Virology

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Second Edition

J. B. Lippincott Company

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Virology

Preface

Virology originated as a discipline to study diseases of major medical or economic importance, such as smallpox, rabies, foot-and-mouth disease, or tobacco mosaic disease. Subsequently, viruses were used as tools for studies of genetics, biochemistry, and molecular biology; as a result, the properties of many viruses are now understood at the molecular level. Viruses are therefore considered in two parallel ways in this book—as very interesting organisms and as agents of disease. Thus, *Virology* discusses both the biological and pathogenic properties of viruses.

The basic properties of the viruses are examined in depth, beginning with bacteriophages, which still allow the deepest insight into the biology of viruses, and following with animal viruses. This information serves as the basis for analyzing the mechanisms by which viruses interact with human or animal cells and organisms and produce disease. Indeed, this material permits an approach to understanding the mechanisms of viral pathogenesis at a molecular level. The properties of the animal cells relevant to this interaction are examined in a separate chapter, and then the viruses infecting humans are examined systematically. Two chapters review the principles of viral oncogenesis and the role of viruses in human cancer. The virus causing AIDS (the human immunodeficiency virus, or HIV), a retrovirus, is considered among them.

Unlike other virology texts, *Virology* applies the principles of basic virology to each viral family. For each family it discusses viral structure, mode of replication, genetic properties, and mechanisms of interaction with the cells. On this basis, problems of pathogenesis, immunology, epidemiology, and control of major viruses can be approached rationally.

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Virology

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The Nature of Viruses

Viruses, as infectious agents responsible for many diseases in humans, animals, and plants are of great medical and economic importance. They were recognized at the end of past century as infectious agents smaller than bacteria ("filterable agents"). Transmission by a cell-free filtrate was demonstrated in 1898 for foot-and-mouth disease, for fowl leukosis in 1908, and for chicken sarcoma in 1911. The discovery of viruses affecting bacteria, made in 1917, made available an important model system for investigations of basic virology.

Distinctive Properties

Passage through the usual bacterial filters, and multiplication only as obligatory parasites in living cells, proved inadequate to distinguish viruses from the smallest bacteria (e.g., rickettsiae). Viruses are distinguished from other microbes in more fundamental ways: their simple organization and their characteristic mode of replication. In addition, animal viruses produce characteristic effects on host cells: death, fusion, or transformation into cancer cells.

The free viral particles, called **virions,** are made up of two essential constituents: a **genome,** which can be DNA or RNA, associated with proteins or polyamines; and a protein coat (**the capsid**), sometimes surrounded by a membranous **envelope.** In addition, some virions have enzymes that are needed in the initial steps of replication of the genome. They may also contain other minor constituents (see below). So a virion is relatively simple: it is little more than a block of genetic material enclosed in a coat. Capsid and envelope protect the genome from the nucleases present in the environment, and they facilitate

its attachment and penetration into the cell in which it will replicate.

Viruses have a unique method of multiplication. Whereas in the replication of other microbes all the constituents are made within the cell envelope, finally causing the microbe to undergo binary fission, virions lack the machinery for using and transforming energy and for making the proteins specified by the viral genes. Accordingly, after the viral genome is released from the coat, it uses the machinery of the host cell to make the constituents of viruses in the cell's cytoplasm or nucleus. Progeny virions are then assembled from these constituents. Although all viruses have this basic mechanism of replication in common, they differ considerably in such characteristics as size and shape, chemical composition of the genome (Fig. 44–1), and the type of cells they infect.

HOST RANGE

Viruses are subdivided into **animal viruses, bacterial viruses (bacteriophages),** and **plant viruses.** Within a class each virus is able to infect only cells of a certain species or of a certain type. The host range is determined in part by the specificity of attachment to the cells, which depends on properties of both the virion's coat and specific receptors on the cell surface. It also depends on the availability of cellular factors required for the replication or transcription of the genome. The host range is broader in **transfection:** infection by the naked nucleic acid, the entry of which does not depend on specific receptors. Limitations determined by intracellular factors, however, persist.

Are Viruses Alive?

When Stanley crystallized tobacco mosaic virus in 1935, there followed extensive debates on whether it was a living being or merely a nucleoprotein molecule. As Pirie pointed out, these discussions showed only that some scientists had a more teleologic than operational view of the meaning of the word *life.* Life can be viewed as a complex set of processes resulting from the actuation of the instructions encoded in the genes; those of viral genes are actuated after the viral genome has entered a susceptible cell; hence, viruses may be considered alive when they replicate in cells. Outside cells, virions are metabolically inert chemicals. Thus, depending on the context, viruses may be regarded both as exceptionally simple microbes and as complex chemicals.

