Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CHAPTER

2

The Structure of Viruses

INTRODUCTION

Virus particles, called virions, contain the viral genome encapsidated in a protein coat. The function of the coat is to protect the genome of the virus in the extracellular environment as well as to bind to a new host cell and introduce the genome into it. Viral genomes are small and limited in their coding capacity, which requires that three-dimensional virions be formed using a limited number of different proteins. For the smallest viruses, only one protein may be used to construct the virion, whereas the largest viruses may use 30 or more proteins. To form a three-dimensional structure using only a few proteins requires that the structure must be regular, with each protein subunit occupying a position at least approximately equivalent to that occupied by all other proteins of its class in the final structure (the principle of quasi-equivalence), although some viruses are now known to violate the principle of quasi-equivalence. A regular three-dimensional structure can be formed from repeating subunits using either helical symmetry or icosahedral symmetry principles. In the case of the smallest viruses, the final structure is simple and quite regular. Larger viruses with more proteins at their disposal can build more elaborate structures. Enveloped viruses may be quite regular in construction or may have irregular features, because the use of lipid envelopes allows irregularities in construction.

Selected families of vertebrate viruses are listed in Table 2.1 grouped by the morphologies of the virions. Also shown for each family is the presence or absence of an envelope in the virion, the triangulation number (defined later) if the virus is icosahedral, the morphology of the nucleocapsid or core, and figure numbers where the structures of members of a family are illustrated. Electron micrographs of five DNA viruses belonging to different families and of five RNA viruses belonging to different families are shown in Fig. 2.1. The viruses chosen represent viruses that are among the largest known and the smallest known, and are all shown to the same scale for comparison. For each virus, the top micrograph is of a virus that has been negatively stained, the middle micrograph is of a section of infected cells, and the bottom panel shows a schematic representation of the virus. The structures of these and other viruses are described next.

HELICAL SYMMETRY

Helical viruses appear rod shaped in the electron microscope. The rod can be flexible or stiff. The best studied example of a simple helical virus is tobacco mosaic virus (TMV). The TMV virion is a rigid rod 18 nm in diameter and 300 nm long (Fig. 2.2B). It contains 2130 copies of a single capsid protein of 17.5 kDa. In the right-hand helix, each protein subunit has six nearest neighbors and each subunit occupies a position equivalent to every other capsid protein subunit in the resulting network (Fig. 2.2A), except for those subunits at the very ends of the helix. Each capsid molecule binds three nucleotides of RNA within a groove in the protein. The helix has a pitch of 23 Å and there are $16^{1}/_{3}$ subunits per turn of the helix. The length of the TMV virion (300 nm) is determined by the size of the RNA (6.4 kb).

Many viruses are constructed with helical symmetry and often contain only one protein or a very few proteins. The popularity of the helix may be due in part to the fact that the length of the particle is not fixed and RNAs or DNAs of different sizes can be readily accommodated. Thus the genome size is not fixed, unlike that of icosahedral viruses.

TABLE 2.1 Listing of Selected Vertebrate Virus Families by Morphology

Morphology of particle/virus family	Enveloped	Triangulation number	Morphology of nucleocapsid	Figure numbers
Icosahedral				
Adenoviridae	No	$T = 25$	Not applicable	Figs. 2.1, 2.12
Reoviridae	No	$T = 13l$[a]	Icosahedral	Figs. 2.1, 2.5, 2.11
Papillomaviridae	No	$P = 7d$[a,b]	Not applicable	Figs. 2.1,[c] 2.5
Polyomaviridae	No	$P = 7d$[a,b]	Not applicable	Figs. 2.1,[c] 2.5, 2.10
Parvoviridor	No	$T = 1$	Not applicable	Figs. 2.1, 2.5
Picornaviridae	No	$P = 3$[b]	Not applicable	Figs. 2.1,[d] 2.5, 2.7, 2.8
Astroviridae	No		Not applicable	
Caliciviridae	No	$T = 3$	Not applicable	
Herpesviridae	Yes	$T = 16$	Icosahedral	Figs. 2.1, 2.5, 2.20
Togaviridae	Yes	$T = 4$	Icosahedral	Figs. 2.5, 2.14, 2.18
Flaviviridae	Yes	$T = 3$	Icosahedral	Figs. 2.5, 2.18
Irregular				
Poxviridae (ovoid)	Yes		Dumbbell	Figs. 2.1, 2.24
Rhabdoviridae (bacilliform)	Yes		Coiled helix	Figs. 2.1, 2.23
Spherical				
Retroviridae	Yes	?	Icosahedral?	Figs. 2.1, 2.21
Round[e]				
Coronaviridae	Yes		Helical or tubular	
Paramyxoviridae	Yes		Helical	Fig. 2.22
Orthomyxoviridae	Yes		Helical	Figs. 2.1, 2.22
Bunyaviridae	Yes		Helical	
Arenaviridae	Yes		Helical	
Hepadnaviridae	Yes	$T = 3$ & 4	Icosahedral	
Filamentous[c]				
Filoviridae	Yes		Helical	Fig. 2.19

[a]Two mirror image structures can be formed using $T = 13$ or $T = 7$, a symmetry referred to as "d" or "l."
[b]Because the subunits are not exactly equivalent, *Papillomaviridae, Polyomaviridae*, as well as poliovirus, have "pseudo-triangulation numbers" so are referred to as $P = 7$, $P = 7$, and $P = 3$ symmetries respectively.
[c]*Papovaviridae*, referred to in Fig. 2.1 have now been separated into *Polyomaviridae* and *Papillomaviridae*.
[d]Enterovirus, referred to in Fig. 2.1 is a genus of the *Picornaviridae*.
[e]Virions are often pleiomorphic.

ICOSAHEDRAL SYMMETRY

Virions can be approximately spherical in shape, based on icosahedral symmetry. Since the time of Euclid, there have been known to exist only five regular solids in which each face of the solid is a regular polygon: the tetrahedron, the cube, the octahedron, the dodecahedron, and the icosahedron. The icosahedron has 20 faces, each of which is a regular triangle, and thus each face has threefold rotational symmetry (Fig. 2.3A). There are 12 vertices where 5 faces meet, and thus each vertex has fivefold rotational symmetry. There are 30 edges in which 2 faces meet, and each edge possesses twofold rotational symmetry. Thus the icosahedron is characterized by twofold, threefold, and fivefold symmetry axes. The dodecahedron, the next simpler regular solid, has the same symmetry axes as the icosahedron and is therefore isomorphous with it in symmetry: the dodecahedron has 12 faces which are regular pentagons, 20 vertices where three faces meet, and 30 edges with twofold symmetry. The three remaining regular solids have different symmetry axes. The vast majority of regular viruses that appear spherical have icosahedral symmetry.

In an icosahedron, the smallest number of subunits that can form the three-dimensional structure is 60 (5 subunits at

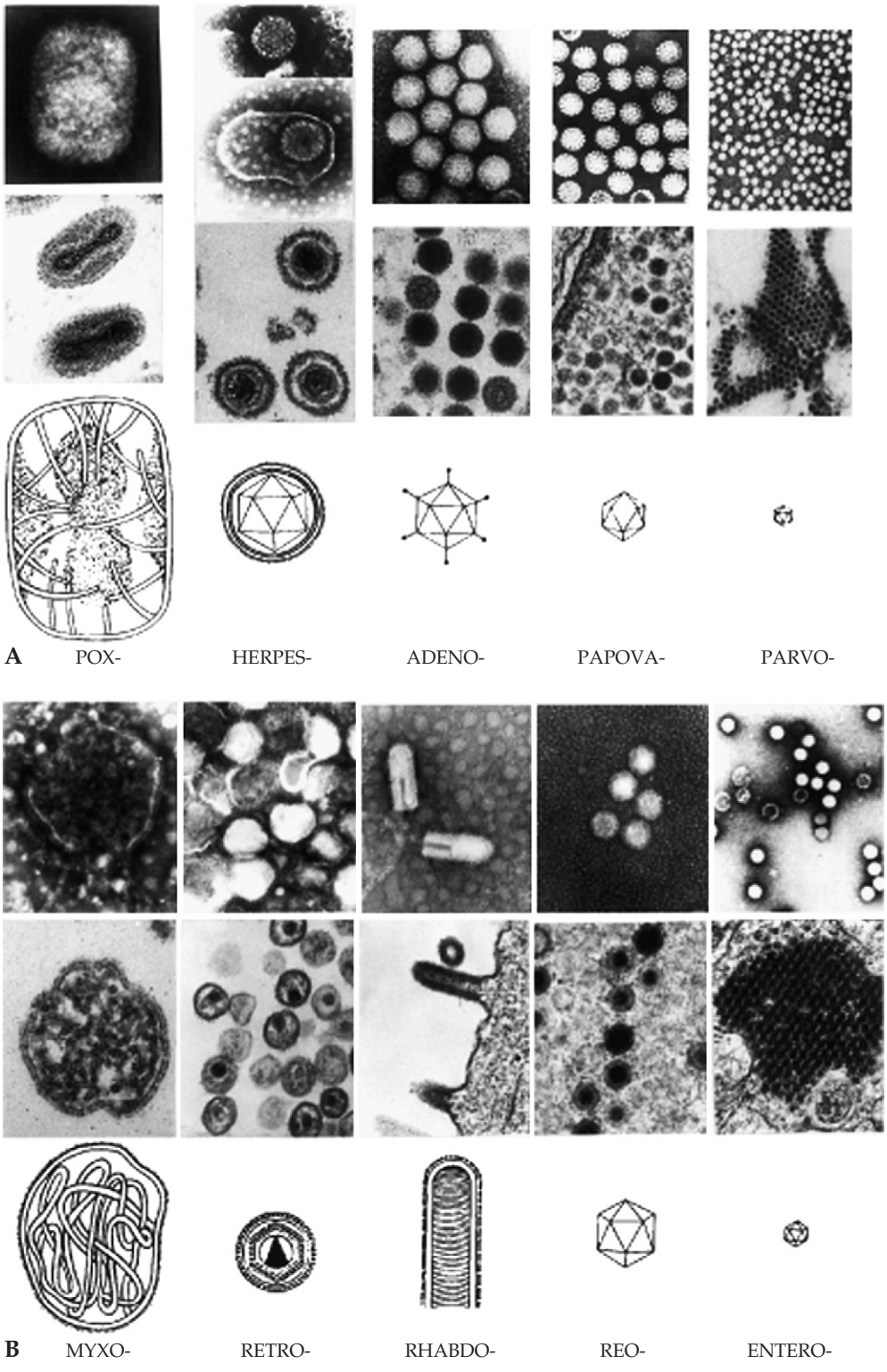


FIGURE 2.1 Relative sizes and shapes of representative (A) DNA- and (B) RNA- containing viruses. In each panel the top row shows negatively stained virus preparations, the second row shows thin sections of virus-infected cells, and the bottom row illustrates schematic diagrams of the viruses. Magnification of the electron micrographs is 50,000. From Granoff and Webster (1999), p. 401.

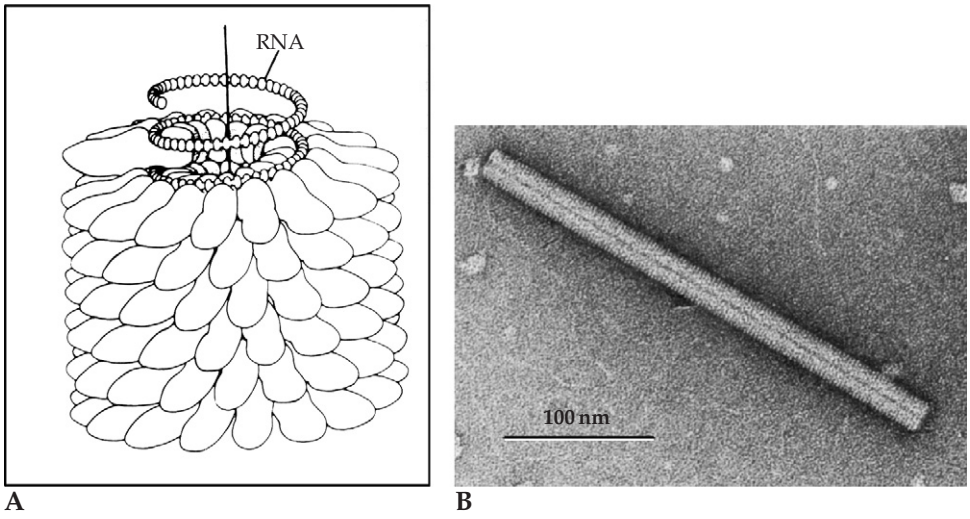


FIGURE 2.2 Structure of TMV, a helical plant virus. (A) Schematic diagram of a TMV particle showing about 5% of the total length. From Murphy *et al*. (1995), p. 434. (B) Electron micrograph of a negatively stained TMV rod. From www.ncbi.nlm.nih.gov.

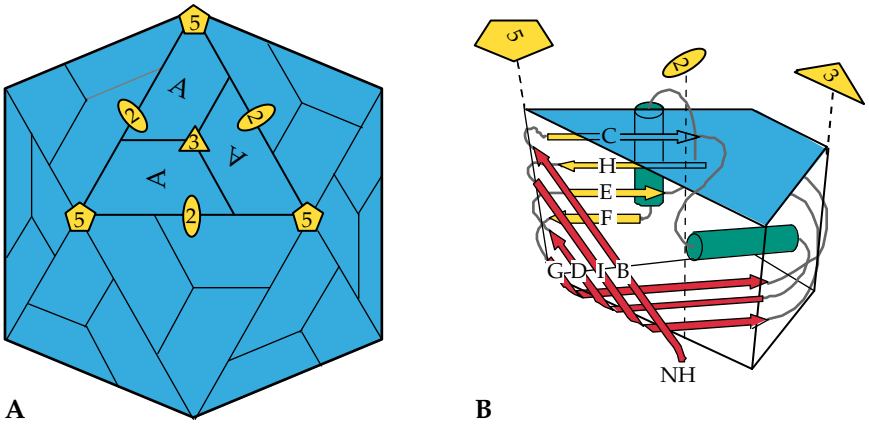


FIGURE 2.3 A simple icosahedral virus. (A) Diagram of an icosahedral capsid made up of 60 identical copies of a protein subunit, shown as blue trapezoids labeled "A." The twofold, threefold, and fivefold axes of symmetry are shown in yellow. This is the largest assembly in which every subunit is in an identical environment. (B) Schematic representation of the subunit building block found in many RNA viruses, known as the eightfold β barrel or β sandwich. The β sheets, labeled B through I from the N terminus of the protein, are shown as yellow and red arrows; two possible α helices joining these sheets are shown in green. Some proteins have insertions in the C–D, E–F, and G–H loops, but insertions are uncommon at the narrow end of the wedge (at the fivefold axis). From Granoff and Webster (1999) Vol. 3, color plate 31. [Originally from J. Johnson (1996)].

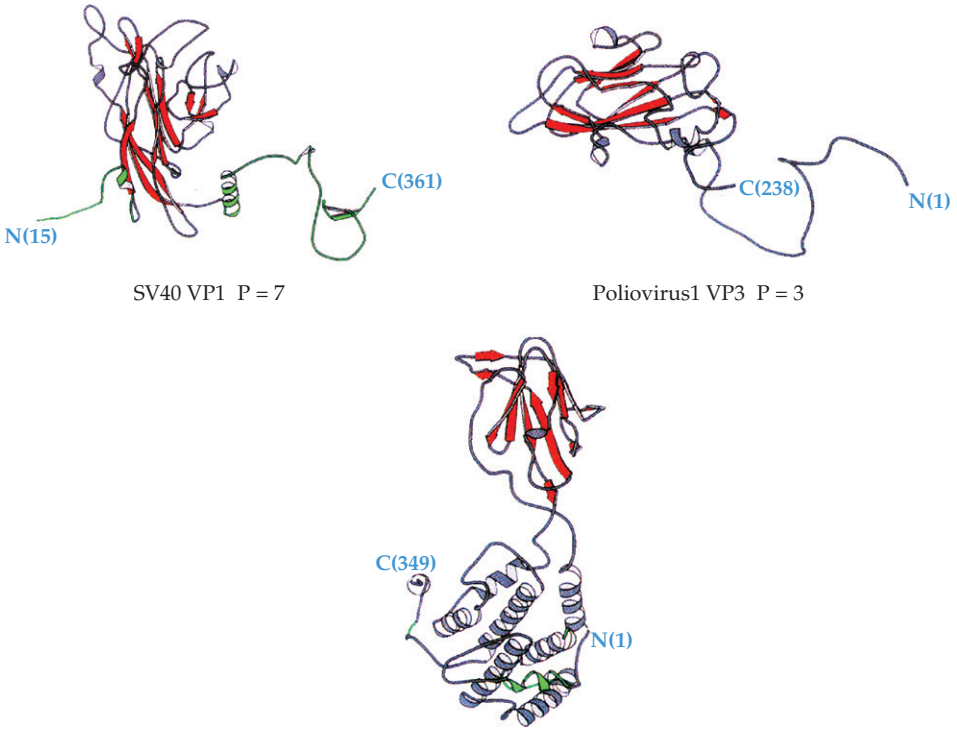
each of the 12 vertices, or viewed slightly differently, 3 units on each of the 20 triangular faces). Some viruses do in fact use 60 subunits, but most use more subunits in order to provide a larger shell capable of holding more nucleic acid. The number of subunits in an icosahedral structure is $60T$, where the permissible values of T are given by $T = H^2 + HK + K^2$, where H and K are integers and T is called the triangulation number. Permissible triangulation numbers are 1, 3, 4, 7, 9, 12, 13, 16, and so forth. A subunit defined in this way is not necessarily formed by one protein molecule, although in most cases this is how a structural subunit is in fact formed. Some viruses that form regular structures that are constructed using icosahedral symmetry principles do not possess true icosahedral symmetry. In such cases they are said to have pseudo-triangulation numbers. Examples are described later.

