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PART VI

The Viruses

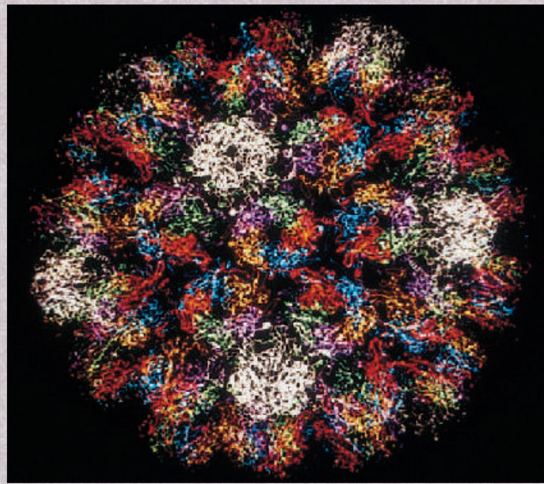
Chapter 16
The Viruses: Introduction and General Characteristics

Chapter 17
The Viruses: Bacteriophages

Chapter 18
The Viruses: Viruses of Eucaryotes

CHAPTER 16

The Viruses: Introduction and General Characteristics



The simian virus 40 (SV-40) capsid shown here differs from most icosahedral capsids in containing only pentameric capsomers (pp. 370–72). SV-40 is a small double-stranded DNA polyomavirus with 72 capsomers. It may cause a central nervous system disease in rhesus monkeys and can produce tumors in hamsters. SV-40 was first discovered in cultures of monkey kidney cells during preparation of the poliovirus vaccine.

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Concepts

1. Viruses are simple, acellular entities consisting of one or more molecules of either DNA or RNA enclosed in a coat of protein (and sometimes, in addition, substances such as lipids and carbohydrates). They can reproduce only within living cells and are obligately intracellular parasites.
2. Viruses are cultured by inoculating living hosts or cell cultures with a virion preparation. Purification depends mainly on their large size relative to cell components, high protein content, and great stability. The virus concentration may be determined from the virion count or from the number of infectious units.
3. All viruses have a nucleocapsid composed of a nucleic acid surrounded by a protein capsid that may be icosahedral, helical, or complex in structure. Capsids are constructed of protomers that self-assemble through noncovalent bonds. A membranous envelope often lies outside the nucleocapsid.
4. More variety is found in the genomes of viruses than in those of procaryotes and eucaryotes; they may be either single-stranded or double-stranded DNA or RNA. The nucleic acid strands can be linear, closed circle, or able to assume either shape.
5. Viruses are classified on the basis of their nucleic acid's characteristics, capsid symmetry, the presence or absence of an envelope, their host, the diseases caused by animal and plant viruses, and other properties.

Great fleas have little fleas upon their backs to bite 'em
And little fleas have lesser fleas, and so on ad infinitum

—*Augustus De Morgan*

Chapters 16, 17, and 18 focus on the viruses. These are infectious agents with fairly simple, acellular organization. They possess only one type of nucleic acid, either DNA or RNA, and only reproduce within living cells. Clearly viruses are quite different from procaryotic and eucaryotic microorganisms, and are studied by **virologists.**

Despite their simplicity in comparison with cellular organisms, viruses are extremely important and deserving of close attention. The study of viruses has contributed significantly to the discipline of molecular biology. Many human viral diseases are already known and more are discovered or arise every year, as demonstrated by the recent appearance of AIDS. The whole field of genetic engineering is based in large part upon discoveries in virology. Thus it is easy to understand why **virology** (the study of viruses) is such a significant part of microbiology.

This chapter focuses on the broader aspects of virology: its development as a scientific discipline, the general properties and structure of viruses, the ways in which viruses are cultured and studied, and viral taxonomy. Chapter 17 is concerned with the bacteriophages, and chapter 18 is devoted to the viruses of eucaryotes.

Viruses have had enormous impact on humans and other organisms, yet very little was known about their nature until fairly recently. A brief history of their discovery and recognition as uniquely different infectious agents can help clarify their nature.

16.1 Early Development of Virology

Although the ancients did not understand the nature of their illnesses, they were acquainted with diseases, such as rabies, that are now known to be viral in origin. In fact, there is some evidence that the great epidemics of A.D. 165 to 180 and A.D. 251 to 266, which severely weakened the Roman Empire and aided its decline, may have been caused by measles and smallpox viruses. Smallpox had an equally profound impact on the New World. Hernán Cortés's conquest of the Aztec Empire in Mexico was made possible by an epidemic that ravaged Mexico City. The virus was probably brought to Mexico in 1520 by the relief expedition sent to join Cortés. Before the smallpox epidemic subsided, it had killed the Aztec King Cuitlahuac (the nephew and son-in-law of the slain emperor, Montezuma II) and possibly 1/3 of the population. Since the Spaniards were not similarly afflicted, it appeared that God's wrath was reserved for the Native Americans, and this disaster was viewed as divine support for the Spanish conquest (**Box 16.1**).

The first progress in preventing viral diseases came years before the discovery of viruses. Early in the eighteenth century, Lady Wortley Montagu, wife of the English ambassador to Turkey, observed that Turkish women inoculated their children against smallpox. The children came down with a mild case and subsequently were immune. Lady Montagu tried to educate the English public about the procedure but without great success. Later in the century an English country doctor, Edward Jenner, stimulated by a girl's claim that she could not catch smallpox because she had had cowpox, began inoculating humans with material from cowpox lesions. He published the results of 23 successful vaccinations in 1798. Although Jenner did not understand the nature of smallpox, he did manage to successfully protect his patients from the dread disease through exposure to the cowpox virus.

Until well into the nineteenth century, harmful agents were often grouped together and sometimes called viruses [Latin virus, poison or venom]. Even Louis Pasteur used the term virus for any living infectious disease agent. The development in 1884 of the porcelain bacterial filter by Charles Chamberland, one of Pasteur's collaborators and inventor of the autoclave, made possible the discovery of what are now called viruses. Tobacco mosaic disease was the first to be studied with Chamberland's filter. In 1892 Dimitri Ivanowski published studies showing that leaf extracts from infected plants would induce tobacco mosaic disease even after filtration to remove bacteria. He attributed this to the presence of a toxin. Martinus W. Beijerinck, working independently of Ivanowski, published the results of extensive studies on tobacco mosaic disease in 1898 and 1900. Because the filtered sap of diseased plants was still infectious, he proposed that the disease was caused by an entity different from bacteria, a filterable virus. He observed that the virus would multiply only in living plant cells, but could survive for long periods in a dried state. At the same time Friedrich Loeffler and Paul Frosch in Germany found that the hoof-and-mouth disease of cattle was also caused by a filterable virus rather than by a toxin. In 1900 Walter Reed began his study of the yellow fever disease whose incidence had been increasing in Cuba. Reed showed that this human disease

Box 16.1

Disease and the Early Colonization of America

Although the case is somewhat speculative, there is considerable evidence that disease, and particularly smallpox, played a major role in reducing Indian resistance to the European colonization of North America. It has been estimated that Indian populations in Mexico declined about 90% within 100 years of initial contact with the Spanish. Smallpox and other diseases were a major factor in this decline, and there is no reason to suppose that North America was any different. As many as 10 to 12 million Indians may have lived north of the Rio Grande before contact with Europeans. In New England alone, there may have been over 72,000 in A.D. 1600; yet only around 8,600 remained in New England by A.D. 1674, and the decline continued in subsequent years.

Such an incredible catastrophe can be accounted for by consideration of the situation at the time of European contact with the Native Americans. The Europeans, having already suffered major epidemics in the preceding centuries, were relatively immune to the diseases they carried. On the other hand, the Native Americans had never been exposed to diseases like smallpox and were decimated by epidemics. In the sixteenth century, before any permanent English colonies had been established, many contacts were made by missionaries and explorers who undoubtedly brought disease with them and infected the natives. Indeed, the English noted at the end of the century that Indian populations had declined greatly but attributed it to armed conflict rather than to disease.

Establishment of colonies simply provided further opportunities for infection and outbreak of epidemics. For example, the Huron Indians decreased from a minimum of 32,000 people to 10,000 in 10 years. Between the time of initial English colonization and 1674, the Narraganset Indians declined from around 5,000 warriors to 1,000, and the Massachusetts Indians, from 3,000 to 300. Similar stories can be seen in other parts of the colonies. Some colonists interpreted these plagues as a sign of God's punishment of Indian resistance: the "Lord put an end to this quarrel by smiting them with smallpox. . . . Thus did the Lord allay their quarrelsome spirit and make room for the following part of his army."

It seems clear that epidemics of European diseases like smallpox decimated Native American populations and prepared the way for colonization of the North American continent. Many American cities—for example, Boston, Philadelphia, and Plymouth—grew upon sites of previous Indian villages.

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was due to a filterable virus that was transmitted by mosquitoes. Mosquito control shortly reduced the severity of the yellow fever problem. Thus by the beginning of this century, it had been established that filterable viruses were different from bacteria and could cause diseases in plants, livestock, and humans.

Shortly after the turn of the century, Vilhelm Ellermann and Oluf Bang in Copenhagen reported that leukemia could be transmitted between chickens by cell-free filtrates and was probably caused by a virus. Three years later in 1911, Peyton Rous from the Rockefeller Institute in New York City reported that a virus was responsible for a malignant muscle tumor in chickens. These studies established that at least some malignancies were caused by viruses.

It was soon discovered that bacteria themselves also could be attacked by viruses. The first published observation suggesting that this might be the case was made in 1915 by Frederick W. Twort. Twort isolated bacterial viruses that could attack and destroy micrococci and intestinal bacilli. Although he speculated that his preparations might contain viruses, Twort did not follow up on these observations. It remained for Felix d'Herelle to establish decisively the existence of bacterial viruses. D'Herelle isolated bacterial viruses from patients with dysentery, probably caused by *Shigella dysenteriae.* He noted that when a virus suspension was spread on a layer of bacteria growing on agar, clear circular areas containing viruses and lysed cells developed. A count of these clear zones allowed d'Herelle to estimate the number of viruses present (plaque assay, p. 368). D'Herelle demonstrated that these viruses could reproduce only in live bacteria; therefore he named them bacteriophages because they could eat holes in bacterial "lawns."

The chemical nature of viruses was established when Wendell M. Stanley announced in 1935 that he had crystallized the tobacco mosaic virus (TMV) and found it to be largely or completely protein. A short time later Frederick C. Bawden and Norman W. Pirie managed to separate the TMV virus particles into protein and nucleic acid. Thus by the late 1930s it was becoming clear that viruses were complexes of nucleic acids and proteins able to reproduce only in living cells.

1. Describe the major technical advances and discoveries important in the early development of virology.
2. Give the contribution to virology made by each scientist mentioned in this section.

16.2 General Properties of Viruses

Viruses are a unique group of infectious agents whose distinctiveness resides in their simple, acellular organization and pattern of reproduction. A complete virus particle or **virion** consists of one or more molecules of DNA or RNA enclosed in a coat of protein, and sometimes also in other layers. These additional layers may be very complex and contain carbohydrates, lipids, and additional proteins. Viruses can exist in two phases: extracellular and intracellular. Virions, the extracellular phase, possess few if any enzymes and cannot reproduce independent of living cells. In the intracellular phase, viruses exist primarily as replicating nucleic acids that induce host metabolism to synthesize virion components; eventually complete virus particles or virions are released.

In summary, viruses differ from living cells in at least three ways: (1) their simple, acellular organization; (2) the presence of either DNA or RNA, but not both, in almost all virions (human cytomegalovirus has a DNA genome and four mRNAs); and (3) their inability to reproduce independent of cells and carry out cell division as procaryotes and eucaryotes do. Although bacteria such as chlamydia and rickettsia (*see sections 21.5 and 22.1*) are obligately intracellular parasites like viruses, they do not meet the first two criteria.

16.3 The Cultivation of Viruses

Because they are unable to reproduce independent of living cells, viruses cannot be cultured in the same way as bacteria and eucaryotic microorganisms. For many years researchers have cultivated animal viruses by inoculating suitable host animals or embryonated eggs—fertilized chicken eggs incubated about 6 to 8 days after laying (**figure 16.1**). To prepare the egg for virus cultivation, the shell surface is first disinfected with iodine and penetrated with a small sterile drill. After inoculation, the drill hole is sealed with gelatin and the egg incubated. Viruses may be able to reproduce only in certain parts of the embryo; consequently they must be injected into the proper region. For example, the myxoma virus grows well on the chorioallantoic membrane, whereas the mumps virus prefers the allantoic cavity. The infection may produce a local tissue lesion known as a pock, whose appearance often is characteristic of the virus.

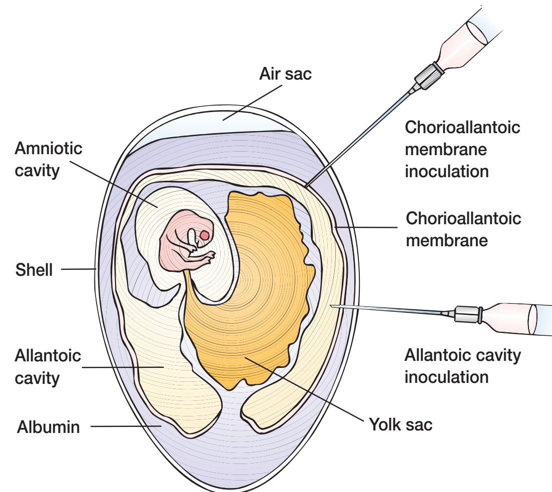


Figure 16.1 Virus Cultivation Using an Embryonated Egg. Two sites that are often used to grow animal viruses are the chorioallantoic membrane and the allantoic cavity. The diagram shows a 9 day chicken embryo.

More recently animal viruses have been grown in tissue (cell) culture on monolayers of animal cells. This technique is made possible by the development of growth media for animal cells and by the advent of antibiotics that can prevent bacterial and fungal contamination. A layer of animal cells in a specially prepared petri dish is covered with a virus inoculum, and the viruses are allowed time to settle and attach to the cells. The cells are then covered with a thin layer of agar to limit virion spread so that only adjacent cells are infected by newly produced virions. As a result localized areas of cellular destruction and lysis called **plaques** often are formed (**figure 16.2**) and may be detected if stained with dyes, such as neutral red or trypan blue, that can distinguish living from dead cells. Viral growth does not always result in the lysis of cells to form a plaque. Animal viruses, in particular, can cause microscopic or macroscopic degenerative changes or abnormalities in host cells and in tissues called **cytopathic effects** (**figure 16.3**). Cytopathic effects may be lethal, but plaque formation from cell lysis does not always occur.

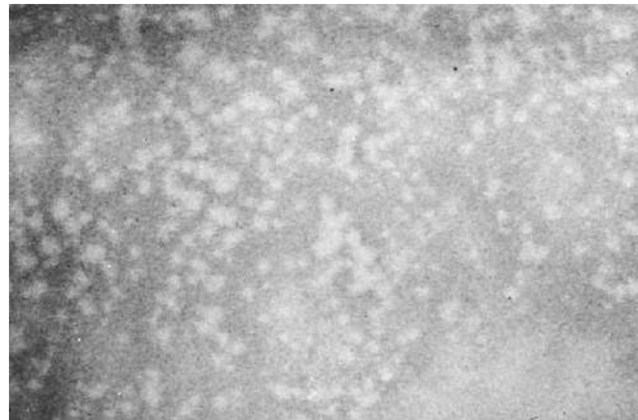


Figure 16.2 Virus Plaques. Poliovirus plaques in a monkey kidney cell culture.

Bacterial viruses or **bacteriophages** (**phages** for short) are cultivated in either broth or agar cultures of young, actively growing bacterial cells. So many host cells are destroyed that turbid bacterial cultures may clear rapidly because of cell lysis. Agar cultures are prepared by mixing the bacteriophage sample with cool, liquid agar and a suitable bacterial culture. The mixture is quickly poured into a petri dish containing a bottom layer of sterile agar. After hardening, bacteria in the layer of top agar grow and reproduce, forming a continuous, opaque layer or "lawn." Wherever a virion comes to rest in the top agar, the virus infects an adjacent cell and reproduces. Eventually, bacterial lysis generates a plaque or clearing in the lawn (**figure 16.4**). As can be seen in figure 16.4, plaque appearance often is characteristic of the phage being cultivated.

Plant viruses are cultivated in a variety of ways. Plant tissue cultures, cultures of separated cells, or cultures of protoplasts (*see section 3.3*) may be used. Viruses also can be grown in whole plants. Leaves are mechanically inoculated when rubbed with a mixture of viruses and an abrasive such as carborundum. When the cell walls are broken by the abrasive, the viruses directly contact the plasma membrane and infect the exposed host cells. (The role of the abrasive is frequently filled by insects that suck or crush plant leaves and thus transmit viruses.) A localized **necrotic lesion** often develops due to the rapid death of cells in

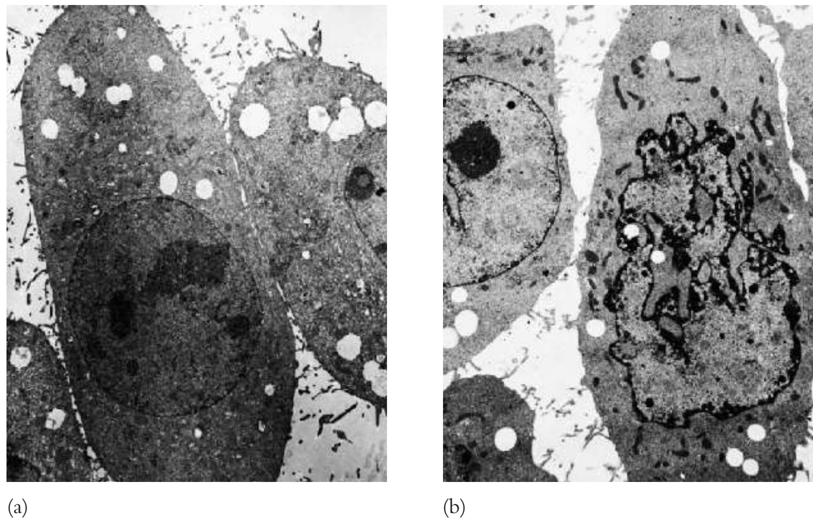


Figure 16.3 The Cytopathic Effects of Viruses. (**a**) Normal mammalian cells in tissue culture. (**b**) Appearance of tissue culture cells 18 hours after infection with adenovirus. Transmission electron microscope photomicrographs (×11,000).

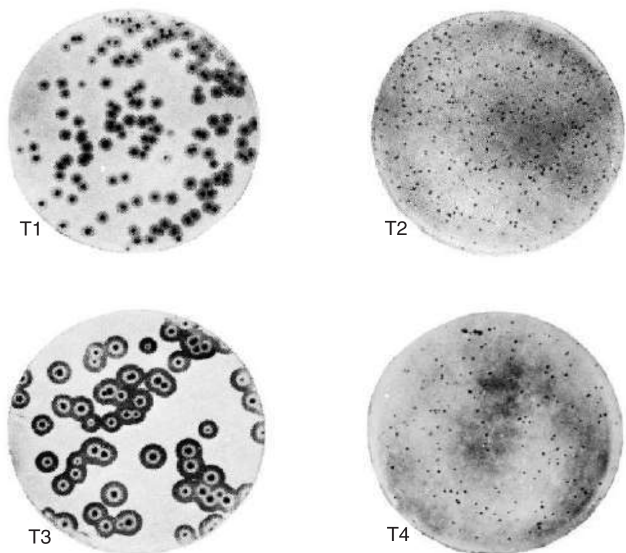


Figure 16.4 Phage Plaques. Plaques produced on a lawn of *E. coli* by some of the T coliphages. Note the large differences in plaque appearance. The photographs are about 1/3 full size.

the infected area (**figure 16.5**). Even when lesions do not occur, the infected plant may show symptoms such as changes in pigmentation or leaf shape. Some plant viruses can be transmitted only if a diseased part is grafted onto a healthy plant.

