Essential Human Virology

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Preface

Essential Human Virology is intended to be an approachable and concise introduction to the field of virology. It focuses on the conceptual framework that is needed for students to understand the replication and transmission of viruses and the diseases they cause in humans. Its goal is to provide the essential foundations necessary to understand more advanced molecular virology and scientific research articles.

This textbook incorporates several techniques to facilitate student learning. The text purposefully uses increasingly complex scientific language as the chapters progress in an effort to gradually introduce students to the virological terms and principles of the field. It incorporates over 250 figures that contain dynamic, full-color illustrations and images to further engage visual learners in the material. Integrated "Study Break" questions allow students to gauge their understanding as they read the chapter, and special "In-Depth Look" sections provide additional, detailed material on related topics of high interest. At the conclusion of each chapter, a "Summary of Key Concepts" reminds students of the entirety of the material covered in the chapter. The "Chapter Review Questions" give students the opportunity to test their recall of the important details and apply them to bigger-picture scenarios. A list of "Flash Card Vocabulary" is also included at the end of each chapter, because a solid grasp of new terminology is paramount for comprehension of virological principles. Two appendices at the end of the book include a glossary and a list of abbreviations, allowing students to easily look up terms whenever necessary.

In order to understand aspects of virology and viral replication, students must already possess an understanding of basic cellular and molecular processes. These topics are usually covered in an introductory biology class and further detailed in advanced cellular and molecular biology classes. For students who may need a refresher on these fundamentals, chapter "Features of Host Cells: Cellular and Molecular Biology Review" reviews the central dogma of molecular biology and notable cellular features that are important for understanding viral replication strategies. Because this information is interwoven throughout many upper-level biology courses, most students—if not all—benefit from a review of these principles.

This book covers several themes related to viruses. Chapters "The World of Viruses," "Virus Structure and Classification," "Features of Host Cells: Cellular and Molecular Biology Review," and "Virus Replication" describe the characteristics of viruses and their detailed replication strategies. Chapters "Virus Transmission and Epidemiology," and "The Immune Response to Viruses" examine the interactions of viruses with individuals and populations, including how viruses are combatted by the host immune system, spread between individuals, and disseminate within a population. Chapters "Detection and Diagnosis of Viral Infections," and "Vaccines, Antivirals, and the Beneficial Uses of Viruses" introduce students to traditional and newer methods of viral diagnosis, outline current and experimental vaccines and antivirals, and discuss the beneficial uses of viruses for gene therapy and anticancer therapeutics. Chapter "Viruses and Cancer" examines those viruses that are associated with the development of cancer. The next six chapters provide an in-depth look into human viruses of clinical significance. These chapters cover the replication strategy, pathobiology, and epidemiology of influenza viruses, human immunodeficiency viruses, the hepatitis viruses, the herpesviruses, poliovirus, and poxviruses. The final chapter highlights examples of emerging diseases and their origins. Actual case studies are incorporated into most chapters in order to give students an opportunity to comprehensively integrate the clinical, diagnostic, and epidemiological aspects of viral infections.

About the Author

Jennifer Louten received her doctoral degree from Brown University Medical School, where she investigated the cellular targets of infection and the induction of type 1 interferons following infection with lymphocytic choriomeningitis virus. Dr. Louten is currently an associate professor of biology at Kennesaw State University, where she has served as a Teaching Fellow and developed courses in virology, biotechnology, immunology, and cell culture techniques. She is presently the biotechnology track coordinator and the director of a scholarship program sponsored by a National Science Foundation S-STEM grant. She is the recipient of a Kennesaw State University Outstanding Early Career Faculty Award and the Student Government Association's Faculty of the Year Award. Before becoming a professor, Dr. Louten performed research in drug discovery at Schering-Plough Biopharma (currently Merck Research Laboratories). She received her Bachelor of Science in biotechnology from the Rochester Institute of Technology.

Instructor Companion Website

The online instructor companion website includes several ideas and examples of activities that can be used to further elaborate upon the topics covered in the textbook. For each chapter, a *Teacher Resources* document provides a summary of the chapter and its student learning objectives, using Bloom's taxonomy action verbs. This document includes a study guide that can be provided to students, as well as many examples of active learning instructional activities, classroom discussion topics, presentation ideas, and related videos and websites. Handouts, hands-on worksheets, and a vocabulary crossword puzzle are also included, as well as links to peer-reviewed research articles that correspond to the material. The instructor can use these activities as a supplement to the textbook for more advanced students or in a "flipped classroom" or blended learning instructional format. In addition, the companion website includes a lecture presentation, image bank, and test bank for each chapter. The website can be found here: www.booksite.elsevier.com/9780128009475.

Chapter 1

The World of Viruses

Consider the following cases:

- In Los Angeles, California, five men ranging in age from 29 to 36 years old are hospitalized with pneumonia (inflammation of the lungs) caused by *Pneumocystis carinii*, a fungus. This sort of pneumonia in previously healthy individuals is extremely rare and most often seen in people with severely suppressed immune systems. All five men die of this condition.
- In Hong Kong, a previously healthy three-year old boy develops a fever, sore throat, and cough. He is hospitalized, and less than a week later he dies of acute respiratory distress syndrome, a condition that prevents sufficient oxygen from getting into the lungs and bloodstream. This was believed to be caused by inflammation in his lungs. Sick poultry are also reported in the area.
- A cruise ship departs on a 21-day trip from Washington to Florida. During the trip, 399 of the 1281 passengers come down with acute gastroenteritis, a sudden stomach illness characterized by nausea, vomiting, and watery diarrhea. The cruise ship is cleaned, but a total of 305 people come down with a similar illness during the next three voyages of the ship.

What do these cases have in common? They are all real cases of illnesses that were caused by viruses. The first case above describes the first documentation of acquired immune deficiency syndrome, or AIDS. Although it sounds like the sick men were suffering from a fungal infection, it was revealed 2 years later, in 1983, that AIDS is caused by a virus, now known as the human immunodeficiency virus (HIV). HIV infects cells of the immune system and causes the immune system to slowly decline until it can no longer fight off pathogens, like the *P. carinii* fungus that infected these men. Worldwide, 36.9 million people are living with HIV, over 1 million people in the United States alone.