Structural studies of viruses have shown that the capsid proteins that form the virions of many plant and animal icosahedral viruses have a common fold. This fold, an eight-stranded antiparallel β sandwich, is illustrated in Fig. 2.3B. The presence of a common fold suggests that these capsid proteins have a common origin even if no sequence identity is detectable. The divergence in sequence while maintaining this basic fold is illustrated in Fig. 2.4, where capsid proteins of three viruses are shown. SV40 (family *Polyomaviridae*), poliovirus (family *Picornaviridae*), and bluetongue virus (family *Reoviridae*) are a DNA virus, a single-strand RNA virus, and a double-strand RNA virus, respectively. Their capsid proteins have insertions into the basic eight-stranded antiparallel β-sandwich structure that serve important functions in virus assembly. However, they all possess a region exhibiting the common β-sandwich fold and may have originated from a common ancestral protein. Thus, once a suitable capsid protein arose that could be used to construct simple icosahedral particles, it may ultimately have been acquired by many viruses. The viruses that possess capsid proteins with this fold may be related by descent from common ancestral viruses, or recombination may have resulted in the incorporation of this successful ancestral capsid protein into many lines of viruses.

Because the size of the icosahedral shell is fixed by geometric constraints, it is difficult for a change in the size of a viral genome to occur. A change in size will require a change in the triangulation number or changes in the capsid proteins sufficient to produce a larger or smaller internal volume. In either case, the changes in the capsid proteins required are relatively slow to occur on an evolutionary timescale and the size of an icosahedral virus is "frozen" for long periods of evolutionary time. For this reason, as well as for other reasons, most viruses have optimized the information content in their genomes, as will be clear when individual viruses are discussed in the following chapters.

Comparison of Icosahedral Viruses

Cryoelectron microscopy has been used to determine the structure of numerous icosahedral viruses to a resolution of 7 to 25 Å. For this, a virus-containing solution on an electron microscope grid is frozen very rapidly so that the sample is embedded in amorphous frozen water. The sample must be maintained at liquid nitrogen temperatures so that ice crystals do not form and interfere with imaging. Unstained, slightly out-of-focus images of the virus are captured on



SV40 VP1 P = 7

Poliovirus1 VP3 P = 3

Bluetongue virus VP7 T = 13

FIGURE 2.4 Structure of three vertebrate virus protein subunits that assemble into icosahedral shells. The N termini and C termini are labeled with the residue number in parenthesis. The β barrels are shown as red arrows, α helices are gray coils, and the subunit regions involved in quasi-symmetric interactions that are critical for assembly are colored green. SV40 and PV have triangulation numbers of "pseudo-*T*=7" or *P* = 7 and "pseudo-*T*=3" or *P* = 3, respectively. Adapted from Granoff and Webster (1999), Vol. 3, plate 32.

film, or more recently captured electronically, using a low dose of electrons. These images are digitized and the density measured. Mathematical algorithms that take advantage of the symmetry of the particle are used to reconstruct the structure of the particle.

A gallery of structures of viruses determined by cryoelectron microscopy is shown in Fig. 2.5. All of the images are to scale so that the relative sizes of the virions are apparent. The largest particle is the nucleocapsid of herpes simplex virus, which is 1250 Å in diameter and has T=16 symmetry (the virion is enveloped but only the nucleocapsid is regular). The rotavirus and reovirus virions are smaller and have T=13. Human papillomavirus and mouse polyomavirus are pseudo-T=7. Ross River virus (RRV) (family *Togaviridae*) is enveloped but has regular symmetry, with T=4. Several examples of viruses with T=3 or pseudo-T=3 are shown (dengue 2, flock house, rhino-, polio-, and cowpea mosaic viruses, of which dengue 2 is enveloped but regular and the

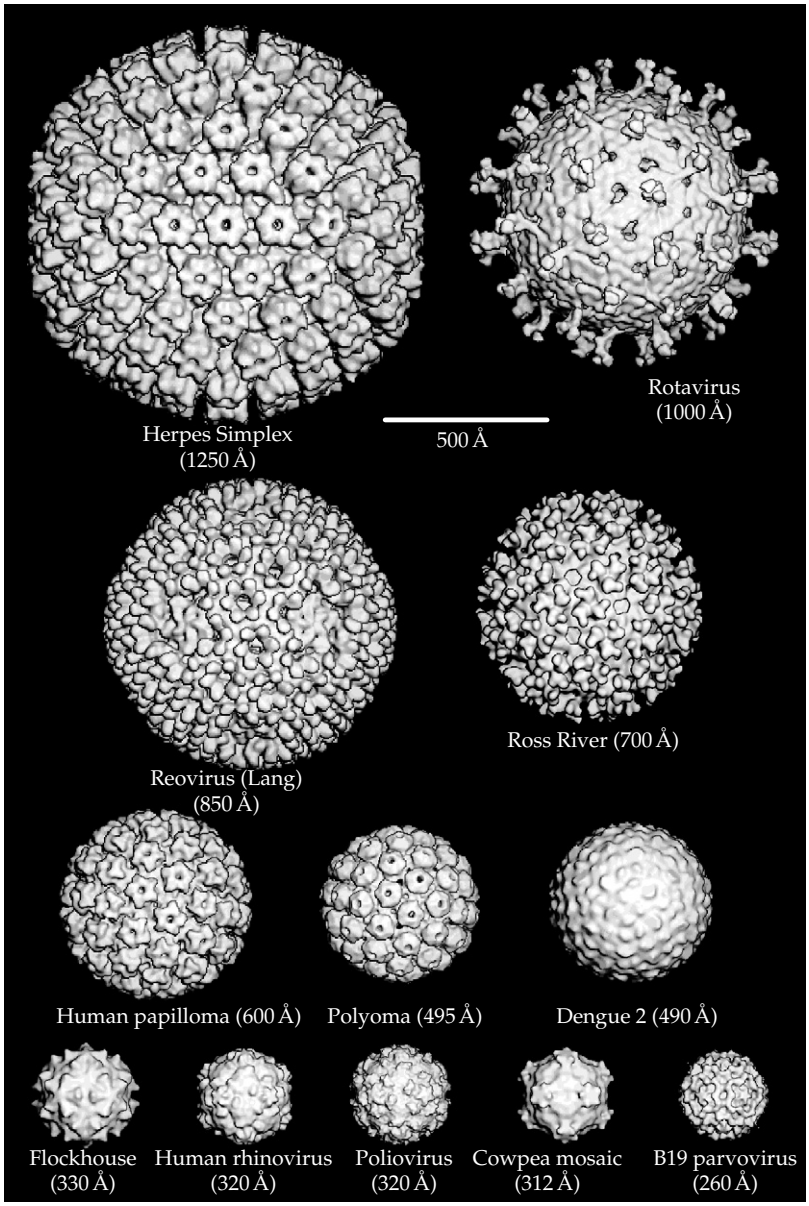


FIGURE 2.5 Gallery of three-dimensional reconstructions of icosahedral viruses from cryoelectron micrographs. All virus structures are surface shaded and are viewed along a twofold axis of symmetry except Ross River, which is viewed along a three-fold axis. All of the images are of intact virus particles except for the herpes simplex structure, which is of the nucleocapsid of the virus. Most of the images are taken from Baker *et al*. (1999), except the images of Ross River virus and of dengue virus, which were kindly provided by Drs. R. J. Kuhn and T. S. Baker.

rest are not enveloped). B19 parvovirus has *T*=1. The general correlation is that larger particles are constructed using higher triangulation numbers, which allows the use of larger numbers of protein subunits. Larger particles accommodate larger genomes.

Atomic Structure of *T*=3 Viruses

Because the simplest viruses are regular structures, they will often crystallize, and such crystals may be suitable for X-ray diffraction. Many viruses formed using icosahedral symmetry principles have been solved to atomic resolution, and a discussion of representative viruses that illustrate the principles used in construction of various viruses is presented here.

Among *T*=3 viruses, the structures of several plant viruses, including tomato bushy stunt virus (TBSV) (genus *Tombusvirus*, family *Tombusviridae*), turnip crinkle virus (TCV) (genus *Carmovirus*, family *Tombusviridae*), and Southern bean mosaic virus (SBMV) (genus *Sobemovirus*, not yet assigned to family), have been solved. All three of these viruses have capsid proteins possessing the eight-stranded antiparallel β sandwich. *T*=3 means that 180 identical molecules of capsid protein are utilized to construct the shell. The structures of two insect viruses that are also simple *T*=3 structures have also been solved. As an example of these simple structures, the *T*=3 capsid of the insect virus, flock house virus (family *Nodaviridae*), is illustrated in Fig. 2.6.

The 180 subunits in these *T*=3 structures interact with one another in one of two different ways, such that the protein shell can be thought of as being composed of an assembly of 60 AB dimers and 30 CC dimers (Fig. 2.6A). The bond angle between the two subunits of the dimer is more acute in the AB dimers than in the CC dimers (Figs. 2.6B and C). For the plant viruses, there are N-terminal and C-terminal extensions from the capsid proteins that are involved in interactions between the subunits and with the RNA. The N-terminal extensions have a positively charged, disordered domain for interacting with and neutralizing the charge on the RNA and a connecting arm that interacts with other subunits. In the case of the CC dimers, the connecting arms interdigitate with two others around the icosahedral threefold axis to form an interconnected internal framework. In the case of the AB conformational dimer, the arms are disordered, allowing sharper curvature. For flock house virus, the RNA plays a role in controlling the curvature of the CC dimers, as illustrated in Fig. 2.6.

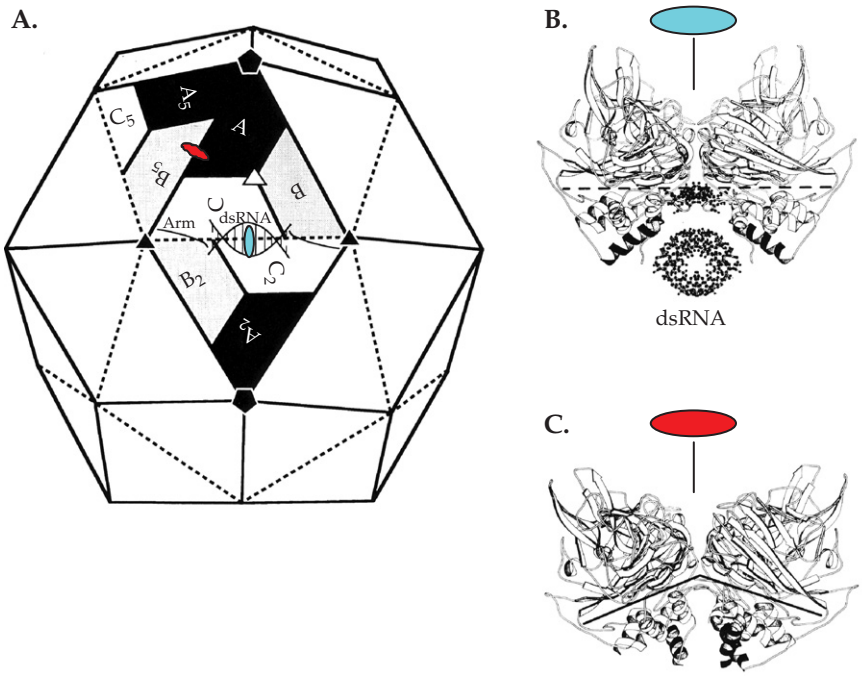


FIGURE 2.6 (A) Diagrammatic representation of a *T*=3 virus, flock house virus. The positions of the three identical proteins that make up a triangular face are only quasi-equivalent. The angle between the A and B_5 units (shown with a red oval and in diagram (C) is more acute than that along the C–C_2 edge, shown with a blue oval, and diagram (B). This difference in the angles is due to the presence of an RNA molecule located under the C–C_2 edge. From Johnson (1996), with permission.

Atomic Structure of Viruses Having Pseudo-*T*=3 Symmetry

The structures of several picornaviruses and of a plant comovirus (cowpea mosaic virus) have also been solved to atomic resolution. The structures of these viruses are similar to those of the plant *T*=3 viruses, but the 180 subunits that form the virion are not all identical. A comparison of the structure of a *T*=3 virus with those of poliovirus and of cowpea mosaic virus is shown in Fig. 2.7. Poliovirus has 60 copies of each of three different proteins, whereas the comovirus has 60 copies of an L protein (each of which fills the niche of two units) and 60 copies of an S protein. All three poliovirus capsid proteins have the eight-stranded antiparallel β-sandwich fold. In the comoviruses, the L protein has two β-sandwich structures fused to form one large protein, and the S protein is formed from one sandwich. The structures of the picornavirus and comovirus virions are called pseudo-*T*=3 or *P*=3, since they are not true *T*=3 structures.

The picornavirus virion is 300 Å in diameter. The 60 molecules of each of the three different proteins have different roles in the final structure, as illustrated in Fig. 2.8, in which the structure of a rhinovirus is shown. Notice that five copies of VP1 are found at each fivefold axis (compare Fig. 2.7 with Fig. 2.8). VP1, VP2, and VP3 are structurally related to one another, as stated, all possessing the common β-sandwich fold. There exists a depression around each fivefold axis of rhinoviruses that has been termed a "canyon." This depression is believed to be the site at which the virus interacts with the cellular receptor during entry, as illustrated in Fig. 2.9. This interaction is thought to lead to conformational changes that open a channel at the fivefold axis, through which VP4 is extruded, followed by the viral RNA.

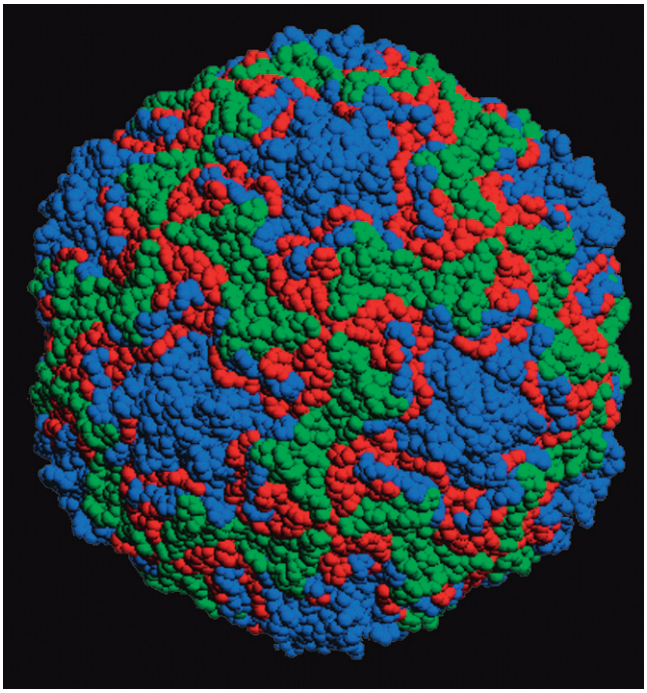


FIGURE 2.8 Three-dimensional space-filling model of the human rhinovirus 14 virion, based upon X-ray crystallographic data. VP1 is shown in blue, VP2 in green, and VP3 in red. VP4 is interior and not visible in this view. This figure was kindly provided by Dr. Michael Rossmann.