1. What is a virus particle or virion, and how is it different from living organisms?
2. Discuss the ways in which viruses may be cultivated. Define the terms pock, plaque, cytopathic effect, bacteriophage, and necrotic lesion.

Figure 16.5 Necrotic Lesions on Plant Leaves. (**a**) Tobacco mosaic virus on *Nicotiana glutinosa.* (**b**) Tobacco mosaic virus infection of an orchid showing leaf color changes.

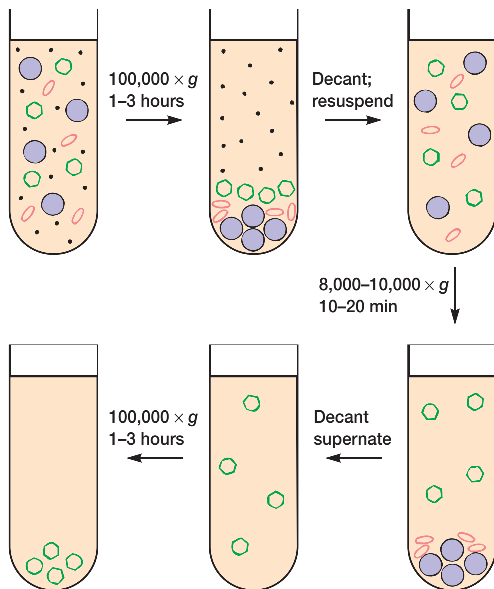


Figure 16.6 The Use of Differential Centrifugation to Purify a Virus. At the beginning the centrifuge tube contains homogenate and icosahedral viruses (in green). First, the viruses and heavier cell organelles are removed from smaller molecules. After resuspension, the mixture is centrifuged just fast enough to sediment cell organelles while leaving the smaller virus particles in suspension; the purified viruses are then collected. This process can be repeated several times to further purify the virions.

16.4 Virus Purification and Assays

Virologists must be able to purify viruses and accurately determine their concentrations in order to study virus structure, reproduction, and other aspects of their biology. These methods are so important that the growth of virology as a modern discipline depended on their development.

Virus Purification

Purification makes use of several virus properties. Virions are very large relative to proteins, are often more stable than normal cell components, and have surface proteins. Because of these characteristics, many techniques useful for the isolation of proteins and organelles can be employed in virus isolation. Four of the most widely used approaches are (1) differential and density gradient centrifugation, (2) precipitation of viruses, (3) denaturation of contaminants, and (4) enzymatic digestion of cell constituents.

1. Host cells in later stages of infection that contain mature virions are used as the source of material. Infected cells are first disrupted in a buffer to produce an aqueous suspension or homogenate consisting of cell components and viruses. Viruses can then be isolated by **differential centrifugation,** the centrifugation of a suspension at various speeds to separate particles of different sizes (**figure 16.6**). Usually the homogenate is first centrifuged at high speed to sediment viruses and other large cellular particles, and the supernatant, which contains the homogenate's soluble molecules, is discarded. The pellet is next resuspended and centrifuged at a low speed to remove substances heavier than viruses. Higher speed centrifugation then sediments the viruses. This process may be repeated to purify the virus particles further.

   Viruses also can be purified based on their size and density by use of **gradient centrifugation** (**figure 16.7**). A sucrose solution is poured into a centrifuge tube so that its concentration smoothly and linearly increases between the top and the bottom of the tube. The virus preparation, often after purification by differential centrifugation, is layered on top of the gradient and centrifuged. As shown in figure 16.7*a,* the particles settle under centrifugal force until they come to rest at the level where the gradient's density equals theirs (isopycnic gradient centrifugation). Viruses can be separated from other particles only slightly different in density. Gradients also can separate viruses based on differences in their sedimentation rate (rate zonal gradient centrifugation). When this is done, particles are separated on the basis of both size and density; usually the largest virus will move most rapidly down the gradient. Figure 16.7*b* shows that viruses differ from one another and cell components with respect to either density (grams per milliliter) or sedimentation coefficient(s). Thus these two types of gradient centrifugation are very effective in virus purification.
2. Viruses, like many proteins, can be purified through precipitation with concentrated ammonium sulfate. Initially, sufficient ammonium sulfate is added to raise its concentration to a level just below that which will precipitate the virus. After any precipitated contaminants are removed, more ammonium sulfate is added and the precipitated viruses are collected by centrifugation. Viruses sensitive to ammonium sulfate often are purified by precipitation with polyethylene glycol.
3. Viruses frequently are less easily denatured than many normal cell constituents. Contaminants may be denatured and precipitated with heat or a change in pH to purify viruses. Because some viruses also tolerate treatment with organic solvents like butanol and chloroform, solvent treatment can be used to both denature protein contaminants and extract any lipids in the preparation. The solvent is thoroughly mixed with the virus preparation, then allowed to stand and separate into organic and aqueous layers. The unaltered virus remains suspended in the aqueous phase while lipids dissolve in the organic phase. Substances denatured by organic solvents collect at the interface between the aqueous and organic phases. Denaturation of enzymes (pp. 163–64)
4. Cellular proteins and nucleic acids can be removed from many virus preparations through enzymatic degradation because viruses usually are more resistant to attack by nucleases and proteases than are free nucleic acids and proteins. For example, ribonuclease and trypsin often degrade cellular ribonucleic acids and proteins while leaving virions unaltered.

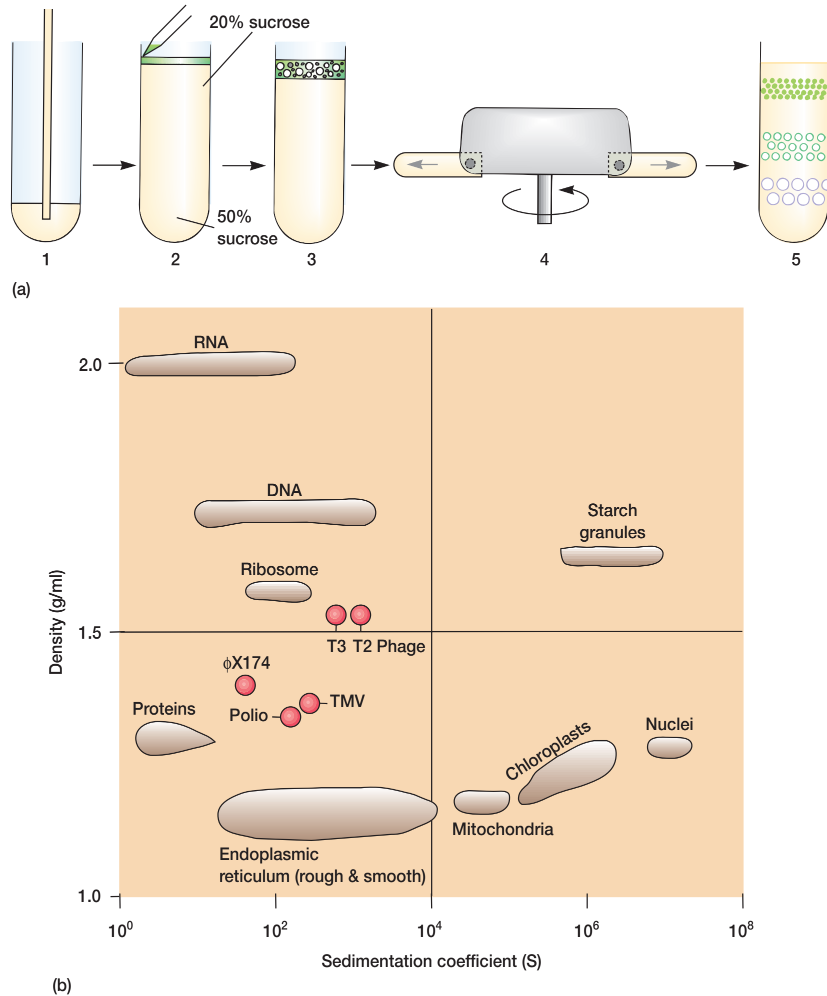


Figure 16.7 Gradient Centrifugation. (**a**) A linear sucrose gradient is prepared, *1*, and the particle mixture is layered on top, *2* and *3*. Centrifugation, *4,* separates the particles on the basis of their density and sedimentation coefficient, (the arrows in the centrifuge tubes indicate the direction of centrifugal force). *5.* In isopycnic gradient centrifugation, the bottom of the gradient is denser than any particle, and each particle comes to rest at a point in the gradient equal to its density. Rate zonal centrifugation separates particles based on their sedimentation coefficient, a function of both size and density, because the bottom of the gradient is less dense than the densest particles and centrifugation is carried out for a shorter time so that particles do not come to rest. The largest, most dense particles travel fastest. (**b**) The densities and sedimentation coefficients of representative viruses (shown in color) and other biological substances.

Virus Assays

The quantity of viruses in a sample can be determined either by counting particle numbers or by measurement of the infectious unit concentration. Although most normal virions are probably potentially infective, many will not infect host cells because they do not contact the proper surface site. Thus the total particle count may be from 2 to 1 million times the infectious unit number depending on the nature of the virion and the experimental conditions. Despite this, both approaches are of value.

Virus particles can be counted directly with the electron microscope. In one procedure the virus sample is mixed with a known concentration of small latex beads and sprayed on a coated specimen grid. The beads and virions are counted; the virus concentration is calculated from these counts and from the bead concentration (**figure 16.8**). This technique often works well with concentrated preparations of viruses of known morphology. Viruses can be concentrated by centrifugation before counting if the preparation is too dilute. However, if the beads and viruses are not evenly distributed (as sometimes happens), the final count will be inaccurate.

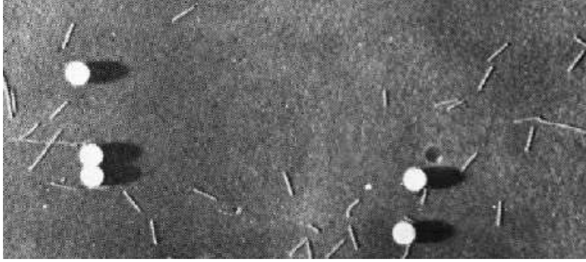


Figure 16.8 Tobacco Mosaic Virus. A tobacco mosaic virus preparation viewed in the transmission electron microscope. Latex beads 264 mm in diameter (white spheres) have been added.

The most popular indirect method of counting virus particles is the **hemagglutination assay.** Many viruses can bind to the surface of red blood cells (*see figure 33.10*). If the ratio of viruses to cells is large enough, virus particles will join the red blood cells together, forming a network that settles out of suspension or agglutinates. In practice, red blood cells are mixed with a series of virus preparation dilutions and each mixture is examined. The hemagglutination titer is the highest dilution of virus (or the reciprocal of the dilution) that still causes hemagglutination. This assay is an accurate, rapid method for determining the relative quantity of viruses such as the influenza virus. If the actual number of viruses needed to cause hemagglutination is determined by another technique, the assay can be used to ascertain the number of virus particles present in a sample.

A variety of assays analyze virus numbers in terms of infectivity, and many of these are based on the same techniques used for virus cultivation. For example, in the **plaque assay** several dilutions of bacterial or animal viruses are plated out with appropriate host cells. When the number of viruses plated out are much fewer than the number of host cells available for infection and when the viruses are distributed evenly, each plaque in a layer of bacterial or animal cells is assumed to have arisen from the reproduction of a single virus particle. Therefore a count of the plaques produced at a particular dilution will give the number of infectious virions or **plaque-forming units (PFU),** and the concentration of infectious units in the original sample can be easily calculated. Suppose that 0.10 ml of a 10–6 dilution of the virus preparation yields 75 plaques. The original concentration of plaque-forming units is

$$\text{PFU/ml} = (75\ \text{PFU}/0.10\ \text{ml})(10^6) = 7.5 \times 10^8.$$

Viruses producing different plaque morphology types on the same plate may be counted separately. Although the number of PFU does not equal the number of virus particles, their ratios are proportional: a preparation with twice as many viruses will have twice the plaque-forming units.

The same approach employed in the plaque assay may be used with embryos and plants. Chicken embryos can be inoculated with a diluted preparation or plant leaves rubbed with a mixture of diluted virus and abrasive. The number of pocks on embryonic membranes or necrotic lesions on leaves is multiplied by the dilution factor and divided by the inoculum volume to obtain the concentration of infectious units.

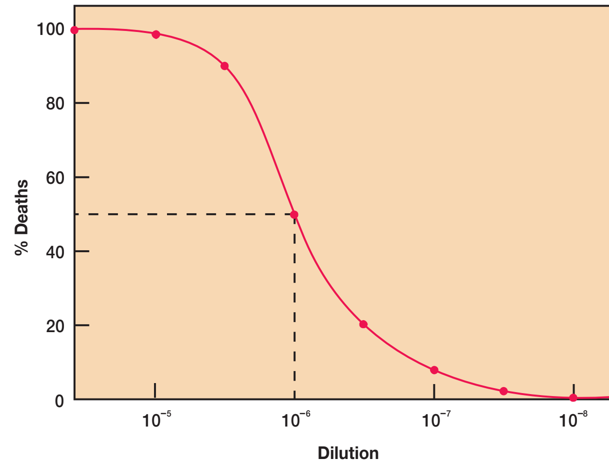


Figure 16.9 A Hypothetical Dose-Response Curve. The LD_{50} is indicated by the dashed line.

When biological effects are not readily quantified in these ways, the amount of virus required to cause disease or death can be determined by the endpoint method. Organisms or cell cultures are inoculated with serial dilutions of a virus suspension. The results are used to find the endpoint dilution at which 50% of the host cells or organisms are destroyed (**figure 16.9**). The **lethal dose (LD_{50})** is the dilution that contains a dose large enough to destroy 50% of the host cells or organisms. In a similar sense, the **infectious dose (ID_{50})** is the dose which, when given to a number of test systems or hosts, causes an infection of 50% of the systems or hosts under the conditions employed.

1. Give the four major approaches by which viruses may be purified, and describe how each works. Distinguish between differential and density gradient centrifugation in terms of how they are carried out.
2. How can one find the virus concentration, both directly and indirectly, by particle counts and measurement of infectious unit concentration? Define plaque-forming units, lethal dose, and infectious dose.

16.5 The Structure of Viruses

Virus morphology has been intensely studied over the past decades because of the importance of viruses and the realization that virus structure was simple enough to be understood. Progress has come from the use of several different techniques: electron microscopy, X-ray diffraction, biochemical analysis, and immunology. Although our knowledge is incomplete due to the large number of different viruses, the general nature of virus structure is becoming clear.

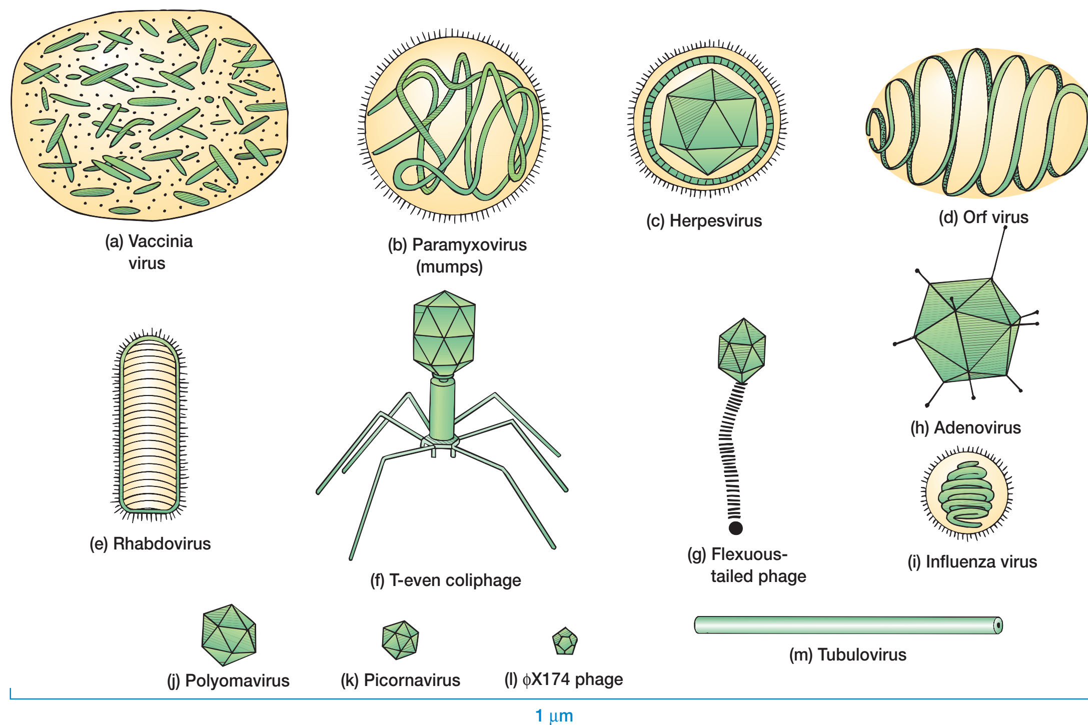


Figure 16.10 **The Size and Morphology of Selected Viruses.** The viruses are drawn to scale. A 1 μm line is provided at the bottom of the figure.

Virion Size

Virions range in size from about 10 to 300 or 400 nm in diameter (**figure 16.10**). The smallest viruses are a little larger than ribosomes, whereas the poxviruses, like vaccinia, are about the same size as the smallest bacteria and can be seen in the light microscope. Most viruses, however, are too small to be visible in the light microscope and must be viewed with the scanning and transmission electron microscopes (*see section 2.4*).

General Structural Properties

All virions, even if they possess other constituents, are constructed around a **nucleocapsid** core (indeed, some viruses consist only of a nucleocapsid). The nucleocapsid is composed of a nucleic acid, either DNA or RNA, held within a protein coat called the **capsid,** which protects viral genetic material and aids in its transfer between host cells.

There are four general morphological types of capsids and virion structure.

1. Some capsids are **icosahedral** in shape. An icosahedron is a regular polyhedron with 20 equilateral triangular faces and 12 vertices (figure 16.10*h,j–l*). These capsids appear spherical when viewed at low power in the electron microscope.
2. Other capsids are **helical** and shaped like hollow protein cylinders, which may be either rigid or flexible (figure 16.10*m*).
3. Many viruses have an **envelope,** an outer membranous layer surrounding the nucleocapsid. Enveloped viruses have a roughly spherical but somewhat variable shape even though their nucleocapsid can be either icosahedral or helical (figure 16.10*b,c,i*).
4. **Complex viruses** have capsid symmetry that is neither purely icosahedral nor helical (figure 16.10*a,d,f,g*). They may possess tails and other structures (e.g., many bacteriophages) or have complex, multilayered walls surrounding the nucleic acid (e.g., poxviruses such as vaccinia).

