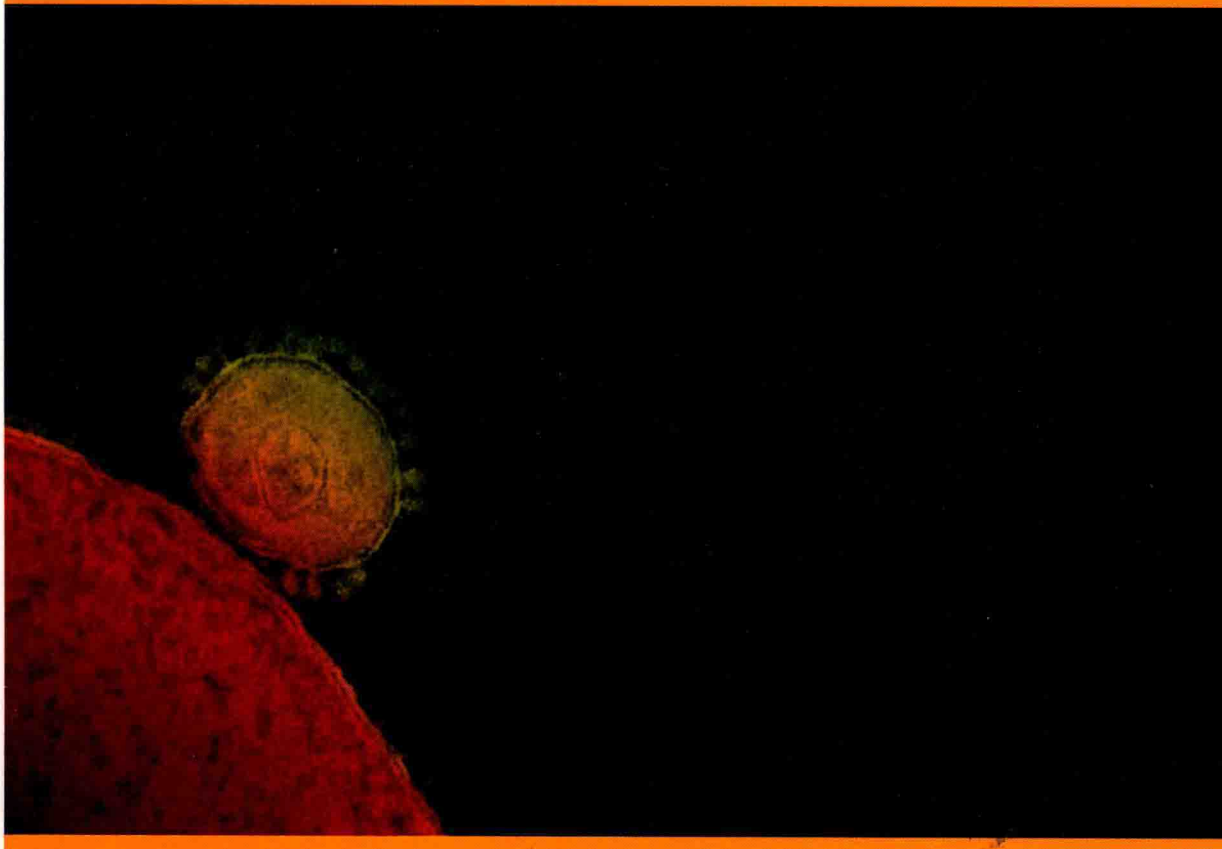
Principles of Molecular Virology

SIXTH EDITION



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Principles of Molecular Virology

Preface to the Sixth Edition

In the age of the Internet, why would anyone write a textbook about virology? Indeed, why would anyone write anything about virology? Virology isn't dead yet (DiMaio, 2014), and neither are books. I encourage everyone to use the wonderful resource of the Internet to improve their knowledge of virology. I encourage my students to use Wikipedia and Google to learn the facts. But as Jimmy Wales said, *Wikipedia is often the best place to start, but the worst place to stop*. The role of this book is not primarily about knowledge but about sense-making—what you can't get from Wikipedia. Virology *explained* by setting facts in a larger context.