In the second case, laboratory tests found that the young boy was infected with the flu, which is caused by the influenza virus. The particular subtype was found to be H5N1 influenza, which had previously been observed only in birds. It can be dangerous when a virus jumps to a new species, because as a population, the new species has never been exposed to the virus and so no individuals will have built up immunity against it. As such, the virus has the potential to spread quickly and cause severe effects. In this case, 18 individuals in Hong Kong ended up with the H5N1 influenza infection, which they acquired through direct interaction with chickens at open-air markets. It was soon discovered that 20% of the chicken population in Hong Kong was infected with this subtype of influenza, and considering the 30% death rate they had so far observed, the government decided to prevent further human exposure to the virus by slaughtering the 1.5 million live chickens found in its markets and poultry farms. This effectively stopped the spread of the virus into any additional humans, but the threat always exists that a similar situation could occur again.

In the final case, passenger stool samples tested positive for Norwalk virus. This type of virus causes diarrhea and vomiting and is very easily transmitted from person to person through the ingestion of contaminated food and water or aerosolized particles, like those that might be generated by flushing a toilet. The virus is difficult to inactivate; alcohol-based hand sanitizers are not completely effective against the virus, so cleaning with a bleach solution is the preferred method. In addition, only a few virus particles need to be ingested to cause illness, which may explain why passengers on subsequent cruises also came down with the illness, even though the ship had been disinfected before their trip. Norwalk virus outbreaks do not occur solely on cruise ships; each year, there are an estimated 20 million cases in the United States of gastroenteritis caused by this virus.

These three cases are just a few examples of the diseases that viruses can cause. In the following chapters, we will explore how viruses replicate, are spread, and cause a variety of diseases in humans.

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In-Depth Look: The Difference Between "Virus" and "Virion"

Many people use the word "virus" and "virion" interchangeably, but the two words have subtle but important differences. The word "virion" is used to describe the infectious virus package that is assembled. It is the extracellular form of the virus, also referred to as a virus particle, that is released from one cell and binds to the surface of another cell.

On the other hand, the word "virus" refers to the biological entity in all its stages and the general characteristics that differentiate it from another infectious entry. Rhinovirus and Epstein–Barr virus are two different entities with different properties. These viruses have different genes, structures, and methods of infection—they have different characteristics that differentiate them from each other and from other viruses.

You may be infected with several different viruses at one time, and you likely have millions of virions present in your body from each one.

An analogy to describe the difference between "virus" and "virion" is FedEx and UPS are shipping companies with different properties. They have different revenues, different administrations, different logos, and different business models. When you see a UPS or FedEx truck passing by, however, you are not seeing the company (the virus)—you are seeing how the company's goods get transported from one location to another (the virions). Each company has thousands of trucks delivering goods at any one time, just as each viral infection will generate millions of virions that spread the virus.

1.1 THE IMPORTANCE OF STUDYING VIRUSES

We study viruses and the diseases they cause for a variety of reasons. First, viruses are everywhere. They are found in all of our surroundings: the air, the ocean, the soil, and in rivers, streams, and ponds. They are present wherever life occurs, and it is thought that every living thing has a virus that infects it.

So how many viruses are there? There are around 3000 documented species of viruses that infect a range of living organisms, although there are thousands of different strains and isolates within these species, and thousands more viruses that remain to be discovered.

As described in the *In-Depth Look*, an infectious virus particle is called a **virion**, and when a cell is infected with a virus, millions of these infectious particles are created and released from the cell to infect other cells. How does the number of individual virions compare to other things found in high abundance around us? There are an estimated 10^{18} grains of sand on Earth and 10^{23} stars in the Universe, yet neither of these numbers compare to the number of virions found on Earth. If you multiplied the number of stars in the Universe 100 million times, you would have the number of infectious virus particles in the world. With an estimated 10^{31} total virions, viruses are the most abundant biological entities on our planet. We know that bacteria are abundant and everywhere, but there are 10 times more virions on Earth than bacteria! It is important that we continue trying to understand the viruses that are constantly around us.

All living organisms have a relationship with viruses. Viruses have been around since the beginning of life on Earth and have shaped the course of human evolution and history. The stela shown in Fig. 1.1A dates back to 1580–1350 BC and shows an Egyptian with a walking stick and foot drop, a condition that prevents dorsiflexion of the foot (lifting of the foot at the ankle). This is a common occurrence in people with poliomyelitis, caused by the poliovirus, which causes this condition by infecting and damaging motor neurons. The mummy of Ramses V, shown in Fig.

FIGURE 1.1 Viruses have existed as long as humans have. (A) An Egyptian stela dating back to 1580–1350 BC depicts a priest with a walking cane and foot-drop deformity, attributed to poliomyelitis. *Photograph by Ole Haupt, courtesy of Ny Carlsberg Glyptotek, Copenhagen.* (B) The mummy of Ramses V, who died in 1157 BC, exhibits a rash on the lower face and neck that is characteristic of a smallpox rash. *Photo courtesy of the World Health Organization.*

1.1B, shows evidence of smallpox-like lesions (seen on the lower face and neck in the photograph), leading scientists to believe he may have died of this poxvirus in 1157 BC. Viruses have been present as long as life has existed, and there is persuasive evidence that viruses may even have existed before life arose.

One of the most compelling reasons we are interested in viruses, however, is because viruses cause diseases. They cause conditions as simple as the common cold or as complex as cancer. A virus can also cause an **epidemic**, an outbreak where the virus infects many more individuals than normal and spreads throughout an area. Some of the world's worst epidemics have been caused by viruses. As mentioned above, the effects of poliovirus have been noted throughout history for thousands of years, but the growth and urbanization of cities provided the conditions that fueled epidemics of the virus (Fig. 1.2A and B). One of the first major epidemics occurred in New York City in 1916 and resulted in over 9000 cases and 2343 deaths. In the early 1950s, over 20,000 cases of paralytic polio (causing temporary or permanent paralysis) occurred each year until, in 1955, the first polio vaccine was introduced and cases dropped precipitously.

A **pandemic** ensues when a virus spreads throughout a much larger area, such as several countries, a continent, or the entire world. Reports and warnings from the Centers for Disease Control and Prevention (CDC) concerning influenza may sometimes seem alarmist, but in late 1918, a strain of influenza originating in the United States spread throughout the entire world, killing 20–50 million people (Fig. 1.2C). In the month of October alone, 195,000 Americans died of this influenza, and when the pandemic was over, over 675,000 Americans had died from the effects of the virus, more Americans than in all the wars of the century combined. Most subtypes of influenza cause mild or moderate respiratory symptoms, but some, like the 1918 influenza, can cause devastating effects. Although medicine has advanced greatly in the last 100 years, surveillance and vaccination is by far the most effective way to prevent another pandemic of this magnitude. Scientific research has led to the development of vaccines that have greatly reduced the burden and death toll of many viral diseases.