Both helical and icosahedral capsids are large macromolecular structures constructed from many copies of one or a few types of protein subunits or **protomers.** Probably the most important advantage of this design strategy is that the information stored in viral genetic material is used with maximum efficiency. For example, the tobacco mosaic virus (TMV) capsid contains a single type of small subunit possessing 158 amino acids. Only about 474 nucleotides out of 6,000 in the virus RNA are required to code for coat protein amino acids. Unless the same protein is used many times in capsid construction, a large nucleic acid, such

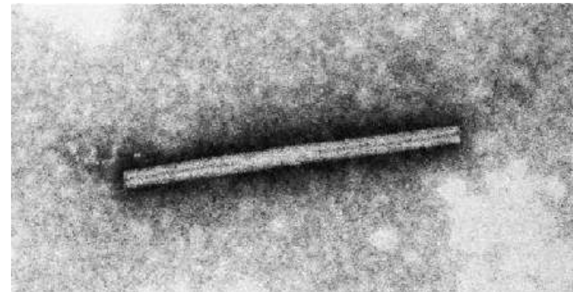
as the TMV RNA, cannot be enclosed in a protein coat without using much or all of the available genetic material to code for capsid proteins. If the TMV capsid were composed of six different protomers of the same size as the TMV subunit, about 2,900 of the 6,000 nucleotides would be required for its construction, and much less genetic material would be available for other purposes. The genetic code and translation (pp. 240–41)

Once formed and exposed to the proper conditions, protomers usually interact specifically with each other and spontaneously associate to form the capsid. Because the capsid is constructed without any outside aid, the process is called self-assembly (*see p. 65*). Some more complex viruses possess genes for special factors that are not incorporated into the virion but are required for its assembly.

Helical Capsids

Helical capsids are shaped much like hollow tubes with protein walls. The tobacco mosaic virus provides a well-studied example of helical capsid structure (**figure 16.11**). A single type of protomer associates together in a helical or spiral arrangement to produce a long, rigid tube, 15 to 18 nm in diameter by 300 nm long. The RNA genetic material is wound in a spiral and positioned toward the inside of the capsid where it lies within a groove formed by the protein subunits. Not all helical capsids are as rigid as the TMV capsid. Influenza virus RNAs are enclosed in thin, flexible helical capsids folded within an envelope (figures 16.10*i* and 16.12*a,b*).

The size of a helical capsid is influenced by both its protomers and the nucleic acid enclosed within the capsid. The diameter of the capsid is a function of the size, shape, and interactions of the protomers. The nucleic acid determines helical capsid length because the capsid does not seem to extend much beyond the end of the DNA or RNA.



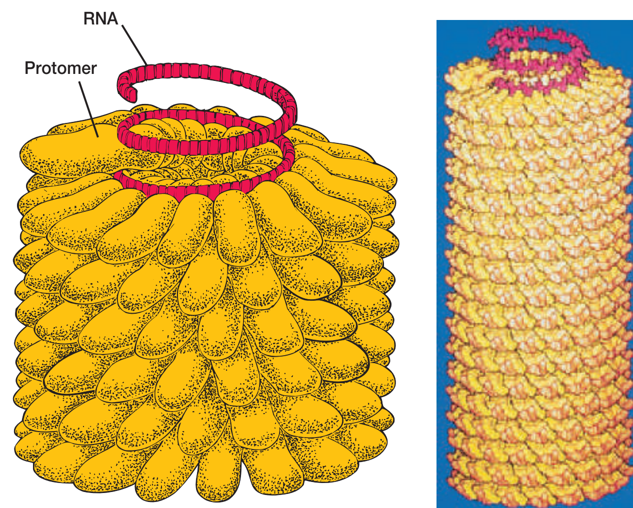


Figure 16.11 Tobacco Mosaic Virus Structure. (**a**) An electron micrograph of the negatively stained helical capsid (×400,000). (**b**) Illustration of TMV structure. Note that the nucleocapsid is composed of a helical array of promoters with the RNA spiraling on the inside. (**c**) A model of TMV.

Icosahedral Capsids

The icosahedron is one of nature's favorite shapes (the helix is probably most popular). Viruses employ the icosahedral shape because it is the most efficient way to enclose a space. A few genes, sometimes only one, can code for proteins that self-assemble to form the capsid. In this way a small number of linear genes can specify a large three-dimensional structure. Certain requirements must be met to construct an icosahedron. Hexagons pack together in planes and cannot enclose a space, and therefore pentagons must also be used.

When icosahedral viruses are negatively stained and viewed in the transmission electron microscope, a complex icosahedral capsid structure is revealed (**figure 16.12**). The capsids are constructed from ring- or knob-shaped units called **capsomers,** each usually made of five or six protomers. **Pentamers (pentons)** have five subunits; **hexamers (hexons)** possess six. Pentamers are at the vertices of the icosahedron, whereas hexamers form its edges and triangular faces (**figure 16.13**). The icosahedron in figure 16.13 is constructed of 42 capsomers; larger icosahedra are made if more hexamers are used to form the edges and faces (adenoviruses have a capsid with 252 capsomers as shown in figure 16.12*g,h*). In many plant and bacterial RNA viruses, both the pentamers and hexamers of a capsid are constructed with only one type of subunit, whereas adenovirus pentamers are composed of different proteins than are adenovirus hexamers. Transmission electron microscopy and negative staining (pp. 30–33)

Protomers join to form capsomers through noncovalent bonding. The bonds between proteins within pentamers and hexamers are stronger than those between separate capsomers. Empty capsids can even dissociate into separate capsomers.

Recently it has been discovered that there is more than one way to build an icosahedral capsid. Although most icosahedral capsids appear to contain both pentamers and hexamers, simian virus 40 (SV-40), a small double-stranded DNA polyomavirus, has only pentamers (**figure 16.14*a***). The virus is constructed of 72 cylindrical pentamers with hollow centers. Five flexible arms

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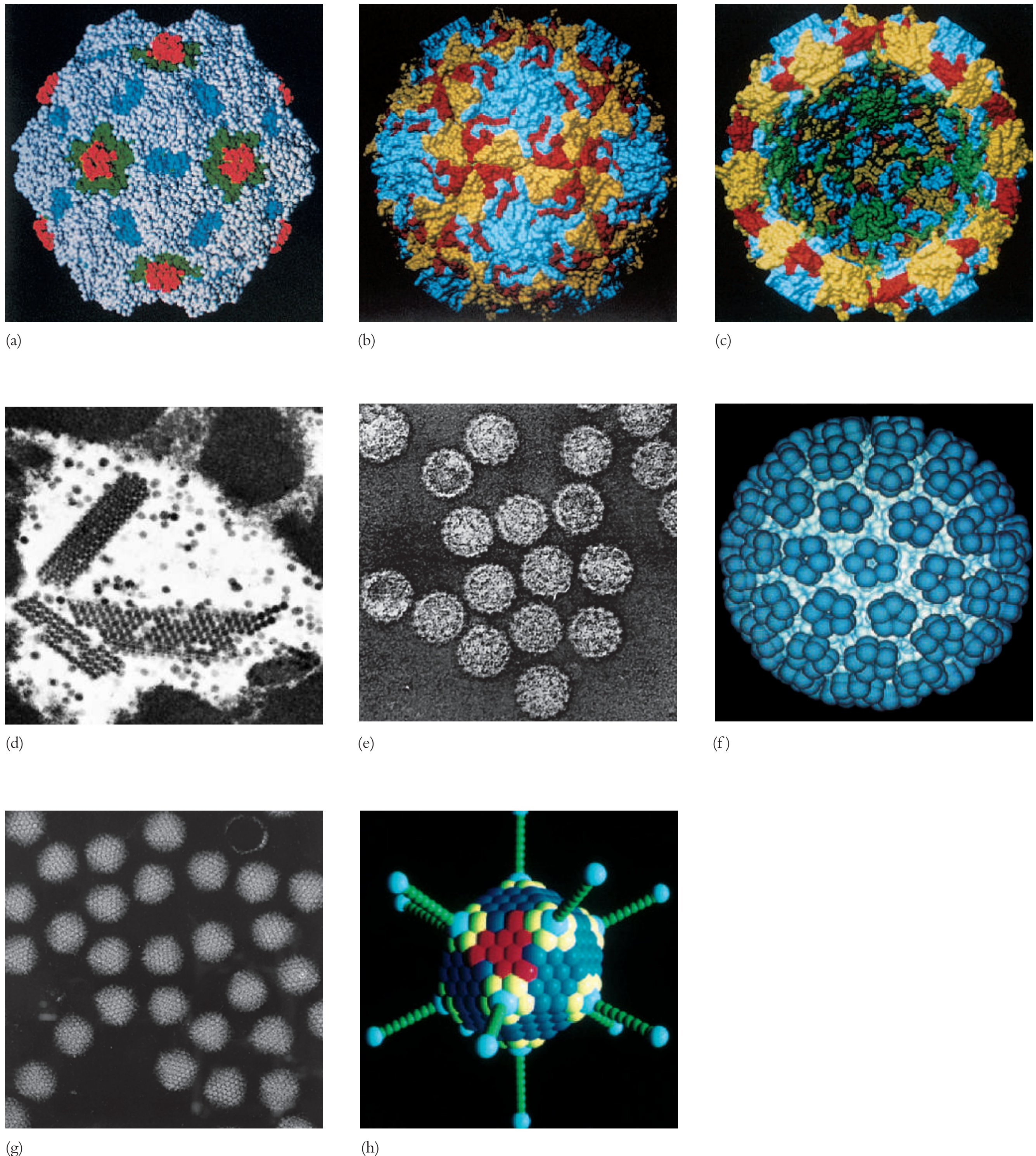


Figure 16.12 Examples of Icosahedral Capsids. (**a**) Canine parvovirus model, 12 capsomers, with the four parts of each capsid polypeptide given different colors. (**b**) and (**c**) Poliovirus model, 32 capsomers, with the four capsid proteins in different colors. The capsid surface is depicted in (*b*) and a cross section in (*c*). (**d**) Clusters of the human papilloma virus, 72 capsomers (×80,000). (**e**) Simian virus 40 (SV-40), 72 capsomers (×340,000). (**f**) Computer-simulated image of the polyomavirus, 72 capsomers, that causes a rare demyelinating disease of the central nervous system. (**g**) Adenovirus, 252 capsomers (×171,000). (**h**) Computer-simulated model of adenovirus.

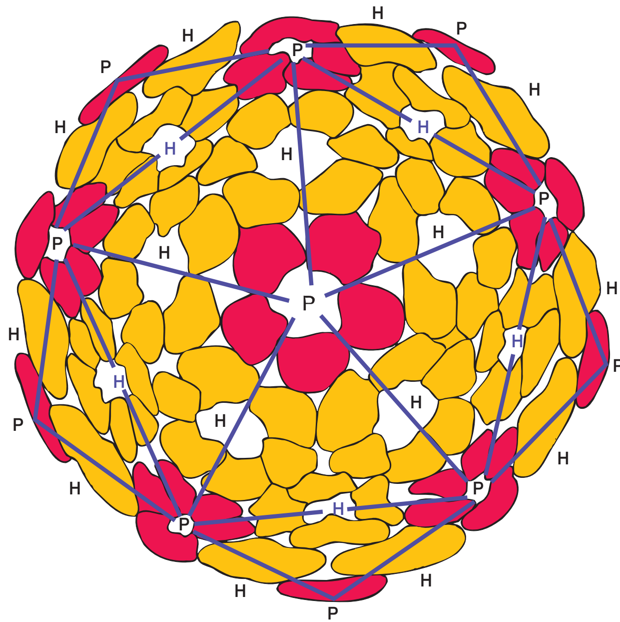
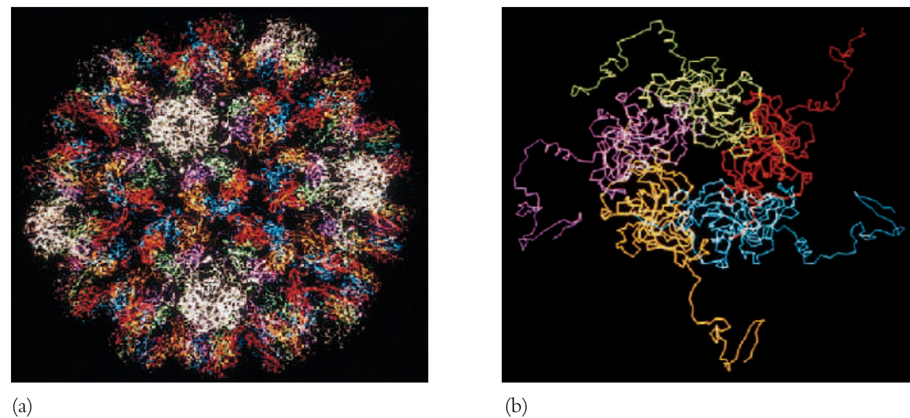


Figure 16.13 The Structure of an Icosahedral Capsid. Pentons are located at the 12 vertices. Hexons form the edges and faces of the icosahedron. This capsid contains 42 capsomers; all protomers are identical.

extend from the edge of each pentamer (figure 16.14*b*). Twelve pentamers occupy the icosahedron's vertices and associate with five neighbors, just as they do when hexamers also are present. Each of the 60 nonvertex pentamers associates with its six adjacent neighbors as shown in figure 16.14*c*. An arm extends toward the adjacent vertex pentamer (pentamer 1) and twists around one of its arms. Three more arms interact in the same way with arms of other nonvertex pentamers (pentamers 3 to 5). The fifth arm binds directly to an adjacent nonvertex pentamer (pentamer 6) but does not attach to one of its arms. An arm does not extend from the central pentamer to pentamer 2; other arms hold pentamer 2 in place. Thus an icosahedral capsid is assembled without hexamers by using flexible arms as ropes to tie the pentamers together.

Nucleic Acids

Viruses are exceptionally flexible with respect to the nature of their genetic material. They employ all four possible nucleic acid types: single-stranded DNA, double-stranded DNA, single-stranded RNA, and double-stranded RNA. All four types are found in animal viruses. Plant viruses most often have single-stranded RNA genomes. Although phages may have single-stranded DNA or single-stranded RNA, bacterial viruses usually contain double-stranded DNA.



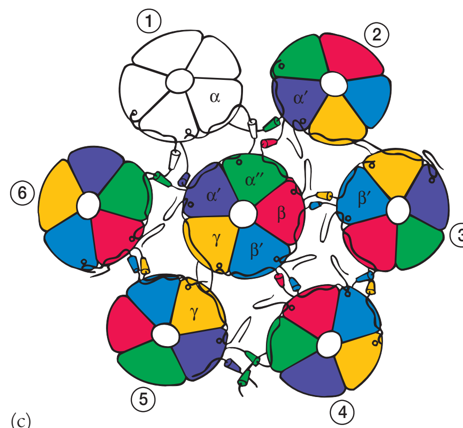


Figure 16.14 An Icosahedral Capsid Constructed of Pentamers. (**a**) The simian virus 40 capsid. The 12 pentamers at the icosahedron vertices are in white. The nonvertex pentamers are shown with each polypeptide chain in a different color. (**b**) A pentamer with extended arms. (**c**) A schematic diagram of the surface structure depicted in part **c.** The body of each pentamer is represented by a five-petaled flower design. Each arm is shown as a line or a line and cylinder (α-helix) with the same color as the rest of its protomer. The outer protomers are numbered clockwise beginning with the one at the vertex.

Table 16.1 Types of Viral Nucleic Acids

Nucleic Acid Type	Nucleic Acid Structure	Virus Examples
DNA		
Single-Stranded	Linear single strand	Parvoviruses
	Circular single strand	ϕX174, M13, fd phages
Double-Stranded	Linear double strand	Herpesviruses (herpes simplex viruses, cytomegalovirus, Epstein-Barr virus), adenoviruses, T coliphages, lambda phage, and other bacteriophages
	Linear double strand with single chain breaks	T5 coliphage
	Double strand with cross-linked ends	Vaccinia, smallpox
	Closed circular double strand	Polyomaviruses (SV-40), papillomaviruses, PM2 phage, cauliflower mosaic
RNA		
Single-Stranded	Linear, single stranded, positive strand	Picornaviruses (polio, rhinoviruses), togaviruses, RNA bacteriophages, TMV, and most plant viruses
	Linear, single stranded, negative strand	Rhabdoviruses (rabies), paramyxoviruses (mumps, measles)
	Linear, single stranded, segmented, positive strand	Brome mosaic virus (individual segments in separate virions)
	Linear, single stranded, segmented, diploid (two identical single strands), positive strand	Retroviruses (Rous sarcoma virus, human immunodeficiency virus)
	Linear, single stranded, segmented, negative strand	Paramyxoviruses, orthomyxoviruses (influenza)
Double-Stranded	Linear, double stranded, segmented	Reoviruses, wound-tumor virus of plants, cytoplasmic polyhedrosis virus of insects, phage ϕ6, many mycoviruses

Modified from S. E. Luria, et al., *General Virology,* 3d edition, 1983. John Wiley & Sons, Inc., New York, NY.

Table 16.1 summarizes many variations seen in viral nucleic acids. The size of viral genetic material also varies greatly. The smallest genomes (those of the MS2 and Qβ viruses) are around 1×10^6 daltons, just large enough to code for three to four proteins. MS2, Qβ, and some other viruses even save space by using overlapping genes (*see section 11.5*). At the other extreme, T-even bacteriophages, herpesvirus, and vaccinia virus have genomes of 1.0 to 1.6×10^8 daltons and may be able to direct the synthesis of over 100 proteins. In the following paragraphs the nature of each nucleic acid type is briefly summarized. Nucleic acid structure (pp. 230–35)

Tiny DNA viruses like ϕX174 and M13 bacteriophages or the parvoviruses possess single-stranded DNA (ssDNA) genomes (table 16.1). Some of these viruses have linear pieces of DNA, whereas others use a single, closed circle of DNA for their genome (**figure 16.15**).

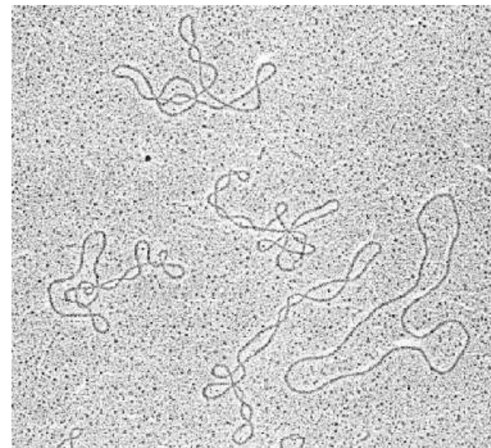


Figure 16.15 Circular Phage DNA. The closed circular DNA of the phage PM2 (×93,000). Note both the relaxed and highly twisted or supercoiled forms.

Most DNA viruses use double-stranded DNA (dsDNA) as their genetic material. Linear dsDNA, variously modified, is found in many viruses; others have circular dsDNA. The lambda phage has linear dsDNA with cohesive ends—single-stranded

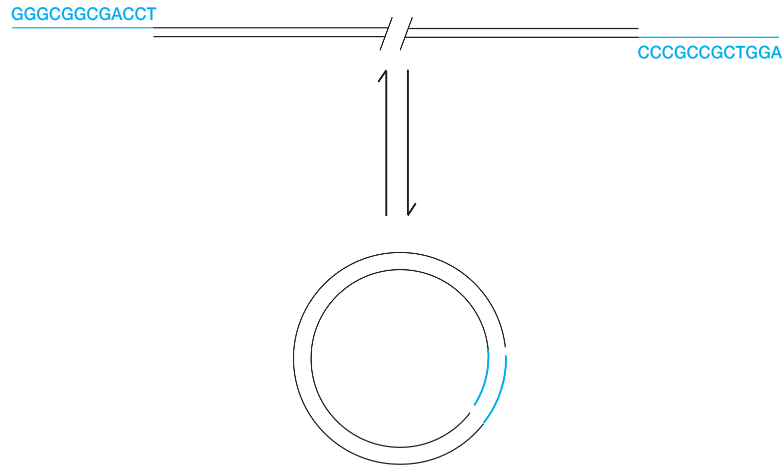


Figure 16.16 Circularization of Lambda DNA. The linear DNA of the lambda phage can be reversibly circularized. This is made possible by cohesive ends (in color) that have complementary sequences and can base pair with each other.

complementary segments 12 nucleotides long—that enable it to cyclize when they base pair with each other (**figure 16.16**).

