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CHAPTER 4

Viral Replication

Viral replication is the central focus of much experimental virology and a significant part of molecular biology. Studies with *bacteriophages* in their prokaryotic host cells in the 1940s and 1950s provided the first insights into the complexities of viral replication. With the development of mammalian cell culture procedures (see Chapter 3), the techniques used for the study of bacteriophages were adapted to animal viruses. Progress has been such that the basic mechanisms of transcription, translation, and nucleic acid replication have been characterized for all the major families of animal viruses and the strategy of gene expression and its regulation clarified. Many important biochemical phenomena, such as splicing and other types of posttranscriptional processing of RNA, posttranslational cleavage and glycosylation of proteins, replication of RNA, reverse transcription, integration, and transposition of

viral genes and cellular oncogenes, were first elucidated by virologists and have general application in cell biology.

Our knowledge of viral replication is now very detailed and is expanding rapidly. Every viral family has a different strategy of replication, and for each family several reviews have been published since 1980. It is neither possible nor appropriate to deal comprehensively with the subject in this book. This chapter provides a general overview; some additional information on particular viral families is provided in Part II. An understanding of viral replication provides a basis for understanding pathogenesis, immunity, chemotherapy, and the role of viruses in cancer.

THE VIRAL REPLICATION CYCLE

The One-Step Growth Curve

Following the pattern established in experiments with bacteriophages, studies of the replication of animal viruses began with the one-step growth experiment. In such experiments, all cells in a culture are infected simultaneously, i.e., at high *multiplicity of infection*. Unadsorbed input virus is removed or neutralized, usually after 1 hour, and the increase in infectious virions over time is followed by titrating cell-free and cell-associated infectivity. Shortly after infection, the inoculated virus "disappears"; infectious particles cannot be demonstrated, even intracellularly. This *eclipse period* (Fig. 4-1) continues until the first progeny virions become detectable some hours later. Nonenveloped viruses mature within the cell and are detectable for some hours as intracellular virions before they are released by cell lysis. Many enveloped viruses, on the other hand, mature by budding from the plasma membrane and are thus immediately released into the medium. The eclipse period ranges from 5 to 15 hours for the various DNA viruses and from 3 to 10 hours for RNA viruses (see Table 4-2).

Early studies, relying on quantitative electron microscopy and assay of infectivity, provided a limited amount of information about the early and the late events in the replication cycle (attachment, penetration, intracellular maturation, and budding) but could not tell us anything about what happened during the eclipse period. Investigation of the expression and replication of the viral genome became possible only with the development of molecular methods, and during the last two decades all the sophisticated techniques of molecular biology have been applied to this problem.

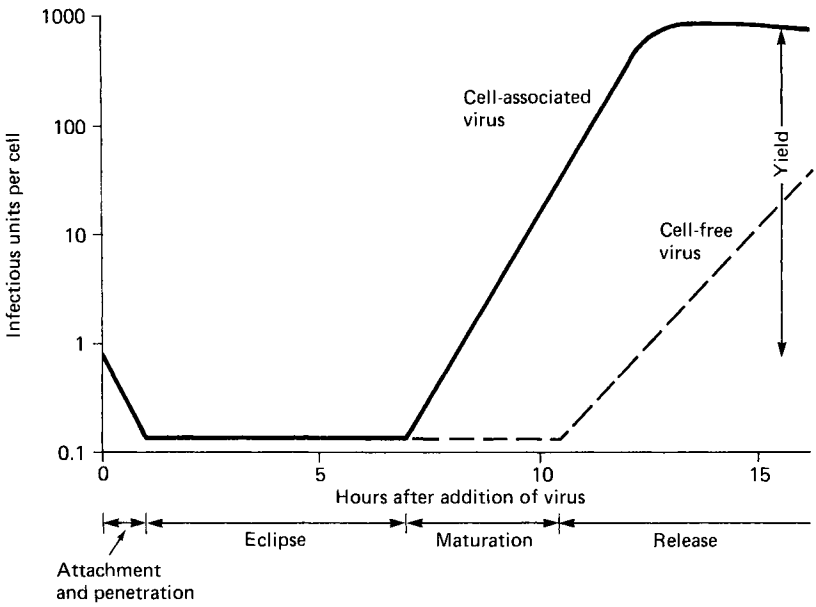

FIG. 4-1. *One-step growth curve of a nonenveloped virus. Attachment and penetration are followed by an eclipse period of 3 to 15 hours (see Table 4-2) during which cell-associated infectivity cannot be detected. This is followed by a period of several hours during which maturation occurs. Virions of nonenveloped viruses are often released late and incompletely, when the cell lyses. Release of enveloped virions occurs concurrently with maturation by budding from the plasma membrane.*

The Replication Cycle

The complete cycle, including what happens during the eclipse period, is illustrated diagrammatically in Fig. 4-2, using an icosahedral DNA virus as an example and ignoring the precise location of intracellular events. Following attachment, the virion penetrates the host cell and is partially uncoated to expose the viral genome. Certain *early viral genes* are transcribed into RNA which may then be processed in a number of ways, including splicing. The early gene-products translated from this *messenger RNA* (*mRNA*) are of two main types: proteins that regulate the expression of the viral and cellular genomes, and enzymes required for the replication of viral nucleic acid (Table 4-1). Following viral nucleic acid replication, *late viral genes* are transcribed. The late proteins are

