

Introduction to Virology

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THE NATURE OF VIRUSES

Virus particles contain:

- A nucleic acid genome (either DNA or RNA)
- A protein coat (**capsid**) that encloses the genome
- In some cases, a lipid membrane (**envelope**)

The infectious virus particle is called a **virion**.

Virus particles are very small: between 20 and 500 nanometers (nm) in diameter.

Viruses are **obligatory intracellular parasites**.

Viruses multiply inside cells by expressing and replicating their genomes.

Viruses need the following machinery provided by cells:

- Enzyme systems that synthesize amino acids, nucleotides, carbohydrates, and lipids
- Enzyme systems that generate usable chemical energy in the form of ATP
- Ribosomes, tRNAs, and enzymes used in protein synthesis
- Membranes that concentrate cellular macromolecules, small molecules, and ions

WHY STUDY VIRUSES?

Viruses are important disease-causing agents.

Probably all different forms of life can be infected by viruses.

Viruses can transfer genes between organisms.

Viruses are important players in the regulation of the Earth's ecology.

Viruses can be engineered to prevent and cure disease.

The study of viruses reveals basic mechanisms of gene expression, cell physiology, and intracellular signaling pathways.

A BRIEF HISTORY OF VIROLOGY

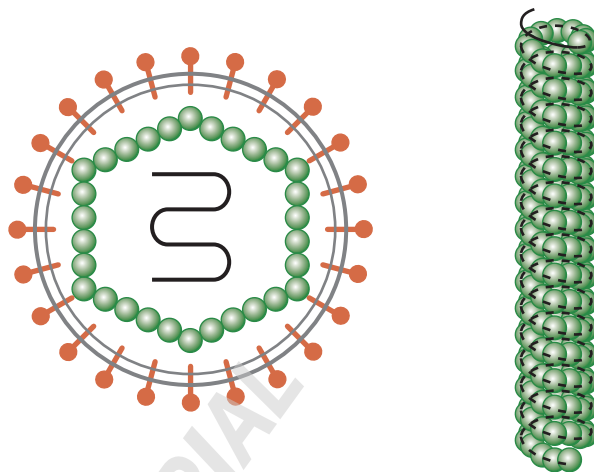
Viruses were first distinguished from other microorganisms by their small size and their ability to pass through fine filters that retain bacteria.

Viruses can be crystallized; they lie on the edge between chemical compounds and life.

The study of bacterial viruses (bacteriophages) by the “phage group” led to understanding of the nature of genes and helped establish the field of molecular biology.

In vitro culture of eukaryotic cells led to rapid advances in the study of viruses and in vaccine production.

The study of tumor viruses led to the discovery of viral and cellular **oncogenes**.



DETECTION AND MEASUREMENT OF VIRUSES

The **plaque assay** is widely used to measure virus infectivity.

Hemagglutination is a cheap and rapid method for detection of virus particles.

Virus particles can be seen and counted by electron microscopy.

The ratio of physical particles to infectious particles is greater than 1.0 for many viruses.

VIRUS REPLICATION CYCLE

1. The virion binds to cell surface receptors.
2. The virion or viral genome enters the cell; the viral genome is uncoated.
3. Early viral genes are expressed (Baltimore classification scheme).
4. Early viral proteins direct replication of the viral genome.
5. Late viral genes are expressed from newly replicated viral genomes.
6. Late viral proteins package genomes and assemble progeny virus particles.
7. Virions are released from the host cell.

THE NATURE OF VIRUSES

Viruses consist of a nucleic acid genome packaged in a protein coat

Viruses are the smallest and simplest forms of life on Earth. They consist of a set of nucleic acid genes enclosed in a protein coat, called a **capsid**, which in some cases is surrounded by or encloses a lipid membrane, called an **envelope** (Figure 1.1). The viral genome encodes proteins that enable it to replicate and to be transmitted from one cell to another and from one organism to another. The complete, infectious virus particle is called a **virion**.

Viruses are dependent on living cells for their replication

Viruses can replicate only within living cells. Another way of saying this is that viruses are **obligatory intracellular parasites**. Viruses depend on cells for their replication because they lack the following basic elements required for growth and replication, which are present in all living cells:

- Enzyme systems that produce the basic chemical building blocks of life: nucleotides, amino acids, carbohydrates, and lipids
- Enzyme systems that generate usable chemical energy, usually in the form of adenosine triphosphate (ATP),

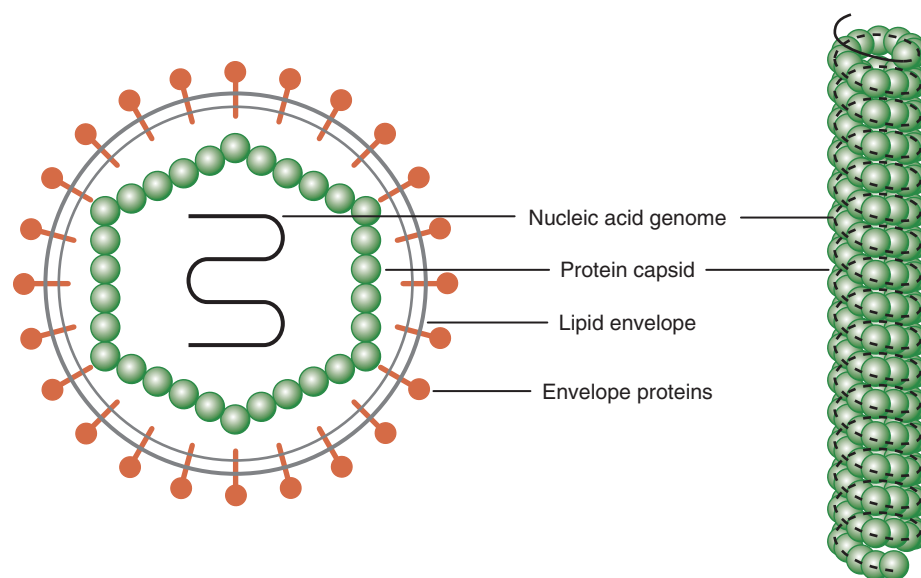


Figure 1.1 Schematic diagram of virus particles. Illustrated are the two most common capsid morphologies: a roughly spherical shell (left) and a tubular rod (right). Some virus particles have an envelope (left) and some do not (right). Nucleic acid genomes are shown as black curved lines, capsid proteins as green spheres, and envelope proteins as orange knobbed spikes.

by photosynthesis or by metabolism of sugars and other small molecules

- Ribosomes, transfer RNAs, and the associated enzymatic machinery that directs protein synthesis
- Membranes that localize and concentrate in a defined space these cellular macromolecules, the small organic molecules involved in growth and metabolism, and specific inorganic ions

Virus particles break down and release their genomes inside the cell

Viruses are not the only obligatory intracellular parasites known. A number of small unicellular organisms, including chlamydiae and rickettsiae, certain other bacterial species, and some protozoa, can multiply only inside other host cells. However, viruses replicate by a pathway that is very different from the mode of replication of these other intracellular parasites.

Virus replication begins with at least partial disintegration of the virus particle and release (**uncoating**) of the viral genome within the cell. Once uncoated, the viral genome can be used as a template for the synthesis of messenger RNAs, which in turn synthesize viral proteins using the enzyme systems, energy, ribosomes, and molecular building blocks that are present in the cell. These viral proteins then direct replication of the viral genome. Viral structural proteins encapsidate the newly replicated genomes to form progeny virus particles.

In contrast, unicellular organisms that replicate inside other cells invariably remain intact and retain their genomes within their own cellular membranes. They replicate not by disintegration and reassembly but by growth and division into daughter cells. Such cellular parasites always contain their own ribosomes and protein synthetic machinery, and their genes code for enzymes that direct many of the basic metabolic pathways.

In summary, viruses in their simplest form contain a nucleic acid genome packaged in a protein coat. To replicate, a virus must transport its genome into a host cell, where the genome directs the synthesis of viral proteins, is replicated, and is packaged. The host cell provides the virus with all of the other biological molecules required for its reproduction.

Virus genomes are either RNA or DNA, but not both

There are many different viruses in the world, and probably all organisms on Earth can be infected by at least one virus. Viruses have a variety of distinct morphologies, genome and particle sizes, and mechanisms of replication. The smallest known viruses are 20 nanometers¹ (nm) in

diameter; their genomes contain fewer than 2000 nucleotides, and they code for as few as 2 proteins. The largest known viruses are some 500 nm in diameter; their genomes can be as large as 2.5 million nucleotides, and they can code for over 2500 proteins. An overview of the variety of known viruses is given in Chapter 3.

All viruses contain genomes made of one and only one type of nucleic acid. Depending on the virus, the genome can be either RNA or DNA, and it can be either single-stranded or double-stranded. Some viral genomes are circular, and others are linear.

