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
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 metapneumoviruses (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 the previous chapter 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.

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.

### 3.1 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 sensitive to infection with human viruses due to the species barrier and the animal immune response.
**General Procedure: One-Step Growth Curves**

**Step 1:** Infect monolayers of tissue culture cells (using a vertical laminar flow biosafety hood) and allow the infection to proceed in a CO₂ incubator.

**Step 2:** Monitor experiments via inverted microscope.

**Step 3:** Collect infected cell lysates at various time points after infection.

**Step 4:** Perform serial dilutions on infected cell lysates and do plaque assays.

**Step 5:** Stain and analyze plaque assays. Record results.

---

**Figure 3-1**

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.

---

**Figure 3-2**

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, 4th edition. Academic Press, 1994.
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 Delbruck 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 than 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 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 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 3-1).

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 3-2a). 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 3-2b 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.

### 3.2 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 3-1 lists examples of viruses and their cellular tropism. Sometimes viruses also display species 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
Virus Replication Cycles

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 3-2 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 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.

### Table 3-1: Viral Cell Tropism

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

### Table 3-2: Cell Surface Receptors Used by Viruses to Attach and Enter Cells

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cell Surface Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A</td>
<td>Sialic acid</td>
</tr>
<tr>
<td>HIV-1</td>
<td>CD4 and chemokine co-receptors (CXCR5, CCR4)</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>Low-density lipoprotein receptor</td>
</tr>
<tr>
<td>Rabies</td>
<td>Acetylcholine receptor, neural cell adhesion molecule, nerve growth factor, gangliosides, phospholipids</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>Intracellular adhesion molecule 1 (ICAM-1)</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>IgA receptor</td>
</tr>
<tr>
<td>Adenovirus Type 2</td>
<td>Integins αβ₁ and αβ₃</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>Immunoglobulin superfamily protein (CD155)</td>
</tr>
</tbody>
</table>

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.
How Are Cellular Receptors Used for Viral Attachment Discovered?

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 3-1 illustrate the general scheme of the various methods employed.

**Figure VF 3-1**

(a) Sialic acid

Host cell

Influenza attaches to sialic acid cell receptors

Influenza virus

(Nucleus)

Treat cells with neuraminidase

(Removes sialic acid)

(b) ICAM-1

Host cell

Nucleus

Rhinovirus

Monoclonal antibody

(c) PVR gene

Poliovirus

Human cells (susceptible to poliovirus infection)

Transfer PVR gene into mouse cells

Mouse cells expressing PVR are susceptible to poliovirus infection

Mouse cells lacking PVR gene are resistant to poliovirus infection
Virus Replication Cycles

CHAPTER 3

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 3-3). (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 3-3a). The fusion at the plasma membrane mode of penetration is pH independent.

In receptor-mediated endocytotic (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 3-3). This endocytotic vesicle may fuse with the lysosomes, which possess an internal acidic pH. In the acidic pH of the endocytic 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 acid pH that the fusion between the viral envelope and the host cell membrane occurs (Figures 3-3b and 3-3c).

Naked Virus Entry

It is more difficult to envision how naked or nonenveloped 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, helps to disassociate the capsid, releasing the nucleic acid genome of the virus into the cytoplasm. Figure 3-4 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.

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 3-2b).

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 3-5). The entire genome may occupy...
3.2 Key Steps of the Viral Replication Cycle

**Figure 3-3**
(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.
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.

Replication of many DNA viruses involves strategies that are familiar in cell biology: DNA replication and mRNA transcription form dsDNA. Viral proteins are translated from the monocistronic mRNAs generated via transcription of viral mRNAs, as shown in Figure 3-6a, 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. In this chapter we also discuss viral taxonomy, including virus families and how they are determined.

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 their 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 3-6a 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 3-6a).

**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 3-6b.
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 squirrels, chipmunks, ducks, geese, chimpanzees, gibbons, and orangutans. Interestingly, HBV-infected cells produce different forms of virus-related particles. Electron microscopy of partially purified virus particle preparations reveals 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 3-7).

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 3-8).