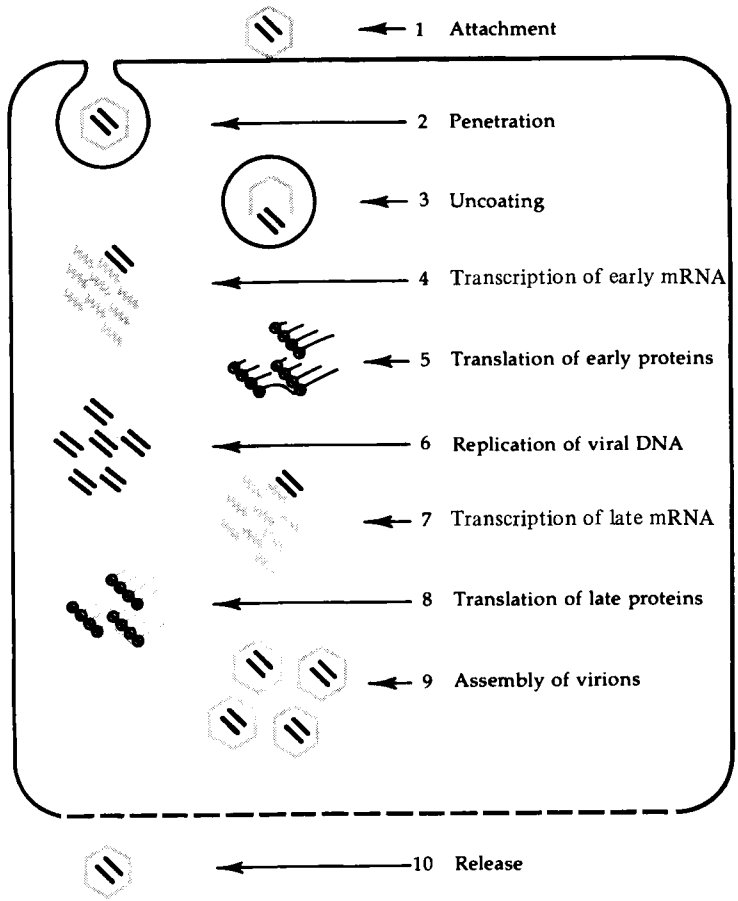

FIG. 4-2. *General features of the viral replication cycle, using a nonenveloped icosahedral DNA virus as a model. No topographical location for any step is implied. One step grades into the next such that, as the cycle progresses, several of these processes are proceeding simultaneously. Release occurs by cell lysis.*

principally viral structural proteins; some of these are subject to post-translational modification, such as glycosylation and/or cleavage. Assembly of icosahedral virions occurs in the nucleus or cytoplasm, depending on the particular family. Enveloped viruses are completed by "budding" through cellular membranes. Each infected cell yields several thousand new virions over a period of several hours.

TABLE 4-1
Kinds of Proteins Coded by the Viral Genome

Kind of gene-product	Description
Early	Nonstructural proteins, mainly enzymes, involved in transcription and replication of viral nucleic acid
Early or late	Proteins affecting cellular macromolecular synthesis Regulatory proteins controlling expression of viral genes
Late	Structural proteins of the virion Virion-associated enzymes

Differences between Families

Many of the processes that can be investigated by morphological studies and infectivity assays differ according to viral family (Table 4-2). There are three principal methods of penetration, and virions may be released by cell lysis or by budding. Some viruses acquire an envelope by budding through plasma membrane, others through nuclear membrane, and still others in the Golgi complex or the endoplasmic reticulum. Some viruses shut down the synthesis of cellular macromolecules very effectively, whereas others do not. Indeed, some viruses are noncytocidal and others actually induce the cell to divide, or even transform it to a tumor cell (see Chapter 6).

Even more significant are the differences in the strategy of expression of the viral genome. Under this heading are subsumed the key processes occurring during the eclipse period: transcription and processing of viral mRNA (Fig. 4-2, steps 4 and 7), translation and processing of viral proteins (steps 5 and 8), and replication of the viral nucleic acid (step 6). Before discussing them, we will describe the earlier events: attachment (step 1), penetration (step 2), and uncoating (step 3).

ATTACHMENT (ADSORPTION)

Because virions and cells are both negatively charged at physiological pH, they tend to repel one another, but random collisions do occur and initial (reversible) attachment may be facilitated by cations. Firm binding requires the presence of specific receptors for the virus on the plasma membrane, to which specific molecules on the surface of the virion

TABLE 4-2
Characteristics of Replication of Selected Viruses

Family	Example	Site of nucleic acid replication	Eclipse period (hours[a])	Budding (membrane)	Cell shutdown[a,b]
Parvoviridae	Rat virus	Nucleus	6	—	+
Papovaviridae	SV40	Nucleus	15	—	+[c]
Adenoviridae	Adenovirus h2	Nucleus	12	—	+
Herpesviridae	Herpes simplex virus	Nucleus	5	Nuclear	+
Unclassified	African swine fever virus	Cytoplasm	5	Plasma	+
Poxviridae	Vaccinia virus	Cytoplasm	5	Golgi	+
Picornaviridae	Poliovirus	Cytoplasm	3	—	+
Caliciviridae	Feline calicivirus	Cytoplasm	3	—	+
Togaviridae	Sindbis virus	Cytoplasm	3	Plasma	+
Flaviviridae	Kunjin virus	Cytoplasm	3	Endoplasmic	+
Coronaviridae	Murine hepatitis virus	Cytoplasm	5	Golgi	+
Paramyxoviridae	Newcastle disease virus	Cytoplasm	4	Plasma	+
Rhabdoviridae	Vesicular stomatitis virus	Cytoplasm	3	Plasma	+
Arenaviridae	Pichinde virus	Cytoplasm	5	Plasma	−
Bunyaviridae	Snowshoe hare virus	Cytoplasm	4	Golgi	+
Orthomyxoviridae	Influenza A virus	Nucleus	3	Plasma	+
Retroviridae	Avian leukosis virus	Nucleus	10	Plasma	−
Reoviridae	Reovirus 3	Cytoplasm	5	—	+
Birnaviridae	Infectious bursal disease virus	Cytoplasm	4	—	−

[a]Differs with multiplicity of infection, strain of virus, cell type, and physiological condition.
[b]Differs markedly in degree and in rapidity, from early and profound to late and partial.
[c]No shutdown in transformation.

attach. Orthomyxoviruses and paramyxoviruses bind via the hemagglutinin, an envelope glycoprotein, to glycoprotein or glycolipid cellular receptors with oligosaccharide side chains terminating in *N*-acetylneuraminic acid. Most enteroviruses of humans, swine, and chickens are highly host cell-specific, because only the homologous cells carry receptors to which the relevant viral capsid protein attachment site can bind.

While there is some specificity about the binding of virions to particular cellular receptors, several different viruses may utilize the same receptor.

PENETRATION

Electron microscopic and other data show that virions can enter cells by at least three different mechanisms: endocytosis, fusion, and translocation. The majority of virions entering a cell fail to initiate infection, many virions taken up by endocytosis being degraded by lysosomal enzymes. However, for some viruses this may be the normal route of penetration, leading to uncoating and productive infection.

