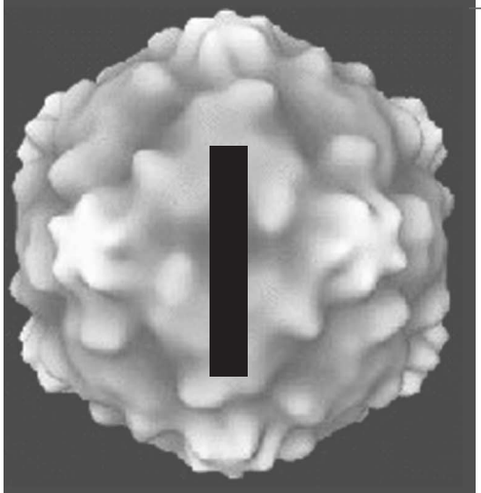
Virology and Viral Disease



PART I

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CHAPTER 1

Introduction – The Impact of Viruses on our View of Life

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The study of viruses has historically provided and continues to provide the basis for much of our most fundamental understanding of modern biology, genetics, and medicine. Virology has had an impact on the study of biological macromolecules, processes of cellular gene expression, mechanisms for generating genetic diversity, processes involved in the control of cell growth and development, aspects of molecular evolution, the mechanism of disease and response of the host to it, and the spread of disease in populations.

In essence, viruses are collections of genetic information directed toward one end: their own replication. They are the ultimate and prototypical example of "selfish genes." The viral genome contains the "blueprints" for virus replication enciphered in the genetic code, and must be decoded by the molecular machinery of the cell that it infects to gain this end. Viruses are, thus, obligate intracellular parasites dependent on the metabolic and genetic functions of living cells.

Submicroscopic, different viruses range in size from smaller than the smallest organelle to just smaller than the simplest cells capable of energy metabolism and protein synthesis, the mycoplasma and simple unicellular algae. Despite their diminutive size, they have evolved and appropriated a means of propagation and replication that ensures their survival in free-living organisms that are between 10 and 10,000,000 times their size and genetic complexity.

The effect of virus infections on the host organism and populations – viral pathogenesis, virulence, and epidemiology

The replication and propagation of a given virus in a population is frequently (but not always) manifest with the occurrence of an infectious disease that spreads between individuals. The study

of effects of viral infection on the host is broadly defined as the study of viral **pathogenesis**. The sum total of the virus-encoded functions that contribute to virus propagation in the infected cell, in the host organism, and in the population is defined as *pathogenicity* of the given virus. This term essentially describes the genetic ability of members of a given specific virus population (which can be considered to be genetically more or less equivalent) to cause a disease and spread through (**propagate** in) a population. Thus, a major factor in the pathogenicity of a given virus is its genetic makeup or **genotype**.

The basis for severity of the symptoms of a viral disease in an organism or a population is complex. It results from an intricate combination of expression of the viral genes controlling pathogenicity, physiological response of the infected individual to these pathogenic determinants, and response of the population to the presence of the virus propagating in it. Taken together, these factors determine or define the **virulence** of the virus and the disease it causes.

A basic factor contributing to virulence is the interaction among specific viral genes and the genetically encoded defenses of the infected individual. It is important to understand, however, that virulence is also affected by the general health and genetic makeup of the infected population, and in humans, by the societal and economic factors that affect the nature and extent of the response to the infection.

The distinction and gradation of meanings between the terms *pathogenesis* and *virulence* can be understood by considering the manifold factors involved in disease severity and spread exhibited in a human population subjected to infection with a disease-causing virus. Consider a virus whose genotype makes it highly efficient in causing a disease, the symptoms of which are important in the spread between individuals—perhaps a respiratory infection with accompanying sneezing, coughing, and so on. This ideal or optimal virus may (and often does) incorporate numerous genetic changes during its replication cycles as it spreads in an individual and in the population. Some viruses generated during the course of a disease may, then, contain genes that are not optimally efficient in causing symptoms. Such a virus is of reduced virulence, and in the extreme case, it might be a virus that has accumulated so many mutations in pathogenic genes that it can cause no disease at all (i.e., has mutated to an **avirulent** or **apathogenic** strain). While an avirulent virus may not cause a disease, its infection may well lead to complete or partial **immunity** against the most virulent genotypes in an infected individual. This is the basis of **vaccination**, which is described in Chapter 8. But the capacity to generate an immune response and the resulting generation of **herd immunity** also means that as a virus infection proceeds in a population, its virulence either must change or the virus must genetically adapt to the changing host.