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 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 use 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...
### 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 3-8
CHAPTER 3  Virus Replication Cycles

Virus Replication Cycles

+ sense ssRNA genome: AUG GCA CGA → met ala arg
− sense ssRNA genome: UAC CGU GCU

Figure 3-9
Differences between positive (+) and negative (−) sense ssRNA viral genomes.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Disease</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus</td>
<td>Gastroenteritis</td>
<td>Reoviridae</td>
</tr>
<tr>
<td>Reovirus</td>
<td>Mild respiratory and gastrointestinal symptoms</td>
<td>Reoviridae</td>
</tr>
</tbody>
</table>

Figure 3-10

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 3-10).

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 particle to form the remaining dsRNA genomic segments (Figure 3-10). 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 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

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 3-9). 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 RNA-dependent RNA polymerase that will synthesize the viral +ssRNA, mRNAs, and −ssRNA viral genomes into the host cell with them.

DNA Viruses

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).

Integrate 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 3-9). 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 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 3-10).

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 particle to form the remaining dsRNA genomic segments (Figure 3-10). 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 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
3.2 Key Steps of the Viral Replication Cycle

<table>
<thead>
<tr>
<th>Virus</th>
<th>Disease</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poliovirus</td>
<td>Poliomyelitis Postpolio syndrome</td>
<td>Picornaviridae</td>
</tr>
<tr>
<td>Rhinovirus (many types)</td>
<td>Common cold</td>
<td>Picornaviridae</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>Hepatitis</td>
<td>Picornaviridae</td>
</tr>
<tr>
<td>Cocksackie Group A Types 21, 24</td>
<td>Common cold</td>
<td>Picornaviridae</td>
</tr>
<tr>
<td></td>
<td>Group A Types 4, 5, 9, 10, 16</td>
<td>Picornaviridae</td>
</tr>
<tr>
<td></td>
<td>Group B Types 1–5</td>
<td>Picornaviridae</td>
</tr>
<tr>
<td></td>
<td>Group B Types 2, 5</td>
<td>Picornaviridae</td>
</tr>
<tr>
<td>Echoviruses Various Types</td>
<td>Diarrhea</td>
<td>Picornaviridae</td>
</tr>
<tr>
<td></td>
<td>Types 1–7, 9, 11, 13–23, 25, 27</td>
<td>Picornaviridae</td>
</tr>
<tr>
<td>Rubivirus</td>
<td>Rubella</td>
<td>Togaviridae</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>Hemorrhagic fever</td>
<td>Flaviviridae</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>Hepatitis liver cancer</td>
<td>Flaviviridae</td>
</tr>
<tr>
<td>Dengue</td>
<td>Dengue fever</td>
<td>Flaviviridae</td>
</tr>
<tr>
<td>West Nile</td>
<td>Fever, rash, myalgia encephalitis</td>
<td>Flaviviridae</td>
</tr>
<tr>
<td>Norovirus</td>
<td>Gastroenteritis</td>
<td>Caliciviridae</td>
</tr>
<tr>
<td>Sapovirus</td>
<td>Gastroenteritis</td>
<td>Caliciviridae</td>
</tr>
</tbody>
</table>

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 polypeptide precursor that is subsequently cleaved into individual viral proteins by viral and cellular proteases (Figure 3-11). 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 3-11). Note that there are exceptions to this replication strategy. Not all ssRNA viruses produce a single polypeptide 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.

**-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 its –ssRNA genome into several different viral monocistronic +ssmRNAs that can be recognized by the host cell machinery. The different +ssmRNAs 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 polypeptide. 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 polymerase is to synthesize the viral/progeny genome using the +ssmRNA as a template. Hence the RNA-dependent RNA polymerase is sometimes referred to having a transcriptase and replicase function (Figure 3-12a).
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 3-12b) 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 dsDNA by a viral RNA-dependent DNA polymerase (reverse transcriptase) to produce an RNA:DNA hybrid, which in turn is converted to dsDNA. The viral dsDNA is inserted into the host chromosomal dsDNA (Figure 3-13). The integrated DNA (provirus) is subsequently transcribed by the host's DNA-dependent RNA polymerase II. The mRNA
transcripts are then spliced and exported into the cytoplasm of the cell, where they will be translated by the cellular protein synthesis machinery. Some full-length +ssRNA transcripts will be packaged into the new retrovirus particles.

