Chapter 2

Virus Structure

Chapter Outline

Viruses are a kind of "nanoparticle" existing in nature. As described in the previous chapter, the viruses in diverse families differ from each other in their morphology. Nonetheless, the virus particles are built on common underlying principles. This chapter will describe the principles of virus particle structure. In addition, modern technologies used for the structural studies of virus particles will be described: electron microscopy (EM), cryo-EM, and X-ray crystallography.

2.1 TERMINOLOGIES

Several terminologies frequently used for description of the virus particles are listed in Table 2.1. First, the viral proteins that constitute the virus particles are called "structural protein," while the viral proteins that are absent in virus particles are called "nonstructural protein." For instance, the proteins that serve as building block of the viral capsid are classified as structural proteins. In particular, "nucleocapsid"[1] is used to refer a viral capsid that is associated with the viral genome. In addition, the envelope proteins are also structural proteins constituting the viral particles. On the other hand, not all virus-coded proteins are found in the virus particles. For instance, many virus-coded enzymes such DNA/RNA polymerase and proteases are frequently not found in the virus particle. These viral proteins are classified as nonstructural proteins, as they do not constitute the virus particle.

2.2 ENVELOPE AND CAPSID

Virus particles can either "enveloped" or "nonenveloped," depending on the presence or absence of the envelope. For instance, poliovirus and norovirus particles do not have an envelope, while influenza virus and yellow fever virus particles do have an envelope (Fig. 2.1). The envelope is composed of a lipid bilayer, which is derived from cell membranes, and virally coded envelope proteins (Fig. 2.2). In addition, in many enveloped particles there is a viral protein, termed matrix protein, that coats the inner leaf of the lipid bilayer. The matrix proteins are involved in the virion assembly of enveloped virus particles. A virus particle that does not have an envelope is aptly called a "naked virus," which hints at the lack of a coat. Inside the envelope, capsid is found that is the protein shell that encloses the viral genome and any other components necessary for to virus structure or function. Importantly, the capsid does play a protective role, sequestering the genome material from physical and chemical damaging agents. In fact, two kinds of capsid structures are found: a spherical capsid and a helical capsid, which will be detailed below.

1. **Nucleocapsid** It refers to a viral capsid that is associated with the viral genome.

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TABLE 2.1 Terminologies Used for Description of the Viral Structures

Term	Description
Structural protein	Viral proteins that make up the viral particles
Nonstructural protein	Viral proteins that is absent in the viral particles
Subunit	Basic assembly unit of viral capsids
Capsid	The protein shell that encloses and protects the nucleic acid genome of a virus
Nucleocapsid	A viral capsid and its associated genome
Envelope	Lipid bilayer that surrounds many type of viruses
Virion	A completely assembled virus particle outside its host cell
Subviral particle	A kind of incompletely assembled virus particle

