Chapter 11

Picornavirus

Chapter Outline

Picornavirus represents the prototype of the positive-strand RNA viruses. In fact, many human pathogenic viruses belong to the family Picornaviridae. This chapter will focus on poliovirus, which is the prototype of the *picornavirus*[1] family (Table 11.1). Poliovirus is the etiologic agent of *poliomyelitis*,[2] an acute flaccid paralysis affecting 1−2% of infected individuals and, on rare occasions, causing death.

11.1 CLASSIFICATION OF PICORNAVIRUSES

Classification: Picornaviruses are associated with diseases of many organs including gastrointestinal tracts, respiratory tracts, neuronal tissue, and muscles. Depending on the tissue tropism, picornaviruses are divided into five genera (Table 11.2). For instance, one that infects gastrointestinal tracts is classified as *Enterovirus*[3] genus. Poliovirus belongs to genus *Enterovirus*. *Rhinovirus*, which causes common colds, also belongs to genus *Enterovirus* of Picornaviridae family. However, common colds caused by rhinovirus infection should not be confused with "flu" caused by influenza virus. The symptoms of the common cold are not as severe as "flu"; nonetheless it still represents a serious disease burden. In addition, *enterovirus 71* and *coxsackie virus*, which cause hand-foot and mouth disease (HFMD) in children, also belongs to genus *Enterovirus*. Finally, *EMCV* (encephalomyocarditis virus) and *hepatitis A virus* (HAV) also belongs to Picornaviridae family.

Animal picornaviruses cause significant veterinary disease in livestock as well. Foot-and-mouth disease virus (FMDV) causes a fatal disease in cows, pigs, sheep, and goats and FMDV epidemic draws significant concern. The FMDV outbreak occurred in 2010 in South Korea resulted in the loss of 3 million livestock, costing 3 billion USD.

Vaccine: Vaccines for poliovirus were developed in the 1950s by Jonas Salk and Albert Sabin, independently (see Box 25.2). Thanks to the poliovirus eradication campaign initiated by the WHO, poliovirus is now on the verge of eradication (see Fig. 25.2).

11.2 THE VIRION AND GENOME STRUCTURE OF POLIOVIRUS

Virus Particles: It is a naked virus having a diameter of only 30 nm (Fig. 11.1). The capsid is composed of 60 subunits (capsomers), each of which comprises three virion proteins (ie, VP1, VP2, and VP3), representing $T = 3$ symmetry. Inside the capsid, the viral RNA genome is enclosed.

1. **Picornavirus** The term "picornavirus" is derived from Latin word for "small"-*pico* + "*RNA*."

2. **Poliomyelitis** The term "poliomyelitis" is derived from Greek word for "gray"-*polio* + for "marrow"-*myelos* + "inflammation"-*titis*. The term implies paralytic poliomyelitis, resulted from destruction of motor neurons within the spinal cord in the CNS.

3. ***Enterovirus*** A genus of picornavirus family that infects gastrointestinal tracts.

Molecular Virology of Human Pathogenic Viruses. DOI: http://dx.doi.org/10.1016/B978-0-12-800838-6.00011-4

TABLE 11.1 The Defining Features of Picornavirus

Genome	Particle Structure	Replication Mechanism
Positive-strand RNA	Nonenveloped	RNA-directed RNA synthesis
VPg-linked genome	Icosahedral symmetry	VPg-primed RNA synthesis
Life cycle	**Host effect**	**Disease**
Cytoplasmic	Translation suppression	Poliomyelitis
IRES	Host shutoff	Common cold

TABLE 11.2 Classification of Picornavirus

Genus	Virus	Host	Tissue	Disease
Aphthovirus	Foot-and-mouth disease virus (FMDV)	Artiodactyla (cow, swine)	Epithelial cell	Foot-and-mouth disease
Cardiovirus	Encephalomyocarditis virus (EMCV)	Human	Heart, CNS	Encephalomyocarditis
Enterovirus	Poliovirus	Human	Gut	Poliomyelitis
	Coxsackie virus	Human	Gut	Hand-foot-mouth disease
	Coxsackie virus A24	Human	Conjunctiva	Conjunctivitis
	Enterovirus 71	Human	Gut	Hand-foot-mouth disease
	Enterovirus 70	Human	Conjunctiva	Conjunctivitis
	Rhinovirus	Human	Upper, lower airway tract	Colds, respiratory diseases
Hepatovirus	Hepatitis A virus (HAV)	Human	Liver	Hepatitis A
Senecavirus	Seneca valley virus	Swine	–	–

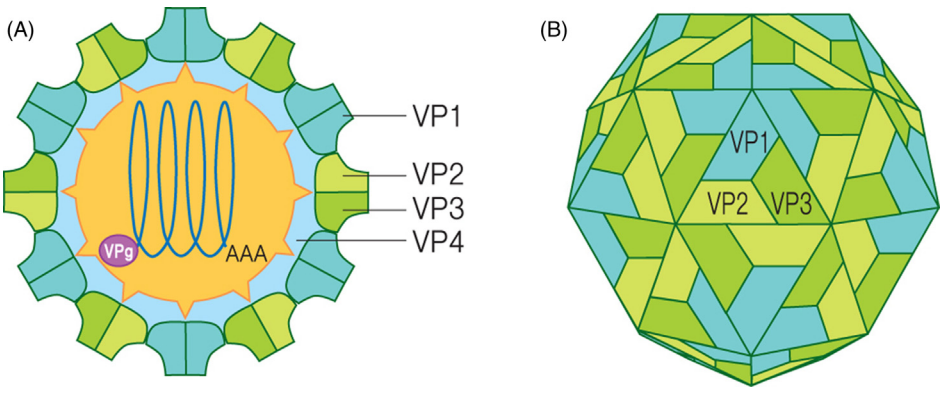


FIGURE 11.1 A diagram showing poliovirus particle. (A) Cross-section view of poliovirus capsid. The viral RNA genome having VPg linked at the 5′ end is packaged inside of the capsid. (B) Schematic diagram of icosahedral capsid structure of poliovirus capsid. VP1, VP2, and VP3, which constitute the assembly subunit, are denoted. VP4 does not contribute to the capsid symmetry.