Viruses are then not organisms in the usual sense: they are parasitic genomes, related to plasmids (see Chap. 8). Moreover, some viral genomes, like certain plasmids, become integrated into the DNA of their host cells, exercising the same form of parasitism displayed by movable DNA elements and by certain repeated sequences abundant in the DNA of eukaryotic cells.

The Viral Particles

GENERAL MORPHOLOGY

Viruses of different families have virions of different morphologies, which can be readily distinguished by electron microscopy. This relationship is useful for diagnosing viral diseases and especially for recognizing new viral agents of infection. For instance, the wheel-shaped virions of rotaviruses in feces of infants with diarrhea could readily be distinguished from other viruses also present in feces, and recognition of paramyxovirus nucleocapsids in thin sections of the brains of patients helped to reveal the viral origin of subacute sclerosing panencephalitis (SSPE).

However, different classes of viruses within the same family have virions of similar morphology. The identification can be refined by the binding of specific antibodies to virions, which is also recognizable by electron microscopy (see Immuno-Electron Microscopy in Chap. 50).

Virions belong to several morphological types (Fig. 44–2 and Table 44–1).

1. **Icosahedral virions** resemble small crystals. Extensive studies, especially by Klug and Caspar, have shown that these virions have an **icosahedral** protein shell **(the capsid)** surrounding a core of nucleic acid and proteins. The capsid and the core form the **nucleocapsid.** Examples are picornaviruses, adenoviruses, papovaviruses, and bacteriophage ϕX174 (Fig. 44–3*A*).

2. **Helical virions,** of which tobacco mosaic virus (see Fig. 44–3*B*) and bacteriophage M13 are examples, form long rods. The nucleic acid is surrounded by a **cylindrical capsid,** in which a helical structure is revealed by high-resolution electron microscopy.

3. **Enveloped virions** contain lipids. In most cases the nucleocapsid—in some viruses icosahedral, in others helical—is surrounded by a membranous **envelope.** Most enveloped virions are roughly spherical but highly **pleomorphic** (i.e., of varying shapes) because the envelope is not rigid. Herpesviruses and togaviruses are examples of **enveloped icosahedral** viruses (see Fig. 44–3*C*). In **enveloped helical** viruses, such as orthomyxoviruses (see Fig. 44–3*D*), the nucleocapsid is coiled within the envelope.

4. **Complex virion** structures belong to two groups. Those illustrated by poxviruses (see Fig. 44–3*E*) do not possess a clearly identifiable capsid but have several coats around the nucleic acid, while certain bacteriophages (see Fig. 44–3*F*) have a capsid to which additional structures are appended.