5. Assembly

It is not always possible to identify the assembly, maturation, and release of virus particles as distinct 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 cycle.

The assembly event occurs when an appropriate concentration of virus proteins and genomic nucleic acids are 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 4) 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 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 (Virecept), Amprenavir (Agenerase), and ABT-378 (Kaletra) target the HIV-encoded protease by preventing the maturation of virions capable of infecting other cells (Figure 3-14).

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 3-15) through

---

<table>
<thead>
<tr>
<th>Virus</th>
<th>Disease</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 and 2</td>
<td>AIDS</td>
<td>Retrovirdae</td>
</tr>
<tr>
<td>HTLV I</td>
<td>T-Lymphocyte Leukemia</td>
<td>Retrovirdae</td>
</tr>
<tr>
<td>HTLV II</td>
<td>?</td>
<td>Retrovirdae</td>
</tr>
</tbody>
</table>

**Figure 3-13** List of ssRNA viruses (that use a DNA intermediate) and their replication strategies. Adapted from Harper, D.R. *Molecular Virology*, 2nd edition. BIOS Scientific Publishers, 1998.
CHAPTER 3  Virus Replication Cycles

Virus Replication Cycles

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.

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 (nonlytic) cycle in which the viral DNA (provirus) becomes inserted into the hosts 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, Herpesviruses).

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.

### 3.3 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 $10^6$ to $10^{11}$ bases. The genome sizes of herpesviruses range from $1.3 \times 10^5$ to $2.0 \times 10^5$ 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-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 hosts 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 3.16.
3.4 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 3-3 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.

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.
### TABLE 3-3
Prevention and Treatment of Human Viral Diseases: Antiviral Drugs

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

(continued)
TABLE 3-3 Prevention and Treatment of Human Viral Diseases: Antiviral Drugs (continued)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Virus/Disease</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enfuvirtide</td>
<td>HIV-1</td>
<td>Binds to HIV-1 gp41 surface protein, inhibiting viral entry.</td>
</tr>
<tr>
<td>Selzentry</td>
<td>HIV-1</td>
<td>Binds to CCR5 co-receptor</td>
</tr>
<tr>
<td>Raltegravir</td>
<td>HIV-1</td>
<td>Integrase strand transfer inhibitor</td>
</tr>
<tr>
<td>Atripla: cocktail of efavirenz, tenofovir, and emtricitabine</td>
<td>HIV-1</td>
<td>A fusion inhibitor and two reverse transcriptase inhibitors</td>
</tr>
<tr>
<td>Ribavirin</td>
<td>Broad spectrum (inhibits DNA and RNA viruses): RSV, Influenza A and B, HCV, HSV-1 and HSV-2, measles, mumps, Lassa fever</td>
<td>mRNA mutagen</td>
</tr>
<tr>
<td>Amantadine</td>
<td>Influenza A</td>
<td>Inhibit penetration and uncoating of virus</td>
</tr>
<tr>
<td>Rimantidine</td>
<td>Influenza A</td>
<td>Neuraminidase inhibitor</td>
</tr>
<tr>
<td>Relenza and Tamiflu</td>
<td>Influenza A</td>
<td>Viruses-mediated membrane fusion, immune modulator?</td>
</tr>
<tr>
<td>Arbidol (not FDA approved)</td>
<td>Influenza A and B, hepatitis C</td>
<td></td>
</tr>
<tr>
<td>Cidofovir</td>
<td>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</td>
<td>DNA polymerase</td>
</tr>
<tr>
<td>Interferons</td>
<td>Hepatitis B &amp; C</td>
<td>Cell defense proteins</td>
</tr>
<tr>
<td>Fomivirsen</td>
<td>CMV</td>
<td>Inhibits viral replication and translation (antisense molecule)</td>
</tr>
</tbody>
</table>

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 (HIV), respiratory syncytial virus (RSV), hepatitis C virus (HCV), human papilloma virus (HPV). Abbreviations of antivirals: zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC). Unless otherwise specified, drugs are approved by the FDA.

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.

---

**Resources**

**Primary Literature and Reviews**


CHAPTER 3  Virus Replication Cycles


Popular Press


**Video Productions**


**eLearning**


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.

---

### 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 3-12a).

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 CDCs *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.