Although viruses can be harmful, research using viruses has resulted in a wealth of information revealing how systems work in living organisms. One very important experiment involving viruses helped to verify that deoxyribonucleic acid (DNA) is the molecule that encodes genetic information. In the earlier half of the 20th century, scientists were uncertain as to whether protein (composed of amino acids) or DNA (composed of nucleotides) was the hereditary instructions for cell development and function. There are 20 different amino acids but only 4 different nucleotides, so it was not unreasonable to think that amino acids could encode more possible information than nucleotides could. In 1944, Oswald Avery, Colin MacLeod, and

FIGURE 1.2 Viruses can cause serious epidemics and pandemics. Public health officials placed quarantine signs, like the one shown in (A), on the houses of people with polio during epidemics. *(Courtesy of the U.S. National Library of Medicine.)* (B) Polio can lead to temporary or permanent paralysis. Muscle weakness or paralysis still present after 12 months is usually permanent. *(Photo courtesy of the CDC/Charles Farmer.)* (C) An influenza ward in a U.S. Army camp hospital in France in 1918, during World War I. *(Courtesy of the U.S. National Library of Medicine.)*

Refresher: Important Biological Molecules

Deoxyribonucleic acid (DNA) is made of many **nucleotides** bonded together. A nucleotide of DNA (Fig. 1.3A) is composed of a sugar, called deoxyribose, with a phosphate group attached at one end of the sugar and a base attached at the other end. There are four different bases in a DNA nucleotide: adenine, guanine, cytosine, and thymine (Fig. 1.3B). DNA is double-stranded in all living organisms, and the bases in one strand bond to the bases in the other strand (Fig. 1.3C). This is known as a **base pair**. Adenine always bonds with thymine, and guanine and cytosine always bond to each other.

Ribonucleic acid (RNA) is also made of nucleotides bonded together, but the sugar used is ribose, rather than deoxyribose. Uracil replaces the base thymine, and RNA is single-stranded, not double-stranded (Fig. 1.3D). In living things, DNA is used as a template for making RNA. DNA and RNA are **nucleic acids**.

Protein is a different biological molecule that is made of **amino acids** bonded together (Fig. 1.3E). There are 20 slightly different amino acids, composed of carbon, hydrogen, oxygen, and nitrogen. Two amino acids, methionine and cysteine, also contain the element sulfur. Enzymes are a very important class of proteins.

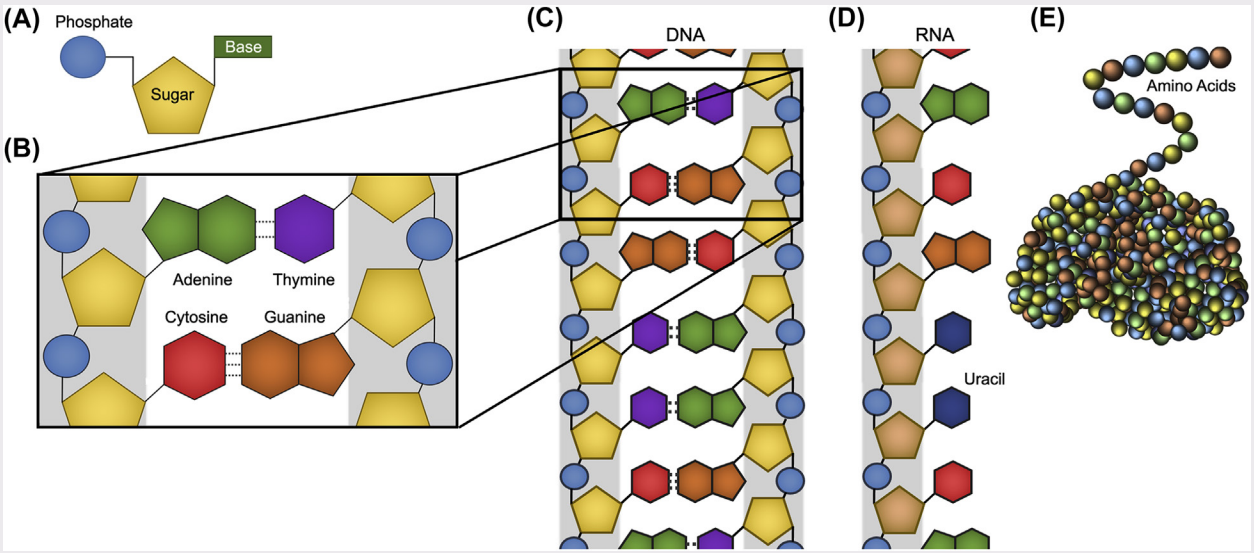

FIGURE 1.3 **Three important biological molecules: (A–C) DNA, (D) RNA, and (E) Protein.**

Maclyn McCarty demonstrated that DNA is the molecule that encodes inheritable traits. They were pursuing a topic of research initially performed by Frederick Griffith, a British medical officer studying two strains of the bacterium *Streptococcus pneumoniae*, which had caused epidemics of pneumonia. The rough (R) strain did not cause disease in mice, but the smooth (S) strain caused pneumonia and killed the mice. In 1928, Griffith noted that if he killed the S bacteria using high heat, there was something in that strain that was taken in by living R bacteria that turned them into the pneumonia-causing S bacteria. He called this the *transforming principle*, although he did not know specifically what molecule was taken in and transformed the R strain.

Avery, MacLeod, and McCarty followed up on Griffith's experiments in an attempt to identify this transforming principle. They isolated the molecule that transformed the bacteria and found it to be DNA. While some scientists believed their results, many were skeptical, arguing that the purified DNA preparations may still have contained some proteins, which they believed to be the true transforming principle. As such, the scientific community was resistant to accepting DNA as the genetic code.

Over the next decade, the field slowly warmed to the idea, and an experiment utilizing viruses conducted in 1952 provided the evidence that was needed to finally convince the world. At Cold Spring Harbor Laboratory on Long Island, New York, Alfred D. Hershey and his laboratory technician Martha Chase were performing research with **bacteriophages** (or **phages**), viruses that infect bacteria (Fig. 1.4). They were working with a bacteriophage called T2 that infects *Escherichia coli*, a bacterium found in the gastrointestinal tract of mammals. T2 attaches to the *E. coli* cell and injects into the chemical instructions to make more T2 bacteriophages.

