9

Viruses and Virology

Bacterial viruses such as the *Escherichia coli* bacteriophage T4 have long been used as model systems for studying viral infection and replication processes.

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Viruses are genetic elements that cannot replicate independently of a living cell, called the **host cell**. However, viruses do possess their own genetic information and are thus independent of the host cell's genome. Viruses rely on the host cell for energy, metabolic intermediates, and protein synthesis. Viruses are therefore obligate intracellular parasites that rely on entering a suitable living cell to carry out their replication cycle. However, unlike genetic elements such as plasmids (Section 6.6), viruses have an extracellular form, the virus particle, that enables them to exist outside the host and that facilitates transmission from one host cell to another. To multiply, viruses must enter a cell in which they can replicate, a process called *infection*.

Viruses can replicate in a way that is destructive to the host cell, and this accounts for the fact that some viruses are agents of disease. We cover a number of human diseases caused by viruses in Chapters 33 and 34. However, viruses may also inhabit a cell and replicate in step with the cell without destroying it. Like plasmids and transposable elements, viruses may confer important new properties on their host cells. These properties will be inherited when the host cell divides if each new cell also inherits the viral genome. These changes are not always harmful and may even be beneficial.

The study of viruses is called *virology*, and we introduce the essentials of the field here. There are four parts in this chapter. The first part introduces basic concepts of virus structure, infection of the host cell, and how viruses can be detected and quantified. The second part deals with the basic molecular biology of virus replication. The third part provides an overview of some key viruses that infect bacteria and animals; further coverage of viral diversity can be found in Chapter 21. The fourth part deals with subviral entities.

Viruses outnumber the living cells on our planet by at least 10-fold, and infect all types of cellular organisms. Therefore, they are interesting in their own right. However, scientists also study viruses for what they reveal about the genetics and biochemistry of cellular processes and, for many viruses, the development of disease. Furthermore, as we shall see in Chapters 10 and 11, viruses are also important in microbial genetics and genetic engineering.

I Virus Structure and Growth

9.1 General Properties of Viruses

Although viruses are not cells and thus are nonliving, they nonetheless possess a genome encoding the information they need in order to replicate. However, viruses rely on host cells to provide the energy and materials needed for replicating their genomes and synthesizing their proteins. Consequently, viruses cannot replicate unless the virus genome has gained entry into a suitable host cell.

Viruses can exist in either extracellular or intracellular forms. In its extracellular form, a virus is a microscopic particle containing nucleic acid surrounded by a protein coat and sometimes, depending on the specific virus, other macromolecules. The virus particle, or **virion**, is metabolically inert and cannot generate energy or carry out biosynthesis. The virus genome moves from the cell in which it was produced to another cell inside the virion. Once in the new cell, the intracellular state begins and the virus replicates. New copies of the virus genome are produced, and the components of the virus coat are synthesized. Certain animal viruses (such as polio and respiratory syncytial virus) may skip the extracellular stage when moving from cell to cell within the same organism. Instead, they mediate the fusion of infected cells with uninfected cells and transfer themselves in this way. However, when moving from one organism to another they are truly extracellular.

Viral genomes are usually very small, and they encode primarily proteins whose functions viruses cannot usurp from their hosts. Therefore, during replication inside a cell, viruses depend heavily on host cell structural and metabolic components. The virus redirects host metabolic functions to support virus replication and the assembly of new virions. Eventually, new viral particles are released, and the process can repeat itself.

Viral Genomes

All cells contain double-stranded DNA genomes. By contrast, viruses have either DNA or RNA genomes. (One group of viruses does use both DNA and RNA as their genetic material but at different stages of their replication cycle.) Virus genomes can be classified according to whether the nucleic acid in the virion is DNA or RNA and further subdivided according to whether the nucleic acid is single- or double-stranded, linear, or circular (**Figure 9.1**). Some viral genomes are circular, but most are linear.

Although those viruses whose genome consists of DNA follow the central dogma of molecular biology (DNA → RNA → protein, Section 6.1), RNA viruses are exceptions to this rule. Nonetheless, genetic information still flows from nucleic acid to protein. Moreover, all viruses use the cell's translational machinery, and so regardless of the genome structure of the virus, messenger RNA (mRNA) must be generated that can be translated on the host cell ribosomes.

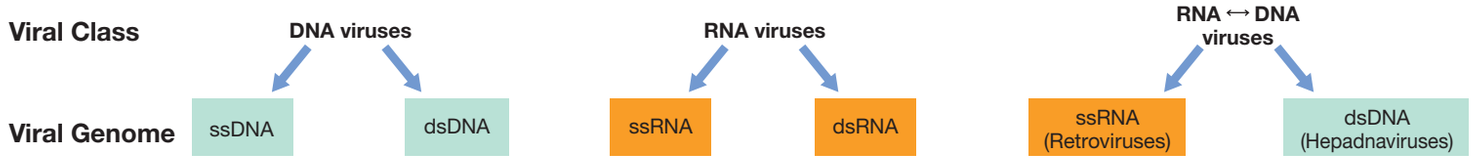


Figure 9.1 Viral genomes. The genomes of viruses can be either DNA or RNA, and some use both as their genomic material at different stages in their replication cycle. However, only one type of nucleic acid is found in the virion of any particular type of virus. This can be single-stranded (ss), double-stranded (ds), or in the hepadnaviruses, partially double-stranded. Some viral genomes are circular, but most are linear.

Viral Hosts and Taxonomy

Viruses can be classified on the basis of the hosts they infect as well as by their genomes. Thus, we have bacterial viruses, archaeal viruses, animal viruses, plant viruses, and viruses that infect other kinds of eukaryotic cells. Bacterial viruses, sometimes called **bacteriophages** (or phage for short; from the Greek *phagein*, meaning "to eat"), have been intensively studied as model systems for the molecular biology and genetics of virus replication. Species of both *Bacteria* and *Archaea* are infected by specific viruses. Indeed, many of the basic concepts of virology were first worked out with bacterial viruses and subsequently applied to viruses of higher organisms. Because of their frequent medical importance, animal viruses have been extensively studied, whereas plant viruses, although of enormous importance to modern agriculture, have been less well studied.

A formal system of viral classification exists that groups viruses into various taxa, such as orders, families, and even genus and species. The family taxon seems particularly useful. Members of a family of viruses all have a similar virion morphology, genome structure, and strategy of replication. Virus families have names that include the suffix *-viridae* (as in *Poxviridae*). We discuss a few of these in Chapter 21.

MiniQuiz

- How does a virus differ from a plasmid?
- How does a virion differ from a cell?
- What is a bacteriophage?
- Why does a virus need a host cell?

9.2 Nature of the Virion

Virions come in many sizes and shapes. Most viruses are smaller than prokaryotic cells, ranging in size from 0.02 to 0.3 μm (20–300 nm). A common unit of measure for viruses is the nanometer, which is one-thousandth of a micrometer. Smallpox virus, one of the largest viruses, is about 200 nm in diameter (about the size of the smallest cells of *Bacteria*). Poliovirus, one of the smallest viruses, is only 28 nm in diameter (about the size of a ribosome). Consequently, viruses could not be properly characterized until the invention of the electron microscope in the 1930s.

Viral genomes are smaller than those of most cells. Most bacterial genomes are between 1000 and 5000 kilobase pairs (kbp) of DNA, with the smallest known being about 500 kbp. (Interestingly, *Bacteria* with the smallest genomes are, like viruses, parasites that replicate in other cells; ↻ Table 12.1.) The largest known viral genome, that of *Mimivirus*, consists of 1.18 Mbp of double-stranded DNA. This virus, which infects protists such as *Amoeba*, is one of a few viruses currently known whose genome is larger than some cellular genomes. More typical virus genome sizes are listed in **Table 9.1**. Some viruses have genomes so small they contain fewer than five genes. Also, as can be seen in the table, the genome of some viruses, such as reovirus or influenza virus, is segmented into more than one molecule of nucleic acid.

Viral Structure

The structures of virions are quite diverse, varying widely in size, shape, and chemical composition. The nucleic acid of the virion is always located within the particle, surrounded by a protein shell called the **capsid**. This protein coat is composed of a number of

Table 9.1 *Some types of viral genomes*[a]

Virus	*Host*	*DNA or RNA*	*Single- or double-stranded*	*Viral genome*		*Size (bases or base pairs)*[a]
				Structure	*Number of molecules*	
H-1 parvovirus	Animals	DNA	Single-stranded	Linear	1	5,176
ϕX174	*Bacteria*	DNA	Single-stranded	Circular	1	5,386
Simian virus 40 (SV40)	Animals	DNA	Double-stranded	Circular	1	5,243
Poliovirus	Animals	RNA	Single-stranded	Linear	1	7,433
Cauliflower mosaic virus	Plants	DNA	Double-stranded	Circular	1	8,025
Cowpea mosaic virus	Plants	RNA	Single-stranded	Linear	2 different	9,370 (total)
Reovirus type 3	Animals	RNA	Double-stranded	Linear	10 different	23,549 (total)
Bacteriophage lambda	*Bacteria*	DNA	Double-stranded	Linear	1	48,514[b]
Herpes simplex virus type I	Animals	DNA	Double-stranded	Linear	1	152,260
Bacteriophage T4	*Bacteria*	DNA	Double-stranded	Linear	1	168,903
Human cytomegalovirus	Animals	DNA	Double-stranded	Linear	1	229,351

[a]The size is in bases or base pairs depending on whether the virus is single- or double-stranded. The sizes of the viral genomes chosen for this table are known accurately because they have been sequenced. However, this accuracy can be misleading because only a particular strain or isolate of a virus was sequenced. Therefore, the sequence and exact number of bases for other isolates may be slightly different. No attempt has been made to choose the largest and smallest viruses known, but rather to give a fairly representative sampling of the sizes and structures of the genomes of viruses containing both single- and double-stranded RNA and DNA.
[b]This includes single-stranded extensions of 12 nucleotides at either end of the linear form of the DNA (see Section 9.10).

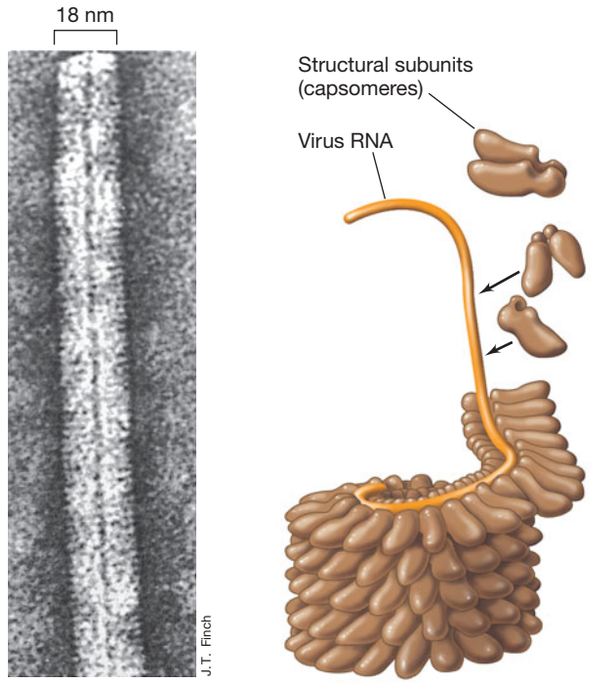


Figure 9.2 **The arrangement of nucleic acid and protein coat in a simple virus, tobacco mosaic virus.** *(a)* A high-resolution electron micrograph of a portion of the virus particle. *(b)* Assembly of the tobacco mosaic virus virion. The RNA assumes a helical configuration surrounded by the protein capsid. The center of the particle is hollow.

individual protein molecules, which are arranged in a precise and highly repetitive pattern around the nucleic acid (**Figure 9.2**).

The small genome size of most viruses restricts the number of different viral proteins that can be encoded. A few viruses have only a single kind of protein in their capsid, but most viruses have several distinct proteins that are associated in specific ways to form assemblies called **capsomeres** (Figure 9.2). The capsomere is the smallest morphological unit that can be seen with the electron microscope. A single virion can have a large number of capsomeres. The information for proper folding and assembly of the proteins into capsomeres is typically contained within the structure of the proteins themselves; hence, the overall process of virion assembly is called *self-assembly*. However, occasional virus proteins, such as the lambda capsid protein, require help from the chaperonin GroE (↻ Section 6.21).

The complete complex of nucleic acid and protein packaged in the virion is called the virus **nucleocapsid**. Inside the virion are often one or more virus-specific enzymes. Such enzymes play a role during the infection and replication processes, as discussed later in this chapter. Some viruses are *naked*, whereas others possess lipid-containing layers around the nucleocapsid called an *envelope* (**Figure 9.3**).

Virus Symmetry

The nucleocapsids of viruses are constructed in highly symmetric ways. Symmetry refers to the way in which the capsomeres are arranged in the virus capsid. When a symmetric structure is

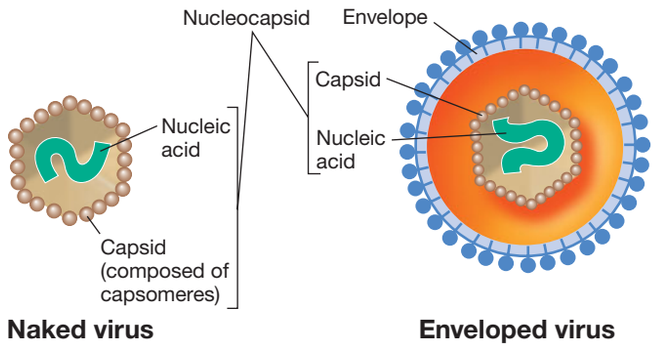


Figure 9.3 Comparison of naked and enveloped virus particles.

rotated around an axis, the same form is seen again after a certain number of degrees of rotation. Two kinds of symmetry are recognized in viruses, which correspond to the two primary shapes, rod and spherical. Rod-shaped viruses have *helical* symmetry, and spherical viruses have *icosahedral* symmetry. In all cases, the characteristic structure of the virus is determined by the structure of the capsomeres of which it is constructed.

A typical virus with helical symmetry is the tobacco mosaic virus (TMV) illustrated in Figure 9.2. It is an RNA virus in which the 2130 identical capsomeres are arranged in a helix. The overall dimensions of the TMV virion are 18 × 300 nm. The lengths of helical viruses are determined by the length of the nucleic acid, but the width of the helical virion is determined by the size and packaging of the capsomeres.

An **icosahedron** is a symmetric structure containing 20 triangular faces and 12 vertices and is roughly spherical in shape (**Figure 9.4**). Icosahedral symmetry is the most efficient arrangement of subunits in a closed shell because it uses the smallest number of capsomeres to build the shell. The simplest arrangement of capsomeres is three per face, for a total of 60 capsomeres per virion. Most viruses have more nucleic acid than can be packed into a shell made of just 60 capsomeres. The next possible structure that permits close packing contains 180 capsomeres, and many viruses have shells with this configuration. Other common configurations contain 240 or 420 capsomeres.

Figure 9.4*a* shows a model of an icosahedron. Figure 9.4*b* shows the same icosahedron viewed from three different angles to illustrate its complex 5-3-2 symmetry. The axes of symmetry divide the icosahedron into segments (5, 3, or 2) of identical size and shape. Figure 9.4*c* shows an electron micrograph of a typical icosahedral virus, human papillomavirus; this virus contains 360 capsomeres clustered into groups of five. Figure 9.4*d* shows a computer model of the same virus, where the five-capsomere clusters are more easily seen.

Enveloped Viruses

Enveloped viruses contain a membrane surrounding the nucleocapsid (**Figure 9.5*a***). Many viruses are enveloped, and most of these infect animal cells (for example, influenza virus), although occasional enveloped bacterial and plant viruses are also known. The viral envelope consists of a lipid bilayer with proteins, usually glycoproteins, embedded in it. The lipids of the viral membrane

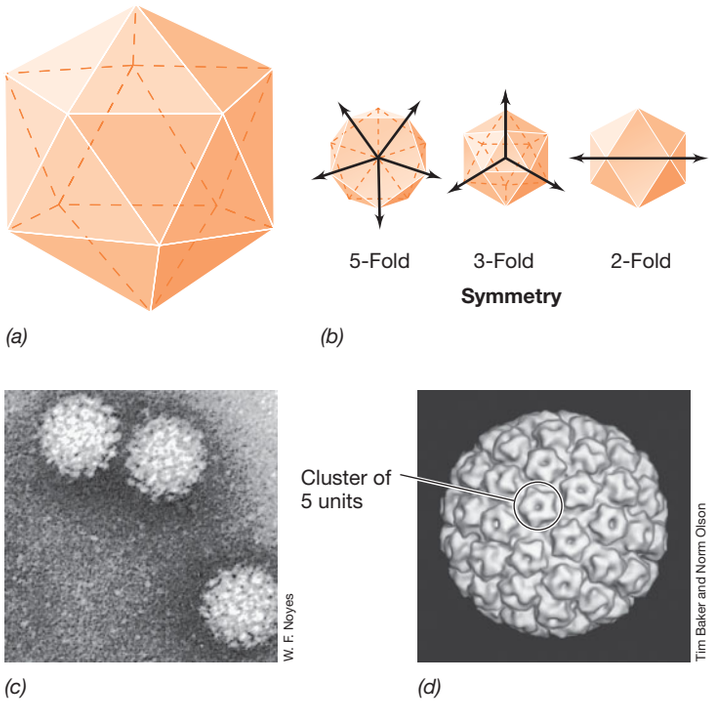


Figure 9.4 **Icosahedral symmetry.** *(a)* A model of an icosahedron. *(b)* Three views of an icosahedron showing the 5-3-2 symmetry. *(c)* Electron micrograph of human papillomavirus, a virus with icosahedral symmetry. The virion is about 55 nm in diameter. *(d)* Three-dimensional reconstruction of human papillomavirus calculated from images of frozen hydrated virions. The virus contains 360 units arranged in 72 clusters of 5 each.

are derived from the membranes of the host cell, but viral membrane proteins that are encoded by viral genes are also embedded in the membrane. The symmetry of enveloped viruses is not expressed by the virion as a whole, but by the nucleocapsid present inside the virus envelope.

