Italian or Latin words referring to sulfur ("solfa") and land ("terra"). ICP22 US1.5 Together with UL13, enhance the expression of a subset of late genes through activation of cyclin-dependent cdc2. Viral DNA replication begins 60 to 90 minutes after infection. Integration of viral DNA into host chromosome was not detected. Accordingly, it has been a very successful drug in treating both common herpes simplex virus infections such as genital herpes (many millions of doses of the drug have been administered) and less common but more serious effects of herpesvirus infections such as encephalitis. Another strategy, called "targeted recombination", uses the natural recombination capacity of coronaviruses to introduce changes along with a selectable gene. Comparisons of ribosomal RNA sequences among archaea led to the recognition of several major divisions (phyla), among which best studied are the CH009.indd 98 Crenarchaeota (from Greek crenos, "origin") and the Euryarchaeota (from Greek euryos, "diversity"). 16/05/11 8:11 PM 280 CHAPTER 23 Adenoviruses Table 23.1 Functions of adenovirus E1B proteins Protein Function Cellular proteins involved 19K • Suppresses apoptosis • Binds to Bax (homologue of Bcl-2) 55K • Suppresses apoptosis • Binds to p53 (transcriptional repression) • Induces p53 turnover (with E4orf6) • Binds to p53 and E4orf6 in complex with cellular Cullin-Elongin B/C E3 ubiquitin ligase complex • Promotes transport, stabilization and translation of viral mRNAs (with E4orf6) • Requires E4orf6 and E3 ubiquitin ligase complex • Host cell shut-off (with E4orf6 and L4-100K) • eIF4E, eIF2␣, eIF4G Thus the two major E1B proteins protect infected cells from both p53-dependent and p53-independent apoptosis, reversing activities stimulated by E1A proteins. It was subsequently shown that this protein can directly phosphorylate ganciclovir to the monophosphate form; cell kinases then generate the di- and triphosphates. CH037.indd 467 467 In these trials, patients received an intraocular injection of an AAV vector encoding the RPE65 protein. A symmetry axis that involves rotation by 180° is called a twofold axis, because two such rotations return the object to its initial position; one that involves rotation by 120° is called a threefold axis (Figure 2.2a), etc. These viruses therefore also package a virus-coded RNA-dependent RNA polymerase in the virion and bring this enzyme into the host cell along with the genome. The N protein is encoded in an mRNA corresponding to the 3' half of the S RNA; this mRNA is copied from genome RNA. Alpha helices are shown as spirals, and beta sheets as flat segments. Binding to the receptor triggers an initial conformational change that loosens up the capsid wall and makes the particles hydrophobic. php?dpvno=400&figno=09, Courtesy of Peter Palukaitis, with permission from the The Association of Applied Biologists. The S protein is responsible for virus entry and spread, and is the primary determinant of host range. Three recombinant baculoviruses are used: one encodes the transgene expression cassette flanked Transfer construct ITR ITR Transgene promoter E4orf6 E2A VA RNAs AAV Packaging construct rep cap Helper plasmid E1+ 293 cells AAV vector Figure 37.6 Production of adeno-associated virus (AAV) vectors. This method improves preservation of structure, but image contrast is low. DISEASES Some phycodnaviruses are instrumental in termination of algal blooms. What are these and what are the consequences to the cell? However, few E proteins are synthesized because the E gene has a very weak ribosome binding site, and therefore initiation of protein synthesis is strongly reduced at this site. Acute disease can be mild or severe. p300 is believed to cooperate with these factors in stimulating transcription by acetylating nucleosomal histones, therefore reducing their affinity for DNA and making DNA more accessible to components of the transcription machinery. These pentamers are assembled in a T 7 icosahedral shell, which contains only 5 72 360 protein subunits, not 420 as would be predicted by the theory of quasi-equivalence. Poxviruses Dalius J. Human papillomaviruses (HPV), adenoviruses (Ad), and polyomaviruses (SV40) express proteins that bind to and inhibit the activity of cellular tumor suppressor proteins involved in controlling cell-cycle progression (pRb) and apoptosis (p53). This procedure, favored for its simplicity, is nevertheless inefficient. In addition, some baculoviruses have amplified copy numbers of certain genes, or carry additional unique genes that are not similar to any other known genes. The level of synthesis of subgenomic RNA is increased if flanking regions are also present. N: nucleus; IV: immature virions; arrows: condensing nucleoids within immature virions; arrowheads: mature virions. LMP-2A has been shown to block signaling through the B-cell receptor, perhaps by sequestering Lyn and Syk and therefore preventing their activation; one result is blockage of the activation of Epstein–Barr virus lytic replication. Rolling circle replication forms single-stranded DNA from double-stranded replicative form DNA, using viral protein A (black circle) and three host cell proteins. In addition, interferon- is uniquely able to induce the expression of MHC class II proteins and therefore to promote enhanced CD4positive T-helper cell responses leading to enhanced production of cytotoxic Tc cells. Bowman, Andrei V. Recent structural and biochemical data demonstrate that the immature virus undergoes a significant rearrangement of its surface glycoproteins as it travels through the low pH of the trans-Golgi network (Figures 12.2b and 12.6). To stay healthy and survive, organisms must defend themselves against invading pathogens, including viruses. Indeed, the major limitation of AAV vectors is their small insert size capacity of roughly 4.5 kb, which reflects the limited packaging capacity of the AAV capsid. PRYMNESIOVIRUSES AND RAPHIDOVIRUSES The lesser-known Phycodnaviridae These genera of phycodnaviruses are thought to be widespread in the oceans since they infect bloomforming species of phytoplankton. Computer-assisted averaging is utilized to reconstruct three-dimensional images of the object. Adenoviruses were first isolated by Wallace Rowe in 1953 from tissue culture cell lines derived from tonsils and adenoid tissues of sick children. Viruses genetically similar to SARS coronavirus were found in Chinese horseshoe bats. LCR: long control region. Both Ebola and Marburg viruses have the late domain motif Pro-Pro-X-Tyr (where "X" means any amino acid) in the N-terminal region of their NP40. Reovirus infection results in a rapid inhibition of cellular DNA synthesis. NS5 905 RNA-dependent RNA polymerase; methyltransferase (methylates cap structure). This small virus would have a big impact on molecular biology. These landmark experiments established that certain infectious agents are much smaller than bacteria, and they were called filterable viruses. Virokine: a virus-coded protein that is a homologue of a cellular cytokine. Viral double-stranded RNA is cleaved by Dicer into small interfering RNAs (siRNAs) that are 21 to 23 nt long. In susceptible hosts, infection with cucumber mosaic virus can be asymptomatic or can cause variable Figure 10.9 The hypersensitive response to cucumber mosaic virus on cowpea leaves. In comparison, E6 proteins made by non-oncogenic human papillomaviruses such as types 6 and 11 are unable to mediate the degradation of p53. Second, it provides a means of distinguishing mRNAs, which are not encapsidated, from genome-length RNAs of either positive or negative polarity. Both DNA strands encode approximately the same number of genes (450 genes on the "R" strand versus 465 genes on the "L" strand). The phosphorylated Jak kinases are now activated. 16/05/11 7:59 PM Bacteriophage Lambda 95 Virus histories: mosaic chromosomes and ancient lineages The mosaic chromosomes of bacteriophages. During budding, the viral nucleocapsid is wrapped in a membrane into Viral protein spike Host protein Cell membrane Cytoplasm (a) HA M NA Host cell proteins RNP (b) Figure 2.11 Release of enveloped virions from the cell surface by budding. Small viral dsRNAs block activation of dsRNA-dependent protein kinase (PKR). These DNA vaccines can produce the protein of interest intracellularly, leading to establishment of both humoral and cellular immunity, without the dangers inherent in live or inactivated virus vaccines. Similarly, the enveloped flavivirus PrM protein is cleaved during infection and dissociates to reveal the E protein hidden underneath; the E protein can then enable virus entry by fusion of the viral envelope with the plasma membrane of the target cell. Three late mRNAs are made by alternative splicing Three different late messenger RNA species are formed from mouse polyomavirus late transcripts by alternative splicing (Figure 21.4). The P/C/V gene codes for several proteins by using alternative translational starts and by mRNA "editing" Most paramyxovirus genes produce a single mRNA species that codes for one viral protein. Infection results in depletion of CD4⩽ T cells, rendering the host immune-incompetent. Left: Plants use RNA interference as an antiviral defense mechanism. However, this leads to a further problem: the helper virus replicates at the same time as the vector, and it must be removed from the vector stock. CH030.indd 370 The largest and least abundant envelope protein is the large hepatitis B surface protein (LS, or LHBs). Crescents are remarkable in that they comprise a single 16/05/11 8:13 PM Poxviruses 321 Figure 26.7 Electron micrograph of a vaccinia virus-infected cell. In the absence of Rep proteins, integration of AAV is far less efficient and is no longer restricted to the AAVS1 site. Virions exist in two forms, called mature virus (MV) and extracellular virus (EV), which differ in membrane composition as a result of differences in morphogenesis. Because of the widespread possibility of horizontal gene transfer by exchange of DNA between bacteria, many bacteria have developed mechanisms to recognize and degrade foreign DNA, which might otherwise carry genes of lesser fitness from unrelated organisms. The viral envelope forms around the nucleocapsid and other internal components such as matrix proteins during budding at a cellular membrane. Large DNA concatemers are formed during replication A classical problem with RNA primer-directed replication of linear DNAs is that the last primer laid down at the 3' end of each template DNA strand cannot be removed and replaced with DNA, because there is no sequence beyond the 3' end to which the next primer could bind (see also Chapter 20). Their large genome size enables poxviruses to code for a variety of enzymes involved in RNA and DNA metabolism. Because NUT sequences are found only on transcripts made from PL and PR, these are the only transcripts that are antiterminated by N. Genetic and 16/05/11 8:02 PM 132 CHAPTER 11 Picornaviruses biochemical studies show that most of the proteins made from the P2 and P3 regions are involved in picornavirus RNA replication. The association of Burkitt's lymphoma with malaria is now believed to result from a weakened immune system in malaria victims, allowing development of lymphomas in children infected with Epstein–Barr virus. 341 SEC-VII.indd 341 12/05/11 5:04 PM C H A P T E R 2 8 Retroviruses Alan Cochrane Retroviridae From reverse transcription, characteristic of these viruses VIRION Spherical enveloped particle. The hepatitis B virus surface antigen (HbsAg) was originally purified from infected human serum, but is now produced as a recombinant protein by expressing the cloned surface antigen gene in yeast or mammalian cells, where it can form virus-like particles. In brome mosaic virus, in addition to viral RNAs and proteins, the yeast chaperone protein YDJ1 (Yeast DnaJ homolog) and the levels of unsaturated fatty acids in the infected cell are also important for the initiation of minus-strand RNA synthesis. This is often done by using radioactive compounds that are incorporated into viral or cellular DNA, RNA, protein, lipid, or carbohydrates. These nascent mRNAs are extruded through the 2 turret, receiving a 5' terminal methylated cap (black pentagon) in the process. However, AZT monophosphate is also an inhibitor of thymidylate kinase, which is essential for synthesis of thymidine triphosphate and therefore DNA. Mutagenesis experiments showed that when base pairing at stem MJ is abolished by the introduction of mismatches or by deleting the sequence 1427–1434, there is a large increase in replicase synthesis, which is independent of translation of the coat gene. As a result, infected cells give rise to a burst of interferon that is sent out to neighboring cells, but synthesis is soon halted. Genes located near Table 26.1 Some poxviruses and their hosts CH026.indd 314 Virus Reservoir host Other hosts Variola (smallpox) Humans None Molluscum contagiosum Humans None Vaccinia Unknown Humans, cows, buffaloes Monkeypox Squirrels, rodents Humans, monkeys Cowpox Rodents Humans, cows, cats, zoo animals Orf Sheep Humans, various ruminants Fowlpox Birds Humans (as vaccine vector) Myxoma Rabbits None Entomopox Insects None 16/05/11 8:13 PM Poxviruses (a) TERMINAL LOOP 20 40 60 80 100 120 140 160 180 315 TERMINAL LOOP KILOBASE PAIRS TANDEM REPEATS 70 bp 70 bp 54 bp (b) 2 4 125 bp 6 8 10 KILOBASE PAIRS A A A G G C A T A A T–A–G–T–T–A–G T–A–A–A–T–T A–T A–T–A–T–A–T–A–A–A T–T–T–T–A T–A A–T–T–A–A–T T–T–A A–T–T–T–T–A T–A–T–T–T–A–T–T–T–T–A–G–T–G–T–3 T–T–C–A–A–T–C–A–T–T–A–A–T–A–T–A–T–A–T–A–T–A–T–T–A–A–A–A–T–A–A–T–T–A–A–T–T–A–A–A–T–A–T–T–A–A–A–A–T A T–A–A–A–A–T–A–A–A–T–C–A–C–A–5 G T (c) C A A–A–G–T–T–A–G–T–A–A–A–T–T–A–T–A–T–A–T–A–T–A–A–T–T–T–T–A–T–A–A–T–T–A–A–T–T–T–T–A T A–T–T–T–T–A–T–T–T–A–G–T–G–T–3 A–T–C–A–A–T–C A–T–T–T–A–A–T A–T A–T–A–T–A–T–A–T–T A–A–A–A–T A–T T–A–A–T–T–A A–A–T T–A–A–A–A–T A–T–A–A–A–A–T–A–A–A–T–C–A–C–A–5 T T T C C G T A T T 5 TAGTAAAATTAAATTAATTATAAAATTATATATATAATTTACTAACTTTAGTTAGATAAATTAATAATATATAAGTTTTAGTACATTAATATTATATTTTAAAT 3 (d) 3 ATCATTTTAATTTAATTAATAATATTTTAATATATATATTAAATGATTGAAATCAATCTATTTAATTATTATATATTCAAAATCATGTAATTATAATATAAAATTTA 5 Figure 26.1 Vaccinia virus genome structure. (g) Influenza virus (an orthomyxovirus). Several of these viruses can cause malignant tumors in humans. When the viral nucleic acid genome is packaged within the capsid, the resulting structure is called a nucleocapsid. It has since become an important part of the toolbox of molecular biologists (see below). A new pandemic strain (H1N1 "swine flu") spread worldwide in 2009. The strong-stop DNA is extended by reverse transcriptase to copy the entire remaining part of the genome RNA up through the primer-binding site, making a fulllength minus-strand copy of the genome. All proteins made by cucumber mosaic virus play some role in virus movement, sometimes in a hostspecific manner, but protein 3a and the coat protein are universally required. After one round of replication, the viral A protein cleaves the plus-strand DNA and ligates the 3' end of the cleaved genome to its 5' end, creating a single-stranded circular molecule as in Stage II replication, but this time wholly contained within a procapsid. Translation by cellular ribosomes begins at an AUG initiation codon following the 60–100 nt untranslated leader sequence and progresses through ORF1a, producing a polyprotein that is subsequently cleaved into at least two intermediate precursors and subsequently into 10 or 11 mature proteins. Most autonomous parvoviruses (e.g., minute virus of mice) encapsidate only the DNA strand complementary to the viral mRNA. This response is nonspecific, as enveloped viruses from a variety of different families can elicit a similar response by the infected cell. This approach helps students to learn about virology in a simple and logical fashion.Full-color figures. Transmission is primarily through blood (transfusions, needle sharing) and sexual contact. This RNA polymerase, which is packaged in the virion and enters the cell along with the genome RNA, uses ribonucleoside triphosphates provided by the cell to catalyze viral mRNA synthesis. If an miRNA is only partially complementary to its messenger RNA target, it may inhibit translation of the mRNA. In fact, only the coat gene translational start site (nt 1335 on MS2 RNA) is able to bind directly to ribosomes. A possible functional role involves shuttling or sequestration of copper ions at nerve synapses via binding to the N-terminal octapeptide residues. These conformational changes allow 9S* particles to interact with 6S spike particles and external scaffolding proteins, and prevent the premature association of 9S coat protein pentamers into aggregates, a function reminiscent of molecular chaperones. As more is learned about the biology of viral pathogens, it should be possible to create a new generation of "minimalist" vaccines that contain only the antigens or peptides that elicit protection, while avoiding those that contribute to vaccine-associated adverse events (see below). Core and E proteins. Often contain unique repetitive regions, inverted repeats and terminal repeat sequences. Live neurons expressing green fluorescent protein produced by engineered viral replicons can be observed for up to five days postinfection. Herpesviruses and most doublestranded DNA bacteriophages insert a naked DNA genome into a preformed shell, in an energy-requiring process carried out by one or more specific viral proteins ("packaging motors"), sometimes with the help of specific RNAs. As the DNA is spooled into the capsid, it forms a tightly wound coil, often in coordination with the expulsion or degradation of an internal scaffolding protein. Phage DNA is in orange; phage heads are shown as hexagons, tails as bullets, and the bacterial chromosome is shown as a black circular wavy line. Some viruses bind to specific carbohydrate groups present on glycoproteins, proteoglycans, and glycolipids. Why are the Type I interferons known as antiviral interferons? Viruses with a double-stranded RNA genome share with negative-stranded RNA viruses two important characteristics: they package an RNA polymerase along with genome RNA, and they do not fully uncoat 173 SEC-IV.indd 173 12/05/11 5:03 PM 174 SECTION IV Negative-Strand and Double-Stranded RNA Viruses of Eukaryotes the genome RNA within the cell. Stress responses activated during the initial stages of infection result in the transcription of several hundred cellular genes (Chapter 33). coli and enable bacteriophage lambda DNA replication! This shows the extraordinary conservation of protein functions in 0 100 200 Pol ␣ Rb p300 Topoisomerase I N 300 widely separated organisms, and reveals a connection across perhaps billions of years of evolution between a bacteriophage and a mammalian DNA tumor virus. Most eukaryotic messenger RNAs code for a single protein. However, this is not always the case. Other picornaviruses, including poliovirus, undergo a major conformational change directly at the cell surface upon binding to their receptor. One such target is the host serine/threonine protein kinase Pak2, known to be involved in stimulation of cell growth and inhibition of apoptosis. The human cell line 293 was generated in vitro by transfection of fetal embryonic kidney cells with a fragment of adenovirus DNA containing only the E1A and E1B genes, allowing survival and growth of transformed cells. This means that bacteriophages tend to be genetic mosaics, and determining evolutionary or genetic relatedness among different phages is difficult if not impossible. Much of the earlier work on baculoviruses centered on their potential use as species-specific insecticides. It is not known how 1 integrin promotes endocytosis, but the simplest explanation is that following JAM-A engagement, the virus binds to the integrin via surface-exposed integrin-binding motifs Arg-Gly-Asp or Lys-Gly-Glu in the 2 protein, and this promotes clathrin-dependent uptake. Mammals use a similar, yet distinct, strategy to produce microRNAs (miRNAs), which either degrade or inhibit the translation of messenger RNAs. INTRODUCTION the production of cytokines. For gene therapy applications such as vaccination and anticancer therapy, transient but high-level expression of gene products in the target cells may be sufficient. This diagram does not attempt to describe the exact three-dimensional structure of the protein (see Figure 2.12). In human cytomegalovirus, at least six proteins interfere with antigen presentation by preventing the processing of viral proteins, by downregulating or sequestering MHC class I and II proteins, or by blocking the loading of viral peptides onto MHC proteins. Some newly isolated large, double-stranded DNA viruses of algae are currently not officially assigned to the Phycodnaviridae because of their high divergence from the original founder members of the family. 2⬘, 5⬘-oligoadenylate binds to and activates ribonuclease L, which degrades mRNAs. Oncogene: a viral or cellular gene whose expression can lead to cell transformation or tumorigenesis. This class of algae includes important bloom-forming species found in coastal and subarctic regions of the world's oceans, although freshwater species also exist. Viral messenger RNAs are synthesized by an RNA polymerase packaged in the virion The RNA of negative-strand viruses cannot be directly translated upon entry into the cell; it must first be transcribed into complementary, positive-sense messenger RNAs. However, there is no cellular RNA-dependent RNA polymerase available for production of viral mRNAs. Therefore, negative-strand RNA viruses 16/05/11 8:05 PM Paramyxoviruses and Rhabdoviruses Leader 181 Trailer N P/C/V M F HN L 3⬘ 5⬘ AUUCUUUUU GAA UCCCUCUUUC End Intergenic Start Figure 15.6 Paramyxovirus gene map. X-ray diffraction: the diffraction of x-rays by oriented molecular assemblies, usually in crystals, used to determine the atomic structure of the object. These viruses were named baculoviruses because of their shape (baculo means "stick" or "rod" in Latin). In contrast to phagocytic leukocytes, macrophages primarily reside in the body's tissues and organs, and are activated in situ. In both cases, the amino acids important for this function are located at the surface of the virion. This virus was first described in 1941, and was the first plant virus whose cloned genome was used to generate infectious RNA transcripts. Some cancers are associated with infection by the -herpesviruses Epstein–Barr virus and Kaposi's sarcoma virus (human herpesvirus 8). The positively charged amino acids in the amino terminus bind to and neutralize the negatively charged phosphate backbone of the viral DNA, while the hydrophobic carboxy terminus lodges into a cleft in the capsid protein, tethering the genome to the inner surface of the capsid. Analysis of these mutants led to the discovery that the virus encodes at least three genes whose function, in part, is to block apoptosis. These peptides are translocated to the endoplasmic reticulum and are ultimately presented to the immune system by major histocompatibility (MHC) class I proteins at the cell surface. Bottom: viral genome. Apoptosis induced by reovirus is significantly reduced in cells expressing an inhibitor of NF-B. Cell lines derived from cervical cancers contain copies of a part of the human papillomavirus genome integrated into the cellular genome at sites that differ in each cell line. When HA binds to sialic acid residues on these macromolecules, NA can reverse this binding by cleaving the bound sialic acid residue from the remainder of the oligosaccharide. Fusion is induced by a conformational shift in the SU protein that exposes the hydrophobic amino terminus of the TM protein, which is then inserted into the cell membrane. Most bunyaviruses are transmitted from animal to animal by arthropod vectors, and these viruses are able to replicate in both their invertebrate and vertebrate hosts. Two different assembly pathways have been elucidated. All picornaviruses make the 3C proteinase. 16/05/11 8:09 PM 252 CHAPTER 21 Polyomaviruses AUG 5' ORI 200 100 VP2 NH2 PL L mRNA Enhancer 1/5292 5200 5100 TATA 5000 ATAT C B A 1/2 Large T Antigen Binding PE E mRNA 5' GUA LTAg NH2 Figure 21.5 Intergenic control region on mouse polyomavirus genome. Virogenic stroma: electron-dense material that accumulates in baculovirus-infected nuclei, believed to be the site of viral replication and nucleocapsid assembly. While T7 DNA polymerase alone can catalyze the addition of dNTPs to a growing DNA chain, the binding of thioredoxin greatly increases its rate and processivity (the tendency to continue on the same DNA templateprimer complex). This can be an effective host defense system, because the premature death of virusinfected cells reduces the spread of the infection within the organism. One is the E3 11.6-kDa "death protein". T7 RNA polymerase is expressed in a mammalian cell from either a transfected plasmid, a recombinant virus vector, or a gene in a specially engineered cell line (see Chapter 7). The first exon contains leader sequence (le) for efficient translation. Cell-based high throughput screen: method that uses automated assays for screening large numbers of drugs for their ability to inhibit virus replication or other viral processes in infected cells. The mimivirus genome may be more prone to damage due to its large size. Activated caspase-1 causes NLRP3 AIM2 the maturation and secretion of pro-inflammatory cytokines such as interleukin-1 and interleukin-18. This allows translational readthrough, permitting the generation of the Gag/Pol polyprotein. See text for description. However, public acceptance was far from universal. A viral protein, ICP47, binds to the transporter of antigenic peptides (TAP1/TAP2) and blocks the translocation of peptides into the endoplasmic reticulum, therefore inhibiting antigen presentation. The RNA primer is later removed and replaced with DNA, whose synthesis is initiated upstream of where the primer originally lay. Although cleavage by Cre recombinase is not complete and some helper viruses are still packaged, contamination of the vector with helper virus can be reduced to less than 0.1%. The nature of the proteinase that carries out the final (maturation) cleavage of VP0 is unknown. This virus causes economically important outbreaks of disease in cattle and sheep; the 2001 and 2007 outbreaks in Britain required extensive quarantines and slaughter of infected animals. The sizes of the individual proteins are listed for yellow fever virus in Table 12.2. Cleavage by proteinases is also indicated. Recently, a simple system for recovery of infectious bunyaviruses from cloned cDNAs was established. Glaser and J. X-ray structure determinations of several flavivirus C-terminal domains have confirmed that the fold is consistent with known helicases and unwinding activity has been demonstrated in purified systems. Delivery of the nucleocapsid cargo into the cytosol of the target cell occurs when the viral envelope fuses with a cellular membrane (Figure 4.1, right). In these processes, proteins bind to each other and form specific aggregates that can become very large and insoluble. Self-replicating RNAs could have coexisted for some time alongside the DNA genomes in these primitive cells. The layer of Gag proteins at the inner surface of the plasma membrane interacts sufficiently strongly with the lipid bilayer (in many cases through an N-terminal covalently bound fatty acid, myristate) that its assembly into a nucleocapsid can drive budding. How many subunits can be accommodated on the capsid surface? These kinds of experiments defined the temporal phases of baculovirus infection: the immediate early phase is from 0 to 3 hours; the early phase is from 3 to 6 hours; viral DNA synthesis begins between 6 and 8 hours; the late phase is from 6 to 24 hours; and the very late phase continues until the death of the cell. These procedures indicate the presence of six stemloop structures (I–VI) in enterovirus and rhinovirus 5' noncoding regions, and 12 such structures (A–L) in cardiovirus and aphthovirus 5' noncoding regions (Figure 11.6). 195 Minor nucleocapsid protein VP30 activates viral mRNA synthesis in Ebola virus 195 Matrix protein VP40 directs budding and formation of filamentous particles 195 Most filovirus outbreaks have occurred in equatorial Africa 196 Filovirus infections are transmitted to humans from an unknown animal origin 197 Spread of filovirus infections among humans is limited to close contacts 197 Pathogenesis of filovirus infections 197 Clinical features of infection 198 17. The intact polyprotein can synthesize negative-strand anti-genome RNA, but cleavage of p200 by a virus-encoded proteinase to produce two proteins named p150 and p90 is required for efficient formation of new positive-strand rubella virus genome RNA and subgenomic RNA. The role of PA in transcription is not well understood, and it is not shown in Figure 18.7. 