Fig. 1.1 A phylogenetic tree of selected species from the three superkingdoms of life, Eukaryotes, Eubacteria, and Archaea. The tree is based upon statistical analysis of sequence variation in seven universally conserved protein sequences: arginyl-t-RNA synthetase, methionyl-t-RNA synthetase, tyrosyl-t-RNA synthetase, RNA pol II largest subunit, RNA pol II second largest subunit, PCNA, and 5′–3′ exonuclease.(Figure based upon Raoult et al. The 1.2-megabase genome sequence of mimivirus. Science 2004;306:1344–1350.)
Fig. 1.2 A phylogenetic tree of selected eukaryotic and archaeal species along with specific large DNA-containing viruses based upon sequence divergence in conserved regions of DNA polymerase genes. (Figure based upon Villarreal and DeFilippis. A hypothesis for DNA viruses as the origin of eukaryotic replication proteins. Journal of Virology 2000;74:7079–7084.)

Fig. 2.1 Dimensions and features of "typical" animal (a), bacteria (b), and plant (c) cells. The dimensions of plant and animal cells can vary widely, but an average diameter of around 50 μm (5×10^{-5} m) is a fair estimate. Bacterial cells also show great variation in size and shape, but the one shown here is *Escherichia coli*, the true "workhorse" of molecular biologists. Its length is approximately 5 μm. Based on these dimensions and shapes of the cells shown, the bacterial cell is on the order of 1/500th of the volume of the eukaryotic cell shown. Virus particles also vary greatly in size and shape, but generally range from 25 to 200 nm ($0.25–2.00\times10^{-7}$ m).
Fig. 2.2 The virus replication cycle. Most generally, virus replication can be broken into the stages shown: (a) initial recognition between virus and cell and introduction of viral genetic material into the host cell; (b) virus gene expression and induction of virus-induced modification of host allowing; (c) virus genome replication. Following this, (d) virus-associated proteins are expressed, and (e) new virus is assembled and released, often resulting in cell death.
Fig. 2.3 The pathogenesis of virus infection. Typically, infection is followed by an incubation period of variable length in which virus multiplies at the site of initial infection. Local and innate immunity including the interferon response counter infection from the earliest stages, and if these lead to clearing, disease never develops. During the incubation period, virus spreads to the target of infection (which may be the same site). The adaptive immune response becomes significant only after virus reaches high enough levels to efficiently interact with cells of the immune system; this usually requires virus attaining high levels or titers in the circulatory system. Virus replication in the target leads to symptoms of the disease in question, and is often important in spread of the virus to others. Immunity reaches a maximum level only late in the infection process, and remains high for a long period after resolution of the disease.
Fig. 2.4 Sites of virus entry in a human. These or similar sites apply to other vertebrates. (Adapted from Mims CA, White DO. Viral pathogenesis and immunity. Boston: Blackwell Science, 1984.)

Fig. 3.1 Some transmission routes of specific viruses from their source (reservoir) to humans. The mode of transmission (vector) is also shown. (Based on Mims CA, White DO. Viral pathogenesis and immunology. Boston: Blackwell Science, 1984.)
Fig. 3.2 Occurrence of respiratory illness in an arctic community (Spitzbergen Island, Norway) that is isolated during the winter months. Following the last boat communication with the European mainland, the number of respiratory illnesses declines from a low number to almost nil. With the first boat arriving in the Spring, new serotypes of respiratory viruses are communicated from the crew and passengers and a "mini-epidemic occurs." As the virus passes through the population, resistance builds and infections decline to a low level. (Based on data originally published by Paul JH, Freese HL. An epidemiological and bacteriological study of the "common cold" in an isolated Arctic community (Spitsbergen [sic]). American Journal of Hygiene 1933;7:517.)
Fig. 3.3 Fictionalized time-line of the spread of SARS virus following its introduction into Toronto, Canada from Hong Kong in early 2003. The data for this figure are based on material presented on the CDC website (http://www.cdc.gov/ncidod/sars/) and in the February 2004 issue of the *Journal of Emerging Infectious Diseases*, which was dedicated to studies on the SARS outbreak of late 2002–early 2003.
Fig. 3.4 The course of experimental poxvirus infection in laboratory mice. Virus is inoculated at day 0 in the footpad of each member of a large group of genetically equivalent mice. Mice are observed daily, and antibody titers in their serum are measured. Selected individuals are then killed, and various organ systems assayed for appearance and presence of virus. Note that symptoms of the disease (rash and swollen foot) only become noticeable after a week.
Fig. 3.5 Visualization of rabies virus-infected neurons in experimentally infected animals. (a) A schematic representation of the pathogenesis of rabies in an experimentally infected laboratory animal. (b) Immunofluorescent detection of rabies virus proteins in neurons of infected animals. As described in Chapters 7 and 12, the ability of an antibody molecule to specifically combine with an antigenic protein can be visualized in the cell using the technique of immunofluorescence. The cell and the antibody bound to it are then visualized in the microscope under ultraviolet light, which causes the dye to fluoresce (a yellow-green color). The top left panel shows replication of rabies virus in a sensory nerve body in a dorsal root ganglion along the spine of an animal infected in the footpad. The bottom left panel shows the virus replicating in a neuron of the cerebellum, while the top right panel shows infected neurons in the cerebral medulla. Infection of the brain leads to the behavior changes so characteristic of rabies infections. Finally, the sensory nerve endings in the soft palate of a hamster infected with rabies virus at a peripheral site contains virus, as shown by the fluorescence in the bottom right panel. This virus can move to the saliva where it can be spread to another animal. The arrows point to selected cells showing the variation in signal intensity that is typical of infections in tissues.
Fig. 3.6 Analysis of the establishment and maintenance of latent HSV infections in mice. A number of mice are inoculated in the footpad, and following the symptoms of primary disease, which includes foot swelling and minor hindquarter paralysis, many mice recover. Those that do not recover have infectious virus in their CNS. The mice that recover are latently infected and no infectious virus can be detected, even with high-sensitivity measurements of nervous and other tissue. HSV genomes, but not infectious virus, can be detected in nuclei of sensory nerve dorsal root ganglia. When these ganglia are cultured with other cells that serve both as an indicator of virus replication and as a feeder layer for the neurons (i.e., explanted), a significant number demonstrate evidence of virus infection and infectious virus can be recovered, as shown on the inset graph (two separate experiments are shown, with essentially the same results).

Fig. 4.1 Virus maintenance in small and large populations. (a) In a small population virus infection can only occur when there is an immunologically naïve individual available. This requires a virus within such a population to be able to maintain itself in an infectious state in individuals long after they have been infected. A favored mode of infection would be from parent to child. Clearly, high mortality rates or severe disease symptoms would be selected against. (b) In a large population, there will be a large number of susceptible individuals appearing at the same time. This can result in local episodic infections of such individuals. The large size of the host population insures that some virus is available from actively infected adults at all times. While persistence is not excluded, it need not be strongly selected for, especially if the course of the acute phase of the disease is relatively long compared to the generation time of the population.
Fig. 4.2 Examples of virus infection of specific organs or organ systems. (a) DNA genome viruses. (b) RNA genome viruses. Blue labels indicate acute infections, while pink labels indicate infections that result in either chronic disease states or carcinomas. Red labels indicate acute infections that can result in severe disease.

Fig. 5.1 A scale of dimensions for biologists. The wavelength of a photon or other subatomic particle is a measure of its energy and its resolving power. An

object with dimensions smaller than the wavelength of a photon cannot interact with it, and thus, is invisible to it. The dimensions of some important biological features of the natural world are shown. Note that the wavelength of ultraviolet (UV) light is between 400 and 280 nm; objects smaller than that, such as viruses and macromolecules, cannot be seen in visible or UV light. The electron microscope can accelerate electrons to high energies; the resulting short wavelengths can resolve viruses and biological molecules. Note that the length of DNA is a measure of its information content, but since DNA is essentially "one-dimensional," it cannot be resolved by light.

Fig. 5.2 The structure and relative sizes of a number of (a) DNA and (b) RNA viruses. The largest viruses shown have dimensions approaching 300–400 nm and can be just resolved as refractile points in a high-quality ultraviolet-light microscope. The smallest dimensions of viruses shown here are on the order of 25 nm. Classifications of viruses based on the type of nucleic acid serving as the genome and the shape of the capsid are described in the text. ss = single stranded; ds = double stranded.

Fig. 5.3 Crystallographic structure of a simple icosahedral virus. (a) The structure of *Desmodium* yellow mottle virus as determined by x-ray crystallography to 2.7-Å resolution. This virus is a member of the tymovirus group and consists of a single positive-strand RNA genome about 6300 nucleotides long. The virion is 25–30 nm in diameter and is made up of 180 copies of a single capsid protein that self-associates in two basic ways: in groups of five to form the 12 pentons and in groups of six to form the 20 hexamers. Two views are shown, panels at left are looking down at a five-fold axis of symmetry and the right-hand panels look at the three-fold and two-fold axes. Note that the individual capsomers arrange themselves in groups of five at vertices of the icosahedra, and in groups of six on the icosahedral faces. Since there are 12 vertices and 20 faces, this yields the 180 capsomers that make up the structure. The axes are outlined in the lower panels. (Courtesy of S Larson and A McPherson, University of California, Irvine.) (b) Schematic diagram of the vertices and faces of a regular icosahedron showing the axes of symmetry. Arrangements of the capsomers described in (a) are also shown.

Fig. 5.4 The structure of a simple icosahedral virus. (a) A space-filling model of the capsid of *Desmodium* yellow mottle virus as determined by x-ray crystallography to 2.7-Å resolution. The assembly of the single capsid protein into 12 pentons and 20 hexons to form the capsid. (b) The structure of the RNA genome inside the capsid as determined by x-ray crystallography. (Courtesy of S. Larson and A. McPherson, University of California, Irvine.)

Fig. 5.5 The virosphere. Classification of a major portion of the currently known genera of viruses (*–viridae*) using criteria defined by the International Committee on the Taxonomy of Viruses. Major groupings are based on the nature of the viral genome and the nature of the host.

Fig. 6.1 (a) The surface of a "typical" animal cell. The lipid bilayer plasma membrane is penetrated by cell surface proteins of various functions. The proteins that extend from the surface (mainly glycoproteins) can be utilized by different viruses as "tether points" or "anchors" for bringing the virus close enough to the cell surface to initiate the entry process. This interaction between a cell surface protein serving as a virus receptor and the virus itself is highly specific between proteins. Integral membrane proteins, such as those mediating transport of small molecules and ions across the plasma membrane, tend not to project as far into the extracellular matrix and can be utilized by retroviruses, especially, as receptors. Some viral receptors are listed in Table 6.1. (b) The interaction between a cellular surface protein (receptor) and a ligand or co-receptor can lead to chemical and structural changes that transmit signals between the exterior and interior of the cell. This is the process of signal transduction. Here, for example, the binding of ligand with two monomeric receptor proteins leads to dimerization, which, in turn, activates a protein kinase in the cytoplasm. This results in phosphorylation of a target protein, producing further changes in the cell.

Fig. 6.2 Schematic of receptor-mediated endocytosis utilized by rhinovirus for entry into the host cell. The endocytotic vesicle forms as a consequence of close association between the rhinovirus–receptor complex and the plasma membrane.

Fig. 6.3 (a) The two basic modes of entry of an enveloped animal virus into the host cell. Membrane-associated viral glycoproteins either can interact with cellular receptors to initiate a fusion between the viral membrane and the cell plasma membrane, or can induce endocytosis. The fate of the input virus membrane differs in the two processes. (b) High-resolution schematic of the process of membrane fusion. The interaction between viral and cellular membrane-associated proteins results in the "clearance" of an area of the two lipid bilayers so that they can become closely juxtaposed leading to fusion. (c) The fusion of pseudorabies virus with the plasma membrane of an infected cultured cell is shown in this series of electron micrographs (scale bars = 150 nm). Although each electron micrograph represents a single event "frozen in time," a logical progression from the initial association between viral envelope glycoproteins and the cellular receptor on the plasma membrane through the fusion event is shown. The final micrograph contains colloidal gold particles bound to antibodies against the viral envelope glycoproteins (dense dots). With them, the envelope can be seen clearly to remain at the surface of the infected cell. (Micrographs reprinted with the kind permission of the American Society for Microbiology from Granzow H, Weiland F, Jöns A, Klupp B, Karger A, Mettenleiter T. Ultrastructural analysis of the replication cycle of pseudorabies virus in cell culture: a reassessment. Journal of Virology 1997;71:2072–2082.) (d) The association of the viral capsid with the intracellular transport machinery following membrane fusion. This process leads to the virion and associated viral genome being transported to its appropriate location inside the cell to initiate the next step of the infection process – the expression of viral genes.

Fig. 6.3 *Continued*

Fig. 6.4 Entry of T4 bacteriophage DNA into an *E. coli* cell. Initial attachment is between the fibers to the ompC lipopolysaccharide receptor on the bacterial cell wall (a). The binding of protein pins on the base plate to the cell wall leads to contraction of the tail fibers and sheath proteins, leading to insertion of the tail tube through the cell wall. As shown in the electron micrograph (b), phage pilot protein allows the highly charged viral DNA genome to penetrate the bacterial plasma membrane and enter the cell. Phage DNA can be seen as shadowy lines emanating from the tail tube. (From Dimmock NJ, Primrose SB. Introduction to modern virology, 4th ed. Boston: Blackwell Science, 1994.)