Other factors not fully correlated with the genetic makeup of a virus also contribute to variations in virulence of a pathogenic genotype. The same virus genotype infecting two **immunologically naive** individuals (i.e., individuals who have never been exposed to any form of the virus leading to an immune response) can cause very different outcomes. One individual might only have the mildest symptoms because of exposure to a small amount of virus, or infection via a suboptimal route, or a robust set of immune and other defense factors inherent in his or her genetic makeup. Another individual might have a very severe set of symptoms or even death if he or she receives a large inoculum, or has impaired immune defenses, or happens to be physically stressed due to malnutrition or other diseases.

Also, the same virus genotype might cause significantly different levels of disease within two more-or-less genetically equivalent populations that differ in economic and technological resources. This could happen because of differences in the ability of one society's support net to provide for effective medical treatment, or to provide for isolation of infected individuals, or to have available the most effective treatment protocols.

Taken in whole, the study of human infectious disease caused by viruses and other pathogens defines the field of **epidemiology** (in animals it is termed **epizoology**). This field requires a good un-

derstanding of the nature of the disease under study and the types of medical and other remedies available to treat it and counter its spread, and some appreciation for the dynamics and particular nuances and peculiarities of the society or population in which the disease occurs.

The interaction between viruses and their hosts

The interaction between viruses (and other infectious agents) and their hosts is a dynamic one. As effective physiological responses to infectious disease have evolved in the organism and (more recently) have developed societally through application of biomedical research, viruses themselves respond by exploiting their naturally occurring genetic variation to accumulate and select mutations to become wholly or partially resistant to these responses. Such resistance has led to periodic or episodic reemergence of diseases thought to have been controlled.

The accelerating rate of human exploitation of the physical environment and the accelerating increase in agricultural populations afford some viruses new opportunities to "break out" and spread both old and novel diseases. Evidence of this is the ongoing **acquired immune deficiency syndrome (AIDS)** epidemic, as well as sporadic occurrences of viral diseases, such as hemorrhagic fevers in Asia, Africa, and southwestern United States. Investigation of the course of a viral disease, as well as societal responses to it, provides a ready means to study the role of social policies and social behavior of disease in general.

The recent worldwide spread of AIDS is an excellent example of the role played by economic factors and other aspects of human behavior in the origin of a disease. The causative agent, **human immunodeficiency virus (HIV)**, may well have been introduced into the human population by an event fostered by agricultural encroachment of animal habitats in equatorial Africa. This is an example of how economic need has accentuated risk.

HIV is not an efficient pathogen; it requires direct inoculation of infected blood or body fluids for spread. In the Euro-American world, the urban concentration of homosexual males with sexual habits favoring a high risk for venereal disease had a major role in spreading HIV and resulting AIDS throughout the male homosexual community. A partial overlap of this population with intravenous drug users and participants in the commercial sex industry resulted in spread of the virus and disease to other portions of urban populations. The result is that in Western Europe and North America, AIDS has been a double-edged sword threatening two disparate urban populations: the relatively affluent homosexual community and the impoverished heterosexual world of drug abusers—both highly concentrated urban populations. In the latter population, the use of commercial sex as a way of obtaining money resulted in further spread to other heterosexual communities, especially those of young, single men and women.

An additional factor is that the relatively solid medical and financial resources of a large subset of the "economic first world" resulted in wide use of whole blood transfusion, and more significantly, pooled blood fractions for therapeutic use. This led to the sudden appearance of AIDS in hemophiliacs and sporadically in recipients of massive transfusions due to intensive surgery. Luckily, the incidence of disease in these last risk populations has been reduced owing to effective measures for screening blood products.

