# Fundamental VIROLOGY

*Second Edition*

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# Preface

The last decade has seen the coming of age of the revolution of biology initiated by the discovery of the importance and role of DNA in heredity. Because of their simplicity, bacterial viruses played a focal role in many of the important developments emerging during the early phase of this period. More recently, animal viruses have had an equally powerful impact on the study of eukaryotic molecular genetics. In conjunction with these fundamental discoveries, the natural history of infectious diseases has seen equally remarkable changes. Smallpox has disappeared and AIDS has appeared. With the increasing use of immunosuppression, many indigenous or "latent" viruses have taken on increasing importance. In addition, many "classic" viral infections have been controlled, in part, by effective vaccines (polio, measles, rubella) while others have resisted effective preventatives (respiratory syncytial virus). With the striking new insights in molecular biology, some important new insights into fundamental features of viruses as infectious agents have resulted. Not only are there new ways to make vaccines, but the biochemistry of viruses has begun to answer classic questions about epidemiology and pathogenesis. The goal of *Virology*[1] is to bring together basic and medical aspects of virology in a more unified, comprehensive presentation than provided by general textbooks. Thus, *Virology* is a reference and textbook for medical and graduate students as well as scientists, physicians, and investigators interested in viruses as they are represented in the biological sciences.

This book, *Fundamental Virology*, consists of a set of chapters reprinted from *Virology, Second Edition*. Its goal is to provide a text for graduate and upper level undergraduate students, researchers, scientists and investigators whose primary interests are directed towards basic aspects of virology rather than its more clinical or applied features. Like the First Edition, this Second Edition is divided into two parts. The first part, Chapters 1 to 15, presents the basic concepts of general virology while the second part, Chapters 16 to 39, describes the biochemistry, molecular biology and cellular aspects of replication of viruses of the different groups.

An enormous explosion in the information about viruses has occurred in the five years since the First Edition was prepared. In addition to updating all chapters, we have included new chapters on virus evolution, latency and persistence, virus-cell interactions, cell transformation, replication of retroviruses, HIV, cytomegalovirus, Epstein-Barr virus, papilloma viruses, and agents causing spongiform encephalopathies. This text will be very useful for courses in general or molecular virology. We hope course instructors will supplement the material in this book with material on pathogenesis of specific viruses from *Virology*, Second Edition to further unify the fields of molecular virology and pathogenesis.

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# Contents

## Part I General Virology

原书缺页

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CHAPTER 1

# Introduction

Bernard N. Fields and David M. Knipe



## INTRODUCTION TO ANIMAL VIROLOGY

Viruses are replicating microorganisms that are among the smallest of all life forms. This simplicity has led to an obligate requirement for intracellular growth and a heavy dependence on host-cell structural and metabolic components. Although attempts to understand the growth of viruses in cells has occupied much of the efforts of virologists in recent years, viruses are still major causes of human disease. Viral diseases are some of the major scourges of humans and include such virulent disorders as AIDS, yellow fever, rabies, and poliomyelitis. Even with the prevention and virtual elimination of some of the more lethal viral diseases of humans (such as smallpox), viruses remain the most common of all human ailments, including the common acute respiratory and gastrointestinal infections, as well as such important chronic infections as hepatitis and genital herpes.

In spite of the visibility of these infections, it is a remarkable and proven fact that the vast majority of viral infections occur without overt symptoms (hence are subclinical). Thus, the distinction between infection (viral multiplication in an infected host) and disease (the illness due to viral multiplication and its resultant tissue injury) is a critical one that must be made for all viral infections. With some viral infections there are few, if any, cases of multiplication of the virus without overt disease (measles is an example of such a virus), whereas with others the inapparent infection rate may be 100 or more cases of viral multiplication for each clinical case.

In addition to the fact that most viral infections are inapparent, it is quite clear that many viruses can cause more than one type of clinical infection. Such infections may involve more than one organ system (brain, respiratory tract) and may vary from mild to fulminant or fatal. Adding further difficulty for the clinician attempting to diagnose a viral infection is the fact that the same clinical picture (e.g., acute respiratory syndrome) may be caused by different viruses. These observations indicate the absolute dependence on the laboratory to identify definitively the etiology of an individual disease, although in the case of certain viral diseases the clinical findings may be quite distinctive.

Much of virology has emphasized the viruses causing human illness; however, viruses, being as simple as they are, have also played central roles in the unraveling of general principles of modern biology. For example, their simplicity has provided simple genetic systems that have been useful for unraveling the structure and function of genes. Viruses have thus been important in the history of the discipline of molecular biology. The goal of this book is to present up-to-date, comprehensive information concerning both medical and molecular features of viruses of medical importance.

