

Chapter 1

Discovery and Classification

Chapter Outline

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The earliest discoveries of viruses and how they have been woven into the history of virology will be recounted first. Then, the elementary facts on virus including the definition and classification will be described. Although this book mainly deals with human viruses, viruses are found in almost all living organisms on this planet and we will take a glimpse of diverse viruses found in other living organisms. Finally, the subviral agents such as prions and viroids will be covered.

1.1 DISCOVERY OF VIRUS

1.1.1 Virus in Ancient History

How long ago did human viruses first appear on Earth? Although human beings are believed to have originated about 3–4 million years ago, the oldest record of virus in history was found only 4000 years ago in ancient Egypt (Fig. 1.1). The victim of poliovirus was inscribed in a stele. In addition, evidence of *smallpox*¹ was found in Egyptian mummies. The earliest physical evidence of it is probably the pustular rash found on the mummified body of Pharaoh Ramses V of Egypt (1149–1145 BC). Smallpox was described in the literature of ancient China (700 BC) as well. It is believed that the collapse of the Inca and Aztec cultures in South America can be attributed to smallpox and *measles*² that were brought by European explorers. After all, viruses have greatly influenced the fates of ancient cultures extinguished in human history.

1.1.2 Discovery of Virus in Plants and Animals

By the end of 19th century, during a time when all the transmissible agents were believed to be microbes, the existence of transmissible agents, which were smaller than a microbe, had begun to be perceived. In 1892, Dimitri Ivanowski, a Russian scientist, reported an unexpected observation during his study on the Tobacco mosaic disease of a plant (Fig. 1.2). He found that the filtrates of the transmissible agent caused the disease. He modestly stated that “according to my experiments, the filtered extract introduced into healthy plants produces the symptoms of the disease just as surely as does the unfiltered sap.” In the year 1898, Martinus Beijerinck, a Dutch scientist, independently made similar observations in his studies on Tobacco mosaic disease of a plant. Further, he speculated that the pathogen exists only in

1. **Smallpox** A fatal infectious disease of human that is caused by a pox virus.

2. **Measles** Measles is an infection of the respiratory system caused by a virus belonging to family *Paramyxoviridae* (see chapter: Other Negative-Strand RNA Viruses).

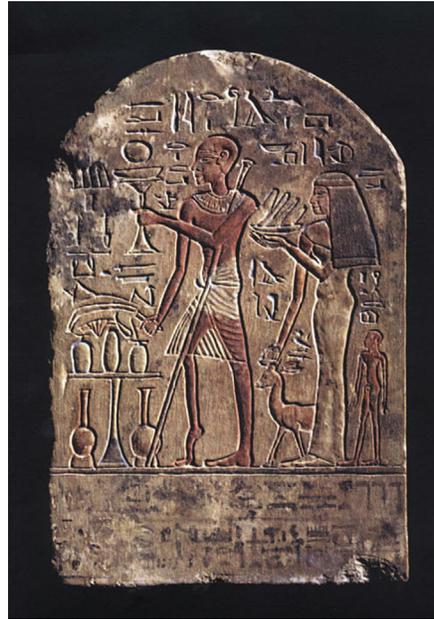
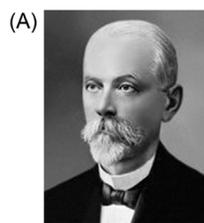
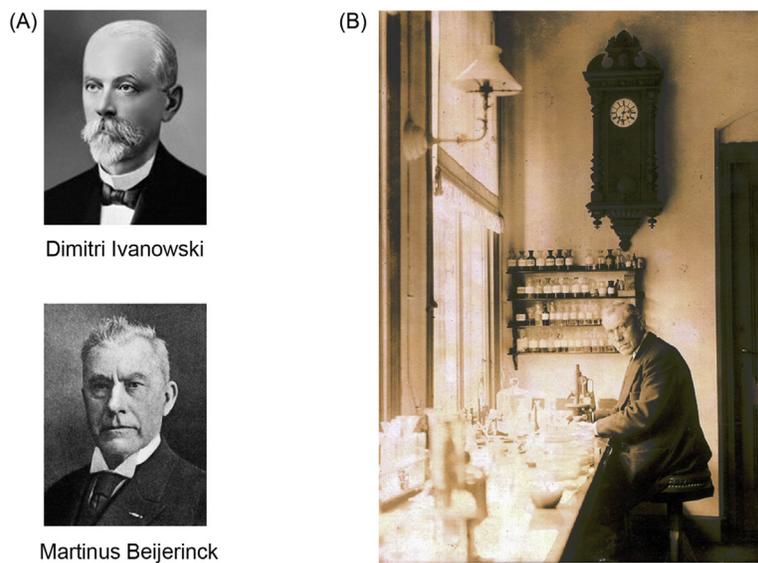


FIGURE 1.1 The historical record of virus. The oldest record of a virus was found in a stele from 13th-century BC Egypt. A man (priest) standing with a stick is believed to be a victim of poliomyelitis.



Dimitri Ivanowski



Martinus Beijerinck



FIGURE 1.2 The photos of two pioneers, who discovered ‘virus’ as a filterable agent. (A) Dimitri Ivanowski (1864–1920), a Russian botanist, the first man to discover virus in 1892 and thus one of the founders of virology (above). In 1898, Martinus Beijerinck (1851–1931), independently reproduced Ivanowski’s filtration experiments and then showed that the infectious agent was able to reproduce and multiply in the host cells of the tobacco plant (below). (B) A photo that captured Martinus Beijerinck in his laboratory in 1921. Martinus Beijerinck coined “virus” to articulate the nonbacterial nature of the causal agent of Tobacco mosaic disease.

living tissues. He named the new pathogen *virus*³ to highlight its nonbacterial nature. Importantly, he articulated two experimental definitions of viruses as the following: the ability to pass through a porcelain filter, and the need for living cells on which to grow. Subsequently, a similar observation was made of an animal virus as well. Loeffler and Frosch, German scientists, found a filterable agent in their studies on *foot-and-mouth disease*⁴ in cows in 1897.

3. **Virus** The word is from the Latin *virus* referring to poison.

4. **Foot-and-mouth disease** Animal disease that is caused by foot-and-mouth disease virus (FMDV), which belongs to family *Picornaviridae*.

Although it was clear that viruses are small entities, significantly smaller than bacteria, the physical identity of viruses remained unclear until the virus particle was crystallized. In the 1930s, Wendell Stanley successfully made crystals of Tobacco mosaic virus (TMV), a finding that implicated that the virus particle constitutes a simple structure, parallel to proteins, because crystallization can be achievable only from molecules or particles having a simple structure. Accordingly, he then speculated that TMV is principally composed of protein only.

1.1.3 Discovery of the Human Viruses

As stated above, viruses were discovered from plants and animals just before the turn of the 20th century. Nonetheless, a human virus had not yet been discovered. As a matter of fact, many human pathogenic microbes had been discovered since 1884, when *Koch's postulates*⁵ for identification of the agent responsible for a specific disease prevailed. Many attempts were made to search for the cause of scourges that considerably threatened human life, including yellow fever, rabies⁶, and poliomyelitis. An etiological agent for these three plagues was discovered soon after the turn of the 20th century. Historical accounts for the discovery of the culprits are revealing, as the following.

Yellow fever was one whose etiology was uncovered first among the human pathogenic viruses (Fig. 1.3). The work of Loeffler and Frosch on animal viruses encouraged Walter Reed and his colleagues, who were working on yellow fever, a terrifying human disease. Walter Reed, who led the U.S. Army Yellow Fever Commission residing in Cuba, was able to demonstrate that an inoculum from an infected individual can infect healthy volunteers even after filtration. It was the moment of discovery of the yellow fever virus (YFV) in 1902—the first human virus ever isolated. In fact, the discovery of YFV involved human volunteers including colleagues of Walter Reed, some of whom unfortunately succumbed to the virus they discovered. In retrospect, the lack of animal models for transmission, which was established almost three decades later, subjected human volunteers to an experimental infection.