Note that the envelope is the component of the virion that makes initial contact with the host cell. The specificity of virus infection and some aspects of virus penetration are thus controlled in part by characteristics of virus envelopes. The virus-specific envelope proteins are critical for attachment of the virion to the host cell during infection or for release of the virion from the host cell after replication.

Complex Viruses

Some virions are even more complex than anything discussed so far, being composed of several parts, each with separate shapes and symmetries. The most complicated viruses in terms of structure are some of the bacterial viruses, which possess icosahedral heads plus helical tails. In some bacterial viruses, such as bacteriophage T4 of *Escherichia coli* (Figure 9.5*b*), the tail itself has a complex structure. The complete T4 tail has almost 20 different proteins, and the T4 head has several more proteins. In such complex viruses, assembly is also quite involved. For instance, in T4 the complete tail is formed as a subassembly, and then the tail is added to the DNA-containing head. Finally, tail fibers formed from another protein are added to make the mature, infectious virion.

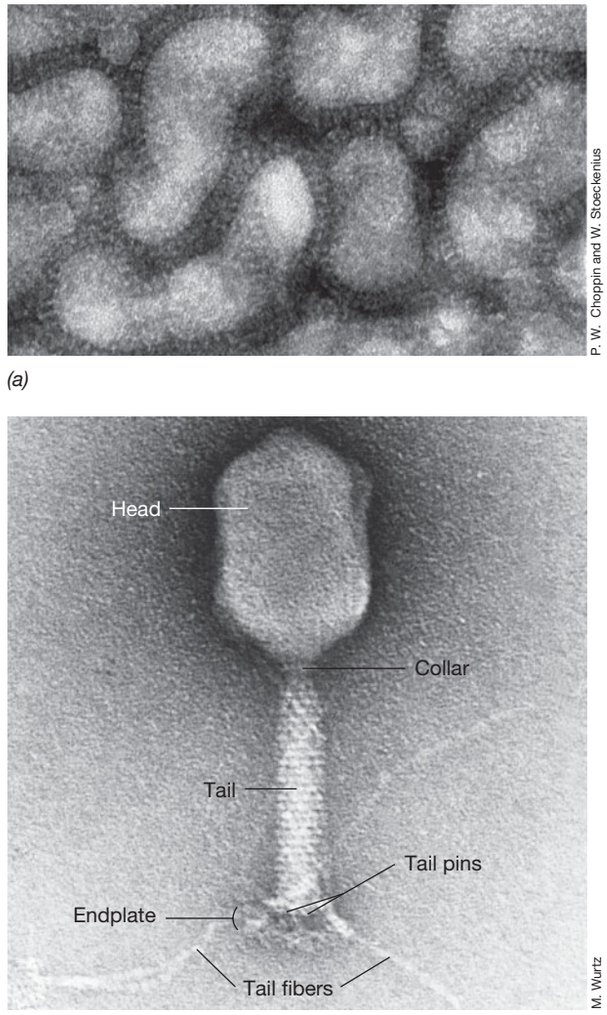


Figure 9.5 **Electron micrographs of animal and bacterial viruses.** *(a)* Influenza virus, an enveloped virus. The virions are about 80 nm in diameter, but have no defined shape (↻ Section 21.9). *(b)* Bacteriophage T4 of *Escherichia coli*. The tail components function in attachment of the virion to the host and injection of the nucleic acid (Figure 9.10). The head is about 85 nm in diameter.

Enzymes in Virions

Virions do not carry out metabolic processes and thus a virus is metabolically inert outside a host cell. However, some virions do contain enzymes that play important roles in infection. Some of these enzymes are required for very early events in the infection process. For example, some bacteriophages contain the enzyme lysozyme (↻ Section 3.6), which they use to make a small hole in the bacterial cell wall. This allows the virus to inject its nucleic acid into the cytoplasm of the host cell. Lysozyme is again produced in large amounts in the later stages of infection, causing lysis of the bacterial cell and release of the new virions.

Many viruses contain their own nucleic acid polymerases for replication of the viral genome and for transcription of virus-specific RNA. For example, retroviruses are RNA viruses that

replicate via DNA intermediates. These viruses possess an RNA-dependent DNA polymerase called *reverse transcriptase* that transcribes the viral RNA to form a DNA intermediate. Other viruses contain RNA genomes and require their own RNA polymerase. These virion enzymes are necessary because cells cannot make DNA or RNA from an RNA template (Sections 6.8 and 6.12).

Some viruses contain enzymes that aid in their release from the host. For example, certain animal viruses contain surface proteins called neuraminidases, enzymes that cleave glycosidic bonds in glycoproteins and glycolipids of animal cell connective tissue, thus liberating the virions. Although most virions lack their own enzymes, those that contain them do so for good reason: The host cell would not be able to produce virions in the absence of these extra enzymes.

MiniQuiz

- What is the difference between a naked virus and an enveloped virus?
- What kinds of enzymes can be found within the virions of specific viruses?

9.3 The Virus Host

Because viruses replicate only inside living cells, the cultivation of viruses requires the use of appropriate hosts. Viruses infecting prokaryotes are typically the easiest to grow in the laboratory. For the study of bacterial viruses, pure cultures are used either in liquid or on semisolid (agar) media. Most animal viruses and many plant viruses can be cultivated in tissue or cell cultures, and the use of such cultures has enormously facilitated research on these viruses. Plant viruses can be more difficult to work with, because their study sometimes requires use of the whole plant. This is a problem because plants grow much slower than bacteria, and plant viruses also often require a break in the thick plant cell wall in order to infect.

Animal cell cultures are derived from cells originally taken from an organ of an experimental animal. Unless blood cells are used, cell cultures are usually obtained by aseptically removing pieces of tissue and dissociating the cells by treatment with an enzyme that degrades the extracellular material that holds animal cells together. The resulting cell suspension is spread over a flat surface, such as the bottom of a culture flask or a Petri dish. The thin layer of cells adhering to the glass or plastic dish, called a *monolayer*, is overlaid with a suitable culture medium and incubated at a suitable temperature. The culture media used for cell cultures are typically quite complex, containing a number of amino acids and vitamins, salts, glucose, and a bicarbonate buffer system. To obtain the best growth, addition of a small amount of blood serum is usually necessary to provide vital nutrients, and several antibiotics are added to prevent bacterial contamination.

Some cell cultures prepared in this way can be subcultured and grown indefinitely as *permanent cell lines*. Cell lines are convenient for virus research because cell material is continuously available. In many cases, a culture will not grow indefinitely, but may remain alive for a number of days. Such cultures, called *primary cell cultures*, may still be useful for growing a virus, although new cultures need to be prepared from fresh sources from time to time, an expensive and time-consuming process. In some cases, primary or permanent cell lines cannot be obtained, but whole organs or pieces of organs can successfully replicate the virus. Such organ cultures may still be useful in virus research because they permit growth of viruses under more or less controlled laboratory conditions.

MiniQuiz

- In virology, what is a host?
- Why is it helpful to use cell culture for viral research?

9.4 Quantification of Viruses

In virology it is often necessary to quantify the number of virions in a suspension. Although one can count virions using an electron microscope (Figures 9.4*c* and 9.5*a*), the number of virions in a suspension can be more easily quantified by measuring their effects on the host. Using such a method, we see that a virus infectious unit is the smallest unit that causes a detectable effect when added to a susceptible host. This can be as few as one virion, although a larger inoculum is more often required. By determining the number of infectious units per volume of fluid, a measure of virus quantity, called a *titer*, can be obtained.

Plaque Assay

When a virion initiates an infection on a layer of host cells growing on a flat surface, a zone of lysis may be seen as a clear area in the layer of growing host cells. This clearing is called a **plaque**, and it is assumed that each plaque originated from the replication of a single virion (**Figure 9.6**).

With bacteriophages, plaques may be obtained when virions are mixed into a small volume of melted agar containing host bacteria that is spread on the surface of an agar medium (Figure 9.6*a*). During incubation the bacteria grow and form a turbid layer that is visible to the naked eye. However, wherever a successful viral infection has been initiated, cells are lysed, forming a plaque (Figure 9.6*b*). By counting the number of *plaque-forming units*, one can calculate the titer, or number of virus infectious units, present in the virus sample.

The plaque assay also permits the isolation of pure virus strains. This is because if a plaque has arisen from a single virion, all the viruses in this plaque should be genetically identical. Some of the virions from this plaque can be picked and inoculated into a fresh bacterial culture to establish a pure virus line. The development of the plaque assay technique was as important for the advancement of virology as Koch's development of solid media (Section 1.8) was for pure culture microbiology.

Plaques may be obtained for animal viruses by using cultured animal cells as hosts. A monolayer of cultured animal cells is prepared on a plate or flat bottle and the virus suspension is overlaid.

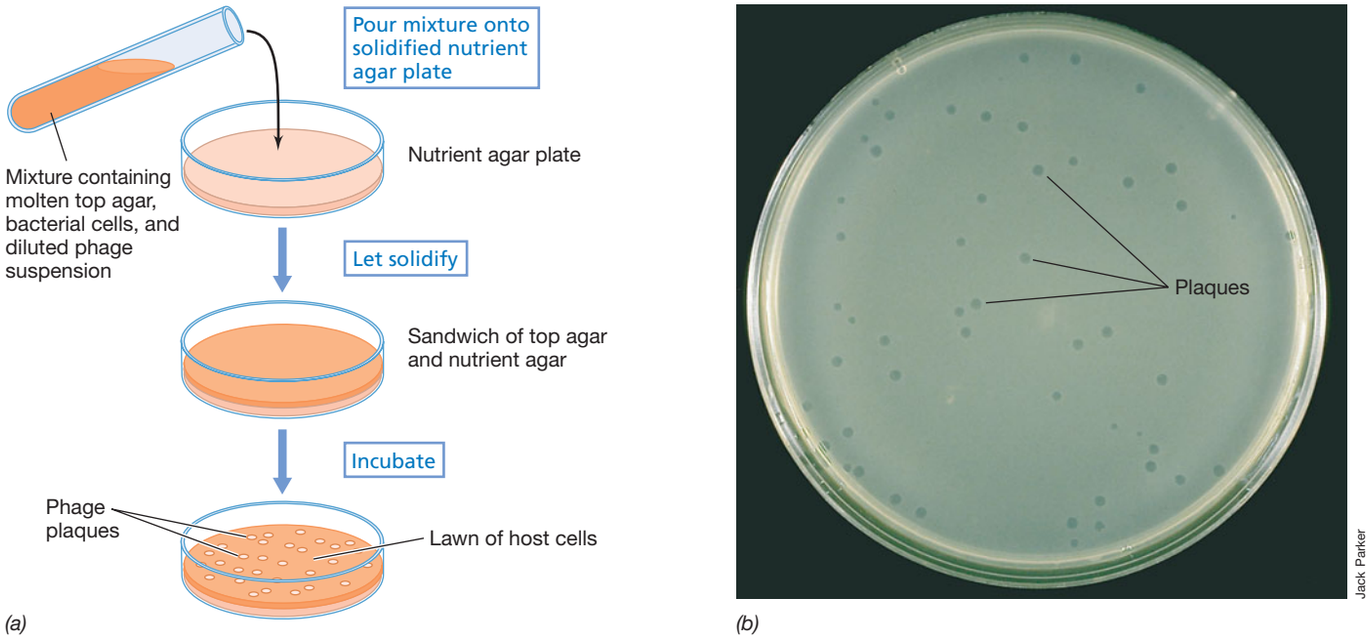


Figure 9.6 Quantification of bacterial virus by plaque assay using the agar overlay technique. *(a)* A dilution of a suspension containing the virus is mixed in a small amount of melted agar with the sensitive host bacteria. The mixture is poured on the surface of an agar plate of the appropriate medium. The host bacteria, which have been spread uniformly throughout the top agar layer, begin to grow, and after overnight incubation form a lawn of confluent growth. Virion-infected cells are lysed, forming plaques in the lawn. The size of the plaque depends on the virus, the host, and conditions of culture. *(b)* Photograph of a plate showing plaques formed by a bacteriophage on a lawn of sensitive bacteria. The plaques shown are about 1–2 mm in diameter.

Plaques are revealed by zones of destruction of the animal cells, and from the number of plaques produced, an estimation of the virus titer can be made (**Figure 9.7**).

Efficiency of Plating

The concept of *efficiency of plating* is important in quantitative virology. In any given viral system, the number of plaque-forming units is always lower than counts of the viral suspension made with an electron microscope. The efficiency with which virions infect host cells is thus rarely 100% and may often be considerably less. Virions that fail to cause infection are often inactive, although this is not always the case. Some viruses produce many incomplete virions during infection. In other cases, especially with RNA viruses, the viral mutation rate is so high that many virions contain defective genomes. However, sometimes a low efficiency of plating merely means that under the conditions used, some virions did not successfully infect cells. Although with bacterial viruses the efficiency of plating is often higher than 50%, with many animal viruses it may be much lower, 0.1% or 1%. Knowledge of plating efficiency is useful in cultivating viruses because it allows one to estimate how concentrated a viral suspension needs to be (that is, its titer) to yield a certain number of plaques.

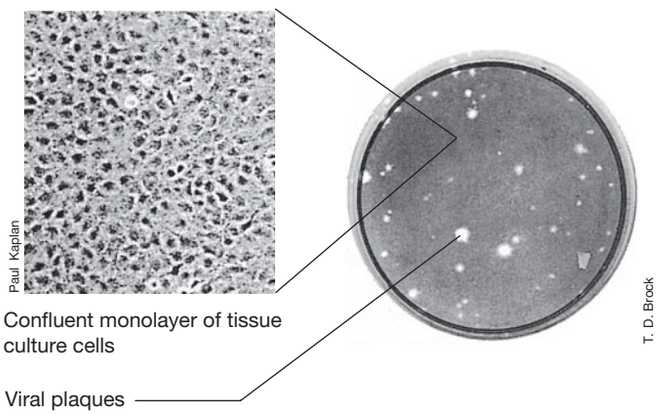


Figure 9.7 Cell cultures in monolayers grown on a Petri plate. Note the presence of plaques. Also shown is a photomicrograph of a cell culture.

Intact Animal Methods

Some viruses do not cause recognizable effects in cell cultures yet cause death in whole animals. In such cases, quantification can be done only by titration in infected animals. The general procedure is to carry out a serial dilution of the virus sample (↻ Section 5.10), generally at 10-fold dilutions, and to inject samples of each dilution into several sensitive animals. After a suitable incubation period, the fraction of dead and live animals at each dilution is tabulated and an end point dilution is calculated. This is the dilution at which, for example, half of the injected animals die (the lethal dose for 50% or LD_{50}, ↻ Section 27.8).

Although using whole animals is much more cumbersome and much less accurate than cell culture methods, it may be essential for the study of certain types of viruses.

MiniQuiz

- Give a definition of efficiency of plating.
- What is a plaque-forming unit?

II Viral Replication

9.5 General Features of Virus Replication

For a virus to replicate it must induce a living host cell to synthesize all the essential components needed to make more virions. These components must then be assembled into new virions that are released from the cell. The viral replication cycle can be divided into five steps (**Figure 9.8**).

1. *Attachment* (adsorption) of the virion to a susceptible host cell.
2. *Penetration* (entry, injection) of the virion or its nucleic acid into the host cell.
3. *Synthesis* of virus nucleic acid and protein by host cell metabolism as redirected by the virus.
4. *Assembly* of capsids (and membrane components in enveloped viruses) and *packaging* of viral genomes into new virions. This whole process is called *maturation*.
5. *Release* of mature virions from the cell.