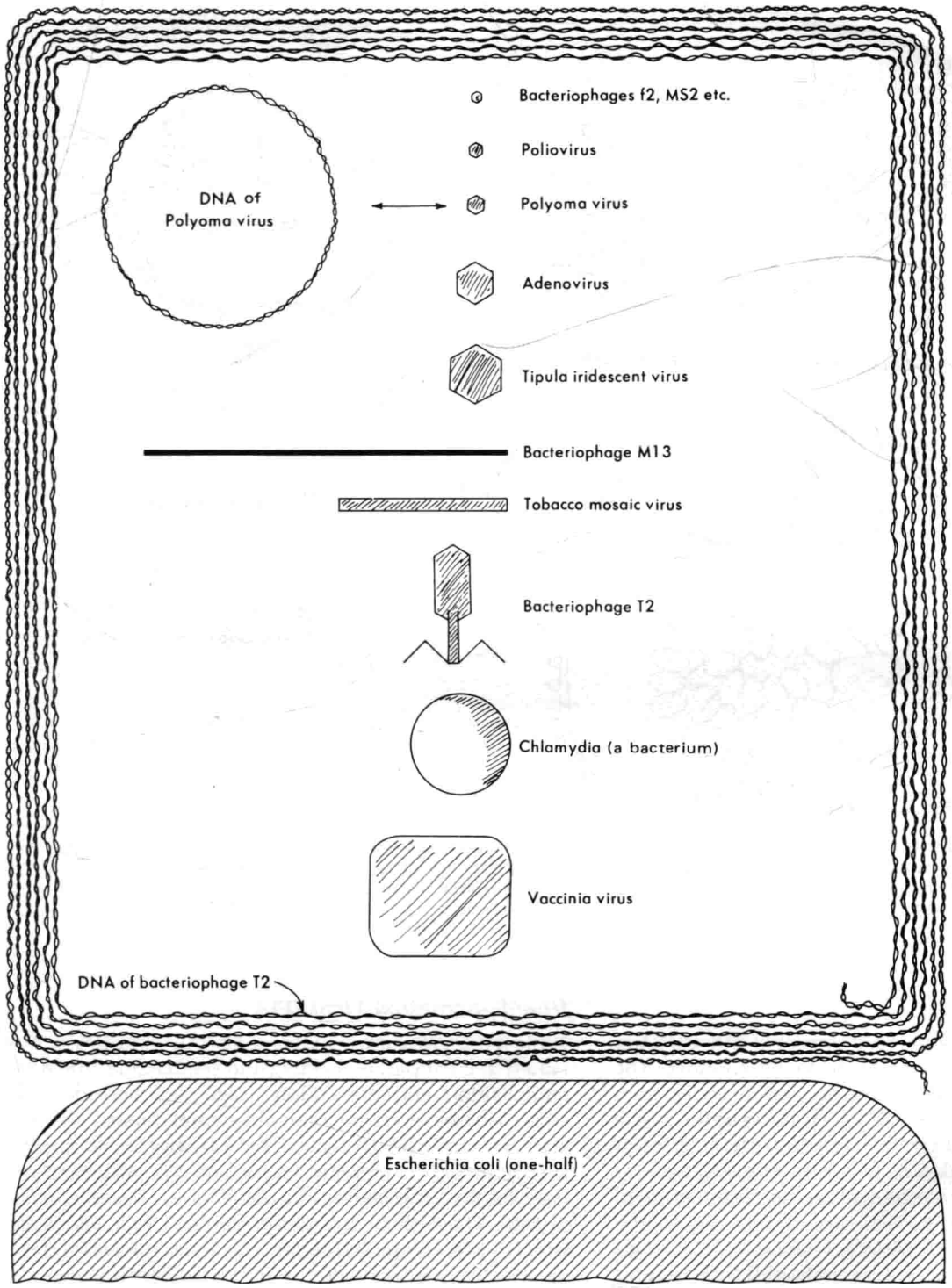

Figure 44–1. Comparative sizes of virions, their nucleic acids, and bacteria. The profiles and the lengths of the DNA molecules are all reproduced on the same scale.