Different societal factors resulted in a different distribution of HIV and AIDS in equatorial Africa and Southeast Asia. In these areas of the world, the disease is almost exclusively found in heterosexual populations and much more frequently in women than in men. This distribution of AIDS occurred because a relatively small concentration of urban commercial sex workers acted as the source of infection of working men living apart from their families. The periodic travel by men to their isolated village homes resulted in the virus being found with increasing frequency in isolated family units. Further spread resulted from infected women leaving brothels and prostitution to return to their villages to take up family life.

Another overweening factor in the spread of AIDS is technology. HIV could not have spread and posed the threat it now does in the world of a century ago. Generally lower population densities and lower concentrations of individuals at risk at that time would have precluded HIV from gaining a foothold in the population. Slower rates of communication and much more restricted travel and migration would have precluded rapid spread; also the transmission of blood and blood products as therapeutic tools was unknown a century ago.

Of course, this dynamic interaction between pathogen and host is not confined to viruses; any pathogen exhibits it. The study and characterization of the genetic accommodations viruses make, both to natural resistance generated in a population of susceptible hosts and to human-directed efforts at controlling the spread of viral disease, provide much insight into evolutionary processes and population dynamics. Indeed, many of the methodologies developed for the study of interactions between organisms and their environment can be applied to the interaction between pathogen and host.

The history of virology

The historic reason for the discovery and characterization of viruses, and a continuing major reason for their detailed study, involve the desire to understand and control the diseases and attending degrees of economic and individual distress caused by them. As studies progressed, it became clear that there were many other important reasons for the study of viruses and their replication.

Since viruses are parasitic on the molecular processes of gene expression and its regulation in the host cell, an understanding of viral genomes and virus replication provides basic information concerning cellular processes in general.

The whole development of molecular biology and molecular genetics is largely based on the deliberate choice of some insightful pioneers of "pure" biological research to study the replication and genetics of viruses that replicate in bacteria: the bacteriophages. (Such researchers include Max Delbrück, Salvadore Luria, Joshua Lederberg, Gunther Stent, Seymour Benzer, Andre Lwoff, François Jacob, Jacques Monod, and many others.)

The bacterial viruses (**bacteriophage**) were discovered through their ability to destroy human enteric bacteria such as *Escherichia coli*, but they had no clear relevance to human disease. It is only in retrospect that the grand unity of biological processes from the most simple to the most complex can be seen as mirrored in replication of viruses and the cells they infect.

The biological insights offered by the study of viruses have led to important developments in biomedical technology and promise to lead to even more dramatic developments and tools. For example, when infecting an individual, viruses target specific tissues. The resulting specific symptoms, as already noted, define their pathogenicity. The normal human, like all vertebrates, can mount a defined and profound response to virus infections. This response often leads to partial or complete immunity to reinfection. The study of these processes was instrumental to gaining an increasingly clear understanding of the immune response and the precise molecular nature of cell–cell signaling pathways. It also provided therapeutic and preventive strategies against specific virus-caused disease. The study of virology has and will continue to provide strategies for the **palliative treatment** of metabolic and genetic diseases not only in humans, but also in other economically and aesthetically important animal and plant populations.

Examples of the impact of viral disease on human history

There is archeological evidence in Egyptian mummies and medical texts of readily identifiable viral infections, including genital papillomas (warts) and poliomyelitis. There are also somewhat imperfect historical records of viral disease affecting human populations in classical and medieval times. While the recent campaign to eradicate smallpox has been successful and it no longer exists

in the human population (owing to the effectiveness of vaccines against it, the genetic stability of the virus, and a well-orchestrated political and social effort to carry out the eradication), the disease periodically wreaked havoc and had profound effects on human history over thousands of years. Smallpox epidemics during the Middle Ages and later in Europe resulted in significant population losses as well as major changes in the economic, religious, political, and social life of individuals. Although the effectiveness of vaccination strategies gradually led to the decline of the disease in Europe and North America, smallpox continued to cause massive mortality and disruption in other parts of the world until after World War II. Despite its apparent control, recently fears have arisen that the high virulence of the virus and its mode of spread might make it an attractive agent for **bioterrorism**.