Fig. 6.5 Expression of a varicella-zoster virus protein following transfection of a cell with the viral gene under the control of a promoter that is active in the uninfected cell. (a) The basic process. The cell membrane is treated with agents that allow it to readily take up large aggregates of protein and nucleic acids by phagocytosis. The transfecting DNA is caused to form aggregates with the use of calcium phosphate ($Ca_3(PO_4)_2$) and then mixed with cells that have been appropriately treated. While most of the DNA taken up by the cell is degraded, some gets to the nucleus by nonspecific cellular transport of macromolecules, and this DNA can be transcribed and any genes within it expressed as proteins. (b) An actual experiment. Cells were made permeable and then transfected with DNA containing the varicella-zoster virus glycoprotein L gene. The protein encoded in this gene was expressed following its transcription into mRNA (see Chapter 13). Cells were treated with fluorescent antibody reactive with the glycoprotein at (clockwise from the top left) 0, 12, and 24 hours after infection. The expression of the glycoprotein in the cytoplasm is clearly evident from the green fluorescence. (See Chapter 12 for a description of the method.) (Photographs courtesy of C. Grose, University of Iowa.)

Fig. 6.6 Assembly of the helical tobacco mosaic virus. Steps in the preassembly of the capsomer disk, insertion of viral RNA, and the translational "screwlike" helix assembly process with sequential addition of more capsomers are shown. (Adapted from Dimmock NJ, Primrose SB. Introduction to modern virology, 4th ed. Boston: Blackwell Science, 1994.)

Fig. 6.7 Assembly of the phage P22 capsid and maturation by insertion of viral genomic DNA. Individual capsomer subunits preassemble into a procapsid around scaffolding protein. This latter protein is recycled with phage P22 but can be proteolytically removed with a maturational protease with other icosahedral viruses. The empty head then associates with viral genomes. Genome insertion requires both energy and a conformational change in the procapsid.

Fig. 6.8 Insertion of glycoproteins into the cell's membrane structures and formation of the viral envelope. The formation of viral glycoproteins on the rough

endoplasmic reticulum parallels that of cellular glycoproteins except that viral mRNA is translated (a). Full glycosylation takes place in the Golgi bodies, and viral glycoproteins are incorporated into transport vesicles for movement to the cell membrane where they are inserted (b). At the same time (c), viral capsids assemble and then associate with virus-modified membranes. This can involve the interaction with virus-encoded matrix proteins that serve as "adapters." Budding takes place (d,e) as a function of the interaction between viral capsid and matrix proteins and the modified cellular envelope containing viral glycoproteins.

Fig. 6.9 Visualization of the budding of an enveloped virion from the plasma membrane of an infected cell. (a) Viral glycoproteins processed in the endoplasmic reticulum and Golgi apparatus are transferred to the plasma membrane forming a virus-modified region of envelope. Depending on the virus, the C-terminal cytoplasmic portions of the viral glycoproteins may associate with other viral proteins of the matrix. The modified region of the plasma membrane can specifically associate with mature virions assembled inside the infected cell. This association leads to budding and release of mature enveloped virions. (b) The appearance of enveloped Murine Leukemia Virus (a retrovirus) at the surface of an infected cell as visualized by atomic force microscopy is also shown. Here the background plasma membrane of the cell has a slightly different appearance due to differences in the membrane-associated proteins present and the budding of the virus at the surface forming enveloped virions is apparent. (Courtesy of Yuri G Kuznetsov and Alex McPherson, University of California, Irvine.)

Fig. 6.10 The envelopment and egress of a herpesvirus. Electron micrographs of exocytosis of pseudorabies virus in the cytoplasm of the infected cell; release of enveloped virions is clearly shown. The bars represent 150 nm. (Micrographs reprinted with the kind permission of the American Society for Microbiology from Granzow H, Weiland F, Jöns A, Klupp B, Karger A, Mettenleiter T. Ultrastructural analysis of the replication cycle of pseudorabies virus in cell culture: a reassessment. Journal of Virology 1997;71:2072–2082.)

Fig. 7.1 Schematic representation showing differences in the intensity and time of appearance of local versus systemic immunity against a typical virus infection in mice. IFN = interferon. (Figure courtesy of DC Bloom.)

Fig. 7.2 The human lymphatic system. The lymphatic system is the principal organ of the immune system. (a) The relationship between the lymphatic circulation and that of the blood. (b) Some of the important components of the lymphatic system as related to the immune response.

Fig. 7.2 *Continued*

Fig. 7.3 T and B cells in immunity. T lymphocytes play the central coordinating role in evoking the immune response. Upon activation by interaction with a specific antigenic determinant with which they can interact, they proliferate and carry out the functions shown. B cells reactive with specific antigens require reactive T cells for their maturation. Upon maturation, they secrete antibody proteins that bind to antigenic determinants.

Fig. 7.4 The antigenic structure of a protein. Specific groups of amino acids (usually hydrated) serve as specific antigenic determinants, or epitopes in an antigenic protein. Some of these are insensitive to the protein's physical structure; others require a specific conformation for presentation.

Fig. 7.5 The processing of a foreign antigen and stimulation of the immune response. As described in the text, an antigenic protein can only stimulate the immune response when it is processed by a macrophage and then presented to cells of the immune system in lymph nodes in the presence of histocompatibility antigens. The processing is relatively rapid and involves partial degradation of the antigenic protein and expression of antigenic portions on the surface of the antigen-presenting cell. (a) MHC-I antigen processing and presentation. (b) MHC-II processing and presentation.

Fig. 7.6 The clonal selection of B lymphocytes. Only the B lymphocytes reactive with a specific epitope can be stimulated to mature by the action of a helper T lymphocyte. Specific mature B cells secrete specific types of antibody molecules, but the same epitope will result in only the stimulation and maturation of B-cell clones reactive with it.

Fig. 7.7 Immune memory. The first exposure to an antigen results in the primary response, which occurs after a week or so. During this time, maturation of immune-reactive cells is taking place. Once the primary response occurs, antibody and reactive T and B cells decline to a low level. Upon restimulation with the same antigen, the memory lymphocytes are rapidly mobilized and a more intense and more rapid immune response follows.

Fig. 7.8 The maturational cascade of serum complement proteins upon binding to an antigen–antibody complex on the surface of a cell. The Fc region, a portion of the antibody molecule that is not involved in binding to the epitope of the antigen, specifically triggers this cascade.

Fig. 7.9 An enzyme-linked immunosorbent assay (ELISA): the method of using a color reaction mediated by an enzyme bound to the Fc region of the antibody molecule. P = colored product; S = substrate.

Fig. 7.10 Antibody neutralization of virus infectivity. Specific types of antibody molecules, called *neutralizing antibodies*, can bind to surface proteins of the virus and block one or another aspect of the early events of virus-cell recognition or effective internalization of the virus.

Fig. 7.11 The hemagglutination inhibition assay for measuring antibody against a virus in serum. The assay is carried out by mixing constant amounts of a known hemagglutinating virus with serial dilutions of serum; then the virus–serum mixture is added to red blood cells. Low dilutions of serum result in sequestering the virus so that it is not available for hemagglutination, and red blood cells in the wells pellet to the bottom under low centrifugal fields. Higher dilutions of the antiserum dilute the antibody concentration to a point where enough virus remains to cause a positive hemagglutinin reaction. If there were more antibody in the serum, a higher dilution would be required to accomplish this. Thus, the hemagglutination inhibition titer of the serum is a measure of how far it can be diluted and still block the hemagglutinin reaction. This is a measure of antibody concentration. In the example shown, a 1:3200 dilution of the original sample (asterisks) was the last one in which agglutination was inhibited. This is the endpoint of the antiserum dilution. Since a 1:3200 dilution was the endpoint, there were 3200 hemagglutination inhibition units in the original stock. (Based on a figure in Dimmock NJ, Primrose SB. Introduction to modern virology, 4th ed. Boston: Blackwell Science, 1994.)

Fig. 8.1 The cascade of events leading to expression of interferon (IFN) and induction of the antiviral state in neighboring cells. The interferon inducer (dsRNA) produced during virus infection leads to an infected cell secreting small numbers of the IFN proteins, which are extremely stable glycoproteins. These interact with neighboring cells to induce the antiviral state in which a number of antiviral effector molecules (AVEMs) are expressed and can be triggered by the presence of dsRNA to alter the cell to markedly reduce the yield of infectious virus. dsRNA = double-stranded RNA; 2′,5′-OAS = 2′,5′-oligoA synthetase; PKR = dsRNA-dependent protein kinase; tscp = transcription.

Fig. 8.2 The structure of some currently effective antiviral drugs.

Fig. 9.1 A schematic comparison of light and electron microscopes. The principles behind the focusing of the image are similar except that magnetic fields must be used to focus electrons. The higher energy of the electrons accelerated through high voltage produces very short wavelengths with resulting high resolving power.

Fig. 9.2 Shadowing specimens for viewing in the electron microscope. (a) A sample of heavy metal is vaporized in a vacuum chamber. This vapor travels in straight lines from the source and forms a layer on all surfaces in its path. (b) Any object in the path will cast a shadow on the grid on which it is supported. (c) A double-shadowed virus in the electron microscope. (d) An icosahedral model is placed in two light beams to show the equivalence of the shadows. This

equivalence occurs because metal particles in vapor travel in straight lines, as does light. (c and d are drawn from photographs originally made by Robley C. Williams.)

Fig. 9.3 Computer-enhanced three-dimensional reconstruction of viral capsids using cryoelectron microscopy. The reconstructions are computed from electron micrographs of capsids preserved by freezing. For this type of electron microscopy, the samples are frozen and irradiated at liquid N_2 temperature with a very low flux of electrons to minimize damage. Information from many individual micrographs of particles is then combined in the computer to produce a reconstruction with a resolution higher than that of any single micrograph. (a) The HSV-1 capsid reconstructed from 60–80 images, which provides a resolution on the order of 2.5 nm, but many more can be combined. Views showing the three major axes of symmetry and a cross section are shown at the top. The bottom figure is a false color rendering of the information. One triangular face of the icosahedral capsid is shown in color. Pentons are orange, hexons red, and triplexes green. VP26, a small protein (molecular weight 12,000) associated with the hexons, is coded in blue. One VP26 molecule is bound to each VP5 molecule in each hexon. No VP26 is present in pentons. More detail concerning herpesvirus capsid structure can be found in Chapter17, Part IV. (Photographs courtesy of J. C. Brown and James Conway.) (b) A schematic rendering of the cyroelectron microscopic structure of the Mimivirus genome reconstructed to 7.5 nm resolution. (Based on work of Xiao et al. Journal of Molecular Biology 2005;353:493–496.)

Fig. 9.4 Atomic force microscopy was used to visualize the protein capsid of a herpes simplex virus on a glass substrate. The virus has lost most of its membrane which forms the large mass to the left. Barely perceptible protein molecules are embedded in the membrane, which is folded back upon itself in places. The capsid is characterized by an icosahedral distribution of protein units which enclose the double-stranded DNA genome.

Fig. 9.5 Assay of influenza virus by hemagglutination. The same number of red blood cells was added to each well, and duplicate samples of a virus stock were added to the wells at the indicated dilutions. Two-fold dilution steps are very convenient to handle and require only a small amount of virus sample. The wells in which there is enough virus present to agglutinate red blood cells have a gelatinous suspension of the colored cells. In wells with no virus, or an amount too low to agglutinate the cells, the red blood cells can be pelleted at the well's bottom with low-speed centrifugation. If more virus particles were in the original suspension, more dilution would be required to lower the concentration below the critical level for the hemagglutination measured. This would result in a *higher* HA titer, which is just the dilution factor required to dilute the agglutination. (Based on Figure 2.5 in Dimmock NJ, Primrose SB. Introduction to modern virology, 4th ed. Boston: Blackwell Science, 1994.)

Fig. 10.1 Generating a primary cell culture. Tissue is surgically removed from an anesthetized animal, and then minced and homogenized. Addition of collagenase breaks down extracellular collagen, but the enzyme does not attack intact cells. The cells are purified by filtration through a coarse mesh to remove large fragments, and then concentrated by deposition under a mild centrifugal field in a low-speed centrifuge. The pelleted cells are washed in various buffered media containing serum, and then can be subjected to differential low-speed centrifugation to partially separate cell types based on sedimentation rates (a function of cell size and density). Various fractions are plated onto culture dishes in the presence of a culture medium containing essential amino acids, vitamins, antibiotics, and serum. Cells grow as loose clumps that can be dispersed with mild trypsin treatment, and individual cell types then can be cultured.

Fig. 10.2 The progression of cells in culture from primary to transformed to continuous lines, and their relationship to tissues in the originating animal.

Fig. 10.3 Apoptosis vs necrosis in cell death

Fig. 10.4 HSV-induced changes in the properties of actin microfilaments of a cultured monkey fibroblast. The cell was stained with a fluorescent dye that reacts with actin fibers so that they can be visualized in ultraviolet light. This technique is similar to immunofluorescence microscopy, which is discussed in Chapter 12. The left panel shows parallel arrangement of the microfibrils in the uninfected cell, while HSV infection (right panel) results in disassociation of the fibrils and diffusion of the actin throughout the cytoplasm. At the same time, the cell loses its spindle-shaped morphology and becomes rounded. The arrows indicate junctions between cells that are also rich in actin fibrils and are not disrupted by HSV infection at this time. (Courtesy of Stephen Rice.)

Fig. 10.5 Visualization of virus plaques. Under appropriate conditions, virus infection can be localized to the vicinity of the originally infected cells. If a limited number of infectious units of virus (PFUs) are incubated in a culture dish or on tissue in which virus can cause a cytopathic effect, virus plaques can be visualized. (a) A continuous line of monkey cells (Vero cells) was grown in the six-well culture dish. When the cells reached confluence, they were infected in duplicate with a series of 10-fold dilutions of a HSV-1 stock. After 48 hours, the cells were partially dehydrated (fixed) with ethanol and stained. Areas of cell death show as white plaques, each representing a single infectious event with the input virus solution. (b) A portion of the surface of a Petri dish containing agar with bacterial nutrient medium. A "lawn" of *E. coli* was grown on the plate's surface, and this layer of cells was infected with a solution containing a genetically "engineered" version of bacteriophage λ that can be used to clone inserted genes. (See Chapter 22, Part V for some general details.) Bacteriophages that contain an inserted gene form clear plaques due to inactivation of an indicator gene (β-galactosidase) and viruses without the insert form dark-colored plaques. (c) Assay of tobacco mosaic virus (TMV). Leaves of a resistant (left) and a susceptible (right) plant that have been infected with small amounts of virus. (d) A higher magnification of plaque development. (Photographs in (c) courtesy of J. Langland.)

