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CHAPTER 1

Structure and Composition of Viruses

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The unicellular microorganisms can be arranged in order of decreasing size and complexity: protozoa, fungi, bacteria, mycoplasmas, rickettsiae, and chlamydiae. These microorganisms, however small and simple, are cells. They always contain DNA as the repository of their genetic information, they contain RNA, and they have their own machinery for producing energy and macromolecules. Microorganisms grow by synthesizing their own macromolecular constituents (nucleic acid, protein, carbohydrate, and lipid), and they multiply by binary fission.

Viruses, on the other hand, are smaller and simpler in construction than unicellular microorganisms, and they contain only one type of nucleic acid—either DNA or RNA, never both. Furthermore, since viruses have no ribosomes, mitochondria, or other organelles, they are completely dependent on their cellular hosts for energy production and protein synthesis. They replicate only within cells of the host that they infect. Indeed, unlike any microorganism, many viruses can, in suitable cells, reproduce themselves from their genome, a single nucleic acid molecule; i.e., their nucleic acid alone is infectious. Are viruses alive? The question is rhetorical. Outside a susceptible cell, the virus particle,

TABLE 1-1
*Contrasting Properties of Unicellular Microorganisms
 and Viruses*

Property	Bacteria	Mycoplasmas	Rickettsiae	Chlamydiae	Viruses
<300 nm diameter	–	±	–	±	+
Growth on nonliving media	+	+	–	–	–
Binary fission	+	+	+	+	–
DNA and RNA	+	+	+	+	–
Nucleic acid infectious	–	–	–	–	+ ^a
Ribosomes	+	+	+	+	–
Metabolism	+	+	+	±	–
Sensitivity to antibiotics	+	+	+	+	–
Sensitivity to interferon	–	–	–	+	+

^aSome, among both DNA and RNA viruses.

like a bacterial spore, is metabolically inert; on the other hand, when replicating in a cell it exhibits all the characteristics of life. The key differences between viruses and microorganisms are listed in Table 1-1.

Several important practical consequences flow from these differences. For example, some viruses (but no microorganisms) may persist in cells by the integration of their DNA (or a DNA copy of their RNA) into the genome of the host cell, and they are not susceptible to antibiotics that act against specific steps in the metabolic pathways of bacteria.

MORPHOLOGY

For many years it has been known that viruses are smaller than unicellular microorganisms. Independently, in 1898–1899, the Dutch plant pathologist Beijerinck, working on tobacco mosaic disease, and the German veterinarians Loeffler and Frosch, working on foot-and-mouth disease of cattle, showed that these diseases could be transmitted by material which could pass through a filter with pores too small to allow passage of bacteria. The new group of “microorganisms” became known as the “filterable viruses.” Filtration studies showed that virus particles (*virions*) range from about the size of the smallest unicellular microorganisms (300 nm) down to objects little bigger than the largest protein molecules (20 nm). For a time they were also called “ultra-

microscopic," since they are too small to be seen with the ordinary light microscope. Only with the advent of the electron microscope did it become possible to study their morphology properly. In 1959, our knowledge of viral ultrastructure was transformed when Brenner and Horne applied *negative staining* to the electron microscopy of viruses. Potassium phosphotungstate, which is electron-dense, fills the interstices of the viral surface, giving the resulting electron micrograph a degree of detail not previously possible. Electron micrographs of negatively stained preparations of the virions of all families of viruses of vertebrates are shown in the relevant chapters of Part II of this book.

Viral Structure

In the simpler viruses the virion consists of a single molecule of nucleic acid surrounded by a protein coat, the *capsid*; the capsid and its enclosed nucleic acid together constitute the *nucleocapsid*. In some of the more complex viruses the capsid surrounds a protein core (Fig. 1-1A), and in other viruses the capsid is surrounded by a lipoprotein *envelope* (Fig. 1-1D). The capsid is composed of morphological units called *capsomers*, which are held together by noncovalent bonds. Individual capsomers, which consist of one or more polypeptide molecules, are usually visible by electron microscopy. In helical nucleocapsids, the viral nucleic acid is folded throughout its length in a specific relationship with the capsomers (Fig. 1-1C), but there is no such specific relationship between RNA and protein in the small icosahedral picornaviruses.

Within an infected cell, the capsomers of the simpler viruses self-assemble to form the capsid. The manner of this assembly is strictly defined by the nature of the bonds formed between individual capsomers, which imparts symmetry to the capsid. Only two kinds of symmetry have been recognized: icosahedral and helical (Fig. 1-1).

Icosahedral Symmetry. The icosahedron is one of the five classical "Platonic solids" of geometry; it has 12 vertices (corners) and 20 faces, each an equilateral triangle. It has axes of two-, three-, and fivefold rotational symmetry, passing through its edges, faces, and vertices, respectively (see Fig. 1-2). The icosahedron is the optimum solution to the problem of constructing, from repeating subunits, a strong structure to enclose a maximum volume. The same principles were applied by the architect Buckminster Fuller to the construction of icosahedral buildings ("geodesic domes").