Atomic Structure of Polyomaviruses

The structures of both mouse polyomavirus and of SV40 virus, two members of the family *Polyomaviridae*, have been solved to atomic resolution. Both viruses possess pseudo-*T*=7 icosahedral symmetry. Although *T*=7

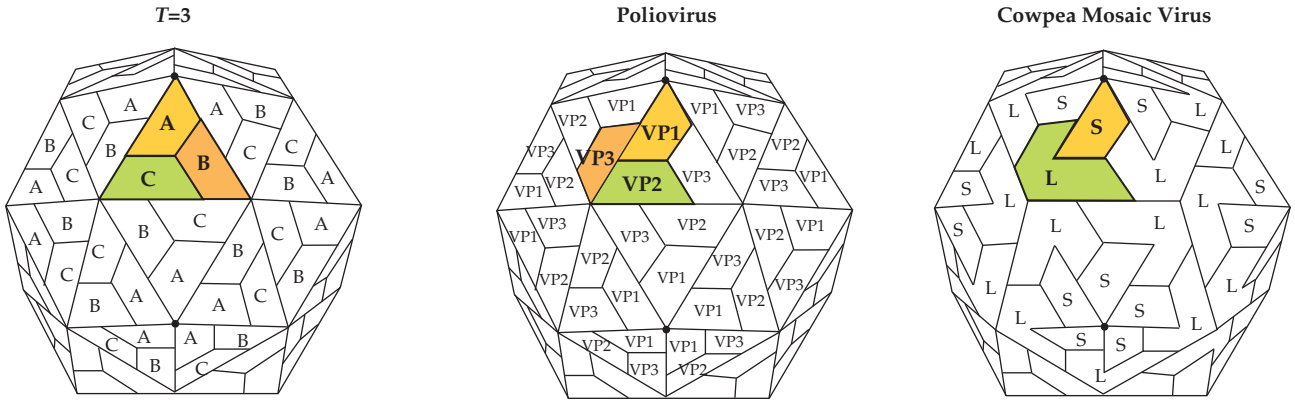


FIGURE 2.7 Arrangement of the coat protein subunits of comoviruses compared with those of simple *T*=3 viruses and picornaviruses. In simple viruses, the asymmetric unit contains three copies of a single protein β sandwich, labeled A, B, and C in order to distinguish them. In picornaviruses such as poliovirus the asymmetric unit is made up of three similar but not identical proteins, all of which have the β-sandwich structure. In comoviruses such as cowpea mosaic virus, two of the β-sandwich subunits are fused to give the L protein. Adapted from Granoff and Webster (1999), p. 287.

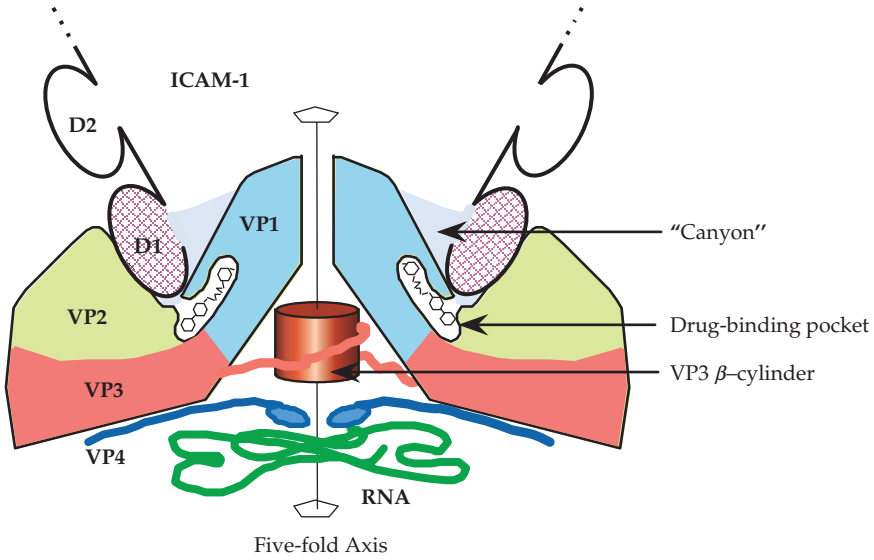


FIGURE 2.9 Binding of the rhinovirus receptor, ICAM-1, to the "canyon" at a fivefold vertex of a virion of a major group human rhinovirus. The colors of the three virion proteins are the same as those shown in the surface view in Fig. 2.8. The distal two domains of ICAM-1 are represented schematically (cross-hatched) as they were in Fig. 1.5. The amino-terminal domains of the five VP3 molecules around the fivefold axis form a five-stranded β cylinder on the virion's interior and are thought to stabilize the pentamer. Below the canyon is the hydrophobic pocket where certain antiviral drugs (indicated schematically in black) are known to bind. Adapted from Kolatkar *et al*. (1999).

symmetry would require 420 subunits, these viruses contain only 360 copies of a major structural protein known as VP1. These 360 copies are assembled as 72 pentamers. Twelve of the 72 pentamers lie on the fivefold axes and the remaining 60 fill the intervening surface in a closely packed array (Fig. 2.10). These latter pentamers are thus sixfold coordinated and the proteins in the shell are not all in quasi-equivalent positions, a surprising finding for our understanding of the principles by which viruses can be constructed. The pentamers are stabilized by interactions of the β sheets between adjacent monomers in a pentamer (Fig. 2.10C). The pentamers are then tied together by C-terminal arms of VP1 that invade monomers in an adjacent pentamer (Figs. 2.10B and C). Because each pentamer that is sixfold coordinated has five C-terminal arms to interact with six neighboring pentamers, the interactions between monomers in different pentamers are not all identical (Fig. 2.10B). Flexibility in the C-terminal arm allows it to form contacts in different ways.

Atomic Structure of Bluetongue Virus

Members of the reovirus family are regular *T*=13 icosahedral particles. They are composed of two or three concentric protein shells. Cryoelectron microscopy has been used to solve the structure of one or more members of three genera within the *Reoviridae*, namely *Reovirus, Rotavirus,* and *Orbivirus*, to about 25-Å resolution. Structures of a reovirus and of a rotavirus are shown in Fig. 2.5. The complete structure of virions has not been determined because of their large size, but in a remarkable feat the atomic structure of the core of bluetongue virus (genus *Orbivirus*) has now been solved. This is the largest structure determined to atomic resolution to date. Solution of the structure was possible because the virus particle had been solved to 25 Å by cryoelectron microscopy, and the structures of a number of virion proteins had been solved to atomic resolution by X-ray diffraction. Fitting the atomic structure of the proteins into the 25-Å structure gave a preliminary reconstruction at high resolution, which allowed the interpretation of the X-ray data to atomic resolution.

Core particles are formed following infection, when the outer layer is proteolytically cleaved (described in more detail in Chapter 5). The structure of the inner surface of the bluetongue virus core is shown in Fig. 2.11A and of the outer surface in Fig. 2.11B. The outer surface is formed by 780 copies of a single protein, called VP7, in a regular *T*=13 icosahedral lattice. The inner surface is surprising, however. It is formed by 120 copies of a single protein, called VP3. These 120 copies have been described as forming a *T*=2

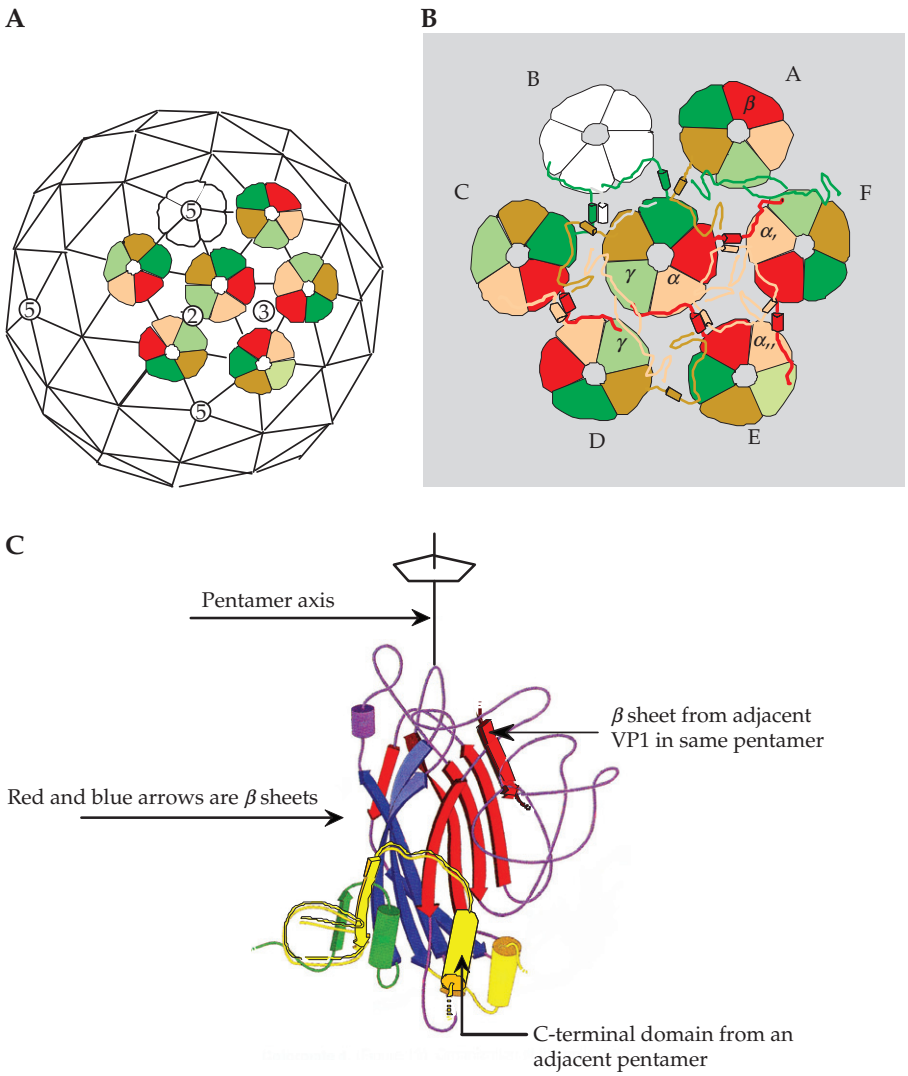


FIGURE 2.10 Organization of the capsid of the polyomavirus SV40. (A) Arrangement of the strict pentamers (white) and quasi pentamers (colored) on the *T*=7d icosahedral lattice. (B) Schematic showing the pattern of interchange of arms in the virion. The central pentamer shares "arms" with six neighboring pentamers. (C) A single VP1 subunit, viewed normal to the pentamer axis. The N-terminal domain is green, the C-terminal domain is yellow, and the β sandwich is shown as arrows of blue and red. The central yellow C-terminal domain (outlined in black) comes from an adjacent pentamer and the β sheet outlined in black comes from the neighboring VP1 within the same pentamer. From Figure 1 Stehle *et al*. (1996) and Fields *et al*. (1996), Color Plate 4.

lattice. Because *T*=2 is not a permitted triangulation number, these 120 copies, strictly speaking, form a *T*=1 lattice in which each unit of the lattice is composed of two copies of VP3. However, the interactions are not symmetrical, leading to the suggested terminology of *T*=2.

It has been suggested that the inner core furnishes a template for the assembly of the *T*=13 outer surface. The reasoning is that a *T*=13 structure may have difficulty in forming, whereas the *T*=2 (or *T*=1) structure could form readily. In this model, the threefold symmetry axis of the inner surface could serve to nucleate VP7 trimers and organize the *T*=13 structure.

Structure of Adenoviruses

Cryoelectron microscopy has also been applied to adenoviruses, which have a triangulation number of 25 or pseudo-25. Various interpretations of the structure of adenoviruses, both schematic and as determined by microscopy or crystallography, are shown in Fig. 2.12. Three copies of a protein called the hexon protein associate to form a structure called a hexon (Fig. 2.12C). The hexon is the basic building block of adenoviruses. Five hexons, called peripentonal hexons, surround each of the 12 vertices of the icosahedron (which, as has been stated, have fivefold rotational symmetry). Between the

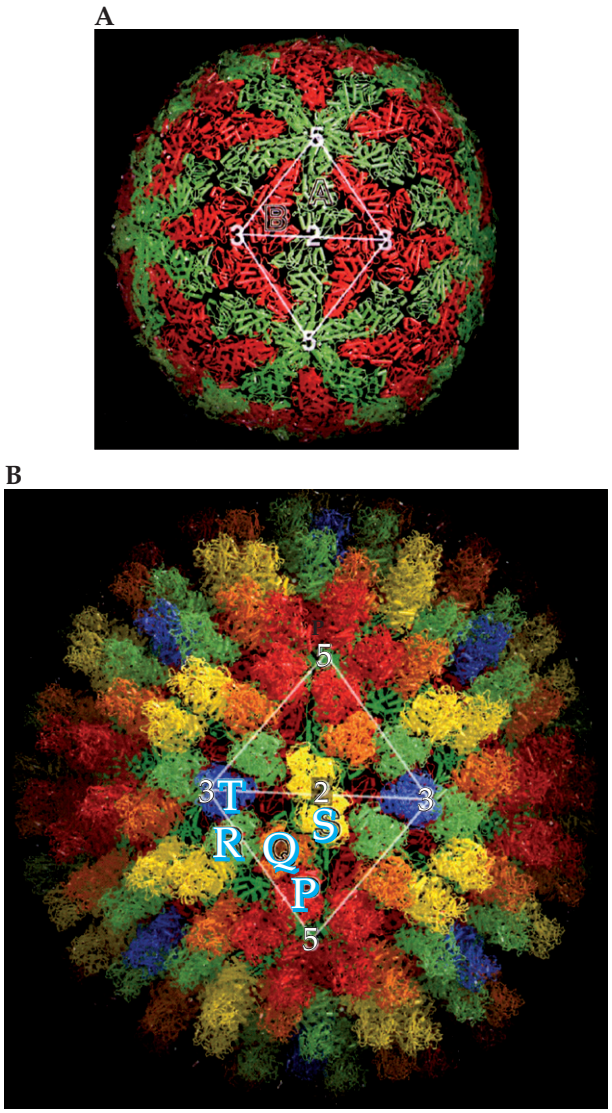


FIGURE 2.11 The essential features of the orbivirus native core particle. The asymmetric unit is indicated by the white lines forming a triangle and the fivefold, threefold, and twofold axes are marked. (A) The inner capsid layer of the bluetongue virus (BTV) core is composed of 120 molecules of VP3, arranged in what has been called *T*=2 symmetry. Note the green subunit A and the red subunit B which fill the asymmetric unit. (B) The core surface layer is composed of 780 copies of VP7 arranged as 260 trimers, with *T*=13 symmetry. The asymmetric unit contains 13 copies of VP7, arranged as five trimers, labeled P, Q, R, S, and T, with each trimer a different color. Trimer "T" in blue sits on the icosahedral threefold axis and thus contributes only a monomer to the asymmetric unit. From Granoff and Webster (1999), Color Plate 17.

groups of peripentonal hexons are found groups of 9 hexons, which are sixfold coordinated. Each group of 9 hexons forms the surface of one of the triangular faces. Thus, there are 60 peripentonal hexons and 180 hexons in groups of nine.

The structure of the hexon trimer has been solved to atomic resolution by X-ray crystallography (Fig. 2.12C). The hexon protein has two eight-strand β sandwiches to give the trimer an approximately sixfold symmetry (and each hexon protein fills the role of two symmetry units). There are long loops that intertwine to form a triangular top. These structures can be fitted uniquely into the envelope of density determined by cryoelectron microscopy, which produces a structure refined to atomic resolution for most of the capsid. The minor proteins can be fitted into this structure.

From the 12 vertices of the icosahedron project long fibers that are anchored in the surface by a unit called the penton base. Each fiber terminates in a spherical extension that forms an organ of attachment to a host cell (Fig. 2.12A). The length of the fiber differs in the different adenoviruses.

NONENVELOPED VIRUSES WITH MORE COMPLICATED STRUCTURAL FEATURES

In addition to the nonenveloped viruses that possess relatively straightforward icosahedral symmetry or helical symmetry, many viruses possess more complicated symmetries made possible by the utilization of a large number of structural proteins to form the virion. The tailed bacteriophages are prominent examples of this (Fig. 2.13). Some of the tailed bacteriophages possess a head that is a regular icosahedron (or, in at least one case, an octahedron) connected to a tail that possesses helical symmetry. Other appendages, such as baseplates, collars, and tail fibers, may be connected to the tail. Other tailed bacteriophages have heads that are assembled using more complicated patterns. For example, the T-even bacteriophages have a large head, which can be thought of as being formed of two hemi-icosahedrons possessing regular icosahedral symmetry, which are elongated in the form of a prolate ellipsoid by subunits arranged in a regular net connecting the two icosahedral ends of the head of the virus.

