PRINCIPLES OF Virology
Fifth Edition

Emphasizing shared principles for teaching introductory virology

Principles of Virology, the leading virology textbook in use, is an extremely valuable and highly informative presentation of virology at the interface of modern cell biology and immunology. This text utilizes a uniquely rational approach by highlighting common principles and processes across all viruses. Using a set of representative viruses to illustrate the breadth of viral complexity, students are able to understand viral reproduction and pathogenesis and are equipped with the necessary tools for future encounters with new or understudied viruses.

This fifth edition was updated to keep pace with the ever-changing field of virology. In addition to the beloved full-color illustrations, video interviews with leading scientists, movies, and links to exciting blogposts on relevant topics, this edition includes study questions and active learning puzzles in each chapter, as well as short descriptions regarding the key messages of references of special interest.

Volume I: Molecular Biology focuses on the molecular processes of viral reproduction, from entry through release. *Volume II: Pathogenesis and Control* addresses the interplay between viruses and their host organisms, on both the micro- and macroscale, including chapters on public health, the immune response, vaccines and other antiviral strategies, viral evolution, and a brand new chapter on the therapeutic uses of viruses. These two volumes can be used for separate courses or together in a single course. Each includes a unique appendix, glossary, and links to internet resources.

Principles of Virology, Fifth Edition, is ideal for teaching the strategies by which all viruses reproduce, spread within a host, and are maintained within populations. This edition carefully reflects the results of extensive vetting and feedback received from course instructors and students, making this renowned textbook even more appropriate for undergraduate and graduate courses in virology, microbiology, and infectious diseases.

About the Authors

Jane Flint is Professor Emerita of Molecular Biology at Princeton University. Dr. Flint's research focused on investigation of the mechanisms by which viral gene products modulate host pathways and antiviral defenses to allow efficient reproduction in normal human cells of adenoviruses, viruses that are used in such therapeutic applications as gene transfer and cancer treatment.

Vincent R. Racaniello is Higgins Professor of Microbiology & Immunology at Columbia University Vagelos College of Physicians & Surgeons. Dr. Racaniello has been studying viruses for over 40 years, including poliovirus, rhinovirus, enteroviruses, hepatitis C virus, and Zika virus. He blogs about viruses at virology.ws and is host of This Week in Virology.

Glenn F. Rall is a Professor and the Chief Academic Officer at the Fox Chase Cancer Center, and is an Adjunct Professor in the Microbiology and Immunology departments at the University of Pennsylvania, as well as Thomas Jefferson, Drexel, and Temple Universities. Dr. Rall studies viral infections of the brain and the immune responses to those infections, with the goal of defining how viruses contribute to disease.

Theodora Hatziioannou is a Research Associate Professor at Rockefeller University and is actively involved in teaching programs at Albert Einstein College of Medicine. Dr. Hatziioannou has worked on multiple viruses with a focus on retroviruses and the molecular mechanisms that govern virus tropism and on the improvement of animal models for human disease.

Anna Marie Skalka is a Professor Emerita and former Senior Vice President for Basic Research at the Fox Chase Cancer Center. Dr. Skalka is internationally recognized for her contributions to the understanding of the biochemical mechanisms by which retroviruses replicate and insert their genetic material into the host genome, as well as her research into other molecular aspects of retrovirus biology.

Instructor resources can be found at www.wiley.com\go\flint\pov5

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PRINCIPLES OF Virology

Flint • Racaniello • Rall
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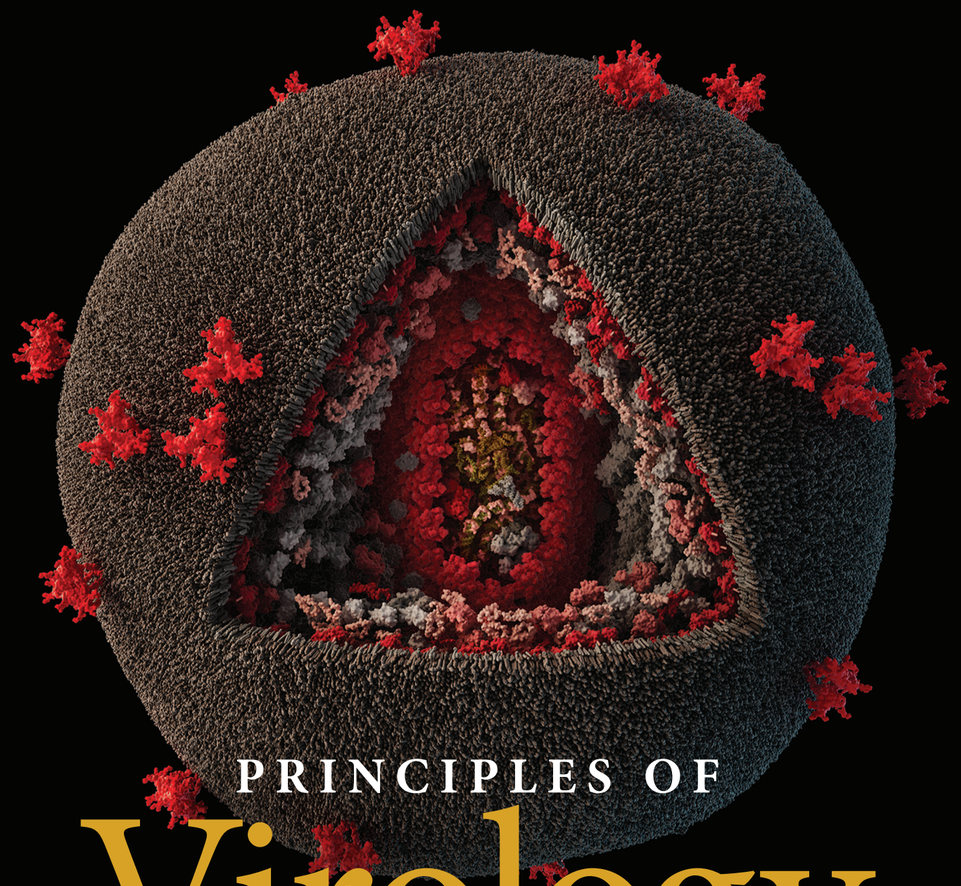
Fifth Edition

ASM PRESS

VOLUME I *Molecular Biology*



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Virology

Fifth Edition

Jane Flint • Vincent R. Racaniello
Glenn F. Rall • Theodora Hatziioannou
Anna Marie Skalka

VOLUME I *Molecular Biology*

PRINCIPLES OF Virology

FIFTH EDITION

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PRINCIPLES OF Virology

Fifth Edition

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We dedicate this book to the students, current and future scientists, physicians, and all those with an interest in the field of virology, for whom it was written.
We kept them ever in mind.

We also dedicate it to our families:
Jonn, Gethyn, and Amy Leedham
Doris, Aidan, Devin, and Nadia
Eileen, Kelsey, and Abigail
Paul, Stefan, and Eve
Rudy, Jeannie, and Chris

Oh, be wiser thou!
Instructed that true knowledge leads to love.

William Wordsworth
Lines left upon a Seat in a Yew-tree
1888

About the Instructor Companion Website

This book is accompanied by a companion website for instructors:

www.wiley.com/go/flint/pov5 

The website includes:

- PowerPoints of figures
- Author podcasts
- Study Questions and Answers

Contents

Preface

The enduring goal of scientific endeavor, as of all human enterprise, I imagine, is to achieve an intelligible view of the universe. One of the great discoveries of modern science is that its goal cannot be achieved piecemeal, certainly not by the accumulation of facts. To understand a phenomenon is to understand a category of phenomena or it is nothing. Understanding is reached through creative acts.

A. D. HERSHEY
Carnegie Institution Yearbook 65

All five editions of this textbook have been written according to the authors' philosophy that the best approach to teaching introductory virology is by emphasizing shared principles. Studying the common steps of the viral reproductive cycle, illustrated with a set of representative viruses, and considering mechanisms by which these viruses can cause disease provides an integrated overview of the biology of these infectious agents. Such knowledge cannot be acquired by learning a collection of facts about individual viruses. Consequently, the major goal of this book is to define and illustrate the basic principles of virus biology.

In this information-rich age, the quantity of data describing any given virus can be overwhelming, if not indigestible, for student and expert alike. The urge to write more and more about less and less is the curse of reductionist science and the bane of those who write textbooks meant to be used by students. In the fifth edition, we continue to distill information with the intent of extracting essential principles, while providing descriptions of how the information was acquired and tools to encourage our readers' exploration of the primary literature. Boxes are used to emphasize major principles and to provide supplementary material of relevance, from explanations of terminology to descriptions of trailblazing experiments. Our goal is to illuminate process and strategy as opposed to listing facts and figures. In an effort to make the book readable, we have been selective in our choice of viruses that are used as examples. The encyclopedic *Fields' Virology* [Knipe DM, Howley PM (ed). 2020. *Fields Virology*, 7th ed. Lippincott Williams & Wilkins, Philadelphia, PA] is recommended as a resource for detailed reviews of specific virus families.

What's New

This edition is marked by a welcome addition to the author team. Our new member, Theodora Hatziioannou, brings expertise in retrovirology, entry, and intrinsic immunity, as well as authority regarding ancient Greek mythology and philosophy that the attentive reader will see is generously sprinkled throughout the text.

We have added an important new chapter in Volume II, "Therapeutic Viruses." While the majority of the chapters define how viruses reproduce and cause mayhem to both cell and host, this new chapter turns the tables to discuss how viruses can be beneficial to eliminate tumor cells, deliver therapeutic genes to specific cells, and expand our arsenal of vaccines for prevention of virus-mediated diseases.

The authors continually strive to make this text accessible and relevant to our readers, many of whom are undergraduates, graduate students, and postdoctoral fellows. Consequently, for this edition, we enlisted the aid of more than twenty of these trainees to provide guidance and commentary on our chapters and ensure that concepts are clearly explained and that the text is compelling to read. This unique group of editors has been invaluable in the design of all of our fully reworked and up-to-date chapters and appendices, and we extend a particular thank-you to them for sharing their perspectives.

A new feature is the inclusion of a set of study questions and/or, in some cases, puzzles, as aids to ensure that the key principles are evident within each chapter. This section complements the Principles that begin each chapter, focusing on unifying core concepts.

Finally, although the SARS-CoV-2 pandemic began as we were preparing to go to press, we have included additions to relevant chapters on the epidemiology, emergence, and replication of this global scourge, as well as some hopeful information concerning vaccine development. What is apparent is that, now more than ever, an appreciation of how viruses impact their hosts is not just an academic pursuit, but rather literally a matter of life and death. We extend our gratitude to all those who serve in patient care settings.

Principles Taught in Two Distinct, but Integrated Volumes

Volume I covers the molecular biology of viral reproduction, and Volume II focuses on viral pathogenesis, control of virus infections, and virus evolution. The organization into two volumes follows a natural break in pedagogy and provides considerable flexibility and utility for students and teachers alike. The two volumes differ in content but are integrated in style and presentation. In addition to updating the chapters and appendices for both volumes, we have organized the material more efficiently, and as noted above, added a new chapter that we believe reflects an exciting direction for the field. Links to Internet resources such as websites, podcasts, blog posts, and movies are provided within each chapter; the digital edition provides one-click access to these materials.

As in our previous editions, we have tested ideas for inclusion in the text in our own classes. We have also received constructive comments and suggestions from other virology instructors and their students. Feedback from our readers was particularly useful in finding typographical errors, clarifying confusing or complicated illustrations, and pointing out inconsistencies in content.

For purposes of readability, references are not included within the text; each chapter ends with an updated list of relevant books, review articles, and selected research papers for readers who wish to pursue specific topics. New to this edition are short descriptions of the key messages from each of the cited papers of special interest. Finally, each volume has a general glossary of essential terms.

These two volumes outline and illustrate the strategies by which all viruses reproduce, how infections spread within a host, and how they are maintained in populations. We have focused primarily on animal viruses, but have drawn insights from studies of viruses that reproduce in plants, bacteria, and archaea.

Volume I: The Science of Virology and the Molecular Biology of Viruses

This volume examines the molecular processes that take place in an infected host cell. Chapter 1 provides a general introduction and historical perspective, and includes descriptions of the unique properties of viruses. The unifying principles that are the foundations of virology,

including the concept of a common strategy for viral propagation, are then described. The principles of the infectious cycle, descriptions of the basic techniques for cultivating and assaying viruses, and the concept of the single-step growth cycle are presented in Chapter 2.

The fundamentals of viral genomes and genetics, and an overview of the surprisingly limited repertoire of viral strategies for genome replication and mRNA synthesis, are topics of Chapter 3. The architecture of extracellular virus particles in the context of providing both protection and delivery of the viral genome in a single vehicle is considered in Chapter 4. Chapters 5 to 13 address the broad spectrum of molecular processes that characterize the common steps of the reproductive cycle of viruses in a single cell, from decoding genetic information to genome replication and production of progeny virions. We describe how these common steps are accomplished in cells infected by diverse but representative viruses, while emphasizing common principles. Volume I concludes with a chapter that presents an integrated description of cellular responses to illustrate the marked, and generally irreversible, impact of virus infection on the host cell.

The appendix in Volume I provides concise illustrations of viral reproductive cycles for members of the main virus families discussed in the text. It is intended to be a reference resource when reading individual chapters and a convenient visual means by which specific topics may be related to the overall infectious cycles of the selected viruses.

Volume II: Pathogenesis, Control, and Evolution

This volume addresses the interplay between viruses and their host organisms. In Chapter 1, we introduce the discipline of epidemiology, and consider basic aspects that govern how the susceptibility of a population is controlled and measured. Physiological barriers to virus infections, and how viruses spread in a host, and to other hosts, are the topics of Chapter 2. The early host response to infection, comprising cell-autonomous (intrinsic) and innate immune responses, are the topics of Chapter 3, while the next chapter considers adaptive immune defenses, which are tailored to the pathogen, and immune memory. Chapter 5 focuses on the classical patterns of virus infection within cells and hosts, and the myriad ways that viruses cause illness. In Chapter 6, we discuss virus infections that transform cells in culture and promote oncogenesis (the formation of tumors) in animals. Next, we consider the principles underlying treatment and control of infection. Chapter 7 focuses on vaccines, and Chapter 8 discusses the approaches and challenges of antiviral drug discovery. In Chapter 9, the new chapter in this edition, we describe the rapidly expanding applications of viruses as therapeutic agents. The origin of viruses, the drivers of viral evolution, and host-virus conflicts are the subjects of Chapter 10. The principles of emerging virus infections, and humankind's experiences with epidemic and pandemic viral infections, are considered in Chapter 11. Chapter 12 is devoted entirely to the "AIDS virus," human immunodeficiency virus type 1, not only because it is the causative agent of the most serious current worldwide epidemic but also because of its unique and informative interactions with the human immune defenses. Volume II ends with a chapter on unusual infectious agents, viroids, satellites, and prions.

The Appendix of Volume II affords snapshots of the pathogenesis of common human viruses. This appendix has been completely re-envisioned in this edition, and now includes panels that define pathogenesis, vaccine and antiviral options, and the course of the infection through the human body. This consistent format should allow students to find information more easily, and compare properties of the selected viruses.

For some behind-the-scenes information about how the authors created the previous edition of *Principles of Virology*, see: http://bit.ly/Virology_MakingOf.

Acknowledgments

These two volumes of *Principles* could not have been composed and revised without help and contributions from many individuals. We are most grateful for the continuing encouragement from our colleagues in virology and the students who use the text. Our sincere thanks also go to colleagues who have taken considerable time and effort to review the text in its evolving manifestations. Their expert knowledge and advice on issues ranging from teaching virology to organization of individual chapters and style were invaluable and are inextricably woven into the final form of the book.

We also are grateful to those who gave so generously of their time to serve as expert reviewers of individual chapters or specific topics in these two volumes: Siddharth Balachandran (Fox Chase Cancer Center), Paul Bieniasz (Rockefeller University), Christoph Seeger (Fox Chase Cancer Center), and Laura Steel (Drexel University College of Medicine). Their rapid responses to our requests for details and checks on accuracy, as well as their assistance in simplifying complex concepts, were invaluable.

As noted in "What's New," we benefited from the efforts of the students and postdoctoral fellows who provided critiques on our chapters and helped to guide our revisions: Pradeep Morris Ambrose, Ruchita Balasubramanian, Mariana Nogueira Batista, Pierre Michel Jean Beltran, Marni S. Crow, Qiang Ding, Florian Douam, Jenna M. Gaska, Laura J. Halsey, Eliana Jacobson, Orkide O. Koyuncu, Robert LeDesma, Rebecca Markham, Alexa McIntyre, Katelynn A. Milora, Laura A. M. Nerger, Morgan Pantuck, Chen Peng, Katrien Poelaert, Daniel Poston, Anagha Prasanna, Pavithran T. Ravindran, Inna Ricardo-Lax, Fabian Schmidt, Andreas Solomos, Nikhila Shree Tanneti, Sharon M. Washio, Riley M. Williams, and Kai Wu.