Fig. 10.6 Some representative morphologies of rat fibroblast cells (F-111) infected with different transforming viruses. (a) Shows normal cells with their characteristic parallel orientation. A focus of transformed cells generated by infection with Rous sarcoma virus (an oncornavirus) is shown in (b). Note the rounded morphology and density of these cells. (c) Shows the subtle difference in morphology when normal F-111 cells are infected with SV40 virus, for which they are nonpermissive. (Based on portions of a photograph in Benjamin J, Vogt PK. Cell transformation in viruses. In: Fields BN, Knipe DM, eds. Fundamental virology, 2nd ed. New York: Raven Press, 1991: Chapter 13.)

Fig. 10.7 Serial 10-fold dilutions of HSV to determine the titer of virus in a stock solution. The details of the infection are as described in the legend to Fig. 10.5(a), and the calculation of the titer is shown in Table 10.1.

Fig. 10.8 Quantal (endpoint dilution) assay of HSV in tissue culture wells. Replicate cultures of rabbit skin fibroblasts were grown to a density of about 5×10^4 cells per well of a 24-well tissue culture plate. Aliquots of the indicated stock virus dilutions were pipetted into the cultures and the plate was incubated for 48 hours and then developed with a stain that indicates black for virus-infected cells. Any well that received at least 1 PFU of virus stained black (two separate experiments are shown). The percentage of positive (infected) wells is shown at each dilution.

Fig. 11.1 Equilibrium density gradient centrifugation of virus-infected cell components to isolate virus particles. A preformed sucrose density gradient is layered with a solution of infected cell material and subjected to centrifugation at high *g* force at 4°C for several days. Virus particles sediment downward until they reach a layer with a density equivalent to their own. At this density, the virus particles will "float" and careful handling of the gradient in a clear plastic tube will reveal a turbid band of virions that can be removed. In the figure shown, the virus was collected by careful dropwise fractionation of the gradient through a hole

in the tube bottom into small tubes. The presence of virus in the appropriate fractions could be confirmed by plaque assay.

Fig. 11.2 Differential centrifugation to purify virions. Infected cells are homogenized and then subjected to varying steps of centrifugation at increasing *g* forces. At low speeds, large cellular components pellet and can be removed. At the proper speed, viral particles sediment to the bottom of the tube.

Fig. 11.3 Denaturing gel electrophoresis of proteins. If proteins are gently denatured in a detergent solution such as sodium dodecyl sulfate (SDS), they will assume globular shapes and a net negative charge due to interaction with the detergent molecules. The proteins then can be fractionated by size on acrylamide gels. The proteins migrate in specific bands, and the amount of mass in each band can be determined with a color reaction that measures protein mass. The intensity of banding is a function of the *total* amount of amino acids (a direct correlate with the total mass) in the band, *not* the number of protein molecules per se. MW=molecular weight.

Fig. 11.4 Electrophoretic fractionation of the capsid proteins isolated from purified poliovirus virions. The icosahedral capsid is made up of 60 capsomers, each containing one copy of each of the four viral proteins. The arrangement is shown schematically. Proteins from purified virions were solubilized in buffer and loaded onto a denaturing SDS-containing acrylamide gel. According to their size, viral proteins migrate in denaturing gel electrophoresis, and the amount of total mass in each band can be measured. The ratio of band intensity demonstrates that all four proteins are present in equimolar amounts. MW=molecular weight.

Fig. 11.5 Electrophoretic fractionation of the capsid proteins isolated from purified adenovirus virions. This complex virion contains many different structural proteins that can be fractionated by denaturing gel electrophoresis. The different band intensities do not correlate with protein size. This result demonstrates that the structural proteins are not present in equimolar amounts.

Fig. 11.6 The famous Kleinschmidt electron micrograph of phage T4 DNA extruded from the capsid. Before this photograph was made, there was controversy about whether the viral genome was a single piece of DNA or multiple pieces – the fragility of large DNA molecules made them difficult to isolate without shearing. Kleinschmidt took purified bacteriophages and very carefully exposed them to low osmotic pressure. Under the proper conditions, viral DNA was gently released from the capsid and visualized in the electron microscope. Note the presence of two ends, showing that the DNA is linear. (Reprinted with the kind permission of the publishers from Kleinschmidt AK, Lang DJ, Jacherts D, Zahn RK. Darstellung und Längenmessungen des Gesamten Desoxyribonucleinsäure-1 haltes von T2-Bakteriophagen. Biochimica et Biophysica Acta 1961;61:857–864.)

Fig. 11.7 Enzymatic sequencing of DNA. The generation of overlapping oligonucleotide sets complementary to a template strand of DNA for sequence analysis was developed by Sanger and colleagues and is described in the text. (a) An outline of the basic method. One major advantage of the method is that it can be used to generate very long sequences with reactions using a single primer site. (b) For example, the gel on the left shows the sequence of a cloned fragment of HSV-1 DNA and the plasmid it is cloned into about 100 bases 3′ of the primer site. The sequence can be read as follows:

5′-ACGTC$_2$T$_2$A$_2$GCTAG$_2$C$_2$G$_2$C$_2$TCGC$_2$ATCG$_2$AG$_5$C$_2$TAGT$_2$CGA$_2$TAGCTA-3′

The right gel shows a comparative analysis of the sequence of a wild-type and mutant promoter region for an HSV-1 capsid protein mRNA. This region is about 300 bases 3′ of the location of the sequencing primer and shows that high resolution is still readily obtainable as long as the reaction products are fractionated under proper conditions, which in this case are long fractionation times under denaturing conditions. The regions of the two sequences that are different are indicated; the sequences read as follows:

Wild type: 5′-TCACAGGGTTGTCTGGGCCCTGC-3′

Mutant: 5′-TCACAGGACCGGCTGACCGCCTGC-3′

Just above (i.e., 3′ of) this region is an example of a typical experimental artifact of this type of sequencing: a spot where there is termination in all reactions due to a structural feature of the sequence in question. Note that the sequence again can be read accurately beyond this point.

Fig. 11.8 Automated DNA sequencing. In a typical sequencing reaction, each of the dideoxynucleotides is labeled with a specific dye that fluoresces to emit a given wavelength of light. Sensors measure the wavelength as the overlapping fragments of DNA in the reaction mixture are separated by electrophoresis and move past the detecting site. The results are recorded and stored in a database for later sequence intepretation.

Fig. 11.9 Use of a method similar to that shown in Fig. 11.6 to spread HSV DNA for comparative contour length measurement. One full-length DNA molecule is extended and its length can be measured and compared to the length of the circular ΦX174 replicative form (RF) DNA molecules included as size standards. A second DNA molecule (or molecules) has formed a tangle around a contaminating protein fragment in the solution.

Fig. 11.10 Electrophoretic separation of bacteriophage λ restriction fragments. Bacteriophage DNA was digested with the restriction enzyme *Bst*EII, which is so named because it was derived from *Bacillus stearothermophilus* (a hot springs-loving organism or **extremophile**). The DNA fragments were fractionated by electrophoresis on 1% agarose gel, and visualized by viewing under ultraviolet light following the addition of ethidium bromide, which specifically binds dsDNA and produces orange fluorescence under ultraviolet light. The migration rate of individual fragments, whose sizes are shown, is plotted against a log of fragment size.

Fig. 11.11 Amplification of DNA with the polymerase chain reaction (PCR). (a) The basic method requires specific primer sets that can anneal to opposite strands of the DNA of interest at sites relatively close to each other. After denaturation, the primers are annealed, and DNA is then synthesized from them. All other DNA in the sample will not serve as a template. Following synthesis, the reaction products are denatured, and more primer is annealed and the process repeated for a number of cycles. The use of heat-stable DNA polymerase allows the reaction to be cycled many times in the same tube. A single copy of a DNA segment of interest could be amplified to 10^9 copies in 30 cycles of amplification. Can you demonstrate this mathematically? (b) The amplified DNA products from a segment of HSV DNA. A total of 1 μg of nonspecific DNA was added to each of a series of tubes, and viral DNA corresponding to the copy numbers shown was added. Following this, primers, heat-stable DNA polymerase, and nucleoside triphosphates were added, and 30 cycles of amplification were carried out in an automated machine. The reaction products were fractionated on a denaturing gel and visualized by autoradiography. The asterisk denotes a longer exposure of the products of the two most dilute samples. The lower gel shows the results of amplification under identical conditions of DNA isolated from two rabbit trigeminal ganglia. One was taken from a control rabbit, and the other was taken from a rabbit that had been infected in the eye with HSV followed by establishment of a latent infection. The use of rabbits to establish HSV latency is shown in Fig. 17.10. Amplified DNA from each sample was fractionated in the lanes shown; in addition to the amplification products, a sample with PCR-amplified HSV DNA as a standard (std) as well as some size markers were fractionated.

Fig. 11.12 Real time PCR amplification of globin DNA in blood macrophages. Five-fold dilutions of DNA from these cells were subjected to multiple cycles of PCR amplification under conditions where the amplified DNA can be measured by measuring fluorescence. As the dilutions increase, the range of cycles in which the amplified signal is logarithmic also increases and, thus, the quantitative measure of the numbers of genes present decreases.

Fig. 12.1 Changes in the proteins synthesized in virus-infected cells with time after infection. The left panel shows an experiment in which HeLa cells were infected with the Sabin (vaccine) strain of poliovirus, and labeled with ^{35}S-labeled methionine for 2-hour pulses at the times (hours post-infection) shown at the top of the gel. Protein was isolated and then fractionated on a denaturing gel, and radioactive proteins were localized by autoradiography (exposure to x-ray film). The capsid proteins are indicated, as are other nonstructural poliovirus-encoded proteins. Some cellular proteins whose synthesis is shutoff following infection are shown with the letter "O," while a couple whose synthesis continues is indicated by "O*." (Photograph courtesy of S. Stewart and B. Semler.) The right panel

shows a similar experiment carried out by labeling HSV-1-infected Vero cells for 30-minute periods at the times shown after infection. Some cellular proteins that are rapidly shutoff are indicated with "C." "C*" marks proteins that do not appear to be shutoff or whose synthesis increases for a period following infection. Viral proteins synthesized early after infection are indicated by "E." Note that there are at least two subsets, E1 and E2, which differ in the length of time that their synthesis continues. Similarly, there are at least two subsets of late proteins ("L"); some are clearly synthesized at the earliest times while others are only synthesized later. In both panels mock-infected cells (M) show the patterns of proteins synthesized in uninfected cells. (Photograph courtesy of S. Silverstein.)

Fig. 12.2 The structure of an antibody molecule, IgG. This molecule is made up of four chains: two heavy and two light. The antigen-combining domains are at the N-terminal of the four chains and are made up of variable amino acid sequences, a specific sequence for each specific antibody molecule. The C-terminal region has a constant amino acid sequence no matter what the antibody's specificity. This is the Fc region.

Fig. 12.3 Generation of monoclonal antibodies by making hybridoma cells between mouse immune B lymphocytes and myeloma cells that are not able to grow in selective (HAT) medium. Antibody-secreting clones are screened by testing with an antigen. Once the hybridoma cell line is made, it can be stored frozen, and then either grown in culture or injected into the peritoneal cavity of a mouse where a tumor grows as a disorganized group of individual cells and fluid (an **ascites**). The ascites cells secrete the monoclonal antibody into the body cavity's fluid where it can be harvested. HGPRT=hypoxanthine-guanine phosphoribosyltransferase; HAT=hypoxanthine, aminopterin, and thymidine.

Fig. 12.4 Outline of immunofluorescence as a means of detecting and localizing an antibody–antigen complex. The antibody specific against the antigen is allowed to react. If it has a fluorescent tag on its Fc region, it can be seen directly when illuminated with ultraviolet light since the tag emits visible light. For indirect immunofluorescence microscopy, a second antibody reactive with the Fc region of the first is used, and this antibody has the florescent tag. This method is somewhat more specific and allows the same tagged antibody preparation to be used with a number of different antibodies of differing specificities.

Fig. 12.5 Confocal microscopy to detect colocalization of antigens. (a) The use of a laser beam and a specific filter to separate the incident laser light from the fluorescence that travels on the same light path. The ability to precisely focus the laser beam onto a single plane in the microscopic field allows one to observe fluorescence from proteins only in that plane. (b) Top: Confocal microscopic visualization of two human cytomegalovirus (HCMV) proteins, IE72 (red) and pp65 (green). Primary aortic endothelial cells were infected with a strain of HCMV isolated from a human patient. This high-magnification view of a cell shows nuclear and cytoplasmic staining of the two HCMV proteins at 8 days following infection. (Photograph courtesy of K. Fish and J. Nelson.) Bottom: A series of three photographs of the identical field viewed with three different filters to localize two specific proteins to the same region. The first panel shows the association of varicella-zoster virus (VZV) glycoprotein E (gE), tagged with a green fluorescent antibody, with the surface of an infected cell. This glycoprotein was expressed in transfected cells. The second panel shows the localization of the red fluorescence due to the transferrin receptor in the same cell, and the third panel shows that both fluorescent signals are located in the same sites on the cell, indicated by the yellow color, seen when a filter that allows both colors to pass is used for viewing. (Photographs courtesy of C. Grose.)

Fig. 12.5 *Continued*

Fig. 12.6 Detection and isolation of proteins reactive with a specific antibody by use of immunoaffinity chromatography. (a) western blot. A mixture of viral and cellular proteins from an infected cell extract was fractionated on a sodium dodecyl sulfate gel, and the proteins blotted onto a membrane filter. The filter was then reacted with a specific antibody and washed, and then the antibody located by using radiolabeled staph A protein. (b) The antibody and antigen mixture is incubated so that specific interaction occurs. This is followed by passing the whole mix through a column with staph A protein bound to the column matrix (sepharose). All antibody molecules bind through their Fc regions, and any antigen bound to them can be eluted with a gentle denaturation rinse that does not cause the staph A protein–Fc binding to be disrupted. (c) A similar approach in which the antibody first is bound to the column matrix, and the proteins are washed over the column for binding. Both methods provide essentially equivalent results.

