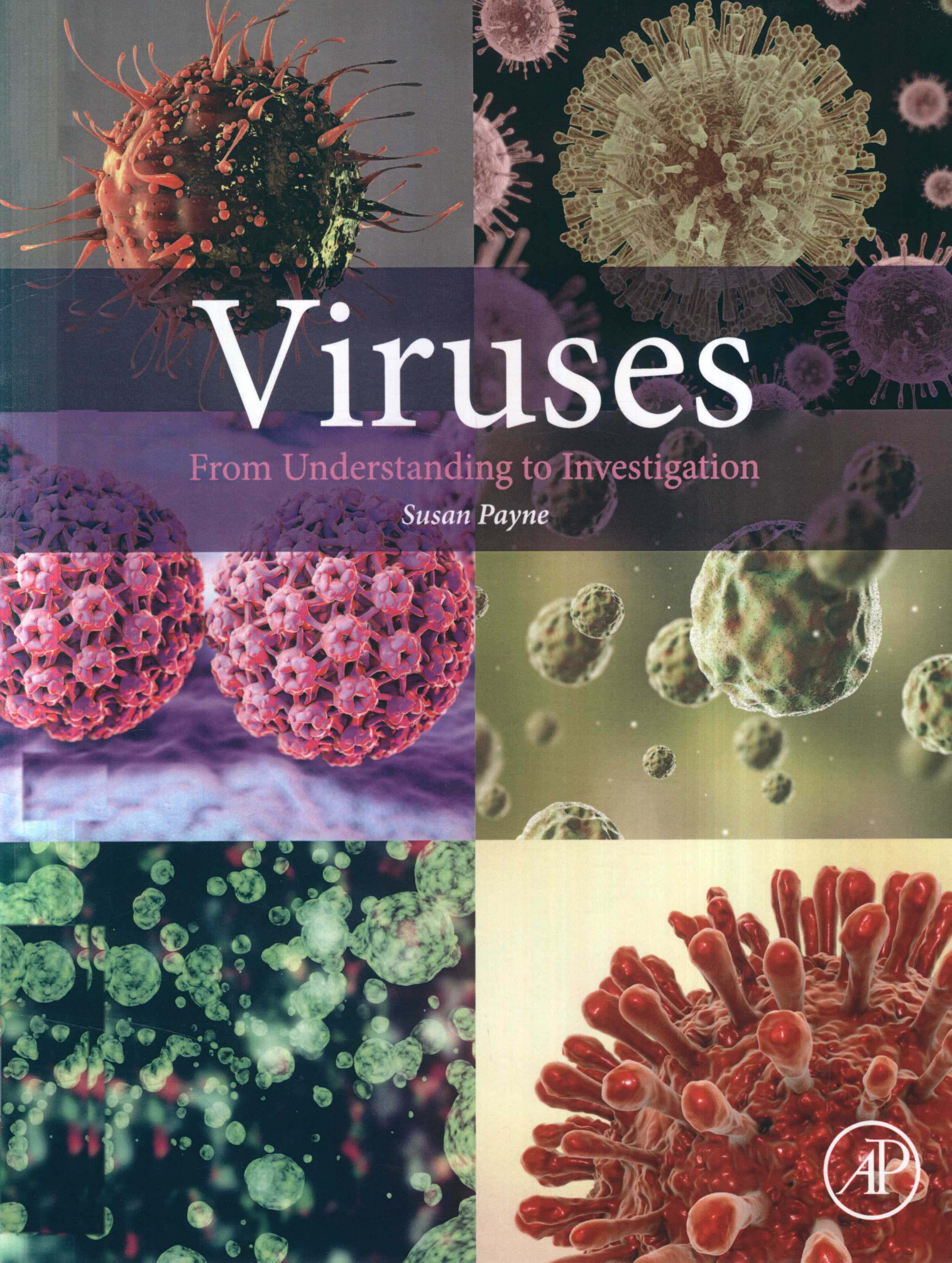

Viruses

From Understanding to Investigation

Susan Payne

VIRUSES

From Understanding to Investigation

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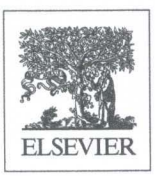

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Preface

This book, *Viruses: From Understanding to Investigation*, was inspired by a long career of teaching and research. My students have included undergraduate, graduate, medical, and veterinary students.