Since the inception of this work, our belief has been that the illustrations must complement and enrich the text. The illustrations are an integral part of the text, and credit for their execution goes to the knowledge, insight, and artistic talent of Patrick Lane of ScEYEnce Studios. A key to common figure elements is provided following the "About the Authors" section. As noted in the figure legends, many could not have been completed without the help and generosity of numerous colleagues who provided original images. Special thanks go to those who crafted figures or videos tailored specifically to our needs, or provided multiple pieces in this latest edition: Jônatas Abrahão (Universidade Federal de Minas Gerais), Mark Andrake (Fox Chase Cancer Center), Irina Arkhipova (Marine Biological Laboratory, Woods Hole), Brian Baker (University of Notre Dame), Ben Beaden (Australia Zoo, Queensland), Paul Bieniasz (Rockefeller University), Kartik Chandran (Albert Einstein College of Medicine), Elliot Lefkowitz (University of Alabama), Joseph Pogliano (University of California,

San Diego), B.V. Venkatar Prasad and Liya Hu (Baylor College of Medicine), Bonnie Quigley (University of the Sunshine Coast, Australia), Jason Roberts (Victorian Infectious Diseases Reference Laboratory, Doherty Institute, Melbourne, Australia), Michael Rout (Rockefeller University), and Nuria Verdaguer (Molecular Biology Institute of Barcelona, CSIC).

The collaborative work undertaken to prepare the fifth edition was facilitated greatly by several authors' retreats. ASM Press generously provided financial support for these as well as for our many other meetings over the three years that this edition has been in preparation. We thank all those who guided and assisted in its production: Christine Charlip (Director, ASM Press) for her enduring support of our efforts; Megan Angelini (Managing Developmental Editor, ASM Press) for steering us through the complexities inherent in a team effort, and for keeping us on track during production; Susan Schmidler for her elegant and creative designs for the layout and cover; and Lindsay Williams (Editorial Rights Coordinator, ASM Press) for obtaining permissions for images and figures.

There is little doubt that in undertaking such a massive effort typographical errors and/or confusing statements still remain; we hope that the readership of this edition will help to remedy any mistakes. Even so, the three authors who have been part of this endeavor since it was first published in 1995, and the two who joined along the way, feel that with each new edition we get closer to our idealized vision of what this book would be. We aspire to convey more than information: we hope to educate, excite, and encourage future generations of science consumers. As Antoine de Saint-Exupéry, author of *The Little Prince*, once said: "If you want to build a ship, don't drum up the workers to gather wood, divide the labor, and give orders. Instead, teach them to yearn for the vast and endless sea."

This often-consuming enterprise was made possible by the emotional, intellectual, and logistical support of our families, to whom the two volumes are dedicated.

About the Authors

L to R: Jane Flint, Vincent Racaniello, Theodora Hatziioannou, Ann Skalka, Glenn Rall

Jane Flint is a Professor Emerita of Molecular Biology at Princeton University. Dr. Flint's research focused on investigation of the molecular mechanisms by which viral gene products modulate host cell pathways and antiviral defenses to allow efficient reproduction in normal human cells of adenoviruses, viruses that are widely used in such therapeutic applications as gene transfer and cancer treatment. Her service to the scientific community includes membership on various editorial boards, several NIH study sections, and the NIH Recombinant DNA Advisory Committee.

Vincent R. Racaniello is Higgins Professor of Microbiology & Immunology at Columbia University Vagelos College of Physicians & Surgeons. Dr. Racaniello has been studying viruses for over 40 years, including poliovirus, rhinovirus, enteroviruses, hepatitis C virus, and Zika virus. He teaches virology to undergraduate, graduate, medical, dental, and nursing students and uses social media to communicate the subject outside of the classroom. His Columbia University undergraduate virology lectures have been viewed by thousands at iTunes University, Coursera, and on YouTube. Vincent blogs about viruses at virology.ws and is host of the popular science program *This Week in Virology*, which, together with six other science podcasts, can be found at microbe.tv.

Glenn F. Rall is a Professor and the Chief Academic Officer at the Fox Chase Cancer Center in Philadelphia. He is an Adjunct Professor in the Microbiology and Immunology departments at the University of Pennsylvania and Thomas Jefferson, Drexel, and Temple Universities. Dr. Rall's laboratory studies viral infections of the brain and the immune responses to those infections, with the goal of defining how viruses contribute to disease in humans. His service to the scientific community includes former membership on the Autism Speaks Scientific Advisory Board, Editor of *PLoS Pathogens*, Career Development Chair and Program Chair of the American Society for Virology, and membership on multiple NIH grant review panels.

Theodora Hatziioannou is a Research Associate Professor at Rockefeller University in New York. Throughout her career, Dr. Hatziioannou has worked on multiple viruses, with a particular focus on retroviruses and the molecular mechanisms that govern virus tropism and on the improvement of animal models for human disease. She is actively involved in teaching programs at the Rockefeller University and the Albert Einstein College of Medicine, is an editor of *Journal of General Virology*, and serves as a reviewer for multiple scientific journals and NIH grant review panels.

Anna Marie Skalka is a Professor Emerita and former Senior Vice President for Basic Research at the Fox Chase Cancer Center in Philadelphia. Dr. Skalka's major research interests are the molecular aspects of retrovirus biology. Dr. Skalka is internationally recognized for her contributions to the understanding of the biochemical mechanisms by which such viruses (including the AIDS virus) replicate and insert their genetic material into the host genome. Both an administrator and researcher, Dr. Skalka has been deeply involved in state, national, and international advisory groups concerned with the broader, societal implications of scientific research. She has also served on the editorial boards of peer-reviewed scientific journals and has been a member of scientific advisory boards including the National Cancer Institute Board of Scientific Counselors, the General Motors Cancer Research Foundation Awards Assembly, the Board of Governors of the American Academy of Microbiology, and the National Advisory Committee for the Pew Biomedical Scholars.

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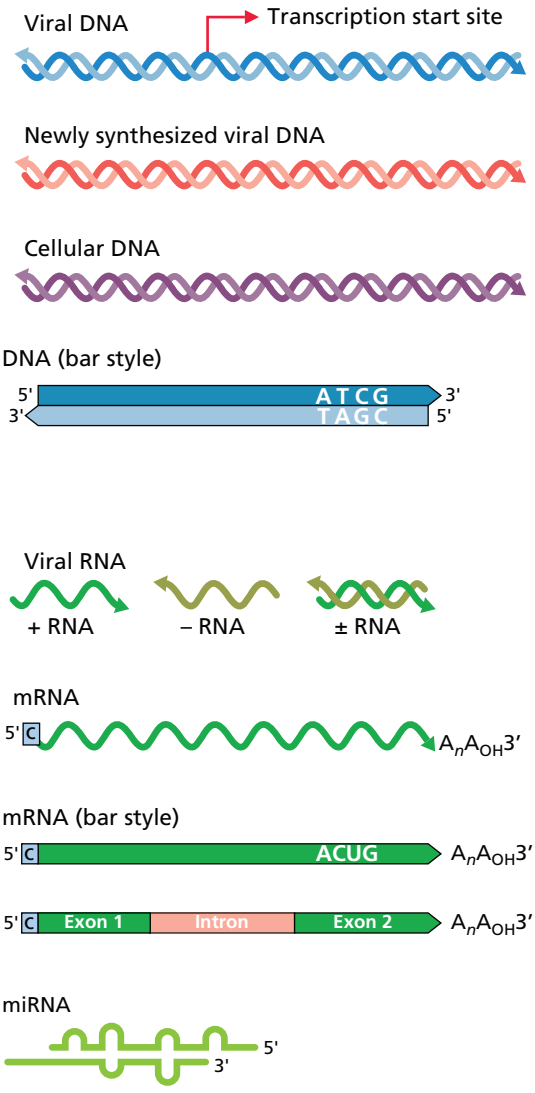
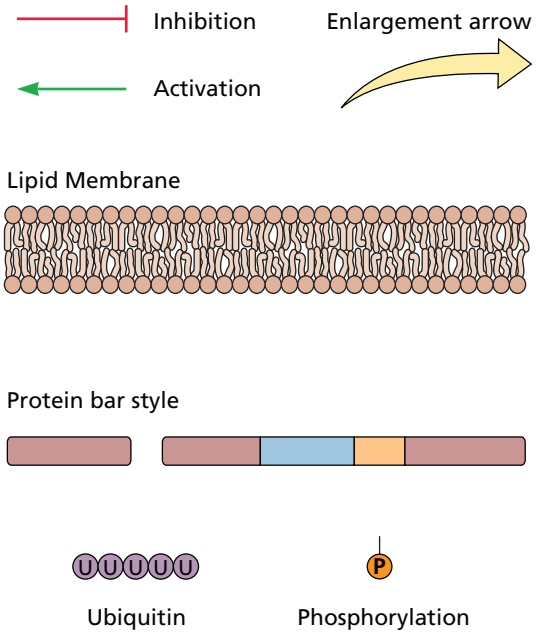
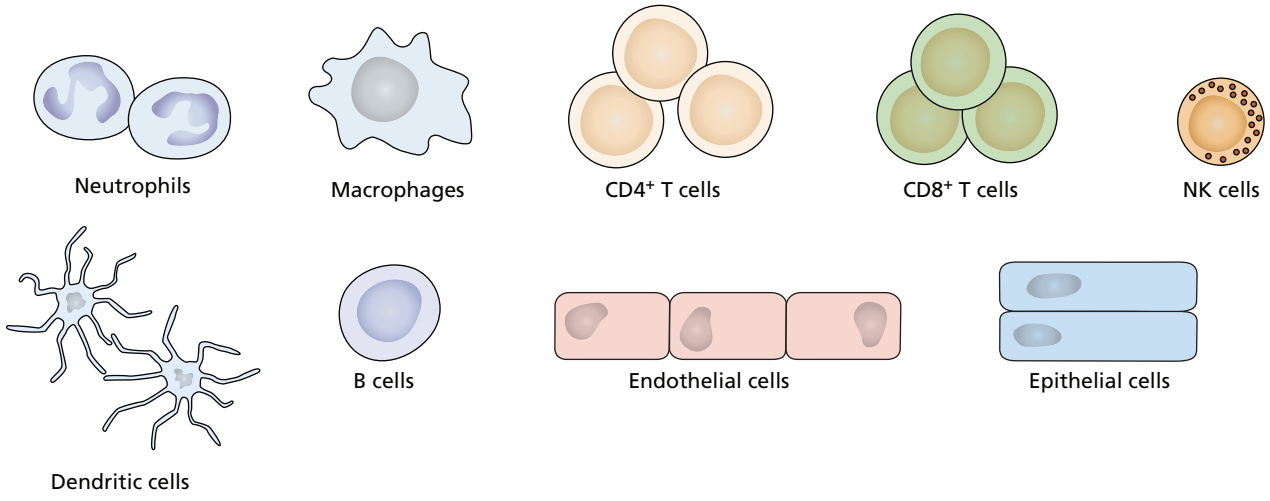
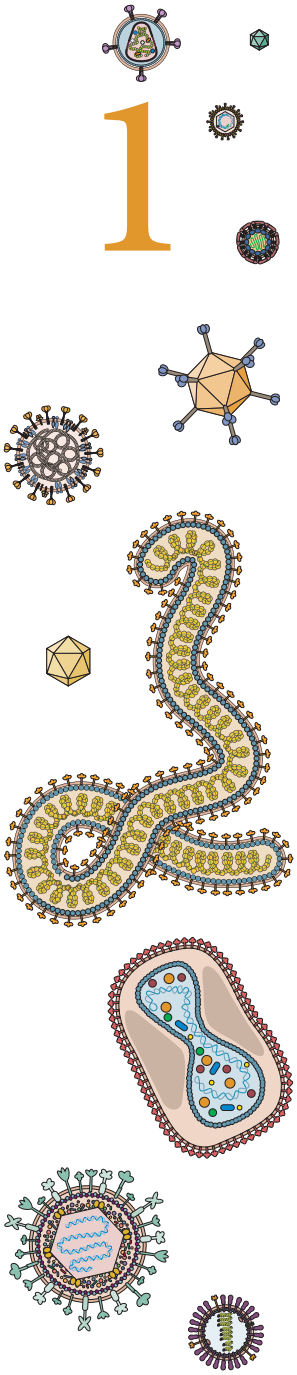






PART I

The Science of Virology



1 Foundations

LINKS FOR CHAPTER 1

- *Video: Interview with Dr. Donald Henderson*
  http://bit.ly/Virology_Henderson
- *This Week in Virology (TWIV): A weekly podcast about viruses featuring informal yet informative discussions and interviews with guests about the latest topics in the field*
  http://www.microbe.tv/twiv
- *Marine viruses and insect defense*
  http://bit.ly/Virology_Twiv301
- *Giants among viruses*
  http://bit.ly/Virology_Twiv261
- *Whiter reefs, fresh breath*
  http://www.microbe.tv/twiv/twiv-391/
- *Latest update of virus classification from the ICTV*
  https://talk.ictvonline.org/taxonomy/
- *The abundant and diverse viruses of the seas*
  http://bit.ly/Virology_3-20-09
- *How many viruses on Earth?*
  http://bit.ly/Virology_9-6-13

Thus, we cannot reject the assumption that the effect of the filtered lymph is not due to toxicity, but rather to the ability of the agent to replicate.

F. Loeffler, 1898

Luria's Credo

"There is an intrinsic simplicity of nature and the ultimate contribution of science resides in the discovery of unifying and simplifying generalizations, rather than in the description of isolated situations—in the visualization of simple, overall patterns rather than in the analysis of patchworks." More than half a century has passed since Salvador Luria wrote this credo in the introduction to the classic textbook *General Virology*.

Despite an explosion of information in biology since Luria wrote these words, his vision of unity in diversity is as relevant now as it was then. That such unifying principles exist may not be obvious considering the bewildering array of viruses, genes, and proteins recognized in modern virology. Indeed, new viruses are being described regularly, and viral diseases such as acquired immunodeficiency syndrome (AIDS), hepatitis, and influenza continue to challenge our efforts to control them. Yet Luria's credo still stands: even as our knowledge of viruses continues to increase, it is clear that their reproduction and survival depend on similar pathways. This insight has been hard-won over many years of observation, research, and debate; the history of virology is rich and instructive.

Viruses Defined

Viruses are microscopic infectious agents that can reproduce only inside a cell that they infect: they are **obligate parasites** of their host cells. Viruses spread from cell to cell via infectious particles called **virions**, which contain genomes comprising RNA or DNA surrounded by a protective protein coat. Upon particle entry and disassociation in a host cell, the viral genome directs synthesis of viral components by cellular systems. Progeny virus particles are formed in the infected cell by *de novo* self-assembly from the newly synthesized components.

As will be discussed in the following chapters, advances in knowledge of the structure of virus particles and the mechanisms by which they are produced in their host cells have been accompanied by increasingly accurate definitions of these unique agents. The earliest pathogenic viruses, distinguished by their small size and dependence on a host organism for reproduction, emphasized the importance of viruses as agents of disease. But there are many other important reasons to study viruses.

Why We Study Viruses

Viruses Are Everywhere

Viruses are all around us, comprising an enormous proportion of our environment, in both number and total mass (Box 1.1). All living things encounter billions of virus particles every day. For example, they enter our lungs in the 6 liters of air each of us inhales every minute; they enter our digestive systems with the food we eat; and they are transferred to our eyes, mouths, and other points of entry from the surfaces we touch and the people with whom we interact. Viral nucleic acids (the **virome**) can be found in the respiratory, gastrointestinal, and urogenital tracts even of normal, healthy individuals (Fig. 1.1). Our bloodstreams harbor up to 100,000 virus particles per milliliter. In addition to viruses that can infect us, our intestinal tracts are loaded with myriad plant and insect viruses, as well as many hundreds of bacterial species that harbor their own constellations of viruses.

PRINCIPLES *Foundations*

- Viruses are obligate intracellular parasites and depend on their host cell for all aspects of their reproduction.
- The field of virology encompasses viral discovery; the study of virus structure and reproduction; and the importance of viruses in biology, ecology, and disease.
- This text focuses primarily on viruses that infect vertebrates, especially humans, but it is important to keep in mind that viruses infect **all** living things including insects, plants, and bacteria.
- Viruses are not solely pathogenic nuisances; they can be beneficial. Viruses contribute to ecological homeostasis, keep our immune responses activated and alert, and can be used as molecular flashlights to illuminate cellular processes.
- Viruses have been part of all of human history: they were present long before *Homo sapiens* evolved, and the majority of human infections were likely acquired from other animals (zoonoses).
- While Koch's postulates were essential for defining many agents of disease, not all pathogenic viruses can be shown to fulfill these criteria.
- Viruses can be described based on their appearance, the hosts they infect, or the nature of their nucleic acid genome.
- All viruses must produce mRNA that can be translated by cellular ribosomes. The Baltimore classification allows relationships among viruses with RNA or DNA genomes to be determined based on the pathway required for mRNA production.
- A common program underlies the propagation of all viruses. This textbook describes that strategy and the similarities and differences in the manner in which different viruses are reproduced, spread, and cause disease.

