

Virus Replication Cycles

Some human and animal viruses are grown in tissue culture cells *in vitro*. This researcher is seeding dishes of tissue culture cell monolayers. The monolayers are grown in a CO₂ incubator before the cells are infected with a laboratory strain of virus. Viruses that can be grown in cell cultures can be well characterized in the research laboratory.



“In the struggle for survival, the fittest win out at the expense of their rivals because they succeed in adapting themselves best to their environment.”

Charles Darwin

OUTLINE

4.1 One-Step Growth Curves

4.2 Key Steps of the Viral Replication Cycle

1. Attachment (Adsorption)
2. Penetration (Entry)
3. Uncoating (Disassembly and Localization)
4. Types of Viral Genomes and Their Replication
5. Assembly
6. Maturation
7. Release

4.3 The Error-Prone RNA Polymerases: Genetic Diversity

4.4 Targets for Antiviral Therapies

RNA Virus Mutagens: A New Class of Antiviral Drugs?

VIRUS FILE 4-1: How Are Cellular Receptors Used for Viral Attachment Discovered?

VIRUS FILE 4-2: Unraveling the Life Cycle of Mimivirus

VIRUS FILE 4-3: Stamping Down Flu Viruses

Refresher: Molecular Biology



CASE STUDY: HUMAN METAPNEUMOVIRUS

The campus day care was recently closed during the peak of the winter flu season because many of the young children were sick with a lower respiratory tract infection. An email announcement was sent to all students, faculty, and staff at the college that stated the closure was due to a **metapneumovirus** outbreak. The announcement briefed the campus community with information about human metapneumonoviruses (hMPVs).

The announcement stated that hMPV was a newly identified respiratory tract pathogen discovered in the Netherlands in 2001. New tests confirm that it is one of the most significant and common viral infections in humans. It is clinically indistinguishable from a viral relative known as respiratory syncytial virus (RSV). Both RSV and hMPV infections occur during the winter. hMPV may account for 2% to 12% or more of previously unexplained pediatric lower respiratory infections for which

samples are sent to diagnostic laboratories, and a lesser percentage in adults.

Both hMPV and RSV cause upper and lower respiratory tract infections associated with serious illness in the young, immunosuppressed, elderly, and chronically ill. Common symptoms include cough, fever, wheezing or exacerbation of asthma, and rhinorrhea (runny nose). It can produce severe enough symptoms to cause intensive care admission and ventilator support. Healthy adults can get a mild form of the disease that is characterized by a cough, hoarseness, congestion, runny nose, and sore throat.

Members of the campus day care staff were doing their best to limit the epidemic spread of the hMPV outbreak at the center. Primary care physicians need up-to-date knowledge and heightened awareness to recognize this new viral disease in patients.

In Chapter 2, you learned that viruses are dependent upon host cells for their reproduction, yet to do so viruses must overcome certain cellular constraints. Only those viruses that have been able to adapt to their hosts have been able to exist in nature. The previous chapter focused on taxonomy and structure of viruses and in summary stated that several different virus families cause similar viral disease syndromes.

This chapter focuses on experiments such as **one-step growth curves**, which are used to study virus–host interactions. These studies have provided information about the events that occur at each step of the infection cycle (**attachment, penetration, uncoating, replication, assembly, maturation, and release**), including the intricate details of the strategies that animal and human viruses use to express and replicate their diverse genomes.

4.1 One-Step Growth Curves

Virologists could not study animal and human viruses well in the laboratory before tissue culture methods were developed by John F. Enders, Thomas H. Weller, and Frederic C. Robbins in the late 1940s. Before their work, viruses were injected into animals and tissues were analyzed for the pathological signs of viral infection. Experimental

animals were difficult to work with and expensive to maintain. Another drawback was that animals were not very permissive to infection with human viruses due to the species barrier and the animal immune response.

Enders, Weller, and Robbins won the 1954 Nobel Prize in Physiology or Medicine for the cultivation of poliovirus in nonnervous tissue cultures (human embryonic skin and muscle cells). Their observations and procedures used to grow viruses *in vitro* contributed to the refinement of tissue culture techniques and played a monumental role in the development of vaccines against poliovirus in the 1950s (Salk vaccine) and 1960s (Sabin vaccine).

One-step or single-step growth curve experiments are used to study a single replication cycle of viruses. Max Delbrück developed the one-step growth curve experiment while using an *Escherichia coli*-T4 bacterial system, which gave faster experimental results than did traditional methods. With the advent of cell culture systems, these experiments were carried out with viruses that infect tissue culture cells. These experiments are performed in special cell culture facilities.

Briefly, **monolayers** (or cell suspensions in liquid medium) of tissue culture cells such as monkey kidney cells are allowed to adhere and form monolayers on the bottom of plastic dishes. The monolayers of cells are subsequently infected with the virus of choice. They are infected at a high **multiplicity of infection (MOI)** to ensure that

General Procedure: One-Step Growth Curves

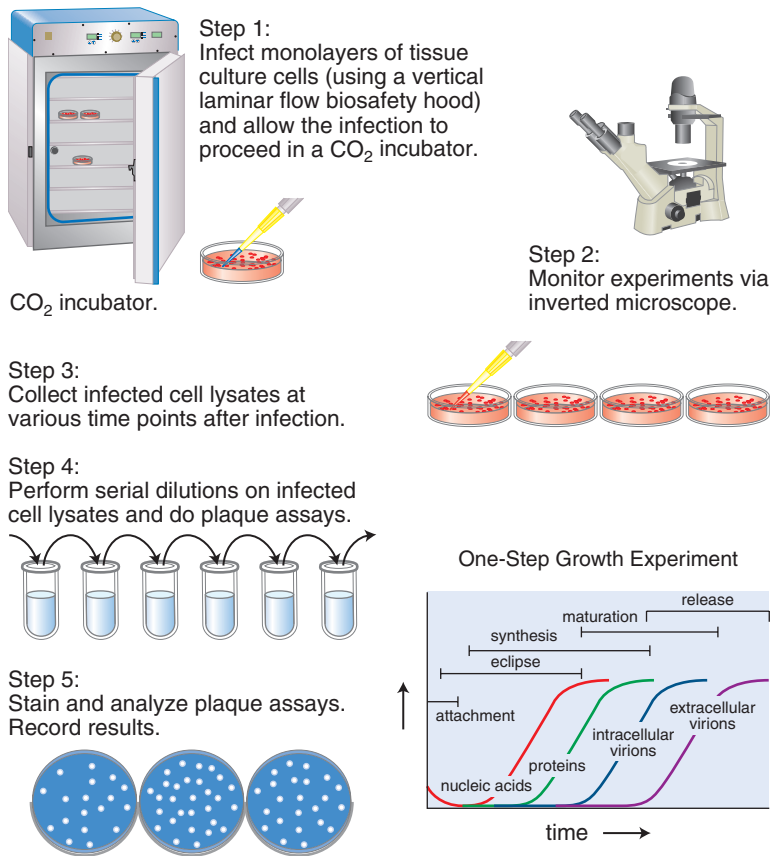


FIGURE 4-1 The diagram briefly outlines the steps involved in performing one-step growth experiments. Step 5 includes a photograph of viral **plaques** (clearings where the virus destroyed the cell monolayer). The plaque assay is a quantitative assay used to determine the number of viruses present in a given sample. The results of these assays can be used to generate a one-step growth curve for a particular virus. For more details about virological methods see Chapter 5, Laboratory Diagnosis of Viral Diseases and Working with Viruses in the Research Laboratory.

every cell of the monolayer is infected simultaneously. The MOI is the average number of viruses/cell. Hence, classic one-step growth experiments usually use an MOI of 10 (10 viruses/cell). The infected cells are maintained in CO₂ incubators and monitored throughout the course of infection. At various times during the infection, infected cells and/or tissue culture fluid are harvested and plaque assays are performed. **Plaque assays** are used to quantitate the number of intracellular or extracellular virus particles present during that point of infection. All viruses should be going through the same step in the viral replication cycle at the same time (**FIGURE 4-1**).

From these experiments, virologists have determined that there is a general pattern observed during the life cycle of a virus that distinguishes it from the life cycle of a bacterium. Shortly after the infection, the input or inoculated virus disappears. No virus particles are detected at this time. This is termed the **eclipse** period (**FIGURE 4-2b**). This continues until progeny viruses are detectable (anywhere from one to several hours or even days depending on the virus), which is termed the productive stage. Figure 4-2b illustrates that there is a lag phase in which few bacteria are detected but there is never a disappearance of bacteria observed

during the life cycle of a typical bacterium. The viral attachment, eclipse, and productive (maturation and release) stages will be discussed in detail as these key steps of viral replication are dissected in the following section.

4.2 Key Steps of the Viral Replication Cycle

1. Attachment (Adsorption)

The first step in the life cycle of a virus is attachment. The virus must be able to attach to its host and enter the “correct” or “target” cell. The attachment event is electrostatic and does not require any cellular energy. This step is a critical step in the viral replication cycle and a great target for antiviral therapies developed to prevent viral infections. If virus attachment is blocked, the infection is prevented. A virus is said to exhibit a tropism for a particular cell type when it targets and infects that cell type. In many cases these cell types are a specific population of cells within organs. **TABLE 4-1** lists examples of viruses and their cellular tropism. Sometimes viruses also display species

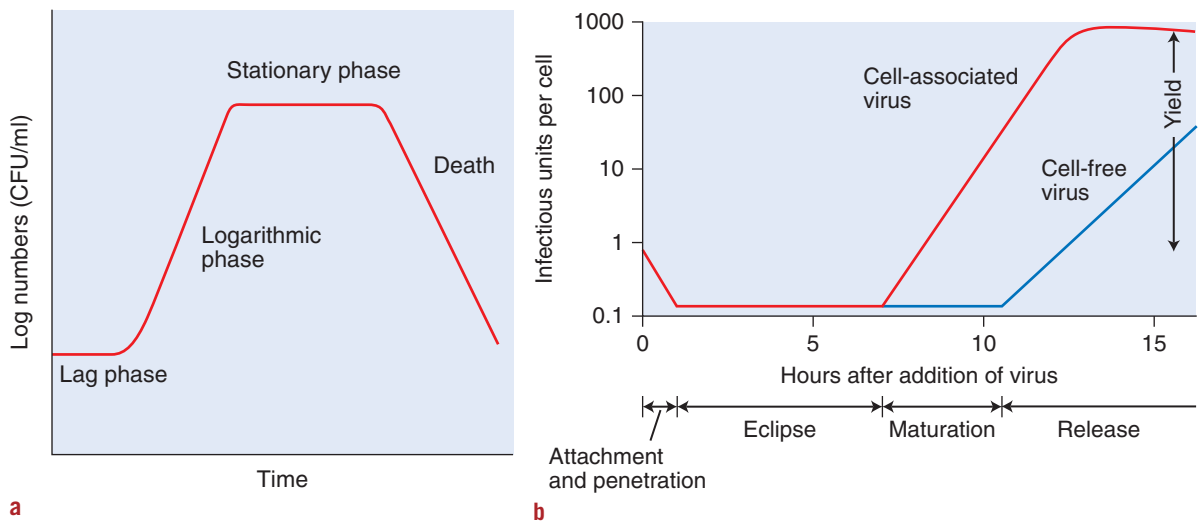


FIGURE 4-2 Typical bacterial growth versus a one-step growth curve of a naked virus. **(a)** Bacterial growth generally proceeds in a series of phases: lag, log (exponential growth in which the rate of multiplication is most rapid and constant), stationary, and death. Viruses require host cells for growth and reproduction. CFU/ml = colony forming units per milliliter. Modified from an illustration by H. Douglas Goff, Ph.D., University of Guelph. **(b)** Viruses are assembled from preformed “parts” when enough of the preformed parts have been made. Adapted from White, D. E., and Fenner, F. J. *Medical Virology*, Fourth Edition. Academic Press, 1994.

tropism. For example, poliovirus infects only primate cells.

Host range is a term that refers to the different types of tissue culture cells or organisms (species) that the virus can infect. The host range may be broad (infecting many different animals or cell lines of different species) or narrow. An example of a broad-range virus is rabies, for which all mammals have varying susceptibility. Human immunodeficiency virus (HIV), which infects humans and monkeys but causes disease only in humans, falls into the narrow range. Human viruses and animal viruses also have preferred routes of entry (e.g., influenza and rhinoviruses enter via the respira-

tory tract). Routes of entry and mechanisms for viral spread in the body are covered in Chapter 6.