Other virus-mediated epidemics had equally major roles in human history. Much of the social, economic, and political chaos in native populations resulting from European conquests and expansion from the fifteenth through nineteenth centuries was mediated by introduction of infectious viral diseases such as measles. Significant fractions of the indigenous population of the western hemisphere died as a result of these diseases.

Potential for major social and political disruption of everyday life continues to this day. As discussed in the final chapter of this book, the "Spanish" influenza of 1918–19 killed millions worldwide and in conjunction with the effects of World War I, came very close to causing a major disruption of world civilization. We do not and may never know what the specific reasons were for the virulence of this disease; surely there is no reason why another could not arise with a similar or more devastating aftermath or **sequela**. Currently a number of infectious diseases could become established in the general population as a consequence of their becoming drug resistant or the human disruption of natural ecosystems. Viral diseases that could play such a role include yellow fever, equine **encephalitis**, dengue fever, Ebola and Rift Valley fevers, *Hantavirus* pathologies, and the newly characterized coronavirus causing severe acute respiratory syndrome (**SARS**).

Animal and plant pathogens are other potential sources of disruptive viral infections. Sporadic outbreaks of viral disease in domestic animals, for example, vesicular stomatitis virus in cattle and avian influenza in chickens, result in significant economic and personal losses. Rabies in wild animal populations in the eastern United States has spread continually during the past half-century. The presence of this disease poses real threats to domestic animals and through them occasionally, to humans. An example of an agricultural infection leading to severe economic disruption is the growing spread of the Cadang-cadang viroid in coconut palms of the Philippine Islands and elsewhere in Oceania. The loss of coconut palms led to serious financial hardship in local populations.

Examples of the evolutionary impact of the virus–host interaction

There is ample genetic evidence that the interaction between viruses and their hosts had a measurable impact on evolution of the host. Viruses provide environmental stresses to which organisms evolve responses. Also, it is possible that the ability of viruses to acquire and move genes between organisms provides a mechanism of gene transfer between lineages.

Development of the immune system, the cellular-based antiviral **interferon (IFN)** response, and many of the inflammatory and other responses that multicellular organisms can mount to ward off infection is the result of successful genetic adaptation to infection. More than this, virus infection may provide an important (and as yet underappreciated) basic mechanism to affect the evolutionary process in a direct way.

There is good circumstantial evidence that the specific origin of placental mammals is the result of an ancestral species being infected with an immunosuppressive proto-retrovirus. It is suggested that this immunosuppression allowed the mother to immunologically accommodate the development of a genetically distinct individual in the placenta during a prolonged period of gestation!

Two current examples provide very strong evidence for the continued role of viruses in the evolution of animals and plants. Certain parasitic wasps lay their eggs in the caterpillars of other insects. As the wasp larvae develop, they devour the host, leaving the vital parts for last to ensure that the food supply stays fresh! Naturally, the host does not appreciate this attack and mounts an immune defense against the invader—especially at the earliest stages of the wasp's embryonic development. The wasps uninfected with a **polydnavirus** do not have a high success rate for their parasitism and their larvae are often destroyed. The case is different when the same species of wasp is infected with a polydnavirus that is then maintained as a persistent genetic passenger in the ovaries and egg cells of the wasps. The polydnavirus inserted into the caterpillar along with the wasp egg induces a systemic, immunosuppressive infection so that the caterpillar cannot eliminate the embryonic tissue at an early stage of development! The virus maintains itself by persisting in the ovaries of the developing female wasps.

An example of a virus's role in development of a symbiotic relationship between its host and another organism can be seen in replication of the ***Chlorella* viruses**. These viruses are found at concentrations as high as 4×10^4 infectious units/ml in freshwater throughout the United States, China, and probably elsewhere in the world. Such levels demonstrate that the virus is a very successful pathogen. Despite this success, the viruses can only infect free algae; they cannot infect the same algae when the algae exist semisymbiotically with a species of paramecium. Thus, the algae cells that remain within their symbiotes are protected from infection, and it is a good guess that existence of the virus is a strong selective pressure toward establishing or stabilizing the symbiotic relationship.