Genome Structure: The poliovirus has a 7.5 kb single-strand RNA with positive polarity (Fig. 11.2). Notably, a viral protein, dubbed *VPg* (virion protein genome linked), is linked to the 5′ end of the RNA genome. The VPg is linked to the 5′ nucleotide residue (pU) via a tyrosine. On the other hand, the 3′ end of the RNA genome is polyadenylated. The virion RNA (vRNA) itself is "*infectious*," as it can lead to the progeny virus production, when transfected into cells (Box 11.1). Note that "infectivity" of the viral genomic RNA is a hallmark of positive-strand RNA viruses. VPg is

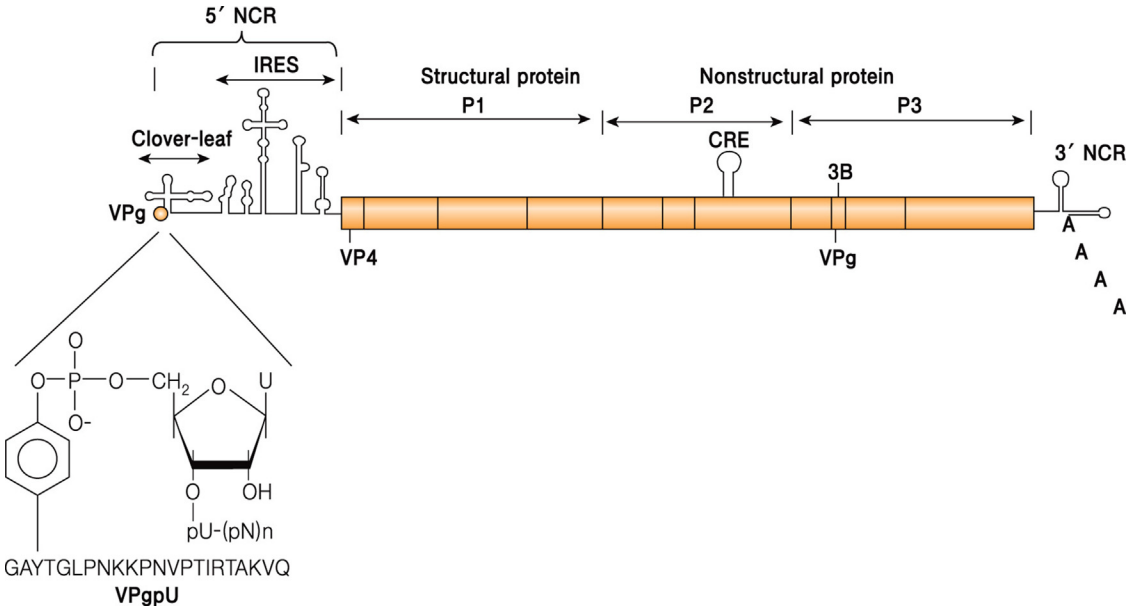
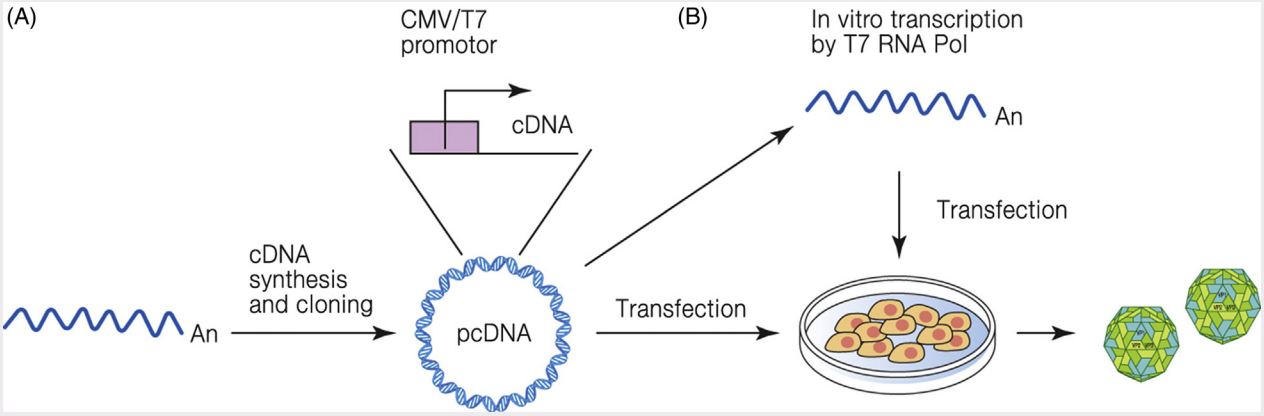


FIGURE 11.2 **RNA genome structure of poliovirus.** The viral RNA genome encodes one large ORF for the polyprotein. The 5′ end of the RNA genome is covalently linked to the viral protein (ie, VPg) and is expanded below for the detail. The amino acid compositions of the VPg peptide are shown. The 3′ end of the RNA genome has poly (A) tail. The 5′ NCR contains two *cis*-acting elements such as a clover-leaf structure and IRES. NCR, noncoding region; CRE, *cis*-acting replication element.

BOX 11.1 Molecular Tools for Studying Picornavirus

How can the RNA genome be manipulated for molecular studies? Advances in molecular biology allowed us to handle RNA as we handled DNA. First, the RNA genome is converted into cDNA, and then it is inserted into an appropriate plasmid vector for transcription. In doing so, one could generate a "replicon" construct, which can produce the progeny virus, when transfected into cells. In principle, the replicon contains all genetic information essential for viral genome replication. In case of picornavirus, the cDNA of the full-length viral RNA genome is inserted downstream of CMV/T7 promoter to construct a replicon. Transfection of animal cells with the replicon will induce transcription of viral genomic RNA from CMV promoter, which leads to production of the progeny virus. Alternatively, the viral genomic RNA can be synthesized in vitro using T7 RNA polymerase. In vitro synthesized RNAs can be used for transfection. Altogether, these molecular approaches that enable us to handle viral genome by molecular technique are termed "Molecular Virology."