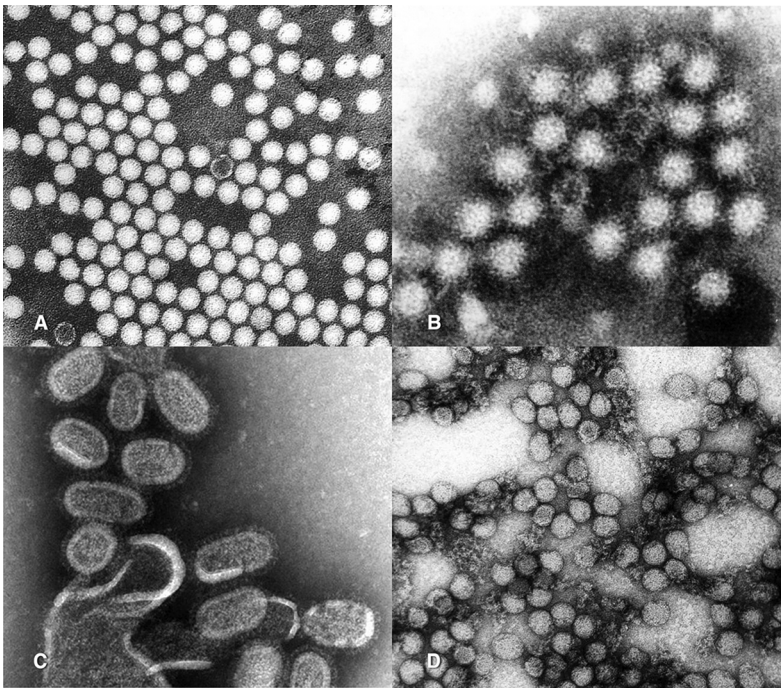


FIGURE 2.1 Electron microscopic views of the major human pathogenic viruses. Transmission electron micrographs of poliovirus (~30 nm diameter) (A), norovirus (27–38 nm in diameter) (B), influenza virus (80–120 nm diameter) (C), and yellow fever virus (D). Poliovirus and norovirus particles are naked (nonenveloped) particles, while influenza and yellow fever virus particles are enveloped particles.

2.3 CAPSID STRUCTURE

Spherical capsids possess an icosahedral structure, while helical capsids possess an elongated capsid structure. Capsids are constituted by numerous building blocks (ie, subunits) in that each subunit is made of one or a few molecules of structural proteins. Although each individual structural protein is not symmetrical, the capsids made of numerous structural proteins are symmetrical. A question raised was "how can a symmetrical structure be made by using unsymmetrical building blocks?" A short answer to this question is to implement the principle of "subunit assembly," by arranging unsymmetrical building blocks in a symmetric manner, which can be either icosahedral symmetry or helical symmetry.

Then, what is the advantage of subunit assembly in the capsid assembly? First, the subunit assembly enables the building of the capsids spontaneously without energy expenditure (ie, self-assembly). Numerous repetitive interactions between subunits can readily derive the capsid assembly, if the capsids are made by only one or a few kinds of subunit.

Second, the subunit assembly minimizes the genome expenditure for coding structural proteins, because only a few genes are required. Third, the subunit assembly allows to construct a more stable capsid, because far more molecular interactions than otherwise are involved in the capsid assembly.

Viruses have adopted "subunit assembly" as a strategy to build robust capsid structures. Conversely, the capsid needs to be dismantled eventually upon infection. In other words, the capsid is not a static shell; rather, it is a dynamic structure comprised of flexible subunits.

Let's consider two symmetrical structures: helical capsids and icosahedral capsids.

Helical capsids: The simplest way to build a symmetric structure is to arrange the building blocks around a circle. Ultimately, a tube-shaped structure can be made by putting multiple layers on the top of the circle. This is the way in which a helical capsid can be built by using unsymmetrical building blocks. The best example of this kind of capsid structure is that of TMV (Fig. 2.3). Cooperative binding of capsid subunits to the helical RNA results in a helical nucleocapsid. Notably, the capsid of TMV assembles spontaneously in vitro without energy expenditure. In addition to TMV, a few animal viruses possess helical nucleocapsid structure as well, including influenza virus, rhabdovirus, and Ebola virus.

Icosahedral capsids: Another way to build a symmetric structure is to construct a three dimensional (3D) symmetric structure. In theory, only five kinds of 3D symmetric structure are possible: ones with 4, 6, 8, 12, and 20 facets. In order to build a 3D symmetric structure with a minimal number of subunits, three subunits can be arranged in a triangular facet. In other words, 24 subunits are needed to build an octahedral structure, while 60 subunits are needed to build an icosahedral structure. Consistent with this theory, electron microscopic examination of viral capsids revealed that the vast majority of spherical vial capsids possess a capsid structure of 20 facets, an icosahedral structure (Fig. 2.4).

