

Chapter 3

Virus Life Cycle

Chapter Outline

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Viruses are obligate intracellular parasites. Therefore, viruses must gain entry into target cells and usurp the host cellular machinery to propagate and to produce progeny viruses. The multiple steps involved in the virus propagation occurring inside cells are collectively termed the “virus life cycle.” The virus life cycle can be divided into three stages—entry, genome replication, and exit. Here, we focus on entry and exit, in which the commonality of mechanisms among viruses prevails. On the other hand, the genome replication, which is a step that is distinct for each of the virus families, is described in Part II to Part IV, where virus families are individually considered.

3.1 STEPS IN VIRUS LIFE CYCLE

A virus encounters multiple obstacles during its journey to enter the host cells. Cellular membranes pose as barriers for the invaders. The plasma membrane represents the first barrier that all animal viruses have to penetrate. The nuclear membrane represents the second barrier to some viruses that replicate their genome in the nucleus. Let’s see how viruses obviate the barriers.

The virus life cycle can be divided into three stages—entry, genome replication, and exit (Fig. 3.1). The virus life cycle can be described in analogy with a businessman’s life; the entry to his way to work, the genome replication to his task at work, and the exit to his way home. The first stage is entry. Entry involves *attachment*, in which a virus particle encounters the host cell and attaches to the cell surface, *penetration*, in which a virus particle reaches the cytoplasm, and *uncoating*, in which the virus sheds its capsid. Following the uncoating, the naked viral genome is utilized for gene expression and viral genome replication. Finally, when the viral proteins and viral genomes are accumulated, they are assembled to form a progeny virion particle and then released extracellularly. Virion assembly and the release from the cell constitute the *exit*.

3.2 VIRAL ENTRY

Entry, the first step of virus infection, involves the recognition of viral receptor by a virus particle. The viral entry can be divided into four steps: attachment, penetration, cytoplasmic trafficking, and uncoating. These steps are often linked to each other so that the division into four steps is obscure, but serves the explanation purpose.

3.2.1 Attachment

The attachment refers to the first encounter of virus particles with host cells, which involves two kinds of host proteins on the plasma membrane: (1) attachment factors and (2) viral receptors. The attachment factor on the cell surface recruits and holds the virus particles, thereby facilitating the interaction of the viral particle with the entry receptor.

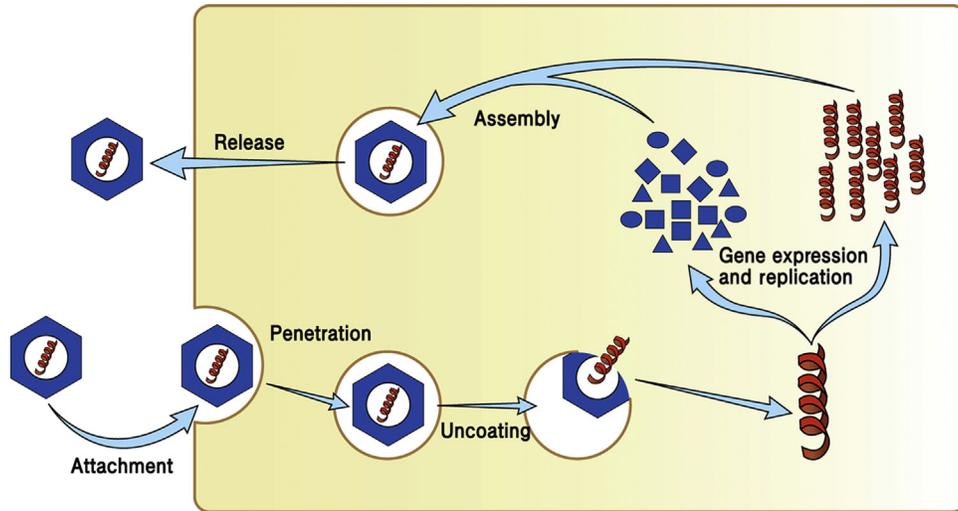


FIGURE 3.1 The life cycle of virus. The virus life cycle could be divided into six steps: attachment, penetration, uncoating, gene expression and replication, assembly, and release. The viral capsid (blue) and genome (brown) are schematically drawn for the purpose of explanation. The nucleus is omitted for clarity.

TABLE 3.1 Viral Receptors for Major Human Viruses

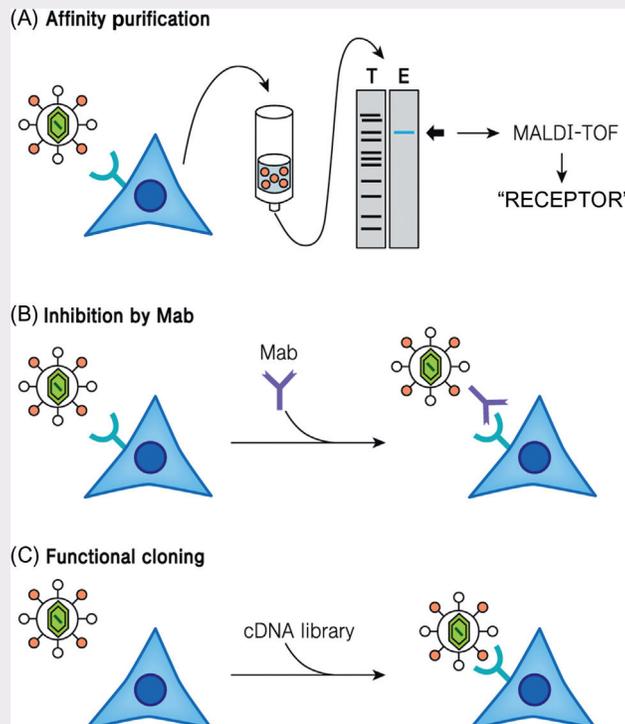
Family: Prototype Virus	Receptor (Coreceptor)	Attachment Factor	Viral Antireceptor
DNA Virus			
Parvovirus: AAV	HSPG (FGFR, integrin)	HSPG	CAP
Polyoma: SV40	GM1 gangliosides	–	VP1
Adenovirus: Ad5	Integrin	CAR	Fiber protein
Herpesvirus: HSV-1	Nectin-1/HVEM, PILR α	HSPG	gD, gB
Herpesvirus: CMV	EGFR	HSPG	
Herpesvirus: EBV	CD21	HSPG	gp350
RNA Virus			
Picornavirus: Poliovirus	PVR/CD155	–	VP1, VP2, VP3
Picornavirus: Coxsackie virus	CAR	–	
Picornavirus: Rhinovirus	ICAM-1 or LDL receptor	DC-SIGN, L-SIGN	VP1, VP2, VP3
Flavivirus: Hepatitis C virus	CD81, Claudin-1, Occludin	SR-B1, LDL receptor	E2
Coronavirus: SARS virus	ACE2	–	Spike
Orthomyxovirus: Influenza virus	Sialic acid	–	HA
Rhabdovirus: VSV	Phosphatidyl serine	–	G protein
Rhabdovirus: Rabies	NCAM-1/CD56	–	G protein
Reovirus: Reovirus	JAM-A	–	Spike protein s1
RT Virus			
Retrovirus: HIV-1	CD4 (CXCR4 or CCR5)	DC-SIGN, L-SIGN	gp120
Hepatitis B virus	NTCP	HSPG	Pre-S1

Note: ACE, angiotensin-converting enzyme; CAR, coxsackie-adenovirus receptor; EGFR, epidermal growth factor receptor; HSPG, heparin sulfate proteoglycan; HVEM, herpes virus entry mediator; ICAM, intercellular adhesion molecule; NTCP, sodium taurocholate cotransporting polypeptide; PVR, poliovirus receptor.