A diagram illustrating molecular approaches in studying picornavirus genome replication. (A) cDNA synthesis: the cDNA is made by reverse transcription of viral RNA genome. The full-length cDNA is then inserted into an appropriate plasmid vector. (B) Transfection: Either transfection of the replicon plasmid itself or in vitro transcribed RNA could lead to production the progeny virus.

TABLE 11.3 Poliovirus Proteins

Precursor	Proteins	Enzyme Activity	Function
P1	VP1, VP2, VP3, VP4	–	Capsid
P2	$2A^{PRO}$	Protease	eIF4G cleavage
	2B	–	–
	2C	ATPase	RNA Synthesis
P3	3A	–	–
	3B/VPg	–	Protein-primer
	$3C^{PRO}(3CD^{PRO})$	Protease	RNA Synthesis
	$3D^{POL}$	RdRp	RNA Synthesis

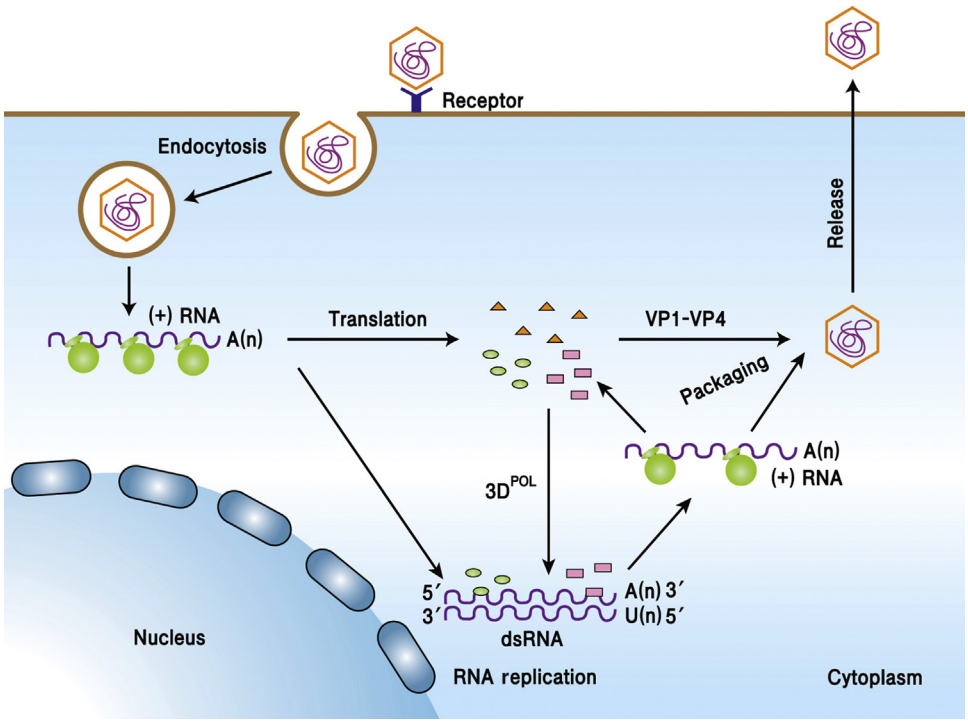


FIGURE 11.3 The life cycle of poliovirus. Poliovirus capsid enters the cell via receptor-mediated endocytosis. The viral genomic RNA itself is utilized as mRNA for translation upon entry. The negative-strand RNA is transiently made during viral genome replication, resulting in double-strand RNA intermediates. The positive-strand RNA is selectively packaged into nascent assembled viral capsid. Note that the life cycle of poliovirus is restricted to the cytoplasm.

dispensable for the infectivity of the RNA genome, whereas the poly (A) tail is indispensable for the infectivity. Few *cis*-acting elements essential for the viral genome replication are located in both 5′ and 3′ *NCR* (noncoding region) of the RNA genome. In particular, a clover-leaf structure and *IRES* (internal ribosome entry site) element at 5′ NCR are essential for the viral genome replication.

Protein Coding: The vRNA itself serves as mRNA, a feature that is a hallmark of positive-sense RNA viruses. The viral genomic RNA encodes one large protein, termed *polyprotein*.[4] Viral proteins are initially synthesized as a polyprotein that is cleaved by the viral protease into individual functional proteins (Table 11.3). The proteolytic processing occurs in two steps: first into three precursors, termed P1, P2, and P3 precursor proteins, and then into individual proteins.

4. **Polyprotein** It refers to a large protein that is later processed to multiple functional proteins.

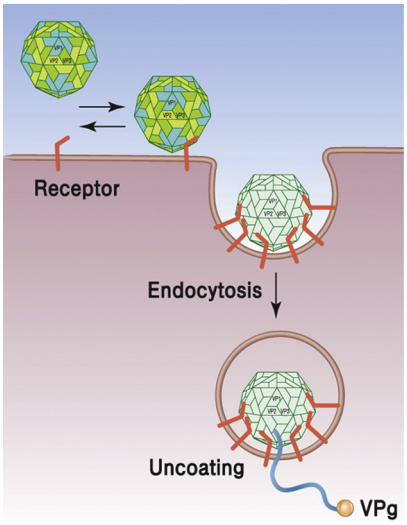


FIGURE 11.4 Steps involved in the entry of poliovirus. Engagement of the receptor (ie, CD155/Pvr) to the viral capsid triggers endocytosis. The native virion undergoes a receptor-mediated conformational transition, as highlighted by distinct color. In the endosome, the acidic pH causes the conformational changes of the viral capsid protein, in particular VP1, resulting in a channel formation such that the RNA genome can pass through. Via the uncoating process, the viral RNA genome penetrates to the cytoplasm. The viral genomic RNA is denoted in blue, while the VPg is denoted in orange.

TABLE 11.4 Cellular Receptors for Picornaviruses

Viruses	Genotype	Receptor	Protein Family
Poliovirus		Pvr or CD155	IgG superfamily
Human Rhinovirus	91	ICAM-1	IgG superfamily
Human Rhinovirus	10	LDL receptor	–
Coxsackie virus	3	ICAM	IgG superfamily
Coxsackie virus	B	CAR	IgG superfamily
EMCV	1	VCAM-1	IgG superfamily
Enterovirus 71	–	SCARB2, PSGL1	–

Cell Tropism: Poliovirus transmits via the fecal−oral route. One ingests the virus and viral replication occurs in the alimentary tract. Poliovirus targets epithelial cells in the intestines. The presence of CD155, which is an entry receptor of poliovirus, defines the animals and tissues that can be infected by poliovirus. CD155 is found only on the cells of humans, higher primates, and Old World monkeys. Poliovirus is however strictly a human pathogen, and does not naturally infect any other species.