In order to infect cells, the attachment proteins located on the outside of the virus must be able to bind to **cellular surface receptors**. Cellular receptors are usually proteins, glycoproteins, carbohydrates, or lipids. **TABLE 4-2** provides examples of viruses and their cellular receptor(s). *Viruses have evolved to use these receptors for attachment and entry to their hosts.* You may ask, “Why haven’t cells evolved to keep up with the evolution of viruses?” The answer is that viruses have evolved to use essential components of the cells as receptors. Without these essential components, the cell can’t exist. Cell surface receptors play important

TABLE 4-1

Viral Cell Tropism

Virus(es)	Cell Type
HIV	CD4+ T lymphocytes, macrophages
Rabies	Muscle, neurons
Human papilloma	Differentiating keratinocytes
Hepatitis A, B, C	Liver (hepatocytes)
Human herpes simplex 1 and 2	Mucoepithelium
Influenza A	Respiratory epithelium
Rotavirus	Intestinal epithelium
Norovirus	Intestinal epithelium
Cytomegalovirus	Epithelium, monocytes, lymphocytes
Rhinovirus	Nasal epithelium
Poliovirus	Intestinal epithelium
Epstein-Barr	B cell

TABLE 4-2

Cell Surface Receptors Used by Viruses to Attach and Enter Cells

Virus	Cell Surface Receptor
Influenza A	Sialic acid
HIV-1	CD4 and chemokine co-receptors (CXCR5, CCR4)
Hepatitis C	Low-density lipoprotein receptor
Rabies	Acetylcholine receptor, neural cell adhesion molecule, nerve growth factor, gangliosides, phospholipids
Rhinovirus	Intracellular adhesion molecule 1 (ICAM-1)
Hepatitis B	IgA receptor
Adenovirus Type 2	Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$
Poliovirus	Immunoglobulin superfamily protein (CD155)



Scientists have developed several techniques to identify cell surface receptors and co-receptors to which viruses attach in order to initiate infection. These approaches may be viral receptor-interference studies or genetic techniques. Parts (a)–(c) of **FIGURE VF 4-1** illustrate the general scheme of the various methods employed.

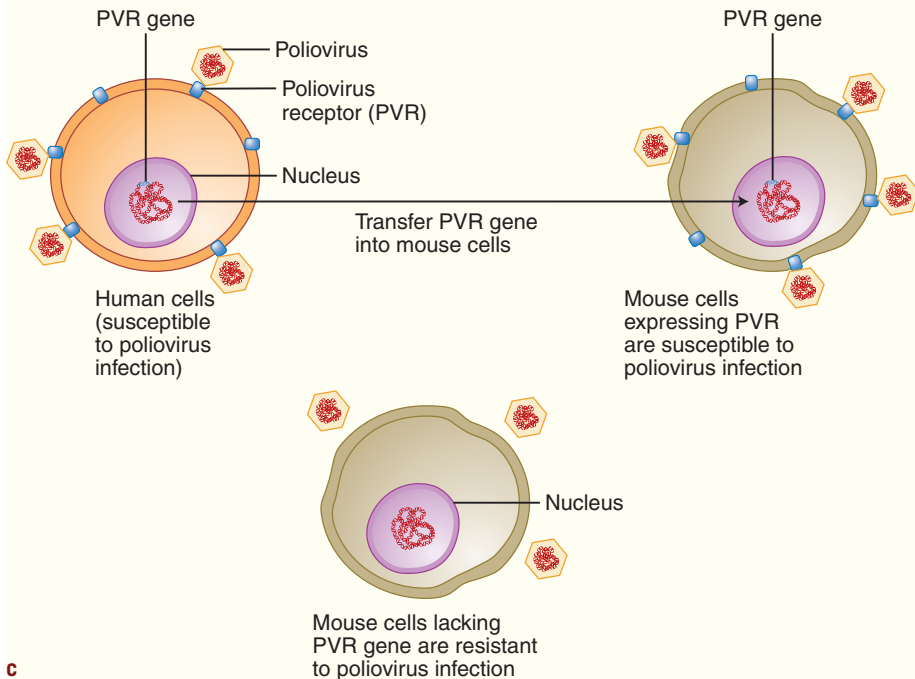
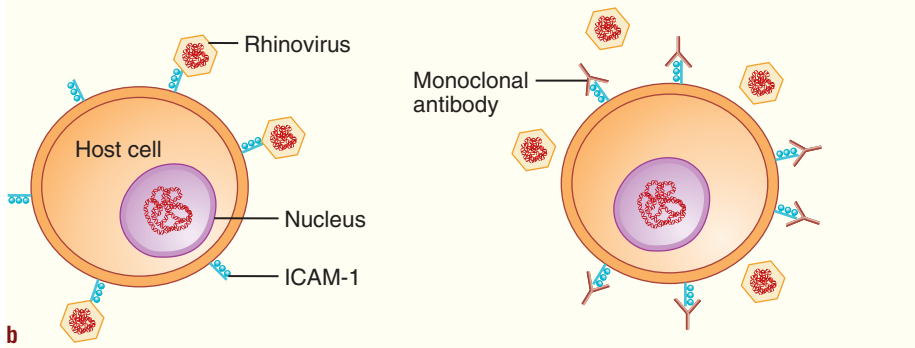
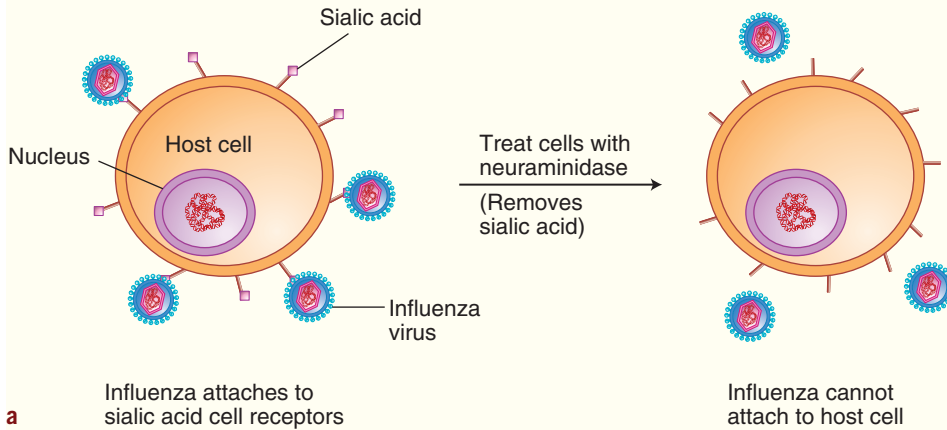


FIGURE VF 4-1 Identification of host cell receptors. **(a)** Removal of cell surface receptors. Adapted from Paulson, J. C., and Rogers, G. N. *Methods Enzymol* (1987):162–168. **(b)** Monoclonal antibodies block cell surface receptors. Adapted from Staunton, D. E., et al. *Cell* 56 (1989):849–853. **(c)** Gene-transfer experiments. Adapted from Mendelsohn, C., et al. *PNAS USA* 20 (1986):7845–7849.

roles in normal cellular activities. We do not know all of the cellular receptors for every virus; however, research continues in this area of host–virus interactions.

There are several factors that may influence the efficiency of viral attachment, such as the density of receptors present on the host cell surface, the density of the **ligands** on the viral surface, and the concentrations of virus and host cells. Temperature, pH, and the presence or absence of specific ions may also play a role in the efficiency of attachment. For some viruses, such as poliovirus, rhinovirus, and influenza virus, a single type of cellular receptor is sufficient for virus attachment. In other cases, including HIV Type 1 (HIV-1) and adenoviruses, one type of cellular receptor is required for the initial attachment and attachment to a **co-receptor** is necessary for viral entry into the cell.

■ 2. Penetration (Entry)

After the animal or human virus attaches to a cellular receptor, it must cross the lipid bilayer plasma membrane (or in some cases the nuclear membrane) of the host cell. Activity at the surface of cellular membranes is dynamic and these membranes are constantly being recycled. **Clathrin**, which is a large, fibrous protein, is instrumental in the formation of specialized regions of the cell membrane called **clathrin-coated pits**. These pits appear as invaginations that are coated with dark material, and are located on the cytoplasmic side of the membrane. The pits are short lived and soon bud off to form **clathrin-coated vesicles**. These vesicles are for transport and are coated with a latticelike network of clathrin. Shortly after formation, the clathrin coat is removed and the resultant vesicles are referred to as **endosomes**. Sometimes these vesicles contain viruses, which can penetrate directly at the plasma membrane or via endosomes. The virus particle must then disassemble to make the viral genome available in the cytoplasm, where it is targeted to the correct location in the cell for genome replication.

Enveloped Virus Entry

Enveloped viruses contain a lipid bilayer, or envelope, that surrounds the nucleocapsid. These viruses enter cells via fusion of the viral and cellular membranes. This process is driven by the viral glycoproteins located on the viral surface. The two basic modes of entry of an enveloped human/animal virus are by **ligand-mediated fusion** of the virus and the cellular plasma membrane or by **receptor-mediated endocytotic entry** of an enveloped virus (**FIGURE 4-3a**). (In

ligand-mediated fusion it is the viral ligand rather than the host receptor that mediates the fusion event.)

In ligand-receptor-mediated fusion, the virus attaches to the plasma membrane of the cell and fusion takes place between the viral and cellular membranes. The **nucleocapsid** of the virus is released inside of the cell. The remaining viral envelope remains as a “patch” on the cellular plasma membrane (**Figure 4-3a**). The fusion at the plasma membrane mode of penetration is **pH independent**.

In receptor-mediated endocytosis (engulfment), the enveloped virus attaches to a receptor on the plasma membrane of the cell and the cell is stimulated to engulf the entire virus, thus forming an endocytotic vesicle (**Figure 4-3b**). This endocytotic vesicle may fuse with the lysosomes, which possess an internal acidic pH. In the acidic pH of the endocytotic vesicles, conformational changes in the viral envelope proteins facilitate the fusion of the viral membrane with the endocytic membrane and the subsequent release of the viral nucleocapsid into the cytoplasm. This mode of viral penetration is **pH dependent** because it is only at an acidic pH that the fusion between the viral envelope and the host cell membrane occurs (**Figures 4-3b and 4-3c**).

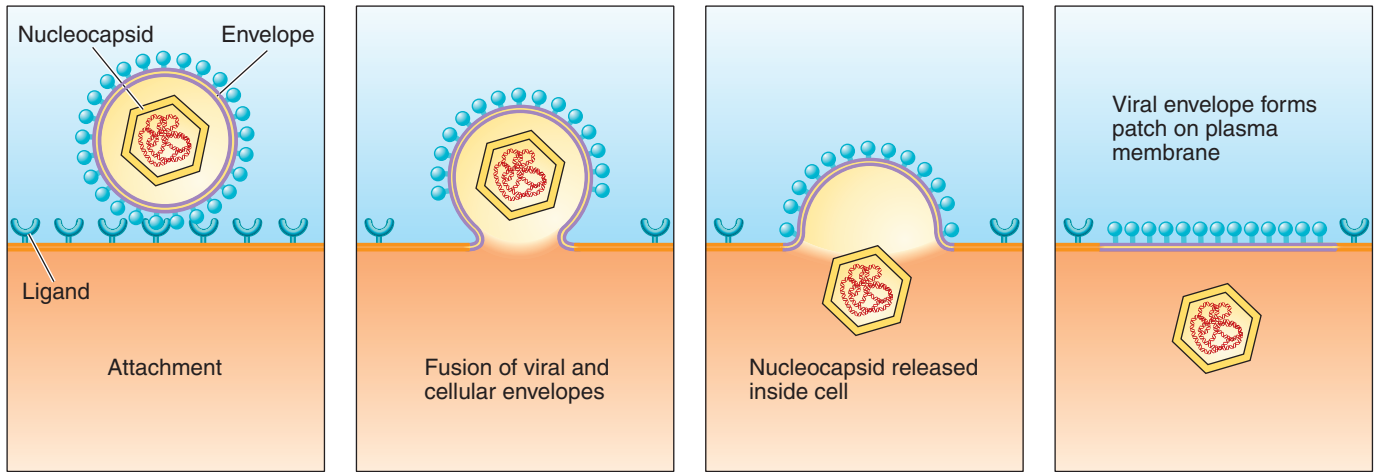
Naked Virus Entry

It is more difficult to envision how naked or non-enveloped viruses cross the cellular membrane, and much remains to be understood about how these viruses enter cells. Studies suggest that the majority of naked viruses enter via receptor-mediated endocytosis. The virus ligand–cell surface receptor interaction causes a clathrin-coated pit formation/invagination at the cell surface. The clathrin-coated pits encase the virus and bud off to form a clathrin-coated vesicle. Within seconds this clathrin coat is shed and the vesicle containing the virus fuses with lysosomes. The low pH, along with proteases in this endocytic vesicle, disassociate the capsid, releasing the nucleic acid genome of the virus into the cytoplasm. **FIGURE 4-4** demonstrates this type of entry.