ENVELOPED VIRUSES

Many animal viruses and some plant viruses are enveloped; that is, they have a lipid-containing envelope surrounding a nucleocapsid. The lipids are derived from the host cell. Although there is some selectivity and reorganization of lipids during virus formation, the lipid composition in general mirrors the composition of the cellular membrane from which the envelope was derived. However, the proteins in the nucleocapsid, which may possess either helical or icosahedral symmetry, and the proteins in the envelope are encoded in the virus. The protein–protein interactions that are responsible for assembly of the mature enveloped virions differ among the different families and the structures of the resulting virions differ. The virions of alphaviruses, and of flaviviruses, are uniform

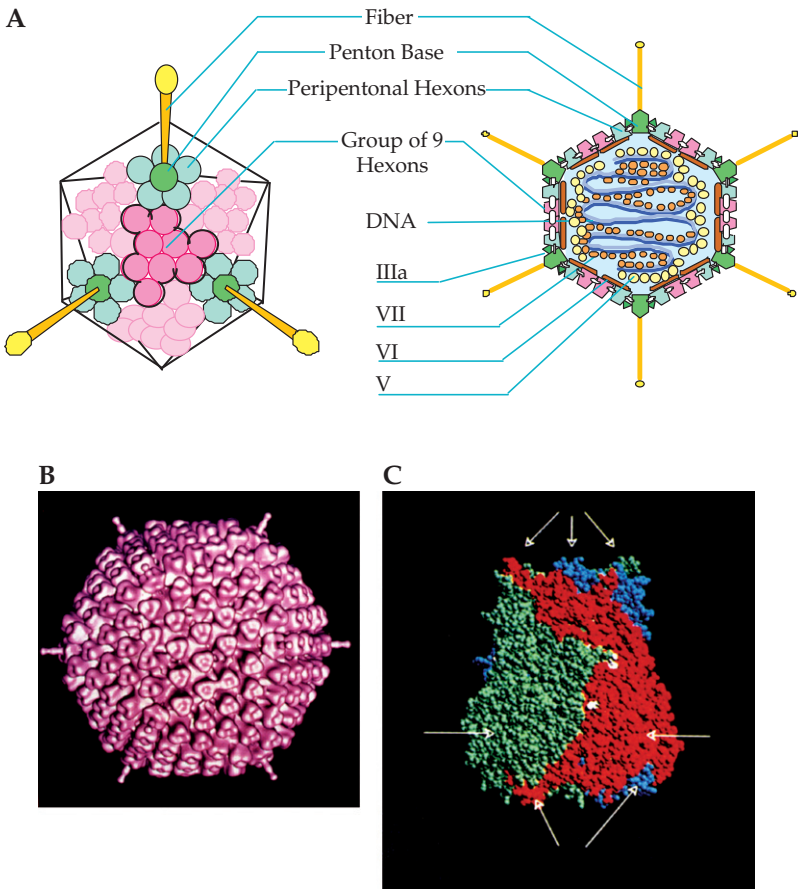


FIGURE 2.12 Structure of adenovirus particles. (A) Schematic drawing of the outer shell of an adenovirus (left), and a schematic cross section through an adenovirus particle, showing the locations of minor polypeptide components (right). The virus is composed of 60 peripentonal hexons at the bases of the fibers at the fivefold vertices, and groups of 9 hexons, one on each triangular face of the icosahedron. (B) Cryoeletron microscopic reconstruction of an adenovirus virion, viewed down the threefold axis. (C) Space-filling model of the hexon trimer, with each subunit in a different color. The atomic structure of the hexon has been solved and fitted into the cryoelectron microscopic reconstruction. The locations of the minor constitutents, indicated schematically in A, were deduced by subtraction. (A) is from Fields *et al*. (1996) p. 80, (B) is from Stewart *et al*. (1991), and (C) is from Athappilly *et al*. (1994).

structures that possess icosahedral symmetry. Poxviruses, rhabdoviruses, and retroviruses also appear to have a regular structure, but there is flexibility in the composition of the particle and the mature virions do not possess icosahedral symmetry. The herpesvirus nucleocapsid is a regular icosahedral structure (Fig. 2.5), but the enveloped herpesvirions are not regular. Other enveloped viruses are irregular, often pleiomorphic, and are heterogeneous in composition to a greater or lesser extent. The structures of different enveloped viruses that illustrate these various points are described next.

The Nucleocapsid

The nucleocapsids of enveloped RNA viruses are fairly simple structures that contain only one major structural protein, often referred to as the nucleocapsid protein or core protein. This protein is usually quite basic or has a basic domain. It binds to the viral RNA and encapsidates it to form the nucleocapsid. For most RNA viruses, nucleocapsids can be recognized as distinct structures within the infected cell and can be isolated from virions by treatment with detergents that dissolve the envelope. The nucleocapsids of alphaviruses, and probably flaviviruses and arteriviruses as well, are regular icosahedral structures, and there are no other proteins within the nucleocapsid other than the nucleocapsid protein. In contrast, the nucleocapsids of all minus-strand viruses are helical and contain, in addition to the major nucleocapsid protein, two or more minor proteins that possess enzymatic activity. As described, the nucleocapsids of minus-strand RNA viruses remain intact within the cell during the entire infection cycle and serve as machines that make viral RNA. The coronaviruses also have helical nucleocapsids, but being plus-strand RNA viruses they do not need to carry enzymes in the virion to initiate infection. The helical nucleocapsids

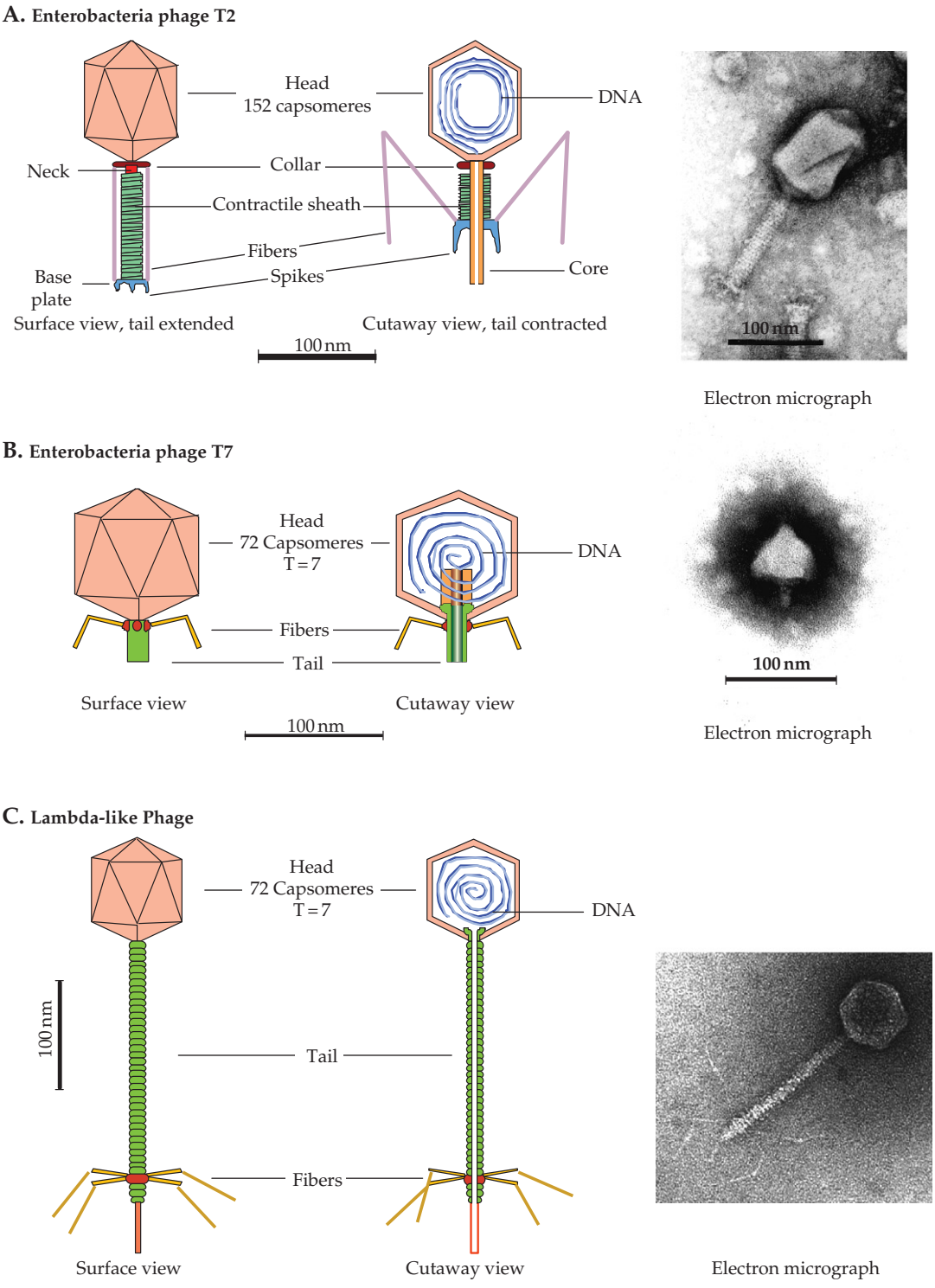


FIGURE 2.13 Morphology of some bacteriophages (members of the *Caudovirales*). (A) Enterobacteria phage T2, in the family *Myoviridae*. The head is an elongated pentagonal structure. (B) Enterobacteria phage T7, a member of the *Podoviridae*. (C) Enterobacteria phage λ, a member of the *Siphoviridae*. All electron micrographs are stained with uranyl acetate, and all bars shown are 100 nm. Phage diagrams are adapted from Murphy *et al*. (1995) pp. 51, 60, 55. Electron micrographs of T2 and T7 were kindly provided by Dr. H.-W. Ackermann, Laval University, Quebec. The electron micrograph of Ur-Lambda (note the long kinked tail fibers) was kindly provided by Dr. Roger Hendrix.

of (–) RNA viruses appear disordered within the envelope of all viruses except the rhabdoviruses, in which they are coiled in a regular fashion (see later).

The nucleocapsids of retroviruses also appear to be fairly simple structures. They are formed from one major precursor protein, the Gag polyprotein, that is cleaved during maturation into four or five components. The precursor nucleocapsid is spherically symmetric but lacks icosahedral symmetry. The mature nucleocapsid produced by cleavage of Gag may or may not be spherical symmetric. The nucleocapsid also contains minor proteins, produced by cleavage of Gag–Pro–Pol, as described in Chapter 1. These minor proteins include the protease, RT, RNase H, and integrase that are required to cleave the polyprotein precursors, to make a cDNA copy of the viral RNA, and to integrate this cDNA copy into the host chromosome.

The two families of enveloped DNA viruses that we consider here, the poxviruses and the herpesviruses, contain large genomes and complicated virus structures. The nucleocapsids of herpesviruses are regular icosahedrons but those of poxviruses are complicated structures containing a core and associated lateral bodies.

Envelope Glycoproteins

The external proteins of enveloped virions are virus-encoded proteins that are anchored in the lipid bilayer of the virus or whose precursors are anchored in the lipid bilayer. In the vast majority of cases these proteins are glycoproteins, although examples are known that do not contain bound carbohydrate. These proteins are translated from viral mRNAs and transported by the usual cellular processes to reach the membrane at which budding will occur. When budding is at the cell plasma membrane, the glycoproteins are transported via the Golgi apparatus to the cell surface. Some enveloped viruses mature at intracellular membranes, and in these cases the glycoproteins are directed to the appropriate place in the cell. Both Type I integral membrane proteins, in which the N terminus of the protein is outside the lipid bilayer and the C terminus is inside the bilayer, and Type II integral membrane proteins, which have the inverse orientation with the C terminus outside, are known for different viruses. Many viral glycoproteins are produced as precursor molecules that are cleaved by cellular proteases during the maturation process.

Following synthesis of viral glycoproteins, during which they are transported into the lumen of the endoplasmic reticulum (ER) in an unfolded state, they must fold to assume their proper conformation, and assume their proper oxidation state by formation of the correct disulfide bonds. This process often occurs very quickly, but for some viral glycoproteins it can take hours. Folding is often assisted by chaperonins present in the endoplasmic reticulum. It is believed that at least one function of the carbohydrate chains attached to the protein is to increase the solubility of the unfolded glycoproteins in the lumen of the ER so that they do not aggregate prior to folding. During folding, the solubility of the proteins is increased by hiding hydrophobic domains within the interior of the protein and leaving hydrophilic domains at the surface.

The glycoproteins possess a number of important functions in addition to their structural functions. They carry the attachment domains by which the virus binds to a susceptible cell. This activity is thought to be related to the ability of many viruses, nonenveloped as well as enveloped, to bind to and agglutinate red blood cells, a process called hemagglutination. The protein possessing hemagglutinating activity is often called the hemagglutinin or HA. The viral glycoproteins also possess a fusion activity that promotes the fusion of the membrane of the virus with a membrane of the cell. The protein possessing this activity is sometimes called the fusion protein, or F. The glycoproteins, being external on the virus, are also primary targets of the humoral immune system, in which circulating antibodies are directed against viruses; many of these are neutralizing antibodies that inactivate the virus.

The glycoproteins of some enveloped viruses also contain enzymatic activities. Many orthomyxoviruses and paramyxoviruses possess a neuraminidase that will remove sialic acid from glycoproteins. The primary receptor for these viruses is sialic acid. The neuraminidase may allow the virus to penetrate through mucus to reach a susceptible cell. It also removes sialic acid from the viral glycoproteins so that these glycoproteins or the mature virions do not aggregate, and from the surface of an infected cell, thereby preventing released virions from binding to it. The viral protein possessing neuraminidase activity may be called NA, or in the case of a protein that is both a neuraminidase and hemagglutinin, HN.

The structure of most enveloped viruses is not as rigorously constrained as that of icosahedral virus particles. The glycoproteins are not required to form an impenetrable shell, which is instead a function of the lipid bilayer. They appear to tolerate mutations more readily than do proteins that must form a tight icosahedral shell and appear to evolve rapidly in response to immune pressure. However, the integrity of the lipid bilayer is essential for virus infectivity, and enveloped viruses are very sensitive to detergents.

Other Structural Proteins in Enveloped Viruses

In some enveloped viruses, there is a structural protein that underlies the lipid envelope but which does not form part of the nucleocapsid. Several families of minus-strand RNA viruses possess such a protein, called the matrix protein. This protein may serve as an adapter between the nucleocapsid and the envelope. It may also have regulatory functions in viral RNA replication. The herpesviruses also have proteins underlying the envelope that form a thick layer called the tegument. The thickness of the tegument is not uniform within a virion, giving rise to some irregularity in its structure. The tegument

proteins perform important functions early after infection of a cell by a herpesvirus (see Chapter 7).

Structure of Alphaviruses

The alphaviruses, a genus in the family *Togaviridae*, are exceptional among enveloped RNA viruses in the regularity of their virions, which are uniform icosahedral particles. Virions of two alphaviruses have been crystallized and the crystals are regular enough to diffract to 30–40-Å resolution. Higher resolution has been obtained from cryoelectron microscopy, which has been used to determine the structures of several alphaviruses to 7–25 Å (Fig. 2.5).