The growth curve resulting from these stages of virus replication is illustrated in **Figure 9.9**. In the first few minutes after infection the virus is said to undergo an *eclipse*. During this period infectious particles cannot be detected in the culture medium. The eclipse begins as soon as infectious particles are removed from the environment by adsorbing to host cells. Once attached to host cells, the virions are no longer available to infect other cells. This is followed by the entry of viral nucleic acid (or intact virion) into the host cell. If the infected cell breaks open at this point, the virion no longer exists as an infectious entity since the viral genome is no longer inside its capsid. *Maturation* begins as the newly synthesized nucleic acid molecules become packaged inside protein coats. During the maturation phase, the titer of active virions inside the host cell rises dramatically. However, the new virus particles still cannot be detected in the culture medium unless the cells are artificially lysed to release them. Because newly synthesized virions have not yet appeared outside the cell, the eclipse and maturation periods together are called the *latent period*.

At the end of maturation, mature virions are released, either as a result of cell lysis or by budding or excretion, depending on the virus. The number of virions released, called the *burst size*, varies with the particular virus and the particular host cell, and can range from a few to a few thousand. The duration of the virus replication cycle varies from 20–60 min (in many bacterial viruses) to 8–40 h (in most animal viruses). Because the release of virions is more or less simultaneous, virus replication

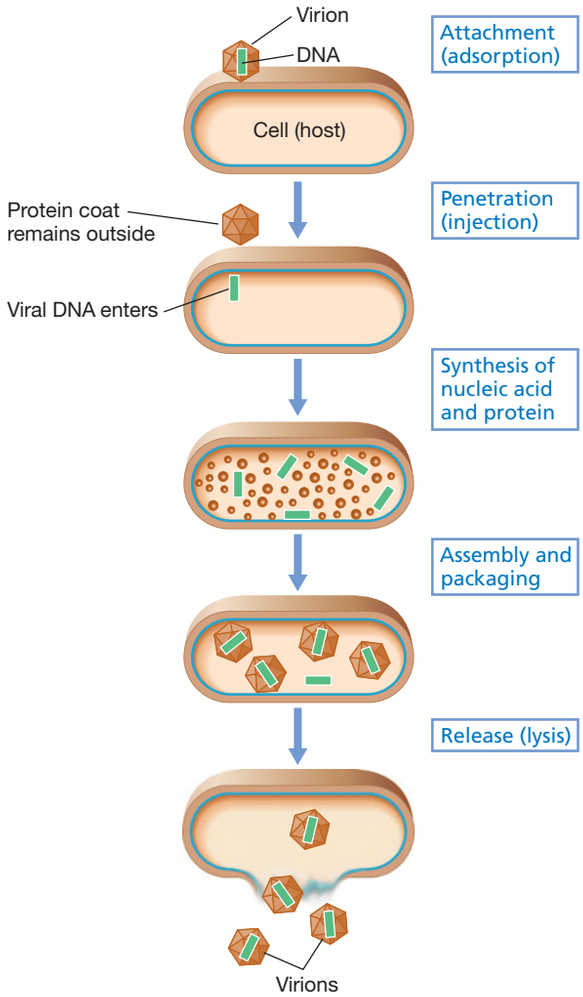


Figure 9.8 The replication cycle of a bacterial virus. Note that the viruses and cell are not drawn to scale.

is typically characterized by a *one-step growth curve* (Figure 9.9). In the next two sections we consider a few key steps of the virus replication cycle in more detail.

MiniQuiz

- What is packaged into the virions?
- Explain the term maturation.
- What events happen during the latent period of viral replication?

9.6 Viral Attachment and Penetration

In this section we focus on virus attachment and penetration, the first steps in the viral life cycle. In addition, we consider the mechanism by which some bacteria react to penetration by bacteriophage DNA.

Attachment

The most common basis for the host specificity of a virus depends upon attachment. The virion itself (whether naked or enveloped) has one or more proteins on its external surface that interact with

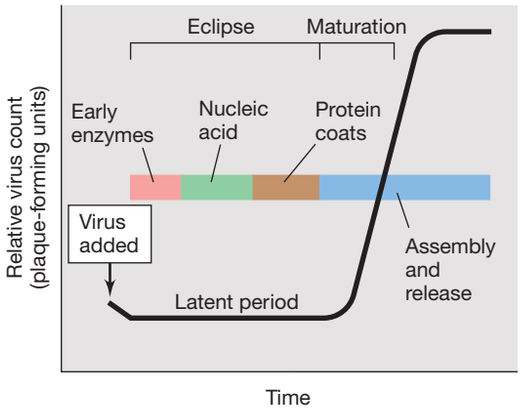


Figure 9.9 **The one-step growth curve of virus replication.** This graph displays the results of a single round of viral replication in a population of cells. Following adsorption, the infectivity of the virus particles disappears, a phenomenon called *eclipse*. This is due to the uncoating of the virus particles. During the latent period, viral nucleic acid replicates and protein synthesis occurs. The maturation period, when virus nucleic acid and protein are assembled into mature virus particles, follows. Finally, the virions are released, either with or without cell lysis. This general picture is amplified for bacteriophage T4 in Figure 9.15.

specific host cell surface components called receptors. These receptors are normal surface components of the host, such as proteins, carbohydrates, glycoproteins, lipids, lipoproteins, or complexes of these, to which the virion attaches. The receptors carry out normal functions for the cell. For example, the receptor for bacteriophage T1 is an iron-uptake protein and that for bacteriophage lambda is involved in maltose uptake. Animal virus receptors may include macromolecules needed for cell–cell contact or by the immune system. For example, the receptors for poliovirus and for HIV are normally used in interactions between human cells.

In the absence of its specific receptor, the virus cannot adsorb and hence cannot infect. Moreover, if the receptor is altered, for example, by mutation, the host may become resistant to virus infection. However, mutants of the virus can also arise that gain the ability to adsorb to previously resistant hosts. In addition, some animal viruses may be able to use more than one receptor, so the loss of one may not necessarily prevent attachment. Thus, the host range of a particular virus is, to some extent, determined by the availability of a suitable receptor that the virus can recognize. In multicellular organisms, cells in different tissues or organs often express different proteins on their cell surfaces. Consequently, viruses that infect animals often infect only cells of certain tissues. For example, many viruses that cause coughs and colds infect only cells of the upper respiratory tract.

Penetration

The attachment of a virus to its host cell results in changes to both the virus and the host cell surface that result in penetration. Viruses must replicate within cells. Therefore, at a minimum, the viral genome must enter the cell (Figure 9.8). Entry of the virus genome into a susceptible cell will not lead to virus replication if the information in the viral genome cannot be read. Consequently, as we mentioned (Section 9.2), for some viruses to replicate, certain viral proteins must also enter the host cell. A cell that allows the complete replication cycle of a virus to take place is said to be *permissive* for that virus.

Different viruses have different strategies for penetration. Uncoating refers to the process in which the virions lose their outer coat and the viral genome is exposed. Some enveloped animal viruses are uncoated at the cytoplasmic membrane, releasing the virion contents into the cytoplasm. However, the entire virion of naked animal viruses and many enveloped animal viruses enters the cell via endocytosis. In such cases the virus must be uncoated inside the host cell so that the genome is exposed and replication can proceed. Some enveloped viruses are uncoated in the cytoplasm. Others (such as influenza) are uncoated at the nuclear membrane and the viral genome then enters the nucleus. In animal cells, wherever uncoating occurs, the viral genome must eventually enter the nucleus to be replicated, except in a few rare cases.

Tailed Bacteriophage Attachment and Penetration

Cells that have cell walls, such as most bacteria, are infected in a manner different from animal cells, which lack cell walls. The most complex penetration mechanisms have been found in viruses that infect bacteria. The bacteriophage T4, which infects *Escherichia coli*, is a good example.

The structure of bacteriophage T4 was shown in Figure 9.5*b*. The virion has a head, within which the viral linear double-stranded DNA is folded, and a long, fairly complex tail, at the end of which is a series of tail fibers and tail pins. The T4 virions first attach to *E. coli* cells by means of the tail fibers (**Figure 9.10**). The ends of the fibers interact specifically with polysaccharides that are part of the outer layer of the gram-negative cell envelope

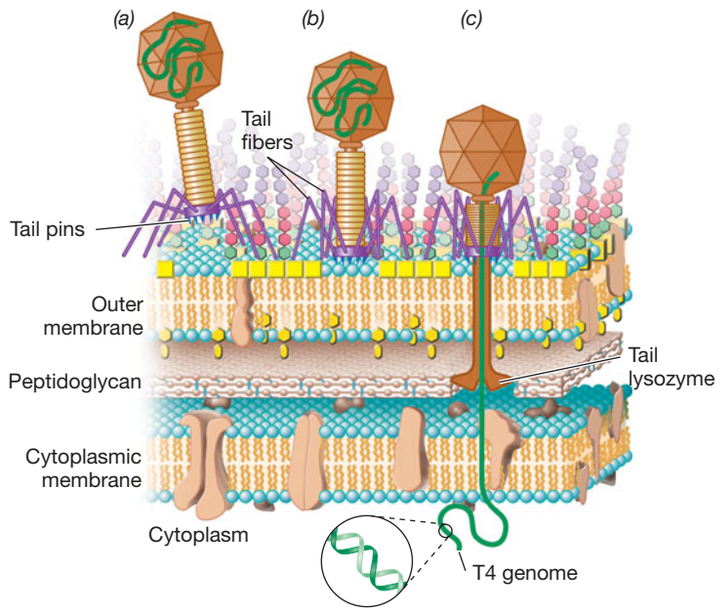


Figure 9.10 **Attachment of bacteriophage T4 to the cell wall of *Escherichia coli* and injection of DNA.** *(a)* Attachment of a T4 virion to the cell wall by the long tail fibers interacting with core lipopolysaccharide. *(b)* Contact of cell wall by the tail pins. *(c)* Contraction of the tail sheath and injection of the T4 genome. The tail tube penetrates the outer membrane, and the tail lysozyme digests a small opening through the peptidoglycan layer.

(↻ Section 3.7). These tail fibers then retract, and the core of the tail makes contact with the cell wall of the bacterium through a series of fine tail pins at the end of the tail. The activity of a lysozyme-like enzyme forms a small pore in the peptidoglycan layer. The tail sheath then contracts, and the viral DNA passes into the cytoplasm of the host cell through a hole in the tip of the phage tail, with the majority of the coat protein remaining outside (Figure 9.10).

Virus Restriction and Modification by the Host

Animals can often eliminate invading viruses by immune defense mechanisms before the viral infection becomes widespread or sometimes even before the virus has penetrated target cells. In addition, eukaryotes, including animals and plants, possess an antiviral mechanism known as RNA interference (↻ Section 7.10). Although they lack immune systems, both *Bacteria* and *Archaea* possess an antiviral mechanism similar to RNA interference, known as CRISPR (↻ Chapter 8, Microbial Sidebar). In addition, prokaryotes destroy double-stranded viral DNA after it has been injected by using restriction endonucleases (↻ Section 11.1), enzymes that cleave foreign DNA at specific sites, thus preventing its replication. This phenomenon is called *restriction* and is part of a general host mechanism to prevent the invasion of foreign nucleic acid. For such a system to be effective, the host must have a mechanism for protecting its own DNA. This is accomplished by specific modification of its DNA at the sites where the restriction enzymes cut (↻ Section 11.1).

Restriction enzymes are specific for double-stranded DNA, and thus single-stranded DNA viruses and all RNA viruses are unaffected by restriction systems. Although host restriction systems confer significant protection, some DNA viruses have overcome host restriction by modifying their own DNA so that they are no longer subject to restriction enzyme attack. Two patterns of chemical modification of viral DNA are known: glucosylation and methylation. For instance, the T-even bacteriophages (T2, T4, and T6) have their DNA glucosylated to varying degrees, which prevents endonuclease attack. Many other viral DNAs can be modified by methylation. However, whether glucosylated or methylated, viral DNAs are modified after genomic replication has occurred by modification proteins encoded by the virus.

Other viruses, such as the bacteriophages T3 and T7, avoid destruction by host restriction enzymes by encoding proteins that inhibit the host restriction systems. To counter this, some bacteria have multiple restriction and methylation systems that help prevent infection by viruses that can circumvent only one of them. Bacteria also contain other DNA methylases in addition to those that protect them from their own restriction enzymes. Some of these methylases take part in DNA repair or in gene regulation, but others protect the host DNA from foreign endonucleases. This is necessary because some viruses encode restriction systems themselves that are designed to destroy host DNA! It is thus clear that viruses and hosts have responded to each other's defense mechanisms by continuing to evolve their own mechanisms to better their chances of infection or survival, respectively.

MiniQuiz

- How does the attachment process contribute to virus–host specificity?
- Why do some viruses need to be uncoated after penetration and others do not?

UNIT 4

9.7 Production of Viral Nucleic Acid and Protein

Once a host has been infected, new copies of the viral genome must be made and virus-specific proteins must be synthesized in order for the virus to replicate. Typically, the production of at least some viral proteins begins very early after the viral genome has entered the cell. The synthesis of these proteins requires viral mRNA. For certain types of RNA viruses, the genome itself is the mRNA. For most viruses, however, the mRNA must first be transcribed from the DNA or RNA genome and then the genome must be replicated. We consider these important events here.

The Baltimore Classification Scheme and DNA Viruses

The virologist David Baltimore, who along with Howard Temin and Renato Dulbecco shared the Nobel Prize for Physiology or Medicine in 1975 for the discovery of retroviruses and reverse transcriptase, developed a classification scheme for viruses. The Baltimore classification scheme (**Table 9.2**) is based on the relationship of the viral genome to its mRNA and recognizes

Table 9.2 *The Baltimore classification system of viruses*

		Examples	
Class	*Description of genome and replication strategy*	*Bacterial viruses*	*Animal viruses*
I	Double-stranded DNA genome	Lambda, T4	Herpesvirus, pox virus
II	Single-stranded DNA genome	ϕX174	Chicken anemia virus
III	Double-stranded RNA genome	ϕ6	Reoviruses (↻ Section 21.10)
IV	Single-stranded RNA genome of plus configuration	MS2	Poliovirus
V	Single-stranded RNA genome of minus configuration		Influenza virus, rabies virus
VI	Single-stranded RNA genome that replicates with DNA intermediate		Retroviruses
VII	Double-stranded DNA genome that replicates with RNA intermediate		Hepatitis B virus

seven classes of viruses. Double-stranded (ds) DNA viruses are in class I. The mechanism of mRNA production and genome replication of class I viruses is the same as that used by the host cell genome, although different viruses use different strategies to ensure that viral mRNA is expressed in preference to host mRNA.

Class II viruses are single-stranded (ss) DNA viruses. Before mRNA can be produced from such viruses, a complementary DNA strand must be synthesized because RNA polymerase uses double-stranded DNA as a template (Section 6.8). These viruses form a dsDNA intermediate during replication that is also used for transcription (**Figure 9.11**). The synthesis of the dsDNA intermediate and its subsequent transcription can be carried out by cellular enzymes (although viral proteins may also be required). The dsDNA intermediate is also used to generate the viral genome; one strand becomes the genome while the other is discarded (Figure 9.11). Until recently, all known ssDNA viruses contained positive-strand DNA, which has the same sequence as their mRNA (see positive-strand viruses below). However, a novel virus is now known that contains circular ssDNA of negative polarity. Torque teno virus (TTV), as this virus is called, is widespread in humans and other animals but causes no obvious disease symptoms. The mode of replication of TTV has not yet been fully investigated.

Positive- and Negative-Strand RNA Viruses

The production of mRNA and genome replication is different for RNA viruses (classes III–VI). Recall that mRNA is complementary in base sequence to the template strand of DNA. By convention in virology, mRNA is of the plus (+) configuration. Its complement is thus of the minus (−) configuration. This convention is used to describe the genome of a single-stranded virus, whether its genome contains RNA or DNA (Figure 9.11). For example, a virus that has a ssRNA genome with the same orientation as its mRNA is a **positive-strand RNA virus**, while a virus whose ssRNA genome is complementary to its mRNA is a **negative-strand RNA virus**.

Cellular RNA polymerases do not normally catalyze the formation of RNA from an RNA template, but instead require a DNA template. Therefore, RNA viruses, whether positive, negative, or double-stranded, require a specific RNA-dependent RNA polymerase. The simplest case is the positive-strand RNA viruses (class IV) in which the viral genome is of the plus configuration and hence can function directly as mRNA (Figure 9.11). In addition to other required proteins, this mRNA encodes a virus-specific RNA-dependent RNA polymerase (also called RNA replicase). Once synthesized, this polymerase first makes complementary minus strands of RNA and then uses them as templates to make more plus strands. These plus strands can either be translated as mRNA or packaged as the genome in newly synthesized virions (Figure 9.11).