■ 3. Uncoating (Disassembly and Localization)

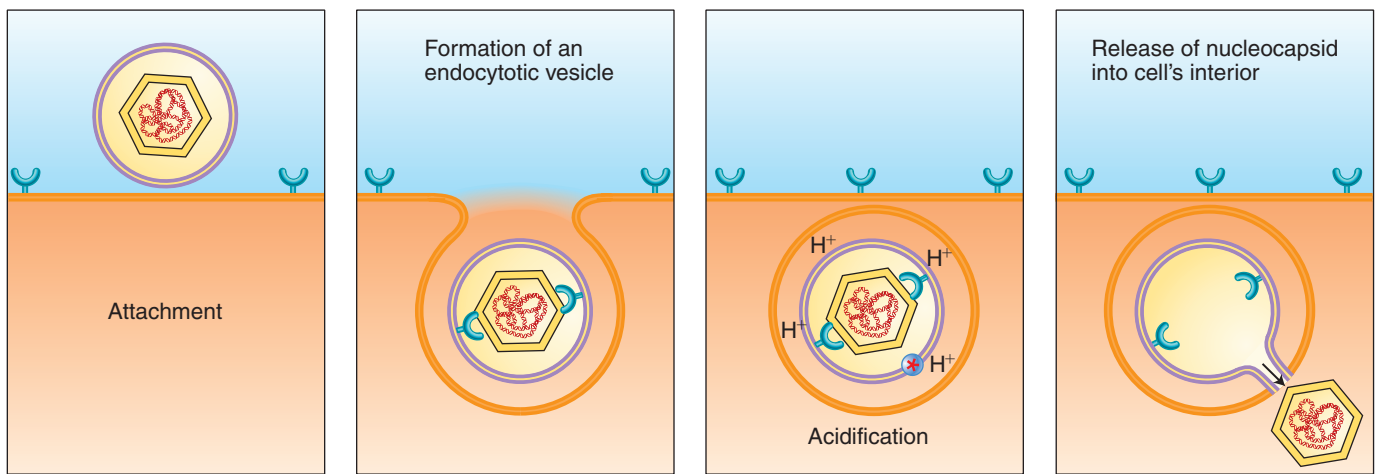
This step refers to the removal or degradation of the capsid (uncoating), thereby releasing the genome into the host cell. The genome is transported to the site where transcription/replication can begin. In some viruses there is no degradation of the capsid because the capsid proteins play a role in viral transcription and replication. For these viruses uncoating refers to changes in the nucleocapsid that make it ready for transcription and/or replication.

Receptor-mediated fusion of an enveloped virus with the plasma membrane

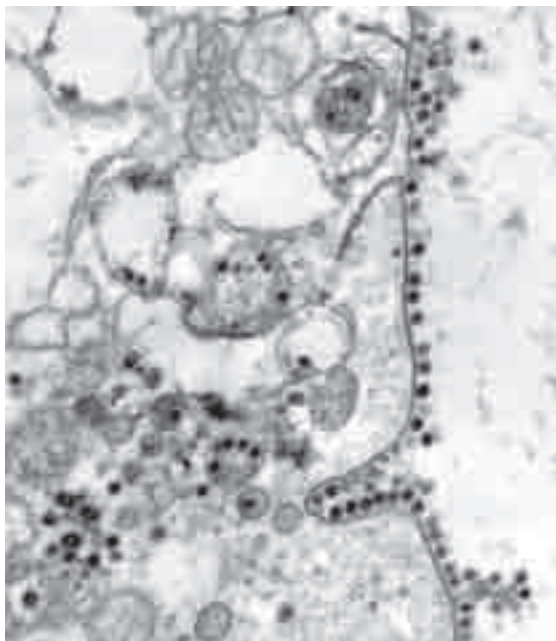


a

Receptor-mediated endocytotic entry of an enveloped virus



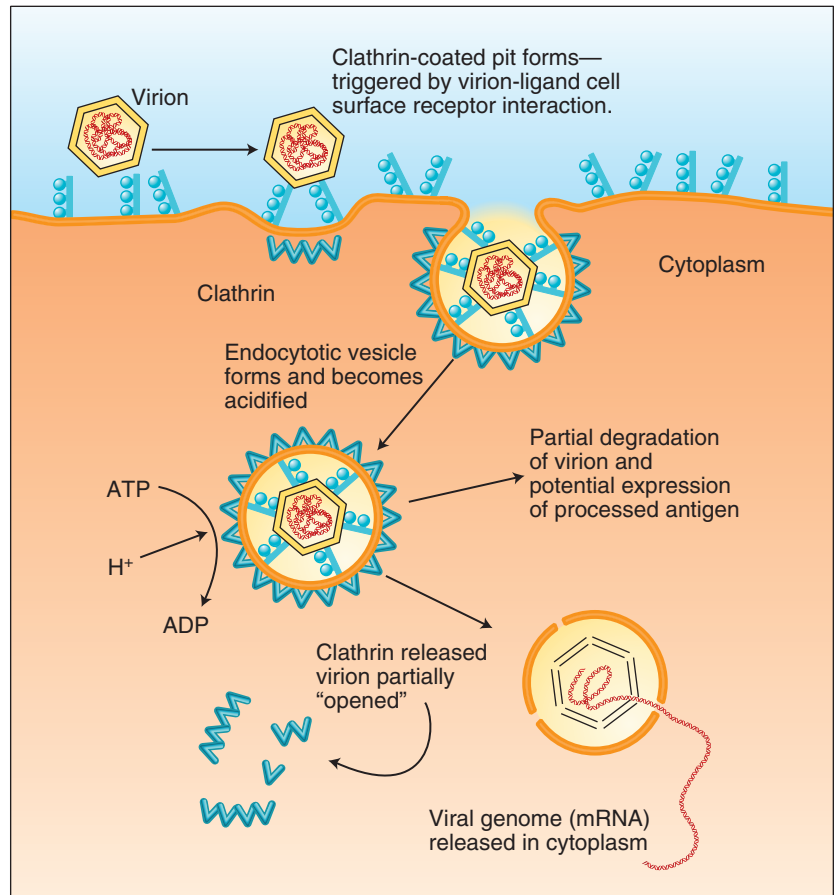
b



c

FIGURE 4-3 (a) Viral entry steps in receptor-mediated fusion. Adapted from Wagner, E. K., and Hewlett, M. J. *Basic Virology*, Second Edition. Blackwell Publishing, 2003. (b) Viral entry steps in a receptor-mediated endocytotic entry of an enveloped virus. Adapted from Wagner, E. K., and Hewlett, M. J. *Basic Virology*, Second Edition. Blackwell Publishing, 2003. (c) Electron micrograph of mouse hepatitis viruses (family Coronaviridae) are absorbed into mouse intestinal cells via receptor-mediated endocytosis. The plasma membrane is invaginated and will release the viruses inside the cell. Magnification 40,000x.

FIGURE 4-4 Steps that naked viruses use to enter cells. Adapted from Wagner, E. K., and Hewlett, M. J. *Basic Virology*, Second Edition. Blackwell Publishing, 2003.



The uncoating step may occur simultaneously with penetration or it may immediately follow penetration of the virus into the host cell. It is a necessary step before replication of the genome can occur. When the nucleic acid genome is uncoated, infectious particles are no longer detected in one-step growth experiments. This is the start of the eclipse phase, which continues until new infectious virus particles are made (see Figure 4-2b).

4. Types of Viral Genomes and Their Replication

When viruses infect cells, two important and separate events must occur:

- the production of virus structural proteins and enzymes, and
- replication of the viral genome.

The genome of a virus may consist of DNA or RNA, which may be single stranded (ss) or double stranded (ds) and linear or circular (FIGURE 4-5). The entire genome may occupy either one nucleic acid molecule (**monopartite** or linear genome) or several nucleic acid molecules (**multipartite** or segmented genome). The different types of genome necessitate different replication strategies.

dsDNA Viruses

The genome replication of most RNA viruses occurs in the cytoplasm of the host. Presumably, this is because their replication is associated with RNA-dependent RNA polymerases that the host cell nucleus cannot provide. In contrast, most DNA viruses replicate their genomes in the nucleus and utilize the host's DNA and RNA synthesizing machinery, along with the host's RNA processing machinery. This means the viral genome must traverse the nuclear membrane to utilize the aforementioned cellular machinery (FIGURE 4-6).

Viral genomes

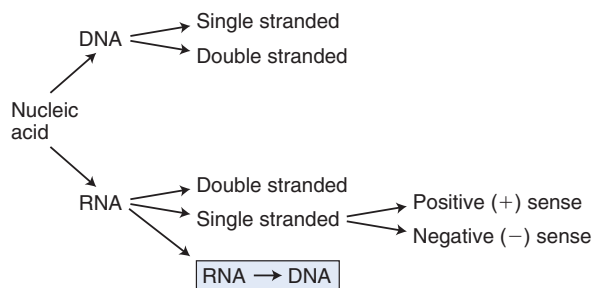


FIGURE 4-5 Types of viral nucleic acid genomes.

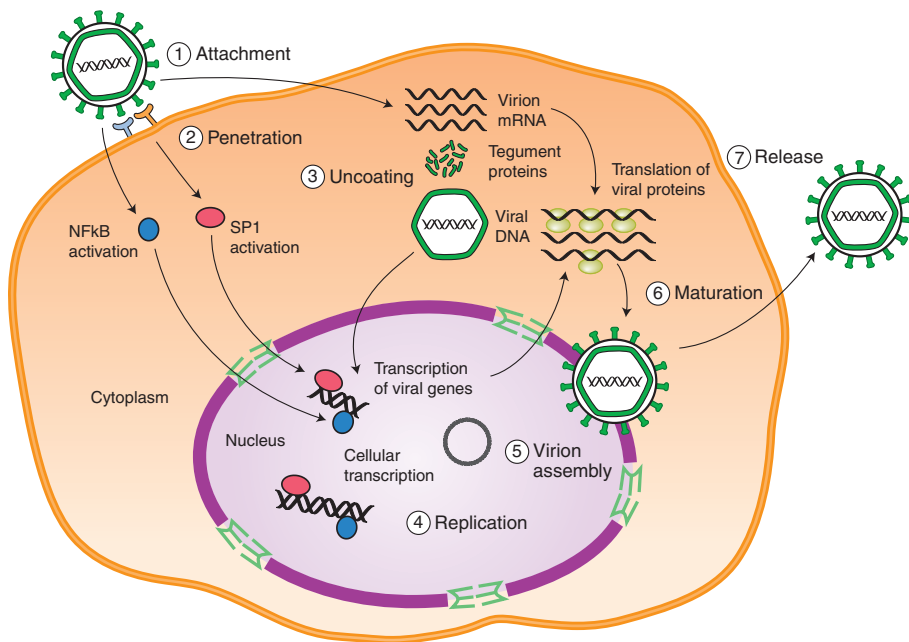


FIGURE 4-6 Example of a life cycle of a dsDNA virus (cytomegalovirus). Cytomegalovirus is a member of the Herpesviridae family. The gH and gB glycoproteins present on the outside of the cytomegalovirus particle bind to the cellular receptors. The attachment event triggers cellular transcription factors SP1 and NF-κB to migrate to the host cell nucleus. After the penetration and uncoating step, the viral cytomegalovirus dsDNA is released and enters the host cell nucleus where the DNA is replicated and transcribed with the help of host cell transcription factors SP1 and NF-κB. Viral mRNAs are exported into the cytoplasm where they are translated by the cellular machinery. Viral dsDNA, and viral and cellular proteins are packaged into the virion. The virion buds from the cell, gaining an envelope from the plasma membrane as the particle is released.

Replication of many DNA viruses involves strategies that are familiar in cell biology: DNA replication and mRNA transcription from dsDNA. Viral proteins are translated from the **monocistronic** mRNAs generated via transcription of viral mRNAs, as shown in **FIGURE 4-7a**, which also lists examples of dsDNA viruses, the diseases they cause, and the families to which they belong. Many DNA viruses have evolved ways to evade host defenses and can cause tumors in animals.

Papovaviruses and herpesviruses are dsDNA viruses and have the most straightforward replication strategy. These viruses utilize the cellular DNA-dependent RNA polymerase II located in the host's nucleus to transcribe the viral mRNAs from the dsDNA viral genome. The host cell must be cycling through the cell cycle for its DNA polymerase to be available for use by these DNA viruses. The viral RNA transcripts are spliced and cleaved via cellular machinery to produce monocistronic mRNAs that are exported into the cytoplasm and translated accordingly by the cell's translation machinery. The viral dsDNA is packaged, along with the necessary structural proteins and enzymes, resulting in the generation of the newly assembled progeny viruses.

Poxviruses differ from the other dsDNA virus families listed in Figure 4-7a in that they replicate solely in the cytoplasm. These viruses carry their own DNA-dependent DNA polymerase (to replicate the viral dsDNA genome) within the virus particle. The genomes of poxviruses are large (ranging from 130–230 kbp, or roughly 100–200 genes), allowing these viruses to be fully equipped with the genes to make them independent of the

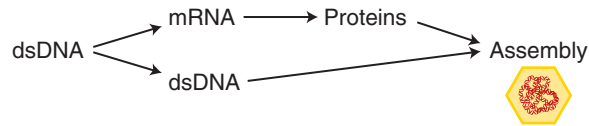
host's nuclear enzymes and machinery. The monocistronic mRNAs are transcribed directly from the viral dsDNA (Figure 4-7a). Refer to Chapter 14 for more details about poxvirus replication.

Six families of viruses comprise a group of viruses collectively designated Nucleo-Cytoplasmic Large DNA Viruses (NCLDV). These families of viruses include major pathogens of humans and other mammals as well as viruses that infect cold-blooded vertebrates, insects, and algal hosts. All of the viruses contain dsDNA genomes and replicate either exclusively in the cytoplasm of the host cells or possess both cytoplasmic and nuclear stages in their life cycle (**TABLE 4-3**).

