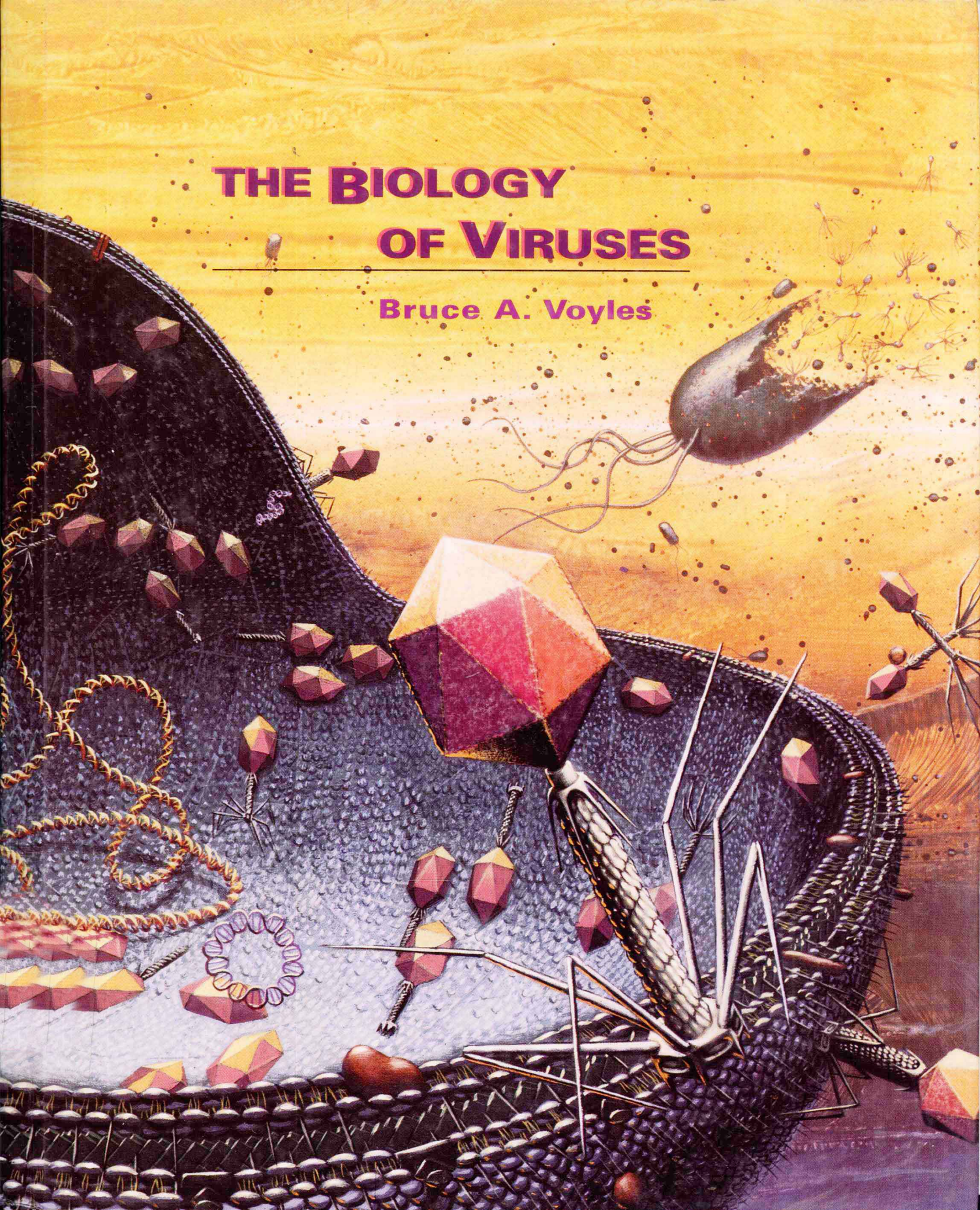
THE BIOLOGY OF VIRUSES

Bruce A. Voyles



THE BIOLOGY OF VIRUSES

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with 195 *illustrations*

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Preface

For 25 years I have been fascinated by the viruses, not just as disease-causing agents, but as organisms that have evolved a wonderful variety of solutions to the problems posed by their reliance on host cells for their reproduction. In the course of studying these organisms, virologists discovered many of the mechanisms by which prokaryotes and eukaryotes replicate and express their own genomes (the bidirectional replication of DNA and the splicing of RNA are just two examples). More recently, bacteriophages like lambda and M13 and animal viruses like vaccinia and the retroviruses have become versatile research tools of molecular biologists. Clearly, the viruses are for everyone, not just the virologist or clinician.

Probably like many first-time authors, I was driven to write this book by frustration. When I wanted to share my fascination with the viruses with undergraduates, I could not find a suitable text. General microbiology texts relegate the viruses to a few examples covered in a handful of pages. Virology books range from "everything-you-ever-wanted-to-know-plus-a-little-bit-extra" encyclopedias that are taxonomic guides to all the viruses to specialized books for medical students or plant pathologists that are narrowly focused on particular viruses or groups of viruses. While such books are wonderful things of their sort, I wanted an introduction to the viruses that illustrated the common features of their life-styles rather than described their every detail; I wanted the forest, but could get only the trees. Hence this book.

SCHEME OF THE BOOK

The core of this book is organized around the features of the life cycle shared by all viruses. How are viruses structured? What strategies do viruses use to enter their host cells? How do they express and replicate their genomes? How do they produce new

virions? How do host cells respond to viral infection? How might the viruses have evolved? The approach is to describe the general strategies that the viruses have evolved to meet particular situations, with illustrations taken from a variety of different viruses, rather than to present each taxonomic group of viruses in its entirety. For this reason, examples are taken across the spectrum of the bacterial, animal, and plant viruses. My hope is that this "theme and variation" approach will allow the nonexpert to appreciate the viruses as biological entities that share many common features. To my colleagues who may think this approach is too idiosyncratic for their courses, I can only say: "Try it! You'll like it!"