Besides the normal nucleotides found in DNA, many virus DNAs contain unusual bases. For example, the T-even phages of *E. coli (see chapter 17)* have 5-hydroxymethylcytosine (*see figure 17.7*) instead of cytosine. Glucose is usually attached to the hydroxymethyl group.

Most RNA viruses employ single-stranded RNA (ssRNA) as their genetic material. The RNA base sequence may be identical with that of viral mRNA, in which case the RNA strand is called the **plus strand** or **positive strand** (viral mRNA is defined as plus or positive). However, the viral RNA genome may instead be complementary to viral mRNA, and then it is called a **minus** or **negative strand.** Polio, tobacco mosaic, brome mosaic, and Rous sarcoma viruses are all positive strand RNA viruses; rabies, mumps, measles, and influenza viruses are examples of negative strand RNA viruses. Many of these RNA genomes are **segmented genomes**—that is, they are divided into separate parts. It is believed that each fragment or segment codes for one protein. Usually all segments are probably enclosed in the same capsid even though some virus genomes may be composed of as many as 10 to 12 segments. However, it is not necessary that all segments be located in the same virion for successful reproduction. The brome mosaic virus genome is composed of four segments distributed among three different virus particles. All three of the largest segments are required for infectivity. Despite this complex and seemingly inefficient arrangement, the different brome mosaic virions manage to successfully infect the same host.

Plus strand viral RNA often resembles mRNA in more than the equivalence of its nucleotide sequence. Just as eucaryotic mRNA usually has a 5′ cap of 7-methylguanosine, many plant and animal viral RNA genomes are capped. In addition, most or all plus strand RNA animal viruses also have a poly-A stretch at the 3′ end of their genome, and thus closely resemble eucaryotic mRNA with respect to the structure of both ends. In fact, plus strand RNAs can direct protein synthesis immediately after entering the cell. Strangely enough, a number of single-stranded plant viral RNAs have 3′ ends that resemble eucaryotic transfer RNA, and the genomes of tobacco mosaic virus will actually accept amino acids. Capping is not seen in the RNA bacteriophages. Eucaryotic mRNA structure and function (pp. 263–64)

A few viruses have double-stranded RNA (dsRNA) genomes. All appear to be segmented; some, such as the reoviruses, have 10 to 12 segments. These dsRNA viruses are known to infect animals, plants, fungi, and even one bacterial species.

Viral Envelopes and Enzymes

Many animal viruses, some plant viruses, and at least one bacterial virus are bounded by an outer membranous layer called an envelope (**figure 16.17**). Animal virus envelopes usually arise from host cell nuclear or plasma membranes; their lipids and carbohydrates are normal host constituents. In contrast, envelope proteins are coded for by virus genes and may even project from the envelope surface as **spikes** or **peplomers** (figure 16.17*a,b,f*). These spikes may be involved in virus attachment to the host cell surface. Since they differ among viruses, they also can be used to identify some viruses. Because the envelope is a flexible, membranous structure, enveloped viruses frequently have a somewhat variable shape and are called pleomorphic. However, the envelopes of viruses like the bullet-shaped rabies virus are firmly attached to the underlying nucleocapsid and endow the virion with a constant, characteristic shape (figure 16.17*c*). In some viruses the envelope is disrupted by solvents like ether to such an extent that lipid-mediated activities are blocked or envelope proteins are denatured and rendered inactive. The virus is then said to be "ether sensitive."

Influenza virus (figure 16.17*a,b*) is a well-studied example of an enveloped virus. Spikes project about 10 nm from the surface at 7 to 8 nm intervals. Some spikes possess the enzyme neuraminidase, which may aid the virus in penetrating mucous layers of the respiratory epithelium to reach host cells. Other spikes have hemagglutinin proteins, so named because they can bind the virions to red blood cell membranes and cause hemagglutination (*see figure 33.10*). Hemagglutinins participate in virion attachment to host cells. Proteins, like the spike proteins that are

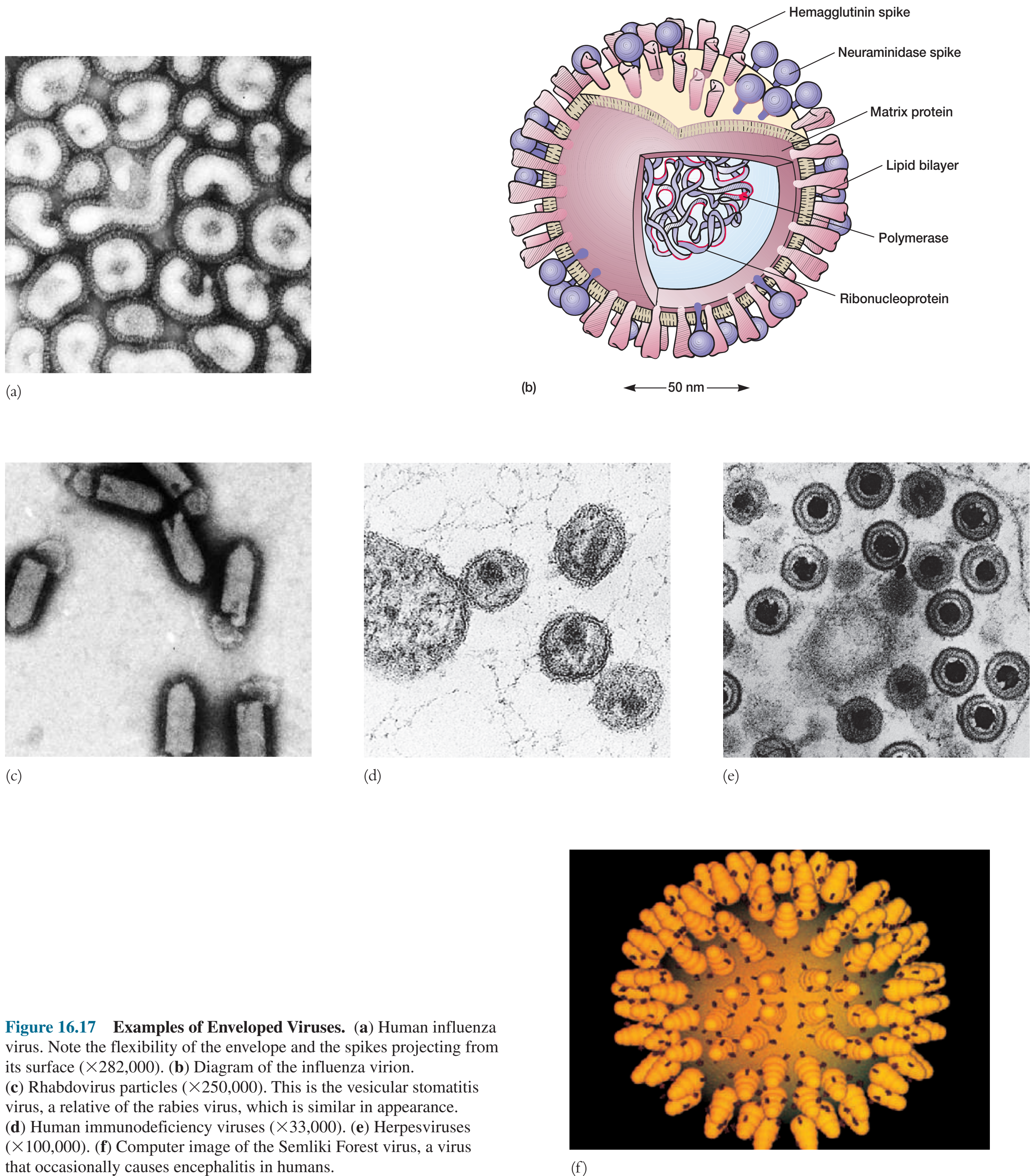


Figure 16.17 Examples of Enveloped Viruses. (**a**) Human influenza virus. Note the flexibility of the envelope and the spikes projecting from its surface (×282,000). (**b**) Diagram of the influenza virion. (**c**) Rhabdovirus particles (×250,000). This is the vesicular stomatitis virus, a relative of the rabies virus, which is similar in appearance. (**d**) Human immunodeficiency viruses (×33,000). (**e**) Herpesviruses (×100,000). (**f**) Computer image of the Semliki Forest virus, a virus that occasionally causes encephalitis in humans.

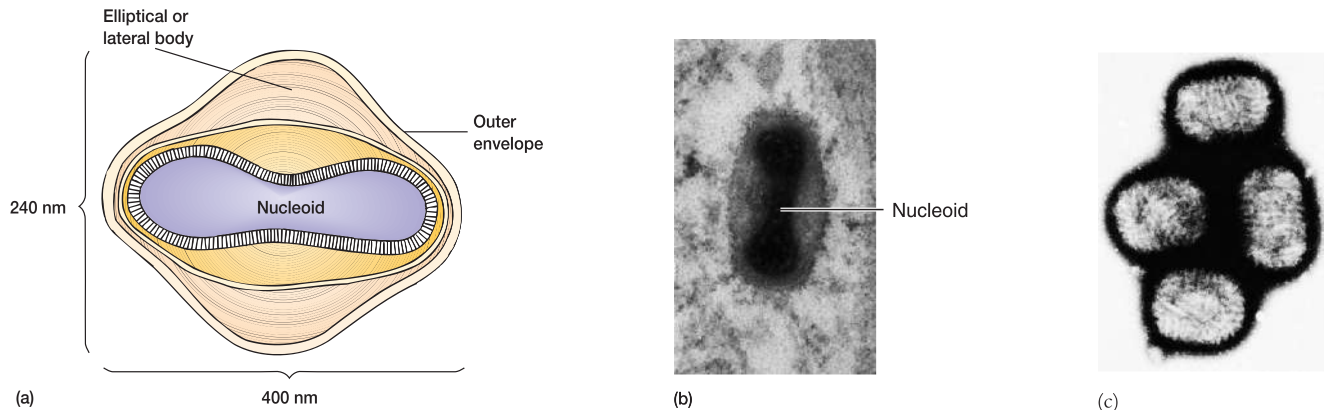


Figure 16.18 Vaccinia Virus Morphology. (**a**) Diagram of vaccinia structure. (**b**) Micrograph of the virion clearly showing the nucleoid (×200,000). (**c**) Vaccinia surface structure. An electron micrograph of four virions showing the thick array of surface fibers (×150,000).

exposed on the outer envelope surface, are generally glycoproteins—that is, the proteins have carbohydrate attached to them. A nonglycosylated protein, the M or matrix protein, is found on the inner surface of the envelope and helps stabilize it.

Although it was originally thought that virions had only structural capsid proteins and lacked enzymes, this has proven not to be the case. In some instances, enzymes are associated with the envelope or capsid (e.g., influenza neuraminidase). Most viral enzymes are probably located within the capsid. Many of these are involved in nucleic acid replication. For example, the influenza virus uses RNA as its genetic material and carries an RNA-dependent RNA polymerase that acts both as a replicase and as an RNA transcriptase that synthesizes mRNA under the direction of its RNA genome. The polymerase is associated with ribonucleoprotein (figure 16.17*b*). Although viruses lack true metabolism and cannot reproduce independently of living cells, they may carry one or more enzymes essential to the completion of their life cycles. Nucleic acid replication and transcription (sections 11.3 and 12.1); Animal virus reproduction (pp. 399–410)

Viruses with Capsids of Complex Symmetry

Although most viruses have either icosahedral or helical capsids, many viruses do not fit into either category. The poxviruses and large bacteriophages are two important examples.

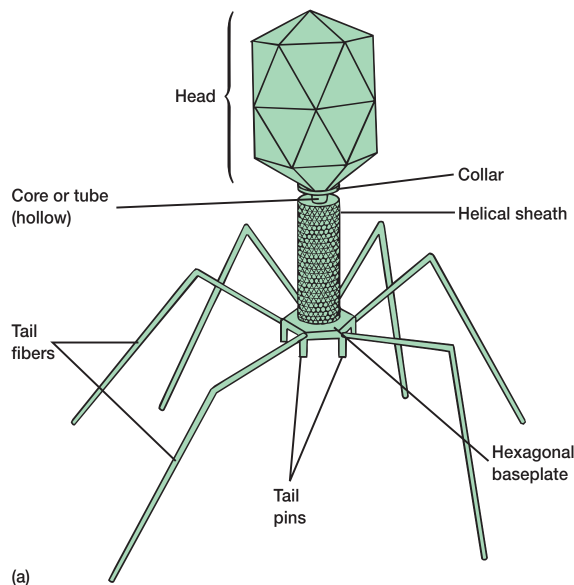
The poxviruses are the largest of the animal viruses (about 400 × 240 × 200 nm in size) and can even be seen with a phase-contrast microscope or in stained preparations. They possess an exceptionally complex internal structure with an ovoid- to brick-shaped exterior. The double-stranded DNA is associated with proteins and contained in the nucleoid, a central structure shaped like a biconcave disk and surrounded by a membrane (**figure 16.18**). Two elliptical or lateral bodies lie between the nucleoid and its outer envelope, a membrane and a thick layer covered by an array of tubules or fibers.

Some large bacteriophages are even more elaborate than the poxviruses. The T2, T4, and T6 phages that infect *E. coli* have been intensely studied. Their head resembles an icosahedron elongated by one or two rows of hexamers in the middle (**figure 16.19**) and contains the DNA genome. The tail is composed of a collar joining it to the head, a central hollow tube, a sheath surrounding the tube, and a complex baseplate. The sheath is made of 144 copies of the gp18 protein arranged in 24 rings, each containing six copies. In T-even phages, the baseplate is hexagonal and has a pin and a jointed tail fiber at each corner. The tail fibers are responsible for virus attachment to the proper site on the bacterial surface (*see section 17.2*).

There is considerable variation in structure among the large bacteriophages, even those infecting a single host. In contrast with the T-even phages, many coliphages have true icosahedral heads. T1, T5, and lambda phages have sheathless tails that lack a baseplate and terminate in rudimentary tail fibers. Coliphages T3 and T7 have short, noncontractile tails without tail fibers. Clearly these viruses can complete their reproductive cycles using a variety of tail structures.

Complex bacterial viruses with both heads and tails are said to have **binal symmetry** because they possess a combination of icosahedral (the head) and helical (the tail) symmetry.

1. Define the following terms: nucleocapsid, capsid, icosahedral capsid, helical capsid, complex virus, protomer, self-assembly, capsomer, pentamer or penton, and hexamer or hexon. How do pentamers and hexamers associate to form a complete icosahedron; what determines helical capsid length and diameter?
2. All four nucleic acid forms can serve as virus genomes. Describe each, the types of virion possessing it, and any distinctive physical characteristics the nucleic acid can have. What are the following: plus strand, minus strand, and segmented genome?
3. What is an envelope? What are spikes or peplomers? Why are some enveloped viruses pleomorphic? Give two functions spikes might serve in the virus life cycle, and the proteins that the influenza virus uses in these processes.
4. What is a complex virus? Binal symmetry? Briefly describe the structure of poxviruses and T-even bacteriophages.



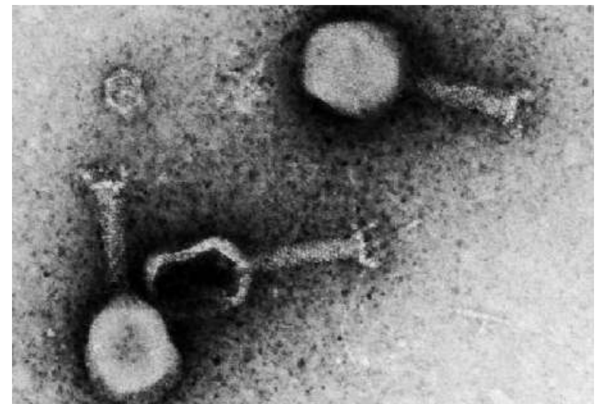


Figure 16.19 T-Even Coliphages. (**a**) The structure of the T4 bacteriophage. (**b**) The micrograph shows the phage before injection of its DNA.

16.6 Principles of Virus Taxonomy

The classification of viruses is in a much less satisfactory state than that of either bacteria or eucaryotic microorganisms. In part, this is due to a lack of knowledge of their origin and evolutionary history (**Box 16.2**). Usually viruses are separated into several large groups based on their host preferences: animal viruses, plant viruses, bacterial viruses, bacteriophages, and so forth. In the past virologists working with these groups were unable to agree on a uniform system of classification and nomenclature. Beginning with its 1971 report, the International Committee for Taxonomy of Viruses has developed a uniform classification system and now divides viruses into three orders, 56 families, 9 subfamilies, 233 genera, and 1,550 virus species. The committee places greatest weight on a few properties to define families: nucleic acid type, nucleic acid strandedness, the sense (positive or negative) of ssRNA genomes, presence or absence of an envelope, and the host. Virus family names end in *viridae;* subfamily names, in *virinae;* and genus (and species) names, in *virus.* For example, the poxviruses are in the family *Poxviridae;* the subfamily *Chorodopoxvirinae* contains poxviruses of vertebrates. Within the subfamily are several genera that are distinguished on the basis of immunologic characteristics and host specificity. The genus *Orthopoxvirus* contains several species, among them variola major (the cause of smallpox), vaccinia, and cowpox.

Viruses are divided into different taxonomic groups based on characteristics that are related to the type of host used, virion structure and composition, mode of reproduction, and the nature of any diseases caused. Some of the more important characteristics are:

1. Nature of the host—animal, plant, bacterial, insect, fungal
2. Nucleic acid characteristics—DNA or RNA, single or double stranded, molecular weight, segmentation and number of pieces of nucleic acid (RNA viruses), the sense of the strand in ssRNA viruses
3. Capsid symmetry—icosahedral, helical, binal
4. Presence of an envelope and ether sensitivity
5. Diameter of the virion or nucleocapsid
6. Number of capsomers in icosahedral viruses
7. Immunologic properties
8. Gene number and genomic map
9. Intracellular location of viral replication
10. The presence or absence of a DNA intermediate (ssRNA viruses), and the presence of reverse transcriptase
11. Type of virus release
12. Disease caused and/or special clinical features, method of transmission

Table 16.2 illustrates the use of some of these properties to describe a few common virus groups. Virus classification is further discussed when bacterial, animal, and plant viruses are considered more specifically, and a fairly complete summary of virus taxonomy is presented in appendix V.

1. List some characteristics used in classifying viruses. Which seem to be the most important?
2. What are the endings for virus families, subfamilies, and genera or species?

Box 16.2

The Origin of Viruses

The origin and subsequent evolution of viruses are shrouded in mystery, in part because of the lack of a fossil record. However, recent advances in the understanding of virus structure and reproduction have made possible more informed speculation on virus origins. At present there are two major hypotheses entertained by virologists. It has been proposed that at least some of the more complex enveloped viruses, such as the poxviruses and herpesviruses, arose from small cells, probably procaryotic, that parasitized larger, more complex cells. These parasitic cells would become ever simpler and more dependent on their hosts, much like multicellular parasites have done, in a process known as retrograde evolution. There are several problems with this hypothesis. Viruses are radically different from procaryotes, and it is difficult to envision the mechanisms by which such a transformation might have occurred or the selective pressures leading to it. In addition, one would expect to find some forms intermediate between procaryotes and at least the more complex enveloped viruses, but such forms have not been detected.

The second hypothesis is that viruses represent cellular nucleic acids that have become partially independent of the cell. Possibly a few mutations could convert nucleic acids, which are only synthesized at specific times, into infectious nucleic acids whose replication could not be controlled. This conjecture is supported by the observation that the nucleic acids of retroviruses (*see section 18.2*) and a number of other virions do contain sequences quite similar to those of normal cells, plasmids, and transposons (*see chapter 13*). The small, infectious RNAs called viroids (*see section 18.9*) have base sequences complementary to transposons, the regions around the boundary of mRNA introns (*see section 12.1*), and portions of host DNA. This has led to speculation that they have arisen from introns or transposons.