To test whether these chemical instructions were composed of DNA or protein, Hershey and Chase used radioactive phosphorus (^{32}P) and radioactive sulfur (^{35}S) isotopes. Phosphorus is found in DNA but not in proteins, and sulfur is found in proteins but not in DNA. Hershey and Chase grew two cultures of bacteriophages, one in ^{32}P and one in ^{35}S (Fig. 1.5). Bacteriophages are composed of DNA surrounded by a protein coat, so the bacteriophages grown in ^{32}P incorporated it into their DNA, while the bacteriophages grown in ^{35}S incorporated it into their protein coats.

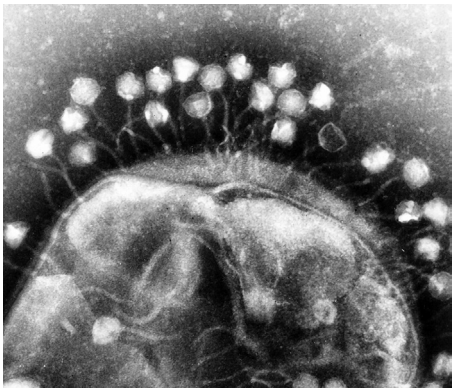

FIGURE 1.4 Bacteriophages. An electron micrograph of bacteriophages attached to a bacterial cell. *Courtesy of Dr. Graham Beards CC-BY-3.0.*

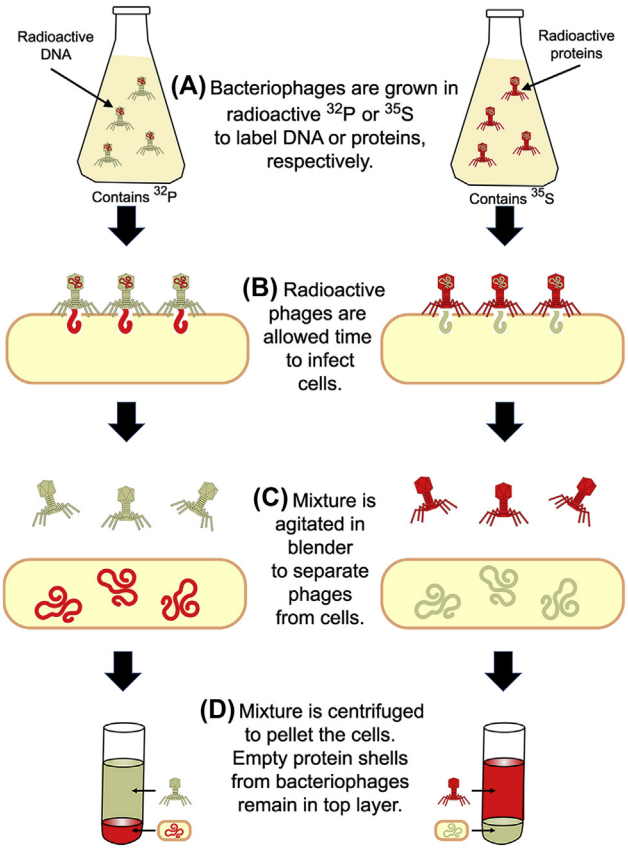

FIGURE 1.5 The Hershey–Chase experiment. (A) Bacteriophages were grown in cultures containing radioactive phosphorus (to label DNA) or radioactive sulfur (to label proteins), and the two phage cultures were allowed time to infect separate bacterial cells (B). After infection, a blender was used to agitate the mixture and separate the phage shells from the cells (C). The radioactive DNA remained in the cells while the radioactive proteins were found with the bacteriophage shells (D), indicating that DNA was the hereditary material.

After creating their two sets of bacteriophages, Hershey and Chase infected the bacteria with the ^{32}P-labeled or ^{35}S-labeled bacteriophages. They allowed time for the bacteriophages to infect and then used a blender to violently agitate the cells, shearing off any of the phage still attached to the cell surface. When they used a centrifuge to separate the cells from the empty phages, they found that the ^{35}S-labeled proteins remained outside the cells, while the ^{32}P-labeled DNA entered the cells. In addition, they noted that some of the ^{32}P was also incorporated into the next set of bacteriophages produced in the cell. This experiment showed that when the bacteriophages attached to the cells, the DNA entered the cell while the protein coat remained outside. The Hershey–Chase experiment used viruses to confirm the Avery, MacLeod, and McCarty's findings that DNA, and not protein, is the genetic material. A year later, in 1953, James Watson and Francis Crick presented their double-helix model of DNA structure. Since that time, numerous important scientific discoveries have been elucidated through research with viruses.

Viruses have also been investigated for their use as therapeutics. Nearly 100 years ago, Felix d'Herelle, the man who coined the term bacteriophages, meaning "bacteria eaters," used these viruses for the treatment of bacterial infections in a time when antibiotics did not yet exist. **Phage therapy** declined after antibiotics were introduced but has undergone a renaissance, as of late, with the evolution of certain antibiotic-resistant strains of bacteria. Viruses are also being used for **gene therapy**, the delivery of DNA into cells to compensate for defective genes. Viruses have evolved ways to deliver their genes into cells; in gene therapy, viruses are engineered to deliver a normal copy of the defective human gene. Gene therapy has great potential to cure many genetic diseases, although there are currently procedural obstacles that must be overcome before it can become a mainstream treatment. These therapies will be discussed in detail in Chapter 8, "Vaccines, Antivirals, and the Beneficial Uses of Viruses."

Study Break

Explain how bacteriophages were used to verify that DNA, and not protein, encodes genetic information.

1.2 VIRUSES ARE NOT ALIVE

Virology is the study of viruses, how they replicate, and how they cause disease. It may seem bizarre that virology is a subset of biology—the study of life—because viruses are not considered to be alive. They are, however, intricately tied to the web of life here on Earth.

In order to understand why viruses are not alive, we must revisit the characteristics of living things. To be considered alive, an organism must satisfy several criteria:

1. It must have a genome, or genetic material.
2. It has to be able to engage in metabolic activities, meaning that it can obtain and use energy and raw materials from the environment.

3. It has to be able to reproduce and grow.
4. It must be able to compensate for changes in the external environment to maintain homeostasis.
5. Populations of living organisms are also able to adapt to their environments through evolution.