Viruses Infect All Living Things

While most of this textbook focuses on viral infections of humans, it is important to bear in mind that viruses also infect pets, domestic and wild animals, plants, and insects throughout the world. They infect microbes such as algae, fungi, and bacteria, and some even interfere with the reproduction of other viruses. Viral infection of agricultural plants and animals can have enormous economic and societal impact. Outbreaks of infection by foot-and-mouth disease and avian influenza viruses have led to the destruction (**culling**) of millions of cattle, sheep, and poultry, including healthy animals, to prevent further spread. Losses in the United Kingdom during the 2001 outbreak of foot-and-mouth disease ran into billions of dollars, and caused havoc for both farmers and the government (Box 1.2). More recent outbreaks of the avian influenza virus H5N1 and other strains in Asia have resulted in similar disruption and economic loss. Viruses that infect crops such as potatoes and fruit trees are common, and can lead to serious food shortages as well as financial devastation.

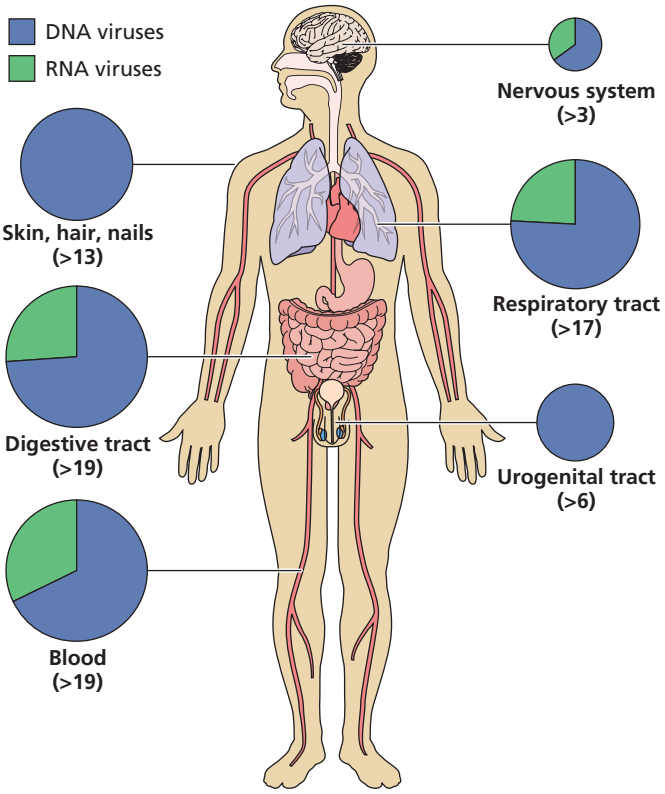


Figure 1.1 The human virome. Our knowledge of the diversity of viruses that can be present in or on a normal human (including some potential pathogens) has increased greatly with the development of high-throughput sequencing techniques and new bioinformatic tools. Current estimates of the numbers of distinct viral families with DNA or RNA genomes in various sites are in parentheses; the > symbol signifies the presence of additional viruses not yet assigned to known families. The numbers may increase as diagnostic tools improve and new viral families are identified. Data from Popgeorgiev N et al. 2013. *Intervirology* 56:395-412; see also http://www.virology.ws/2017/03/23/the-viruses-in-your-blood/.

BOX 1.1

BACKGROUND

Some astounding numbers

- Viruses are the most abundant entities in the biosphere. The biomass on our planet of bacterial viruses *alone* exceeds that of all of Earth's elephants by more than 1,000-fold. There are more than 10^{30} particles of bacterial viruses in the world's oceans, enough to extend out into space for 200 million light-years if arranged head to tail (http://www.virology.ws/2009/03/20/the-abundant-and-diverse-viruses-of-the-seas/; http://www.phagehunter.org/2008/09/how-far-do-those-phages-stretch.html).
- Whales are commonly infected with a member of the virus family *Caliciviridae* that causes rashes, blisters, intestinal problems, and diarrhea, and that can also infect humans. Infected whales excrete more than 10^{13} calicivirus particles daily.
- The average human body contains approximately 10^{13} cells, but almost an equal number of bacteria, and as many as 100-fold more virus particles.
- With about 10^{16} human immunodeficiency virus type 1 (HIV-1) genomes on the planet today, it is highly probable that somewhere there exist HIV-1 genomes that are resistant to every one of the antiviral drugs that we have now or are likely to have in the future.

Viruses reside in Earth's vast oceans and everywhere else on our planet. Courtesy of NASA's Earth Observatory, Suomi NPP satellite image courtesy of NASA/GSFC.

BOX 1.2

DISCUSSION

The first animal virus discovered remains a scourge today

Foot-and-mouth disease virus infects domestic cattle, pigs, and sheep, as well as many species of wild animals. Although mortality is low, morbidity (illness) is high and infected farm animals lose their commercial value. The virus is highly contagious, and the most common and effective method of control is by the slaughter of entire herds in affected areas.

Outbreaks of foot-and-mouth disease were widely reported in Europe, Asia, Africa, and South and North America in the 1800s. The largest epidemic ever recorded in the United States occurred in 1914. After entry into the Chicago stockyards, the virus spread to more than 3,500 herds in 22 states. This calamity accelerated epidemiological and disease control programs, eventually leading to the field- and laboratory-based systems maintained by the U.S. Department of Agriculture to protect domestic livestock from foreign animal and plant diseases. Similar control systems have been established in other Western countries, but this virus still presents a formidable challenge throughout the world. A 1997 outbreak of foot-and-mouth disease among pigs in Taiwan resulted in economic losses of greater than $10 billion.

In 2001, an epidemic outbreak in the United Kingdom spread to other countries in Europe and led to the slaughter of more than 6 million infected and uninfected farm animals. The associated economic, societal, and political costs jolted the British government. Images of mass graves and horrific pyres consuming the corpses of dead animals (see figure) sensitized the public as never before. Minor outbreaks that occurred later in the United Kingdom and parts of Asia were also controlled by culling. But in 2011, South Korea was reported to have destroyed 1.5 million pigs, roughly 12% of its population, to curb a more serious outbreak spread of the virus.

Hunt J.3 January 2013. Foot-and-mouth is knocking on Europe's door. *Farmers Weekly*. http://www.fwi.co.uk/articles/03/01/2013/136943/foot-and-mouth-is-knocking-on-europe39s-door.htm.

Murphy FA, Gibbs EPJ, Horzinek MC, Studdert MJ. 1999. *Veterinary Virology*, 3rd ed. Academic Press, Inc, San Diego, CA.

Mass burning of cattle carcasses during the 2001 foot-and-mouth disease outbreak in the United Kingdom. Courtesy of Dr. Pamela Hullinger, California Department of Food and Agriculture.

Viruses Can Cause Human Disease

With such constant exposure, it is nothing short of amazing that the vast majority of viruses that infect us have little or no impact on our health or well-being. As described in Volume II, we owe such relative safety to our elaborate immune defense systems, which have evolved under the selective pressure imposed by microbial infection. When these defenses are compromised, even the most common infection can be lethal. Despite such defenses, some of the most devastating human diseases have been or still are caused by viruses; these diseases include smallpox, yellow fever, poliomyelitis, influenza, measles, and AIDS. Viral infections can lead to life-threatening diseases that impact virtually all organs, including the lungs, liver, central nervous system, and intestines. Viruses are responsible for approximately 15% of the human cancer burden, and viral infections of the respiratory and gastrointestinal tracts kill millions of children in the developing world each year. As summarized in Volume II, Appendix, there is no question about the biomedical importance of these agents.

Viruses Can Be Beneficial

Despite the appalling statistics from human and agricultural epidemics, it is important to realize that viruses can also be beneficial. Such benefit can be seen most clearly in the marine ecosystem, where virus particles are the most abundant biological entities (Box 1.1). Indeed, they comprise 94% of all nucleic acid-containing particles in the oceans and are 15 times more abundant than *Bacteria* and *Archaea*. Viral infections in the ocean kill 20 to 40% of marine microbes daily, converting these living organisms into particulate matter. In so doing they release essential nutrients that supply phytoplankton

at the bottom of the ocean's food chain, as well as carbon dioxide and other gases that affect the climate of the earth. Pathogens can also influence one another: infection by one virus can have an ameliorating effect on the pathogenesis of a second virus or even bacteria. For example, mice latently infected with some murine herpesviruses are resistant to infection with the bacterial pathogens *Listeria monocytogenes* and *Yersinia pestis*. The idea that viruses are solely agents of disease is giving way to an appreciation of their positive, even necessary, effects, and a realization that their unique properties can actually be harnessed for human benefit (Volume II, Chapter 9).

Viruses "R" Us

Every cell in our body contains viral DNA. Human endogenous retroviruses, and elements thereof, make up about 8% of our genome. Most are inactive, fossil remnants from infections of germ cells that occurred over millions of years during our evolution. Some of them are suspected to be associated with specific diseases, but the regulatory sequences and protein products of other endogenous retroviruses have been coopted during our evolution for their unique functions. For example, retroviral gene products may play a role in the regulation of pluripotency in germ cells, in transmission of signals at neuronal synapses, and clearly in the way that we give birth. The development of the human placenta depends on cell fusion promoted by a retroviral protein. If not for these endogenous retroviruses, we might be producing our young in eggs, like birds and reptiles.

Recent genomic studies have revealed that our viral "heritage" is not limited to retroviruses. Human and other vertebrate genomes harbor sequences derived from several other RNA and DNA viruses. As many of these insertions are estimated to have occurred some 40 million to 90 million years ago, this knowledge has provided unique insight into the ages and evolution of their currently circulating relatives. The conservation of some of these viral sequences in vertebrate genomes suggests that they may have been selected for beneficial properties over evolutionary time.

Viruses Can Cross Species Boundaries

Although viruses generally have a limited host range, they can and do spread across species barriers. As the world's human population continues to expand and impinge on the wilderness, cross-species (**zoonotic**) infections of humans are occurring with increasing frequency. In addition to the AIDS pandemic, the highly fatal Ebola hemorrhagic fever, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS) are recent examples of viral diseases to emerge from zoonotic infections. The influenza virus H5N1 continues to spread among poultry and wild birds in areas of the Middle East and Asia. The virus is deadly to humans who catch it from infected birds. The frightening possibility that it could gain the ability to spread among humans is a major incentive for monitoring for person-to-person transmission in case of infection by this and other pathogenic avian influenza viruses. Given the eons over which viruses have had the opportunity to interact with various species, today's "natural" host may simply be a way station in viral evolution.

Viruses Are Unique Tools To Study Biology

Because viruses are dependent on their hosts for propagation, studies that focus on viral reprogramming of cellular mechanisms have provided unique insights into genetics, cellular biology, and functioning of host defenses. Groundbreaking studies of viruses that infect bacteria (called **bacteriophages**) in the mid-20th century established the molecular basis of genetic inheritance. Through development and use of stringent, quantitative methods with these relatively simple biological entities, this research confirmed that DNA encodes genes and genes encode proteins. General mechanisms of genetic recombination, repair, and control of gene expression were also elucidated, laying the foundations of modern molecular biology and recombinant DNA technology. Subsequent studies of animal viruses established many fundamental principles of cellular function, including the presence of intervening sequences in eukaryotic genes. The study of cancer (transforming) viruses established the genetic basis of this disease.

With the development of recombinant DNA technology and our increased understanding of viral systems, it has become possible to use viral genomes as vehicles for the delivery of genes to cells and organisms for both scientific and therapeutic purposes. The use of viral vectors to introduce genes into various cells and organisms to study their function has become a standard method in biology. Viral vectors are also being used to treat human disease, for example, via "gene therapy," in which functional genes delivered by viral vectors compensate for faulty genes in the host cells (Volume II, Chapter 9).

The study of viruses has contributed in a unique way to the field of anthropology. As ancient humans moved from one geographic area to another, the viral strains unique to their original locations came along with them. The presence of such strains can be detected by analysis of viral nucleic acids, proteins, and antibodies from ancient human specimens and in modern populations. Together with archeological information, identification of these virological markers has been used to trace the pathways by which humans came to inhabit various regions of our planet (Fig. 1.2).

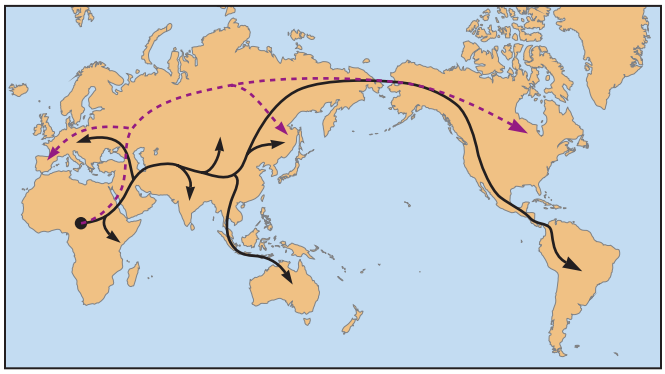


Figure 1.2 Tracking ancient human migrations by the viruses they carried. The polyomavirus known as JC virus is transmitted among families and populations and has coevolved with humans since the time of their origin in Africa. This virus produces no disease in normal, healthy people. Most individuals are infected in childhood, after which the virus establishes a persistent infection in the gastrointestinal tract and is shed in urine. Analysis of the genomes of JC virus in human populations from different geographic locations has suggested an expansion of ancient humans from Africa via two distinct migrations, each carrying a different lineage of the virus. Results from these studies are consistent with analyses of human DNAs (shown by the solid line). They also suggest an additional route that was undetectable in the human DNA analyses (indicated by the dashed line). Data from Pavesi A. 2005. *J Gen Virol* 86:1315–1326.

Virus Prehistory

Although viruses have been known as distinct biological entities for only about 120 years, evidence of viral infection can be found among the earliest recordings of human activity, and methods for combating viral disease were practiced long before the first virus was recognized. Consequently, efforts to understand and control these important agents of disease began only in the last century.

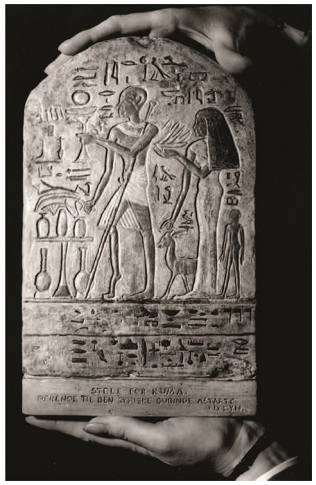
Viral Infections in Antiquity

Reconstruction of the prehistoric past to provide a plausible account of when or how viruses established themselves in human populations is challenging. However, extrapolating from current knowledge, we can deduce that some modern viruses were undoubtedly associated with the earliest precursors of mammals and coevolved with humans. Other viruses entered human populations only recently. The last 10,000 years of history was a time of radical change for humans and our viruses: animals were domesticated, the human population increased dramatically, large population centers appeared, and commerce and technology drove worldwide travel and interactions among unprecedented numbers of people.

Viruses that established themselves in human populations were undoubtedly transmitted from animals, much as still happens today. Early human groups that domesticated and lived with their animals were almost certainly exposed to different viruses than were nomadic hunter/gatherer societies. Similarly, as many different viruses are **endemic** in the tropics, human societies in that environment must have been exposed to a greater variety of viruses than societies established in temperate climates. When nomadic groups met others with domesticated animals, human-to-human contact could have provided new avenues for virus spread. Even so, it seems unlikely that viruses such as those that cause measles or smallpox could have entered a permanent relationship with small groups of early humans. Such highly virulent viruses, as we now know them to be, either kill their hosts or induce lifelong immunity. Consequently, they can survive only when large, interacting host populations offer a sufficient number of naive and permissive hosts for their continued propagation. Such viruses could not have been established in human populations until large, settled communities appeared. Less virulent viruses that enter into a long-term relationship with their hosts were therefore more likely to be the first to become adapted to reproduction in the earliest human populations. These viruses include the modern retroviruses, herpesviruses, and papillomaviruses.

Evidence for knowledge of several diseases that we now know to be caused by viruses can be found in ancient records. The Greek poet Homer characterizes Hector as "rabid" in *The Iliad* (Fig. 1.3A), and Mesopotamian laws that outline the responsibilities of the owners of rabid dogs date from before 1000 B.C.E. Their existence indicates that the communicable nature of this viral disease was already well-known by that time. Egyptian hieroglyphs illustrate what appear to be the consequences of poliovirus infection (a withered leg typical of poliomyelitis [Fig. 1.3B]). Pustular lesions characteristic of smallpox have also been found on Egyptian mummies. The smallpox virus was probably endemic in the Ganges River basin by the fifth century B.C.E. and subsequently spread to other parts of Asia and Europe. This viral pathogen has played an important part in human history. Its introduction into the previously unexposed native populations of Central and South America by colonists in the 16th century led to lethal **epidemics**, which are considered an important factor in the conquests achieved by a small number of European soldiers. Other viral diseases known in ancient times include mumps and, perhaps, influenza. Europeans have described yellow fever since they discovered Africa, and it has been suggested that this scourge of the tropical trade was the basis for legends about ghost ships, such as the *Flying Dutchman*, in which an entire ship's crew perished mysteriously.