Along with updating the facts and smoothing some of the rough edges, I have noticed a big scientific change in writing this edition. Open Access scientific publishing has finally made its impact felt. In this updated edition the reading recommendations at the end of each chapter I have been able, in *almost* all cases, to recommend freely available peer-reviewed content for readers. You may have to hunt around to find it—a good working knowledge of PubMed and Google Scholar is at least as useful as Google and Wikipedia—but it is now possible to access much of the scientific literature the public has paid for. But there is still the question of interpretation. In writing this book I have tried to do my part. The rest is up to the reader.

As with previous editions, I am grateful to the staff of Elsevier, in particular Halima Williams and Jill Leonard, for their patience with me.

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December 2014

Reference

DiMaio, D., 2014. Is virology dead? mBio 5 (2), e01003–e01014.

Contents

CHAPTER 1

Introduction

Intended Learning Outcomes

On completing this chapter you should be able to:

- Define how viruses are different from other biological organisms.
- Explain how the development of virology led us to our present understanding of viruses.
- Be able to discuss how technology has influenced the study of viruses in recent years.

CONTENTS

This book is about "molecular virology," that is, the molecular basis of how viruses work. It looks at the protein–protein, protein–nucleic acid, and protein–lipid interactions which control the structure of virus particles, the ways viruses infect cells, and how viruses replicate themselves. Later we will also examine the consequences of virus infection for host organisms, but it is important to consider the basic nature of viruses first. To understand how our present knowledge of viruses was achieved, it will be useful to know a little about the history of virology. This helps to explain how we think about viruses and what the current and future concerns of virologists are.

There is more biological diversity between different viruses than in all the rest of the bacterial, plant, and animal kingdoms put together. This is the result of the success of viruses in parasitizing all known groups of living organisms, and understanding this diversity is the key to comprehending the interactions of viruses with their hosts. The principles behind some of the experimental techniques mentioned in this chapter may not be well known to all readers. That is why it may be helpful to you to use the further reading at the end of this chapter to become more familiar with these methods or you will not be able to understand the current research literature you read. In this and the subsequent chapters, terms in the text in **bold red print** are defined in the glossary at the end of the book (Appendix 1).

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WHAT ARE VIRUSES?

Viruses are submicroscopic, obligate intracellular parasites. Most are too small to be seen by optical microscopes, and they have no choice but to replicate inside host cells. This simple but useful definition goes a long way toward describing viruses and differentiating them from all other types of organism. However, this short definition is not completely adequate. It is not a problem to differentiate viruses from multicellular organisms such as plants and animals. Even within the broad scope of microbiology, covering **prokaryotic** organisms as well as microscopic **eukaryotes** such as algae, protozoa, and fungi, in most cases this simple definition is enough. A few groups of prokaryotic organisms also have specialized intracellular parasitic life cycles and overlap with this description. These are the *Rickettsiae* and *Chlamydiae*-obligate intracellular parasitic bacteria which have evolved to be so cell-associated that they can exist outside the cells of their hosts for only a short period of time before losing viability.

A common mistake is to say that viruses are smaller than bacteria. While this is true in most cases, size alone does not distinguish them. The largest virus known (currently *Pithovirus sibericum*) is 1,200 nm long, while the smallest bacteria (e.g., *Mycoplasma*) are only 200–300 nm long. Nor does genetic complexity separate viruses from other organisms. The largest virus genome (Pandoravirus, 2.8 Mbp—million base pairs—approximately 2,500 genes) is twenty times as big as smallest bacterial genome (*Tremblaya princeps*, at 139 kbp—thousand base pairs—and with only 120 protein coding genes), although it is still shorter than the smallest **eukaryotic** genome (the parasitic protozoan *Encephalitozoon*, 2.3 Mbp). For these reasons, it is necessary to go further to produce a definition of how viruses are unique:

- Virus particles are produced from the assembly of preformed components, while other biological agents grow from an increase in the integrated sum of their components and reproduce by division.
- Virus particles (**virions**) do not grow or undergo division.
- Viruses lack the genetic information that encodes the tools necessary for the generation of metabolic energy or for protein synthesis (ribosomes).