There is no question that viruses share some of these characteristics. Every virus has genetic material, or a **genome**, although viruses are a bit different because, unlike living organisms that only have DNA genomes, viruses can have genomes composed of DNA or RNA, depending upon the virus. Many viruses also have high mutation rates that lead to the evolution of the virus. For instance, if a person with HIV is treated with one antiviral drug, the virus quickly evolves into a strain that is no longer affected by the drug. The influenza virus continuously acquires small mutations, which is why the flu vaccine you received last year may not protect you from this year's flu. In fact, because viruses mutate so quickly, they function as a great model for studying and observing evolutionary change, which takes much longer in living organisms.

Viruses do not, however, engage in their own metabolic activities. **Metabolism** refers to the collective set of biochemical reactions that takes place within a cell. Biochemical reactions that break down substances to generate energy are constantly taking place within each cell of a living organism. This energy is used in other parts of the cell for thousands of other reactions that are necessary for the survival of the cell. Viruses, however, are unable to perform these metabolic reactions while outside a cell. In essence, they are inert particles that do not have the ability to generate their own energy. They use the cell's energy and machinery to synthesize new virus particles.

This brings us to the next characteristic of living things: the ability to reproduce independently. To reproduce, a cell makes a copy of its DNA, expands in size, and divides the DNA and cell in two. This is known as **binary fission** in prokaryotes and **mitosis** in eukaryotes. Viruses, however, do not reproduce in this way (Fig. 1.6). When a virus particle enters the cell, it completely disassembles. The viral nucleic acid encodes the instructions, and the cell's machinery will be used to make new infectious virus particles (virions). The replication cycle of viruses functions in the same way that a manufacturing factory (the cell) receives a package with instructions (the virus) on how to mass produce a new product, entirely from scratch, that is then shipped to other locations after manufacturing. All cells of living organisms arise from the growth and division of a previously existing cell, and viruses do not reproduce in this manner.

Homeostasis is a steady internal condition that is exhibited by living organisms. For example, if energy levels fall too low within a cell, it will compensate by breaking down stored molecules to generate energy. If you go outside in the winter with a T-shirt on, you start shivering so your muscles generate heat to warm you up, and goose bumps on your skin raise the hairs on your arm in an attempt to keep that heat near your skin. Living organisms have mechanisms to regulate internal highs and lows to maintain homeostasis, but viruses do not. As inert particles, they are unable to compensate for changes in their external environment.

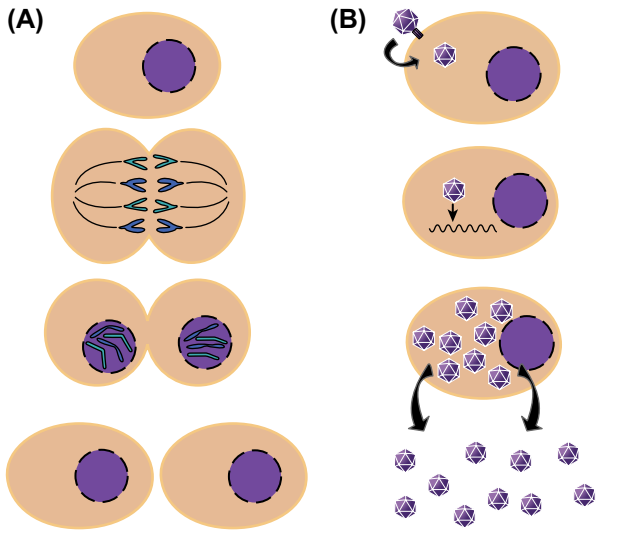

FIGURE 1.6 Cell division versus viral replication. (A) Eukaryotic cells make a copy of their genetic information and divide into two cells through the process of mitosis. All cells arise from the growth and division of previously existing cells. (B) Viruses, on the other hand, attach to cells and disassemble within the cell. New virions are assembled from newly made components and are released from the cell.

Despite not being alive, viruses still share many similarities with living organisms. They are composed of the same biological substances, such as nucleic acids or amino acids, and their proteins are translated by ribosomes much in the same way as living organisms. They play a role in the cycling of energy and matter within ecosystems, and some can quickly evolve, as described above. Within the cell, viruses are far from inert: although they use the cell's energy, raw materials, and organelles, their nucleic acid genome encodes the instructions to assemble new infectious virions that are able to carry on the "life cycle" of a virus.

It is interesting to note that viruses are thought to have been around since the beginning of life itself. In fact, one current hypothesis states that viruses were some of the precursors to life on Earth as we know it.

Study Break

What characteristics do viruses share with living organisms? Why are they not considered to be alive?

1.3 THE ORIGIN OF VIRUSES

The question of how viruses arose is a difficult and much debated issue. It is generally accepted that viruses appeared around the same time that life began, and when new information is discovered concerning the origin of life, the hypotheses about the origin of viruses are also revisited. The Earth was a very different environment when life is thought to have originated, around 3.5 billion years ago, and it is a formidable challenge to uncover evidence from that time period. More often, scientists reveal current or past biological processes that help to explain how viruses may have evolved over time. Based upon the evidence that we currently have, there are three viable hypotheses on how viruses originated:

1. The precellular hypothesis (or "virus-first" hypothesis)
2. The escape hypothesis
3. The regressive hypothesis

The **Precellular Hypothesis**, also known as the "virus-first hypothesis," proposes that viruses existed before or alongside cells and possibly contributed to the development of life as we know it. It is now thought that life may have developed in an "RNA World" where RNA, instead of DNA, was the first genetic material. RNA is easier to create than DNA from the precursor chemicals that are thought to have existed on the early Earth, and in present-day cells, the sugar found in DNA, deoxyribose, is made from ribose, the sugar found in RNA. To replicate, DNA also requires complex protein enzymes, but RNA has the unique property that it can encode genetic material and in some cases, like RNA ribozymes, catalyse reactions much like a protein enzyme does. In this way, RNA could have functioned as an enzyme that copied an early RNA genome.

Many important molecules in the cell include RNA or parts of it, such as ATP (used for energy) or the ribosome, which assembles proteins and is composed of RNA. It is thought that these might be conserved remnants of the creation of cells in an RNA World. Similarly, it is conceivable that RNA viruses also originated in this RNA world, either before or alongside RNA-based cells (Fig. 1.7A). All known viruses require a host cell to replicate, however, so it seems more likely that they developed alongside these primitive cells, rather than as precursors of them.