Only certain arrangements of the repeating morphological units, the capsomers, can fit into the faces, edges, and vertices. In adenovirus particles, for example, capsomers on the faces and edges bond to six

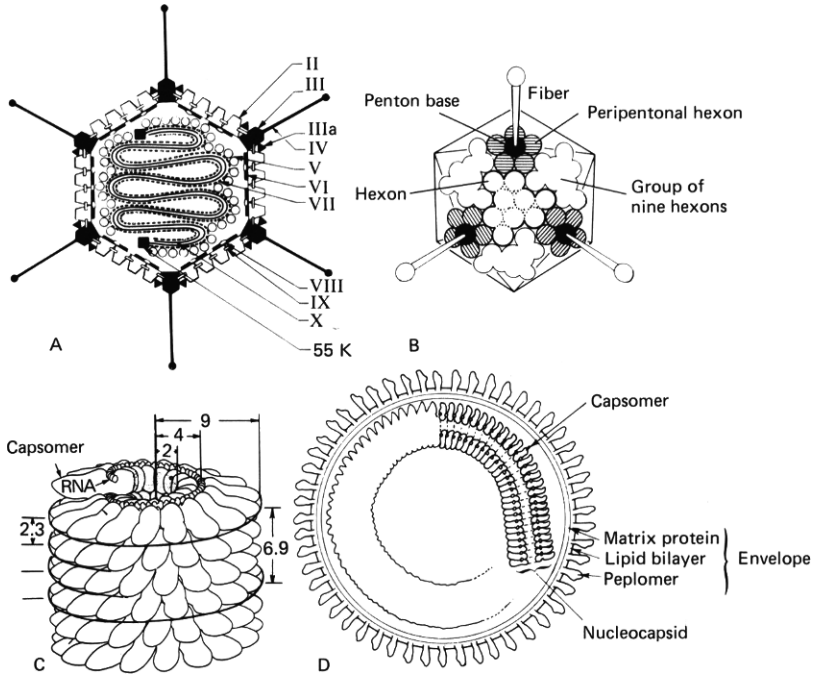


FIG. 1-1. Features of virion structure, exemplified by adenovirus (A,B), tobacco mosaic virus (C), and paramyxovirus (D). Not to scale. (A,B) Icosahedral structure of adenovirus. All hexon capsomers are trimers of the same polypeptide (II), distinguished as "group of nine" or "peripentonal," respectively, only by their location in the capsid. The penton base is a pentamer of polypeptide III; the fiber is a trimer of polypeptide IV. Several other viral polypeptides occur just beneath the capsid (VI, VIII, IX) and others again in the core (V, VII, 55K), where they are intimately associated with the viral DNA. (C) The structure of helical nucleocapsids has been elucidated by studies of a nonenveloped plant virus, tobacco mosaic virus, but the principles apply to animal viruses with helical nucleocapsids, all of which are enveloped. In tobacco mosaic virus a single polypeptide is folded to form a capsomer. A total of 2130 capsomers assemble in a helix with a pitch of 2.3 nm and an axial repeat of 6.9 nm (49 subunits in each three turns). The 6-kb RNA genome sits in a groove on the inner part of the capsomer, and is wound to form an RNA helix of the same pitch, 8 nm in diameter, which extends the length of the virion. The virion is 300 nm long and 18 nm in diameter, with a hollow cylindrical core 4 nm in diameter. (D) All animal viruses with a helical nucleocapsid and some of those with an icosahedral capsid are enveloped. The envelope consists of a virus-specified matrix protein (M; absent in *Arenaviridae*, *Bunyaviridae*, and *Coronaviridae*, as well as in the enveloped viruses with icosahedral capsids), beneath a lipid bilayer in which are inserted numerous glycoprotein peplomers. [A, from H. S. Ginsberg, In "Comprehensive Virology" (H. C. Fraenkel-Conrat and R. R. Wagner, eds.), Vol. 13, p. 409. Plenum Press, New York, 1979. B, by John Mack, from R. M. Burnett, In "Biological Macromolecules and Assemblies: Virus Structures" (F. Journak and A. McPherson, eds.), Vol. 1, p. 337. Wiley, New York, 1984; C, from C. F. T. Mattern, In "Molecular Biology of Animal Viruses" (D. P. Nayak, ed.), Vol. 1, p. 5. Dekker, New York, 1977; and D, modified from D. L. D. Caspar et al., Cold Spring Harbor Symp. Quant. Biol. 27, 49 (1962).]