It is possible that viruses have arisen by way of both mechanisms. Because viruses differ so greatly from one another, it seems likely that they have originated independently many times during the course of evolution. Probably many viruses have evolved from other viruses just as cellular organisms have arisen from specific predecessors. The question of virus origins is complex and quite speculative; future progress in understanding virus structure and reproduction may clarify this question.

Table 16.2 Some Common Virus Groups and Their Characteristics

Nucleic Acid	Strandedness	Capsid Symmetry[a]	Presence of Envelope	Size of Capsid (nm)[b]	Number of Capsomers	Virus Group	Host Range[c]
RNA	Single	I	–	22–30	32	*Picornaviridae*	A
		I	+	40–70(e)	32	*Togaviridae*	A
		I?	+	100(e)		*Retroviridae*	A
		H	+	9(h), 80–120(e)		*Orthomyxoviridae*	A
		H	+	18(h), 125–250(e)		*Paramyxoviridae*	A
		H	+	14–16(h), 80–160(e)		*Coronaviridae*	A
		H	+	18(h), 70–80 × 130–240 (bullet shaped)		*Rhabdoviridae*	A
		I,B	–	26–35; 18–26 × 30–85		*Bromoviridae*	P
		H	–	18 × 300		*Tobamovirus*	P
		I	–	26–27	32	*Leviviridae* [Qβ]	B
RNA	Double	I	–	70–80	92	*Reoviridae*	A,P
		I	+	100(e)		*Cystoviridae*	B
DNA	Single	I	–	20–25	12	*Parvoviridae*	A
		I	–	18 × 30 (paired particles)		*Geminiviridae*	P
		I	–	25–35		*Microviridae*	B
		H	–	6 × 900–1,900		*Inoviridae*	B
DNA	Double	I	–	40	72	Polyomaviridae	A
		I	–	55	72	*Papillomaviridae*	A
		I	–	60–90	252	*Adenoviridae*	A
		I	+	130–180		*Iridoviridae*	A
		I	+	100, 180–200(e)	162	*Herpesviridae*	A
		C	+	200–260 × 250–290(e)		*Poxviridae*	A
		H	+	40 × 300(e)		*Baculoviridae*	A
		C	+	28 (core), 42(e)	42	*Hepadnaviridae*	A
		I,B	–	50; 30 × 60–900		*Caulimoviridae*	P
		I	–	60		*Corticoviridae*	B
		Bi	–	80 × 110, 110[d]		*Myoviridae*	B

[a]Types of symmetry: I, icosahedral; H, helical; C, complex; Bi, binal; B, bacilliform.

[b]Diameter of helical capsid (h); diameter of enveloped virion (e).

[c]Host range: A, animal; P, plant; B, bacterium.

[d]The first number is the head diameter; the second number, the tail length.

Summary

1. Europeans were first protected from a viral disease when Edward Jenner developed a smallpox vaccine in 1798.
2. Chamberland's invention of a porcelain filter that could remove bacteria from virus samples enabled microbiologists to show that viruses were different from bacteria.
3. In the late 1930s Stanley, Bawden, and Pirie crystallized the tobacco mosaic virus and demonstrated that it was composed only of protein and nucleic acid.
4. A virion is composed of either DNA or RNA enclosed in a coat of protein (and sometimes other substances as well). It cannot reproduce independently of living cells.
5. Viruses are cultivated using tissue cultures, embryonated eggs, bacterial cultures, and other living hosts.
6. Sites of animal viral infection may be characterized by cytopathic effects such as pocks and plaques. Phages produce plaques in bacterial lawns. Plant viruses can cause localized necrotic lesions in plant tissues.
7. Viruses can be purified by techniques such as differential and gradient centrifugation, precipitation, and denaturation or digestion of contaminants.
8. Virus particles can be counted directly with the transmission electron microscope or indirectly by the hemagglutination assay.
9. Infectivity assays can be used to estimate virus numbers in terms of plaque-forming units, lethal dose (LD_{50}), or infectious dose (ID50).
10. All virions have a nucleocapsid composed of a nucleic acid, either DNA or RNA, held within a protein capsid made of one or more types of protein subunits called protomers.
11. There are four types of viral morphology: naked icosahedral, naked helical, enveloped icosahedral and helical, and complex.
12. Helical capsids resemble long hollow protein tubes and may be either rigid or quite flexible. The nucleic acid is coiled in a spiral on the inside of the cylinder (**figure 16.11*b***).
13. Icosahedral capsids are usually constructed from two types of capsomers: pentamers (pentons) at the vertices and hexamers (hexons) on the edges and faces of the icosahedron (**figure 16.13**).
14. Viral nucleic acids can be either single stranded or double stranded, DNA or RNA. Most DNA viruses have double-stranded DNA genomes that may be linear or closed circles (**table 16.1**).
15. RNA viruses usually have ssRNA that may be either plus (positive) or minus (negative) when compared with mRNA (positive). Many RNA genomes are segmented.
16. Viruses can have a membranous envelope surrounding their nucleocapsid. The envelope lipids usually come from the host cell; in contrast, many envelope proteins are viral and may project from the envelope surface as spikes or peplomers.
17. Although viruses lack true metabolism, some contain a few enzymes necessary for their reproduction.
18. Complex viruses (e.g., poxviruses and large phages) have complicated morphology not characterized by icosahedral and helical symmetry. Large phages often have binal symmetry: their heads are icosahedral and their tails, helical (**figure 16.19*a***).
19. Currently viruses are classified with a taxonomic system placing primary emphasis on the host, type and strandedness of viral nucleic acids, and on the presence or absence of an envelope.

Key Terms

bacteriophage *364*
binal symmetry *376*
capsid *369*
capsomers *390*
complex viruses *369*
cytopathic effects *364*
differential centrifugation *366*
envelope *369*
gradient centrifugation *366*
helical *369*
hemagglutination assay *368*
hexamers (hexons) *370*
icosahedral *369*
infectious dose (ID_{50}) *368*
lethal dose (LD_{50}) *368*
minus strand or negative strand *374*
necrotic lesion *364*
nucleocapsid *369*
pentamers (pentons) *370*
phage *364*
plaque *364*
plaque assay *368*
plaque-forming units (PFU) *368*
plus strand or positive strand *374*
protomers *369*
segmented genome *374*
spike or peplomer *374*
virion *363*
virologist *362*
virology *362*
virus *363*

Questions for Thought and Review

1. In what ways do viruses resemble living organisms?
2. Why might virology have developed much more slowly without the use of Chamberland's filter?
3. What advantage would an RNA virus gain by having its genome resemble eucaryotic mRNA?
4. A number of characteristics useful in virus taxonomy are listed on page 377. Can you think of any other properties that might be of considerable importance in future studies on virus taxonomy?

Critical Thinking Questions

1. Many classification schemes are used to identify bacteria. These start with Gram staining, progress to morphology/ arrangement characteristics, and include a battery of metabolic tests. Build an analogous scheme that could be used to identify viruses. You might start by considering the host, or you might start with viruses found in a particular environment, such as a marine filtrate.
2. Consider the different perspectives on the origin of viruses in Box 16.2. Discuss whether you think viruses evolved before the first procaryote, or whether they have coevolved, and are perhaps still coevolving with their hosts.

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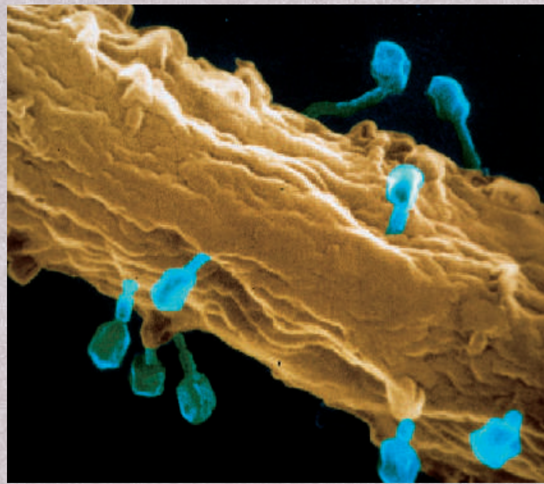
CHAPTER 17

The Viruses: Bacteriophages



A scanning electron micrograph of T-even bacteriophages infecting *E. coli.* The phages are colored blue.

Outline

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- Adsorption to the Host Cell and Penetration 384
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- Release of Phage Particles 388

17.3 Reproduction of Single-Stranded DNA Phages 388
17.4 Reproduction of RNA Phages 389
17.5 Temperate Bacteriophages and Lysogeny 390

Concepts

1. Since a bacteriophage cannot independently reproduce itself, the phage takes over its host cell and forces the host to reproduce it.
2. The lytic bacteriophage life cycle is composed of four phases: adsorption of the phage to the host and penetration of virus genetic material, synthesis of virus nucleic acid and capsid proteins, assembly of complete virions, and the release of phage particles from the host.
3. Temperate virus genetic material is able to remain within host cells and reproduce in synchrony with the host for long periods in a relationship known as lysogeny. Usually the virus genome is found integrated into the host genetic material as a prophage. A repressor protein keeps the prophage dormant and prevents virus reproduction.

You might wonder how such naive outsiders get to know about the existence of bacterial viruses. Quite by accident, I assure you. Let me illustrate by reference to an imaginary theoretical physicist, who knew little about biology in general, and nothing about bacterial viruses in particular. . . . Suppose now that our imaginary physicist, the student of Niels Bohr, is shown an experiment in which a virus particle enters a bacterial cell and 20 minutes later the bacterial cell is lysed and 100 virus particles are liberated. He will say: "How come, one particle has become 100 particles of the same kind in 20 minutes? That is very interesting. Let us find out how it happens! . . . Is this multiplying a trick of organic chemistry which the organic chemists have not yet discovered? Let us find out."

—Max Delbrück

Chapter 16 introduces many of the facts and concepts underlying the field of virology, including information about the nature of viruses, their structure and taxonomy, and how they are cultivated and studied. Clearly the viruses are a complex, diverse, and fascinating group, the study of which has done much to advance disciplines such as genetics and molecular biology.

Chapters 17 and 18 focus on virus diversity. This chapter is concerned with bacterial viruses or **bacteriophages;** the next surveys animal, plant, and insect viruses. The taxonomy, morphology, and reproduction of each group are covered. Where appropriate, the biological and practical importance of viruses is emphasized (**Box 17.1**), even though viral diseases are examined in chapter 38. Since the bacteriophages (or simply phages) have been the most intensely studied viruses and are best understood in a molecular sense, this chapter is devoted to them.

17.1 Classification of Bacteriophages

Although properties such as host range and immunologic relationships are used in classifying phages, the most important are phage morphology and nucleic acid properties (**figure 17.1**). The genetic material may be either DNA or RNA; most known bacteriophages have double-stranded DNA. Most can be placed in one of a few morphological groups: tailless icosahedral phages, viruses with contractile tails, viruses with noncontractile tails, and filamentous phages. There are even a few phages with envelopes. The most complex forms are the phages with contractile tails, for example, the T-even phages of *E. coli.*

1. Briefly describe in general terms the morphology and nucleic acids of the major phage types.

17.2 Reproduction of Double-Stranded DNA Phages: The Lytic Cycle

After DNA bacteriophages have reproduced within the host cell, many of them are released when the cell is destroyed by lysis. A phage life cycle that culminates with the host cell bursting and re-

Box 17.1

An Ocean of Viruses

Microbiologists have previously searched without success for viruses in marine habitats. Thus it has been assumed the oceans probably did not contain many viruses. Recent discoveries have changed this view radically. Several groups have either centrifuged seawater at high speeds or passed it through an ultrafilter and then examined the sediment or suspension in an electron microscope. They have found that marine viruses are about 10 times more plentiful than marine bacteria. Between 10^6 and 10^9 virus particles per milliliter are present at the ocean's surface. It has been estimated that the top one millimeter of the world's oceans could contain a total of over 3×10^{30} virus particles!

Although little detailed work has been done on marine viruses, it appears that many contain double-stranded DNA. Most are probably bacteriophages and can infect both marine heterotrophs and cyanobacteria. Up to 70% of marine procaryotes may be infected by phages. Viruses that infect diatoms and other major algal components of the marine phytoplankton also have been detected.

Marine viruses may be very important ecologically. Viruses may control marine algal blooms such as red tides (p. 580), and bacteriophages could account for 1/3 or more of the total aquatic bacterial mortality or turnover. If true, this is of major ecological significance because the reproduction of marine bacteria far exceeds marine protozoan grazing capacity. Virus lysis of procaryotic and algal cells may well contribute greatly to carbon and nitrogen cycling in marine food webs. It could reduce the level of marine primary productivity in some situations.

Bacteriophages also may greatly accelerate the flow of genes between marine bacteria. Virus-induced bacterial lysis could generate most of the free DNA present in seawater. Gene transfer between aquatic bacteria by transformation (*see pp. 305–7*) does occur, and bacterial lysis by phages would increase its probability. Furthermore, such high phage concentrations can stimulate gene exchange by transduction (*see pp. 307–9*). These genetic exchanges could have both positive and negative consequences. Genes that enable marine bacteria to degrade toxic pollutants such as those in oil spills could spread through the native population. On the other hand, antibiotic resistance genes in bacteria from raw sewage released into the ocean also might be dispersed (*see section 35.7*).

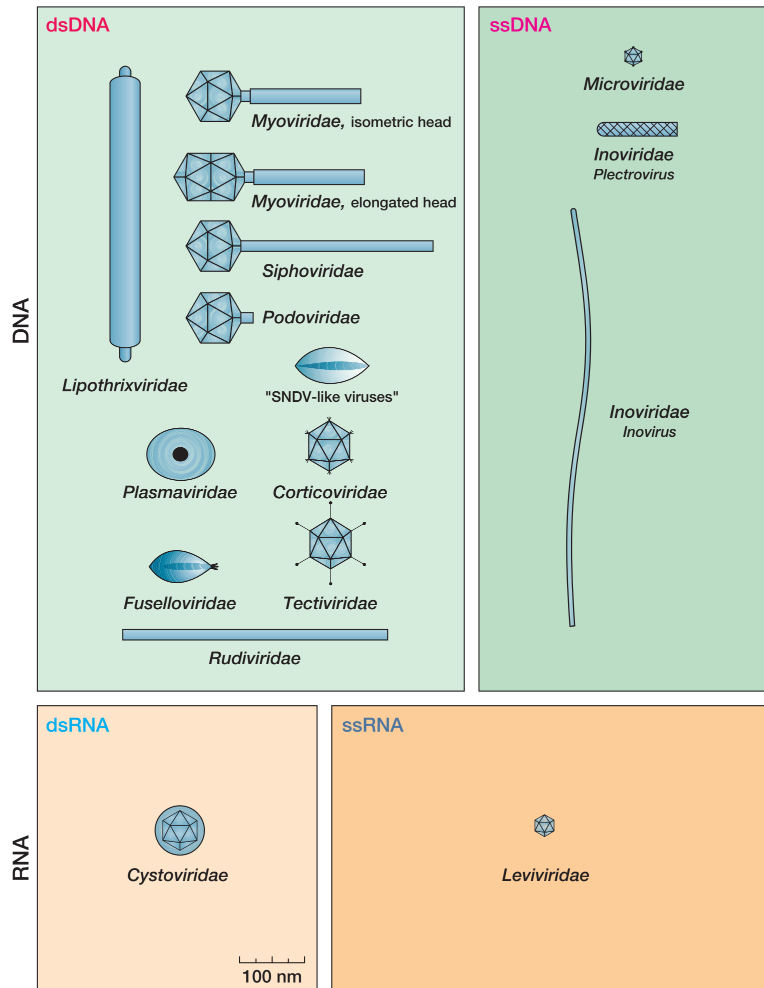


Figure 17.1 Major Bacteriophage Families and Genera. The *Myoviridae* are the only family with contractile tails. *Plasmaviridae* are pleomorphic. *Tectiviridae* have distinctive double capsids, whereas the *Corticoviridae* have complex capsids containing lipid.

leasing virions is called a **lytic cycle.** The events taking place during the lytic cycle will be reviewed in this section, with the primary focus on the T-even phages of *E. coli.* These are double-stranded DNA bacteriophages with complex contractile tails and are placed in the family *Myoviridae.* They are some of the most complex viruses known. The structure of T-even coliphages (p. 376)

The One-Step Growth Experiment

The development of the one-step growth experiment in 1939 by Max Delbrück and Emory Ellis marks the beginning of modern bacteriophage research. In a **one-step growth experiment,** the reproduction of a large phage population is synchronized so that the molecular events occurring during reproduction can be followed. A culture of susceptible bacteria such as *E. coli* is mixed with bacteriophage particles, and the phages are allowed a short interval to attach to their host cells. The culture is then greatly diluted so that any virus particles released upon host cell lysis will not immediately infect new cells. This strategy works because phages lack a means of seeking out host cells and must contact them during random movement through the solution. Thus phages are less likely to contact host cells in a dilute mixture. The number of infective phage particles released from bacteria is subsequently determined at various intervals by a plaque count (*see section 16.4*).

A plot of the bacteriophages released from host cells versus time shows several distinct phases (**figure 17.2**). During the **latent period,** which immediately follows phage addition, there is no release of virions. This is followed by the **rise period** or **burst,** when the host cells rapidly lyse and release infective phages. Finally, a plateau is reached and no more viruses are liberated. The total number of phages released can be used to calculate the **burst size,** the number of viruses produced per infected cell.

The latent period is the shortest time required for virus reproduction and release. During the first part of this phase, host bacteria do not contain any complete, infective virions. This can be shown by lysing them with chloroform. This initial segment of the latent period is called the **eclipse period** because the virions

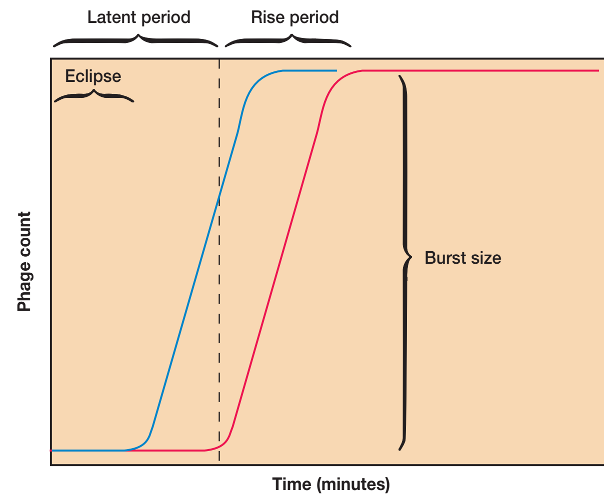


Figure 17.2 The One-Step Growth Curve. In the initial part of the latent period, the eclipse period, the host cells do not contain any complete, infective virions. During the remainder of the latent period, an increasing number of infective virions are present, but none are released. The latent period ends with host cell lysis and rapid release of virions during the rise period or burst. In this figure the blue line represents the total number of complete virions. The red line is the number of free viruses (the unadsorbed virions plus those released from host cells). When *E. coli* is infected with T2 phage at 37°C, the growth plateau is reached in about 30 minutes and the burst size is approximately 100 or more virions per cell. The eclipse period is 11–12 minutes, and the latent period is around 21–22 minutes in length.

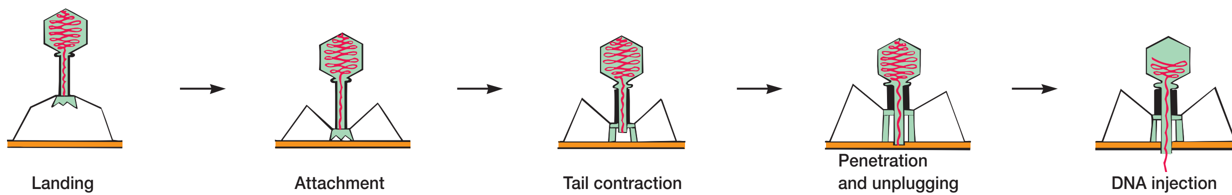


Figure 17.3 T4 Phage Adsorption and DNA Injection. See text for details.

were detectable before infection but are now concealed or eclipsed. The number of completed, infective phages within the host increases after the end of the eclipse period, and the host cell is prepared for lysis.