Viruses are the only known forms of life that can have genomes made of RNA. All cellular organisms store the information required to sustain life, to grow, and to reproduce exclusively in DNA molecules, and all RNA molecules in these organisms are transcribed from DNA sequences. RNA-containing viruses are therefore unique, and they face two related problems as a result of their RNA genomes: (1) they must synthesize messenger RNAs from an RNA template, and (2) they must replicate their genome RNA. Most RNA viruses encode their own RNA-dependent RNA polymerases to carry out both of these functions.

WHY STUDY VIRUSES?

Viruses are important disease-causing agents

As living organisms arose and evolved during the past 4 billion years on Earth, they were probably always accompanied by viruses that could replicate within cells and pass from cell to cell. Some of these viruses interfere with normal cellular processes and cause disease (although many other viruses infect their host organisms without causing overt disease). Some of the most feared, widespread, and devastating human diseases are caused by viruses (see Table 1.1). These include smallpox, influenza, **poliomyelitis**, yellow fever, measles, COVID-19, and AIDS (acquired immunodeficiency syndrome). Viruses are responsible for many cases of human **encephalitis**, meningitis, pneumonia, **hepatitis**, and cervical cancer, as well as warts and the common cold. Viruses causing respiratory infections, **gastroenteritis**, and diarrhea in young children lead to millions of deaths each year in less-developed countries.

A number of newly emerging human diseases are caused by viruses. In addition to the worldwide AIDS epidemic that started in the early 1980s, there have been localized outbreaks in Africa of the highly fatal Marburg and Ebola **hemorrhagic fevers** during the past 30 years, a short-lived epidemic in southern Asia and Canada of severe acute respiratory syndrome (SARS) in 2003, and spread of acute and chronic hepatitis via both hepatitis B and C viruses. An invasion of North America by the West Nile virus, transmitted by mosquitoes, began in 1999 and,

¹ 1 nanometer = 10⁻⁹ meter or 10⁻⁶ millimeter.

Table 1.1 Some human diseases caused by viruses

Disease	Virus	Family
Acquired immunodeficiency syndrome (AIDS)	HIV-1	Retrovirus
Cervical carcinoma	Human papillomavirus types 16, 18, and 31	Papillomavirus
Chickenpox	Varicella virus	Herpesvirus
“Cold sores”	Herpes simplex virus type 1	Herpesvirus
Common cold	Adenoviruses	Adenovirus
	Coronaviruses	Coronavirus
	Rhinoviruses	Picornavirus
Diarrhea	Norwalk virus	Calicivirus
	Rotaviruses	Reovirus
Genital herpes	Herpes simplex virus type 2	Herpesvirus
Hemorrhagic fevers	Dengue virus	Flavivirus
	Ebola and Marburg viruses	Filovirus
	Lassa fever virus	Arenavirus
Hepatitis	Hepatitis A virus	Picornavirus
	Hepatitis B virus	Hepadnavirus
	Hepatitis C virus	Flavivirus
Influenza	Influenza A and B virus	Orthomyxovirus
Measles	Measles virus	Paramyxovirus
Mononucleosis	Epstein-Barr virus	Herpesvirus
	Cytomegalovirus	Herpesvirus
Mumps	Mumps virus	Paramyxovirus
Poliomyelitis	Poliovirus types 1, 2, and 3	Picornavirus
Rabies encephalitis	Rabies virus	Rhabdovirus
Severe acute respiratory syndrome (SARS)	SARS coronavirus	Coronavirus
Smallpox	Variola virus	Poxvirus
Warts	Human papillomavirus types 1, 2, and 4	Papillomavirus
Yellow fever	Yellow fever virus	Flavivirus

fortunately, has only caused disease and death in a limited number of victims. A pandemic of SARS caused by the coronavirus SARS CoV-2 began in late 2019 and led to disruption of the world economic system and over seven million deaths by 2023. There are fears that a new deadly pandemic of human influenza could occur if a recently emerged, highly pathogenic strain of avian influenza virus mutates to a form that is easily transmitted among humans.

Viruses can infect all forms of life

Viruses also infect animals, plants, and insects of importance to humans. Outbreaks of virus diseases in domesticated animals can lead to the destruction of thousands or millions of animals to avoid even more widespread epidemics. These diseases include avian influenza; foot-and-mouth disease of cattle; infectious gastroenteritis and bronchitis in pigs, cattle, and chickens; sheep lung tumors caused by a retrovirus; canine distemper; and feline immunodeficiency disease. Virus diseases affecting domesticated plants such as potatoes, tomatoes, tobacco, coconut trees, and citrus trees are

common and widespread. Insect viruses that kill silkworms, used for centuries in Asia and Europe to produce silk, have plagued that industry over the ages. Viruses can also infect and kill bacteria, archaea, algae, fungi, and protozoa.

Viruses are the most abundant form of life on Earth

Recent studies of soil and seawater have revealed that bacterial viruses, also called bacteriophages, are much more numerous than previously imagined. There are 10–50 million bacteriophages on average per ml of seawater, and even more in many soils. Given the enormous volume of the oceans, scientists have calculated that there may be as many as 10^{31} bacteriophages in the world. This is about 10-fold greater than the estimated number of bacteria. In terms of mass, this many phages would weigh about 100 million tons, or the equivalent of 1 million blue whales (the largest animal on Earth). More astonishingly, these 10^{31} phages, if lined up head-to-tail, would stretch some 200 million light years into space—that is, far into the universe beyond many of our known neighboring galaxies (see text box on page 6)!

More important is the ecological role played by bacteriophages and viruses that infect unicellular eukaryotic organisms such as algae and cyanobacteria. From 95 to 98% of the biomass in the oceans is microbial (the remaining 2–5% being made up of all other forms of life, including fish, marine invertebrates, marine mammals, birds, and plants), and roughly half of the oxygen in the Earth's atmosphere is generated by the photosynthetic activity of marine microbes. It has been estimated that 20% of the microbes in the Earth's oceans are destroyed each day by virus infections. Therefore, these viruses play a major role in the carbon and oxygen cycles that regulate our atmosphere and help feed the world's population.

The study of viruses has led to numerous discoveries in molecular and cell biology

Because viruses replicate within cells but express a limited number of viral genes, they are ideal tools for understanding the biology of cellular processes. The intensive study of bacteriophages led to the discovery of some of the fundamental principles of molecular biology and genetics. Research on animal, insect, and plant viruses has shed light on the functioning of these organisms, their diseases, and molecular mechanisms of replication, cell division, and signaling pathways. For example:

Phages lined up through the universe

Scientists estimate that there are approximately 10^{31} tailed bacteriophages on Earth. Each phage measures approximately 200 nm (0.2 μm) in length from top of head to base of tail. Aligned head-to-tail, these phages would therefore cover the following distance:

$$\begin{aligned} 10^{31} \times 0.2 \mu\text{m} &= 0.2 \times 10^{25} \text{ m} = 2 \times 10^{24} \text{ m} \\ &= \mathbf{2 \times 10^{21} \text{ km.}} \end{aligned}$$

Because 1 light year (the distance traveled by light in one year) = 10^{13} km,

$$\begin{aligned} 2 \times 10^{21} \text{ km} &= 2 \times 10^{21} / 10^{13} \text{ light years} \\ &= \mathbf{2 \times 10^8 \text{ light years}} \text{ (200 million light years)} \end{aligned}$$

Note that our Milky Way galaxy measures approximately 100,000 light years edge to edge, and the furthest visible galaxies in the universe are approximately 10 billion (10×10^9) light years distant.

- The study of gene expression in small DNA viruses led to the identification of promoters for eukaryotic RNA polymerases.
- Research on the replication of bacteriophage and animal virus DNAs laid the foundations for understanding the enzymes involved in cellular DNA replication.

- RNA splicing in eukaryotic cells was first discovered by studying messenger RNAs of DNA viruses.
- The study of cancer-producing viruses led to the isolation of numerous cellular **oncogenes** and the understanding that cancer is caused by their mutation or unregulated expression.

Given this track record, the study of viruses will undoubtedly continue to shed light on many important aspects of cell and molecular biology.

A BRIEF HISTORY OF VIROLOGY: THE STUDY OF VIRUSES

The scientific study of viruses is very recent

Although viruses were probably present among the first forms of life and have evolved over several billion years, humans only began to understand the nature of viruses near the end of the nineteenth century (see Table 1.2). It had been appreciated for some time that infectious diseases were transmitted by air, water, food, or close contact with sick individuals. Many diseases were considered to be caused by mysterious elements in fluids termed *virus* (“poison” in Latin), but the distinction between what are now called viruses and cellular microorganisms was not clear. Scientists had begun to use light microscopes to discover and describe fungi and bacteria in the first half of the nineteenth century. Louis Pasteur and Robert Koch firmly established the science of bacteriology in the latter part of that century by isolating and growing a variety of bacteria, some of which were shown to cause disease (e.g., tuberculosis). Even though effective vaccines against smallpox (Edward Jenner 1798) and rabies (Louis Pasteur 1885) were developed, there was no understanding of the nature of these disease agents, which we now know to be viruses.