For negative-strand RNA viruses (class V), the situation is more awkward. The incoming RNA is the wrong polarity to serve as mRNA, and therefore mRNA must be synthesized first. Because cells do not have an RNA polymerase capable of this, these viruses must carry some of this enzyme in their virions, and the enzyme enters the cell along with the genomic RNA. The complementary plus strand of RNA is synthesized by this RNA-dependent RNA polymerase and is then used as mRNA. This plus-strand mRNA is also used as a template to make more negative-strand genomes (Figure 9.11). The dsRNA viruses (class III) face a similar problem. Although the virion does contain plus-strand RNA, this is part

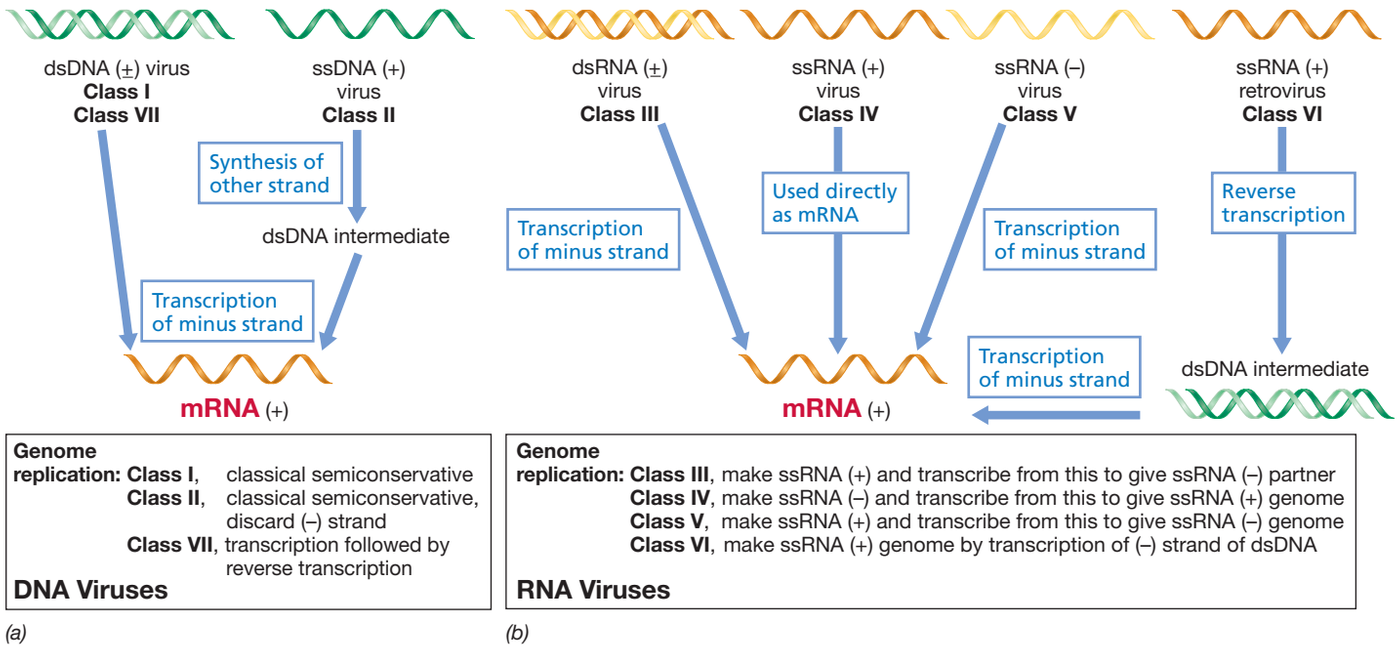


Figure 9.11 **Formation of mRNA and new genomes in *(a)* DNA viruses and *(b)* RNA viruses.** By convention, mRNA is always considered to be of the plus (+) orientation. Examples of each class of virus are given in Table 9.2.

of the dsRNA genome and cannot be released to act as mRNA. Consequently, the virions of dsRNA viruses must also contain RNA-dependent RNA polymerases that transcribe the dsRNA genome to produce plus-strand mRNA upon entry into the host cell.

Retroviruses

The **retroviruses** are animal viruses that are responsible for causing certain kinds of cancers and acquired immunodeficiency syndrome, AIDS. Retroviruses have ssRNA in their virions but replicate through a dsDNA intermediate (class VI). The process of copying the information found in RNA into DNA is called **reverse transcription**, and thus these viruses require an enzyme called **reverse transcriptase**. Although the incoming RNA of retroviruses is the plus strand, it is not used as mRNA, and therefore these viruses must carry reverse transcriptase in their virions. After infection, the virion ssRNA is converted to dsDNA via a hybrid RNA–DNA intermediate. The dsDNA is then the template for mRNA synthesis by normal cellular enzymes.

Finally, class VII viruses are those that have double-stranded DNA in their virions but replicate through an RNA intermediate. These unusual viruses also use reverse transcriptase. The strategy these viruses use to produce mRNA is the same as that of class I viruses (Figure 9.11), although their DNA replication is very unusual because, as we will see later, the genome is only partially double-stranded (Section 21.11).

While the Baltimore scheme covers most possibilities, there are exceptions. For example, ambiviruses contain a ssRNA genome, half of which is in the plus orientation (and can thus be used as mRNA) and half in the minus configuration (which cannot). A complementary strand must be synthesized from the latter half before the genes there can be translated. Evolution has clearly pushed viral genome diversity to the limits!

Viral Proteins

Once viral mRNA is made (Figure 9.11), viral proteins can be synthesized. These proteins can be grouped into two broad categories:

1. Proteins synthesized soon after infection, called **early proteins**, which are necessary for the replication of virus nucleic acid
2. Proteins synthesized later, called **late proteins**, which include the proteins of the virus capsid

Generally, both the timing and amount of virus proteins are highly regulated. Early proteins are typically enzymes that act catalytically and are therefore synthesized in smaller amounts. By contrast, late proteins are typically structural components of the virion and are made in much larger amounts.

Virus infection upsets the regulatory mechanisms of the host because there is a marked overproduction of viral nucleic acid and protein in the infected cell. In some cases, virus infection causes a complete shutdown of host macromolecular synthesis, whereas in other cases, host synthesis proceeds concurrently with virus synthesis. In either case, regulation of virus synthesis is under the control of the virus rather than the host. Several aspects of this control resemble the regulatory mechanisms discussed in Chapter 8, but there are also some uniquely viral regulatory mechanisms. We discuss these regulatory mechanisms next when we consider some well-studied viruses.

MiniQuiz

- Why must some types of virus contain enzymes in the virion in order for mRNA to be produced?
- Distinguish between a positive-strand RNA virus and a negative-strand RNA virus.
- Both positive-strand RNA viruses and retroviruses contain plus configuration RNA genomes. Contrast mRNA production in these two classes of viruses.

UNIT 4

III Viral Diversity

9.8 Overview of Bacterial Viruses

Bacteriophages are quite diverse, and examples of the various classes are illustrated in **Figure 9.12**. Most bacterial viruses that have been investigated in detail infect well-studied bacteria, such as *Escherichia coli* and *Salmonella enterica*. However, viruses are known that infect a wide range of *Bacteria* and *Archaea*.

Most known bacteriophages contain dsDNA genomes, and this type of bacteriophage is thought to be the most common in nature. However, many other kinds are known, including those with ssRNA genomes, dsRNA genomes, and ssDNA genomes (Figure 9.11). In fact, this remarkable diversity of genomes may have been an important factor in the evolution of nucleic acid function in cellular organisms (see the Microbial Sidebar, "Did Viruses Invent DNA?").

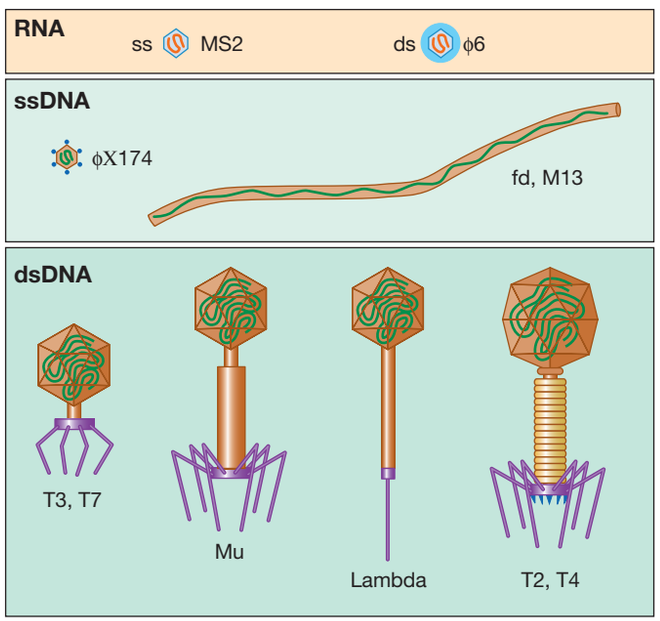


Figure 9.12 Schematic representations of the main types of bacterial viruses. Sizes are to approximate scale. The nucleocapsid of ϕ6 is surrounded by a membrane.

Did Viruses Invent DNA?

The three-domain theory of cellular evolution divides living cells into three lineages, *Bacteria*, *Archaea*, and *Eukarya*, based on the sequence of their ribosomal RNA (↻ Section 16.9). In addition, molecular analyses of the cellular components required for transcription and translation support this scheme rather well. However, when molecular analyses of the components required for DNA replication, recombination, and repair are considered, the three-domain scheme does not hold up so well. For example, class II topoisomerases of the *Archaea* are more closely related to those of the *Bacteria* than to those of the *Eukarya*. In addition, viral DNA-processing enzymes show erratic relationships to those of cellular organisms. For example, the DNA polymerase of bacteriophage T4 is more closely related to the DNA polymerases of eukaryotes than to those of its bacterial hosts.

Another major problem in microbial evolution is where viruses fit into the universal tree of life. Did they emerge relatively late as rogue genetic elements escaping from cellular genomes, or were they around at the same time as the very earliest cells? A related issue is when DNA entered the evolutionary scene and took over from RNA as the genetic material. The scenario of the RNA world (↻ Section 16.2) proposes that RNA was the original genetic material of cells and that DNA took over relatively early because it was a more stable molecule than RNA. Hence, in this scheme, the last universal common ancestor (LUCA) to the three domains of life was a DNA-containing cell. But how did the LUCA obtain its DNA?

Recently, Patrick Forterre of the Institut Pasteur has suggested a novel evolutionary scenario for how cells obtained DNA that also explains how the cellular machinery that deals with DNA originated in cells in the first place. Forterre argues that minor improvements in genetic stability would not have been sufficiently beneficial to select for the upheaval of converting an entire cellular genome from RNA to DNA. Instead, he suggests that viruses invented DNA as a modification mechanism to protect their genomes from host cell enzymes designed to destroy them (Figure 1). Viruses are known today that contain genomes of RNA, DNA, DNA containing uracil instead of thymine, and DNA containing hydroxymethylcytosine in place of cytosine (Figure 1*a*). Moreover, modern cells of all three domains contain systems designed to destroy incoming foreign DNA or RNA.

Forterre's hypothesis starts with an RNA world consisting of cells with RNA genomes plus viruses with RNA genomes. Viruses with DNA genomes were then selected because this protected them from degradation by cellular nucleases. This would have occurred before the LUCA (also containing an RNA genome) split into the three domains (Figure 1*b*). Then three nonvirulent DNA viruses ("founder viruses") infected the ancestors of the three domains. The three DNA viruses replicated inside their host cells as DNA plasmids, much as a P1 prophage replicates inside *Escherichia coli* today. Furthermore, two of the founder viruses were more closely related to each other (and these infected the ancestors of today's *Archaea* and *Eukarya*) than to the third founder virus (which infected the ancestor of *Bacteria*). Gradually, cells converted their genes from RNA into DNA due to its greater stability. Reverse transcriptase is believed to be an enzyme of very ancient origin, and it is conceivable that it was involved in the conversion of RNA genes to DNA, as occurs in retroviruses today.

To recap the hypothesis, the LUCA diverged into the three cellular ancestors to the three domains of life, and this laid the groundwork for the transcription and translation machinery in cells—that is, those functions that involve RNA (but not DNA). However, the use of DNA as a storage system for genetic information—now a universal property of cells—was provided by a family of DNA viruses that infected cells eons ago. Because DNA is a more stable molecule than RNA, cells with RNA genomes that were not infected by DNA viruses never became DNA-based cells and eventually became extinct (Figure 1*b*).

The Forterre model explains the origin of DNA in cells and provides a mechanism for the gradual replacement of RNA genomes with DNA. And, importantly, it also explains the noncongruence of the DNA replication, recombination, and repair machinery of cells of the different domains as compared with the transcription and translation machinery. Although this hypothesis does not wholly explain the origin of viruses, it does explain their diversity of replication systems and the very ancient structural similarities between certain families of DNA and RNA viruses.

A few bacterial viruses have lipid envelopes, but most are naked (that is, they have no further layers outside the capsid). However, many bacterial viruses are structurally complex. All examples of bacteriophages with dsDNA genomes shown in Figure 9.12 have heads and tails. The tails of bacteriophages T2, T4, and Mu are contractile and function in DNA entry into the host (Figure 9.10). By contrast, the tail of phage lambda is flexible.

Although tailed bacterial viruses were first studied as model systems for understanding general features of virus replication, some of them are now used as convenient tools for genetic engineering. Understanding bacterial viruses is not only valuable as background for the discussion of animal viruses but is also essential for the material presented in the chapters on microbial genetics (Chapter 10) and genetic engineering (Chapter 11).

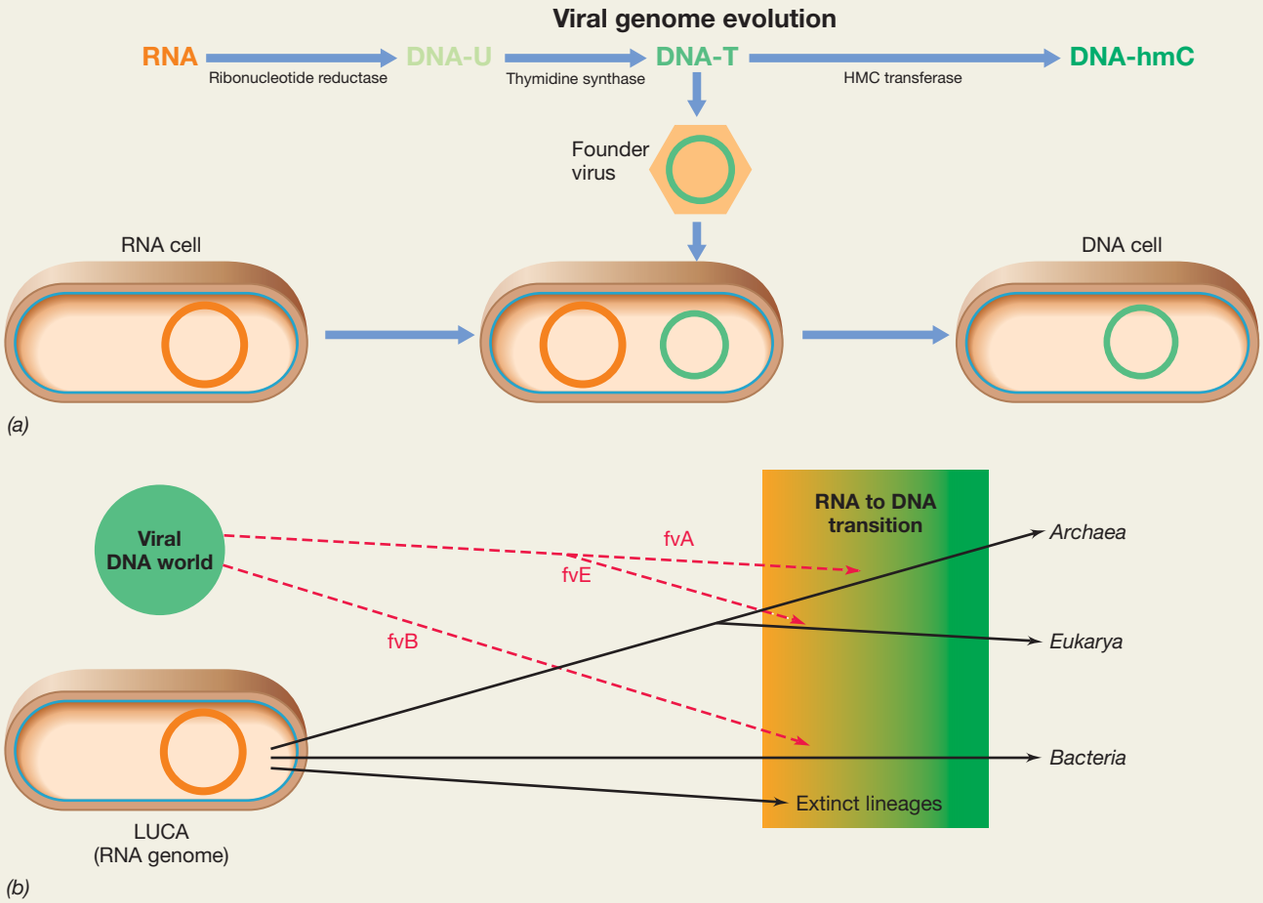


Figure 1 **Hypothesis of viral origin of DNA.** *(a)* Several successive cycles of mutation and selection resulted in the appearance of viral nucleic acids more resistant to degradation by the host cell: DNA-U, DNA with uracil; DNA-T, DNA with thymine (i.e., normal DNA); DNA-hmC, DNA with 5-hydroxymethylcytosine. All four types of nucleic acid are found in present-day viruses, although DNA-U and DNA-hmC are rare. Conversion of RNA cellular genomes to DNA postulates lysogeny by a DNA "founder virus" followed by movement of host genes onto the DNA genome. *(b)* Three founder viruses, fvB, fvA, fvE, are hypothesized to have infected the ancestors of the *Bacteria*, *Archaea*, and Eukarya, respectively. Note that viruses fvA and fvE are more closely related to each other than to fvB. As a result of viral infection, the genomes of these three ancestral lines were eventually converted from RNA to DNA. Presumably, other cellular lineages derived from the last universal common ancestor (LUCA) that retained RNA genomes are extinct.