Fig. 12.6 *Continued*

Fig. 12.7 Use of immunoaffinity chromatography to isolate HSV envelope proteins from infected cells. Total infected cell protein was labeled by incubation with radioactive amino acids. The protein then was mixed with a polyclonal antibody monospecific for viral envelope proteins. The reactive proteins were isolated as described in Fig. 12.6 and fractionated on a denaturing gel. The third column shows the results of a similar experiment where a virus unable to express glycoprotein C was used. wt=wild type.

Fig. 12.8 Separation of HSV DNA from cellular DNA based on differences in base composition. The percentage of G+C residues in a given fragment of dsDNA will determine its buoyant density in CsCl. In the experiment shown, three DNA samples were mixed with a CsCl solution. One sample has a very high G+C content and serves as a density marker. HSV DNA has a lower density, but is significantly higher in G+C content than cellular DNA (approximately 67% versus 48%). For this reason it has a greater buoyant density in an equilibrium gradient of CsCl. Unlike the equilibrium sucrose gradient shown in Fig. 11.1, CsCl solutions are so dense that the gradient will form under the centrifugal force available in an ultracentrifuge. Therefore, the mixture of DNA and CsCl is made and placed in a centrifuge rotor, and the mixture is allowed to form a density gradient by high-speed centrifugation. Following equilibrium, the various DNA fragments can be isolated by careful dropwise collection of the gradient. The graph shows the position of the three DNA species at equilibrium.

Fig. 12.9 Different viral mRNA molecules are encoded by different regions of a viral genome. The diagram shows the 150,000-base-pair HSV genome and the location of three cloned DNA fragments that can be used to hybridize to total infected cell RNA. More detailed information concerning the HSV genome and specific genes can be found in Chapter 17, Part IV. A number of fractionation gels are shown. (a) The total viral mRNA species expressed at 5 hours following infection. The RNA was isolated and fractionated, and a northern blot made of the RNA. This was hybridized with radioactive viral DNA to locate the viral mRNA species. (b) The RNA species expressed in region 1 by hybridization with radioactive DNA from this region only. (c) The different RNAs seen with a probe for region 2. (d) The RNA expressed from region 2 changes in character between 3 and 8 hours following infection (at the intermediate time shown in c, all species are being expressed). The lanes marked "SS" contain radioactive ribosomal RNA included as a size standard.

Fig. 12.10 In situ hybridization of human neurons latently infected with HSV. The trigeminal nerve ganglion was taken at autopsy from a middle-aged man killed in an automobile accident. The tissue was sectioned and individual slices incubated with labeled probe DNA from either region 1 or region 3 of the HSV genome shown in Fig. 12.9 under hybridization conditions. The left panel shows no hybridization; the dark spot in the neuronal nucleus is the nucleolus, which is the site of ribosomal RNA synthesis. The right panel shows positive hybridization due to the expression and nuclear localization of the HSV latency-associated transcript.

Fig. 12.11 In situ hybridization of sections of suckling mice infected with polyomavirus. A stained section showing the location of major organs of the mouse is shown in the center. Fluororadiographs of sections showing tissues in which virus is replicating are shown above and below this section. (Photographs courtesy of L. P. Villarreal.)

Fig. 12.12 Characterization of isolated viral mRNA by in vitro translation (IVT). Total protein labeled in a 1-hour pulse was isolated at 6 hours after infection from HSV-infected cells and fractionated on a denaturing gel. The capsid antibody (Ab) used in this experiment reacted specifically with only the 155,000-dalton major capsid protein. The third lane shows the fractionation of protein synthesized in vitro using a rabbit reticulocyte system and mRNA hybridizing to DNA from region 1 of the HSV genome shown in Fig. 12.9. Two proteins are seen: one migrating at 155,000 daltons and the other at 35,000 daltons. Demonstration that the large protein is, indeed, the major capsid protein is made by use of the antibody, as shown in the other lanes.

Fig. 12.13 The application of microarrays or the study of viral products produced in an HSV-infected cell. A DNA microarray for analysis of mRNA in HSV-

infected cells. Oligonucleotides complementary to each viral transcript are bound to a glass slide along with oligonucleotides complementary to a number of diagnostic cellular transcripts. Samples of mRNA are isolated from cells under different conditions of infection, and cDNA copies are made using a dye-substituted deoxy-base; a different fluorescent dye is used for each condition. Then the cDNA is hybridized to the chip, unhybridized material washed away, and bound material is localized by scanning with a laser of a wavelength that only excites one or the other of the two dyes. The color and intensity of the signal in each spot can then be directly related to the amount of mRNA present in the original sample.

Fig. 14.1 Some general features of viruses containing RNA genomes that use RNA-directed RNA transcription in their replication. (a) The general relationship between viruses containing a genome that can be translated as the first step in the expression of viral genes versus those viruses that first must carry out transcription of their genome into mRNA utilizing a virion-associated transcriptase. (b) The basic rules for RNA-directed RNA replication. As with DNA-directed RNA and DNA synthesis, the new (nascent) strand is synthesized 5′ to 3′ antiparallel to the template, and the Watson–Crick base-pairing rules are the same, with U substituting for T. However, the very high thermal stability of dsRNA leads to complications. The major complication is that newly synthesized RNA must be denatured and removed from the template strand to avoid its "collapsing" into a double-stranded form. Formation of such dsRNA is an effective inducer of interferon (see Chapter 8, Part II), and it appears to be refractory to serving as a template when free in the cytoplasm. A second complication is that in order to generate a ssRNA molecule of the same coding sense as the virion genome, *two* replicative intermediates (RIs) must be generated. These intermediates are dynamic structures of ribonucleoprotein containing a full-length template strand, and a number of newly synthesized product RNA molecules growing from virion-encoded replicase that is traversing the template strand. RI-1 generates RNA complementary to the virion genomic RNA. This serves as a template for new virion genome RNA in RI-2.

Fig. 14.2 (a) Poliovirus, a typical picornavirus. The 30-nm-diameter icosahedral capsid comprises 60 identical subunits – each a pentamer of subunits (often called protomers) containing a single copy of VP1, VP2, VP3, and VP4. The map of the approximately 7700-nucleotide (nt) single-stranded RNA genome that serves as mRNA in the initial stages of replication is also shown. Unlike cellular mRNA, poliovirus genomic RNA has a viral protein (VPg) at its 5′ end instead of a methylated nucleotide cap structure. The RNA has a c. 740-nt sequence at the 5′ end that encodes no protein, but assumes a complex secondary structure to aid ribosome entry and initiation of the single translational reading frame. The single precursor protein synthesized from the virion RNA is cleaved by internal proteases (2A and 3C) initially into three precursor proteins, P1, P2, and P3. Protein P1 is then proteolytically cleaved in a number of steps into the proteins that assemble into the precapsid, VP0, VP1, and VP3. Proteins P2 and P3 are processed into replicase, VPg, and a number of proteins that modify the host cell, ultimately leading to cell lysis. With three exceptions, all proteolytic steps are accomplished by protease 3C, either by itself or in association with protein 3D. Protease 2A carries out the first cleavage of the precursor protein into P1 and P2 as an intramolecular event. It also mediates cleavage of the protease 3CD precursor into protease 3C and protein 3D. It is not known how the third cleavage that does not utilize protease 3C occurs. This is the maturation of the capsomers by the cleavage of VP0 into VP2 and VP4. The VP4 protein is modified by the addition of a myroistyl residue at the amino terminus (myr=myroistyl). (b) The structure of the poliovirus internal ribosome entry site (IRES). The diagram is a schematic of the predicted secondary structure in the 5′ proximal region of the poliovirus genome. The shaded secondary structure features make up the IRES. Note that one of the mutations associated with attenuation of the Sabin vaccine strains is located in this region. The site of the AUG at which initiation of the large polyprotein occurs is also indicated.

Fig. 14.2 *Continued*

Fig. 14.3 The poliovirus replication cycle. The schematic representation is broken into discrete steps. Viral entry is by receptor-mediated endocytosis during which the virion proteins are sequentially removed, releasing virion-associated positive-sense RNA. Viral entry involves attachment to a cell surface receptor, followed by rearrangement of the capsid with insertion of the myroistylated-VP4 terminus into the cell membrane, releasing the positive-sense RNA genome into the cytoplasm of the cell. This RNA is translated into a large polyprotein. Viral replicase released from the precursor protein then mediates generation of RI-1 and RI-2 to generate more mRNA that, unlike the original genomic RNA, has the VPg protein cleaved off. As infection proceeds, the replication complexes become associated with cellular membrane structures into replication compartments. Newly synthesized positive-sense RNA is also translated and the process repeats many times until sufficient capsid protein precursors are formed to allow assembly of the procapsid. Procapsids associate with newly synthesized positive-sense RNA still containing VPg at its 5′ end, and entry of viral genomes results in capsid maturation. As the process continues, virions accumulate in the cytoplasm until viral proteins induce cell lysis and virus release occurs. The entire process can take place in the absence of a nucleus.

Fig. 14.4 The steps in the assembly of the poliovirus virion. Precursor proteins associate to form 5s protomers, which then assemble to form pentamers. Twelve of these assemble to form the procapsid into which virion RNA is incorporated. Final cleavage of VP0 into VP2 and VP4 takes place to form the mature capsid that has a diameter of 28–30 nm.

Fig. 14.5 The yellow fever virus (a flavivirus) and its genome. This flavivirus has a replication cycle very similar in broad outline to that detailed for poliovirus. Unlike poliovirus, flaviviruses encode a single envelope glycoprotein, and its approximately 10,000-nucleotide (nt) genome is capped, although not polyadenylated. Also in contrast to poliovirus, the yellow fever virus precursor polyprotein is cleaved into a large number of products as it is being translated, so the very large precursor proteins of poliovirus replication are not seen. The enveloped capsid is larger than that of poliovirus, with a diameter of 40–50 nm. ER=endoplasmic reticulum.

Fig. 14.6 Sindbis virus – a typical togavirus. The virion (60–70 nm in diameter) and genetic map are shown. The Sindbis genome contains two translational reading frames; only the upstream (5′) one can be translated from the approximately 11,000-nucleotide (nt) capped and polyadenylated 49s (positive) virion-associated genomic RNA. This upstream translational frame encodes nonstructural proteins via expression of two precursor proteins. The larger, which contains the polymerase precursor, is translated by suppression of an internal stop codon in the reading frame.

Fig. 14.7 The early stages of Sindbis virus infection. (a) The first step is receptor-mediated endocytosis, leading to fusion of the viral membrane with that of the endocytotic vesicle, which leads to release of the Sindbis virus genome (mRNA) into the infected cell's cytoplasm. As outlined in Chapter 6, Part II, internalization of the enveloped virion within an endocytotic vesicle is followed by acidification and covalent changes in membrane proteins. This results in fusion of the viral membrane with that of the endocytotic vesicle and release of the viral genome. (b) Translation of the virion RNA results in expression of the precursors to the nonstructural replicase and other viral proteins encoded in the 5′ translational reading frame. These proteins mediate replicase, capping, and protease functions.

Fig. 14.8 (a) The replication of Sindbis virus genome, and generation of the subgenomic 26s mRNA. This mRNA is expressed by an internal start site for viral replicase, and is translated into structural proteins since it encodes only the open reading frame (ORF) that was cryptic in the 49s positive-sense virion RNA. (b) Sindbis virus structural proteins are translated as a single precursor. When the N-terminal capsid protein is cleaved from the precursor, a signal sequence consisting of a stretch of aliphatic amino acids associates with the endoplasmic reticulum. This association allows the membrane protein portion of the precursor to insert into the lumen of the endoplasmic reticulum. As the protein continues to be inserted into the lumen, it is cleaved into smaller product proteins by cellular enzymes. Cellular enzymes also carry out glycosylation.

Fig. 14.8 *Continued*

Fig. 14.9 A schematic representation of the coronavirus virion. This is the only known group of positive-sense RNA viruses with a helical nucleocapsid. The

name of the virus is derived from appearance of the glycoproteins projecting from the envelope, which gives the virus a crown-like shape. The diameter of the spherical enveloped virion ranges between 80 and 120 nm depending on experimental conditions in visualization. The 30,000-nucleotide (nt) capped and polyadenylated positive-sense genome encodes five translational reading frames that are expressed through translation of the genomic RNA and six subgenomic positive-sense mRNAs. These capped and polyadenylated subgenomic mRNAs each have the same short 5′ leader and share nested 3′ sequences. Although two models exist for the production of this nested set, the most likely at this time appears to be that they are derived by transcription of subgenomic negative-sense templates, produced by discontinuous copying of the viral genomic RNA.

Fig. 14.10 The replication cycle of a coronavirus. Replication is entirely cytoplasmic. Infection is initiated by receptor-mediated membrane fusion to release the genomic mRNA. This RNA is translated into the very large (>200 kd) polymerase/capping enzyme. The interaction between full-length virion positive-sense RNA and replicase generates the templates for the mRNAs. Two models are proposed for the synthesis of subgenomic mRNA: leader-primed synthesis and discontinuous negative-strand synthesis. The second of these two models is shown in the figure. The result of both models is the synthesis of a nested set of mRNAs that contain the same 5′ leader sequence and overlapping 3′ ends. Translation of the various subgenomic mRNAs leads to synthesis of the various structural and nonstructural proteins encoded by interior translational reading frames. The mature virions assemble and become enveloped by budding into intracytoplasmic vesicles; these exocytotic vesicles then migrate to the cell surface where virus is released. At later times, cell lysis occurs.