In fact, glycoaminoglycans, such as heparins, serve as the attachment factor for diverse viruses, revealing the broader specificity of the attachment factors (Table 3.1). Unlike attachment factors, viral receptors, upon binding to the virus particles, promote the penetration of virus particles into cells. Further, the viral receptors are virus-specific and more importantly, determine cell tropism. For example, $CD4^1$ is specifically recognized by HIV, which infects $CD4$ -expressing T lymphocytes.

BOX 3.1 Strategies to Discovery Viral Receptor

What are the experimental approaches to unveil viral receptors on the cell surface of the susceptible cells? Three experimental methods have been successfully used. The first approach is to identify the receptors by biochemical purification of cellular proteins on the cell surface that bind to the viral antireceptors (ie, viral structural proteins). Affinity purification of plasma membrane proteins using the viral structural proteins as a ligand is feasible. Alternatively, immunoprecipitation of plasma membrane proteins that bind to the viral structural protein could lead to many candidate proteins in SDS-PAGE gel. Subsequently, each protein band on the gel is subjected to mass spectroscopy (eg, MALDI-TOF) for identification. The second approach is to use *monoclonal antibodies*² (Mab) against plasma membrane proteins. It exploits the fact that an antibody specific to the receptor could block the entry. A set of the Mab is prepared against the plasma membrane of the susceptible cell in the first place. Then, each individual Mab is tested for its ability to block the viral infection. If the virus infection is blocked by a specific Mab, the ligand for the Mab is a potential candidate for the viral receptor. Ultimately, the ligand can be identified by immunoprecipitation, followed by mass spectroscopy. The third approach is to identify the receptor by *functional cloning*, which exploits the cDNA expression library. An individual cDNA of the library is transfected into a nonsusceptible cell, then tested as to whether the cell becomes infected. This method necessarily involves high-throughput robotics, as the human cDNA library is composed of over 30,000 genes. Recently, this modern technology has been successfully implemented to clone the HCV receptor. Once the potential molecules for the viral receptors are identified by one of above three approaches, the functionality of the receptors needs to be validated. This validation is to confirm whether the cDNA transfection to a nonsusceptible cell is necessary and sufficient to convert the nonsusceptible cell to a susceptible cell.



(A) Affinity purification. (B) Inhibition by Monoclonal antibodies (Mab). (C) Functional cloning.

- CD4** A glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells. CD4 is best known as a cellular marker for T helper lymphocyte.
- Monoclonal antibody** Monoclonal antibodies (mAb) are monospecific antibodies that are made by identical immune cells that are all clones of a unique parent cell.

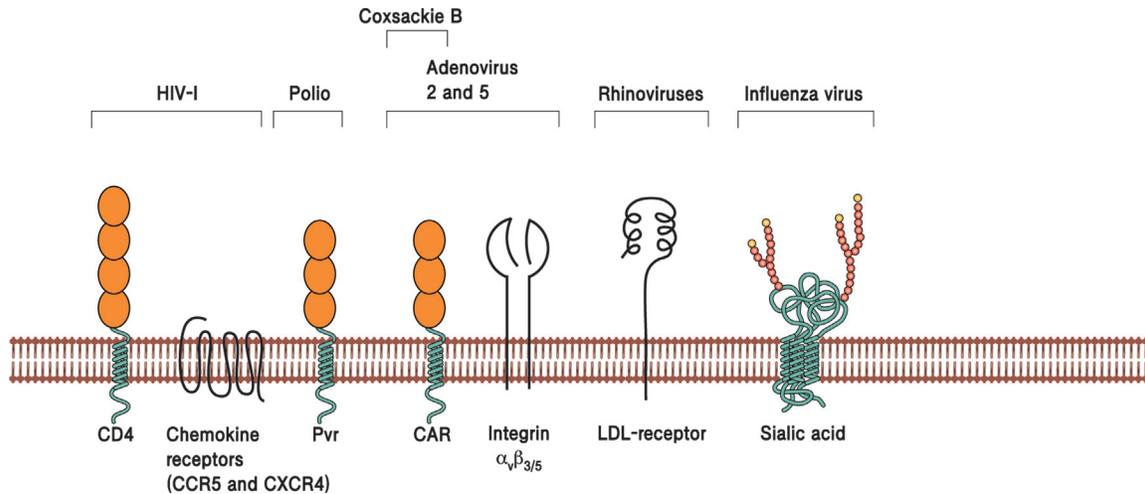


FIGURE 3.2 The viral receptors of representative human pathogenic viruses. The ectodomain of the receptor molecules is recognized by virus particles. Immunoglobulin (IgG) fold, which is shared by members of immunoglobulin superfamily (ie, CD4, Pvr, and CAR), is drawn in a circle. The sialic acid moiety of glycan in membrane glycoproteins is marked by yellow. Pvr, poliovirus receptor; CAR, coxsackie virus and adenovirus receptor.

Almost all viral receptors for the major human pathogenic viruses have been identified during the past three decades (Box 3.1). Advances in molecular biology made since the 1970s played a critical role for the discovery. Importantly, a few intriguing points stand out regarding the attributes of viral receptors. First, the molecular nature of the viral receptors is quite diverse, ranging from glycoproteins to phospholipids (see Table 3.1). Even the carbohydrate moiety of membrane glycoproteins is utilized as the viral receptor. For instance, *sialic acid*³ residue of glycans is the entry receptor for influenza virus. Second, most of the viral entry receptors have their own cellular functions. For instance, the physiological function of LDL receptor, an entry receptor of a certain picornavirus, is to uptake LDL particles into cells, while epidermal growth factor receptor (EGFR), an entry receptor of a certain herpesvirus, is an EGFR (Fig. 3.2). In other words, viruses subvert the cellular proteins that have their physiological functions, and utilize them as entry receptors. Third, many of the viral receptors belong to the immunoglobulin superfamily, such as CD4 and CAR (see Fig. 3.2). Fourth, some viruses require coreceptors for their entry, in addition to the main receptors for entry. For example, HIV requires chemokine receptors, such as *CCR5* or *CXCR4*, as a coreceptor for efficient entry (see Fig. 17.4).

Importantly, the presence of the receptor in a given cell is a determinant for the susceptibility to a certain virus. Thus, cell *tropism*⁴ is largely determined by the receptor. For instance, the presence of CD4 in T helper lymphocyte confers the susceptibility to HIV infection. A HeLa cell that is otherwise resistant to HIV infection becomes susceptible to HIV infection if CD4 is experimentally expressed. On the other hand, the viral proteins that recognize the receptors are ones on the surface of virus particles. In the case of enveloped viruses, the envelope glycoproteins on the viral envelope bind to the receptors. For instance, *gp120* of the HIV virion particle binds to the CD4 molecule of the target cells (see Fig. 17.4). In case of naked viruses, capsid proteins may directly bind to the receptor. For instance, the fiber protein of the adenovirus particle binds to the *CAR*⁵ molecule on the target cells.

Although the presence or absence of a receptor is a key determinant for the susceptibility of a certain virus, cell tropism can also be determined by *host restriction factors*, which limit the virus infection. For instance, *TRIM5 α* of monkey cell restricts HIV infection of monkey cell, whereas *TRIM5 α* of human cell restricts simian immunodeficiency virus infection of human cell (see Fig. 17.17). Thus, *TRIM5 α* is a host restriction factor that is responsible for determining host range of primate lentivirus.