Phycodnaviruses are members of the Phycodnaviridae family. The family name is derived from two distinguishing features: “phyco” from their algal hosts and “dna” because all of these viruses have dsDNA genomes. The phycodnaviruses are recognized as important ecological **virio**plankton in aquatic ecosystems. Virioplankton now represent the most abundant viruses in natural waters, surpassing the number of bacteria by an order of magnitude. The phycodnaviruses, along with other viruses, including bacteriophages, play roles in nutrient recycling (refer to Chapter 1, Viruses and Aquatic Ecosystems) and algal blooms. There are currently extensive **metagenomics** studies underway to determine aquatic **viromes**. Metagenomics is the genomic analysis of all DNA applied to entire communities of microbes and/or viruses, bypassing the need to isolate and culture individual microbes or viruses in the laboratory. A virome is the genomes of all viruses that inhabit a particular environment.

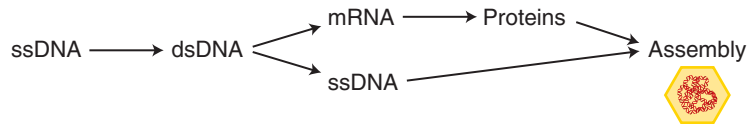
FIGURE 4-7 (a) List of dsDNA viruses and their replication strategy. Adapted from Harper, D. R. *Molecular Virology*, Second Edition. BIOS Scientific Publishers, 1999. **(b)** List of ssDNA viruses. Adapted from Harper, D. R. *Molecular Virology*, Second Edition. BIOS Scientific Publishers, 1999.

dsDNA Viruses		
Virus	Disease	Family
Herpes simplex Type 1 Type 2	Cold sores Genital herpes	Herpesviridae
Adenovirus	Respiratory infections	Adenoviridae
Cytomegalovirus	Infectious mononucleosis	Herpesviridae
Variola	Smallpox	Poxviridae
Human Papillomavirus *Types 16 and 18 *Types 6 and 11 *Types 1, 2, and 4	Cervical cancer Genital warts Plantar warts	Papovaviridae
*common types		



a

ssDNA Viruses		
Virus	Disease	Family
Human parvovirus B19	Fifth disease (slapped-cheek syndrome)	Parvoviridae
Transfusion transmitted virus (TTV)	Hepatitis?	Circoviridae



b

ssDNA Viruses

Parvoviruses are the smallest of the human viruses (only 20–25 nm in diameter). In contrast to the dsDNA virus genomes, these ssDNA viruses contain very small linear genomes (the genome of the human parvovirus B19 is 5 kb). Parvoviruses do not carry any enzymes in the virus particle. These viruses infect cells that are in the cell cycle because they are dependent upon the host's DNA polymerase to synthesize the viral ssDNA and the cell's DNA-dependent RNA polymerase II to transcribe

the viral dsDNA into viral mRNA in the nucleus. The cellular splicing machinery is also used in the production of the viral mRNAs. The general outline of their replication strategy is illustrated in Figure 4-7b.

ss/dsDNA Viruses (Using an RNA Intermediate)

Hepadnaviruses replicate via a very unique and somewhat complicated mechanism. This textbook focuses on hepatitis B virus (HBV) because it specifically infects humans. Other members of the Hepadnaviridae family infect woodchucks, ground

TABLE 4-3

Host Range and Replication Sites of Selected Families of the Eukaryotic Nucleo-Cytoplasmic Large DNA Viruses

Virus Family	Replication Site	Host Range
Ascoviridae	Nucleus and Cytoplasm	Insects, mainly noctuids (night-flying moths)
Asfarviridae	Cytoplasm	Mammals
Iridoviridae	Nucleus and Cytoplasm	Insects, cold-blooded vertebrates (reptiles, amphibians, and fish)
Mimiviridae	Cytoplasm	Acanthamoeba
Phycodnaviridae	Nucleus and Cytoplasm	Chlorella-like green algae, algal symbionts of paramecia and hydras
Poxviridae	Cytoplasm	Insects, mammals, birds, reptiles

squirrels, chipmunks, ducks, geese, chimps, gibbons, and orangutans. Interestingly, HBV-infected cells produce different forms of virus-related particles. Electron microscopy of partially purified virus particle preparations reveal three types of particles: a 42- to 47-nm mature spherical virus particle (known as **Dane particles**, named after their discoverer); 22-nm spherical particles, which are found in 10,000- to 100,000-fold excess over the Dane particle; and filamentous particles that are 22 nm in diameter and of varying lengths. All three forms contain the same surface protein, called the hepatitis B surface antigen (HbsAg). The Dane particle is the only infectious particle of HBV. The 22-nm spheres and filaments do not contain nucleic acid (**FIGURE 4-8**).

The genome of the Dane particles consists of a 3.2-kb linear DNA that is arranged in a relaxed circle. Some parts of the genome are dsDNA, whereas others consist of ssDNA regions or gaps. This partially duplexed DNA consists of a full-length (-) sense ssDNA and a shorter length (+) sense

ssDNA. As a result, the gapped regions contain only (-) sense ssDNA. After the HBV has entered its host cell and the virus is partially uncoated, the partial dsDNA genome of the Dane particle migrates to the nucleus, where it is completed or repaired by a viral reverse transcriptase. The dsDNA enters the nucleus and the ends are ligated by cellular enzymes, forming a circular **episome**. (The term episome applies to a viral genome that is maintained in cells by autonomous replication.) Next, the repaired viral dsDNA associates with cellular histones and is transcribed into separate viral mRNA transcripts and a full-length ssRNA pre-genome (**FIGURE 4-9**).

The viral mRNAs are translated to yield the hepatitis B core antigens and the viral reverse transcriptase. The RNA pre-genome associates with the viral reverse transcriptase and is packaged with the core proteins to form an **immature** virus particle in the cytoplasm of the cell. The viral **reverse transcriptase** synthesizes the (-) sense ssDNA strand using the ssRNA intermediate as a template (see *Refresher: Molecular Biology*, which

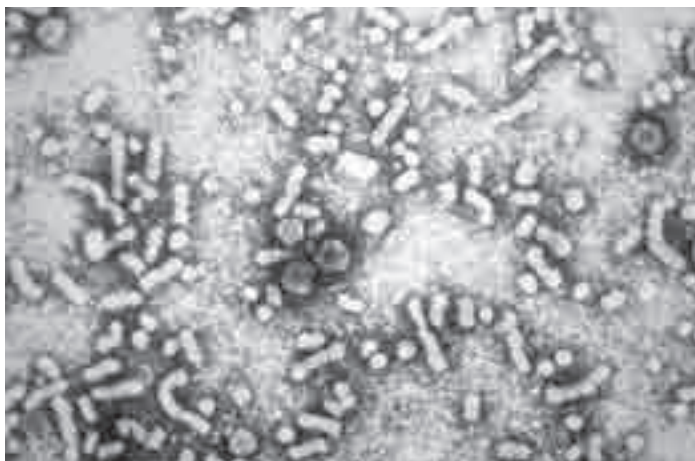


FIGURE 4-8 (a) HBV infection results in the formation of three different types of virus particles: 42- to 47-nm intact infectious Dane particles, 22-nm spheres, and 22-nm filaments of varying lengths. **(b)** Illustration depicting the different forms of HBV particles. Mature hepatitis B Dane particles contain dsDNA with associated protein, but their mode of replication is different from the other dsDNA viruses and their replication strategy. Adapted from University of South Carolina, School of Medicine. "Virology: Hepatitis Viruses." *Microbiology and Immunology*. University of South Carolina, 2008. <http://pathmicro.med.sc.edu/virol/hepatitis-virus.htm>.

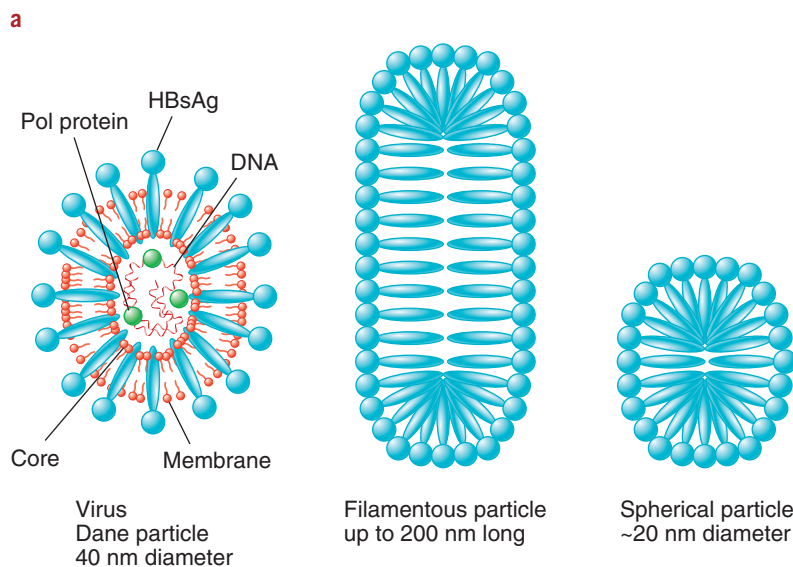
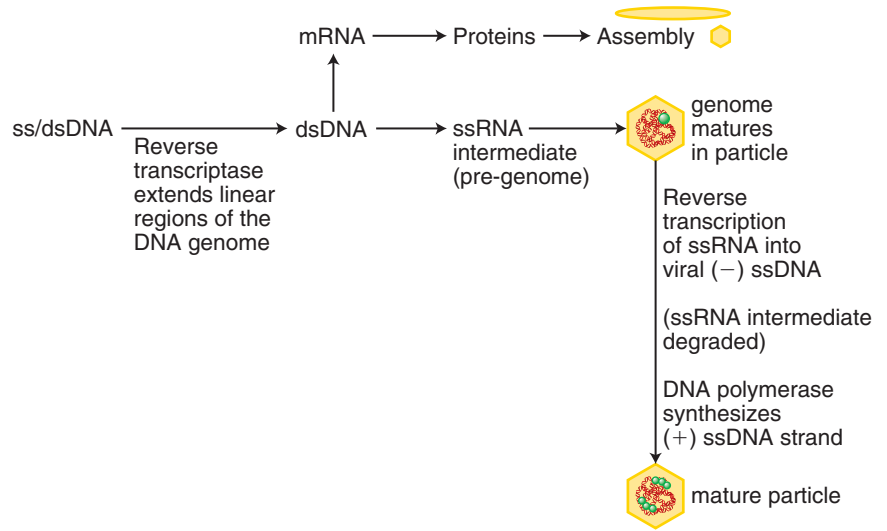
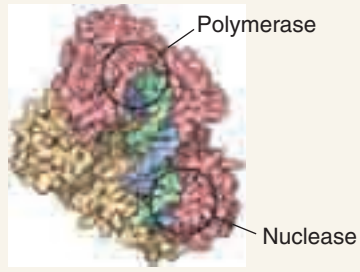


FIGURE 4-9 ssDNA/dsDNA virus (that uses ssRNA as an intermediate) and its replication strategy. Adapted from Harper, D. R. *Molecular Virology*, Second Edition. BIOS Scientific Publishers, 1999.

Virus	Disease	Family
Hepatitis B	Hepatitis associated with liver cancer	Hepadnaviridae

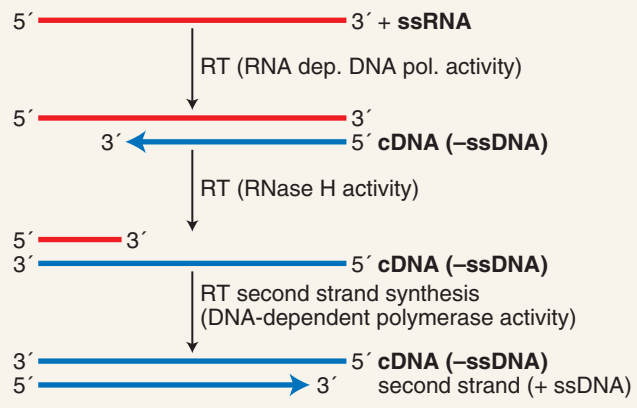


Refresher Molecular Biology



What is reverse transcriptase?
Reverse transcriptase (RT) has three distinct enzymatic activities:

1. RNA-dependent DNA polymerase
2. RNase H activity (cleaves/degrades RNA from RNA/DNA hybrids)
3. DNA-dependent DNA polymerase



Retroviruses and hepadnaviruses utilize RT in their life cycles.

FIGURE RB 4-1 Reverse Transcriptase Courtesy of David S. Goodsell, Scripps Research Institute. Protein Data Bank: 2hmi. Reproduced from J. Ding, et al., *J. Mol. Biol.* 284 (1998): 1095–1111.

is about reverse transcriptase functions). The pre-genome is degraded by the RNase H activity of the reverse transcriptase enzyme, but it leaves a short sequence of RNA at its 5' end that acts as a primer for DNA polymerase to synthesize a complementary (+) DNA strand in the **mature** particle.