Then, one wonders about the advantages of having an icosahedral symmetrical structure. Compared to other symmetrical structures, an icosahedral structure is the symmetric structure that can be built using the minimal kinds and the maximal numbers of subunits. An icosahedral structure is the most stable structure that can be built by multiple subunits

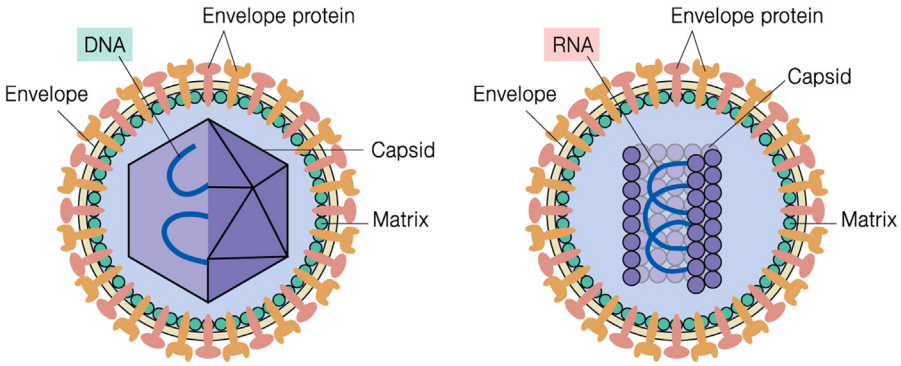


FIGURE 2.2 Schematic diagram of virus particles. (A) A typical envelope virus particle with a spherical capsid. The left side of the capsid is uncovered to show the viral genome inside (eg, DNA). (B) A typical envelope virus particle with a helical capsid. A part of the nucleocapsid is uncoated to show the viral genome inside (eg, RNA). Three kinds of virus structural proteins are denoted: envelope glycoproteins, capsid protein, and matrix protein. The viral structural protein that cover the inner leaf of viral envelope is often referred as matrix protein.

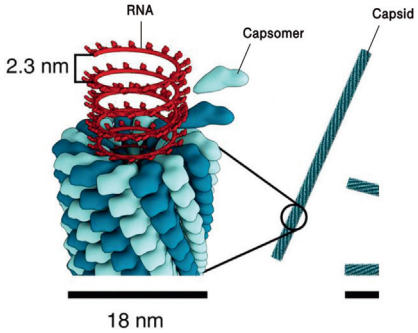


FIGURE 2.3 The helical capsid structure of TMV. The helical structure is made by the cooperative binding of the subunits protein (ie, capsomer) to the RNA genome of TMV. Note that all subunits bind to the RNA in equivalent manner.

(Fig. 2.5). Moreover, the use of a few genes to code for structural proteins is a strategy to minimize the genome expenditure.

How can the subunits be put together to build icosahedral capsid? In fact, the majority of viral capsids are composed by the multiplicity of 60 subunits. The simplest icosahedral structure is one with three subunits in each facet, resulting in 60 subunits (Fig. 2.6A). To build a more complex structure, each facet of an icosahedral structure can be divided into four small triangles, in which three subunits in each triangle are positioned. As such, 240 subunits (12 subunits × 20 facets) can be

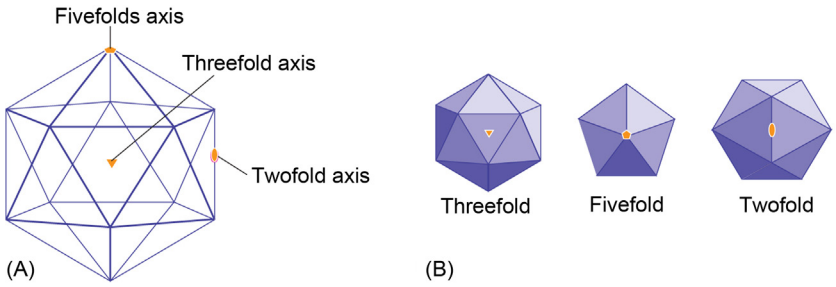


FIGURE 2.4 Schematic diagram of icosahedral structure. (A) An icosahedral structure is composed of 20 triangular facets and 12 vertices. (B) An icosahedral structure has a threefold axis of symmetry in the middle of facet, fivefold axis of symmetry at vertices, and twofold axis of symmetry.