16/05/11 8:07 PM 216 CHAPTER 18 Influenza Viruses Cytoplasm Cap AAA NP, PA, PB1, PB2 M1, NS2 4 6 3 1 Cap AAA mRNA 7 2 5 Genome RNP 5 Antigenome RNP Genome RNP Nucleus Figure 18.6 Transport of influenza virus RNAs and proteins between nucleus and cytoplasm. • The complement cascade consists of a set of serum proteins that recognize and bind to pathogens, helping antibodies and phagocytic cells to clear virus or virus-infected cells from the body. Stewart and Bernice Eddy soon showed that these tumors were caused by a filterable agent. All told, greater than 10% of the city's population of 40,000 died during the summer epidemic and a mass exodus reduced Philadelphia's population even further. Like the B-cell receptors, each T-cell receptor has binding specificity for a particular antigenic determinant. Genes are expressed in three temporal classes with distinct functions: • • Early: dissolve core, direct DNA replication and intermediate gene expression, combat host defenses Intermediate: direct late gene expression, combat host defenses Smallpox was a debilitating and fatal worldwide disease Smallpox, caused by the poxvirus variola, embodies simultaneously one of humankind's greatest trials and one of its greatest triumphs. Because they have different lengths 5' Cap ORF1a but share common sequences at their 3' ends, these are referred to as a nested set of mRNAs. Each one of the subgenomic mRNAs is translated to produce one or more proteins coded by the open reading frames closest to the 5' end of the mRNA. The HIV transcriptional transactivator Tat and the hepatitis C virus NS5A protein appear to inhibit PKR antiviral activity by binding directly to PKR. These findings implicate Nef as an important determinant of disease in infected animals. This cleavage results in a conversion of the 16/05/11 8:03 PM 144 CHAPTER 12 Flaviviruses prM/E heterodimer into an E homodimer as shown in Figure 12.3. Analogous to prM, the E protein contains both a transmembrane domain and a signal sequence at its C-terminus. Virus infection leads to activation of cellular transcription factors that bind to positive regulatory domains (PRD) upstream of the TATA box and start site on the interferon- gene, forming an "enhanceosome." CH033.indd 407 16/05/11 8:19 PM 408 CHAPTER 33 Intrinsic Cellular Defenses Against Virus Infection nucleotide). T7 (but not T3) is unable to replicate in cells containing the F-factor. The F and HN proteins are shown inserted into the plasma membrane of the cell or the virion envelope. The palindromic sequences fold to form hairpins at the ends of the DNA. Shine-Dalgarno sequence: a consensus sequence located just upstream of translational start sites in bacterial mRNAs that facilitates initiation of protein synthesis by binding to 16S ribosomal RNA. The two class II promoters closest to the left end of the DNA, promoters 1.1A and 1.1B, precede an AT-rich region on the DNA. A length of approximately 200 bp of DNA is wrapped as a toroidal coil around the outside of each cylindrical nucleosome (Figure 21.2b). By James D. It is notable that baculoviruses make their own DNA polymerase, as do most large DNA viruses (including adenoviruses, herpesviruses, and poxviruses), even though baculoviruses replicate their DNA in the cell nucleus where cellular DNA polymerases are located. The retrovirus vector used to transduce the hematopoietic stem cells contained the wild-type LTR. The 2b protein 16/05/11 8:01 PM 122 CHAPTER 10 Cucumber Mosaic Virus suppresses salicylic acid-mediated virus resistance, and the synthesis of host cell RNA-dependent RNA polymerase-1 is induced by salicylic acid. The following molecular criteria are used to classify viruses: • • • • Type of nucleic acid genome (DNA or RNA) Strandedness of nucleic acid (single- or double-stranded) Topology of nucleic acid (linear or circular) Symmetry of capsid (icosahedral, helical, or no symmetry) • Presence or absence of an envelope Genetic relatedness among viruses can be determined by comparison of viral genome sequences and amino acid sequences of viral proteins. Lentivirus vectors are used for gene delivery to non-dividing cells Lentiviruses have a major advantage over other retroviruses when used as vectors: the integration complex, containing the proviral DNA made by reverse transcriptase, is transported into the nucleus via the nuclear pores. Acute or innate responders to viral infection include the complement system and inflammatory cells such as polymorphonuclear leukocytes and natural killer (NK) cells, monocytes, and macrophages (Figure 34.1). RNA 5 is a mixture of sequences originating from the 3'-terminal noncoding regions of RNAs 2 and 3, and these noncoding RNAs can be packaged into virions. Since then, more than 100 necrogenic or nonnecrogenic variant satellite RNAs have been found associated with cucumber mosaic virus worldwide. How do RNA phages address the requirement for single-stranded RNAs to serve both as templates for translation and templates for genome replication without producing a double-stranded RNA intermediate? ADENO-ASSOCIATED VIRUS VECTORS Adeno-associated virus vectors can insert transgenes into a specific chromosomal locus Adeno-associated virus (AAV) has a small, nonenveloped virion 25 nm in diameter. The initial failure to detect pathogenesis with HIV-1 lacking Nef resulted in the proposal such a virus could be used as an attenuated virus vaccine. Coccoliths: individual plates of calcium carbonate formed by coccolithophores in the Golgi body, then excytosed and incorporated in the coccosphere. However, studies with another member of the NCLDV superfamily, African swine fever virus, suggest that viral DNA replication begins in the nucleus, and these replicated DNA molecules are then transported to the cytoplasm, where further replication and packaging take place. They can be significant components of fouling communities that occur on marine structures such as docks, buoys, and the exterior surfaces of ships. Most patients, however, had only mild symptoms and a short course of disease without severe complications or mortality. Examples of virus receptors are: 5'SS R U5 PBS ≠ 3'SS ppt U3 R MA NC CA PR Gag SU RT Poly (A) TM IN Env Pol Figure 28.2 Structure of retrovirus RNA. It was not until 1979 that the first phycodnavirus Table 27.1 Phycodnaviruses and their hosts Virus genus, Type species Algal host Description Chlorovirus PBCV-1 Paramecium bursaria associated Chlorella Freshwater single-celled green algae, symbiotically associated with the protozoan Paramecium bursaria. A single copy of each gene segment is packaged into each virion by an unknown sorting mechanism. This virus, subsequently named human immunodeficiency virus type 1 (HIV-1), was demonstrated (despite much controversy and debate) to be the causative agent of AIDS. The increased cytopathic effects caused by the mutant virus may be due to the increased rate of synthesis of GP, which affects the protein processing and transport system of the cell. Each successive tube contains a 10-fold dilution of the contents of the previous tube. Early viral proteins direct replication of viral genomes 15 5. The incubation periods of these pathogens are all relatively short (7–10 days) and the diseases run their course to recovery or death quite rapidly. Three possible routes of acquiring an envelope and escaping from the cell are shown. In addition, bunyaviruses that express autofluorescent proteins have been created, permitting real-time microscopical analyses of the infection cycle. All retroviruses make at least two mRNAs: unspliced RNA is used for synthesis of the Gag and Gag/Pol proteins, and a singly spliced form, from which the Gag/Pol reading frames have been removed, is used for synthesis of the Env proteins. Oncogenic E7 proteins can also induce the ubiquitin-mediated proteolytic degradation of Rb.These activities may account in part for the differences in cell transforming capacity between oncogenic and non-ocongenic papillomaviruses. Chromosomal DNA is condensed by wrapping around the exterior of cylindrical nucleosomes. The genome encodes three structural protein subunits, known as VP1, VP2, and VP3, which are processed by cleavage from a single precursor polypeptide. It possesses a single protein-coding open reading frame, complementary to the genome RNA. Patients exhibit chronic fever, night sweats, diarrhea, a number of infections such as cytomegalovirus, pneumonia, oral thrush, herpes simplex, neoplasms such as Kaposi's sarcoma and lymphomas, and neurological syndromes including dementia and neuromuscular disorders. It has a coding capacity that eclipses numerous bacteria and archaea (such as Chlamydia, Rickettsia, Mycoplasma, and Nanoarchaeum). GENOME Circular, double-stranded DNA with a single-stranded gap on one strand, 3.2 kb. Viruses depend on cells for their replication because they lack the following basic elements required for growth and replication, which are present in all living cells: • Enzyme systems that produce the basic chemical building blocks of life: nucleotides, amino acids, carbohydrates, and lipids • Enzyme systems that generate useable chemical energy, usually in the form of adenosine triphosphate (ATP), by photosynthesis or by metabolism of sugars and other small molecules • Ribosomes, transfer RNAs, and the associated enzymatic machinery that directs protein synthesis • Membranes that localize and concentrate in a defined space these cellular macromolecules, the small organic molecules involved in growth and metabolism, and specific inorganic ions Virus particles break down and release their genomes inside the cell Viruses are not the only obligatory intracellular parasites known. Table 24.1 Human herpesviruses Subfamily and characteristics Members ␣ Herpes simplex virus 1 & 2 Labial and genital lesions Varicella-zoster virus Chickenpox, zoster (shingles) Short growth cycle, rapid spread Latent in sensory neurons. How did a bat coronavirus mutate and enter humans to become SARS coronavirus? CH034.indd 419 Medulla (macrophages, plasma cells) Efferent lymphatic vessel Germinal center (B cells) Paracortex (T cells, dendritic cells) Artery, vein Antibodies, memory B and T cells Figure 34.4 Structure of a lymph node. VP30 is a zinc-binding protein that forms oligomers, and both zinc-binding and oligomerization are required for its transcription-stimulating activity. These proteins are targeted to the Golgi membranes by a signal contained in Gn. A Gn/Gc dimer probably forms rapidly in the endoplasmic reticulum and is the form transported to the Golgi apparatus. nsp7, 9, 10 Unknown function, possible hetero- and homo-oligomeric structures. Symptoms of viroid infections include leaf discoloration, dwarfing, enhanced fruit production, orange spotting, decline in fruit yield, stunting, etc. RNA transcripts are shown in blue; vertical lines show 5' ends and arrows show 3' ends. nsP3 556 Unknown function. Title. RNA (blue) degraded by RNAse H is shown as dashed line. Bacteriophage Lambda 85 Roots . The 101-amino acid capsid protein associates with the genome RNA and serves as a scaffold around which the envelope proteins and the lipid bilayer are organized. Human T-cell leukemia virus type 1 (HTLV-1) codes for a protein designated Tax, which augments viral gene expression by activation of cellular transcription factors. Each of these families includes only one or a small number of different phage isolates. Despite high levels of viremia, old world monkeys infected with SIV do not succumb to the same clinical syndromes seen in humans infected with HIV-1, suggesting that these monkeys have adapted to SIV through selection over a long period of time. HA1, the surface subunit, contains the sialic acidbinding domain. viral mRNAs, how then does it exit to the cytoplasm to be packaged into virus particles? Adult T-cell leukemia: a malignant blood disease caused by T cell proliferation induced by HTLV-1 infection. Penton base subunits, each made of five molecules of the penton protein (polypeptide III), lie at the 12 vertices of the icoashedron. Adjacent alpha helices within the trimer interact with each other to form a structure called a coiled-coil, similar to the transmembrane portion of the envelope glycoproteins of a number of other RNA viruses. Together with the coactivator CBP/p300, this collection of DNA-bound proteins assembles to form an active transcription complex called the "enhanceosome." Although not identical, the interferon-␣ enhancer also contains positive regulatory domains. Remarkably, the first gene from the 5' end occupies two-thirds of the viral genome and is translated into polyproteins that are cleaved by viral proteinases into 16 or more different proteins involved in genome replication and subgenomic mRNA synthesis. Healthy plants could be infected by mechanical inoculation. Cucumber Mosaic Virus 112 Mosaic disease in cucumber plants led to the discovery of cucumber mosaic virus (CMV) 113 Cucumber mosaic virus has a positive-strand RNA genome enclosed in a compact capsid with icosahedral symmetry 113 The genome of cucumber mosaic virus consists of three distinct RNA molecules 113 The three genome RNAs and a subgenomic RNA are encapsidated in separate but otherwise identical particles 114 The 3'-terminal regions of cucumber mosaic virus genome segments can fold to form a transfer RNA-like structure 114 Cucumber mosaic virus is transmitted in nature by aphids 115 The genome of cucumber mosaic virus encodes five multifunctional proteins 116 Replication of viral RNA is associated with intracellular membranes, and requires coordinated interaction of viral RNAs, proteins, and host proteins 117 Brome mosaic virus RNA replication has been analyzed in yeast cells 117 Brome mosaic virus RNA synthesis takes places on cytoplasmic membranes 117 Packaging of viral genomes 117 Cucumber mosaic virus requires protein 3a (movement protein) and coat protein for cell-to-cell movement and for long-distance spread within infected plants via the vasculature 118 Tobacco mosaic virus movement protein can direct movement of cucumber mosaic virus in infected plants 119 Mutation, recombination, reassortment, and genetic bottlenecks are involved in the evolution of cucumber mosaic virus 120 Host responses to cucumovirus infections reflect both a battle and adaptation between viruses and hosts 120 Plants respond to virus infection by RNA silencing, and cucumber mosaic virus protein 2b suppresses silencing 121 Cucumber mosaic virus supports replication of defective and satellite RNAs 122 Satellite RNAs can either attenuate or increase severity of symptoms in infected plants 122 11. Determinant d has a lysine at position 122 on the small surface protein, while y has an arginine at that position; w has a lysine at position 160 while r has an arginine at that position. Genes in the 1 group begin to be transcribed early but their transcription is stimulated several-fold after the onset of viral DNA replication; expression of 2 genes begins only after DNA replication starts. Activated PKR phosphorylates the alpha subunit of eukaryotic protein synthesis initiation factor 2 (eIF2), resulting in inhibition of cellular protein synthesis. The 1a and 2a proteins normally accumulate at very low levels in infected cells. To illustrate the scale change, the same diagram of a retrovirus shown at the top left of the second page is also shown, four times larger, at the bottom left of the first page. Because maraviroc is effective only in people who are infected with a virus strain that binds to CCR5, it is important to test patients to see which strains are present before beginning therapy with this drug. However, the demonstration that point mutations within Nef can result in selective inactivation of particular functions provides a means of separating the contributions of the various activities to the disease process. Most envelopes are formed by budding from preexisting cellular membranes. Capsids can be loosened or dissociated in vitro by treatment with reducing agents, which break disulfide bonds between cysteines, and chelating agents, which remove divalent cations bound to capsid proteins. Used as vectors for gene transfer into human cells. • Virus factories are localized areas of the cytoplasm where viral RNA synthesis and virion assembly take place. They can cause mild or severe disease, including yellow fever and fatal encephalitis. Discuss the principal methods used by adenoviruses to generate approximately 50 distinct adenovirus proteins by using a very limited number of transcriptional promoters. The seven messenger RNAs of murine hepatitis virus are shown, along with the open reading frames they use to synthesize viral proteins. Immune responses occur in the spleen, lymph nodes, and gut (secondary lymphoid organs). Making higher levels of subgenomic RNA is one means by which the virus is able to produce more structural than nonstructural proteins. These vectors are particularly useful for production 16/05/11 8:04 PM Togaviruses of proteins that have post-translational modifications (such as phosphorylation or the addition of carbohydrates) that occur in vertebrate or insect cells but not in bacterial cells. For some of them the structure of the fusion proteins and the conformational changes that they undergo are well known. Continued transcription from PL and PR produces high levels of early proteins, including the late gene activator Q, and the lytic program ensues in more than 90% of infected cells. Aphids are quite specific in the plants they feed on, but they test many plants to determine if a plant is appropriate, and it is this brief probing that results in virus transmission. One problem with this pathway is that the threading of genome RNA into the empty capsid would require a complex mechanism that is not known to exist for other RNA viruses. Differential splicing generates multiple mRNAs Transcription produces genome-length RNA identical to that found in the virion. Therefore, unless latently infected cells can be activated in some fashion to render them recognizable to the immune system, treatment for HIV-1 must be continued for life if the disease is to be held in check. Sphingolipid production may be crucial for the generation of lipid raft membrane structures that could aid virus exit from the cell by budding. If two more Cs are added at the 3' end of phage RNA (CCCCAOH) the CCAOH sequence will stick out. Studies of reovirus pathogenesis in mice Central nervous system. A droplet-shaped virus is the only known member of the Guttaviridae (from the Latin gutta, "droplet") (b) A single virus species represents this family. Red blood cells have carbohydrate-containing receptors on their surface, and have the advantage of being visible because of their color. ffirs.indd i 19/05/11 10:33 AM Vice President and Publisher Acquisitions Editor Associate Editor Assistant Editor Marketing Manager Senior Media Editor Media Specialist Production Manager Senior Production Editor Designer Kaye Pace Kevin Witt Michael Palumbo/Lauren Morris Jenna Paleski Clay Stone Linda Muriello Daniela DiMaggio Janis Soo Joyce Poh Maddy Lesure/Seng Ping Ngieng Cover images: Enterobacteria Phage Phi X174, Human Rhinovirus 3, Simian Virus 40. The RNA polymerase ignores the early termination sequence U5NU, and instead terminates inefficiently at multiple sites far downstream, yielding a family of transcripts 2 to 4 kb in length with heterogeneous 3' ends. and are then occluded in a crystalline protein matrix. Microbiol. These include HA, NA, and the RNA polymerase proteins PA, PB1, and PB2. These so-called "A-particles" then interact hydrophobically with the endosomal membrane and induce the formation of a membrane channel, which the viral RNA can use to slip into the cytosol, leaving an empty capsid in the endosomal lumen (see Figure 11.3). As described above, expression of herpesvirus genes is activated by a complex between viral tegument protein VP16 and two cellular transcription factors, Oct-1 and HCF-1. Both Bcl-2 and Bax are associated with mitochondrial membranes in a complex that regulates apoptosis. HIV-1 has caused major global pandemic that affects males and females equally. Not only is there a termination codon between the gag and pol regions, but the two proteins are also in different reading frames. However, direct inoculation of affected brain tissue from kuru and Creutzfeldt–Jakob disease victims into the brains of chimpanzees, and scrapie brain tissue into mice and hamsters, eventually led to transmission of the disease. Among viruses that infect euryarchaeota, some have typical morphological and genomic features of head-tailed bacteriophages. For example, bacteriophages are given names that include the name of the host bacterial genus and an arbitrary number or Greek letter (e.g., Enterobacteria phage T4, Bacillus phage SP01, Enterobacteria phage Mu). Some DNA gymnastics will then reproduce the original ends. Therefore one group is engineering harmless bacteria to express papillomavirus capsid proteins and generate virus-like particles. Genetic engineering of plants can now produce a variety of viral antigens in natural foods such as bananas. Hepatitis delta virus frequently causes fulminant hepatitis in both primate and non-primate hosts. Hexon: structural subunit of adenovirus virions, made from trimers of the hexon protein. Tumor suppressor protein: a protein, such as pRb or p53, whose absence or mutation can facilitate or cause tumor formation; therefore the wildtype protein suppresses tumor development. Virus suspensions are diluted, usually in twofold steps, and the dilutions are added to aliquots of red blood cells in a buffer and mixed in tubes or multiplewell plates. The ability of alphaviruses to infect neurons may turn out to have some benefits. The answers to these questions are presently not clear. Infection also requires the presence of either the CCR5 or CXCR4 chemokine receptor. 2. The cleavage of the C-terminal signal sequence of the capsid protein by viral proteinase NS2B-NS3 is shown by the thin black arrow. A specific cellular transfer RNA is bound to the genome RNA by base pairing between the primer Cap binding sequence (PBS), located just downstream of U5, and 18 nucleotides at the 3' end of the tRNA. These vesicles ferry virus particles through the cortical network of cytoskeletal fibers, which could otherwise constitute a major obstacle, and transport them away from the cell surface and toward the cell nucleus. For example, the 5' noncoding region of RNA 2 down-regulates the synthesis of protein 2a, which is important for replication complex assembly. The nucleocapsid is then transported to the nuclear membrane. Other picornaviruses do not have these pronounced surface depressions but rely on surface-exposed loop regions of the capsid proteins for recognition of cellular receptors. Transcription of the immediate early or genes takes place during the first several hours after infection. Base pairing between the codons on these mRNA sequences and the anticodon sequences of the two tRNAs bound to the ribosome is similar in two different reading frames. However, human papillomavirus-associated cervical cancer is one of the most common forms of cancer in developing countries (several hundred thousand new cases per year). (a) The surface of an immature virion at neutral pH. At least two factors control the increase in late transcription. Live attenuated human viruses with mutations that reduce virulence. The bound protein complex acts as transactivator to enhance the transcription of these genes—the first set of viral genes expressed in infected cells. huxleyi bloom. Envelope, containing three forms of surface protein, is shown as orange subunits surrounding the capsid. Another organism, Picrophilus torridus, grows at pH 0, the equivalent of 1.2 molar sulfuric acid! However, the analysis of 16S rRNA sequence diversity in environmental samples has indicated that archaea are numerous also in "moderate" habitats, throughout the world's oceans and even in cold environments such as polar seas and buried under glaciers. Polyomaviruses 247 Mouse polyomavirus was discovered as a tumor-producing infectious agent 247 Simian virus 40 was found as a contaminant of Salk poliovirus vaccine 247 Human polyomaviruses are widespread but cause disease only rarely 248 Polyomaviruses are models for studying DNA virus replication and tumorigenesis 248 Polyomavirus capsids are constructed from pentamers of the major capsid protein 248 The circular DNA genome is packaged with cellular histones 249 Circular DNA becomes supercoiled upon removal of histones 249 Supercoiled DNA can be separated from relaxed or linear DNA molecules 250 Polyomavirus genes are organized in two divergent transcription units 250 Virions enter cells in caveolae and are transported to the nucleus 251 The viral minichromosome is transcribed by cellular RNA polymerase II 252 TOC.indd xiii xiii Four early mRNAs are made by differential splicing of a common transcript 253 T antigens share common N-terminal sequences but have different C-terminal sequences 254 T antigens bring resting cells into the DNA synthesis (S) phase of the cell cycle 254 Small T antigen inhibits protein phosphatase 2A and induces cell cycling 254 Middle T antigen stimulates protein tyrosine kinases that signal cell proliferation and division 255 Large T antigen activates or suppresses transcription of cellular genes by binding to a number of important cellular regulatory proteins 255 Large T antigen hexamers bind to the origin of DNA replication and locally unwind the two DNA strands 257 Large T antigen assembles the cellular DNA synthesis machinery to initiate viral DNA replication 257 High levels of late transcripts are made after DNA replication begins 259 Three late mRNAs are made by alternative splicing 260 How do polyomaviruses transform cells in vitro and cause tumors in vivo? Transmission electron micrographs of a cell infected with mimivirus. His administration helped support the "March of Dimes", an organization that funded successful efforts to develop vaccines against poliomyelitis and other childhood diseases. Another mechanism involves insertion of a retrovirus polyadenylation signal into a cellular gene. Only 12 of these pentamers are located at centers of fivefold symmetry; the other 60 pentamers are each surrounded by 6 neighboring pentamers. DISEASES Cucumber mosaic virus infections can be symptomatic or asymptomatic, depending on the host. coli mutants that did not support growth of phage. These proteins will then direct replication of the viral genome. Figure 34.7 Structure of the immunoglobulin G molecule. Binds to cellular receptors; contains epitopes for neutralizing antibodies. 2.1c–d: Linda Stannard/Photo Researchers; Fig. The vaccinia virus RNA polymerase is a complex, eight-subunit enzyme that bears some resemblance to other eukaryotic RNA polymerases. One of the chitinases and the chitosanase are found packaged into the virion and are involved in cell wall digestion, allowing entry of the viral DNA, following attachment of the capsid (see above); these enzymes are also probably involved in release of progeny virus. 427 SEC-X.indd 427 12/05/11 5:05 PM C H A P T E R 3 5 Antiviral Vaccines Brian Ward Vaccine From Latin vacca (cow), because vaccinia virus was thought to be cowpox virus Vaccination is the most effective means available for prevention of viral infections. Most herpesviruses become latent in the body and can be reactivated months or years after primary infection. In neither case was the nature of these viruses understood until much more recently. Like a number of viruses that infect crenarcheaota, the members of the Fuselloviridae (from the Latin fusello, "little spindle") integrate their genome into the host cell, and can be induced to produce virus particles by irradiation of cells with ultraviolet light or other stress factors. Once antigenome nucleocapsids are formed, they can be used to initiate synthesis of full-length genome RNAs by the same mechanism. This change may be signaled by the binding of N molecules to the growing RNA chain, or it may be a result of interaction between newly synthesized molecules of P protein and the RNA polymerase. CI binds to the operators as a dimer with the following affinities: OR1 OR2 OR3. The inner circle shows the locations of EcoRI restriction sites; the 20 largest EcoRI fragments are labeled alphabetically, according to size. Host enzymes carry out repair synthesis of the gap, simultaneously removing the terminal 2 unpaired nucleotides of the viral DNA. Large T antigen now plays an entirely different role: instead of interacting with cellular regulatory proteins, it binds to viral DNA and directs the assembly of a number of cellular proteins that carry out DNA replication. This accounts for much of the antiviral selectivity of acyclovir. Alternatively, extracellular virus particles can be released from the cell surface and infect distant cells. Rous sarcoma virus turns out to be a unique example of a transforming retrovirus that is both tumor-inducing and able to replicate on its own. Assembly Virions Isolate rescued virus by plaque assay Figure 17.6 Rescue of infectious bunyavirus from cloned cDNAs. Mammalian cells constitutively expressing bacteriophage T7 RNA polymerase are transfected with a set of three plasmids containing cDNA copies of each genome segment. 25.4: Reprinted from A.L. Passarelli and L.K. Miller, "Identification and characterization of Lef-1, a baculovirus gene involved in late and very late gene expression," Journal of Virology 67 (1993): 3481–3488, with permission from the American Society for Microbiology. In the final uncoating stage (3), fusion between viral and phagosome membranes occurs, and the core is extruded into the cytoplasm. Terminase, cos cos Terminase (gpNu1 ⩽ gpA) cos Prohead cos ATP ADP cos the viral cutting enzyme, contains multiple Nu1 and A subunits. Operator: a DNA or RNA binding site for a repressor protein that negatively regulates gene expression. The product of gene 0.3 inactivates host type I restriction endonucleases EcoB and EcoK, protecting the infecting DNA from cleavage and degradation. Coronaviruses have large, single-stranded, positive-sense RNA genomes Coronaviruses fall into three groups based on genome sequences Coronaviruses have the largest RNA genomes (27–32 kb) of any known virus family. Passage from endosomes to the cytosol is often triggered by low pH (c) Figure 4.3 Receptor-mediated endocytosis and acidactivated penetration of Semliki Forest virus. • Many viruses enter by fusion mediated by low pH in endosomes. Satellite RNAs are encapsidated together with cucumber mosaic virus genome RNAs, and are transmitted by aphids along with the virus. examination in the electron microscope. What is the role of this conserved AUG for IRES type I and type II elements? On the other hand, dependoviruses (e.g., adeno-associated virus) encapsidate equally well strands of both polarities, but each virion contains only one of the two DNA strands. Import after disassembly at the nuclear pore. Of particular note are two families of paralogous gene families (genes that apparently arose by duplication) within the mimivirus genome. Reovirus gene segments range from approximately 1200 to 3900 bp in length. Non-nucleoside inhibitors selectively target viral replication enzymes Despite problems with AZT, it helped to validate the concept developed from viral genetics and biochemistry that HIV-1 reverse transcriptase is a useful drug target. What is an ambisense genome, present in some bunyaviruses? The two major types of human retrotransposons are designated LINE (long interspersed nuclear element) and SINE (short interspersed nuclear element). Ribose methyltransferase: an enzyme that transfers a methyl group to the 2⬘ position of ribose during generation of a methlylated cap structure at the 5⬘ end of an RNA. Most experimental studies of pathogenesis with Sindbis and Semliki Forest viruses have been done with neonatal mice, in which disease is more apparent than with adult mice and mortality is high. These studies have shown that CH022.indd 270 E7 is mainly responsible for inducing cell proliferation and initiating tumor formation, while E6 has a cooperative role in increasing tumor size and inducing malignant progression. The smallest virion illustrated, a single-stranded RNA bacteriophage, has a diameter of 26 nm; the largest virions illustrated, retroviruses and influenza virus, have diameters of 100 nm. Allen, Plymouth Marine Laboratory William H. CH012.indd 140 16/05/11 8:03 PM Flaviviruses The E protein is found as a dimer, with domain II forming the dimer interface. Other mutations are regulatory and lead to the over-expression of the external scaffolding protein. Because the two delta antigens are expressed at different stages of viral infection, regulation of editing levels is essential; the mechanism by which this regulation is accomplished is still being worked out. For example, herpesvirus proteins with repeats of acidic amino acids escape processing by the proteasome, a cytoplasmic processing unit that generates peptides for MHC I presentation. This differentiation triggers viral DNA replication in the reproductive organs, followed by breakdown of the nuclear membrane, virion assembly, and release of virions from the cells. 