As regards the book title, my intent is to lead students of virology from a basic understanding to an interest in the investigations that have provided the information contained herein. The focus of this textbook is on animal and human viruses, only because these have been the focus of my research and teaching for many years. The viruses of plants, fungi, bacteria, and single-celled organisms are certainly no less interesting.

There is a huge amount of information about viruses available online, in journals, books, websites, and blogs. So why the need for another virology textbook? My intent was to organize and present a thoughtful, understandable, and up-to-date summary of the volumes of information available for consumption elsewhere. While every textbook, including this one, contains many facts, I have tried to emphasize general concepts.

With 38 chapters, this book contains more than enough material for a semester long course in introductory virology. The book is geared toward students with some background in cell biology, microbiology, immunology, and/or biochemistry, and I hope that it will be useful for both undergraduate and beginning graduate students. I also hope that no instructor will try to cover all of the material contained herein during a single semester. The book is organized into two parts, the first nine chapters cover topics including an introduction to viruses (containing information on replication cycle, diversity, taxonomy, and outcomes of virus infection), structure, interactions with the host cell, methods for studying viruses, immunity to viruses, and introductions to viral epidemiology, evolution, and pathogenesis. There are also chapters that serve as introductions to RNA and DNA viruses. I imagine that this will be more than enough information for many instructors and students.

The remaining chapters present viruses by family, with information about structure, genome organization, replication strategies, and disease. I have tried to be up-to-date and include virus families that are relatively new (hence these chapters are short). While each chapter includes basic information about a particular virus family, I am fond of narratives that tie the molecular basis of virus replication to pathogenesis, and have provided examples from a variety of animals, including human animals. The inclusion of "animal diseases" specifically serves as a reminder that companion and food animals play integral roles in human health and well-being. (As do plant and bacterial viruses, but those are subjects for other authors to address.)

I encourage instructors to review the material on virus families and choose a handful of these chapters to use in their courses. Positive-strand RNA viruses are presented first followed by negative and dsRNA viruses. The DNA viruses are presented from the smallest to the largest. Last, but certainly not least, are chapters covering the reverse transcribing retroviruses and hepadnaviruses. I have included some taxonomic information in each chapter, sometimes more, sometimes less. I imagine this to be a reference resource and starting point for students who wish to know more. (And I ask my colleagues not to make these boxes a giant exercise in memorization.)

Throughout the book, I have included brief discussions of both a historical nature (for example, oncogenic retroviruses and an account of the discovery of hepatitis B virus) and current issues such as the recent initiative of the World Health Organization and the World Organization for Animal Health (OIE) to collaborate to reduce human deaths by rabies virus in underdeveloped countries. In the mix are also topics relevant to basic research such as use of vesicular stomatitis virus G protein for pseudotyping and lymphocytic choriomeningitis virus (LCMV) as a model for pathogenesis.

For instructors and colleagues, a final word. You will find the depth of coverage somewhat mixed throughout, and I may have neglected your favorite virus or disease. I am also quite sure that I have presented ideas with which you disagree. Share these with your students, start a conversation, and call me out if necessary. My research manuscripts have always been improved by thoughtful criticism, and if this book is to have a life beyond the first edition, I expect that the same will be true in this case.

Acknowledgments

I cannot overstate the contributions of my illustrator, and wonderful sister, Marcy Edelstein. As I expected, she went "above and beyond" in assisting with this project. In addition to creating illustrations, she learned virology and gently pushed this project to completion with constant help and advice. Many thanks are also owed to my husband, Ross Payne, and to my mom for their patience and understanding during this project. I also wish to thank Texas A&M University, United States, for providing an incredible work and learning environment and my virology colleagues at the College of Veterinary Medicine and Biomedical Sciences and the Health Science Center for their support and inspiration. Finally, many thanks to my mentors over the years and to the dedicated and imaginative researchers who work to unravel the complex and beautiful world of viruses.