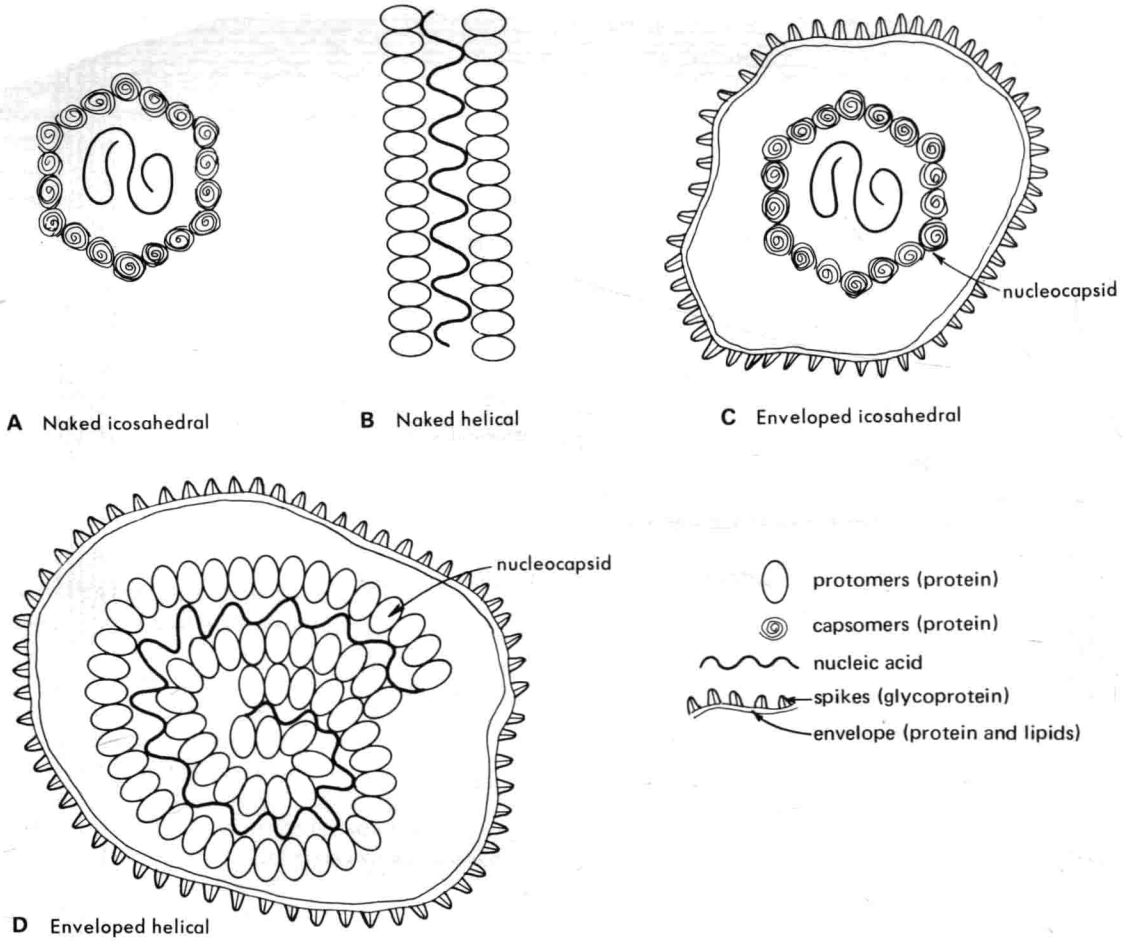

Figure 44–2. Simple forms of virions and of their components. The naked icosahedral virions (*A*) resemble small crystals; the naked helical virions (*B*) resemble rods with a fine regular helical pattern in their surface. The enveloped icosahedral virions (*C*) are made up of icosahedral nucleocapsids surrounded by the envelope; the enveloped helical virions (*D*) are helical nucleocapsids bent to form a coarse, often irregular coil within the envelope.

VIRAL GENOMES

In a given virus the genome may consist of either DNA or RNA, which may be either single or double stranded. The amount of genetic information per virion varies from about 3 to 300 kilobases (Kb). If 1 Kb is taken as the size of an average gene, small viruses contain perhaps three or four genes, and large viruses several hundred. The diversity of virus-specific proteins synthesized in the infected cells varies accordingly. With the exception of retroviruses (see Chap. 65), virions contain only a single copy of the genome; that is, they are **haploid.** The virions of some plant viruses contain only a fraction of the genome, and several virions, collectively containing the whole genome, must enter the same cell for viral multiplication to take place.

Double-Stranded Viral DNA

Table 44–2 gives the lengths of various viral DNAs, obtained in great part by cloning and sequencing. The base composition and the codon usage vary considerably: some viruses even contain **abnormal bases.** For instance, cytosine is replaced by 5-hydroxymethyl-cytosine in T-even coliphages (see Chap. 45), and substitutions for thymine are found in *Bacillus* and *Pseudomonas* phages. These differences from the host cells allow the viral DNA to escape the action of cellular nucleases or to be selectively recognized by virus-specified enzymes.

Many viral DNAs have **special features** related to their methods of replication. These features avoid the difficulty of complete replication of the ends of a linear molecule from an internal initiation (see Fig. 45–10). To

TABLE 44–1. Characteristics of Viruses

Morphological Class	*Nucleic Acid**	*Example*		*Size of Capsid (nm)*	*No. of Capsomers*	*Size of Virions (Enveloped Viruses) (nm)*	*Special Features*
		Virus Family	*Virus*				
Helical capsid							
Naked	DNA	Coliphage f1, M13		5 × 800			Single-stranded cyclic DNA
	RNA	Many plant viruses	Tobacco mosaic	17.5 × 300			
			Beet yellow	10 × 1200			
Enveloped	RNA	Orthomyxoviruses	Influenza	9 (diameter)		90–100	Segmented RNA
		Paramyxoviruses	Newcastle disease	18 (diameter)		125–250	
		Rhabdoviruses	Vesicular stomatitis			68 × 175	Bullet shaped
Icosahedral capsid							
Naked	DNA	Parvoviruses	Adeno-associated	20	12		Single-stranded linear DNA
		Coliphage ϕx174		22	12		Single-stranded cyclic DNA
		Papovaviruses	Polyoma	45	72		Cyclic DNA
			Papilloma	55	72		Cyclic DNA
		Adenoviruses		60–90	252		
	RNA	Coliphage F2 and others		20–25			
		Picornaviruses	Polio	28	32		
		Many plant viruses	Turnip yellow	28	32		
		Reoviruses		70	92		Segmented; double-stranded RNA
Enveloped	DNA	Herpesviruses	Herpes simplex	100	162	180–200	
		Hepadnaviruses	Hepatitis B	27	42		
Capsids of binal symmetry (i.e., some components icosahedral, others helical)							
Naked	DNA	Large bacteriophages	T2,T4,T6	Modified icosahedral head: 95 × 65; helical tail: 17 × 115			
Complex virions	DNA	Poxviruses	Variola, Vaccinia			250 × 300	Brick shaped
			Contagious pustular dermatitis of sheep			160 × 260	

* DNA double stranded, RNA single stranded, unless specified in last column

this purpose some viral DNA are **cyclic,** and therefore without ends, whereas others are made to become cyclic after entering cells. Others have **terminal redundancies,** which enable incompletely replicated molecules to complete each other by recombination. Some viral DNAs have **palindromes** or **terminal proteins** at the ends, which act as primers during replication. These characteristics and their roles will be considered in greater detail together with DNA replication in Chapter 50 and in the Chapters on specific viruses (45, 46, 52–65).