Humans have not only been subject to viral disease throughout much of their history but have also manipulated these agents, albeit unknowingly, for much longer than might be imagined. One classic example is the cultivation of marvelously patterned tulips, which were of enormous value in



Here this firebrand, rabid Hector, leads the charge.
Homer, *The Iliad*,
translated by Robert Fagels
(Viking Penguin)

Figure 1.3 References to viral diseases from the ancient literature. (A) An image of Hector from an ancient Greek vase. Courtesy of the Penn Museum, object 30-44-4. **(B)** An Egyptian stele, or stone tablet, from the 18th dynasty (1580–1350 B.C.E.) depicting a man with a withered leg and the "drop foot" syndrome characteristic of poliomyelitis. Image courtesy of SPL/Science Source.

17th-century Holland. Such efforts included deliberate spread of a virus (tulip breaking virus or tulip mosaic virus) that we now know causes the striping of tulip petals so highly prized at that time (Fig. 1.4). Attempts to control viral disease have an even more venerable history.

The First Vaccines

Measures to control one viral disease have been used for the last millennium. The disease is smallpox (Fig. 1.5), and the practice is called **variolation**. The process entails taking material directly from the smallpox lesions of an infected individual and scratching it onto the skin of healthy individuals with a lancet. Widespread in China and India by the 11th century, variolation was based on the recognition that smallpox survivors were protected against subsequent bouts of the disease. Variolation later spread to Asia Minor, where its value was recognized by Lady Mary Wortley Montagu, wife of the British ambassador to the Ottoman Empire. She introduced this practice into England in 1721, where it became quite widespread following the successful inoculation of children of the royal family. George Washington is said to have introduced the practice among Continental Army soldiers in 1776. However, the consequences of variolation were unpredictable and never pleasant: serious skin lesions invariably developed at the site of inoculation and were often accompanied by more generalized rash and disease, with a fatality rate of 1 to 2%. From the comfortable viewpoint of

Figure 1.4 *Three Broken Tulips*. A painting by Nicolas Robert (1624–1685), now in the collection of the Fitzwilliam Museum, Cambridge, United Kingdom. Striping patterns (color breaking) in tulips were described in 1576 in western Europe and were caused by a viral infection. This beautiful image depicts the remarkable consequences of infection with the tulip mosaic virus. © Fitzwilliam Museum, Cambridge.

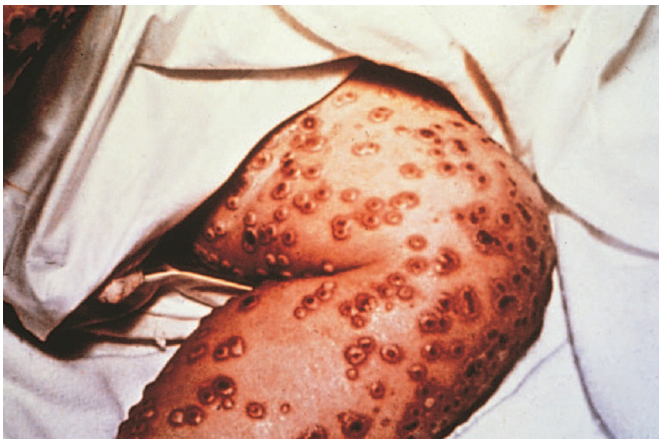


Figure 1.5 Characteristic smallpox lesions in a young victim. Illustrations like these were used as examples to track down individuals infected with the smallpox virus (variola virus) during the World Health Organization campaign to eradicate the disease. Courtesy of CDC/Dr. Robinson (CDC PHIL ID#10398). See also the interview with Dr. Donald Henderson: http://bit.ly/Virology_Henderson.

an affluent country in the 21st century, such a death rate seems unacceptably high. However, in the 18th century, variolation was perceived as a much better alternative than naturally contracting natural smallpox, a disease with a fatality rate of 25% in the whole population and 40% in babies and young children.

In the 1790s, Edward Jenner, an English country physician, established the principle on which modern methods of viral immunization are based, even though viruses themselves were not to be identified for another 100 years. Jenner himself was variolated with smallpox as a young boy and was undoubtedly familiar with its effects and risks. Perhaps this experience spurred his abiding interest in this method. Although it is commonly asserted that Jenner's development of the smallpox vaccine was inspired by his observations of milkmaids, the reality is more prosaic. As a physician's apprentice at age 13, Jenner learned about a curious observation of local practitioners who had been variolating farmers with smallpox. No expected skin rash or disease appeared in farmers who had previously suffered a bout with cowpox. This lack of response was typical of individuals who had survived earlier infection with smallpox and were known to be immune to the disease. It was supposed therefore that, like smallpox survivors, these nonresponding farmers must somehow be immune to smallpox. Although the phenomenon was first observed and later reported by others, Jenner was the first to appreciate its significance fully and to follow up with direct experiments. From 1794 to 1796, he demonstrated that inoculation with material from cowpox lesions induced only mild symptoms in the recipient but protected against the far more dangerous disease. It is from these experiments that we derive the term **vaccination** (*vacca* = "cow" in Latin); Louis Pasteur coined this term in 1881 to honor Jenner's accomplishments.

Initially, the only way to propagate and maintain the cowpox-derived vaccine was by serial infection of human subjects. This method was eventually banned, as it was often associated with transmission of other diseases such as syphilis and hepatitis. By 1860, the vaccine had been passaged in cows; later, horses, sheep, and water buffaloes were also used. The origin of the current vaccine virus, vaccinia virus, is now thought to be horsepox virus (Box 1.3).

The first rabies vaccine was made by Louis Pasteur, although he had no idea at the time that the relevant agent was a virus. In 1885, he inoculated rabbits with material from the brain of a cow suffering from rabies and then used aqueous suspensions of dried spinal cords from these animals to infect other rabbits. After several such passages, the resulting preparations were administered to human subjects, where they produced mild disease but effective immunity against rabies.

Today, viral vaccine strains selected for reduced virulence are called **attenuated**, a term derived from the Latin prefix *ad*, meaning "to," and *tenuis*, meaning "weak." Safer and more efficient methods for the production of larger quantities of these first vaccines awaited the recognition of viruses as distinctive biological entities and parasites of cells in their hosts. Indeed, it took almost 50 years to discover the next antiviral vaccines: a vaccine for yellow fever virus was developed in 1935, and an influenza vaccine was available in 1936. These advances became possible only with radical changes in our knowledge of living organisms and of the causes of disease.

Microorganisms as Pathogenic Agents

The 19th century was a period of revolution in scientific thought, particularly in ideas about the origins of living things. The publication of Charles Darwin's *The Origin of Species* in 1859 crystallized startling (and, to many people, shocking) new ideas about the origin of diversity in plants and animals, until then generally attributed directly to the hand of God. These insights permanently undermined the perception that humans were somehow set apart from all other members of the animal kingdom. From the point of view of the science of virology, the most important changes were in ideas about the causes of disease.

The diversity of macroscopic organisms has been appreciated and cataloged since the dawn of recorded human history. However, a vast new world of organisms too small to be visible to the naked eye was revealed through the microscopes of Antony van Leeuwenhoek (1632–1723). Van Leeuwenhoek's vivid and exciting descriptions of living microorganisms, the "wee animalcules" present in such ordinary materials as rain or seawater, included examples of protozoa, algae, and bacteria. By the early 19th century, the scientific community had accepted the existence of microorganisms and turned to the question of their origin, a topic of fierce debate. Some believed that microorganisms arose spontaneously, for example, in decomposing matter, where they were especially abundant. Others held the view that all were generated by their reproduction, as are macroscopic organisms. The death knell of the spontaneous-generation hypothesis was sounded with the famous experiments of Pasteur. He demonstrated that boiled (i.e., sterilized) medium remained free of microorganisms as long as it was maintained in special flasks with curved, narrow necks designed to prevent entry of airborne microbes (Fig. 1.6). Pasteur also established that distinct microorganisms were associated with specific processes, such as fermentation, an idea that was crucial in the development of modern explanations for the causes of disease.

From the earliest times, poisonous air (miasma) was generally invoked to account for **epidemics** of contagious

BOX 1.3

DISCUSSION

Origin of vaccinia virus

Over the years, many hypotheses have been advanced to explain the curious origin of vaccinia virus. However, recent investigations into this mystery by collaborators in the United States, Germany, and Brazil indicate that horsepox, not cowpox, was the likely precursor of vaccine strains of vaccinia virus.

The proverbial smoking gun was an original wooden and glass container that held capillaries with the smallpox vaccine produced in 1902 by H.K. Mulford in Philadelphia (a company that merged with Sharpe and Dohme in 1929). Sequence analysis of the DNA showed that the core genome of the virus in that vial had the highest degree of similarity (99.7%) to horsepox virus. A review of the historical record shows that during the 19th century, pustular material derived from both cowpox and horsepox lesions was used to immunize against smallpox. The latter technique was called **equination**. Although the disease is now rare in horses and was never reported in the Americas, it was prevalent in Europe, where most vaccine samples were obtained at the time.

Most smallpox vaccines used in the United States, Brazil, and many European countries were produced in the United States from calves inoculated with material collected in 1866 from spontaneous cases of cowpox in France. Genetic analysis of existing samples of these early vaccines indicates that they contained a virus more similar to horsepox and vaccinia viruses than to cowpox virus. While naturally occurring vaccinia viruses are found today only in India (in buffalos) and Brazil (in cows), they can infect horses and people, producing pustular lesions similar to those caused by horsepox and cowpox viruses. One hypothesis is that the ancestor of the current vaccine strain was a naturally occurring vaccinia virus present in the widely distributed French preparation. Alternatively, the vaccine strain may have evolved from horsepox virus during animal passage.

The original wooden (top) and glass (bottom) containers that held capillaries containing the Mulford 1902 smallpox vaccine. Photo kindly provided by Dr. Jose Esparza, Institute of Human Virology, University of Maryland School of Medicine, Baltimore. ©Merck Sharp & Dohme Corp., Merck & Co., Inc.

It is important to consider that development of the smallpox vaccine took place more than a century before modern concepts of virology were established. One can think of other scenarios to explain why the vaccine strain of vaccinia virus is closely related to horsepox and not cowpox, as originally supposed.

- The milkmaid with lesions that were the source of Jenner's original inoculum in 1796 was infected with horsepox, not cowpox. Horsepox can be transmitted to cows, and both animals are common on farms.
- Cows from which pustular material was obtained for vaccination were most often infected with horsepox, transmitted by their handlers or by rodents.

The student is invited to conjure up other plausible explanations.

Damaso CR. 2018. Revisiting Jenner's mysteries, the role of the Beaugency lymph in the evolutionary path of ancient smallpox vaccines. *Lancet Infect Dis* **18:**e55–e63.

Esparza J, Schrick L, Damaso CR, Nitsche A. 2017. Equination (inoculation of horsepox): an early alternative to vaccination (inoculation of cowpox) and the potential role of horsepox virus in the origin of the smallpox vaccine. *Vaccine* **35:**7222–7230.

Schrick L, Tausch SH, Dabrowski PW, Damaso CR, Esparza J, Nitsche A. 2017. An early American smallpox vaccine based on horsepox. *N Engl J Med* **377:**1491–1492.

TWIV 478: A pox on your horse. http://www.microbe.tv/twiv/twiv-478/.

diseases, and there was little recognition of the differences among causative agents. The association of particular microorganisms, initially bacteria, with specific diseases can be attributed to the ideas of the German physician Robert Koch. He developed and applied a set of criteria for identification of the agent responsible for a specific disease (a **pathogen**), articulated in an 1890 presentation in Berlin. These criteria, **Koch's postulates**, can be summarized as follows.

- The organism must be regularly associated with the disease and its characteristic lesions.
- The organism must be isolated from the diseased host and grown in culture.
- The disease must be reproduced when a pure culture of the organism is introduced into a healthy, susceptible host.
- The same organism must be reisolated from the experimentally infected host.

Modern technology has allowed some of Koch's principles to be amended by the application of other types of evidence (Box 1.4). However, by applying his criteria, Koch demonstrated that anthrax, a common disease of cattle, was caused

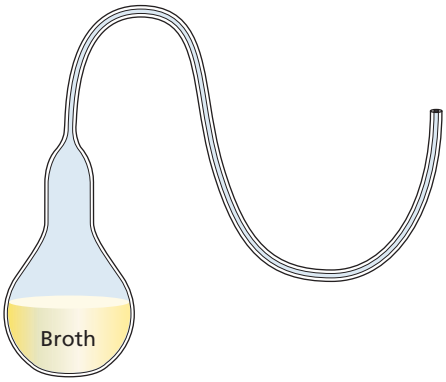


Figure 1.6 Pasteur's famous swan-neck flasks provided passive exclusion of microbes from the sterilized broth. Although the flask was freely open to the air at the end of the long, curved stem, the broth remained sterile, provided that microbe-bearing dust that collected in the neck of the stem did not reach the liquid.

by a specific bacterium (designated *Bacillus anthracis*) and that a second, distinct bacterial species caused tuberculosis in humans. Guided by these postulates and the methods for the sterile culture and isolation of pure preparations of bacteria developed by Pasteur, Joseph Lister, and Koch, many pathogenic bacteria (as well as yeasts and fungi) were identified and classified during the last part of the 19th century (Fig. 1.7). From these beginnings, investigation into the causes of infectious disease was placed on a secure scientific foundation, the first step toward rational treatment and ultimately control. Furthermore, during the last decade of the 19th century, failures of the paradigm that bacterial or fungal agents are responsible for **all** diseases led to the identification of a new class of infectious agents—submicroscopic pathogens that came to be called **viruses**.

Discovery of Viruses

The first report of a pathogenic agent smaller than any known bacterium appeared in 1892. The Russian scientist Dimitrii Ivanovsky observed that the causative agent of tobacco mosaic disease was not retained by the unglazed filters used at that time to remove bacteria from extracts and culture medium (Fig. 1.8A). Six years later in Holland, Martinus Beijerinck independently made the same observation. More importantly, Beijerinck made the conceptual leap that this must be a distinctive agent, because it was so small that it could pass through filters that trapped all known bacteria. However, Beijerinck thought that the agent was an infectious liquid. It was two former students and assistants of Koch, Friedrich Loeffler and Paul Frosch, who in the same year (1898) deduced that such infectious filterable agents comprised small particles: they observed that while the causative agent of foot-and-mouth disease (Box 1.2) passed through filters that held back bacteria, it could be retained by a finer filter.

Not only were the tobacco mosaic and foot-and-mouth disease pathogens much smaller than any previously recognized microorganism, but also they could only reproduce in their host organisms. For example, extracts of an infected tobacco plant diluted into sterile solution produced no additional infectious agents until introduced into leaves of healthy plants, which subsequently developed tobacco mosaic disease. The serial transmission of infection by diluted extracts established that these diseases were not caused by a

BOX 1.4

DISCUSSION

New methods amend Koch's principles

While it is clear that a microbe that fulfills Koch's postulates is almost certainly the cause of the disease in question, we now know that microbes that do not fulfill such criteria may still represent the etiological agents of disease. In the latter part of the 20th century, new methods were developed to associate particular viruses with disease based on immunological evidence of infection, for example, the presence of antibodies in blood. The availability of these methods led to the proposal of modified "molecular Koch's postulates" based on the application of molecular techniques to monitor the role played by virulence genes in bacteria.

The most revolutionary advances in our ability to link particular viruses with disease (or benefit) come from the more recent development of high-throughput nucleic acid sequencing methods and bioinformatics tools that allow detection of viral genetic material directly in environmental or biological samples, an approach called viral metagenomics. Based on these developments, alternative "metagenomic Koch's postulates" have been proposed in which (i) the definitive traits are molecular markers such as genes or full genomes that can uniquely distinguish samples obtained from diseased subjects from those obtained from matched, healthy control subjects and (ii) inoculating a healthy individual with a sample from a diseased subject results in transmission of the disease as well as the molecular markers.