No known virus has the biochemical or genetic means to generate the energy necessary to drive all biological processes. They are absolutely dependent on their host cells for this function. Lacking the ability to make ribosomes is one factor which clearly distinguishes viruses from all other organisms. Although there will always be some exceptions and uncertainties in the case of organisms that are too small to see easily and in many cases difficult to study, the above guidelines are sufficient to define what a virus is.

A number of virus-like agents possess properties that confuse the above definition yet are clearly more similar to viruses than other organisms. These are the subviral elements known as **viroids**, **virusoids**, and **prions**. Viroids are small (200–400 nucleotide), circular RNA molecules with a rod-like secondary structure. They have no **capsid** or **envelope** and are associated with certain plant diseases. Their replication strategy is like that of viruses—they are obligate intracellular parasites. Virusoids are satellite, viroid-like molecules, a bit larger than viroids (approximately 1,000 nucleotides), which are dependent on the presence of virus replication for their multiplication (the reason they are called "**satellites**"). They are packaged into virus capsids as passengers. **Prions** are infectious protein molecules with no nucleic acid component. Confusion arises from the fact that the prion protein and the gene that encodes it are also found in normal "uninfected" cells. These agents are associated with diseases such as Creutzfeldt–Jakob disease in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE) in cattle. Chapter 8 deals with these subviral infectious agents in more detail.

Genome analysis has shown that more than 10% of the **eukaryotic** cell genome is composed of mobile retrovirus-like elements (**retrotransposons**), which may have had a considerable role in shaping these complex genomes (Chapter 3). Furthermore, certain **bacteriophage** genomes closely resemble bacterial **plasmids** in their structure and in the way they are replicated. Research has revealed that the evolutionary relationship between viruses and other living organisms is perhaps more complex than was previously thought.

ARE VIRUSES ALIVE?

As discussed earlier, viruses do not reproduce by division but are assembled from preformed components, and they cannot make their own energy or proteins. A virus-infected cell is more like a factory than a womb. One view is that inside their host cell viruses are alive, whereas outside it they are only complex arrangements of metabolically inert chemicals. Chemical changes do occur in extracellular virus particles, as explained in Chapter 4, but these are not in the "growth" of a living organism. This is a bit problematic—alive at sometimes but not at others. Viruses do not fit into most of the common definitions of "life"—growth, respiration, etc. Ultimately, whether viruses are alive or not is a matter of personal opinion, but it is useful to make your decision after considering the facts. Some of the reading at the end of this chapter will help you consider the evidence.

BOX 1.1 ARE VIRUSES ALIVE? WHO CARES?

Viruses don't care (can't care) if we think they are living or not. And I don't care much either, because as far as I'm concerned it is much more important to understand how viruses replicate themselves and interact with their hosts. But you might care, either because you are a philosophical person who likes thinking about these things, or because you have to write an essay or answer an exam question on the subject. In that case, it is important to consider how you define what a living organism is and how viruses are similar or different to microorganisms we consider to be alive (you're going to make life hard for yourself if you start comparing them to humans). This is not a simple question, and any simple answer is, quite simply, wrong.

THE HISTORY OF VIROLOGY

Human knowledge of virus diseases goes back a long way, although it is only much more recently that we have become aware of viruses as distinct from other causes of disease. The first written record of a virus infection is a hieroglyph from Memphis, the capital of ancient Egypt, drawn in approximately 3700 BC, which depicts a temple priest showing typical clinical signs of paralytic poliomyelitis. Pharaoh Ramses V, who died in 1196 BC and whose well-preserved mummified body is now in a Cairo museum, is believed to have died from smallpox—the comparison between the pustules on the face of this mummy and those of more recent patients is startling.