The one-step growth experiment with *E. coli* and phage T2 provides a well-studied example of this process. When the experiment is carried out with actively growing cells in rich medium at 37°C, the growth curve plateau is reached in approximately 30 minutes. Bacteriophage reproduction is an exceptionally rapid process, much faster than animal virus reproduction, which may take hours.

Adsorption to the Host Cell and Penetration

Bacteriophages do not randomly attach to the surface of a host cell; rather, they fasten to specific surface structures called **receptor sites.** The nature of these receptors varies with the phage; cell wall lipopolysaccharides and proteins, teichoic acids, flagella, and pili can serve as receptors. The T-even phages of *E. coli* use cell wall lipopolysaccharides or proteins as receptors. Variation in receptor properties is at least partly responsible for phage host preferences. The structure of cell walls, flagella, and pili (pp. 55–61, 62–66)

T-even phage adsorption involves several tail structures (*see figure 16.19*). Phage attachment begins when a tail fiber contacts the appropriate receptor site. As more tail fibers make contact, the baseplate settles down on the surface (**figures 17.3** and **17.4**).

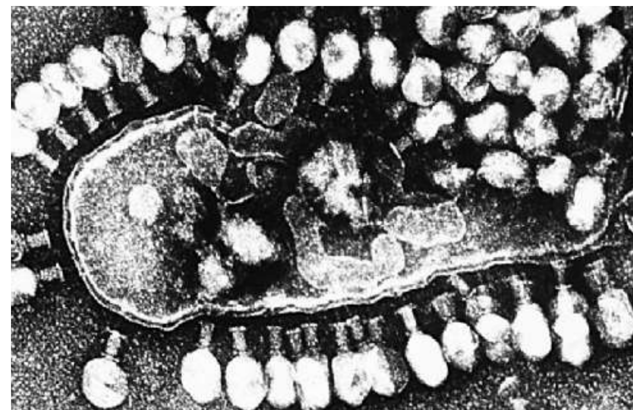
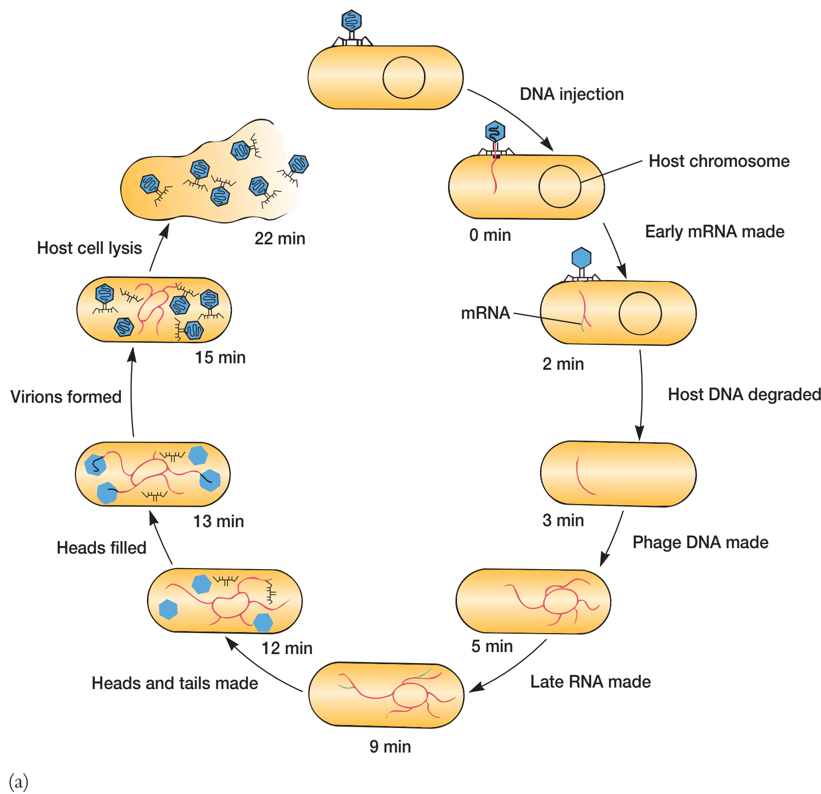
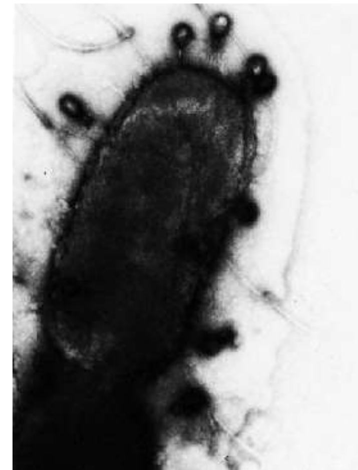


Figure 17.4 Electron Micrograph of *E. coli* Infected with Phage T4. Baseplates, contracted sheaths, and tail tubes can be seen (×36,500).

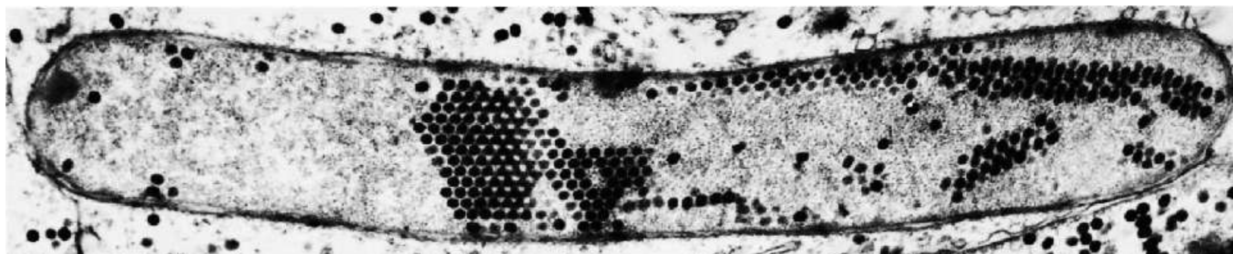
Binding is probably due to electrostatic interactions and is influenced by pH and the presence of ions such as Mg^{2+} and Ca^{2+}. After the baseplate is seated firmly on the cell surface, conformational changes occur in the baseplate and sheath, and the tail sheath reorganizes so that it shortens from a cylinder 24 rings long (*see p. 376*) to one of 12 rings. That is, the sheath becomes



(a)



(b1)



(b2)

Figure 17.5 The Life Cycle of Bacteriophage T4. (**a**) A schematic diagram depicting the life cycle with the minutes after DNA injection given beneath each stage. mRNA is drawn in only at the stage during which its synthesis begins. (**b**) Electron micrographs show the development of T2 bacteriophages in *E. coli.* (*b1*) Several phages are near the bacterium, and some are attached and probably injecting their DNA. (*b2*) By about 30 minutes after infection, the bacterium contains numerous completed phages.

shorter and wider, and the central tube or core is pushed through the bacterial wall. Finally, the DNA is extruded from the head, through the tail tube, and into the host cell. The tube may interact with the plasma membrane to form a pore through which DNA passes. The penetration mechanisms of other bacteriophages often appear to differ from that of the T-even phages but have not been studied in much detail.

Synthesis of Phage Nucleic Acids and Proteins

Since the T4 phage of *E. coli* has been intensely studied, its reproduction will be used as our example (**figure 17.5**). Soon after phage DNA injection, the synthesis of host DNA, RNA, and protein is halted, and the cell is forced to make viral constituents. *E. coli* RNA polymerase (*see section 12.1*) starts synthesizing phage mRNA within 2 minutes. This mRNA and all other **early mRNA** (mRNA transcribed before phage DNA is made) direct the synthesis of the protein factors and enzymes required to take over the host cell and manufacture viral nucleic acids. Some early virus-specific enzymes degrade host DNA to nucleotides, thereby simultaneously halting host gene expression and providing raw material for virus DNA synthesis. Within 5 minutes, virus DNA synthesis commences. Promoters and transcription (pp. 261–63)

Virus gene expression follows an orderly sequence because of modifications of the RNA polymerase and changes in sigma factors. Initially T4 genes are transcribed by the regular host RNA polymerase and the sigma factor σ^{70}. After a short interval, a virus enzyme catalyzes the transfer of an ADP-ribosyl group from NAD to an α-subunit of RNA polymerase. This helps inhibit the transcription of host genes and promotes virus gene expression. Then

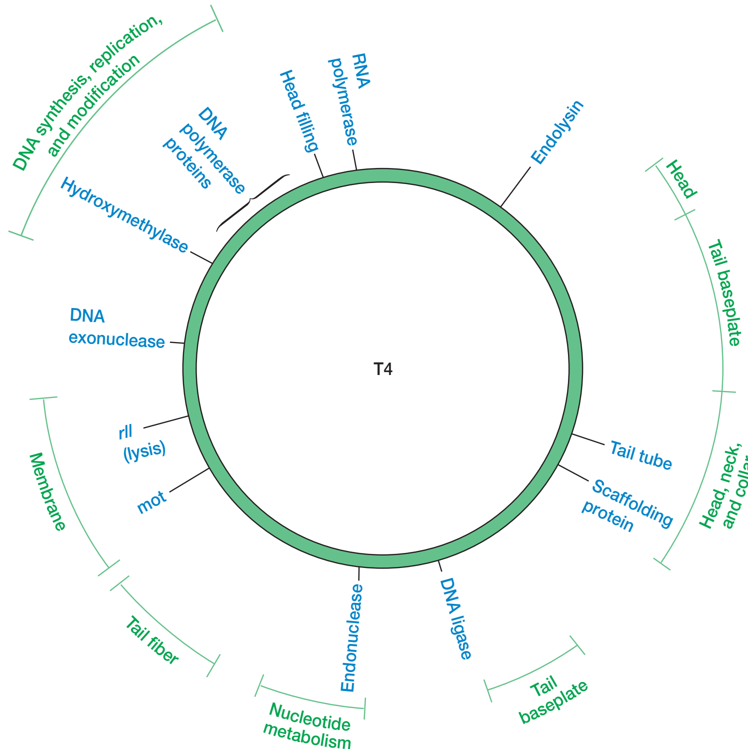


Figure 17.6 A Map of the T4 Genome. Some of its genes and their functions are shown. Genes with related functions tend to be clustered together.

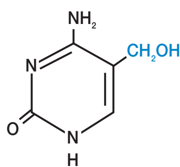


Figure 17.7 5-Hydroxymethylcytosine (HMC). In T4 DNA, the HMC often has glucose attached to its hydroxyl.

the second α-subunit receives an ADP-ribosyl group and this turns off some of the early T4 genes. The product of one early gene, *motA,* stimulates transcription of somewhat later genes, one of which produces the sigma factor gp55. This sigma factor helps RNA polymerase bind to late promoters and transcribe late genes, which become active around 10 to 12 minutes after infection.

It is clear from the sophisticated control of RNA polymerase and the precise order in which events occur in the reproductive cycle that the expression of T4 genes is tightly regulated. Even the organization of the genome appears suited for efficient control of the life cycle. As can be seen in **figure 17.6,** genes with related functions—such as the genes for phage head or tail fiber construction—are usually clustered together. Early and late genes also are clustered separately on the genome; they are even transcribed in different directions—early genes in the counterclockwise direction and late genes, clockwise. Since transcription always proceeds in the 5′ to 3′ direction, the early and late genes are located on different DNA strands (*see sections 11.5 and 12.1*).

Considerable preparation is required for synthesis of T4 DNA because it contains **hydroxymethylcytosine (HMC)** instead of cytosine (**figure 17.7**). HMC must be synthesized by two phage-encoded enzymes before DNA replication can begin. After T4 DNA has been synthesized, it is glucosylated by the addition of glucose to the HMC residues. Glucosylated HMC residues protect T4 DNA from attack by *E. coli* endonucleases called **restriction enzymes,** which would otherwise cleave the viral DNA at specific points and destroy it. This bacterial defense mechanism is called **restriction.** Other groups also can be used to modify phage DNA and protect it against restriction enzymes. For example, methyl groups are added to the amino groups of adenine and cytosine in lambda phage DNA for the same reason. The replication of T4 DNA is an extremely complex process requiring at least seven phage proteins. Its mechanism resembles that described in chapter 11. Restriction enzymes and genetic engineering (pp. 320–21)

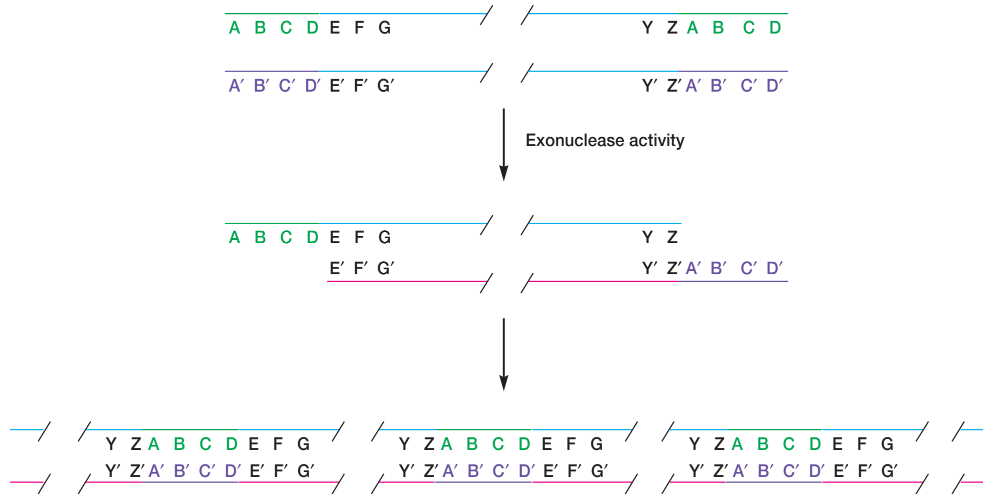
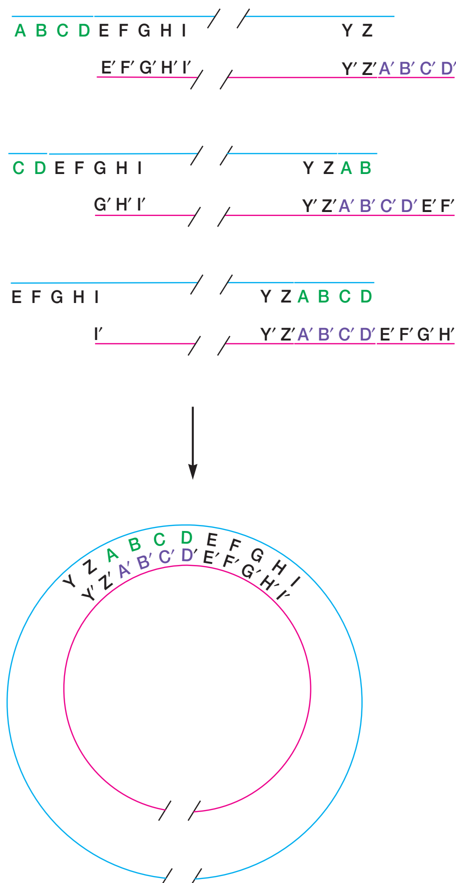


Figure 17.8 An Example of Terminal Redundancy. The gene sequences in color are terminally redundant; they are repeated at each end of the DNA molecule. This makes it possible to join units together by their redundant ends forming a concatemer. For example, if the 3′ ends of each unit were partially digested by an exonuclease, the complementary 5′ ends would be exposed and could base pair to generate a long chain of repeated units. The breaks between terminal sequences indicate that the DNA molecules are longer than shown here.



T4 DNA shows what is called terminal redundancy; that is, a base sequence is repeated at both ends of the molecule (**figure 17.8**). When many DNA copies have been made, about 6 to 10 copies are joined by their terminally redundant ends with the aid of several enzymes (figure 17.8). These very long DNA strands composed of several units linked together with the same orientation are called **concatemers.** During assembly, concatemers are cleaved in such a way that the genome is slightly longer than the T4 gene set. The genetic map is therefore drawn circular (figure 17.6) because T4 DNA is circularly permuted (**figure 17.9**). The sequence of genes in each T4 virus of a population is the same but starts with a different gene at the 5′ end. If all the linear pieces of DNA were coiled into circles, the DNA circles would have identical gene sequences.

The Assembly of Phage Particles

The assembly of the T4 phage is an exceptionally complex self-assembly process. **Late mRNA,** or that produced after DNA replication, directs the synthesis of three kinds of proteins: (1) phage structural proteins, (2) proteins that help with phage assembly without becoming part of the virion structure, and (3) proteins involved in cell lysis and phage release. Late mRNA transcription begins

Figure 17.9 Circularly Permuted Genomes Cut from a Concatemer. The concatemer formed in figure 17.8 can be cut at any point into pieces of equal length that contain a complete complement of genes, even though different genes are found at their ends. If each piece has single-stranded cohesive ends as in figure 17.8, it will coil into a circle with the same gene order as the circles produced by other pieces.

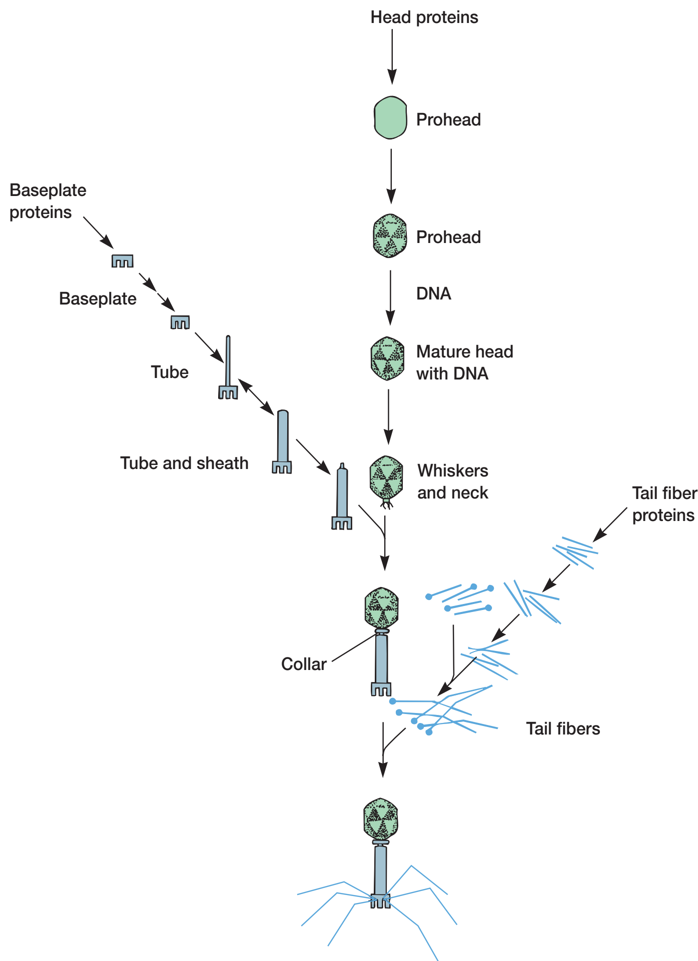


Figure 17.10 The Assembly of T4 Bacteriophage. Note the subassembly lines for the baseplate, tail tube and sheath, tail fibers, and head.

about 9 minutes after T4 DNA injection into *E. coli*. All the proteins required for phage assembly are synthesized simultaneously and then used in four fairly independent subassembly lines (**figure 17.10**). The baseplate is constructed of 15 gene products. After the baseplate is finished, the tail tube is built on it and the sheath is assembled around the tube. The phage prohead or procapsid is constructed separately of more than 10 proteins and then spontaneously combines with the tail assembly. The procapsid is assembled with the aid of **scaffolding proteins** that are degraded or removed after construction is completed. A special portal protein is located at the base of the procapsid where it connects to the tail. The portal protein is part of the DNA translocating vertex, a structure that helps initiate head assembly and aids in DNA movement into and out of the head. Tail fibers attach to the baseplate after the head and tail have come together. Although many of these steps occur spontaneously, some require special virus proteins or host cell factors.