Hepatitis B is one of a few known nonretroviral viruses that uses reverse transcription as part of its replication process. Other viruses that utilize reverse transcriptase are retroviruses such as human T-cell leukemia virus (HTLV) and HIV, which possess an RNA genome. For these retroviruses reverse transcription is one of the first steps in viral replication, whereas for hepatitis B reverse transcription occurs during maturation (the latter steps) in making new virus particles. In addition, in contrast to retroviruses, HBV does not have **integrase** activity. The DNA of hepatitis B is usually not integrated into cellular DNA; it is found as an independent episome. Integrated parts of the hepatitis B genome, however, are found in the chromosomes of hepatocellular tumors from cancer patients. Retroviruses have integrase activity (see Chapter 10).

RNA Viruses

RNA viruses are unique because their genetic information is encoded in RNA. The genomes of RNA viruses are diverse [ss or ds, (+) or (-) sense, linear or segmented]. The type of RNA genome determines if the first step after uncoating will be translation, transcription, or RNA replication.

Viruses that contain +ssRNA genomes have genomes that can be directly translated using the host cell machinery because the +ssRNA acts like an mRNA (**FIGURE 4-10**). These +ssRNA viruses, however, do need to carry the gene that encodes the replicase that produces the viral genomic RNA.

All other types of RNA viruses (-ssRNA, dsRNA, linear, segmented) must be transcribed into mRNA before translation can occur. Eukaryotic host cells do not contain RNA-dependent RNA polymerases (see Section 2.3, Molecular Constraints of the Host Cell), and as a result these viruses must carry an

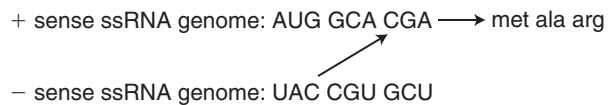


FIGURE 4-10 Differences between positive (+) and negative (-) sense ssRNA viral genomes.

RNA-dependent RNA polymerase that will synthesize the viral +ssRNA, mRNAs, and -ssRNA viral genomes into the host cell with them.

dsRNA Viruses

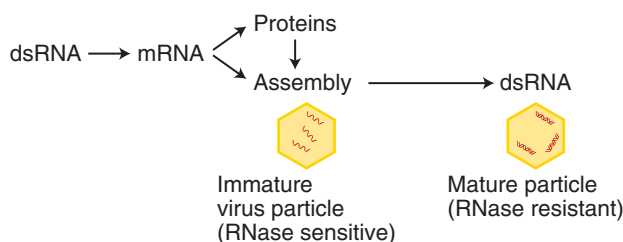
Rotaviruses have emerged as the main agent of acute gastroenteritis in infants and children worldwide. These viruses have dsRNA **segmented** genomes. The rotavirus particle contains 11 segments or pieces of the viral dsRNA. The host does not produce RNA-dependent RNA polymerases; thus, the virus carries its own RNA-dependent RNA polymerase and the replication cycle occurs solely in the cytoplasm.

A rotavirus particle is nonenveloped and icosahedral with a double-capsid. One of its two layers is removed but the other is not; the transcription takes place inside of this single capsid and the mRNAs are released in the cytoplasm for translation. After attachment, entry, and uncoating, the virus synthesizes a +ssRNA from each of the 11 dsRNA segments (using the -ssRNA strands of the dsRNA genome as a template) via a viral RNA-dependent RNA polymerase. These viral ssRNAs are also capped via a viral capping enzyme. The RNAs are not polyadenylated. Half of the newly synthesized capped +ssRNAs strands (mRNAs) are translated by the cellular machinery in the cytoplasm. The remaining strands are packaged into a viral capsid during assembly (**FIGURE 4-11**).

At this stage of the life cycle, the RNAs inside the particles are sensitive to RNase treatment. During maturation of the virus particle, the complementary -ssRNA strands are synthesized using the capped +ssRNAs as a template within the virus

Virus	Disease	Family
Rotavirus	Gastroenteritis	Reoviridae
Reovirus	Mild respiratory and gastrointestinal symptoms	Reoviridae

FIGURE 4-11 List of dsRNA viruses and their replication strategies. Adapted from Harper, D. R. *Molecular Virology*, Second Edition. BIOS Scientific Publishers, 1999.



4.2 Key Steps of the Viral Replication Cycle

particle to form the remaining dsRNA genomic segments (Figure 4-11). These final dsRNA segments are resistant to RNase treatment. There are still several remaining questions about the replication cycle of rotaviruses and other viruses of the Reoviridae family; for example, how does the virus particle manage to contain only one of copy of each of the 11 mRNAs?

+ssRNA Viruses

The +ssRNA viruses include several families of viruses. Members of the Picornaviridae, Flaviviridae, and Caliciviridae families, in particular, are ubiquitous in nature and cause a wide range of diseases. Their success and widespread distribution suggest that their replication strategy is very effective. The RNA in the virus particle itself functions as mRNA. This genomic RNA is a polycistronic mRNA that is recognized by cellular machinery and translated as one open reading frame into a single polyprotein precursor that is subsequently cleaved into individual viral proteins by viral and cellular proteases (FIGURE 4-12). One of the viral encoded proteins is an RNA-dependent RNA polymerase that replicates the viral genome. It transcribes the viral +ssRNA into a –ssRNA replicative intermediate, which in

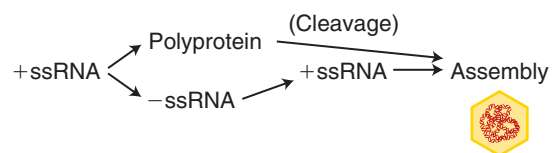
turn serves as a template for the genomic +ssRNA (Figure 4-12). Note that there are exceptions to this replication strategy. Not all ssRNA viruses produce a single polyprotein that is cleaved by proteases into individual proteins. Some produce more than one mRNA, allowing greater control of the production of individual proteins; for example, early replication proteins and later structural proteins are produced at different times during the viral replication cycle (FIGURE 4-13).

–ssRNA Viruses

Viruses in the Paramyxoviridae, Rhabdoviridae, and Filoviridae families contain –ssRNA nonsegmented genomes. All of these viruses encode their own RNA-dependent RNA polymerases that transcribe the –ssRNA genome into several different viral monocistronic +ssmRNAs that can be recognized by the host cell machinery. The different +ssRNAs are made by a complicated start–stop type of mechanism. In other words, a range of viral mRNAs are each translated to make different viral proteins rather than a polyprotein. All of the proteins are not produced to the same level, and a number of control mechanisms are used. The second function of the viral RNA-dependent RNA

FIGURE 4-12 List of +ssRNA viruses and their replication strategies. Adapted from Harper, D. R. *Molecular Virology*, Second Edition. BIOS Scientific Publishers, 1999.

Virus	Disease	Family
Poliovirus	Poliomyelitis Postpolio syndrome	Picornaviridae
Rhinovirus (many types)	Common cold	Picornaviridae
Hepatitis A	Hepatitis	Picornaviridae
Cocksackie		Picornaviridae
Group A Types 21, 24	Common cold	
Group A Types 4, 5, 9, 10, 16	Hand, foot, and mouth disease	
Group B Types 1–5	Myocarditis	
Group B Types 2, 5	Hand, foot, and mouth disease	
Echoviruses		Picornaviridae
Various Types	Diarrhea	
Types 1–7, 9, 11, 13–23, 25, 27	Aseptic meningitis	
Rubivirus	Rubella	Togaviridae
Yellow fever	Hemorrhagic fever	Flaviviridae
Hepatitis C	Hepatitis liver cancer	Flaviviridae
Dengue	Dengue fever	Flaviviridae
West Nile	Fever, rash, myalgia encephalitis	Flaviviridae
Norovirus	Gastroenteritis	Caliciviridae
Sapovirus	Gastroenteritis	Caliciviridae



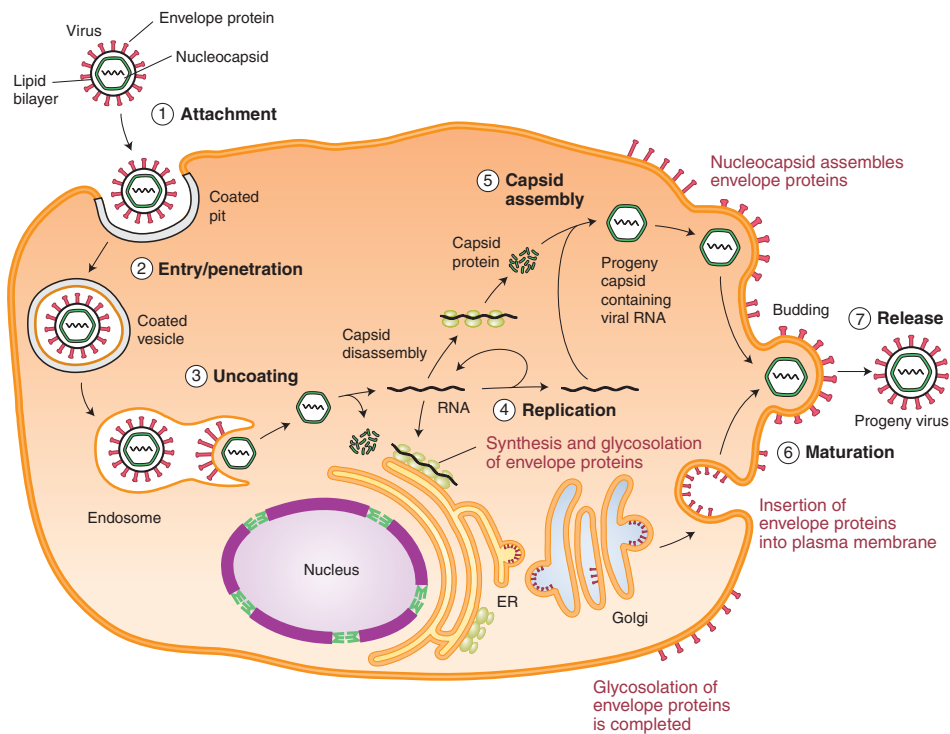


FIGURE 4-13 Example of a life cycle of a +ssRNA enveloped virus. All steps of the life cycle take place in the cytoplasm. Like a typical eukaryotic mRNA, the viral +ssRNA genome is directly translated by the cellular ribosomal machinery in the cytoplasm. Viral glycoproteins are synthesized on and inserted into the rough endoplasmic reticulum (ER) membrane, where they are subsequently transported to the trans Golgi network and then inserted into the plasma membrane. A viral polymerase synthesizes the viral genome. Newly synthesized capsid proteins bind to the replicated genome, forming a nucleocapsid that buds out of the plasma membrane to form the final enveloped virion.

polymerase is to synthesize the viral/progeny genome using the +ssRNA as a template. Hence, the RNA-dependent RNA polymerase is sometimes referred to as having a **transcriptase** and **replisome** function (**FIGURE 4-14a**).

The –ssRNA viruses containing segmented genomes also encode their own RNA-dependent RNA polymerase that functions as a transcriptase and replicase. Each segment produces a monocistronic mRNA or an RNA that is differentially spliced to make monocistronic mRNAs. The genomes of the viruses in the *Arenaviridae* and *Bunyaviridae* families are more complicated in that at least one of the viral ssRNA genomic segments are **ambisense** [the ssRNA is both (+) and (–) sense on the same ssRNA segment]. The process of replication (Figure 4-14b) and translations of these RNAs is not completely understood.

Viruses with ssRNA Genomes That Use a dsDNA Intermediate to Replicate

The *Retroviridae* family contains viruses that have been identified in virtually all organisms including invertebrates. This suggests that these viruses have an evolutionarily successful design. Their biology is quite unique.

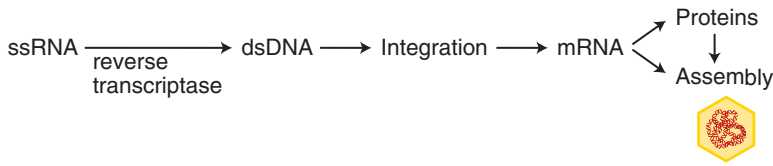
The main focus on retroviruses has been on the avian (chicken) or human retroviruses: Rous sarcoma virus (RSV, discovered in 1911, see Chapter 10), HIV (discovered in 1983), and HTLVs (discovered in 1981). Retrovirus infections cause a wide spectrum of diseases including cancer, immune deficiencies, and neurological disorders. Most retroviral infections, however, occur without having any detectable, deleterious damage to the host.

The replication cycle of retroviruses includes the integration of the viral complementary DNA (cDNA) into the chromosomal DNA of the host cell. The result of this integration event is that the retroviral DNA is inherited from parent to offspring of the infected host if germline cells (sperm and egg) contain the integrated viral genome. These are termed **endogenous retroviruses** or **proviruses**, and their biologic properties and functions are still under investigation. Approximately 8% to 12% of the human genome consists of sequences of **human endogenous retroviruses (HERVs)**. Retroviruses that are not integrated in germline cells of their hosts are called exogenous retroviruses (or external viruses).