3. **Sialic acid** It is a generic term that refers to the N-acetyl neuraminic acid, an amino sugar, which is terminally linked to glycans on the cell membrane.

4. **Tropism** The term *tropism* is derived from Greek word for “a turning”—*tropos*—indicating growth or turning movement of a biological organism. It refers to the cell specificity of viral infection in this context.

5. **CAR (Coxsackie-Adenovirus Receptor)** CAR is exploited by two unrelated viruses for entry (ie, coxsackie type B3 and adenovirus type 5).

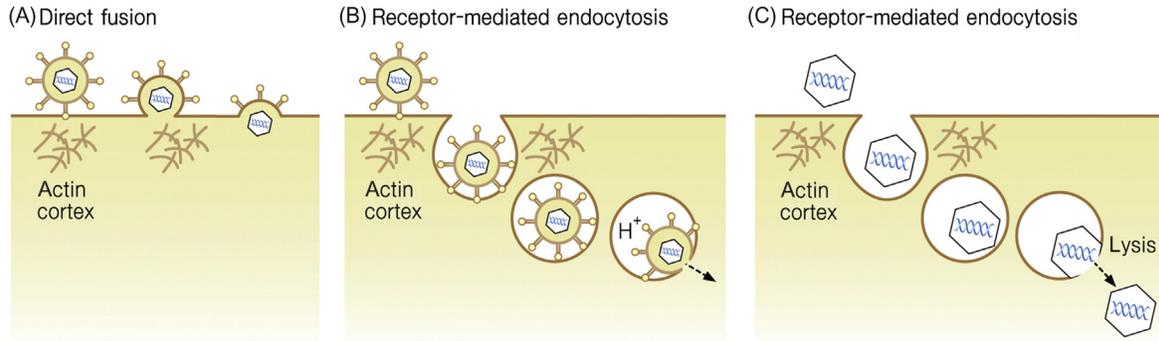


FIGURE 3.3 Three modes of viral penetration. (A) Direct fusion. The viral nucleocapsids enter the cell by the fusion between viral envelope and plasma membrane. (B) Receptor-mediated endocytosis for enveloped viruses. The virus particle acts as a ligand for the endocytosis. The virus particle is located inside endosomes. The fusion takes place between the viral envelope and endosomal membrane. Note that actin cortex (a mechanical support of the plasma membrane), which serves a physical barrier for the entry, is bypassed through the endocytosis. (C) Receptor-mediated endocytosis for nonenveloped naked viruses. A component of viral nucleocapsid triggers the lysis of endosomal membrane necessary for the release of the viral genome to the cytoplasm.

3.2.2 Penetration

Following attachment of the virus particle on the target cells, the next step is the penetration into the cytoplasm. The mechanism for the penetration differs, whether enveloped or not. For enveloped viruses, one of the following two mechanisms is used: *direct fusion* and *receptor-mediated endocytosis*. For nonenveloped naked viruses, receptor-mediated endocytosis is used for penetration.

Direct fusion: Direct fusion, as its name implies, is a mechanism in which two membranes (ie, the viral envelope and cell membrane) fuse (Fig. 3.3A). In this case, the viral nucleocapsid is directly delivered to the cytoplasm, leaving the viral envelope behind on the plasma membrane. Retrovirus is a representative that penetrates by direct fusion (see Fig. 17.4).

Receptor-mediated endocytosis: Although some viruses, as described above, penetrate into the cytosol directly through the plasma membrane, most viruses depend on endocytic uptakes, a process termed *receptor-mediated endocytosis* (Fig. 3.3B). Following the engagement of viral particles on the receptor, the virus particle-receptor complex triggers the endocytosis by forming a coated pit on the plasma membrane, leading to endosome formation. As a result, the virus particle becomes located inside the endosome. The next step is to breakdown the endosome to penetrate to the cytoplasm. The process of endosome breakdown differs whether enveloped or not. For enveloped viruses, the membrane fusion between the viral envelope and the endosomal membrane triggered by acidic pH at early endosome causes the endosome breakdown. More precisely, the fusion peptide embedded on the envelope glycoprotein becomes exposed (ie, activated) as a consequence of conformational change upon low pH; then the fusion is triggered by the fusion peptide (Box 3.2). For nonenveloped naked viruses, the endosome lysis is induced by one of the capsid proteins. In other words, membrane fusion is the mechanism of penetration for envelope viruses (see Fig. 3.3B), while membrane lysis is the mechanism of penetration for nonenveloped viruses (Fig. 3.3C).

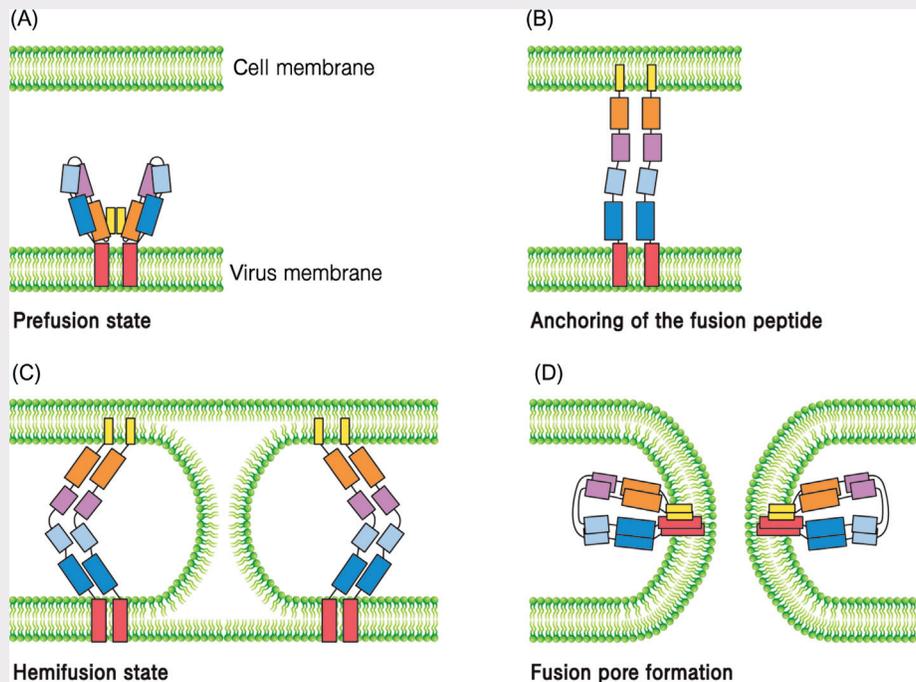
As stated above, most viruses enter the cell via receptor-mediated endocytosis. What would be the advantages of receptor-mediated endocytosis, as opposed to direct fusion? Unlike direct fusion, evidently, receptor-mediated endocytosis bypasses the actin cortex or the meshwork of microfilaments in the cortex that presents an obstacle for the penetration (see Fig. 3.3). Moreover, by being taken up by endocytosis, animal viruses can avoid leaving the viral envelope glycoprotein on the plasma membrane, thus likely causing a delay in detection by immune system.

Typically, receptor-mediated endocytosis proceeds via a clathrin-dependent manner (Fig. 3.4). Receptor-mediated endocytosis is the mechanism intrinsic to the cells, which is utilized to take extracellular molecules into the cells. *Clathrin-mediated*⁶ endocytosis, which is also the pathway utilized for uptake of LDL, is employed by many viruses, such as influenza virus and adenovirus. Upon the binding of the virus particle with the receptor, a clathrin-coated pit is formed, as clathrins are recruited near the plasma membrane. Following the formation of an endocytic vesicle, the vesicles are fused with early endosomes. The virus particles are now located inside the early endosomes.