Endocytosis

The majority of mammalian cells are continuously engaged in *receptor-mediated endocytosis*, a specific process for the uptake of essential macromolecules. Viruses may use receptor-mediated endocytosis to initiate infection (Plate 4-1). Following attachment to receptors, virions move down into *coated pits*. These pits, coated with clathrin, fold inward to produce coated vesicles that enter the cytoplasm and fuse with a lysosome to form a phagolysosome. With enveloped viruses, the envelope of endocytosed virions fuses with the lysosomal membrane, releasing the viral nucleocapsid into the cytoplasm. In this way a virion can be uncoated by a lysosome but escape total degradation by the lysosome's hydrolytic enzymes. Recent studies with influenza virus have identified a pH 5-mediated conformational change in the hemagglutinin molecule which enables fusion to occur between the viral envelope and the membrane of the phagolysosome.

Fusion with Plasma Membrane

The F (fusion) glycoprotein of paramyxoviruses, in its cleaved form, enables the envelope of these viruses to fuse directly with the plasma membrane, even at pH 7. This may allow the nucleocapsid to be released directly into the cytoplasm. Although a number of other enveloped viruses display a capacity to fuse cells or to lyse erythrocytes, it is not clear whether this is the normal way in which they infect cells.

Translocation

Some nonenveloped icosahedral viruses appear to be capable of passing directly through the plasma membrane.

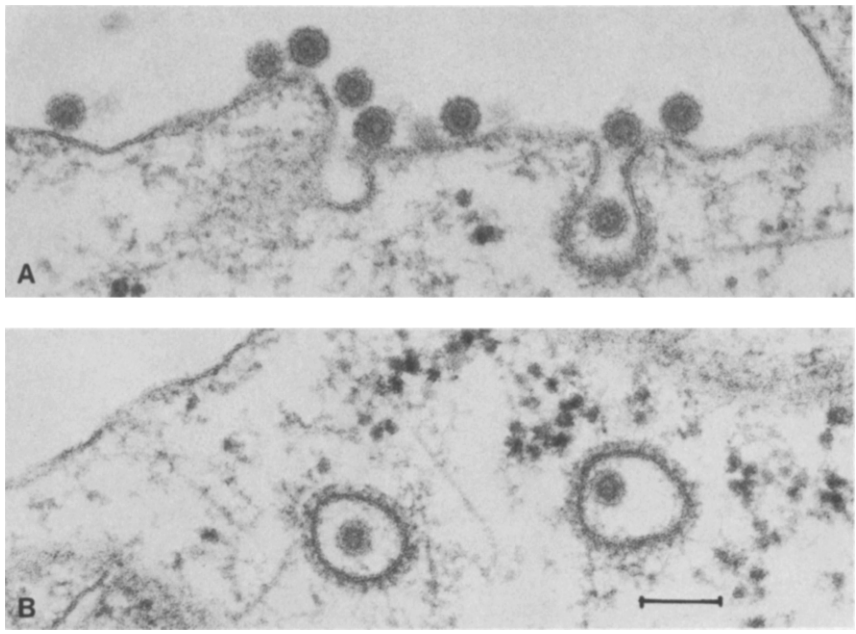

PLATE 4-1. *Penetration by a togavirus. (A) Attachment and movement into a coated pit. (B) Endocytosis, coated vesicle (bar = 100 nm). [A, from E. Fries and A. Helenius,* Eur. J. Biochem. **8,** *213 (1979); B, from K. Simons* et al., Sci. Am. **246,** *46 (1982), Courtesy Dr. A. Helenius.]*

UNCOATING

In order that at least the early viral genes may become available for transcription, it is necessary that the virion be at least partially uncoated. With viruses that enter the cell by fusion of their envelope with either the plasma membrane or the membrane of a phagolysosome, the nucleocapsid is discharged directly into the cytoplasm. In the case of viruses with helical nucleocapsids, transcription begins from viral RNA while it is still associated with nucleoprotein. In the case of the icosahedral reoviruses only certain capsid proteins are removed and the viral genome expresses all its functions, even though it is never fully released from the core ("subviral particle"; Plate 4-2). Poxviruses are uncoated in two stages: first, to a core, from which half the genome is transcribed; then completely, following the synthesis of a virus-coded uncoating protein. With the picornaviruses, the process of attachment of the virion to the cell leads to a conformational change in the capsid,

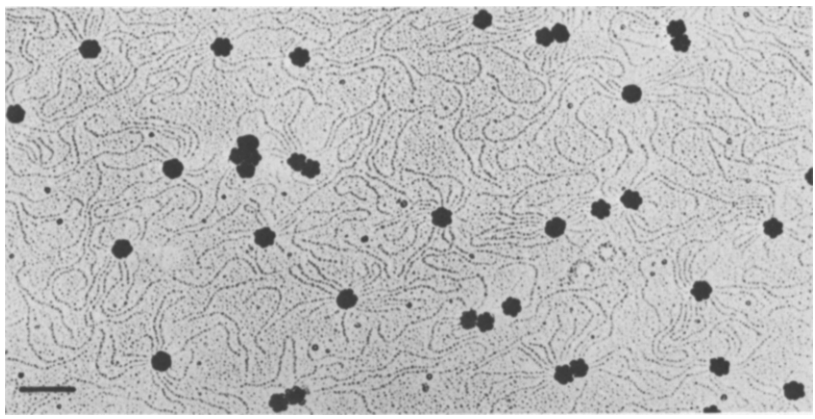

PLATE 4-2. *Reovirus messenger RNA (bar = 200 nm). Reovirus "cores" that have synthesized mRNA for 8 minutes at 37°C were prepared for electron microscopy by the Kleinschmidt technique, stained with uranyl acetate, and shadowed at a low angle with platinum–palladium, showing the fine fibrils of mRNA being extruded from the cores or occurring free around them. The results of polyacrylamide gel electrophoretic analysis of such mRNA molecules at various times during the replication cycle are illustrated in Fig. 4-6. [From N. M. Bartlett, S. C. Gillies, S. Bullivant, and A. R. Bellamy,* J. Virol. **14,** *315 (1974), courtesy Dr. A. R. Bellamy.]*

resulting in the loss of capsid proteins VP4 and VP2 and rendering the particle susceptible to proteases; the attachment step itself triggers the process of uncoating. For some viruses that replicate in the nucleus there is evidence that the later stages of uncoating occur there, rather than in the cytoplasm.