FEATURES

I have tried to create a book that is "user friendly" so that students will actually read it. To this end, I have incorporated a number of features into the book to make it easier to follow the development of the material and its presentation:

- In order to emphasize concepts, the material in each chapter is presented to the student in the form of questions opening each section. The text then provides the answers to the questions.
- Since many of the illustrations depict the organization or expression of genetic material, the book uses a set of standard conventions that allows the student to identify immediately the exact nature of the molecules being shown and their relationships to each other.
- Each chapter concludes with a summary and an annotated list of suggested readings from readily available sources. These include *Scientific American* articles, reviews of broad topics, as well as some of the classic works in the field.
- Since throughout the text there are frequent discussions on how virologists know what they know, an appendix, The Virologist's Toolkit, presents brief descriptions of the experimental techniques widely used in virology.
- Since the body of the text does not use a taxonomic approach, a second appendix, Characteristics of Selected Viruses, provides brief summaries (keyed to the text) of the features and life cycles of the viral groups discussed in the book.

ACKNOWLEDGMENTS

Like Blanche Dubois, authors must frequently "rely on the kindness of strangers" in the preparation of their books. I am especially grateful to a fine panel of reviewers who read the manuscript carefully and gave me invaluable assistance both by their brickbats and their bouquets: Jean Acton, James Madison University; Richard Adler, University of Michigan-Dearborn; Penny Amy, University of Nevada-Las Vegas; Lee Beldon, University of Wyoming-Laramie; Katherine J. Denniston, Towson State University; Christina Lee Frazier, Southeast Missouri State University; Gerald Goldstein, Ohio Wesleyan University; Judith Kandel, California State University-Los Angeles; Ruth Kibbler, San Jose State University; John Knesek, Texas Woman's University; Ken Platt, Iowa State University-Ames; Gerry Silverstein, University of Vermont-Burlington, William Steinhart, Bowdoin College. Any errors remaining in the text are

the result of my own cussedness. Other "kind strangers" include the scientists who permitted me to use original photographs or illustrations from their papers in this book. The credit line on each illustration is only a token of my thanks for their courtesy and assistance.