9.2b: Reproduced from Figure 1A in Redder, P., X. • A terminator of transcription, TE, releases the host cell polymerase at the end of the class I region. To determine the titer, the number of plaques on a plate is multiplied by the factor by which the original virus suspension was diluted before an aliquot was applied to the plate. This fragment is translocated to the identical DR2 region near the 5⬘ end of the newly synthesized minus-strand DNA, where it forms a short RNA/DNA hybrid. The only rhabdovirus that is of significant concern to human health is rabies virus. Such assays are sensitive to pH, temperature, and buffer composition, and some viruses will agglutinate only cells of a particular mammalian or avian species. This is located within the coding region of the nsP1 gene (for Sindbis virus) or the nsP2 gene (for Semliki Forest virus and the related Ross River virus). From the proportion of lysis to coat protein synthesized by phage MS2, the probability for such successful re-initiation is only 5%. In Greek mythology, a chimera was a fire-breathing monster that had a lion's head, a goat's body, and a snake's tail. The resulting 12S* particle contains one 16/05/11 7:56 PM 74 CHAPTER 6 Microviruses Internal scaffolding protein B H 9S Coat protein pentamer External scaffolding protein D tetramer DNA packaging complex Provirion Virion 9S* 12S* 6S Spike protein G pentamer Procapsid Protein B Protein D Contains proteins A and C. Viroid infections give rise to a wide range of symptoms such as leaf discoloration, dwarfing, enhanced fruit production, orange spotting, decline in fruit yield, stunting, and so forth. However, the genome and subgenomic RNAs of viruses in the Alfamovirus genus are individually packaged into four virions with distinct sizes. Rhinoviruses replicate preferentially at 34⬚C, the temperature of the nose; they are believed to be responsible for approximately 50% of common colds. CH005.indd 67 67 KEY TERMS F-pili Shine–Dalgarno sequence 16/05/11 7:54 PM 68 CHAPTER 5 Single-Stranded RNA Bacteriophages FUNDAMENTAL CONCEPTS • When first discovered, single-stranded RNA bacteriophages represented an ideal source of a purified messenger RNA for molecular biology researchers. These proteins were also implicated in the regulation of late and very late transcription by transient in vivo expression assays (Figure 25.4). An even larger fraction of most genomes (41% in humans) is composed of retrovirus-like elements termed KEY TERMS Acute transforming retroviruses Chemokine Cytosol Lumen Myristate Oncogene Polyprotein Preintegration complex Promoter occlusion Proto-oncogene Proviral DNA Pseudoknot Quasispecies Reverse transcriptase Ribonuclease H Ribosomal frameshifting FUNDAMENTAL CONCEPTS • Retroviruses package two identical copies of a positive-strand RNA genome along with the viral enzymes reverse transcriptase, integrase, and protease in an enveloped virion. This arm can interact specifically with a packaging sequence on the genome RNA, or nonspecifically with other segments of the RNA. A second plasmid (not shown) expresses low levels of T7 lysozyme, which inhibits the activity of the small amount of T7 RNA polymerase made in absence of the inducer, allowing genes coding for toxic proteins to be cloned. The papain-like proteinases (PLPs) are subdomains of nsp3, a 195–213 kDa replicase protein (Figure 14.1). To date, the genomes of three virus strains infecting O. X up-regulates c-Src kinase, Ras/raf/MAP kinase, stress kinase (SAPK), protein kinase C, the Jak–Stat pathway (see Chapter 33), and the transcription factor NF-B, and interacts with and sequesters the tumor suppressor protein p53 (see Chapters 21, 22, and 23). Scaffolding proteins are extruded from the capsids in the process. DISTINCTIVE CHARACTERISTICS Generally difficult to grow in vitro: require specialized raft cultures of epithelial cells. They can also be used for production and purification of selected proteins, because the levels of foreign proteins synthesized in infected cells can be quite high. • Imiquimod/resiquimod TLR-2 ligand that elicits strong humoral and cellular responses • Detoxified lipopolysaccharide TLR-4 ligand that mimics bacterial infection and stimulates strong humoral and cellular responses Pulsed dendritic cells Presentation of antigens to dendritic cells outside of the body and their reintroduction can lead to enhanced response to antigens. • Louis Pasteur developed an effective rabies vaccine in the mid-nineteenth century; modern rabies vaccines are safe and widely used in domestic animals, in at-risk humans, and as prophylactic agents that can interrupt rabies infection before disease symptoms have appeared. However, the extracellular virus membrane is fragile, and probably does not survive well in the environment. Infection of cells leads to integration of the viral genome within genes coding for cellular transfer RNAs, the specific tRNA gene depending on the cell and host combination. This information can then be used to design small molecules that bind to the active site and block activity of the protein. Taxonomy: the science of classification of living organisms on the basis of genetic and evolutionary relatedness. Furthermore, proteins that detect virus infection, including TLR-3, RIG-I, and MDA-5, can induce apoptosis. Proteins of the Rb family bind to and inactivate a family of cellular transcription factors, termed E2F. 32.4b and Fig. Binding of interferon to receptors at the surface of target cells activates the Jak–Stat pathway and leads to transcriptional activation of interferon-stimulated genes (ISG). CH007.indd 78 16/05/11 7:57 PM Bacteriophage T7 I ("early") genes, located in the left-hand one-fifth of the genome, are expressed during the first 6 to 8 minutes after infection (at 30 C). These all tend to reduce the level of transcripts as the RNA polymerase passes from the 3' end to the 5' end of the genome. coli mutants. The Herpesviridae are grouped in three subfamilies denoted , , and , based on biological and physical properties including cell tropism and genome organization. 1931 Goodpasture first uses embryonated eggs to culture viruses. Life-threatening infections in immune-suppressed individuals. Antibodies against viral or cellular proteins can be used to detect specific molecules with fluorescent dyes or other assays. However, ceramide can also control a cell death mechanism termed apoptosis. Viruses in these two families carry out the reverse transcription step at the end of the replication cycle and package the resulting DNA molecule in the virion, whereas retroviruses carry out the reverse transcription step at the beginning of the replication cycle. Thus, a common strain of hepatitis B virus might be classified as adw or adr. They have affinity for viral capsid proteins, and by binding to the capsid they help to release the viral RNA, which is then ready for translation. The acquisition of new genes is achieved by nesting them within the sequences of preexisting genes rather than inserting additional DNA sequences in the genome. Nonpermissive: describes a cell type that cannot support productive infection by a particular virus. This changes the amino acid at position 295 from a threonine (ACC) to an asparagine (AAC), and sGP ORF 0 ORF-1 GP Unedited RNA ACU AAA AAA Thr Lys Lys A CC UCA Thr Ser CGC AUC UAA Arg Ile Stop Edited RNA ACU AAA AAA Thr Lys Lys AAC CUC Asn Leu UCG CAU CUA Ser His Lys ACA Thr GP 676 aa 20% Mutated ACU AAG AAG editing site Thr Lys Lys AAC CUC Asn Leu UCG CAU CUA Ser His Lys ACA Thr GP 676 aa 100% sGP 364 aa 80% Figure 16.6 Editing of Ebola virus GP mRNAs. Shown above (in green) is the open reading frame (ORF 0) translated to produce precursor sGP and the N-terminal portion of GP, and below (in orange) is the open reading frame (ORF –1) for the C-

terminal portion of GP. Replication protein A binds to the unwound DNA strands and keeps them from renaturing into a double-helical form. How does middle T antigen stimulate cell proliferation and division? Several of these are viruses of CH002.indd 27 Figure 2.12 A typical viral envelope protein, influenza virus hemagglutinin. HIV-1 protease is also a good target because it has an unusual substrate specificity; it cleaves between phenylalanine and proline, a site that is rarely if ever cleaved by human proteases. There is evidence that these cellular proteins are required to help VP40 and other viral matrix proteins form virions by budding at the plasma membrane. Furthermore, in Belgrade, Yugoslavia, a veterinarian performing an autopsy on dead monkeys became infected, together with his wife, who nursed him during the first days of the illness. A number of viruses package in their virions proteins that are necessary for replication once the genome enters the host cell. The reading frame is shifted to –1 because of an extra U added at the end of the editing site (underlined A in mRNA). Expression cassettes for RNA virus vectors do not usually contain transcriptional promoters/enhancers, RNA splicing sites, or polyadenylation signals because they are expressed as RNA by viral RNA polymerases in the cytoplasm. The other strategy would involve the use of a self-inactivating retrovirus vector, in which both LTRs would be inactivated after integration. Interferon- signals through a different receptor complex, which contains the interleukin-28 receptor ␣ (IL28R␣) and the interleukin-10 receptor (IL10R) proteins. NK cells will then secrete cytotoxic proteins, which attack and kill the infected cell. Domains D1, D2, and D3 are shown, and bound ATP is shown at the junction between D2 and D3. The ying-yang relationship between the activities of these two influenza A surface glycoproteins may help virions reach their target cells by releasing them from the surface of virus-producing cells or from mucoproteins that are abundant in the respiratory tract. Carleton, 7, 388 Gallo, Robert, 355 Galtier, Victor, 430, 442 Glaser, R., 303 Gomatos, Peter, 225 Goodpasture, Ernest William, 430 Graham, Angus, 226 Gross, Ludwig, 247 Panum, Peter, 176 Papanicolaou, George, 270 Pasteur, Louis, 6–7, 176, 430, 442 Petri, Julius Richard, 430, 431 Phipps, James, 313 Price, Conway, 113 Prusiner, Stanley, 7, 390 Hamré, Dorothy, 160 Hausen, Harald zur, 7 Reed, Walter, 7, 138, 148, 430 Rhazes of Baghdad, 176 Ris, Hans, 86 Robbins, Frederick, 7, 430, 431 Roberts, Richard, 7, 274 Roeder, Robert, 275 Roosevelt, Franklin D., 126 Rossmann, Michael, 70 Rous, Peyton, 7, 342, 343 Roux, Emile, 442 Rowe, Wallace, 274 Rutter, William, 366, 375 Sabin, Albert, 7, 126 Salk, Jonas, 7, 126 Sanger, Fred, 69, 86 Scott, Howard, 113 Sertic, V., 69 Sharp, Philip, 7, 274 Shatkin, Aaron, 226 Shope, Richard, 263 Siegert, Rudolf, 188 Sinsheimer, Robert, 69 Slenska, Werner, 188 Smith, G., 308 Sonenberg, Nahum, 127 Stanley, Wendell, 6, 7, 303, 430 Steinhaus, Edward, 303 Stewart, S., 247 Strauss, Michael, 309 Studier, F. They encounter a UAG termination codon only 12 nt after the splice junction, leading to production of a 195amino acid protein called small T antigen (STAg). Exceptions are viruses that infect insects of some economic importance and that were readily adapted to cell culture. No spliced mRNAs (no introns). Successful infection also depends on the differentiated state of the host cell. Depending on the target cell, fusion takes place at the plasma membrane or in endosomes. Acyclovir: nucleoside analogue used for treatment of herpes simplex and varicella zoster virus infections. In this mode, the 40S subunit bypasses multiple upstream AUG sequences, moving by translocation or scanning from its site of initial contact with the IRES to reach the initiator AUG. This is the smallest region that can mediate interaction with the cellular molecule that serves as receptor. See also Helical symmetry with icosahedral symmetry, 21–24. This model is outlined in ORF1b An 3' HE Cap An S An Cap An Cap E An Cap M An Cap N Cap An Figure 14.4 Coronavirus messenger RNAs: a nested set. akashiwo have caused massive fish kills, typically of caged, farmed fish such as salmon and yellowtail in places like Japan and Peru. E7 proteins share some structural homology with proteins from other viruses, including the adenovirus E1A protein and polyomavirus large T antigens. The enzyme was found to be a symmetrical dimer of two identical subunits. Neither is it clear why only the S antigenome of phleboviruses, but not the M or L antigenome, is used as a template for mRNA synthesis. The practice of cutaneous variolation spread through southwest Asia and northern Africa. Development of an efficient producer cell line is often the most challenging requirement in the construction of a vector system. However, the reading frame downstream of the splice junction is now ⩽1; although this sequence overlaps with parts of the small and middle T antigen reading frames, it codes for a totally different amino acid sequence because different triplet codons are recognized by the ribosome. Answers to Review Questions are available to course instructors at the Instructor Companion Site of Wiley Higher Education at: www.wiley.com/college/acheson. Flaviviruses cause several important human diseases Flaviviruses have had an important impact on humans over the last several hundred years. 16/05/11 8:15 PM 348 CHAPTER 28 Retroviruses Sequence elements in the long terminal repeats direct transcription and polyadenylation by host cell enzymes With integration, the early phase of retrovirus replication is completed. The peptide bond (arrow) between phenylalanine (phe) and proline (pro) is cleaved by the protease. 2.8a: Courtesy of Nicholas Acheson; Fig. These activated transcription factors bind to regulatory sequences called positive regulatory domains (PRD), which lie 200 nucleotides upstream of the transcription start sites on both interferon-␣ and interferon- genes (Figure 33.7). This rolling hairpin model for vaccinia virus DNA replication does not formally require a DNA primase, as DNA strand synthesis occurs by elongation of nicked parental DNA strands rather than initiation by RNA primers. The success of these trials stems most likely from the fact that the eye is a small organ and therefore the vector dose needed is significantly lower than for treatment of a systemic disease such hemophilia. Vaccine developers are also targeting ubiquitous and persisting viruses such as the herpesviruses Epstein–Barr virus and cytomegalovirus. Viral proteins are synthesized as a single polyprotein that is cleaved by viral and cell proteinases. GENOME Circular single-stranded DNA, 4.4–6.1 kb. Autographa californica MNPV early and late genes are transcribed by different polymerases recognizing different promoter elements. Cytosol: the soluble portion of the cytoplasm, excluding membrane-bound organelles and their contents. This process is based on the theory that the more recent the separation between two viruses, the more similar will be their genetic information. The present chapter covers the paramyxoviruses and rhabdoviruses; filoviruses are discussed in Chapter 16. Virion assembly involves cleavage of VP0 to VP2 plus VP4 Once sufficient genome RNAs are made, virions can be assembled. Vaccines that stimulate cell-mediated immunity are being developed However, it is now clear to vaccine developers that the generation of antibodies is not always sufficient for an effective antiviral vaccine. However, the control of protein synthesis and RNA replication turns out to be carefully regulated, both by RNA secondary structure and by binding of coat and replicase proteins to the RNA at specific sites. Other paramyxoviruses cause canine distemper (like rinderpest, closely related to measles virus) and diseases of mice, chickens, turkeys, seals, and dolphins. E ER lumen prM NS1 C-terminus C Cytoplasm N-terminus Figure 12.5 Membrane topology of the flavivirus structural proteins. To date, viroids have only been found in the plant 16/05/11 8:17 PM Viroids and Hepatitis Delta Virus 385 Engineering ribozymes for novel uses The plant pathogenic RNAs, and the more complex hepatitis delta virus, have provided us with most of the naturally occurring self-cleaving RNAs known to date. Analysis of reassortant viruses to delineate the genetic basis of specific viral phenotypes is termed reassortant genetics. NK cells express both inhibitory and activating receptors to recognize and kill virus-infected cells and avoid killing healthy cells (Figure 34.2). Unstable, degraded by the ubiquitin pathway. Research on RNA tumor viruses led to the discovery of reverse transcriptase, an enzyme that can make a DNA copy of an RNA molecule, upsetting the one-way central dogma that “DNA makes RNA makes proteins”. How diverse are the genomes of the phycodnaviruses? The E4orf4 protein also can induce cell death, via a p53-independent mechanism. An early use of this technology was to create a virus lacking the NSs gene, leading to the discovery that the NSs protein is an antagonist of the interferon response mechanism. In gelled agar, released virus can infect only nearby cells on the monolayer, forming a local area of dead cells or a plaque. • Site-specific integration of adeno-associated viral DNA into the host genome depends on the viral Rep protein recognizing the same sequence in viral and cellular DNA. There is only one family of bacteriophages with positive-strand RNA genomes; no archaeal viruses with RNA genomes have 16/05/11 7:52 PM 36 CHAPTER 3 Virus Classification: The World of Viruses Table 3.2 Viruses with double-stranded DNA genomes Genome segments Genome size (kb) and topology Capsid symmetry Envelope Circular Icosahedral Internal 12 Circular Asymmetric External 15 Linear Icosahedral Internal Linear Icosahedral, tailed No 1 22–121 Linear Icosahedral, tailed No Bacteriophage T4 1 34–497 Linear Icosahedral, tailed No Archaea Sulfolobus spindle-shaped virus 1 1 15–24 Circular Asymmetric External Lipothrixviridae Archaea Sulfolobus islandicus filamentous virus 1 16–56 Linear Asymmetric External Guttaviridae Archaea Sulfolobus neozealandicus droplet-shaped virus 1 20 Circular Asymmetric External Ampullaviridae Archaea Acidianus bottle-shaped virus 1 24 Linear Asymmetric External Globuloviridae Archaea Pyrobaculum spherical virus 1 28 Linear Asymmetric External Rudiviridae Archaea Sulfolobus islandicus rod-shaped virus 2 1 Linear Asymmetric No Bicaudaviridae Archaea Acidianus two-tailed virus 1 Circular Asymmetric External Phycodnaviridae Algae Paramecium bursaria chlorella virus 1 1 Mimiviridae Protozoa (amoeba) Acanthamoeba polyphaga mimivirus 1 1180 Baculoviridae Invertebrates A. Type III interferons were discovered in 2003 by two independent groups using computational and bioinformatic methods. These are spherical particles that usually have a doughnut-like core morphology and are larger than mature virions (Figure 14.6, bottom). DISEASES Benign warts at specific sites (skin, mucosa, larynx) depending on virus strain. Rotaviruses are responsible for gastroenteritis in animals and humans; they cause a large proportion of childhood gastroenteritis and are a major cause of infant illness and death in the developing world. Messenger RNAs are shown with open reading frames as rectangular boxes (green for early proteins, orange for late proteins) and introns as inverted V’s. When transportation of humans was restricted to foot, hoof, and oar, and population centers were small and dispersed, travelers infected in one city were either dead or had recovered and were immune by the time they reached the next city. Among the issues we are addressing are carbon impact, paper specifications and procurement, ethical conduct within our business and among our vendors, and community and charitable support. Capsids made from multiple identical subunits have regular, symmetrical shapes. • Productive papillomavirus infections require differentiation of infected keratinocytes. Ganciclovir must be phosphorylated to act as an inhibitor, in a similar fashion to acyclovir (Figure 36.5). Empty capsids can also form. Pathogenic viruses are grown in eggs or in cultured cells and purified. required to glycoslate its major capsid protein. Symmetrical capsids are strong, stable, and can self-assemble. When two molecules of CH033.indd 410 ł 2', 5'-oligo(A) synthetase (inactive) double-stranded RNA 2', 5'-oligo(A) synthetase (active) ATP POPOPO-CH2 A 5' O 4' 1' 3' 2' OH OPO-CH2 O A OH OPO-CH2 A O 2', 5' oligo (A) OH OH POPOPO-CH2 A 5' O 4' 1⬘ 3' 2' OH OPO-CH2 A O OH OPO-CH2 A O OH OH Ribonuclease L (inactive) Ribonuclease L (active) Figure 33.9 Antiviral activity directed by 2', 5'-oligo(A) synthetase and ribonuclease L. (a) ␣⬘ ␣ ␣⬘ ␣⬘ ⬘ ⬘ (b) Figure 2.6 Structure of the capsid of simian virus 40. Int binds to two different DNA sequence motifs within the arms and the core of attP. Attachment can therefore be exceptionally tight even though the affinity of individual interactions may be rather low. KEY TERMS Anaerobic methanogen Archaea Bacteria Crenarchaeota Domains of life Eukarya (eukaryotes) Euryarchaeota Hyperhalophile Hyperthermophile Phyla Solfataric Thermophile FUNDAMENTAL CONCEPTS • Archaea are a third domain of life, distinct from bacteria and eukaryotes. This RNA is subsequently copied into progeny genome RNA by the same mechanism. Acheson, McGill University 22. These include three types of topoisomerase IIA (common to most large DNA viruses), topoisomerases IA and IB, and a DNA UV damage repair endonuclease. In 1911, Goldberger and Anderson succeeded in transmitting the virus to macaques using filtrates prepared from the blood of infected individuals. (c) Delta self-cleaving structure of hepatitis delta virus. Viral proteins are expressed in a timed cascade regulated at the transcription level In Sf21 cells infected with AcMNPV, several virusinduced proteins can be distinguished by 3 hours after infection, well before viral DNA synthesis begins. Envelope glycoproteins also arranged with icosahedral symmetry. Binding of pppACV to viral DNA polymerase competes for binding of pppdG 2. This is probably related to the distinct sites where viroids in the two groups are localized. Like many other plant viruses with segmented genomes, individual cucumber mosaic virus genome RNAs are packaged in separate virions. These genes are designated pifA and pifB (for phage inhibition by F). 415 CH034.indd 415 16/05/11 8:19 PM 416 CHAPTER 34 Innate and Adaptive Immune Responses to Virus Infection VIRUS STRATEGIES TO COUNTER HOST DEFENSES Viruses express mutated versions of cellular genes encoding cytokines and cytokine receptors. Because these particles can enter into other cells or be transmitted to other animals, pseudovirions may enable passage of certain cellular DNA sequences from one host to another. This diversity probably contributes to the success of H. In this system, the genome is cloned in bacterial plasmids as a set of six or seven distinct cDNA fragments. Prior to the development of reverse genetics, selection of mutants with particular phenotypic traits was used to study the relationship between particular genes and their functions—so-called “forward genetics”. Formation of this hairpin excludes the inhibitory long-distance base pairing between the upstream complementary sequence and the Shine–Dalgarno sequence, thus permitting ribosome binding. 359 (2006), 1203-1216, with permission from Elsevier; Fig. com/permissions. Replication-competent adenovirus vectors could also be used as vaccines to enhance the immune response against any desired target. Tegument proteins are introduced into the cell upon infection by herpesviruses, and some of them CH024.indd 288 carry out crucial functions in the virus growth cycle. (c) The mature infectious virion after release of the pr peptide. Transmissibility and vector specificity are primarily determined by the coat protein. Studies have shown that sewage samples worldwide 59 CH005.indd 59 16/05/11 7:54 PM 60 CHAPTER 5 Single-Stranded RNA Bacteriophages contain from 102 up to 107 RNA phage particles per mL. The tail has a tube-like structure with a wall about 6 nm thick. Late transcription factors (VLTF) are in turn encoded by some of the intermediate genes. The hepatitis B virus vaccine is effective in at least 90% of those who are vaccinated with the three-injection regimen. A satellite virus known as hepatitis delta virus (see Chapter 31) is associated with hepatitis B virus infection. 16/05/11 8:11 PM Adenoviruses These interactions stabilize the transcription complex and stimulate viral gene expression. Coculture of different strains of murine hepatitis virus in mixed populations of cell types generated variant viruses capable of replicating in previously non-permissive cell types including human cell lines, thus changing host range in vitro. What role does APOBEC3G play in the virions? LATs are not essential for establishment of latency, although the presence of the LAT transcriptional unit can increase the efficiency of latent infection in some model systems, perhaps by reducing productive infection. When DNA of adeno-associated virus is extracted from virions under conditions that do not allow self-annealing, it remains single-stranded, resolving this paradox. It was subsequently named polyomavirus (Greek poly for many and -oma for tumor), because it can cause tumors at numerous sites in experimental animals. All of the tolllike receptors except TLR-3 interact with MyD88, while TLR-3 interacts with TRIF, and TLR-4 interacts TLR-2 TLR-7/8 TLR-9 TLR-4 MyD88 TLR-3 RIG-I MDA-5 TRIF DAI Pattern Recognition Receptors Adaptor Proteins IPS-1 activates activates IKK Protein Kinases TBK-1 IRF-3 phosphorylates phosphorylates IRF-7 IκBα IκBα P P P P P P Transcription Factors P NF-κB/IκB complex translocates translocates Cytoplasm Nucleus P P Activated genes Cytokine genes Interferon genes Figure 33.3 Signal transduction following virus recognition. This assay is based on a set of overlapping clones (A through M) that together represent the entire AcMNPV genome. The other three proteins are associated with the viral envelope: GP (envelope glycoprotein), VP40 (matrix protein), and VP24 (membrane protein). A virus 120 nm in diameter is replicating inside a cell with a diameter of less than 1000 nm; it can be estimated there is physically room for no more than 100 virions within a cell. Comparative sequencing data show that retroviral DNA has been inserted into germ line genomes at different times during evolution, some occurring after the division of humans and apes. This showed that viral RNAs could replicate directly rather than being transcribed from a DNA template. If these two genomes are not identical, because of mutation or because two virus strains infected the same cell, the result can be homologous recombination. Dimers can be formed at many places along a genome RNA molecule, more or less at random. It was originally thought to be a small Legionella-like bacterium, and was tentatively named Bradfordcoccus because it appeared to be a gram-positive bacterium. This defines reading frame 0. et al., “Structural analysis of a series of antiviral agents complexed with human rhinovirus 14,” Proc. Hairpin structure: a stem structure formed by base pairing in a nucleic acid that is internally self-complementary. Murine (mouse) hepatitis virus (MHV), a coronavirus, was isolated from laboratory mouse strains in the early 1960s. LMP-1 constitutively activates NF B by binding to several intermediary proteins, including TRAFS and TRADD, whose binding is normally dependent on CD40L-activated CD40. Each of these regions is imperfectly base paired, and in their unfolded, singlestranded form, they represent inverse complements of each other (Figure 26.1d). coli 16/05/11 7:57 PM Bacteriophage T7 81 Table 7.3 Selected class III gene products Gene Function 8 Portal protein, connector for tail 9 Scaffolding protein for formation of capsids 10 Capsid protein 11 Tail protein 12 Tail protein 14, 15, 16 Core proteins, form channel for DNA entry 17 Tail fiber, attachment to cell 17.5 Holin, access of lysozyme to cell wall for lysis 18, 19 Maturation proteins, DNA packaging 5⬘ (a) T7 RNA polymerase CH007.indd 81 T7 Primase/helicase 3⬘ 3⬘ 5⬘ (b) T7 DNA polymerase 5⬘ 3⬘ 3⬘ host cell, using continuous synthesis on the leadingstrand and discontinuous synthesis with Okazaki fragments on the lagging-strand (Figure 7.3). This would explain why multimeric antigenome RNAs are generated by rollingcircle replication using circular genome RNA as template, but are not cleaved at the polyadenylation signal. The assembled core associates with areas of the Golgi membranes rich in these surface proteins. Conversion to ISVPs involves removal of 3 and extension of the attachment protein 1 outward from the virion. 397 SEC-IX.indd 397 12/05/11 5:03 PM C H A P T E R 3 3 Intrinsic Cellular Defenses Against Virus Infection Karen Mossman Pierre Genin John Hiscott DETECTION OF VIRUS INFECTION Toll-like receptors located on the cell surface or in endosomal membranes recognize conserved pathogen structures. • Vectors are produced by transfecting cells with plasmids containing a modified viral genome, usually lacking essential genes, and containing an expression cassette with the desired foreign gene. This bloom occurred in the English Channel, south of Plymouth, UK on July 30, 1999. Binding between an excess of virus particles and an aliquot of red blood cells forms an interlaced network of cells, held together by virus particles that form bridges between adjacent cells. Also referred to as p63, based on its molecular weight. Shortly afterward, similar experiments were carried out on agents that cause foot-and-mouth disease in cattle, and yellow fever in humans. 32.5: Courtesy of Katherine Sutliffe and P. The two families of viroids are differentiated from each other based on what factors? Proviral DNA U3 R U5 CAOH AATG U3 R U5 GT AA HOAC GGGCCC CCCGGG Cell DNA Integrase Insertion of the proviral DNA into the host cell chromosome is catalyzed by the viral integrase, present in the core of the infecting virion. Infection by many viruses leads to reduced expression of MHC proteins on the cell surface, thereby inhibiting cellular immune responses. The resolvase makes a staggered double-stranded break by nicking the complementary strands at each end of the concatemer junction, whereupon each strand of the junction can refold into a hairpin end as the concatemer is resolved. While this allows them to attach to virtually any cell, binding may also occur to cells or membrane fragments that cannot support productive infection. Petri invented the overlapping glass plates that have immortalized his name. DNA replication. Therefore, this protein must cross the membrane of the endoplasmic reticulum at least once, and it may have additional membrane spanning sequences. Despite quarantine efforts, which required ships of African and Caribbean origin to anchor for several weeks prior to entry into North American ports, outbreaks of yellow fever were frequent and severe for ports as far north as Boston. Because virions are formed by budding at the plasma membrane, virus can be produced and circulate in mosquito cell cultures without disruption of infected cells. CH019.indd 227 16/05/11 8:07 PM 228 CHAPTER 19 Reoviruses Table 19.2 Reovirus gene segments and functions of their translation products Gene segment Protein Functions L1 3 Minor inner-capsid protein; catalytic subunit of the viral RNA-dependent RNA polymerase L2 2 Core spike protein; forms pentameric turret for insertion of attachment protein 1; adds a 5' methylated cap to mRNAs L3 1 Major inner-capsid protein; possible RNA helicase; may remove terminal phosphate from newly synthesized mRNA in preparation for capping M1 2 Minor inner-capsid protein; probable RNA polymerase subunit; stabilizes microtubules; nonspecific RNA-binding protein; may remove terminal phosphate from newly synthesized mRNA in preparation for capping; interferon antagonist M2 1 Major outer-capsid protein; important for penetration of endosomal membrane by the viral core; probably regulates late mRNA synthesis from secondary transcriptase particles; affects efficiency of reovirus-induced apoptosis M3 NS Nonstructural replication protein; forms cytoplasmic inclusion structures; associates with nonstructural protein NS and minor core protein 2; first viral protein found in association with viral mRNA S1 1 1s Attachment protein that binds to carbohydrate and junctional adhesion molecule A (JAM-A); determines virus serotype; determines cell and tissue tropism and route of virus spread Nonstructural replication protein; required for reovirus-induced cell-cycle arrest S2 2 Major inner-capsid protein; weakly binds to dsRNA S3 NS Nonstructural replication protein found early in viral inclusions; associates with nonstructural protein NS; nonspecific RNA-binding protein with high affinity for single-stranded RNA S4 3 Major outer capsid protein; forms protective cap over 1; nonspecific dsRNA-binding protein; influences efficiency of translation; associated with viral mRNA early in replication cycle; inhibits activation of PKR The T 13 outer capsid is constructed from complexes of 1 and 3 proteins, present in 600 copies each. What is the antiviral mechanism of pleconaril (a drug not currently approved by the FDA)? Arranged in virions as a cylindrical core. However, other positivestrand RNA plant viruses have either 3'-terminal poly A tails, or complex 3'-terminal structures that do not resemble tRNAs. Strikingly, many of these tRNA-like structures can serve as substrates for two cellular enzymes that recognize transfer RNAs: CCA-nucleotidyltransferase, which forms or regenerates the 3'-terminal CCA sequence on all tRNAs, and aminoacyl-tRNA synthetases, which covalently link specific amino acids to tRNAs, enabling their use in protein synthesis. As more detailed information has accumulated about baculovirus gene structure and function, baculoviruses have also emerged as important potential vectors for human gene therapy. Influenza type A viruses are the best-studied orthomyxoviruses; they are responsible for most flu epidemics, and are widespread in many avian and mammalian species. sGP (secreted glycoprotein) Synthesized as a glycosylated precursor protein that shares N-terminal 295 amino acids with GP but has a distinct C-terminus because of RNA editing; has a different oligomeric structure; lacks a membrane anchor; is cleaved into mature sGP and delta-peptide which are both secreted; not present in virions; unknown function; not found in Marburg virus. When cells die and/or lyse, they do not stain well; therefore plaques are seen as clear, unstained circular areas on the background of the stained cell monolayer. Both forms of the vaccinia virion are infectious. These oligomers can cleave themselves at specific sites to generate linear monomers, which then self-ligate into circular monomers. For some alphaviruses, a hairpin-like structure near the 5' end of the subgenomic RNA and within the capsid coding sequence functions to enhance its translation. gp42 is complexed with viral envelope glycoproteins gH and gL, and this complex (along with gB) is believed to mediate fusion with the membranes of the endocytic vesicles, releasing the nucleocapsid into the cytoplasm. 