6. **Clathrin** A protein that plays a major role in the formation of coated vesicles.

BOX 3.2 Fusion peptide

The entry of enveloped viruses into the cell involves membrane fusion between the plasma membrane and the viral envelope. Membrane fusion promotes the penetration of the viral capsid into the cytoplasm. A question is how is membrane fusion, which is seemingly a difficult biochemical reaction, carried out? A short answer to this question is that the viral envelope proteins (eg, fusion proteins) harbor a “fusion peptide,” that triggers the membrane fusion. How does the fusion peptide promote membrane fusion? The fusion protein, which is intrinsically “metastable,” is present in its prefusion conformation in the virion particles. In this state, the fusion peptides are buried inside the viral fusion proteins. Various triggers, such as acidic pH and receptor binding, induce conformational rearrangements, resulting in the anchoring of the fusion peptide in the juxtaposing cellular membrane. Anchoring leads to concurrent formation of a complementary amphipathic domain in the prehairpin extended intermediates. These newly exposed domains are unstable and refold to form more energetically favorable structure. The enthalpy associated with these conformational changes forces mixing of the outer leaflet of the viral membrane with the outer layer of the cellular membrane, resulting in formation of the “hemifusion” stalk. The inner leaflet of the lipid bilayers then come into contact and begin mixing, opening a pore (fusion pore) between viral and cellular membranes as the trimeric structures refold into a highly stable postfusion conformation.



The fusion process between viral and cellular membranes. The fusion peptide (yellow) is buried inside the fusion protein, which is in an energetically unfavorable metastable prefusion state (A). The conformational change induced by triggers (eg, acidic pH and receptor binding) results in anchoring the fusion peptide in the cell membrane (B), forcing the viral membrane and cell membrane into a hemifusion state (C). Subsequently refolding of the fusion protein into a highly stable postfusion conformation leads to a fusion pore formation (D). For simplicity, only dimers are represented, but the fusion proteins are always trimeric: HA of influenza virus and F (fusion) protein of paramyxovirus.

It is worth noting some features of membrane fusion. First, only one viral factor (ie, the fusion protein) drives membrane fusion, but no cellular factors are involved in membrane fusion. This feature contrasts with that of budding, which also involves membrane fusion. Specifically, numerous cellular factors of the MVBs pathway are involved in budding (see Box 3.4). Secondly, membrane fusion, which is essentially the mixing of two lipid bilayers, is thermodynamically driven. In other words, membrane fusion is accompanied with the conformational changes of the fusion protein that is poised to change from a metastable prefusion state to a highly stable postfusion state.

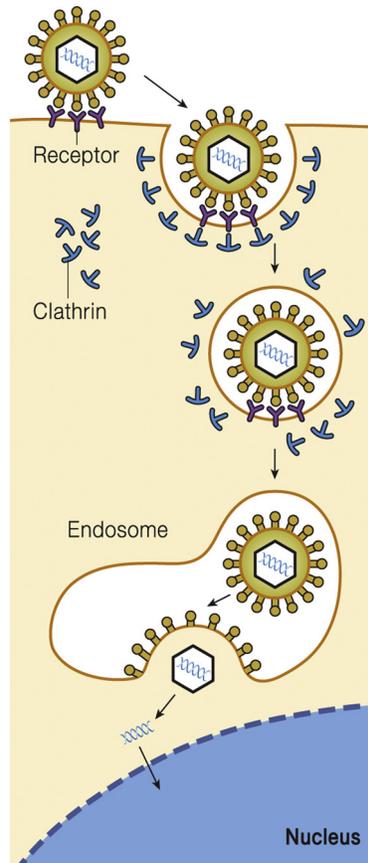


FIGURE 3.4 Clathrin-mediated endocytosis. Upon the binding of virus particles to the receptor, clathrins are recruited to form the clathrin-coated pit via its interaction with AP-2 adapter. Clathrin-coated pits are pinched off by dynamins. After the vesicle coat is shed, the uncoated endocytic vesicle fuses with the early endosome. The capsids are released from the endosome by membrane fusion between viral envelope and endosome that is triggered by low pH inside the endosome.

In addition to receptor-mediated endocytosis, a few other endocytic mechanisms are utilized by animal viruses (Fig. 3.5). For instance, caveolin-mediated⁷ endocytosis is used for the entry of polyomaviruses, such as SV40 (see Fig. 6.3). In this case, caveolin, instead of clathrin, serves as a coat protein; otherwise it is similar to *clathrin-mediated endocytosis*. *Macropinocytosis*⁸ is utilized for the entry of particles with a larger size, such as vaccinia virus and herpes viruses. The virus particle first activates the signaling pathways that trigger actin-mediated membrane ruffling and blebbing. The formation of large vacuoles (macropinosomes) at the plasma membrane is followed by the internalization of virus particles and penetration into the cytosol by the viruses or their capsids.

3.2.3 Intracellular Trafficking

Following successful penetration inside cells, the virus particles need to get to an appropriate site in the cell for genome replication. This process is termed *intracellular trafficking*. In fact, the biological importance of the cytoplasmic trafficking was not realized until the invention of live cell imaging technology. For viruses that replicate in the cytoplasm, the viral nucleocapsids need to be routed to the site for replication. In fact, *microtubule-mediated transport* coupled with receptor-mediated endocytosis is the mechanism for the transport (Fig. 3.6). In addition, for viruses that replicate in the nucleus, the viral nucleocapsids need to enter the nucleus. For many DNA viruses, the viral nucleocapsids are routed to the perinuclear area via microtubule-mediated transport. In this process, a *dynein motor* powers the movement of virus particles. As an analogy, the viral nucleocapsids can be envisioned as a train in a railroad.

7. **Caveolins** A family of integral membrane proteins which are the principal components of caveolae membranes.

8. **Macropinocytosis** An endocytic mechanism normally involved in fluid uptake.

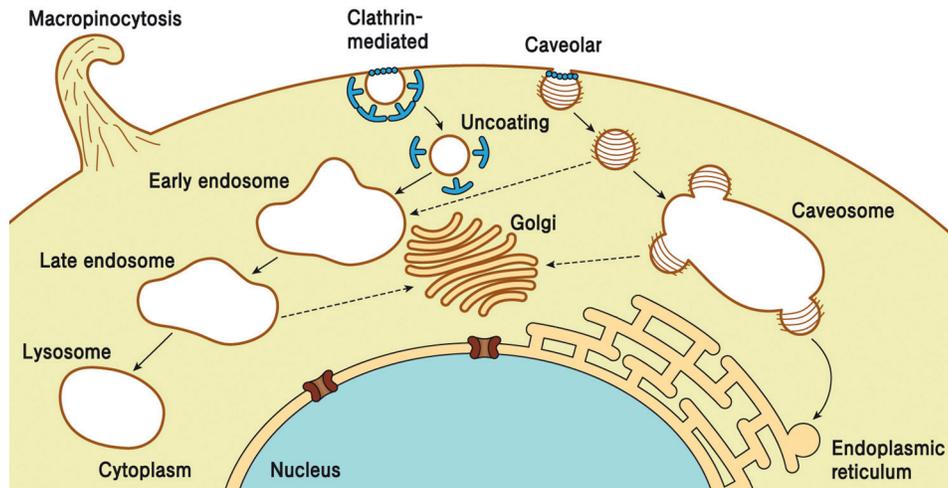


FIGURE 3.5 Diverse pathways for the receptor-mediated endocytosis. *Clathrin-mediated endocytosis*. This pathway is the most commonly observed uptake pathway for viruses. The viruses are transported via the early endosome to the late endosome and eventually to the lysosome. *Caveolin/lipid-raft-mediated endocytosis*. The caveola pathway brings viruses to caveosomes. By the second vesicle transport step, viruses are transported to Golgi, and then to ER. *Macropinocytosis*. Macropinocytosis is utilized for the entry of particles with larger size, such as vaccinia viruses and herpes viruses.