Viruses were first distinguished from other microorganisms by filtration

In the last decade of the nineteenth century, Russian scientist Dimitrii Ivanovski and Dutch scientist Martinus Beijerinck independently showed that the agent that causes tobacco mosaic disease could pass through fine earth or porcelain filters, which retain bacteria. Shortly afterward, similar experiments were carried out on agents that cause foot-and-mouth disease in cattle and yellow fever in humans. These landmark experiments established that certain infectious agents are much smaller than bacteria, and they were called **filterable viruses**. For some time, it was not clear whether viruses were soluble small molecules (“infectious living fluid”) or simply very small particles, similar to bacteria but too small to be retained by these filters.

Table 1.2 Some milestones in virology research

Discovery	Date	Scientist	Nobel Prize awarded
Smallpox vaccine	1798	Edward Jenner	
Rabies vaccine	1885	Louis Pasteur	
Filterable viruses: tobacco mosaic virus	1892	Dimitrii Ivanovski	
	1898	Martinus Beijerinck	
Foot-and-mouth disease (cattle)	1898	Friedrich Loeffler and Paul Frosch	
Yellow fever (humans: transmitted by mosquitoes)	1900	Carlos Finlay and Walter Reed	
Discovery of Rous Sarcoma virus	1911	Peyton Rous	1966
Discovery of bacteriophages and the plaque assay	1915	Frederick Twort	
	1917	Felix d'Herelle	
Vaccine against yellow fever	1930s	Max Theiler	1951
Crystallization of tobacco mosaic virus	1935	Wendell Stanley and John Northrup	1946
Studies with bacteriophages	1940s	Max Delbruck and Salvador Luria	1969
Growth of poliovirus in cultured cells	1949	John Enders, Frederick Robbins, and Thomas Weller	1954
Bacteriophage lambda and lysogeny	1950s	Andre Lwoff	1965
Bacteriophage genes are DNA	1952	Alfred Hershey and Martha Chase	1969
Discovery of interferon	1957	Alick Isaacs and Jean Lindenmann	
Poliovirus vaccines:			
killed	1955	Jonas Salk	
live	1960	Albert Sabin	
Studies on polyomavirus: a tumor virus	1960s	Renato Dulbecco	1975
Kuru is caused by an infectious agent	1965	D. Carleton Gajdusek	1976
Discovery of hepatitis B virus	1968	Baruch Blumberg	1976
Reverse transcriptase in retroviruses	1971	Howard Temin and David Baltimore	1975
Virus vectors and genetic engineering	1970s	Paul Berg	1980
Cellular oncogene is part of a retrovirus genome	1976	Michael Bishop and Harold Varmus	1989
RNA splicing in adenovirus	1977	Phillip Sharp and Richard Roberts	1993
Prions: infectious proteins	1975–1990	Stanley Prusiner	1997
Human papillomaviruses cause cervical cancer	1972–1984	Harald zur Hausen	2008
Discovery of AIDS virus (HIV-1)	1983	Luc Montagnier and Françoise Barré-Sinoussi	2008
Discovery and identification of hepatitis C virus	1968, 1998	Harvey Alter, Michael Houghton, and Charlie Rice	2021
Discovery of Marburg and Ebola viruses	1963, 1976	Werner Slenczka and Rudolf Siebert Peter Piot and Jean-Jacques Muyembe	
Identification and sequencing of SARS-CoV genome	2003	Marco Marra Paul Rota	
Discovery of MERS	2012	Ali Mohamed Zaki, Ron Fouchier	
Identification and sequencing of SARS-CoV-2 genome	2020	Zhang Yongzhen	
Modified mRNA vaccine technology applied to COVID-19	2005, 2020	Katalin Kariko and Drew Weissman	2023

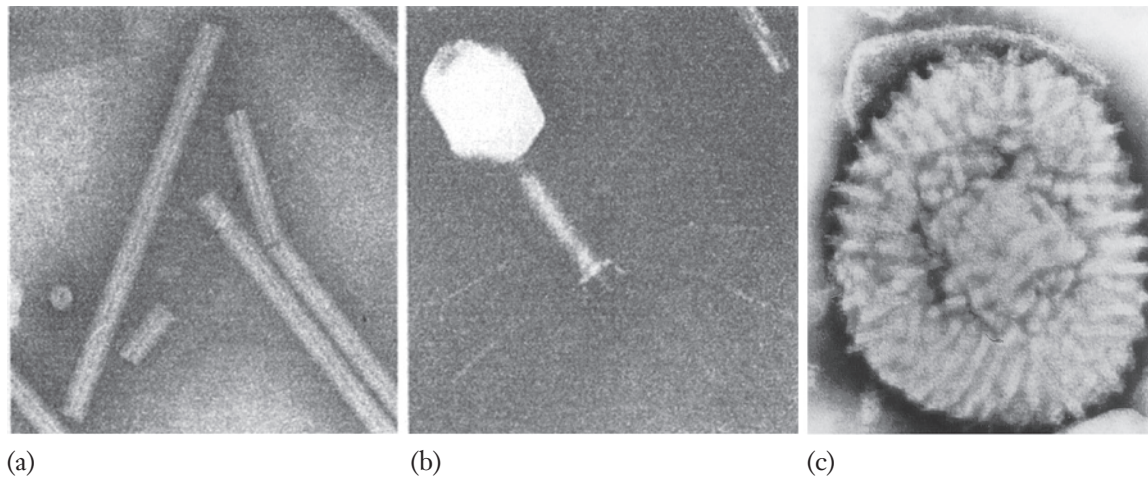


Figure 1.2 Electron micrographs of some representative virus particles. (a) Tobacco mosaic virus. (b) Bacteriophage T4. (c) Vaccinia virus.

Using filtration as a diagnostic tool, numerous viruses infecting humans, other vertebrate animals, plants, insects, and bacteria were described during the first half of the twentieth century. A tumor virus, Rous Sarcoma virus, was isolated from sarcomas of chickens in 1911 and was only many years later recognized as a representative of the important retrovirus family, of which human immunodeficiency virus (HIV) is a member. Scientists working in England and France discovered in 1915–1917 that bacterial cultures could be lysed by filterable agents, the first known bacterial viruses. Vaccines against the important human pathogens responsible for influenza and yellow fever were developed during the 1930s.

The crystallization of tobacco mosaic virus challenged conventional notions about genes and the nature of living organisms

Wendell Stanley found in the mid-1930s that highly purified tobacco mosaic virus could form crystals. This discovery shook the scientific world because it placed viruses at the edge between living organisms and simple chemical compounds like sodium chloride. It posed the question: Are viruses living or inanimate? We now know that viruses are inanimate when their genomes are packaged in virions, but they share many attributes of life, including the ability to mutate, evolve, and reproduce themselves, when they enter cells that can support their replication.

Studies by Stanley and others showed that viruses contain both proteins and nucleic acids. At that time, most scientists believed that genes were made of proteins,

not nucleic acids, because only proteins were believed to be sufficiently complex to encode genetic information. The development of the electron microscope in the late 1930s allowed scientists for the first time to actually see viruses—tobacco mosaic virus (a long rod-shaped virus), bacteriophages with their polygonal heads and tubular tails, and vaccinia virus, one of the largest animal viruses (Figure 1.2).

The “phage group” stimulated studies of bacteriophages and helped establish the field of molecular biology

During the late 1930s and early 1940s, a group of scientists led by German physicist Max Delbrück, American biologist Emory Ellis, and Italian biologist Salvador Luria decided that the study of bacterial viruses (bacteriophages) could lead to understanding of the basic processes of life at a molecular level. They reasoned that bacteriophages show heritable traits, and therefore must contain and express genes as do all other organisms. Because bacteriophages are small and simple and can be propagated easily in bacterial cultures, they would be a fertile terrain for scientific discovery.

These scientists formed an informal network called the “phage group,” which stimulated studies of bacteriophages and their host bacteria by numerous physicists, chemists, and biologists. These studies led to the isolation and analysis of phage genomes, the mapping of phage and bacterial genes by genetic crosses, and the elucidation of phage replication cycles. The phage group helped to establish the field of molecular biology, which developed rapidly during the 1950s and 1960s.