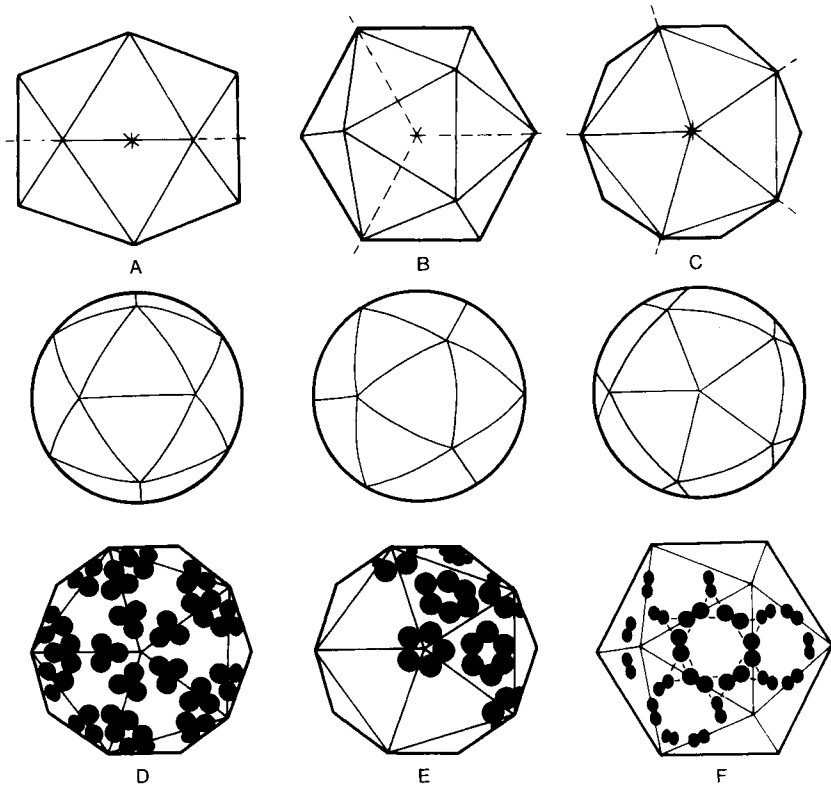


FIG. 1-2. Features of icosahedral structure. A regular icosahedron viewed along two-fold (A), threefold (B), and fivefold (C) axes of symmetry. In negatively stained electron micrographs, virions may appear hexagonal in outline (upper row) or apparently spherical (middle row). Various clusterings of capsid polypeptides give characteristic appearances of the capsomers in electron micrographs (lower row). For example, they may be arranged as 60 trimers (D), capsomers being then difficult to define, as in poliovirus; or they may be grouped as 12 pentamers and 20 hexamers (E), which form bulky capsomers as in parvoviruses; or as dimers on the faces and edges of the triangular facets (F), producing an appearance of a bulky capsomer on each face, as in caliciviruses.

neighboring capsomers and are called *hexamers*; those at the vertices bond to five neighbors and are called *pentamers* (Fig. 1-1A,B; Plate 1-1A). In some viruses both hexamers and pentamers consist of the same polypeptide; in others they are different. The varied arrangements of hexamers between pentamers have been systematically codified by Caspar using terms such as "P-number" and "T-number" (see references). Some possible arrangements of capsomers are shown in Fig. 1-2D, E, and F.

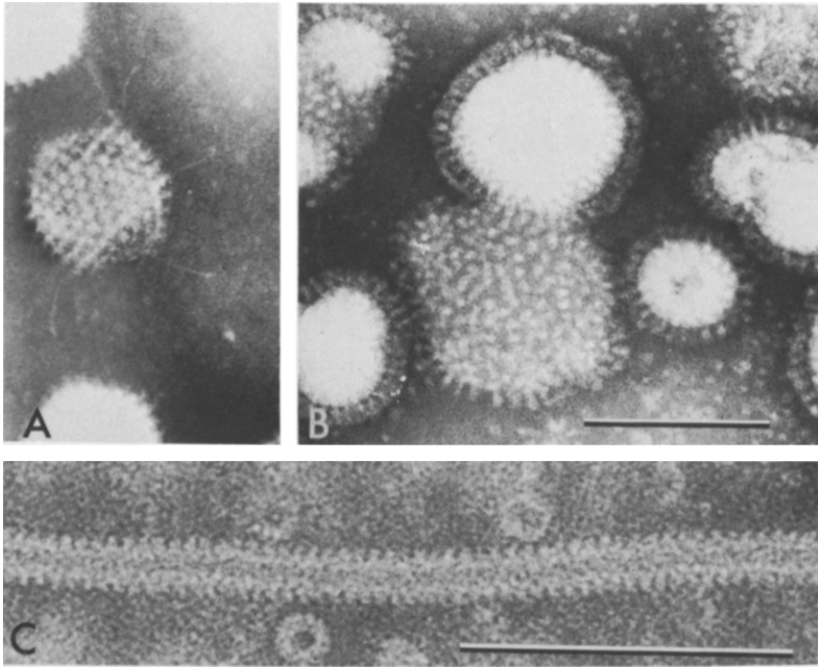


PLATE 1-1. Morphological features of viral structure revealed by negative staining and electron microscopy (bars = 100 nm). (A) Icosahedral structure of adenovirus capsid. At each of the 12 vertices there is a penton base capsomer from which projects a fiber with a small terminal knob; each of the 20 triangular facets contains 18 identical hexon capsomers, of which 6 are unshared and 12 shared with adjacent facets. The capsid encloses a protein core with which the DNA is associated. (B) Envelope of influenza virus (family: Orthomyxoviridae). The peplomers are of two morphological types: the hemagglutinin is a rod-shaped trimer and the neuraminidase is a stud-shaped tetramer. Both are embedded in lipid, beneath which there is a matrix protein; this lipoprotein envelope encloses a helical nucleocapsid. (C) Nucleocapsid of parainfluenza virus (family: Paramyxoviridae). The RNA is wound within and protected by a helix composed of identical capsomers. The complete nucleocapsid is 1000 nm (1 μm) long, but in the intact particle is folded within a roughly spherical envelope about 180 nm in diameter. (A and B, courtesy Dr. N. G. Wrigley; C, courtesy Dr. A. J. Gibbs.)

In a practical sense, the examination of negatively stained icosahedral virions in the electron microscope, and analysis of their capsomer arrangement, can often provide immediate and unambiguous information for the identification of a virus as a member of a known family—or, in very rare instances, as a candidate prototype for a new family. For example, the visualization of a nonenveloped virion with a row of four

hexamers in line between vertex pentamers would identify a virus as an adenovirus (Plate 1-1A).