It is presumed that DNA, being a more stable molecule, was selected for and eventually replaced RNA. An interesting thought related to the precellular hypothesis is that DNA first originated in RNA viruses, thereby giving rise to DNA viruses, and that cells with DNA originated from the infection of an RNA cell with a DNA virus (Fig. 1.7B). In this scenario, a DNA virus might have infected an RNA cell and the genome of the virus continued to persist within it, in the same way that the genomes of some currently existing DNA viruses can. Eventually, the viral DNA might have picked up some of the host cell's genes and became the cell's chromosome, resulting in a cell with a DNA genome. Alternatively, the mechanisms used to create DNA within the virus could have been adopted by the cell.

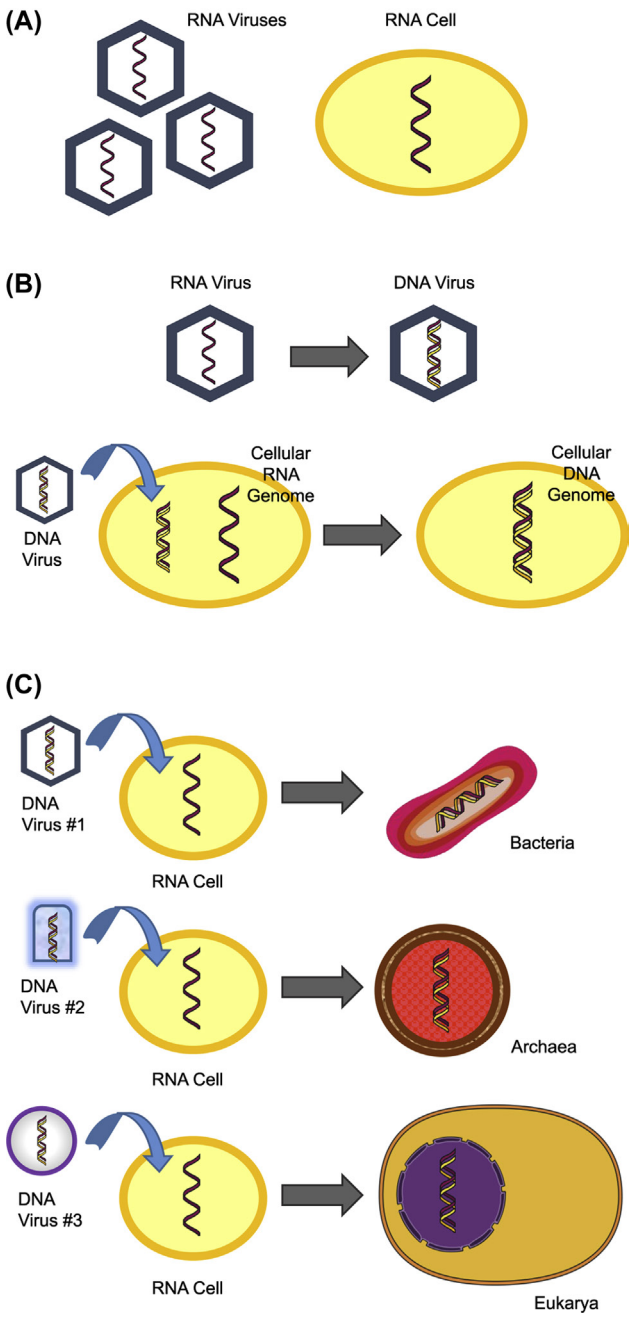

FIGURE 1.7 Variations of the virus-first hypothesis. The precellular (or virus-first) hypothesis proposes that viruses initially developed before or alongside cells in an RNA-based world (A). A variation of this hypothesis (B) proposes that RNA viruses evolved into DNA viruses that infected RNA cells, which eventually gained a DNA genome by using the viral DNA or viral DNA-generating mechanisms. (C) A related version speculates that the three Domains of life (*Bacteria*, *Archaea*, and *Eukarya*) may have arisen from infection of cells with three distinct DNA viruses.

Support for this idea came when the DNA polymerase from an algae-infecting virus was found to be related to a DNA polymerase found in eukaryotic cells. Taking this one step further, it has been suggested that the three domains of life—*Bacteria, Archaea,* and *Eukarya*—each arose independently from the infection of cells with three distinct DNA viruses (Fig. 1.7C).

Critics of the precellular hypothesis point out that all viruses are parasitic and require a cellular host. Therefore, it is unlikely that viruses could have existed before cells because they would not have had a reliable source of the materials they need to replicate. In addition, the majority of viral genes are not found in cells, and one should expect to see more similarities between cells and viruses if a DNA virus was the origin of a cell's genetic material.

The other two hypotheses for the origin of viruses presume that cells existed before viruses. The first of these is called the **Escape Hypothesis**. This hypothesis proposes that viruses are pieces of cells that broke away at one point in time (hence they "escaped" from the cell) and gained the ability to travel from cell to cell. By extension, the viruses of Bacteria, Archaea, and Eukarya may have arisen from distinct escape events within those three domains (Fig. 1.8).

The Escape Hypothesis gained popularity when transposable elements were discovered. **Transposable elements**, or transposons, are pieces of DNA that can physically move from one location to another in the genome of a living organism. Some are only a few hundred nucleotides long, while others span thousands of nucleotides. Initially thought to be "junk DNA" with no apparent function, these transposable elements make up nearly half of the human genome, although many are no longer functional. Some of these transposable elements have similarity to **retroviruses**, such as HIV, which incorporate into the host's DNA upon entering a cell. Supporters of the escape hypothesis point out that retroviruses may have originated from the escape of these transposable elements from the cell. Many retrovirus genomes are also found permanently integrated into cellular genomes as relics of past periods. Critics, however, emphasize that the great majority of viral genes have no **homologous** (evolutionarily similar) cellular counterpart, so if viruses originated from escaped cellular genes, why are not more cellular genes found in viruses, and where did all these unique viral genes come from? It is more likely that retroviruses infected cells and integrated into their genomes, rather than retroviruses being derived from them.