Some viral DNAs have features that show their **relatedness to transposons** (see Chap. 8), such as **terminal repeats.** The DNAs of some herpesviruses are made up of two unequal transposons joined together, each with its own terminal repeats (see Chap. 53); each transposon undergoes frequent inversion independently of the other, so a population of virions contains four, equally frequent, kinds of DNA: ⟶ →, ⟵ →, ⟶ ←, ⟵ ←. Some DNAs have single-strand nicks at characteristic places, which define special genomic segments during

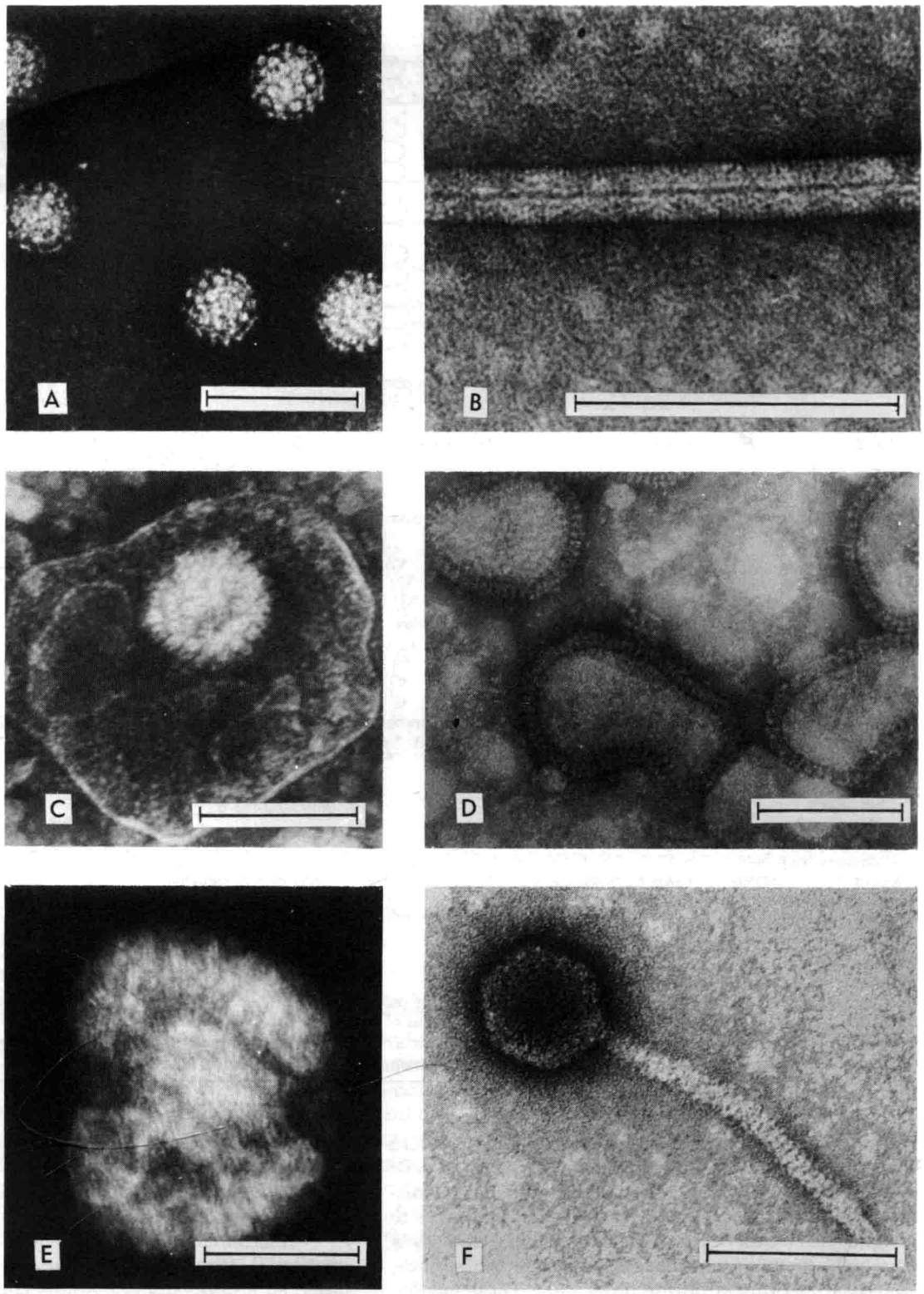

Figure 44–3. Electron micrographs of representative virions were obtained by negative staining; that is, suspending the virions in an electron-opaque salt solution so that structures are transparent on a dark background. Markers under each micrograph are 100 nm. (*A*) Naked icosahedral: human wart virus (papovavirus, Chap. 65). (*B*) Naked helical: segment of tobacco mosaic virus. (*C*) Enveloped icosahedral: herpes simplex virus (herpesvirus, Chap. 53). (*D*) Enveloped helical: influenza virus (orthomyxovirus, Chap. 56). (*E*) Complex virus: vaccinia virus (poxvirus, Chap. 54). (*F*) Coliphage λ (Chap. 46). (*A*, Noyes WF: Virology 23:65, 1964; *B*, Finch JT: J Mol Biol 8:872, 1964. Copyright by Academic Press, Inc. [London] Ltd.; *C*, courtesy of P. Wildy; *D*, Choppin PW, Stockenius W: Virology 22:482, 1964; *E*, courtesy of R. W. Horne; *F*, courtesy of F. A. Eiserling)

TABLE 44–2. Characteristics of Viral Nucleic Acids

Type of Nucleic Acid	Representative Virus	Mol. wt. (in 10^6 daltons)	Kilobases per Strand*	No. of Segments	Polarity
DNA, DOUBLE STRANDED					
Hepatitis B (cyclic)		1.6	2.5		
Papovavirus (cyclic)	Polyoma	3.5	5.0		
	Papilloma	6	9		
Pseudomonas phage PMS2 (cyclic)		6	9		
Adenovirus	Types 12,18	21	32		
	Types 2,5	23	35		
Coliphages T3,T7		25	38		
Coliphage Mu		26	39		
Coliphage λ		31	47		
Coliphage T5		77	117		
Herpesvirus	Herpes simplex	100	151		
Coliphages T2,T4,T6		110	167		
Bacillus subtilis phage SP8		130	197		
Poxvirus	Vaccinia	160	242		
DNA, SINGLE STRANDED					
Parvovirus	Adeno-associated†	1.5	4.5		
Coliphage ϕx174 (cyclic)		1.7	5.2		
Coliphage M13 (cyclic)		2.4	7.3		
RNA, DOUBLE STRANDED					
Rotaviruses		15[3]	23	10	
Rice dwarf virus		15[3]	23	10	