DNA packaging within the T4 head is still a somewhat mysterious process. In some way the DNA is drawn into the completed shell so efficiently that about 500 μm of DNA are packed into a cavity less than 0.1 μm across! It is thought that a long DNA concatemer enters the procapsid in an ATP-dependent process until it is packed full and contains about 2% more DNA than is needed for the full T4 genome. The concatemer is then cut, and T4 assembly is finished. The first complete T4 particles appear in *E. coli* at 37°C about 15 minutes after infection.

Release of Phage Particles

Many phages lyse their host cells at the end of the intracellular phase. The lysis of *E. coli* takes place after about 22 minutes at 37°C, and approximately 300 T4 particles are released. Several T4 genes are involved in this process. One directs the synthesis of an endolysin that attacks the cell wall peptidoglycan. Another phage protein called a holin produces a plasma membrane lesion that stops respiration and allows the endolysin to attack the peptidoglycan. Presumably it forms holes in the membrane.

17.3 Reproduction of Single-Stranded DNA Phages

Thus far, only double-stranded DNA phage reproduction has been discussed, with the lytic phage T4 as an example. The reproduction of single-stranded DNA phages now will be briefly reviewed. The phage of φX174, family *Microviridae,* is a small ssDNA phage using *E. coli* as its host. Its DNA base sequence is the same as that of the viral mRNA (except that thymine is substituted for uracil) and is therefore positive; the genome contains overlapping genes (*see figure 11.20*b). The phage DNA must be converted to a double-stranded form before either replication or transcription can occur. When φX174 DNA enters the host, it is immediately copied by the bacterial DNA polymerase to form a double-stranded DNA, the **replicative form** or **RF** (**figure 17.11**). The replicative form then directs the synthesis of more RF copies, mRNA, and copies of the +DNA genome. The phage is released by host lysis through a different mechanism than used by the T4 phage.

The filamentous ssDNA bacteriophages behave quite differently in many respects from φX174 and other ssDNA phages. The fd phage, family *Inoviridae,* is one of the best studied and is shaped like a long fiber about 6 nm in diameter by 900 to 1,900 nm in length (figure 17.1). The circular ssDNA lies in the center of the filament and is surrounded by a tube made of a small coat protein organized in a helical arrangement. The virus infects male *E. coli* cells by attaching to the tip of the pilus; the DNA enters the host along or possibly through the pilus with the aid of a special adsorption protein. A replicative form is first synthesized and then transcribed. A phage-coded protein then aids in replication of the phage DNA by use of the rolling-circle method (*see section 11.3*).

The filamentous fd phages do not kill their host cell but establish a symbiotic relationship in which new virions are continually released by a secretory process. Filamentous phage coat

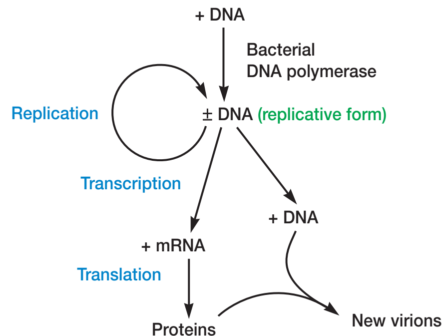


Figure 17.11 The Reproduction of ϕX174, a + Strand DNA Phage. See text for details.

proteins are first inserted into the membrane. The coat then assembles around the viral DNA as it is secreted through the host plasma membrane (**figure 17.12**). The host bacteria grow and divide at a slightly reduced rate.

1. How is a one-step growth experiment carried out? Summarize what occurs in each phase of the resulting growth curve. Define latent period, eclipse period, rise period or burst, and burst size.
2. Be able to describe in some detail what is occurring in each phase of the lytic dsDNA phage life cycle: adsorption and penetration, nucleic acid and protein synthesis, phage assembly, and phage release. Define the following terms: lytic cycle, receptor site, early mRNA, hydroxymethylcytosine, restriction, restriction enzymes, concatemers, replicative form, late mRNA, and scaffolding proteins.
3. How does the reproduction of the ssDNA phages ϕX174 and fd differ from each other and from the dsDNA T4 phage?

17.4 Reproduction of RNA Phages

Many bacteriophages carry their genetic information as single-stranded RNA that can act as a messenger RNA and direct the synthesis of phage proteins. One of the first enzymes synthesized is a viral **RNA replicase,** an RNA-dependent RNA polymerase

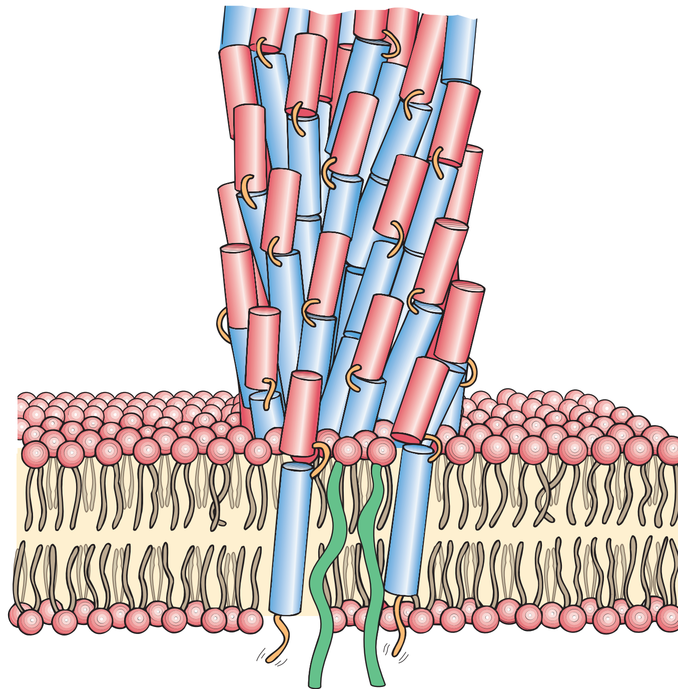


Figure 17.12 Release of the Pf1 Phage. The Pf1 phage is a filamentous bacteriophage that is released from *Pseudomonas aeruginosa* without lysis. In this illustration the blue cylinders are hydrophobic α-helices that span the plasma membrane, and the red cylinders are amphipathic helices that lie on the membrane surface before virus assembly. In each protomer the two helices are connected by a short, flexible peptide loop (yellow). It is thought that the blue helix binds with circular, single-stranded viral DNA (green) as it is extruded through the membrane. The red helix simultaneously attaches to the growing virus coat that projects from the membrane surface. Eventually the blue helix leaves the membrane and also becomes part of the capsid.

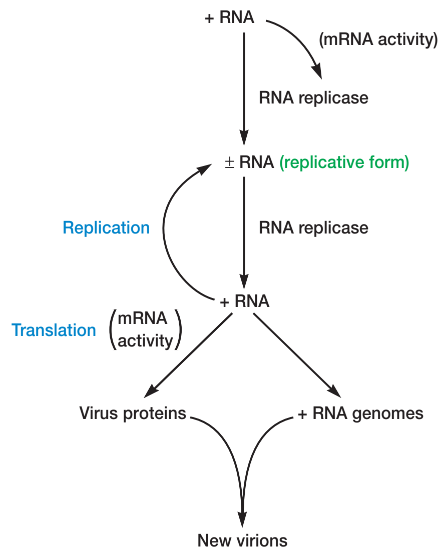


Figure 17.13 The Reproduction of Single-Stranded RNA Bacteriophages.

(**figure 17.13**). The replicase then copies the original RNA (a plus strand) to produce a double-stranded intermediate (±RNA), which is called the replicative form and is analogous to the ±DNA seen in the reproduction of ssDNA phages. The same replicase next uses this replicative form to synthesize thousands of copies of +RNA. Some of these plus strands are used to make more ±RNA in order to accelerate +RNA synthesis. Other +RNA acts as mRNA and directs the synthesis of phage proteins. Finally, +RNA strands are incorporated into maturing virus particles. The genome of these RNA phages serves as both a template for its own replication and an mRNA.

MS2 and Qβ, family *Leviviridae,* are small, tailless, icosahedral ssRNA phages of *E. coli,* which have been intensely studied (figure 17.1). They attach to the F-pili of their host and enter by an unknown mechanism. These phages have only three or four genes and are genetically the simplest phages known. In MS2, one protein is involved in phage adsorption to the host cell (and possibly also in virion construction or maturation). The other three genes code for a coat protein, an RNA replicase, and a protein needed for cell lysis.

Only one dsRNA phage has been discovered, the bacteriophage ϕ6 of *Pseudomonas phaseolicola* (figure 17.1). It is also unusual in possessing a membranous envelope. The icosahedral capsid within its envelope contains an RNA polymerase and three dsRNA segments, each of which directs the synthesis of an mRNA. It is not yet known how the dsRNAs are replicated.

1. How are ssRNA phages reproduced, and what role does RNA replicase play in the process?
2. What is peculiar about the structure of phage ϕ6?

17.5 Temperate Bacteriophages and Lysogeny

Up to this point many of the viruses we have discussed are **virulent bacteriophages;** these are phages that lyse their host cells during the reproductive cycle. Many DNA phages also can establish a different relationship with their host. After adsorption and penetration, the viral genome does not take control of its host and destroy it while producing new phages. Instead the viral genome remains within the host cell and replicates with the bacterial genome to generate a clone of infected cells that may grow and divide for long periods while appearing perfectly normal (*see figure 13.18*). Each of these infected bacteria can produce phages and lyse under appropriate environmental conditions. They cannot, for reasons that will become clear later, be reinfected by the same virus—that is, they have immunity to superinfection. This relationship between the phage and its host is called **lysogeny.** Bacteria having the potential to produce phage particles under some conditions are said to be **lysogens** or **lysogenic,** and phages able to enter into this relationship are **temperate phages.** The latent form of the virus genome that remains within the host but does not destroy it is called the **prophage.** The prophage usually is integrated into the bacterial genome but sometimes exists independently. **Induction** is the process by which phage reproduction is initiated in a lysogenized culture. It leads to the destruction of infected cells and the release of new phages—that is, induction of the lytic cycle. Lysogeny was briefly described earlier in the context of transduction and genetic recombination, but will be discussed in more detail here. Generalized and specialized transduction (pp. 307–9)

Most bacteriophages that have been studied are temperate, and it appears that there are advantages in being able to lysogenize bacteria. Consider a phage-infected culture that is becoming dormant due to nutrient deprivation. Before bacteria enter dormancy, they degrade their own mRNA and protein. Thus the phage is faced with two problems: it can only reproduce in actively metabolizing bacteria, and phage reproduction is usually permanently interrupted by the mRNA and protein degradation. This predicament can be avoided if the phage becomes dormant (lysogenic) at the same time as its host; in fact, nutrient deprivation does favor lysogeny. Temperate phages also have an advantage in situations where many viruses per cell initiate an infection—that is, where there is a high multiplicity of infection (MOI). When every cell is infected, the last round of replication will destroy all host cells. Thus there is a risk that the phages may be left without a host and directly exposed to environmental hazards for months or years. This prospect is avoided if lysogeny is favored by a high MOI; some bacteria will survive, carry the virus genome, and synthesize new copies as they reproduce. Not surprisingly a high MOI does stimulate lysogeny.

A temperate phage may induce a change in the phenotype of its host cell that is not directly related to completion of its life cycle. Such a change is called a **lysogenic conversion** or a conversion and often involves alterations in bacterial surface characteristics or pathogenic properties. For example, when *Salmonella* is infected by an epsilon phage, the structure of its outer lipopolysaccharide layer (*see pp. 58–60*) may be modified. The phage changes the activities of several enzymes involved in con-

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struction of the lipopolysaccharide carbohydrate component and thus alters the antigenic properties of the host. These epsilon-induced changes appear to eliminate surface phage receptors and prevent infection of the lysogen by another epsilon phage. Another example is the temperate phage β of *Corynebacterium diphtheriae,* the cause of diphtheria. Only *C. diphtheriae* that is lysogenized with phage β will produce diphtheria toxin (*see sections 34.3 and 39.1*) because the phage, not the bacterium, carries the toxin gene.

The lambda phage, family *Siphoviridae,* that uses the K12 strain of *E. coli* as its host is the best-understood temperate phage and will serve as our example of lysogeny. Lambda is a double-stranded DNA phage possessing an icosahedral head 55 nm in diameter and a noncontractile tail with a thin tail fiber at its end (**figure 17.14**). The DNA is a linear molecule with cohesive ends—single-stranded stretches, 12 nucleotides long, that have complementary base sequences and can base pair with each other. Because of these cohesive ends, the linear genome cyclizes immediately upon infection (**figure 17.15**). *E. coli* DNA ligase then seals the breaks, forming a closed circle. The lambda genome has been carefully mapped, and over 40 genes have been located (**figure 17.16**). Most genes are clustered according to their function, with separate groups involved in head synthesis, tail synthesis, lysogeny and its regulation, DNA replication, and cell lysis. DNA ligase (p. 239)

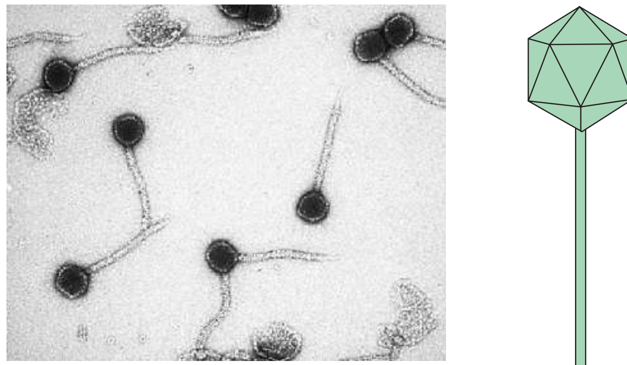


Figure 17.14 Bacteriophage Lambda.

Lambda phage can reproduce using a normal lytic cycle. Immediately after lambda DNA enters *E. coli,* it is converted to a covalent circle, and transcription by the host RNA polymerase is initiated. As shown in figure 17.16, the polymerase binds to both a rightward promoter (PR) and a leftward promoter (PL) and begins to transcribe in both directions, copying different DNA strands. The first genes that are transcribed code for regulatory proteins that control the lytic cycle: leftward gene *N* and rightward genes *cro* and *cII* (figure 17.16). These and other regulatory genes ensure that virus proteins will be synthesized in an orderly time sequence and will be manufactured only when needed during the life cycle. Regulation of transcription (pp. 275–78)

Lambda DNA replication and virion assembly are similar to the same processes already described for the T4 phage. One significant difference should be noted. Although initially bidirectional DNA replication is used and theta-shaped intermediates are seen (*see section 11.3*), lambda DNA is primarily synthesized by way of the rolling-circle mechanism to form long concatemers that are finally cleaved to give complete genomes (*see figure 11.12*).

The establishment of lysogeny and the earlier-mentioned immunity of lysogens to superinfection can be accounted for by the presence of the **lambda repressor** coded for by the *cI* gene. The repressor protein chain is 236 amino acids long and folds into a dumbbell shape with globular domains at each end (**figure 17.17**). One domain is concerned with binding to DNA, while the other binds with another repressor molecule to generate a dimer (the most active form of the lambda repressor). In a lysogen the repressor is synthesized continuously and binds to the operators O_L and O_R, thereby blocking RNA polymerase activity (figure 17.18*c*). If another lambda phage tries to infect the cell, its mRNA synthesis

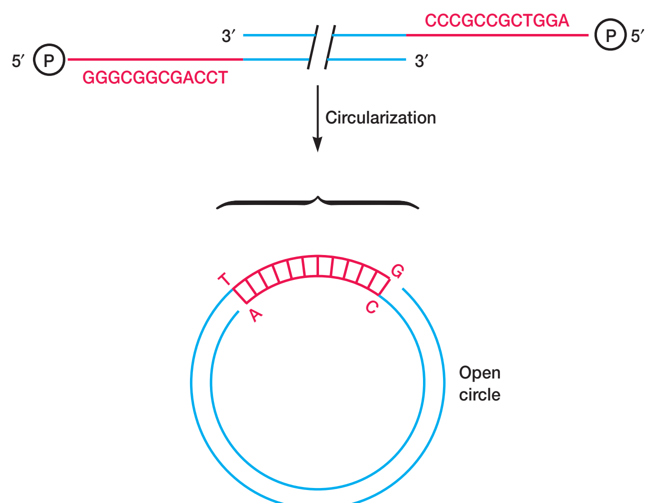


Figure 17.15 Lambda Phage DNA. A diagram of lambda phage DNA showing its 12 base, single-stranded cohesive ends (printed in red) and the circularization their complementary base sequences make possible.

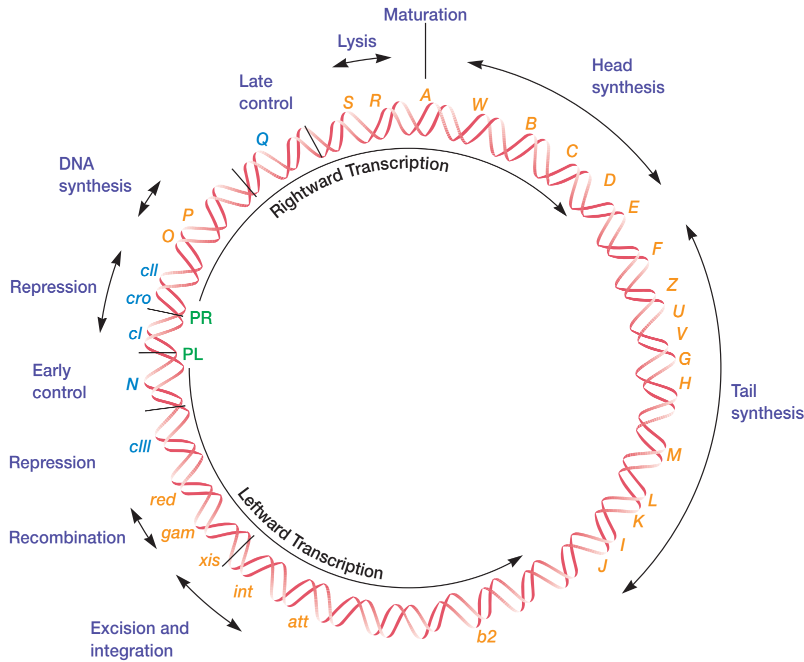
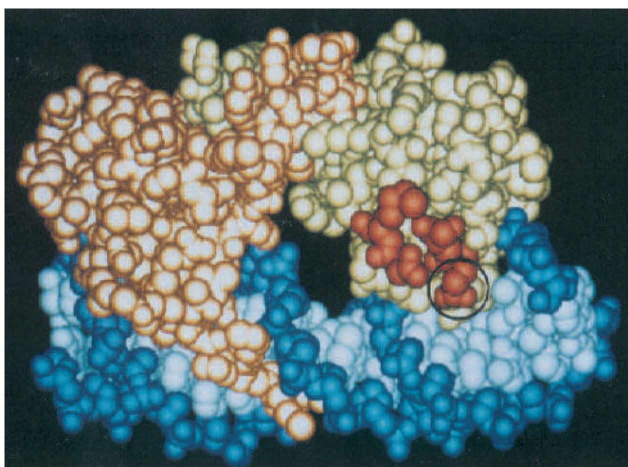
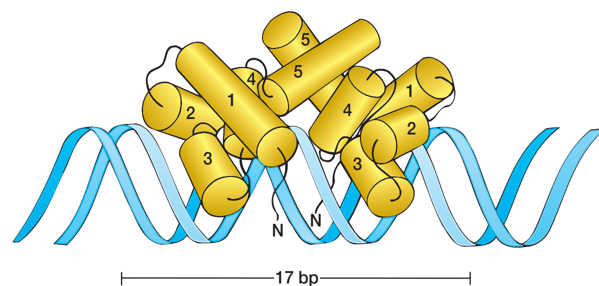


Figure 17.16 The Lambda Phage Genome. The direction of transcription and location of leftward and rightward promoters (PL and PR) are indicated on the inside of the map. The positions of major regulatory sites are shown by lines on the map and regulatory genes are in blue. Lambda DNA is double stranded, and transcription proceeds in opposite directions on opposite strands.