11.3 THE LIFE CYCLE OF POLIOVIRUS

The life cycle of poliovirus is confined to the cytoplasm (Fig. 11.3).

Entry: The poliovirus enters the cells via receptor-mediated endocytosis using *CD155* (Pvr) as an entry receptor (Fig. 11.4). Many cellular receptors for the picornavirus family belong to immunoglobulin superfamily (Table 11.4). For instance, *ICAM-1* (intracellular adhesion molecule) molecule is a receptor for human rhinovirus genotype 91, while *LDL receptor* is a receptor for human rhinovirus genotype 10. Notably, coxsackie virus genotype B utilizes *CAR* (coxsackie virus adenovirus receptor) as a receptor, which is also used as a receptor for adenovirus (see Fig. 8.5). Note that cellular membrane proteins having an intrinsic function are co-opted for the viral entry. Upon the engagement on the receptor, the capsid penetrates into the cytoplasm, and becomes located inside endosomes. Upon an acidic pH (pH 5.5) of the endosomes, the capsid undergoes structural changes involving the exposure of the hydrophobic region of the

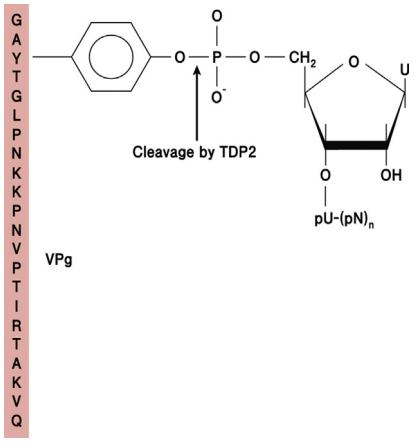


FIGURE 11.5 **Cleavage of VPg-RNA linkage.** Following entry, the VPg that is linked to the 5′ terminus of the polioviral RNA is removed by the cleavage catalyzed by cellular TDP2. The phosphodiester linkage between the tyrosine (Y) residue of VPg and the first U residue of the viral RNA is cleaved.

capsid protein, which is poised for interaction with the endosomal membrane (see Fig. 11.4). Consequently, a channel is formed in the capsid, from which the vRNA is released. Intriguingly, the vRNA exits from the capsid from its 5′ end. Note that the uncoating mechanism of the poliovirus is different from endosome lysis that is used by adenovirus or membrane fusion, a process that occurs for almost all enveloped viruses (see Fig. 3.3).

Following the release from the capsid, the VPg of the RNA genome is removed by so-called VPg unlinkage activity. For over three decades, the identity of this cellular activity responsible for the cleavage of the VPg-RNA linkage remained elusive. Recently, the VPg unlinkage activity was identified to be 5′-tyrosyl-DNA phosphodiesterase 2 (*TDP2*)[5] (Fig. 11.5). Intriguingly, the DNA repair enzyme is involved in the cleavage of the tyrosine-RNA linkage. Currently, the biological importance of the removal of VPg by TDP2 during a picornavirus infection is unclear. The removal of VPg by TDP2 may be required to stimulate efficient viral RNA replication by inhibiting premature vRNA packaging, because only VPg-linked RNA is encapsidated.

Following the removal of VPg from the 5′ end, the genomic RNA itself serves as mRNA. The clover-leaf structure at 5′ NCR is necessary and sufficient to protect the RNA from nucleases following the removal of VPg.

Translation: Translation in eukaryotic cells proceeds in a cap-dependent manner (Box 11.2). However, the RNA genome of picornavirus does not have a cap structure at the 5′ end. So a question arises, how is translation initiation of the picornavirus RNA accomplished? A short answer to this question is that ribosome recognizes the IRES element on the viral RNA and enters the RNA internally, as opposed to the 5′ end, for translation. This mode of translation initiation is termed "cap-independent translation." As the polyprotein is being translated, it is processed by viral $2A^{PRO}$ to yield P1 and P2 polypeptides, and then by $3C^{PRO}$ to yield multiple individual proteins (Fig. 11.6).

Genome Replication: The RNA genome replication of poliovirus follows a strategy of the positive-strand RNA viruses (see Fig. Part III-1). The negative-strand RNA is synthesized by using the vRNA as a template, and then the nascent negative-strand RNA serves as a template for the positive-strand RNA synthesis. The genomic RNA is a template for RNA genome replication as well as for translation. The mechanism that streamlines these apparently competitive processes has been described (Box 11.3).

Negative-Strand RNA Synthesis: One salient feature of picornavirus RNA genome replication is that the RNA synthesis is initiated by *protein-priming*[6] (Fig. 11.7). In other words, a protein (ie, VPg), rather than nucleotide, acts as a primer for RNA synthesis by $3D^{POL}$. Specifically, the hydroxyl group of a tyrosine residue of VPg peptide is linked to the first nucleotide UTP, a process dubbed *VPg-uridylylation*. As a result, VPg-linked oligonucleotide (ie, VPg-pUpUOH) is synthesized. Following the cleavage of the 3AB precursor to VPg by $3C^{PRO}$, the RNA synthesis continues to yield a full-length negative-strand RNA. It is notable that protein-priming occurs in association with the vesicular membrane, where the 3AB precursor is attached. The membrane-associated RNA genome replication occurs for almost all positive-strand RNA viruses (see Fig. 12.7).