The genome of retroviruses contains two copies of a +ssRNA molecule that is reverse transcribed into

Virus	Disease	Family
HIV-1 and 2	AIDS	Retroviridae
HTLV I	T-Lymphocyte Leukemia	Retroviridae
HTLV II	?	Retroviridae

FIGURE 4-15 List of ssRNA viruses (that use a DNA intermediate) and their replication strategies. Adapted from Harper, D. R. *Molecular Virology*, Second Edition. BIOS Scientific Publishers, 1999.



and separate stages of the viral life cycle. Virus assembly is a key step in the replication cycles of viruses. It involves the process in which the immature virus particle is formed. Despite the structural diversity of virus particles, the repertoire of assembly mechanisms is limited. All of the components of the virus must be assembled to create a stable structure. At the same time, the newly assembled virus must accomplish disassembly to start a new infectious life cycle.

The assembly event occurs when an appropriate concentration of virus proteins and genomic nucleic acids is reached and localized at

specific sites within the infected cell. The genomic nucleic acids are packaged into preexisting shells that form via **self-assembly** (spontaneous assembly, also refer to Chapter 3) of viral capsid proteins, or are coated with capsid proteins, or are co-assembled with capsid proteins. Assembly sites (for example, the cytoplasm, nucleus, on the inner surface of the plasma membrane of cells) differ according to the virus and have some influence on how the virus particle is released.

Historically, research directed toward virus assembly mechanisms has received less attention because of more interest in the mechanisms of viral

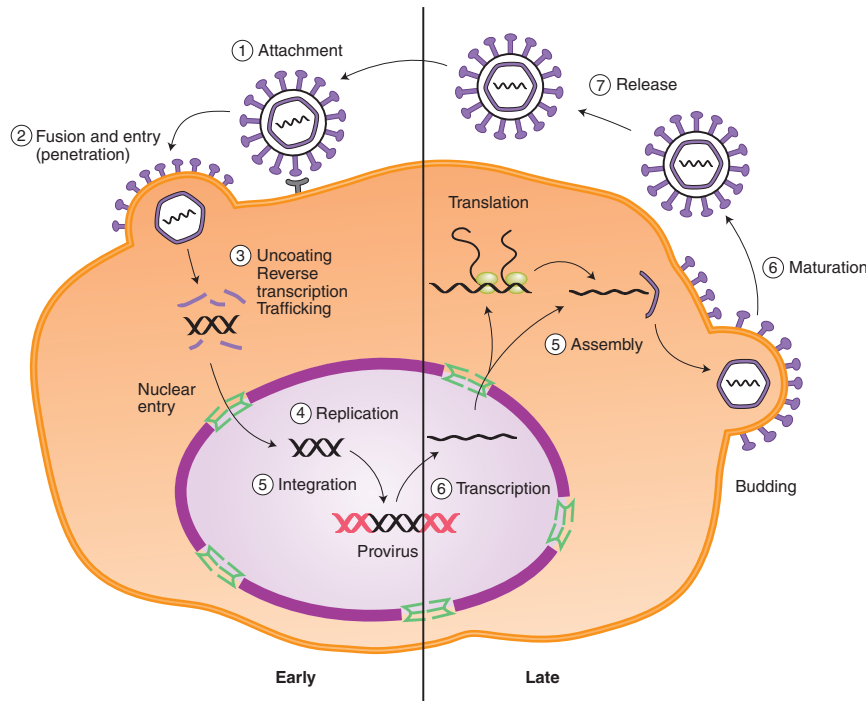


FIGURE 4-16 Basic life cycle of a typical retrovirus. Retroviruses undergo either a latent (early) phase in which the virus begins the steps of a typical virus life cycle: virus attaches to a host cell receptor(s) followed by uncoating of the virus particle in which the genome is translocated (also referred to as trafficking) to the nucleus where the genome is reverse transcribed into dsDNA that is integrated into the host's chromosome. At this point, the retroviral DNA is replicated along with the host chromosome. This early stage is latent in that no infectious particles are made while the retroviral DNA has integrated into the chromosomal DNA. After an "activation" event, the integrated retroviral DNA is transcribed. Viral mRNAs are exported to the cytoplasm where the cellular translational machinery produces the viral proteins. The capsid proteins and retroviral RNA is assembled/packaged into a nucleocapsid that buds from the plasma membrane, forming the final particle that undergoes a maturation step before the virus is fully infectious. Modified from an illustration by Kate Bishop, MRC National Institute for Medical Research, UK.

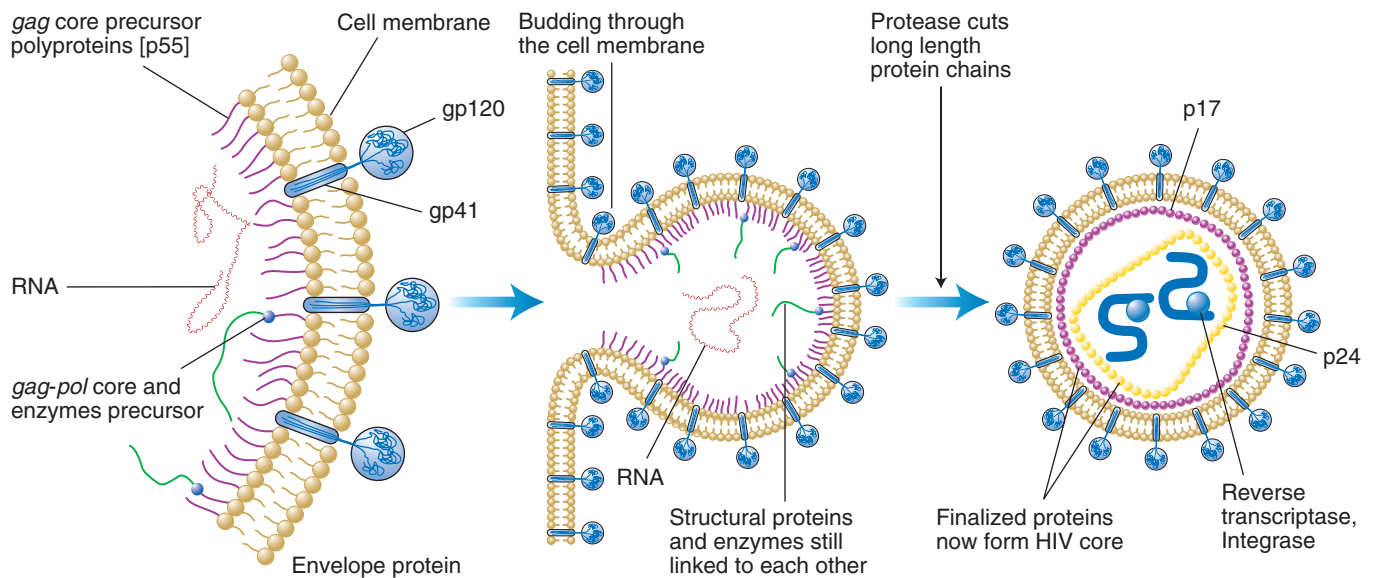


FIGURE 4-17 The structural proteins within the immature HIV virus particle must be cleaved by a viral protease inside of the particle in order for the virus to be infectious. Adapted from Vella, S., et al., *AIDS Soc.* 4 (1996): 15–18.

gene expression and replication. There is now renewed interest in understanding virus assembly because of the development of new molecular technologies and the success of therapeutic agents designed to inhibit virus-specific reactions involved in the production of infectious virus particles. These advances in understanding continue at an accelerated pace.

6. Maturation

This is the stage of the virus life cycle in which the virus becomes infectious. Viral or cellular proteases are often involved in maturation. One or more **capsid** or **envelope** proteins may undergo specific proteolytic cleavage within the particle. The cleavage event results in a subtle structural change of the virus particle, which may give it increased stability.

Virus-encoded proteases are attractive targets for antiviral therapies; for example, the protease inhibitors Saquinavir mesylate (Invirase), Saquinavir (Fortovase), Ritonavir (Norvir), Indinavir (Crixivan), Nelfinavir (Viracept), Amprenavir (Agenerase), and ABT-378 (Kaletra) target the HIV-encoded protease by preventing the maturation of virions capable of infecting other cells (**FIGURE 4-17**).

7. Release

Newly formed viruses are either released to the outside environment upon lysis, escaping the cell as it disintegrates (**lytic viruses**), or are released by budding (**FIGURE 4-18**) through the plasma membrane of the cell (as is the case with retroviruses, togaviruses,

orthomyxoviruses, paramyxoviruses, bunyaviruses, coronaviruses, rhabdoviruses, and hepadnaviruses). Viruses that are released via budding may damage the cell (as is the case with paramyxoviruses, rhabdoviruses, and togaviruses) or they may not (as is the case with retroviruses). Some viruses bud from other membranes and are released from the cell via a secretory-like mechanism.

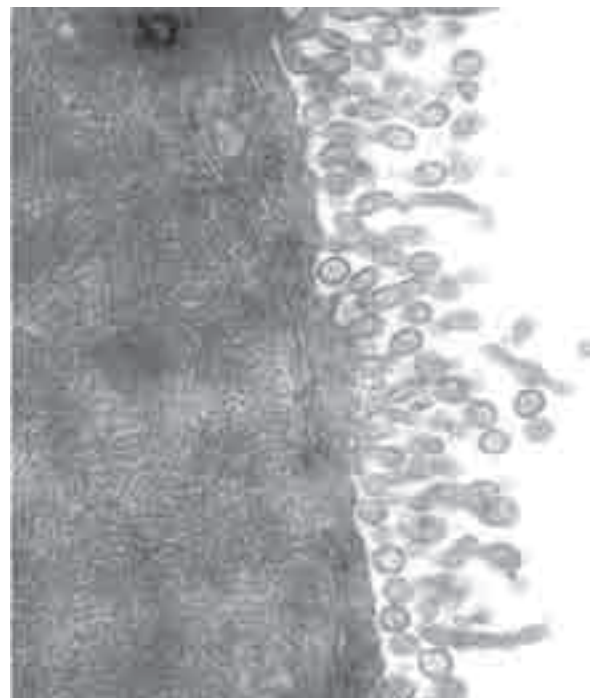


FIGURE 4-18 Transmission electron micrograph of measles virus released by budding.

Lytic Viruses

Most naked viruses are released when infected cells break open (cell lysis/destruction) due to the activity of viral enzymes, rather than distention. Poliovirus is an example of a lytic virus. A lytic life cycle is one that kills the host cell. Many enveloped viruses also do this.

Latent Eukaryotic Viruses

Retroviruses such as HIV-1 undergo a **latent (non-lytic) cycle** in which the viral DNA (provirus) becomes inserted into the host's DNA. In certain cell types, the proviral DNA replicates "silently" along with the cellular DNA, and the virus is undetected for many years. The provirus, however, can be activated at any time, allowing a productive infection or lytic cycle (one that produces infectious exogenous particles) to occur. Note that the

viral DNA remains integrated and does not excise itself from the chromosome as is done in bacteriophages (see Chapter 21). **Latency** is considered to be one of the biggest reasons why drug therapy fails to eradicate HIV from patients.

Herpesviruses enter and migrate down neurons, where they become latent in the body of neurons. They do not integrate their DNA; it remains as an episome. Subsequent activation of the latently infected neurons by a variety of factors (such as extreme temperatures, physical trauma, emotional stress, and immune suppression) enables the viruses to migrate back up the nerve cell and replicate again in the epithelial cells. Two common herpesviruses are herpes simplex virus type 1 (HSV-1), which usually causes fever blisters or oral herpes, and herpes simplex virus type 2 (HSV-2), which usually causes genital herpes (see Chapter 15).

VIRUS FILE 4-2

Unraveling the Life Cycle of Mimivirus



The discovery of Mimivirus in 2003 sent a shockwave through the community of virologists and evolutionists. The virus was 750 nm in size—gargantuan compared to typical viruses—and was termed a **Girus** (see Chapter 3, Viruses That Challenge the Definition of a Virus). It contained the largest known viral genome at a whopping 1,181,404 base pairs of dsDNA, coding for 911 gene products. It contained many genes not found before in other viruses such as **aminoacyl-tRNA synthetases** that are central to components of cellular translation machinery, genes that are components of cellular replication and transcriptional machinery, and genes associated with metabolic pathways, and four of its genes contained **introns**. *But Mimivirus still appears to be absolutely dependent on its host cells for synthesis of its proteins.*

Following the deciphering of the genome sequence of Mimivirus, efforts began to elucidate the life cycle of the virus. Research teams led by Didier Raoult or Abraham Minsky resulted in extensive ultrastructural studies to determine the life cycle of Mimivirus. In their studies, Mimiviruses were "fed" and allowed to infect *Acanthamoeba polyphaga* at a cell-virus ratio of 1:10. At various times post-infection within a 24-hour period, the infected cells were collected and prepared for transmission electron microscopy. Electron microscopy demonstrated that within 30 minutes, the Mimiviruses appeared to enter the amoeba cells by phagocytosis. Upon engulfment, the Mimiviruses underwent uncoating steps by fusing with the host phagosomes. During the fusion event, the viral capsids morphed into 5-fold star-shaped structures that acted like a "stargate," or portal, through which viral DNA was released into the cytoplasm of the host cell. Subsequently, the viral DNA was imported into the host nucleus where its first round of replication began. Like poxviruses, Mimiviruses possess their own transcriptional apparatus (refer to Chapter 14).