More detailed reconstructions of Sindbis virus and Ross River virus (RRV) have been derived from a combination of cryoelectron microscopy of the intact virion and X-ray crystallography of alphavirus structural proteins. A cutaway view of RRV at about 25-Å resolution is shown in Fig. 2.14A. The nucleocapsid, shown in red and yellow, has a diameter of 400 Å, and is a regular icosahedron with T=4 symmetry. It is formed from 240 copies of a single species of capsid protein of size 30 kDa. Note the fivefold and sixfold

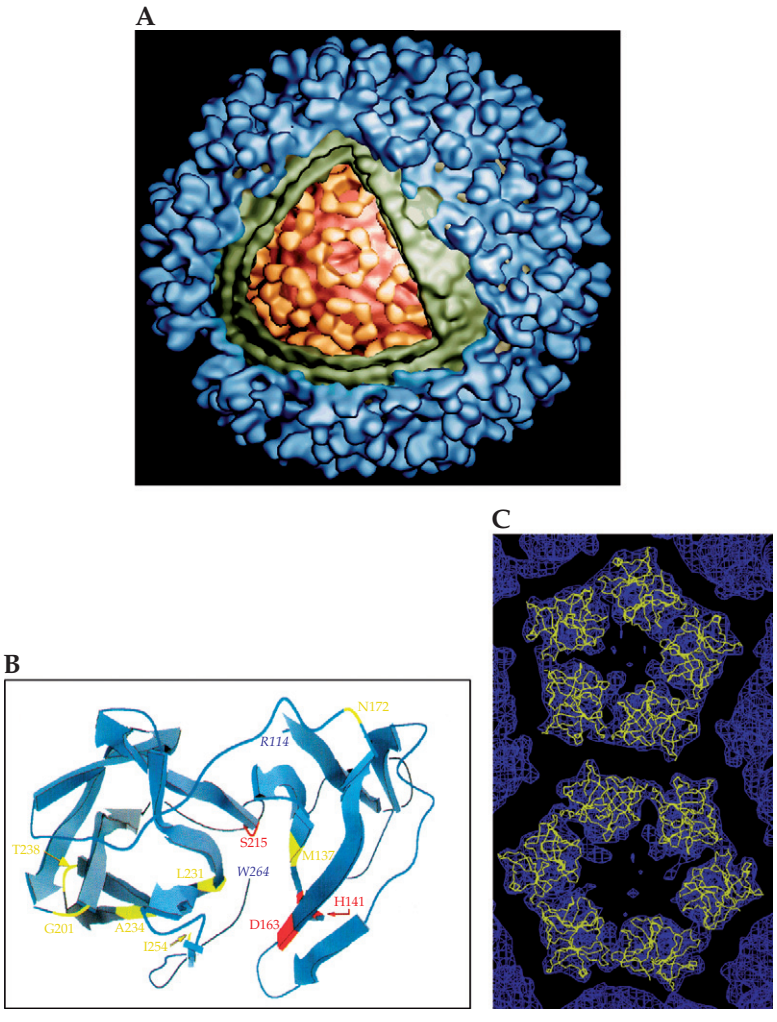


FIGURE 2.14 Structure of Ross River virus reconstructed from cryoelectron microscopy. (A) Cutaway view of the cryoelectron reconstruction illustrating the multilayered structure of the virion. Envelope glycoproteins are shown in blue, the lipid bilayer in green, the ordered part of the nucleocapsid in yellow, and the remainder of the nucleocapsid in orange. (B) Ribbon diagram of the X-ray crystallographic structure of the Sindbis virus capsid protein, with β sheets represented by large arrows. Only the carboxy-terminal domain, which starts at Arg-114, is ordered in crystals. The active site residues of the autoprotease, Ser-215, His-141, and Asp-163, are shown in red. The carboxy-terminal Trp-264, which is the P1 residue of the cleavage site, lies within the active site of the enzyme. The seven residues shown in yellow-green may interact with the cytoplasmic domain of glycoprotein E2 during budding of the nucleocapsid. (C) Fit of the Sindbis capsid protein Cα trace (yellow) into the electron density of Ross River virus (blue) determined by cryoelectron microscopy. (A) and (B) were adapted from Strauss *et al*. (1995), Figures 4 and 3, respectively, and (C) was kindly provided by Richard J. Kuhn.

coordinated pedestals in yellow that rise above the red background of RNA and unstructured parts of the protein. Each of these pedestals is formed by the ordered domains of one capsid protein molecule. The lipid bilayer is shown in green and is positioned between the capsid and the external shell of glycoproteins, shown in blue. The glycoproteins are also icosahedrally arranged with T=4 symmetry. The complete structure is therefore quite regular and the virion has been described as composed of two interacting protein shells with a lipid bilayer sandwiched between.

The structure of the ordered part of the capsid protein of Sindbis virus has been solved to atomic resolution by conventional X-ray crystallography and this structure is shown in Fig. 2.14B. The first 113 residues are disordered and the structure is formed by residues 114–264. This ordered domain has a structure that is very different from the eight-fold β sandwich described earlier (compare Fig. 2.14B with Figs. 2.3B and 2.4). Instead, its fold resembles that of chymotrypsin, and it has an active site that consists of a catalytic triad whose geometry is identical to that of chymotrypsin. The capsid protein is an active protease that cleaves itself from a polyprotein precursor. After cleavage, the C-terminal tryptophan-264 remains in the active site and the enzymatic activity of the protein is lost.

The interactions between the capsid protein subunits that lead to formation of the T=4 icosahedral lattice have been deduced by fitting the electron density of the capsid protein at 2.5-Å resolution into the electron density of the nucleocapsid found by cryoelectron microscopy. Such a reconstruction, based on a cryoEM structure of Sindbis virus at a resolution of better than 10 Å, is shown in Fig. 2.14C. The fit of the capsid protein is unique and the combined approaches of X-ray crystallography and cryoelectron microscopy thus define the structure of the shell of the nucleocapsid to atomic resolution.

The envelopes of alphaviruses contain 240 copies of each of two virus-encoded glycoproteins, called E1 and E2. E2 is first produced as a precursor called PE2. E1 and PE2 form a heterodimer shortly after synthesis, and both span the lipid bilayer as Type I integral membrane proteins (having a membrane-spanning anchor at or near the C terminus). The C-terminal cytoplasmic extension of PE2 interacts in a specific fashion with a nucleocapsid protein so that there is a one-to-one correspondance between a capsid protein and a glycoprotein heterodimer. The 240 glycoprotein heterodimers form a T=4 icosahedral lattice on the surface of the particle by interacting with one another and with the capsid proteins. Because of the glycoprotein–capsid protein interactions, the icosahedral lattices of the nucleocapsid and the glycoproteins are coordinated.

At some time during transport of the glycoprotein heterodimers to the cell surface, PE2 is cleaved by a cellular protease called furin to form E2. E1 and E2 remain associated as a heterodimer. If cleavage is prevented, noninfectious particles are produced that contain PE2 and E1.

In the virion, three glycoprotein heterodimers associate to form a trimeric structure called a spike, easily seen in Figs. 2.5 and 2.14A. It is not known if the spike assembles during virus assembly or if heterodimers trimerize during their transport to the cell surface. A reconstruction of a spike of Sindbis virus at a resolution better than 10 Å is shown in Fig. 2.15A. In this reconstruction, the electron density of E1 has been replaced by the E1 structure of the related Semliki Forest virus determined to atomic resolution by X-ray crystallography. The three copies of E1 project upwards at an angle of about 45° and are shown in three colors because they have slightly dif-

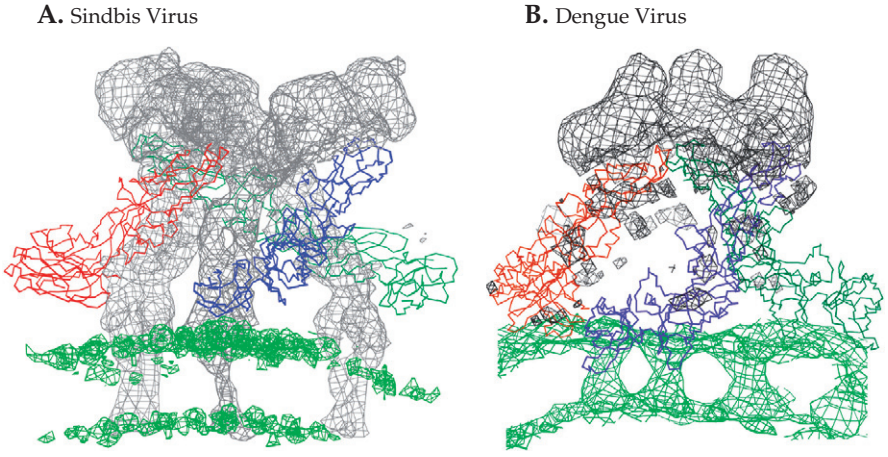


FIGURE 2.15 Comparison of (A) the spike structure of mature Sindbis virus (an alphavirus) with (B) the spike of immature dengue virus (a flavivirus). The Cα backbones of the three E1 (Sindbis) and E (dengue) glycoprotein ectodomains are shown in red, green, and blue, as they were fitted into the cryoelectron density envelope. The E1 and E densities have been zeroed out, leaving the gray envelope that corresponds to E2 for Sindbis and prM for dengue. The density corresponding to the lipid bilayer is shown in bright green. Adapted from Figure 5 in Y. Zhang *et al*. (2003) with permission.

ferent environments. The electron density in gray that remains after subtracting the density due to E1 is thus the electron density of E2. E2 projects further upward than does E1 and covers the apex of E1, which has the fusion peptide. Thus, E2 covers the fusion peptide with a hydrophobic pocket so that it does not interact with the hydrophilic environment. The apex of the E2 spike contains the domains that attach to receptors on a susceptible cell. Both E1 and E2 have C-terminal membrane-spanning anchors that traverse the lipid bilayer shown in green. The C-terminal domain of E1 is not present in the protein whose structure has been determined because hydrophobic domains do not easily crystallize. Thus, the electron density shown traversing the lipid bilayer arises from both E1 and E2 and shows that the two membrane spanning anchors go through as paired α helical structures (Fig. 2.16).

Upon entry of an alphavirus into a cell, the acidic pH of endosomal vesicles causes disassembly of E2/E1 heterodimers and trimerization of E1 to form homotrimers. The fusion peptide is exposed and penetrates the target bilayer of the host endosomal membrane. Fusion follows by methods discussed in Chapter 1.

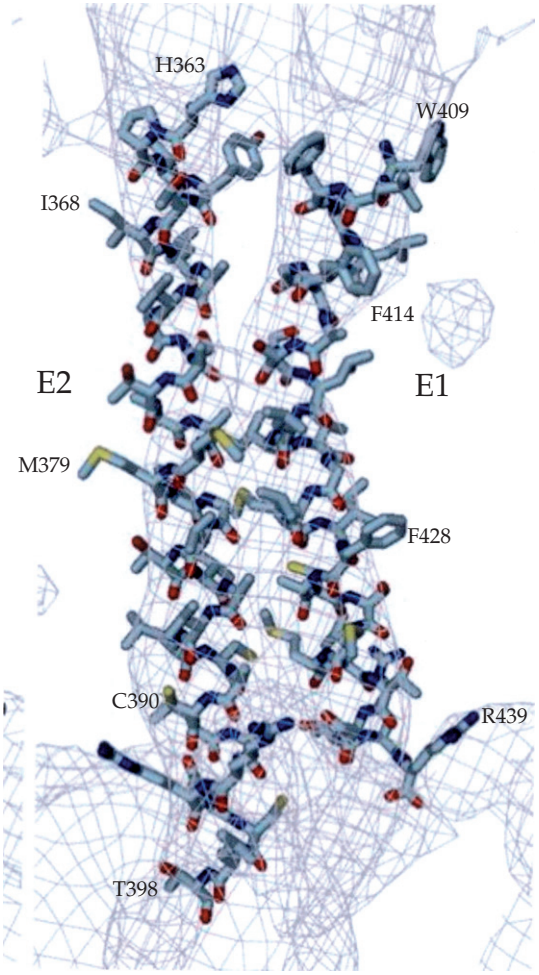


FIGURE 2.16 The E1 and E2 transmembrane helices of Sindbis (an alphavirus) determined from a 9Å resolution cryoelectron microscopy reconstruction. Shown are E1 residues from 409 to 439 and E2 residues 363 to 398 fitted into the transmembrane density. This is Figure 6 from Mukhopadhyay *et al*. (2006), reprinted with permission.

Structure of Flaviviruses

Flaviviruses also possess a regular icosahedral structure (Fig. 2.5) that has been solved by methods similar to those used to determine the structure of alphaviruses. The structures of alphaviruses and flaviviruses are related and have descended from a common ancestral structure. Like alphaviruses, flaviviruses produce two structural glycoproteins, called E and prM (for precursor to M). E is homologous to E1 of alphaviruses. Although no sequence identity is detectable, the structures of the two proteins are virtually identical and are formed with a similar fold (Fig. 2.17). prM and E form a heterodimer and immature particles can be formed if cleavage of prM is prevented. The glycoprotein heterodimers in these immature particles trimerize to form spikes whose structure is very similar to the spikes of alphaviruses (Fig. 2.15B). The arrangement of the glycoproteins on the immature virus particle is illustrated in Fig. 2.18A and a cryoEM reconstruction that illustrates the surface of the immature dengue virus particle is shown in Fig. 2.18D. The major differences between the immature flavivirus particle and the alphavirus particle are that there are 180 copies of the heterodimer in the flavivirus particle arranged in a T=3 icosahedral structure rather than 240 heterodimers arranged in a T=4 structure in alphaviruses; that prM is a smaller molecule than PE2 so that in the flavivirus spike there is but a thin trace of density that projects downward, parallel to E, from the cap that shields the fusion peptide (Fig. 2.15B) rather than a substantial trace of density in the alphavirus spike (Fig. 2.15A); and that the C-terminal regions of prM and E that enter the membrane do so independently and do not emerge from the internal side of the membrane (illustrated in Fig. 2.19B), unlike the alphavirus membrane spanning regions (Fig. 2.16). There is no evidence that the membrane glycoproteins interact with the nucleocapsid in flaviviruses, and the flavivirus nucleocapsid, assuming it is a regular icosahedral structure, is not coordinated with the icosahedral structure formed by the spikes, which is again different from alphaviruses where the C-terminal domain of PE2 interacts with the nucleocapsid.

Following cleavage of prM by furin to form M, there is a dramatic rearrangement of the flavivirus glycoproteins such that the final virion structure is very different from the alphavirus structure. The heterodimers dissociate and E-E homodimers are formed that collapse over the lipid bilayer (Fig. 2.19). The E-E homodimers occur in two totally different environments, either perpendicular to the twofold axis where they interact with homodimers in side-to-side interactions, forming a herringbone pattern, or parallel to a twofold axis where they interact at fivefold and threefold axes (Fig. 2.18B). A comparison of the immature flavivirus particle,

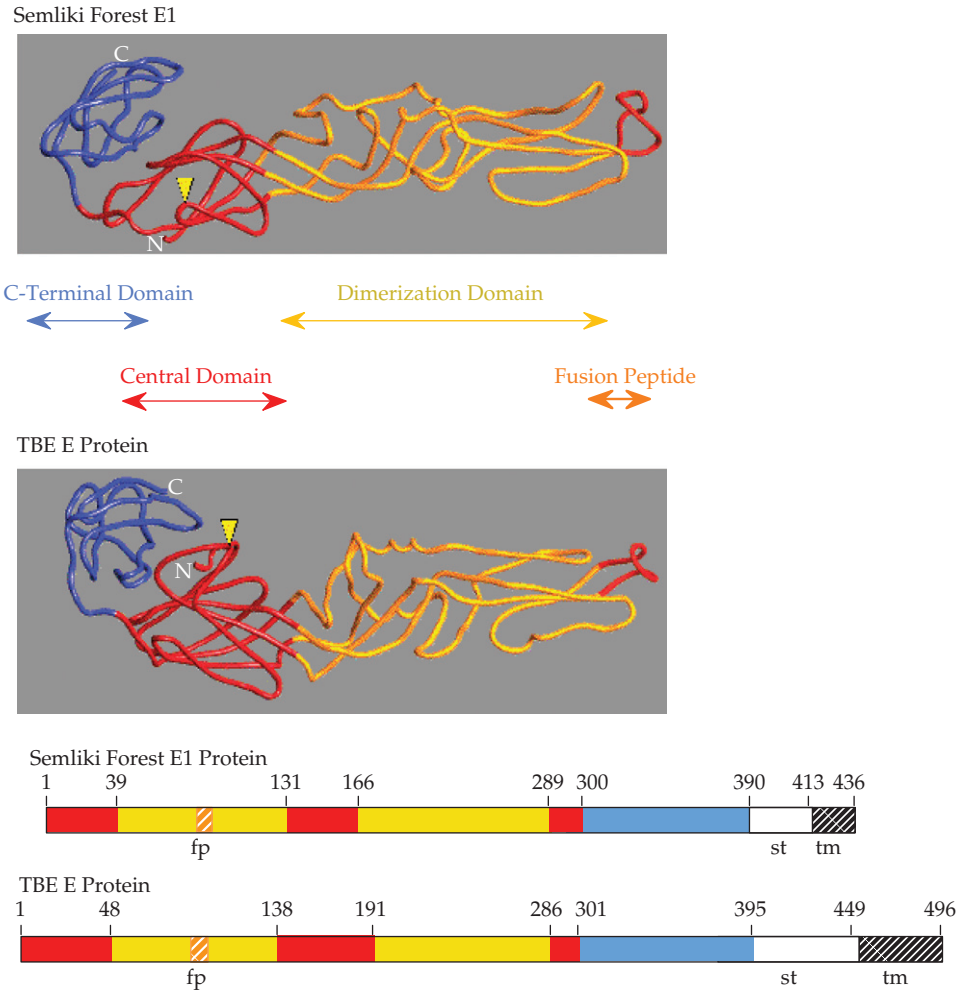


FIGURE 2.17 Comparison of the folds of the Semliki Forest (alphavirus) E1 protein and the tick-borne encephalitis (flavivirus) E protein. At the top are shown the X-ray crystal structures of the two proteins. At the bottom is a schematic of the linear amino acid sequences, color coded to indicate which amino acids contribute to each of the domains. Adapted from Figure 2 in Lescar *et al.* (2001) with permission.

the mature flavivirus particle, and the alphavirus particle is shown in Fig. 2.18. The mature flavivirus is smooth, with no surface projections, and is 50 nm in diameter (Fig. 2.18E). The immature particle is ragged in appearance and is 60 nm in diameter (Fig. 2.18D). The alphavirus virion shows conspicuous spikes and is 70 nm in diameter (Fig. 2.18F). The arrangement of E or E1 in the three particles is also shown (Figs. 2.18A, B, C), illustrating the differences in their arrangement.