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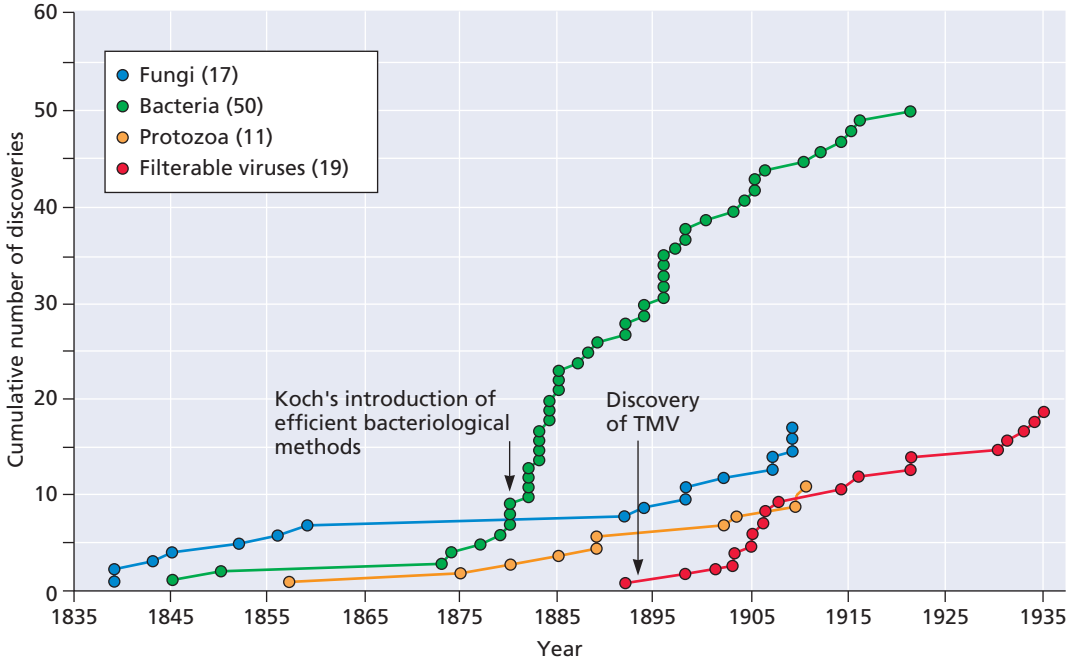


Figure 1.7 The pace of discovery of new infectious agents in the dawn of virology. Koch's introduction of efficient bacteriological techniques spawned an explosion of new discoveries of bacterial agents in the early 1880s. Similarly, the discovery of filterable agents launched the field of virology in the early 1900s. Despite an early surge of virus discovery, only 19 distinct human viruses had been reported by 1935. TMV, tobacco mosaic virus. Data from Burdon KL. 1939. *Medical Microbiology* (Macmillan Co, New York, NY).

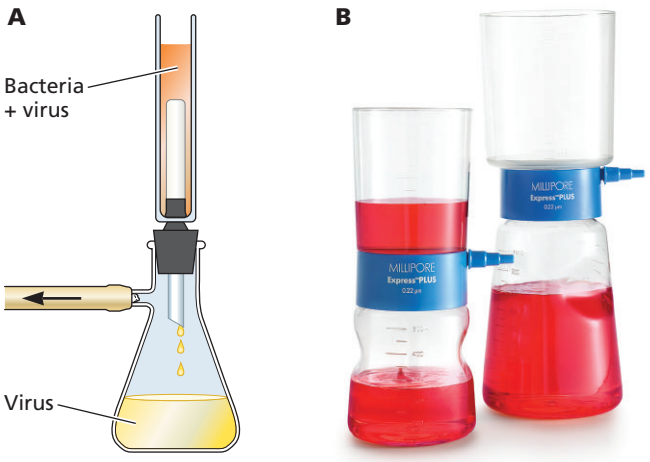


Figure 1.8 Filter systems used to characterize/purify virus particles. (A) The Berkefeld filter, invented in Germany in 1891, was a "candle"-style filter comprising diatomaceous earth (called Kieselguhr), pressed into a hollow candle shape. The white candle in the upper chamber is open at the top to receive the liquid to be filtered. The smallest pore size retained bacteria and let virus particles pass through. Such filters were probably used by Ivanovsky, Loeffler, and Frosch to isolate the first viruses. **(B)** Modern-day filter systems are made of disposable plastic with the upper and lower chambers separated by a biologically inert membrane, available in a variety of pore sizes. Such filtration approaches may have limited our detection of giant viruses. Image courtesy of EMD Millipore Corporation.

bacterial toxin present in the original preparations derived from infected tobacco plants or cattle. The failure of both pathogens to multiply in solutions that readily supported the growth of bacteria, as well as their dependence on host organisms for reproduction, further distinguished these new agents from pathogenic bacteria. Beijerinck termed the submicroscopic agent responsible for tobacco mosaic disease *contagium vivum fluidum* to emphasize its infectious nature and distinctive reproductive and physical properties. Agents passing through filters that retain bacteria came to be called ultrafilterable viruses, appropriating the term *virus* from the Latin for "poison." This term was simplified eventually to "virus."

The discovery of the first virus, tobacco mosaic virus, is often attributed to the work of Ivanovsky in 1892. However, he did not identify the tobacco mosaic disease pathogen as a distinctive agent, nor was he convinced that its passage through bacterial filters was not the result of some technical failure. It may be more appropriate to attribute the founding of the field of virology to the astute insights of Beijerinck, Loeffler, and Frosch, who recognized the distinctive nature of the plant and animal pathogens they were studying more than 120 years ago.

The pioneering work on tobacco mosaic and foot-and-mouth disease viruses was followed by the identification of viruses associated with specific diseases in many other organ-

isms. Important landmarks from this early period include the identification of viruses that cause leukemias or solid tumors in chickens by Vilhelm Ellerman and Olaf Bang in 1908 and Peyton Rous in 1911, respectively. The study of viruses associated with cancers in chickens, particularly Rous sarcoma virus, eventually led to an understanding of the molecular basis of cancer (Volume II, Chapter 6).

The fact that bacteria could also be hosts to viruses was first recognized by Frederick Twort in 1915 and Félix d'Hérelle in 1917. d'Hérelle named such viruses **bacteriophages** because of their ability to cause their bacterial host cells to rupture (a phenomenon called **lysis**; "phage" is derived from the Greek for "eating"). In an interesting twist of serendipity, Twort made his discovery of bacterial viruses while testing the smallpox vaccine virus to see if it would grow on simple media. He found bacterial contaminants, some of which proved to be infected by a bacteriophage. As discussed below, investigation of bacteriophages established not only the foundations for the field of molecular biology but also fundamental insights into how viruses interact with their host cells.

The Defining Properties of Viruses

Throughout the early period of virology when many viruses of plants, animals, and bacteria were cataloged, ideas about the origin and nature of these distinctive infectious agents were quite controversial. Arguments centered on whether viruses originated from parts of a cell or were built from unique components. Little progress was made toward resolving these issues and establishing the definitive properties of viruses until the development of new techniques that allowed their visualization or propagation in cultured cells.

The Structural Simplicity of Virus Particles

Dramatic confirmation of the structural simplicity of virus particles came in 1935, when Wendell Stanley obtained crystals of tobacco mosaic virus. At that time, nothing was known of the structural organization of **any** biologically important macromolecules, such as proteins and DNA. Indeed, the crucial role of nucleic acids as genetic material had not even been recognized. The ability to obtain an infectious agent in crystalline form, a state that was more generally associated with inorganic material, created much wonder and speculation about whether a virus is truly a life form. In retrospect, it is obvious that the relative ease with which this particular virus could be crystallized was a direct result of its structural simplicity.

The 1930s saw the introduction of the instrument that rapidly revolutionized virology: the electron microscope. The great magnifying power of this instrument (eventually more than 100,000-fold) allowed direct visualization of virus particles for the first time. It has always been an exciting experience for investigators to obtain images of viruses, especially as they appear to be remarkably elegant (Fig. 1.9). Images of many different virus particles confirmed that these agents are very small (Fig. 1.10) and that most are far simpler in structure than any cellular organism. Many appeared as regular helical or spherical particles. The description of the morphology of virus particles made possible by electron microscopy also opened the way for the first rational classification of viruses.

The Intracellular Parasitism of Viruses

Organisms as Hosts

A defining characteristic of viruses is their absolute dependence on a living host for reproduction: they are **obligate parasites**. Transmission of plant viruses such as tobacco mosaic virus can be achieved readily, for example, by applying extracts of an infected plant to a scratch made on the leaf of a healthy plant. Furthermore, as a single infectious particle of many plant viruses is sufficient to induce a characteristic lesion (Fig. 1.11), the concentration of the infectious agent could be measured. Plant viruses were therefore the first to be studied in detail. Some viruses of humans and other species could also be propagated in laboratory animals, and methods were developed to quantify them by determining the lethal dose. The transmission of yellow fever virus to mice by Max Theiler in 1930 was an achievement that led to the isolation of an attenuated strain, still considered one of the safest and most effective ever produced for the vaccination of humans.

After specific viruses and appropriate host organisms were identified, it became possible to produce sufficient quantities of virus particles for study of their physical and chemical properties and the consequences of infection for the host. Features such as the incubation period, symptoms of infection, and effects on specific tissues and organs were investigated. Laboratory animals remain an essential tool in investigations of the pathogenesis of viruses that cause disease. However, real progress toward understanding the mechanisms of virus reproduction was made only with the development of cell culture systems. The first and the simplest, but crucial to both virology and molecular biology, were cultures of bacterial cells.

Lessons from Bacteriophages

In the late 1930s and early 1940s, the bacteriophages, or "phages," received increased attention as a result of controversy centering on how they might have arisen. John Northrup, a biochemist at the Rockefeller Institute in Princeton, NJ, championed the theory that a phage was a metabolic product of a bacterium. On the other hand, Max Delbrück, in his work with Emory Ellis and later with Salvador Luria, regarded phages as autonomous, stable, self-replicating entities characterized by heritable traits. According to this paradigm,

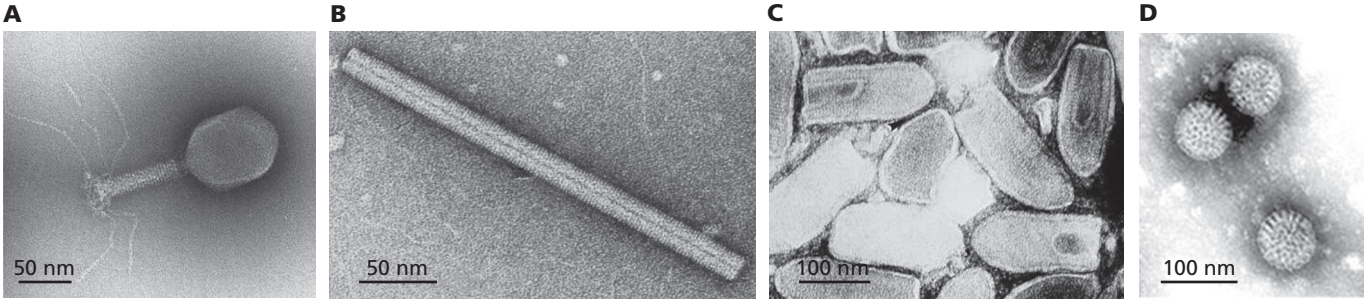


Figure 1.9 Electron micrographs of virus particles following negative staining. **(A)** The complex, nonenveloped virus bacteriophage T4. Note the intricate tail and tail fibers. Reproduced with permission from Dr. Robert L. Duda, University of Pittsburgh, Pittsburgh, PA. **(B)** The helical, nonenveloped particle of tobacco mosaic virus. Courtesy of Plant Resistance Gene Wiki (http://prgdb.crg.eu/wiki/Species:Tobacco_mosaic_virus), licensed under CC BY-SA 3.0. **(C)** Enveloped particles of the rhabdovirus vesicular stomatitis virus. Courtesy of CDC/Dr. Fred. A. Murphy (CDC PHIL ID#5611). **(D)** Nonenveloped, icosahedral human rotavirus particles. Courtesy of F. P. Williams, U.S. Environmental Protection Agency, Washington, DC.

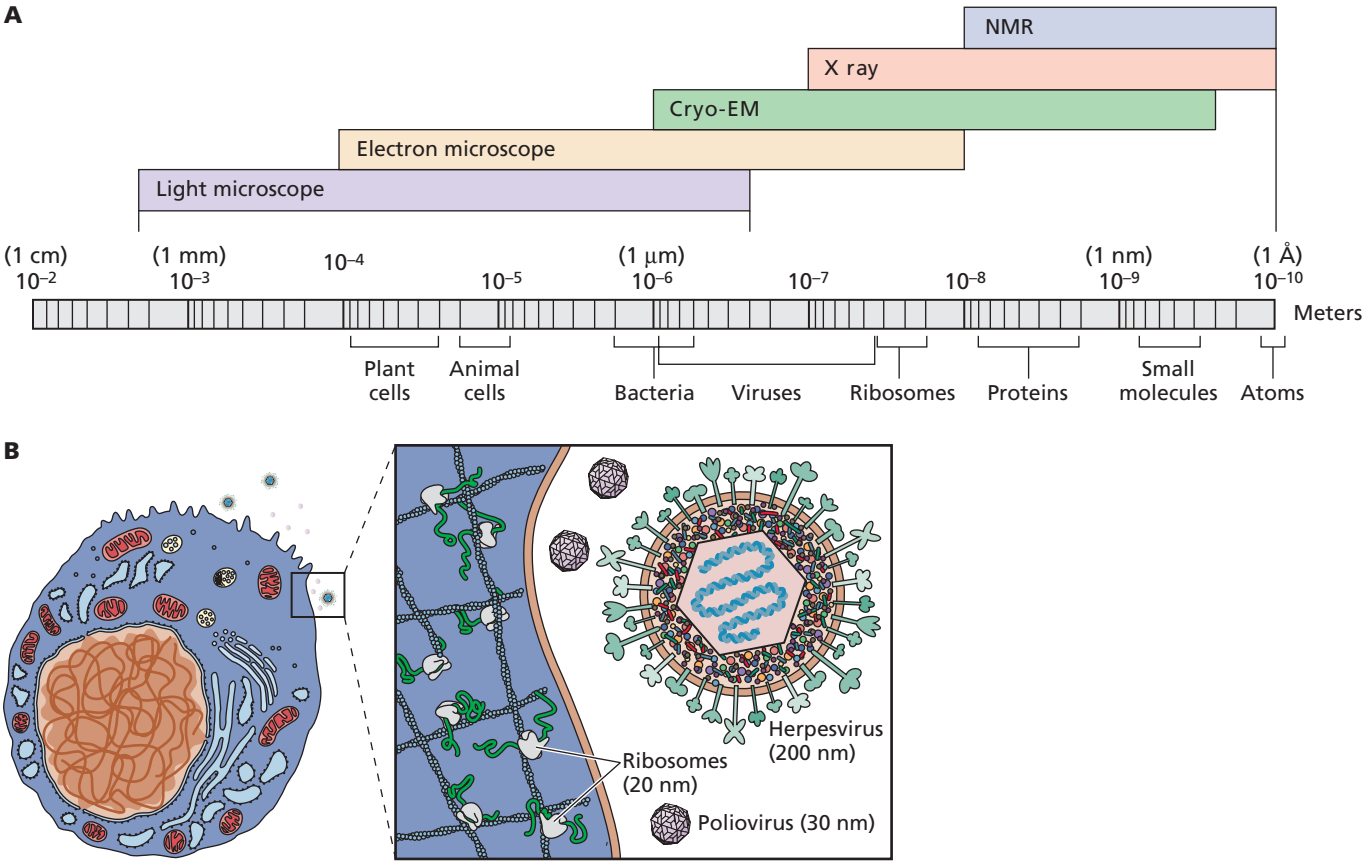


Figure 1.10 Size matters. **(A)** Sizes of animal and plant cells, bacteria, viruses, proteins, molecules, and atoms are indicated. The resolving powers of various techniques used in virology, including light microscopy, electron microscopy, cryo-electron microscopy (Cryo-EM), X-ray crystallography, and nuclear magnetic resonance (NMR) spectroscopy, are indicated. Viruses span a broad range from that equal to some small bacteria to just above ribosome size. The units commonly used in descriptions of virus particles or their components are the nanometer (nm [10^{-9} m]) and the angstrom (Å [10^{-10} m]). **(B)** Illustration of the size differences among two animal viruses and a typical eukaryotic host cell.

Figure 1.11 Lesions induced by tobacco mosaic virus on an infected tobacco leaf. In 1886, Adolph Mayer first described the characteristic patterns of light and dark green areas on the leaves of tobacco plants infected with tobacco mosaic virus. He demonstrated that the mosaic lesions could be transmitted from an infected plant to a healthy plant by aqueous extracts derived from infected plants. Following application of the preparation to healthy plant leaves, the number of characteristic lesions containing dead cells is directly proportional to the number of infectious particles in the test sample. Courtesy of USDA Forest Service, under license CC BY 3.0.

phages were seen as ideal tools with which to investigate the nature of genes and heredity. Probably the most critical early contribution of Delbrück and Ellis was the perfection of the "one-step growth" method for synchronization of the reproduction of phages, an achievement that allowed analysis of a single cycle of phage reproduction in a population of bacteria. This approach introduced highly quantitative methods to virology, as well as an unprecedented rigor of analysis. The first experiments showed that phages indeed multiplied in the bacterial host and were liberated in a "burst" following disruption of the cell.

Delbrück was a zealot for phage research and recruited talented scientists to pursue the fundamental issues of what is now known as the field of molecular biology. This cadre of scientists focused their attention on specific phages of the bacterium *Escherichia coli*. Progress was rapid, primarily because of the simplicity of the phage infectious cycle. By the mid-1950s it was evident that viruses from bacteria, animals, and plants share many fundamental properties. However, the phages provided a far more tractable experimental system. Consequently, their study had a profound impact on the field of virology.