The recent demonstration by X-ray crystallography of the atomic resolution structure of two picornaviruses (poliovirus and rhinovirus) has provided a remarkable insight into the organization and assembly of their virions, the location of the antigenic sites involved in neutralization, and aspects of their penetration into cells. Similar detail can be expected as these new technical capabilities are applied to other viruses and to problems of replication, assembly, and pathogenesis.

Helical Symmetry. The nucleocapsids of several RNA viruses have a different type of symmetry: the capsomers and nucleic acid molecule(s) self-assemble as a helix (Fig. 1-1C,D; Plate 1-1C). In all such viruses each capsomer consists of a single polypeptide molecule. The plant viruses with helical nucleocapsids are rod shaped and naked (nonenveloped). However, in all animal viruses helical nucleocapsids are wound into a coil and enclosed within a lipoprotein envelope (see Plate 27-1), possibly to give the very long nucleocapsids stability.

Viral Envelopes. Viral envelopes are acquired at host cell membranes—some at the plasma membrane, others at internal cell membranes such as the nuclear membrane, endoplasmic reticulum, and Golgi complex—during the maturation of the virus by the process known as “budding.” The lipids of the viral envelope are derived directly from the cell, but the proteins in the envelope are virus coded. One kind is the glycoprotein *peplomer* (*peplos* = envelope) or spike. These peplomers can often be seen clearly in electron micrographs as projections from the outer surface of the envelope (Plate 1-1B). The other kind of envelope protein, *matrix protein*, is nonglycosylated and is found on the inside of the envelope of virions of several families; it provides added rigidity. The envelope of rhabdoviruses is closely applied to a bullet-shaped matrix protein that encloses a helical nucleocapsid. Arenaviruses, bunyaviruses, and coronaviruses have no matrix protein and consequently are rather more pleomorphic than other enveloped viruses.

Envelopes are not restricted to viruses of helical symmetry; some icosahedral viruses (ranaviruses, African swine fever virus, herpesviruses, togaviruses, flaviviruses, and retroviruses) have envelopes. The infectivity of most enveloped viruses depends on the integrity of the envelope, but some poxviruses have an envelope which is not necessary for infectivity.

CHEMICAL COMPOSITION

The essential components of infectious viral particles are nucleic acid (the genome) and protein. In addition, all enveloped viruses contain

lipid in the envelope and carbohydrate in their glycoprotein peplomers (as well as that in the nucleic acid). The largest and most complex viruses (poxviruses, ranaviruses, and African swine fever virus) also have lipids associated with other parts of the virion.

Nucleic Acid

Any particular virus contains only a single kind of nucleic acid. However, this may be DNA or RNA; indeed, the RNA viruses provide the only instance in nature in which RNA is the exclusive repository of genetic information. All viral genomes are *haploid*, i.e., they contain only one copy of each gene, except for retrovirus genomes, which are *diploid*. Viral DNA or RNA can be *double-stranded (ds)* or *single-stranded (ss)*. Since 1978 the genomes of many of the smaller animal viruses have been sequenced, and there are now no insuperable technical impediments to the sequencing of any viral genome. By 1985, the largest genome to be completely sequenced was that of a herpesvirus (EB virus), which consist of 172,000 base pairs (172 kilobase pairs, kbp).

When carefully extracted from the virion, the nucleic acid of viruses of certain families of both DNA and RNA viruses is infectious; i.e., when introduced into a cell it can initiate a complete cycle of viral replication, with the production of a normal yield of progeny virions. In these cases, messenger RNA (mRNA) is transcribed from the viral DNA in the nucleus, by a cellular transcriptase, or in the case of RNA viruses the genomic RNA itself functions as mRNA. In other cases, the isolated nucleic acid is not infectious even though it contains all the necessary genetic information. Among DNA viruses, failure to infect occurs if transcription requires a viral rather than a cellular transcriptase; among RNA viruses failure occurs when the viral RNA is of minus (–) sense or is double-stranded; its transcription to produce plus (+) sense mRNA then requires a virion-associated transcriptase. The (+) sense RNA of retroviruses is not infectious, because replication of the RNA occurs only after the production of a DNA provirus by a virion-associated reverse transcriptase (see Table 1-3).

DNA. The genome of all DNA viruses consists of a single molecule, which is double-stranded except in the case of the parvoviruses, and may be linear or circular.

The DNA of papovaviruses and hepadnaviruses is circular. Within the virion, the circular DNA of the papovaviruses, like that of mitochondria and bacterial plasmids, is a supercoiled circle, known as a superhelix (Plate 1-2A). When an enzyme relieves the tension by introducing a nick into one strand, the molecule becomes a relaxed circle (Plate 1-2B). One

