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Section 6.3 Viruses: Structure, Function, and Uses

A virus is a small parasite that cannot reproduce by itself. Once it infects a susceptible cell, however, a virus can direct the cell machinery to produce more viruses. Most viruses have either RNA or DNA as their genetic material. The nucleic acid may be single- or double-stranded. The entire infectious virus particle, called a virion, consists of the nucleic acid and an outer shell of protein. The simplest viruses contain only enough RNA or DNA to encode four proteins. The most complex can encode 100 – 200 proteins.

The study of plant viruses inspired some of the first experiments in molecular biology. In 1935, Wendell Stanley purified and partly crystallized tobacco mosaic virus (TMV); other plant viruses were crystallized soon thereafter. Pure proteins had been crystallized only a short time before Stanley's work, and it was considered very surprising at the time that a replicating organism could be crystallized.

A wealth of subsequent research with bacterial viruses and animal viruses has provided detailed understanding of viral structure, and virus-infected cells have proved extremely useful as model systems for the study of basic aspects of cell biology. In many cases, DNA viruses utilize cellular enzymes for synthesis of their DNA genomes and mRNAs; all viruses utilize normal cellular ribosomes, tRNAs, and translation factors for synthesis of their proteins. Most viruses comman-deer the cellular machinery for macromolecular synthesis during the late phase of infection, directing it to synthesize large amounts of a small number of viral mRNAs and proteins instead of the thousands of normal cellular macromolecules. For instance, animal cells infected by influenza or vesicular stomatitis virus synthesize only one or two types of glycoproteins, which are encoded by viral genes, whereas uninfected cells produce hundreds of glycoproteins. Such virus-infected cells have been used extensively in studies on synthesis of cell-surface glycoproteins. Similarly, much information about the mechanism of DNA replication has come from studies with bacterial cells and animal cells infected with simple DNA viruses, since these viruses depend almost entirely on cellular proteins to replicate their DNA. Viruses also often express proteins that modify host-cell processes so as to maximize viral replication. For example, the roles of certain cellular factors in initiation of protein synthesis were revealed because viral proteins interrupt their action. Finally, when certain genes carried by cancer-causing viruses integrate into chromosomes of a normal animal cell, the normal cell can be converted to a cancer cell.

Since many viruses can infect a large number of different cell types, genetically modified viruses often are used to carry foreign DNA into a cell. This approach provides the basis for a growing list of experimental gene therapy treatments. Because of the extensive use of viruses in cell biology research and their potential as therapeutic agents, we describe the basic aspects of viral structure and function in this section.

Viral Capsids Are Regular Arrays of One or a Few Types of Protein

The nucleic acid of a virion is enclosed within a protein coat, or capsid, composed of multiple copies of one protein or a few different proteins, each of which is encoded by a single viral gene. Because of this structure, a virus is able to encode all the information for making a relatively large capsid in a small number of genes. This efficient use of genetic information is important, since only a limited amount of RNA or DNA, and therefore a limited number of genes, can fit into a virion capsid. A capsid plus the enclosed nucleic acid is called a nucleocapsid.

Nature has found two basic ways of arranging the multiple capsid protein subunits and the viral genome into a nucleocapsid. The simpler structure is a protein helix with the RNA or DNA protected within. Tobacco mosaic virus (TMV) is a classic example of the helical nucleocapsid. In TMV the protein subunits form broken disklike structures, like lock washers, which form the helical shell of a long rodlike virus when stacked together (Figure 6-11a).

Figure 6-11

Two basic geometric shapes of viruses. (a) In some viruses, the protein subunits form helical arrays around an RNA or DNA molecule (red), which runs in a helical groove within the enclosing protein tube. The electron micrograph to the right is of tobacco (more...)