Cytoplasmic polyhedrosis of silkworms		15[3]	23	10	
RNA, SINGLE STRANDED					
Satellite necrosis virus†		0.4	1.2	1	
Coliphage R17		1.3	4	1	+
Tobacco mosaic virus		2	6	1	+
Turnip yellow mosaic virus		2	6	1	+
Picornavirus	Polio	2.5	7.5	1	+
Bunyavirus		3‡	9	3	−
Retrovirus§	Rous sarcoma virus	3.5	10.5	1	+
Alphavirus	Sindbis	4	13	1	+
Rhabdovirus	Vesicular stomatitis virus	4	13	1	−
Orthomyxovirus	Influenza	6‡	18	8	−
Paramyxovirus	Newcastle disease	6	18	1	−

+, positive; −, negative

* A kilobase (1000 bases) corresponds to a molecular weight of about 700,000 for double-stranded and 350,000 for single-stranded nucleic acid; it can specify about 33,000 daltons of protein. The number of genes is approximately equal to the number of kilobases; ϕx174 has fewer kilobases because **some genes overlap each other** (see Chap. 45).

† These viruses are defective and multiply only in cells infected by a helper virus (adenovirus or tobacco necrosis virus, respectively). They probably specify only their own capsid, perhaps with another small protein.

‡ This value, as for other virions with segmented genomes, is the aggregate of all fragments.

§ Retroviruses have diploid virions.

entry into cells (Phage T5; see Regulation of Transcription in Chap. 45).

Control Elements of DNA Viruses

Genes contained in viral DNAs are controlled essentially like the genes of the host cells, and they have the corresponding characteristic sequences. DNAs of bacterial viruses, like bacterial genes, have promoters, operators, and ribosome-binding sites. DNAs of eukaryotic viruses have control regions comparable to those of eukaryotic genes, enhancers (see Chap. 64), and a TATA box for locating the exact initiation of the transcripts; they also have poly(A) addition sites at their 3′ ends where the messengers terminate. In poxviruses, however, the transcription signals do not conform to those of the host cells and are recognized by enzymes specified by viral genes. The structure of the genome is suitable for poly-

cistronic transcription in bacterial viruses and for monocistronic transcription in eukaryotic viruses. The DNA of eukaryotic viruses encodes intervening sequences, whereas those of bacterial viruses usually do not. (For an exception in bacteriophage T4, see Posttranscriptional Regulation in Chap. 45.)