(a)



(b)

Figure 17.17 Lambda Repressor Binding. (**a**) A computer model of lambda repressor binding to the lambda operator. The lambda repressor dimer (brown and tan) is bound to DNA (blue and light blue). The arms of the dimer wrap around the major grooves of the double helix. (**b**) A diagram of the lambda repressor-DNA complex. The repressor binds to a 17 bp stretch of the operator. The α3-helices make closest contact with the major grooves of the operator (the helices are labeled in order, beginning at the N terminal of the chain).

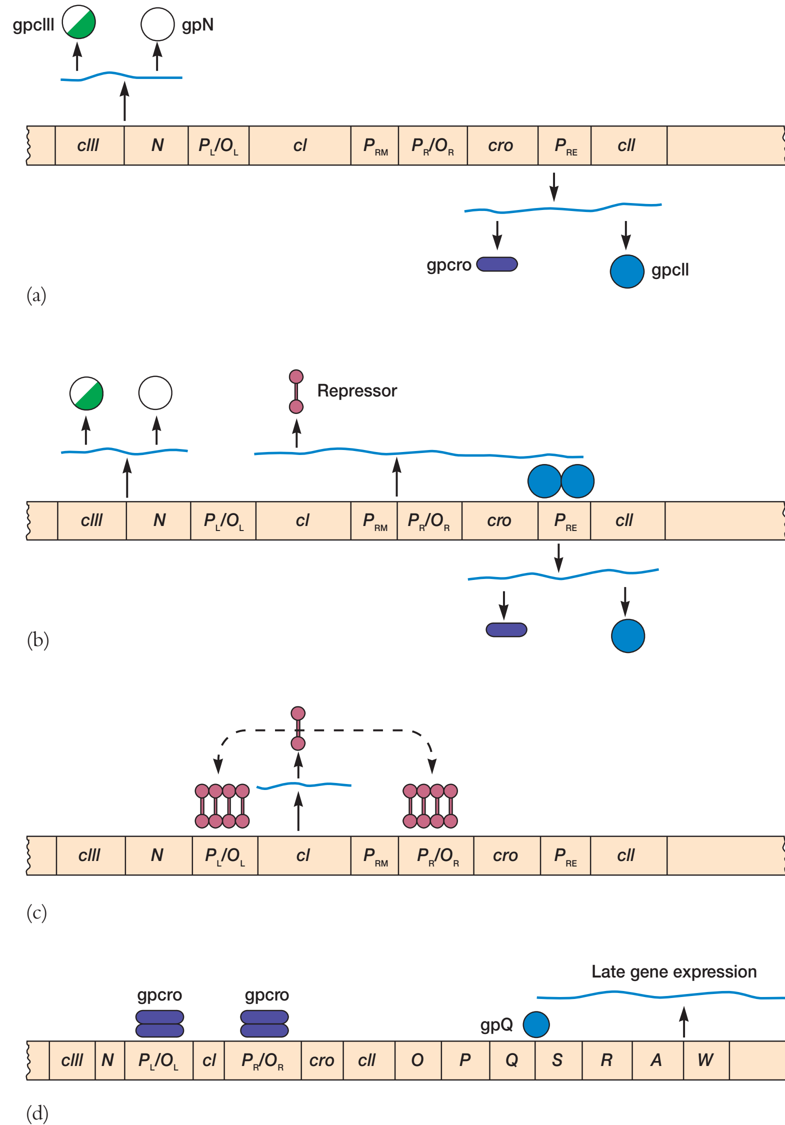


Figure 17.18 Choice Between Lysogeny and Lysis. Events involved in the choice between the establishment of lysogeny and continuation of the lytic cycle. The action of gpN is ignored for the sake of simplicity, and the scale in part (*d*) differs from that in parts (*a*) to (*c*). The abbreviation gp stands for gene product (gpcro is the product of the *cro* gene). (**a**) and (**b**) illustrate the initial steps leading to lambda repressor synthesis. (**c**) represents the situation when repressor production overcomes cro synthesis and establishes lysogeny. In part (**d**) the cro protein has accumulated more rapidly than lambda repressor and cro protein dimers (the active form) have bound to O_L and O_R. This blocks both *cI* and *cro* gene function, but not late gene expression, since gpQ has already accumulated and promoted late mRNA synthesis. See text for further details.

also will be inhibited. It should be noted that immunity always involves repressor activity. A potential host cell might remain uninfected due to a mutation that alters its phage receptor site. In such an instance it is said to be resistant, not immune, to the phage.

The sequence of events leading to the initial synthesis of repressor and the establishment of lysogeny is well known. Immediately after lambda DNA has been circularized and transcription has commenced, the cII and cIII proteins accumulate (**figure 17.18*a***). The cII protein binds next to the promoter for the *cII* gene (P_{RE}, RE stands for repressor establishment), and stimulates RNA polymerase binding (figure 17.18*b*). The cIII protein protects cII from degradation by a host enzyme, the HflA protease. Lambda

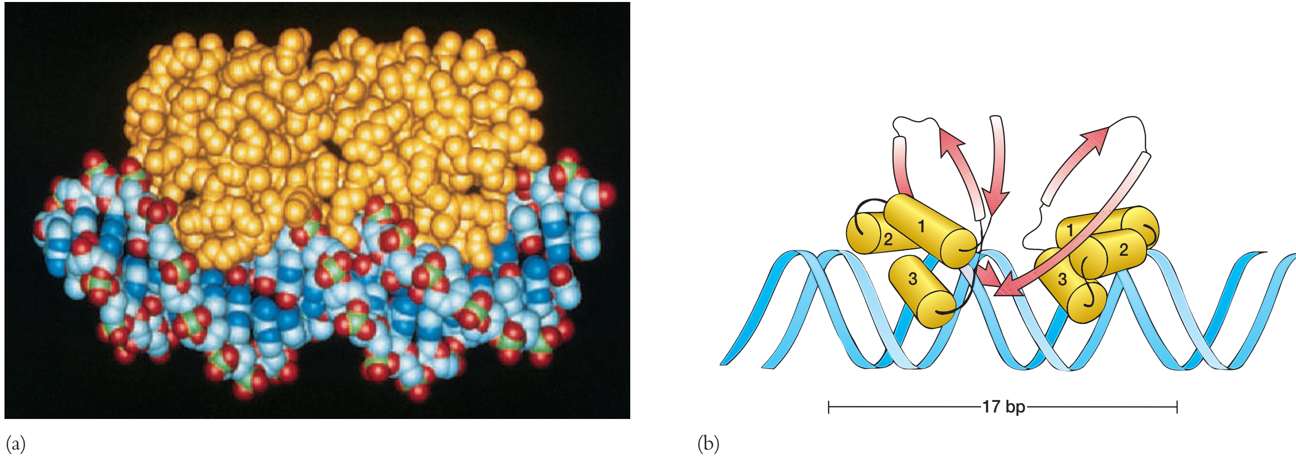


Figure 17.19 Cro Protein Binding. (**a**) A space-filling model of the cro protein–DNA complex. The cro protein is in yellow. (**b**) A diagram of the cro protein dimer–DNA complex. Like the lambda repressor protein, the cro protein functions as a dimer and binds to two adjacent DNA major grooves.

repressor (gpcI) is rapidly synthesized and binds to O_R and O_L, thus turning off mRNA synthesis and the production of the cII and cIII proteins (figure 17.18*c*). The *cI* gene continues to be transcribed at a low rate because of the activity of a second promoter (P_{RM}, RM stands for repressor maintenance) that is activated by the repressor itself. This control circuit in which lambda repressor stimulates its own synthesis ensures that lysogeny will normally be stable when once established.

One might expect that lysogeny would be established every time but this is not the case. During this period the **cro protein** (gpcro) has also been accumulating. The cro protein binds to O_R and O_L, turns off the transcription of the repressor gene (as well as inhibiting the expression of other early genes), and represses P_{RM} function (figure 17.18*d* and **figure 17.19**). Because the lambda repressor can block *cro* transcription, there is a race between the production of lambda repressor and that of the cro protein. Although cro protein synthesis begins before that of the lambda repressor, gpcro binds to O_R more weakly and must rise to a higher level than the repressor before repressor synthesis is blocked and the lytic cycle started (figure 17.18*d*). The details of this competition are not yet completely clear, but it has been shown that a number of environmental factors influence the outcome of the race and the choice between the lytic and lysogenic pathways.

If the lambda repressor wins the race, the circular lambda DNA is inserted into the *E. coli* genome as first proposed by Alan Campbell. **Integration** or insertion is possible because the cII protein stimulates transcription of the *int* gene at the same time as that of the *cI* gene. The *int* gene codes for the synthesis of an **integrase** enzyme, and this protein becomes plentiful before lambda repressor turns off transcription. Lambda DNA has a phage attachment site (the *att* site) that can base pair with a bacterial attachment site located between the galactose or *gal* operon and the biotin operon on the *E. coli* chromosome. After these two sites match up, the integrase enzyme, with the aid of a special host protein, catalyzes the physical exchange of viral and bacterial DNA strands (**figure 17.20**). The circular lambda DNA is integrated into the *E. coli* DNA as a linear region next to the *gal* operon and is called a prophage. As can be seen in figure 17.20, the linear order of phage genes has been changed or permuted during integration.

The lambda prophage will leave the *E. coli* genome and begin the production of new phages when the host is unable to survive. The process is known as induction and is triggered by a drop in lambda repressor levels. Occasionally the repressor will spontaneously decline and the lytic cycle commence. However, induction usually is in response to environmental factors such as UV light or chemical mutagens that damage host DNA. This damage causes the recA protein, which normally plays a role in genetic recombination in *E. coli (see section 11.8),* to act as a protease and cleave the repressor chain between the two domains. The separated domains cannot assemble to form the normal active repressor dimer, and the lytic cycle genes become active again. There is some recent evidence that activated recA protein may not directly cleave the repressor. RecA may instead bind to the lambda repressor and stimulate it to proteolytically cleave itself. An early gene located next to the *int* gene, the *xis* gene, codes for the synthesis of an **excisionase** protein that

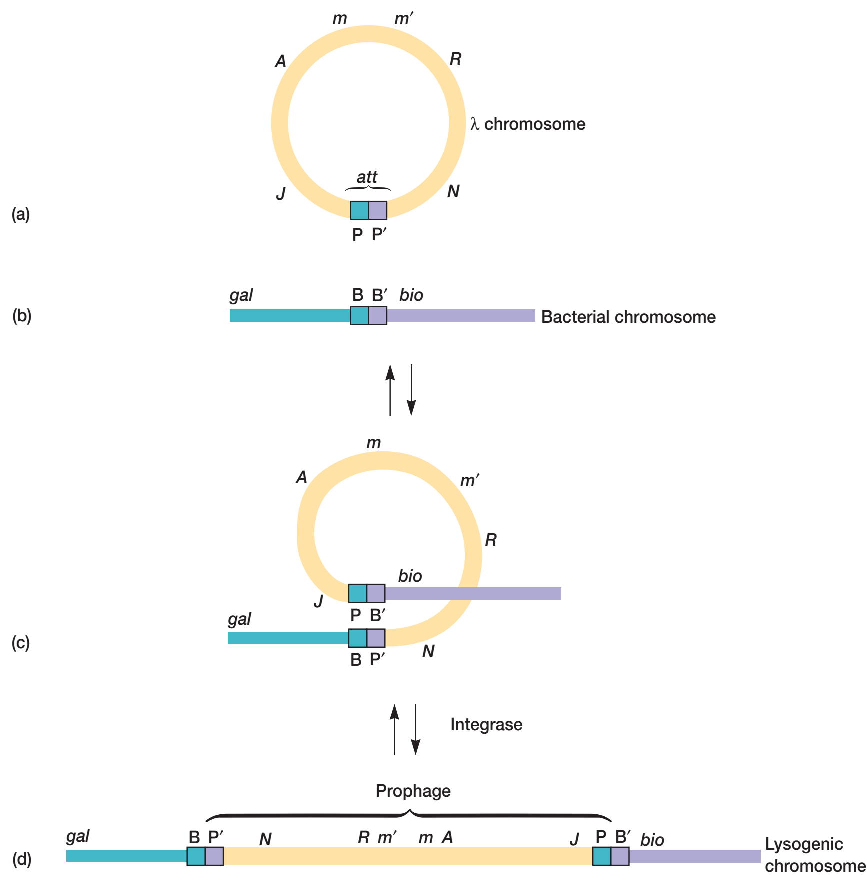


Figure 17.20 Reversible Insertion and Excision of Lambda Phage. After circularization,the *att* site P, P′ (**a**) lines up with a corresponding bacterial sequence B, B′ (**b**) and is integrated between the *gal* and *bio* operons to form the prophage, (**c**) and (**d**). If the process is reversed, the circular lambda chromosome will be restored and can then reproduce.

binds to the integrase and enables it to reverse the integration process and free the prophage (figure 17.20). The lytic cycle then proceeds normally.

Most temperate phages exist as integrated prophages in the lysogen. Nevertheless, integration is not an absolute requirement for lysogeny. The *E. coli* phage P1 is similar to lambda in that it circularizes after infection and begins to manufacture repressor. However, it remains as an independent circular DNA molecule in the lysogen and is replicated at the same time as the host chromosome. When *E. coli* divides, P1 DNA is apportioned between the daughter cells so that all lysogens contain one or two copies of the phage genome.

1. Define virulent phage, lysogeny, temperate phage, lysogen, prophage, immunity, and induction.
2. What advantages might a phage gain by being capable of lysogeny?
3. Describe lysogenic conversion and its significance.
4. Precisely how, in molecular terms, is a bacterial cell made lysogenic by a temperate phage like lambda?
5. How is a prophage induced to become active again?
6. Be able to describe the roles of the lambda repressor, cro protein, the recA protein, integrase, and excisionase in lysogeny and induction.
7. How does the temperate phage P1 differ from lambda phage?

Summary

1. There are four major morphological groups of phages: tailless icosahedral phages, phages with contractile tails, those with noncontractile tails, and filamentous phages (**figure 17.1**).
2. The lytic cycle of virulent bacteriophages is a life cycle that ends with host cell lysis and virion release.
3. The phage life cycle can be studied with a one-step growth experiment that is divided into an initial eclipse period within the latent period, and a rise period or burst (**figure 17.2**).
4. The life cycle of the dsDNA T4 phage of *E. coli* is composed of several phases. In the adsorption phase the phage attaches to a specific receptor site on the bacterial surface. This is followed by penetration of the cell wall and insertion of the viral nucleic acid into the cell (**figure 17.3**).
5. Transcription of T4 DNA first produces early mRNA, which directs the synthesis of the protein factors and enzymes required to take control of the host and manufacture phage nucleic acids (**figure 17.5**).
6. T4 DNA contains hydroxymethylcytosine (HMC) in place of cytosine, and glucose is often added to the HMC to protect the phage DNA from attack by host restriction enzymes.
7. T4 DNA replication produces concatemers, long strands of several genome copies linked together.
8. Late mRNA is produced after DNA replication and directs the synthesis of capsid proteins, proteins involved in phage assembly, and those required for cell lysis and phage release.
9. Complete virions are assembled immediately after the separate components have been constructed. This is a self-assembly process, but does require participation of the bacterial membrane and a few extra proteins.
10. T4, ϕX174, and many other phages are released upon lysis of the host cell.
11. The replication of ssDNA phages proceeds through the formation of a double-stranded replicative form (RF) (**figure 17.11**). The filamentous ssDNA phages are continually released without host cell lysis.
12. When ssRNA bacteriophage RNA enters a bacterial cell, it acts as a messenger and directs the synthesis of RNA replicase, which then produces double-stranded replicative forms and, subsequently, many +RNA copies (**figure 17.13**).
13. The ϕ6 phage is the only dsRNA phage known. It is also unusual in having a membranous envelope.
14. Temperate phages, unlike virulent phages, often reproduce in synchrony with the host genome to yield a clone of virus-infected cells. This relationship is lysogeny, and the infected cell is called a lysogen. The latent form of the phage genome within the lysogen is the prophage (**figure 13.18**).
15. Lysogeny is reversible, and the prophage can be induced to become active again and lyse its host.
16. A temperate phage may induce a change in the phenotype of its host cell that is not directly related to the completion of its life cycle. Such a change is called a conversion.
17. Two of the first proteins to appear after infection with lambda are the lambda repressor and the cro protein. The lambda repressor blocks the transcription of both cro protein and those proteins required for the lytic cycle, while the cro protein inhibits transcription of the lambda repressor gene (**figure 17.18**).
18. There is a race between synthesis of lambda repressor and that of the cro protein. If the cro protein level rises high enough in time, lambda repressor synthesis is blocked and the lytic cycle initiated; otherwise, all genes other than the lambda repressor gene are repressed and the cell becomes a lysogen.
19. The final step in prophage formation is the insertion or integration of the lambda genome into the *E. coli* chromosome; this is catalyzed by a special integrase enzyme (**figure 17.20**).
20. Several environmental factors can lower repressor levels and trigger induction. The prophage becomes active and makes an excisionase protein that causes the integrase to reverse integration, free the prophage, and initiate a lytic cycle.

Key Terms

bacteriophages *382*
burst size *383*
concatemer *387*
cro protein *394*
early mRNA *385*
eclipse period *383*
excisionase *394*
hydroxymethylcytosine (HMC) *386*
induction *390*
integrase *394*
integration *394*
lambda repressor *391*
late mRNA *387*
latent period *383*
lysogen *390*
lysogenic *390*
lysogenic conversion *390*
lysogeny *390*
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one-step growth experiment *383*
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replicative form (RF) *388*
restriction *386*
restriction enzyme *386*
rise period or burst *383*
RNA replicase *389*
scaffolding proteins *388*
temperate phage *390*
virulent bacteriophage *390*

Questions for Thought and Review

1. Explain why the T4 phage genome is circularly permuted.
2. Can you think of a way to simplify further the genomes of the ssRNA phages MS2 and Qβ? Would it be possible to eliminate one of their genes? If so, which one?
3. No temperate RNA phages have yet been discovered. How might this absence be explained?
4. How might a bacterial cell resist phage infections? Give those mechanisms mentioned in the chapter and speculate on other possible strategies.

Critical Thinking Questions

1. The choice between lysogeny and lysis is influenced by many factors. How would external conditions such as starvation or crowding be "sensed" and communicated to the transcriptional machinery and influence this choice?
2. If you were a doctor charged with curing a bacterium of its viral infection, what target would you choose for "chemotherapy" and why?
3. We don't know exactly how double-stranded RNA is replicated. Propose two possible models, and design experiments that would distinguish between them.
4. The most straightforward explanation as to why the endolysin of T4 is expressed so late in infection is that its promoter is recognized by the gp55 alternative sigma factor. Propose a different explanation.

Additional Reading

Chapter 16 references also should be consulted, particularly the introductory and advanced texts.

General

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