Authors also require the kindness of nonstrangers. My thanks to my colleagues in the Biology Department of Grinnell College, especially Charles Sullivan, for their patience and forbearance as I worked on the book, to the division secretaries for their assistance in so many ways, and to Carolyn Bosse for her help in preparing photographs, mailing requests for permissions, and for patiently listening to my moanings and groanings. Great thanks are also due to my editors at Mosby, especially my developmental editor Laura Edwards. Over the past 2 years Laura has been "the other woman in my life," albeit at a safe 300-mile remove, as she provided handholding and support, or the lash to my back, as the situation required. This is really "our" book.

Finally, thanks beyond measure to my wife Martha and my children Paul and Erin. Their loving support made it possible—and we're still together to enjoy the royalties, if any!

Bruce A. Voyles

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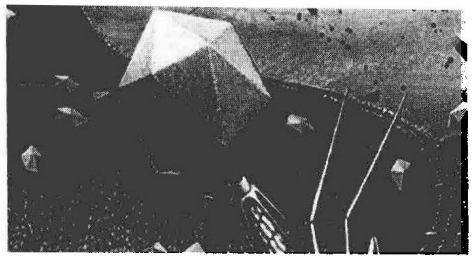

CHAPTER ONE

Viruses and Host Cells

- What is a virus?
- What are the physical properties of viruses?
- What structural features of cells are important in their role as viral hosts?
- What genetic features of cells are important in the virus–host cell interaction?
- What are the basic techniques used to study the virus–host cell interaction?
- What are the general features of the interaction between a virus and its host cell?
- How are viruses named?
- What is a virus?

WHAT IS A VIRUS?

Virus. The word itself is unadorned Latin for "poison," dramatic evidence of the long association of these entities with the medical woes of humankind. As the germ theory of disease became established as a central paradigm of medical science at the end of the nineteenth century, a great blossoming of medical microbiology occurred. The causative agents of numerous diseases were isolated, observed under the microscope, cultured on artificial media, and demonstrated to cause their particular pathologies. The causative agents of some diseases, however, could not be studied in that fashion. Among these were the viruses. Since they did not "follow the rules," these mysterious entities came to be described in terms that were all essentially negative in their construction and connotations.

First, viruses were *small.* Unlike bacteria, they could not be seen under the light microscope. More significantly, they were so small they could pass through the filters used to sterilize solutions by removing bacteria and other contaminants. This quality

of smallness is not a defining characteristic, however, since it is not unique to the viruses. Some bacteria like the mycoplasma are also small enough to pass through the filters used for bacterial sterilization.

Second, *viruses could not be cultivated on artificial media* in the same fashion as other organisms like bacteria. This characteristic is also not the exclusive property of the viruses, since a number of bacteria cannot be cultivated on artificial media. Despite more than a century of searching, no artificial medium has yet been found to support the growth of the bacterium that causes syphilis, for example, so it still must be cultured within the tissues of a host organism or in conjunction with animal cells in in vitro culture systems.

Viruses are even more demanding than the syphilis organism, however. They must be cultivated not only in a host organism, but within a host *cell.* Viruses are *intracellular parasites* that require the metabolic activities of a host cell to support their growth. This, too, is not a characteristic unique to the viruses. Two groups of bacteria, the rickettsia and the chlamydia, are also intracellular parasites and require specific metabolites from their host cells for growth.