In the next two sections we examine two contrasting viral life cycles: *virulent* and *temperate*. In the virulent (or lytic) mode, viruses lyse or kill their hosts after infection, whereas in the temperate (or lysogenic) mode, viruses replicate their genomes in step with the host genome and without killing their hosts. A similar phenomenon is seen with viruses that infect higher organisms. When animal viruses divide in step with host cells, this is known as a "latent" infection.

MiniQuiz

- What type of nucleic acid is thought to be most common in bacteriophage genomes?
- What is the role of the contractile tails found in many bacteriophages?
- How do the virulent and temperate lifestyles of a bacteriophage differ?

9.9 Virulent Bacteriophages and T4

Virulent viruses kill their hosts after infection. The first such viruses to be studied in detail were bacteriophages with linear, dsDNA genomes that infect *Escherichia coli* and a number of related *Bacteria*. Virologists studied these viruses as model systems for virus replication and used them to establish many of the fundamental principles of molecular biology and genetics. These phages were designated T1, T2, and so on, up to T7, with the "T" referring to the tail these phages contain. We have already briefly mentioned how one of these viruses, T4, attaches to its host and how its DNA penetrates the host (Section 9.6 and Figure 9.10). Here we consider this virus in more detail to illustrate the replication cycle of virulent viruses.

The Genome of T-Even Bacteriophages

Bacteriophages T2, T4, and T6 are closely related, but T4 is the most extensively studied. The virion of phage T4 is structurally complex (Figure 9.5*b*). It consists of an elongated icosahedral head whose overall dimensions are 85 × 110 nm. To this head is attached a complex tail consisting of a helical tube (25 × 110 nm) to which are connected a sheath, a connecting "neck" with "collar," and a complex end plate carrying long, jointed tail fibers (Figure 9.5*b*). Altogether, the virus particle contains over 25 distinct types of structural proteins.

The genome of T4 is a linear dsDNA molecule of 168,903 base pairs that encodes over 250 different proteins. Although no known virus encodes its own translational apparatus, T4 does encode several of its own tRNAs. The T4 genome has a unique linear sequence, but the actual genomic DNA molecules in different virions are not identical. This is because the DNA of phage T4 is *circularly permuted*. Molecules that are circularly permuted appear to have been linearized by opening identical circles at different locations. In addition to circular permutation, the DNA in each T4 virion has repeated sequences of about 3–6 kbp at each end, called *terminal repeats*. Both of these factors affect genome packaging.

When T4 DNA enters a host cell, it is first replicated as a unit, and then several genomic units are recombined end to end to form a long DNA molecule called a *concatemer* (**Figure 9.13**). During the packaging of T4 DNA, the DNA is not cut at a specific sequence. Instead, a segment of DNA long enough to fill a phage head is cut from the concatemer. Because the T4 head holds slightly more than a genome length, this "headful mechanism" leads to circular permutation and terminal redundancy. T4 DNA contains the modified base *5-hydroxymethylcytosine* in place of cytosine (**Figure 9.14**). These residues are glucosylated (Section 9.6), and DNA with this modification is resistant to virtually all known restriction enzymes. Consequently, the incoming T4 DNA is protected from host defenses.

Events During T4 Infection

Things happen rapidly in a T4 infection. Early in infection T4 directs the synthesis of its own RNA and also begins to replicate its unique DNA. About 1 min after attachment and penetration of the host by T4 DNA, the synthesis of host DNA and RNA ceases and transcription of specific phage genes begins. Translation of viral mRNA begins soon after, and within 4 min of infection, phage DNA replication has begun.

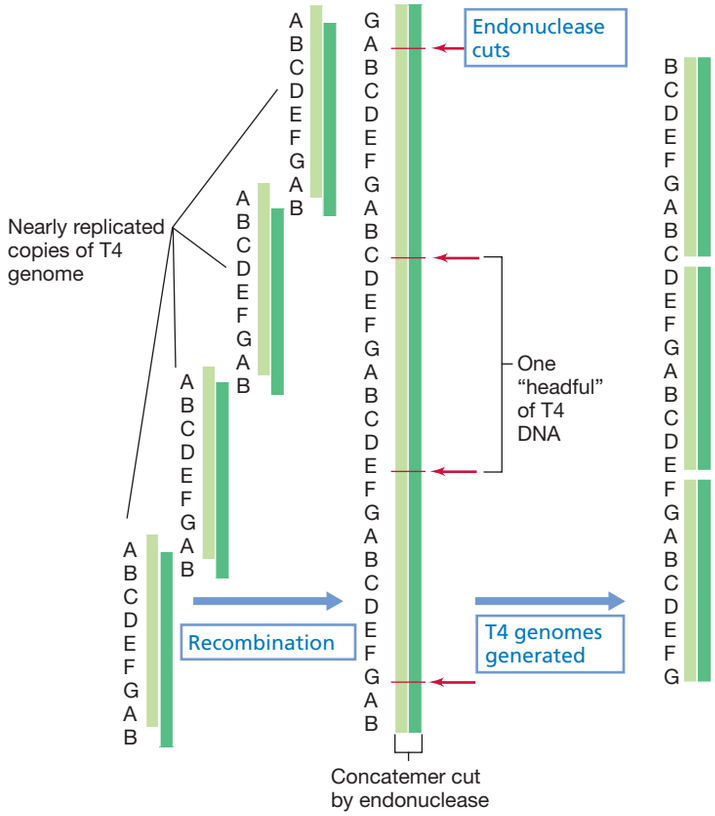


Figure 9.13 **Circular permutation.** Generation of virus-length T4 DNA molecules with permuted sequences by an endonuclease that cuts off constant lengths of DNA without regard to the sequence. Left: nearly replicated copies of infecting T4 genome are recombined to form a concatemer. Middle: red arrows, sites of endonuclease cuts. Right: genome molecules generated. Note how each of the T4 genomes formed on the right contains genes A–G, but that the termini are unique in each molecule.

The T4 genome can be divided into three parts, encoding *early proteins*, *middle proteins*, and *late proteins*, respectively (**Figure 9.15**). The early and middle proteins are primarily enzymes needed for DNA replication and transcription, whereas the late

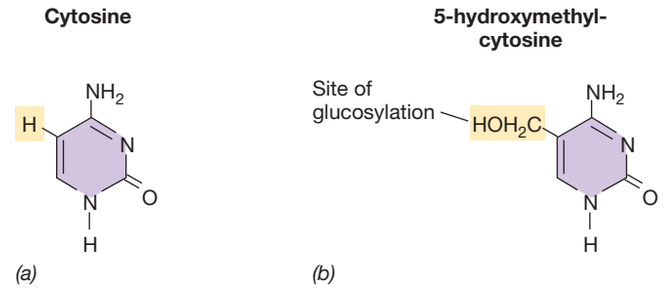


Figure 9.14 **The unique base in the DNA of the T-even bacteriophages, 5-hydroxymethylcytosine.** *(a)* Cytosine. *(b)* 5-Hydroxymethylcytosine. DNA containing glucosylated 5-hydroxymethylcytosine is resistant to cutting by restriction enzymes.

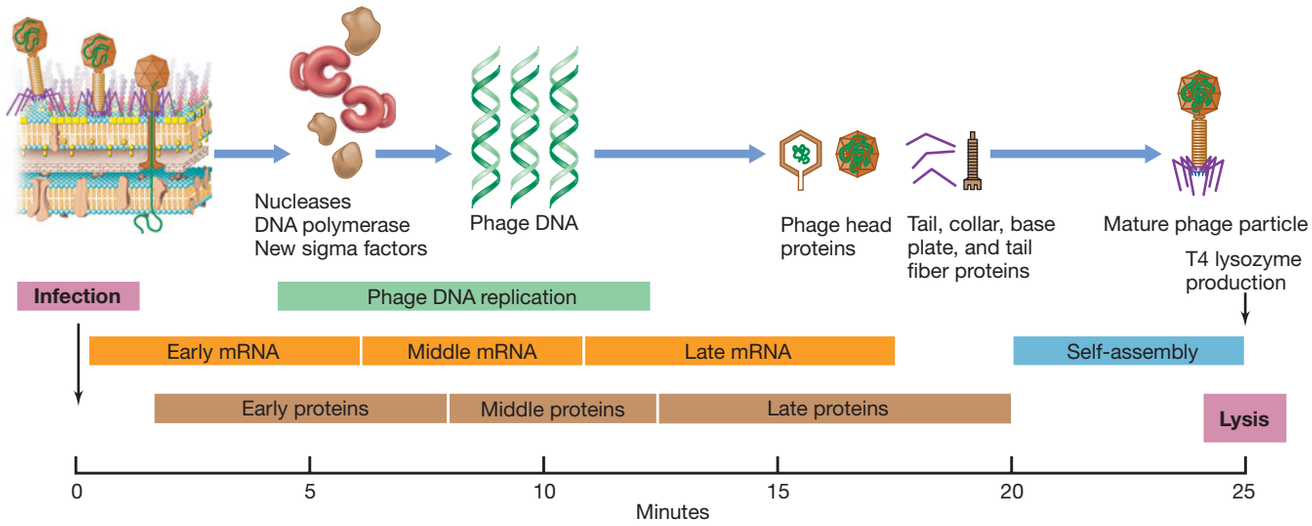


Figure 9.15 **Time course of events in phage T4 infection.** Following injection of DNA, early and middle mRNA is produced that codes for nucleases, DNA polymerase, new phage-specific sigma factors, and other proteins needed for DNA replication. Late mRNA codes for structural proteins of the phage virion and for T4 lysozyme, which is needed to lyse the cell and release new phage particles.

proteins are the head and tail proteins and the enzymes required to liberate the mature phage particles from the cell. The time course of events during T4 infection is shown in Figure 9.15.

Although T4 has a very large genome for a virus, it does not encode its own RNA polymerase. The control of T4 mRNA synthesis requires the production of proteins that sequentially modify the specificity of the host RNA polymerase so that it recognizes phage promoters. The early promoters are read directly by the host RNA polymerase and require the host sigma factor. Host transcription is shut down shortly after this by a phage-encoded anti-sigma factor that binds to host σ^{70} (↻ Section 6.13) and interferes with its recognition of host promoters.

Phage-specific proteins encoded by the early genes also covalently modify the host RNA polymerase α-subunits (↻ Section 6.12), and a few phage-encoded proteins also bind to the RNA polymerase. These modifications change the specificity of the host RNA polymerase so that it now recognizes T4 middle promoters. One of the T4 early proteins, MotA, recognizes a particular DNA sequence in T4 middle promoters and guides RNA polymerase to these sites. Transcription from the late promoters requires a new T4-encoded sigma factor. Sequential modification of host cell RNA polymerase as described here for phage T4 is used to regulate gene expression by many other bacteriophages as well.

T4 encodes over 20 new proteins that are synthesized early after infection. These include enzymes for the synthesis of the unusual base 5-hydroxymethylcytosine (Figure 9.14) and for its glucosylation, as well as an enzyme that degrades the normal DNA precursor deoxycytidine triphosphate. In addition, T4 encodes a number of enzymes that have functions similar to those of host enzymes in DNA replication, but that are formed in larger amounts, thus permitting faster synthesis of T4-specific DNA. Additional early proteins include those involved in the processing of newly replicated phage DNA (Figure 9.13).

Most late genes encode structural proteins for the virion, including those for the head and tail. The assembly of heads and tails is independent. The DNA is actively pumped into the head until the internal pressure reaches the required level, which is over ten times that of bottled champagne! The tail and tail fibers are added after the head has been filled (Figure 9.15). The phage encodes an enzyme, T4 lysozyme, which degrades the peptidoglycan layer of the host cell. The virus exits when the cell is lysed. After each replication cycle, which takes only about 25 min (Figure 9.15), over 100 new virions are released from each host cell, which itself has now been almost completely destroyed.

MiniQuiz

- What does it mean that the bacteriophage T4 genome is both circularly permuted and has terminal repeats?
- Explain how T4 ensures that its genes, rather than those of the host, are transcribed.

9.10 Temperate Bacteriophages, Lambda and P1

Bacteriophage T4 is virulent. However, some other viruses, although able to kill cells through a virulent cycle, also possess an alternative life cycle that results in a stable relationship with the host. Such viruses are called **temperate viruses**. Such viruses can enter into a state called **lysogeny**, where most virus genes are not expressed and the virus genome, called a **prophage**, is replicated in synchrony with the host chromosome. It is expression of the viral genome that harms the host cell, not the mere presence of viral DNA. Consequently, host cells can harbor viral genomes without harm, provided that the viral genes for lytic functions are not expressed. In cells that harbor a temperate virus, called **lysogens**, the phage genome is replicated in step with the host

genome and, during cell division, is passed from one generation to the next. Under certain stressful conditions temperate viruses may revert to the **lytic pathway** and begin to produce virions.

The two best-characterized temperate phages are lambda and P1. Both have contributed significantly to the advance of molecular genetics and are used in bacterial genetics (phage P1, Section 10.8) and molecular cloning (lambda, Section 11.9). Lysogeny is also of ecological importance because most bacteria isolated from nature are lysogens for one or more bacteriophages. Lysogeny can confer new genetic properties on the bacterial host cell, and we will see several examples in later chapters of pathogenic bacteria whose virulence depends on the lysogenic bacteriophage they harbor. Many animal viruses persist in their host cells in ways that resemble lysogeny.

The Replication Cycle of a Temperate Phage

Temperate phages may enter the virulent mode after infecting a host cell or they may establish lysogeny. An overall view of the life cycle of a temperate bacteriophage is shown in **Figure 9.16**.

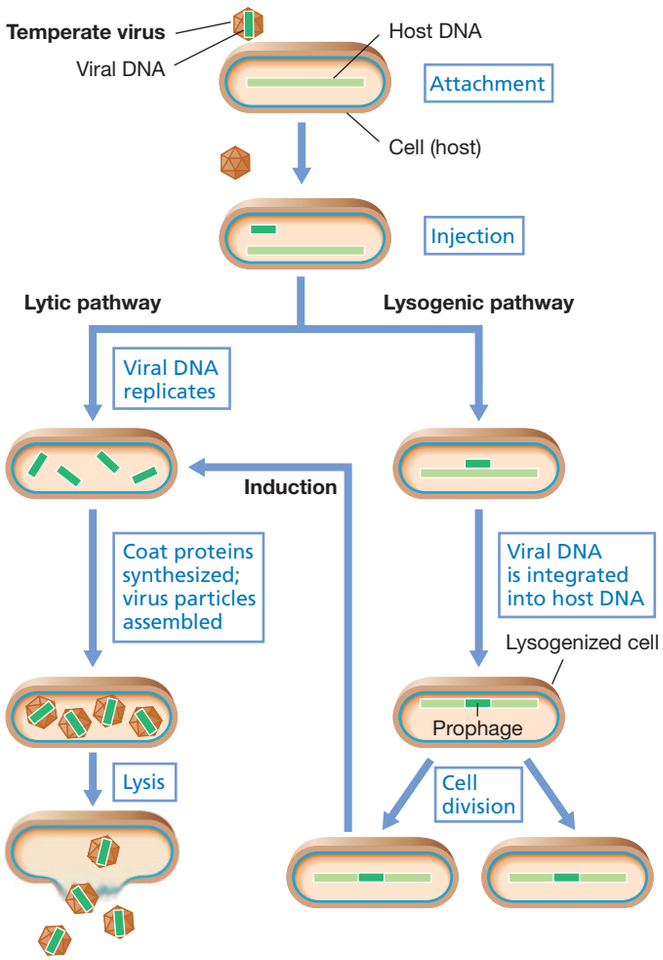


Figure 9.16 The consequences of infection by a temperate bacteriophage. The alternatives upon infection are replication and release of mature virus (lysis) or lysogeny, often by integration of the virus DNA into the host DNA, as shown here. The lysogen can be induced to produce mature virus and lyse.

During lysogeny, the temperate virus does not exist as a virus particle inside the cell. Instead, the virus genome is either integrated into the bacterial chromosome (e.g., bacteriophage lambda) or exists in the cytoplasm in plasmid form (e.g., bacteriophage P1). In either case, it replicates in step with the host cell as long as the genes activating its virulent pathway are not expressed. These forms of the virus are known as prophages. Typically, this control is due to a phage-encoded repressor protein (clearly, the gene encoding the repressor protein must be expressed). The virus repressor protein not only controls genes on the prophage, but also prevents gene expression by any identical or closely-related virus that tries to infect the same host cell. This results in the lysogens having immunity to infection by the same type of virus.