One critical lesson came from a definitive experiment that established that viral nucleic acid carries genetic information. It was known from studies of the "transforming principle" of pneumococcus by Oswald Avery, Colin MacLeod, and Maclyn McCarty (1944) that nucleic acid was both necessary and sufficient for the transfer of genetic traits of bacteria. However, in the early 1950s, protein was still suspected to be an important component of viral heredity. In a brilliantly simple experiment that included the use of a common kitchen food blender, Alfred Hershey and Martha Chase showed that this hypothesis was incorrect; DNA, not protein, carries the information for virus reproduction (Box 1.5).

Bacteriophages were originally thought to be lethal agents, invariably killing their host cells after infection. In the early 1920s, a previously unknown interaction was discovered, in which the host cell not only survived the infection but also stably inherited the genetic information of the virus. It was also observed that certain bacterial strains could lyse spontaneously and produce bacteriophages after a period of growth in culture. Such strains were called **lysogenic**, and the phenomenon, **lysogeny**. Studies of lysogeny revealed many previously unrecognized features of virus-host cell interactions (Box 1.6). Recognition of this phenomenon came from the work of many scientists, but it began with the elegant experiments of André Lwoff and colleagues at the Institut Pasteur in Paris. Lwoff showed that a viral genome exists in lysogenic cells in the form of a silent genetic element called the **prophage**. This element determined the ability of lysogenic bacteria to produce infectious bacteriophages. Subsequent studies of the *E. coli* bacteriophage lambda established a paradigm for one mechanism of lysogeny, the integration of a phage genome into a specific site on the bacterial chromosome.

Bacteriophages became inextricably associated with the new field of molecular biology. Their study established many fundamental principles: for example, control of the decision to enter a lysogenic or a lytic pathway is encoded in the genome of the virus. The first mechanisms discovered for the control of gene expression, exemplified by the elegant operon theory of Nobel laureates François Jacob and Jacques Monod, were deduced in part from studies of lysogeny by phage lambda. The biology of phage lambda provided a fertile ground for work on gene regulation, but study of virulent T phages (T1 to T7, where T stands for "type") of *E. coli* paved the way for many other important advances. As we shall see, these systems also provided an extensive preview of mechanisms of animal virus reproduction (Box 1.7).

Animal Cells as Hosts

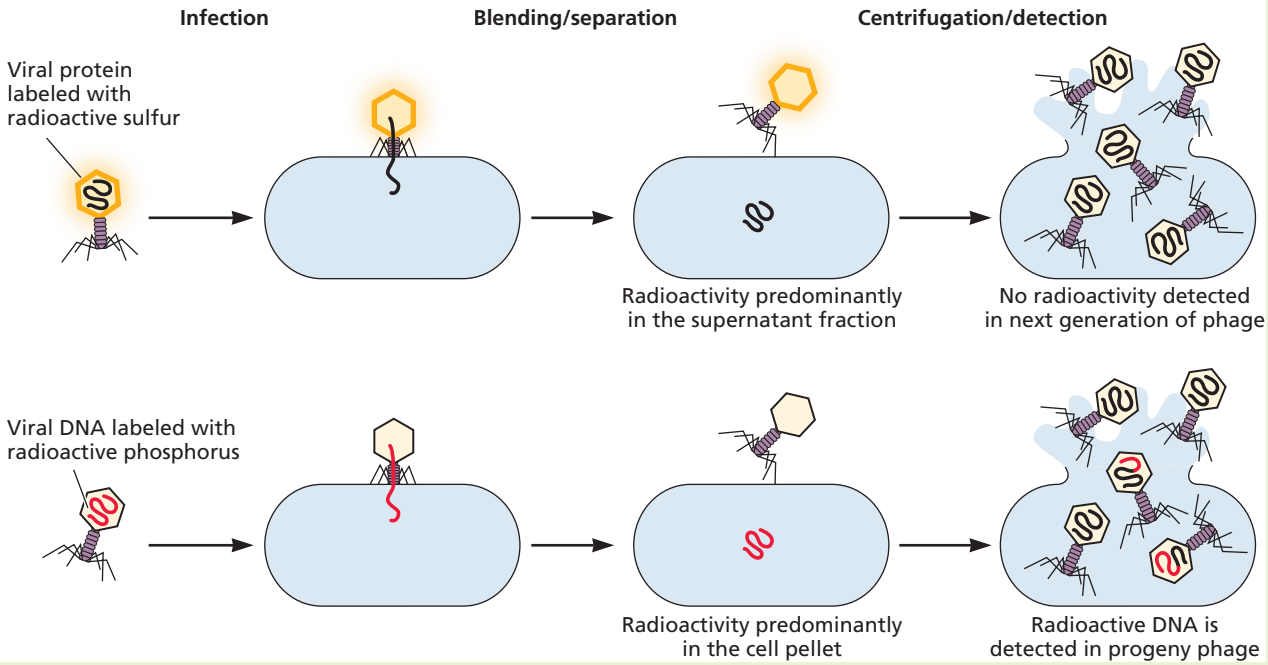
The culture of animal cells in the laboratory was initially more of an art than a science, restricted to cells that grew out of organs or tissues maintained in nutrient solutions under sterile conditions. Cells so obtained from living tissues, called **primary cells**, have a finite life span. Their dependence for growth on natural components in their media such as lymph, plasma, or chicken embryo extracts, and the technical demands

BOX 1.5

EXPERIMENTS

The Hershey-Chase experiment

By differentially labeling the nucleic acid and protein components of virus particles with radioactive phosphorus (^{32}P) and radioactive sulfur (^{35}S), respectively, Alfred Hershey and Martha Chase showed that the protein coat of the infecting virus could be removed soon after infection by agitating the bacteria for a few minutes in a blender. In contrast, ^{32}P-labeled phage DNA entered and remained associated with the bacterial cells under these conditions. Because such blended cells produced a normal burst of new virus particles, it was clear that the DNA contained all of the information necessary to produce progeny phages.



of sterile culture prior to the discovery of antibiotics, made reproducible experimentation very difficult. However, by 1955, the work of many investigators had led to a series of important methodological advances. These included the development of defined media optimal for growth of mammalian cells, incorporation of antibiotics into cell culture media, and development of immortal cell lines such as the mouse L and human HeLa cells that are still in widespread use. These advances allowed growth of animal cells in culture to become a routine, reproducible exercise.

The availability of a variety of well-characterized animal cell cultures had several important consequences for virology. It allowed the discovery and propagation of new human viruses, such as adenovirus, measles virus, and rubella virus, for which animal hosts were not available. In 1949, John Enders and colleagues used cell cultures to propagate poliovirus, a feat that led to the development of polio vaccines a few years later. Cell culture technology revolutionized the ability to investigate the reproduction of viruses. Viral infectious cycles could be studied under precisely controlled conditions by employing the analog of the one-step growth cycle of bacteriophages and simple methods for quantification of infectious particles described in Chapter 2.

Our current understanding of the molecular basis of viral parasitism, the focus of this volume, is based almost entirely on analyses of one-step growth cycles in cultured cells. Such studies established that viruses depend absolutely on the biosynthetic machinery of their host cells for synthesis of the components from which progeny viral particles are built. In contrast to cells, viruses are not reproduced by growth and division. Rather, the infecting genome contains the information necessary to redirect cellular systems to the production of many copies of all the components needed for the *de novo* assembly of new virus particles. It is remarkable, however, that while viruses lack the complex energy-generating and biosynthetic systems necessary

BOX 1.6

BACKGROUND

Properties of lysogeny shared with animal viruses

Lytic versus Lysogenic Response to Infection

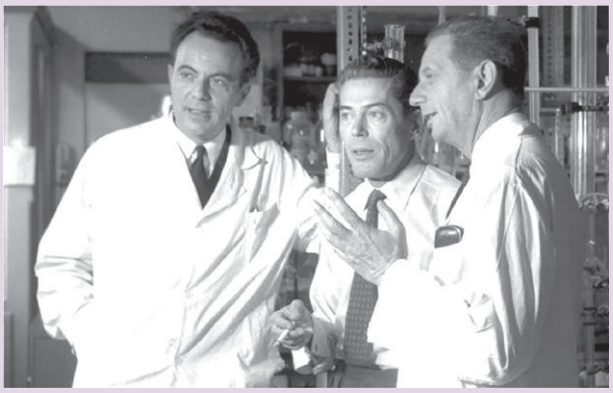
Some bacterial viruses can enter into either destructive (lytic) or relatively benign (lysogenic) relationships with their host cells. Such bacteriophages were called temperate. In a lysogenic bacterial cell, viral genetic information persists but viral gene expression is repressed. Such cells are called lysogens, and the quiescent viral genome, a prophage. By analogy with the prophage, an integrated DNA copy of a retroviral genome in an animal genome is termed a provirus.

Propagation as a Prophage

For some bacteriophages like lambda and Mu (Mu stands for "mutator"), prophage DNA is integrated into the host genome of lysogens and passively replicated by the host. Virally encoded enzymes, known as integrase (lambda) and transposase (Mu), mediate the covalent insertion of viral DNA into the chromosome of the host bacterium, establishing it as a prophage. The prophage DNA of other bacteriophages, such as P1, exists as a plasmid, a self-replicating, autonomous chromosome in a lysogen. Both forms of propagation have been identified in certain animal viruses, for example, retroviruses and a lethal herpesvirus.

Insertional Mutagenesis

Bacteriophage Mu inserts its genome into many random locations on the host chromosome, causing numerous mutations by disrupting host DNA sequences. This process is called insertional mutagenesis and is a phenomenon observed with retroviruses.



Pioneers in the study of lysogeny: Nobel laureates François Jacob, Jacques Monod, and André Lwoff, 1965. Courtesy of the U.S. National Library of Medicine.

Gene Repression and Induction

Prophage gene expression in lysogens is turned off by the action of viral proteins called repressors. Expression can be turned on when repressors are inactivated (a process called induction). The discovery that genes can be regulated by such *trans*-acting proteins, and elucidation of their mechanism, set the stage for later investigation of the control of gene expression with other viruses and their host cells.

Transduction of Host Genes

Bacteriophage genomes can pick up cellular genes and deliver them to new cells (a process known as transduction). For example, occasional mistakes in excision of the lambda prophage from its host chromosome after induction result in production of unusual progeny phages that have lost some of their own DNA but have acquired the bacterial DNA adjacent to the prophage. The acute transforming retroviruses also arise via capture of genes in the vicinity of their integration as proviruses (Volume II, Chapter 6). These cancer-inducing cellular genes are then transduced along with viral genes during subsequent infection.

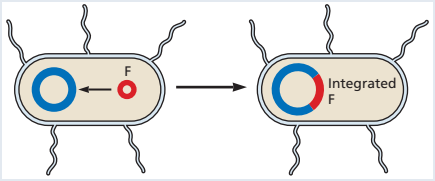
BOX 1.7

TERMINOLOGY

The episome

In 1958, François Jacob and Elie Wollman realized that lambda prophage and the *E. coli* F sex factor had many common properties. This remarkable insight led to the definition of the episome.

An episome is an exogenous genetic element that is not necessary for cell survival. Its defining characteristic is the ability to reproduce in two alternative states: while integrated in the host chromosome or autonomously. However, this term is now most commonly applied to genomes that can be maintained in cells by autonomous replication and never integrate, for example, the DNA genomes of certain animal viruses.



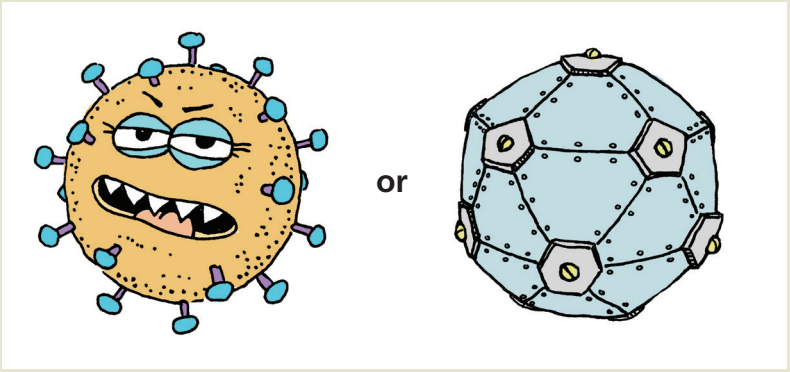
BOX 1.8

DISCUSSION

Are viruses living entities? What can/can't they do?

Viruses can be viewed as microbes that exist in two phases: an inanimate phase, the virion; and a multiplying phase in an infected cell. Some researchers have promoted the idea that viruses are bona fide living entities. According to this notion, inanimate virions may be viewed as "spores" that transform the infected cell into a novel type of organism (termed a virocell), dedicated to the production of new virions. The nature of viruses has long been a topic of intense discussion, stimulated most recently by the discovery of giant viruses such as the mimiviruses and Pandoraviruses, which encode more functions that previously ascribed to viral genomes.

Apart from attributing "life" to viruses, many scientists have succumbed to the temptation of ascribing various **actions** and **motives** when discussing them. While remarkably effective in enlivening a lecture or an article, anthropomorphic characterizations are inaccurate and also quite misleading. Infected cells and hosts respond in many ways after virus infection, but viruses, which are totally at the mercy of their environment, lack the capacity for intentional, goal-directed activity. Therefore, viruses cannot employ, ensure, synthesize, induce, display, destroy, deploy, depend, avoid, retain, evade, exploit, generate, etc.



As virologists can be very passionate about their subject, it is exceedingly difficult to purge such anthropomorphic terms from virology communications. Indeed, hours were spent doing so in the preparation of this textbook, though undoubtedly there remain examples in which actions are attributed to viruses. Should you find them, let us know!

Check out what the contemporary general public feels about this topic at http://www.virology.ws/are-viruses-alive/.

Forterre P. 2016. To be or not to be alive: how recent discoveries challenge the traditional definitions of viruses and life. *Stud Hist Philos Biol Biomed Sci* **59:**100–108.

van Regenmortel MHV. 2016. The metaphor that viruses are living is alive and well, but it is no more than a metaphor. *Stud Hist Philos Biol Biomed Sci* **59:**117–124.

for independent existence (Box 1.8), they are **not** the simplest biologically active agents: **viroids**, which are infectious agents of a variety of economically important plants, comprise a single small molecule of noncoding RNA, whereas agents called **prions**, which cause neurological disease in humans and animals, are thought to be aggregates of single protein molecules (Volume II, Chapter 13).

Cataloging Animal Viruses

As new viruses were being discovered and studied by electron microscopy, the virus world was seen to be a veritable zoo of particles with different sizes, shapes, and compositions. With no standard rules for naming isolates, the viral lexicon was, and still is, idiosyncratic (Box 1.9). Constructing a rational scheme by which these agents could be classified became a subject of colorful and quite heated controversy. A traditionalist camp argued that it was impossible to infer, from the known properties of viruses, anything about their evolutionary origin or their relationships to one another—the major goal of classical taxonomy. Others maintained that despite such limitations, there were significant practical advantages in grouping viruses with similar properties. A major sticking point, however, was finding agreement on **which** properties should be considered most important in constructing a scheme for virus classification.

The Classical System

Lwoff, Robert Horne, and Paul Tournier, in 1962, advanced a comprehensive scheme for the classification of all viruses under the classical Linnaean hierarchical system consisting of phylum, class, order, family, genus, and species. Although a subsequently formed international committee on the nomenclature of viruses did not adopt this system *in toto*, its designation of orders, families, genera, and species is used for the classification of animal viruses.

One of the most important principles embodied in the system advanced by Lwoff and his colleagues was that viruses should be grouped according to **their** shared properties rather than those of the cells or organisms they infect. A second principle was a focus on the nature of the nucleic acid

BOX 1.9

TERMINOLOGY

Complexities of viral nomenclature

No consistent system for naming viral isolates has been established by their discoverers. For example, among the vertebrate viruses, some are named for the associated diseases (e.g., poliovirus, rabies virus), for the specific type of disease they cause (e.g., murine leukemia virus), or for the sites in the body that are affected or from which they were first isolated (e.g., rhinovirus and adenovirus). Others are named for the geographic locations from which they were first isolated (e.g., Sendai virus [Sendai, Japan] and Coxsackievirus [Coxsackie, NY]) or for the scientists who first discovered them (e.g., Epstein-Barr virus). In these cases, the virus names are capitalized. Some viruses are even named for the way in which people imagined they were contracted (e.g., influenza, for the "influence" of bad air), how they were first perceived (e.g., the giant mimiviruses [Box 1.10], for the fact that they "mimic" bacteria), or totally by whimsy (e.g., Pandoravirus, after Pandora's jar [later box] of Greek mythology). Finally, combinations of the above designations are also used (e.g., Rous sarcoma virus).

genome as the primary criterion for classification. The importance of the genome had become clear when it was inferred from the Hershey-Chase experiment that viral nucleic acid alone can be infectious (Box 1.5). Four characteristics are used in the taxonomic classification of all viruses:

1. Nature of the nucleic acid in the virus particle (**DNA** or **RNA**)
2. Symmetry of the protein shell (**capsid**)
3. Presence or absence of a lipid membrane (**envelope**)
4. Dimensions of the virion and capsid

The elucidation of evolutionary relationships by analyses of nucleic acid and protein sequence similarities is now **the** standard method for assigning viruses to a particular family and ordering members within a family. For example, hepatitis C virus was classified as a member of the family *Flaviviridae* and MERS was assigned to the *Coronaviridae* based on their genome sequences. However, as our knowledge of molecular properties of viruses and their reproduction has increased, other relationships have become apparent. *Hepadnaviridae*, *Retroviridae*, and some plant viruses are classified as different families on the basis of the nature of their genomes. Nevertheless, they are all related by the fact that reverse transcription is an essential step in their reproductive cycles, and the viral polymerases that perform this task exhibit important similarities in amino acid sequence. Another example is the classification of the giant protozoan *Mimiviridae* as members of a related group called nucleocytoplasmic large DNA viruses (NCLDVs), which includes the *Poxviridae* that infect vertebrates (Box 1.10).