Smallpox was **endemic** in China by 1000 BC. In response, the practice of **variolation** was developed. Recognizing that survivors of smallpox outbreaks were protected from subsequent infection, people inhaled the dried crusts from smallpox lesions like snuff or, in later modifications, inoculated the pus from a lesion into a scratch on the forearm. Variolation was practiced for centuries and was shown to be an effective method of disease prevention, although risky because the outcome of the inoculation was never certain. Edward Jenner was nearly killed by variolation at the age of seven. Not surprisingly, this experience spurred him on to find a safer alternative treatment. On May 14, 1796, he used cowpox-infected material obtained from the hand of Sarah Nemes, a milkmaid from his home village of Berkeley in Gloucestershire, England, to successfully vaccinate 8-year-old James Phipps. Although initially controversial, **vaccination** against smallpox was almost universally adopted worldwide during the nineteenth century.

This early success, although a triumph of scientific observation and reasoning, was not based on any real understanding of the nature of infectious agents. This arose separately from another line of reasoning. Antony van Leeuwenhoek (1632–1723), a Dutch merchant, constructed the first simple microscopes and with these identified bacteria as the "animalcules" he saw

in his specimens. However, it was not until Robert Koch and Louis Pasteur in the 1880s jointly proposed the "germ theory" of disease that the significance of these organisms became apparent. Koch defined four famous criteria which are now known as Koch's postulates and still generally regarded as the proof that an infectious agent is responsible for a specific disease:

1. The agent must be present in every case of the disease.
2. The agent must be isolated from the host and grown *in vitro*.
3. The disease must be reproduced when a pure culture of the agent is inoculated into a healthy susceptible host.
4. The same agent must be recovered once again from the experimentally infected host.

Subsequently, Pasteur worked extensively on rabies, which he identified as being caused by a "virus" (from the Latin for "poison"), but despite this he did not discriminate between bacteria and other agents of disease. In 1892, Dimitri Iwanowski, a Russian botanist, showed that extracts from diseased tobacco plants could transmit disease to other plants after being passed through ceramic filters fine enough to retain the smallest known bacteria. Unfortunately, he did not realize the full significance of these results. A few years later (1898), Martinus Beijerinick confirmed and extended Iwanowski's results on tobacco mosaic virus (TMV) and was the first to develop the modern idea of the virus, which he referred to as *contagium vivum fluidum* ("soluble living germ"). Freidrich Loeffler and Paul Frosch (1898) showed that a similar agent was responsible for foot-and-mouth disease in cattle, but, despite the realization that these new-found agents caused disease in animals as well as plants, people would not accept the idea that they might have anything to do with human diseases. This resistance was finally dispelled in 1909 by Karl Landsteiner and Erwin Popper, who showed that poliomyelitis was caused by a "filterable agent"—the first human disease to be recognized as being caused by a virus.

Frederick Twort (1915) and Felix d'Herelle (1917) were the first to recognize viruses that infect bacteria, which d'Herelle called **bacteriophages** ("eaters of bacteria"). In the 1930s and subsequent decades, pioneering virologists such as Salvador Luria, Max Delbruck, and others used these viruses as model systems to investigate many aspects of virology, including virus structure (Chapter 2), genetics (Chapter 3), and replication (Chapter 4). These relatively simple agents have since proved to be very important to our understanding of all types of viruses, including those of humans which can be much more difficult to propagate and study. The further history of virology is the story of the development of experimental tools and systems with which viruses could be examined and which opened up whole new areas of biology, including not only the biology of the viruses themselves but inevitably also the biology of the host cells on which they are dependent.

LIVING HOST SYSTEMS

In 1881, Louis Pasteur began to study rabies in animals. Over several years, he developed methods of producing **attenuated** virus preparations by progressively drying the spinal cords of rabbits experimentally infected with rabies which, when inoculated into other animals, would protect from disease caused by virulent rabies virus. In 1885, he inoculated a child, Joseph Meister, with this, the first artificially produced virus **vaccine** (since the ancient practice of **variolation** and Jenner's use of cowpox virus for **vaccination** had relied on naturally occurring viruses). Whole plants have been used to study the effects of plant viruses after infection ever since TMV was first discovered by Iwanowski in 1892. Usually such studies involve rubbing preparations containing virus particles into the leaves or stem of the plant to cause infection.