A year after the discovery of YFV, an etiologic agent for rabies was discovered (see Fig. 1.3). Rabies spread in the 1880s in Europe as dogs became a popular pet animal. Louis Pasteur had earlier used rabbits for transmission of rabies for the development of rabies vaccine (see Box 25.1). Therefore, unlike yellow fever, an animal model for transmission was already available before the discovery of the etiologic agent. Nonetheless, Louis Pasteur failed to isolate and cultivate a rabies microbe in a media, in which bacterium are expected to grow. Almost two decades later in 1903, Paul Remlinger demonstrated that the filtrates of rabies inoculum transmit the disease to animals. Thus, rabies virus, a filterable agent that causes rabies in animals, was discovered. In 1908, Karl Landsteiner, an Austrian scientist, reported the

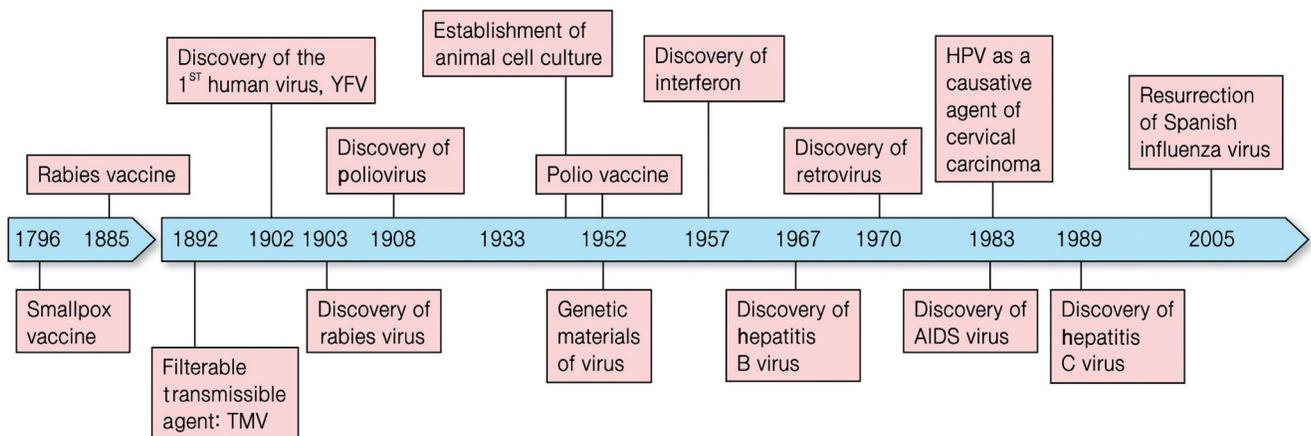


FIGURE 1.3 Chronicle of the major discoveries in virus research. The major milestones in virus research are shown. Small pox vaccine (by Edward Jenner) and rabies vaccine (by Louis Pasteur) were developed even prior to the discovery of virus by Ivanowski in 1892, as demarcated by a separate arrow bar. In the first decade of the 20th century, three human viruses (YFV, Rabies virus, and Poliovirus) were discovered. YFV, yellow fever virus; HPV, human papillomavirus.

5. **Koch's postulates** It refers to a set of criteria that Koch developed to establish a causative relationship between a microbe and a disease. Koch applied the postulates to describe the etiology of cholera and tuberculosis.

6. **Rabies** It refers to a viral disease that causes acute encephalitis in animals. Rabies virus belongs to a family *Rhabdoviridae* (see chapter: Rhabdovirus). The rabies is a Latin word for “madness.”

transmission of poliomyelitis to monkeys, revealing the etiological agent of poliomyelitis. Overall, during the first decade of the 20th century, the etiological agents of three major viral scourages, YFV, rabies virus, and poliovirus, were discovered (see Fig. 1.3).

1.1.4 Discovery of Genetic Materials

One big question that remained unanswered until the 1950s was what is the genetic material of life? A prevailing view was that protein rather than nucleic acid was more likely to be the genetic materials responsible for inheritance due to its higher diversity (ie, 20 amino acids versus 4 bases). In fact, the experiment that proved nucleic acids to be the genetic material was carried out by an experiment using *bacteriophages*⁷ (ie, a virus of bacteria). Bacteriophages, which propagate rapidly in bacterial hosts, became a favorite experimental subject during 1940–1960. In 1952, when the identity of the genetic material was still in debate, Hershey clearly demonstrated that the nucleic acid component of bacteriophage T2 was the genetic material (Fig. 1.4). In his experiment, nucleic acids and proteins were radiolabeled distinctively either with ³²P and ³⁵S, respectively. Such a prepared T2 phage was used to infect a bacterium, and examined to see whether ³²P or ³⁵S was detectable in the cell pellet after centrifugation. Since T2 phage was known to inject the genetic material into the host cell, what is detected in the cell pellet represents the genetic materials. Indeed, ³²P (nucleic acid) was abundantly detected in the pellet, while ³⁵S (protein) was detected in the supernatant, revealing that

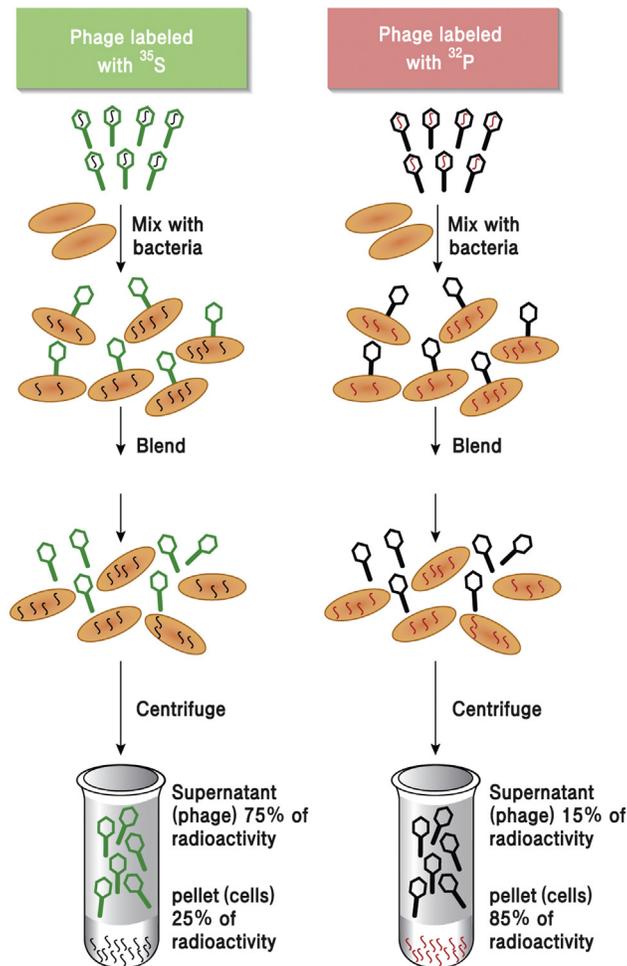


FIGURE 1.4 A seminal experiment, which demonstrated that DNA is the genetic material. T2 phage, that was propagated either in the presence of ³²P (purple) or ³⁵S (green), was used to infect *Escherichia coli*, a host. The mixture was subjected to blending to detach the phage from the host cells. Following centrifugation, the amount of ³²P and ³⁵S was measured from cell pellet (*E. coli*) and supernatants (T2 phage).

7. **Bacteriophage** A bacteriophage (informally, *phage*) is a virus that infects and replicates within bacteria. The term is derived from Greek word *phagein* “to eat.”