VIRUSES

About the Author

Dr. Susan Payne is an associate professor in the Department of Veterinary Pathobiology at the Texas A&M University, United States. During her career, she has mentored graduate and undergraduate students at three universities and has taught virology to undergraduate, graduate, medical, and veterinary students. Those courses are the basis for this textbook. She has also had an active research career and has written over 40 peer reviewed research and review articles. She serves as an ad hoc reviewer for several virology journals. She currently lives in Caldwell, Texas with her husband, mom, five cats, one dog, nine goats, one donkey, eight chickens (if the dog has not eaten one recently), and eight guinea fowls. She is most easily available in email at SPayne@cvm.tamu.edu.

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Contents

CHAPTER

1

Introduction to Animal Viruses

OUTLINE

After studying this chapter, you should be able to:

- Provide a meaningful definition of a virus.
- Explain difference between cell division and virus replication.
- Explain the correct usage of "virion" versus "virus."
- Describe the basic steps in a virus replication-cycle.
- Draw, label, and describe each part of a "one-step" growth curve.
- List possible outcomes of a virus infection (1) at the level of the individual cell and (2) at the level of the host animal.
- Define the term "host range" as regards viruses.

WHAT IS A VIRUS?

Most of us are familiar with the term virus and know viruses as disease causing agents, transmitted from one person or animal to another. We are familiar with "cold" and "flu" viruses; we fear a worldwide pandemic of Ebola. We may even be aware that viruses are used to deliver genes to cells for the purposes of gene therapy or genetic engineering. But what are viruses?

- Viruses are infectious agents that are *not* cellular in nature.
- Viruses must enter a living host cell in order to replicate, thus *all* viruses are obligate intracellular parasites. Synthesis of the proteins and nucleic acids (DNA and RNA) for assembly into new virus particles (virions) requires an energy source (ATP), building materials (amino acids and nucleotides), and protein synthesis machinery (ribosomes) supplied by the host cell. The cell also provides scaffolds (microtubules, filaments, membranes) on which virus particles replicate their genomes and assemble. Thus the cell is a factory providing working machinery and raw materials. The infected cell may or may not continue normal cellular processes (host cell mRNA and protein synthesis) during a viral infection.
- Viruses have nucleic acid genomes that are surrounded by and protected by protein coats called capsids. Capsids protect genomes from environmental hazards and are needed for efficient delivery of viral genomes into new host cells. Some viruses have lipid membranes, called envelopes that surround the capsid (Fig. 1.1).
- Viruses are structurally much simpler than cells. Some viruses can be crystalized. Viruses do not increase in number by cell division; instead they assemble from *newly synthesized* protein and nucleic