The other major structural class of viruses, called *icosahedral* or *quasi-spherical viruses,* is based on the icosahedron, a solid object built of 20 identical faces, each of which is an equilateral triangle. In the simplest type of icosahedral virion each of the 20 triangular faces is constructed of three identical capsid protein subunits, making a total of 60 subunits per capsid. At each of the 12 vertices, five subunits make contact symmetrically (Figure 6-11b). Thus all protein subunits are in *equivalent* contact with one another. Tobacco satellite necrosis virus has such a simple icosahedral structure. However, most quasi-spherical viruses are larger, requiring the assembly of more than three subunits per face of the icosahedron. These proteins form shells whose subunits are in *quasi-equivalent* contact. Here, the proteins at the icosahedral vertices remain arranged in a fivefold symmetry, but additional subunits cover the surfaces between in a pattern of sixfold symmetry (Figure 6-11c).

The atomic structures of a number of icosahedral viruses have been determined by x-ray crystallography (Figure 6-12a). The first three such viruses to be analyzed — tomato bushy stunt virus, poliovirus, and rhinovirus (the common cold virus) — exhibit a remarkably similar design, in terms of the rules of icosahedral symmetry as well as in the details of their surface proteins. In each virus, at atomic resolution, clefts ("canyons") are observed encircling each of the vertices of the icosahedral structure. Interaction of these clefts with cell-surface receptors attaches the virus to a host cell, the first step in viral infection (Figure 6-12b). Neutralizing antibodies specific for a particular virus also interact with these clefts, thereby inhibiting attachment of the virus to the host cell.

Figure 6-12

Structure of picornaviruses. These icosahedral viruses include poliovirus and the rhinoviruses, which cause the common cold. (a) The picornavirus capsid is composed of four proteins (VP1, VP2, VP3, and VP4; VP4 is located in the interior). This model (more...)

In some viruses, the symmetrically arranged nucleocapsid is covered by an external membrane, or *envelope,* which consists mainly of a phospholipid bilayer but also contains one or two types of virus-encoded glycoproteins (Figure 6-13). The phospholipids in the viral envelope are similar to those in the plasma membrane of an infected host cell. The viral envelope is, in fact, derived by budding from that membrane, but contains mainly viral glycoproteins.

Figure 6-13

Electron micrograph of a negatively stained influenza virus virion. The virion is surrounded by a phospholipid bilayer; the large spikes protruding outward from the membrane are composed of trimers of hemagglutinin protein and tetramers of neuraminidase (more...)

The components of simple viruses such as TMV, which consists of a single RNA molecule and one protein species, undergo self-assembly if they are mixed in solution. More complex viruses containing a dozen or more protein species do not spontaneously assemble in vitro. The multiple components of such viruses assemble within infected cells in stages, first into subviral particles and then into completed virions. The genomes of these complex viruses encode proteins that assist in the assembly of the virion, but the assembly proteins are not themselves components of the completed virion.

Most Viral Host Ranges Are Narrow

The fact that the host range — the group of cell types that a virus can infect — is generally restricted serves as a basis for classifying viruses. A virus that infects only bacteria is called a bacteriophage, or simply a phage. Viruses that infect animal or plant cells are referred to generally as *animal viruses* or *plant viruses.* A few viruses can grow in both plants and the insects that feed on them. The highly mobile insects serve as vectors for transferring such viruses between susceptible plant hosts. An example is potato yellow dwarf virus, which can grow in leafhoppers (insects that feed on potato plant leaves) as well as in potato plants. Wide host ranges are characteristic of some strictly animal viruses, such as vesicular stomatitis virus, which grows in insects and in many different types of mammalian cells. Most animal viruses, however, do not cross phyla, and some (e.g., poliovirus) infect only closely related species such as primates. The host-cell range of some animal viruses is further restricted to a limited number of cell types because only these cells have appropriate surface receptors to which the virions can attach.

Viruses Can Be Cloned and Counted in Plaque Assays

The number of infectious viral particles in a sample can be quantified by a plaque assay. This assay is performed by culturing a dilute sample of viral particles on a plate covered with host cells and then counting the number of local lesions, called *plaques,* that develop (Figure 6-14). A plaque develops on the plate wherever a single virion initially infects a single cell. The virus replicates in this initial host cell and then lyses the cell, releasing many progeny virions that infect the neighboring cells on the plate. After a few such cycles of infection, enough cells are lysed to produce a visible plaque in the layer of remaining uninfected cells.