Bacteriophages Can Be Used as Targeted Antibiotics Against Bacterial Diseases

Bacteriophages may be called upon to play an important role in the fight against bacterial diseases in humans and animals. Because bacteriophages can attack and kill specific bacteria, they have long been considered as possible alternatives to antibiotics in treating disease. Bacteriophages specific to a variety of pathogenic bacteria have been isolated and characterized. There is a long history of their medical use, particularly in the Republic of Georgia, in an institute cofounded by one of the discoverers of bacteriophages, Felix d'Herelle. However, their use as antibacterial agents in humans is not accepted in most countries; there are significant unsolved problems, not the least of which is the induction of immune reactions to the bacteriophage. Future research and development may well reveal situations in which their use will be able to control otherwise runaway infections by bacteria that have developed resistance to many commonly used antibiotics.

One outcome of the phage group's work was the demonstration, by Alfred Hershey and Martha Chase in 1952, that the DNA of a bacteriophage is injected into the host cell and the protein coat remains outside the cell. This strongly backed up the chemical and enzymatic data published eight years earlier by Oswald Avery, Maclyn McCarty, and Colin MacLeod, showing that a bacterial gene was made of DNA. Thus, studies of a bacteriophage were important in proving the chemical nature of genes. Hershey and Chase's paper was followed a year later by the proposal by James Watson (a student of Luria) and Francis Crick of the double-helical model of DNA, which galvanized thinking and research throughout biology, but particularly in virology and molecular biology.

Study of tumor viruses led to discoveries in molecular biology and understanding of the nature of cancer

Virus research underwent an explosive development in the second half of the twentieth century that led to the discovery of many new viruses and basic concepts in cell and molecular virology. Among the most important were the discovery and intensive study of DNA tumor viruses (polyomaviruses, papillomaviruses, adenoviruses, and some herpesviruses; see Chapters 23–26) and RNA tumor viruses (retroviruses; see Chapters 28 and 29). Research on DNA tumor viruses led to the discovery of viral oncogenes, whose protein products (tumor antigens) interact

with numerous cell signaling pathways to stimulate cell growth and division.

Research on RNA tumor viruses led to the discovery of **reverse transcriptase**, an enzyme that can make a DNA copy of an RNA molecule, upsetting the one-way central dogma that “DNA makes RNA makes proteins.” Numerous cellular **oncogenes** were discovered, and many were found to be incorporated into retrovirus genomes. These oncogenes are normal cellular regulatory genes whose mutation and/ or overexpression can lead to the development of cancer; their protein products are involved in a variety of cellular signaling pathways. The study of viral and cellular oncogenes has led to major advances in the detection and treatment of cancer.

Virus Vectors Can Replace Defective Genes, Serve as Vaccines, and Combat Cancer

Advances in molecular cloning have allowed the construction of numerous **virus vectors**. These are viruses in which some or all viral genes are removed by genetic engineering and are replaced by foreign genes. Because viruses can efficiently target specific cell types and express their genes at high levels, they can be used as vectors for the expression of a variety of genes. The introduction of virus vectors into host cells or organisms can correct genetic diseases in which specific gene products are missing or defective (see Chapter 42). Vectors can also be used as vaccines that generate **immune responses** against a variety of unrelated pathogens (see Chapter 39). In a new twist, virus vectors have recently been used to combat cancers by expressing proteins that specifically kill tumor cells. These agents are called **oncolytic** viruses (see Chapter 41).

Epidemics and pandemics drive the production and deployment of new vaccines

As is usually the case, necessity drives invention. The field of vaccinology has been driven by the occurrence of **epidemics** and **pandemics** throughout history. **Variolation** was a “risky” form of vaccination originally practiced using powdered smallpox scabs by the Chinese in the tenth century and the Turks in the seventeenth century. The very first “safe” viral vaccines were developed against smallpox (1798) and rabies (1885) using attenuated cowpox virus by Jenner and inactivated rabies virus by Pasteur, respectively. There was a long *hiatus* between these immunizing agents and the modern vaccines developed in the twentieth and twenty-first centuries. Globalization, increased urbanization, population crowding, and wars drove many of the epidemics. The Spanish–American War along with the construction of the Panama

Canal brought yellow fever to the forefront. Intense research directed against yellow fever proved that the infectious agent was a mosquito-borne virus (1900), which culminated with the development of an effective live-attenuated vaccine by Max Theiler (1934).

On another front, the Spanish flu pandemic of 1918 killed an estimated 50 million individuals, and the first inactivated influenza virus was produced in fertile hens' eggs by several famous virologists, including Ernest Goodpaster and Macfarlane Burnet. The vaccine was administered to soldiers and sailors in WWII, but its efficacy was limited and still is, due to genetic variation of the virus. The great polio epidemics throughout North America and Europe in the 1950s ignited the March of Dimes, which financed the development of the inactivated Salk vaccines (1955) and the live-attenuated Sabin vaccine (1961). These immune agents effectively controlled polio. Other attenuated childhood vaccines were developed against measles, rubella, mumps, and chickenpox by researchers like John Enders (polio, measles) and Maurice Hilleman (mumps, rubella, chickenpox). These attenuated viruses were developed through continued passage of wild-type viruses in cultured cells (human, monkey, chicken) in the laboratory. Live-attenuated viruses convey superior T-cell immunity against their parental pathogens compared to subunit protein or inactivated viral vaccines. However, **live-attenuated vaccines** and **subunit vaccines** take time to produce and test. The COVID-19 pandemic spurred the development of **mRNA vaccines**, which were fast-tracked in the fight against SARS-CoV-2. The idea for mRNA vaccines was born in the 1980s using mRNA molecules manufactured through *in vitro* transcription and T7 RNA polymerase. However, this lab-made mRNA was recognized as foreign and triggered potentially harmful immune responses due to host cell innate immunity. Natural mRNAs found in mammalian cells contained modified nucleotides (pseudouridine, 5'-methyluridine, N6-methyladenosine), 5' type 1 cap structures, and poly-A tails of around 80 nucleotides. Katalin Kariko and Drew Weissman discovered that these modifications, along with encapsulation of the mRNA in lipid bi-layered **nanoparticles**, facilitated efficient delivery and translation of mRNA in the target cell following transfection. By 2015, these lipid nanoparticle mRNA vaccines were showing promising results in influenza clinical trials. During the COVID-19 pandemic, similar vaccines containing mRNA for the viral spike protein were produced by Pfizer/BioNTech and Moderna Pharmaceuticals. Over a million doses were manufactured within six weeks of sequencing the genome of SARS-CoV-2. Clinical trials of the new mRNA vaccines were fast-tracked and found to be effective. This advance saved many lives and was awarded the Nobel Prize in Physiology and Medicine in 2023.

DETECTION AND TITRATION OF VIRUSES

Most viruses were first detected and studied by infection of intact organisms

Many viruses cause disease in the host organism, and this is how scientists and medical doctors usually become aware of their existence. The original methods for the study of such viruses relied on the inoculation of animals or plants with filtered extracts from infected individuals and their observation to detect the effects of virus infection. However, this is expensive and time-consuming work, and in most cases, it is no longer ethically acceptable when applied to humans. Experimental laboratory animals such as suckling mice, in which many animal viruses are able to replicate, were adopted for use because they are relatively easy and inexpensive to raise. A number of animal viruses have also been adapted to grow in embryonated chicken eggs, which are readily available from farms; this has reduced both the expense and the time of virus assays.

The plaque assay arose from work with bacteriophages

Bacterial viruses can be easily studied by inoculating bacteria grown in tubes or on Petri dishes on the lab bench. Intact bacteria diffract visible light, and therefore dense liquid cultures appear cloudy. Many bacteriophages lyse their host cell, and this **lysis** causes a loss in light diffraction leading to clearing of the bacterial culture; "clear lysis" serves as an indicator of phage replication.

A simpler and more quantitative application of cell lysis is to spread bacteria on the surface of nutrient agar in a Petri dish and to apply dilutions of a phage suspension. Wherever a phage binds to a bacterial cell and replicates, that cell releases the progeny phage particles, which are then taken up by neighboring cells and further replicated. After several such cycles, all the cells in a circular area surrounding the original infected cell are lysed. The lysis area can be seen as a clear "plaque" against the cloudy background of the uninfected cells, which grow in multiple layers on the surface of the agar in the Petri dish. The use of this **plaque assay** (Figure 1.3) allows scientists to count the number of infectious virus particles in a suspension with a high degree of precision and reproducibility. The chance observation of such plaques played an important role in the discovery of bacteriophages.