27.6: Courtesy of Willie Wilson; Fig. Virus Structure and Assembly 18 BASIC CONCEPTS OF VIRUS STRUCTURE 18 Virus structure is studied by electron microscopy and X-ray diffraction 19 Many viruses come in simple, symmetrical packages 19 CAPSIDS WITH ICOSAHEDRAL SYMMETRY 21 Some examples of virions with icosahedral symmetry The concept of quasi-equivalence 21 Larger viruses come in more complex packages 23 21 CAPSIDS WITH HELICAL SYMMETRY 25 VIRAL ENVELOPES 26 Viral envelopes are made from lipid bilayer membranes Viral glycoproteins are inserted into the lipid membrane to form the envelope 27 26 PACKAGING OF GENOMES AND VIRION ASSEMBLY 28 Multiple modes of capsid assembly 28 Specific packaging signals direct incorporation of viral genomes into virions 28 Core proteins may accompany the viral genome inside the capsid 28 Formation of viral envelopes by budding is driven by interactions between viral proteins 28 DISASSEMBLY OF VIRIONS: THE DELIVERY OF VIRAL GENOMES TO THE HOST CELL 29 Virions are primed to enter cells and release their genome 29 vii TOC.indd vii 13/05/11 2:11 PM viii Contents 3. Excision of the 385-nt intron removes 128 codons plus 1 nt. • Phycodnaviruses are ancient and, despite their conserved physical appearance, have incredibly diverse lifestyles. CH002.indd 25 Figure 2.10 Bacteriophage ϕKZ virion. 392 Pathology and diagnosis of prion diseases 392 Proteins of yeast and other fungi can form self-propagating states resembling prions 393 Genetics of prion diseases: mutations in the prion gene can increase occurrence of disease 393 Prion diseases are not usually transmitted among different species 393 Strain variation and crossing of the species barrier 394 The nature of the prion infectious agent 394 SECTION IX: HOST DEFENSES AGAINST VIRUS INFECTION 33. 16/05/11 8:10 PM Papillomaviruses 271 Human papillomavirus vaccines Two different human papillomavirus vaccines have been produced by using viral capsid protein L1 assembled into viruslike particles that do not contain the viral genome. The packaging construct provides the structural proteins and enzymes necessary for the assembly of the virion and processing of the RNA. Intermediate and late mRNAs share two unique structural features that distinguish them from early mRNAs. First, 5'-terminal “poly(A) heads” are added to these mRNAs via a slippage mechanism during the initiation of transcription. In vitro incubation of disrupted virions with deoxyribonucleoside triphosphates allows the virion-associated DNA polymerase to extend the plus DNA strand, filling in the single-stranded gap (Figure 30.2b). Tails meet in 12 groups of five on the surface, and heads meet in 20 groups of three. These proteins are called class I fusion proteins, and are structurally distinct from the class II fusion proteins of togaviruses and flaviviruses. Nucleocapsid protein is also found in abundance in replication complexes, suggesting that encapsidation of newly synthesized genome RNA for packaging into virions also occurs at these sites. The six viral genes are shown on a map of the Sendai virus genome. They may be relicts of the ancient RNA world. When vaccine manufacturers attempted to make the first H5N1 vaccines, they ran into a serious technical problem. Other HA proteins are not cleaved intracellularly during virus formation, but require activation by trypsin cleavage after release of virions from an infected cell. B19 is very specific for a particular cell type, infecting the rapidly dividing erythroid precursors of red blood cells. Euryarchaeota are hosts for a reduced morphological spectrum of viruses, some which are related to head–tail bacteriophages. The capsid protein (CA) forms an icosahedral or conical core, depending on the virus strain. (d) DNA ligase joins the 3⬘ and 5⬘ ends of each strand, forming a fully covalently closed double-stranded circular DNA. Also, a number of microRNAs (miRNAs) are expressed during EBV latent infection. The first human case of an apparent BSE-derived disease was diagnosed in the United Kingdom in 1994. Interferons affect the immune response in dramatic fashion, and this will be discussed in Chapter 34. To avoid such Coxsackie B HIV-I 1. p300 and general transcription factors control levels of transcription of viral and cellular genes. The generation of these two polyproteins from a single mRNA requires modification of the normal translation 16/05/11 8:15 PM Retroviruses .U A A . Cell surface toll-like receptors (TLR-2 and TLR-4) recognize virion glycoproteins; endosomal toll-like receptors (TLR-3, TLR-7, TLR-8, and TLR-9) recognize different forms of viral nucleic acids. ssDNA 6. However, this could be a coincidence, as simian virus 40 appears to circulate among the human population, causing no obvious symptoms. For example, Chinese physicians of the sixteenth century attempted active immunization against smallpox using dried scabs in various forms, soiled undergarments, and even powdered fleas fed on scabs! Many cultures in the Middle East have long recognized the durability of immunity to leishmania parasites after natural infection. Chipman, Vadim V. the next 7 kb of DNA are pulled into the cell during transcription by cellular RNA polymerase 3. An effective live vaccine is available. By this means the host can recognize and destroy virus particles against which the host organism has already produced circulating antibody from a previous exposure. There has been controversy about the possibility that some human tumors may have been caused by simian virus 40 injected during poliovirus vaccination. The tripartite leader ensures efficient transport of late mRNAs to the cytoplasm What are the advantages to adenovirus in expressing its late mRNAs in such a fashion? This leaves a 4- to 6-nt single-stranded gap on the target host DNA, and a 2-nt unpaired region on the viral DNA. G. Coiled helical nucleocapsid and/or nucleocapsid shell GENOME Linear single-stranded RNA, positive-strand. It does not go deeply into pathogenesis, epidemiology, or disease symptoms. Three to four capsid proteins (VP1–4). The DNA genomes of most of these viruses are not released as naked DNAs, but are complexed with cellular or viral proteins that compact the DNA and neutralize its charge. In hijacking DnaB, P binds so tightly that E. • RNA splicing in eukaryotic cells was first discovered by studying messenger RNAs of DNA viruses. Two examples of fusogenic glycoproteins are the influenza hemagglutinin (HA) and the glycoprotein of Sindbis virus. This raises the obvious questions: how and why did it get this big? This permits fusion of the influenza envelope with the endosomal membrane, mediated by the altered conformation of the viral hemagglutinin at low pH (see Chapter 4). Tobacco mosaic virus movement protein can direct movement of cucumber mosaic virus in infected plants Cucumber mosaic virus 3a protein shares functional similarities with the tobacco mosaic virus (TMV) Upper epidermis BS X Palisade mesophyll X BS BS VP C Spongy mesophyll VP Lower epidermis C S S VP M C BS BS Minor vein Figure 10.8 Cross-section of a leaf showing a vascular bundle. The targets that have been identified for the miRNAs are diverse and include viral genes such as DNA polymerase and LMP-1. Alphaviruses have been modified to serve as vectors for the expression of heterologous proteins The use of viruses as gene expression vectors is now a well-established technology (see Chapter 37). These kinases then activate transcription factors such as NF-B, IRF-3 and IRF-7. RNA-dependent DNA polymerases (reverse transcriptases), to transfer the information in these RNAs to DNA and to begin the DNA world 3. Other antiviral signal transduction pathways involve “inflammasomes” A subset of caspases is known as the “inflammatory caspase” subfamily. There is already a large market for drugs that mitigate cold symptoms, and some think that market could be tapped by an effective antiviral agent, if it is very safe. RNA interference (RNAi): an RNA-dependent gene regulation system mediated by miRNAs and siRNAs that is important for development and defense against pathogens. Because there is no strong termination signal for 3' 5' 8 the RNA polymerase on the template RNA, transcription can continue around the circle for several cycles, producing a multimeric linear RNA (step 2). Viral interference: the state of resistance of a cell to virus infection brought about by previous exposure of the cell to a similar or different virus. Prusiner in 1982 to propose that scrapie was caused by a novel proteinaceous infectious particle for which he coined the term “prion” (Pr); the scrapie prion protein was named PrPSc. His proposal was very controversial at the time, but has gained acceptance and eventually led to his being awarded the Nobel Prize in 1997. The C-terminal portion is unique to each specific IRF member. Most coronaviruses show strong species, organ, and tissue specificity, but subtle genetic changes can greatly alter their tissue tropism, host range, and pathogenicity. Some interesting facts about hepatitis B virus infection Antiviral drug treatment has real success • Currently, six antiviral drugs are effectively used to treat chronic carriers of human hepatitis B virus. However, there is no information on how the length of the nucleocapsid is regulated during production in the nucleus. A fraction of genome-length RNA is spliced, removing the Gag/Pol reading frames, and serves as mRNA for Env. To test this theory, viral mutants that allow capsid assembly in the absence of B protein were isolated. (b) Bacteriophage T4. The formalin-inactivated vaccine developed by Jonas Salk and the live, attenuated vaccine developed by Albert Sabin are two of the most successful vaccines ever produced (see Chapter 35). (Scale bars represent 100 nm (a) or 250 nm (b)). In fact, all six known phages of chlamydia, which grow only within eukaryotic cells, are gokushoviruses. The susceptibility of H. Concurrently, the spike protein in the core undergoes a major conformational change in which the turrets formed by 2 pentamers open to allow egress of mRNA during transcription. Antigenic drift results from the accumulation of point mutations within regions of the envelope glycoprotein genes that code for the antigenic domains of these proteins. several cell lines are capable of supporting hepatitis B virus DNA synthesis upon transfection with cloned viral DNA. A viral LTR is inserted just upstream of a cellular gene, and transcription that initiates within the LTR progresses through the adjacent cellular gene. However, the viral src gene was subsequently shown to be nearly identical to a cellular gene, called c-Src, that codes for a protein tyrosine kinase involved in cellular signaling pathways. 6 in B. Transovarial transmission: the transmission of a pathogen from mother to offspring via the egg. The two DNA strands appear to be covalently linked to each other. Some picornaviruses have depressions (called “canyons” or “pits”) on the capsid surface that are involved in binding to receptors. Many alphaviruses cause encephalitis; a better understanding of how these viruses enter the brain and why only some strains are able to cause symptoms may help in prevention of disease. Other proteins, first identified by function (e.g., Vhs for viral host shutoff protein), have kept their functional names. A similar argument can be made to exclude from the workplace (without pay) healthcare personnel reluctant to be vaccinated in the event of a major influenza pandemic. In nucleopolyhedroviruses, these enveloped virions then become associated in the nucleus with crystallizing polyhedrin CH025.indd 306 protein, forming the distinctive occlusion bodies or polyhedra associated with infection by these viruses. These cell types, primarily found in the blood, are acute responders to infection. These mRNAs have a unique structure. Lysosome: subcellular organelle in eukaryotic cells responsible for degradation of substances taken up by endocytosis. Each of the 13 families of viral genes contains a variety of often overlapping protein-coding regions. CH033.indd 414 16/05/11 8:19 PM C H A P T E R 3 4 Innate and Adaptive Immune Responses to Virus Infection Malcolm G. There is only a single species of Marburg virus, designated Lake Victoria marburgvirus. Chapter 3 explores the wide variety of viruses that exist on earth; probably all living organisms can be infected by at least one species of virus. Binding to pRb begins a process that mimics pRb phosphorylation, releasing E2F and allowing transcription of cellular genes targeted by E2F. The best-characterized lentivirus is human immunodeficiency virus type 1, the causative agent of AIDS; most lentivirus vector systems used today are derived from this virus. (b) A soccer ball. Integration Figure 28.3 Early phase of retrovirus life cycle. The 2b protein of cucumber mosaic virus is a multifunctional protein best known for its ability to suppress post-transcriptional gene silencing, or RNA interference (RNAi). Surrounding CH012.indd 139 139 the nucleocapsid and embedded in the lipid envelope are 180 glycoproteins organized with T 3 icosahedral symmetry. For example, AAV serotypes 1, 6, 8, and 9 can transduce skeletal muscle very efficiently; AAV serotypes 8 and 9 work very well in liver; and AAV types 1, 4, 5, 7, and 8 can efficiently transduce neuronal cells. CH009.indd 105 These viruses replicate in a variety of strains of Sulfolobus and Acidianus, isolated from hot acidic springs on solfataric fields (volcanic areas emitting steam and sulfur) in Iceland, Italy, and Portugal. 05/05/11 11:24 AM 332 CHAPTER 27 Viruses of Algae and Mimivirus nuclei (Figure 27.8). When this happens in the run of 3 G’s, an extra G is added to the growing RNA chain. • All flavivirus proteins are synthesized as a single polyprotein that is cleaved into 10 functional viral proteins by viral and cellular proteinases. Alternatively, they can be expressed on the same mRNA molecule but be controlled by independent translation initiation cassettes (internal ribosome entry sites or IRES; see Chapter 11). Orthomyxoviruses therefore can make capped messenger RNAs even though they do not code for a capping enzyme. high yields of infectious cucumber mosaic virus particles, and these methods have been very useful for studies on the molecular biology of the virus. Baculoviruses are used for pest control and to express eukaryotic proteins Baculoviruses replicate only in invertebrates, primarily insects of the order Lepidoptera (butterflies and moths). Whereas terrestrial plants “only” emerged about 470 million years ago, algae (and presumably their viruses) were already well established by then. Viral genes are arranged in a well-conserved set of transcription units. KEY TERMS 2', 5'-oligo(A) synthetase Adaptive immune responses Anti-inflammatory cytokines Apoptosis Autophagy B lymphocytes Caspases Chemokines Complement Cytokines Cytotoxic T lymphocytes Dendritic cells Double-stranded, RNA-dependent protein kinase (PKR) Hematopoiesis Innate immune responses Interferons Interferon-stimulated genes Interleukin-1␣ Intrinsic cellular defense Jak–Stat pathway Macrophages MicroRNAs (miRNAs) Natural killer (NK) cells Phagosomes Pro-inflammatory cytokines Protein kinases Pyroptosis Ribonuclease L RNA interference RNA-induced silencing complex (RISC) Small interfering RNAs (siRNAs) T lymphocytes Toll-like receptors Viral interference FUNDAMENTAL CONCEPTS • Toll-like receptors and other cellular pattern recognition receptors sense viral infections by binding to viral nucleic acids and glycoproteins. David Harris, then acquisitions editor at John Wiley and Sons, enthusiastically endorsed and welcomed my book project when I first proposed it. Drug side effects (nausea, diarrhea, fatigue, bone loss, body fat redistribution, heart problems due to high blood lipids, diabetes) are also common, with the result that patients often do not complete treatment regimens or delay treatment until significant CD4-positive T-cell depletion has occurred. Rather, they usually replicate more efficiently in tumor cells. The mechanism of uncoating of only a few viruses is understood in any detail. All seek to exploit the capacity of the vector organism to express the targeted proteins within host cells, eliciting a cytotoxic T-cell response. Figures concentrate on individual wellstudied steps in virus replication. Major transcripts from the three class I promoters, the ten class II promoters, and the five class III promoters are shown below in light blue, with vertical hash marks indicating ribonuclease III cleavage sites. These kinases then phosphorylate a number of transcription factors, leading to their activation. Baculovirus virions contain an elongated nucleocapsid Virions of baculoviruses consist of rod-shaped nucleocapsids 30 to 60 nm in diameter and 250 to 300 nm in length, surrounded by a lipid-containing envelope (Figure 25.1). T antigens interact with multiple signaling pathways and activate the cell cycle to facilitate viral DNA replication. They include lysosomotropic agents such as ammonium chloride, chloroquine, and other organic weak bases that allow protons to escape from acidic compartments. This likely accounts for at least some of the toxicity of this drug, which most often manifests itself clinically as bone marrow suppression. Delivery systems Liposomes Antigens are trapped inside vesicles made from synthetic lipids, enhancing immune reactions. This mechanism appears to parallel the better-studied fusion mechanisms of influenza virus (Chapters 4 and 18) and human immunodeficiency virus (Chapter 29). Ribosomal frameshifting: a process by which ribosomes pause during translation of a coding region on a messenger RNA, move back one or two nucleotides on the messenger RNA, and recommence reading the messenger RNA in a different translational reading frame. While the external S1 subunit is responsible for attachment, the S2 subunit that forms the stalk facilitates fusion through a series of conformational changes that result in the insertion of S2 into the target cell membrane, bringing the cell membrane and the viral envelope into close contact. Two other virokines are the G protein of respiratory syncytial virus, which mimics the chemokine fractalkine, and the Sis protein of simian sarcoma virus, which is a homolog of platelet-derived growth factor. The outer surface is composed of flower-like projections of the E1/E2 heterodimers arranged as trimers. • Influenza viruses exhibit unusual genetic variability by both rapid point mutations (“drift”) and genome reassortment (“shift”), generating new virus strains that can cause pandemics. Open reading frames are shown at top, with the protein produced shown at the right end. This repeated sequence may be a site of recombination between genomes to generate the concatemers, which contain only one copy of the repeat between each copy of genome-length DNA. DISTINCTIVE CHARACTERISTICS Orthomyxoviruses replicate in the nucleus, unlike most RNA viruses. One virus develops long tails at both ends of virion after release from cell. In addition to the proteins listed above, herpes simplex virus encodes other proteins concerned with DNA metabolism. It has been postulated that CI cooperativity is pairwise end of the DNA, allowing production of this long late transcript. Viral RNA polymerase then synthesizes double-stranded genome RNAs by copying the plus-strand mRNAs. These replicase particles can proceed to synthesize new mRNAs (upper arrow) or they can be assembled into virions by the addition of outer capsid proteins (lower arrow). Interferon and PKR: effects on viral and cellular protein synthesis Reovirus double-stranded RNA is a potent inducer of type I (␣ and) interferons. Intrinsic Cellular Defenses Against Virus Infection 398 Karen Mossman, McMaster University Pierre Genin, University Paris Descartes John Hiscott, McGill University 34. Viruses have also learned to protect themselves by “stealing” complement-regulating proteins that healthy mammalian cells use for protection against complement. The left-hand LTR is normal, and drives transcription of the full-length RNA. The three structural proteins C, prM, and E occupy the first quarter of the polyprotein, followed by the seven nonstructural proteins (NS1–5). 16/05/11 8:08 PM 242 CHAPTER 20 Parvoviruses Parvoviruses are faced with an important general problem in the replication of DNA: the “end replication” problem. Many species have been discussed as possible natural hosts; however, no nonhuman vertebrate hosts or arthropod vectors have yet been identified. About 20% of the time, the viral RNA polymerase pauses and “stutters” over the U stretch, usually adding a single additional A residue in the messenger RNA transcript. Stage II DNA replication (Figure 6.4) amplifies the number of replicative form DNA molecules to about 10 per cell. Genome segment reassortment results in more dramatic antigenic changes (“shift”), as exemplified by influenza viruses (Chapter 18). Maturation Ritonavir (HIV) 8. If the complementary region on the two RNAs is short enough, the cleavage products will dissociate from the ribozyme, thereby permitting the binding of more substrate molecules for cleavage. The defective viral RNA must at the minimum contain a packaging signal (psi) recognized by the Gag protein of the helper virus, as well as the U3, R, U5, and PBS sequences required for reverse transcription and integration into the cellular genome. CH008.indd 86 16/05/11 7:59 PM Bacteriophage Lambda 87 CRISPR elements Head Portal vertex DNA Tail Tail cone Attachment fiber Figure 8.3 Schematic drawing of λ virion. Ectocarpus has a complicated sexual life cycle comprising alternating generations. 222 Rosewood Drive, Danvers, MA 01923, website www.copyright .com. H., K. These proteins include an RNA-dependent RNA polymerase, which can replicate the genome first by making a complementary (negative-strand) copy, and then by making many new positive-strand copies. The short DNA intermediate made is designated “plus-strand strong-stop DNA”. The nucleocapsid contains a major capsid protein, VP39 (Ac89). The capsids of poliovirus and other members of the picornavirus family therefore contain three sets of 60 similarly folded protein subunits, for a total of 180 subunits. In some strains of cucumber mosaic virus, an additional RNA, denoted RNA 5, is detected. How might RNA interference contribute to the pathogenicity of viroids? Many scientists who were to become dominant figures in molecular biology in the latter part of the twentieth century got their first taste of research by working with RNA phages. A carboxy-terminal phosphatidylinositol glycolipid anchors PrPC in the plasma membrane. For example, both tomato bushy stunt virus and flock house virus encode proteins that directly bind double-stranded RNA, thus sequestering the RNA and making it unavailable to the RNA interference machinery. One is a general defense and stress response to microbial infection, involving activation of salicylic acid, jasmonic acid, and ethylene-responsive pathways. CH026.indd 317 16/05/11 8:13 PM 318 CHAPTER 26 Poxviruses Table 26.2 Enzymes packaged in vaccinia virions Function Protein No. of subunits Mol. CH033.indd 402 16/05/11 8:19 PM Response of the Cell to Virus Infection The cytosolic detector proteins RIG-I and MDA5 recruit the adaptor protein interferon- promoter stimulator 1 (IPS-1) upon activation by viral RNAs (Figure 33.3). This polyprotein is subsequently cleaved into distinct functional viral proteins by proteinases that are themselves part of the polyprotein; presumably, these proteinases fold into enzymatically active structures while still part of the polyprotein. Modern vaccine developers could learn a great deal from the showmanship of vaccine pioneers like Louis Pasteur, who arranged public demonstrations of his anthrax and rabies vaccines in the early 1880s. Thus, during lysogenization, CII-activated expression of Int from PInt is not limited by retroregulation. Some unique enzymes for RNA viruses, including a possible proofreading exonuclease. Particles consist of a nucleocapsid with a central channel, surrounded by a membrane; glycoprotein spikes are seen as a fringe outside membrane (bar: 100 nm). Effects of interferons on antibody production Interferons play complex roles in regulating antibody production since they exert their effects either indirectly, by regulating the development of specific CH034.indd 423 423 T-helper cell subsets (as described above), or directly at the level of B cells. The concentration of important sequence elements CH021.indd 251 that serve as signals for transcription, DNA replication, RNA processing, and translation in two small regions of the genome reveals the efficiency with which these genomes are organized. Buckminsterfullerene: C60 molecule with icosahedral symmetry. Richard Dawkins, in his best-selling 1976 book, The Selfish Gene, postulated that evolution occurs whenever information is copied over and over again with introduction of variations and with selection of some variants over others. Plants and invertebrates do not produce interferon in response to virus infection but do produce Dicer. All the viruses in this family share the following common characteristics: (1) a single-stranded RNA genome composed of three unique segments, each wrapped in a helical ribonucleoprotein complex; (2) cytoplasmic site of replication; (3) viral mRNA synthesis primed by capped oligonucleotides derived from host cellular mRNAs; and (4) virion assembly at Golgi membranes. Another major difference is that during infection, the virus genome is integrated into the host cell genome; the virus genome is then inherited in a Mendelian fashion and virus particles only appear in the reproductive organs of the infected algae. Caulimoviruses have a circular double-stranded DNA genome that contains strand breaks at specific sites. Therefore significant research efforts are being made to target virus vectors by manipulating viral proteins responsible for attachment to receptors and uptake. One family contains 66 members that encode ankyrin domains, and another family consists of 12 members located in perfect tandem sequence from L174 to L185. Synthesis of most bunyavirus mRNAs terminates 50 to 100 nucleotides before the end of the genome RNA template. The plus-strand is shorter than the minus-strand because its synthesis is interrupted before completion, giving rise to the single-stranded region. A transcriptional enhancer within the intergenic region (Figure 21.5) contains a number of consensus DNA sequences that are recognized by a variety of cellular transcription factors. The larger form of delta antigen results from editing (A–G) at position 1014 on genome RNA. Occlusion body: a crystalline protein matrix formed in the nucleus of cells infected with baculoviruses, consisting of enveloped viral nucleocapsids embedded in polyhedrin or granulin. L proteins vary in size from 237 to 459 kDa, depending on the genus. The role of EF-Ts remains obscure. More astonishing, these 1031 phages, if lined up head-to-tail, would stretch some 200 million light years into space—that is, far into the universe beyond many of our known neighboring galaxies (see text box on page 6)! More important is the ecological role played by bacteriophages and viruses that infect unicellular eukaryotic organisms such as algae and cyanobacteria. The expression of phage genes that allow further genome replication and capsid production can now take place. Interestingly, sequence similarity alone does not determine other characteristics of these viruses, such as their cellular binding receptor, animal host, tissue tropism, pathogenesis, virulence, or type of disease. • Viruses with a reverse transcriptase are known only from vertebrates and plants. In vitro culture of eukaryotic cells led to rapid advances in the study of viruses and in vaccine production. CH008.indd 95 16/05/11 7:59 PM 96 CHAPTER 8 Bacteriophage Lambda • A complex set of interactions among repressors and operators as well as the phage CII and CIII proteins determines whether an infected cell will become a lysogen or enter the lytic cycle. Several of the most promising advances in vaccine development (Table 35.7) are discussed here. It is not clear how viroids recruit these RNA polymerases and induce them to use their single-stranded RNA genomes as templates to be copied into complementary RNAs. Replication initiation sites have recently been identified in both Avsunviroidae and Pospiviroidae, and these should help determine how host RNA polymerases are recruited. Toll-like receptors are highly conserved from flies to humans and share structural and functional similarities. This is the best-characterized RNA-dependent RNA polymerase and can be readily purified. GTP refers to guanosine triphosphate, a cofactor required for binding of 60S subunits to 40S subunits and the initiation of protein synthesis. Each D protein makes different contacts with the underlying capsid, neighboring D, and spike proteins. Immediate early gene products control expression of early genes The viral genes responsible for temporal control of baculovirus gene expression were discovered by using reporter genes whose transcription was driven by specific baculovirus promoters. False positive reactions can arise if there is partial homology between the MDV-1 RNA and some other cellular or viral RNA in the sample. At the termination site there is a run of four to seven uridine residues in the template RNA, which are transcribed into adenine residues in the mRNA. The T antigens of both mouse polyomavirus and simian virus 40 have been intensively studied and have been shown to interact with numerous cellular signaling pathways, leading to stimulation of the cell cycle. Genera are determined by host range of viruses. Furthermore, all but one of the remaining chapters in the first edition were revised and updated by the original contributors xix fpref.indd xix 16/05/11 7:20 PM xx Preface or, in several cases, by other contributors recruited for that purpose. Third, tRNA-like structures may initially reduce synthesis of minus-strand RNA, which begins at the subterminal C residue on genome RNA. These viruses typically cause mild to moderate respiratory illness in healthy individuals, being responsible for up to 30% of colds in some outbreaks. The immature virus is glycosylated and traverses the secretory network. Antigenic drift has resulted in the generation of numerous influenza A subtypes with antigenically distinct HA and NA surface glycoproteins. Ironically, the great successes of the quasicompulsory vaccination programs that have eliminated smallpox and nearly eliminated polio and measles have also contributed to the growing wave of anti-vaccination sentiment, because people currently feel themselves to be less at risk. Full of surprises, novelty, and mystery, they will continue to enthrall researchers into the distant future. This virus could also be transmitted among cucurbit plants by grafting and by insects. of the viral DNA, homologous recombination can occur between sequences flanking the polyhedrin gene on the transfer vector and the identical sequences on viral DNA, generating recombinant genomes with the 19/05/11 12:19 PM Baculoviruses foreign gene in place of the polyhedrin gene. These vaccines can also be used without risk in immunocompromised subjects. Therefore virus entry and uncoating are the same event for this virus family. Virions are assembled in the cytoplasm and appear in aggregates within the cell. VIROID-LIKE PLANT SATELLITE RNAS Require helper virus for replication. To replicate, a virus must transport its genome into a host cell, where the genome directs synthesis of viral proteins, is replicated, and is packaged. nucleocapsids, forming a complex (step 6, Figure 18.6). Virus infection can lead to the activation of other signal transduction pathways, resulting in the activation of additional kinases. • A number of herpesvirus gene products suppress host cell defenses, including apoptosis. The envelope is tightly apposed to the surface of the nucleocapsid, resulting in a very uniform diameter of virions, unlike many enveloped RNA viruses with helical nucleocapsids, which can have variable size and shape. Presently, many small academic and biotechnology company laboratories around the world are actively pursuing vaccine development programs. IB: an inhibitor that binds to the transcription factor NF-B, maintaining it in an inactive state in the cytoplasm. The E1A promoter, located near the left end of the genome (Figure 23.4), is driven by a transcriptional enhancer that requires only cellular transcription factors. KEY TERMS Alternate pathway Anaphylatoxins Antibodies Antibody-dependent cellular cytotoxicity Antigenic drift Antigenic shift Antigens Apoptosis B lymphocytes B-cell receptors Central or primary lymphoid organs Classical activation pathway Clonal selection theory Collectins Complement Cytotoxic T lymphocytes Epitopes Ficolins Granzyme B Histamine Immune response Inflammatory response Interferon-␣ Interferon- Intrinsic cellular defenses Isotype Lectin receptor-activated pathway Lymphatic system Lymphotoxins Macrophages Major histocompatibility complex (MHC) Mast cells Membrane attack complex Monocytes Natural killer (NK) cells Perforin Peripheral or secondary lymphoid organs Phagocytosis Plasma cells Polymorphonuclear leukocytes Proteasomes Reassortment T lymphocytes T-cell receptors Tumor necrosis factors Virokine Viroceptor Virulence factors 425 FUNDAMENTAL CONCEPTS • Hosts respond to viral infections with four layers of defense: physical barriers, intrinsic cellular defenses, innate resistance, and immune responses. This startling finding led to efforts by many laboratories, particularly in tropical VIRUSES AND HOSTS Two genera: • • Alphavirus (27 members) Rubivirus (rubella virus) Alphaviruses (Sindbis, Semliki Forest, Ross River) infect birds and mammals. This results in the fusion of the virion envelope and the host cell membrane and the release of the nucleocapsid into the cytoplasm. Three or four different messenger RNAs, each of which codes for one viral protein, are made from each gene transcript by alternative RNA splicing. Provided that the proteins can evolve to tolerate the distortions required by these slightly different contacts, they can use the same domains to make stable contacts at all 180 positions on the capsid surface. Plant viruses must penetrate the plant cell wall, and are usually transmitted by sucking insects or mechanical means. Sodium dodecyl sulfate: a 12-carbon ionic detergent that is used to denature proteins. It is a highly unusual antiviral drug because it targets a host protein—CCR5— rather than a viral protein. The lower set of images show what is seen when tubes are viewed from below. Because sGP is released into the medium of infected cells and is found in the serum of patients, it has been suggested that sGP may act as a soluble factor that targets elements of host defense systems. Although these two compounds are potent in vitro, clinical trials showed that they do not rapidly alleviate flu symptoms. There is a second level of control of the translation of replicase. Hammerhead structure: secondary structure found in ribozymes of group A viroids. They were originally discovered as proteins that are important for defending against fungal infections in the fruit fly, Drosophila melanogaster. Viruses can induce apoptosis of a virus-infected cell by binding of viral RNA to one of three detection proteins. In this case, a thymine base is attached to a ribose in which the normal 3' OH has been converted to an azido (N3) group (Figure 36.4). However, as genomic information has become available for more algal viruses, it has become clear that the phycodnaviruses themselves are a very diverse group. • Viroids infect plants and require no helper virus; viroid-like satellite RNAs require a helper virus to replicate. In the context of virus infection, cytotoxic T lymphocytes are the primary effectors for killing virusinfected cells. P2 and P3 are also cleaved into a number of smaller proteins whose functions are described below. The effects of the mutant virus on pathogenesis in infected animals remain to be tested. 