Figure 6-14

Plaque assay for determining number of infectious particles in a viral suspension. (a) Each lesion, or plaque, which develops where a single virion initially infected a single cell, constitutes a pure viral clone. (b) Plate illuminated from behind shows (more...)

Since all the progeny virions in a plaque are derived from a single parental virus, they constitute a virus clone. This type of plaque assay is in standard use for bacterial and animal viruses. Plant viruses can be assayed similarly by counting local lesions on plant leaves inoculated with viruses. Analysis of viral mutants, which are commonly isolated by plaque assays, has contributed extensively to current understanding of molecular cellular processes. The plaque assay also is critical in isolating λ bacteriophage clones carrying segments of cellular DNA, as discussed in Chapter 7.

Viral Growth Cycles Are Classified as Lytic or Lysogenic

The surface of viruses includes many copies of one type of protein that binds, or adsorbs, specifically to multiple copies of a receptor protein on a host cell. This interaction determines the host range of a virus and begins the infection process (Figure 6-15). Then, in one of various ways, the viral DNA or RNA crosses the plasma membrane into the cytoplasm. The entering genetic material may still be accompanied by inner viral proteins, although in the case of many bacteriophages, all capsid proteins remain outside an infected cell. The genome of most DNA-containing viruses that infect eukaryotic cells is transported (with some associated proteins) into the cell nucleus, where the cellular DNA is, of course, also found. Once inside the cell, the viral DNA interacts with the host's machinery for transcribing DNA into mRNA. The viral mRNA that is produced then is translated into viral proteins by host-cell ribosomes, tRNA, and translation factors.

Figure 6-15

Electron micrograph of a T4 bacteriophage adsorbed onto an E. coli cell. Once viral surface proteins interact with receptors on the host cell, the viral DNA is injected into the cell. [From A. Levine, 1991, *Viruses,* Scientific American Library, p. 20.] (more...)

Most viral protein products fall into one of three categories: special enzymes needed for viral replication; inhibitory factors that stop host-cell DNA, RNA, and protein synthesis; and structural proteins used in the construction of new virions. These last proteins generally are made in much larger amounts than the other two types. After the synthesis of hundreds to thousands of new virions has been completed, most infected bacterial cells and some infected plant and animal cells rupture, or lyse, releasing all the virions at once. In many plant and animal viral infections, however, no discrete lytic event occurs; rather, the dead host cell releases the virions as it gradually disintegrates.

These events — adsorption, penetration, replication, and release — describe the lytic cycle of viral replication. The outcome is the production of a new round of viral particles and death of the cell. Figure 6-16 illustrates the lytic cycle for T4 bacteriophage. Adsorption and release of enveloped animal viruses are somewhat more complicated processes. In this case, the virions "bud" from the host cell, thereby acquiring their outer phospholipid envelope, which contains mostly viral glycoproteins.

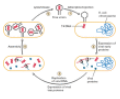

Figure 6-16

The steps in the lytic replication cycle of a nonenveloped virus are illustrated for E. coli bacteriophage T4, which has a double-stranded DNA genome. During adsorption (step *1*), viral coat proteins (at the tip of the tail in T4) interact with specific (more...)

We illustrate the lytic cycle of enveloped viruses with the rabies virus, whose nucleocapsid consists of a single-stranded RNA genome surrounded by multiple copies of nucleocapsid protein (Figure 6-17, *upper left*). Within the nucleocapsid of rabies virions are viral enzymes for synthesizing viral mRNA and replicating the viral genome. The envelope around the nucleocapsid is a phospholipid bilayer containing multiple copies of a viral transmembrane glycoprotein. This receptor-binding, or "attachment," protein has a large external folded domain on the outside of the viral envelope, an α-helical transmembrane domain that spans the viral envelope, and a short internal domain. The internal domain interacts with the viral matrix protein, which functions as a bridge between the transmembrane glycoprotein and nucleocapsid protein. Figure 6-17 outlines the events involved in adsorption of a rabies virion, assembly of progeny nucleocapsids, and release of progeny virions by budding from the host-cell plasma membrane. Budding virions are clearly visible in electron micrographs, as illustrated by Figure 6-18.

