Virus

By- Dr. Ekta Khare

Department of Microbiology,

Chhatrapati Shahu Ji Maharaj University, Kanpur

Viruses

- 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.
- 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.

Cultivation

- Because they are unable to reproduce independent of living cells, viruses cannot be cultured in the same way as bacteria and eucaryotic organisms.
- For many years researchers have cultivated animal viruses by inoculating suitable host animals or embryonated eggs.
- More recently animal viruses have been grown in tissue (cell) culture on monolayers of animal cells.
- 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 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.**
- Cytopathic effects may be lethal, but plaque formation from cell lysis does not always occur.

...Cultivation

- Bacterial viruses or **bacteriophages (phages for short) are** cultivated in either broth or agar cultures of young, actively growing bacterial cells.
- 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.
- 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:

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

- Plant viruses are cultivated in a variety of ways. Plant tissue cultures, cultures of separated cells, or cultures of protoplasts may be used.
- Viruses also can be grown in whole plants.

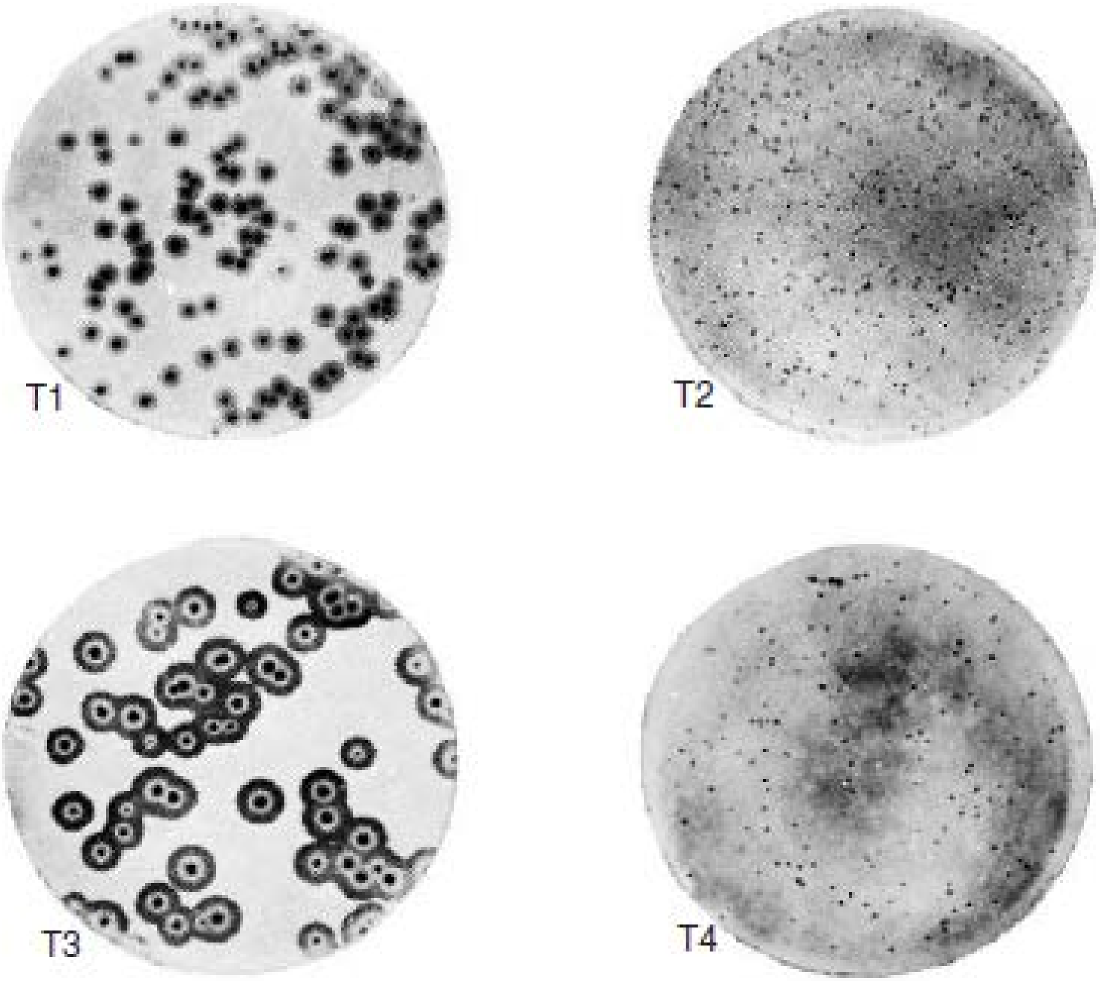


Figure 16.4 **Phage Plaques.** Plaques produced on a lawn of *E. coli* by some of the T coliphages. Note the large differences in plaque appearance. The photographs are about 1/3 full size.

Virus Structure

- **Virion Size**
- Virions range in size from about 10 to 300 or 400 nm in diameter.
- The smallest viruses are a little larger than ribosomes, whereas the poxviruses, like vaccinia, are about the same size as the smallest bacteria.
- There are four general morphological types of capsids and virion structure:
  - **Icosahedral**
  - **Helical**
  - **Enveloped**
  - **Complex viruses**

- **Icosahedral Capsids:** An icosahedron is a regular polyhedron with 20 equilateral triangular faces and 12 vertices (figure 16.10*h,j–l).*
- **Helical and shaped like hollow protein** cylinders, which may be either rigid or flexible (figure 16.10*m).*
- **Enveloped virus**, 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).*
- **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).