STRATEGIES OF VIRAL REPLICATION

The key events in viral replication are the synthesis of viral proteins, the replication of the viral genome, and the assembly of the new components into virions. To synthesize viral proteins, viral mRNAs must be produced in a form capable of being recognized and translated on cellular ribosomes. Eukaryotic cells synthesize their own mRNA in the nucleus by transcription of the cellular DNA followed by processing of the transcript. They lack the enzymes necessary for synthesizing mRNA off a viral RNA genome and they cannot transcribe viral DNA located in the cytoplasm. Therefore, only those DNA viruses that replicate in the nucleus utilize the cellular machinery for transcription. All other viruses

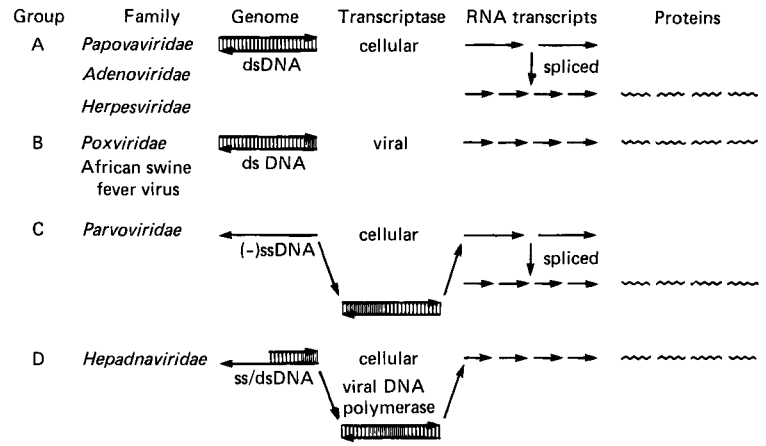

FIG. 4-3. *Simplified diagram showing essential features of the strategy of expression of the genome of DNA viruses. The sense of each nucleic acid molecule is indicated by an arrow [(+), to the right; (−) to the left]. The number of mRNA and protein species for each virus has been arbitrarily shown as four. See text for details.*

provide their own enzymes to produce mRNAs. Eukaryotic cells have a further constraint, namely, that the protein-synthesizing machinery apparently cannot recognize internal initiation sites within polycistronic mRNAs. Hence viruses must synthesize a separate (*monocistronic*) mRNA corresponding to each gene in their genome, or, alternatively, a *polycistronic* mRNA must be translated into a large precursor "polyprotein" which is then cleaved into individual proteins.

The diverse strategies followed by viruses of different families for transcription and translation are illustrated diagrammatically in Fig. 4-3 (for DNA viruses) and Fig. 4-5 (for RNA viruses). Necessarily, the processes summarized in these figures and the descriptions of them involve major oversimplifications. We will describe in turn transcription, translation, and replication of the viral nucleic acid.

TRANSCRIPTION

The viral RNA of (+) sense ssRNA viruses binds directly to ribosomes and is translated in full or in part without the need for any prior transcriptional step. With all other classes of viral genomes, mRNA must be transcribed. In the case of DNA viruses that replicate in the nucleus, the cellular DNA-dependent RNA polymerase II performs this function. All other viruses require unique and specific transcriptases which are virus-

coded and are an integral component of the virion. Cytoplasmic dsDNA viruses carry a DNA-dependent RNA polymerase, whereas dsRNA viruses have dsRNA-dependent RNA polymerase, and (−) sense ssRNA viruses carry a ssRNA-dependent RNA polymerase (see Tables 2-1 and 2-2).

DNA Viruses

For all DNA viruses, mRNA must be transcribed by a DNA-dependent RNA polymerase. Transcription of the viral DNA is programmed such that not all genes are expressed simultaneously or continuously throughout the replication cycle. Particular parts of the genome are transcribed in sequence, the so-called *early genes* first, and the *late genes* later in the cycle. Viruses of different families differ according to whether a cellular or a viral transcriptase is employed, correlating with a nuclear or cytoplasmic site of replication. There are four classes of strategy of expression of the viral genome (Fig. 4-3A–D), described below.

dsDNA; Cellular Transcriptase (Fig. 4-3A). This group comprises the papovaviruses, adenoviruses, and herpesviruses, and has in one respect the most straightforward strategy: the viral DNA is transcribed within the nucleus by a cellular DNA-dependent RNA polymerase. There are at least two temporally separated cycles for adenoviruses and herpesviruses; in each instance the structural proteins of the virion are made from mRNAs produced in the last cycle of transcription. Polycistronic but subgenomic RNA transcripts (corresponding to several genes but less than the whole genome) undergo cleavage and splicing to produce monocistronic mRNAs, introns being removed in the process.

dsDNA; Virion Transcriptase (Fig. 4-3B). The poxviruses and African swine fever virus, which replicate in the cytoplasm, carry their own transcriptase. It appears that monocistronic mRNAs are transcribed directly from the viral DNA. There are at least three cycles of transcription. The transcripts are translated directly into proteins, some of which need to undergo posttranslational cleavage to yield functional molecules.

ssDNA; Cellular Transcriptase (Fig. 4-3C). The (−) sense ssDNA of the parvoviruses requires the synthesis of a complementary strand to form dsDNA; this is then transcribed in the nucleus and the transcripts are processed to produce mRNAs, before export to the cytoplasm for translation.

ds/ssDNA; Cellular Transcriptase, Virion DNA Polymerase (Fig. 4-3D). The ssDNA portion of the genome of hepadnaviruses is first repaired by