Fig. 14.11 The approximately 25-nm-diameter icosahedral capsid of positive-sense RNA bacteriophage Qβ. The positive-sense RNA genome contains three separate open reading frames (ORFs). These ORFs can be independently translated from the full-length virion RNA because unlike the situation in eukaryotic viruses, bacterial ribosomes can initiate translation at interior start signals provided that the ribosome can interact with them. With this bacteriophage, ribosome attachment and translation require active transcription to allow the nascent positive-sense RNA to be unfolded so that the translation start is accessible.

Fig. 14.12 Coupled transcription-translation of bacteriophage Qβ RNA results in opening the blocked translational start site for the A (maturational) and coat proteins. As the replicase enzyme passes the region containing the translation start site on the negative-sense template (which is a GUG for the A protein), the nascent positive-sense mRNA can interact with a ribosome before it has a chance to fold into a structure in which this initiator codon is sterically blocked. Multiple ribosome entry results in translation of a large number of copies of the maturational and coat proteins being synthesized. High levels of coat protein specifically inhibit translation of replicase from full-length genomic RNA so that replicase is only synthesized at early times in the replication cycle. For this reason, it is often termed an "early" protein or gene product.

Fig. 15.1 The vesicular stomatitis virus (VSV) virion. All rhabdoviruses have this characteristic bullet shape that appears to be due to the P (formally called NS) and L proteins interacting with the envelope in a specific way. The 70×180-nm VSV virion contains enzymes for RNA transcription that can be activated by mild detergent treatment and incubation with nucleoside triphosphates in vitro. The genetic map of VSV is also shown. The 11,000-nucleotide (nt) virion negative-sense strand RNA encodes five individual mRNAs; each is capped and polyadenylated by virion enzymes. Note, because the genomic RNA serves as a template for mRNA synthesis, it is shown in 3′ to 5′ orientation instead of the conventional 5′ to 3′ orientation. G=envelope glycoprotein; L=part of the replication enzyme; M=matrix; N=nucleocapsid; P (or NS)=also part of the replication enzyme.

Fig. 15.2 The VSV replication cycle. (a) Early events in infection begin with virus attachment to the receptor followed by receptor-mediated endocytosis and partial uncoating to virion ribonucleoprotein (RNP). This is transcribed into mRNAs that are translated in the cytoplasm. (b) Later, as protein synthesis proceeds, levels of the N (nucleocapsid) protein increase, and some nascent positive-sense strand from RI-1 associates with it. This association with N protein blocks the polyadenylation and cleavage of individual mRNAs, and the growing positive-sense strand becomes a full-length positive-sense strand complement to the viral genome that serves as a template for negative-sense RNA synthesis via RI-2. (c) At still later times in the replication cycle, viral proteins associate with the nucleocapsids made up of newly synthesized negative-sense genomic RNA and N protein. These migrate to the surface of the infected cell membrane, which has been modified by the insertion of viral G protein translated on membrane-bound polyribosomes. M protein aids the association of the nucleocapsid with the surface envelope and virions form by budding from the infected cell surface.

Fig. 15.2 *Continued*

Fig. 15.3 A higher-resolution schematic of the generation of positive-sense strand mRNA from genomic negative-sense strand RNA template in the presence of low levels of N protein. Polymerase associates with the template at the extreme 3′ end, and "tunnels" or "burrows" under the N protein. Transcription begins with capping of the nascent mRNA, and proceeds through the first gene on the template (the N protein) gene. At the end of this gene, the transcriptase encounters an intergenic "pause" or stutter site. The enzyme pauses here and adds a number of A residues to the nascent mRNA, which is then released. The transcriptase then either dissociates from the template and begins the process over again at the extreme 3′ end, or continues on to synthesize a transcript encoding the next gene on the genomic template. At the end of this gene, the same process occurs. Since the transcriptase has a higher probability of returning to the extreme 3′ end of the template, the mRNAs are synthesized in decreasing amounts, with those encoding N protein>P (NS) protein>M protein>G protein>L protein.

Fig. 15.4 The genetic map and virion structure of Sendai virus, a typical paramyxovirus. The Sendai virion is a flexible, helical nucleocapsid that contains the 15,000-nucleotide (nt) genome and is about 18 nm in diameter and 1000 nm in length. The roughly spherical enveloped virion is about 150–200 nm in diameter. The gene marked "HN" is a membrane protein that contains both neuraminidase and hemagglutination activity. The replication strategy is similar to that outlined for VSV. Also like VSV, the negative-sense strand genomic RNA is shown 3′ to 5′ instead of in the conventional 5′ to 3′ orientation.

Fig. 15.5 The structure of influenza virus A. The virion is about 120 nm in diameter, and the genome is made up of eight helical nucleocapsid segments that total about 13,600 nucleotides of negative-sense strand RNA. The virus requires the nucleus for replication. Although these virions also exhibit neuraminidase and hemagglutinin, the glycoproteins responsible are separate.

Fig. 15.6 An outline of the replication cycle of influenza. Following virus attachment to its cellular receptor(s) and endocytosis, the envelope fuses with vesicular membrane. The released ribonucleoprotein (RNP) capsid segments, each containing a specific negative-sense genomic segment, migrate to the nucleus where transcription of positive-sense RNA takes place using virion-associated transcriptase. The transcription and formation of mRNA require the "snatching" or "stealing" of caps of nascent cellular mRNA by a *trans*-splicing mechanism. Two of the pre-mRNAs generated in this way are further subjected to one of two alternative *cis*-splicing reactions using cellular machinery, so that each generates two separate mRNAs. Translation of viral proteins leads to proteins that modify the cell and its plasma membrane. The viral proteins associated with the nucleocapsid RNPs migrate to the nucleus where they mediate the synthesis of full-length positive-sense template and synthesis of negative-sense strand genomic RNA. Viral membrane-associated proteins are translated on the rough endoplasmic reticulum and processed in the Golgi apparatus. New virions form by the association of the nucleocapsids with virus-modified membrane and budding. Influenza A virus does not control this aspect of packaging; therefore, phenotypic mixing is frequent following mixed infection. HA=hemagglutinin; M=matrix protein; NA=neuraminidase.

Fig. 15.7 Antigenic changes in the surface glycoproteins of influenza A virus between 1918 and 1980. Abrupt changes in these antigens (antigenic shifts) are the result of mixed infections and random assortment of nucleocapsids to generate novel genotypes. Such shifts, which occur with random frequency, lead to epidemics worldwide. Strain designations at the bottom of the figure indicate hemagglutinin (H) and neuraminidase (N) genotypes.

Fig. 15.8 The bunyavirus virion. The three ribonucleoprotein (RNP) segments, each associated with both L and N protein, are contained within a well-defined envelope made up of two glycoproteins. The virion diameter ranges from 80 to 120 nm. The size of the RNPs as determined by their sedimentation rates (see Chapter 11) and the size of the RNA genomes and the proteins encoded by the various members of the Bunyaviridae are shown in Table 15.3. The general scheme of gene expression and genome replication of La Crosse virus is also shown. Expression and replication take place in the cytoplasm, but have many similarities to the process outlined for influenza virus. The positive-sense strand mRNA expressed from the S genomic segment contains two partially overlapping translational reading frames that are out of phase with each other. Alternative recognition of one or the other translation initiation codons by the cellular ribosomes leads to the expression of two proteins with a completely different amino acid sequence.
Fig. 15.9 The ambisense strategy of gene expression exhibited by some bunyaviruses and by arenaviruses. The expression of the small genomic segment of a tospovirus as phlebovirus is shown. With these viruses, full gene expression requires the generation of a subgenomic mRNA of same sense as the genomic RNA. Thus, even though the genomic RNA is nominally negative sense, it has regions of positive-sense information in it! This strategy is referred to as *ambisense* since both senses are present in the genome.
Fig. 15.10 The 60-nm-diameter human reovirus with its double shell. The 10 segments of the reovirus genome and the proteins encoded are shown. Note that the S1 segment encodes two overlapping translation frames. Like the situation with the La Crosse virus mRNA encoded by the S genomic fragment, these proteins are expressed by alternate initiation sites for translation. Thus, the virus encodes 11 proteins. The total size of the genome is 23,549 base pairs.
Fig. 15.11 The reovirus replication cycle. Virus attachment is followed by receptor-mediated endocytosis. Virion "core" particles are formed by the degradation of the outer shell in the endosome, and this core particle expresses capped mRNA using a virion transcriptase. Various viral proteins are translated and structural proteins assemble around newly synthesized viral mRNA. This process is apparently random, since random assortment of genetic markers following mixed infection is readily observed (see Chapter 3, Part I). The complementary strand of the double-stranded genomic RNAs is synthesized in the immature capsid while morphogenesis proceeds. Virus release is by cell lysis.
Fig. 15.12 The three RNAs of hepatitis delta virus found in infected liver cells. The genomic negative-sense RNA, which is replicated by means of RNA polymerase II, encodes the antigenomic positive-sense RNA, which is the template for genomes, and a subgenomic positive-sense mRNA. This mRNA is cleaved from the antigenomic RNA by RNA self-cleavage. Further, the RNA can be edited by cellular enzymes so that the first translational terminator can be altered. With such edited RNA, a protein 19 amino acids larger than that expressed from unedited RNA is produced.
Fig. 15.13 The potato spindle tuber viroid genome. Various pathogenic strains range from 250 to 360 nucleotides in length. This circular RNA does not encode a protein, but the sequences indicated as pathogenic are required to cause the disease. Modification of these sequences leads to a viroid that is nonpathogenic and can protect the plant from pathogenesis by the original viroid. Viroid RNA is replicated with cellular RNA polymerase, forming large multimeric structures of both positive and negative sense. Individual viroid RNA is released by RNA self-cleavage.

Fig. 16.1 Polyomavirus and the genetic and transcript map of SV40 virus. (a) The 60 pentameric subunits of the capsid proteins are arranged in an unusual fashion so that the packaging of individual capsomers is not equivalent in all directions. The drawing is based on computer-enhanced analysis using the electron microscope and x-ray diffraction methods (see Chapter 5) published by Salunke et al (Cell 1986;46:895–904). The 5243-base pair dsDNA genome is condensed with host cell histones and packaged into the 45-nm-diameter icosahedral capsid. (b) The early and late promoters, origin of replication, and bidirectional cleavage/polyadenylation signals are shown along with the introns and exons of the early and late transcripts. A high-resolution schematic of the approximately 500-base pair control region with the early and late promoters is also provided. Two early promoter enhancers, one containing the 21-base pair repeats and the other containing the 72-base pair repeats, are shown. The origin of replication (ori) is situated between the enhancers and the early promoter, and the three binding sites for large T antigen (T) are indicated. (c) A higher-resolution schematic of the processing of early viral mRNAs. Splice sites, translational reading frames, and other features are indicated by sequence number. Details are described in the text. Note that the 3′ end of the pre-mRNA occurs just beyond the early polyadenylation site (2590) that is situated in the 3′ transcribed region of the late pre-mRNA. (d) A higher-resolution schematic of the processing of late viral mRNAs. Splice sites, translational reading frames, and other features are indicated by sequence number. Details are described in the text. Note that the 3′ end of the pre-mRNA occurs just beyond the late polyadenylation site (2650) and is situated in the 3′ transcribed region of the early pre-mRNA. T-Ag=large T antigen; t-Ag=small t antigen.
Fig. 16.1 *Continued*
Fig. 16.2 The replication cycle of SV40 virus in a permissive cell. The replication is divided into two phases, early and late. During the early stages of infection, virus attaches and viral genomes with accompanying cellular histones are transported to the nucleus via receptor-mediated endocytosis. RNA polymerase II (pol II) recognizes the enhanced early promoter, leading to transcription of early pre-mRNA, which is processed into mRNAs encoding small t (t-Ag) and large T antigen (T-Ag). These mRNAs are translated into their corresponding proteins. Large T antigen migrates to the nucleus where it carries out a number of functions, including inactivation of the cellular growth control proteins p53 and Rb, and binding of the SV40 origin of DNA replication (ori). Viral DNA replication takes place by the action of cellular DNA replication enzymes, and each round of DNA replication requires large T antigen to bind to the ori.

As genomes are replicated, the late stage of infection begins. High levels of large T antigen suppress the expression of early pre-mRNA and stimulate expression of late pre-mRNA. This is processed into two late mRNAs; the smaller encodes both VP2 and VP3 while the larger encodes VP1. At very late times, some transcripts are expressed and can be translated into the small agnoprotein. Viral capsid proteins migrate to the nucleus where they assemble into capsids with newly synthesized viral DNA. Finally, progeny virus is released by cell lysis.
Fig. 16.3 The replication of SV40 DNA. The closed circular DNA has no end problem, unlike the replication of linear DNAs. Structures of the replication fork and growing points are essentially identical to those in replicating cellular DNA, and use cellular DNA replication enzymes and accessory proteins. Replication results in the formation of two covalently closed and interlinked daughter genomes that are nicked and religated into individual viral genomes by the action of cellular topoisomerase and other helix-modifying enzymes. T-Ag=large T antigen; ori=origin of replication.
Fig. 16.4 Representation of the two steps in transformation of a nonpermissive cell by SV40. The infection begins as described in Fig. 16.2 and early mRNA is expressed into early proteins. The infection is abortive in that DNA replication and late gene expression cannot occur in the nonpermissive cell. Still, the large T antigen (T-Ag) is able to interfere with cellular growth control (tumor suppressor) proteins, leading to cell replication. Stable transformation requires a second step, the integration of the viral DNA. This is a random (stochastic) occurrence with SV40, and integration is random throughout the genome. A similar path is followed in the transformation of nonpermissive cells by other polyomaviruses. t-Ag=small t antigen.
Fig. 16.5 The human papillomavirus (HPV)-16 genome. The 7-kbp circular genome contains a number of translational reading frames that are expressed from spliced mRNAs. Unlike the related polyomaviruses, papillomaviruses encode all proteins on the same DNA strand. The actual details of mRNA expression also appear to differ among different papillomaviruses. For example, HPV-16 has only one known promoter, which appears to control expression of both early and late transcripts. The locations of cleavage/polyadenylation signals for early and late transcripts are shown. All mRNAs appear to be derived by splicing of one or two pre-mRNAs. The characterization of transcripts has required heroic efforts of isolating small amounts of RNA from infected tissue, generating cDNA clones by use of reverse transcriptase and polymerase chain reaction, and then sequence analysis. This is necessary because many are present in very small amounts in

tissue and the virus does not replicate in cultured cells. The transcripts shown are three of nine that have been fully characterized, and it can be expected that others are also expressed. The region marked "LCR" encodes both the constitutive (plasmid) origin of replication and an enhancer. Location of the vegetative origin of replication is not known. Specific details of papillomavirus replication are described in the text.