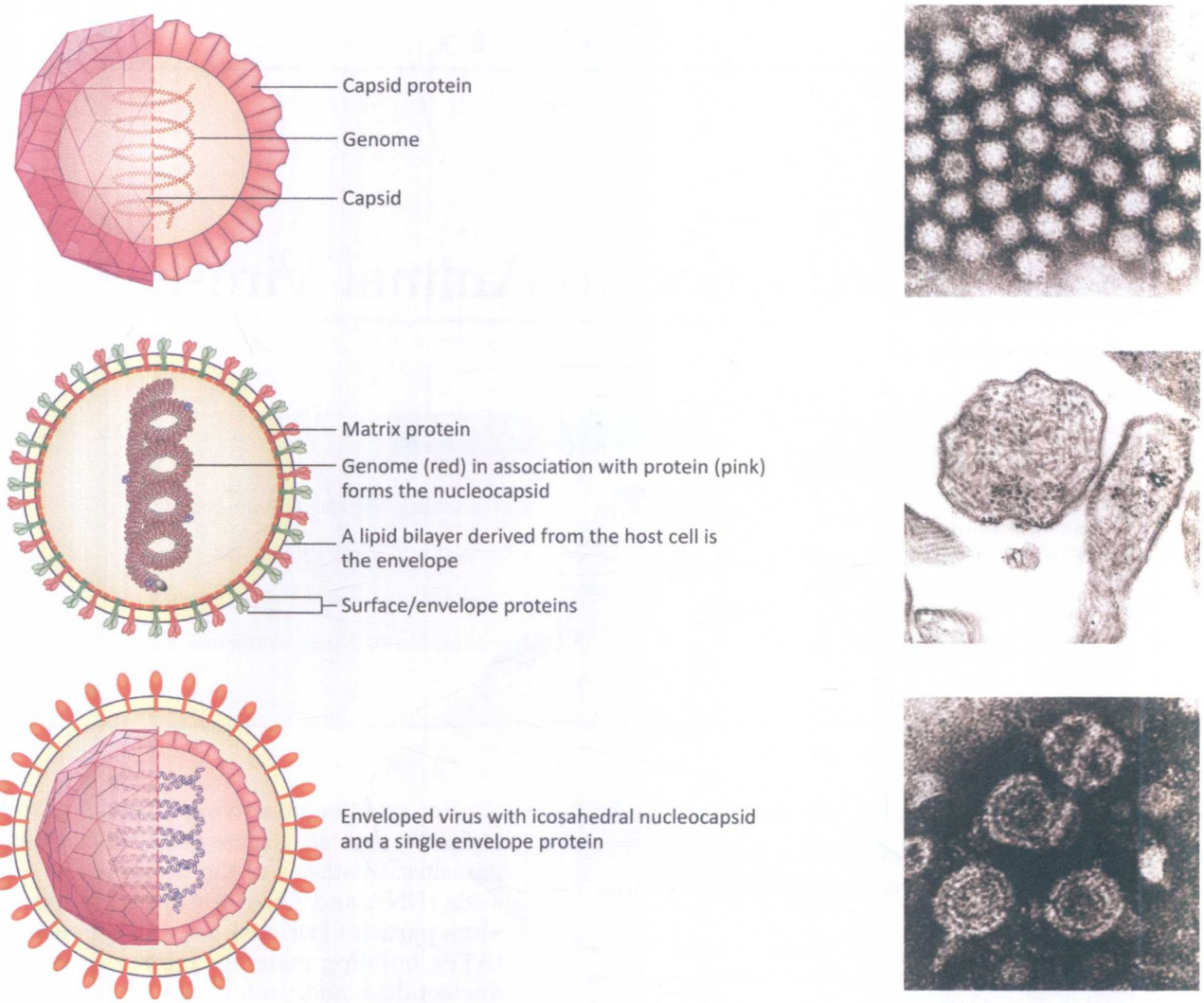

FIGURE 1.1 Basic features of virions. Panel A. On left, simple diagram of an unenveloped virus with icosahedral symmetry; on right, electron micrograph of calicivirus (Chapter 12: Family *Caliciviridae*). Panel B. On left, simple diagram of enveloped virus with a helical nucleocapsid; on right, electron micrograph of measles virus, a paramyxovirus (Chapter 20: Families *Paramyxoviridae* and *Pneumoviridae*). Panel C. On left a simple diagram of an enveloped virus with an icosahedral nucleocapsid; on right, electron micrograph of hepadnavirus (Chapter 38: Family *Hepadnaviridae*).

acid parts (building blocks). As viruses are not cells, they have none the organelles associated with cells. A sample of purified virions has no metabolic activity.

- Viruses are packages designed to deliver nucleic acids to cells; they are excellent examples of "selfish genes."

The preceding description might suggest uninteresting, inanimate particles, but examining virus replication strategies and interactions with host cells provides a diverse and dynamic view into cellular and molecular processes. Viruses are not a homogenous group. They are an extremely diverse group of infectious agents. It is highly unlikely that they arose from a single common ancestor (Box 1.1).

DIVERSITY IN THE WORLD OF VIRUSES

- All viruses have nucleic acid genomes, but some utilize DNA as genetic material, while others have RNA genomes. Viral genomes are not always double-stranded molecules; there are single-stranded viral RNA and DNA genomes. There are viral genomes that consist of a single molecule of nucleic acid, but some genomes are segmented. For example, reoviruses (Chapter 26: Family *Reoviridae*) package 11–12 different pieces of double-stranded RNA and each genome segment encodes a different gene.
- Some viruses have lipid envelopes in addition to a genome and protein coat. Viral envelopes are not

BOX 1.1

WHAT IS IN A DEFINITION?