The International Committee on Taxonomy of Viruses (ICTV), founded by André Lwoff, authorizes and organizes the classification and establishes nomenclature for all viruses. Freely available as a periodically updated, online resource (https://ictv.global/taxonomy), the 2018 report lists orders, families, genera, and species for all known viruses. In addition, it describes numerous viruses that are not yet classified and probably representatives of new genera and/or families. The ICTV catalog also includes descriptions of subviral agents (**satellites**, viroids, and prions) and a list of viruses for which information is still insufficient to make assignments. The pace of discovery of new viruses has been accelerated greatly with the application of **metagenomic analyses**, direct sequencing of genomes from environmental samples, suggesting that we have barely begun to chart the viral universe.

The ICTV nomenclature has been applied widely in both the scientific and medical literature, and therefore we adopt it in this text. In this nomenclature, the Latinized virus family names are recognized as starting with capital letters and ending with *-viridae*, as, for example, in the family name *Parvoviridae*. These names are used interchangeably with their common derivatives, as, for example, parvoviruses (see additional examples in the Appendix).

Classification by Genome Type: the Baltimore System

Francis Crick conceptualized the central dogma for flow of information from the DNA genome in all living cells:

$$\text{DNA} \rightarrow \text{mRNA} \rightarrow \text{protein}$$

As intracellular parasites that depend on the host cell's translational machinery for protein production, all viruses must direct the synthesis of mRNAs. But viral genomes comprise both DNA and RNA in a variety of conformations. Appreciation of the essential role of the translational machinery

BOX 1.10

DISCUSSION

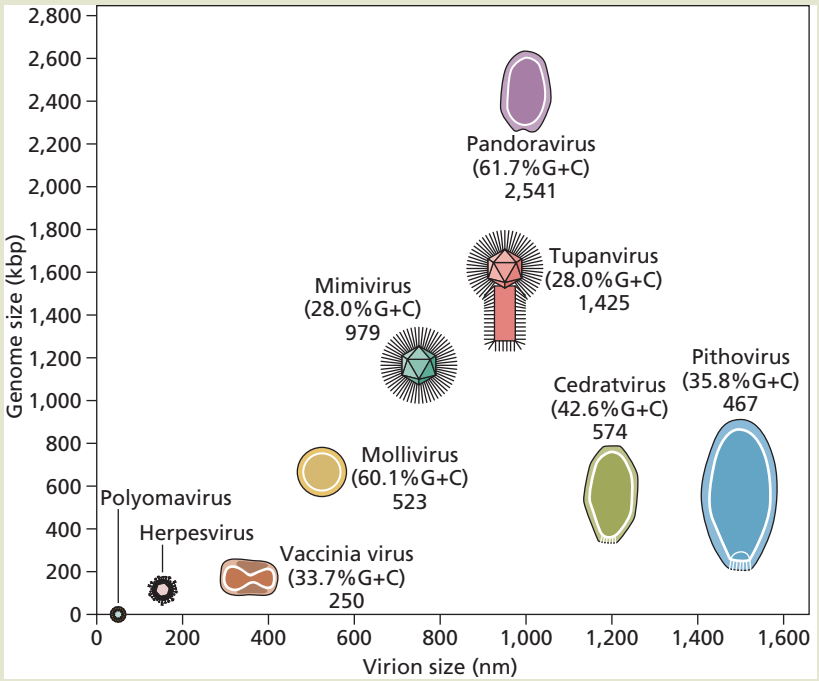
Giant viruses discovered in amoebae

The mimivirus virion, the prototype member of the *Mimiviridae*, was the first giant virus of amoebae to be discovered. Isolated from water in a cooling tower in England in 1992, it is large enough to be visible in a light microscope and was initially thought to be an intracellular bacterium within its host. Not until publication of a brief note in 2003 did it become apparent that this giant was really a virus. The mimivirus genome of 1.2 Mbp was much larger than that of any known virus at the time, exceeding that of some bacteria. This giant encodes more than 900 proteins, many of which are components of the protein translational apparatus, a function for which other viruses rely entirely on the host.

Since reports of the first giant viruses, the use of different strains of amoebae to screen soil and water samples from diverse environments and geographic locations has yielded more than 50 isolates, assigned to nine distinct families. Among the most spectacular is a Pandoravirus isolate, discovered in saltwater off the coast of Chile in 2013. The genome of this giant is twice the size of the mimivirus genome, and contains ~2,500 putative protein-coding sequences, most of them never seen before. Furthermore, while mimivirus has a more or less familiar icosahedral capsid, the Pandoravirus has no regular capsid. Instead, the genomes of these viruses are surrounded by an ovoid envelope, with a pore at the apex that allows delivery of the internal components into the cytoplasm of its host. The following year two additional giant amoeba viruses, a circular mollivirus and ovoid pithovirus, were discovered in a sample of Siberian permafrost more than 30,000 years old.

The unusual properties of the giant viruses of amoebae have prompted the somewhat controversial speculation that they might represent a separate branch in the tree of life, or that they arose by reductive evolution from the nucleus of a primitive cellular life form. However, the discovery in 2017 of another group of these viruses, by metagenomic analysis of samples from a sewer in Klosterneuburg, Austria, has suggested a more pedestrian origin. While the new group, called Klosneuviruses, encode numerous components of translational machinery, comprehensive phylogenetic analyses indicate that these genes were captured from a cellular host by a smaller, precursor virus during evolution of Klosneuviruses. If this is a general phenomenon, the 2018 description of tailed mimivirus relatives, isolated from the extreme environments of an alkaline soda lake in Brazil and from deep in the Atlantic Ocean, must be considered an extraordinary example of such capture. The genomes of these odd-looking isolates, called Tupanviruses, contain nearly all of the necessary translation-associated genes, lacking only ribosomes for protein synthesis. It would seem that there is still much to ponder concerning the evolution of these giant viruses.

For illustrations of giant amoeba virus structures, see http://viralzone.expasy.org/all_by_species/670.html. See also TWiV 261: Giants among viruses. Interview with Drs. Chantal Abergel and Jean-Michel Claverie at http://www.microbe.tv/twiv/twiv-261-giants-among-viruses/.



Properties of some of the largest currently known giants, all of which infect amoebae, with representative vertebrate-infecting DNA viruses, of which poxviruses are the largest. The broad range of nucleic acid composition among the amoeba viral genomes is illustrated by the substantial differences in their G+C content. The number of known or putative coding genes in each viral genome is listed. Examples of small, medium, and large mammalian viruses (poliovirus, herpesvirus, and vaccinia virus, respectively) are included for comparison.

Abrahão J, Silva L, Silva LS, Khalil JYB, Rodrigues R, Arantes T, Assis F, Boratto P, Andrade M, Kroon EG, Ribeiro B, Bergier I, Seligmann H, Ghigo E, Colson P, Levasseur A, Kroemer G, Raoult D, La Scola B. 2018. Tailed giant Tupanvirus possesses the most complete translational apparatus of the known virosphere. *Nat Commun* **9:**749–761.

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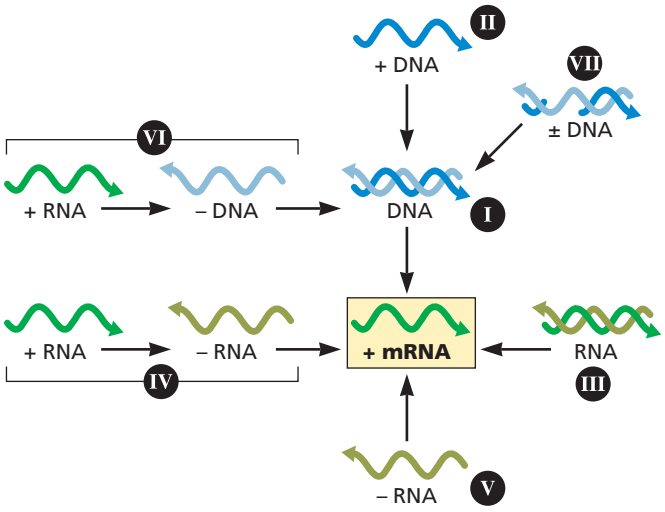

Figure 1.12 The Baltimore classification. The Baltimore classification assigns viruses to seven (I to VII) distinct classes on the basis of the nature and polarity of their genomes. Because all viruses must produce mRNA that can be translated by cellular ribosomes, knowledge of the composition of a viral genome provides insight into the pathways required to produce mRNA, indicated by arrows. See also Baltimore D. 1971. *Bacteriol Rev* 35:235–241.

in virus reproduction inspired David Baltimore, in 1971, to devise a classification scheme for viruses, based on the steps that would be required to produce mRNA from their diverse genomes (Fig. 1.12).

By convention, mRNA is defined as a **positive [(+)] strand** because it contains immediately translatable information. In the Baltimore classification, a strand of DNA that is of equivalent sequence is also designated a (+) strand. The RNA and DNA complements of (+) strands are designated **negative [(–)] strands**.

As originally conceived, the Baltimore scheme included six classes of viral genomes (designated I to VI). When the gapped DNA genome of hepadnaviruses (e.g., hepatitis B virus) was discovered, these viruses were assigned to a seventh class (VII). The DNA and RNA descriptors for the viral classes [single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), (+) RNA, or (–) RNA, etc.], but not the Roman numeral designations, have been adopted universally and are a valuable complement to classical taxonomy. The information embodied in classification by genome type provides virologists with immediate insight into the steps that must take place to initiate the replication and expression of any viral genome.

Because the viral genome carries the entire blueprint for virus propagation, molecular virologists have long considered it the most important characteristic for classification purposes. Although individual virus families are known by their classical designations, they are commonly grouped according to their genome type. In the ICTV compilation, all viral families are assigned to one of the seven classes described in the Baltimore system (Fig. 1.13).

A Common Strategy for Viral Propagation

The basic thesis of this textbook is that **all** viral propagation can be described in the context of three fundamental properties.

- Viral genomes are packaged inside particles that mediate their transmission from host to host.
- The viral genome contains the information for initiating and completing an infectious cycle within a susceptible, permissive cell.
- An infectious cycle includes attachment and entry, decoding of genome information, genome replication, and assembly and release of particles containing the genome.
- Viral propagation is ensured by establishment in a host population.

Perspectives

The study of viruses has increased our understanding of the importance and ubiquitous existence of these diverse agents and, in many cases, yielded new and unexpected insight into the molecular biology of host cells and organisms. Indeed, because viruses are obligate molecular parasites, every tactical solution encountered in their reproduction and propagation must of necessity tell us something about the host as well as the virus. Some of the important landmarks and achievements in the field of virology are summarized in Fig. 1.14. It is apparent that much has been discovered about the biology of viruses and about host defenses against them. Yet the more we learn, the more we realize that much is still unknown.

In the first edition of this textbook (published in 2000), we noted that the most recent (1995) report of the ICTV listed 71 different virus families, which covered most new isolates. We speculated therefore that: "As few new virus families had been identified in recent years, it seems likely that a significant fraction of all existing virus families are now known." In the intervening years, this prediction has been shattered, not only by the discovery of new families of viruses, including giant viruses with genome sizes that surpass those of some bacteria, but also by results from metagenomic analyses. For example, the fact that a high percentage (93%) of protein-coding sequences in the genomes of the giant Pandoraviruses have **no** homologs in the current databases was totally unexpected. The unusual morphological features and atypical reproduction process of these viruses

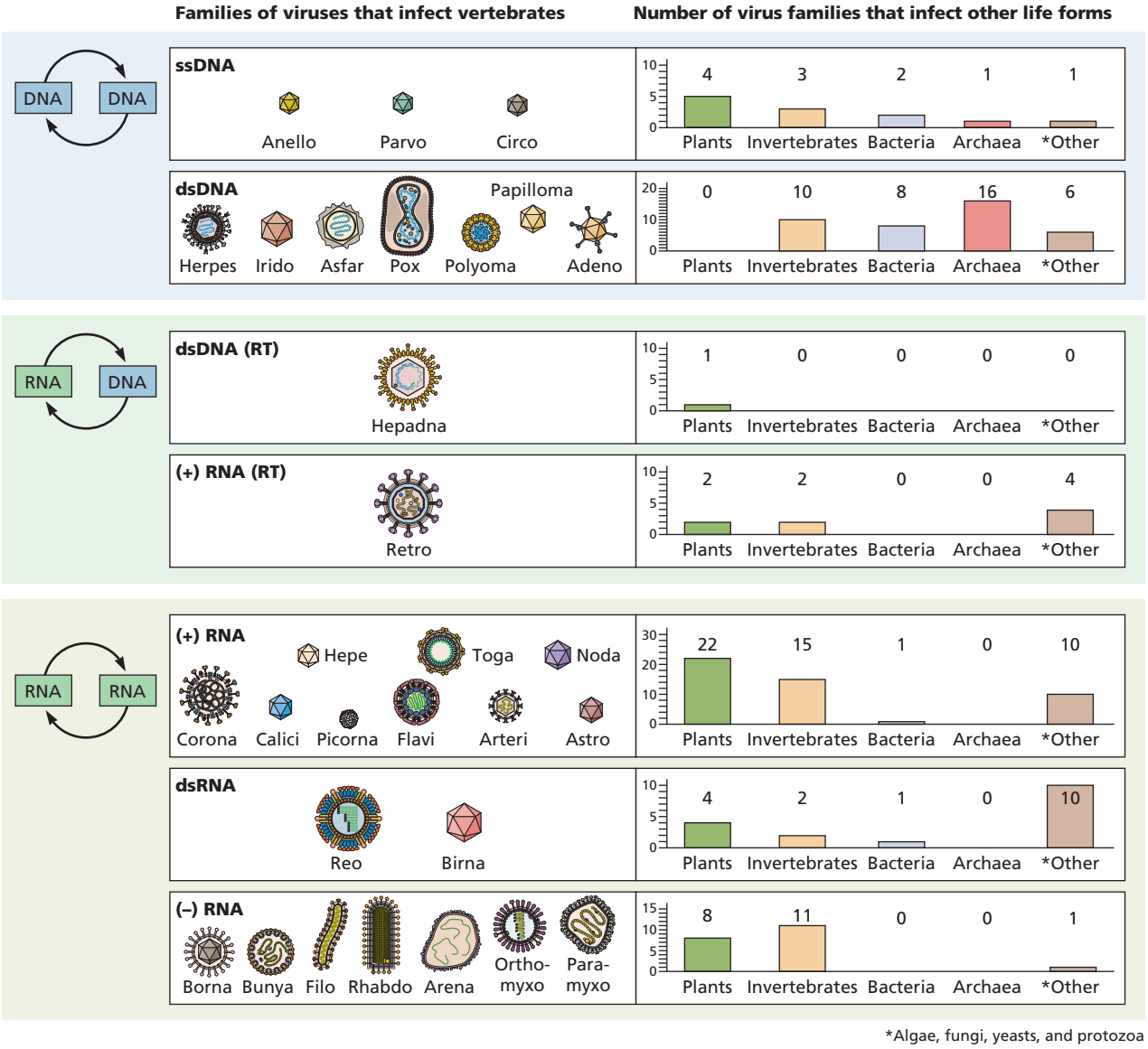

Figure 1.13 Viral families sorted according to the nature of the viral genomes. A wide variety of sizes and shapes are illustrated for the families of viruses that infect vertebrates. Families are identified by Latinized names and organized in seven distinct classes, based on the nature of their genomes. Genome replication cycles are illustrated in the column at the left. Similar diversity exists for the families of viruses that infect other life forms, but the chart lists only the approximate number found to date in each class. As noted in the 9th and 10th ICTV Reports, in some cases there are as yet no examples. Data from King AMQ et al. 2012. *Virus Taxonomy: The Classification and Nomenclature of Viruses* (https://talk.ictvonline.org/ictv-reports/), with assistance from Dr. Elliot J. Lefkowitz, Department of Microbiology, Director of Informatics, UAB Center for Clinical and Translational Science, Birmingham, AL (http://www.uab.edu/bioinformatics/).

were also surprising. In addition, it is mind-boggling to contemplate that of almost 900,000 viral sequences identified in samples of only one type of ecosystem (raw sewage), more than 66% bore **no** relationship to any viral family in the current database. From these analyses, and similar studies of other ecosystems (i.e., oceans and soil), it has been estimated that only a minor percentage of extant viral diversity has been explored to date. Clearly, the viral universe is far more vast and diverse than suspected only a decade ago, and there is much fertile ground for gaining a deeper understanding of the biology of viruses and their host cells and organisms.