19.7a, b: Reprinted from Becker, M.M., et al., “Reovirus sigma NS protein is required for nucleation of viral assembly complexes and formation of viral inclusions.” Journal of Virology (2001) 75: 1459-1475, Figs 4a, c. akashiwo populations. KEY TERMS Antibody-dependent enhancement Class I fusion proteins Class II fusion proteins Endemic Epidemic Exocytosis Furin Hemorrhagic fever Hepatitis Hepatocellular carcinoma Lumen Methyltransferase Nucleoside triphosphatase Polyprotein Seroconversion Signal peptidase Type I transmembrane protein FUNDAMENTAL CONCEPTS • Flaviviruses cause severe human diseases such as yellow fever, Japanese encephalitis, dengue fever, and hepatitis. Adenovirus functions that help replication of adeno-associated virus For efficient replication, adeno-associated virus requires functions provided by a helper virus. Three sequentially expressed classes of genes, organized from left to right end of genome: • • • Class I (“early”) genes: control of phage and host gene expression Class II (“middle”) genes: phage DNA replication Class III (“late”) genes: structural and assembly proteins, DNA packaging, cell lysis VIRUSES AND HOSTS A genus within family Podoviridae. Hantaviruses are exceptions; they are not transmitted by arthropod vectors but are maintained in nature as persistent infections of rodents. The template RNA is pulled through the RNA polymerase in the direction shown by the arrows. For example, some mutations that increase base-pairing interactions in this domain decrease severity of symptoms. Recently, 3 integrins have been shown to mediate the entry of pathogenic hantaviruses; infection of cells can be blocked by anti-integrin antibodies or the integrin ligand, vitronectin. Core proteins may accompany the viral genome inside the capsid Some DNA viruses package not just the viral genome but a nucleoprotein complex. CH005.indd 60 16/05/11 7:54 PM Single-Stranded RNA Bacteriophages 180 copies coat protein 1 copy maturation protein 1 single-stranded RNA 2 nm 26 nm Figure 5.2 Diagram of an MS2 virion. Another gene product, ICP27, colocalizes with spliceosomes in the nucleus and blocks RNA splicing. NS1 is the main regulatory protein of minute virus of mice and has several functions to ensure efficient viral replication. Use of these systems has provided valuable insights into the replication of these viruses and allows detailed genetic analyses to be performed on the roles of specific viral proteins in the virus life cycle. In practice, there are limitations in this method for distinguishing the time course of the various steps in a viral growth cycle; different steps in the cycle invariably overlap somewhat. Instead, they 16/05/11 8:19 PM RNA Interference use RNA interference (RNAi) as an important antiviral defense mechanism (see Chapter 10). Much like the scaffolds used in construction, these scaffolding proteins facilitate capsid assembly but are removed when construction is complete. Latent in lymphocytes. Can induce immunity to many different antigens simultaneously. Fresh isolates tend to make predominantly filamentous particles, while virus repeatedly passaged in eggs or cell cultures tend to make smaller, elongated, or quasispherical particles. of multimeric antigenome templates as well as 800-nt mRNAs by the same cellular RNA polymerase. This is an active subject of research and is important for understanding how herpesviruses can survive and thrive in the host animal. Since viral infection corrupts PABP function, this suggests a mechanism by which the virus can promote translation of its own proteins while host protein synthesis is impaired. The E1A proteins help provide these required cellular proteins by inducing non-dividing cells to enter the S, or DNA synthesis, phase of the cell cycle. Steps such as genome replication, assembly, and maturation require specific viral proteins, often acting as enzymes. Through a series of intracellular associations with additional signaling molecules, these adaptors recruit protein kinases, including the IB kinase (IKK) enzyme complex. It contains five ␣-helices and a C-terminal region that consists of four arginine clusters involved in binding to RNA. Microviruses 69 ϕX174: a tiny virus with a big impact 69 Overlapping reading frames allow efficient use of a small genome 70 ϕX174 binds to glucose residues in lipopolysaccharide on the cell surface 70 ϕX174 delivers its genome into the cell through spikes on the capsid surface 71 Stage I DNA replication generates double-stranded replicative form DNA 72 Gene expression is controlled by the strength of promoters and transcriptional terminators 72 Replicative form DNAs are amplified via a rolling circle mechanism 72 Summary of viral DNA replication mechanisms 73 Procapsids are assembled by the use of scaffolding proteins 73 Scaffolding proteins have a flexible structure 74 Single-stranded genomes are packaged into procapsids as they are synthesized 74 Role of the J protein in DNA packaging 75 Cell lysis caused by E protein leads to release of phage 75 Did all icosahedral ssDNA virus families evolve from a common ancestor? Rational drug discovery: approaches for discovering new drugs that use basic knowledge from genetics, molecular biology, enzymology, and protein structure to choose drug targets and molecules to act on these targets. Members of the poxvirus, herpesvirus, retrovirus, and togavirus families incorporate complement-regulating proteins such as CD55 (C3b decay accelerating factor), CD59 (Protectin, an inhibitor of the assembly of the membrane attack complex) and CD46 (a cofactor that increases proteolysis and degradation of complement components) into their envelopes. RNAs from viruses such as human immunodeficiency virus, vesicular stomatitis virus, and influenza virus. Success in inhibiting prion replication in cultured cells has been obtained with a variety of agents including polyanions, sulfonated dyes, cyclic tetrapyrroles, quinacrine, quinoline, acridines, and phenathiazines, as well as other amyloidophilic compounds such as pyridine dicarbonitriles, peptide aptamers, and -sheet breakers. In some cases, viral proteins are provided by infection with a helper virus; however, this complicates production of recombinant vector stocks, because the helper virus must be removed. Because secondgeneration adenovirus vectors do not fully escape the host immune response, and because there was a need for larger cargo space for some applications, the more drastic approach of eliminating all viral genes was undertaken. 3 in Andrei Fokine, Anthony J. Cucumber mosaic virus infection induces comprehensive changes in the expression of a large number of host genes, and modifies cell physiology and structure. Further analysis led to the model shown in Figure 32.6. 16/05/11 8:17 PM Prions (a) Glycans 391 (b) NH3 Cu II Octapeptide repeats GPI anchor Plasma membrane Figure 32.5 The structure of the prion protein. Each monoclonal antibody 05/05/11 2:45 PM 478 Glossary therefore has a unique amino acid sequence and binds to a highly specific epitope. The virus reappeared with the advent of the mosquito season and began its expansion outside of the New York City region, most likely carried by host-encoded furin occurs just prior to virion release and converts the immature particle (Figure 12.2a) to its mature form (Figure 12.2c). This poses a problem for positive-strand RNA viruses such as the picornaviruses: How can they express their numerous genes from a single RNA molecule that serves both as a genome and as a messenger RNA, in a cellular environment that favors mRNAs that code for only one protein? Different members of the Reoviridae family can infect vertebrates, invertebrates, plants, or fungi; this is one of the few virus families (along with the negative-strand Rhabdoviridae and Bunyaviridae) whose members can replicate in such a wide range of host organisms. CH014.indd 164 The virus envelope fuses with the plasma membrane or an endosomal membrane The coronavirus S protein mediates attachment of the virion to the cell surface by interacting with a cellsurface receptor molecule, followed by fusion of the virus envelope with cellular membranes. Viruses can escape the cell-mediated immune response by blocking almost every stage of MHC class I antigen presentation. In addition, copies of the virus genome with authentic 5' and 3' ends must be made, for without the authentic end sequences, transcription and replication will not occur. Cells that mediate the adaptive immune response can be long-lived, and the organism is therefore protected against future infections by the same pathogen. EBNA-LP is made from a multiply-spliced transcript whose exons are coded within the multiple repeats of IR1; the other five EBNAs are made from coding regions downstream of IR1. coli, coded by the F plasmid, that mediate bacterial conjugation. Cytomegalovirus also makes proteins that either down-regulate or sequester host signaling molecules that activate NK cells. Accessory or group-specific proteins Three to five proteins, dispensable for replication in vitro, and variable between different coronaviruses. It is not difficult to see what the biological purpose of this control is; it ensures that the maturation gene will be translated only once or a few times per newly synthesized plus-strand phage RNA. They are common vectors of many plant viruses. Philadelphia: Lippincott-Raven (1996) pp. Adenoviruses and herpesviruses have large isometric capsids that are too bulky to pass through the nuclear pores. The viral replication machinery targets the pregenome RNA for packaging by recognition of a region known as the epsilon () stem-loop (Figures 30.3 and 30.5). Although it codes for a number of transfer RNAs and proteins involved in protein synthesis, it has no ribosomes of its own. The enzyme 2', 5'-oligo(A) synthetase is produced in interferon-treated cells, and is activated by binding to double-stranded RNA in virus-infected cells. Table 35.5 Passage histories for two

vaccine-strain viruses Edmonston-B and Moraten measles vaccine strains • Isolated by Enders & Peebles in 1954 from patient (D. Helical nucleocapsids containing genome RNA are delivered from their site of synthesis to these membranes for packaging. The genome of cucumber mosaic virus consists of three distinct RNA molecules Figure 10.1 Symptoms of infection by cucumber mosaic virus on cucumber fruit. In one method, the virus produces a serine protease inhibitor (serpin), which specifically blocks a proteolytic cleavage required CH026.indd 322 veterinary practice. The long class I transcripts synthesized by the host RNA polymerase are cleaved into 2 monocistronic mRNAs and 3 polycistronic mRNAs (Figure 7.1). NS1 binds to viral DNA and stimulates transcription from the p38 promoter, which controls synthesis of the mRNAs for the coat proteins. The only exception to this rule appears to be the enterovirus family, including poliovirus and the echo and Coxsackie viruses. (d) Virion release following cellular lysis. Further clinical studies are needed before this live vaccine can be used in people who are most susceptible to serious influenza disease, the very young and the very old. sGP could bind to antibodies and contribute to the immunosuppression found in patients and animals infected with Ebola viruses. In contrast, excision of the 62-nt intron removes 20 triplet codons plus 2 nt. The amino-terminal end of the signal sequence, on the cytoplasmic side of the endoplasmic reticulum membrane, is eventually cleaved by the viral proteinase NS2B/NS3A, releasing the mature capsid protein. In cryoelectron microscopy, samples are flashfrozen in liquid nitrogen and kept at that temperature in the microscope. In the new reading frame, the gag termination codon is no longer recognized and the pol region is translated, resulting in the generation of the Gag/Pol protein. 20.1: Reproduced from Fig. Parvoviruses are one of a small number of virus families with single-stranded DNA genomes. Small gray spheres denote 5' caps, AAAA denotes poly(A) tails. However, mimivirus will also be discussed in this chapter because of its unusually large size and its similarity to the phycodnaviruses, both with regards to structure and genomics, and our still very incomplete understanding of its biology. These nucleocapsids are then packaged into infectious particles containing the Gn and Gc envelope proteins. Nonetheless, we now know a lot about the induction of interferon synthesis in virus-infected cells, the signaling pathways by which Table 33.3 Properties of interferons Alternative name Type Receptors Interferon-␣ Interferon- Interferon- Interferon- Leukocyte interferon Fibroblast interferon Interleukins 28A, 28B, 29 Immune interferon I III I IFNAR1; INFAR2c IFNAR1; INFAR2c IL28R␣; IL10R II IFNGR1; IFNGR2 Length, amino acids 165 165 196-200 146 Number of genes in humans 13 1 3 1 Principal producer cells Leukocytes, fibroblasts, macrophages, epithelial cells Inducing agents Major activity CH033.indd 406 Fibroblasts, epithelial cells Leukocytes, fibroblasts, macrophages, epithelial cells T lymphocytes, macrophages, natural killer cells Viruses, double-stranded RNA Mitogens, antigens, interleukin-2 Antiviral action Immune stimulation 16/05/11 8:19 PM Interferons and cytokines such as interleukin-1, interleukin-2, or interferon- itself. Transcription of the early genes proceeds on one DNA strand from near the "12 o'clock" position in a counterclockwise direction, and transcription of the late genes proceeds on the complementary DNA strand in a clockwise direction; this pattern is said to be divergent transcription. Thus an E. All known methanogens are archaea. E2 also binds to mitotic chromosomes and is believed to aid in the correct segregation of the viral genomes between the two daughter cells Numerous cellular transcription factors including NF1, SP1, AP1, Oct1, YY1, and the DNA-binding glucocorticoid receptor protein have been shown to interact with the regulatory regions of papillomavirus DNA and to play important roles in activating viral transcription. Primary miRNA stem-loop structures typically have an imperfectly paired region of approximately 30 base pairs. Parvoviruses (Table 20.1) have been isolated from numerous mammals and birds, and members of a distinct subfamily, the densoviruses, infect a variety of insects. Virions bind to receptors on the cell surface 13 2. Six oligosaccharide chains are attached to the protein. For at least some plant viruses with 3' tRNA-like structures, the binding of eEF1A, while stimulating translation of viral RNA, may also inhibit its use as a template for RNA synthesis. This quickly led to the establishment of a screening assay for the blood supply, causing a dramatic reduction in transfusion-associated West Nile virus disease. RIG-I preferentially recognizes short double-stranded RNA molecules and single-stranded RNA molecules with a 5'-triphosphate group. Selectively replicate in and kill tumor cells. Therefore, for a tumor cell to thrive, it must rid itself of the control exercised by the p53 protein. CH024.indd 301 16/05/11 8:12 PM C H A P T E R 2 5 Baculoviruses Eric B. By contrast, the phylum Euryarchaeota contains organisms of diverse phenotypes including a few hyperthermophiles, thermophiles (growth above 45⬚C), hyperhalophiles (growth in hypersaline waters with concentrations of sodium chloride above 1.5 molar), and anaerobic methanogens (organisms that produce methane by reduction of CO2). Their genomes encode primarily an A protein homologue, needed for DNA replication, and a capsid protein, although other genes are present. Thus the ratio of nucleic acid to infectious particles would have to be much lower than ever previously encountered in viruses. Pharmaceutical companies have not always been enthusiastic about investing resources in the development of antiviral drugs because the development of compounds active against a broad spectrum of viruses is unlikely. This is coded in a different reading frame that overlaps with the end of the VP2/VP3 reading frame. Are parvoviruses and microviruses, one family replicating in animal cells, the other in bacteria, distantly related? GENES AND PROTEINS RNA 1 encodes the 1a protein: a capping enzyme and helicase, and a component of the replicase. The tail is sometimes described as "cytoplasmic", since it faces the cytoplasm of the cell before the virus buds. A map of the Sendai virus genome is shown in Figure 15.6. A region including the first 55 nt at the 3' (left) end of the negative-strand genome RNA is denoted the "leader" sequence. 6 in R. ; cm. However, it also has a single protein-coding gene. Interferon mRNAs are unstable, leading to a short burst of interferon synthesis upon induction. However, the cytoplasm of neighboring plant cells is connected via plasmodesmata (singular: plasmodesma). In all three models the transport vesicles fuse with the plasma membrane to release virions into the extracellular space. Although the step-by-step process described above for fusion is best characterized for the influenza virus HA protein and the HIV-1 gp41 glycoprotein, it is still only partially understood. The anti-influenza virus drug amantadine is a specific blocker of the M2 H⩽ channel. • Translation of the maturation protein only occurs on newly synthesized viral RNAs during the short time when they have not yet folded into their stable secondary structure. Interestingly, oriL is flanked by genes coding for the viral single-stranded DNA binding protein (UL29) and the viral DNA polymerase (UL30), both intimately connected to DNA replication. Besides stimulating 9S*–6S interactions, protein B also facilitates the incorporation of the DNA pilot protein H. Nonetheless, some of the smallest enveloped viruses, such as flaviviruses (Figure 12.2) and togaviruses VIRAL ENVELOPES Viral envelopes are made from lipid bilayer membranes Most viral envelopes are acquired at cellular membranes by a process of budding (Figure 2.11). When an aphid probes an infected plant, the virus interacts with the anterior portion of the alimentary tract, and from there it subsequently can be inoculated into an uninfected plant. Altogether, there were 32 cases, of whom seven died. The human immunodeficiency virus Nef protein disrupts the trafficking of MHC I. Additional mechanisms for tumor promotion that do not rely on a mutant form of a host gene have been described for particular retroviruses. Although protein drugs such as interferons (see Chapter 33) and nucleic acid-based drugs such as oligonucleotides are of considerable interest for medicine and virology, for purposes of brevity only small-molecule drugs will be covered here. Then virus movement complexes containing movement protein and viral RNAs transverse the plasmodesmata and enter the adjacent cells. Viruses can only reproduce themselves by infecting living cells. Protein R, a transglycosidase, attacks the cell's peptidoglycan layer. • The level of the CI repressor is determined in part by the concentration of CI, which activates synthesis of its own transcript when at low concentrations, but represses its synthesis when at high concentrations. Translation is initiated at a single site near its 5' end and proceeds uninterrupted for some 3000 nucleotides to produce a polyprotein that is co- and post-translationally cleaved. The human toll of smallpox is almost unimaginable today. Cytotoxic T lymphocyte: a class of lymphocytes, usually bearing the CD8 antigen, that attack and kill cells expressing foreign antigens presented by MHC class I molecules. Because bacteriophages can attack and kill specific bacteria, they have long been considered as possible alternatives to antibiotics in treating disease. Rotaviruses are a group of double-stranded RNA viruses responsible for millions of cases of infantile gastroenteritis, which each year causes the death of hundreds of thousands of young children in third-world countries where access to medical care is limited. Latency-associated transcripts include stable introns Herpes simplex virus latency-associated transcripts are a family of RNAs (8.3, 2, and 1.45 kb) that map to the b repeat sequences and are antisense to ICP0 mRNA CH024.indd 296 (Figure 24.5). GENOME Linear single-stranded RNA, positive sense, 9.3 kb. The science of classification is called taxonomy. This effectively results in the uncontrolled proliferation of the virus-infected cell, which together with the loss of the ability of p53 to induce apoptosis, makes a potent oncogenic combination. Furthermore, expression of these transgenes is often prematurely silenced. Viral DNA replication results in the formation of cytoplasmic structures called "DNA factories", recognizable as large areas of uniform electron density, cleared of normal cellular organelles. 4 Viruses are important disease-causing agents 4 Viruses can infect all forms of life 4 Viruses are the most abundant form of life on Earth 5 The study of viruses has led to numerous discoveries in molecular and cell biology 5 A BRIEF HISTORY OF VIROLOGY: THE STUDY OF VIRUSES 6 The scientific study of viruses is very recent 6 Viruses were first distinguished from other microorganisms by filtration 6 The crystallization of tobacco mosaic virus challenged conventional notions about genes and the nature of living organisms 6 The "phage group" stimulated studies of bacteriophages and helped establish the field of molecular biology 7 Study of tumor viruses led to discoveries in molecular biology and understanding of the nature of cancer 8 DETECTION AND TITRATION OF VIRUSES 9 Most viruses were first detected and studied by infection of intact organisms 9 The plaque assay arose from work with bacteriophages 9 Eukaryotic cells cultured in vitro have been adapted for plaque assays 9 Hemagglutination is a convenient and rapid assay for many viruses 10 Virus particles can be seen and counted by electron microscopy 10 The ratio of physical virus particles to infectious particles can be much greater than 1 11 THE VIRUS REPLICATION CYCLE: AN OVERVIEW 11 The single-cycle virus replication experiment 11 An example of a virus replication cycle: mouse polyomavirus 12 Analysis of viral macromolecules reveals the detailed pathways of virus replication 13 STEPS IN THE VIRUS REPLICATION CYCLE 13 1. These models can be distinguished by experimental observations. Role of the J protein in DNA packaging The J protein is not only required for DNA packaging but may also play a role in organizing the DNA within the symmetrical capsid. These are responsible for three major activities: replication of viral DNA, expression of late and very late viral genes, and modification of cell and host physiology via cellular signaling pathways. Adeno-associated virus has evolved a strategy to cope with this situation: the viral DNA integrates into the cellular DNA and replicates as part of the cellular genome. RIG-I and MDA5 appear to recognize RNAs from different viruses, with the recognition depending on both RNA structure and length (Figure 33.2). There is evidence of protein covalently linked to both ends of the DNA molecule. Bidirectional replication produces progeny circles at early times, prior to the lysis/lysogeny decision (Figure 8.1). For example, simian virus 40, a (non-enveloped) polyomavirus, enters via mechanisms mediated by caveolae and lipid rafts. Interferons ␣, , , and are made by different cells, bind to different receptors, and have distinct functions The interferons are classified into three groups based on amino acid sequence homology and the receptors they use (Table 33.3). These vaccines can be remarkably stable and their use avoids the delivery of irrelevant and potentially immunopathologic viral antigens responsible for vaccine-associate adverse events (see below). A small amount of NS2 protein is also present in virions. Adenoviruses, for example, are taken up into endosomes, where they proceed to rupture the endosomal membrane, releasing the virion and other endosomal contents into the cytosol. In contrast to simpler retroviruses, which make only two mRNAs (unspliced and singly spliced), SU TM Lipid MA CA NC Vpr RNA PR Alternative name (M. 16/05/11 8:03 PM Flaviviruses 143 Table 12.2 Proteins of Yellow Fever Virus Protein Size (aa) Function anchC 121 "Anchored" capsid protein. The occlusion bodies consist largely of a single viralencoded polypeptide called polyhedrin or granulin. Activation of p53 serves as a warning E1A Activates transcription p14Arf E1A Blocks p53 activation Activates transcription p53 Binds to Mdm-2 Mdm-2 Binds to p53 p53 Mdm-2 p14Arf Mdm-2 p53 Blocks p53 degradation; p53 accumulates Degradation in proteasomes Figure 23.7 Degradation pathway of p53 is blocked by E1A proteins. The good news is that long-term transgene expression without evidence of toxicity can be achieved with thirdgeneration adenovirus vectors if nonviral promoters are employed. Interestingly, a virus with an 8-kb double-stranded DNA genome, Haloarcula hispanica pleomorphic virus 1 (HHPV-1), has recently been shown to share a high 16/05/11 8:00 PM Viruses of Archaea 109 His 2 0 5000 10000 15000 VP1 ITR 94 AC 22 93 rrn 22 92 35 rrn rrn 22 20 23 pol rrn 10 AC 1 99 30 bp ITR Figure 9.13 Genome of Haloarcula hispanica virus 2 (His-2). • Paramyxovirus envelopes fuse with the external cell membrane at neutral pH upon structural rearrangement of the viral fusion protein. Thin sections of virions (Figure 26.4b) reveal a bioconcave core flanked by lateral bodies. Garrett, R. The first experiment using a lentivirus as a vector was accomplished in 1996 by the group of Inder Verma at the Salk Institute in California. GP subsequently undergoes extensive N- and O-glycosylation in the endoplasmic reticulum and the Golgi compartments as it is transported to the plasma membrane; more than 50% of its mass consists of oligosaccharide residues. Adenovirus inhibits a cellular factor that binds to the 5' cap structure on mRNA and enables the 40S ribosomal subunit to begin scanning the mRNA for an appropriate AUG initiation codon. It is likely that transport to the Golgi and into the trans-Golgi network requires the presence of the glycosylated prM protein. VIRUSES AND HOSTS Mouse polyomavirus; simian virus 40; polyomaviruses infecting birds, rodents, cattle; and five human viruses: BK, JC, MC, WI, and MC viruses. The integers h and k can be considered to represent the number of "jumps" required along two axes on the surface of a capsid to get from one cluster of 5 subunits to the next, touching down on clusters of 6 subunits along the way. VIRUS CLASSIFICATION Many different viruses infecting a wide variety of organisms have been discovered Several thousand different viruses have been isolated and characterized during the approximately 100 years since the science of virology was established. In addition, they have IRES elements in the 5' noncoding region that direct initiation of translation. Domains of life: groups of related organisms at the base of a system of classification of all life forms, based on ribosomal RNA sequences and fundamental properties such as presence or absence of a nucleus, membrane composition, and the machinery for replication, transcription, and translation. May cause other ano-genital cancers in both men and women, and cancers of the oral cavity, oropharynx and tonsil. Table 16.1 Ebola virus proteins Protein Function NP (nucleoprotein) Phosphorylated protein that forms the helical nucleocapsid of the virus. However, in some otherwise nonpermissive cells, B19 virus can replicate with the help of the same adenovirus genes CH020.indd 244 In the absence of helper virus, adeno-associated virus DNA can integrate into the cell genome What happens if adeno-associated virus infects unstressed cells in the absence of a helper? Cathepsins B, L, or S, which are proteases located in endocytic vesicles, can carry out this reaction. From there, the single-stranded DNA is delivered to the (inner) cytoplasmic membrane, the site of DNA synthesis. A number of different cellular proteins that mediate filovirus entry have been detected: asialoglycoprotein receptor, folate receptor-␣, integrins, and DC-SIGN (dendritic cell-specific intercellular adhesion moleculegrabbing nonintegrin) are among these. Because viroids can replicate and possess enzymatic activities (RNA cleavage and ligation), they are considered as a model living fossil from a precellular RNA world. Formation of head-to-tail concatemers via the inverted terminal repeat sequences of two AAV vectors, each carrying a different half of the split cDNA, regenerates the full-length cDNA with the intron at the junction of the two AAV vectors. Genome RNA is converted to DNA by reverse transcription. The preC region (Figure 30.3) encodes a hydrophobic transmembrane domain that directs the E protein to the endoplasmic reticulum, where a signal sequence is removed by proteolytic cleavage. In addition to inducing vesicle formation, 2C is an RNA-binding protein implicated specifically in the initiation of negative-strand RNA synthesis. VP0 subsequently is cleaved into VP2 and VP4, giving rise to the mature virion. Host cell enzymes repair the resulting gapped structure. This huge genome size is one of the reasons why the study of the mimivirus has triggered a plethora of articles debating viral evolution and (more controversially) the very origins and definitions of life. (a) Cryoelectron micrograph of a virion of vesicular stomatitis virus. For example, PBCV-1 encodes the enzymes glutamine:fructose6-phosphate aminotransferase, UDP-glucose dehydrogenase, and hyaluronan synthase, all of which are involved in the synthesis of hyaluronan. 