By the late 19th century, the term "virus" was used to describe infectious agents that could pass through filters designed to remove bacteria from liquids. Thus viruses were "smaller than bacteria" and size became an important part of the definition of a virus. Today we know of a few viruses that are larger than many bacteria, so the trend has been to drop "smallness" from the definition. Another part of the definition of a virus is that they are all obligate intracellular parasites. This is certainly true of all viruses, but there are also bacteria and protozoan parasites that are obligate intracellular parasites. When the biochemical nature of viruses was discovered, it became clear that viruses lack many of the complex structures common to cells. This resulted in definitions of viruses based on comparisons to cells. While these comparisons emphasize the many ways that viruses are different from cells, they do not help us understand these unique infectious agents. So, what are viruses? Very simply, they are genes packaged within a protein coat. Their replication process begins when the virion delivers its genome to a cell. The viral genome encodes proteins required for the synthesis of new viral genomes. New viral proteins plus new viral genomes assemble to form new particles or virions, and so the cycle continues.

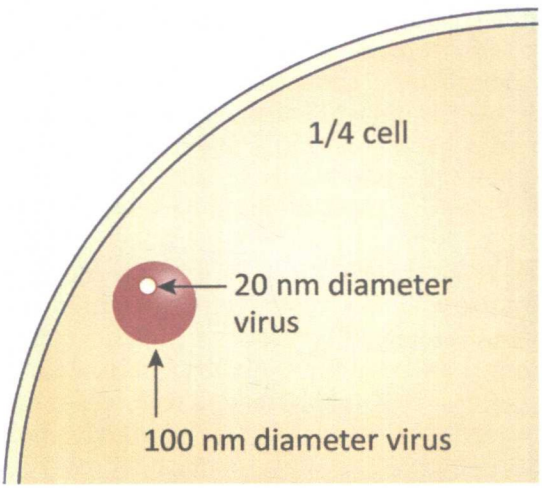

FIGURE 1.2 Relative sizes of an animal cells and virions.

homogenous. Different types of host membranes may be utilized, and their specific lipid and protein components can differ.

- Viruses range in size from 10 to 1000 nm is size (Fig. 1.2).
- Viral genomes range in size from 3000 nucleotides (nt) to over 1,000,000 base pairs.
- Outcomes of viral infections are diverse. Infection does not always result in cell or host death. Some host genes are derived from viruses and have played key roles in evolution. (Some plant viruses are beneficial in extreme environments.)
- Some viruses complete their replication cycles in minutes while others take days. Some viruses are transiently associated with an infected host (days or weeks) while others (for example, herpesviruses) are life-long residents.
- Where did viruses come from? Three general scenarios for virus evolution have been proposed:
  - Retrograde evolution: Intracellular parasites lost the ability for independent metabolism keeping only those genes necessary for replication. Poxviruses are very large complex viruses that *may* have evolved in this manner.
  - Origins from cellular DNA and RNA components: Some DNA genomes resemble plasmids or episomes. Did these DNAs acquire protein coats and the ability to be transferred from cell to cell efficiently?
  - Descendants of primitive precellular life forms: Viruses originated and evolved along with primitive, self-replicating molecules. This is the likeliest origin of the RNA viruses described in this text.

For the most part, names of specific viruses have been omitted in this section, to emphasize the general subject of viral diversity. Throughout this text, the details will be forthcoming. But I hope that now, when reading about any virus, you will want to learn its place in the complex world of viruses. (Big? small? friend? foe? transient visitor? life-long partner?)

ARE VIRUSES ALIVE?

Viruses parasitize every known form of life on this planet and they have both short-term and long-term impacts on their hosts. But are viruses alive? This question is the subject of ongoing debate, but the