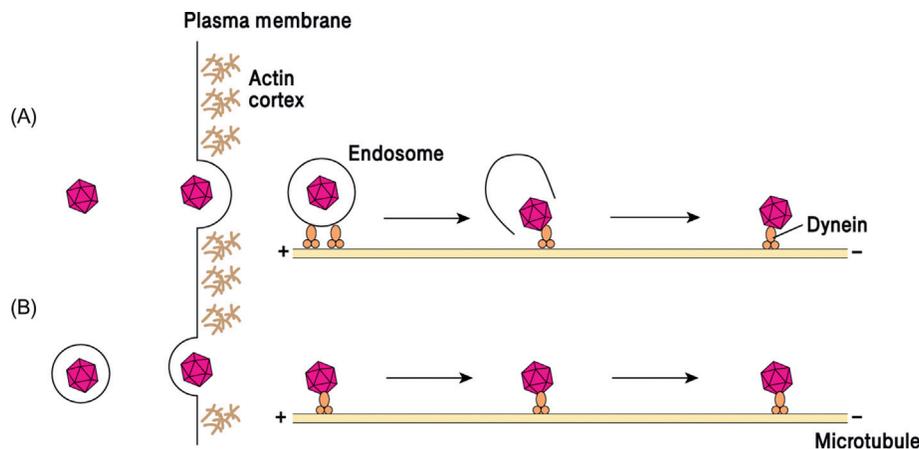


FIGURE 3.6 Cytoplasmic trafficking. Two distinct viruses are used to explain how the entry is linked to cytoplasmic trafficking: (A) adenovirus (naked) and (B) herpes virus (enveloped). Incoming viruses can enter cells by endocytosis (A) or direct fusion (B). Following penetration into cytoplasm, either endocytic vesicles or viral capsids exploit dynein motors to traffic toward the minus ends of microtubules. Either the endocytic vesicles (A) or the capsids (B) interact directly with the microtubules. The virus can also lyse the endocytic membrane, releasing the capsid into the cytosol (A).

3.2.4 Uncoating

As the virus particles approach to the site of replication, from the cell periphery to the perinuclear space, the viral genome becomes exposed to cellular machinery for viral gene expression, a process termed uncoating. Uncoating is often linked with the endocytic route or cytoplasmic trafficking (see Fig. 3.6).

For viruses that replicate in the nucleus, the viral genome needs to enter the nucleus via a nuclear pore. Multiple distinct strategies are utilized, largely depending on their genome size (Fig. 3.7). For the virus with a smaller genome, such as polyomavirus, the viral capsid itself enters the nucleus. For viruses with a larger genome, the docking of nucleocapsids to a nuclear pore complex causes a partial disruption of the capsid (eg, adenovirus) or induces a minimal change in the viral capsid (eg, herpes virus), allowing the transit of DNA genome into the nucleus.

3.3 VIRAL GENE EXPRESSION AND GENOME REPLICATION

The viral genome replication strategies are distinct from each other among the virus families. In fact, the genome replication mechanism is the one that defines the identity of each virus family. Furthermore, the extent to which each virus family relies on host machinery is also diverse, ranging from one that entirely depends on host machinery to one that is

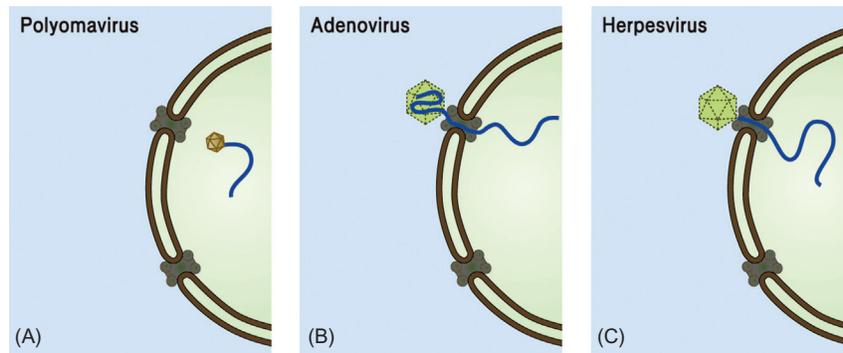


FIGURE 3.7 Strategies for the nuclear entry of DNA viruses. (A) Polyomavirus capsids are small enough to enter the nucleus directly via the nuclear pore complex without disassembly. Uncoating of the polyomavirus genome takes place in the nucleus. (B) The adenovirus capsids are partially disrupted upon binding to the nuclear pore complex, allowing the transit of the DNA genome into the nucleus. (C) For herpesvirus, the nucleocapsids are minimally disassembled to allow transit of the DNA genome into the nucleus.

quite independent. However, all viruses, without exception, entirely rely on host translation machinery, ribosomes, for their protein synthesis. This stage of the virus life cycle will be covered in some detail from Part II to Part IV.

3.4 EXIT

Exit can be divided into three steps: capsid assembly, release, and maturation.

3.4.1 Capsid Assembly

The capsid assembly follows as the viral genome as well as the viral proteins abundantly accumulates. The capsid assembly can be divided into two processes: capsid assembly and genome packaging. Depending on viruses, these two processes can occur sequentially or simultaneously in a coupled manner. Picornavirus is an example of the former, while adenovirus is an example of the latter (Fig. 3.8). In the case of picornavirus, the capsids (ie, immature capsid or procapsid) are assembled first without the RNA genome. Subsequently, the RNA genome is packaged or inserted via a pore formed in the procapsid structure. By contrast, in the case of adenovirus, the capsid assembly is coupled with the DNA genome packaging. Then, a question that arises is how does the virus selectively package the viral genome? A *packaging signal*,⁹ a *cis*-acting element present in the viral genome, is specifically recognized by the viral capsid proteins, which selectively package either RNA or DNA.

3.4.2 Release

For naked viruses, the virus particles are released via cell lysis of the infected cells. Thus, no specific exit mechanism is necessary, because the cell membrane that traps the assembled virus particles are dismantled. Examples of naked viruses are polyomavirus (ie, SV40) and adenovirus. By contrast, in cases of enveloped viruses, *envelopment*, a process in which the capsids become surrounded by lipid bilayer, takes place prior to the release. With respect to the relatedness of the capsid assembly to the envelopment, two mechanisms exist. First, the envelopment can proceed after the completion of capsid assembly (Fig. 3.9A). In this sequential mechanism, the fully assembled capsids are recruited to the membrane by interaction of the viral capsids with viral envelope glycoprotein. Examples of this include herpesvirus and hepatitis B virus. Alternatively, the envelopment can occur simultaneously with the capsid assembly (Fig. 3.9B). Retrovirus is the representative of this coupled mechanism.