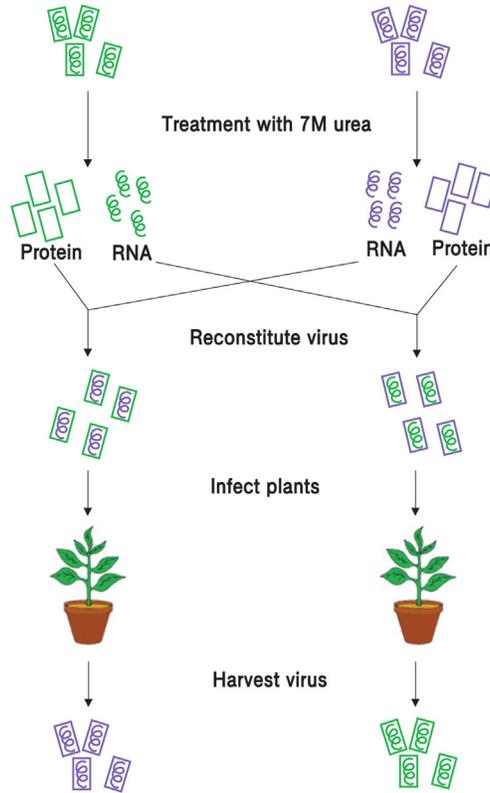


FIGURE 1.5 A reconstitution experiment, which proved that RNA is the genetic material of TMV. This experiment exploited the fact that TMV can be reconstituted *in vitro*, following fractionation into proteins and RNA by 7M urea treatment. Two strains of TMV were colored differently by either green or purple.

the nucleic acids are the genetic materials. This work represents a seminal discovery that clearly proved that “DNA is the genetic material” (see Fig. 1.3).

A few years later, in 1957, Fraenkel-Conrat and Singer confirmed that the nucleic acid (RNA) is the genetic material by an experiment using TMV. In their experiment, they explored the fact that TMV can be reconstituted *in vitro* by mixing proteins and RNA components following fractionation of TMV particles (Fig. 1.5). Briefly, two strains of TMV, that exhibits distinct lesions on the leaf, were employed. After separating into proteins and RNA, two hybrid viruses were reconstituted by mixing two components. These hybrid viruses were used to infect the host plant. Remarkably, the phenotype of the reconstituted hybrid viruses was determined by the RNA, but not the proteins. This reconstitution experiment confirmed that “nucleic acid (RNA) is the genetic material.”

As stated above, RNA as well as DNA was discovered to be the genetic material of viruses.

1.2 DEFINITION OF VIRUS

What are the defining features of a “virus”? As stated above, unlike the other pathogens known in those days, a “virus” is a filterable transmissible agent. In addition, it is submicroscopic and the physical size of the most of animal viruses ranges from 30 to 300 nm in diameter (Fig. 1.6). Hence, a virus can be aptly said to be a “nanoparticle” in nature. On the other hand, a “virus” can be viewed as a molecular complex constituted of the nucleic acids and the protein shells that encompass the nucleic acids. The nucleic acids can be either RNA or DNA. Although all living organisms on the globe have a DNA genome, viruses are the only organisms, if you may, that still employs RNA as genome. The role of protein shells, also called capsids, is to protect the viral genome from biochemical damage. In addition, some viruses have an envelope (ie, a lipid bilayer) that coats the capsids.

What are the common features that are shared by viruses? Because viruses are found in almost all living organisms on earth, their biological properties should be as diverse as their host organisms. Nonetheless, five common features of viruses, regardless of their hosts, are perceived. A defining feature is that a “virus” replicates only inside living cells. In other words, a “virus” is simply a physical entity outside of cells, as it cannot reproduce outside of cells. More precisely speaking, what a virus really belongs to is an interface between living and nonliving matter (Box 1.1). Since a virus

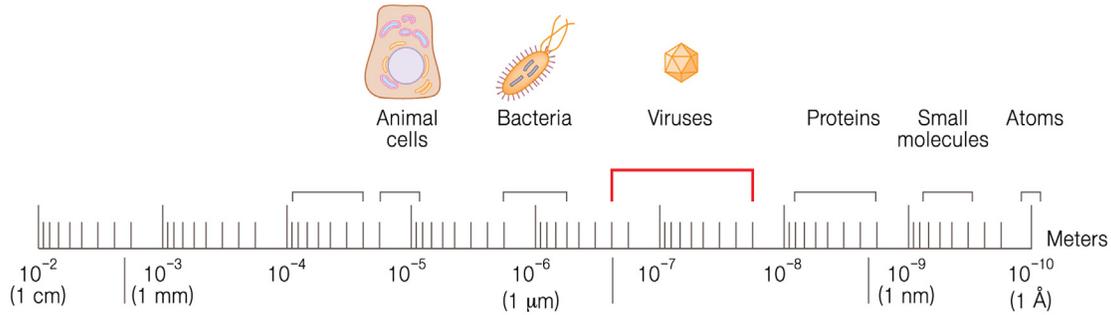


FIGURE 1.6 Comparison of the size of virus particles to other living organisms. The size of most animal viruses ranges from 30 to 300 nm in diameter, as marked by red bracket. The ruler is drawn in logarithmic scale. It can be said that the animal viruses are about 100–1000 times smaller than their host cell.

BOX 1.1 Are Viruses Alive?

“Are viruses alive or not?” This question always provokes lively discussion. Since we are now well aware of the molecular details of virus, this question is no longer a scientific question but a somewhat philosophical question. Viruses share many features with a living organisms, such as the ability to replicate (reproduce), and the possession of inheritable materials (genome). Nonetheless, viruses can be regarded as nonliving entities, since they cannot replicate outside of host cells.

can propagate only inside host cells, it has long been referred to as “a parasite living in cells.” Second, a virus is “an infectious agent,” in that it is transmissible from an infected host to uninfected hosts. Third, a virus propagates itself via assembly. In other words, the assembly of its components in infected cell is the way of multiplication, not by division as per cells. Fourth, a virus could rapidly cope with environmental changes (eg, host cell, drug, and antibodies), a property that is attributable to its higher mutation rate (see [Box 1.3](#)). Fifth, a virus is a unique organism that delivers its genome to the host cells via the process called “infection.” This special feature of viruses is exploited as vehicles in gene therapy (see chapter: Virus Vectors).

The vast majority of viruses are associated with diseases, because they were discovered as etiological agents for infectious diseases, such as yellow fever, rabies, and poliomyelitis. One might wonder whether the disease-causing properties are common property of all viruses. This is not the case. There are some viruses that are nonpathogenic to their hosts, for example, adeno-associated virus (AAV) is not pathogenic to its host, human (see chapter: Other DNA Viruses). A widely accepted view is that the aim of virus evolution is not to cause disease in its host, but is to maximize its spread (see [Box PVI.1](#)).

1.3 ADVANCES IN VIROLOGY

Let’s consider the major discoveries made in the past century, including the early discoveries of viruses (see [Fig. 1.3](#)). In fact, it was Louis Pasteur, who first started virus research in the laboratory setting. He successfully developed a rabies vaccine, presuming the microbial cause in 1885, which is even before the virus was officially discovered as a filterable agent. Following the discovery of TMV as a filterable agent, the first human virus, YFV, was discovered. Soon after, rabies virus was discovered as a cause of rabies, and poliovirus was discovered as a cause of poliomyelitis. It is worth noting that these discoveries were made even before DNA was discovered to be the genetic material in 1952 (see [Fig. 1.3](#)).

Until 1950s, animal viruses could not be experimentally studied in a laboratory due to the lack of animal cell culture. Instead, bacteriophages, a virus of bacteria, had greatly advanced “virology” as a discipline of experimental science, primarily because bacteriophages can be readily propagated in bacterial culture (see [Fig. 1.4](#)). A breakthrough of that advanced animal virus research was the successful establishment of animal cell culture in the early 1950s (see [Fig. 1.3](#)). Needless to say, animal cell culture was instrumental for the development of the poliovirus vaccine by Salk in 1956 (see [Box 25.2](#)), which saved thousands of lives. A cell line derived from monkey kidney was used to propagate poliovirus.

Another breakthrough that greatly contributed to the advance of the discipline of virology was the advance of recombinant DNA technology that was established in the early 1970s. Recombinant DNA technology has drastically changed the way of studying viruses from classical experimental science to modern biology. It was exemplified by the discovery of the retrovirus in 1970 by Howard Temin and David Baltimore that led down the road to the molecular era (see Fig. 1.3 and Box 17.1). Undoubtedly, the discovery of retrovirus was the cornerstone for the discovery of the AIDS virus, HIV, in 1983.