What, then, are viruses? We see from these examples that viruses cannot be defined by negatives, that is, by how they fail to fit the characteristics of other organisms like the bacteria. Their small size and their requirement for a host cell to support their growth are not unique. What characteristics do define the viruses? To address that question we shall begin by considering several sets of hypothetical experimental data in order to develop a positive picture of the virus and how it differs from the cells that may be its hosts. These will be "generic" data that will enable us to consider the properties of a "typical" bacterial virus and a "typical" bacterial host cell such as *Escherichia coli.*

The first experiment involves a biochemical analysis of our generic bacterial virus and its host cell. The results of this analysis are presented in Table 1-1. The differences are striking. The virus contains only a single type of nucleic acid (DNA in this case), while the host cell contains both DNA and RNA. The remainder of the virus is protein or glycoprotein. It contains none of the lipids, glycolipids, simple sugars,

TABLE 1-1 **Comparison (In Percentages) of the Biochemical Composition of a Bacterial Virus and a Bacterium**

Component	Host Cell	Virus
DNA	3	40
RNA	21	0
Protein and glycoprotein	55	60
Lipid	12	0
Polysaccharide	5	0
Small molecules and ions	4	0

Host cell values are modified from data given in *Growth of the Bacterial Cell* by Ingraham, Maaloe, and Neidhardt, 1983; virus values are from *The Genetics of Bacteria and Their Viruses* by Hayes, 1964.

polysaccharides, nucleotides like ATP and ADP, free amino acids, and other small molecules that occur in its bacterial host cell. Clearly this bacterial virus is fundamentally simpler than its host cell at the biochemical level.

The second experiment involves determining growth curves for our "typical" bacterial virus and bacterium. The growth curve for a bacterium like *E. coli* is shown in Figure 1-1, *A*. One bacterium was introduced into a suitable nutrient broth and allowed to grow. At closely spaced intervals, an exceptionally able scientist carefully determined the exact number of organisms present and plotted those numbers using the conventional semilogarithmic plot, which relates the number of organisms to time. The data fall on a straight line on this semilogarithmic plot, which means that growth in the culture is *exponential;* that is, a geometric progression resulted as that one cell produced two, those two produced four, those four produced eight, and so forth. Eventually there is no further increase in cell number and growth appears to cease in the culture. If our scientist continues this experiment by setting the *E. coli* culture aside to sit on a shelf for 1 year and at the end of that time examines a bacterium taken from it, she will not be able to detect any metabolic activity of any kind in the cell. Furthermore, when a bacterium from that aged culture is transferred into fresh medium, nothing happens; the bacterium appears no longer able to initiate growth.