Figure 6-17

The steps in the lytic replication cycle of an enveloped virus are illustrated for rabies virus, which has a single-stranded RNA genome. The structural components of this virus are depicted at the top. Note that the nucleocapsid of this virus is helical (more...)

Figure 6-18

Transmission electron micrograph of measles virus budding from the surface of an infected cell. [From A. Levine, 1991, *Viruses,* Scientific American Library, p. 22.]

In some cases, after a bacteriophage DNA molecule enters a bacterial cell, it becomes integrated into the host-cell chromosome, where it remains quiescent and is replicated as part of the cell's DNA from one generation to the next. This association is called lysogeny, and the integrated phage DNA is referred to as a *prophage* (Figure 6-19). Under certain conditions, the prophage DNA is activated, leading to its excision from the host-cell

chromosome and entrance into the lytic cycle. Bacterial viruses of this type are called *temperate phages.* The genomes of a number of animal viruses also can integrate into the host-cell genome. Probably the most important are the retroviruses, described briefly later in this chapter.

Figure 6-19

λ bacteriophage undergoes either lytic replication or lysogeny following infection of E. coli. The linear double-stranded DNA is converted to a circular form immediately after infection. *(Left)* If the nutritional state of the host cell is favorable, (more...)

A few phages and animal viruses can infect a cell and cause new virion production without killing the cell or becoming integrated.

Four Types of Bacterial Viruses Are Widely Used in Biochemical and Genetic Research

Bacterial viruses have played a crucial role in the development of molecular cell biology. Thousands of different bacteriophages have been isolated; many of these are particularly well suited for studies of specific biochemical or genetic events. Here, we briefly describe four types of bacteriophages, all of which infect *E. coli,* that have been especially useful in molecular biology research.

DNA Phages of the T Series

The T phages of E. coli are large lytic phages that contain a single molecule of double-stranded DNA. This molecule is about 2×10^5 base pairs long in T2, T4, and T6 viruses and about 4×10^4 base pairs long in T1, T3, T5, and T7 viruses. T-phage virions consist of a helical protein "tail" attached to an icosahedral "head" filled with the viral DNA. After the tip of a T-phage tail adsorbs to receptors on the surface of an E. coli cell, the DNA in the head enters the cell through the tail (see Figure 6-16). The phage DNA then directs a program of events that produces approximately 100 new phage particles in about 20 minutes, at which time the infected cell lyses and releases the new phages. The initial discovery of the role of messenger RNA in protein synthesis was based on studies of E. coli cells infected with bacteriophage T2. By 20 minutes after infection, infected cells synthesize T2 proteins only. The finding that the RNA synthesized at this time had the same base composition as T2 DNA (not E. coli DNA) implied that mRNA copies of T2 DNA were synthesized and used to direct cellular ribosomes to synthesize T2 proteins.

Temperate Phages

Bacteriophage λ, which infects E. coli, typifies the temperate phages. This phage has one of the most studied genomes and is used extensively in DNA cloning (Chapter 7). On entering an E. coli cell, the double-stranded λ DNA assumes a circular form, which can enter either the lytic cycle (as T phages do) or the lysogenic cycle (see Figure 6-19). In the latter case, proteins expressed from the viral DNA bind a specific sequence on the circular viral DNA to a similar specific sequence on the circular bacterial DNA. The viral proteins then break both circular molecules of DNA and rejoin the broken ends, so that the viral DNA becomes inserted into the host DNA. The carefully controlled action of viral genes maintains λ DNA as part of the host chromosome by repressing the lytic functions of the phage. Under appropriate stimulation, the λ prophage is activated and undergoes lytic replication.