As stated above, virology, as a discipline that studies the diverse aspects of viral infection of host cells and its consequence, became established during the early 20th century. The discipline of virology can be divided into a few subdisciplines such as viral epidemiology, clinical virology, viral immunology, and molecular virology. Viral epidemiology investigates the mode of viral transmission, and the risk factors for disease. Clinical virology develops the diagnostic methods for detecting viral infection. Viral immunology studies the consequence of host immune response to viral infection. Molecular virology studies the molecular mechanism of viral replication in the context of virus life cycle. In fact, the division into four subdisciplines is somewhat vague, and the breadths of each subdiscipline inevitably overlap. This book, as the title implies, is inclined toward molecular virology.

What are the aims of virus research? One of the important reasons is to gain knowledge that is instrumental in controlling viral diseases. As stated above, viruses have made a great impact on human life throughout history. The Spanish flu pandemic is the best example that clearly shows the magnitude of the impact of viral diseases on human life (Fig. 1.7). It was one of the deadliest natural disasters in human history, which killed 30–50 million people between 1918 and 1919. The HIV epidemic is another example of a viral disease that has significantly impacted our life. It has killed more than 30 million people in the past three decades. Thanks to intensive research, the HIV epidemic is more or less under control, at least in the Western hemisphere (see chapter: HIV and AIDS).

The second aim of virus research is to exploit viruses as tools for academic research. In the early period of molecular biology in the 1970s, the virus was a favorite experimental model, because it is easier to manipulate in laboratory due to its small genome size. Consequently, many important findings on eukaryotic molecular biology were made by using virus, such as the discovery of introns, enhancers, and so on. Moreover, oncogenes and tumor suppressor genes were mainly discovered through studies on RNA tumor viruses and DNA tumor viruses, respectively (see chapter: Tumor Viruses). In fact, the field of molecular biology itself owes much to the earlier discoveries made by using animal viruses as experimental models.

Viruses are also of interest to pharmaceutical industries. Diagnostics, vaccines, and therapeutic drugs are just few kinds of products that drug industries are interested in that are related to virus research. We will see how these products are made by pharmaceutical industries and clinically used in chapter “Diagnosis and Methods” on viral diagnostics, in chapter “Vaccines” on vaccines, and in chapter “Antiviral Therapy” on antiviral therapy.

Lastly, viruses are often exploited as gene delivery vehicles or gene therapy vectors (see chapter: Virus Vectors). One outstanding feature of viruses is that they have the ability to deliver their genome to target cells. A few animal

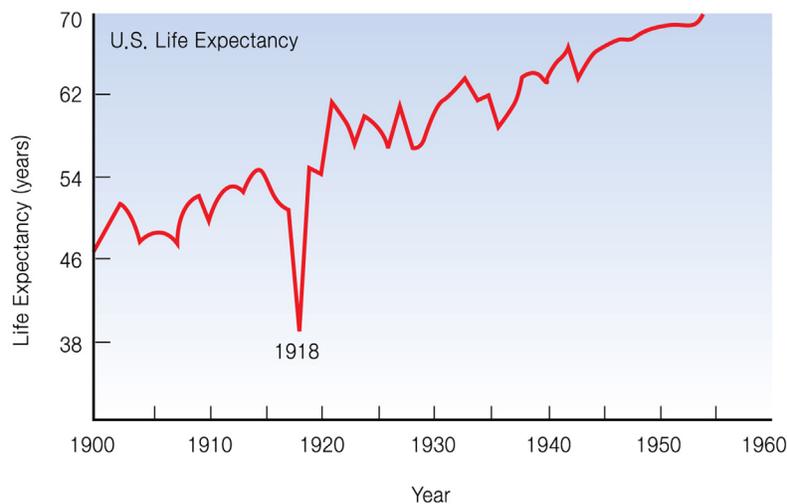


FIGURE 1.7 The impact of viral diseases on the human life expectancy. Life expectancy gradually increased during the past century in U.S.A. Spanish flu during 1918–1919 at the end of World War I was the only episode that adversely affected the incremental increase of life expectancy in the past century.

viruses, such as retrovirus and adenovirus, have been extensively developed for therapeutic purpose. Therapeutic utilization of otherwise pathogenic viruses is a wise strategy to “exploit an enemy to conquer an enemy”, a quote from an ancient Chinese literature, although therapeutic application is not yet in practice.

1.4 CLASSIFICATION OF VIRUSES

In the early days, viruses were discovered as the etiological agents of the disease they caused. Then, viruses were named after, and often classified based on, the diseases that they caused, such as yellow fever virus, and rabies virus. Since the advent of molecular technologies, the genome-based classification was established. The nucleotide sequence relatedness allows a more precise classification of virus species. Furthermore, the genome-based classification allows to predict the mode of viral genome replication. For instance, SARS virus, a new emerging virus in 2003, was immediately identified as a new member of the coronavirus family by the nucleotide sequence analysis.

1.4.1 Genome-Based Classification

The nature of the nucleic acids in the genome is the criteria of the genome-based classification. Viruses that have RNA as a genome are called “RNA viruses,” whereas viruses that have DNA as a genome are called “DNA viruses” (Fig. 1.8). An exception to this rule comprises viruses that replicate via reverse transcription, which are grouped separately as “reverse transcribing (RT) viruses,” regardless of whether the genome is RNA or DNA. As a result, animal viruses can be largely classified into three groups based on the nucleic acids of the virus genome (Table 1.1).

Animal viruses can be further classified into seven groups, based on the genome features, which pertain to the mode of genome replication (Fig. 1.9). In addition to the nucleic acids species (whether DNA or RNA), whether it is a positive-strand or negative-strand (ie, *polarity*⁸) and whether it is a single-strand or double-strand are considered. This genome-based classification is also called the “Baltimore Classification,” named after the prominent scientist who envisioned the genome-based classification (Box 1.2).

According to the Baltimore classification, animal viruses are subdivided into seven groups: DNA viruses (Group I and II), RNA viruses (Group III, IV, and V), and RT viruses (Group VI and VII). The schematic diagram in Fig. 1.9 illustrates how viruses in each group differently synthesize their mRNAs. After all, distinct mRNA transcription strategies represent the hallmark of each virus group. Group I is represented by viruses containing a double-stranded DNA genome. Group I viruses synthesize mRNA by transcription from the DNA genome template. Group II is represented by viruses containing a single-stranded DNA genome. Group II viruses first convert their single-stranded DNA genome to double-stranded DNA, which is then used as a template for mRNA transcription. Group III is represented by viruses containing a double-stranded RNA genome. Group III viruses synthesize mRNA by transcription from their double-stranded RNA template. Group IV is represented by viruses containing a positive-stranded RNA genome. Group IV viruses utilize the genomic RNA directly as mRNA (denoted by dotted lines in the figure). Group V is represented by viruses containing a negative-stranded RNA genome. Group V viruses synthesize mRNA by transcription from their RNA genome template.

Group VI and VII are “reverse transcribing (RT) viruses” viruses. Although they have either RNA or double-stranded DNA genome, these RT viruses are not classified as either RNA or DNA viruses. An important feature that is shared by the RT viruses is that the viral DNAs are synthesized via reverse transcription. Note that although Group VI viruses contain an RNA genome, the genomic RNA does not serve as mRNA, unlike those of Group IV.

Overall, the Baltimore classification enables us to classify all animal viruses to the extent that the genome replication strategy is precisely predictable.

1.4.2 Taxonomy of Virus

Viral species are officially classified and named by an international committee, the International Committee on Taxonomy of Virus (ICTV)⁹. Viral species can be placed in a ranked hierarchy, starting with orders, which are divided into families, then genera (singular: genus), and then species (singular: species) (Table 1.2). Species can be further

8. **Polarity** It is a terminology used in referring to one of two strands in nucleic acids. The strand that is the same sense as mRNA is called “positive-strand,” while the complement is called “negative-strand,” by definition.

9. **International Committee on Taxonomy of Virus (ICTV)** <http://www.ictvdb.org/>.