Single-Stranded Viral DNA

The DNA is single-stranded and cyclic in some very small bacteriophages (the icosahedral ϕX174 and the helical f1 and M13) and in one family of animal viruses (parvoviruses). The phages have DNA molecules of the same polarity in all virions; parvoviruses have strands of both polarities, but in different virions. Parvoviruses have also inverted terminal repeats that can form hairpins, important for replication.

Viral RNAs

Some RNA viral genomes are **double stranded** (in reovirus, in a phage, and in some viruses of lower animals, insects, yeasts, and plants). Other genomes are **single stranded.** Single-stranded genomes belong to two classes: **positive-strand** genomes that, on entering the cells, can directly act as messengers for protein synthesis; and **negative-strand** genomes that are not of messenger polarity and must be transcribed into messengers. Positive-strand RNAs of eukaryotic cells have the general organization of eukaryotic mRNAs: they have a cap at the 5′ end, and they end with a poly(A) chain at the other end. Picornaviruses are an exception: they do not have a cap but have a small protein covalently linked to the 5′ terminal uridylate. Negative-strand RNAs do not have caps, but each is terminated at the 5′ end with a nucleoside triphosphate.

Of these viruses, the retroviruses are closely related to transposons: their genome is flanked by two repeats, and it integrates into the cellular DNA where it is flanked by two short repeated cellular sequences. It is propagated by reverse transcriptions (RNA → DNA), like transposons of Drosophila and yeast.

Segmentation of the Genome

The genomes of double-stranded RNA viruses, and of those of some negative-strand viruses, have a peculiarity: they are made of separate segments. For instance, the double-stranded reoviruses have ten segments, and the negative-stranded orthomyxoviruses have eight. The segmentation of the genome is probably a mechanism for avoiding polycistronic messengers, because eukaryotic cells rarely initiate protein synthesis internally in a messenger. A segment, however, may specify two proteins.

Control Sequences for RNA Viruses

Control sequences in RNA genomes have the function of interacting with the replication or transcription apparatus, with initiation factors for protein synthesis and ribosomes, and with the capsid. Short sequences with these functions, present near the ends of genomes and at the ends of genes, are recognized because they are conserved among related viruses.

Exceptional arrangements are found in some viral genomes. For instance, the positive-strand phage MS2 lacks a ribosome entry site for a lysis gene. This gene uses ribosomes that translate the upstream coat gene, with which it partly overlaps but in a different phase. Ribosomes that accidentally go out of phase in the coat gene can enter the lysis gene. This arrangement ensures that the lysis protein is made in much smaller amounts than the coat protein, so that the cells lyse only after enough virus is made. The polymerase gene of retroviruses has a similar arrangement. These are examples of gene overlap with regulatory function.

Origin of Viral Genomes

Because viral genomes could not have evolved readily except by replication within cells, it is logical to assume that viral genomes are ultimately derived from the genomes of host cells. A step in this evolution may be the incorporation of cellular genes into the genomes of transducing bacteriophages (see Chap. 46) and retroviruses (see Chap. 65). The evolutionary separation, however, is long, for homologies between viral genomes and cellular genes are rare. One is found in vaccinia virus, which has a gene with some homology with a cellular gene for a growth factor.

More stringent evidence for a cellular origin is found in positive-strand RNA viruses, the genomes of which resemble cellular messengers. The RNAs of some plant viruses terminate at the 3′ end with sequences that fold into the tertiary structure of tRNAs and can be aminoacylated by specific tRNA aminoacylases. At its 5′ position this cistron is connected to a poly(A) sequence from a cistron with all the features of a cellular mRNA. These genomes were evidently derived from the recombination of a cellular mRNA with a tRNA-like polynucleotide.

THE VIRION'S COATS

The Capsid

The study of the organization of the capsid is important because it uncovers the principles by which biological macromolecules assemble into complex structures. The capsid encloses the genome and gives the virions their characteristic shapes. It accounts for a large part of the virion's mass and is made up of protein molecules, which are specified by viral genes. Since viruses have small genomes, they cannot afford too many genes for specifying capsid proteins; hence, the capsid must be formed by the association of many protein units of a single kind or of relatively few kinds (**protomers**). For instance, poliovirus RNA (7 Kb) can specify at most 250,000 daltons of protein altogether; some of the pro-

teins must be used for replication. Yet the poliovirus capsid weighs about 6×10^6 daltons. In fact, it contains only four unique proteins. The shape and dimensions of the capsid depend on characteristics of the constituent protomers and, for helical capsids, on the length of the viral nucleic acid.

The repeated protomers forming the capsid must be arranged in a regular architecture that utilizes bonds between the same pairs of chemical groups. This goal is attained in different ways in the icosahedral and helical capsids, as shown by extensive x-ray crystallographic studies.