By 3 hours post-infection, Mimivirus DNA exited the host nucleus to form cytoplasmic viral replication factories surrounded by mitochondria. By 5 to 8 hours post-infection, these viral factories increased 50% in size and viral proteins were detected. In addition to electron microscopy, direct fluorescent staining was performed on the infected cells in order to study the Mimivirus factories. The viral factories appeared to have three zones: an electron-dense inner replication center, an intermediate assembly zone, and the peripheral zone

(continued)

4.2 Key Steps of the Viral Replication Cycle



where particles matured and acquired fibrils. At 8 hours after the infection, the viral factories contained empty, fiberless capsids that were partially assembled as well as some icosahedral capsids undergoing DNA packaging through a transient “stargate” or aperture. By 10 to 12 hours post-infection, mature fibril-coated particles budded from the viral factories and were released through cell lysis (FIGURE VF 4-2). Overall, the takeover of the cellular machinery by Mimivirus was rapid and efficient. Many of the viral events in the life cycle took place in a giant volcano-like viral factory.

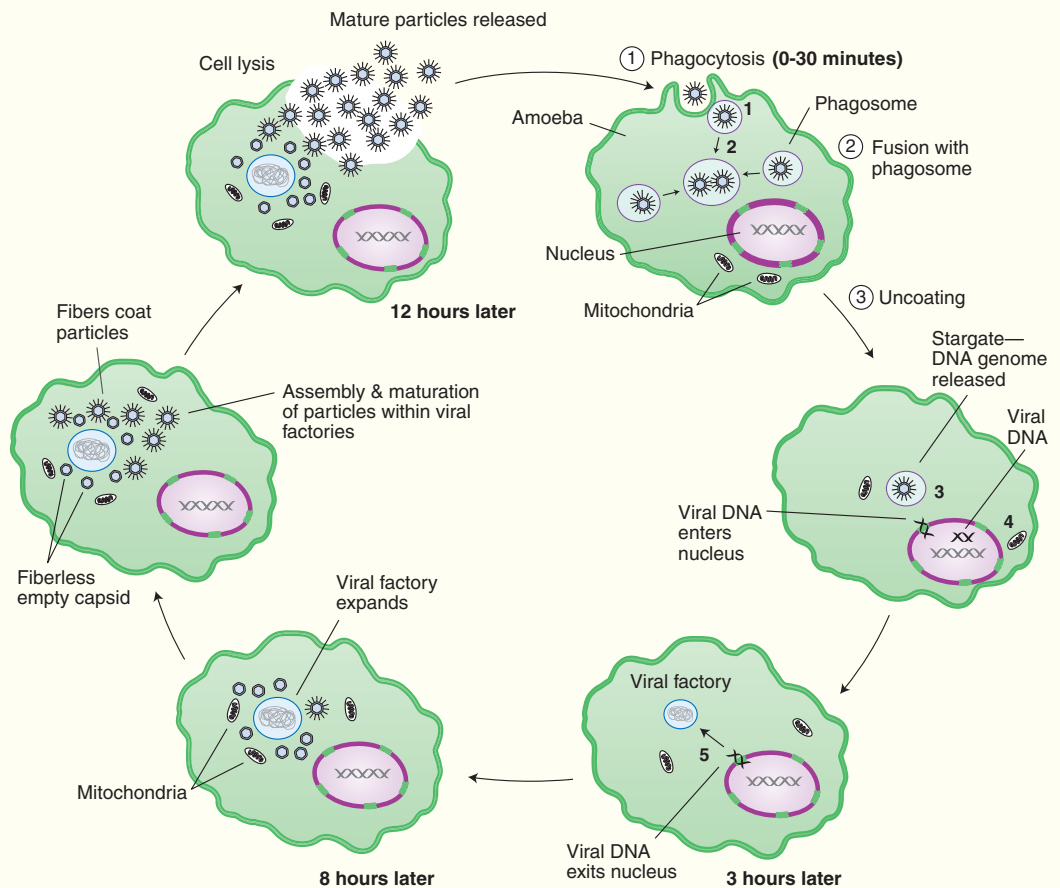


FIGURE VF 4-2 The Mimivirus life cycle based on observations of infected amoeba cells at various times after infection. Adapted from Suzan-Monti, M., et al. 2007. “Ultrastructural characterization of the giant volcano-like virus factory of *Acanthamoeba polyphaga* Mimivirus.” *PLoS ONE* 3:e328. Figure 8.

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Claverie, J. M., and Abergel, C., 2009. “Mimivirus and its viroplasm.” *Ann Rev Gen* 43:49–66.

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Zauberman, N., et al. 2008. “Distinct DNA exit and packaging portals in the virus *Acanthamoeba polyphaga* Mimivirus.” *PLoS Biol* 6:1104–1114.

Why Don't the Viruses Get Stuck on the Cellular Receptors as They Are Released?

The release of virus particles poses an interesting dilemma. Viruses are designed to enter rather than leave cells. Some viruses, such as influenza A, produce a protein during their life cycle to destroy the cellular receptors as they exit the cell. The protein, **neuraminidase**, cleaves the sialic-acid receptors on the outside of cells as the infectious particles are released (see Chapter 12). As a result, the viruses do not aggregate at the cell surface.

4.3 The Error-Prone RNA Polymerases: Genetic Diversity

Viruses replicate rapidly. During the process of replication, an error, or point mutation, may occur. The mutation rate of DNA viruses is usually similar to those of their cellular hosts because most of these polymerases used to replicate DNA genomes possess proofreading ability; for example, the mutation rate of herpesviruses (with **proofreading ability**) is one error in every 10^8 to 10^{11} bases. The genome sizes of herpesviruses range from 1.3×10^5 to 2.0×10^5 base pairs in length. As a result, herpesviruses potentially evolve very slowly because few mutations will be made, if any, during the infection cycle of the virus.

RNA viruses possess mutation rates as high as one error in 10^3 to 10^4 bases. The RNA-dependent RNA polymerases and RNA-dependent DNA polymerases (for example, reverse transcriptases) used by the RNA viruses for genome replication do not possess proofreading ability. Hence, the presence of mutants in a virus population during each replication cycle of the virus occurs much more rapidly than in DNA viruses or cellular organisms. HIV and coronaviruses (such as severe acute respiratory syndrome-associated coronavirus, or SARS-CoV) are excellent examples of RNA viruses with high mutation rates. These viruses misincorporate a nucleotide into their genome once in 10^3 to 10^4 bases. For coronaviruses, this means that there are three mutations that occur during the replication of one viral genome. For HIV, this means one to two mutations in every genome copied. The high mutation rate probably limits the size of most RNA virus genomes to approximately 10^4 nucleotides.

Many mutations are lethal because the mutated virus is unable to replicate. Nonlethal mutations may give the mutated virus a selective advantage. Mutations have been associated with the development of antiviral drug resistance (such as the drug-



FIGURE 4-19 A Vietnamese woman weighs a chicken at a market in Hanoi, Vietnam (January 2004). At the time, the avian influenza virus infected several humans who had direct contact with chickens. The virus infected millions of chickens, raising fears that it might mutate to a strain that could readily pass from human to human, leading to the next human influenza pandemic.

resistant strains of HIV), changes in virulence (for example, the 1997 Hong Kong avian influenza A was highly virulent), changes that allow the virus to evade the host's immune system (such as the influenza viruses and antigenic drift, which will be discussed in Chapter 12), and changes in host range.

A good example of a change in host range adaptation occurred in 1978. Canine parvovirus suddenly began killing large numbers of dogs globally in 1978. The parvovirus originally had infected only cats, foxes, raccoons, and minks. A small number of changes in the capsid genes adapted the virus for efficient spread among dogs (**FIGURE 4-19**).

4.4 Targets for Antiviral Therapies

Modern technology allows scientists to deliberately design drugs. To do this, they need to understand “the enemy.” Knowledge of the life cycle of viruses and the mapping of them using computer-aided design (CAD) is applied toward antiviral development by scientists at pharmaceutical companies. Any of the seven stages of the viral life cycle can be targeted for antiviral intervention. The stages are:

1. Attachment specificity
2. Penetration
3. Uncoating
4. Replication
5. Assembly
6. Maturation
7. Release

TABLE 4-4 lists the current antiviral therapies available along with a list of which viruses they target and their mechanism of action. Key to antiviral drug development is that the drug must *target a process essential for viral replication and it must be active against the virus without being “toxic” to the host*

organism. It has been difficult to develop antivirals that have no toxic side effects for the host because viruses use some of the host cellular processes for replication. Hence, drugs cannot target those cellular processes because of toxicity problems for the host.

TABLE 4-4

Prevention and Treatment of Human Viral Diseases: Antiviral Drugs

Drug	Virus/Disease	Target
Idoxuridine Trifluridine	Herpes simplex keratoconjunctivitis	Viral and cellular DNA synthesis
Vidarabine	HSV-1, HSV-2	Viral DNA polymerase
Acyclovir	HSV-1, HSV-2, VZV, EBV, CMV	Virus DNA polymerase
Tromantadine	HSV	Viral DNA polymerase
Famciclovir	HSV-1, HSV-2, VZV, some activity against EBV, CMV, and HBV	Viral thymidine kinase and DNA polymerase
Penciclovir	HSV-1, HSV-2	Viral DNA polymerase
Valacyclovir	HSV-1, HSV-2, VZV, modestly active against EBV and CMV	Viral DNA polymerase
Gancyclovir	CMV retinitis in HIV patients	Virus polymerase
Foscarnet	Acyclovir-resistant HSV/VZV strains Ganciclovir-resistant CMV HSV-1, HSV-2, HHV-6, EBV, VZV, parainfluenza virus CMV retinitis in HIV disease	Viral DNA polymerase and reverse transcriptase
Docosanol (10% topical cream)	HSV-labialis episodes	Fusion inhibitor
Brivudine (approved for use in Germany and other European countries)	HSV-1 and VZV	Viral thymidine kinase and DNA polymerase
Entecavir, Adefovir	Hepatitis B	Hepatitis B reverse transcriptase
Abacavir	HIV-1 and HIV-2	HIV-1 reverse transcriptase
Didanosine (ddl)		
Emtricitabine (FTC)		
Lamivudine (3TC)		
Lamivudine + zidovudine (Combivir)		
Stavudine (d4T)		
Tenofovir + emtricitabine (Truvada)		
Tenofovir DF		
Zalcitabine (ddC)		
Zidovudine (AZT)		
Nevirapine	HIV-1	HIV-1 reverse transcriptase
Delavirdine	HIV-1	HIV-1 reverse transcriptase
Efavirenz	HIV-1	HIV-1 reverse transcriptase
Amprenavir	HIV-1	HIV-1 protease
Atazanavir		
Fosamprenavir		
Saquinavir		
Lopinavir + ritonavir		
Indinavir		

(continued)

TABLE 4-4

Prevention and Treatment of Human Viral Diseases: Antiviral Drugs (continued)

Drug	Virus/Disease	Target
Darunavir	HIV-1	HIV-1 protease
Nelfinavir		
Ritonavir		
Tipranavir		
Enfuvirtide	HIV-1	Binds to HIV-1 gp41 surface protein, inhibiting viral entry
Selzentry	HIV-1	Binds to CCR5 co-receptor
Raltegravir	HIV-1	Integrase strand transfer inhibitor
Atripola: cocktail of efavirenz, tenofovir, and emtricitabine	HIV-1	A fusion inhibitor and two reverse transcriptase inhibitors
Ribavirin	Broad spectrum (inhibits DNA and RNA viruses): RSV, Influenza A and B, HCV, HSV-1 and HSV-2, measles, mumps, Lassa fever	mRNA mutagen
Amantadine	Influenza A	Inhibits penetration and uncoating of virus
Rimantidine		
Relenza and Tamiflu	Influenza A	Neuraminidase inhibitor
Peramivir (not FDA approved)	Influenza A (H1N1)	Neuraminidase inhibitor
Arbidol (not FDA approved)	Influenza A and B, hepatitis C	Virus-mediated membrane fusion, immune modulator?
Cidofovir	Broad spectrum (inhibits DNA viruses): HSV-1, HSV-2, VZV, CMV, EBV, adenovirus, HPV, CMV retinitis in HIV patients, experimentally used to treat poxvirus infections	DNA polymerase
Interferons	Hepatitis B & C Hairy cell leukemia, HPV, respiratory viruses	Cell defense proteins activated
Fomivirsen	CMV	Inhibits viral replication and translation (antisense molecule)
Podophyllotoxin	HPV genital warts	Binds E2 and inhibits E2/E3 interaction

Abbreviations of viruses: Herpes simplex type 1 (HSV-1), herpes simplex type 2 (HSV-2), varicella zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), hepatitis B virus (HBV), hepatitis C virus (HCV), human herpes Type 6 virus (HHV-6), human immunodeficiency virus Type 1 (HIV-1), respiratory syncytial virus (RSV), hepatitis C virus (HCV), human papillomavirus (HPV). Abbreviations of antivirals: zidovudine (AZT), didanosine (ddl), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC). Unless otherwise specified, drugs are approved by the FDA.