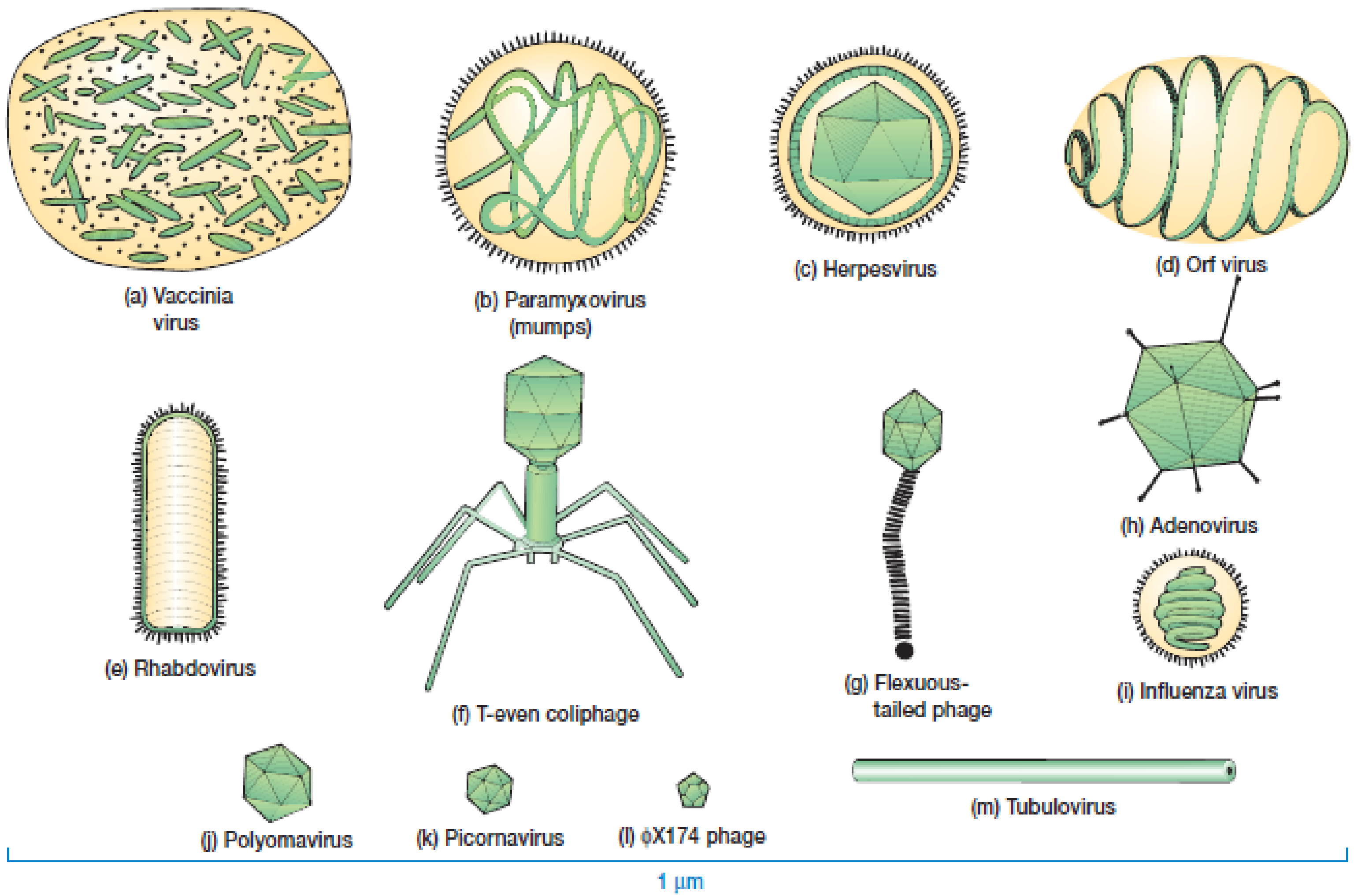


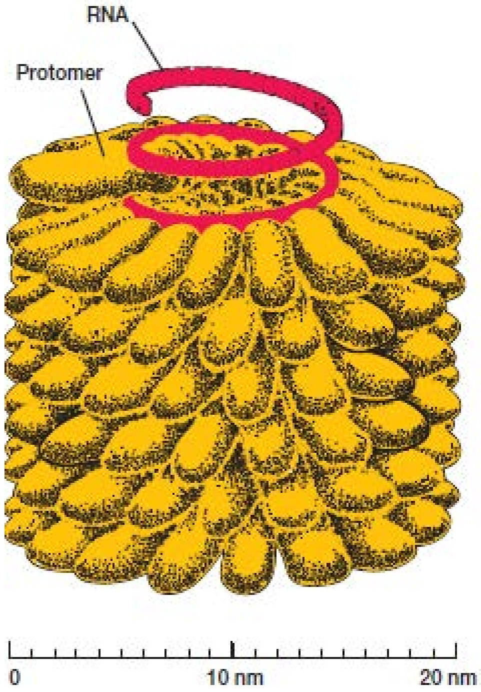
Figure 16.10 **The Size and Morphology of Selected Viruses.** The viruses are drawn to scale. A 1 µm line is provided at the bottom of the figure.

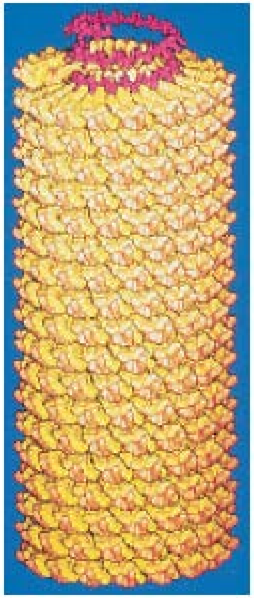
Protomers

- Both helical and icosahedral capsids are large macromolecular structures constructed from many copies of one or a few types of protein subunits or **protomers.**
- 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.
- 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.

Helical Capsids

- Helical capsids are shaped much like hollow tubes with protein walls. eg,. TMV
- **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.
- 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.