The origin of viruses

Although there is no geological record of viruses (they do not form fossils in any currently useful sense), the analysis of the relationship between the amino acid sequences of viral and cellular proteins and that of the nucleotide sequences of the genes encoding them provide ample genetic evidence that the association between viruses and their hosts is as ancient as the origin of the hosts themselves. Some viruses (e.g., retroviruses) integrate their genetic material into the cell they infect, and if this cell happens to be a germ line, the viral genome (or its relict) can be maintained essentially forever. Analysis of the sequence relationship between various retroviruses found in mammalian genomes demonstrates integration of some types before major groups of mammals diverged.

While the geological record cannot provide evidence of when or how viruses originated, genetics offers some important clues. First, the vast majority of viruses do not encode genes for ribosomal proteins or genetic evidence of relicts of such genes. Second, this same vast majority of viruses do not contain genetic evidence of ever having encoded enzymes involved in energy metabolism. This is convincing evidence that the viruses currently investigated did not evolve from free-living organisms. This finding distinctly contrasts with two eukaryotic organelles, the mitochondrion and the chloroplast, known to be derived from free-living organisms, and is convincing evidence that the viruses currently investigated did not evolve from free-living organisms.

Genetics also demonstrates that a large number of virus-encoded enzymes and proteins have a common origin with cellular ones of similar or related function. For example, many viruses containing DNA as their genetic material and viral-encoded DNA polymerase are clearly related to all other DNA polymerase isolated from plants, animals, or bacteria. The reverse transcriptase enzyme encoded by retroviruses, and absolutely required for converting genetic information contained in RNA to DNA, is related to an important eukaryotic enzyme involved in reduplicating the telomeric ends of chromosomes upon cell division.

Such considerations are consistent with a model that places virus origin in the cells they infect. Thus, the relationship between certain portions of the replication cycle of retroviruses and mecha-

nisms of gene transposition in cells suggests that retroviruses may have originated as types of retrotransposons, which are circular genetic elements that can move from one chromosomal location to another. Some plant viruses may have arisen as the result of gene capture by the self-replicating RNA molecules seen in some plants. Other viruses may have arisen by more bizarre mechanisms.

A major complication to a complete and satisfying scheme for the origin of viruses is that a large proportion of viral genes have no known cellular counterparts, and viruses themselves may be a source of much of the genetic variation seen between different free living organisms. In an extensive analysis of the relationship between groups of viral and cellular genes, L. P. Villarreal points out that the deduced size of the **Last Universal Common Ancestor (LUCA)** to eukaryotic and prokaryotic cells is on the order of 300 genes—no bigger than a large virus, and provides some very compelling arguments for viruses having provided some of the distinctive genetic elements that distinguish cells of the eukaryotic and prokaryotic kingdoms. In such a scheme, precursors to both viruses and cells originated in pre-biotic environment hypothesized to provide the chemical origin of biochemical reactions leading to cellular life.

At the level explored here, it is probably not terribly useful to spend great efforts to be more definitive about virus origins beyond their functional relationship to the cell and organism they infect. The necessarily close mechanistic relationship between cellular machinery and the genetic manifestations of viruses infecting them makes viruses important biological entities, but it does not make them organisms. They do not grow, they do not metabolize small molecules for energy, and they only "live" when in the active process of infecting a cell and replicating in that cell. The study of these processes, then, must tell as much about the cell and the organism as it does about the virus. This makes the study of viruses of particular interest to biologists of every sort.

Viruses have a constructive as well as destructive impact on society

Often the media and some politicians would have us believe that infectious diseases and viruses are unremitting evils, but to quote Sportin' Life in Gershwin's *Porgy and Bess*, this "ain't necessarily so." Without the impact of infectious disease, it is unlikely that our increasingly profound understanding of biology would have progressed as it has. As already noted, much of our understanding of the mechanisms of biological processes is based in part or in whole on research carried out on viruses. It is true that unvarnished human curiosity has provided an understanding of many of the basic patterns used to classify organisms and fostered Darwin's intellectual triumph in describing the basis for modern evolutionary theory in his *Origin of Species*. Still, focused investigation on the microscopic world of pathogens needed the spur of medical necessity. The great names of European microbiology of the nineteenth and early twentieth centuries—Pasteur, Koch, Ehrlich, Fleming, and their associates (who did much of the work with which their mentors are credited)—were all medical microbiologists. Most of the justification for today's burgeoning biotechnology industry and research establishment is medical or economic.