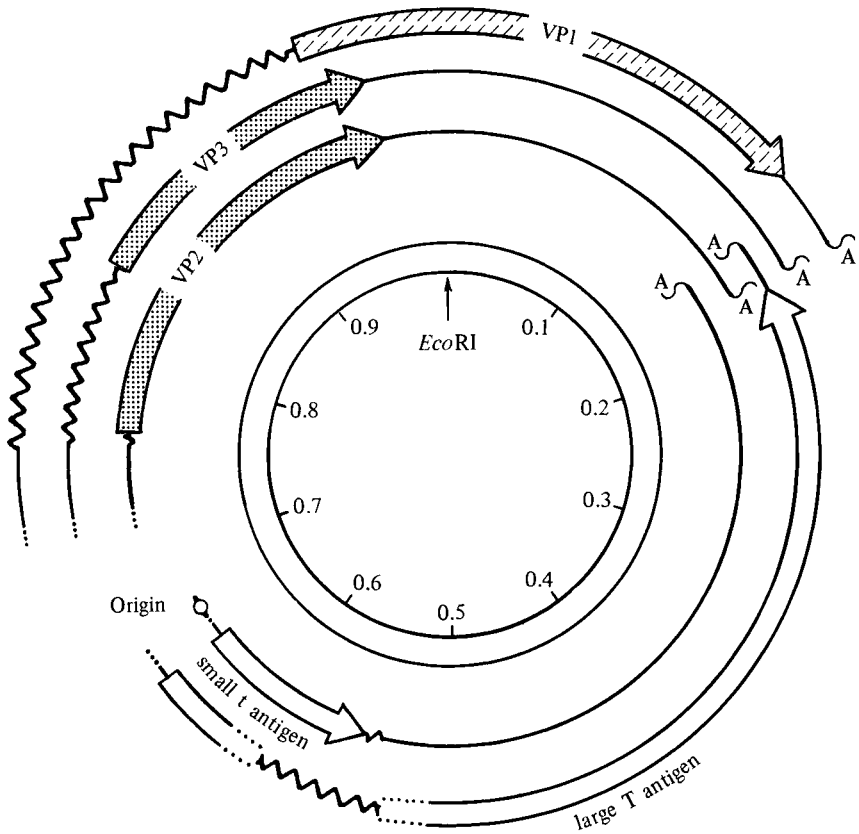

FIG. 4-4. *Transcription map of the DNA of the papovavirus SV40. The circular dsDNA is oriented with the* EcoRI *restriction endonuclease cleavage site at zero and the origin of DNA replication (origin) at map position 0.66. The direction of transcription of the early genes is counterclockwise on one DNA strand (open arrows), and that of the late genes is clockwise on the other strand (stippled and shaded arrows). The thin lines indicate regions of the primary RNA transcript that are not translated into protein, while the wavy lines indicate regions of the transcript that are spliced out (introns). The 3'-terminal poly(A) tail of each mRNA is labeled A. The coding regions of the primary transcript are shown with large arrows. The genes for the early proteins, small-t and large-T overlap, as do those for the late proteins VP1, VP2, and VP3. Large-T is coded by two noncontiguous regions of DNA. The amino acid sequence of VP3 corresponds with the C-terminal half of VP2. However, VP1 shares no part of its amino acid sequence with VP2 or VP3, even though the VP1 gene overlaps VP2 and VP3, because its mRNA is transcribed in a different reading frame. [Modified from W. Fiers* et al., Nature (London) **273,** *113 (1978).]*

a virion-associated DNA polymerase, and the DNA then converted into a supercoiled dsDNA. Transcription of mRNA by cellular RNA polymerase II then occurs.

Expression of a DNA Genome. Analysis of the 5224-bp sequence of the circular dsDNA of the papovavirus SV40 and its transcription program have provided insights into these processes (Fig. 4-4). The following points should be noted:

1. The early genes and the late genes are transcribed by the host cell RNA polymerase II in opposite directions, from different strands of the DNA.
2. Certain genes overlap and are translated in the same frame, so that their protein products have some amino acid sequences in common.
3. Some regions of the viral DNA are read in overlapping but different reading frames, so that two completely different amino acid sequences are obtained.
4. At least 15% of the viral DNA consists of *intervening sequences* (*introns*), which are transcribed but not translated, because they are excised from the primary transcript.
5. Up to three distinct proteins can be produced from mRNAs derived from a primary transcript by different splicing protocols.

Regulation of Transcription. Studies with adenoviruses have elucidated the mechanisms that regulate the expression of viral genomes, which operate principally, but not exclusively, at the level of transcription. Because of the complications arising from posttranscriptional cleavage of mRNA and posttranslational cleavage of precursor proteins in eukaryotic cells, it is no longer adequate to talk of a "gene" and its "gene-product." More appropriate perhaps is to think in terms of a *transcription unit*, i.e., that region of the genome beginning with the transcription initiation site, extending right through to the transcription termination site, and including all introns and exons in between. "Simple" transcription units may be defined as those encoding only a single protein, whereas "complex" transcription units code for more than one. There are many adenovirus transcription units. At different stages of the viral replication cycle—"pre-early," "early," "intermediate," and "late"—the various transcription units are transcribed in a given temporal sequence. A product of the early-region E1A induces the other early regions including E1B, but following viral DNA replication, there is a 50-fold increase in the rate of transcription from the major late promoter relative to early promoters such as E1B, and a decrease in E1A mRNA levels. A second control operates at the point of termination of transcription.

Transcripts that terminate at a particular point early in infection are read through this termination site later in infection to produce a range of longer transcripts with different polyadenylation sites.

Processing of RNA Transcripts. Primary RNA transcripts from eukaryotic DNA are subject to a series of posttranscriptional alterations in the nucleus, known as *processing,* prior to export to the cytoplasm as mRNA. First, a *cap,* consisting of 7-methylguanosine (m^7Gppp) is added to the 5′ terminus. The function of this *poly(A) tail* is uncertain, but it may act as a recognition signal for processing and for transport of mRNA from the nucleus to the cytoplasm, and it may stabilize mRNA against degradation in the cytoplasm. Third, a methyl group is added at the 6 position to about 1% of the adenylate residues throughout the RNA (methylation). Fourth, introns are removed from the primary transcript and the *exons* are linked together in a process known as *splicing;* the precise mechanism is not known but may involve excision of the introns by endonucleases, followed by ligation. Splicing is an important mechanism for regulating gene expression in nuclear DNA viruses. A given RNA transcript can have two or more splicing sites and be spliced in several different ways to produce a variety of mRNA species coding for distinct proteins; both the preferred poly(A) site and the splicing pattern may change in a regulated fashion as infection proceeds. The rate of degradation of mRNA provides another level of regulation. Not only do different mRNA species have different half-lives, but the half-life of a given mRNA species may change as the replication cycle progresses.