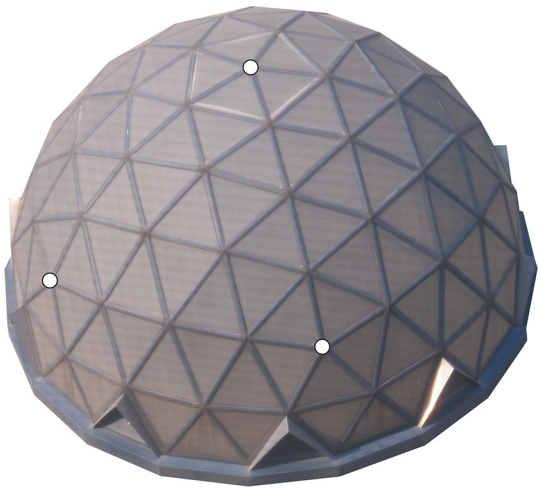


FIGURE 2.5 An icosahedral sculpture. An icosahedral sculpture is often used by architects. A dome structure built in roof of a building at Yonsei University in Korea. A triangular facet is evident by linking three fivefold axes of symmetry (the white dot).

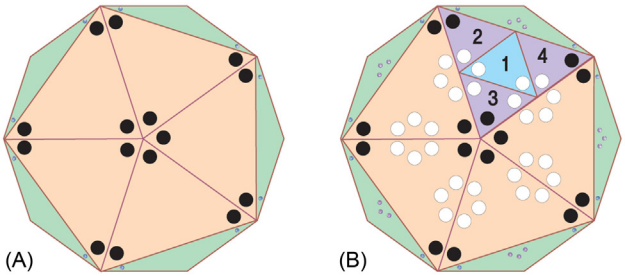
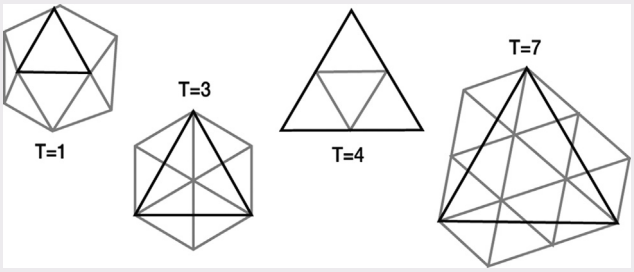


FIGURE 2.6 The arrangement of subunits in icosahedral structures. (A) An icosahedral structure with T number of 1. The structure is made of 60 subunits, having 20 triangular facets (3 subunits per facet). (B) An icosahedral structure with T number of 4. The structure is made of 240 subunits, having 20 facets (12 subunits per facet). In particular, 5 subunits (black dot) constitute the fivefold axis of symmetry (12 vertices), whereas 6 subunits (white dot) constitute newly formed vertices. Although all subunits appear to be equally arranged, some of them are arranged as a pentagon, while others are arranged as a hexagon. In other words, a subunit is said to be "quasi-equivalent" (Box 2.1).

BOX 2.1 Triangulation Number

An icosahedral symmetrical structure constitutes 20 equilateral triangle facets, each of which can be made of 3 subunits. In other words, 3 asymmetric subunits (ASU) can make up the facet. Thus, an icosahedral capsid could be made by at least 60 subunits. In reality, most viral capsids are composed of more than 60 subunits. To account for the principle of viral capsid assembly, Drs Casper and Klug proposed "Quasi-equivalence theory." The theory states that, in the case where the capsids are composed of more than 60 subunits, each subunit is not topologically equivalent; however, if they are considered to be equivalent, they can be assembled into an icosahedral structure under the same principle. According to this theory, each facet (ie, triangle) is constituted by an integer number of triangles, in which the number is dubbed "triangulation (T) number." Furthermore, T number can be obtained by an equation: $T = H^2 + HK + K^2$, where H and K are the position of a fivefold axis of symmetry in vector space.

On the other hand, the numbers of subunits that constitute the capsid are calculated by the following equation: the subunit number $= 12 \times 5 + 10(T - 1) \times 6 = 60$ T. For instance, a capsid having $T = 4$ symmetry is composed of 240 subunits. T number can be obtained by high resolution EM image of capsids. Therefore, the number of subunits that make up a capsid from an EM image of the capsid can be estimated without biochemical analysis.