Eukaryotic cells cultured *in vitro* have been adapted for plaque assays

In vitro culture of human, animal, insect, and plant cells was achieved in the mid-twentieth century and allowed for more convenient and cheaper growth and titration of

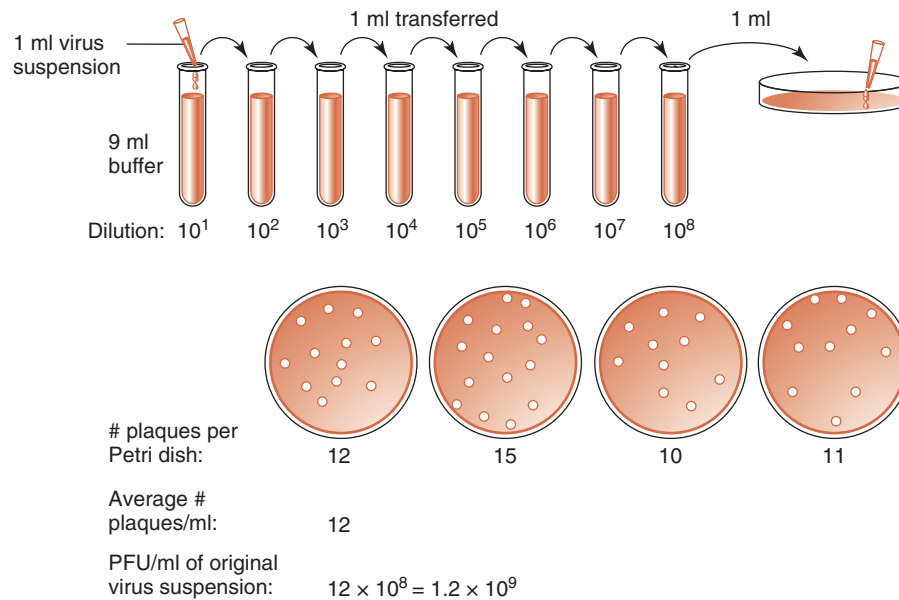


Figure 1.3 Plaque assay: an example. A virus suspension was subjected to 10-fold serial dilutions by adding 1 ml of the original suspension to 9 ml of a dilution buffer. After mixing, 1 ml of that dilution was added to 9 ml of fresh dilution buffer and mixed; these steps were repeated a total of eight times. Each successive tube contains a 10-fold dilution of the contents of the previous tube. The eighth tube is therefore diluted by a factor of 10^8 compared with the original virus suspension. One-ml aliquots from this 10^8 -fold dilution were applied to four different Petri dishes of susceptible cells and plaques (bottom) were allowed to develop. Plaque-forming units (PFU) per ml are calculated as shown.

many viruses. In the case of animal viruses, cells of many different tissues (especially from embryos or newborn animals) can be induced to grow in a monolayer on a glass or plastic surface underneath liquid media. Cultured cells also facilitated the production of numerous antiviral vaccines (see Chapter 39), starting with the vaccines against poliovirus that were developed in the 1950s.

Plaque assays were subsequently developed for animal, plant, and insect viruses using cells cultured *in vitro*. When cells growing in a monolayer are infected, the progeny virus is released into the medium and can travel to distant sites, infecting other cells. To restrict diffusion of the progeny virus, the infected cells are overlaid with nutrient medium in melted agar, which solidifies on cooling. In gelled agar, the released virus can infect only nearby cells on the monolayer, forming a local area of dead cells or a plaque.

Because cell monolayers are too thin to diffract light well, plaques in cultured animal or insect cells are usually visualized by staining the cells. When cells die and/or lyse, they do not stain well; therefore, plaques are seen as clear, unstained circular areas on the background of the stained cell monolayer. Virus present in individual plaques can be isolated by sampling with a needle or Pasteur pipette, allowing the “cloning” of progeny virus derived from a single virion that initiated the infection leading to the plaque.

When a plaque assay is used to measure the infectious titer of a virus suspension, the results are usually expressed as **plaque-forming units (PFUs)** per ml of suspension. To determine the titer, the number of plaques

on a plate is multiplied by the factor by which the original virus suspension was diluted before an aliquot was applied to the plate. For an example, see Figure 1.3.

Hemagglutination is a convenient and rapid assay for many viruses

A number of animal viruses bind to **sialic acid** residues or other carbohydrates on cell surface proteins and lipids. Red blood cells have carbohydrate-containing receptors on their surface and have the advantage of being visible because of their color. Furthermore, they can be easily isolated from the blood of a variety of animals, are sturdy during manipulation, and can be stored for days or weeks. This makes red blood cells an ideal substrate for assaying viruses.

Virus particles have multiple copies of receptor-binding proteins on their surface, and red blood cells contain many copies of surface receptors. Binding between an excess of virus particles and an aliquot of red blood cells forms an interlaced network of cells, held together by virus particles that form bridges between adjacent cells. These “agglutinated” red blood cells, when allowed to settle, form a light pink hemispherical shell in the bottom of a tube or plastic well. In contrast, individual red blood cells slide to the bottom of the tube and form a compact, dark-red pellet (Figure 1.4). This is the basis of **hemagglutination assays** for viruses.

Virus suspensions are diluted, usually in twofold steps, and the dilutions are added to aliquots of red blood cells in a buffer and mixed in tubes or multiple-well plates. After

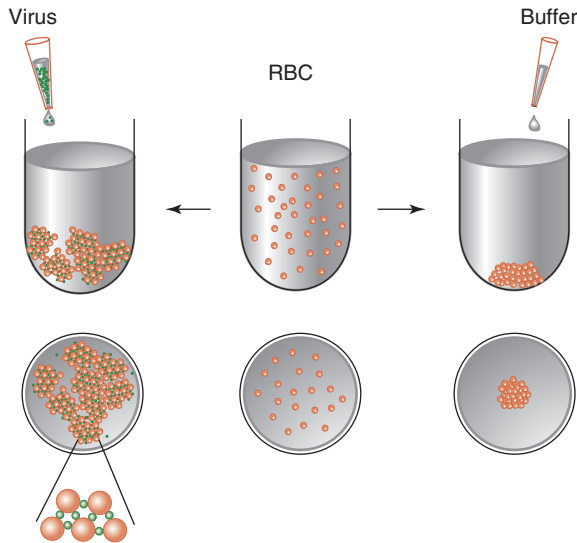


Figure 1.4 Hemagglutination assay. Red blood cells (RBC; small orange circles in central tube) are mixed with virus (small green spheres), or with buffer, and are allowed to settle. Individual red blood cells settle to form a compact pellet in the bottom of the tube (right), but when agglutinated, form a thin shell on sides and bottom of tube (left). The lower set of images shows what is seen when tubes are viewed from below. An enlargement on left shows red blood cells bound together by virus particles.

allowing the cells to settle, the tubes or plates are examined; the highest dilution that will agglutinate the aliquot of cells is considered to have one hemagglutinating unit (HAU) of virus. Such assays are sensitive to pH, temperature, and buffer composition, and some viruses will agglutinate only cells of a particular mammalian or avian species.

Because many (approximately 10^5) red blood cells are used in each tube, one HAU represents 10^5 or more virus particles. Therefore, hemagglutination assays are much less sensitive than plaque assays, but they are rapid and cheap. They can also be used to detect antibodies that bind to viral surface antigens, because addition of such antibodies will inhibit hemagglutination.

Virus particles can be seen and counted by electron microscopy

A variety of staining or shadowing methods can be used to detect virus particles by electron microscopy. One of the simplest methods is to mix a virus suspension with an electron-dense stain, usually phosphotungstate or uranyl acetate, and spread the mixture on a grid for examination under the electron microscope. The stain tends to form electron-dense pools around virus particles; virus particles exclude the stain and therefore show up as light images against a dark background, and much fine surface detail can be seen (Figure 1.2). This technique is called “negative staining.” Measured aliquots of dilutions of virus suspensions can be applied to grids, and the number

of virus particles in a given area can be counted. Standard suspensions of tiny latex spheres or other small uniform objects are often added to the virus suspension to help establish absolute numbers of virus particles per unit volume.

Virus genome copy equivalents can be determined by quantitative polymerase chain reaction (qPCR)

Quantitative **polymerase chain reaction** (qPCR) utilizes Taq DNA polymerase and reverse transcriptase chemistry to amplify viral DNA or viral RNA. Amplification produces sufficient concentrations of DNA for detection and quantification of viral genomes by fluorescence. Quantification by qPCR relies on serial dilutions of standards of known concentrations that are analyzed in parallel with the unknown samples. Quantitative detection can be achieved using different fluorescence detection methods, including sequence-specific fluorescent probes (TaqMan Beacons/Scorpion), which bind specifically to the amplified viral DNA being produced, or non-specific fluorescent dyes for DNA, such as SYBR Green, which bind to all double-stranded DNA. To analyze RNA virus genomes, it is necessary to first convert the RNA to a DNA template using reverse transcriptase, prior to DNA amplification.