During the Spanish–American War of the late nineteenth century and the subsequent building of the Panama Canal, the number of American deaths due to yellow fever was colossal. The disease also appeared to be spreading slowly northward into the continental United States. In 1900, through experimental transmission of the disease to mice, Walter Reed demonstrated that yellow fever was caused by a virus spread by mosquitoes. This discovery eventually enabled Max Theiler in 1937 to propagate the virus in chick embryos and to produce an attenuated vaccine—the 17D strain—which is still in use today. The success of this approach led many other investigators from the 1930s to the 1950s to develop animal systems to identify and propagate pathogenic viruses.

Cultures of **eukaryotic** cells can be grown in the laboratory and viruses can be propagated in these cultures, but these techniques are expensive and technically demanding. Some viruses such as influenza virus will replicate in the living tissues of developing embryonated hens' eggs. Egg-adapted strains of influenza virus replicate well in eggs and very high virus **titers** can be obtained. Embryonated hens' eggs were first used to propagate viruses in the early decades of the twentieth century. This method proved to be highly effective for the isolation and culture of many viruses, particularly strains of influenza virus and various poxviruses (e.g., vaccinia virus). Counting the "pocks" on the chorioallantoic membrane of eggs produced by the replication of vaccinia virus was the first quantitative assay for any virus. Animal host systems still have their uses in virology:

- To produce viruses that cannot be effectively studied *in vitro* (e.g., hepatitis B virus).
- To study the pathogenesis of virus infections (e.g. human immunodeficiency virus, HIV, and its near relative, simian immunodeficiency virus, SIV).
- To test vaccine safety (e.g., oral poliovirus vaccine).

Nevertheless, they are increasingly being discarded for the following reasons:

- Breeding and maintenance of animals infected with pathogenic viruses is expensive.
- Animals are complex systems in which it is sometimes difficult to isolate the effects of virus infection.
- Results obtained are not always reproducible due to host variation.
- Unnecessary or wasteful use of experimental animals is morally unacceptable.

With the exception of studying pathogenesis, the use of animals is generally being overtaken by molecular biology methods which are faster and cheaper. In the 1980s the first **transgenic** animals were produced which carried the genes of other organisms. Inserting all or part of a virus **genome** into the DNA of an embryo (typically of a mouse) results in expression of virus **mRNA** and proteins in the animal. This allows the pathogenic effects of virus proteins, individually and in various combinations, to be studied in living hosts. "Humanized" mice have been constructed from immunodeficient animals transplanted with human tissue. These mice form an intriguing model to study the pathogenesis of HIV as there is no real alternative to study the properties of HIV *in vivo*. Similarly, transgenic mice have proved to be vitally important in understanding the biology of **prion** genes. While these techniques raise the same moral objections as "old-fashioned" experimental infection of animals by viruses, they are immensely powerful new tools for the study of virus pathogenicity. A growing number of plant and animal viruses genes have been analyzed in this way, but the results have not always been as expected, and in some cases it has proved difficult to equate the observations obtained with those gathered from experimental infections. Nevertheless, this method has become quite widely used in the study of important diseases where few alternative models exist.

BOX 1.2 WHAT'S THE PROBLEM WITH TRANSGENICS?

For thousands of years farmers have transferred genes from one species of plant into another by crossing two or more species. This is the way that wheat was created over 10,000 years ago. There was no control, other than trial and error, over which genes were transferred or the properties the resulting offspring possessed. In the 1980s it became possible to genetically modify plants and animals by transferring specific genes or groups of genes from another species. And so the controversy over GM crops arose—were they the saviors of humanity, feeding the starving and reducing pollution, or heralds of environmental doom? At about the same time, the first transgenic mice were made. Although there was an outcry at the time, this was dwarfed by the controversy over the first transgenic monkey in 2001. Genetically modified versions of our human relatives seemed too close to home for some people, reminding them of eugenics, the selective breeding of humans with its negative political and moral associations. In truth, science and technology are neutral, and it is societies who ultimately decide how they are used. Should we use these new technologies to feed the world and cure disease, or abandon them for fear of misuse? It's not the technology, it's what we do with it that matters.