T numbers of icosahedral structures. The bold lines, linking the fivefold axis of symmetry of each structure, make up one triangle (facet). For T = 4, 4 subunits make up the facet. For T = 7, it is considered that seven subunits make up a facet, although it is not evident in the diagram shown here.

TABLE 2.2 T Number of the Major Animal Viruses

Virus Species	Family	T Number	Subunit Copies
Parvovirus	Parvovirus	1	60
Poliovirus, Rhinovirus	Picornavirus	3	180
Hepatitis B virus	Hepadnavirus	4	240
SV40	Polyomavirus	7	420
Reovirus	Reovirus	13	780
HSV-1	Herpesvirus	16	960
Adenovirus type 2	Adenovirus	25	1500

arranged (Fig. 2.6B). Likewise, an icosahedral structure having an increasing number of subunits can be made. *Triangulation number*[2] (T), a parameter that pertains to the topological features, can estimate the precise number of subunits constituting an icosahedral structure (Box 2.1). T numbers of the majority of animal viruses are known (Table 2.2). In general, the bigger the capsids are, the higher T numbers are. T numbers can be acquired from a high-resolution electron microscope image of a viral capsid structure.

2. **Triangulation number** It refers to the number of subunits that constitute a triangular facet of icosahedral capsid.

2.4 ROLES OF VIRUS STRUCTURE

What are the roles of virus structure? First, one of the most important roles of virus structure is a protective role. The capsid structure protects the viral genome from physicochemical damage, such as nucleases, and radiation (eg, ultraviolet). Second, the role of the virus structure is to recognize the cellular receptor for the entry. Specifically, one of the viral structural proteins (either an envelope glycoprotein for the enveloped viruses or a capsid protein for the nonenveloped viruses) directly binds to the cellular receptor, for the viral entry. Third, the viral capsids play a role in delivering the viral genome to the site of genome replication. For instance, for the viruses that replicate in the nucleus, the viral capsids play a critical role in the nuclear entry of the viral genomes (see Fig. 3.7).

2.5 TOOLS USED FOR VIRUS STRUCTURE RESEARCH

As stated above, virus particles represent a "nanoparticle" existing in nature, mostly ranging from 30 to 300 nm in size, which can be viewed only by an electron microscope. Thus, optical instruments, such as the electron microscope, are essential to examine the morphology of virus particles. Three kinds of modern technologies that are utilized for the visualization of virus particles will be briefly described: electron microscopy (EM), cryo-electron microscopy, and X-ray crystallography.

2.5.1 Electron Microscopy

The electron microscope was invented in the early 1930s to overcome the limitations of light microscopes. It was originally invented to view nonbiological materials such as metals. Light microscopes at that time could magnify specimens as high as 1000 times. However, instead of light rays, electron microscopes use a beam of electrons focused by magnets to resolve minute structures (Fig. 2.7). With electron microscopy (EM), it is possible to magnify a structure 100,000 times. Two kinds of electron microscopic technologies have been developed: transmission electron microscopy (*TEM*) and scanning electron microscopy (*SEM*). TEM is a microscopic technique in which a beam of electrons is transmitted