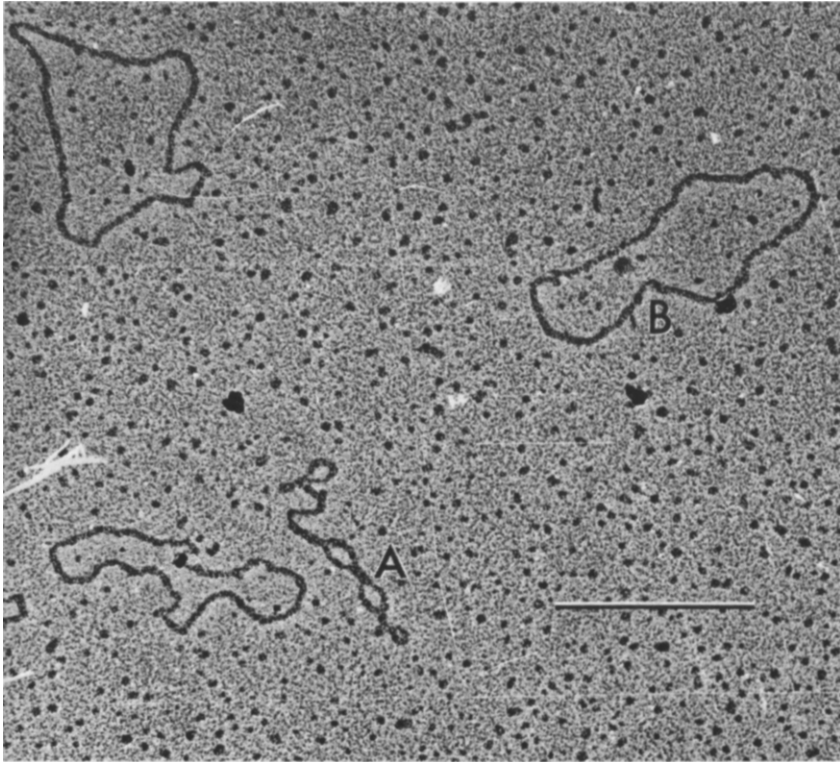


PLATE 1-2. DNA molecules extracted from the papovavirus SV40 (bar = 500 nm). Molecules of SV40 DNA exist in two major forms. (A) When it is isolated from the virions, most of the DNA occurs as a double-stranded, supercoiled, circular molecule (superhelix). (B) If one of the DNA strands is nicked, the superhelix becomes a circle. (Courtesy Dr. P. Sharp.)

strand of the circular DNA of hepadnaviruses is shorter than the other; the genome is thus only partially double-stranded.

Most of the linear DNAs from viruses of other families have characteristics which enable them to adopt a circular configuration temporarily, presumably during replication. The two strands of poxvirus DNA are covalently cross-linked at each end, so that on denaturation, the molecule becomes a large single-stranded circle (Fig. 1-3C). The linear dsDNA of some herpesviruses (and the linear ssRNA of retroviruses) contains *repeat sequences* at the ends of the molecule. Following partial digestion of both DNA strands from their 5' ends by an exo-

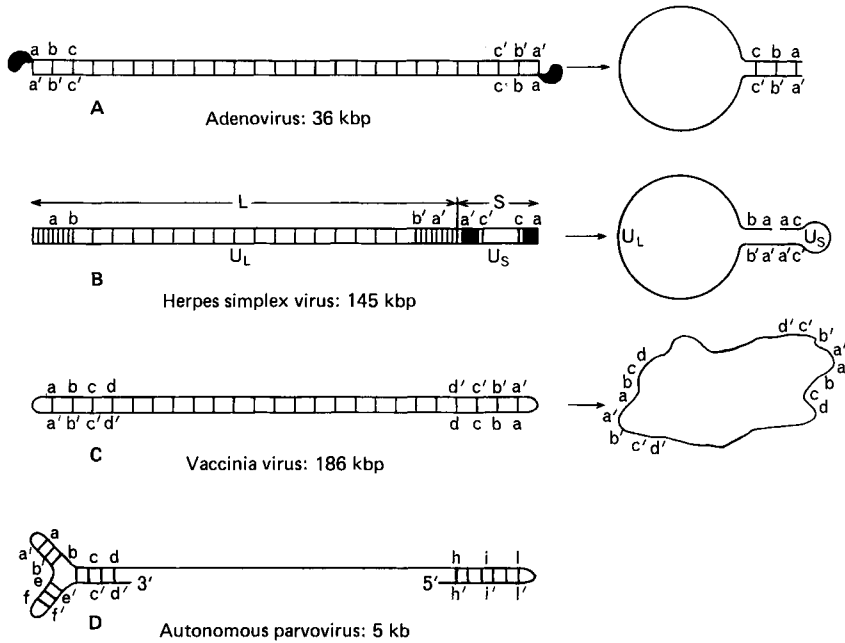


FIG. 1-3. Specialized arrangements at the termini of linear DNA viral genomes. Not to scale. (A) Adenovirus DNA has inverted terminal repeats, with a covalently linked protein located at each end of the molecule. Termini of single strands anneal to form "saucerpan" structures, as shown on the right. (B) Herpes simplex virus DNA consists of two covalently linked components, long (L) and short (S), each of which consists of a large unique sequence (U_L and U_S, respectively) flanked by inverted repeats. In a viral population, four isomeric forms differing in the orientation of the unique regions relative to each other occur in equimolar amounts. Intact single strands anneal as shown on the right. (C) Vaccinia virus DNA has inverted terminal repeats and each end is covalently closed, so that on denaturation it forms a large, single-stranded circular molecule. (D) Parvovirus DNA is single-stranded, with a palindromic sequence at the 3' end that folds back to form a Y-shaped hairpin structure stabilized by hydrogen bonding. The 5'-terminal sequence is also palindromic, but the sequence is unrelated to that at the 3' end.

nuclease, the exposed single-stranded ends are complementary in their nucleotide sequences, thus providing "cohesive" or "sticky" ends, so that, if the molecule is melted, it will reanneal as a circular dsDNA. In the case of the adenoviruses, these terminal repeats are inverted; hence, even without enzymatic digestion, denatured molecules self-anneal to form single-stranded circles (Fig. 1-3A). Inverted terminal repeat sequences, which give rise to "hairpin" structures, are also a feature of the ssDNA parvoviruses (Fig. 1-3D).