If the phage repressor is inactivated or if its synthesis is prevented, the prophage is induced (Figure 9.16). New virions are produced, and the host cell is lysed. Altered conditions, especially damage to the host cell DNA, induce the lytic pathway in some cases (e.g., in bacteriophage lambda). If the virus loses the ability to leave the host genome because of mutation, it becomes a *cryptic virus*. Genomic studies have shown that many bacterial chromosomes contain DNA sequences that were clearly once part of a viral genome. Thus, the establishment and breakdown of the lysogenic state is likely a dynamic process in prokaryotes.

www.microbiologyplace.com **Online Tutorial 9.1: A Temperate Bacteriophage**

Bacteriophage Lambda

Bacteriophage lambda, which infects *Escherichia coli*, has been studied in great detail. As with other temperate viruses, both the virulent and the temperate pathways are possible (Figure 9.16). Lambda virions resemble those of other tailed bacteriophages, although no tail fibers are present in the commonly used laboratory strains (**Figure 9.17** and Figure 9.12). Wild-type lambda does have tail fibers. The lambda genome consists of linear dsDNA. However, at the 5′ terminus of each strand is a single-stranded region 12 nucleotides long. These single-stranded cohesive ends are complementary, and when lambda DNA enters the host cell

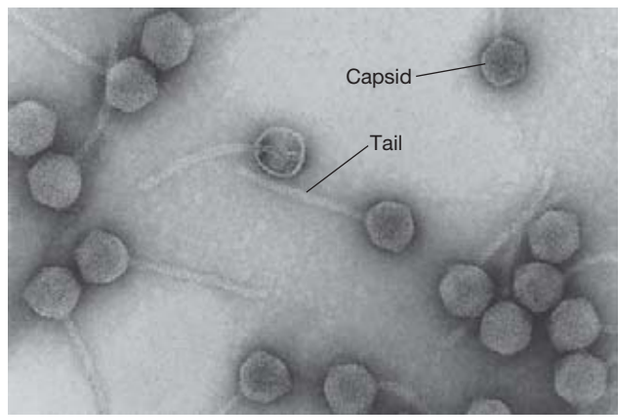


Figure 9.17 Bacteriophage lambda. Electron micrograph by negative staining of phage lambda virions. The head of each virion is about 65 nm in diameter and contains linear dsDNA.

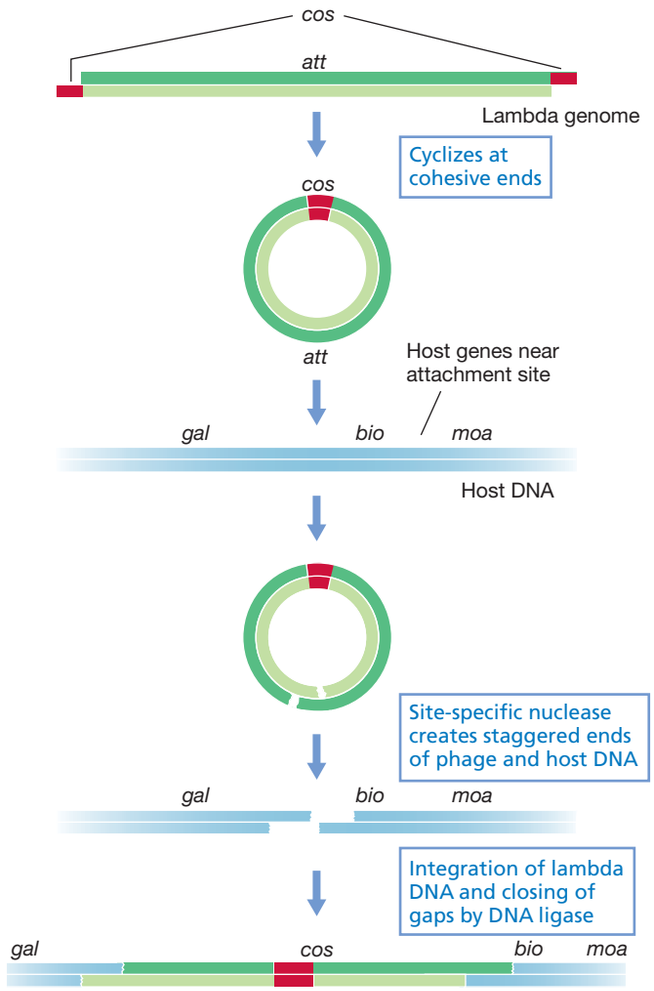


Figure 9.18 Integration of lambda DNA into the host. Integration always occurs at specific attachment sites (*att* sites) on both the host DNA and the phage. Some host genes near the attachment site are given: *gal* operon, galactose utilization; *bio* operon, biotin synthesis; *moa* operon, molybdenum cofactor synthesis. A site-specific enzyme (integrase) is required, and specific pairing of the complementary ends results in integration of phage DNA.

and circularizes, they base-pair, forming what is known as the *cos* site (**Figure 9.18**). The DNA is then ligated, forming a double-stranded circle.

When lambda is lysogenic, it integrates into the *E. coli* chromosome at a unique site known as the lambda attachment site, *att*λ. Integration requires the enzyme lambda integrase, which recognizes the phage and bacterial attachment sites (labeled *att* in Figure 9.18) and catalyzes integration. The integrated lambda DNA is then replicated along with the rest of the host genome and transmitted to progeny cells.

When lambda enters the virulent (lytic) pathway, it synthesizes long, linear concatemers of DNA by rolling circle replication (**Figure 9.19**). In contrast to semiconservative replication, this mechanism is asymmetrical and occurs in two stages. In the first stage, one strand of the circular lambda genome is nicked. Then a long single-stranded concatemer is made using the intact strand

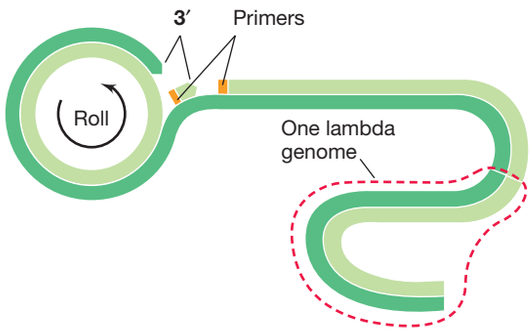


Figure 9.19 Rolling circle replication of the lambda genome. As the dark green strand rolls out, it is being replicated at its opposite end. Note that this synthesis is *asymmetric* because one of the parental strands continues to serve as a template and the other is used only once.

as a template. In the next stage, a second strand is made using the single-stranded concatemer as a template. Finally, the double-stranded concatemer is cut into genome-sized lengths at the *cos* sites, resulting in cohesive ends. The linear genomes are packaged into phage heads and the tails are added; the host cell is then lysed by phage-encoded enzymes. Many DNA and RNA viruses and some plasmids use variants of rolling circle replication. In some cases, single-stranded concatemers are cut and packaged; in other cases, the complementary strand is made before packaging, as in lambda.

Lambda: Lysis or Lysogeny?

Whether lysis or lysogeny occurs during lambda infection depends on an exceedingly complex genetic switch. The key elements are two repressor proteins, the lambda repressor, or cI protein (Figure 9.20), and the repressor protein Cro. To establish lysogeny, two events must happen: (1) The production of late proteins must be prevented; and (2) a copy of the lambda genome must be integrated into the host chromosome. If cI is made, it represses the synthesis of all other lambda-encoded proteins and lysogeny is established. Conversely, Cro indirectly represses the expression of the lambda cII and cIII proteins, which are needed to maintain lysogeny, by inducing synthesis of the cI. Thus, when Cro is made in high amounts, lambda is committed to the lytic pathway. The degradation of cII by a host cell protease (FtsH protein) is also critical. The cIII protein protects cII against protease attack and stabilizes it. A summary of the steps controlling lambda lysis and lysogeny is shown in **Figure 9.20**. The final outcome is determined by whether Cro protein or cI dominates in a given infection. If Cro dominates regulatory events, the outcome is lysis, whereas if cI dominates, lysogeny will occur.

MiniQuiz

- What are the two pathways available to a temperate virus?
- What is a lysogen?
- What events need to happen for lambda to become a prophage?

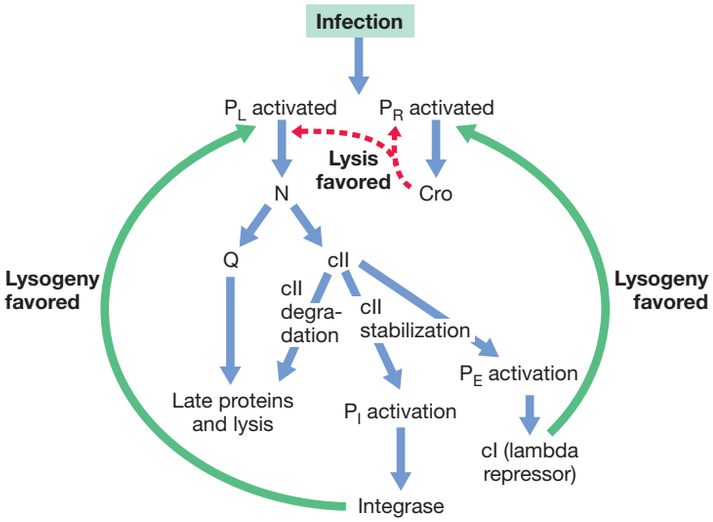


Figure 9.20 Summary of the steps in lambda infection. Lysis versus lysogeny is governed by whether or not the lambda repressor (cI) is made. High Cro activity prevents transcription (red dashed arrows) from the lambda leftward promoter, P_L, and the lambda rightward promoter, P_R. This prevents the synthesis of N protein, which in turn results in a decrease of both Q protein and cII protein. The lack of cII prevents synthesis of cI protein, and the result is lysis. The level of cII also depends on its degradation by host proteases versus its protection by lambda cIII protein (not shown). If sufficient cII is present, the promoters for cI (P_E) and integrase (P_I) are activated (green arrows) and both cI and integrase are made. This results in integration and lysogeny.

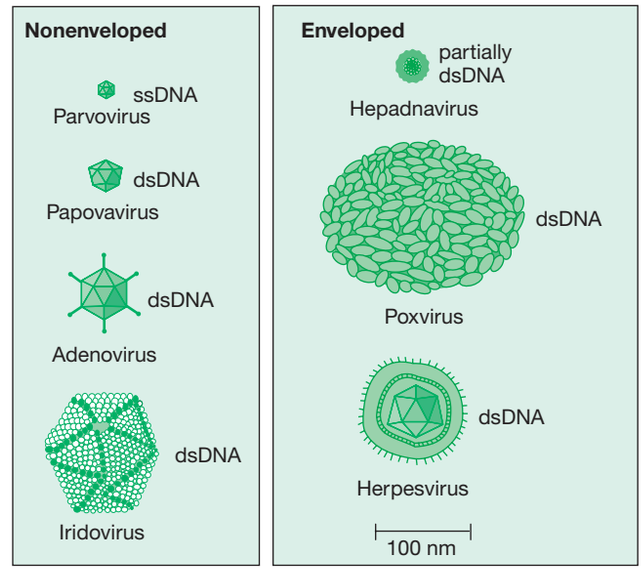
9.11 Overview of Animal Viruses

The first few sections of this chapter were devoted to general properties of viruses, and little was said about animal viruses. Here we consider animal viruses. It is important to remember that the bacteriophage host is a bacterial cell, whereas the host of an animal virus is a eukaryotic cell. We will expand on these important differences in Chapter 21, where we discuss several types of animal virus in more detail. However, the key points are that (1) unlike in prokaryotes, the entire virion typically enters the animal cell, and (2) eukaryotic cells contain a nucleus, where many animal viruses replicate.

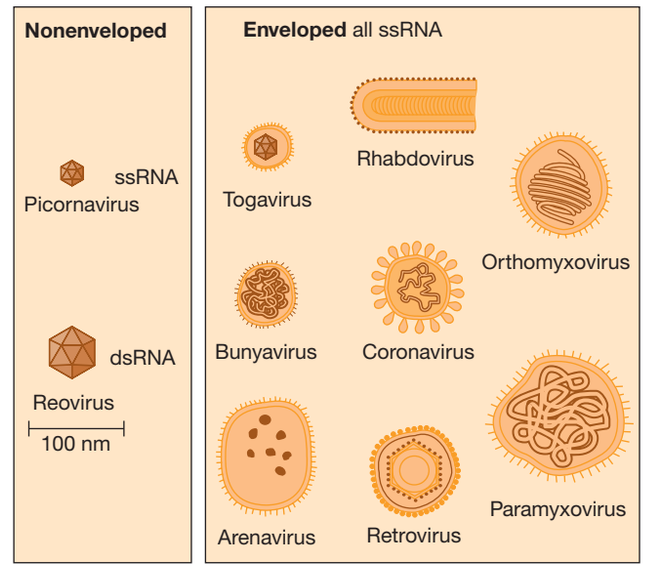
Classification of Animal Viruses

Various types of animal viruses are illustrated in **Figure 9.21**. We discussed the principles of virus classification in Section 9.7. As for bacterial viruses, animal viruses are classified according to the Baltimore classification system (Table 9.2), which classifies viruses by genome type and reproductive strategy. Animal viruses are known in all replication categories, and an example of each is discussed in Chapter 21. Most animal viruses that have been studied in detail are those that can replicate in cell cultures (Section 9.3).

Note that there are many more kinds of enveloped animal viruses than enveloped bacterial viruses (Section 9.8). This relates to the differences in host cell exteriors. Unlike prokaryotic cells, animal cells lack a cell wall, and thus viruses are more easily released from the cell. Many animal viruses are enveloped and when these exit, they remove part of the animal cell's lipid bilayer as they pass through the membrane.



(a) **DNA viruses**



(b) **RNA viruses**

Figure 9.21 Diversity of animal viruses. The shapes and relative sizes of the major groups of vertebrate viruses. The hepadnavirus genome has one complete DNA strand and part of the complementary strand (Section 21.11).

Consequences of Virus Infection in Animal Cells

Viruses can have several different effects on animal cells. Virulent infection results in the destruction of the host cell (**Figure 9.22**). With enveloped viruses, however, release of virions, which occurs by a kind of budding process, may be slow, and the host cell may not be lysed. The infected cell may therefore remain alive and continue to produce virus indefinitely. Such infections are called persistent infections (Figure 9.22).

Viruses may also cause latent infection of a host. In a latent infection, there is a delay between infection by the virus and host cell lysis. Fever blisters (cold sores), caused by the herpes simplex virus (Section 21.14), are a typical example of a latent viral infection;

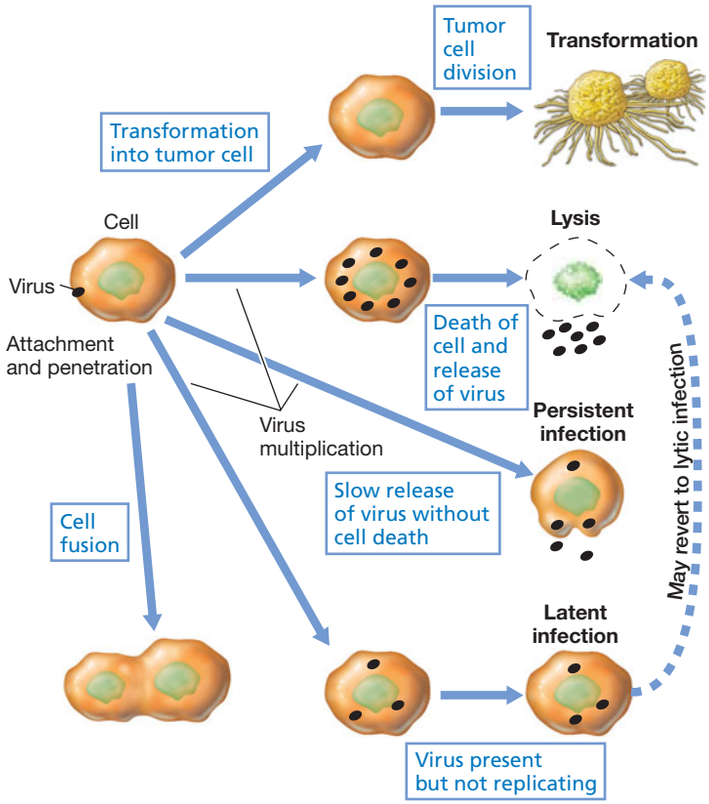


Figure 9.22 Possible effects that animal viruses may have on cells they infect. Most animal viruses are lytic, and only very few are known to cause cancer.

the symptoms (the result of lysed cells) reappear sporadically as the virus emerges from latency. The latent stage in viral infection of an animal cell is not usually due to integration of the viral genome into the host genome, as often happens with lysogenic infections by temperate bacteriophages. Instead, herpesviruses exist in a relatively inactive state within nerve cells. A low level of transcription continues, but the viral DNA does not replicate.