---

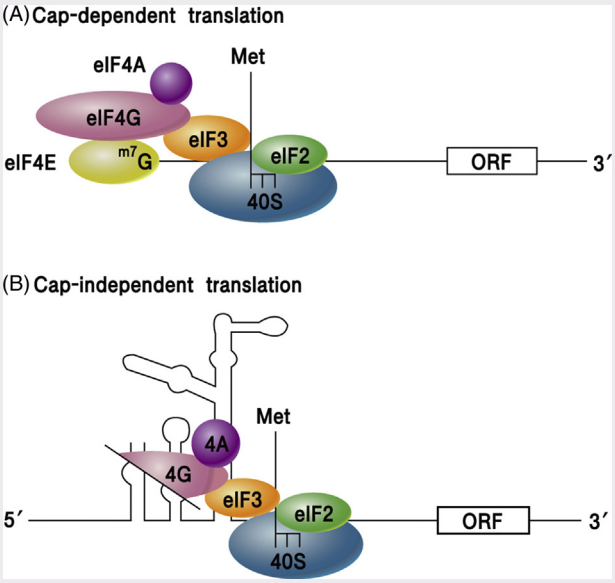
5. **TDP2 (5′-tyrosyl-DNA phosphodiesterase 2)** An enzyme that is known to cleave a tyrosine-DNA phosphodiester linkage found in topoisomerase II-DNA adducts.

6. **Protein-priming** Refers to RNA synthesis that is initiated by protein-primer.

BOX 11.2 IRES

The 5′ end of eukaryotic mRNA harbors the cap structure, which plays a pivotal role in translation. In fact, ribosome engages the mRNA via recognition of the cap structure. Specifically, eIF4F (eIF4A + eIF4G + eIF4E), an eukaryotic translation initiation factor, is associated with mRNA via the interaction of the cap with eIF4E, a cap-binding factor. Then, the 40S ribosomal subunit, which is associated with eIF3, engages mRNA via eIF3-eIIF4G interaction, and begins translation. This process represents a typical translation mechanism, so-called "cap-dependent translation."

On the other hand, the 5′ end of picornavirus RNA genome lacks the cap structure. Instead, the picornavirus RNA harbors an IRES element at 5′ NCR. It was shown that the ribosome could enter mRNA directly via interaction with IRES without the cap structure. This kind of translation mechanism is termed "*cap-independent translation*." In cells that are infected by poliovirus, the N-terminal domain of eIF4G is cleaved by viral $2A^{PRO}$ protease so that cap-dependent translation of cellular mRNAs is suppressed. This suppression is the underlying mechanism for the "*host shutoff*" function induced by picornavirus. By contrast, a ribosome could enter the viral RNA because the N-terminal domain of eIF4G is dispensable for binding to IRES, thereby following the cap-independent translation.



(A) Cap-dependent translation. The eIF3, bound to mRNA via eIF4F (eIF4A + eIF4G + eIF4E), recruits 40S ribosome subunit that is associated with eIF2-methionine (Met) charged tRNA (fork shape) for translation initiation. (B) Cap-independent translation. The eIF4G binds directly to the IRES, thereby recruiting eIF3-40S ribosome. Following the cleavage of eIF4G by $2A^{PRO}$ (as denoted by *solid line*), the remaining eIF4G domain is sufficient to recruit eIF3/40S ribosome complex.

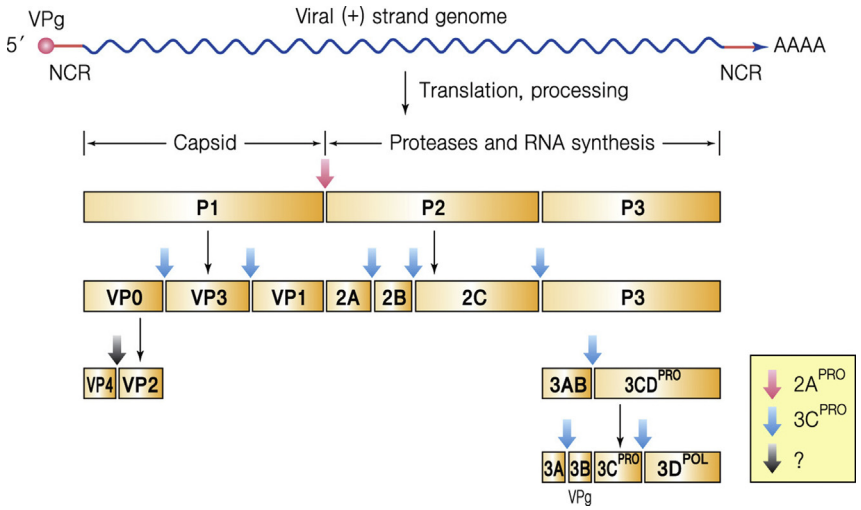
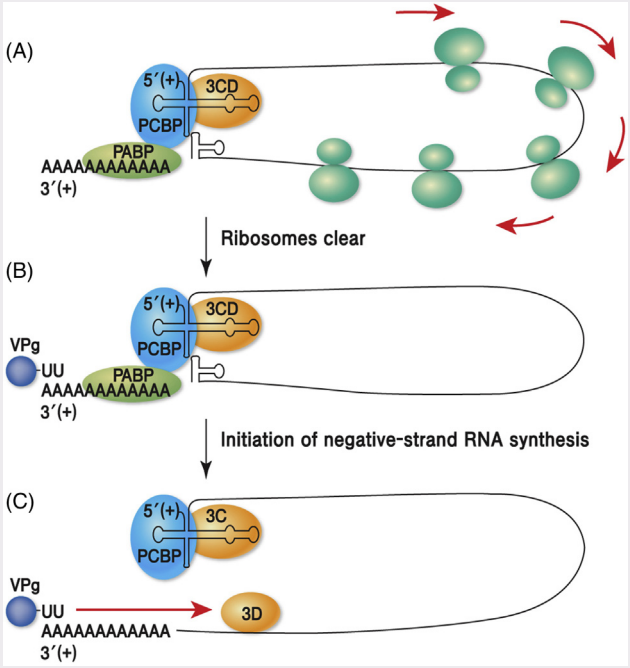


FIGURE 11.6 Proteolytic processing of the poliovirus polyprotein. The polyprotein is proteolytically processed into three precursors (P1, P2, and P3), and then further processed to generate multiple individual proteins. The cleavage sites for $2A^{PRO}$ and $3C^{PRO}$ are denoted. The protease responsible for the cleavage of VP0 to VP4 and VP2 is unknown. Superscript denotes the biochemical activities (eg, $3CD^{PRO}$ = protease and $3D^{POL}$ = polymerase).