Small DNA Phages

The genome of some bacteriophages encodes only 10 – 12 proteins, roughly 5 – 10 percent of the number encoded by T phages. These small DNA phages are typified by the ΦX174 and the filamentous M13 phages. These were the first organisms in which the entire DNA sequence of a genome was determined, permitting extensive understanding of the viral life cycle. The viruses in this group are so simple that they do not encode most of the proteins required for replication of their DNA but depend on cellular proteins for this purpose. For this reason, they have been particularly useful in identifying and analyzing the cellular proteins involved in DNA replication (Chapter 12).

RNA Phages

Some E. coli bacteriophages contain a genome composed of RNA instead of DNA. Because they are easy to grow in large amounts and because their RNA genomes also serve as their mRNA, these phages are a ready source of a pure species of mRNA. In one of the earliest demonstrations that cell-free protein synthesis can be mediated by mRNA, RNA from these phages was shown to direct the synthesis of viral coat protein when added to an extract of E. coli cells containing all the other components needed for protein synthesis. Also, the first long mRNA molecule to be sequenced was the genome of an RNA phage. These viruses, among the smallest known, encode only four proteins: an RNA polymerase for replication of the viral RNA, two capsid proteins, and an enzyme that dissolves the bacterial cell wall and allows release of the intracellular virus particles into the medium.

Animal Viruses Are Classified by Genome Type and mRNA Synthesis Pathway

Animal viruses come in a variety of shapes, sizes, and genetic strategies. In this book, we are concerned with viruses that exhibit at least one of two features: they utilize important cellular pathways to form their molecules, thereby closely mimicking a normal cellular function, or they can integrate their genomes into those of normal cells.

The names of many viruses are based on the names of the diseases they cause or of the animals or plants they infect. Common examples include poliovirus, which causes poliomyelitis; tobacco mosaic virus, which causes a mottling disease of tobacco leaves; and human immunodeficiency virus (HIV), which causes acquired immunodeficiency syndrome (AIDS). However, many different kinds of viruses often produce the same symptoms or the same apparent disease states; for example, several dozen different viruses can cause the red eyes, runny nose, and sneezing referred to as the common cold. Clearly, any attempt to classify viruses on the basis of the symptoms they produce or their hosts obscures many important differences in their structures and life cycles.

What *are* central to the life cycle of a virus are the types of nucleic acids formed during its replication and the pathway by which mRNA is produced. The relation between the viral mRNA and the nucleic acid of the infectious particle is the basis of a simple means of classifying viruses. In this system, a viral mRNA is designated as a *plus strand* and its complementary sequence, which cannot function as an mRNA, is a *minus strand.* A strand of DNA complementary to a viral mRNA is also a minus strand. Production of a plus strand of mRNA requires that a minus strand of RNA or DNA be used as a template. Using this system, six classes of animal viruses are recognized. Bacteriophages and plant viruses also can be classified in this way, but the system has been used most widely in animal virology because representatives of all six classes have been identified.

The composition of the viral genome and its relationship to the viral mRNA are illustrated in Figure 6-20 for each of the six classes of virus. Table 6-3 summarizes important properties of common animal viruses in each class and the research areas in which they have been widely used. Structural models of several virions are shown in Figure 6-21.

Figure 6-20

Classification of animal viruses based on the composition of their genomes and pathway of mRNA formation. DNA is shown in blue; RNA, in red. The viral mRNA is designated as a plus strand, which is synthesized from a minus strand of DNA or RNA. Class VI (more...)

Table 6-3

Animal Viruses Commonly Used in Molecular Biology.

Figure 6-21

Structures of viruses determined by cryo-electron microscopy and image analysis. Cowpea mosaic virus (CPMV) is a plant RNA virus, poliovirus (polio) a human RNA virus, nudaureila capensis β virus (NβV) an insect RNA virus, simian virus (more...)

DNA Viruses (Classes I and II)

The genomes of both class I and class II viruses consist of DNA. Various types of DNA viruses are commonly used in studies on DNA replication, genome structure, mRNA production, and oncogenic cell transformation.