Entry of flaviviruses follows pathways similar to those used by alphaviruses. The acidic pH of the endosome causes the E-E homodimers to reorganize to form E homotrimers. These trimers must reorient so that the exposed fusion peptide is projected upwards where it penetrates the host membrane.

Structure of Other Enveloped Viruses with Round Nucleocapsids

The arteriviruses possess icosahedral nucleocapsids, but the mature virion does not appear to be regular in structure. Detailed structures of these particles are not available.

The herpesviruses are large DNA viruses that have a T=16 icosahedral nucleocapsid (Fig. 2.5). A schematic diagram of an intact herpesvirion is shown in Fig. 2.20A. Underneath the envelope is a protein layer called the tegument. The tegument does not have a uniform thickness, and thus the virion is not uniform. Two electron micrographs of herpesvirions are shown in Figs. 2.20B and 2.20C that illustrate the irregularity of the particle and the differing thickness of the tegument in different particles.

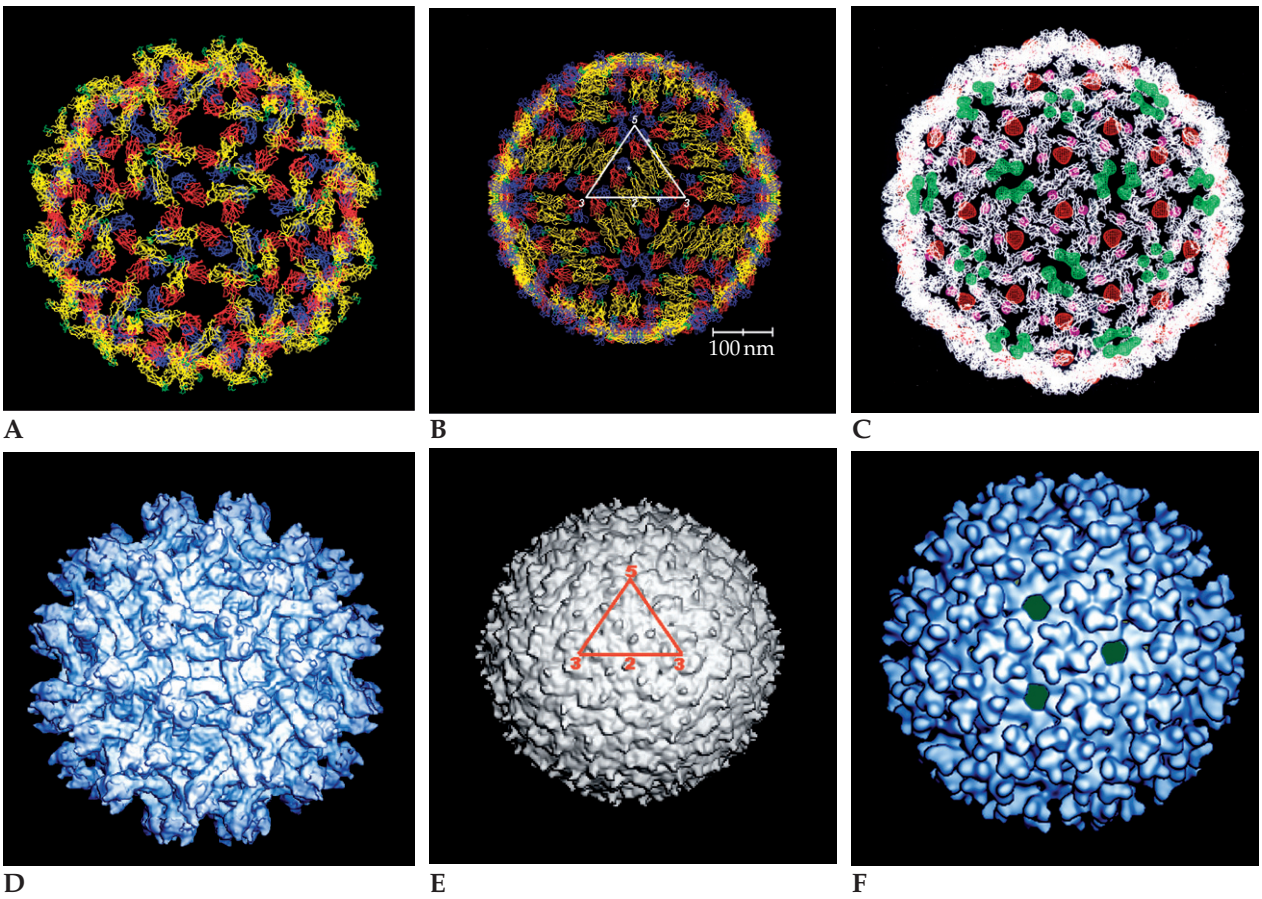


FIGURE 2.18 Fitting the X-ray crystal structures of flavivirus E protein and alphavirus E1 protein into the respective cryoelectron density envelopes of the virions. (A) Dengue E protein fitted into the cryoelectron density of immature prM-containing particle; (B) dengue E protein fitted into the envelope of the mature virion; (C) the fit of Sindbis alphavirus E1 into the Sindbis virus envelope; (D) cryoelectron reconstruction of the immature dengue prM-containing particle at 16-Å resolution; (E) cryoelectron reconstruction of the mature dengue virion at 12-Å resolution; (F) cryoelectron reconstruction of Ross River alphavirus at 25-Å resolution. Panels A and C were provided by Richard J. Kuhn; panel B is adapted from Figure 3c in Kuhn *et al*. (2002) with permission; panel D is adapted from Figure 3b in Y. Zhang *et al*. (2003) with permission; panel E is reprinted from Figure 1a in W. Zhang *et al*. (2003) with permission; panel F is adapted from Figure 4 in Strauss *et al*. (1995) with permission.

The retroviruses have a nucleocapsid that forms initially using spherical symmetry principles. Cleavage of Gag during virus maturation results in a nucleocapsid that is not icosahedral and that is often eccentrically located in the virion. Fig. 2.21A presents a schematic of a retrovirus particle that illustrates the current model for the location of the various proteins after cleavage of Gag and Gag–Pol. Figs. 2.21B, C, and D show electron micrographs of budding virus particles and of mature extracellular virions for three genera of retroviruses. Betaretrovirus particles usually mature by the formation of a nucleocapsid within the cytoplasm that then buds through the plasma membrane. This process is shown in Fig. 2.21B for mouse mammary tumor virus. In the top micrograph in Fig. 2.21B, preassembled capsids are seen in the cytoplasm. In the middle micrograph, budding of the capsid through the plasma membrane is illustrated. In the bottom micrograph, a mature virion with an eccentrically located capsid is shown.

In gammaretroviruses, the capsid forms during budding, and the nucleocapsid is round and centrally located in the mature virion. This process is illustrated in Fig. 2.21C for murine leukemia virus. The top micrograph shows a budding particle with a partially assembled capsid. The bottom micrograph shows a mature virion.

In the lentiviruses, of which HIV is a member, the capsid also forms as a distinct structure only during budding. In the top panel of Fig. 2.21D is shown a budding particle of bovine immunodeficiency virus. After cleavage of Gag to form the mature virion, the capsid usually appears cone shaped or bar shaped (bottom panel of Fig. 2.21D).

Mason–Pfizer monkey virus is a betaretrovirus whose capsid is cone shaped and centrally located in the mature virion. A single amino acid change in the matrix protein MA determines whether the capsid preassembles and then buds, or whether the capsids assemble during budding.

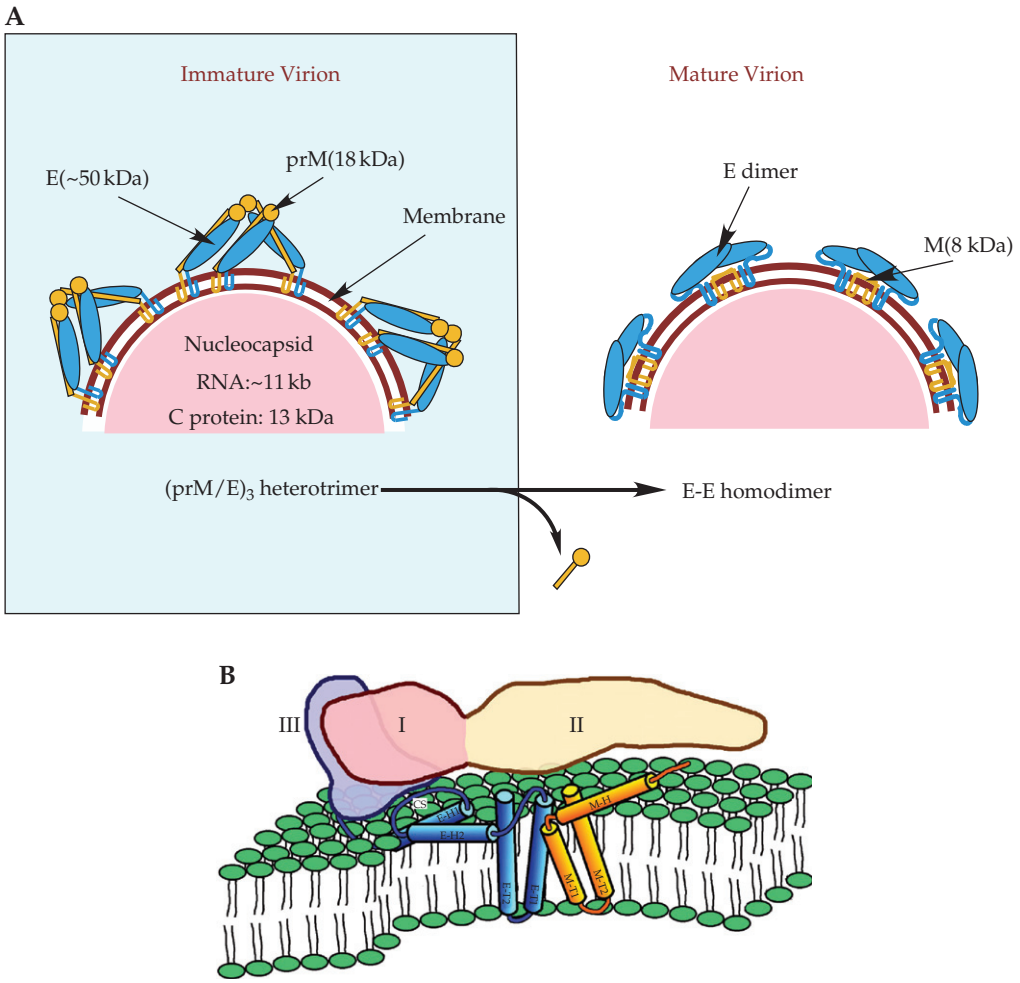


FIGURE 2.19 (A) Schematic representation of maturation of dengue virus. In the immature particle, as shown in panels (A) and (D) of Fig. 2.18, three heterodimers of E and prM come together to form a heterotrimer. Upon cleavage of prM to M and pr, E collapses onto the surface of the mature particle (panels B and E in Fig. 2.18) as homodimers. (B) Diagram of the dengue virus E protein in the mature particle as derived from cryoEM. This shows the three domains colored in the same way as in Figs. 2.17 and 2.18 as well as the locations of the transmembrane and intramembrane helices in blue and orange for E and M, respectively. Reprinted from Figure 4a in W. Zhang *et al*. (2003) with permission.

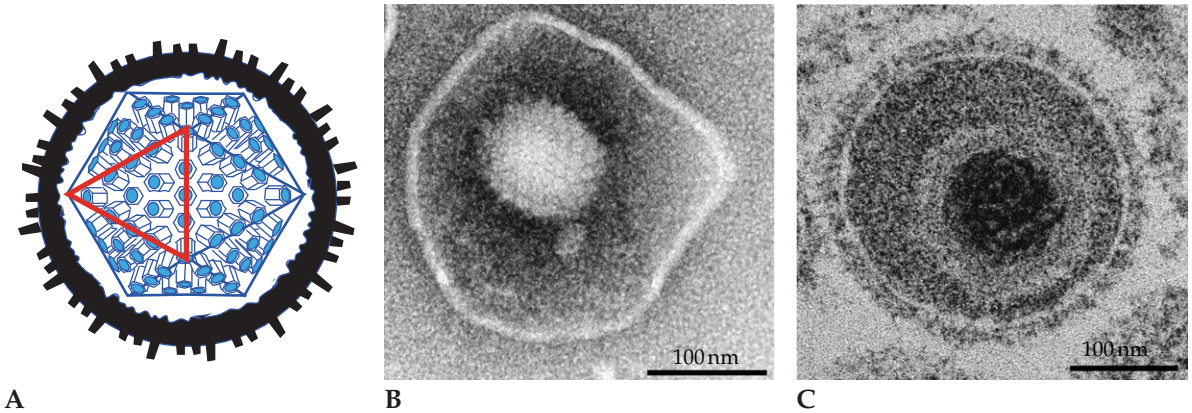


FIGURE 2.20 Two views of herpes simplex virus. (A) Cutaway schematic representation showing the outer envelope with projecting spikes, the irregular inner margin of the envelope due to the tegument, and the icosahedral core containing 162 capsomeres in a *T*=16 arrangement. One of the triangular faces of the icosahedron is outlined. Adapted from Murphy *et al*. (1995) p. 114. (B) Negatively stained electron micrograph of an intracellular particle of bovine herpesvirus. (C) Section through a bovine herpesvirion. Images in (B) and (C) were kindly provided by Dr. Peter Wild.

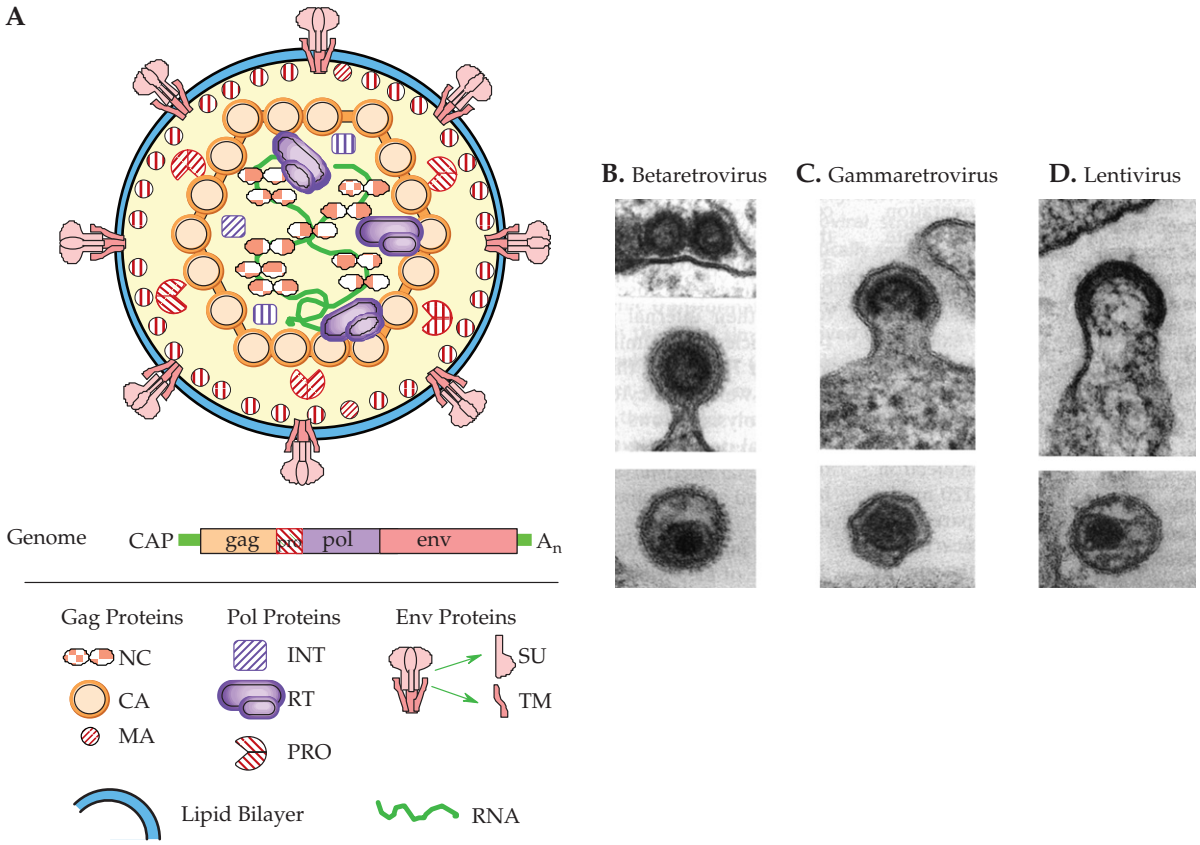


FIGURE 2.21 Structure of retrovirus particles. (A) Schematic cross section through a retrovirus particle. The lipid bilayer surrounds the particle and has imbedded in it trimeric spikes composed of surface (SU) and transmembrane (TM) envelope proteins. The internal nonglycosylated proteins are encoded by the *gag* gene and include NC, the nucleocapsid protein complexed with the genomic RNA, CA, the major capsid protein, and MA, the matrix protein that lines the inner surface of the membrane. Other components include RT, the reverse transcriptase, IN, the integrase, and PR, the protease. Adapted from Coffin *et al*. (1997), *Retroviruses*, p. 2. (B) Electron micrographs of mouse mammary tumor virus particles. Top: intracytoplasmic particles; middle: budding particles; bottom: mature extracytoplasmic particles. (C) Electron micrographs of murine leukemia virus particles. Top: budding particles; bottom: mature extracytoplasmic particles. (D) Electron micrographs of bovine immunodeficiency virus. Top: budding particles; bottom: mature extracytoplasmic particles. Adapted from Coffin *et al*. (1997), *Retroviruses*, p. 30.