BOX 11.3 Switch from Translation to Genome Replication

The positive-strand RNA of picornaviruses serves a dual role in that it is mRNA for translation, but also it is a template for RNA genome replication. These two processes could not occur simultaneously in a given RNA, since ribosomes move from 5′ to 3′ direction, while RNA polymerase moves from 3′ to 5′ direction. Otherwise, they could collide in the middle of the RNA genome, which would be futile. How can these two otherwise competitive processes be streamlined? A clue to this puzzle was hinted at in a recent finding, supporting the circularization of the positive-strand RNA. Intriguingly, the sequence element at the 5′ end contributes to the event occurring at the 3′ end of the RNA via circularization. Two observations supported this notion. First, $3CD^{PRO}$ binds not only to 3′ NCR but also 5′ NCR. Second, *PCBP* [poly (rC) binding protein], which binds to the clover-leaf structure at 5′ NCR, interacts with *PABP* [poly (rA) binding protein] at 3′ NCR. According to this scenario, the circularization of the RNA, which is mediated by the cross-talk between the 5′ and 3′ ends of the RNA via protein–protein interaction, is instrumental for the switch from translation to RNA genome replication. Intriguingly, the circularization of RNA genome was also reported in other positive-strand RNA viruses such as flaviviruses (eg, dengue virus, hepatitis C virus, and Japanese encephalitis virus). Moreover, the circular form of flavivirus RNA genome (eg, dengue virus) was visualized by atomic force microscopy, providing physical evidence for the circularized RNA. After all, the circularization of RNA genome became a rule, rather than an exception among positive-strand RNA viruses.



Circularization of the RNA genome is instrumental for the switch. (A) Blockade of ribosome entry. Circularization via a cross-talk between the 5′ and 3′ ends prevents the entry of ribosome, thereby clearing ribosomes in the RNA. (B) Initiation of RNA synthesis. The initiation by VPg-uridylylation starts using 3′ poly (A) tail as a template. (C) Elongation. The processing of $3CD^{PRO}$ into $3D^{POL}$ is accompanied by elongation.

Positive-Strand RNA Synthesis: Next, we consider positive-strand RNA synthesis, which is templated by the nascent full-length negative-strand RNA (Fig. 11.8). Intriguingly, a novel *cis*-acting element, dubbed *CRE* (*cis*-acting replication element), is essential for positive-strand RNA synthesis; note that, however, this element is not essential for negative-strand RNA synthesis. Similar to that of negative-strand RNA synthesis, the VPg-linked oligonucleotide (ie, VPg-pUpUOH) is first synthesized via protein-priming. Unlike negative-strand RNA synthesis, the synthesis of VPg-UU primer is templated by a CRE element, which lies in the middle of the RNA genome. Then, a VPg-UU primer is translocated to the 3′ end of negative-strand RNA, and then resumes the RNA synthesis.

One notable feature is that RNA synthesis is accomplished in an asymmetric manner in that positive-strand RNA synthesis occurs more abundantly than negative-strand RNA synthesis. The asymmetry is simply achieved by CRE-templated VPg-UU synthesis being more efficient than poly (A) tail-templated VPg-UU synthesis. The asymmetry makes sense in a viral perspective, because only positive-strand RNA counts.

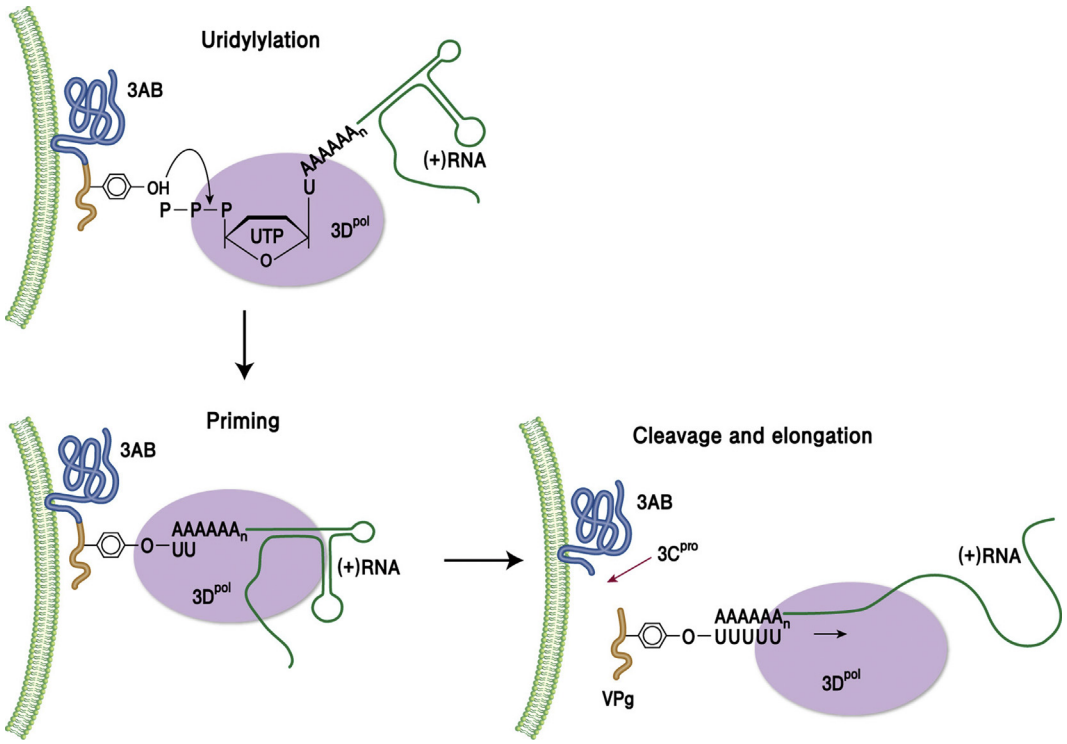


FIGURE 11.7 **Protein-priming by poliovirus 3D^{POL}.** The initiation of (−) RNA synthesis by 3D^{POL} is primed by 3AB precursor, which is associated with the vesicular membrane (eg, endoplastic reticulum). The nucleophilic attack by a hydroxyl group of a tyrosine residue covalently links the 3AB precursor to the first U residue. VPg-linked RNA is released from the membrane following the cleavage by 3C^{PRO}.