Icosahedral Capsids

- Transmission electron microscope revealed a complex icosahedral capsid structure is revealed.
- 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.
- **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.
- 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.
- Protomers join to form capsomers through noncovalent bonding.
- There is more than one way to build an icosahedral capsid.

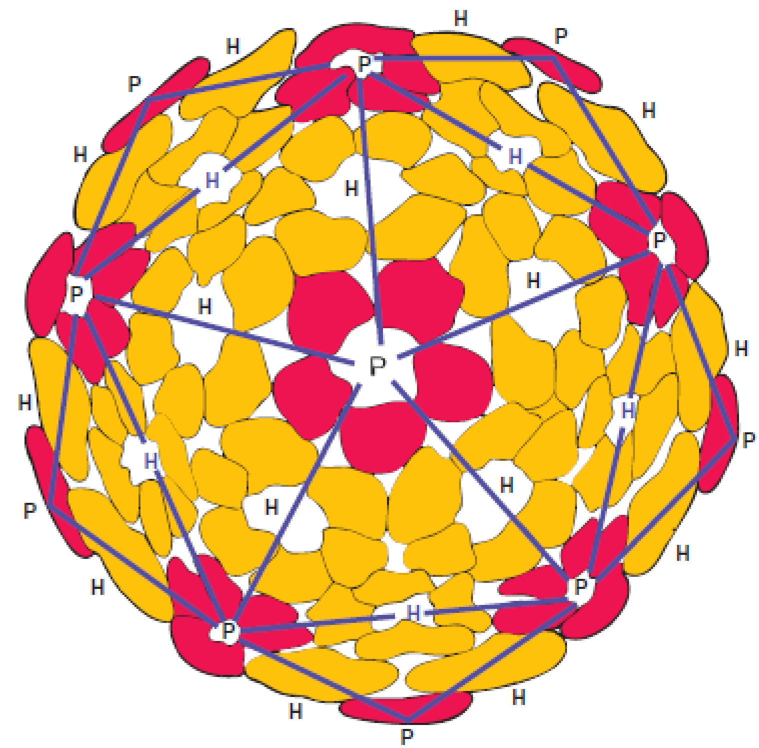


Figure 16.13 The Structure of an Icosahedral Capsid. Pentons are located at the 12 vertices. Hexons form the edges and faces of the icosahedron. This capsid contains 42 capsomers; all protomers are identical.

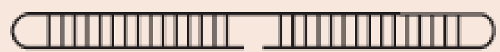
•The bonds between proteins within pentamers and hexamers are stronger than those between separate capsomers.

•Empty capsids can even dissociate into separate capsomers.

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.

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.

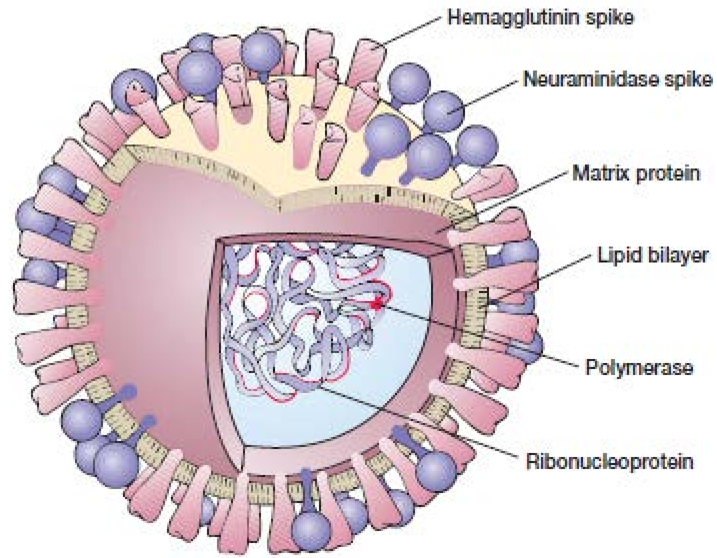
- The lambda phage has linear dsDNA with cohesive ends—single-stranded complementary segments 12 nucleotides long—that enable it to cyclize when they base pair with each other.
- Besides the normal nucleotides found in DNA, many virus DNAs contain unusual bases. Eg., the T-even phages of *E. coli* have 5-hydroxymethylcytosine 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**.
- However, the viral RNA genome may instead be complementary to viral mRNA, and then it is called a **minus or negative strand.**
- 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.
- 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.