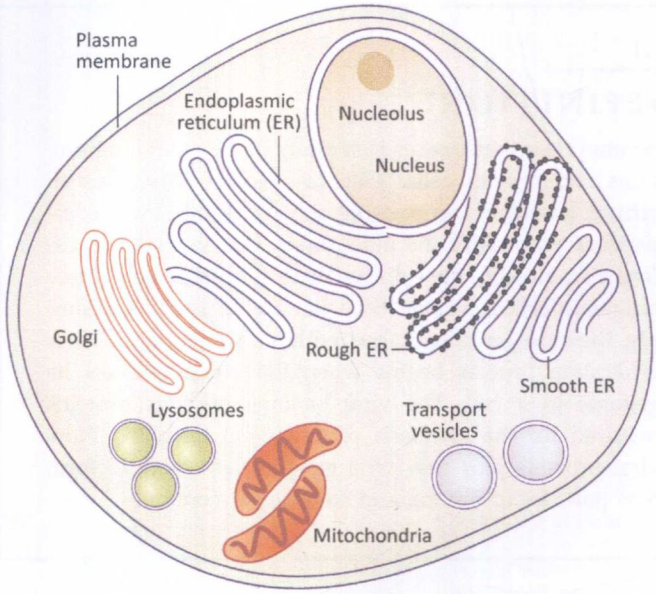

FIGURE 1.3 Simple schematic of a eukaryotic cell identifying some major organelles.

answer does not change the *nature* of the virus. As we discuss and describe viruses it is easy to assume that they are alive. They replicate to increase in number and the terms "virus replication-cycle" and "virus life-cycle" are often used interchangeably. Viruses also evolve (change their genomes), sometimes very rapidly. In this manner they adapt to new hosts and environments.

In contrast, the virion (the physical package that we view with an electron microscope) has no metabolic activity. Some viruses can be assembled simply by mixing purified genomes and proteins in a test tube. The genomes may have been synthesized by machine and the viral proteins may have been produced in bacteria. If those component parts combine under suitable conditions, a fully infectious virion can be produced. To avoid the question of living versus nonliving, the term "infectious agent" is both appropriate and descriptive. We can then speak of *infectious* virions that are *capable of entering a cell and initiating a replication-cycle*, or inactivated virions that cannot

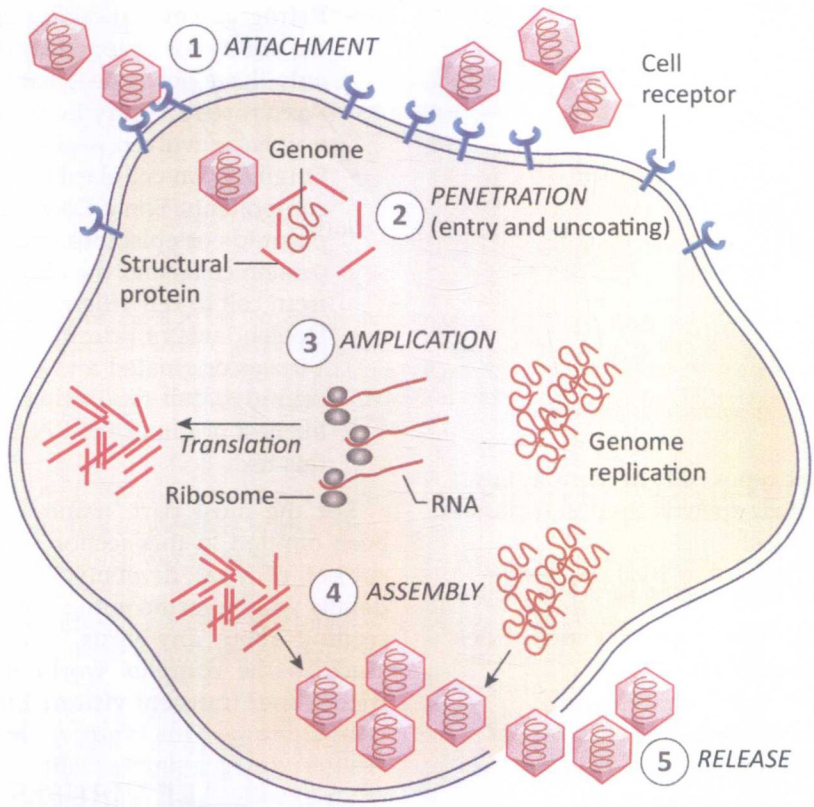

FIGURE 1.4 The basic virus life-cycle is shown in a generic cells. (For simplicity no cell organelles are shown but the processes of virus replication are intimately associated with cell organelles and structures.) The basic virus life-cycle begins with: (1) Attachment of the virion to receptors on a cell. (2) The genome is delivered into cytoplasm (penetration). (3) Viral proteins and nucleic acids are synthesized (amplification). (4) Genomes and proteins assemble to form new virions. (5) Virions are released from the cell.

complete a replication-cycle. As we will see in later chapters, the difference between an infectious and a noninfectious virion may be as small as the cleavage of a single peptide bond.