The ratio of physical virus particles to infectious particles can be much greater than 1

Measurement of the number of infectious virus particles by use of plaque assays, and of the number of physical virus particles in the same virus suspension by electron microscopy, allows calculation of the ratio of physical particles to infectious particles. Naively, one would expect that most intact virus particles are infectious. This is true for some bacteriophages and for a small number of animal viruses. However, for many viruses, the ratio of physical particles to infectious particles can be 10, 100, or even 1000! There are several possible reasons for the low infectivity of virus preparations:

- *Not all virus particles may be intact.* For example, virus envelopes are fragile and can be disrupted, rendering the particle non-infectious. Some viral surface protein molecules can be denatured and therefore unable to bind to the cell receptor. However, virions contain numerous copies of receptor-binding proteins on their surface, so the loss or denaturation of a few protein molecules should not lead to loss of infectivity.
- *Some virus particles may contain defective genomes.* Mutations, including deletions in viral genes, occur frequently during genome replication, and such defective genomes are often incorporated into virus particles. In extreme cases, 90% or more of a virus preparation consists of particles with defective genomes.

- “Empty” capsids that contain no viral genome can be made in large numbers. Some viruses can form capsids in the absence of the viral genome. Others incorporate cellular DNA or RNA instead of the viral genome into the capsid. However, many viruses have specific packaging signals that ensure the incorporation of only viral nucleic acid into virions.
- Cells have antiviral defense mechanisms. Many virus preparations consist of fully intact virions that contain infectious genomes. However, cells have a variety of defense mechanisms that can interfere with many steps in virus infection. Therefore, even though a cell takes up an intact and potentially infectious virion, it may not produce any progeny virus. This can be a major cause of the high ratio of physical particles to infectious particles of some viruses.

THE VIRUS REPLICATION CYCLE: AN OVERVIEW

The single-cycle virus replication experiment

The use of the plaque assay enables the quantitative study of the kinetics of virus replication. To understand the time course of events taking place during the replication cycle, scientists usually study cultures containing thousands to millions of infected cells, because only then can sufficient viral nucleic acids or proteins be isolated and analyzed. All cells must be infected simultaneously, and with some luck, the events of the virus replication cycle will be synchronized so that similar steps will be taking place at the same time in all cells. To ensure simultaneous infection, cell cultures are infected with a sufficient number of virus particles such that each cell receives at least one infectious particle.

These considerations led to the concept of **multiplicity of infection (m.o.i.)**. This is defined as the number of infectious virus particles added per susceptible cell. An m.o.i. of 10–100 PFUs per cell is often used in studies of bacterial or animal viruses. Nearly all cells in a culture are infected simultaneously, and there remain very few uninfected cells.

In practice, there are limitations in this method for distinguishing the time course of the various steps in a viral growth cycle; different steps in the cycle invariably overlap somewhat. However, the single-cycle approach does simplify the study of virus replication and is nearly universally used. Some bacteriophages complete their replication cycles in as little as 20 minutes; some animal viruses can take several days to complete one replication cycle. Certain viruses do not always undergo a productive growth cycle but instead lodge one or more “silent” copies of their genome in the host cell until conditions are appropriate for a lytic cycle (lysogeny or **latent infection**; see Chapters 9, 10, 26, 28, and 34).

An example of a virus replication cycle: mouse polyomavirus

An idealized example of a single-cycle growth curve for mouse polyomavirus (see Chapter 23), a small DNA virus that replicates in cultured baby mouse kidney cells, is shown in Figure 1.5. Each 6-cm-diameter Petri dish contains approximately 5 million cells, and 0.5 ml of a suspension containing 100 million (10^8) PFU/ml of polyomavirus is used to infect the cells (m.o.i. = 10 PFU/cell).

After 1 hour, fresh medium is added to each culture, and the cells are incubated at 37 °C in a humidified chamber with a 5% CO₂ atmosphere. At intervals, samples of the infected cells or of the medium are harvested and analyzed for infectious virus, viral mRNA, viral proteins, or viral DNA. The results are expressed as PFU/cell for virus (logarithmic scale on left) or as a percent of the maximum amount of the viral macromolecule (linear scale on right).

During the first hour or two after adding virus, most of the infecting virions are taken up into the cells or are subsequently washed away by changing the medium. This leads to an initial loss in the titer of virus detected in the medium. Eventually, many of the virus particles taken up into the cells are uncoated, rendering intracellular virus non-infectious, and thus the titer of infectious intracellular virus also drops. This phase has been called the “latent phase” of infection because the infecting virus has disappeared and no new progeny virus has yet been made.

Sometime later (in this case, starting at 18–20 hours after infection), new progeny virus begins to appear. Polyomavirus particles are assembled in the cell nucleus and are not efficiently released from the cell until after cell death. This means that most progeny viruses can be detected only by lysing the cells after harvesting them. A much lower virus titer is detected in the medium surrounding the cells (not shown). This is true of many non-enveloped viruses that replicate and assemble in the nucleus or in the **cytoplasm** of eukaryotic cells, as well as of most bacteriophages. In contrast, many enveloped viruses form virions at the cell surface, where they acquire a lipid envelope, and therefore newly assembled virus particles are immediately released into the medium.

There is often a long period during which progeny virus is being produced, but at some point, virus replication stops, often because the host cell dies. At this point, no further virus is made, but release can take some time as cells slowly disintegrate after death.

Analysis of viral macromolecules reveals the detailed pathways of virus replication

This analysis of the virus growth cycle is very elementary and gives only an overview of the beginning and the end of steps in virus replication. Many important events of the virus replication cycle take place during the latent period. These steps are studied by extracting from the infected cell the macromolecules that constitute viral genomes,

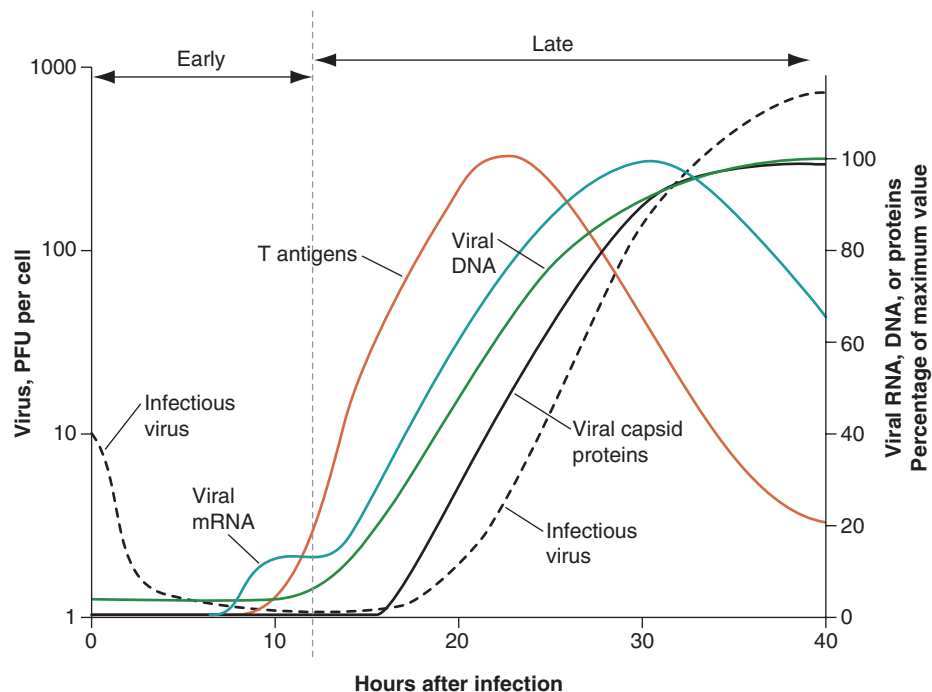


Figure 1.5 Replication cycle of mouse polyomavirus. The time course of appearance of infectious virus particles, viral mRNA, DNA, and proteins during a typical replication cycle of mouse polyomavirus in baby mouse kidney epithelial cells.

messenger RNAs, and proteins. Their interactions with cellular macromolecules and organelles can be analyzed, and their size, number per cell, and rates of synthesis and turnover can be studied.

This is often done by using radioactive compounds that are incorporated into viral or cellular DNA, RNA, proteins, lipids, or carbohydrates. Antibodies against viral or cellular proteins can be used to detect specific molecules with fluorescent dyes or other assays. Molecular hybridization with oligonucleotide probes, or the polymerase chain reaction (PCR), can be used to detect specific viral DNA or RNA molecules. Macromolecules are often separated from each other by electrophoresis on polyacrylamide or agarose gels.