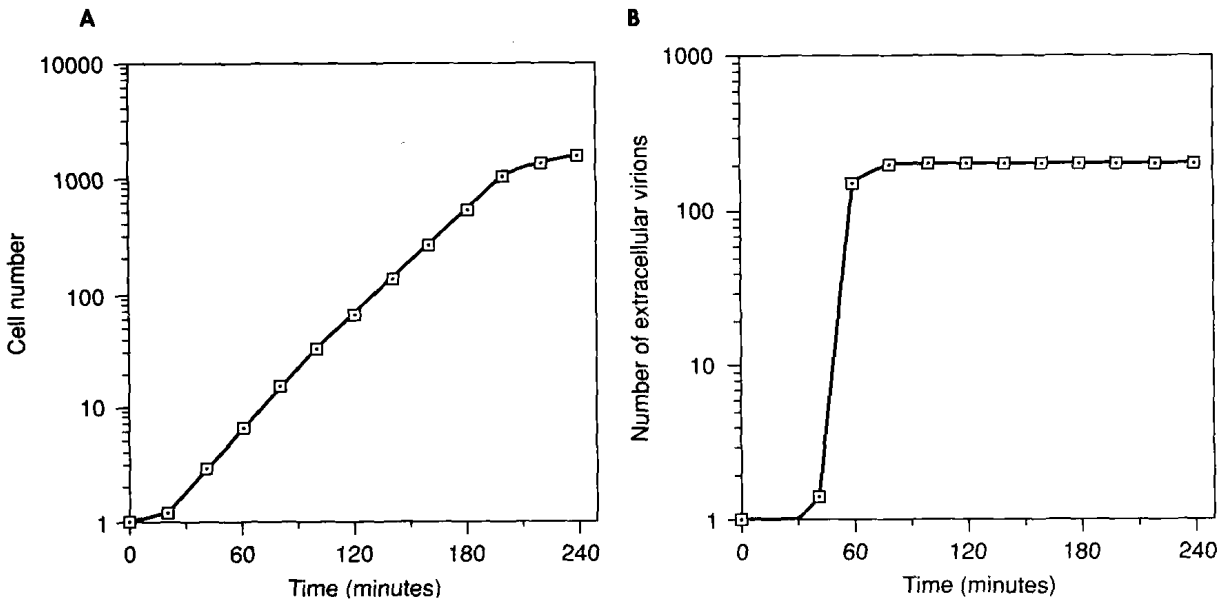

FIGURE 1-1 Growth curves for a "typical" bacterium and a "typical" bacterial virus. A, Growth of a bacterium. The number of cells in this culture doubles about every 20 minutes until exhaustion of the medium causes the rate to slow. B, Growth of a bacterial virus. The virus put into the culture at the beginning of the experiment disappears and no extracellular viruses are detected until an abrupt increase occurs at about 60 minutes. There is no further increase after that sharp rise.

The graph in Figure 1-1, *B*, shows a similar experiment performed with a "typical" bacterial virus. The pattern is completely different. When the virus is introduced to a culture containing suitable host cells (since we have already established that a host is necessary for growth of the virus), our scientist initially sees no increase whatsoever in the number of viruses. In fact, when the scientist searches very, very diligently within that culture, she is not even able to find the virus she initially introduced. It seems to have disappeared even from within its host cell! Then, suddenly, there is a dramatic, almost instantaneous, increase in the number of viruses in the culture. One virus appears to have given rise to 150 new viruses all at once rather than by means of a geometric progression. If no additional host cells are available, there is no further change in the number of viruses. Addition of new host cells, however, causes the cycle to repeat itself. When this experiment is continued in parallel with the first, with the culture being allowed to sit for a year untouched, and then one of the viruses is examined carefully, our scientist again detects no metabolic activity, just as with the bacterium. When a virus that shows no metabolic activity is introduced into a culture containing live host cells, however, the same pattern of growth occurs as in the first instance. Clearly the absence of metabolic activity means something quite different in a virus and a bacterium.

What can we conclude from comparison of these experiments? First, it is obvious that the nature of growth of the bacterial virus is fundamentally different from the nature of growth of the bacterium itself. The bacterium appears to multiply by a process of cell division since its numbers increase exponentially. The virus does not appear to multiply by cell division since its growth is not exponential, but rather shows a pattern of plateaus and very sharp rises. Secondly, the lack of any detectable metabolic activity in a bacterial cell and a bacterial virus indicates fundamentally different states. Since the bacterium cannot grow again, it appears to have changed from a living state to a nonliving state. Although the virus would also appear to be in that same nonliving state, it nonetheless could initiate growth when introduced into the appropriate conditions. Although questions about the nature of life itself properly belong to the realm of metaphysics, it is clear that viruses and host cells differ in some fundamental fashion in this context as well.

WHAT ARE THE PHYSICAL PROPERTIES OF VIRUSES?

Our experiments on a "typical" bacterial virus and its host cell suggested that viruses are very different in structure from the cells that host them. We noted that the virus that was the subject of those experiments contained only DNA, while the host cell had both DNA and RNA. In many other viruses the single nucleic acid type present is *RNA*, not DNA. The only other biochemical constituents of the simple bacterial virus in our example were proteins and glycoproteins. The host cell, in contrast, contained proteins and glycoproteins, lipids, and glycolipids, simple sugars and polysaccharides, as well as a vast array of small biochemical molecules like nucleotides (especially ATP and ADP) and amino acids. Clearly the virus must possess a much simpler architecture than its host cell. Equally clearly, since the sole nucleic acid pre-

present can be RNA rather than DNA, viruses can function with different genetic systems than their host cells.