BASIC STEPS IN THE VIRUS REPLICATION-CYCLE

The first step in a virus life-cycle is attachment (or binding) to the host cell (Figs. 1.3 and 1.4). Attachment results from very specific interactions between viral proteins and molecules on the surface of the host cell. The interactions are usually hydrophobic and ionic, rather than covalent bonds. Thus attachment is influenced by environmental conditions such as pH and salt concentration. Attachment becomes stronger as many copies of a viral surface protein interact with multiple copies of the host cell receptor molecules.

The next step in the virus life-cycle is penetration of the viral genome into the host cell cytoplasm or nucleoplasm. After penetration, there may be a further rearrangement of viral proteins to release the viral genome, a process called uncoating. Penetration and uncoating are two distinct steps for some viruses while for others the viral genome is uncoated during the process of penetration. The processes of penetration and uncoating are irreversible, the infecting virion cannot reassemble.

The next phase in the virus life-cycle is synthesis of the new viral proteins and genomes. This is a complex process that requires transcription (synthesis of mRNA), translation (protein synthesis), and genome replication to generate the parts that will assemble into new virions. Synthesis of viral proteins and genomes occurs in close association with, and depends upon, many host cell proteins and structures. The great diversity among viruses will be evident as we examine processes that regulate transcription, translation, genome replication and the specific virus–host cell interactions that shape these processes.

The next step in the virus replication-cycle is assembly of new virions. New particles assemble from the genome and protein components that accumulate in the infected cell. Viruses are assembled at different sites in host cells; sometime large areas of the cell become virus factories, concentrated regions of viral proteins and genomes from which host cell organelles are excluded.

The final step(s) in the virus replication-cycle are release from the host cell and maturation of the released virions. Virion release may occur upon cell rupture or lysis. Enveloped viruses must acquire their envelopes from cellular membranes in a process called budding. Some enveloped viruses bud through the plasma membrane, but budding can occur at other, intracellular membranes. The budding process can, but does not always, kill the host cell. Other viruses obtain their lipid enveloped by budding into cellular vesicles. These vesicles then fuse with the plasma membrane to release the virions; this is process called exocytosis.

Maturation is the term used to describe changes in virus structure that occur after a virus is released from the host cell. Maturation may be required before a virus is able to infect a new cell; maturation may involve cleavage or rearrangement of viral proteins. Viruses assemble in the cell (under conditions of favorable energy) but when the released virions encounter new cells they must be able to disassemble (uncoating). Maturation events that occur after virus release set the stage for a productive encounter with the next cell. Maturation processes are well understood for several important animal viruses and examples will be presented in future chapters.

It is important to stress that each step in the virus replication-cycle requires specific interactions between viral proteins and host cell proteins. Some viruses can infect many different cell types and organisms because they interact with proteins found on, and in, many cell types. These viruses are said to have a broad host range. Other viruses have a very narrow host range due to their need to interact with specific cellular proteins that are expressed only in a few cell types. Factors that impact virus replication include the presence or absence of receptors, the metabolic state of the cell, the presence or absence of any number of intracellular proteins required to complete the virus replication-cycle.

Another way to view the replication-cycle of a virus is the one-step growth curve (Fig. 1.5). This graph illustrates the concept that penetration of a virus into the host cell is not reversible. During the so-called eclipse phase infectious virions cannot be detected, even if cells are broken open (lysed), there are no infectious particles to be found!

GROWING VIRUSES

Viruses are obligate intracellular parasites; they replicate only within living cells. Thus in the laboratory, susceptible cells or organisms are required to study virus replication. For the virologist, ideal host cells are easily grown and maintained in the laboratory. Animal virologists often use cell and (less often) organ cultures. To culture animal cells, tissues or organs are harvested and disrupted (using mechanical and enzymatic methods) to obtain individual cells. Often cells are derived from tumors that grow robustly in culture. Cells circulating in the blood, such as