Virology is a new field. It is worth noting that during the 19th century, before the development of bacteriologic techniques, all infectious agents were "vi-

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ruses," and hence crude inoculations were one of the few techniques available for studying the causes of infectious diseases. It was only with the developments by Koch, who used pure culture techniques, that it became possible to isolate bacteria as the causative agent of many infectious diseases. Once bacteria could be established as etiologic agents, it became possible to distinguish bacterial from nonbacterial diseases. In fact, the earliest descriptions of those agents that were likely to be viruses followed the concept of filterable "agents." Such agents were those that appeared infectious, but could not be identified as bacteria by the techniques developed during the late 1800s, and were transmitted after passage through a filter that retained bacteria.

## HISTORY OF VIROLOGICAL TECHNIQUES AND CONCEPTS

### Animal Host Systems

One way of looking at the progressive increase in understanding of viruses that occurred during the 20th century is to review some of the techniques that have been used in virology between the late 1800s and modern times as well as the concepts that played a role in understanding viruses. It was Pasteur, in 1881, who introduced the systematic use of laboratory animals for the recognition and study of rabies virus (22). The use of small laboratory animals for studying viruses and the inoculation of rabies materials directly into the brain of rabbits were the forerunners of later studies whereby a viral agent could be inoculated directly into a highly susceptible organ or tissue. Another important landmark occurred around the turn of the century with the experiments of the U.S. Army Yellow Fever Commission under the direction of Walter Reed (23). Their studies established that yellow fever virus, the first recognized human virus, was present in the blood of patients during the first 3 days of the febrile illness, that the virus could be transmitted by mosquito bites, and that a number of days had to elapse before the mosquito could further transfer infection, thus defining an extrinsic incubation period. It took the studies of Max Theiler some 30 years later to develop and systematically use mice as susceptible animal host to open the modern phase of work on yellow fever (32). This approach ultimately led to other virus isolations, culminating in the isolation by Daldorf and Sickles, in 1948, of the coxsackie group of viruses in suckling mice (8). In addition to mice, the use of chicken embryos was introduced during the early 1930s, and this provided yet another source of susceptible tissues for virus inoculation and growth, especially for the poxvirus group.

As all these experimental systems were developing, quantitative techniques were introduced first during the 1920s for testing lymph containing vacciniavirus in humans and subsequently for assays of other viruses starting with the work of Harvey and Acton in 1923 with rabies virus (13).

### Cell Culture

Modern virology has been heavily dependent on the development of cell culture. Cell cultures were initially introduced during the late 1920s and were extended during the 1940s to studies of encephalitis viruses; in the landmark study of Enders et al. in 1949, cultured cells were shown to be capable of supporting the growth of poliovirus (12). The growth of poliovirus ushered in the modern era of virology and initiated a number of studies that led to the isolation of many of the viruses involved in important human disease. Thus, the era of the 1950s and 1960s was a golden age of medical virology. The isolation of a number of enteric viruses (coxsackie, ECHO) and respiratory viruses (respiratory syncytial virus, adenoviruses) identified the etiologies of a large number of diseases previously only hypothesized to be viral in origin. In parallel with these clinical studies, quantitative virology was adapted to cell culture systems when, in 1952, Dulbecco introduced the plaque assay to animal virology (11). The plaque assay was a direct extension of studies involving bacteria and bacteriophages.

### Bacterial Viruses and Genetics

The finding that bacteria could be infected by viruses began in 1917 with the discovery of bacterial viruses or bacteriophages. d'Herrell thought that virus infection of bacteria might serve a useful therapeutic purpose (10). He was never able to prove this idea or convince his peers of its potential. However, his discovery of the bacteriophage took on a new impetus when during the late 1930s a group of scientists began to study bacteriophages as models for understanding virus–cell interactions in precise physicochemical and genetic terms. Delbruck and Luria, among others, introduced quantitative methodology, carefully developed systems for studying lytic interactions of the bacteriophage, and helped to define the meaning of mutation and many of the techniques and technical approaches needed to fully evaluate important genetic concepts (9,20). Subsequently, studies by Hershey and Chase proved that the genetic information of bacterial viruses was encoded in their nucleic acids (14). Following this observation, it was found that viruses possess either RNA or DNA. Ultimately, many of the principles on which modern molecular biology is based

were established using studies of bacteriophage infections of bacteria. In addition to the lytic interactions whereby a bacterial virus causes lysis of an infected bacteria, Lwoff introduced the concept of a vegetative viral infection whereby the virus enters into the bacterial host without causing lysis (21). Such lysogenic or vegetative infections have provided important insights into how viruses enter host chromosomes, and they helped form the conceptual basis concerning how tumor viruses and latent viruses may interact with cells.