Another type of terminal structure occurs in adenoviruses, hepadnaviruses, parvoviruses, and the ssRNA picornaviruses and caliciviruses, in all of which a protein is covalently linked to the 5' terminus. This has an essential function in replication of the genome.

The DNA of certain iridoviruses (genus *Ranavirus*) contains a high proportion of 5-methylcytosine instead of cytosine.

The size of viral DNA genomes ranges from 4.5 kilobases (kb) (molecular mass, $M_r = 1.5 \times 10^6$) for the small ssDNA parvoviruses to over 200 kbp ($M_r = 185 \times 10^6$) for the large dsDNA poxviruses. As 1 kb or 1 kbp contains enough genetic information to code for about one average-sized protein, we recognize as an approximation that viral DNAs contain from about 4 to 200 genes and code for 4 to 200 proteins. However, the relationship between any particular nucleotide sequence and its protein product is not as straightforward as this.

First, the DNA of most of the larger viruses—like that of cells—contains what appears to be redundant information, in the form of (1) *repeat (reiterated) sequences* and (2) *introns*, i.e., regions which are *spliced* out and discarded from the RNA transcript. On the other hand, a single such RNA transcript may be spliced and/or cleaved in several different ways to yield several distinct mRNAs, which may be translated into different proteins. Furthermore, a given mRNA sequence may be read in two different reading frames (theoretically, up to three, because each *codon* is a triplet), giving rise to two (or three) proteins with different amino acid sequences. These fascinating examples of genetic economy are well illustrated by the papovaviruses (see Fig. 4-4) and will be discussed in Chapter 4. Suffice it to say at this point that nowadays we cannot always talk in terms of a direct one-to-one relationship between a “gene” and its “gene-product,” although such a relationship does sometimes occur.

Viral DNAs contain several kinds of noncoding sequences, in addition to introns and various types of terminal repeat sequences, described above. Consensus sequences, which tend to be conserved through evolution because they serve vital functions, include those of RNA splice sites, polyadenylation sites, RNA polymerase recognition sites and promoters, initiation codons for translation, and termination codons.

RNA. The genome of RNA viruses may also be single-stranded or double-stranded. Furthermore, while some occur as a single molecule, others are segmented. Arenavirus and birnavirus RNAs consist of 2 segments, bunyavirus RNA of 3, orthomyxovirus RNA of 7 or 8 (in different genera), and reovirus 10, 11, or 12 (in different genera). Each of these molecules is unique (often a single “gene”). All viral RNAs are linear; none is a covalently closed circle. However, the ssRNAs of arenaviruses and bunyaviruses have sticky ends, hence these molecules

occur as circles. The genomes of ssRNA viruses have considerable secondary structure, regions of base pairing probably serving as signals controlling transcription, translation, and/or packaging into the capsid.

Single-stranded viral nucleic acid, which is generally RNA, can also be defined according to its *sense* (also known as *polarity*). If it is of the same sense as mRNA, it is said to have *positive (+) sense*. This is the case with picornaviruses, caliciviruses, togaviruses, flaviviruses, coronaviruses, and retroviruses. If, on the other hand, its nucleotide sequence is complementary to that of mRNA, it is said to have *negative (-) sense*. Such is the case with the paramyxoviruses, orthomyxoviruses, rhabdoviruses, arenaviruses, and bunyaviruses, all of which have an RNA-dependent RNA polymerase (*transcriptase*) in the virion, in order that mRNA can be transcribed. With the arenaviruses and at least one genus of bunyaviruses one of the RNA segments is *ambisense*, i.e., part (+) sense, part (-) sense.

Where the viral RNA is of (+) sense, it is usually polyadenylated at its 3' end (in picornaviruses, caliciviruses, togaviruses, and coronaviruses, but not in flaviviruses) and capped at its 5' end (togaviruses, flaviviruses, coronaviruses) (see Chapter 4). The picornaviruses and caliciviruses have a protein attached to the 5' end of the viral RNA.

The size of ssRNA viral genomes varies from 7.5 to 18 kb ($M_r = 2.5$ to 7×10^6) and that of the dsRNA viruses from 7 to 22 kbp ($M_r = 4.8$ to 15×10^6)—a much smaller range than seen with the DNA viruses. Accordingly they code, in general, for fewer than a dozen proteins. In the case of the segmented RNA genomes of orthomyxoviruses and reoviruses, one can consider most of the segments to be individual genes, each coding for one unique protein. No such simple relationship applies to the other RNA viruses. For example, the picornavirus genome [(+) sense ssRNA] is directly translated into a single "polyprotein," which is subsequently cleaved to give the several viral polypeptides.

The essential features of the genomes of viruses of vertebrates are summarized in Table 1-2. Their remarkable variety is reflected in the diverse ways in which the information encoded in the viral genome is transcribed to RNA, then translated into proteins, and the ways in which the viral nucleic acid is replicated (see Chapter 4).

Viral preparations often contain some particles with an atypical content of nucleic acid (see Chapter 5). Host cell DNA is found in some papovavirus particles, and cellular ribosomes are incorporated in arenaviruses. Several copies of the complete viral genome may be enclosed within a single particle, or viral particles may be formed that contain no nucleic acid (empty particles) or that have an incomplete genome (*defective interfering particles*).