The Genome

The genome of host cells occurs only in the form of a double-stranded molecule of DNA. In prokaryotic cells the single molecule of the genome is arranged as a closed circle, which obviously has neither beginning nor end. In eukaryotic cells the multiple molecules of the genome are linear rather than circular and have characteristic repeated sequences of nucleotides at their ends (the telomers).

In contrast to the regularity seen in the prokaryotics and eukaryotes, diversity is the rule in the viruses, almost as if nature were playing with all the possibilities for arrangements of nucleic acids. Figure 1-2 illustrates some of these possibilities. DNA genomes can be single-stranded as well as double-stranded, and each of these can form either a linear or circular molecule. More unusual forms, such as linear double-stranded DNA with nicks at various points in the chain, or double-stranded DNA with closed ends, occur as well. The size of viral DNAs ranges over about two orders

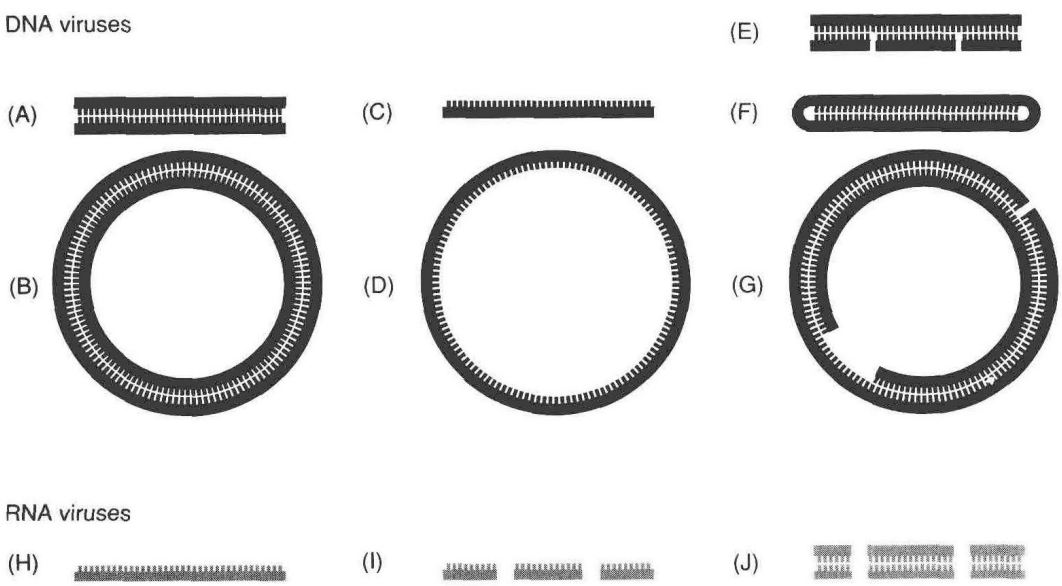

FIGURE 1-2 The organization of viral nucleic acids. In addition to the double-stranded molecules seen in eukaryotic and prokaryotic cells that are linear (**A**), or circular (**B**), respectively, viral DNAs can be single-stranded linear (**C**), or circular (**D**). Other unusual forms of viral DNA are double-stranded linear with breaks in the phosphodiester backbone in one strand (**E**), linear double-stranded with closed ends (**F**), or circular with large gaps in each strand (**G**). Unlike their host cells, viruses can also use RNA as their genetic material. All RNA viral genomes are linear, but they may be one single-stranded molecule (**H**), segmented single-stranded molecules (**I**), or segmented double-stranded molecules (**J**).