These phage studies were also at the center of the revolution of biology that led to molecular biology. Whereas classic genetic studies did not involve biochemical manipulations, the ability to use bacteriophages as genetic tools allowed a blending of genetics with biochemistry that is at the heart of molecular biology. With the discovery of the structure of DNA during the 1950s, bacteriophages have repeatedly played a key role in developing new insights and new techniques as to the organization of genes, the transcription of DNA, the translation of messenger RNAs, the genetic code, and most recently in the use of viruses as vectors for recombinant DNA. Technologies involving molecular genetics have led to the complete sequencing of viral genes and have provided levels of understanding barely dreamed of a short while before (see Chapter 7).

### Tumor Induction by Viruses

The concept that viruses could be involved in tumors originated when it was shown that Rous sarcoma virus caused tumors in chickens (24) and with the discovery during the 1930s of mammalian tumor viruses (29). Such studies proved that viruses could cause tumors. Subsequently, other viruses were shown to be capable of producing tumors in various small rodents and numerous other animal species; moreover, within the last few years direct proof has been obtained that viral agents are capable of causing tumors in humans (see Chapters 14, 52, 59, and 68). The components of viruses responsible for causing tumors are the "viral oncogenes." This field of study has had additional implications for molecular biology of eukaryotic cells and treatment of human disease. The viral oncogenes have provided some of the best model systems to study the mechanisms of oncogenic transformation of mammalian cells (3) (see Chapter 13). These studies have defined the role and significance of protein kinases and nuclear proteins in cell growth control (6). The discovery that human tumor cells have mutations in genes closely related to those used by RNA tumor viruses to transform cells has given the first insight into the defects in these tumor cells responsible for tumor induction. The study of these protooncogenes is a new and important branch of the field of oncology and is one of the most exciting fields in cancer research (19,28).

### Biochemistry, Molecular Biology, and Cell Biology

Historically, much of virology has focused on identifying viruses in disease situations by studying the pathology of the disease and determining whether a virus is the causative agent. However, plant and animal viruses have also played a role in disciplines other than those involved in human or animal disease. For example, viruses have played an important role in the discipline of biochemistry.

In 1935 a plant virus, tobacco mosaic virus, was crystallized. This provided a powerful tool for thinking of viruses as simple chemicals, consisting solely of protein and ribonucleic acid (30). Other plant viruses were soon crystallized, but it was not until 1955 that the first human virus, poliomyelitis, was crystallized. It is only during the 1970s and 1980s that the fine details of some of these crystal structures have been solved and have begun to help explain the structure and function of important viral proteins. For example, it has become feasible to crystallize individual viral proteins such as the influenza hemagglutinin and neuraminidase, as well as entire intact virions, such as the plant virus tomato bushy stunt, rhinovirus, and poliovirus (see Chapters 3 and 4). These studies have led to correlations of the biochemical features of individual proteins with the precise physical structure and function of the entire virion.

During the 1960s, the techniques of density gradient and velocity gradient centrifugation allowed investigators to purify viruses and identify their individual components and to begin to study in more detail the pathways of viral assembly. In addition, polyacrylamide gel electrophoresis was introduced and was helpful in resolving multiple protein species, initially with poliovirus and eventually in analyzing the more complex protein and nucleic acids of viruses and cultured cells.

During the 1950s, virus particles were thought of as packages of nucleic acids and proteins. The "inert" nature of the viral particle was, however, not fully consistent with the neuraminidase activity of influenza and the lysozyme-like enzymes in bacteriophage tails. During the 1960s and early 1970s, a number of viral enzymes were discovered, most notably the RNA polymerase in vaccinia virus (16) and in a number of RNA viruses (17). The most remarkable enzyme was the reverse transcriptase found in retroviruses capable of transcribing RNA to DNA (1,31). More recently, en-

zymes capable of performing capping of mRNA have been detected (27).

The study of eukaryotic viral gene expression by recent techniques of nucleic acid biochemistry has also yielded fundamental information about eukaryotic molecular biology. For example, the polyadenylated 3′ ends (16) and splicing of mRNAs (2) were first observed with animal viruses. The sequence signals regulating transcription and translation of eukaryotic mRNAs have been defined first for viral genes and mRNAs in many cases (see Chapter 7).

Virus infection of cells provides a probe of the structural organization of the cell because viruses interact so intimately with the host-cell macromolecular machinery. The study of the cell biology of viral gene products has provided insight into the mechanisms of membrane protein localization and nuclear localization, for example. Chapter 12 describes the study of virus interactions with the host cell and the multiple kinds of information that derive from these studies.