Thus, the point at which capsids assemble does not reflect a fundamental difference in retroviruses. Preassembly of capsids or assembly during budding appears to depend on the stability of the capsid in the cell. Stable capsids can preassemble. Unstable capsids require interactions with other viral components to form as a recognizable structure.

Enveloped Viruses with Helical Nucleocapsids

The coronaviruses and the minus-strand RNA viruses have nucleocapsids with helical symmetry. The structures of the mature virions are irregular, with the exception of the rhabdoviruses, and the glycoprotein composition is not invariant. Because of the lack of regularity in these viruses, as well as the lack of symmetry, detailed structural studies of virions have not been possible. The lack of regularity arises in part because in these viruses there is no direct interaction between the nucleocapsid and the glycoproteins. The lack of such interactions permits these viruses to form pseudotypes, in which glycoproteins from other viruses substitute for those of the virus in question. Pseudotypes are also formed by retroviruses.

The structures of paramyxoviruses and orthomyxoviruses are illustrated schematically in Fig. 2.22. The helical nucleocapsids contain a major nucleocapsid protein called N or NP, and the minor proteins P (NS1) and L (PB1, PB2, PA), as shown. There is a matrix protein M (M1) lining the inside of the lipid bilayer and also two glycoproteins anchored in the bilayer that form external spikes. The two glycoproteins, called F and HN in paramyxoviruses and HA and NA in orthomyxoviruses, do not form heterodimers but rather form homooligomers so that there are two different kinds of spikes on the surface of the virions. HA in the orthomyxoviruses forms homotrimers whereas NA forms homotetramers, and the two types of spikes can be distinguished in the electron microscope if the

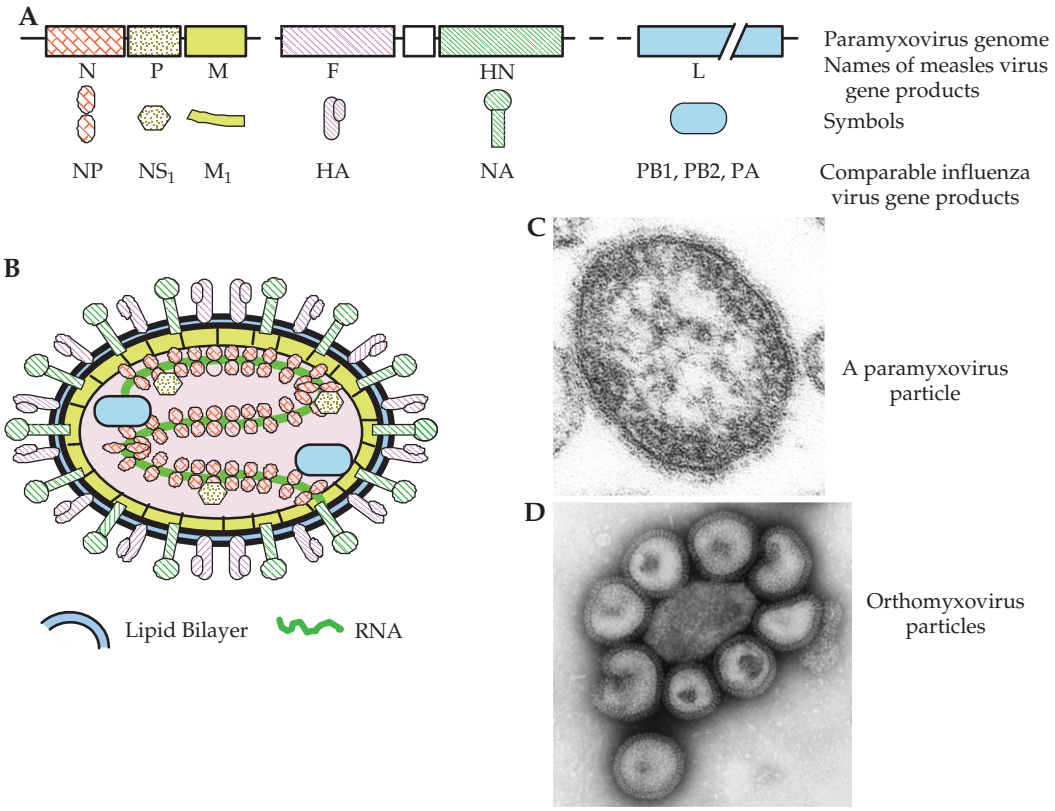


FIGURE 2.22 Morphology of orthomyxoviruses and paramyxoviruses. (A) Schematic of the genome organization of a paramyxovirus, Sendai virus. The names of the gene products and symbols to be used in the diagram are indicated. Also shown are the comparable gene products of influenza virus, an orthomyxovirus. (B) Schematic cutaway view of an orthomyxovirus or paramyxovirus particle. The nucleocapsid consists of a helical structure made up of the RNA complexed with many copies of the nucleocapsid protein. This internal structure also contains a few molecules of the RNA polymerase L (or PB1, PB2, PA in influenza virus), and P (or NS1). The nucleocapsid is enveloped in a lipid bilayer derived from the host cell in which are embedded two different glycoproteins, F and HN (or HA and NA in influenza virus) and which is lined on the inner surface with the matrix protein M. (C) Electron micrograph of a thin section of a measles particle. This photo was taken by Cynthia S. Goldsmith and obtained from the Public Health Image Library (PHIL). (D) Electron micrograph of a negatively stained influenza virus particles. This photo was taken by Fred Murphy and obtained from PHIL.

resolution is high enough. As occurs in many enveloped RNA viruses, F and HA are produced as precursors that are cleaved by furin during transport of the proteins. Cleavage is required to activate the fusion peptide of the virus, which is found at the N terminus of the C-terminal product (see Fig. 1.6). Electron micrographs of virions are shown in Figs. 2.22C and D. The particles in the preparations shown are round and reasonably uniform, but in other preparations the virions are pleomorphic baglike structures that are not uniform in appearance. In fact, clinical specimens of some orthomyxoviruses and paramyxoviruses are often filamentous rather than round, illustrating the flexible nature of the structure of the virion. The micrograph of the paramyxovirus measles virus shown in Fig. 2.22C is a thin section and illustrates the lack of higher order structure in the internal helical nucleocapsid. The micrograph of the orthomyxovirus influenza A virus shown in Fig. 2.22D is of a negative-stained preparation and illustrates the spikes that decorate the virus particle.

The structures of rhabdoviruses and filoviruses are illustrated in Fig. 2.23. The rhabdoviruses assemble into bullet-shaped or bacilliform particles in which the helical nucleocapsid is wound in a regular elongated spiral conformation (Figs. 2.23B and C). The virus encodes only five proteins (Fig. 2.23A), all of which occur in the virion (Fig. 2.23B). The nucleocapsid contains the major nucleocapsid protein N and the two minor proteins L and NS. The matrix protein M lines the inner surface of the envelope, and G is an external glycoprotein that is anchored in the lipid bilayer of the envelope. Budding is from the plasma membrane (Fig. 2.23D).

The filoviruses are so named because the virion is filamentous. A schematic diagram of a filovirus is shown in Fig. 2.23E, and electron micrographs of two filoviruses, Marburg virus and Ebola virus, are shown in Figs. 2.23F and G. Notice that in the electron microscope, filovirus virions often take the shape of a shephard's crook or the number 6.

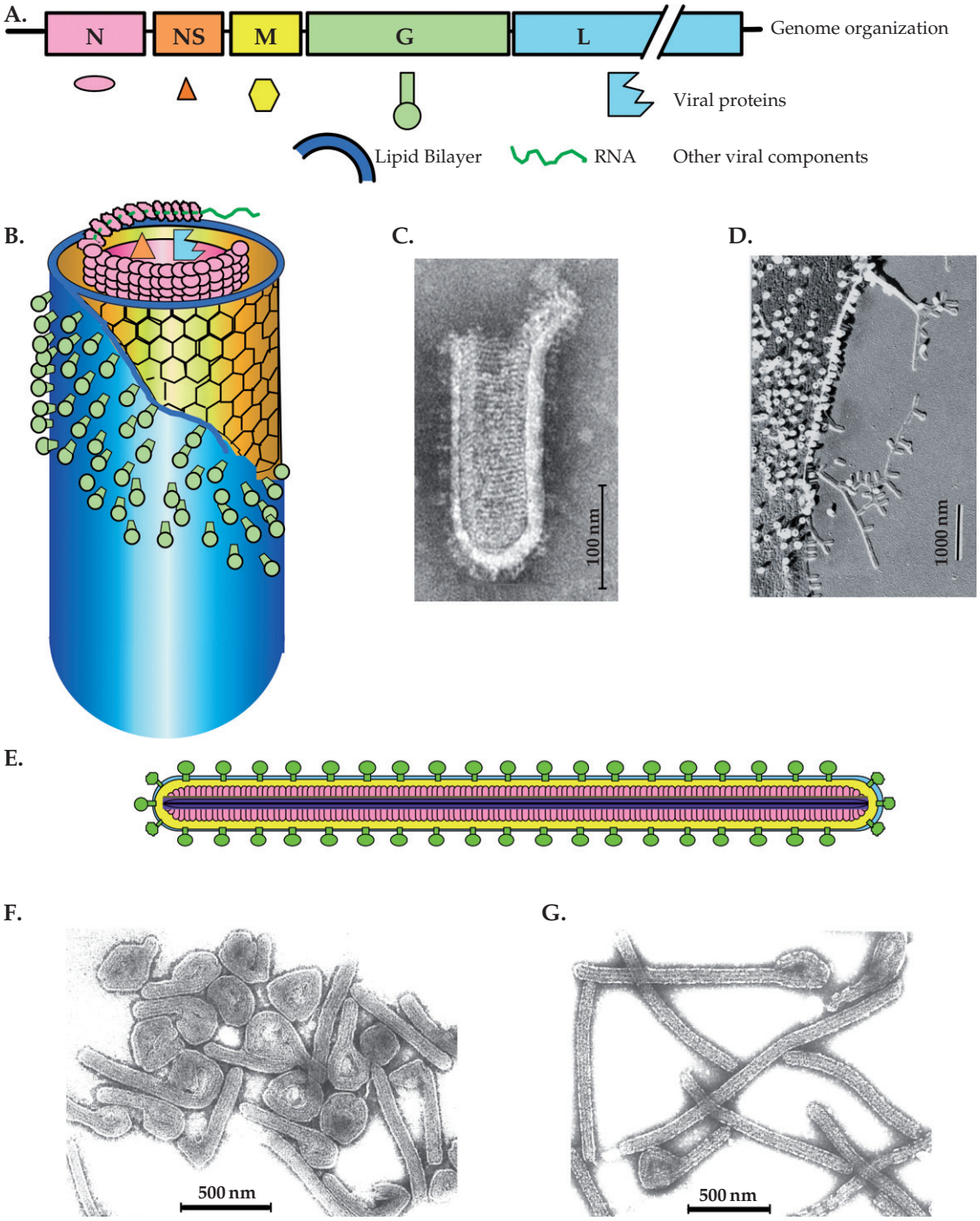


FIGURE 2.23 Morphology of the *Rhabdoviridae* and *Filoviridae*. (A) Genome organization of vesicular stomatitis virus (VSV), a *Vesiculovirus*, with the symbols for the various viral components shown below. (B) Cutaway diagram of a VSV particle. (C) A negatively stained electron micrograph of VSV virions. (D) Surface replica of a chicken embryo fibroblast infected with VSV. Note that the magnification is approximately 1/10 of that shown in (C). (E) Diagram of a filovirus, using the same color code for the components. (F) Negatively stained preparation of Marburg virus. (G) Filamentous forms of Ebola (Reston) virus. (B) is from Simpson and Hauser (1966); (F) and (G) are from Murphy *et al*. (1995) p. 289; and (D) is from Birdwell and Strauss (1975).

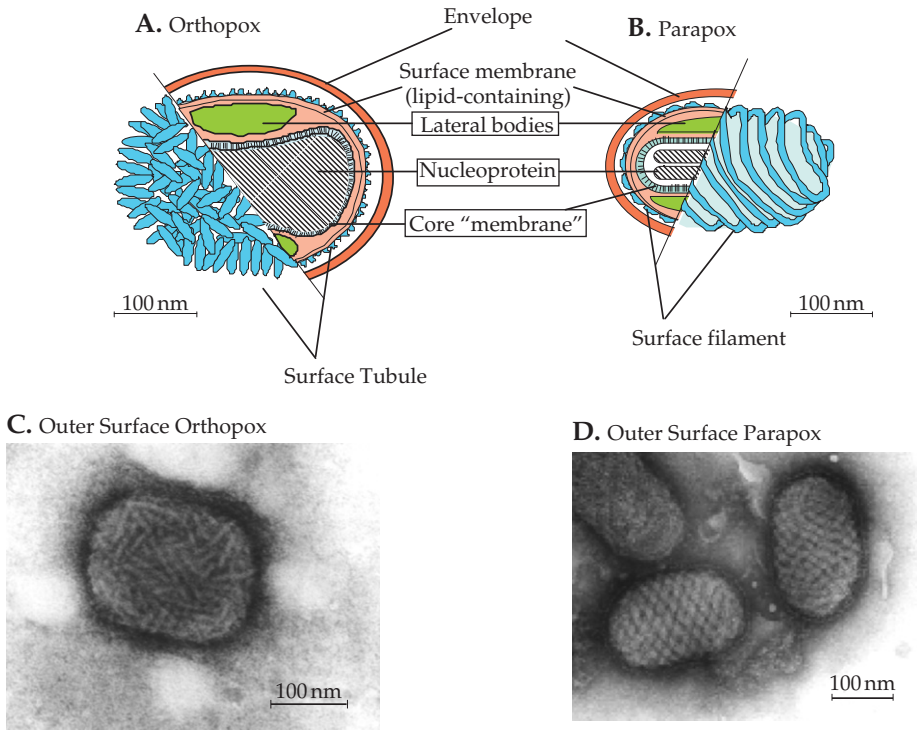


FIGURE 2.24 Morphology of orthopox and parapox virions. (A) and (B) Diagrams of orthopox and parapox virions. At the far left and right are shown the surfaces of the particles as they are isolated from infected cells, with the outer tubules or the outer filament shown in turquoise. The inner parts of each diagram show the enveloped particle in cross section illustrating the core membrane, the lateral bodies, and the nucleoprotein. Adapted from Fenner and Nakano (1988). (C) Purified vaccinia virus negatively stained with phosphotungstate; (D) Image of the outer surface of Orf parapox virus. Images in (C) and (D) were kindly provided by Prof. Stewart McNulty, Veterinary Sciences Division, Queens University of Belfast.