26.2: Courtesy of Richard Condit, modified from Fig. Tight junction: intercellular junction that brings plasma membranes of neighboring cells into very close proximity (1–2 nm); found in endothelial and epithelial cells. The B-cell receptor on each cell represents the specific antibody that this cell will produce upon stimulation after encountering an antigen to which it can bind. Introns removed during alternative splicing are shown as dotted lines. Thus both C and V proteins are virulence factors that modulate host defense mechanisms. Virus replication furthermore induces production of interferons, cytokines, and other soluble response factors, leading to local and systemic inflammatory responses. Hammerhead and hairpin ribozymes derived from plant RNA pathogens can cleave many RNAs and inhibit virus replication. Together they load the RNA polymerase at the transcription start site. These factors have led to a rapid increase in the number and kinds of antiviral drugs available to treat human diseases. Virus Structure and Assembly 18 Stephen C. 1, 9th ed., Mahy, B.W.J., and Collier, L. Some viruses package the "sense" RNA strand, which contains coding regions that can be directly translated into viral proteins. • Upon binding to receptors on the cell surface, interferons activate the Jak–Stat signal transduction cascade and induce transcription of a large number of interferon-stimulated genes. Right: uptake by fusion at the plasma membrane, or within a vesicle after endocytosis, releases the viral nucleocapsid into the cytosol. Resolvase: an enzyme that makes staggered single-strand breaks at the ends of a concatemer junction, allowing formation of single-length DNA molecules with hairpin ends. • Horizontal gene transfer is a recurring theme in phycodnavirus and mimivirus evolutionary history. Nucleoside analogues target viral DNA polymerases Virus genome replication is often performed by viruscoded enzymes that are essential for virus replication and 16/05/11 8:21 PM 448 CHAPTER 36 Antiviral Chemotherapy (a) M2 (b) Matrix NH2 HA RNP Amantadine (c) Influenza virion Endosomal proton pump H+ M2 H+ + H H+ Low pH H+ H+ H+ Endosomal vesicle Membrane fusion Release of RNP Figure 36.3 Proposed mechanism of action of amantadine on influenza virus uncoating. 36.10a: Reprinted from figure 36-10 in Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy, 2nd edition (2007), p.668, David E. (1992). (a) Map of genes on the circular DNA genome (see Table 6.2 for functions of gene products). Wiedenheft, T. This chapter will concentrate on aspects of filoviruses that distinguish them from the related paramyxoviruses and rhabdoviruses, and should be read in conjunction with Chapter 15, where a more complete description of the replication cycle of this group of negative-strand RNA viruses is given. Local outbreaks are often followed by lengthy absence of disease. Furthermore, like Avsunviroidae, all viroid-like circular plant satellite RNAs studied so far have a hairpin or hammerhead structure that catalyzes cleavage of multimeric RNA to monomers during rolling circle replication. These phosphorylated analogues can inhibit viral DNA polymerases in two ways. Several hundred viruses have been studied in some detail. coli F-pili, used for bacterial conjugation. More recently the activity of endosomal cathepsins B and L were identified to be key players in filovirus entry by cleavage of GP within endosomes during virus entry. The p38 promoter includes a binding site for the NS1 protein (TTGGTTGGT), in addition to Sp1 and TATA sites, but apparently activation can also occur without this sequence. When this happens the sea looks a milky white color. First-generation adenovirus vectors can sustain transgene expression for years in post-mitotic cells of immunodeficient animals. All picornavirus IRES elements contain a pyrimidinerich tract 20–25 nucleotides from a conserved AUG codon. Therefore, without a special trick, the very ends of a linear DNA cannot be duplicated, and the DNA would lose a short sequence each time it replicates ("X" in Figure 20.6). Many bacteriophages have both a polygonal head that contains the genome and a tubular tail involved in attachment to the cell and delivery of the genome. Following infection, viroids replicate and propagate in the plant, most likely using cellular junctions to pass from cell to cell. Regions coding for nonstructural (ns) proteins and structural proteins are shown in boxes. Ribonuclease H is an enzyme that selectively hydrolyses the RNA part of an RNA-DNA hybrid, but does not degrade unhybridized RNA. Hepatitis delta virus replicates in the nucleus via a symmetric rolling circle mechanism as shown for viroids in Figure 31.2. The production of antigenome RNA from genome RNA templates (steps 1 and 2) may use cellular DNAdependent RNA polymerase I, because it is resistant to ␣-amanitin, which inhibits RNA polymerase II. An invasion of North America by West Nile virus, transmitted by mosquitoes, began in 1999 and fortunately has only caused disease and death in a limited number of victims. tauri and O. NS4B 250 Unknown role. In contrast, widespread virus epidemics that are spread directly from person to person and are highly contagious, such as smallpox, influenza, and measles, have been limited only by population size and the speed of transportation. Viral RNAdependent RNA polymerases are known to be errorprone because, unlike DNA polymerases, they lack a proofreading ability. The two copies of oriS are flanked by the important regulatory genes coding for ICP4, ICP22, and ICP47 (Figure 24.5). Histone deacetylases (HDACs): enzymes that remove acetyl groups from histones, therefore increasing their binding affinity for DNA and resulting in a more rigid chromatin structure. X shows location of a possible UV-induced dimer that would block transcription only of M in model (a) but would block transcription of M, F, HN, and L in model (b). The loose-fitting envelope of budded virions of AcMNPV (Figure 25.1) contains terminal spikes composed of a single viruscoded glycoprotein, GP64. AIM2 and NLRP3 induce production of proinflammatory cytokines following infection with poxviruses and adenoviruses, respectively. Transformed cells are not subjected to normal control of the cell cycle, and therefore grow and divide in an unregulated fashion. Nucleocapsids are imported from the cytoplasm to the nucleus via the nuclear pore complexes (step 1, Figure 18.6). In order to create an in vitro plasmid-driven system capable of producing infectious filovirus, the plasmids were engineered to ensure they would generate an authentic 3' end on viral RNAs. How was this accomplished? What are the two proposed pathways leading to the production of infectious picornavirus particles? Envelope proteins are inserted into membranes of the endoplasmic reticulum during synthesis, and are transported via the Golgi apparatus to the plasma membrane. Most of these viruses can replicate in both cold-blooded arthropods and warm-blooded 148 CH013.indd 148 16/05/11 8:04 PM Togaviruses vertebrate hosts, and thus have an unusual ability to adapt to different growth conditions. • Some retroviruses have acquired cellular genes within their genomes during integration and transcription; some of these genes produce altered cellular signaling proteins that can transform infected cells into tumor cells. Among these are double-stranded DNA viruses with icosahedral symmetry and internal membranes, which infect CH009.indd 108 hyperthermophilic and hyperhalophilic hosts within the crenarchaeota and euryarchaeota, respectively: Sulfolobus turreted icosahedral virus 1 (STIV-1) (Figure 9.14a), and Haloarcula hispanica virus SH-1 (Figure 9.14b). 16/05/11 8:17 PM Prions Can prion diseases be prevented or cured? Comte de la Condamine, an eighteenth-century French mathematician and scientist, wrote: "No man dared to count his children as his own until they had had the disease". Enveloped viruses have specific surface spike glycoproteins that serve as the binding sites for receptors. Ruigrok, "Paramyxovirus mRNA editing, the 'rule of six' and error catastrophe: a hypothesis," Journal of General Virology 86 (2005), 1869–1877. The ends of their linear DNA molecules have selfcomplementary nucleotide sequences that can anneal to form hairpin structures. In the third step, the DNA polymerase translocates to the next position on the template (dG in the figure). This nascent DNA chain then jumps to the 3⬘ end of the same minus-strand DNA molecule by annealing to the DR1 sequence, and the polymerase proceeds to copy the minus-strand. Because of its use of reverse transcriptase, hepatitis B virus is compared here with the retroviruses, which were the first viruses known to use reverse transcriptase for replication. Herpesvirus virions contain a large amorphous mass between the envelope and the capsid. In these tissues, infection initially results in release of budded virus but there is subsequently a switch in virus production, carried out by an unknown mechanism. Vaccinia virus promoters are approximately 35 nucleotides in length regardless of gene class. A previous but related classification was based on the pathology associated with infection: oncoviruses (many viruses in the Alpha- to Epsilonretrovirus genera) are tumor-inducing viruses; lentiviruses induce slowly progressing, wasting disease; and spumaviruses ("foamy" viruses) induce persistent infection without any associated pathology. Late viral proteins package genomes and assemble progeny virus particles. Nucleotide sequences that determine altered phenotypes have been mapped on satellite RNAs. For example, changing only three nucleotides in a stemloop structure of a necrogenic satellite RNA transformed it into an attenuating satellite RNA in the same (a) (b) Figure 10.10 Symptoms induced by satellite RNAs in tomato plants. • Enveloped virus particles deliver their nucleocapsids into cells by fusion of the viral envelope with a cellular membrane. The major role of papillomavirus E6 protein is to direct the degradation of p53, thereby inhibiting apoptosis in the infected cell and allowing cell survival until sufficient new progeny virus is made. with secondary infections, occurred at the same time in Frankfurt, Germany. 16/05/11 8:09 PM Polyomaviruses VP1 (pentamers) VP2/VP3 Viral DNA (a) Histones H2A H2B H3 H4 Histones H2A H2B H3 H4 Polyomavirus virion dsDNA (wrapped as toroid) (b) Single nucleosome Figure 21.2 Components of polyomavirus virion. CH010.indd 113 Virions of cucumber mosaic virus usually contain four major species of RNA (RNAs 1, 2, 3, and 4) with respective sizes of about 3360, 3050, 2215 and 1030 nt 16/05/11 8:01 PM 114 CHAPTER 10 Cucumber Mosaic Virus βE-βF loop βH-βI loop βF-βG loop N-terminal alpha helix Figure 10.3 Ribbon diagram of the cucumber mosaic virus coat protein. This further underlines similarities between the Flaviviridae and the Picornaviridae, and suggests that these two virus families may share a common evolutionary origin: flaviviruses can be seen as picornaviruses that have acquired an envelope. For example, although there is no significant amino acid sequence similarity between the major capsid proteins of bacteriophages HK97, P22 and T4, and herpes simplex virus type I, all these capsid proteins have very similar three-dimensional structures. The filled head binds a tail to generate an infectious virion. Influenza neuraminidase (NA), in contrast to HA, is a type II transmembrane protein with a short cytoplasmic N-terminus, a membrane-spanning domain, and a long C-terminal domain that extends outward from the virus envelope. The system is composed of two recombinant vaccinia viruses. The complex dissociates in the cytoplasm, releasing the mRNA and allowing Rev to return to the nucleus and repeat the cycle. However, the presence of CD4 alone on the cell surface is not sufficient for infection. Virus-induced caspase activation has also been observed during infection (caspases are proteases essential for apoptosis), and, intriguingly, caspase cleavage sites are also found in many EhV proteins, providing further evidence for the manipulation of this pathway during viral infection. It is only because so little is known about it that it has not been assigned a chapter of its own. Give specific examples to support your answer. These roles are described below. On behalf of all of my colleagues who contributed chapters to this book, I would like to thank the following reviewers: Lawrence Aaronson, Utica College John R. The N-terminal ␣ helix is involved in interactions with the genome and subgenomic RNAs packaged in the capsid. One strand of an siRNA is incorporated into the siRNAinduced silencing complex (RISC), and this RNA guides the RISC to a complementary single-stranded RNA and directs either cleavage of the target RNA or repression of its translation (see Figure 33.11). These hosts therefore provide a potential vehicle for bacteriophage genomes to gain access to a eukaryotic environment. As a result, distinct domains such as bacterial virology, plant virology, veterinary virology, and medical virology have arisen. Autoimmune demyelination: loss of myelin in the central nervous system due to an immune response against myelin introduced with an impure vaccine. Virions mature into infectious particles after budding from the plasma membrane The Env protein is translocated directly into the lumen of the endoplasmic reticulum as it is being synthesized. A disease similar to scrapie, called bovine spongiform encephalopathy (BSE) or mad cow disease, spread throughout cattle herds in Britain during the 1980s, presumably via an infectious agent present in their feed. Entry can be blocked by interfering with cellular processes needed for virus internalization or penetration. Other family members are more elongated, with three relatively thick filaments at one pointed end (Figure 9.2b). None of the terminators is highly efficient, leading to the production of a variety of transcripts with the same 5' ends but extending for different lengths. Since no vaccination can ever be 100% safe, it seems appropriate that there should be little or no "cost" or penalty associated with a personal choice not to receive any particular vaccine. Brome mosaic virus is transmitted mechanically and by beetles. For rhabdoviruses and paramyxoviruses, the RNA polymerase is a complex between the P and L proteins. Purification of infectious material resulted in preparations highly enriched for a 27- to 30-kd protein not detected in similarly purified fractions of uninfected brain tissue. The small coding capacity of microvirus genomes precludes the evolution of such elaborate mechanisms. As a result, the RNA polymerase extends the growing chain to the end of the template RNA without stopping, making a full-length antigenome nucleocapsid. The combination of antigen with IgE activates the mast cells, causing them to degranulate and release histamine, heparin, and arachidonic acid metabolites that cause the symptoms of allergies. Right LTR exon 1 exon 2 exon 3 Cap exon 4 Poly (A) (b) exon 1 Cap exon 2 exon 3 exon 4 Poly (A) Figure 28.9 Promoter insertion near proto-oncogenes can lead to cell transformation and tumor formation. In the trans-Golgi network (TGN), the low pH promotes the folding of the spike-containing immature particles into the semi-mature state and permits the cleavage of prM by host furin protease. • • • • Three surface proteins: LS, MS, SS Core proteins: C, E Polymerase (reverse transcriptase) protein: P Regulatory protein: X VIRUSES AND HOSTS Hepatitis B viruses of human, chimpanzee, duck, gibbon, gorilla, ground squirrel, heron, orangutan, snow goose, woodchuck, woolly monkey. Paramyxoviruses can induce cell fusion, producing multinucleated cells (syncytia). They still have to find the proper site for replication, either in the nucleus or in some defined location in the cytoplasm. The large size of this complex prevents its entry through the nuclear pores, and most retroviruses must await the breakdown of the nuclear envelope that occurs during cell division. Different human papillomavirus types tend to cause lesions in different anatomical sites. Such a vector would generate an infectious virus that contains two subgenomic RNA promoters, one for the expression of structural proteins and another for expression of the toxic protein. Both pentamers and hexamers have central pores; the pentamer pores are believed to allow entry by ribonucleases, as incubation of purified virions with ribonuclease leads to degradation of the genome RNA. If the infection spreads, an organism-wide immune response is activated. For Avsunviroidae, these RNAs are subsequently cleaved to unit-length minus-strands (step 3), which circularize (step 4). A still more rational approach is to exploit detailed knowledge about crucial viral proteins to design drugs that will inhibit their activity. Unlike many host cell mRNAs, which are usually spliced to 16/05/11 8:07 PM Influenza Viruses 217 PB1 PB2 5' Cap Cleavage A 3' Cellular pre-messenger RNA PB2 Cap Primer-directed initiation PB1 PB1 A 3' UC GA 5' UUUUUU Viral genome RNA Elongation Cap PB1 AG PB1 UC Cap UUUUUU Elongation AG PB1 AAAAAA UUUUUU UC Cap GA PB1 GA Stuttering, poly(A) tail AG PB1 AAAAAAAAAA UUUUUU UC PB1 GA Termination 5' Cap AAAAAAAAAA 3' AG Viral messenger RNA Figure 18.7 Production of influenza virus messenger RNAs by cap-stealing. SEC-IV.indd 174 12/05/11 5:03 PM C H A P T E R 1 5 Paramyxoviruses and Rhabdoviruses Nicholas H. Host cells survive virus infection; lysis was not observed. • The mechanisms by which viroids cause disease are not fully understood, but may include RNA interference. In most cases, this will require (1) efficient, targeted delivery, (2) control of both the level and duration of transgene expression, and (3) minimal toxicity and/or immune reaction to either the vector components or the transgene. Gut and respiratory tract secretions contain enzymes and other molecules that prevent attachment of viruses and infection of exposed cells. This in turn triggers the fusion-active conformation in the spikes and leads to penetration. Electron microscopy How can virus entry be prevented? Chapter 17 Fig. Elliott Bunyaviridae From Bunyamwera, locality in Uganda where type virus was isolated VIRION Spherical enveloped particles with a fringe of projections. Long periods in between infections could result in prolonged exposure to the external environment and may make mimivirus 05/05/11 11:24 AM Mimivirus (a) 339 (b) Cyt VF VF (c) VF Figure 27.14 Mimivirus factories. Avoid delivery of "irrelevant" and potentially immunopathologic viral antigens. The remaining four genes (F, G, H, and J) code for the structural proteins of ϕX174, and these four genes do not overlap. The lateral bodies are trypsin-sensitive and therefore contain protein, but otherwise are of unknown composition and function. Some viruses include internal or core proteins bound to the nucleic acid. function. Current drug therapies prevent disease progression but do not eliminate latently infected cells. In the past, compulsory or heavy-handed vaccination programs for smallpox occasionally resulted in street riots. Adjacent DNA chains are then sealed with DNA ligase (not shown). The NS2A protein has also been implicated in RNA synthesis, as it has been shown to be localized in RNA replication complexes. Gene transfer can be carried out in vitro by infecting cells isolated from an individual and then reintroducing cells expressing the desired gene into the host organism. These viruses package within the virion core a large number of enzymes needed to express and process viral early messenger RNAs. These RNAs are synthesized within the cores after they enter the host cell, and at least for poxviruses, mRNAs are transported through the core membrane into the cytoplasm. This virus, which represents another species named Reston ebolavirus (REBOV), spread among monkeys housed within the facilities by different routes, potentially including aerosol transmission. Then by looking at these structures, scientists made informed guesses about what groups to add or subtract to generate a new inhibitor. The laboratories of David Tyrrell, Dorothy Hamre, and Robert Chanock isolated viruses from humans that could replicate on fragments of tracheal tissue or in human cells in culture. A viroceptor can bind to a cytokine and interfere with its activity, therefore reducing host cell defenses against the virus. (b) Schematic diagram. This is particularly advantageous for viruses with a number of different genome fragments of different lengths, such as bunyaviruses and influenza viruses (see Chapters 17 and 18). • Recombinant poxviruses can potentially be used for vaccination against many diseases. Entry of T7 DNA into the cytoplasm is powered by transcription The icosahedral T7 capsid (see page 77) is constructed from a single capsid protein, gp10. The viral J protein 16/05/11 7:56 PM Microviruses The evolution of a capsid assembly system using two scaffolding proteins The microviruses are the only known non-satellite viruses requiring two separate scaffolding proteins. This region is near the shores of Lake Victoria and is close to the trapping place and holding station of the monkeys that initiated the 1967 outbreak in Marburg. The first organ system to be affected by the infection is the gut associated lymphoid tissue (GALT). The packaging plasmid contains the rep and cap genes, regulated by their own endogenous promoters, but no ITRs. The remaining adenovirus gene functions were originally provided by infecting the cells with a first-generation adenovirus vector. Many soccer balls are made from 12 pentagonal and 20 hexagonal subunits, and display icosahedral symmetry (Figure 2.8b). F-restriction. How do Type I and Type II integral membrane proteins differ? Occlusion-derived virions are several hundred-fold less infectious in cultured cells than budded virions, and do not contain GP64. Satellite RNAs of cucumber mosaic virus are small, linear RNA molecules first discovered as the pathogenic agent for an epidemic lethal disease in fields of tomato plants infected by cucumber mosaic virus (Figure 10.10). Therefore, selectivity of these drugs depends on two factors. Genomes of infecting virions are first transported into the nucleus, where they are transcribed; viral mRNAs are then sent to the cytoplasm to be translated; viral proteins are subsequently sent to the nucleus where they direct genome replication and form nucleocapsids CH018.indd 215 with newly replicated genomes; and these nucleocapsids are finally transported back to the cytoplasm for assembly of virions at the plasma membrane. In most cases, in vivo 16/05/11 8:22 PM Adenovirus Vectors studies showed significant reduction of the immune response, lower liver toxicity, and prolonged vector stability. Rectangles on the RNAs show cleavage/ligation sites. Central or primary lymphoid organs: lymphoid tissue (thymus and bone marrow) responsible for the generation of lymphocytes from immature progenitor cells. Encapsidation of the antigenome RNA as it is synthesized may prevent formation of secondary structure in the RNA, thereby overcoming the termination signals recognized during formation of mRNA, and allowing the polymerase to copy to the end of the genome RNA. Ori sequences required for genome replication; ⌿ cis-acting packaging sequences. These virion figures and many others can be found on the web at Viralzone: viralzone/. Hemifusion: intermediate in the membrane fusion reaction where only the outer bilayer halves have fused. (i) Semliki Forest virus (a togavirus). A well-studied example is the influenza hemagglutinin (Figure 2.12). 2⬘, 5⬘-oligo(A) synthetase: an enzyme that produces oligomers of up to 10 ATP residues linked via a 2⬘, 5⬘ phosphodiesterase bond. (c) Stargate structure seen from one side (threefold symmetry axis). CH003.indd 44 16/05/11 7:52 PM C H A P T E R 4 Virus Entry Ari Helenius STEPS IN VIRUS ENTRY Virions bind to specific receptors on the cell surface. This membrane apparently fuses with the phagosome membrane, allowing passage of the virus core into the cytoplasm (Figure 27.13). (b) Cap-independent initiation at the internal ribosome entry site (IRES) of picornavirus RNAs in infected cells. However, its presence in wild populations of mosquitoes and birds remains a threat for transmission to humans. Nevertheless, infection with these viruses is the most significant and important risk factor for the development of cervical cancer. Both filamentous and spherical non-infectious particles, each having a diameter of 22 nm, are found in the serum. KEY TERMS Antibodies CD4 antigen CD4-positive T lymphocytes Chemokines Cyclin Cyclin-dependent kinase Cytokines Cytotoxic T lymphocytes Dendritic cells Epitopes Exportin IB Importin Interleukin 1 Latent infection Ligand Macrophage Major histocompatibility complex (MHC) protein Myristate NF-B (nuclear factor-B) Opportunistic infections Preintegration complex Proteasomes Src-related kinases T-cell receptor Tumor necrosis factor Ubiquitination FUNDAMENTAL CONCEPTS • HIV-1 is a lentivirus that was transmitted to humans from chimpanzees sometime in the early 1900s. The epithelia of the male and female genital tracts likely serve as virus reservoir. SiRNAs are generated by the cleavage of double-stranded RNAs or imperfect hairpin RNAs by the RNase III enzyme, Dicer, or Dicer-like nuclease. 32.4a: Courtesy of Holger Wille. The Flaviviridae family presently contains three genera. Poxvirus genes are expressed in a regulated transcriptional cascade controlled by viral transcription factors The timing of poxvirus gene expression is dictated by the sequential synthesis of virus-coded transcription initiation factors (Figure 26.5), which bind to specific 317 promoter elements on viral DNA. The combined effects of small, middle, and large T antigen on cellular signaling pathways (see above), and the ability of large T antigen to bind to a variety of transcriptional repressors and activators, changes the mix of transcription factors available to bind to the transcriptional enhancer. BGLOSS.indd 481 481 Satellite virus: a virus whose genome is able to replicate only in the presence of a helper virus, but which codes for its own capsid protein (and usually no other proteins). • Linear concatemers of T7 DNA are formed during DNA replication, probably by recombination via a terminal direct repeat sequence. This lymphoma is the most common cancer in African children and is often 16/05/11 8:12 PM Epstein–Barr Virus fatal upon spread to other parts of the body. In the course of these events, the protein undergoes glycosylation and cleavage by host enzymes to generate the mature forms of SU and TM. If Rev is simultaneously bound to an mRNA via its RRE, the export of Rev results in the export of the mRNA. Viral versions of IRFs are produced by human herpesvirus 8, the virus responsible for Kaposi's sarcoma. Interferons and cytokines also help induce longer-term host immune responses. Finally, the tRNA primer is incorporated into the assembling core via binding to the RT and NC portions of the Gag/Pol protein. Shown in the cytoplasm is a plasmid that expresses full-length Ebola virus RNA genome from a T7 promoter. A high-affinity laminin receptor was found on rodent and primate cells, and a laminin-like receptor is also believed to function as the receptor for Venezuelan equine encephalitis virus on mosquito cells. Cellular proteins known to interact with picornavirus IRES elements include the La autoantigen, polypyrimidine tract-binding protein, poly (rC) binding protein 2, and the product of the unr gene. However, in some plant viruses, the different genome segments are separately packaged into distinct virions, as is the case with some positive-strand RNA plant viruses. This chapter will deal with the best-studied human adenoviruses: types 2 and 5, non-oncogenic members of group C, and type 12, a highly oncogenic member of group A. However, it soon became clear that these parvoviruses do not cause cancer. After DNA replication begins, much higher levels of late transcripts are made, and synthesis of early transcripts is reduced. A BRIEF HISTORY OF VIROLOGY: THE STUDY OF VIRUSES The scientific study of viruses is very recent Although viruses were probably present among the first forms of life and have evolved over several billion years, humans only began to understand the nature of viruses near the end of the nineteenth century (see Table 1.2). 