### Electron Microscopy and Ultrastructure

Although "spores of micrococci" were noted in vaccine lymph as early as 1886, it was not until the use of electron microscopy in 1940 that the complex structure of bacteriophage was reported (25). Although details of ultrastructure of viral particles were not seen in the early studies, the sizes of virus particles were measured and compared to the measurements obtained by filtration and other techniques. Although there was some variability in measurements, it was clear that the largest viruses were only slightly smaller than bacteria whereas others were extremely small. The use of the negative-contrast staining method was a powerful advance that allowed unfixed material and crude virus suspensions to be analyzed (4). By this time, certain basic principles of virus assembly had been proposed, and it was becoming clear that viral capsids were largely helical or icosahedral (5,7) (see Chapter 3).

An interesting application of electron microscopy was appreciated only later when this method was used to identify viruses that were fastidious in their growth properties, such as the causative agents of viral gastroenteritis (rotaviruses and Norwalk agents) and hepatitis. In addition to these common diseases, electron microscopy played a central role in identifying the etiologic agents of subacute sclerosing panencephalitis and progressive multifocal leukoencephalitis.

### Immunology

One of the goals of virology has always been to learn how to prevent viral diseases. Consistent with progress toward this goal have been the increasing insights into the two forms of host immunity: humoral and cellular (see Chapter 14). The techniques of immunology during the early part of the 20th century were very important in classifying viruses and determining the role of viruses and nature of immunity against viruses. Three techniques have played central roles in studying and classifying viruses. An important immunological technique helpful for rapidly identifying viral agents, hemagglutination, was discovered by Hirst during the 1940s (15). Hemagglutination, the clumping and aggregation of red blood cells, is a rapid physical measure of the presence of a virus whose surface proteins cause agglutination of red blood cells. This technique has had wide impact on the study of viruses and their assay. During the 1960s, the introduction of radioimmune assays similarly had a powerful impact on the ability to rapidly, and with great sensitivity, identify viruses and assay them by immunological means. The use of monoclonal antibodies introduced within the last decade has also provided a most powerful tool for identifying specific regions, or epitopes, on viral proteins and for providing very specific probes for individual viral proteins (18).

Studies involving the ability to grow and clone various T lymphocytes have begun to have important impact on virology. The ability to grow pure T cells has allowed immunologists to identify the role of different cell types in the defense against viral infections and, in addition, has provided cells that are useful for isolating and identifying viruses that grow specifically in these types of lymphoid cell (e.g., human retroviruses; see Chapter 28). The ability to combine monoclonal antibody techniques with molecular cloning and recombinant DNA techniques has provided a powerful approach for isolating and identifying the T-cell receptor (26). This finding has helped elucidate the fact that T cells recognize small peptides in association with class I or class II molecules on the surface of infected cells.

### Prevention and Treatment

Although the various approaches for studying virology range from the most fundamental ones concerned with issues of structure and function of macromolecules to the most practical ones involving identification of viral agents in disease, it is clear that the prevention and treatment of viral infection is one of the major goals of all of virology and certainly the one with the most practical impact on human disease. The first human vaccine introduced by Jenner against smallpox 200 years ago eventually led to the eradication of this viral disease. Other vaccines, such as the yellow fever vaccine, reduced and controlled other important major epidemic diseases. Certain vaccines,

such as those against polio, measles, mumps, and rubella, have dramatically modified or reduced some of the most important of modern diseases. However, it is clear that there remain a number of viral agents to which effective vaccines are not available (e.g., respiratory syncytial virus) or to which effective vaccines are limited in their uses (e.g., hepatitis virus). During the 1980s, a number of new approaches have emerged that have created great optimism among virologists that there will be a new set of antiviral vaccines (see Chapter 15). A number of new technologies have been introduced, including (a) recombinant DNA with production of individual proteins, (b) insertion of genes into viruses such as vaccinia and herpes that can encode additional immunogenic proteins, and (c) simulation of viruses using immunologic means or "anti-idiotypes." All these approaches have been shown under various experimental conditions to have the potential to generate candidate vaccines. Thus, it is likely that we are at the threshold of a new era in the development of newer and safer vaccines. In addition, many of the technologies used for fundamental studies are increasingly being adapted to the study of how viral infections are caused at the most fundamental level (see Chapter 10). The emergence of the AIDS epidemic in the last few years (see Chapter 28) has highlighted how important it is to couple fundamental studies to clinical studies in attempting to control a viral disease. It is likely that, as these studies are extended to more and more viruses, new ways of thinking about the treatment of viral diseases should emerge. Although, in general, viral infections have been more resistant than bacterial infections to chemotherapy, the last several years have seen the use of several drugs that have effective antiviral action. These areas of basic and medical virology pose the problems of the future for animal virology.

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