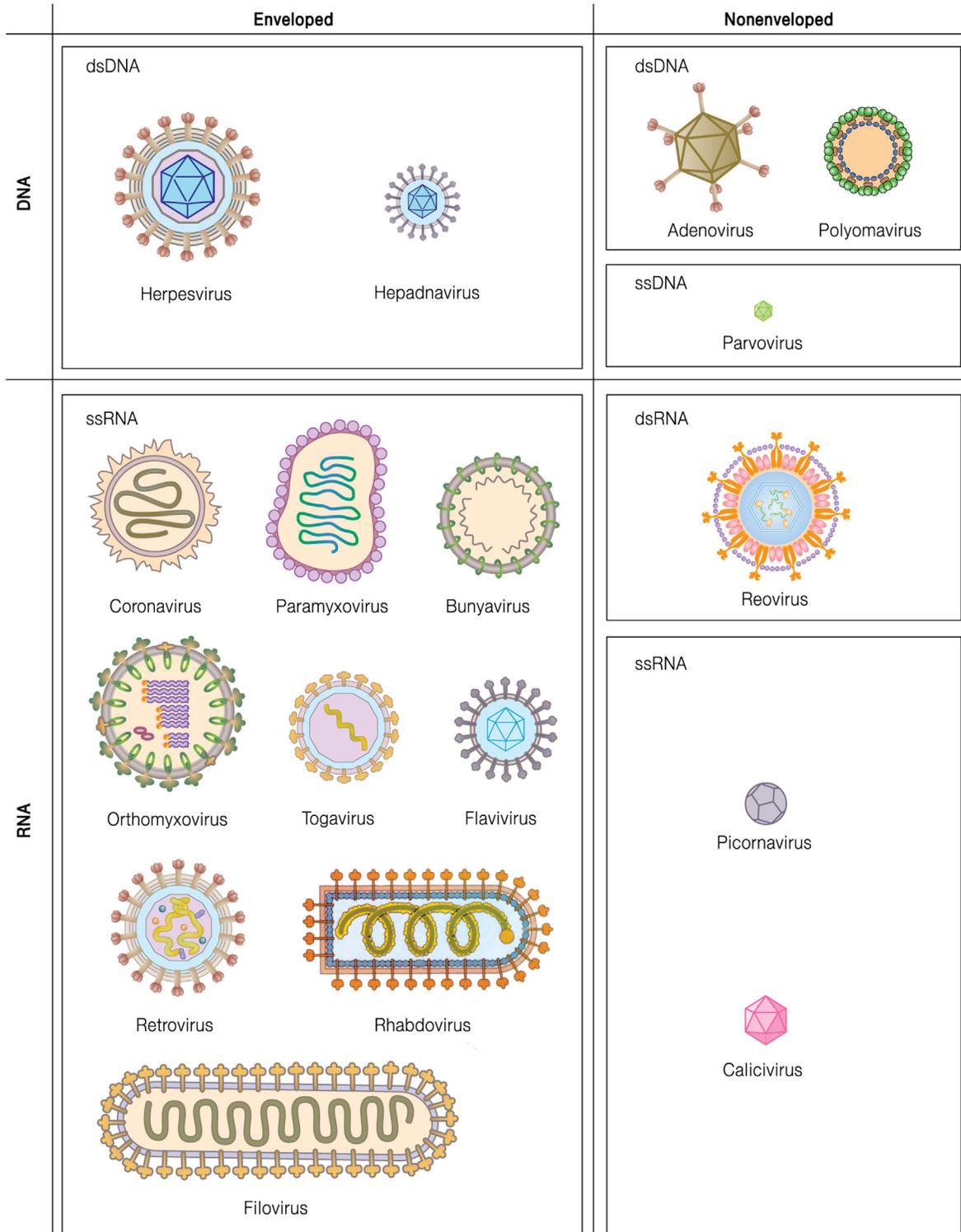


FIGURE 1.8 Classification of viruses. Animal viruses can be classified by the nature of the nucleic genome (ie, DNA or RNA). In addition, viruses are often called by their morphological features. For instance, according to the existence of envelope, it can be called either an enveloped virus or naked (nonenveloped virus). Family names are given below. *dsDNA*, double-strand DNA; *ssDNA*, single-strand DNA; *dsRNA*, double-strand RNA; *ssRNA*, single-strand RNA.

TABLE 1.1 Classification of Animal Viruses

Family Name	Genome Structure	Genome Size (kb)*	Prototypes	Chapter
DNA Viruses				
Parvovirus	ss Linear	5	Adeno-associated virus (AAV)	Adenoviruses
Polyomavirus	ds Circular	5–6	Polyomavirus, SV40	Polyomaviruses
Papillomavirus	ds Circular	7–8	Papillomavirus	Papillomaviruses
Adenovirus	ds Linear	42	Adeonovirus	Herpesviruses
Herpesvirus	ds Linear	120–200	Herpes simplex virus, EBV, VZV	Other DNA Viruses
RNA Viruses				
<i>(+) Single-Stranded RNA Viruses:</i>				
Picornavirus	ss Linear	7–8	Poliovirus, Rhinovirus, Hepatitis A virus	Picornaviruses
Flavivirus	ss Linear	9–10	Yellow fever virus, Hepatitis C virus	Flaviviruses
Togavirus	ss Linear	10–12	Sindbis virus, Semliki Forest virus (SFV)	Other Positive-Strand RNA Viruses
Coronavirus	ss Linear	30	Mouse hepatitis virus (MHV), SARS	Other Positive-Strand RNA Viruses
<i>(–) Single-Stranded RNA Viruses:</i>				
Rhabdovirus	ss Linear	11	Vesicular stomatitis virus (VSV)	Rhabdovirus
Orthomyxovirus	ss Linear, segmented	8 × (0.8–2.3)*	Influenza virus	Influenza Viruses
Paramyxovirus	ss Linear	15	Sendai virus, Measles virus	Other Negative Strand-RNA Viruses
<i>Double-Stranded RNA Viruses:</i>				
Reovirus	ds Linear, segmented	10 × (1.2–3.8)*	Reovirus, Rotavirus	Retroviruses
Viruses with Reverse Transcriptase				
Retrovirus	ss Linear RNA	8–10	Murine leukemia virus (MLV), HIV	Hepadnaviruses
Hepadnavirus	Circular, ds DNA	3.2	Hepatitis B virus (HBV)	Virus Vectors

**The range of genome sizes of segment genome are denoted within parenthesis.*

subdivided into “genotype” or “subtype.” According to the ICTV’s rule, the name of virus families is italicized and ends with the Latin suffix *-viridae*, and the name of the genera ends with the Latin suffix *-virus*. However, in this book, virus family names are often referred in plain English for sake of simplicity.

1.4.3 Mutation and Evolution

All living organisms on earth employ DNA as a genome. In contrast, viruses employ either DNA or RNA as a genome. Having a more flexible RNA molecule as a genome, RNA viruses exhibit a higher mutation rate (Box 1.3). Consequently, RNA viruses can mutate or evolve rapidly upon antiviral stresses, such as host immune response and treatment of antiviral drugs. Not surprisingly, the majority of newly emerging viruses are RNA viruses (see chapter: New Emerging Viruses).