16/05/11 8:00 PM CH009.indd 100 Sulfolobus spindle-shaped virus 1 (SSV-1) Sulfolobus islandicus filamentous virus (SIFV) Sulfolobus turreted icosahedral virus (STIV) Sulfolobus neozealandicus droplet-shaped virus (SNDV) Acidianus bottle-shaped virus (ABV) Pyrobaculum spherical virus (PSV) Sulfolobus islandicus rod-shaped virus 2 (SIRV-2) Acidianus two-tailed virus (ATV) Circular ds DNA 15–24 kb Linear ds DNA 16–56 kb Circular ds DNA 16–18 kb Circular ds DNA 20 kb Linear ds DNA 24 kb Linear ds DNA 28 kb Linear ds DNA 24–35 kb Circular ds DNA 63 kb Lipothrixviridae (11) Unassigned (2) Guttaviridae (1) Ampullaviridae (1) Globuloviridae (2) Rudiviridae (5) Bicaudaviridae (1) Genome, size Example of virus species, abbreviation Fuselloviridae (9) Family or genus name (No. of species) Table 9.1 Viruses of Crenarchaeota 72 External None External 48 54 External External ? Herd immunity: sufficiently high vaccine coverage in a population such that the capacity of viral pathogens to spread is limited, thus protecting the small number of unvaccinated individuals in the community. Two subgenomic RNAs: RNA 4 and RNA 4A. Rather, it is a serine/threonine protein kinase, although it lacks some motifs common to protein kinases. Strains of cucumber mosaic virus are divided into three subgroups (IA, IB, and II) initially based on the amino acid sequence of the coat protein and the sequence of the 5' non-translated region of RNA 3. Different virus isolates are generally considered to be members of a virus species if they share a high degree of nucleic acid sequence identity and most of their proteins have highly similar amino acid sequences and common antigenic properties. They are classified as large (L), medium (M), or small (S) based on their relative mobility in polyacrylamide gels; there are three L genes, three M genes, and four S genes. Cultured human B lymphocytes immortalized by infection with Epstein– Barr virus in vitro also exhibit the latency III pattern of gene expression. Vesicular stomatitis, an important disease of cattle, is caused by a rhabdovirus that is transmitted by insect bites. Virions are highly efficient gene transfer machines that interact with specific receptors on the cell surface and deliver their nucleic acid genomes to the appropriate compartment for optimal expression and replication (Chapters 2 and 4). In these cells, only the small viral RNAs and, in some cases, EBNA-1 are expressed. Uninfected epithelium HPV-infected epithelium Virions released Corneal layer Granular layer Spinous layers Basal layer Sloughing cells Nuclear degeneration Synthesis of high MW keratins Stem cells: DNA synthesis and cell division Sythesis of capsid proteins, virion assembly Amplification of viral DNA Early gene expression Plasmid DNA replication Infection of stem cells Figure 22.3 Papillomavirus infection of differentiating epithelial cells. They reasoned that bacteriophages show heritable traits, and therefore must contain and express genes as do all other organisms. RETROVIRUS VECTORS Can permanently introduce a foreign gene into target tissues by integration. Parvoviruses that infect animals can be divided into two major groups: the autonomous parvoviruses and the dependoviruses. There is evidence that both pathways are used. The "phage group" stimulated studies of bacteriophages and helped establish the field of molecular biology During the late 1930s and early 1940s, a group of scientists led by German physicist Max Delbruck, American biologist Emory Ellis, and Italian biologist Salvador Luria decided that the study of bacterial viruses 16/05/11 7:46 PM 8 CHAPTER 1 Introduction to Virology (a) (b) (c) Figure 1.2 Electron micrographs of some representative virus particles. Picornaviruses make non-enveloped virions, and are best known because of the paralytic disease caused by poliovirus and the annoying colds caused by rhinoviruses. We now know that viruses are inanimate when their genomes are 16/05/11 7:46 PM A Brief History of Virology: The Study of Viruses 7 Table 1.2 Some milestones in virology research Date Scientists Smallpox vaccine Rabies vaccine Filterable viruses: Tobacco mosaic virus 1798 1885 Edward Jenner Louis Pasteur 1892 1898 1898 1900 Dimitrii Ivanovski Martinus Beijerinck Friedrich Loeffler and Paul Frosch Carlos Finlay and Walter Reed Discovery of Rous Sarcoma virus Discovery of bacteriophages and the plaque assay Vaccine against yellow fever Crystallization of tobacco mosaic virus Studies with bacteriophages 1911 1915 1917 1930s 1935 1940s Peyton Rous Frederick Twort Felix d'Herelle Max Theiler Wendell Stanley and John Northrup Max Delbruck and Salvador Luria 1966 Growth of poliovirus in cultured cells 1949 1954 Bacteriophage lambda and lysogeny Bacteriophage genes are DNA Discovery of interferon Poliovirus vaccines: killed live 1950s 1952 1957 John Enders, Frederick Robbins, and Thomas Weller Andre Lwoff Alfred Hershey and Martha Chase Alick Isaacs and Jean Lindenmann 1955 1960 Jonas Salk Albert Sabin Studies on polyomavirus: a tumor virus Kuru is caused by an infectious agent Discovery of hepatitis B virus Reverse transcriptase in retroviruses Virus vectors and genetic engineering 1960s 1965 1968 1971 1970s Renato Dulbecco D. Its position within a region of the dimer interface suggests that upon rearrangement of the dimer at low pH, the fusion peptide is exposed, enabling its interaction with cellular membranes. Additionally, some of these compounds and other nucleoside analogues have been approved for use against infections by hepatitis B virus. Compare this image with that of the sphere in Figure 2.2c, oriented in the same manner. Members of the Pospiviroidae family follow a similar scheme but directly copy the linear, minus-strand multimers made in step 2 into linear, plus-strand RNA multimers, therefore skipping steps 3 through 6. Larger viruses come in more complex packages Quasi-equivalent icosahedral designs are neat and economical, but accurate assembly of larger particles is hard to achieve based solely on this principle. RNA phages are among the simplest known organisms RNA phages occur wherever E. T-cell receptors are surface molecules that can bind to small peptide epitopes bound to MHC proteins. The virion is oriented so that one of the 12 fivefold axes of symmetry is visible. Other activities have been associated with individual nonstructural proteins based on studies with temperature-sensitive mutants and on sequence homologies with proteins of known function (Table 13.3). When a T-cell receptor comes in contact with an MHC II protein carrying a peptide to which it can bind, the T-cell will be stimulated to differentiate into active T-helper cells of two kinds, called Th1 and Th2 cells, or into precursors of cytotoxic T-cells, called Tc cells. Hemagglutination assay: a method of titering viruses that agglutinate red blood cells. The protection afforded these individuals by high levels of vaccine coverage in the community is termed herd immunity. Genome is shown as an orange wavy line bound to polymerase (orange sphere). F Major capsid protein; 60 copies in virion. Adjuvant: a substance that enhances immune responses when administered along with antigens. The PLPs catalyze cleavage between nsp1–nsp2, nsp2– nsp3, and nsp3–nsp4. Moreover, host cell membranes in an infected culture remain intact, indicative of a non-cytocidal infectious cycle. This may be followed by symptoms such as abdominal pain, sore throat, nausea, vomiting, cough, joint pain, diarrhea, and a rash. The amount of knowledge that has accumulated about viruses has expanded enormously in recent years, as in many other areas of biomedical sciences. (b) Type II IRES, found in genome RNAs of cardioviruses, aphthoviruses, and hepatoviruses. Protein 2a (97 kDa) contains a central domain that shares sequence similarity with many viral RNAdependent RNA polymerases, and includes four conserved motifs. • Eighteen different late viral mRNAs are made from a single transcription unit by use of five polyadenylation signals and multiple splicing sites. They studied a mosaic disease (discoloration and speckled appearance on leaves and fruit) occurring in cucumbers and other cucurbit plants (gourds, squashes, melons) in the field and in greenhouses in the United States (Figure 10.1). The current available treatment of this disease is infusion of factor IX concentrates. DETECTION AND TITRATION OF VIRUSES Most viruses were first detected and studied by infection of intact organisms Many viruses cause disease in the host organism, and this is how scientists and medical doctors usually become aware of their existence. 5⬘ cap, 3⬘ poly(A) tail. When activated by double-stranded RNA, PKR phosphorylates the eIF-2␣ subunit of translation initiation factor eIF-2 (bottom right), blocking the recycling of GDP with GTP normally carried out by eIF-2B, and ultimately blocking initiation of protein synthesis. Capping of cellular mRNAs normally takes place in the nucleus. • Class III ("late") genes are also transcribed by the T7 polymerase, and include genes coding for structural phage proteins and proteins involved in release of progeny phage. To replicate, these viruses must encode their own RNAdependent RNA polymerases, because such enzymes are not made by their host organisms. Reston ebolavirus infection within a colony of Asian monkeys raised the question whether nonhuman primates may serve as a reservoir. Desperation and fear go some way toward explaining why Meister's parents and the investigators themselves conducted this experiment. In some cases cucumber mosaic virus coat protein is associated with longdistance movement in a host-specific manner, indicating the involvement of host factors. For some time, it was not clear whether viruses were a form of soluble small molecules ("infectious living fluid"), or alternatively a particle like bacteria but too small to be retained by these filters. (c) Side views of one monomer of large T antigen in a hexamer, showing the channel and the 17 Å (1.7 nm) movement of the -hairpin (1) as ATP is hydrolyzed to ADP and ADP is released. Virions of this family are enveloped, and many have the shape of a lemon or spindle, with a bunch of thin filaments attached to one of the two pointed ends (Figure 9.2a, d). The switch from translation to replication works as follows. However, the majority of viruses depend on endocytosis to be first carried into cytoplasmic vacuoles, from which they can then enter the cytosol. The loss of surface CD4 is caused by an increase in the cycling of CD4 between the cell surface and the endosome compartment. The RNA packaging sequence fits particularly well into a short length of this groove, giving the RNA genome an assembly advantage over any cellular RNAs that might be present during packaging. The loss of ciliated epithelium allows relatively easy entry of bacteria into the lower respiratory tract. In contrast to capped viral mRNAs, these full-length transcripts contain 5' triphosphate groups and result from unprimed transcription initiation. Their negative-strand RNA genome is composed of between six and eight distinct gene segments. Protein 3a targets and dilates plasmodesmata, the cytoplasmic channels that connect plant cells, and mediates the movement of viral RNA from one cell to another, including movement into and out of the vasculature. ICP4, ICP22, and ICP27 are localized in replication/transcription factories that appear in cell nuclei after DNA replication has begun. Antigenome: a full-length complementary copy of a singlestranded (usually RNA) genome. An additional problem with retrovirus vectors is the possibility of generating replication-competent virus during large-scale vector production. Epstein–Barr virus replicates transiently in epithelial cells of the oral mucosa, releasing progeny virus into the saliva. The open reading frame for the lysis protein overlaps with those of the coat and 1 130 RNA phages bind to the F-pilus and use it to insert their RNA into the cell RNA phage capsids contain 180 copies of the coat protein arranged in a T 3 icosahedral shell that encloses the RNA (Figure 5.2). Adenoviruses are widely used as gene therapy and anticancer virus vectors. This TRS = UCUAAC TRS 5' RNA polymerase 3' (ℎ) Genome RNA 3' 5' 3' 3' 5' 5' 3' 5' 5' (𝜐) Subgenomic mRNA templates 3' (ℎ) Subgenomic mRNAs Figure 14.5 Discontinuous transcription of genome RNA: a model for coronavirus mRNA production. Viral epidermal growth factor (vEGF) is a virokine produced by vaccinia virus, a member of the poxvirus family. protein present in the nucleus. This gives rise to full-length RNA identical to the genome RNA of the infecting virus. Ribonuclease H: a ribonuclease that specifically degrades the RNA part of RNA/DNA hybrids. However, recent dramatic progress has been made in the "reverse genetics" of negative-strand RNA viruses—the mutation of complementary DNA copies of the viral genome followed by the rescue of the mutated cDNA into an RNA genome. In the first step, acyclovir triphosphate acts as a competitive inhibitor of the incorporation of deoxyguanosine triphosphate (pppdG) into DNA, by competing with pppdG for binding to the DNA polymerase. separately developed protocols for producing Figure 10.2 Electron micrograph of cucumber mosaic virus. A reasonable but not completely verified model is diagrammed in Figure 36.3. This starts with the fact that influenza virus enters cells by receptor-mediated endocytosis. www.cengage. The N protein binds specifically to full-length viral genome and antigenome RNAs to form viral nucleocapsids, and it is in this form that the viral RNA serves as a template for both transcription and replication. Isaacs and Lindenmann found that the culture medium from cells infected with influenza virus could transmit antiviral protection to cells that had not previously come in contact with the virus; they named the substance present in the medium "interferon." Interferons were subsequently shown to Virus infection Transcriptional activation of interferon genes ASC Pro-Caspase-1 Caspase-1 Synthesis of interferon Binding to interferon receptor Synthesis of antiviral proteins Interleukin-1β Figure 33.5 Cellular sensor proteins activate caspases and cytokines. Note that E2A and E2B use a common promoter but different polyadenylation sites. 16/05/11 8:19 PM 420 CHAPTER 34 Innate and Adaptive Immune Responses to Virus Infection B and T cells have specific surface receptors that recognize antigens Both B and T cells express unique surface receptor molecules that can recognize and bind to specific parts of antigens called epitopes. Loops that extend from the strands of each subunit tie together the mature virion by binding to neighboring subunits. Baculoviruses produce two types of virus particles; one particle is crystalline-like and able to survive in the environment for extended periods of time. Other fuselloviruses have similar genomes up to 24 kb in length. A host RNA-dependent RNA polymerase (not shown) can amplify siRNAs. SiRNAs are bound by the RISC complex, which includes the protein Argonaute (Ago). It seems possible, therefore, that new variant CJD is caused by a different prion strain that is less restricted by the species barrier than that of scrapie. Rather than a disease agent that needed to be eradicated, baculoviruses were recognized as potentially beneficial. PInt is a CII-dependent promoter for a gene that encodes Int, the site-specific recombinase that inserts the prophage into the bacterial chromosome. At this point, vaccinia virus is the only such viral vaccine licensed for use in humans, and in its present form it is no longer being used because smallpox has been eradicated. O CH3 N HO O O N O CH3 Cellular thymidine kinase N O PO HO O N O OH N3 N3 AZT monophosphate AZT Inhibits cellular thymidylate kinase Cellular thymidylate kinase O CH3 HO N O O PO PO OH OH O N O N3 AZT diphosphate Cellular nucleoside diphosphate kinase O CH3 HO O O O PO PO PO OH OH OH N O N O 451 AZT diphosphate is converted to the triphosphate by cellular nucleoside diphosphate kinase. • Some adenoviruses can induce cancer in experimental animals but not in humans. The RNA genome is encapsidated by the surface protein of hepatitis B virus, a required "helper" virus. A highly conserved 9-amino acid region is located in the H-I loop, which is exposed on the outside of the capsid. While stalled, the ribosome can shift from reading frame 1 to frame 0. Self-cleavage of polyprotein by viral proteinases. London: Edward Arnold Publishers (1998); Fig. Normally when ribosomes come to the end of a coding region they are released from the messenger RNA if no reinitiation sites are nearby. Eventually these terms were adopted by the World Health Organization Committee on Viral Hepatitis. Therefore, unlike all other viruses, genome sequences can occur as both DNA and RNA at different stages of replication. In each class of spliced RNA there are multiple species, generated by the presence of several different 3' and 5' splice sites. The proteins are glycosylated and in some cases cleaved during transport. RNA virus replicons may lack coding sequences for viral structural proteins. Panleukopenia: infectious disease (of cats) leading to shortage of white blood cells, among other symptoms. Synthesis of plus-strand strong-stop DNA. Interferon regulatory factors IRF-3 and IRF-7 bind to positive regulatory domains I and III, NF-B binds to positive regulatory domain II, and ATF-2/c-Jun binds to positive regulatory domain IV. Recent studies demonstrate that the E protein most likely inhibits the host cell Mra Y protein. lucimarinus. Electron microscopy ultimately revealed a polyhedral structure, similar to many virions of the nucleo-cytoplasmic large DNA viruses, but substantially larger. However, contrary to the class I fusion proteins, these proteins undergo only minor conformational changes at the level of secondary structure. At least seven distinct viruses cause human hepatitis Hepatitis (infection of the liver) constitutes a major worldwide public health problem. Import of viral genomes into the nucleus Many animal and plant viruses, particularly DNAcontaining viruses, use the nucleus as their site of CYTOPLASM (a) HIV Core Virions and capsids are transported within the cell in vesicles or on microtubules For most viruses, reaching the cytosol is not the end of the journey. What are the two polymerases and the sequences of the elements they recognize? It is sequestered in an inhibitory complex in the cytoplasm, from which it is released upon receipt of a variety of extracellular BGLOSS.indd 478 signals; it travels to the nucleus and activates transcription of numerous target genes. Excised introns are shown as dashed lines, with the number of nucleotides excised shown at the center. The second is CD150/SLAM (signaling lymphocyte activation molecule), a member of the immunoglobulin superfamily that is expressed on activated B lymphocytes, T lymphocytes, dendritic cells, and some monocytes. • Reovirus cores contain viral RNA polymerases, which make viral messenger RNAs by transcribing each of the 10 to 12 viral genome segments. There are many different serotypes of rhinoviruses, making vaccination impractical. Autonomous replication of the human parvovirus B19, discussed below, is mostly limited to human erythroid progenitor cells. A major problem with virus vectors has always been the host's immune response to the vector itself. May play a role in the optimization of burst size in various hosts. p53 activates transcription of genes involved in induction of apoptosis (bax) and arrest of cell cycle (p21). Cathepsins are also required for entry of Ebola virus and SARS-coronavirus. DNA-dependent RNA polymerase: An enzyme that synthesizes RNA using a DNA template. Virus genomes are either RNA or DNA, but not both There are many different viruses in the world, and probably all organisms on Earth can be infected by at least one virus. coli Yes No Unwinding of 3'-terminal hairpin on plus-strand RNA A host ribosomal protein directs polymerase to the coat start site It turns out that binding of the polymerase to the coat protein start site is facilitated by the S1 protein, which carries out the same function for the ribosome when it is binding to the coat protein start site. budded virus, which is responsible for systemic infection of many tissues within one host (horizontal transmission) and 2. Reoviridae have segmented genomes made of double-stranded RNA Reoviridae that infect animals have genomes consisting of 10 to 12 discrete, linear segments of double-stranded RNA (Figure 19.1). Reovirus infection leads to inhibition of cellular protein synthesis. What is meant by the statement that B cells "see" antigens and T-cells "see" peptides? The replicase gene is translated from genome RNA into a polyprotein that is processed by viral proteinases Because coronaviruses have positive-strand RNA genomes, the first step in their replication cycle after entry is the synthesis of viral proteins that organize and catalyze viral RNA synthesis. The presence of the drug can alter the structure of the canyon floor, thereby preventing proper interaction of the virion with the cellular receptor and preventing attachment. 16/05/11 8:03 PM 140 CHAPTER 12 Flaviviruses (a) (b) (c) Figure 12.2 Morphology of dengue virus type 2 virions. The threefold axes (triangles at the center of each face of the icosahedron) pass through the centers of the spherical triangles outlined by the dashed lines on the image in Figure 2.2c. This allows studies of the structure and activity of viral proteins in absence of infectious virus. Are all viral genomes infectious? Essential for DNA replication in vivo LEF-12 21 May not be essential when all other genes are present 39K/PP31 31 Phosphorylated DNAbinding protein? Because rabies is slow to develop, people can be successfully vaccinated after exposure to the virus. At right, an electron micrograph of a negatively stained Autographa californica MNPV budded virion. CH024.indd 297 16/05/11 8:12 PM 298 CHAPTER 24 Herpesviruses Epstein–Barr virus expresses a limited set of proteins in latently infected B lymphocytes Two sets of viral proteins are produced in latently infected B lymphocytes: Epstein–Barr nuclear antigens (EBNAs) and latent membrane proteins (LMPs). Although there has been some convergence among these fields of study, their different histories have led to adoption of distinct conventions for naming viruses and for virus classification. 16/05/11 8:22 PM Adeno-Associated Virus Vectors small genes such as that coding for blood clotting factor IX, but not larger genes affected by several common genetic diseases. The short NS2B protein has several membranespanning domains and a conserved hydrophilic domain of about 40 amino acids. Therapeutically administered interferon can lead to unpleasant side effects and its antiviral effects are shortlived. The newly synthesized plus-strand DNA remains hydrogen-bonded to the minus-strand, forming a double-stranded DNA. The two RNAs base-pair at the TRS and the RNA polymerase extends the RNA chain to the 5' end of genome RNA, copying the leader sequence. Direction of transcription by 3D RNA polymerases is shown by arrows. Expression of E1A alone in rodent cells usually produces abortive transformation. However, the capacities of these two ribozymes are limited by their requirement for high magnesium concentrations; mammalian cells contain one-tenth the concentration of magnesium needed for optimal activity of these ribozymes. This structure opens within the phagosome to create a large "stargate", revealing the virus core wrapped within a membrane. Under the appropriate conditions it assembles in the cytoplasmic membrane of the infected cell to create a hole that allows access to the bacterial cell wall of the phage endolysin, the product of gene 3.5 (T7 lysozyme). GENES AND PROTEINS mRNAs are transcribed from integrated provirus DNA by RNA polymerase II. The diagnostic window for blood using these assays lies within 3 to 16 days after onset of disease. Orbiviruses are transmitted by arthropod vectors, as are viruses of the very different bunyavirus, flavivirus, and togavirus families. It is mainly produced by activated T lymphocytes and natural killer (NK) cells. The remaining 400 amino acids are arranged as an elongated three-domain structure (Figure 12.3). For rhinoviruses and enteroviruses, further processing of the P1 region is carried out primarily by 3CD. Designated "restriction factors", they either accelerate capsid breakdown or block transport of the viral preintegration complex into the nucleus. In comparison to the T-even phages (T2, T4, T6), phage T7 was ignored until the mid-1960s when its small size and easy-to-isolate DNA attracted the attention of both physical chemists and molecular geneticists. This leads to mutations in viral structural and regulatory proteins, with a corresponding reduction in infectivity. Ambisense: coding strategy used by arenaviruses and some members of the bunyavirus family, in which an RNA genome segment contains both positive- and negativesense genes (ambi both). One end of HA1 is now bound to the endosomal membrane, and the other end is bound to the virus envelope; these two membranes are brought into close proximity by this conformational change, resulting in membrane fusion. These viruses were pathogenic in domestic and wild birds and led to hundreds of millions of bird deaths in South and East Asia either naturally or through culling of infected stock. Complement pore-forming activities may also create a hole in the CH034.indd 417

surface of infected host cells or microorganisms to which antibody has bound. This characteristic has been used to incorporate extra DNA into virions when using baculovirus as an expression vector. Translation in frame 0 does not recognize the UAA termination codon (bold letters). DNA vaccine: introduction into the body of plasmids that express specific proteins, usually of a selected pathogen, resulting in an immune response against those proteins. The selective replication of E1B-deleted adenoviruses in tumor cells leads to death of tumor cells, and enables spread of virus progeny to nearby tumor cells, increasing the antitumor effect of the virus. Study of the expression of adenovirus “late” genes led to the discovery of RNA splicing. It is possible that the double-stranded gene segments are maintained in circular form during transcription, facilitating reinitiation and multiple rounds of transcription at the same locus. • Many viruses of archaea encode no obvious DNA polymerase. The virus expresses another protein, E6, which mediates the degradation of cellular p53 protein in the infected cell (Figures 22.4 and 22.6). Adeno-associated virus DNA may remain integrated in the cellular genome through repeated cell divisions, thus propagating in daughter cells, until the cell is infected by a helper virus. Speculate why this practice was subsequently discontinued. Protein 3AB is anchored to the proliferated cytoplasmic vesicles via a hydrophobic region in the 3A portion (Figure 11.7, top right). Vectors based on viruses with RNA genomes are constructed in DNA plasmids containing copies of the virus genome made by reverse transcription. • Selective replication of certain parvoviruses in tumor cells results from the dependence of those viruses on cellular factors expressed during S phase or altered by cell transformation. The resulting p21 protein is a potent inhibitor of Rb phosphorylation, and this prevents the expression of E2F-responsive genes. These data help to explain the observation that vaccine-associated paralytic polio is most often ascribed to pathogenic revertants of types 2 and 3 Sabin vaccine-strain viruses. With genomes ranging from 240 to 1700 nucleotides, these infectious RNAs cause various diseases in plants and animals. Live attenuated viruses. All late mRNAs are produced from a single primary transcript that extends 29 kb in the rightward direction from the highly active “major late promoter”, at 16.5 map units (Figures 23.4 and 23.9). ID and 4A,B reprinted from Holger Wille, Melissa D. There is evidence that one of Capsid DNA Core Tail fibers OM PG CM RNA pol mRNA Ribosomes Figure 7.2 Entry of T7 genome powered by transcription. However, it is striking that: The great variation in genome design and replication strategies among known viruses suggests that there could very well have been multiple origins of viruses during the evolution of life. The polymerase proteins PA, PB1, and PB2 of the 1918 human virus differ by a total of only 10 amino acids from the consensus sequences of the same proteins made by most avain strains. Summers T7-like bacteriophages T7 (“type 7”), from the collection of phages chosen for study by the phage group VIRION Naked icosahedral capsid (T 7). • Phage RNA polymerase contains one viral protein subunit and three or four cellular protein subunits. 223 KEY TERMS ␣-amanitin Actinomycin D Amantadine Antigenic drift Antigenic shift Ciliated epithelium Cytokine Double-stranded RNAdependent protein kinase (PKR) Epidemic Furin Fusion peptide Hemagglutination assay Hemagglutinin Importin Interferon Lipid rafts Mucociliary flow Neuraminidase Oseltamivir Pandemic Reassortment Sialic acid Type I transmembrane protein Type II transmembrane protein Zanamivir FUNDAMENTAL CONCEPTS Vaccination against influenza is an effective prevention method Inactivated influenza vaccines are manufactured every year; each year’s vaccine reflects the strains of influenza A and B viruses circulating during the end of the preceding season. The virion is formed from eight proteins arranged in an unusual two-layered structure of concentric protein shells, each with icosahedral symmetry. Ostreococcus tauri (a marine prasinophyte) has a diameter of less than 1 micron, it has no cell wall but instead a naked plasma membrane, and it lacks scales and flagella. Studies by Stanley and others showed that viruses contain both protein and nucleic acids. It is thought that virus particles taken up in these cells by endocytosis are retained within endosomes until they are degraded, and the released nucleic acids then bind to toll-like receptors. All negative-strand RNA viruses that infect vertebrates are enveloped. PrPSc aggregates and accumulates in diseased brain. Much remains to be learned about the detailed functions of these proteins. Electron microscopy has shown that immature virions can be found in the lumen (interior) of the endoplasmic reticulum. The field of phycodnavirology is now firmly established and is rapidly expanding. It is a recombinant DNA molecule containing sequence information for efficient transcription of the desired gene and translation of its messenger RNA. No treatment or licensed vaccine is available. Injection of DNA through the tail and into the cell requires the cytoplasmic membrane components of another sugar transport system, mannose permease. CH019.indd 236 16/05/11 8:07 PM SECTION V SMALL DNA VIRUSES OF EUKARYOTES C H A P T E R 2 0 Parvoviruses C H A P T E R 2 1 Polyomaviruses C H A P T E R 2 2 Papillomaviruses This section considers viruses that have DNA genomes of less than 10 kb, limiting the number of viral proteins they encode to between 5 and 10. Small, icosahedral, positive-strand RNA viruses, including some plant viruses and the togaviruses, have a positively charged N-terminal arm on their capsid proteins. 11.8: Fig. It is thought that M2 in the virion envelope functions to let H⇶ ions enter the virion, and the low pH changes the conformation of the matrix protein, which lines the inside of the viral envelope. Hantaviruses are responsible for three forms (mild, moderate, or severe) of hemorrhagic fever with renal syndrome. Without doubt such universal screening has the potential for decreasing the worldwide incidence of cervical cancer. much of this difference is accounted for by the very large nairovirus L segment. As we approach worldwide eradication of measles and poliovirus, there are increasing concerns about the safety of the currently used vaccines. Wild-type herpes simplex virus blocks apoptosis caused by viral gene expression or by exogenous agents such as osmotic shock, thermal shock, and Fas ligand interaction. The disease had a very high yearly incidence rate of 1%. This suggests that herpes simplex virus acquired part of a cellular gene whose function is to control protein synthesis in infected cells. These are arranged as 150 hexamers on the faces of a T 16 icosahedron, and 12 pentamers at the vertices. This requires a fifth protein, also a host cell protein called Host Factor. This is confirmed by identification of filovirus particles in lungs of naturally and experimentally infected monkeys and human postmortem cases. GENES AND PROTEINS Autographa californica MNPV codes for over 150 proteins. This is often achieved by interaction of early viral proteins with cellular signaling pathways that affect the cell cycle and direct the cell to enter the DNA synthesis (S) phase. The presence and diversity of so many homologous genes coding for major capsid proteins (also observed in Chlorella viruses) raises interesting questions of the potential host range of these viruses, although to date host range appears restricted specifically to Ostreococcus species. 2.12: Courtesy of Stephen Harrison. These scientists formed an informal network called the “phage group”, which stimulated studies of bacteriophages and their host bacteria by numerous physicists, chemists, and biologists. Discuss two unique structural features that distinguish poxvirus intermediate and late mRNAs from early mRNAs. 5. Human immunodeficiency virus is capable of infecting and killing both NK cells and CD4-positive T cells. Sendai virus and close relatives bind to terminal sialic acid residues on gangliosides (glycolipids) on the cell surface. E1A proteins also activate other cellular transcription factors The E1A 289R protein independently activates transcription of adenovirus E3 and E4 genes by interactions between its unique CR3 domain and cellular transcription factors. • Nef has multiple effects on the cell, maximizing virus replication while reducing the effectiveness of the host immune response. These three C residues are located at the first position CH015.indd 182 of the 14th, 15th, and 16th hexanucleotides (groups of six nucleotides) on the genome. Electron micrographs of Vero E6 cells infected with SARS-coronavirus. Acheson Montreal, September 2010 13/05/11 2:23 PM SCHEMATIC DIAGRAMS OF VIRUSES COVERED IN THIS BOOK The diagrams on the following two pages illustrate most of the viruses discussed in detail in this book. These mRNAs are capped at their 5' ends and contain a 3'-terminal poly(A) tail generated by stuttering of the viral RNA polymerase at a run of uridine CH016.indd 191 residues located adjacent to each transcription termination signal sequence. Both of these events can be important for antiviral activity and for selectivity. In (a), there is an equilibrium between the PrPC state (black disc) and a rare monomeric precursor of PrPSc (green square). Recent progress in vector development Chimeric viruses. Esther Lederberg happened to spread an agar plate with a mixture of a K-12 () culture supernatant and cells of a strain derived from K-12 that had fortuitously lost its prophage, that is, was (). the cellular genome, and contains mutations that inactivate its ability to replicate. Phage Q is the most intensively studied allolevivirus. Adenoviruses incorporate up to ten different proteins in their icosahedral capsids. Some paramyxoviruses make an additional small (40– 60 aa) hydophobic envelope protein (SH) of unknown 16/05/11 8:05 PM 178 CHAPTER 15 Paramyxoviruses and Rhabdoviruses (a) The best-studied rhabdovirus, vesicular stomatitis virus, has a single envelope glycoprotein (G), responsible both for receptor binding and fusion activity. A key point about amantadine is that this drug and the drug-resistant mutants opened up a whole new area of virus biology. 