VIRUS FILE 4-3

Stamping Down Flu Viruses



On a global scale, there are about 3.5 million severe cases of influenza illness and 300,000–500,000 deaths annually. Most at risk are the elderly, young children, and immunocompromised patients. Influenza virus can be controlled in two ways: vaccination and use of antivirals. It takes at least 6 months to produce sufficient vaccine to vaccinate a large proportion of the population upon the emergence of a new strain. For this reason, antivirals are necessary to mitigate an influenza pandemic.

Currently, two types of anti-influenza drugs are available: M2 ion channel blockers (amantadine and rimantadine) and neuraminidase inhibitors: tamiflu (oseltamivir phosphate) and relenza (zanamivir). M2 ion channel blockers prevent the uncoating step of the life cycle of influenza viruses. After influenza A viruses bind to sialic acid receptors, the virus is internalized by receptor-mediated endocytosis. The low pH of the endosome causes the viral and endosomal membranes to fuse and an influx of H⁺ ions enter the M2 ion channels present on the influenza virus particle, allowing the viral RNA segments to be released into the cytoplasm of the host cell (uncoating).

Antivirals that block the M2 ion channel prevent this uncoating step (**FIGURE VF 4-3**).

(continued)

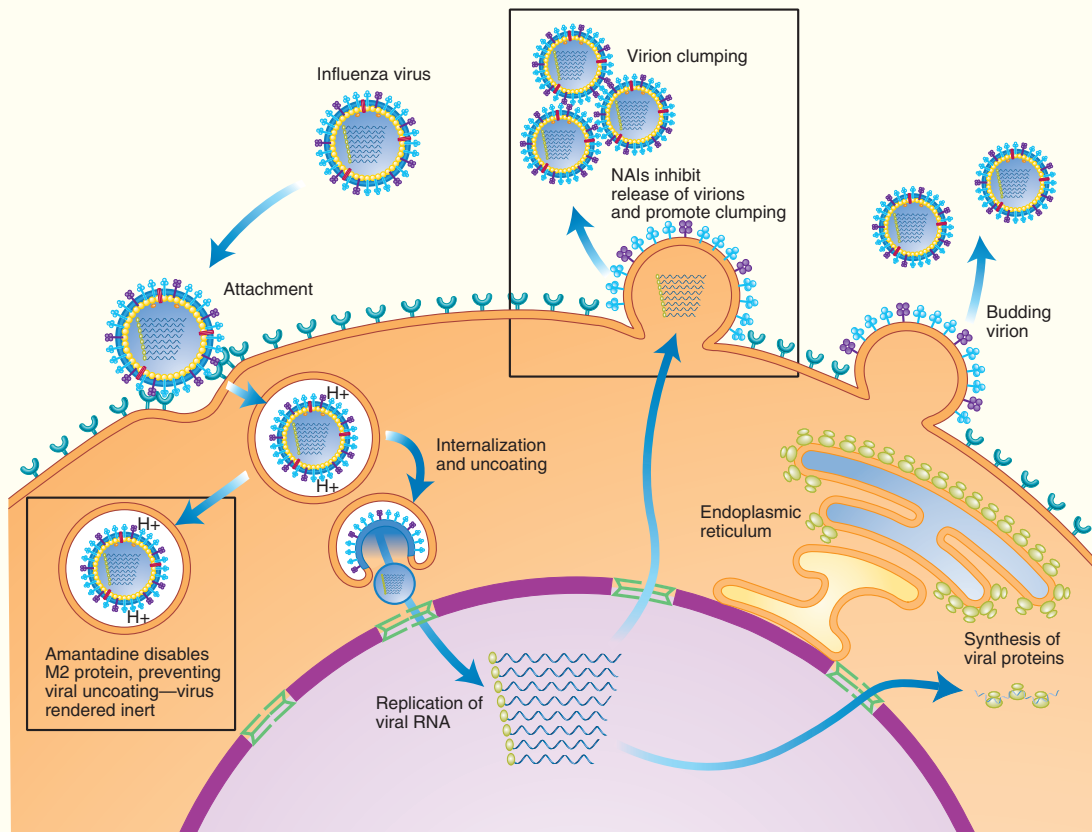


FIGURE VF 4-3 Influenza antivirals block the M2 ion channel or neuraminidase activity of influenza virus.

Antiviral drugs that block neuraminidase cause influenza viruses to clump at the surface of host cells, preventing its spread to other host cells at the end of the viral life cycle. Neuraminidase is an influenza protein present on the outside of the virus particle. As newly assembled viruses bud from host cells and are released, neuraminidase cleaves or removes the host cell sialic acid receptors present on the outside of host cells. When neuraminidase is blocked, influenza viruses stick to the sialic acid receptors present on the plasma membrane of the infected host cells, causing viruses to clump and not spread to other cells of the respiratory tract (Figure VF4-3).

Unfortunately, resistant strains of M2 inhibitors rapidly emerge. Resistant strains of oseltamivir (Tamiflu) are also emerging. It is believed that indiscriminate use of these drugs has led to the appearance of drug-resistant viruses. In response to the need for new anti-influenza drugs, scientists are developing and testing promising new antiviral candidates. Structure-based design, targeting the conserved active site amino acids of neuraminidase, is being used to synthesize new high-affinity neuraminidase inhibitors.

Two such candidates are CS-8958 and peramivir. Both of these are neuraminidase inhibitors. During the 2009 H1N1 pandemic, emergency authorization was given to use the FDA-unapproved peramivir to treat severe cases of H1N1 influenza. Patients receiving peramivir were hospitalized with life-threatening influenza including such complications as kidney failure. Peramivir could only be used in an injectable form.

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■ RNA Virus Mutagens: A New Class of Antiviral Drugs?

The mechanism of action of the antiviral drug ribavirin was a mystery for over 30 years. Drs. Joseph T. Witkowski and Roland K. Robins synthesized ribavirin in the laboratory in 1970. In 1972, it was reported that ribavirin inhibited a wide spectrum of viruses *in vitro* but its mechanism was unknown. Today it is used to treat hepatitis C virus infections in combination with interferon- α , SARS-CoV infections, human rabies (as in the case of the Wisconsin patient in 2004), Lassa fever, and some herpesvirus infections. It is used experimentally to treat HBV, Hantaan, Dengue, and parainfluenza virus infections.

In 2001 the mechanism of action was proposed and demonstrated by Shane Crotty, Craig E. Cameron, and Raul Andino. Their poliovirus experiments suggested that ribavirin's antiviral activity is exerted through lethal mutagenesis. In other words, the drug overwhelms the virus with a high mutation rate that in turn drives the virus into a genetic meltdown. Interestingly, this high mutation rate does not allow the virus to escape the inhibitory effects of the drug. This discovery provides pharmaceutical companies with an entirely new drug strategy: RNA virus mutagens.

Summary

With the development of animal cell culture techniques, scientists have been able to study virus replication via one-step growth experiments. The knowledge today is very detailed and continues to progress so rapidly that it is impossible to cover the viral replication strategies used by every family of viruses in a single chapter. This chapter presents an overview of viral replication that includes the seven key steps in the life cycle of a virus. They are:

1. Attachment specificity
2. Penetration
3. Uncoating

4. Replication
5. Assembly
6. Maturation
7. Release

Virus attachment occurs when a virus particle attaches to a target cell surface receptor and is the first event in the viral life cycle. Viral receptors are discovered via experiments that involve:

- *the removal of surface receptors,*
- *the use of monoclonal antibodies to block cell surface receptors, and*
- *gene-transfer experiments.*

Viruses enter their target cells via fusion or receptor-mediated endocytosis. ADE has been shown to be an alternative mechanism of viral entry into host cells. This phenomenon has been observed with Dengue, West Nile, HIV-1, HIV-2, and Influenza A viruses.

Replication strategies are carried out based on the nature of the viral genome (DNA or RNA, ss or ds, segmented or nonsegmented). It is often difficult to distinguish between the steps of assembly, maturation, and release of virus particles.

Some viruses destroy their host cells (lytic viruses), whereas others do not. Some viruses have developed ways to overcome the conundrum of entry versus exiting cell surfaces that are coated with viral receptors.

Viruses are masters of mutation. Mutations can be lethal or nonlethal. Nonlethal mutations may give rise to mutants that may increase their survivorship; for example, mutations may increase their infectivity, result in viral drug resistance, antagonize the host immune system, or broaden the host range of the virus.

Scientists are applying the knowledge obtained through molecular biology toward the design of antiviral drugs. These antivirals must target the virus specifically to be effective in eliminating the virus without causing harm to its host.



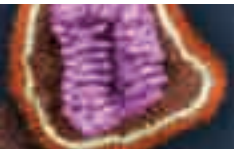
CASE STUDY QUESTIONS

These questions relate to the Case Study presented at the beginning of the chapter.

1. hMPV and RSV belong in the Paramyxoviridae family. What type of nucleic acid genome do they have? Do they encode their own viral polymerase? (see Figure 4-14a).
2. Draw a flowchart of the viral life cycle of hMPV.
3. How many genes does hMPV have in its genome?
4. The first report of a fatal encephalitis case associated with hMPV was published in the CDC's *Emerging and Infectious Diseases* publication in 2005. The authors recommended screening for patients, especially children with encephalitis symptoms of unknown origin. What is encephalitis?
5. Have neurological symptoms been associated with other viruses in the Paramyxoviridae family? If so, which virus(es)?

Note: You may need to do further research to find the answers to these questions. The following references will provide some helpful information.

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CASE STUDY: 1 RABIES

In February 2009, a 17-year-old Texas teenager arrived at a hospital emergency room presenting with symptoms of a severe headache, neck pain, dizziness, **photophobia**, nausea and vomiting, and tingling of her face and forearms. A **lumbar puncture** was performed, which revealed a very high white blood cell count suggesting the teen might be fighting a bacterial infection. She was treated intravenously with the broad-spectrum antibiotic ceftriaxone. No bacteria were cultured from her cerebral spinal fluid so the antibiotic was discontinued. After a 3-day hospital stay, she was released because her symptoms had resolved. About a week later, she returned to the hospital with the same symptoms. She also had a rash on her arms and back. **Magnetic resonance imaging** (MRI) was performed on her head. The MRI showed enlarged lateral ventricles inside her brain. The size was abnormal for her age and she was given the diagnosis of **encephalitis**. She was hospitalized and treated with ceftriaxone and antibiotics used to treat tuberculosis.

Four days later, she continued to weaken. She became agitated and "combative." At this time, clinicians did an extensive workup, collecting the patient's history, and created a list of possible etiologies to explain the encephalitis/aseptic meningitis. A breakthrough came when the teenager mentioned she had been hiking and spelunking during December (2008) and had brushed up against some bats but didn't recall being bitten. Serum, saliva, and cerebral spinal fluid were drawn or collected from the patient, a **nuchal skin biopsy** was performed, and the clinical specimens were sent to the CDC to test for rabies antibodies, rabies antigens, or the RNA genome of rabies virus.

The next day, no rabies RNA or virus antigens were detected. Four serum samples tested positive for anti-rabies antibodies by direct fluorescent antibody tests, however. Anti-rabies antibodies were also found in the cerebral spinal fluid. Four days later, the teenager was given 1 dose of rabies vaccine and 1,500 international units (IU) of human rabies immunoglobulin.

CASE STUDY: 1 RABIES (continued)

Eight days later, her symptoms resolved and she was released from the hospital. Another week passed and she returned to the emergency room of a hospital with recurring headache but left before a lumbar puncture was performed. A few more days passed. Again, she had a recurring headache. A lumbar puncture was performed. Her headache resolved and she has not been rehospitalized since. Only her boyfriend met the criteria of requiring a series of rabies vaccinations.

1. Create a hypothesis as to why this teenager survived a rabies virus infection.
2. What do the CDC researchers speculate as to why this patient survived rabies? Refer to the CDC report related to this case published February 26, 2010 (*MMWR* 59[7]; *MMWR* can be found at: <http://www.cdc.gov/mmwr/>).

3. Assuming the teenager was not bitten, what could be another plausible explanation for how the teenager contracted this infection?
4. Rabies virus is a member of the Rhabdoviridae family. What are the molecular characteristics of this family of viruses (e.g., particle characteristics, life cycle)?
5. How many genes do rabies viruses carry and what are their function(s)?
6. What types of cells do rabies viruses replicate in (hint: Table 4-1)?
7. Rabies bind to what host receptor(s)?
8. Who should receive a rabies vaccination? (hint: <http://www.cdc.gov/rabies/>)

For more information see Chapter 13 (Rabies).

Resources

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The site features eLearning, an online review area that provides quizzes and other tools to help you study for your class. You can also follow useful links for in-depth information, or just find out the latest virology and microbiology news.