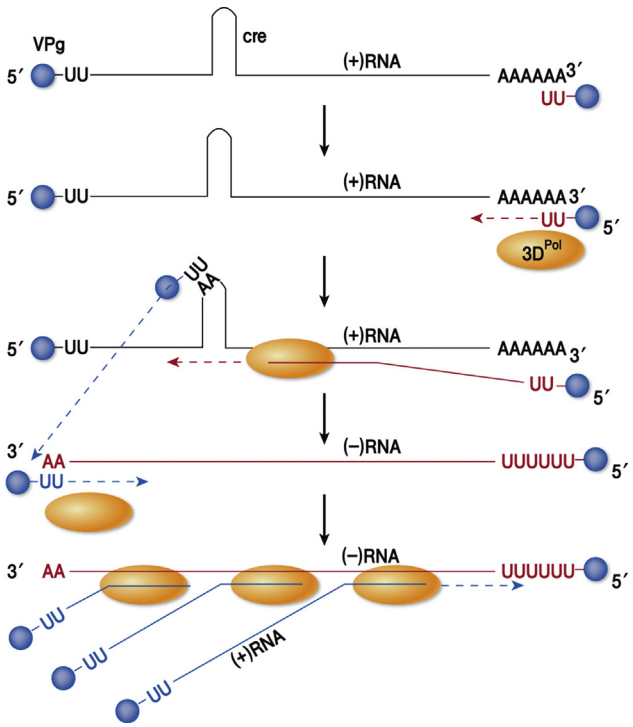


FIGURE 11.8 **The RNA genome replication of the poliovirus.** The initiation of (−) RNA synthesis is templated by 3′ poly (A) tail, whereas the initiation of (+) RNA synthesis is templated by CRE element. The translocation of VPg-UU primer from CRE to the 3′ end of (−) RNA is denoted by dashed lines. CRE element, which holds a hairpin structure, encodes "AA" residues on the loop region of the hairpin. Nascent (−) RNA is colored by red, while nascent (+) RNA is colored by blue.

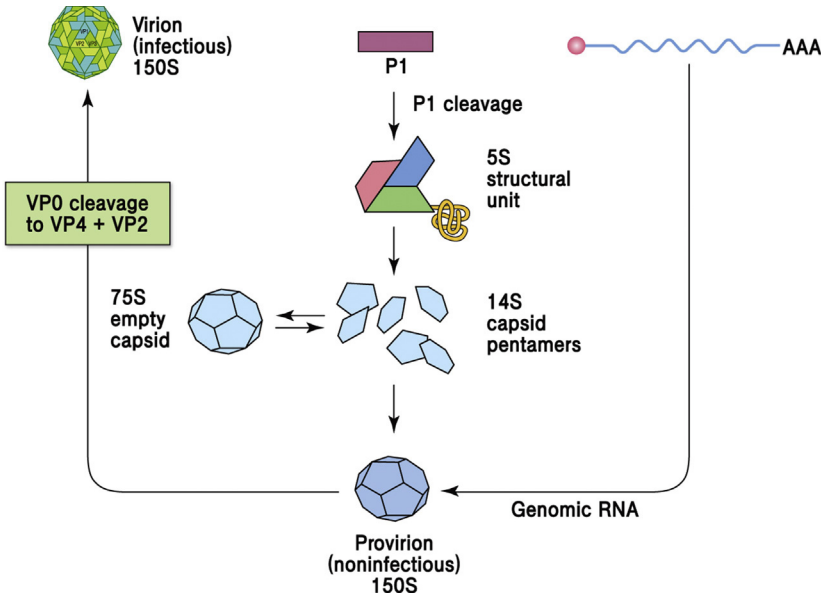


FIGURE 11.9 Steps involved in the capsid assembly of the poliovirus. Multiple assembly intermediates are formed in poliovirus capsid assembly. Note that the 5S subunit (ie, VP0 + VP3 + VP1) is the basic assembly subunit. The 14S subunit could be self-assembled reversibly to the 75S empty capsid when the RNA genome is absent. The 150S provirion is noninfectious, unless it is converted to the 150S virion by the cleavage of VP0 to VP4 and VP2 (S, Svedberg sedimentation coefficient).

Assembly and Release: Capsid assembly occurs, when building blocks for the capsid assembly accumulate. The proteolytic cleavage of the P1 precursor leads to the formation of a *5S* structural subunit, dubbed *protomer*,[7] which is composed of VP0 + VP3 + VP1 (Fig. 11.9). Next, 14S pentamers are assembled by arranging five 5S units. The recognition of the genomic RNA by 14S pentamers drives not only the RNA packaging, but also the 150S provirion assembly. The provirion, although it contains the RNA genome, is noninfectious. The assembled poliovirus particles (ie, provirion) are released through cell lysis. Finally, the proteolytic cleavage of VP0 to VP4 + VP2 occurs after the viral release. This process is called "*maturation*," which confers the virion "infectivity."

11.4 EFFECTS ON HOST

The productive infection by poliovirus leads to cell lysis, leaving a plaque on the monolayer cells in culture.

Translation Suppression: Notably, cellular translation function is severely impaired in poliovirus infected cells. For instance, the viral $2A^{PRO}$ protease cleaves *eIF4G*, thereby leading to the blockade of cap-dependent translation (see Box 11.2). In contrast, cap-independent translation of IRES-containing poliovirus mRNA is still to occur. In other words, poliovirus subverts the host translation machinery for viral protein synthesis. This is one example of showing how viruses cunningly explore host functions for the benefit of their own purpose.

Suppression of IFN Action: Viral infection evokes an innate immune response, leading to interferon (IFN) production. IFN induces and activates *PKR*,[8] which phosphorylates *eIF2α* and thereby leads to the inhibition of translation (see Fig. 5.8). In principle, the antiviral effect of IFN would equally affect the translation of both viral mRNA and cellular mRNA. Nonetheless, the host cells are less vulnerable, since host proteins have already accumulated to some extent. Nonetheless, poliovirus successfully manages to lead to the productive infection under these circumstances. Then, one wonders how poliovirus avoids the antiviral action of IFN. Recently, it was shown that picornaviruses avoid the antiviral effect of IFN by switching from earlier eIF2α-dependent translation to *eIF2α-independent translation* in the late phase of infection. After all, picornaviruses co-opt host translation machinery to complete their life cycle.