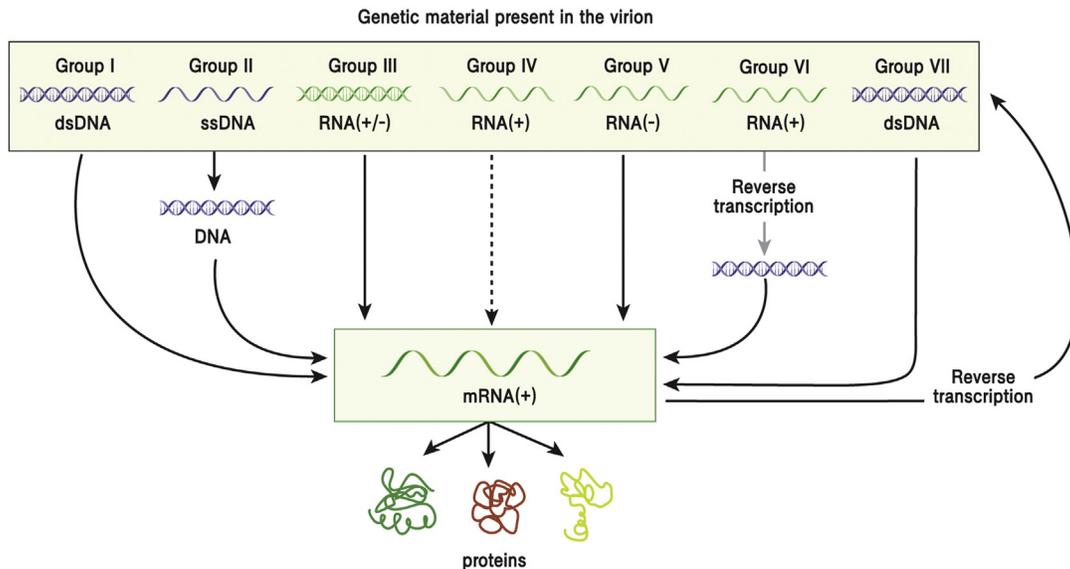


FIGURE 1.9 Classification of viruses based on the genome structure. Animal viruses are divided into three groups: DNA viruses (Group I and II), RNA viruses (Group III, IV, and V), and RT viruses (Group VI and VII). The box on the top contains the viral genome structure in each group, highlighting the nature of nucleic acids (DNA or RNA), the strandness (*ss*: single-strand or *ds*: double-strand), and the polarity (plus or minus). The relationship of the genome to mRNA is indicated either by solid line (transcription) or dotted line (no transcription). *RT*: reverse transcription.

BOX 1.2 Baltimore Classification

David Baltimore was the one who proposed the genome-based classification of animal viruses. It was a truly visionary insight that foretold the era of molecular virology. Undoubtedly, the Baltimore classification serves as a gold standard of virus classification. In addition to his contribution as a virologist, Baltimore has profoundly influenced international science, including key contributions to immunology, virology, cancer research, biotechnology, and recombinant DNA research, through his accomplishments as a researcher, administrator, educator, and public advocate for science and engineering. He shared the Nobel Prize in 1975 with Howard Temin for the discovery of reverse transcriptase (see Box 17.1) at the age of only 37. Even afterward, he continued to be productive as a research scientist not only in virology but also in the field of immunology. He is also well known as the discoverer of transcription factor NF- κ B. In addition, his contribution extended to his role as a university administrator. He has served as the president of California Institute of Technology (Caltech) from 1997 to 2006. He is currently the President Emeritus and Robert Andrews Millikan Professor of Biology at Caltech.

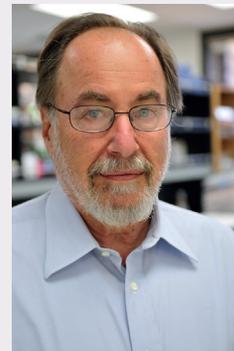


Photo of David Baltimore (1938–).

1.5 VIRUSES IN OTHER ORGANISMS

As described earlier, this book deals only with human viruses. In fact, viruses are discovered in almost all organisms on earth, including animals, plants, insects, amoeba, plankton, and bacteria. Here, it is worth noting what kinds of viruses are found in such diverse organisms. Table 1.3 includes only one or two viral species per organism for brevity, which is only the tip of the iceberg. For instance, plant viruses are found in many agricultural products, such as rice, corns, potato, and tobacco (Fig. 1.10). Pathogenic plant viruses damage the crops, resulting in significant economic loss in the farming industry. Interestingly, viruses do not always cause disease in host organisms. For instance, tulip breaking virus

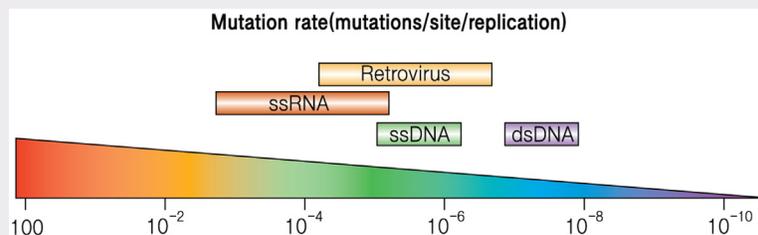
TABLE 1.2 ICTV Nomenclature of Some Representative Viruses

Family	Genus	Species
<i>Picornaviridae</i>	<i>Enterovirus</i>	Poliovirus 1
<i>Flaviviridae</i>	<i>Hepacivirus</i>	Hepatitis C virus
<i>Herpesviridae</i>	<i>Simplexvirus</i>	Herpes simplex virus 1
<i>Retroviridae</i>	<i>Lentivirus</i>	HIV

BOX 1.3 Viral Genome and Mutation

One of the salient features that distinguish viruses from other organisms is the higher rate of mutation. The mutation rates of eukaryotic organisms are considerably lower, ranging from 10^{-8} to 10^{-10} , since proofreading capability (10^{-3}) takes largely care of most of the errors. In the case of viruses, the mutation rates are significantly higher than host organisms, because viral DNA/RNA polymerases are not equipped with proofreading capability. Moreover, the genomic features also affect the mutation rates. For instance, the mutation rates of RNA viruses (10^{-3} to 10^{-5}) are significantly higher than those of DNA viruses (10^{-6} to 10^{-8}). In other words, the RNA virus with 10 kb genome size has at least one or more mutation per genome per replication cycle. RNA being more flexible than DNA, RNA viruses have intrinsically higher mutation rates. On the other hand, in the case of DNA virus, single-stranded DNA viruses have higher mutation rates than double-stranded DNA viruses. Note that the mutation rate of retroviruses are lower than that of single-stranded RNA viruses, but comparable to that of single-stranded DNA viruses.

What is the biological implication of having a higher mutation rate? A living organism has to cope with environmental changes via a process called “adaptation” or “evolution.” Mutation is the driving force for evolution. In other words, mutations are not always harmful, but can be beneficial to an organism. Being random events, the vast majority of mutations lead to the loss of gene function. Some of the mutations, at least, can be advantageous for the virus to survive in challenging environments. The process of outgrowth of a virus having such advantageous mutations is termed “selection” or “adaptation.” Therefore, the emergence of viral mutants (ie, variants) is the consequence of not only mutation but also selection. Selection constitutes an important concept in understanding viral evolution.



Mutation rate of virus genomes. The mutation rate of virus genomes are largely determined by nucleic acids of virus genomes: whether single- or double-stranded, and whether DNA or RNA.

does not cause pathogenic lesion on the host plant but leaves a beautiful stripe pattern on the flower that is appreciated by people (Box 1.4). Notably, many plant viruses constitute “families” that do not have a counterpart in animal viruses. For instance, TMV is classified in the “Virgavirus family,” and cauliflower mosaic virus is classified in the “Caulimovirus family,” neither of which have any animal members (Table 1.3).

In addition to higher eukaryotes, viruses are also found in unicellular organisms such as amoeba, yeasts, and bacteria. Recently, *giant viruses*¹⁰, which are an extraordinary size (400 nm in diameter), were discovered in amoeba (Box 1.5). The genome size of “Mimivirus,” the first giant virus discovered, is about 1200 kb, which is five times larger than any other known virus (ie, 230 kb of cytomegalovirus). It even rivals some bacteria in the genome size. After all, the discovery of giant viruses in amoeba makes the distinction between virus and organism blurred.

10. **Giant virus** A novel virus found in amoeba, which is bigger than any other known viruses (400 nm in diameter, 1200 kb in genome).