The third current hypothesis to explain the origin of viruses is the **Regressive Hypothesis**, which suggests that viruses were once independent intracellular organisms that *regressed* back to a less-advanced state where they were unable to replicate independently. Two organelles currently found within cells, namely the mitochondrion and the chloroplast, are thought to have originated in this manner. Precedent for this idea also comes from the world of bacteria,

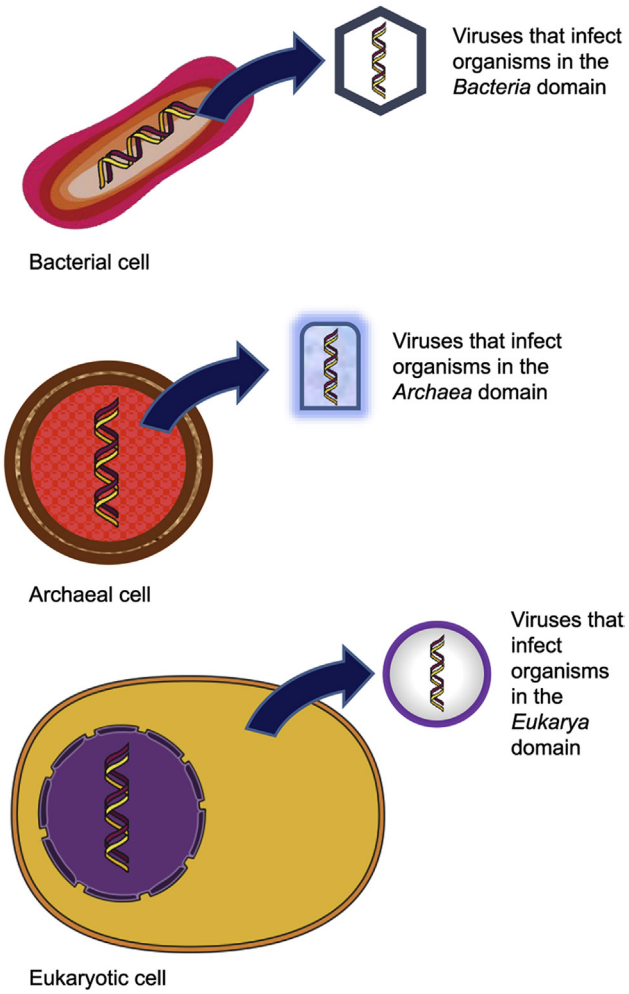

FIGURE 1.8 The escape hypothesis. The escape hypothesis proposes that viruses arose from portions of cells that gained the ability to travel from cell to cell. Viruses that infect cells within the three Domains of life (*Bacteria*, *Archaea*, and *Eukarya*) may have arisen from cells within each of those domains.

where certain bacteria such as *Chlamydia* and *Rickettsia* require the intracellular environment of the cell to replicate. Similarly, perhaps viruses were once living intracellular organisms that dissolved their membranes to facilitate easier access to cellular equipment and materials.

The discovery of a giant amoeba-infecting virus, in 2003, lended support to the regressive hypothesis. Mimivirus, short for "microbe-mimicking virus," is so named because of its size: at approximately 750 nm in diameter, the virus was initially thought to be a small bacterium (Fig. 1.9A). It was the largest virus discovered at the time, and a handful of larger viruses have since been characterized. Mimivirus has one of the largest known viral genomes, at over a million base pairs; by comparison, the average virus has a genome composed of thousands or tens of thousands of nucleotides. Its physical and genome size are strikingly large, and several of the genes in the mimivirus genome

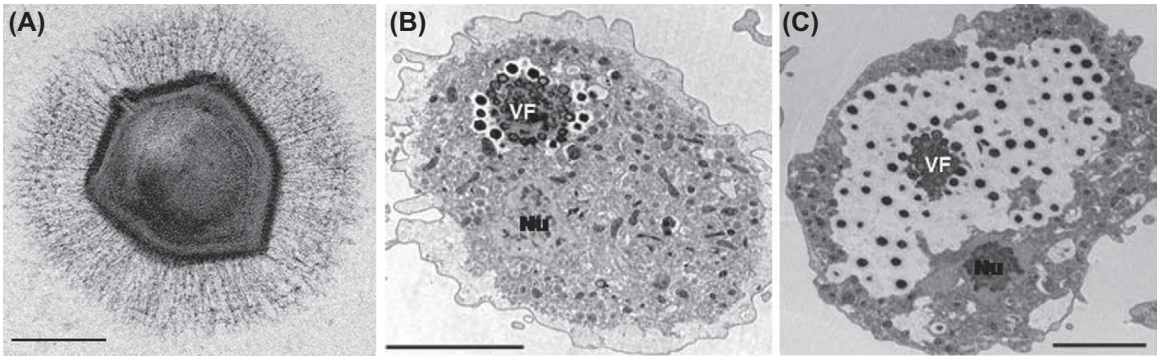

FIGURE 1.9 The giant mimivirus. Mimivirus was first discovered in 1992 and initially thought to be a bacterium, due to its large size. Including the protein filaments that project from its surface, mimivirus is about 750 nm in diameter (A). Scale bar = 200 nm. *(Image courtesy of Ghigo, E., Kartenbeck, J., Lien, P., et al., 2008. Ameobal pathogen mimivirus infects macrophages through phagocytosis. PLoS Pathog. 4 (6), e1000087,* http://dx.doi.org/10.1371/journal.ppat.1000087.*)* (B and C) Mimivirus, like other large complex DNA viruses, sets up virus factories (VF) within the cytoplasm of a cell to facilitate the replication of the virus. Scale bar = 5 µm (B) and 3 µm (C). *(Image courtesy of Suzan-Monti, M., La Scola, B., Barrassi L., Espinosa L., Raoult D., 2007. Ultrastructural characterization of the giant volcano-like virus factory of Acanthamoeba polyphaga Mimivirus. PLoS One 2 (3), e328.)*

resemble genes for creating proteins, suggesting that the virus may have been able to create its own proteins at one point in evolutionary time.

After infecting a cell, mimivirus and many large complex DNA viruses set up so-called **virus factories** made of cellular membranes, where the replication and assembly of virions takes place (Fig. 1.9B and C). These factories contain the enzymes necessary to copy the viral genome and either contain or are in close proximity to ribosomes (for making proteins) and mitochondria (for supplying energy in the form of ATP). This scenario is reminiscent of the *Chlamydia* reticulate body, a structure that the bacteria form within a cell that is used as a factory to develop new bacteria. The new bacteria infect new cells, and again form reticulate body factories within the cell, similar to the viral factories observed with infection by large complex DNA viruses. It is possible that our characterization of viruses as inert biochemical packages is too simplistic, and when we observe inert virions outside the cell, we may actually only be observing the infectious portion of the viral life cycle, much in the same way that *Chlamydia* have an inert infectious phase but new bacteria are produced in the intracellular reticulate body.