Pathogenesis: Poliovirus transmits via the fecal−oral route and viral replication occurs in the alimentary tract. In 95% of cases, only a primary and transient presence of viremia (virus in the blood) occurs, and the poliovirus infection is asymptomatic. In about 5% of cases, the virus spreads and replicates in other sites such as muscle. The sustained viral replication causes secondary viremia and leads to the development of minor symptoms such as fever, headache, and sore throat. Paralytic poliomyelitis occurs in less than 1% of poliovirus infections. Paralytic disease

7. **Protomer** It refers to a structural subunit of poliovirus capsid. It is also called 5S structural unit, based on its sedimentation coefficient (ie, Svedberg sedimentation coefficient).

8. **PKR (protein kinase R)** A serine/threonine protein kinase that is activated by double-strand RNA.

occurs when the virus enters the central nervous system (CNS) and replicates in motor neurons within the spinal cord, brain stem, or motor cortex, resulting in the selective destruction of motor neurons leading to temporary or permanent paralysis. However, the underlying mechanism by which poliovirus invades the CNS and causes poliomyelitis remains unknown.

11.5 PERSPECTIVES

Since its discovery in 1908, poliovirus has been intensively studied to better understand and control this formidable pathogen. Poliovirus vaccines developed in the 1950s have been paramount in controlling the pathogen (see chapter: Vaccines). The history of poliovirus research is not, however, limited to the fight against the disease. Poliovirus replication studies also have played importantly roles in the development of modern virology. Poliovirus was, for example, the first animal RNA virus to have its complete genome sequence determined; the first animal RNA virus of which an infectious clone was constructed; and along with the related rhinovirus, the first human virus for which its three-dimensional structure was solved by X-ray crystallography. In particular, the studies on poliovirus have contributed greatly to our current understanding of eukaryotic translation. For instance, the IRES element first discovered in the poliovirus was instrumental for our current understanding on cap-independent translation, one that is distinct from conventional cap-dependent translation. Importantly, enteroviruses other than poliovirus have emerged as serious threats to public health. These include *enterovirus 71*, responsible in infants and young children for hand, foot, and mouth disease with the potential for severe nervous system complications, and *enterovirus D68*, detected in children hospitalized with severe lower respiratory symptoms and asthma. In addition, many other members of the picornavirus family present serious health concerns. For instance, HAV is responsible for epidemic hepatitis in the Western Hemisphere (see chapter: Hepatitis Viruses). It is hoped that knowledge learned from poliovirus as the prototype should be instrumental in better understanding other related picornaviruses that represent unmet medical needs.

11.6 SUMMARY

- *Picornavirus*: Picornaviruses are found in diseases of many organs including gastrointestinal tracts, respiratory tracts, neuronal tissue, and muscles. Poliovirus is a prototype of picornavirus family possessing a single-strand RNA genome.
- *Virion structure*: It is a nonenveloped capsid with $T = 3$ symmetry. The capsid is composed of 60 subunits, each of which constitutes three virion proteins (ie, VP1, VP2, and VP3).
- *Viral genome*: The virion RNA (vRNA) is a positive-strand RNA about 7.5 kb in length. VPg is linked to the 5′ end of the vRNA. The IRES element located at 5′ NCR allows "cap-independent translation." The RNA genome encodes one large polyprotein, which is subsequently processed into individual viral proteins.
- *RNA genome replication*: The RNA genome replication is initiated via protein-priming by using VPg as a primer.
- *Host effect*: Poliovirus infection causes cell lysis, resulting in plaque formation. The cleavage of eIF4G by $2A^{PRO}$ blocks cap-dependent translation.

STUDY QUESTIONS

11.1 A novel RNA virus was discovered. Intriguingly, the 5′ terminus of the RNA genome is covalently linked to a viral protein. (1) Please hypothesize the role of the 5′-linked viral protein. (2) How would you test your hypothesis?

11.2 Poliovirus progeny particles are produced if the viral genomic RNA is transfected into appropriate cells. Please state whether the progeny virus is produced when the respective viral RNA is transfected. (1) VPg-free RNA, (2) Δ clover-leaf structure-RNA, (3) Δ IRES-RNA, (4) Δ CRE-RNA, (5) poly (A) tail-free RNA.

11.3 Compare and contrast the differences between (−) RNA synthesis and (+) RNA synthesis of picornaviruses.

SUGGESTED READING

Feng, Z., Hensley, L., McKnight, K.L., Hu, F., Madden, V., Ping, L., et al., 2013. A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. Nature. 496 (7445), 367–371.

Leveque, N., Semler, B.L., 2015. A 21st century perspective of poliovirus replication. PLoS Pathog. 11 (6), e1004825.

Nagy, P.D., Pogany, J., 2012. The dependence of viral RNA replication on co-opted host factors. Nat. Rev. Microbiol. 10 (2), 137–149.

Virgen-Slane, R., Rozovics, J.M., Fitzgerald, K.D., Ngo, T., Chou, W., van der Heden van Noort, G.J., et al., 2012. An RNA virus hijacks an incognito function of a DNA repair enzyme. Proc. Natl Acad. Sci. USA. 109 (36), 14634−14639.

Walsh, D., Mohr, I., 2011. Viral subversion of the host protein synthesis machinery. Nat. Rev. Microbiol. 9 (12), 860−875.

JOURNAL CLUB

- Feng, Z., Hensley, L., McKnight, K.L., Hu, F., Madden, V., Ping, L., et al., 2013. A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. Nature 496 (7445), 367−371.

  Highlight: Picornavirus has a naked capsid lacking an envelope component. Intriguingly, this article showed that hepatitis A virus (HAV), a picornavirus causing acute hepatitis, is cloaked in host-derived membranes, thereby protecting the virion from antibody-mediated neutralization. These enveloped viruses ("eHAV") resemble exosomes and are fully infectious and circulate in the blood of infected humans. Thus, membrane hijacking by HAV blurs the classical distinction between "enveloped" and "nonenveloped" viruses and has broad implications for host immune response.