On the other hand, regarding the membrane for envelopment, two cellular membranes are exploited. The plasma membrane is the site of envelopment for some viruses, such as retrovirus and influenza virus, whereas endosomes, such as endoplasmic reticulum (ER) and Golgi bodies, are the site of envelopment for others, such as herpesvirus (see Fig. 9.11) and hepatitis B virus (see Fig. 18.4).

Then, how are the viruses released from the infected cells? Most enveloped viruses are released extracellularly via *exocytosis*¹⁰; often, this process is also called *budding*, as an analogy of buds in plants. Via budding, the envelopment

9. **Packaging signal** A sequence element in the viral genome that is essential for the genome packaging.

10. **Exocytosis** The process in which a cell directs the contents of secretory vesicles out of the cell membrane into the extracellular space.

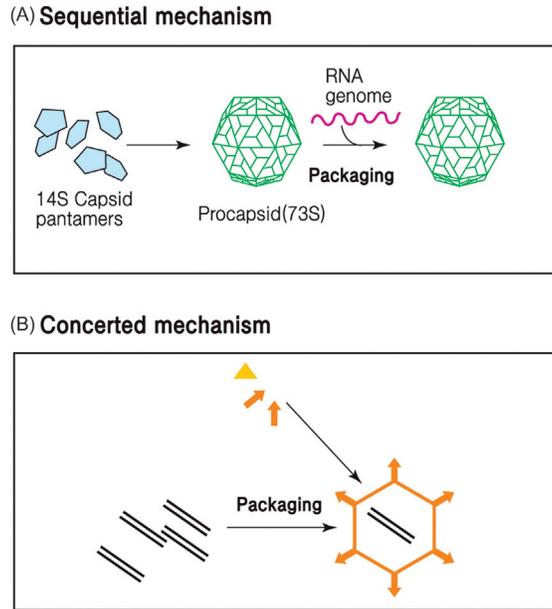


FIGURE 3.8 Relationship between capsid assembly and genome packaging. (A) Sequential mechanism. For picornavirus, the procapsid, a precursor of the capsids, is preassembled without RNA genome. Subsequently, the RNA genome penetrates into the procapsid via a pore. (B) Coupled mechanism. For adenovirus, the DNA genome is packaged into the capsid during capsid assembly.

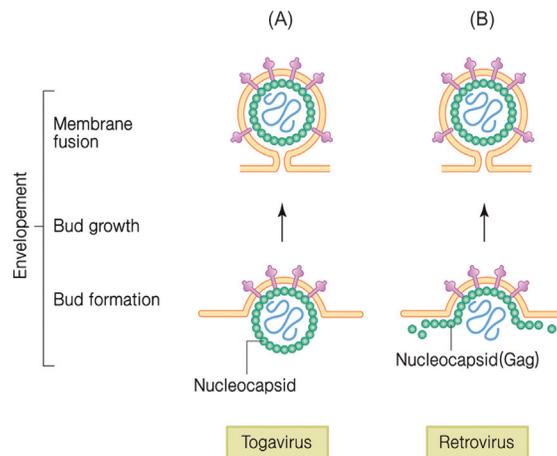


FIGURE 3.9 Relationship between capsid assembly and envelopment. (A) Sequential mechanism. The capsid assembly occurs prior to the envelopment. The assembled capsid is then targeted to the membrane for envelopment. Togavirus constitutes a family of positive-strand RNA viruses (see Table 13.2). (B) Coupled mechanism. Capsid proteins and the viral genome are recruited together to the budding site on the membrane. Capsid assembly and the envelopment of the capsid proceeds simultaneously. The envelopment process can be divided into three steps: a bud formation, a bud growth, and finally membrane fusion.

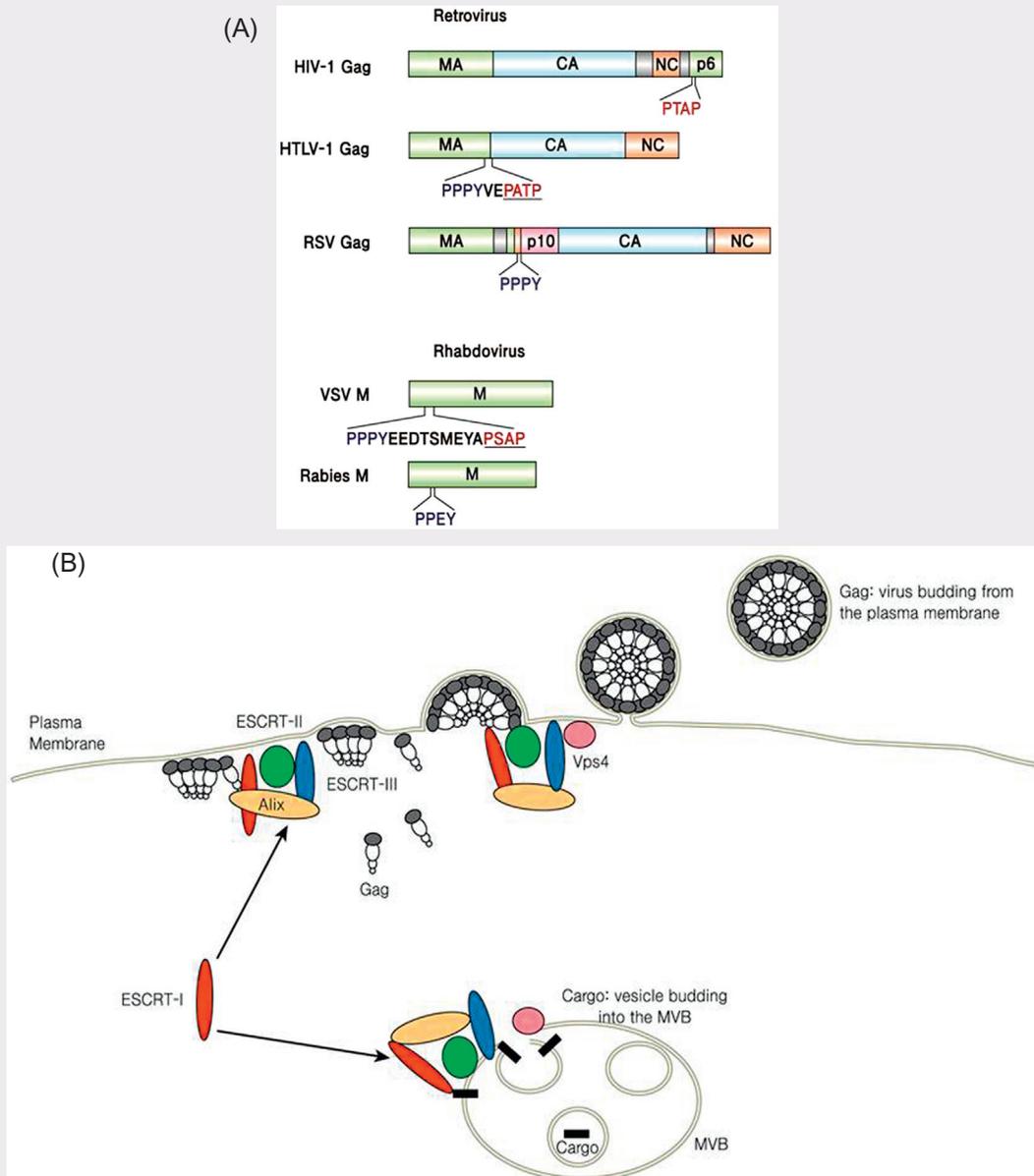
proceeds in a linked manner with extracellular release. Then a question that arises is how mechanistically is budding triggered? The clue for this was revealed by the identification of a peptide motif termed *late (L) domain*,¹¹ which is instrumental in triggering the budding process (Box 3.3). For instance, the retroviral Gag protein encodes “PTAP” motif as a late domain. Briefly, Gag protein, via its late domain, recruits cellular factors involved in the *multivesicular bodies*¹² (MVBs) pathway and subverts the MVB pathway for budding. After all, it is intriguing to learn how viruses exploit cellular mechanisms to produce their own progeny extracellularly.