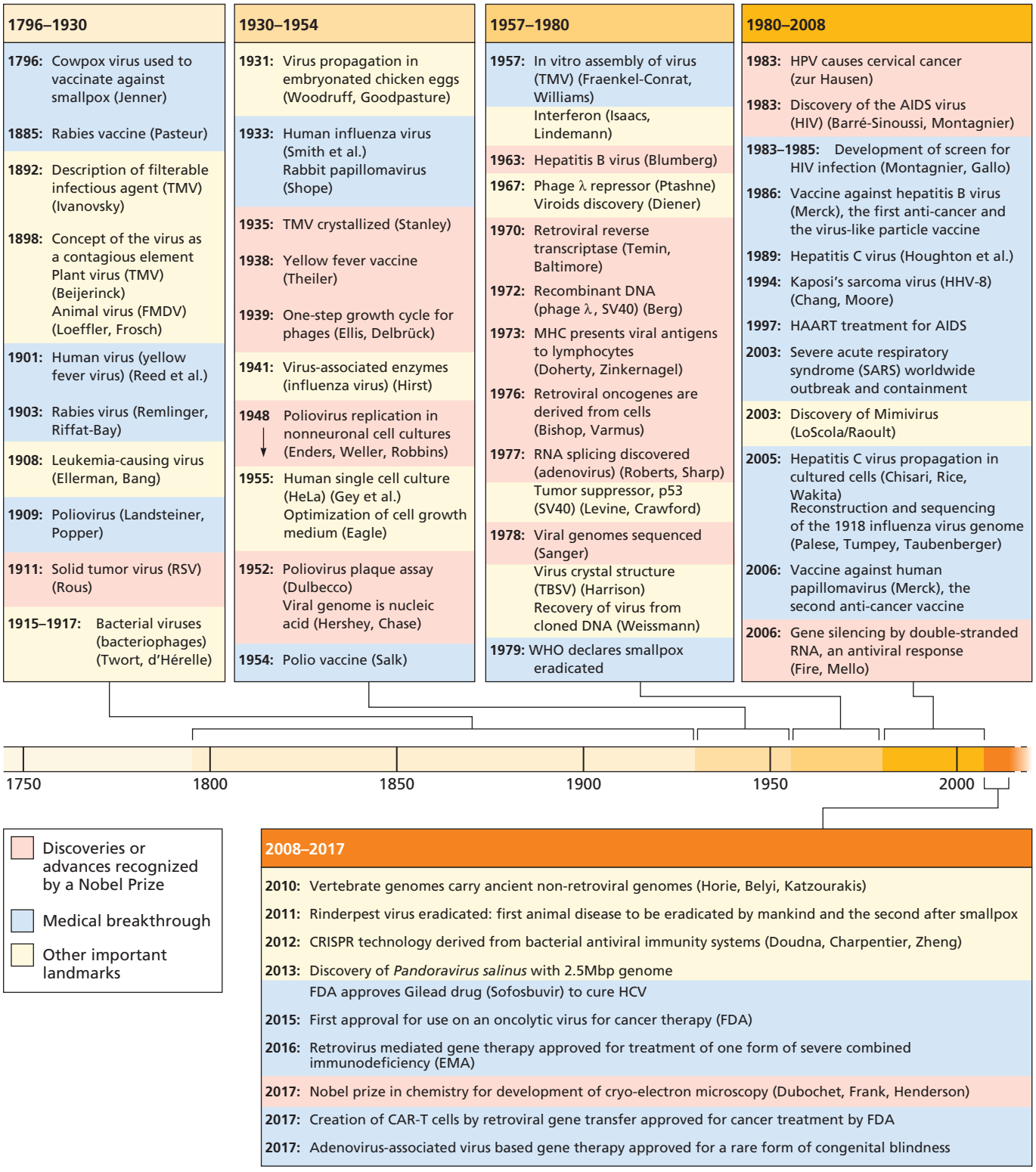

Figure 1.14 Landmarks in the study of viruses. Key discoveries and technical advances are listed for each time interval. The pace of discovery has increased exponentially over time. Abbreviations: AAV, adenovirus-associated virus; EU, European Union; EMA, European Medical Association; FDA, U.S. Food and Drug Administration; FMDV, foot-and-mouth disease virus; HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; HHV-8, human herpesvirus 8; HIV-1, human immunodeficiency virus type 1; HPV, human papillomavirus; MHC, major histocompatibility complex; RSV, Rous sarcoma virus; SV40, simian virus 40; TBSV, tomato bushy stunt virus; TMV, tobacco mosaic virus; WHO, World Health Organization.

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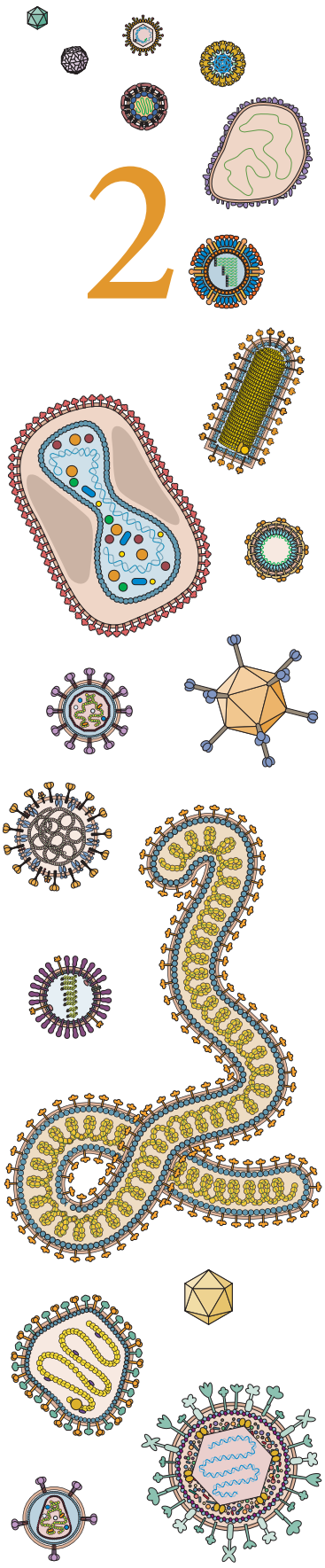
http://ictvonline.org/ ICTV-approved virus names and other information as well as links to virus databases can be downloaded.

http://microbe.tv/twiv A weekly podcast about viruses featuring informal yet informative interviews with guest virologists who discuss their recent findings and other topics of general interest.

STUDY QUESTIONS

1. What is the definition of a virus?
2. Which is a key property first discovered about viruses that distinguished them from other microorganisms?
   - **a.** They were too large to pass through a 0.2-micron filter
   - **b.** They could reproduce only in broth
   - **c.** They made tobacco plants sick
   - **d.** They were small enough to pass through a 0.2-micron filter
   - **e.** None of the above
3. All of us carry many different viruses throughout our daily lives. Why don't they make us sick?
4. Why do we care that viruses comprise the most biodiversity on the planet?
5. The first viruses were discovered near the end of the 1800s. How was this done?
   - **a.** By transmitting a disease to tobacco plants using a cell-free filtrate of diseased leaves
   - **b.** Pasteur showed that viruses could reproduce in a sterile medium
   - **c.** Leeuwenhoek saw viruses in his microscope
   - **d.** Robert Koch showed that viruses grown in broth could cause disease
   - **e.** All of the above
6. Why were the bacteriophage systems so useful for elucidating principles of viral reproduction? What important features of virus-host interactions were discovered from these studies?
7. How are viruses classified?
8. How does the discovery of new viruses today differ from 100 years ago?
9. Which host cell function is essential for the reproduction of all viruses?
10. What is the basis of the Baltimore classification system? How many genome types are sufficient to describe all viral families in this system?



2 The Infectious Cycle

LINKS FOR CHAPTER 2

- ***Video: Interview with Dr. Thomas Hope***
  http://bit.ly/Virology_Hope
- ***Cloning HeLa cells with Philip I. Marcus***
  http://bit.ly/Virology_Twiv197
- ***Ode to a plaque***
  http://bit.ly/Virology_Twiv68
- ***Movie 2.1: Plaque formation by vesicular stomatitis virus***
  http://bit.ly/Virology_VZVGFP
- ***Think globally, act locally***
  http://bit.ly/Virology_Twim90

You know my methods, Watson.
SIR ARTHUR CONAN DOYLE

Introduction

Viruses are unique: often made up of nothing more than a nucleic acid molecule wrapped in protein, they parasitize the cellular machinery to produce thousands of progeny. This simplicity is misleading: viruses can infect all known life forms, and they comprise a variety of structures and genomes. Despite such variety, viruses are amenable to study because all viral propagation can be described in the context of three fundamental properties, as noted in Chapter 1: viral genomes are packaged inside particles that mediate their transmission from cell to cell; the viral genome contains the information for initiating and completing an infectious cycle; viruses establish themselves in a host population to ensure virus survival.

How viruses enter individual cells, their genomes are replicated, and new infectious particles are assembled are some of the topics of research in virology. These studies are usually carried out with cell cultures because they are a much simpler and more homogeneous experimental system than animals. Cells can be infected in such a way as to ensure that a single reproduction cycle occurs synchronously in every infected cell, called **one-step growth**. A full understanding of viral infectious cycles also requires knowledge of cell biology. Consequently, to reproduce the diversity of cells and architectures that are typical of tissues and organs, three-dimensional culture systems have been developed. In this chapter we begin with a brief overview of the infectious cycle, followed by a discussion of methods for cultivating and assaying viruses and detecting viral proteins and genomes and a consideration of viral reproduction and one-step growth analysis.

The Infectious Cycle

The production of new infectious particles can take place only within a cell (Fig. 2.1). Virologists divide viral infectious cycles into discrete steps to facilitate their study, although in virus-infected cells no such artificial boundaries occur. The infectious cycle comprises attachment and entry of the particle, production of viral mRNA and its translation by host ribosomes, genome replication, and assembly and release of progeny particles containing the genome. New virus particles produced during the infectious cycle may then infect other cells. The term **virus reproduction** is another name for the sum total of all events that occur during the infectious cycle.

Some events are common to virus replication in animals and in cells in culture, but there are also many important differences. While virus particles readily attach to cells in culture, in nature they must encounter a host, no mean feat for nanoparticles without any means of locomotion. After encountering a host, the virus particle must pass through physical host defenses, such as dead skin, mucous layers, and the extracellular matrix. Such barriers and other host defenses, such as antibodies and immune cells, which exist to combat virus infections, are not found in cell cultures. Virus infection of cells in culture has been a valuable tool for understanding viral infectious cycles, but the dissimilarities with infection of a living animal must always be considered.

The Cell

Viral reproduction requires many different functions of the host cell. Examples include the machinery for translation of viral mRNAs, sources of energy, and enzymes for genome replication. The cellular transport apparatus brings viral genomes to the correct cellular compartment and ensures that viral subunits reach locations where they may be assembled into virus particles. Subsequent chapters include a discussion of

PRINCIPLES ***The infectious cycle***

- Many distinct functions of the host cell are required to complete a viral infectious cycle.
- The synthesis of new virus particles (i.e., a productive infection) requires target cells that are both susceptible (i.e., allow virus entry) and permissive (i.e., support virus reproduction).
- Viral nucleic acids must be shielded from harsh environmental conditions in extracellular particles but be readily accessible for replication once inside the cell.
- Viruses may be studied by propagation in cells within a laboratory animal or in cells in culture.
- The plaque assay is the major way to determine the concentration of infectious virus particles in a sample.
- Methods for quantifying and characterizing virus particles evolve rapidly, based on developments in detection, ease, cost, safety, utility in the field, and amenability to large-scale implementation.
- Relationships among viruses can be deduced from phylogenetic trees generated from protein or nucleic acid sequences.
- Viral reproduction is distinct from cellular or bacterial replication: rather than doubling with each cycle, each single cell cycle of viral reproduction is typically characterized by the release of many (often thousands) of progeny virions.
- The multiplicity of infection (MOI) is the number of infectious units added per cell; the probability that any one target cell will become infected based on the MOI can be calculated from the Poisson distribution.
- Global analysis of viral, cell, and host responses to virus infection can implicate particular cellular pathways in viral reproduction and can reveal signatures of virus-induced lethality or immune protection.

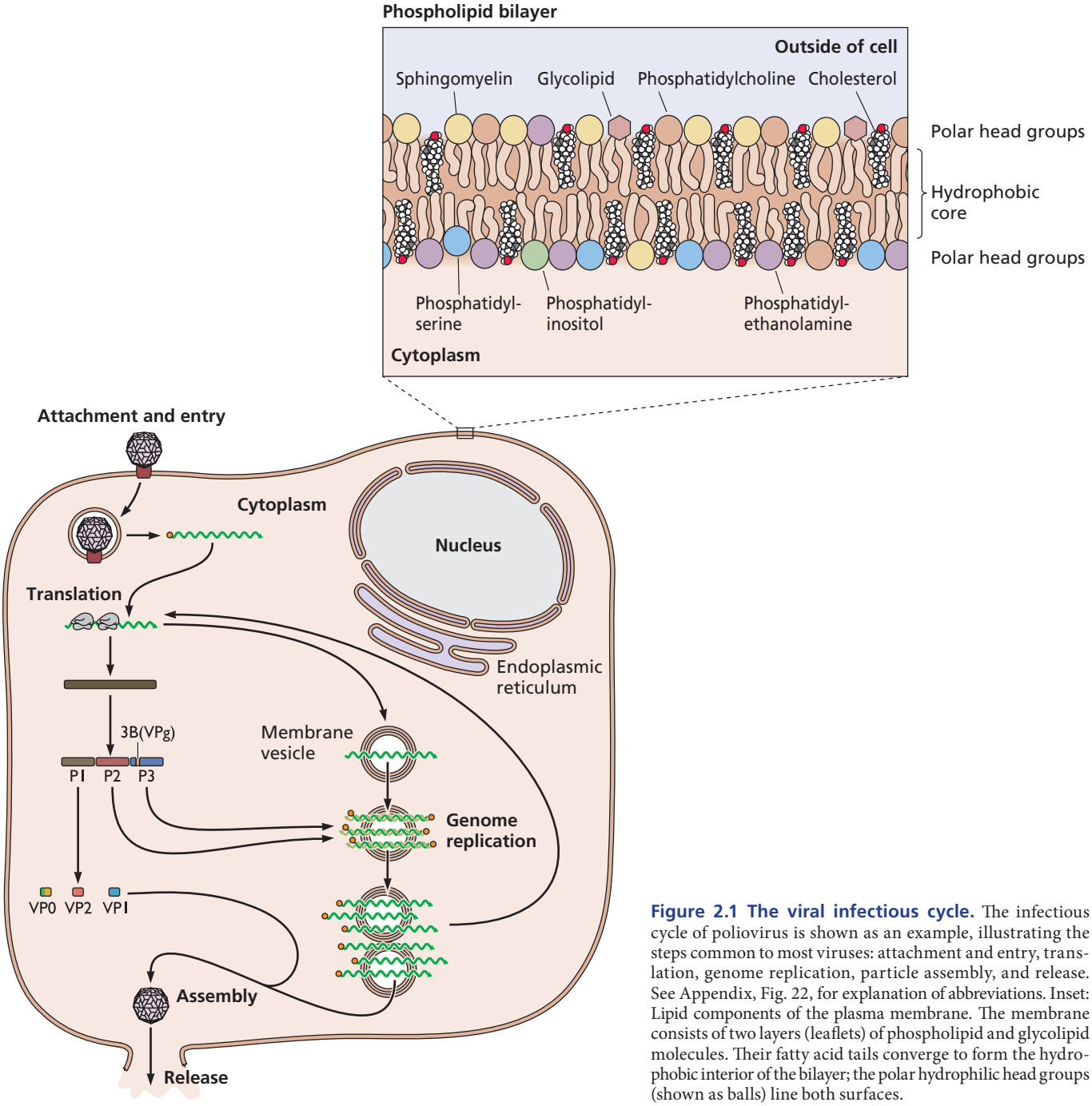


Figure 2.1 The viral infectious cycle. The infectious cycle of poliovirus is shown as an example, illustrating the steps common to most viruses: attachment and entry, translation, genome replication, particle assembly, and release. See Appendix, Fig. 22, for explanation of abbreviations. Inset: Lipid components of the plasma membrane. The membrane consists of two layers (leaflets) of phospholipid and glycolipid molecules. Their fatty acid tails converge to form the hydrophobic interior of the bilayer; the polar hydrophilic head groups (shown as balls) line both surfaces.

cellular functions that are important for individual steps in the viral infectious cycle.

Entering Cells

Viral infection is initiated by a collision between the virus particle and the cell, a process that is governed by chance. A virion may not infect every cell it encounters: it must first come in contact with the tissues that contain cells to which it can bind. Such cells are normally recognized by means of the specific interaction of a virus particle with a cell surface receptor. These cellular molecules do not exist for the benefit of viruses: they all perform functions for the cell. Virus-receptor interactions can be either promiscuous or highly selective, depending on the virus and the distribution of the cell receptor. The presence