TABLE 1-2
Structure of the Genome in Viruses of Different Families^a

Family	Structure of nucleic acid
<i>Papovaviridae</i>	Circular superhelical dsDNA (see Plate 1-2)
<i>Adenoviridae</i>	Linear dsDNA with inverted terminal repeats and a covalently bound protein (see Fig. 1-3A)
<i>Herpesviridae</i>	Linear dsDNA; two unique sequences flanked by reiterated sequences; isomeric configurations occur (see Fig. 1-3B)
<i>Poxviridae</i>	Linear dsDNA; both ends covalently closed, with inverted terminal repeats (see Fig. 1-3C)
African swine fever virus	
<i>Parvoviridae</i>	Linear ssDNA, (-) sense; with repeated sequences and a hairpin structure at one end (see Fig. 1-3D)
<i>Hepadnaviridae</i>	Circular dsDNA with ss region
<i>Picornaviridae</i>	Linear ssRNA, (+) sense; serves as mRNA; 3' end polyadenylated (except <i>Flaviviridae</i>); 5' end capped, or protein covalently bound (<i>Picornaviridae</i> , <i>Caliciviridae</i>)
<i>Caliciviridae</i>	
<i>Togaviridae</i>	
<i>Flaviviridae</i>	
<i>Coronaviridae</i>	
<i>Paramyxoviridae</i>	Linear ssRNA, (-) sense
<i>Rhabdoviridae</i>	
<i>Orthomyxoviridae</i>	Segmented genome; 7 or 8 molecules of linear ssRNA, (-) sense
<i>Arenaviridae</i>	Segmented genome; 2 molecules of ssRNA, (-) sense or ambisense ^b ; "sticky ends" allow circularization
<i>Bunyaviridae</i>	Segmented genome; 3 molecules of ssRNA, (-) sense or ambisense; "sticky ends" allow circularization
<i>Retroviridae</i>	Diploid genome, dimer of linear ssRNA, (+) sense; hydrogen bonded at 5' ends; terminal redundancy; both 3' termini polyadenylated, both 5' ends capped; may carry oncogene
<i>Reoviridae</i>	Segmented genome; 10, 11, or 12 molecules of linear dsRNA
<i>Birnaviridae</i>	Segmented genome; 2 molecules of linear dsRNA

^aThere is considerable variation within some families, e.g., *Herpesviridae*, *Reoviridae*.

^bAmbisense indicates that part of molecule is (+) and part (-) sense.

Protein

Some virus-coded proteins are *structural*, i.e., they are part of the virion; some are *nonstructural* and are concerned with various aspects of the replication cycle. A major role of structural proteins is to provide the viral nucleic acid with a protective coat. The virions of all viruses of vertebrates contain several different proteins, the number ranging from 3 in the case of the simplest viruses to over 100 in the case of the complex poxviruses. In *isometric* viruses, the structural proteins form an icosahedral capsid which sometimes encloses a polypeptide core that is inti-

mately associated with the nucleic acid. Some virions, e.g., those of reoviruses, appear to have two concentric capsids.

The capsid proteins are assembled in the virion in groups, to form the capsomers visible in electron micrographs. Each capsomer is composed of one to six molecules of polypeptide, usually of the same kind (homopolymers) but sometimes different (heteropolymers). Capsomers from the vertices and the faces are usually composed of different polypeptides. A few viruses have a double capsid, each being composed of a different set of polypeptides. Other proteins, invariably glycoproteins, make up the peplomers projecting from the envelope; a second type of envelope protein is the nonglycosylated matrix protein that occurs as a layer at the inner surface of the lipid envelope of orthomyxoviruses, paramyxoviruses, and rhabdoviruses. One or more of the proteins on the surface of the virion has a specific affinity for complementary receptors present on the surface of susceptible cells; the same viral protein contains the antigenic determinants against which neutralizing antibodies are made. Virions of several families carry a limited number of enzymes, transcriptases being the most important (Table 1-3).

Lipid

Lipid constitutes about 30–35% of the dry weight of enveloped viruses, the viral envelope being derived from cellular lipids. As a consequence, the composition of lipids of particular viruses differs according to the composition of the membrane lipids of the cells in which they have replicated. About 50–60% of the envelope lipid is phospholipid, and most of the remainder is cholesterol.

The poxviruses, ranaviruses, and African swine fever virus contain cellular lipid in their envelopes, and other lipids in the inner part of the virion. Lipid occurs in the outer membrane of poxviruses, and has a different composition from that of host cell lipids. In ranaviruses and African swine fever virus the additional viral lipid occurs within the icosahedral capsid.

Carbohydrate

Apart from that associated with viral nucleic acid, carbohydrate occurs as a component of viral glycoproteins, which usually occur as peplomers, with their hydrophobic ends buried in the lipid bilayer of the envelope, while their glycosylated hydrophilic ends project into the medium. Poxviruses also contain internal glycoproteins, in the membrane of the core, and one of the outer capsid proteins of rotaviruses is glycosylated.