RNA Viruses

Transcription is more complicated for the RNA viruses than for rhe DNA viruses, which is perhaps not surprising, since they are the only forms of life that utilize RNA as the repository of genetic information. There are, broadly, three main strategies: (1) the virion RNA of most viruses with (+) sense RNA is itself infectious, because it functions as mRNA, (2) viruses with (−) sense ssRNA, or with dsRNA, carry a virion-associated RNA-dependent RNA polymerase which transcribes mRNA from the viral RNA, and (3) the (+) sense virion RNA of retroviruses is transcribed into DNA, which serves as a template for transcription of viral mRNAs by a cellular transcriptase. These three general strategies can be further subdivided on the basis of more subtle differences to give seven groups (Fig. 4-5A–G).

ssRNA; (+) Sense (Fig. 4-5A,B,C). In these groups the (+) sense virion RNA is itself infectious. In the picornaviruses and flaviviruses the ge-

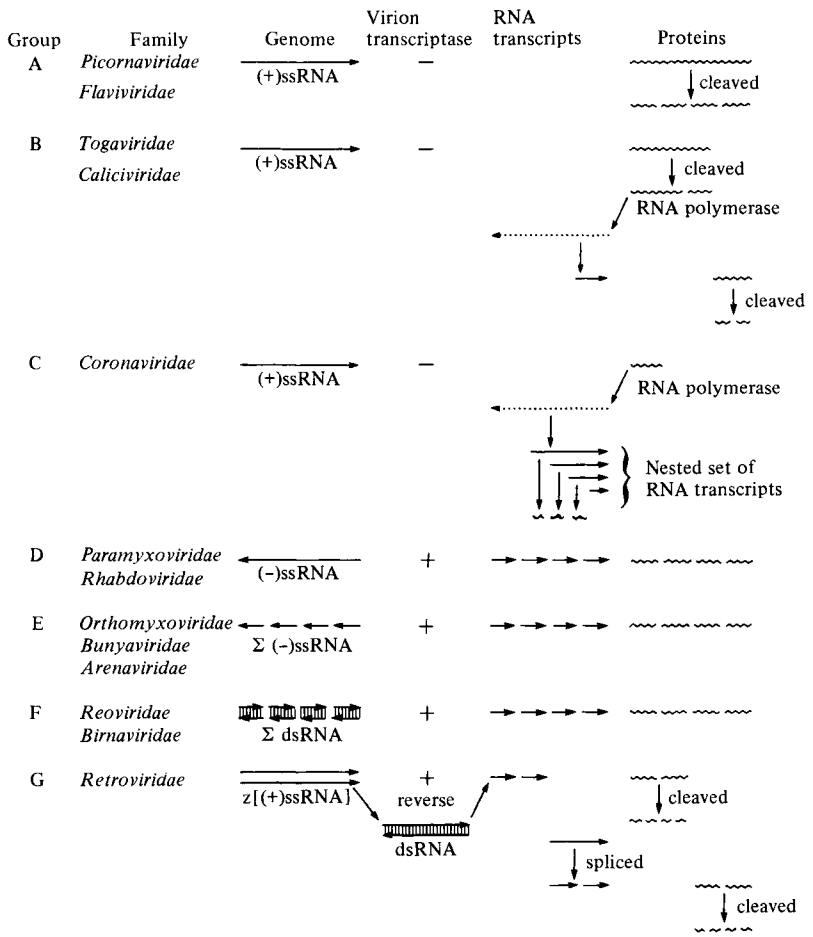

FIG. 4-5. *Simplified diagram showing the essential features of the strategy of expression of the genome of RNA viruses. The sense of each nucleic molecule is indicated by an arrow [(+) to the right; (−) to the left]. The number of segments of segmented genomes, mRNA molecules, and protein molecules has been arbitrarily shown as four. See text for details.*

nome, acting as a single polycistronic mRNA, is translated into a single polyprotein which is subsequently cleaved to give the individual viral polypeptides (Fig. 4-5A). Togaviruses of the genus *Alphavirus* also contain a single polycistronic (+) sense ssRNA molecule, but only about two-thirds of the viral RNA (the 5′ end) is translated; the resulting polyprotein is cleaved into four nonstructural proteins, two of which form the RNA polymerase. This enzyme then copies a full-length (−)

sense strand, from which two species of (+) sense strand are copied: full-length virion RNA destined for encapsidation, and a one-third length RNA, which is colinear with the 3′ terminus of the viral RNA and is translated into a polyprotein from which three or four structural proteins are produced by cleavage. The caliciviruses have not been so extensively studied, but also produce both genome-length and subgenomic mRNA species.

Flaviviruses were recently accorded the status of a family separate from the togaviruses. They do not produce subgenomic mRNAs, and translation of the (+) sense virion RNA initiates with the capsid protein near the 5′ end of the genome and proceeds sequentially through the genome to produce one precursor polyprotein. This is rapidly cleaved during the process of translation, so that the complete polyprotein is never seen.

Coronaviruses have a unique strategy. Initially, in a step about which little is known, part of the virion RNA acts as mRNA and is translated to produce an RNA polymerase, which then synthesizes a genome-length (−) sense strand. From this, a "nested set" of overlapping subgenomic RNAs is transcribed, of which only the unique (nonoverlapping) sequence in each is translated (see Chapter 28).

ssRNA; (−) Sense; Virion Transcriptase (Fig. 4-5D,E). Primary transcription from the (−) sense ssRNA viruses occurs in the cytoplasm, when the virion RNA is still within the helical nucleocapsid, in association with the nucleoprotein as well as the transcriptase. Particular sequences of 10 to 20 nucleotides, located at or near the termini of each RNA molecule, may serve as recognition signals for transcriptase binding.

The paramyxoviruses and rhabdoviruses have similar transcription strategies, as well as similar consensus sequences at the 3′ and 5′ termini of their viral RNA, suggestive of a common ancestry. The (−) sense virion RNA is copied in two distinct ways: the replication mode and the transcription mode. Copying in the replication mode produces a full-length (+) sense strand which is used as a template for the synthesis of new virion RNA. In the transcription mode, five subgenomic (+) sense RNAs are produced; each is capped and polyadenylated and serves as a monocistronic mRNA. It is still not certain what dictates whether the polymerase reads right through from 3′ to 5′ end of the (−) sense RNA template (replication mode), ignoring internal termination signals which are obeyed in the transcription mode to produce the family of five monocistronic mRNAs. There is some evidence that the polymerase may have only a certain probability of "falling off" its template as it reaches a termination codon; the five mRNAs are made in decreasing molar amounts, reading from the 3′ end of the parental RNA.