Vaccinia Virus

The poxviruses, large DNA-containing viruses, also have lipid envelopes. In fact, they may have two lipid-containing envelopes. The structures of poxviruses belonging to two different genera, *Orthopox* and *Parapox,* are illustrated in Fig. 2.24. Electron micrographs of the orthopox virus vaccinia virus and of a parapox virus are also shown.

Vaccinia virus has been described as brick shaped. The interior of the virion consists of a nucleoprotein core and two (in vertebrate viruses) proteinaceous lateral bodies. Surrounding these is a lipid-containing surface membrane, outside of which are several virus-encoded proteins present in structures referred to as tubules. This particle is called an intracellular infectious virion. As its name implies, it is present inside an infected cell, and if freed from the cell it is infectious. A second form of the virion is found outside the cell and is called an extracellular enveloped virion. This second form has a second, external lipid-containing envelope with which is associated five additional vaccinia proteins. This form of the virion is also infectious.

Parapox virions are similar to orthopox virions. However, their morphology is detectably different, as illustrated in Fig. 2.24.

ASSEMBLY OF VIRIONS

Self Assembly

Virions self-assemble within the infected cell. In most cases, assembly appears to begin with the interaction of one or more of the structural proteins with an encapsidation signal in the viral genome, which ensures that viral genomes are preferentially packaged. After initiation, encapsidation continues by recruitment of additional structural protein molecules until the complete helical or icosahedral structure has been assembled. Thus, packaging of the viral genome is coincident with assembly of the virion, or of the nucleocapsid in the case of enveloped viruses. The requirement for a packaging signal may not be absolute. In many viruses that contain an encapsidation signal, RNAs or DNAs lacking

such a signal may be encapsidated, but with much lower efficiency. For some viruses, there is no evidence for an encapsidation signal.

Assembly of the TMV rod (Fig. 2.2) has been well studied. Several coat protein molecules, perhaps in the form of a disk, bind to a specific nucleation site within TMV RNA to initiate encapsidation. Once the nucleation event occurs, additional protein subunits are recruited into the structure and assembly proceeds in both directions until the RNA is completely encapsidated. The length of the virion is thus determined by the size of the RNA.

The assembly of the icosahedral turnip crinkle virion has also been well studied. Assembly of this T=3 structure is initiated by formation of a stable complex that consists of six capsid protein molecules bound to a specific encapsidation signal in the viral RNA. Additional capsid protein dimers are then recruited into the complex until the structure is complete.

It is probable that most other viruses assemble in a manner similar to these two well-studied examples. At least some viruses deviate from this model, however, and assemble an empty particle into which the viral genome is later recruited. It is also known that many viruses will assemble empty particles if the structural proteins are expressed in large amounts in the absence of viral genomes, even if assembly is normally coincident with encapsidation of the viral genome in infected cells.

Enveloped Viruses

The nucleocapsids of most enveloped viruses form within the cell by pathways assumed to be similar to those described above. They can often be isolated from infected cells, and for many viruses the assembly of nucleocapsids does not require viral budding or even the expression of viral surface glycoproteins. After assembly, the nucleocapsids bud through a cellular membrane, which contains viral glycoproteins, to acquire their envelope. Budding retroviruses were illustrated in Fig. 2.21 and budding rhabdoviruses in Fig. 2.23. A gallery of budding viruses belonging to other families is shown in Fig. 2.25. The membrane chosen for budding depends on the virus and depends, in part if not entirely, on the membrane to which the viral glycoproteins are directed by signals within those glycoproteins. Many viruses bud through the cell plasma membrane (Figs. 2.25B–F); in polarized cells, only one side of the cell may be used. Other viruses, such as the coronaviruses and the bunyaviruses, use the endoplasmic reticulum or other internal membranes. The herpesviruses replicate in the nucleus and the nucleocapsid assembles in the nucleus; in this case, the first budding event is through the nuclear membrane (Fig. 2.25A).

Although the nucleocapsid of most enveloped viruses assembles independently within the cell and then buds to acquire an envelope, exceptions are known. The example of retroviruses, some of which assemble a nucleocapsid during virus budding, was discussed earlier. In these viruses, morphogenesis is a coordinated event.

The forces that result in virus budding are not well understood for most enveloped viruses. In the case of the alphaviruses, there is evidence for specific interactions between the cytoplasmic domains of the glycoproteins and binding sites on the nucleocapsid proteins. The model for budding of these viruses is that the nucleocapsid first binds to one or a few glycoprotein heterodimers at the plasma membrane. By a process of lateral diffusion, additional glycoprotein heterodimers move in and are bound until a full complement is achieved and the virus is now outside the cell. Additional free energy for budding is furnished by lateral interactions between the glycoproteins, which form a contiguous layer on the surface (Fig. 2.18C). This model accounts for the regularity of the virion, the one-to-one ratio of the structural proteins in the virion, and the requirement of the virus for its own glycoproteins in order to bud.

In other enveloped viruses, however, there is little evidence for nucleocapsid–glycoprotein interactions. The protein composition of the virion is usually not fixed, but can vary within limits. In fact, glycoproteins from unrelated viruses can often be substituted. In the extreme case of retroviruses, noninfectious virus particles will form that are completely devoid of glycoprotein. The matrix proteins appear to play a key role in the budding process, as do other protein–protein interactions that are yet to be determined.

Maturation Cleavages in Viral Structural Proteins

For most animal viruses, there are one or more cleavages in structural protein precursors during assembly of virions that are required to activate the infectivity of the virion. Interestingly, these cleavages may either stabilize or destabilize the virion in the extracellular environment, depending on the virus. Many of these cleavages are effected by viral proteases, whereas others are performed by cellular proteases present in subcellular organelles. Virions are formed by the spontaneous assembly of components in the infected cell, sometimes with the aid of assembly factors (“scaffolds”) that do not form components of the mature virion. For most nonenveloped viruses, complete assembly occurs within the cell cytoplasm or nucleoplasm. For enveloped viruses, final assembly of the virus occurs by budding through a cellular membrane. In either case, the virion must subsequently disassemble spontaneously on infection of a new cell. The cleavages that occur during assembly of the virus potentiate penetration of a susceptible cell after binding of the virus to it, and the subsequent disassembly of the virion on entry into the cell. A few examples will be described that illustrate the range of cleavage events that occur in different virus families.

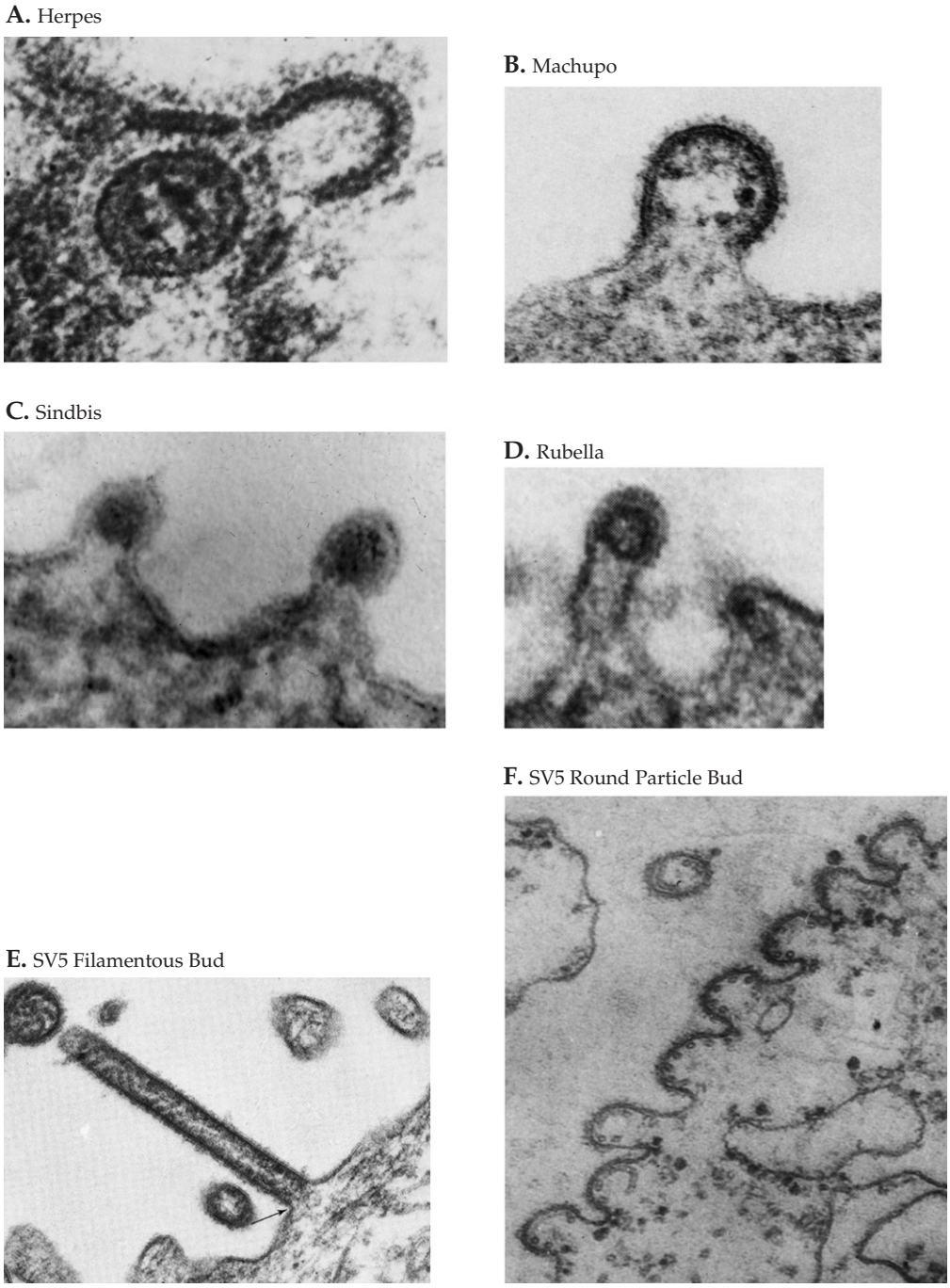


FIGURE 2.25 Gallery of budding figures of viruses representing several different families. (A) Thin section of a herpes simplex virion (*Herpesviridae*) in an infected Hep-2 cell. The particle is apparently coated with an inner envelope, and is in the process of acquiring its outer envelope from the nuclear membrane. From Roizman (1969). (B) Machupo virus (*Arenaviridae*) budding from a Raji cell. From Murphy *et al*. (1969). (Magnification: 120,000×). (C) Sindbis virus (*Togaviridae*) budding from the plasma membrane of an infected chicken cell. From Strauss *et al*. (1995). (160,000×). (D) Rubella virions (*Togaviridae*) budding from the surface of a BHK cell. From Higashi *et al*. (1976). (190,000×). (E) A portion of the cell surface with SV5 filaments (*Paramyxoviridae*) in the process of budding. From Compans and Choppin (1973). (45,000×). (F) A row of SV5 virions budding from the surface of a monkey kidney cell. Cross sections of the nucleocapsid can be seen within several of the particles. From Compans *et al*. (1966). (87,000×).

In the picornaviruses, a provirion is first formed that is composed of the viral RNA complexed with three viral proteins, called VP0, VP1, and VP3. During maturation to form the virion, VP0 is cleaved to VP2 and VP4. No protease has been found that performs this cleavage, and it has been postulated that the virion RNA may catalyze it. Cleavage to produce VP4, which is found within the interior of the capsid shell, as illustrated schematically in Fig. 2.9, is required for the virus to be infectious. As described in Chapter 1, VP4 appears to be required for entry of the virus into the cell. This maturation cleavage has another important consequence. Whereas the provirion is quite unstable, the mature virion is very stable. The poliovirus virion will survive treatment with proteolytic enzymes and detergents, and survives exposure to the acidic pH of less than 2 that is present in the stomach. Only on binding to its receptor (Figs. 1.5 and 2.9) is poliovirus destabilized such that VP4 can be released for entry of the viral RNA into the host cell.

Similarly, the insect nodaviruses first assemble as a procapsid containing the RNA and 180 copies of a single protein species called α (44 kDa). Over a period of many hours, spontaneous cleavage of α occurs to form β (40 kDa) and γ (4 kDa). This cleavage is required for the particle to be infectious. These events in nodaviruses have been well studied because it has been possible to assemble particles *in vitro,* and the structures of both cleaved and uncleaved particles have been solved to atomic resolution.

Rotaviruses, which form a genus in the family *Reoviridae*, must be activated by cleavage with trypsin after release from an infected cell in order to be infectious. Trypsin is present in the gut, where the viruses replicate, and activation occurs normally during the infection cycle of the virus in animals. When the viruses are grown in cultured cells, however, trypsin must be supplied exogenously.

A different type of cleavage event occurs during assembly of retroviruses and adenoviruses, as well as of a number of other viruses. During assembly of retroviruses, the Gag and Gag–Pol precursor polyproteins are incorporated, together with the viral RNA, into a precursor nucleocapsid. These polyproteins must be cleaved into several pieces by a protease present in the polyprotein if the virus is to be infectious. These cleavages often visibly alter the structure of the particle as seen in the electron microscope (Fig. 2.21). An analogous situation occurs in adenoviruses, where a viral protease processes a protein precursor in the core of the immature virion.

In most enveloped viruses, one of the envelope proteins is produced as a precursor whose cleavage is required to activate the infectivity of the virus. This cleavage may occur prior to budding, catalyzed by a host enzyme called furin, or may occur after release of the virus, catalyzed by other host enzymes. The example of the hemagglutinin of influenza virus was described in Chapter 1. This protein is produced as a precursor called HA0, which is cleaved to HA1 and HA2 (Fig. 1.6). Cleavage is required to potentiate the fusion activity present at the N terminus of HA2. As a second example, alphaviruses produce two envelope glycoproteins that form a heterodimer and one of the glycoproteins is produced as a precursor, as described before. The heterodimer containing the uncleaved precursor is quite stable, so that a particle containing uncleaved heterodimer is not infectious. The cleaved heterodimer, which is required for virus entry, is much less stable and dissociates readily during infection. Thus, in contrast to the poliovirus maturation cleavage, the alphavirus cleavage makes the virion less stable rather than more stable. Maturation cleavages also occur in the envelope glycoproteins of retroviruses, paramyxoviruses, flaviviruses, and coronaviruses.

Neutralization of Charge on the Virion Genome

DNA or RNA has a high net negative charge, and there is a need for counterions to neutralize this charge in order to form a virion. In many viruses, positively charged polymers are incorporated that neutralize half or so of the nucleic acid charge. The DNA in the virions of the polyomaviruses is complexed with cellular histones. The viral genomes in these viruses have been referred to as minichromosomes. In contrast, the adenoviruses encode their own basic proteins that complex with the genome in the core of the virion. Another strategy is used by the herpesviruses, which incorporate polyamines into the virion. Herpes simplex virus has been estimated to incorporate 70,000 molecules of spermidine and 40,000 molecules of spermine, which would be sufficient to neutralize about 40% of the DNA charge. Among RNA viruses, the nucleocapsid proteins are often quite basic and neutralize part of the charge on the RNA. As one example, the N-terminal 110 amino acids of the capsid protein of Sindbis virus have a net positive charge of 29. The positive charges within this domain of the 240 capsid proteins in a nucleocapsid would be sufficient to neutralize about 60% of the charge on the RNA genome. This charged domain is thought to penetrate into the interior of the nucleocapsid and complex with the viral RNA.

STABILITY OF VIRIONS

Virions differ greatly in stability, and these differences are often correlated with the means by which viruses infect new hosts. Viruses that must persist in the extracellular environment for considerable periods, for example, must be more stable than viruses that pass quickly from one host to the next. As an example of such requirements, consider the closely related polioviruses and rhinoviruses, members of two different genera of the family *Picornaviridae*. These viruses shared a common ancestor in the not too distant

past and have structures that are very similar. The polioviruses are spread by an oral–fecal route and have the ability to persist in a hostile extracellular environment for some time where they may contaminate drinking water or food. Furthermore, they must pass through the stomach, where the pH is less than 2, to reach the intestinal tract where they begin the infection cycle. It is not surprising, therefore, that the poliovirion is stable to storage and to treatments such as exposure to mild detergents or to pH < 2. In contrast, rhinoviruses are spread by aerosols or contaminated mucus, and spread normally requires close contact. The rhinovirion is less stable than the poliovirion. It survives for only a limited period of time in the external environment and is sensitive to treatment with detergents or exposure to pH 3.

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