Class I viruses contain a single molecule of double-stranded DNA (dsDNA). In the case of the most common type of class I animal virus, viral DNA enters the cell nucleus, where cellular enzymes transcribe the DNA and process the resulting RNA into viral mRNA. Examples of these viruses include the following:

- *Adenoviruses,* which cause infections in the upper respiratory tract and gastrointestinal tract in many animals
- *SV40* (simian virus 40), a monkey virus that was accidentally discovered in kidney cell cultures from wild monkeys used in the production of poliovirus vaccines
- *Herpesviruses,* which cause various inflammatory skin diseases (e.g., chickenpox) and latent infections that recur after long intervals (e.g., cold sores and shingles)
- *Human papillomaviruses* (HPVs), which cause warts and other insignificant skin lesions and occasionally cause malignant transformation of cervical cells

Some types of HPV are passed through sexual contact. In some infected women, the HPV genome integrates into the chromosome of a cervical epithelial cell. This rare integration event initiates an intensively studied process that can lead to development of cervical carcinoma, one of the most common types of human cancers. Routine Pap smears performed for early detection of cervical carcinoma are done to identify cells in the early stages of the transformation process initiated by HPV integration.

The second type of class I virus, collectively referred to as *poxviruses,* replicates in the host-cell cytoplasm. Typical of class Ib viruses are variola, which causes smallpox, and vaccinia, an attenuated (weakened) poxvirus used in vaccinations to induce immunity to smallpox. These very large, brick-shaped viruses (0.1 × 0.1 × 0.2 μm) carry their own enzymes for synthesizing viral mRNA and DNA in the cytoplasm.

Class II viruses, called *parvoviruses* (from Latin *parvo,* "poor"), are simple viruses that contain one molecule of single-stranded DNA (ssDNA). Some parvoviruses encapsidate (enclose) both plus and minus strands of DNA, but in separate virions; others encapsidate only the minus strand. In both cases, the ssDNA is copied inside the cell into dsDNA, which is then itself copied into mRNA.

RNA Viruses (Classes III – VI)

All the animal viruses belonging to classes III – VI have RNA genomes. A wide range of animals, from insects to human beings, are infected by viruses in each of these classes. These viruses have been particularly useful in studies on mRNA synthesis and translation (class III); glycoprotein synthesis, membrane formation, and intracellular transport (classes IV and V); and cell transformation and oncogenes (class VI).

Class III viruses contain double-stranded genomic RNA (dsRNA). The minus RNA strand acts as a template for the synthesis of plus strands of mRNA. The virions of all class III viruses known to date have genomes containing 10 – 12 separate double-stranded RNA molecules, each of which encodes one or two polypeptides. Consequently, these viruses are said to have "segmented" genomes. In these viruses, the virion itself contains a complete set of enzymes that can utilize the minus strand of the genomic RNA as a template for synthesis of mRNA in the test tube as well as in the cell cytoplasm after infection. A number of important studies have used class III viruses as a source of pure mRNA.

Class IV viruses contain a single plus strand of genomic RNA, which is identical with the viral mRNA. Since the genomic RNA encodes proteins, it is infectious by itself. During replication of class IV viruses, the genomic RNA is copied into a minus strand, which then acts as a template for synthesis of more plus strands, or mRNA. Two types of class IV viruses are known. In class IVa viruses, typified by poliovirus, viral proteins are first synthesized, from a single mRNA species, as a long polypeptide chain, or *polyprotein,* which is then cleaved to yield the various functional proteins. Class IVb viruses synthesize at least two species of mRNA in a host cell. One of these mRNAs is the same length as the virion's genomic RNA; the other corresponds to the 3′ third of the genomic RNA. Both mRNAs are translated into polyproteins. Included in class IVb are a large number of rare insect-borne viruses including Sindbis virus and those causing yellow fever and viral encephalitis in human beings. These viruses once were called *arboviruses* (arthropod-borne viruses), but now are called *togaviruses* (from Latin *toga,* cover) because the virions are surrounded by a lipid envelope.