Fig. 16.6 The formation of a wart by cell proliferation caused by infection of basement epithelial cells with human papillomavirus (HPV). Early gene expression leads to stimulation of cell division and terminal differentiation. This results in late gene expression and virus replication in a terminally differentiated, dying cell, which produces large quantities of keratin.

Fig. 16.7 The genetic and transcription map of the 36-kbp adenovirus genome. There are three kinetic classes of transcripts. The E1 transcripts are controlled by enhanced promoters and require no modification of the host cell because some functions of their expression are similar to those of T antigen in SV40 virus replication. These functions include stimulating cellular transcriptional activity and cell replication. Early in infection, only early transcripts are expressed. These include mRNAs encoding viral DNA polymerase and terminal proteins. There are a number of early promoters and transcription units. The E2 transcription unit also has a 72-kd DNA-binding protein (72KDBP) that shuts off early transcription. Two primary transcripts, E2A and E2B, are expressed from the same E2 promoter. The mRNA for the DNA-binding protein continues to be expressed late because there is a second promoter upstream of the E2 promoter that is not shut off by the DNA-binding protein. The major late promoter at map position 15 is always "on," but polyadenylation and splicing patterns change markedly as infection proceeds. Late in infection, the late transcription unit extends to one of five polyadenylation signals and differential splicing results in generation of a myriad of late mRNAs encoding structural proteins as well as proteins involved in host cell modification and virus maturation.

Fig. 16.8 Adenovirus DNA replication. The 5′ ends of the viral genome have 50,000-dalton terminal proteins bound to them. Adenovirus does not have discontinuous strand synthesis, and exhibits other features that are at variance with the general scheme for viral DNA replication outlined in Chapter 14. Of major interest is the fact that there is no discontinuous strand synthesis. The process is marked by the accumulation of a large amount of single-stranded DNA (unusual in eukaryotic DNA replication). Further, the initial priming event requires the first nucleotide of the new DNA strand being covalently bound to the 80,000-dalton precursor of the 50,000-dalton terminal protein. Following complete second strand synthesis, the precursor end proteins are proteolytically cleaved to form the mature terminal proteins. TBP=precursor to terminal binding protein.

Fig. 16.9 The 5000-nucleotide (nt) linear genome of adeno-associated virus (AAV). This ssDNA has repeated sequences on both ends that allow it to form a "hairpin" structure. This serves as the template for conversion into dsDNA by cellular enzymes. Cellular enzymes also mediate replication of the viral genome. Three families of coterminal mRNAs are expressed from the three AAV promoters; the genome encodes replication proteins and a capsid protein but depends on cell replication for its ability to replicate its genome. This cellular replication is induced by a helper virus such as adenovirus in the animal, but the virus can replicate in cultures of some actively replicating cells. Other groups of parvoviruses, such as minute virus of mice (MVM), are able to replicate in some actively replicating cells of their natural host.

Fig. 16.10 The capsid structure and compressed genome of bacteriophage ΦX174. The capsid is made up of three proteins: major capsid, major spike, and minor spike. In all, a total of 10 genes are compressed into 3.4 kb of ssDNA! This is accomplished by very short intergenic regions, and two completely overlapping genes. The functions of the proteins encoded by these genes are listed.

Fig. 17.1 Electron micrograph of an enveloped HSV-1 virion revealing specific features, especially glycoprotein spikes projecting from the envelope. The capsid has a diameter of about 100 nm and encapsidates the 152,000-base pair viral genome. The interior of the capsid does not contain any cellular histones, in contrast to smaller DNA viruses. Rather, it contains relatively high levels of polyamines such as spermidine and putrescine, which serve as counterions to allow compact folding of the viral DNA needed in the packaging. (Photograph courtesy of Jay Brown.)

Fig. 17.2 The HSV-1 genetic and transcription map. Specific features of the genome are discussed in the text, and tabulated in Table 17.1. Individual transcripts are controlled by their own specific promoters, and splicing is uncommon. Each transcript is headed by its own promoter, and most are terminated with individual cleavage/polyadenylation signals. The time of expression of the various transcripts is roughly divided into immediate-early (α), early (β), late (βγ), and strict late (γ). This is, in turn, based on whether the transcripts are expressed in the absence of viral protein synthesis (α), before viral DNA replication and shutoff following this (β), before viral DNA replication but reaching maximum levels following this (βγ), or only following viral DNA replication (γ). The genome is about 152,000 base pairs and contains extensive regions of duplicated sequences.

Fig. 17.3 The programmed cascade of HSV transcription at different stages of the infection cycle. The details of this cascade are virtually identical for HSV-1 and HSV-2. The linear viral genome with its repeat and unique regions are shown at the top, and the levels of gene expression during the three basic phases of transcription as determined by DNA microarray analysis (see Chapter 13) are shown. Note – transcription from only one copy of each repeat region is shown for clarity. Immediately following infection, and prior to the expression of any viral proteins, five transcripts are expressed as immediate early transcripts (red). The expression of these immediate-early transcripts is ensured by virtue of the interaction between their enhancers, the viral α-TIF, and cellular Octamer binding protein These function during the early period and in the absence of any viral genome replication to allow relatively high levels of transcription of a number of early genes involved in replicating viral DNA as well as certain responses to host defense (green). The replication of the viral genome results in high levels of expression of the late transcripts (blue), which encode structural proteins and other responses to host defenses. The only clustering of genes into kinetic class is seen with the immediate-early transcripts, which are concentrated in the repeat regions. In addition to the classic late transcripts, several other transcripts (mauve) encoded in the long repeats and corresponding to the latency associated transcription unit can be detected late in infection. While a number of these transcripts are normally expressed abundantly during latency in neurons, the significance of their expression during lytic infection is unknown. This may be important in reactivation from latency.

Fig. 17.4 The entry of HSV-1 into a cell for the initiation of infection. (a) Outline of the process. The initial association is between proteoglycans of the surface and glycoprotein C (gC); this is followed by a specific interaction with one of several cellular receptors collectively termed herpesvirus entry mediators (HVEMs). These are related to receptors for nerve growth factors (NGFs) and tumor necrosis factor (TNF). The association requires the specific interaction with glycoprotein D (gD). Fusion with the cellular membrane follows; this requires the action of a number of viral glycoproteins including gB, gH, gI, and gL. An electron micrographic study of herpesvirus fusion with the infected cell is shown in Fig. 6.3. The viral capsid with some tegument proteins then migrate to nuclear pores utilizing cellular transport machinery. This "docking" is thought to result in the viral DNA being injected through the pore while the capsid remains in the cytoplasm. Some tegument proteins, such as α-TIF, also enter the nucleus with the viral genome. (b) Electron micrographic analysis of pseudorabies virus capsid "docking" and genome injection at the nuclear pore. A logical sequence is shown progressing from the (dark) full capsid to an empty one. The process is quite similar to injection of bacteriophage DNA into a bacterial cell (see Chapter 6, Part II). (Micrographs reprinted with the kind permission of the American Society for Microbiology from Granzow H, Weiland F, Jons A, Klupp BG, Karger A, Mettenleiter TC. Ultrastructural analysis of the replication cycle of pseudorabies virus in cell culture: a reassessment. Journal of Virology 1997;71:2072–2082.)

Fig. 17.4 *Continued*

Fig. 17.5 The HSV-1 productive and latent infection cycles. In productive infection, the viral genome becomes circular but does not associate with chromatin proteins (1). This is followed by immediate-early transcription that requires the association of cellular factors (Oct-1) binding to the **TATGARAT sequence**

element within the immediate-early promoter enhancers and with α-TIF to enhance transcription of immediate-early transcripts (2). These are controlled with promoters with specific enhancers. This process results in transcriptional activation that leads to early transcription, and ultimately, to viral genome replication (3, 4). Viral genome replication is accompanied by rearrangement of nuclear structures and late transcription (5), and this is followed by capsid assembly (6). In latent infection, the earliest transcription does not occur and the viral genome becomes associated with histones to form a mini-chromosome (L-1). This essentially shuts down productive transcription but allows expression of the latency-associated transcript (LAT) (L-2). LAT facilitates the stress-induced reactivation of virus by an unknown mechanism (L-3). Reactivation reinitiates the productive cascade. vhs=virion-associated host shutoff protein; α-TIF=alpha-trans-inducing factor protein; CTF, SpI, TAFs, TFIID, TFIIA-J, and TBC are all components of eukaryotic transcription machinery as explained in Chapter 13.

Fig. 17.6 Replication and encapsidation of viral genomes. (a) HSV DNA initiates rounds of DNA replication at one of three origins of replication (ori). (b) The genome is circular in the cell, during latency, and possibly the initial rounds of lytic replication, which leads to a structure that is nicked to form a rolling circle. Long concatemeric strands of progeny DNA are encapsidated by the interaction of cleavage/packaging proteins with the specific packaging signals at the end of the viral genomes (the "a" sequences).

Fig. 17.7 Immune fluorescence analysis of the rearrangement of nuclear structures following HSV-1 infection. (a) The localization of an antibody to the early single-stranded DNA-binding protein. The antibody is found diffusely distributed in the nucleus of infected cells at 3 hours after infection, but it (and the associated replicating viral DNA molecules) rapidly becomes concentrated in "replication factories" following this. (Photograph courtesy of R. M. Sandri-Goldin.) (b) Confocal microscopy of infected cell nuclei. In this series of views, an uninfected (mock infected) cell, or a cell at 3 hours or 7 hours following infection, was incubated with two antibodies bound to different chromophores. The first (green) is specific for newly synthesized RNA. This trick is accomplished by incubating the infected cells in medium containing a modified nucleotide that is incorporated into RNA. The base (5-Br-uridine) is antigenic, and there is a good commercial antibody available against it. The second (red) is an antibody specific to a component of the pre-initiation complex TATA-associated factor TFIID, and will react with this complex as it forms at the transcription start site. The merged colors (yellow) show that RNA and the transcription complex are confined to localized areas in the infected cell nucleus at the late time. Confocal microscopy is described in Chapter 12.

Fig. 17.8 Maturation of the HSV capsid and its envelopment by tegument and virus-modified nuclear membrane. (a) The procapsid assembles around scaffolding proteins that are then digested away and the empty capsid incorporates DNA by means of the action of cleavage/packaging proteins. (b) The filled capsid then migrates through the double nuclear membrane by first budding into the intercisternal space between the inner and outer membranes, and then fusing of this initial membrane with the outer nuclear envelope, releasing the capsids into the cytoplasm. Final enveloping is by budding through the walls of the exocytotic vesicle, and final release of the enveloped virion is by fusion of this vesicle with the cytoplasmic membrane as shown in Fig. 6.9.

Fig. 17.8 *Continued*

Fig. 17.9 The "decision" made by HSV upon infection of epidermal tissue enervated with sensory neurons. Productive infection follows infection of peripheral tissue, but entry of the virus into neurons leads to latent infection in a significant proportion of them.

Fig. 17.10 The expression of HSV transcripts during latent infection and reactivation in the rabbit. A standard approach toward experimental investigation, as was briefly described in Chapter 3, Part I, is outlined. If a latently infected rabbit is killed and RNA in the trigeminal ganglion extracted and subjected to reverse transcription–polymerase chain reaction (PCR) using primers specific for the latent-phase transcript (LAT), or several productive cycle transcripts (α4, α27, DNA polymerase, or VP5), the only signal seen is with the LAT primer pair. This indicates that only the LAT region is being transcribed with any frequency. Following induction of the rabbit with epinephrine, however, all transcripts are expressed, suggesting that at least some viral genomes are induced to enter productive cycle replication.

Fig. 17.11 The Epstein–Barr virus genome and the latency transcripts. (a) The 170,000-bp EBV genome contains five regions of unique sequence separated by regions of DNA containing numerous repeated sequence elements. The IR1 region contains a number of copies of a basic repeat element. The virus has two origins of replication, the OriP, which mediates latent genome replication and segregation in concert with cellular chromosome replication, and two copies of the OriLyt which functions during productive infection for vegetative DNA replication. Three regions of the genome contain genes homologous to those expressed during the productive replication of the alpha-herpesviruses. The structure of these genes is similar in the two classes of herpesviruses with each gene being expressed under the control of its own cognate promoter. (b) During the latent phase of infection, the genome circularizes and becomes a histone-associated mini-chromosome. A number of latent transcripts are expressed, one family, the EBNAs are derived by alternative splicing of a very large precursor transcript which extends from one of two promoters (Cp and Wp) rightward to the end of the IR3 region. The LMP transcripts are expressed from their own cognate promoters, and LMP-2A/B are also derived by alternate splicing of a small precursor RNA. The EBERs are expressed from RNA pol III promoters.

Fig. 18.1 The vaccinia virus virion. The structure of poxviruses is the most complex known among the animal viruses, and rivals that of some bacterial ones. The particles are on the order of 400 nm in their longest dimension. The virion contains numerous enzymes involved in RNA expression from the viral genome concentrated as a nucleoprotein complex in the core. The lateral bodies have no known function. The inset shows an atomic force micrograph of mature vaccinia virions in which the lateral bodies are observable as "bulges" on the surface of the virion. (Photo courtesy of A McPherson modified from a figure in A Malkin, A McPherson, and P Gershon. Structure of intracellular mature vaccinia virus visualized by in situ atomic force microscopy. Journal of Virology 2003;77:6332–6340.)