Today, we see the promise of adapting many of the basic biochemical processes encoded by viruses to our own ends. Exploitation of virus diseases of animal and plant pests may provide a useful and regulated means of controlling such pests. While the effect was only temporary, the introduction of **myxoma** virus—a pathogen of South American lagomorphs (rabbits and their relatives)—had a positive role in limiting the predations of European rabbits in Australia. Study of the adaptation dynamics of this disease to the rabbit population in Australia taught much about the coadaptation of host and parasite.

The exquisite cellular specificity of virus infection is being adapted to generate biological tools for moving therapeutic and palliative genes into cells and organs of individuals with genetic and degenerative diseases. Modifications of viral-encoded proteins and the genetic manipulation of viral genomes are being exploited to provide new and (hopefully) highly specific **prophylactic** vaccines as well as other therapeutic agents. The list increases monthly.

Viruses are not the smallest self-replicating pathogens

Viruses are not the smallest or the simplest pathogens able to control their self-replication in a host cell—that distinction goes to **prions**. Despite this, the methodology for the study of viruses and the diseases they cause provides the basic methodology for the study of all subcellular pathogens.

By the most basic definition, viruses are composed of a genome and one or more proteins coating that genome. The genetic information for such a protein coat and other information required for the replication of the genome are encoded in that genome. There are genetic variants of viruses that have lost information either for one or more coat proteins or for replication of the genome. Such virus-derived entities are clearly related to a parental form with complete genetic information, and thus, the mutant forms are often termed **defective virus particles**.

Defective viruses require the coinfection of a **helper virus** for their replication; thus, they are parasitic on viruses. A prime example is hepatitis delta virus, which is completely dependent on coinfection with hepatitis B virus for its transmission.

The hepatitis delta virus has some properties in common with a group of RNA pathogens that infect plants and can replicate in them by, as yet, obscure mechanisms. Such RNA molecules, called **viroids**, do not encode any protein, but can be transmitted between plants by mechanical means and can be pathogens of great economic impact.

Some pathogens appear to be entirely composed of protein. These entities, called **prions**, appear to be cellular proteins with an unusual folding pattern. When they interact with normally folded proteins of the same sort in neural tissue, they appear to be able to induce abnormal refolding of the normal protein. This abnormally folded protein interferes with neuronal cell function and leads to disease. While much research needs to be done on prions, it is clear that they can be transmitted with some degree of efficiency among hosts, and they are extremely difficult to inactivate. Prion diseases of sheep and cattle (scrapie and "mad cow" disease) recently had major economic impacts on British agriculture, and several prion diseases (**kuru** and **Creutzfeldt-Jacob disease [CJD]**) infect humans. Disturbingly, passage of sheep scrapie through cattle in England has apparently led to the generation of a new form of human disease similar to, but distinct from, CJD.

The existence of such pathogens provides further circumstantial evidence for the idea that viruses are ultimately derived from cells. It also provides support for the possibility that viruses had multiple origins in evolutionary time.

QUESTIONS FOR CHAPTER 1

1 Viruses are a part of the biosphere. However, there is active debate concerning whether they should be treated as living or nonliving.

a Briefly describe one feature of viruses that is *also found* in cell-based life forms.

b Briefly describe one feature of viruses that *distinguishes* them from cell-based life forms.

2 Why is it likely that viruses have not evolved from free-living organisms?

3 Give examples of infectious agents that are smaller self-replicating systems than viruses.

4 Ebola virus is a deadly (90% case-fatality rate for some strains) infectious agent. Most viruses, however, are not nearly as lethal. Given the nature of viruses, why would you expect this to be so?

5 Given that viruses are a part of the biosphere in which other organisms exist, what might be the kinds of selective pressure that viruses exert on evolution?