Critics of the regressive hypothesis point out that although a few mimivirus genes resemble genes in cells, the majority are unlike any genes found in bacteria or eukaryotic cells. If viruses were once parasitic cells, then more viral genes should show similarity to the genes of currently existing cells because they would have shared a common ancestor at one point in time. Perhaps the few viral genes that resemble cellular genes are not artifacts of a free-living organism that evolved into a virus, but were instead stolen from the cell's DNA at one point in time. Known as **horizontal gene transfer**, there are many examples of viral genes that are thought to have originated in this way.

Another criticism of the regressive hypothesis deals with the manner in which viruses are replicated. As described above and in Fig. 1.6, viruses are assembled completely from scratch, rather than splitting in two like cells do. Even the *Chlamydia* reticulate body divides repeatedly to generate the new bacteria that are released from the cell. If viruses were once free-living parasites, what situation could have caused such a major modification, different from every other living thing?

It is understandably much easier to brainstorm than test new hypotheses concerning the origin of viruses. These hypotheses will continue to be refined and modified as more information is revealed from the characterization of known viruses and the discovery of new viruses. The first viruses that existed may have only remotely resembled the plentitude of highly evolved present-day viruses. In any case, it is a distinct possibility that viruses have been continuously evolving alongside life since it began, over three billion years ago.

1.4 THE DISCOVERY OF VIRUSES

Several important discoveries have contributed to the identification of viruses as novel biological entities (Fig. 1.10). In the mid-1800s, the French chemist Louis Pasteur (Fig. 1.11A) performed some simple yet elegant experiments to show that life does not arise through spontaneous generation, the belief at the time that living organisms could spontaneously arise from nonliving matter. In one experiment, Pasteur sterilized beef broth by boiling it in a swan-neck flask, so named for the similarity of the flask's neck to that of a swan (Fig. 1.11B). The curved neck of the flask allowed air to enter but trapped any dust or particles that might have contained microorganisms, and so the broth remained clear and free of any microbes, even after prolonged incubation. In contrast, the broth became contaminated if the top of the neck of the flask was broken off

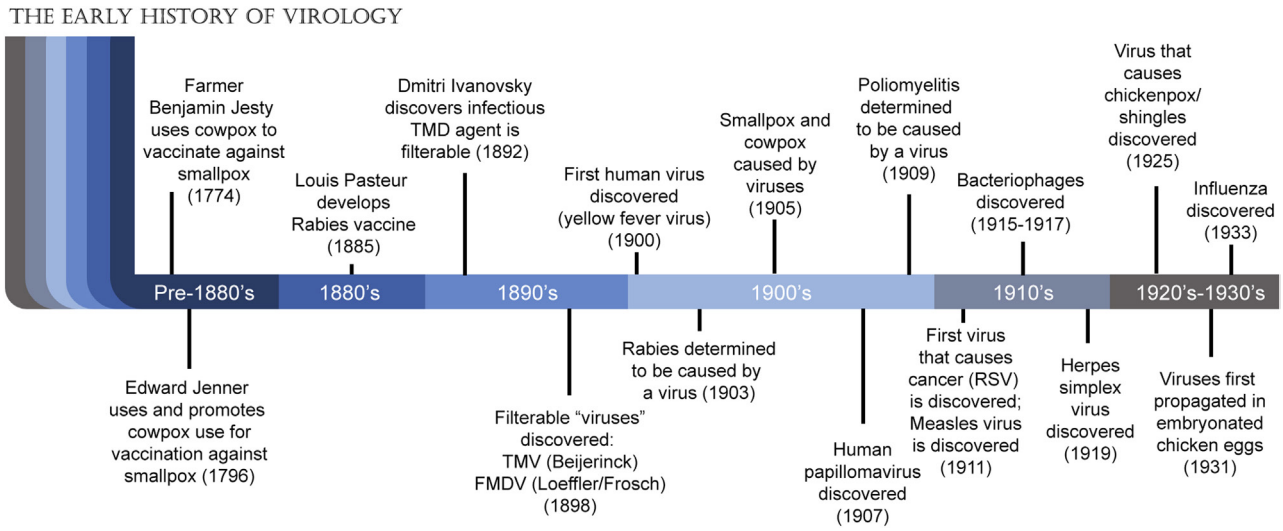

FIGURE 1.10 Timeline of important discoveries in the early history of virology.

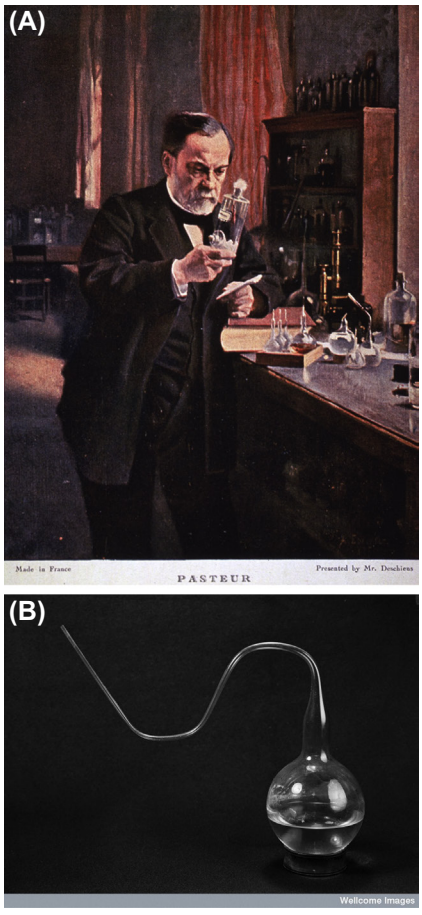

FIGURE 1.11 Louis Pasteur and the germ theory. French microbiologist Louis Pasteur (A) conducted experiments with swan-neck flasks (B) in the mid-1800s that convinced his contemporaries that life does not arise by spontaneous generation. *Image of Pasteur courtesy of the U.S. National Library of Medicine; swan-neck flask image courtesy of the Wellcome Library, London.*

FIGURE 1.12 Robert Koch. German physician and microbiologist Robert Koch, considered the founder of bacteriology, developed and published "Koch's Postulates" in 1890 to aid in establishing whether a microorganism is the direct cause of a disease. *Image courtesy of the U.S. National Library of Medicine.*

and the bacteria-laden particles within the air were allowed to enter the flask, thereby showing that microorganisms do not spontaneously generate but are able to enter areas by traveling through the air. At this point, the **germ theory**, which states that infectious diseases are caused by microorganisms, gained support and was further substantiated by the discovery of several disease-causing bacteria, such as those that cause anthrax, cholera, and tuberculosis.

The German physician and microbiologist Robert Koch (Fig. 1.12) was the first person to identify that the causative