Fig. 18.2 The replication cycle of vaccinia virus. Following viral attachment to cellular receptors, internalization is thought to be by receptor-mediated endocytosis. Virions are partially uncoated in the vesicles and core particles are then released into the cytoplasm where early mRNA synthesis and expression of early viral proteins occur. These proteins function to continue the uncoating of the core and to replicate viral DNA. Late mRNA expression from replicated genomes leads to expression of structural and other proteins involved with virus maturation. Viral gene expression and genome replication cease by approximately 6 hours after infection, but morphogenesis of the complex virion requires a further 14–16 hours.

Fig. 18.3 The structure and genetic map of T7 bacteriophage. (a) The 40-kb gene map shows that genes are clustered according to function, with those involved in the earliest stages of infection shown to the left. Transcription begins as the DNA is injected into the host, so the left portion of the genome must be injected first. The early genes include an RNA polymerase that transcribes later genes from the viral genome. (b) Negative stained electon micrograph of purified T7 phage. (Photo courtesy of Ian Molineux.)

Fig. 18.4 The genetic map and structure of bacteriophage T4. By convention, this map is divided into kilobases instead of map units. Since the viral DNA ends are redundant, the starting point shown is entirely arbitrary. Considerably larger than T7, the phage particles have similar head shapes, but T4 has a contractile sheath and a base plate important in attachment (see Chapter 6, Part II). The viral genetic map is as complex, but not more so, than that of HSV shown in Fig. 17.2. Note that unlike HSV but similar to T7, many genetic elements are functionally clustered.

Fig. 18.5 Rolling circle replication and packaging of phage T4 DNA. The process has similarities to that of HSV shown in Fig. 17.5, but there is no specific packaging signal in the viral genome. Packaging begins at random sites and once the phage head is filled with an amount of DNA that is equivalent to 110% or so of the full genome, the ends are cleaved and packaging is completed. This results in the encapsidated DNA ends being redundant. This redundancy leads to this linear DNA molecule producing a circular genetic map, as shown in Fig. 18.4.

Fig. 18.6 Time of appearance of various functions encoded by T4 bacteriophage. Each class of transcripts is transcribed by modified *E. coli* RNA polymerase. The modifications are sequential: first one and then the second alpha (α) subunit of the RNA polymerase is modified by covalent linkage of an ADP molecule; then various phage-encoded proteins displace first the host sigma (σ) factor and then one another to generate enzymes of altered specificity.
Fig. 18.7 The assembly of T4 bacteriophage. Note that assembly of the phage head is similar to the process seen with HSV. Other components of the virion are assembled as "subassemblies" brought together sequentially to form the full phage. (This figure is drawn from work reviewed and presented by Wood WB. Bacteriophage T4 assembly and the morphogenesis of subcellular structure. Harvey Lectures 1979;73:203–223.)
Fig. 18.8 The bacteriophage λ genetic map. Specific clustered functions are indicated. The primary decision of whether to replicate or integrate involves the single question of whether leftward or rightward transcription occurs first. This process is entirely stochastic (random). If transcription takes place to the left, cI repressor is expressed and blocks lytic replication. At the same time, integrase and recombination functions lead to the phage DNA being integrated into the host bacteria's genome.
Fig. 18.9 The earliest events in the infection of a bacteria by phage λ. Details of the biochemical decision concerning lysogeny or vegetative replication are outlined here and described in the text. (This figure is based, in part, on work described by Ptashne M, in: A genetic switch: gene control and phage λ. Palo Alto: Blackwell Science and Cell Press, 1986. Especially Chapters 2 and 3.)
Fig. 18.10 A phylogenetic tree of selected large DNA-containing virus families based upon sequence divergence in conserved regions of DNA polymerase genes. Compare with Fig. 1.2. (Figure based upon analyses described in the Phycodnaviridae section of Virus taxonomy, eighth report (Fauquet et al. eds). Elsevier, Amsterdam; 2005.

Fig. 19.1 The structures of an oncornavirus and a mature lentivirus. Virion diameter varies between 60 and 100 nm for different oncornaviruses, and lentiviruses are slightly larger with a diameter of about 119 nm. Virion proteins are all derived by proteolytic processing of the Gag precursor protein, while envelope glycoproteins are all derived by processing of the Env protein. The approximate molar amounts of viral structural proteins are indicated by the numbers of copies shown. The "cone" shape of the mature lentivirus capsid is a result of a structural change as proteolytic processing occurs in the immature capsid budded from the infected cell.
Fig. 19.2 Genetic maps of various retroviruses. Specific examples are discussed in the text. Avian leukosis virus (ALV) and murine leukemia virus (MLV) are slow-transforming oncornaviruses. Note, the Env protein of ALV has a short region at its N-terminal that is the same as the N-terminal of Gag. Rous sarcoma virus (RSV) and mouse mammary tumor virus (MMTV) are rapid-transforming oncornaviruses; they have an additional v-*onc* gene. In the MMTV genome, the v-*sag* gene is encoded in the U_3 region. Human T-cell leukemia virus (**HTLV**) is an example of a slow-transforming oncornavirus that encodes extra regulatory proteins in addition to Gag, Pol, and Env. HIV is discussed in detail in Chapter 20.
Fig. 19.3 The replication cycle of a typical retrovirus. Adsorption and penetration by receptor-mediated membrane fusion (1) result in partial uncoating of the viral capsid. The generation of cDNA takes place by action of virion reverse transcriptase and RNase-H. The generation of cDNA results in formation of two copies of the long terminal repeat (LTR) made up of the R, U3, and U5 regions. The cDNA now joins with viral integrase and certain host proteins to form the pre-integration complex (PIC) (2). This is followed by integration of the proviral cDNA into the genome by the action of virion integrase. Migration of cDNA to the nucleus and integration of the proviral DNA of oncornaviruses require cell division, but cell division is not required for nuclear transport of lentivirus cDNA where integrase has a major role in transit across the intact nuclear membrane. (3) The integrated provirus acts as its own gene that is transcribed from the viral promoter contained in the LTR. Transcription terminates at the other LTR at the end of the provirus (4). Transcription of viral genes and splicing lead to expression of viral mRNAs, some of which are translated into structural proteins (5). The immature capsids are assembled and bud from the cell membrane. Following this, the final stages of capsid maturation (6) occur in the virion by means of encapsidated protease after release from the infected cell.
Fig. 19.4 The detailed mechanism for formation of proviral cDNA from viral RNA. The individual steps shown in the diagram are discussed in the text.
Fig. 19.5 Splicing patterns of various retrovirus RNAs to generate subgenomic mRNAs. The genes that can be translated in each mRNA are shown. Note: In each case the unspliced genomic RNA serves as the mRNA to encode the Gag and Gag-Pol proteins. ALV=avian leukosis virus; MLV=murine leukemia virus; MMTV=mouse mammary tumor virus; HTLV=human T-cell leukemia virus.
Fig. 19.6 Cell division and oncogenes. (a) In the normal cell, division is controlled in a variety of ways, including signals from growth factors (such as platelet-derived growth factor, PDGF) that trigger phosphorylation if cSrc family proteins or signals received by G-protein linked receptors that modulate cAMP levels. Cell division is inhibted by proteins such as p53. (b) Different retroviruses have "pirated" cellular genes to allow them to short-circuit control of cell division. Sis mimics PDGF, erb B mimics epidermal growth factor (EGF), vSrc bypasses the cSrc family of proteins, Ras acts as a continuously active G-protein, and T-antigen (TAg), a gene product of SV-40 virus, blocks the action of p53.
Fig. 19.7 The genomic structure of yeast Ty1. The similarity to a retrovirus is evident.

Fig. 20.1 The HIV-1 genome, viral particle, and transcripts. The figure shows the HIV-1 genome, the location of virion proteins, and the mRNA used to express each gene. The virion is 100–120 nm in diameter. The "cone" shaped mature capsid forms following proteolytic processing of the immature capsid after the virion buds from the infected cell. As in the case of other retroviruses, the final *gag*, *pol*, and *env* gene products are derived by proteolytic processing of precursor proteins. HIV-1 transcripts are processed into three size-groups of mature mRNA molecules as shown.
Fig. 20.2 HIV-1 entry in detail. HIV-1 binds cell surface CD4 via its surface glycoprotein, gp120. Following CD4 binding, gp120 undergoes a conformational change that exposes the coreceptor binding site. HIV-1 then binds its coreceptor, CCR5 or CXCR4 or a related molecule, which triggers a second conformational change. This causes the virion transmembrane protein to extend into the target cell membrane and then fold back on itself to initiate fusion of the viral and cytoplasmic membranes.
Fig. 20.3 The HIV-1 life cycle. After receptor-mediated membrane fusion at the cell surface (1), partial uncoating of the viral capsid forms the pre-integration complex (PIC). The generation of cDNA takes place by action of virion reverse transcriptase and RNase-H (2). The generation of cDNA results in formation of two copies of the long terminal repeat (LTR) made up of the R, U3, and U5 regions. This is followed by integration of the proviral cDNA into the genome by the action of virion integrase (3). Cell division is not required for nuclear transport of HIV-1 cDNA where integrase has a major role in transit across the intact nuclear membrane. The integrated provirus acts as its own gene that is transcribed from the viral promoter contained in the 5′ LTR. Transcription terminates at the other LTR at the 3′ end of the provirus (4). Transcription of viral genes and splicing lead to expression of viral mRNAs, some of which are translated into structural proteins (5). The immature capsids are assembled and bud from the cell membrane. Following this, the final stages of capsid maturation (6) occur in the virion by means of encapsidated protease after release from the infected cell.
Fig. 20.4 Vif and APOBEC action. APOBEC (red) is incorporated into the virion in the virus-producing cell. Vif (green) blocks APOBEC incorporation into virions by targeting it for proteasomal degradation. If APOBEC enters the virion and subsequently reaches a target cell, it deaminates cytidine residues in the first strand of retroviral cDNA (blue). The resulting uracil residues function as a template for the incorporation of adenine, which, in turn, can result in strand-

specific C/G to T/A transition mutations that affect virus viability. Uracil residues also trigger degradation of the retroviral DNA before it can integrate into the host-cell genome.

Fig. 20.5 Tat activation of HIV-1 transcription. A tripartite complex consisting of Tat, cyclin-T1, and CDK-9 binds to the *trans*-activation response region (TAR), which is a stem loop that forms at the 5′ end of HIV-1 transcripts. Subsequently, CDK-9 phosphorylates the C-terminal domain of RNA polymerase II and stimulates elongation of HIV-1 transcripts by increasing the processivity of RNA polymerase II.

Fig. 20.6 Rev binds to the RRE to mediate nuclear export of unspliced and singly spliced HIV-1 RNA molecules. The HIV-1 Rev protein binds to a complex multi-stem-loop Rev response element (RRE) that forms in unspliced and singly spliced HIV-1 RNA molecules. Rev mediates transport of these molecules from the nucleus to the cytoplasm then discharges its cargo and returns to the nucleus. This is needed because these longer HIV-1 RNAs contain nuclear retention signals (NRS) that retain them in the nucleus in the absence of Rev. By contrast, doubly spliced HIV-1 mRNA molecules do not contain NRS or RRE and are therefore exported to the cytoplasm in the absence of Rev.

Fig. 20.7 Nef and Vpu downregulate CD4 expression and Nef downregulates MHC-I expression in HIV-1 infected cells. HIV-1 Nef protein mediates internalization of CD4 by binding the cytoplasmic domain of CD4 and linking it to clathrin adaptor protein-2 (AP-2) and β-COP, leading to its internalization and degradation. By contrast, Vpu associates with CD4 in the endoplasmic reticulum (ER) during modification and transport. Vpu causes CD4 to be ubiquitinated, released from complexes with gp120, and degraded. Nef also mediates internalization of MHC-I molecules using clathrin adaptor protein-1 (AP-1) and phosphofurin acidic cluster sorting protein-1 (PACS-1).

Fig. 20.8 The pathogenesis of HIV infection leading to AIDS in a young person. The patient was infected near or at birth and the progression of virus infection and accompanying symptoms is shown. PGL=persistent generalized lymphadenopathy (swollen glands); CMV=cytomegalovirus. (Adapted from Dimmock NJ, Primrose SB. Introduction to modern virology, 4th ed. Cambridge: Blackwell Science, 1994, p 299.)

Fig. 20.9 Triphasic decay of HIV-1 in plasma following initiation of effective antiviral therapy. The decay of HIV-1 in plasma following initiation of effective treatment occurs in three phases. The first phase has a half-life of approximately 2 days and results from the death of infected T lymphocytes. The second phase of viral decay has a half-life of about 2 weeks. During this phase, virus is released from infected macrophages and from resting, latently infected CD4 T cells stimulated to divide and develop productive infection. The third phase has a half-life of 44 months and results from the reactivation of integrated provirus in memory T cells and other long-lived reservoirs of infection. This reservoir of latently infected cells may require more than 60 years of treatment to eliminate.

Fig. 21.1 A diagram of the virion structure and a genomic and genetic map of human hepatitis B virus. The virion has a lipid envelope with a single exterior coat protein and a single interior capsid protein, which encloses the viral DNA genome. The virion DNA is partially double stranded and was derived by incomplete reverse transcription of full-length virion RNA transcribed during infection. The genetic map shows the compressed arrangement of the hepatitis virus genes and the transcripts expressed from the hepatitis B virus genome. The genome contains three specific promoters. All transcripts terminate at the same site in the interior of the core protein gene.

Fig. 21.2 The genome of cauliflower mosaic virus. The three breaks in the genome are indicated by Greek letters. The translational reading frames are indicated by Roman numerals. The two viral mRNAs are also indicated.

Fig. 22.1 The impact of molecular understanding of viral and host genes on the interactions between virus and host. Different stages of the virus replication cycle and the interaction between virus and host are illustrated along with the outcomes of such interactions. Understanding the molecular basis of such interactions are important in controlling disease, aiding recovery and immunity, prevention of lasting sequelae. These interactions also can be exploited in biotechnology and medicine.