11. **Late domain** A peptide motif (four amino acid), that involves in the budding of enveloped viruses. It is composed of four amino acids such as “PTAP” or “PPXY” residues (see Box 3.3).

12. **Multivesicular bodies (MVBs)** An intracellular structure that is generated by the inward vesiculation in late endosomes. MBV plays a large role in the transport of ubiquitinated proteins and receptors to a lysosome.

BOX 3.3 Late Domain

Late domain, which was first discovered in the Gag polyproteins of retroviruses and M (matrix) proteins of rhabdoviruses, is involved in the budding process of the enveloped viruses. It is composed of four amino acid residues (ie, PTAP, PPPY, and PPEY) encoded in either the rhabdovirus matrix protein or the p6 subdomain of HIV Gag polyprotein (panel A). Intriguingly, a substitution mutation of the late domain motif results in virions attached to the plasma membrane without being released, as if the viral release is blocked at a late stage of budding, a phenotype that is reflected in the nomenclature. It was found that the late domains are involved in recruiting cellular factors involved in the MVBs pathway (panel B). For instance, PTAP motif is critical for the interaction of Gag polyprotein with *Tsg101*,¹³ which is a component of *ESCRT-I*.¹⁴ Importantly, the budding of retrovirus particles is topologically equivalent to MVB biogenesis: in both cases budding is directed away from the cytoplasm. In other words, the retroviral late domains mediate the viral egress from the plasma membrane by coopting the cellular machinery for MVB biogenesis.



Late domain and its function in MVBs pathway. (A) Three kinds of L domain found in enveloped viruses such as retroviruses (ie, HIV, HTLV-1, and RSV) and rhabdoviruses (ie, vesicular stomatitis virus and rabies virus): PTAP motif, PPPY motif, and PPEY motif. A (alanine), E (glutamic acid), P (proline), T (threonine), and Y (tyrosine). (B) L domain and ESCRT complexes involved in MVBs pathway. The budding of retroviral Gag is facilitated by ESCRT complexes, which are normally involved in the MVB pathway. In HIV, Gag interacts with *Tsg101* and Alix, an adapter protein, leading to recruitment of the additional components of the MVB pathway, that is, ESCRT-II (green), ESCRT-III (purple), to assemble into a functional complex. Vps4 (red) is involved in recycling the MVB machinery.

13. **Tsg101 (tumor suppressor gene)** Vps23p, the yeast ortholog of *Tsg101*, is a component of the ESCRT-I complex, which is involved in the MVB pathway.

14. **ESCRT (endosomal sorting complex required for transport)** ESCRT machinery is made up of cytosolic protein complexes referred to as ESCRT-0, -I, -II, and -III. Together with a number of accessory proteins, these ESCRT complexes enable a unique mode of membrane remodeling that results in membranes bending/budding away from the cytoplasm.

3.4.3 Maturation

The last step of the virus particle assembly is “maturation,” a process that occurs extracellularly following release. For picornavirus and retrovirus, maturation is an essential step to acquire infectivity. In case of retrovirus, the cleavage of the Gag polyprotein by the viral PR protein (aspartate protease) occurring in the released virion is accompanied with a considerable morphological transition such as the condensation of the capsid structure (see Fig. 17.10). Importantly, such a maturation process confers the particle its infectivity.

3.5 TYPES OF VIRUS INFECTION

Above, we learned the steps involved in the virus life cycle, starting from attachment to target cells to progeny production. However, the virus life cycle is not always fully executed, because the invading virus encounters many obstacles, such as host immune response and host factors, that restrict the viral propagation. Depending on whether a progeny virus is produced or not, virus infection can be divided into “productive infection” or “nonproductive infection,” respectively (Fig. 3.10). *Productive infection* refers to a successful execution of the virus infection that leads to the production of progeny virus. Productive infection includes *lytic infection* and *persistent infection*. Specifically, lytic infection produces a progeny virus via cell lysis, thus the virus genome replication cannot persist (eg, adenovirus and influenza virus). In contrast, persistent infection continues to produce a progeny virus for a long period either without cell death [eg, hepatitis B virus and hepatitis C virus (HCV)] or with cell death but leaving long-lasting reservoir cells (eg, HIV) (see Fig. 22.9).

On the other hand, nonproductive infection refers to the type of virus infections that do not lead to the production of a progeny virus. Nonproductive infection includes *latent infection*, *transforming infection*, and *abortive infection*. Latent infection (eg, herpesvirus and HIV) maintains the viral genomes stably in the infected cell without producing a progeny virus. However, a progeny virus can be produced upon the activation of latently infected cells. Transforming infection (eg, human papillomavirus) harbors the viral genome as a chromosomally integrated form without producing a progeny virus. Instead, the infected cells are transformed to cancerous cells. In addition, virus infection is not always successfully executed. For instance, the viral genome replication may not occur after entry to target cells due to strong host immune response or host restriction factors. This type of infection is termed “abortive infection”.

In addition, depending on the clinical symptoms, there are a few other types of infections. *Symptomatic infection* refers to a viral infection with clinical symptoms, whereas *asymptomatic infection* refers to a viral infection without any clinical symptoms. In fact, asymptomatic infection is not uncommon. In poliovirus infection, only one out of

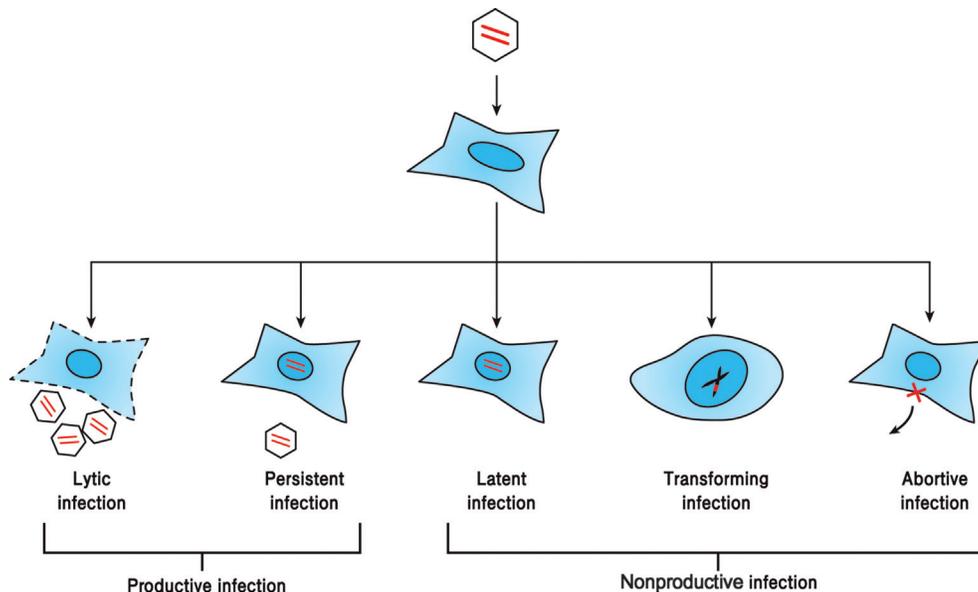


FIGURE 3.10 Types of virus infection. Five types of virus infections are illustrated with emphasis on the progeny virus production and the state of the viral genome (red). The virus life cycle including viral genome replication is fully executed in productive infection, while the virus life cycle is not fully executed in nonproductive infection. The type of virus infection is determined by the intricate interplay between virus and host interaction.