The orthomyxoviruses, bunyaviruses, and arenaviruses have segmented genomes, and each segment is transcribed to yield an mRNA which is translated into one or more proteins (Fig. 4-5E). In the case of the orthomyxoviruses, most of the segments can be regarded as single genes, for they encode single proteins. Special mention needs to be made of a phenomenon known colloquially as "cap-snatching," which is required by orthomyxoviruses for the initiation of mRNA synthesis. A virion-associated endonuclease enters the nucleus and removes a short segment from the capped 5′ terminus of cell mRNA; this is transported back to the cytoplasm, where it binds to the virion RNA and serves as a primer to initiate transcription.

In general, each viral RNA segment of the genomes of the bunyaviruses and arenaviruses codes for more than one protein. Furthermore, the S segment, at least, of arenaviruses and the *Phlebovirus* genus of bunyaviruses is ambisense. The replication strategy of ambisense RNA viruses, like the sense of their genomes, is mixed, with features of both (+) sense and (−) sense ssRNA viruses (see Chapters 29 and 34). Bunyavirus mRNAs also carry nonviral sequences at their 5′ termini, presumably derived from cellular mRNA primers.

dsRNA; Virion Transcriptase (Fig. 4-5F). The two families of viruses with dsRNA (*Birnaviridae* and *Reoviridae*) have segmented genomes and each segment is separately transcribed in the cytoplasm by a virion-associated RNA-dependent RNA polymerase. With reoviruses, each of the 10, 11, or 12 dsRNA segments corresponds to a single gene. Monocistronic mRNAs are transcribed from each segment within the partly uncoated subviral particle (see Plate 4-2); these RNAs complex with a protein before each is copied to produce a dsRNA, which serves as the template for further mRNA transcription.

ssRNA; (+) Sense; Virion Reverse Transcriptase (Fig. 4-5G). In the retroviruses the viral RNA is (+) sense, but instead of functioning as mRNA it is transcribed into DNA by a viral RNA-dependent DNA polymerase, and the resulting RNA–DNA hybrid molecule is converted to dsDNA and integrated into the cellular DNA. Transcription of RNA then occurs from the integrated viral DNA via the cellular transcriptase, followed by splicing of the RNA transcript as well as cleavage of the resulting proteins (see Chapters 12 and 31).

Regulation. Transcription from RNA viral genomes is generally not as rigorously regulated as with DNA viruses. In particular, the temporal separation into early genes transcribed before the replication of viral nucleic acid and late genes transcribed thereafter is not nearly so clear. Figure 4-6 shows that although the rate of synthesis of viral mRNAs

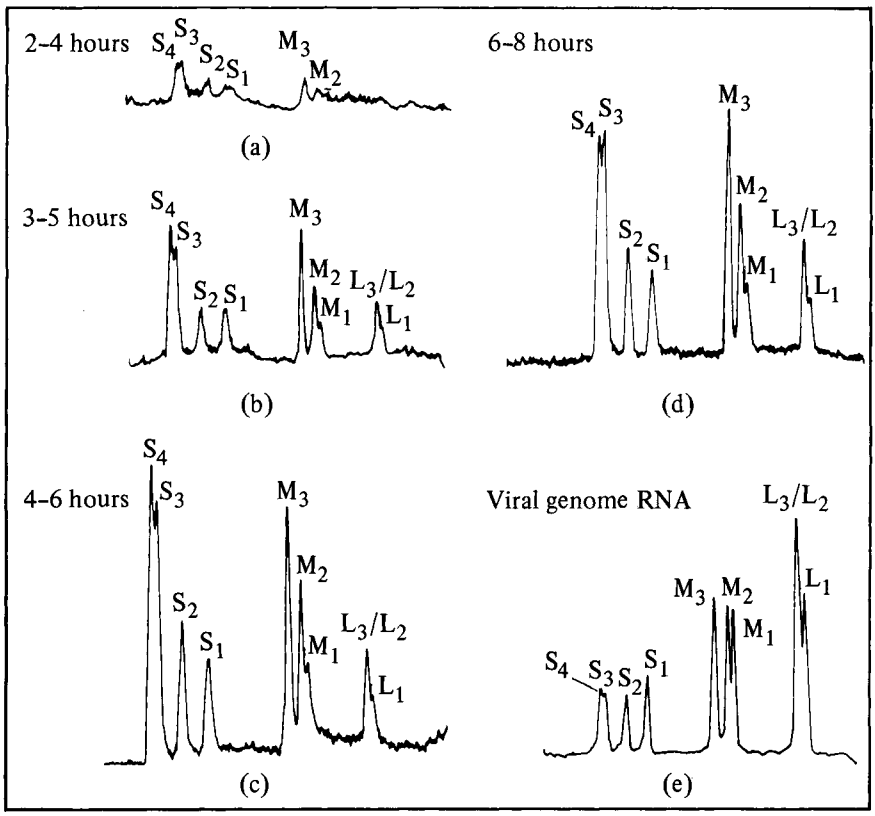

FIG. 4-6. *Transcription of mRNAs from dsRNAs of reovirus by the viral transcriptase. Tracings from polyacrylamide gel autoradiograms showing relative rates of formation of reovirus mRNA species during the replication cycle (a–d), as determined by hybridizing labeled mRNAs extracted from the cytoplasm of infected cells to genome RNAs; compared with the tracing of genome RNAs derived from virions (e). [From H. J. Zweerink and W. K. Joklik,* Virology **41,** *501 (1970).]*

increases steadily during the first 6 hours of reovirus infection as more template becomes available, the relative amounts of each of the 10 mRNA species remain unchanged. With some viruses, however, a subtle form of control can modulate the relative abundance of mRNAs for different proteins. For instance, in the case of the (−) sense ssRNA rhabdoviruses and paramyxoviruses, where the whole genome is transcribed into five monocistronic mRNA species, each coding for one of the five structural proteins, the "polarity" of the linear transcription by

the viral transcriptase, described earlier, results in favored synthesis of mRNA for the proteins coded by the 3′ end of the viral RNA.

TRANSLATION

Capped, polyadenylated, and processed monocistronic viral mRNAs bind to ribosomes and are translated into protein in the same fashion as cell mRNAs. The sequence of events has been closely studied for reovirus. Each monocistronic mRNA molecule binds via its capped 5′ terminus to the 40 S ribosomal subunit, which then moves along the mRNA molecule until stopped at the initiation codon. The 60 S ribosomal subunit then binds, together with methionyl tRNA and various initiation factors, after which translation proceeds. Despite the fact that mRNA is transcribed from each of the 10 monocistronic dsRNA reovirus segments in equimolar amounts, there are pronounced differences in the amounts of each protein made, indicating the existence of a regulatory mechanism at the level of translation.