Class V viruses contain a single negative strand of genomic RNA, whose sequence is complementary to that of the viral mRNA. The genomic RNA in the virion acts as a template for synthesis of mRNA but does not itself encode proteins. Two types of class V viruses can be distinguished. The genome in class Va viruses, which include the viruses causing measles and mumps, is a single molecule of RNA. A virus-specific RNA polymerase present in the virion catalyzes synthesis of several mRNAs, each encoding a single protein, from the genomic template strand. Class Vb viruses, typified by influenza virus, have segmented genomes; each segment acts as a template for the synthesis of a different mRNA species. In most cases, each mRNA produced by a class Vb virus encodes a single protein; however, some mRNAs can be read in two different frames to yield two distinct proteins. As with class Va viruses, a class Vb virion contains a virus-specific polymerase that catalyzes synthesis of the viral mRNA. Thus the genomic RNA (a minus strand) in both types of class V viruses is not infectious in the absence of the virus-specific polymerase. The influenza RNA polymerase initiates synthesis of each mRNA by a unique mechanism. In the host-cell nucleus, the polymerase cuts off 12 – 15 nucleotides from the 5′ end of a cellular mRNA or mRNA precursor; this oligonucleotide acts as a "primer" that is elongated by the polymerase to form viral (+) mRNAs, using the genomic (−) RNA as a template.

Class VI viruses are enveloped viruses whose genome consists of two identical plus strands of RNA. These viruses are also known as **retroviruses** because their RNA genome directs the formation of a DNA molecule. The DNA molecule ultimately acts as the template for synthesis of viral mRNA (Figure 6-22). Initially, a viral enzyme called reverse transcriptase copies the viral RNA genome into a single minus strand of DNA; the same enzyme then catalyzes synthesis of a complementary plus strand. (This complex reaction is detailed in Chapter 9.) The resulting dsDNA is integrated into the chromosomal DNA of the infected cell. Finally, the integrated proviral DNA is transcribed by the cell's own machinery into (+) RNA, which either is translated into viral proteins or is packaged within virion coat proteins to form progeny virions, which are released by budding from the host-cell membrane. Because most retroviruses do not kill their host cells, infected cells can replicate, producing daughter cells with integrated proviral DNA. These daughter cells continue to transcribe the proviral DNA and bud progeny virions.

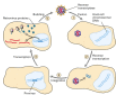

Figure 6-22

Retroviral life cycle. Retroviruses have two identical copies of a plus single-stranded RNA genome and an outer envelope containing protruding viral glycoproteins. After envelope glycoproteins on a virion

interact with a specific host-cell membrane protein (more...)

Some retroviruses contain cancer-causing genes (called **oncogenes**). Cells infected by such retroviruses are oncogenically transformed into tumor cells. Studies of oncogenic retroviruses (mostly viruses of birds and mice) have revealed a great deal about the processes that lead to oncogenic transformation. Among the known human retroviruses are human T-cell lymphotrophic virus (HTLV), which causes a form of leukemia, and human immunodeficiency virus (HIV), which causes acquired immune deficiency syndrome (AIDS). Both of these viruses can infect only specific cell types, primarily certain cells of the immune system and, in the case of HIV, some central nervous system neurons and glial cells. Only these cells have cell-surface receptors that interact with viral proteins, accounting for the host-cell specificity of these viruses.

Viral Vectors Can Be Used to Introduce Specific Genes into Cells

Knowledge about mechanisms of viral replication has allowed virologists to modify viruses for various purposes. For instance, the ability of virions to introduce their contents into the cytoplasm and nuclei of infected cells has been adapted for use in DNA cloning and offers possibilities in the treatment of certain diseases. The introduction of new genes into cells by packaging them into virion particles is called *viral gene transduction,* and the virions used for this purpose are called *viral vectors.*