Some enveloped viruses promote fusion between multiple animal cells, creating giant cells with several nuclei (Figure 9.22). Not surprisingly, such fused cells fail to develop correctly and are short-lived. Cell fusion allows viruses to avoid exposure to the immune system by moving between host cell nuclei without emerging from the host cells. Finally, certain animal viruses can convert a normal cell into a tumor cell, a process called **transformation**. We discuss cancer-causing viruses in Sections 21.11 and 21.14.

Many different animal viruses are known. But of all the viruses listed in Figure 9.21, one group stands out as having an absolutely unique mode of replication. These are the retroviruses. We explore them next as an example of a complex and highly unusual animal virus with significant medical and evolutionary implications.

MiniQuiz

- Differentiate between a persistent and a latent viral infection.
- Contrast the ways in which animal viruses enter cells with those used by bacterial viruses.

9.12 Retroviruses

Retroviruses contain an RNA genome that is replicated via a DNA intermediate (Section 9.7 and Figure 9.11). The term *retro* means "backward," and the name retrovirus is derived from the fact that these viruses transfer information from RNA to DNA. Retroviruses employ the enzyme reverse transcriptase to carry out this interesting process. The use of reverse transcriptase is not restricted to the retroviruses. Hepatitis B virus (a human virus) and cauliflower mosaic virus (a plant virus) also use reverse transcription during their life cycles (↻ Section 21.11). However, these other viruses carry the DNA version of their genome in the virion whereas retroviruses carry RNA.

Retroviruses are interesting for several other reasons. For example, they were the first viruses shown to cause cancer and have been studied for their carcinogenic characteristics. Also, one retrovirus, human immunodeficiency virus (HIV), causes acquired immunodeficiency syndrome (AIDS). This virus infects a specific kind of white blood cell (T-helper cell) in humans that is vital for proper functioning of the immune system. In later chapters we discuss the medical aspects of AIDS (↻ Section 32.6).

Retroviruses are enveloped viruses (**Figure 9.23*a***). There are several proteins in the virus envelope and typically seven internal proteins, four of which are structural and three of which are enzymatic. The enzymes found in the virion are reverse transcriptase, integrase, and a protease. The virion also contains specific cellular tRNA molecules used in replication (discussed later in this section).

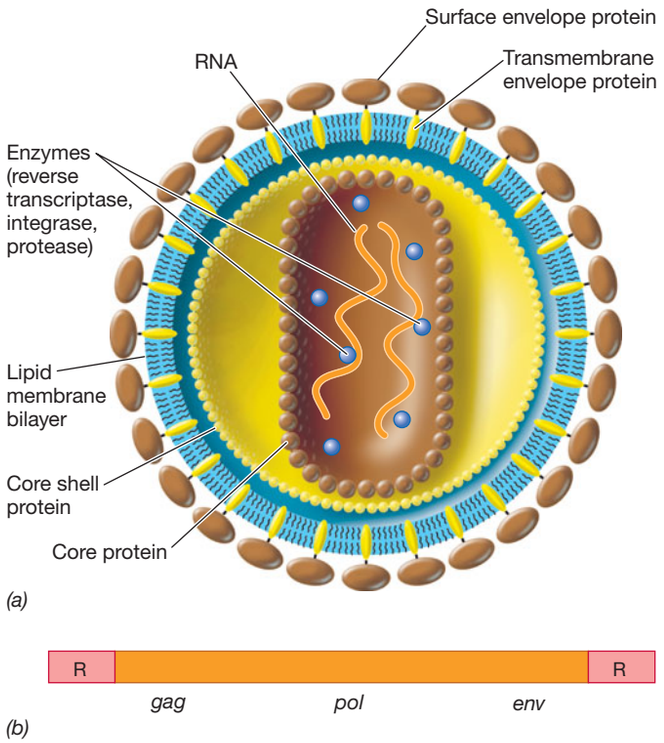


Figure 9.23 Retrovirus structure and function. *(a)* Structure of a retrovirus. *(b)* Genetic map of a typical retrovirus genome. Each end of the genomic RNA contains direct repeats (R).

Features of Retroviral Genomes and Replication

The genome of the retrovirus is unique. It consists of two identical single-stranded RNA molecules of the plus (+) orientation. A genetic map of a typical retrovirus genome is shown in Figure 9.23*b*. Although there are differences between the genetic maps of different retroviruses, all contain the following genes arranged in the same order: *gag*, encoding structural proteins; *pol*, encoding reverse transcriptase and integrase; and *env*, encoding envelope proteins. Some retroviruses, such as Rous sarcoma virus, carry a fourth gene downstream from *env* that is active in cellular transformation and cancer. The terminal repeats shown on the map are essential for viral replication.

The overall process of replication of a retrovirus can be summarized in the following steps (**Figure 9.24**):

1. Entry into the cell by fusion with the cytoplasmic membrane at sites of specific receptors
2. Removal of the virion envelope at the cytoplasmic membrane, but the genome and virus-specific enzymes remain in the virus core
3. Reverse transcription of one of the two identical genomic RNA molecules into a ssDNA that is subsequently converted by reverse transcriptase to a linear dsDNA molecule, which then enters the nucleus
4. Integration of retroviral DNA into the host genome
5. Transcription of retroviral DNA, leading to the formation of viral mRNAs and viral genomic RNA
6. Assembly and packaging of the two identical genomic RNA molecules into nucleocapsids in the cytoplasm
7. Budding of enveloped virions at the cytoplasmic membrane and release from the cell

Activity of Reverse Transcriptase

A very early step after the entry of the RNA genome into the cell is reverse transcription: conversion of RNA into a DNA copy using the enzyme reverse transcriptase present in the virion. The DNA formed is a linear double-stranded molecule and is synthesized in the cytoplasm within an uncoated viral core particle. Details of this process can be found in Section 21.11. Reverse transcriptase is a type of DNA polymerase and, like all DNA polymerases, must have a primer (↻ Section 6.8). The primer for retrovirus reverse transcription is unusual in being a specific tRNA encoded by the host cell. The type of tRNA used as primer depends on the virus and is packaged into the virion from the previous host cell.

The overall process of reverse transcription generates a product that has long terminal repeats (LTRs, Figure 9.24) that are longer than the terminal repeats on the RNA genome itself (Figure 9.23). This entire dsDNA molecule enters the nucleus along with the integrase protein; here the viral DNA is integrated into the host DNA. The LTRs contain strong promoters of transcription and participate in the integration process. The integration of the retroviral DNA into the host genome is analogous to the integration of phage DNA into a bacterial genome to form a lysogen, except that the retrovirus cannot excise its DNA from the host genome. Thus, once integrated, the retroviral DNA, now

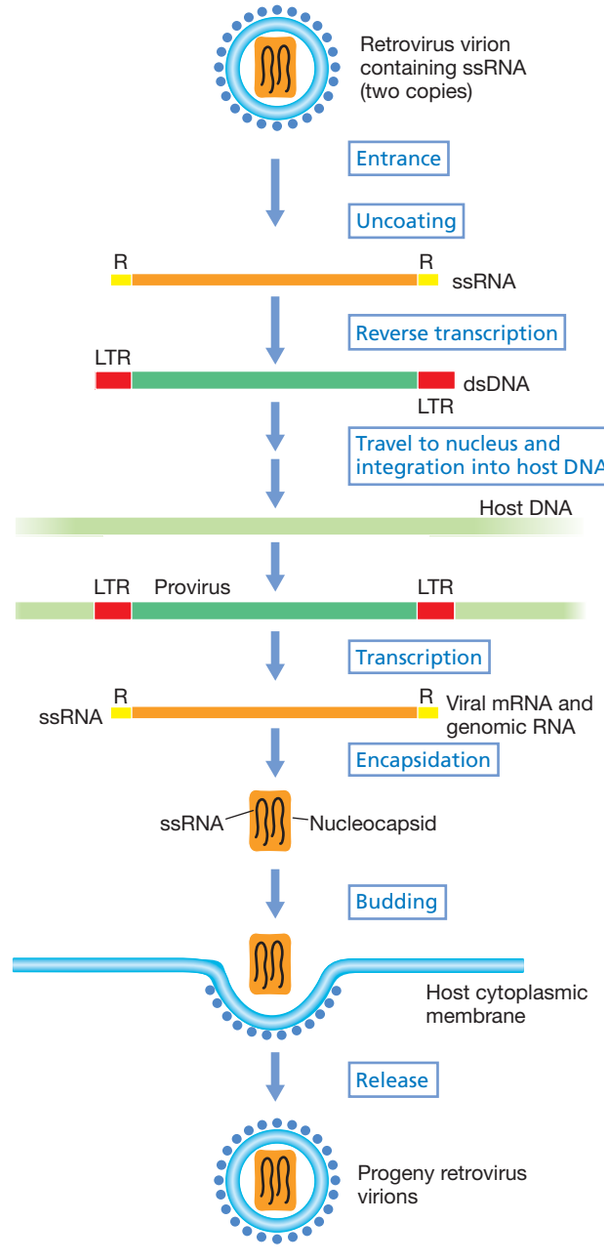


Figure 9.24 Replication process of a retrovirus. R, direct repeats; LTR, long terminal repeats. For more details on the conversion of RNA to DNA (reverse transcription, step 3), refer to Section 21.11.

called a **provirus**, becomes a permanent insertion into the host genome (Section 9.10). Viral DNA can be integrated anywhere in the host chromosomal DNA. Indeed, many higher eukaryotic genomes have high numbers of endogenous retroviral sequences. An estimated 8% of the sequences of the human genome are of retroviral origin.

If the promoters in the right-hand LTR are activated, the integrated proviral DNA is transcribed by a host cell RNA polymerase into RNA transcripts. These RNA transcripts may either be packaged into virus particles (as the genome) or may act as mRNA and be translated into virus proteins. Some virus proteins

are made initially as a large primary *gag* protein that is split by proteolysis into the capsid proteins. Occasionally ribosomes fail to terminate at the *gag* stop codon (due either to inserting an amino acid at a stop codon or a shift in reading frame by the ribosome). This leads to the low-level translation of *pol*, the reverse transcriptase gene, which yields reverse transcriptase for insertion into virions. Note that reverse transcriptase is needed in much lower amounts than the retrovirus structural proteins.

When virus structural proteins have accumulated in sufficient amounts, nucleocapsids are assembled. Encapsidation of the RNA genome leads to the formation of mature nucleocapsids, which move to the cytoplasmic membrane for final assembly into the enveloped virions. As nucleocapsids bud through the cytoplasmic membrane they are sealed and then released and may infect neighboring cells (Figure 9.24).

MiniQuiz

- Why are some viruses known as retroviruses?
- How does the replication cycle of a temperate bacteriophage differ from that of a retrovirus?

IV Subviral Entities

We have defined a virus as a genetic element that subverts normal cellular processes for its own replication and that has an infectious extracellular form. There are several infectious agents that resemble viruses but whose properties are at odds with this definition, and are thus not considered viruses. Defective viruses are clearly derived from viruses but have become dependent on other, complete, viruses to supply certain gene products. In contrast, two of the most important subviral entities, viroids and prions, are not viruses at all, but differ in fundamental ways from viruses. They both illustrate the unusual ways that genetic elements can replicate and the unexpected ways they can subvert their host cells. However, prions stand out among all the entities we have considered in this chapter because the infectious transmissible agent lacks nucleic acid.

9.13 Defective Viruses

Some viruses cannot infect a host cell alone and rely on other viruses, known as **helper viruses**, to provide certain functions. Some of these so-called **defective viruses** merely rely on intact helper viruses of the same type to provide necessary functions. Far more interesting are those defective viruses, referred to as *satellite viruses*, for which no intact version of the same virus exists; these defective viruses rely on unrelated viruses as helpers.

Many defective viruses are known. For example, bacteriophage P4 of *Escherichia coli* can replicate, but its genome does not encode the major capsid protein. Instead, it relies on the related phage P2 as a helper to provide capsid proteins for the phage particle. However, P4 does encode an external scaffold protein that takes part in capsid assembly.

Satellite viruses are found in both animals and plants. For example, adeno-associated virus (AAV) is a satellite virus of humans that depends on adenovirus as a helper. AAV and adenovirus belong to two quite different virus families. Thus, AAV is not just a defective mutant of adenovirus, but is an unrelated virus that inhabits the same host cells. Because it causes little or no damage to the host, AAV is now being used as a eukaryotic cloning vector in gene therapy (Section 15.17). In this system, AAV can be used to carry replacement genes to specific host tissues without causing disease itself.

MiniQuiz

- What is a helper virus?
- What is a satellite virus?

9.14 Viroids

Viroids are infectious RNA molecules that differ from viruses in lacking a capsid. Despite this lack, they have a reasonably stable extracellular form that travels from one host cell to another. Viroids are small, circular, single-stranded RNA molecules that are the smallest known pathogens. They range in size from 246 to 399 nucleotides and show a considerable degree of sequence homology to each other, suggesting that they have common evolutionary roots. Viroids cause a number of important plant diseases and can have a severe agricultural impact (**Figure 9.25**). A few well-studied viroids include coconut cadang-cadang viroid (246 nucleotides), citrus exocortis viroid (375 nucleotides), and potato spindle tuber viroid (359 nucleotides). No viroids are known that infect animals or prokaryotes.

Viroid Structure and Function

The extracellular form of the viroid is naked RNA; there is no protein capsid of any kind. Although the viroid RNA is a single-stranded, covalently closed circle, there is so much secondary

Yijun Qi and Biao Ding

Figure 9.25 Viroids and plant diseases. Photograph of healthy tomato plant (left) and one infected with potato spindle tuber viroid (PSTV) (right). The host range of most viroids is quite restricted. However, PSTV infects tomatoes as well as potatoes, causing growth stunting, a flat top, and premature plant death.

Figure 9.26 Viroid structure. Viroids consist of single-stranded circular RNA that forms a seemingly double-stranded structure by intra-strand base pairing.

structure that it resembles a short double-stranded molecule with closed ends (**Figure 9.26**). This apparently makes the viroid sufficiently stable to exist outside the host cell. Because it lacks a capsid, the viroid does not use a receptor to enter the host cell. Instead, the viroid enters a plant cell through a wound, as from insect or other mechanical damage. Once inside, viroids move from cell to cell via the plasmodesmata, which are the thin strands of cytoplasm that link plant cells (**Figure 9.27**).

Even more curious, the viroid RNA molecule contains no protein-encoding genes, and therefore the viroid is almost totally dependent on host function for its replication. The viroid is replicated in the host cell nucleus or chloroplast by one of the plant RNA polymerases. The result is a multimeric RNA molecule consisting of many viroid units joined end to end. The viroid does contribute one function to its own replication; part of the viroid itself has ribozyme activity (↻ Section 7.8). This ribozyme activity is used for self-cleavage of the multimeric RNA molecule, which releases individual viroids.

Viroid Disease

Viroid-infected plants can be symptomless or develop symptoms that range from mild to lethal, depending on the viroid (Figure 9.25). The mechanisms by which viroids cause plant diseases remain unclear. Most severe symptoms are growth

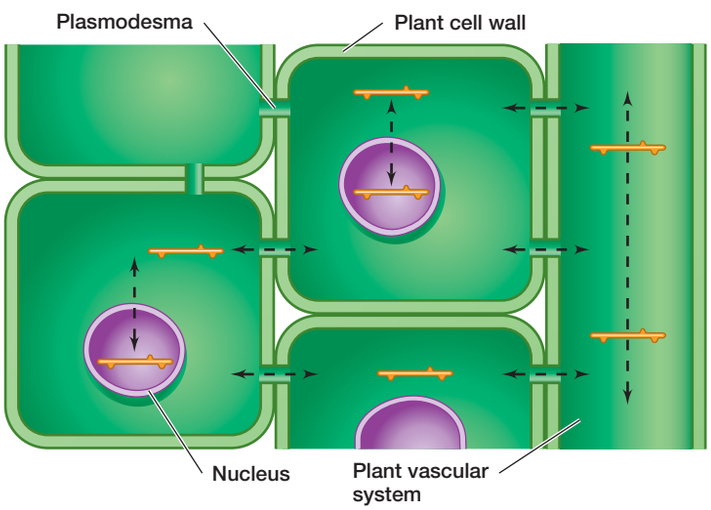


Figure 9.27 Viroid movement inside plants. After entry into a plant cell, viroids (orange) replicate either in the nucleus (shown here in purple) or in the chloroplast (not shown). Viroids can move between plant cells via the plasmodesmata (thin threads of cytoplasm that penetrate the cell walls and connect plant cells). In addition, on a larger scale, viroids can move around the plant via the plant vascular system.

related, suggesting that viroids mimic or interfere in some way with small regulatory RNAs (↻ Section 7.11), examples of which are widely known in plants. Thus, viroids could themselves be derived from regulatory RNAs that have evolved away from carrying out beneficial roles in the cell to inducing destructive events. Recent data suggest that viroids give rise to siRNA (↻ Section 7.10) as a side product during replication. It has been proposed that these siRNAs may then act via the RNA interference silencing pathway to suppress the expression of plant genes that show some homology to the viroid RNA. However, this is still unproven.