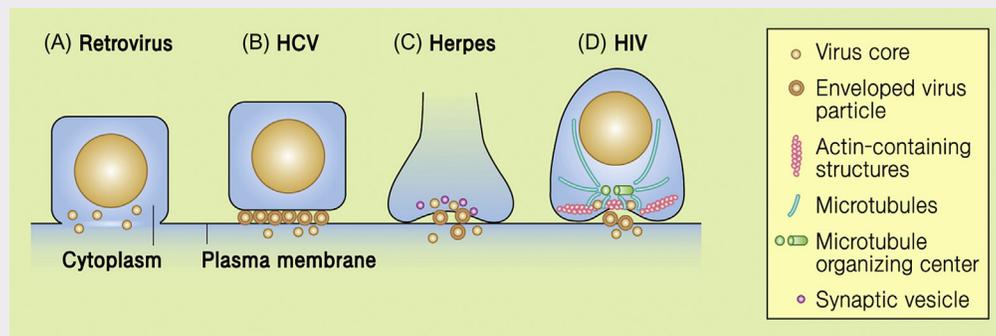
approximately 200 infected people manifests clinical symptoms. In this case, the viral life cycle is executed in a limited way, but an infected individual often ends up having antibodies. Thus, this type of infection is also called *inapparent infection*.

3.6 PERSPECTIVES

Viruses, being intracellular parasites, rely on hosts for their propagation. Through evolution, viruses have acquired the abilities to subvert host functions to comply with their needs. In this regard, similarities are notable with respect to entry, penetration, assembly, and exit stages of the virus life cycle. In fact, many steps in the virus life cycle have been extensively studied in the past three decades. Nevertheless, some novel steps in virus life cycles have only begun to be unraveled. In particular, cell–cell transmission (Box 3.4) is one novel mechanism that draws significant attention,

BOX 3.4 Cell–Cell Transmission

It was believed that viruses could infect neighboring cells only by being released extracellularly from the infected cells. In contrast to this belief, a novel mechanism has been described, in which a virus could infect the neighboring cell without being released. This new mode of infection is termed cell–cell transmission or cell-to-cell spread. Four distinct modes of cell–cell transmission mechanisms have been described. First, cell–cell transmission is mediated by plasma membrane fusion between two cells. The viral capsids are transmitted from an infected cell to uninfected cells without being enveloped. This mode of cell–cell transmission is described in retrovirus and herpesvirus. Second, cell–cell transmission occurs across a *tight junction*.¹⁵ The virus exits basolaterally from an infected cell and is trapped between the infected and uninfected cell membranes at the tight junctions. Using viral entry receptors on the target cell, virions enter the uninfected target cells. This mode of cell–cell transmission is described in herpesvirus and HCV. Third, cell-to-cell spread occurs across a neural synapse. Virions, either mature or incomplete (naked core), assemble in either the postsynaptic or presynaptic cell depending on the virus, and either bud through the membrane into the synaptic space or are released from synaptic vesicles into the cleft. Virions then either fuse directly with the opposing synaptic cells or are endocytosed. Rhabdovirus, herpes viruses, and paramyxoviruses move across neural synapses. Fourth, cell–cell transmission occurs across a virological synapse. Immune cells can be polarized via cell contact, which is termed an *immunological synapse*.¹⁶ Likewise, an infected cell can polarize viral budding toward the receptor-expressing target cell in a structure called a “virological synapse.” Virions bud from the infected cell into a synaptic cleft, from which they fuse with the target cell. HIV and HTLV-1, lymphotropic retrovirus, are examples for this mode of viral transmission.



Four modes of cell-to-cell spread. (A) Via plasma membrane fusion. (B) Across tight junction. (C) Across a neural synapse. (D) Across a virological synapse.

(Continued)

15. Tight junction Tight junctions are the closely associated areas of two cells whose membranes join together forming a virtually impermeable barrier to fluid. They help to maintain the polarity of cells by preventing the lateral diffusion of integral membrane proteins between the apical and lateral/basal surfaces, allowing the maintenance of specialized functions of each surface.

16. Immunological synapse An immunological synapse is the interface between an antigen-presenting cell or target cell and a lymphocyte, such as an effector T cell, which is named as an analogy to a neural synapse.

BOX 3.4 (Continued)

What is the advantage of cell–cell transmission? It facilitates the viral spread from infected cell to uninfected neighboring cells via direct contact without diffusion. More importantly, the viruses associated with cells are physically protected from neutralizing antibodies. In fact, cell–cell transmission was uncovered by an experiment characterizing the neutralizing antibodies-resistant viral transmission. Notably, cell–cell transmission was found only in enveloped viruses, but not in naked viruses. Perhaps, cell–cell transmission is useless for naked viruses, where a bulk of virion is abruptly released upon cell lysis.

because of its implication in viral pathogenesis. It is hoped that our better understanding on cell-to-cell spread could be exploited for the treatment of chronic viral diseases, such as AIDS and viral hepatitis.

3.7 SUMMARY

- *Virus life cycle*: Virus life cycle can be divided into three stages: entry, genome replication, and exit. Entry can be subdivided into attachment, penetration, and uncoating. Exit can be subdivided into virion assembly and release.
- *Attachment*: Two kinds of molecules on cell surface are involved: attachment factors and viral receptors. Glycoaminoglycans, such as heparins, act as attachment factors for many viruses, while membrane proteins that belong to immunoglobulin superfamily act as cellular receptors for the viral entry.
- *Penetration*: Receptor-mediated endocytosis is exploited for the entry of most viruses. Alternatively, direct fusion is used for some viruses.
- *Cytoplasmic trafficking*: Following penetration, microtubule-mediated transport is used to deliver the virus particle to appropriate sites in the cell.
- *Exit*: Naked viruses exit cells via cell lysis, while enveloped viruses exit cells via budding through cellular membranes.

STUDY QUESTIONS

- 3.1** Describe three distinct strategies that could be exploited for the discovery of virus receptors for entry. Compare the pros and cons of three strategies. How would you validate the biological function of the newly identified receptors?
- 3.2** The viral genome needs to get to the nucleus for the virus that replicates in the nucleus. In other words, the viral nucleocapsid has to overcome two barriers (ie, plasma membrane and nuclear membrane). Compare and contrast the mechanisms by which the viruses penetrate the two membranes.
- 3.3** The consequence of virus infection depends on the interplay between host and virus. (1) List two types of productive infection and three types of nonproductive infection. (2) Describe to what extent the virus life cycle is executed.

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Highlight: During cell entry, capsids of incoming influenza viruses must be uncoated before viral ribonucleoproteins (vRNPs) can enter the nucleus for replication. After membrane fusion in late endocytic vacuoles, the vRNPs and the matrix proteins dissociate from each other and disperse within the cytosol. A question is how the vRNPs that disperse in the cytoplasm could make it to the nucleus. This paper revealed that influenza virus subverts “aggresome” formation machinery by mimicking misfolded protein aggregates by carrying unanchored ubiquitin chains.