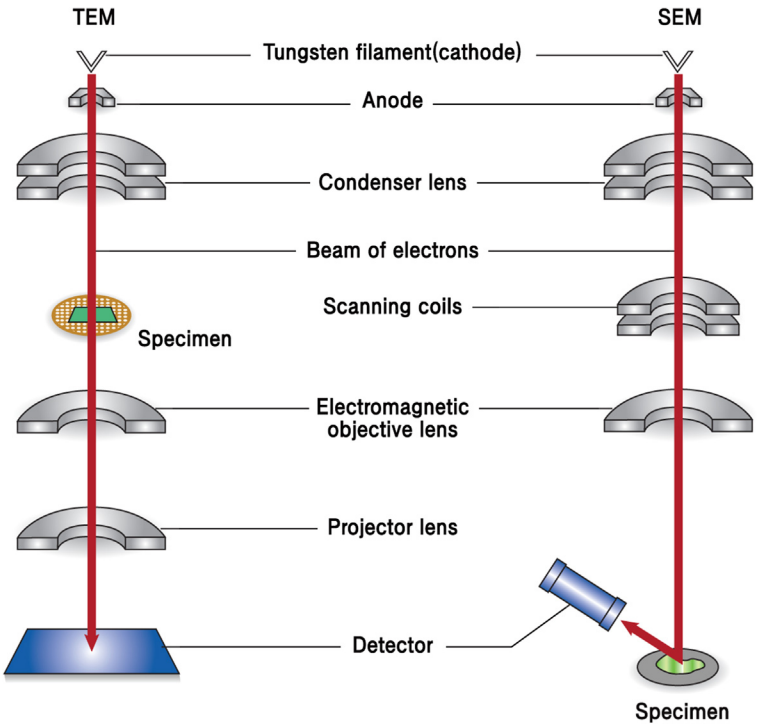


FIGURE 2.7 Principles of electron microscope. The principle of TEM and SEM is illustrated for comparison. Electron beam lines are indicated by red line. TEM generates the image by electrons that transmit the specimen, while SEM generates the image by electrons that diffract from the specimen.

through an ultra-thin specimen, interacting with it as the beam passes through. TEM relies on negative staining of purified virus particles with an electron-dense material, such as uranyl acetate or phosphotungstate. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto a layer of photographic film (Fig. 2.8). What can be learned from TEM images of virus particles? TEM reveals the overall morphology (whether viruses are spherical or elongated particles and whether they are naked or enveloped). In addition, even the symmetric parameter (ie, T number) of an icosahedral capsid could be obtained from a high resolution image.

On the other hand, SEM is a type of electron microscopic technique that produces images of a sample by scanning it with a focused beam of electrons (see Fig. 2.7). The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the topography and composition of the sample's surface. As you can see in Fig. 2.8, more realistic 3D images are obtained.

2.5.2 Cryo-Electron Microscopy

Cryo-electron microscopy (Cryo-EM) is a kind of TEM where the sample is examined at cryogenic temperatures (generally lower than $-160^{\circ}C$). A feature of cryo-EM is the ability to freeze the specimen rapidly so that water molecules in the specimen turn into transparent ice crystals. The ice crystal of the solvent transforms the biomolecules (ie, virus particle) into a rigid state so that a high resolution image can be acquired. Numerous images are captured from the specimen on grid that is kept frozen. Then, such acquired images are digitally processed to reconstruct a high resolution image (Fig. 2.9). One outstanding feature of cryo-EM is that it examines the specimen in its intact state without artificial treatments such as fixing, dehydration, and staining. In other words, the cryo-EM images are more likely to represent the native state of biological structures. More importantly, the analysis of cryo-EM image yields even interior structure of capsids as well as exterior structure (see Fig. 2.9).

What kinds of objects (ie, viral particles) are suitable for the analysis with cryo-EM? Primarily, the symmetry of the particles is an important element to acquire a high resolution image. In addition, the rigidity and conformational homogeneity of the objects are also important for obtaining a high resolution. Notably, the resolution of cryo-EM images has been greatly improved by recent advances in image reconstruction technology. For instance, some virus structures

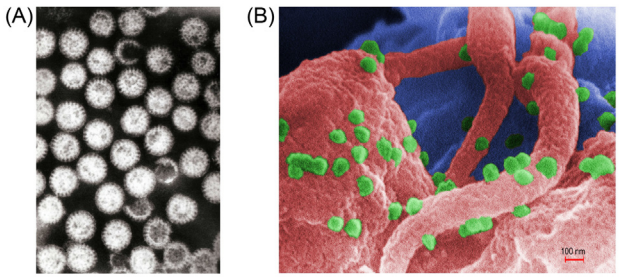


FIGURE 2.8 Electron microscopic image of virus particles. (A) Transmission electron micrograph of rotavirus particles (~70 nm in diameter). (B) Scanning electron microscope image of HIV particles (green) budding from the infected T lymphocytes (pink and blue).