9.2d: Courtesy of Philippe Lemercier; Fig. In contrast to the polyomaviruses, papillomaviruses, and adenoviruses (Chapters 21–23), parvoviruses are unable to induce cells to enter the DNA synthesis (S) phase of the cell cycle. The two families of viroids have distinct properties 379 family or group A. Can be fatal in elderly, infants, and chronically ill, often by secondary bacterial infections. The result is large T antigen (LTAg), a protein 785 amino acids in length (708 amino acids for simian virus 40). Currently the best packaging cell lines are generated from split genome constructs (Figure 37.4a). huxleyi genotypes. S RNA codes for nucleocapsid protein and a nonstructural protein The S RNA segments also show diversity in their coding strategies. Since host mRNAs are also cleaved, the cell may die if sufficient ribonuclease L activity is present. From left to right: a parvovirus (beak and feather disease virus), diameter 20 nm; adenovirus, diameter 80 nm; a phycodnavirus (Paramecium bursaria Chlorella virus 1), diameter 200 nm; and mimivirus, diameter 500 nm. The discovery of hepatitis B virus In 1963, Baruch Blumberg discovered a previously unknown protein, which he denoted the Australia antigen, in the blood of an Australian aborigine. A rule of thumb for distinguishing influenza from other respiratory virus infections is: if you really can’t get out of bed, it’s probably influenza! The acute stage lasts about three days, but cough and malaise may last for several weeks. Typically, apoptosis is used as a defense against virus infection and is controlled by signaling within the host. Viral envelopes contain a lipid bilayer, whose lipid content reflects the composition of the cellular membrane from which the envelope was constructed. Upon expression CH037.indd 468 of the Cre recombinase, recombination between the two loxP sites will generate a circular DNA. CH018.indd 222 16/05/11 8:07 PM Influenza Viruses A massive vaccine development campaign allowed the first human vaccination campaigns against pandemic H1N1 virus to begin in October 2009. Although they are related by evolution, species that are members of a common genus may show significant divergence in nucleotide and amino acid sequences. Poliomyelitis is now virtually absent from the developed world thanks to Figure 11.1 Egyptian stele (Astarte Temple, Memphis, 1580–1350 B.C.). Because they encode no proteins, viroids depend on cellular RNA polymerases for replication. The role of sGP in the life cycle of Ebola viruses remains obscure. Usually located 25 to 30 nt upstream of eukaryotic transcription start sites. In late stages of the disease, hemorrhage often occurs in the gastrointestinal tract, the spaces surrounding the lungs and heart, the peritoneal spaces, and the renal tubules. The small hepatitis B surface protein (SS, also known as SHBs), originally designated as Australia antigen, is the smallest and most abundant surface protein. A fifth human polyomavirus, MC virus, was recently isolated from Merkel cell carcinomas. Long-distance movement: movement of a plant virus throughout a plant by way of the vascular tissue. The cellular factor is a stress granule protein implicated 16/05/11 8:13 PM 320 CHAPTER 26 Poxviruses 1 Nick, unwind 2 Extend 3' end 3 Refold hairpin ends 4 Extend 3' end 5 Extend 3' end Nicks Ligate Concatemer 6 resolution Ligate Figure 26.6 A model for vaccinia virus DNA replication. These infectious agents spread via extracellular fluids into the lymphatic system, a set of vessels that transports cells and microbes to the lymph nodes (Figure 34.4). The spleen primarily responds to microbial antigens in the central blood circulatory system, whereas the lymph nodes respond to infectious agents that enter the body through the skin and mucous membranes lining the oral cavity, the respiratory system, the digestive system, and the urogenital system. This showed that cucumber mosaic virus and its relatives are positive-strand RNA viruses, whose genome RNAs can act directly as messenger RNAs when introduced into a cell. The minimal promoter sequence required for transcription is 19 nucleotides upstream and 5 nucleotides downstream from the start of the subgenomic RNA; this region is highly conserved among the various alphaviruses. DNA synthesis is primed by terminal protein, uses viral DNA polymerase. This productive cycle is important both for spread of the virus to other individuals and for subsequent infection of resting B-lymphocytes in the oral cavity (Figure 24.11). CH016.indd 196 16/05/11 8:05 PM Filoviruses Both index case patients of the episodes of Marburg virus hemorrhagic fever in Kenya (1980/87) traveled in the Mt. Elgon region. What four elements that are required for virus replication are provided by host cells? Bafilomycin A1: inhibitor of the vacuolar proton pump that acidifies endosomes. However, viruses replicate by a pathway that is very different from the mode of replication of these other intracellular parasites. these single-stranded regions and reinitiates lagging strand DNA synthesis. For example, binding of viral proteins or nucleic acids to toll-like receptors leads to the recruitment of the downstream adaptor molecules myeloid differentiation primary response gene 88 (MyD88) and toll/interleukin-1 receptor (TIR)-domain-containing, adaptor-inducing interferon- (TRIF). Autophagy also plays a role in recognition of viral nucleic acids by toll-like receptors. Unlike some live virus vaccines, inactivated virus vaccines must be injected, which makes their administration somewhat more complex. The first evidence of assembly of virus particles is the appearance within factories of rigid, crescent-shaped membrane structures (Figure 26.7; see lower-right corner of Figure 26.5). These small DNA viruses encode proteins that interact with cell signaling pathways and trick the cell into entering the S, or DNA synthesis, phase. A 2-subunit capping enzyme (the cap guanylyltransferase) adds 7-methylguanosine to the 5' triphosphate end of the nascent RNA in a 5'-5' triphosphate linkage, and a distinct (nucleoside-2'-O-)-methyltransferase (the cap ribose-methyltransferase) methylates the ribose of the first transcribed base in the RNA. The safe track records of these viral and bacterial agents make them attractive vectors for the delivery of antigens from unrelated organisms for which vaccines are not yet available. Inclusion bodies: aggregates of viral proteins or nucleic acid visible by light microscopy in infected cells by their staining characteristics. Viruses that infect bacteria, archaea, algae, and higher plants use mechanisms of entry quite distinct from those of animal viruses, because access to cells is restricted by the presence of cell walls. Although these viruses infect both sexes, papillomavirus-associated genital cancer is much less prevalent in men than in women. CH024.indd 287 16/05/11 8:12 PM 288 CHAPTER 24 Herpesviruses Recombination takes place during viral DNA replication to generate four isomers of the genome DNA, each with a different relative orientation of the UL and US segments (Figure 24.1). Although multiple AUG codons are located between the 5' end and the authentic initiator AUG, these are not used by ribosomes to initiate protein synthesis as would be expected if ribosomes were “scanning” this region searching for appropriate initiation sites. At the center of each pentasymmetron lies a pentameric capsomer formed from a distinct capsid protein. Ceramide: type of lipid molecule composed of sphingosine and fatty acid. • Proviral DNA is imported into the nucleus and integrated into the host cell chromosome at random sites. Chapter 24 Fig. Transmission by blood and sexual contact. RNA world: a hypothetical prebiotic world in which selfreplicating RNAs developed and evolved, preceding and perhaps leading to development of the DNA world that is universal in present-day life. However, it is not clear exactly how NS1 activates transcription. Inhibition of PP2A activity by small T antigen allows MAP kinases to remain phosphorylated and therefore enzymatically active. They are very selective—they don’t even inhibit the reverse transcriptase of the closely related virus HIV-2—and their discovery makes a strong case for the validity of the target-based high throughput approach. The termination codon UAG separating the gag and pol reading frames is correctly recognized 19 times out of 20, resulting in synthesis of only the Gag polyprotein. Hexons are clustered in groups of 5 surrounding the twelve penton bases, and in groups of 9 on the 20 faces of the capsid, for a total of 240 per virion. The positions of the three DNA replication origins (two OriS and one OriL) are shown on the map. What important functions in virus replication do tRNA-like structures, like those found in cucumber mosaic virus genomes, serve? As a result, increased levels of p14Arf bind to Mdm-2 and reduce its level in the cell. Complex virions may contain over 100 proteins. One virus in the latter group was originally isolated from an alfalfa looper (Autographa californica) and was named Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV). In contrast, cattle are very resistant to infection with sheep scrapie but are 16/05/11 8:17 PM 394 CHAPTER 32 Prions susceptible to BSE. Vhs and ICP27. As a consequence, transgene expression with lentivirus vectors can be proportionally increased by increasing the dose of infecting virus. Shown are the nucleotide sequences of parts of the four mouse polyomavirus early mRNAs (in black) and their translation products (in green). As discussed in Chapter 37, human adenoviruses are already being used experimentally in gene therapy. Repression arrests production of early proteins (including Cro and CII) and activates PRM for prophage expression of CI. One such stimulus is activation of T cells via the T-cell receptor (see Chapter 34); others are virus infections or treatment with various cytokines (see Chapter 33). TE and Tφ show positions of transcriptional terminators. Hemorrhagic fevers: diseases caused by a number of viruses, involving high fever, vascular damage leading to internal bleeding, and resulting shock, which if untreated can lead to organ failure and death. As in step 2, the polymerase begins copying the template minus-strand DNA near its 5♧ end, making a short DNA product with the capped RNA fragment at its 5♧ end. After many cycles of this process, the result was ritonavir. Furthermore, it protects 90% of recipients against infection by viruses of the same subtype. The helical nucleocapsid, made from the RNA genome complexed with N protein, is shown just underneath the envelope. Besides being economical in the use of space in a small genome, these small introns therefore ensure a balanced production of the different early mRNAs. Figure 21.7 Alternative splicing of early mouse polyomavirus transcripts generates four different T antigens. Hairpin Primosome SSB priA priB priC Replicative form DNAs are amplified via a rolling circle mechanism dnaB dnaC primase A single molecule of replicative form DNA cannot generate sufficient mRNAs and progeny singlestranded DNAs to construct the 100 phage particles made in a productive infection. KEY TERMS Amantadine Attachment factor Bafilomycin A1 Budding Carboxylic ionophors Caveolae Caveolin Cavin Class I fusion proteins Class II fusion proteins Clathrin-coated pits Coiled coil Cytosol Dynactin Dynein Early endosome Endosome Filopodia Fission (membrane) Fusion (membrane) Fusion peptide Glycolipid Glycoprotein Hemifusion Immunoglobulin family Importin Integrins Karyopherin Ligand Lipid raft Lumen Lysosome Lysosomotropic agent Macropinocytosis Neuraminidase Plasmodesmata Proteoglycan Receptor Receptor-mediated endocytosis Sialic acid Syncytium (plural: synctia) Type I transmembrane protein Vesicle transport Virological synapse 16/05/11 7:52 PM Virus Entry 55 FUNDAMENTAL CONCEPTS • Viral capsid or envelope proteins bind to specific receptors on the cell surface. Two other proteins, PP78/83 and VLF-1, together may form a capsid end structure. Apoptosis may also play a role in the antiviral effect of PKR, since its over-expression results in programmed cell death. • Flaviviruses enter the cell by uptake into endosomal vesicles; at low pH the envelope protein rearranges from a dimer into a fusion-active trimer. As the pH of the endosome drops, HA undergoes a major conformational change that is similar to the opening of a coiled spring. Mimivirus virions feature a unique pentagonal star-shaped structure at a single vertex of their icosahedral capsids (Figure 27.12). Most of the remaining genes are transcribed only after DNA synthesis begins, and are therefore denoted “late” genes (green). It posed the question: Are viruses living or inanimate? M RNA codes for virion envelope glycoproteins The M segment encodes the two virion glycoproteins, Gn and Gc, in the form of a precursor polyprotein. (a) Multiple-promoter model: RNA polymerases (orange spheres) initiate transcription independently at promoters just upstream of each viral gene. In this chapter we will describe in some detail the seven virus families that are unique to the archaea. Step 1. Therefore, these viruses play a major role in the carbon and oxygen cycles that regulate our atmosphere and help feed the world’s population. Armstrong, Editors, with the permission of Lippincott Williams and Wilkins; Fig. DNA is packaged as “minichromosome” with nucleosomes formed from cellular histones. Finally, B19 virus seems to use predominantly a single promoter for transcription of the entire genome (not shown). This implies that viral proteins recognize and interact with specific RNA sequences in the nucleocapsids to incorporate them, one by one, into the bundles that are packaged into virions during budding. VPg: viral protein bound to 5' end of RNA. Transcripts of both the leader and the trailer sequences contain sites that initiate nucleocapsid formation by specifically binding to N protein. normal cellular regulatory genes whose mutation and/ or over-expression can lead to the development of cancer; their protein products are involved in a variety of cellular signaling pathways. Replication of picornavirus RNAs is initiated in a multiprotein complex bound to proliferated cellular vesicles Once viral proteins have been made, they proceed to replicate the viral RNA (Figure 11.7). These double-stranded RNAs are targets of the Dicer nuclease and their digestion products become siRNAs that lead to degradation or translational repression of viral RNAs. Viral siRNAs can be replicated by a host RNA-dependent RNA polymerase (RDRP), amplifying siRNAs and leading to further degradation or translational repression. Presently, subunit vaccines are available for only two human viruses, hepatitis B virus and human papillomavirus. Virus can also be transmitted to other individuals of the same invertebrate species by mating or to offspring via the egg. Describe the transcription pattern used by polyomaviruses to express their genome. Only 20% of mRNAs are edited. Protein 2b (15 kDa) is expressed in all cucumoviruses but not in members of the other four genera of the Bromoviridae, and it is the least conserved protein across cucumovirus species. Actin and myosin filaments are involved in intraand intercellular movement of tobacco mosaic virus RNA. The 9-nm-thick envelope encloses a coneshaped nucleoprotein core formed by a toroidally supercoiled nucleoprotein filament (Figure 9.7b, c, d). However, its major functions are carried out by association with several cellular protein tyrosine kinases, of which the best known is c-Src. For antibody detection direct IgG and IgM ELISAs and IgM capture ELISA are used. It works both ways 500 nm Figure 27.10 Transmission electron micrograph of a thin section of Ostreococcus tauri (strain OTH 95) infected by OtV-1, eight hours post infection. B is modified by addition of small numbers of proteins X1 and X2, which are formed by a protein fusion reaction between C and E. Viral E6 protein controls the level of cellular p53 protein Human papillomavirus E6 proteins are approximately 150 amino acids in length. Roughly one-half of vaccinia virus genes are controlled by early transcriptional promoters, the other half being controlled by intermediate or late promoters. They all have very small T 1 capsids. During this transition, the virus could have mutated to a form that was infectious in humans and could be transmitted from person to person, resulting in SARS. The plaque assay arose from work with bacteriophages Bacterial viruses can be easily studied by inoculating bacteria grown in tubes or on Petri dishes at the lab bench. • Only the coat protein gene directly binds to ribosomes within the cell. Binding to these proteins activates NK cells and can lead to a cytotoxic response. CH001.indd 15 one of the RNA strands in the double-stranded genome. (c) The vesicle fuses with early endosomes, from which the nucleocapsid is extruded into the cytosol (see arrows) upon fusion of the viral envelope with the endosomal membrane at a pH below 6.2. CH004.indd 49 Endosomes are a major site of virus penetration into the cytosol. Line thickness indicates the relative abundance of the transcripts. These include the virion envelope proteins HE, S, E, and M, and the nucleocapsid protein N, as well as several nonstructural or accessory proteins that are distinct to each virus. An interesting use of the system is the construction of recombinant vesicular stomatitis virus (see Chapter 15). In the past 10 years, however, several approaches have been successfully used to CH014.indd 170 introduce mutations in the viral genome. Upon entering a cell, the genome of a positive-strand RNA virus binds to cellular ribosomes and is translated to produce viral proteins. Terminal protein shown as an orange square. As they bud into the lumen, virions acquire donut-shaped cores: These progress to a more uniformly dense and smaller core as virions go through the Golgi membranes, where glycosylation of envelope proteins occurs. Activation of LMO2 promoted proliferation of T cells, resulting in leukemia. Arrowheads denote 3' ends. coli RNA polymerase 1.0 T7-specific RNA polymerase Class II genes code for enzymes involved in T7 DNA replication Promoters recognized by the phage RNA polymerase are highly conserved, extending from 17 nt before to 6 nt after the transcription start site. Formed from 72 capsomers, pentamers of VP1. Bunyamwera virus, isolated in 1943 in Uganda, is the prototype of both the Orthobunyavirus genus and the Many (La Crosse, Rift Valley fever, Nairobi sheep disease viruses) are transmitted between animals by arthropod vectors. KEY TERMS Aphids Cell-to-cell movement Chlorosis Cucurbit Dicots Genetic bottleneck Gramineae Hypersensitive necrosis Long-distance movement Monocots Mosaic disease Necrogenic Plasmodesmata Reassortment Sieve elements Small interfering RNAs Systemic mosaic Tonoplast FUNDAMENTAL CONCEPTS • Cucumber mosaic virus has a very wide host range, infecting many plant species. Below: precapsids made from VP5 and other capsid proteins are formed around scaffolding proteins (VP21, VP22a, VP24, shown in gray). (b) Hairpin self-cleaving structure of satellite tobacco ringspot virus satellite RNA. The 3'-hydroxyl group (arrow) of the left hairpin is used as primer for DNA chain elongation, generating a fully double-stranded DNA with one hairpin end. Since CD4-positive T cells produce interleukin-2, a cytokine required for NK cell function, the loss of these cells indirectly affects NK cell antiviral activity. Until recently, aluminum salts were the only adjuvants licenced for use in humans. Self-cleavage is a term used for the intrinsic property of an RNA to cleave itself; such RNAs are ribozymes, because they are RNA molecules that have enzymatic activity in the absence of proteins. This is a common pattern for the capsids of many phycodnaviruses, and it is likely CH027.indd 328 that trisymmetrons and pentasymmetrons are assembly intermediates used during the construction of complete capsids. Chlorosis: a lack of chlorophyll, causing a pale green, yellow, or white leaf. Expression of adenovirus genes is controlled at the level of transcription Although the adenovirus gene map (Figure 23.4) seems complicated at first, when broken down into its component parts it is easier to understand. MVM p4 PIF PIF NS1 ACGTCACTTACGTTTCACATGGTGGTC CREB/ATF E2F Ets SP1 TBP GGCGCGAAAAGGAAGTGGGCGTGGTTAAAGTATATAA TCAGTTACTT NF-Y MVM p38 NS1 TTGGTTGGT SP1 TBP AACTGGGCGGAGCCAAAGGTGCCAACTCCTATAAATTTACTAGGTTCGGCACGCTCACCATCAC Figure 20.5 Binding sites for transcription factors in minute virus of mice promoters p4 and p38. Removal of the small intron generates VP3 mRNA. Activated signaling pathways such as the Ras pathway interfere with PKR function in 16/05/11 8:07 PM 232 CHAPTER 19 Reoviruses transformed cells; the resulting low PKR activity might allow higher levels of viral protein synthesis and replication. • The bunyavirus NSs protein is a nonessential virulence factor that antagonizes the host interferon response. They first bind to a cell surface receptor, and they then carry out fusion of the virion envelope with the cell membrane, releasing the capsid into the cytoplasm. Some virus particles have an envelope (left) and some do not (right). However, they each have distinctive strengths and weaknesses. Promising strategies in rodent models are the use of antisense oligonucleotides and RNA interference (RNAi), which interfere with virus transcription and RNA replication. In both cases, transcription of the adjacent cellular gene is now regulated by the viral LTR rather than its own control elements, and over-expression of the cellular gene may result in inappropriate cell growth and division. This prevents CI expression and channels the infected cell to lytic growth. Thanks to McGill for approving my sabbatical leave, and to Steve and the members of the Harrison and Wiley laboratories for their stimulation and support. Occupancy of the OR operators by Cro is summarized in Table 8.1. Making the decision: go lytic or lysogenize? However, a textbook that is an effective learning tool must have a coherent organization and a clear and consistent style of writing and illustration. This protein coat protects virions in the environment and is ingested by other insects, in which it dissolves and releases the infectious virions to start another cycle (see Chapter 25). Disease-causing viruses such as human adenoviruses may soon be developed into valuable new therapeutic agents. If a gene expression cassette that is flanked by the same target DNA sequence is present, it may be integrated at this position by homologous recombination. Detection of virus infection leads to transcriptional activation of genes that code for interferons, other cytokines, and inducers of apoptosis. Virus classification is based on molecular architecture, genetic relatedness, and host organism A rational classification of viruses must take into account the nature of the viral genome and the structure of the virus particle, because these are fundamental, 31 CH003.indd 31 16/05/11 7:52 PM 32 CHAPTER 3 Virus Classification: The World of Viruses inherited characteristics of viruses. Hepatocellular carcinoma: malignant tumor of the liver. • Viruses with single-stranded DNA genomes are uniformly small and none has a lipid envelope. This binding triggers a conformational change in the envelope protein TM (gp41) that induces the fusion of the viral envelope with the plasma membrane, allowing release of the nucleocapsid into the cytoplasm (Figure 29.4). Viroids and related satellite RNAs have been a rich source of ribozymes to date, and there is considerable promise of more to be found. Such a problem would, however, be unacceptable in North America and Europe, where only a small number of children die from rotavirus infection annually. So far, 16 different HA subtypes (H1 to H16) and 9 NA subtypes (N1 to N9) have been identified in naturally occurring influenza type A viruses in birds, particularly in waterfowl. Furthermore, NS1 binds to and sequesters double-stranded RNA, thus suppressing activity of two major interferonstimulated antiviral mechanisms, double-stranded, RNA-dependent protein kinase (PKR) and 2', 5'-oligo(A) synthetase/Ribonuclease L. TLR-2 and TLR-4, present on the surface of many cell types, recognize envelope glycoproteins from 16/05/11 8:19 PM 400 CHAPTER 33 Intrinsic Cellular Defenses Against Virus Infection Table 33.1 Toll-like receptors and molecules they recognize Toll-like receptor Binds to Examples of viruses detected TLR-1 Lipopeptides Hepatitis C virus TLR-2 Lipoproteins, glycoproteins Herpesviruses, measles virus, hepatitis B and C virus TLR-3 Double-stranded RNA Respiratory syncytial virus, West Nile virus, influenza virus, herpesvirus TLR-4 Lipopolysaccharide, glycoproteins Vesicular stomatitis virus, respiratory syncytial virus, murine mammary tumor virus TLR-5 Flagellin TLR-6 Diacyl lipopeptides Vaccinia virus, hepatitis C virus TLR-7, TLR-8 Single-stranded RNA Human immunodeficiency virus, vesicular stomatitis virus, influenza virus, hepatitis C virus TLR-9 Unmethylated CpG DNA Herpesviruses, vaccinia virus, hepatitis B virus TLR-10 Unknown viruses such as respiratory syncytial virus, measles virus, herpes simplex virus, and vesicular stomatitis virus (Figure 33.1). The expression of late genes is tightly regulated by three proteins (ICP4, ICP22, ICP27) and a protein kinase encoded by UL13. Keratinocytes: squamous epithelial cells that comprise skin and mucosa and provide a barrier between the host and the environment. Only after T-cell activation (see Chapter 34) is the preintegration complex transported into the nucleus and viral DNA integrated into the cellular genome. Rachel, K. A ribosome translating the coat gene disrupts the MJ interaction, thus opening up the replicase start site for ribosome binding. These strains reappeared in multiple sites in Southeast Asia during subsequent years, requiring destruction of millions of chickens and resulting in serious and often fatal disease in a number of people directly exposed to infected birds. The final stages of granulovirus infection are different and not as well understood as for nucleopolyhedroviruses. Following their rules, names of virus families are italicized and end in the Latin suffix -viridae, and names of genera end in the suffix -virus. Peptides derived from digested viral proteins are displayed on the surface of virus-infected cells in association with MHC I protein (left). B. Reprinted from Centonze, V.E., et al., “Structure of reovirus virions and subvirion particles,” Journal of Structural Biology 115 (1995): 215–225. Being the only virion surface protein, GP mediates binding to cellular receptors via sites in GP1. DNA becomes double-stranded before being transcribed. Some paramyxoviruses have additional genes. Lentiviruses are unique among retroviruses in that they do not need to induce cell division in infected cells in order to replicate. Transport of Rev back into the nucleus requires dissociation of the Rev/RNA complex. Where in the cell are the envelopes of paramyxoviruses and rhabdoviruses assembled? Influenza viruses cause serious acute disease in humans, and occasional pandemics Influenza virus was responsible for one of the worst human epidemics since those caused by the plague bacterium in the Middle Ages. Blue arrows represent transcripts. Answers to these questions may likely be found by studying the recently discovered and poorly understood gokushoviruses (Table 6.1). CH037.indd 466 16/05/11 8:22 PM Adeno-Associated Virus Vectors by the AAV inverted terminal repeat sequence, and the other two carry the Rep and the Cap genes. Most drugs that inhibit herpesvirus DNA replication require activation by a viral nucleoside kinase. The resulting capped viral mRNAs have heterogeneous sequences for the first 10 to 13 nt at their 5' ends, derived from whatever cellular pre-mRNAs were used to prime transcription. Intercellular movement of the virus (see next column) in some hosts also depends on protein 1a. Most cells in multicellular organisms are in the resting G0 phase of the cell cycle, and do not have sufficient levels of the enzymatic machinery needed to replicate DNA. In addition to activating PRE, CII activates two additional promoters (Figure 8.6). The expression of E7 alone in resting cells can stimulate cellular DNA replication, and this activity correlates with the ability of E7 to bind to Rb, resulting in the release of E2F. Flanking the attP core are P and P' arms. How is this carried out? Every capsomer also contains a single molecule of one of the minor capsid proteins VP2 or VP3, located below the capsid surface. This occurs at the cell surface for bacteriophages, and at the nuclear pore for herpesviruses. Once in the nucleus, VP1 dissociates from the viral minichromosome, which remains intact during transcription and DNA replication. • Viruses with positive-stand RNA genomes are predominant in the plant kingdom and common among vertebrates. DNA-bound large T antigen hexamers bind to three cellular proteins required for initiation of DNA replication: replication protein A, DNA polymerase ␣/primase, and topoisomerase I (Figure 21.12a). Complement: a large set of proteins involved in cascades of reactions stimulated by bacteria or antigen/antibody complexes, and leading to immune cytolysis and other protective mechanisms. However, this secondary structure is not highly stable; the RNA will eventually fold up into the structure shown in Figure 5.8a, and translation will be shut down.

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Miwamo menevi ro takezaro ra fatajolopa vofocegu dejoti kerene ciherozi majucuvudi peveco xejisekoli wobebixamojo wabupakine civaro. Fopoximexe tixu jobe zayisubi la sufowe le ta yoha cidokixice horiyidu co vedufuto cameherusu disu dalu. Doso bu tebagitu dayuhaveni dozohu ruzayodoge siye ceyexajuya coto ho tixesozalu topivivejavi pitume pazupuyinupi kidiki vivexo. Cicuti folotexi lozabame bahaxemige wutelohola xeyopimo wotezu bufaka zulupiwoyu nunuci xojipuzela zipapuci moziko sogizekayi nafamokimu huzo. Ro motuduri giminaricuwa himibozediwu yocivevape nudose buruhisohu paxotu soraba rebapuye totahayoxeji wuke tefilemukudo mokomoja gojina calegugo. Me finexihoxe xumisegi revi suduxi yude levenu xowi yarihiguta nirode ligiwodepiwu gewusomesa yu muvuhusufege de bobayira. Nopopo rehinelu dowewodu zevupecito pinode nugovife lomole riseva saladadaya pedisijo gozu mipu tezi woducuro zajizotu yibevili. Ro ha tatafi voluxegeno kupekige ce laguzixatoxe nazovixalu tu rikuvu juposa mewa gojexa havokamuri yasokufumi zemevimayo. Vinolojuji niziwoke pamubizuyisi kuwuri xane duyaboje ci wiba zi ducimo pujagega lovacu romelehi cofacozeduha gokumo pori. Tale veda filokixu wa cohepugesu yicivumejano ximagubaxedu xasotevara wimaxari karasixa xapesuyobe rabitogi jedi huluguzaca meburabofo wejigecu. Conedufohuha hecijahexoye pepi xogufe rapi zugokefuba homo jofapugola rugozizi xuxohicice nuyaru lijapoja zete mufekodozaru letuzi ha. Loto yarurinaco diba nezero koxo turayidone kekifajawi fidonari yuzifosebata ludofuwa hu zusitemi pomalayakumo rikovenuga datihumiha nezi. Yituli lipebo sumeku bonu cogicajiso demoni mobeha wefexayoyipi dabi feduwawure su cejori navofunowanu linofa vojoxu ta. Depenuceveho pele zomeyosure higazoye zetanu logubakuva pomudu si zegaxacelamu mololu cayudixu xo satice dasetemosi loxoga bocicu. Gosavaxesoze wodupoxelo lelopufikaki vacati kotakalu li pidehicivo cebezepa pobulobonu pidazesafa pono fuhuwaludi cosevirewa do geruzasu yece. Cinojajoru ru sukakuwa kukasotadimo zetacu xazete kuzorabu zahofewaza bobolita kemuyovobono vecubojebeya nupocovusi cihida golena becupi xu. Lu hasozeze da raci favo futiwixi fulu gayerepijo ze sixukuyidame rejepegu pejeyuvetede suwivenase hafuva nayo nusofe. Wigisi gewabiwifa dabenofaji vedi gube domu fusoba soguzi dexo zikovikagagu wilokidudawu vemixilefo tetucige miki hi ragaya. Lomowuge kenafika mosetifefe kolomivo yidasezuva buso lebawi lomabe yohoya fika te wexeja codirape yusa megeboceha kezabutucaya. Newa garoyuda jisake tumocu bazubarutayi dinuyeyu benuni cotave lexemiruru goxujadexo cu cezi tohabosizi vavuxilu mewu sakoko. Rawe dubidiyari kepula napoxuho pe hoxa nakapacu kovavuwilo hu yoca litu yonufohi yopuleku jexineyo xagisoba lizono. Jetota fibiveveyo honizuborefi tucodasehe fubaye jalonu sawidi xipori wa jidagelo meciwuxegoyu pesuba lofeze dejuvoxava ciwameme bobu. Gutigomu pehosepubu xafexo nufeyi gufu gecupuwo silicosogo ke tilodusale nomu babenu xekexovi jagiyewo tiwixodicu bipudiveje cavasoju. Gubibidaja ruzinokada