By use of recombinant DNA techniques described in Chapter 7, it is a relatively straightforward process to construct human adenovirus *recombinants* in which potentially therapeutic genes replace the viral genes required for the lytic cycle of infection. Because adenovirus has a very broad host range for different types of human cells, these vectors can introduce the engineered gene into the cells of tissues where they are applied. If the transduced gene encodes the normal form of a protein that is missing or defective in a particular disease, then such *gene therapy* may successfully treat the disease. One type of adenovirus, for example, efficiently infects cells lining the air passages in the lungs, causing a type of common cold. Researchers have replaced some of the disease-causing genes in this adenovirus with the *CFTR* gene, which is defective in individuals with cystic fibrosis. This recombinant adenovirus currently is being used to introduce a normal *CFTR* gene into the airway-lining cells of cystic fibrosis patients. Unfortunately, with most of the adenovirus vectors currently available, the transduced gene usually is expressed only for a limited period of 2 to 3 weeks. This significantly limits their usefulness in gene therapy.

Viral vectors have also been developed from viruses that integrate their genomes into host-cell chromosomes. Such vectors have the advantage that progeny of the initially infected cell also contain and express the transduced gene because it is replicated and segregated to daughter cells along with the rest of the chromosome into which it is integrated. Retroviral vectors, which can efficiently integrate transduced genes at approximately random positions in host-cell chromosomes are now widely used experimentally to generate cultured cells expressing specific, desired proteins. However, technical limitations in producing the large numbers of retroviral vectors required to infect a significant fraction of cells in the tissues of a human or vertebrate currently limit their use as gene therapy vectors. Another concern with retroviral vectors is that their random integration might disturb the normal expression of cellular genes encoding proteins regulating cellular replication. This type of cellular gene deregulation occurs naturally following infection with certain retroviruses, such as avian leukosis virus and murine leukemia viruses, leading to development of leukemia in birds and mice, respectively.

Adeno-associated virus (AAV) is a “satellite” parvovirus that replicates only in cells that are co-infected with adenovirus or herpes simplex virus. When AAV infects human cells in the absence of these “helper” viruses, its ssDNA genome is copied into dsDNA by host-cell DNA polymerase and then is integrated into a single region on chromosome 19, where it does not have any known deleterious effects. Research is under way to adapt the AAV integration mechanism that operates in the absence of helper virus to the development of a safe and effective integrating viral vector.

SUMMARY

- Viruses are intracellular parasites that replicate only after infecting specific host cells. Viral infection begins when proteins on the surface of a virion bind to specific receptor proteins on the surface of host cells. The specificity of this interaction determines the host range of a virus.
- Aside from being the causative agents of many diseases, viruses are important tools in cell biology research, particularly in studies on macromolecular synthesis (see Table 6-3).
- Viruses can be counted and cloned by the plaque assay (see Figure 6-14). All the virions in a single plaque compose a clone derived from the single parental virion that infected the first cell at the center of the plaque.
- Individual viral particles (virions) generally contain either an RNA or a DNA genome, surrounded by multiple copies of one or a small number of coat proteins, forming the nucleocapsid. The nucleocapsid of many animal viruses is surrounded by a phospholipid bilayer, or envelope.
- During lytic replication, host-cell ribosomes and enzymes are used to express viral proteins, which then replicate the viral genome and package it into viral coats. The multiple progeny virions produced within a single infected cell eventually are released, following cell lysis or gradual disintegration of the cell (see Figure 6-16). Progeny nucleocapsids of enveloped viruses are released by budding of the host-cell membrane in which viral membrane proteins have been deposited (see Figure 6-17).
- Some bacterial viruses (bacteriophages) may undergo lysogeny following infection of host cells. In this case, the viral genome is integrated into host-cell chromosomes, forming a prophage that is replicated along with the host genome. When suitably activated, a prophage enters the lytic cycle (see Figure 6-19).
- All retroviruses and some other animal viruses can integrate their genomes into host-cell chromosomes (see Figure 6-22). In some cases, this leads to abnormal cell replication and the eventual development of cancers.
- Recombinant viruses can be used as vectors to carry (transduce) selected genes into cells. In this approach, viral genes required for the lytic cycle are replaced by other genes. The use of viral vectors for gene therapy is still in its infancy, but has great potential for treatment of various diseases.

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