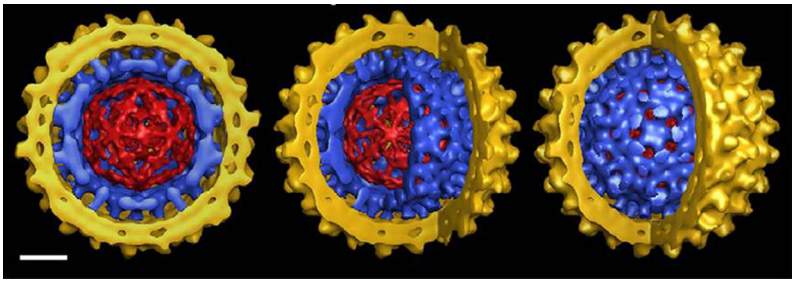


FIGURE 2.9 High-resolution image obtained by cryo-electron microscope. Cross-section and two cut away views of a hepatitis B virus (HBV) virion was obtained by cryo-electron microscope. HBV virion is comprised of a T = 4 icosahedral capsid (blue) with 120 spikes and an outer envelope with projections of envelope glycoproteins (yellow). The image analysis of the capsid shell yields an interior dodecahedral cage of density, which is ascribed to ordered mature double-strand DNA.

obtained by cryo-EM are already at a resolution that can be interpreted in terms of an atomic model. For instance, a high resolution (~3.5 angstrom) image of adenovirus particles, which provides sufficient resolution to trace polypeptide chains in the capsid structure, was recently achieved by cryo-EM. It should be noted that the structural analysis of adenovirus particles represents a formidable challenge, since it has a gigantic capsid particle having a 150-megadalton capsid containing nearly 1 million amino acid residues (ie, equivalent to having over 3000 molecules of a protein composed of 300 amino acids residues). Now, it can be said that cryo-EM rivals X-ray crystallography, when applied to large, homogenous, and highly symmetric objects.

2.5.3 X-ray Crystallography

X-ray crystallography is a tool used for determining the atomic and molecular structure of a crystal. The underlying principle is that the crystalline atoms cause a beam of X-rays to diffract into many specific directions (Fig. 2.10). By measuring the angles and intensities of these diffracted beams, a crystallographer can produce a 3D picture of the density of electrons within the crystal. From this electron density image, the mean positions of the atoms in the crystal can be determined, as well as their chemical bonds, their disorder, and various other information. The method revealed the structure and function of many biological molecules, including vitamins, drugs, proteins, and nucleic acids, such as DNA. Note that the double helix structure of DNA discovered by James Watson and Francis Crick was revealed by X-ray crystallography. Recent advances in image reconstruction technology have made X-ray crystallography amenable to the structural analysis of much larger complexes, such as virus particles (Fig. 2.11). The major shortcoming of X-ray crystallography is that it is difficult to obtain a crystal of virus particles, which is a prerequisite for X-ray crystallography. Another shortcoming is that X-ray crystallography generally requires placing the samples in nonphysiological environments, which can occasionally lead to functionally irrelevant conformational changes.

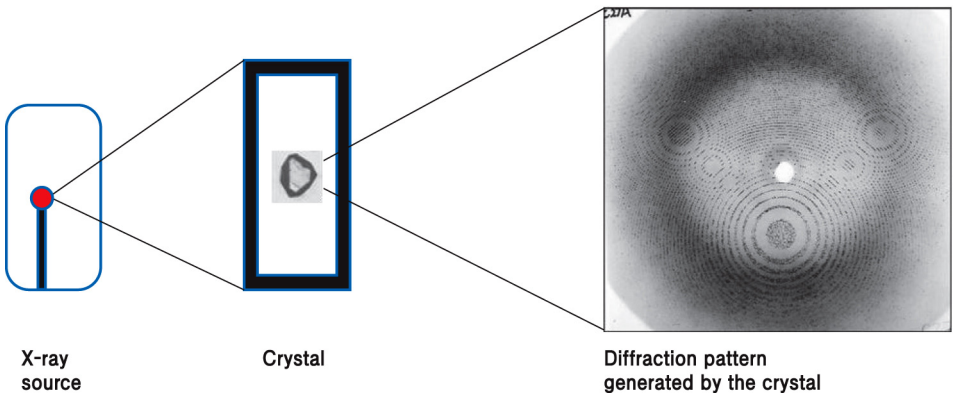


FIGURE 2.10 Principle of X-ray crystallography. High-resolution capsid structure can be obtained from the diffraction pattern generated by crystals of the virus particles (eg, human rhinovirus 14).