Fig. 22.2 Complementation. Neither of two mutant viruses shown can replicate because each contains a lethal mutation in a required gene encoding an enzyme or structural protein. Still, if the two mutant viruses are infected into the same cell, each can supply functions missing in the other. This means that the infected cell will have all the necessary viral gene products for the replication of both mutant viruses – they can complement each other's growth in a mixed infection.

Fig. 22.3 The example shows replicas of virus plaques that were developed under different selective and screening conditions as described in the text. Permissive temperature is 34°C, nonpermissive temperature is 39°C, and the antiviral drug is a nucleoside analogue.

Fig. 22.4 Mapping restriction endonuclease cleavage sites on a viral genome. The basic methods for mapping restriction sites resemble those used in putting a puzzle together. Essentially, all possible combinations are tried until one is found that satisfies the results of all combinations of cuts with the restriction enzymes being mapped. (a) The eight possible arrangements of sites for enzyme A that cuts the genome once and enzyme B that cuts the genome twice. Since the two enzymes can cut the DNA into three pieces, there are 2^3, or eight possible arrangements. (b) Cutting the DNA with both enzymes together followed by measuring the size of the resulting fragments eliminates all but two of the possible arrangements posited in (a). (c) Finally, a marker specific for one region of the DNA will allow choice of a single arrangement of sites.

Fig. 22.4 *Continued*

Fig. 22.5 Three widely used cloning plasmids that replicate in *E. coli*. These plasmids contain drug-resistance markers that can be used for screening or selection, high-copy-number origins of replication, and genetic markers for screening. Different variants of pUC-based plasmids have different restriction sites in their multiple cloning sites. The pGEM plasmid contains two bacteriophage promoter elements that can be used in conjunction with commercially available bacteriophage-encoded RNA polymerases to make specific transcripts from the cloned sequences. MCS=multiple cloning site.

Fig. 22.6 Isolation of a specific restriction fragment of viral DNA cloned into a **bacterial plasmid**. (a) Outline of the process of cloning a DNA fragment into a plasmid using selection and screening. (b) Cloning of a 6.3-kbp HSV DNA fragment. The electrophoretic separation of 20-μg aliquots of *Sal*I-digested HSV DNA, *Hin*dIII-digested bacteriophage λ DNA, and a pBR322 plasmid containing the cloned fragment are shown. The digestion of λ DNA with *Hin*dIII generates the six fragments ranging from 23 to 2 kbp in size, and these serve as a convenient size marker, indicated by M in the figure. The screening of individual ampicillin-resistant colonies of bacteria that were formed by transformation (transfection) of a sensitive strain with pBR322, which had been ligated with a mixture of HSV DNA fragments, is shown. The cloned DNA fragment was isolated by *Sal*I digestion of plasmid DNA isolated from a bacterial colony that is ampicillin resistant but tetracycline sensitive.

Fig. 22.6 *Continued*

Fig. 22.7 Phage λ-based cloning. The use of bacteriophage λ in cloning is shown on the left. Only the genes on the left and right ends of the linear λ genome are required for vegetative (lytic) replication. These ends can be isolated following restriction digestion of intact phage DNA, and ligated to appropriately digested DNA fragments to be cloned. The *cos* sites at the ends of the arms will mediate packaging of the recombinant viral genome provided that the total size of arms and inserts ranges between 78% and 105% of the intact λ genome. This packaged DNA can then be propagated by infection of *E. coli* with the recombinant virus. The cos sites alone in a bacterial plasmid cloning vector (a *cosmid*) allows the packaging of relatively large fragments (≥50 kbp) of DNA into replication incompetent λ virions (*phagemids*); these allow the efficient introduction of cloned DNA into bacteria where it can be propagated as a plasmid.

Fig. 22.8 A togavirus expression vector. Semliki Forest virus (a togavirus) has been adapted for use as a protein expression vector. The replication cycle involves the translation of viral replication proteins from the genomic 49S (+) strand and the translation of viral structural proteins from the subgenomic 26S (+) RNA transcribed during replication (see Fig. 14.8(a)). For protein expression, two cloned DNA fragments are generated by reverse transcription of the viral (+) RNAs followed by insertion into expression vectors containing T7 or Sp6 phage promoters (see Chapter 18). The recombinant clone containing the DNA compliment of the 49S RNA is engineered to contain the gene of interest in the position of the structural proteins, while the helper clone contains the 26S RNA complement encoding structural proteins. These cloned fragments are expressed as RNA by addition of phage transcriptase and nucleoside triphosphates, and the RNA is transfected into a packaging cell. This transfected cell then generates replication-incompetent virions which, when infected into a suitable cell line, will express the protein of interest from subgenomic RNA generated from replicated input virion RNA.
Fig. 22.9 Directed mutagenesis of viral DNA. (a) Single-stranded DNA containing the gene of interest inserted into phage M13 is isolated from mutant *E. coli* cells, which incorporated deoxyuridine into DNA in place of thymidine. This DNA is used as a template for synthesis of dsDNA using an oligonucleotide primer in which a single base has been changed (in the example shown, a "T" is substituted for a "C"). This dsDNA is used to transfect *wt E. coli*, and the presence of "U" residues in the template strand results in the preferred use of the mutated strand as a replication template. Newly replicated DNA is then isolated, amplified by transfection and replication, sequence confirmed, and then utilized for recombination back into the virus under study. (b) Synthesis of an "artificial" gene containing a mutation or mutations of choice. A series of single-stranded synthetic oligonucleotides are synthesized. These are constructed so as to have short regions on their ends, which are complementary to other synthetic oligonucleotides containing more of the gene in question. The oligonucleotides at the ends of the construct are often engineered to contain a restriction site for cloning and sequences complementary to viral sequences flanking the insertion site for the modified gene to ease recombination. A whole series of such short, synthetic DNA sequences can be ligated together and dsDNA generated by incubation with exonuclease free DNA polymerase and DNA ligase. If a restriction site is incorporated in the terminal ends, a long synthetic gene can be generated, which can be cloned into a suitable vector, amplified, and used for generation of recombinant virus.
Fig. 22.9 *Continued*
Fig. 22.10 Generating and isolating recombinant viruses. (a) As outlined in Chapter 6, Part II, transfection of infectious viral DNA into a permissive cell leads to gene expression. If a full-length viral genome is transfected into a cell, production of infectious virus will ensue. If a fragment of homologous DNA containing a modified or foreign gene is included in the transfection, recombination can occur. While this is a rare event, the appropriate combination of selection and screening for recombinant virus can result in isolation of pure stocks. (b) One approach toward screening for a recombinant virus. In this example, hybridization was used to detect the presence of virus containing the bacterial β-galactosidase gene. This requires physically picking plaques from an infected dish and testing the viral DNA present. The presence of the desired gene was confirmed by the fact that insertion of the 4-kbp β-galactosidase gene results in formation of an altered restriction fragment that can be identified by Southern blot hybridization. (c) The DNA fragment was inserted into a 3.5-kb *Sal*I fragment of HSV-1. The altered fragment size can be seen by hybridization of blots of electrophoretically separated *Sal*I-digested DNA isolated from recombinant viruses. Hybridization was either with the DNA sequences specific for the region of viral DNA used for the homologous recombination, or with a probe specific for the inserted β-galactosidase gene. Hybridization of the blot with the latter probe, however, does not produce a signal with the *wt* fragment into which the gene was inserted.
Fig. 22.10 *Continued*
Fig. 22.10 *Continued*

Fig. 23.1 Construction of a transgenic mouse. The desired DNA (here the human poliovirus receptor CD155) is injected into the pronucleus of mouse eggs and the resulting embryos are implanted into a number of **pseudo-pregnant females**. A certain proportion of such implanted embryos will result in offspring that contain the desired transgene inserted into the mouse's genome. The mice are screened for these offspring by PCR analysis of tail clippings or blood for the presence of the transgene. Once this first-generation transgenic mouse (founder) is identified, it is then mated with sibling transgenic mice until a pure or homozygous line is produced that now can be infected with polio.
Fig. 23.2 Use of β-galactosidase as a reporter to trace viral infections. (a) An HSV-1 recombinant has been engineered that contains the *E. coli LacZ* gene inserted into its genome. When the virus infects cells, it will express the *lacZ* gene and produce β-galactosidase. (b) When the HSV-1 lacZ recombinant is used to infect mice on the nose or eye, the virus will spread to the trigeminal ganglion (shown). Use of the chromogenic substrate x-gal stains cells (indicated by arrow) that are expressing β-galactosidase blue/black. (c) A thin section (6μm) of the ganglion allows individual cells (indicated by arrows) to be visualized.
Fig. 23.3 (a) MRI images of a mouse infected with HSV-1 at 10 days p.i. (post infection), 1 month p.i., and 10 months. Arrow represents the injection site. The light regions 10 days p.i. represent the inflammation resulting from the initial infection, which resolves with time. (Images courtesy of S Rabkin; from Journal of Virology 73(8):6319–6326, 1999.) (b) Use of real-time bioluminescence to monitor the spread of HSV-1 in wild-type and interferon-deficient mice. An HSV-1 strain engineered to express firefly luciferase was used to infect the right eye of mice, either wild-type (WT), or three different strains of interferon KO mice (IFNγR$^{-/-}$, IFNα/βR$^{-/-}$, or IFNα/β/γR$^{-/-}$). The mice were imaged for bioluminescence at 1, 2, 3, and 4 days post infection. The intensity of bioluminescence, reflecting the spread of the infection, is represented by pseudocolors with dark blue being the least intense, a red being the most intense. In the wild-type virus and the IFNγR$^{-/-}$ mice, the luminescence indicates that the active infection is largely confined to the area surrounding the eye. By contract, the infection of the IFNα/βR$^{-/-}$, or IFNα/β/γR$^{-/-}$ mice is not controlled efficiently, and the virus disseminates to the brain as well as to the spinal cord and abdominal cavity, as indicated by the enhanced luminescence. (Images courtesy of Luker and Leib; from *Journal of Virology* 77(20):11082–11093; 2003.)

Fig. 24.1 Growth of the genomic databases. Data taken from the National Center for Biotechnology Information (NCBI) at the National Library of Medicine, National Institutes for Health, is displayed here. From 1982 to 1993, very little expansion of the database is seen. However, with the advent of rapid sequencing technologies, dramatic increases in the sequences deposited in the databases took place. (Data courtesy NCBI: http://www.ncbi.nlm.nih.gov/Genbank/genbankstats.html)
Fig. 24.2 Results of a BLAST Search for a Nucleotide Sequence. (a) Initial BLAST input page, with a 50 nucleotide "unknown" sequence entered. Notice that the database filter has been set to "Others." (b) Result of the search is shown in graphic format at the top, along with the first ten "hits" from the database. The search algorithm calculates a score for each hit, along with a significance parameter (E-value). The lower the E-value, the more significant is the match. In this example, the "unknown" sequence is actually 50 bases from the human herpes virus 6 p41 gene.
Fig. 24.3 Random vs. scale-free networks. (a) A random network, in which the five nodes that have the most links are connected to 27% of the nodes. (b) A scale-free network, in which the five nodes that have the most links are connected to 60 of the nodes. (From Alberts R, et al. Error and attack tolerance of complex networks. *Nature* 2006;406:378–382, reprinted with permission from Nature Publishing.)
Fig. 24.4 Yeast two-hybrid detection system. Transformation vectors are created containing hybrid sequences in which the DNA binding domain and the activation domain of a gene activator are present in separate plasmids (plasmid #1 and plasmid #2). In each case, the sequence of a test protein is inserted such

that it is expressed in phase with the transcriptional activator domain. The two plasmids are used to cotransform a cell that also contains a reporter gene downstream from a promoter that is sensitive to control by the transcriptional regulator. When the two test proteins are able to bind together sufficiently well so that the two domains of the transcriptional activator can bind to and enhance transcription of the reporter gene, then and positive interaction is scored. Such assays can be done using microarrays, as described in Chapter 12.

Fig. 24.5 Protein interaction map (PIM) for the round worm, *Caenorhabditis elegans* (From Li S, et al, A map of the interactome network of the metazoan *C. elegans.* Science 2004;303:540–543, reprinted with permission from AAAS.)

Fig. 24.6 Protein interaction map of Kaposi's sarcoma, human herpesvirus and the human interactome. (a) Global view of the interplay between KSHV and a human interaction network. (b) A local view of the interactions identified between these two networks from the analysis in (a). (c) A statistical analysis of these identified interactions shows the power of such an analysis in identifying interplay between networks. From Uetz P, et al. Herpesviral protein networks and their interaction with the human proteome. Science 2006;311:239–242, reprinted with permission from AAAS.)

Fig. 25.1 Mortality from infectious diseases in the United States between 1900 and the present. The data show a continuing decline associated with improvements in public health measures and antiseptic methods in hospitals through the end of World War II. The already low rate was then further lowered by the use of antibiotics. The two increases seen are due to the 1918 Spanish influenza worldwide pandemic and the onset of HIV-induced AIDS in the early 1980s. The rate may continue to increase as antibiotic-resistant bacterial strains become established in the population. (Data are from the US Centers for Disease Control and Prevention.)

Fig. 25.2 Infection of a rat brain with an "engineered" replication-deficient HSV bearing the bacterial β-galactosidase gene as a marker. An amount of virus ($\alpha 4^-$ virus able to form 10^6 plaques on complementing cells) was injected into the hippocampus of male Sprague–Dawley rats. (a) After 4 days, the rats were killed and the brain sectioned, fixed with paraformaldehyde, and then stained with X-gal, which forms a blue precipitate in the presence of the enzyme. (b) A 40× magnification of the area indicated by the arrow in (a). The cells were counterstained with another dye (pyronin Y). Note that the virus is concentrated in pyramidal neuronal cells and their dendritic processes. (Photographs courtesy of D. Bloom.)