ICOSAHEDRAL CAPSIDS. The icosahedral shape of many viruses is of considerable interest because the only closed shell that can be made with identical protomers is icosahedral. The simplest icosahedron is a regular solid with 12 vertices and 20 triangular faces (Fig. 44–4). To

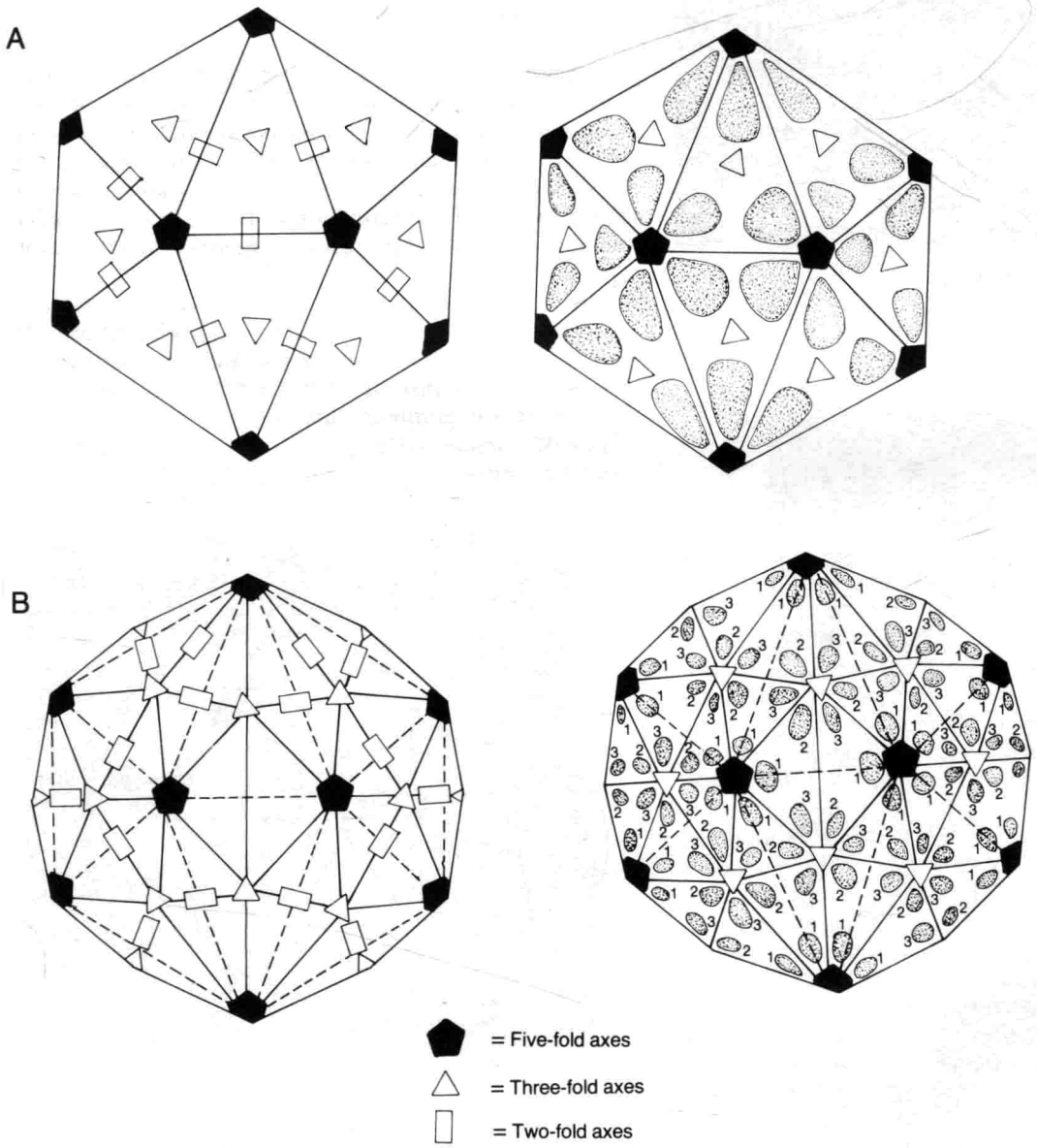

Figure 44–4. (*A*) The basic icosahedron. The drawing at left shows the triangular faces, with pentons at each vertex; the drawing at right shows the positions of the monomers around the fivefold axis. (*B*) A derived icosahedron (T = 3; see Appendix). Each triangular face of the icosahedron shown in *A* is subdivided into six half-triangles. The corners of the inscribed faces are solid lines; those of the basic faces are dashed lines. Monomers are arranged in pentons around the fivefold axes and in hexons around the threefold axes. *1, 2,* and *3* indicate quasi-equivalent monomers.