Staining of macromolecules can be observed in fixed or living cells by microscopy, especially by using computerized methods that allow visualization of horizontal layers of thickness 1 micrometer (μm) or less within individual cells. These views, revealed by **confocal microscopes** using focused laser light sources, show the localization of viral macromolecules within the nucleus, or in association with organelles such as the endoplasmic reticulum, lysosomes, or the plasma membrane. Other localization methods use the higher-resolution electron microscope coupled with specific staining, for example, using colloidal gold particles linked to antibodies.

The study of virus replication cycles by these and other methods has led to a general understanding of the various steps in virus replication. In the case of polyomavirus, Figure 1.5 shows that small amounts of “early” viral messenger RNAs are made beginning about

8–10 hours after infection, and early viral proteins, known as T antigens, begin to appear shortly afterward. This is followed by the beginning of viral DNA replication at 12–15 hours after infection. Once viral DNA replication has begun, much larger amounts of viral messenger RNAs can be detected, and these “late” messenger RNAs are copied from a different set of viral genes. Mature progeny virions begin to appear by 18–20 hours after infection, and virus titers increase slowly for the next 24 hours, by which time most of the infected mouse cells are dead.

Virus replication cycles vary greatly, depending on the host cell, genome type, and complexity of the virus. However, there is logic to the progression of steps in virus replication. The following section gives an outline and analysis of the replication pathway starting with the initial infection of a cell and progressing to the release of progeny virions (Figure 1.6). Designation and naming of these steps is somewhat arbitrary, but it is useful to help understand the important elements of the replication cycles of all viruses.

STEPS IN THE VIRUS REPLICATION CYCLE

1. Virions bind to receptors on the cell surface

Viruses must first recognize and bind to the cells that they infect. Virus-coded proteins on the surface of the virion bind to specific proteins, carbohydrates, or lipids on the cell surface. Structural surface proteins of many

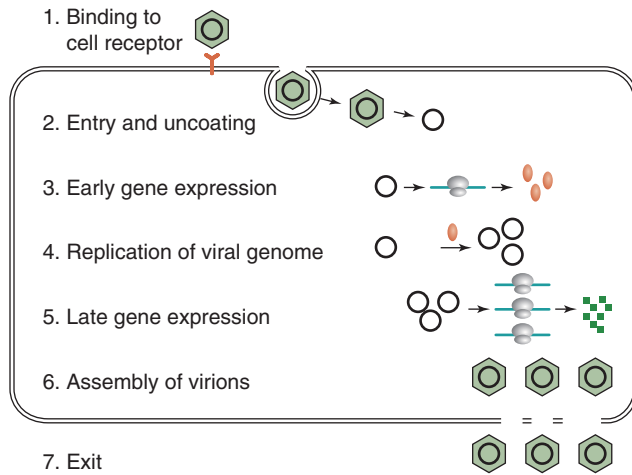


Figure 1.6 Steps in virus replication cycle. A schematic diagram showing a simplified virus replication cycle in seven steps, as described in the text. The host cell is shown as a rounded box. Capsids are represented as green icosahedra, genomes as black circles, messenger RNAs as blue lines with translating ribosomes, viral replication proteins as orange ovals, and viral structural proteins as green squares.

viruses bind to carbohydrate residues found in surface **glycoproteins** and **glycolipids** that are widely distributed on many cell types. Other viruses bind to cell surface proteins that are found only on specific cell types and are often species-specific, thus limiting the **tropism** of the virus to a particular tissue or organism. Many viruses first bind to a relatively non-specific primary receptor, such as a carbohydrate, and subsequently bind to a specific cell surface protein that serves as a secondary receptor.

2. The virion (or the viral genome) enters the cell

Once they have located an appropriate cell, bacteriophages and plant viruses are presented with the problem of passing through a rigid cell wall as well as the outer cell membrane(s). Many bacteriophages have specialized tails that drill holes in the cell wall and membranes and serve as a conduit for the passage of the DNA genome through the hole into the cell. Plant viruses often penetrate as a result of damage to the cell wall caused by abrasion or a wound caused by an insect.

Some enveloped animal viruses fuse their lipid envelopes directly with the plasma membrane of the cell, releasing the viral capsid and genome into the cell. Many other animal viruses are taken up into the cytoplasm in vesicles formed at the plasma membrane. These vesicles then release the virion or its genome into the cytoplasm, and in some cases, the genome is transported to the nucleus. Release can happen by disintegration of the vesicle membrane or by fusion of the viral envelope with

the vesicle membrane. In cases where the capsid is released into the cell, a variety of pathways lead to disintegration of the capsid and release of the genome, called uncoating. The entry of animal viruses into cells is discussed in detail in Chapter 4.

3. Early viral genes are expressed: the Baltimore classification of viruses

Once in the appropriate compartment of the cell, the viral genome must direct the expression of “early” proteins that will enable genome replication. The molecular pathways that lead to the synthesis of early viral proteins from viral messenger RNAs depend on the chemical nature and strandedness of the genome. David Baltimore first recognized that all viruses can be divided into six (now seven) groups based on the pathways leading to mRNA and protein synthesis; this has come to be known as the *Baltimore classification system* (see Figure 1.7). One advantage of this system is that it draws our attention to the distinct kinds of RNA polymerases needed by viruses in each group and indicates whether these enzymes are available in the cell or must be provided by the virus.

Viruses with single-stranded RNA genomes fall into two categories. Some viruses package the “sense” RNA

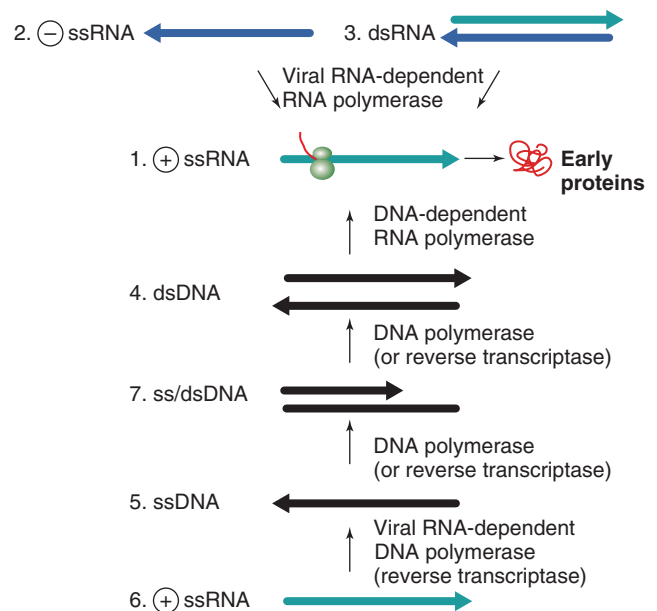


Figure 1.7 Baltimore classification of viruses. Seven categories of viruses are distinguished based on the nature of the viral genome and the pathway leading to the synthesis of early messenger RNAs. Numbering is arbitrary. RNA and DNA polymerases that carry out different steps are shown. DNAs are shown as black lines and RNAs as light blue (positive strand) or dark blue (negative strand) lines. ss, single-stranded; ds, double-stranded; +, positive-strand RNA; -, negative-strand RNA. Messenger RNA is shown being translated by a ribosome.

strand, which contains coding regions that can be directly translated into viral proteins. By convention, this is labeled the **positive** (or **plus**) **strand**. Other viruses package the “antisense” strand, which cannot be translated because it does not contain meaningful coding regions; only its complementary copy codes for viral proteins. This is labeled the **negative** (or **minus**) **strand**. Viruses in these two groups differ fundamentally in how their replication cycle begins.