TABLE 1-3
Virion-Associated Enzymes and Their Functions

Family	Virus	Function
<i>Enzymes affecting interaction of virions with the host cell surface</i>		
Neuraminidase	Orthomyxovirus, paramyxovirus	Cleaves N-acetylneuraminic acid from surface polysaccharides
<i>Enzymes transcribing the viral genome into mRNA^a</i>		
DNA-dependent RNA polymerase	Poxvirus, African swine fever virus	Transcribes RNA
dsRNA-dependent RNA polymerase	Viruses with dsRNA	Transcribes RNA
ssRNA-dependent RNA polymerase	Viruses with (-) sense ssRNA	Transcribes RNA
<i>Enzymes adding specific terminal groups to viral mRNA</i>		
Nucleotide phosphohydrolase	Viruses synthesizing mRNA in virions (e.g., poxvirus, reovirus)	Converts terminal 5' triphosphate to diphosphate as prelude to guanylylation
Guanylyltransferase	Viruses synthesizing mRNA in virions (e.g., poxvirus, reovirus)	Adds guanylyl residue to 5' end diphosphate in mRNA
RNA methylases	Viruses synthesizing mRNA in virions (e.g., poxvirus, reovirus)	Methylate guanylyl residue at 5' end of mRNA and some riboses in 2' position
Poly(A) polymerase	Viruses synthesizing mRNA in virions (e.g., poxvirus, reovirus)	Synthesizes poly(A) tail at 3' end of mRNA
<i>Enzymes involved in copying virion RNA into DNA</i>		
RNA-dependent DNA polymerase (reverse transcriptase)	Retrovirus	Makes DNA-RNA hybrids
RNase H (an activity of the reverse transcriptase)	Retrovirus	Breaks down RNA strand in RNA-DNA hybrid
Polynucleotide ligase	Retrovirus	Closes ss breaks in dsDNA
<i>Enzymes for nucleic acid replication or processing</i>		
DNA-dependent DNA polymerase	Hepadnavirus	Synthesizes dsDNA
Deoxyribonuclease (exo- and endo-)	Poxvirus, retrovirus, adenovirus	Break DNA chains and cross-links
Endoribonuclease	Poxvirus	Processing of mRNA
<i>Other enzymes</i>		
Protein kinases	Retrovirus, orthomyxovirus, paramyxovirus, herpesvirus, adenovirus	Phosphorylate proteins
tRNA aminoacylases	Retrovirus	Aminoacylate tRNA

^aAlso called transcriptases.

PRESERVATION OF VIRAL INFECTIVITY

In general, viruses are more sensitive than bacteria or fungi to inactivation by physical and chemical agents. A knowledge of their sensitivity to environmental conditions is therefore important for ensuring the preservation of the infectivity of viruses as reference reagents, and in clinical specimens collected for diagnosis, as well as for their deliberate inactivation for such practical ends as sterilization, disinfection, and the production of inactivated vaccines (see Chapters 14 and 16).

The principal environmental condition that may adversely affect the infectivity of viruses in clinical specimens is too high a temperature; other important conditions are pH and lipid solvents.

Temperature

Viruses vary considerably in heat stability. Surface proteins are denatured within a few minutes at temperatures of 55° to 60°C, with the result that the virion is no longer infectious, because it is no longer capable of normal cellular attachment and/or uncoating. At ambient temperature the rate of decay of infectivity is slower but significant, especially in hot summer weather or in the tropics in any season. Viral preparations must therefore be stored at low temperature; 4°C (ice or a refrigerator) is usually satisfactory for a day or so, but longer term preservation requires temperatures well below zero. Two convenient temperatures are -70°C, the temperature of frozen CO₂ ("dry ice") and of some freezers, or -196°C, the temperature of liquid nitrogen. As a rule of thumb, the half-life of most viruses can be measured in seconds at 60°C, minutes at 37°C, hours at 20°C, days at 4°C, and years at -70°C or lower. The enveloped viruses are more heat labile than nonenveloped viruses. Some enveloped viruses, notably respiratory syncytial virus, tend to be inactivated by the process of freezing and subsequent thawing, probably as a result of disruption of the virion by ice crystals. This poses problems in the collection and transportation of clinical specimens. The most practical way of avoiding such problems is to deliver specimens to the laboratory as rapidly as practicable, packed without freezing, on ice (see Chapter 13).

In the laboratory, it is often necessary to preserve stocks of viable virus for years. This is achieved in one of two ways: (1) rapid freezing of small aliquots of virus suspended in medium containing protective protein and/or dimethyl sulfoxide, followed by storage at -70°C or -196°C; (2) freeze-drying (lyophilization), i.e., dehydration of a frozen viral suspension under vacuum, followed by storage of the resultant powder at

4°C or -20°C. Freeze-drying prolongs viability significantly even at ambient temperatures, and is important in enabling live viral vaccines to be used in tropical countries.

Ionic Environment and pH

On the whole, viruses prefer an isotonic environment at physiological pH, but some virions tolerate a wide ionic and pH range. For example, whereas most enveloped viruses are inactivated at pH 5-6, adenoviruses and many picornaviruses survive the acidic pH of the stomach.

Lipid Solvents

The infectivity of enveloped viruses is readily destroyed by lipid solvents such as ether or chloroform, or detergents like sodium deoxycholate, so that these agents must be avoided in laboratory procedures concerned with maintaining the viability of viruses. On the other hand, detergents are commonly used by virologists to solubilize viral envelopes and liberate proteins for use as vaccines or for chemical analysis. Sensitivity to lipid solvents is also employed as a preliminary screening test in the identification of new viral isolates, especially by arbovirologists.

FURTHER READING

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