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.
- 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.**
- These spikes may be involved in virus attachment to the host cell surface.
- 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.
- 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."**

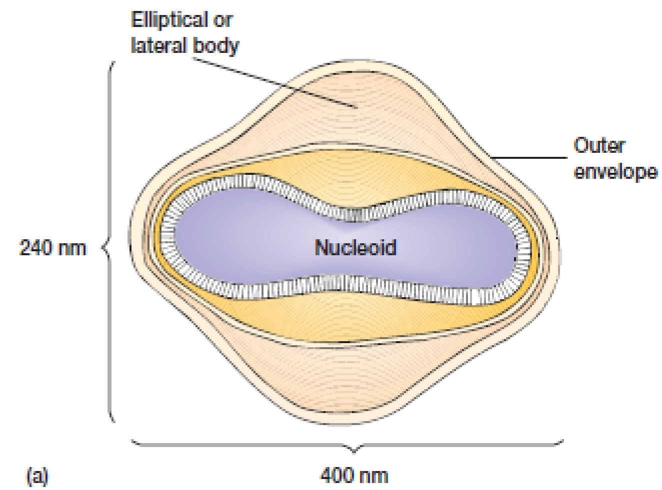
Influenza virus

- Some spikes possess the enzyme neuraminidase, which may aid the virus in penetrating mucous layers of the respiratory epithelium to reach host cells.
- Hemagglutinins participate in virion attachment to host cell.
- A nonglycosylated protein, the M or matrix protein, is found on the inner surface of the envelope and helps stabilize it.
- 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.



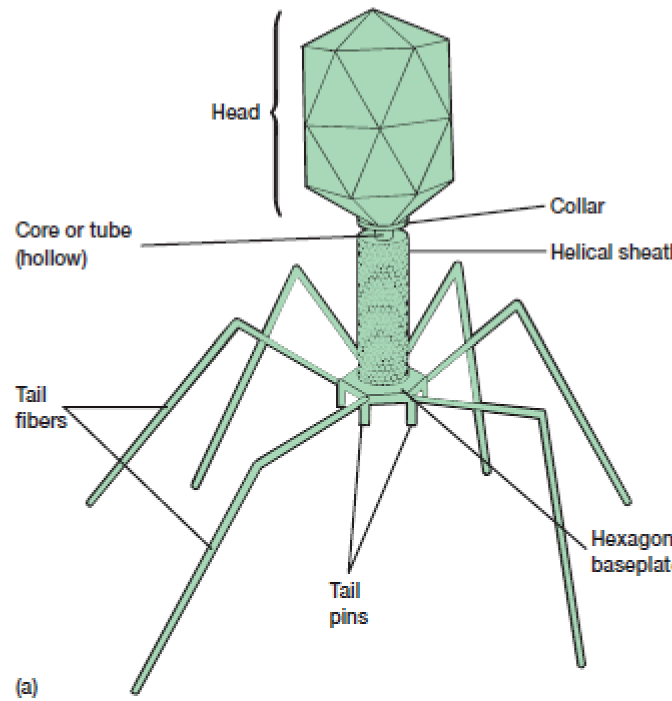
Viruses with Capsids of Complex Symmetry

- 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).
- They possess an exceptionally complex internal structure with an ovoid- to brickshaped 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.



The T2, T4, and T6 phages that infect *E. coli*

- Their head resembles an icosahedron elongated by one or two rows of hexamers in the middle 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.
- 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 bacterial surface.
- 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.



(a)

- Complex bacterial viruses with both heads and tails are said to have **binal symmetry**