The seven groups in the Baltimore classification system

1. *Viruses with positive-strand RNA genomes* bring their messenger RNA directly into the cell in the form of the genome. This RNA can bind to ribosomes and is translated into viral proteins as the first step in virus replication. These proteins will then direct the replication of the viral genome.
2. *Viruses with negative-strand RNA genomes* must first synthesize complementary positive-strand copies of the genome, which serve as viral messenger RNAs. However, the cell does not produce an enzyme able to carry out this step, so the virus itself must supply this enzyme. Negative-strand RNA viruses package a virus-coded **RNA-dependent RNA polymerase** within the virion, and this enzyme accompanies the negative-strand RNA genome as it enters the host cell. The first intracellular step in their replication cycle is therefore the synthesis of positive-sense viral mRNAs by this viral RNA-dependent RNA polymerase, using the negative-sense genome as a template.
3. *Viruses with double-stranded RNA genomes* are faced with the same problem as those with negative-strand RNA genomes: the cell does not produce an enzyme that can generate an mRNA by transcribing one of the RNA strands in the double-stranded genome. It is theoretically possible that the double-stranded genome RNA could be denatured within the cell, allowing the positive-strand RNA to be translated; however, this is energetically unlikely and does not occur. These viruses therefore also package a virus-coded RNA-dependent RNA polymerase in the virion and bring this enzyme into the host cell along with the genome. This RNA polymerase can specifically recognize the double-stranded RNA genome and transcribe its negative strand into positive-strand messenger RNAs.
4. *Viruses with double-stranded DNA genomes* must first transcribe their genomes into messenger RNAs, using a **DNA-dependent RNA polymerase**. Cellular messenger RNAs are synthesized in exactly the same way, and therefore all cells contain such enzymes. Most DNA viruses use a host cell RNA polymerase for this purpose, but some of the larger DNA viruses bring their own RNA polymerase into the cell along with their genome, or make an additional RNA polymerase later in the cycle to transcribe a subset of their genes.
5. *Viruses with single-stranded DNA genomes* must first convert their DNA into a double-stranded form, as there are no known enzymes that will transcribe single-stranded DNA directly into RNA. This conversion is carried out by a cellular DNA polymerase. The resulting double-stranded DNA is then transcribed by a cellular RNA polymerase to generate viral mRNAs. Note that either of the complementary DNA strands can be packaged in virions. Most viruses preferentially package only one of the two DNA strands, but some viruses package both strands, making two kinds of virions that are identical except for the polarity of their single-stranded DNA genomes. In all cases, the single-stranded DNA is converted into a double-stranded form before being transcribed, so the polarity of the packaged strand is of little importance.
6. *Retroviruses have a single-stranded, positive-sense RNA genome*, but they follow a pathway different from other positive-strand RNA viruses. Retroviruses package a virus-coded *reverse transcriptase* (an **RNA-dependent DNA polymerase**) within the virion, and use this enzyme to produce a double-stranded DNA copy of their RNA genome after they enter the cell. Once the double-stranded DNA is made (and integrated into the cellular genome; see Chapter 28 for details), it is transcribed into viral messenger RNAs by host cell RNA polymerase II, as for most DNA viruses.
7. There remains a maverick category of viruses in the Baltimore classification system, of which the best-known representatives are the hepadnaviruses (see Chapter 30). These viruses package a circular DNA genome that is partly double-stranded, with a single-stranded gap of variable length on one strand. Hepadnaviruses can be seen as a special case of the category of viruses that have single-stranded DNA genomes (#5 above) because, like those viruses, their genomes are made fully double-stranded by cellular DNA polymerases after entering the cell. This double-stranded DNA is subsequently transcribed into messenger RNAs by cellular RNA polymerase, as for other DNA viruses. However, like retroviruses and unlike other DNA viruses, hepadnaviruses code for a reverse transcriptase. In the case of the hepadnaviruses, this reverse transcriptase is used to synthesize the partially single-stranded DNA genome that is packaged in virions. This DNA is made by reverse transcribing a genome-length RNA molecule that is one of the transcription products of the fully double-stranded DNA. See Chapter 30 for a complete description of how this occurs.

4. Early viral proteins direct replication of viral genomes

Once early viral proteins are made, they act to promote replication of the viral genome. All RNA viruses (except retroviruses) must synthesize an RNA-dependent RNA polymerase to replicate their genomes, as this enzyme is not present in host cells. A number of other early proteins, as well as a variety of cellular proteins, help form **RNA replication complexes** in the cell. These replication complexes are often associated with cellular membranes.

The early proteins of many DNA viruses induce the production of a number of cellular enzymes that are involved in the synthesis of DNA and its building blocks, deoxyribonucleoside triphosphates. This is often achieved by the interaction of early viral proteins with cellular signaling pathways that affect the cell cycle and direct the cell to enter the DNA synthesis (S) phase. Small DNA viruses usually use host cell DNA polymerases to replicate their genomes; larger DNA viruses often code for their own DNA polymerases as well as other enzymes involved in DNA replication.

Hundreds to tens of thousands of copies of the viral genome can be made in each cell. These progeny genomes can be used as templates for the synthesis of more viral messenger RNAs or for further genome replication. At this stage, the cell has become a factory for the expression and replication of viral genomes.

5. Late messenger RNAs are made from newly replicated genomes

Many viruses synthesize a distinct set of “late” messenger RNAs after genome replication has begun. Because of genome replication, there can be many templates for late messenger RNA synthesis, and these mRNAs may therefore be abundant. The mechanisms that control the switch between early and late messenger RNA synthesis have been extensively studied as paradigms for understanding how gene expression is controlled in the host cell.

6. Late viral proteins package viral genomes and assemble virions

Structural proteins used to package viral genomes and to assemble the capsid are usually the most abundant viral proteins made in an infected cell. The simplest virus capsids consist of one protein that forms either a closed shell or a helical tube within which the viral genome is packaged (Figure 1.1). Larger and more complex viruses may have numerous capsid proteins, and some viruses make **scaffolding proteins** that are involved in virion assembly but are subsequently discarded and do not form part of the mature virion. Many bacteriophages have both a polygonal head that contains the genome and a tubular tail involved in attachment to the cell and delivery of the genome.

Enveloped viruses code for glycoproteins that are inserted into lipid membranes and direct the formation of the viral envelope by a process called **budding**. For a detailed discussion of virion structure and assembly, see Chapter 2.

7. Progeny virions are released from the host cell

Once formed, virions leave the cell to find and infect new host cells and reinitiate the replication cycle. For viruses that infect unicellular organisms, this usually involves death and lysis of the host cell, and many viruses code for specialized late proteins that lead to cell death. However, some viruses of bacteria and archaea are released by extrusion from the cell membrane and do not kill their host cells.

For viruses that infect multicellular organisms, the problem becomes more complex. Viruses can spread from one cell to another within an organism, or they can move from one organism to another; virions that follow these two pathways are not necessarily identical. For example, many viruses of higher plants spread from cell to cell throughout a plant by passage through intracellular channels (**plasmodesmata**), using specific viral proteins to enhance the passage of unencapsidated viral genomes. However, spread from plant to plant is often carried out by insects that feed on plant juices, ingesting virions, and reinjecting them into other plants.

Some insect viruses spread as individual virions from cell to cell within the host organism, but wrap one or more virions in a specialized protein coat that is released when the insect dies. This protein coat protects virions in the environment and is ingested by other insects, in which it dissolves and releases the infectious virions to start another cycle (see Chapters 35 and 36).

Release of animal viruses can occur through a number of pathways. Some viruses accumulate within host cells and are released only on cell death. Certain viral proteins function to retard cell death, prolonging the period of virus replication, and other viral proteins can kill the host cell, leading to virus release. Many enveloped viruses are assembled by budding at the plasma membrane and can be released with little or no effect on the host cell. Others bud at internal cell membranes or form their envelopes *de novo*; many of these viruses use cellular transport vesicles to reach the cell membrane and to exit the cell.

KEY TERMS

Budding	Envelope
Capsid	Epidemic
Confocal microscope	Filterable virus
Cytoplasm	Gastroenteritis
DNA-dependent RNA polymerase	Glycolipid
Encephalitis	Glycoprotein
	Hemagglutination assay

Hemorrhagic fever	Poliomyelitis
Hepatitis	Polymerase chain reaction (PCR)
Immune response	Positive (or plus) strand
Latent infection	Replication complex
Live-attenuated vaccine	Reverse transcriptase
Lysis	RNA-dependent DNA polymerase
mRNA vaccines	RNA-dependent RNA polymerase
Multiplicity of infection (m.o.i.)	Scaffolding protein
Nanoparticles	Sialic acid
Negative (or minus) strand	Subunit vaccine
Obligatory intracellular parasite	Tropism
Oncogenes	Uncoating
Oncolytic	Variolation
Pandemic	Virion
Plaque assay	Virus vectors
Plaque-forming units (PFU)	
Plasmodesmata	

FUNDAMENTAL CONCEPTS

- A virus particle, or virion, consists of a nucleic acid genome enclosed in a protein coat and, in some cases, a lipid-containing envelope.
- Viruses depend on cells for molecular building blocks, energy, and the machinery for protein synthesis.
- Viruses can have genomes made of either RNA or DNA.
- Viruses replicate by uncoating and expressing the viral genome inside the cell, directing the synthesis of viral proteins, replicating the genome, and encapsidating it to form new progeny virus particles.
- Viruses can be detected and enumerated by plaque assays, hemagglutination assays, and electron microscopy.
- The sequence of steps during virus replication can be studied by infecting all cells in a culture at a high multiplicity of infection.
- Seven different classes of viruses can be distinguished based on the pathway to synthesis of early mRNAs; this is known as the Baltimore classification system.

REVIEW QUESTIONS

1. State two problems faced by viruses with RNA genomes and explain how these viruses address these challenges.
2. How were viruses first distinguished from other microorganisms, and what did this ultimately reveal about viruses?
3. Viruses can replicate only within living cells. What four elements that are required for virus replication are provided by host cells?
4. Are viruses living or inanimate?