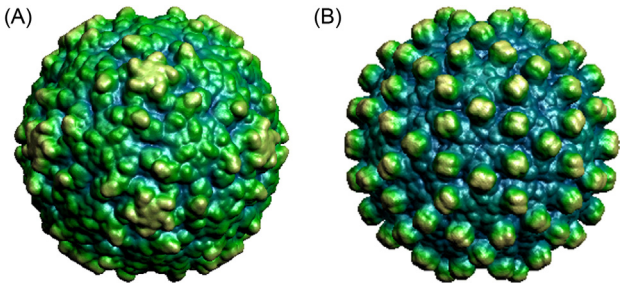


FIGURE 2.11 Viral capsid structure obtained by X-ray crystallography. (A) Poliovirus capsid with T = 3 symmetry. (B) Hepatitis B virus capsid with T = 4 symmetry (http://viperdb.scripps.edu/).

2.6 PERSPECTIVES

The study of viruses involves the microscopic examination and the structural analysis of the virus particles. In fact, the structural analysis of virus particles played an important role in the early advances in virus research and contributed to the establishment of "virology" as a discipline. The crystal structure of TMV had been resolved in 1935, when virology was still in its infancy. Electron microscopy was applied to virus particles soon after the first instrument was constructed in the early 1930s. Importantly, the electron microscope significantly transformed the science of virology. It provided a powerful approach for rapid diagnosis, and became an exquisitely sensitive tool to define new viral agents, especially when other methods failed. Recent advances in digital image processing have provoked the renaissance of "structural virology." The advances have allowed the structural analysis of larger virus particles, such as adenovirus. In particular, the advances in cryo-EM made the analysis of virus particles more feasible. Notably, not only the shell structure but also the structure of molecules present inside the capsid particles such as the viral genomes (or noncapsid viral proteins) has been obtained. It is expected that tools for structural virology will make a greater contribution to advance our knowledge on many aspect of virology, and eventually help us to design better strategies to combat the pathogens. Remember that "seeing is believing."

2.7 SUMMARY

- *Virus structure:* It is a kind of nanoparticle found in nature. It can be visualized only by an electron microscope. Virus particles are composed of the capsid that encompasses the virus genome and the viral envelope. The viral envelope is absent in some viruses, known as nonenveloped viruses.
- *Capsid structures:* Two kinds of the viral capsid structure are found: spherical icosahedral capsid and cylinder-shaped helical capsid. The icosahedral capsid has 20 facets, each of which represents a triangular shape.
- *T number:* T number defines the topological feature of the icosahedral structure. It indicates the number of subunits that constitute a triangular facet made of three fivefold axes of symmetry.
- *Tools for the virus structure analysis:* Three kinds of technologies that are utilized for the visualization of virus particles are the following: electron microscopy (EM), cryo-EM, and X-ray crystallography.

STUDY QUESTIONS

2.1 Describe the biological roles of the viral capsids in the virus life cycle.

2.2 Estimate the T number of the sculpture shown in Fig. 2.5. Estimate how many sheets of glass are needed to assemble the sculpture.

2.3 Describe two methods that are utilized to obtain a high resolution image of a virus particle structure. And compare the pros and cons of the two methods.

SUGGESTED READING

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  Highlight: Single particle cryo-EM showed that the RNA, which has never been observed by any other technology, is clearly visible both inside and outside the capsids. Further, the RNA being released from the capsid during uncoating was caught in the act of exiting. Advances in imaging technology have allowed acquiring the dynamic movement of subjects in action, as opposed to still images of the subjects acquired from fixed specimen.