TABLE 1.3 Viruses in Other Organisms

Host Range	Major Virus	Infectious Disease	Family Name	Genome	Chapter	
Vertebrates						
Primates	Chimpanzee	Simian immunodeficiency virus	AIDS-like	Retrovirus	ssRNA(RT)	Retroviruses
	Gorilla	Ebola virus	Fatal	Filovirus	(-)RNA	Other Negative-Strand RNA Viruses
	Monkeys	Simian virus 5	–	Paramyxovirus	(-)RNA	Other Negative-Strand RNA Viruses
Mammalians	Cows, Pigs	Foot-and-mouth disease virus	Blisters	Picornavirus	(+)RNA	Picornavirus
	Rabbits	Rabbit hemorrhagic disease virus	Fatal	Calicivirus	(+)RNA	Other Postive-strand RNA Viruses
	Cats	Feline parvovirus	Fatal	Parvovirus	ssDNA	Adenoviruses
	Dogs	Canine parvovirus	Diarrhea	Parvovirus	ssDNA	Other DNA Viruses
	Bats	Bat coronavirus	–	Coronavirus	(+)RNA	Other Positive-Strand RNA Viruses
Rodents	Mouse	Mouse hepatitis virus	Fatal	Coronavirus	(+)RNA	Other Positive-Strand RNA Viruses
	Mouse	Minute virus of mice	–	Parvovirus	ssDNA	Other DNA Viruses
	Chicken	Newcastle disease virus	Fatal	Paramyxovirus	(-)RNA	Other Negative-Strand RNA Viruses
	Pigeons	Pigeon coronavirus	–	Coronavirus	(+)RNA	Other Positive-Strand RNA Viruses
Fishes	Salmons	Infectious salmon anemia virus	Fatal	Orthomyxovirus	(-)RNA	Influenza Viruses
	Trouts	Infectious hematopoietic necrosis virus	Fatal	Rhabdovirus	(-)RNA	Rhabdovirus
	Breams	Red sea bream iridovirus	Fatal	Iridovirus	dsDNA	–
	Flounders	Viral hemorrhagic septicemia virus	Sepsis	Rhabdovirus	(-)RNA	Rhabdovirus
Reptiles	Turtles	Turtle herpes virus	Wart	Herpesvirus	dsDNA	Herpesviruses
	Snakes	Snake adenovirus	–	Adenovirus	dsDNA	Adenoviruses
	Lizards	Lizard adenovirus	–	Adenovirus	dsDNA	Adenoviruses
Amphibians	Frogs	Frog virus 3	Fatal	Iridovirus	dsDNA	–
Invertebrates						
Arthropods	Shrimps	White spot syndrome virus	Fatal	Nimavirus	dsDNA	–
Shellfishes	Oysters, Clams	Ostreid herpesvirus-1	–	Herpesvirus	dsDNA	Herpesviruses
Insects	Mosquitoes	Sindbis virus	–	Togavirus	(+)RNA	Other Positive-Strand RNA Viruses

(Continued)

TABLE 1.3 (Continued)

Host Range		Major Virus	Infectious Disease	Family Name	Genome	Chapter
	Moths	Nuclear polyhedrosis virus	–	Baculovirus	dsDNA	Virus Vectors
	Honey bees	Israeli acute paralysis virus	Fatal	Picornavirus-like	(+)RNA	Picornavirus
	Beetles	Flock house virus	–	Nodavirus	(+)RNA	Other Positive-Strand RNA Viruses
	Cricket	Cricket paralysis virus	Fatal	Picornavirus-like	(+)RNA	Picornavirus
Plants						
	Tobacco	Tobacco mosaic virus	Necrosis	Virgavirus	(+)RNA	–
	Tulip	Tulip breaking virus	Stripes	Potyvirus	(+)RNA	–
	Grass	Brome mosaic virus	Necrosis	Alphavirus-like	(+)RNA	Other Positive-Strand RNA Viruses
	Potato	Potato spindle tuber viroid	–	Viroid	ssRNA	Subviral Agents and Prions
	Rice	Rice dwarf virus	–	Reovirus	dsRNA	Other Negative-Strand RNA Viruses
	Maize	Maize streak virus	–	Geminivirus	ssDNA	–
	Cauliflower	Cauliflower mosaic virus	Necrosis	Caulimovirus	dsDNA(RT)	–
Unicellular Organisms						
	Amoeba	Mimivirus	–	Mimivirus	dsDNA	Discovery and Classification
	Yeasts	Saccharomyces L-A virus	–	Totivirus	dsRNA	–
	Fungi	Botrytis porri RNA virus 1	–	Mycovirus	dsRNA	–
	Bacteria	λ phage, T2 phage	Cell lysis	–	dsDNA	–

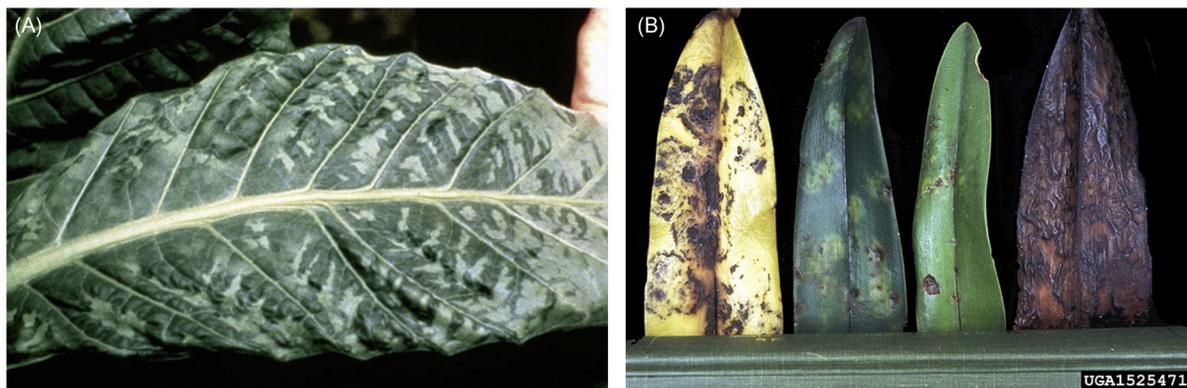


FIGURE 1.10 Tobacco mosaic virus, a plant virus. TMV has a very wide host range and has different effects depending on the host being infected. (A) Tobacco mosaic virus symptoms on tobacco. (B) Tobacco mosaic virus symptoms on orchid.

BOX 1.4 Tulip Breaking Virus

Tulip breaking virus (TBV), also known as tulip mosaic virus, is a plant virus. In peculiar, TBV infection of tulip leaves a stripe pattern without pathogenic lesions on the host. Tulips with the stripe pattern were once sold at extraordinarily high prices, which was about 10 times the annual income of average workers during the so-called Tulip mania period during the 17th century in the Netherlands. Of course, the stripe pattern was highly valued for its artistic beauty, without knowing it was the result of a viral infection. In fact, TBV belongs to the potyvirus family (see Table 1.3). The stripe pattern is believed to be the result of bleaching caused by TBV infection.



The portrait of tulip named “Semper Augustus.” The tulip was sold at the higher price in the market during 17th century in the Netherlands under “tulip mania.” The effects of the TBV are seen in the striking streaks of white in its red petals.

1.6 SUBVIRAL AGENTS

In addition, some filterable agents, which do not comply with the classical definition of “virus,” were discovered. They are so-called subviral agents¹¹ or virus-like transmissible agents. These subviral agents have long been considered to be “virus,” since they are transmissible, pathogenic to their host, and filterable. These subviral agents will be described in more detail in chapter “Subviral Agents and Prions.”