MiniQuiz

- If viroids are circular molecules, why are they usually drawn as compact rods?
- In what part of the host cell are viroids replicated?

9.15 Prions

Prions represent the other extreme from viroids. They have a distinct extracellular form, which consists entirely of protein. The prion particle contains neither DNA nor RNA. Nonetheless, it is infectious, and prions are known to cause diseases in animals, such as scrapie in sheep, bovine spongiform encephalopathy (BSE or "mad cow disease") in cattle, chronic wasting disease in deer and elk, and kuru and Creutzfeldt–Jakob disease (CJD) in humans. No prion diseases of plants are known, although prions have been found in yeast. Collectively, animal prion diseases are known as **transmissible spongiform encephalopathies (TSEs)**. In 1997 the American scientist Stanley B. Prusiner won the Nobel Prize for Physiology or Medicine for his pioneering work with these diseases and with the prion proteins.

In 1996 it became clear from disease tracking in England that the prion that causes BSE in cattle can also infect humans, resulting in a novel type of CJD called variant CJD (vCJD). Because transmission was from consumption of contaminated beef products, vCJD quickly became a worldwide health concern, with a major impact on the animal husbandry industry (↻ Section 36.12). Most such instances of BSE occurred in the United Kingdom or other European Union (EU) countries and were linked to improper feeding practices in which protein supplements containing rendered cattle and sheep (including nervous tissues) were used to feed uninfected animals. Since 1994, this practice has been banned in all EU countries, and cases of BSE have dropped dramatically. Thus far, TSE transmission via other domesticated animals, such as swine, chicken, or fish, has not been found.

Forms of the Prion Protein

As prions lack nucleic acid, how is the protein they consist of encoded? The host cell contains a gene, *Prnp* (standing for "*Prion p*rotein") which encodes the native form of the prion protein, known as PrP^C (*Pr*ion *P*rotein *C*ellular), that is primarily found in the neurons of healthy animals, especially in the brain. The pathogenic form of the prion protein is designated

PrP^{Sc} (prion protein *Sc*rapie), because the first prion disease to be discovered was scrapie in sheep. PrP^{Sc} is identical in amino acid sequence to PrP^{C} from the same species, but has a different conformation. Prion proteins from different species of mammals are very similar, but are not identical in amino acid sequence. Susceptibility to infection depends on the protein sequence in a manner not fully understood. For example, PrP^{Sc} from cattle can infect humans, although at a very low frequency. However, PrP^{Sc} from sheep have never been observed to infect people. Native prions consist largely of α-helical segments, whereas pathogenic prions have less α-helix and more β-sheet regions instead. This causes the prion protein to lose its normal function, to become partially resistant to proteases, and to become insoluble, leading to aggregation within the neural cell (**Figure 9.28**). In this state, prion protein accumulates and neurological symptoms commence.

Prion Diseases and the Prion Infectious Cycle

When a pathogenic prion enters a host cell that is expressing native prion protein, it promotes the conversion of PrP^{C} protein into PrP^{Sc}. Thus the pathogenic prion does not subvert host enzymes or genes as a virus does; rather, it "replicates" by converting native prion proteins that already exist in the host cell into the pathogenic form. As the pathogenic prions accumulate, they form insoluble aggregates in the neural cells (Figure 9.28). This leads to disease symptoms that are invariably neurological and, in most cases, are due to destruction of brain or related nervous tissue (↬ Section 36.12). Whether the destruction of brain tissue is directly due to the accumulation of aggregated PrP^{Sc} is uncertain. PrP^{C} functions in the cell as a cytoplasmic membrane glycoprotein, and it has been shown that membrane attachment of pathogenic prions is necessary for disease symptoms to commence. Mutant versions of PrP^{Sc} that can no longer attach to nerve cell cytoplasmic membranes may still aggregate, but no longer cause disease.

Prion disease occurs by three distinct mechanisms, although all lead to the same result. In *infectious prion disease*, as described above, PrP^{Sc} is transmitted between animals or humans. In *sporadic prion disease*, random misfolding of a PrP^{C} molecule occurs in a normal, uninfected individual. This change is propagated as for infectious prion disease, and eventually PrP^{Sc} accumulates until symptoms appear. In humans this occurs in about one person in a million. In *inherited prion disease*, a mutation in the prion gene yields a prion protein that changes more often into PrP^{Sc}. Several different mutations are known whose symptoms vary slightly.

What happens if an incoming PrP^{Sc} protein finds no PrP^{C} to alter? The answer is that no disease results. This may seem surprising, but is logical given the mechanism of prion action. Mice that have been engineered with both copies of the *Prnp* gene disrupted and thus do not produce PrP^{C} are resistant to infection with pathogenic prions. Interestingly, such mice also live for a normal time and do not show any obvious behavioral abnormalities. This leaves wide open the puzzling question of what role PrP^{C} plays in brain cells.

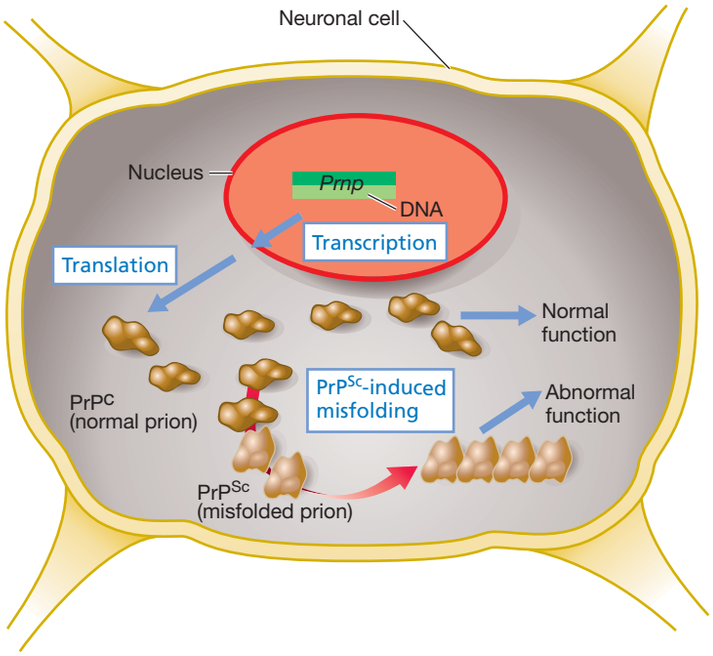


Figure 9.28 Mechanism of prion misfolding. Neuronal cells produce the native form of the prion protein. The pathogenic form of the prion protein catalyzes the refolding of native prions into the pathogenic form. The pathogenic form is protease resistant, insoluble, and forms aggregates in neural cells. This eventually leads to destruction of neural tissues and neurological symptoms.

Non-mammalian Prions

Are prions only found in mammals? Other vertebrates, including amphibians and fish, possess genes that are clearly homologous to the Prnp gene of mammals and that are expressed in nervous tissue. However, the proteins encoded by these genes do not have misfolded pathogenic versions and are therefore, by definition, not prions.

Curiously, proteins that fit the prion definition of an inherited self-perpetuating change in protein conformation are found in certain fungi, although they do not cause disease. Instead they adapt the fungal cells to altered conditions. In yeast, for example, the [URE3] prion is a transcription factor that regulates nitrogen metabolism. The normal, soluble form of this protein represses genes for using poor nitrogen sources. When the [URE3] prion accumulates, it forms insoluble aggregates, just as for mammalian prion protein. However, in yeast there is no pathogenic effect, instead the genes for nitrogen metabolism are derepressed.

MiniQuiz

- What is the difference between the native and pathogenic forms of the prion protein?
- How does sporadic prion disease differ from the transmitted form?
- How does a prion differ from a viroid?

UNIT 4

Big Ideas

9.1
A virus is an obligate intracellular parasite that cannot replicate without a suitable host cell. A virion is the extracellular form of a virus and contains either an RNA or a DNA genome inside a protein shell. The virus genome may enter a new host cell by infection. The virus redirects the host metabolism to support virus replication. Viruses are classified by their nucleic acid and type of host.

9.2
In the virion of a naked virus, only nucleic acid and protein are present, with the nucleic acid on the inside; the whole unit is called the nucleocapsid. Enveloped viruses have one or more lipoprotein layers surrounding the nucleocapsid. The nucleocapsid is arranged in a symmetric fashion, with a precise number and arrangement of structural subunits surrounding the virus nucleic acid. Although virus particles are metabolically inert, one or more key enzymes are present within the virion in some viruses.

9.3
Viruses can replicate only in certain types of cells or in whole organisms. Bacterial viruses have proved useful as model systems because the host cells are easy to grow and manipulate in culture. Many animal and plant viruses can be grown in cultured cells.

9.4
Although only a single virion is required to initiate an infectious cycle, not all virions are equally infectious. One of the most accurate ways of measuring virus infectivity is by the plaque assay. Plaques are clear zones that develop on lawns of host cells. Theoretically, each plaque is due to infection by a single virus particle. The virus plaque is analogous to the bacterial colony.

9.5
The virus replication cycle can be divided into five stages: attachment (adsorption), penetration (injection), protein and nucleic acid synthesis, assembly and packaging, and virion release.

9.6
The attachment of a virion to a host cell is a highly specific process requiring complementary receptors on the surface of a susceptible host cell and its infecting virus. Resistance of the host to infection by the virus can involve restriction–modification systems that recognize and destroy foreign double-stranded DNA.

9.7
Before viral nucleic acid can replicate, new virus proteins are needed, and these are encoded by mRNA transcribed from the virus genome. In some RNA viruses, the viral genomic RNA is also the mRNA. In other viruses, the virus genome is a template for the formation of viral mRNA, and in certain cases, essential transcriptional enzymes are contained in the virion.

9.8
Bacterial viruses, or bacteriophages, are very diverse. The best-studied bacteriophages infect bacteria such as *Escherichia coli* and are structurally quite complex, containing heads, tails, and other components.

9.9
After a virion of T4 attaches to a host cell and the DNA penetrates into the cytoplasm, the expression of viral genes is regulated so as to redirect the host synthetic machinery to the production of viral nucleic acid and protein. New virions are then assembled and are released by lysis of the cell. T4 has a double-stranded DNA genome that is circularly permuted and terminally redundant.

9.10
Lysogeny is a state in which lytic events are repressed. Viruses capable of entering the lysogenic state are called temperate viruses. In lysogeny the virus genome becomes a prophage, either by integration into the host chromosome or by replicating like a plasmid in step with the host cell. However, lytic events can be induced by certain environmental stimuli.

9.11
There are animal viruses with all known modes of viral genome replication. Many animal viruses are enveloped, picking up portions of host membrane as they leave the cell. Not all infections of animal host cells result in cell lysis or death; latent or persistent infections are common, and a few animal viruses can cause cancer.

9.12
Retroviruses are RNA viruses that replicate via a DNA intermediate. The retrovirus human immunodeficiency virus (HIV) causes AIDS. The retrovirus particle contains an enzyme, reverse transcriptase, that copies the information from its RNA genome into DNA. The DNA is then integrated into the host chromosome in the manner of a temperate virus. The retrovirus DNA can be transcribed to yield mRNA (and new genomic RNA) or may remain in a latent state.

9.13
Defective viruses are parasites of intact helper viruses. The helper viruses supply proteins that the defective virus no longer encodes. Some defective viruses rely on closely related but intact helper viruses. However, satellite viruses rely on unrelated intact viruses that infect the same host cells to complete replication events.

9.14
Viroids are circular single-stranded RNA molecules that do not encode proteins and are dependent on host-encoded enzymes, except for the ribozyme activity of the viroid molecule itself. Viroids are the smallest known pathogens that contain nucleic acids.

9.15
Prions consist of protein, but have no nucleic acid. Prions exist in two conformations, the native cellular form and the pathogenic form. The pathogenic form "replicates" itself by converting native prion proteins into the pathogenic conformation.

Review of Key Terms

Bacteriophage a virus that infects prokaryotic cells
Capsid the protein shell that surrounds the genome of a virus particle
Capsomere the subunit of a capsid
Defective virus a virus that relies on another virus, the helper virus, to provide some of its components
Early protein a protein synthesized soon after virus infection and before replication of the virus genome
Helper virus a virus that provides some necessary components for a defective virus
Host cell a cell inside which a virus replicates
Icosahedron a three-dimensional figure with 20 triangular faces
Late protein a protein synthesized later in virus infection, after replication of the virus genome
Lysogen a bacterium containing a prophage
Lysogeny a state in which a viral genome is replicated as a prophage along with the genome of the host
Lytic pathway a series of steps after virus infection that leads to virus replication and destruction of the host cell
Negative-strand virus a virus with a single-stranded genome that has the opposite sense to the viral mRNA
Nucleocapsid the complex of nucleic acid and proteins of a virus
Plaque a zone of lysis or growth inhibition caused by virus infection of a lawn of sensitive host cells
Positive-strand virus a virus with a single-stranded genome that has the same complementarity as the viral mRNA
Prion an infectious protein whose extracellular form contains no nucleic acid
Prophage the lysogenic form of a bacterial virus
Provirus the genome of a temperate or latent virus when it is replicating in step with the host chromosome
Retrovirus a virus whose RNA genome is replicated via a DNA intermediate
Reverse transcriptase the enzyme that makes a DNA copy using RNA as template
Reverse transcription the process of copying information found in RNA into DNA
Temperate virus a virus whose genome can replicate along with that of its host without causing cell death, in a state called lysogeny
Transformation in eukaryotes, a process by which a normal cell becomes a cancer cell
Transmissible spongiform encephalopathy (TSE) a degenerative disease of the brain caused by prion infection
Virion the infectious virus particle; the viral genome surrounded by a protein coat and sometimes other layers
Virulent virus a virus that lyses or kills the host cell after infection; a nontemperate virus
Virus a genetic element containing either RNA or DNA surrounded by a protein capsid and that replicates only inside host cells
Viroid a small, circular, single-stranded RNA that causes certain plant diseases

Review Questions

1. In what ways do viral genomes differ from those of cells (Section 9.1)?
2. Define virus. What are the minimal features needed to fit your definition (Section 9.2)?
3. Define the term "host" as it relates to viruses (Section 9.3).
4. Describe the events that occur on an agar plate containing a bacterial lawn when a single bacteriophage particle causes the formation of a bacteriophage plaque (Section 9.4).
5. Under some conditions, it is possible to obtain nucleic acid–free protein coats (capsids) of certain viruses. Under the electron microscope, these capsids look very similar to complete virions. What does this tell you about the role of the virus nucleic acid in the virus assembly process? Would you expect such particles to be infectious? Why (Section 9.5)?
6. Describe how a restriction endonuclease might play a role in resistance to bacteriophage infection. Why could a restriction endonuclease play such a role whereas a generalized DNase could not (Section 9.6)?
7. One can divide the replication process of a virus into five steps. Describe the events associated with each of these steps (Sections 9.6 and 9.7).
8. Specifically, why are both the life cycle and the virion of a positive-strand RNA virus likely to be simpler than those of a negative-strand RNA virus (Section 9.8)?
9. In terms of structure, how does the genome of bacteriophage T4 resemble and differ from that of *Escherichia coli* (Section 9.9)?
10. Many of the viruses we have considered have early genes and late genes. What is meant by these two classifications? What types of proteins tend to be encoded by early genes? What types of proteins by late genes? For bacteriophage T4 describe how expression of the late genes is controlled (Section 9.9).
11. Define the following: virulent, lysogeny, prophage (Section 9.10).
12. A strain of *Escherichia coli* that is missing the outer membrane protein responsible for maltose uptake is resistant to bacteriophage lambda infection. A lambda lysogen is immune to lambda infection. Describe the difference between resistance and immunity (Section 9.10).
13. Describe and differentiate the effects animal virus infection can have on an animal (Section 9.11).
14. Typically, tRNA is used in translation. However, it also plays a role in the replication of retroviral nucleic acid. Explain this role (Section 9.12).
15. What does a helper virus provide that allows a satellite virus to replicate (Section 9.13)?
16. What are the similarities and differences between viruses and viroids (Section 9.14)?
17. What are the similarities and differences between prions and viruses (Section 9.15)?

Application Questions

1. What causes the viral plaques that appear on a bacterial lawn to stop growing larger?
2. The promoters for mRNA encoding early proteins in viruses like T4 have a different sequence than the promoters for mRNA encoding late proteins in the same virus. Explain how this benefits the virus.
3. One characteristic of temperate bacteriophages is that they cause turbid rather than clear plaques on bacterial lawns. Can you think why this might be? (Remember the process by which a plaque develops in a lawn of bacteria.)
4. Suggest possible reasons why viroids infect only plants and not animals or bacteria.
5. Contrast the enzyme(s) present in the virions of a retrovirus and a positive-strand RNA bacteriophage. Why do they differ if each has plus configuration single-stranded RNA as their genome?
6. Since viral infection leads to more viral particles being formed, explain why the "growth curve" for viruses is stepped rather than smooth (as seen with bacterial multiplication).
7. What might be the advantage to bacterial host cells of carrying temperate viruses?

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