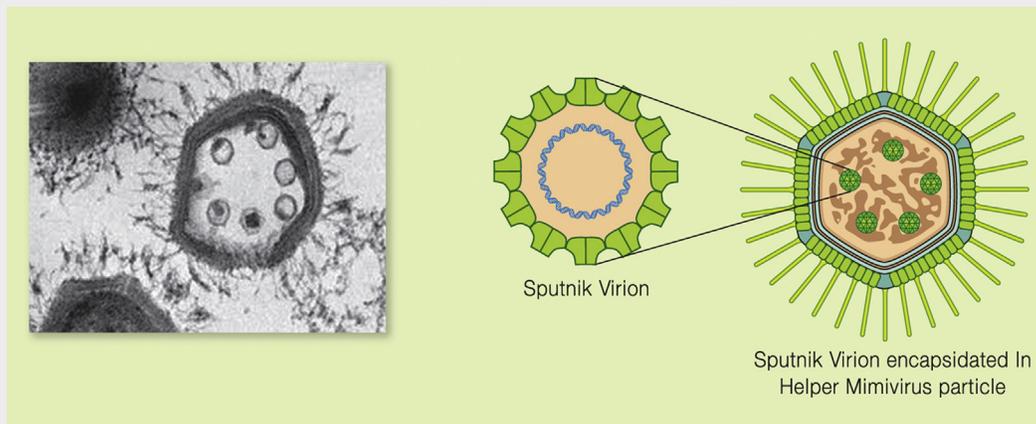
Subviral agents comprise three kinds: satellite viruses, viroids, and prions (Table 1.4). A satellite virus is morphologically indistinguishable from a regular virus, and composed of nucleic acids and capsid proteins. However, one important distinction is that its replication depends on another virus (ie, a host virus). Since the satellite virus cannot replicate in the absence of a host virus, it can be said to be “a parasite of parasite.” The second kind of subviral agent is the “viroids” that are found only in plants. A viroid contains a small RNA molecule (~0.3 kb circular RNA) only, but is devoid of proteins. In other words, the RNA itself is the transmissible agent. The third kind of subviral agent is the “prions” that are associated with *TSE* (transmissible spongiform encephalopathy) or scrapie. In contrast to the viroids, prions are composed of proteins only, but devoid of nucleic acids. The “prions hypothesis” which states that protein, devoid of nucleic acids, is the only etiologic agent for TSE, had not been accepted in the science community until recently (see Box 20.1).

11. **Subviral agent** It refers to virus-like transmissible agents, which do not comply with the classical definition of “virus.”

BOX 1.5 Giant Virus and Virophage

Attention is paid to the discovery of two giant viruses in amoeba that lie beyond the classical definition of virus. The first giant virus discovered in 2003 was named “mimivirus.” The name derived from “mimicking microbes.” It was initially mistaken by Jean-Michel Claverie as a microbe and left in his freezer for 10 years, since its size nearly parallels the microbe (~400 nm) under microscope. In fact, its genome size is 1200 kb, which is five times larger than herpesvirus, which is known to have the largest genome among viruses. And it encodes 1200 genes. Considering the genome size and the number of genes it encodes, mimivirus is closer to a microbe than a virus.

Recently, Jean-Michel Claverie discovered another giant virus, named “mamavirus.” An interesting twist in this discovery is that another virus was found inside the giant mamavirus. In other word, a satellite virus of giant virus was discovered, which is packaged inside the host virus. Although satellite viruses were previously reported in other organisms, this is the first example where the satellite virus is found packaged inside host virus particles. The satellite virus is now called “Sputnik,” named after the first man-made satellite. It has a 18 kb DNA genome, which encodes 21 genes (Table Box 1.5). Interestingly, Sputnik-infected mamavirus was somewhat attenuated in progeny production and morphologically distorted. Briefly put, Sputnik makes its host sick. Moreover, the satellite virus of the giant virus is now dubbed a “virophage,” as an analogy to the bacteriophage. Jean-Michel Claverie wittily added that “The discovery of virophage makes ‘virus’ more living organisms.” Paradoxically, “being sick” can be said to be the evidence of “living.” It was the first report that a virus particle was found packaged inside a host virus, which is already acting as a parasite of the host organism.



Mamavirus and virophage. (A) Transmission electron microscopy. Sputnik particles (six) are clearly visible inside mamavirus capsids. (B) Illustration of mamavirus and its satellite Sputnik virus.

TABLE BOX 1.5 Giant Virus and Virophage

Host Organism	Giant Virus (Genome Size)	Virophage (Genome Size)
Amoeba	Mimivirus (~1200 kb)	–
Amoeba	Mamavirus (~1200 kb)	Sputnik (18 kb)

1.7 PERSPECTIVES

A virus is physically smaller than a cell, the basic unit of life, and it propagates only inside cells, and often causes diseases. Strenuous efforts were made in the past century to control scourges caused by viruses. Since the discovery of the virus as a filterable agent at the dawn of the 20th century, the study of viruses has been established and has advanced a great deal during the past century. In retrospect, the achievements made in virus research are truly remarkable. Currently, almost all viral etiologic agents of human infectious diseases are believed to be identified, and have been extensively subjected to experimental analysis. For instance, viruses are now precisely classified by genome features rather than the diseases that they cause. The Baltimore classification now enables us to predict the

TABLE 1.4 Outstanding Features of Subviral Agents

Features	Satellite Virus	Viroid	Prion
Genome (Nucleic acid)	○	○	X
Protein coding	○	X	X
Particle protein	○ (Capsid)	X	○ (PrP)

genome replication mechanism of a novel virus immediately upon the availability of nucleotide sequence. On the other hand, subviral agents, such as viroids and prions, are extraordinary in the sense that RNA or protein itself is an infectious agent, as detailed in chapter “Subviral Agents and Prions.” Another extraordinary infectious agents are giant viruses discovered in amoeba. These two extraordinary infectious agents seem to blur and challenge the classical definition of the virus.

1.8 SUMMARY

- **Discovery:** Historical records on the scourges caused by viruses have been found in ancient relics. The virus was first described as a filterable transmissible agent that causes disease in plants and animals.
- **Definition:** A virus is a “submicroscopic and intracellular parasite.” Viruses are found in almost all living organisms on earth.
- **Virology:** Virology is a discipline, which studies the diverse aspects of virus replication and its consequences to the host cell. Virology, the study of viruses, has been established and has advanced a great deal over the past century.
- **Classification:** Animal viruses are classified into three groups: DNA viruses, RNA viruses, and reverse transcribing (RT) viruses. They are further classified into seven groups, according to their genome or by the Baltimore classification.
- **Subviral agent:** Virus-like transmissible agents, which do not comply with the classical definition of a “virus” are termed “subviral agents.” Satellite viruses, viroids, and prions are the tree types of subviral agents.

STUDY QUESTIONS

- 1.1** “Computer virus” is coined as an analogy to “virus.” State in what respects these two seemingly unrelated entities are related.
- 1.2** According to the Baltimore classification, viruses that belong to two distinct groups contain a positive-stranded RNA genome. List these two groups and describe the differences of the genome replication mechanisms between two groups.
- 1.3** List three kinds of subviral agents and state why each of these subviral agents does not comply with the classical definition of a “virus.”

SUGGESTED READING

- Duffy, S., Shackelton, L.A., Holmes, E.C., 2008. Rates of evolutionary change in viruses: patterns and determinants. *Nat. Rev. Genet.* 9 (4), 267–276.
- Enquist, L.W., Editors of the *Journal of Virology*, 2009. Virology in the 21st century. *J. Virol.* 83 (11), 5296–5308.
- Raoult, D., Forterre, P., 2008. Redefining viruses: lessons from Mimivirus. *Nat. Rev. Microbiol.* 6 (4), 315–319.
- Villarreal, L.P., 2004. Are viruses alive? *Sci. Am.* 291 (6), 100–105.

JOURNAL CLUB

- Enquist, L.W., 2009. Virology in the 21st century. *J. Virol.* 83 (11), 5296–308.
Highlight: An interesting perspective on Virology in the 21st century, which is written by Prof. Enquist, a leading virologist and an Editor-in-Chief of the *Journal of Virology*.

BOOK CLUB

- Boose, J. and August, M.J., 2013. *To Catch a Virus*, ASM Press, Washington, DC.
Highlight: Historical accounts of earlier discovery on human pathogenic viruses are vividly described from early work of Louis Pasteur on rabies vaccine to the more recent Barre-Sinoussi and Luc Montagnier's work on the discovery of HIV. It is a must-read for anyone who is interested in the historical account of the virus discovery.
- Crotty, S., 2001. *Ahead of the Curve: David Baltimore's Life in Science*, University of California Press.
Highlight: A compelling biography of David Baltimore, which details the life and work of one of the most brilliant, powerful, and controversial scientists of our time.

INTERNET RESOURCES

- International Committee on Taxonomy of Virus (ICTV): <http://www.ictvdb.org/>
- Viral Zone (<http://viralzone.expasy.org/>); a Swiss Institute of Bioinformatics web resource for all viral genus and families, providing general molecular and epidemiological information, along with virion and genome figures.
- The official web site of Nobel Prize: <http://www.nobelprize.org/>