Early Proteins

The proteins translated from the early transcripts of DNA viruses include enzymes and other proteins required for the replication of viral nucleic acid, as well as proteins that suppress host cell RNA and protein synthesis. However, the function of most early viral proteins of the large DNA viruses is still unknown.

Late Proteins

The late viral proteins are translated from late mRNA, most of which is transcribed from progeny viral nucleic acid molecules. Most of the late proteins are viral structural proteins, and they are often made in considerable excess. Some of them also double as regulatory proteins, modulating the transcription or translation of cellular or early viral genes.

Regulation

The temporal order and amount of synthesis of particular proteins of DNA viruses is regulated mainly at the level of transcription. With RNA viruses it is also usual for nonstructural proteins to be made early and structural proteins later, but the control is generally not as rigorous as for the DNA viruses and occurs at the level of translation. For instance, in the case of caliciviruses, coronaviruses, and togaviruses, only the 5′ end of the (+) sense viral RNA, which codes for the nonstructural pro-

teins, including the RNA polymerase, is translated early, hence production of complementary (−) sense RNA can commence. This then serves as the template for transcription of subgenomic RNA corresponding to the 3′ end of the viral RNA, from which are translated the structural proteins required in abundance later in infection.

Posttranslational Cleavage of Polyproteins

In the picornaviruses, the polycistronic viral RNA is translated directly into a single polyprotein which carries protease activity. This virus-coded protease cleaves the polyprotein at defined recognition sites into smaller proteins. The first cleavage steps are carried out while the polyprotein is still bound to the polyribosome. Some of the larger intermediates exist only fleetingly; others are functional but are subsequently cleaved to smaller proteins with alternative functions.

Posttranslational cleavage occurs in several other RNA virus families but is a less prominent feature in the overall production of individual proteins. In the case of the togaviruses and caliciviruses, polyproteins corresponding to only part, albeit a large part, of the genome are translated from polycistronic mRNA and then cleaved. With viruses of several other families, cleavage of particular proteins late in the replication cycle is essential for the production of infectious virions.

Migration of Proteins

Newly synthesized viral proteins must migrate to the various sites in the cell where they are needed. e.g., back into the nucleus in the case of viruses that replicate there. The mechanisms controlling such migration are unknown, but presumably resemble those used for cellular proteins and possibly involve the cytoskeleton. Migration is doubtless intimately dependent on the structural features of particular proteins. In the case of glycoproteins, the polypeptide is translated on membrane-bound ribosomes, i.e., on rough endoplasmic reticulum; various co- and posttranslational modifications, including acylation, proteolytic cleavage, and addition and subtraction of sugars, occur sequentially as the protein moves in vesicles to the Golgi complex and thence to the plasma membrane (see below).

REPLICATION OF VIRAL NUCLEIC ACID

DNA Replication

Different mechanisms of DNA replication are employed by each family of DNA viruses. We can give only a brief overview here.

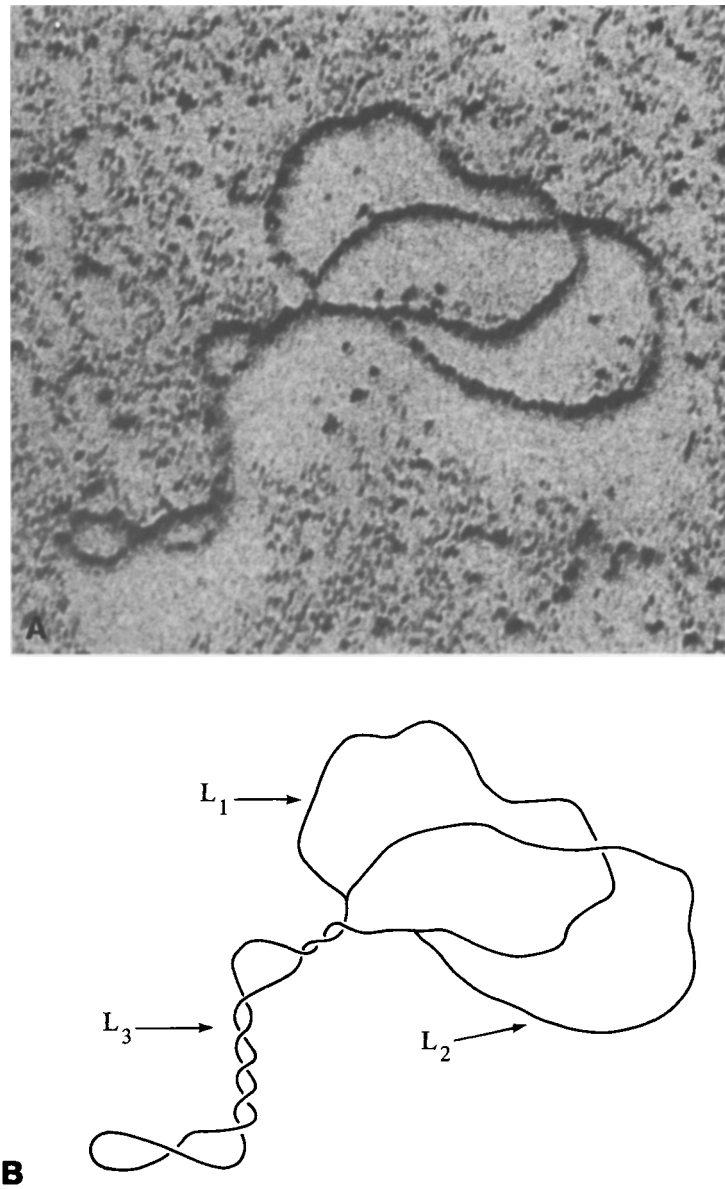

FIG. 4-7. *Replication of circular viral DNA. (A) Electron micrograph of a replicating molecule of the papovavirus SV40 DNA (Magnification: 1.5 × 10^5). In an interpretative drawing of the molecule (B) the two replicating branches are designated L_1 and L_2. The superhelical unreplicated section is designated L_3. [From E. D. Sebring* et al., J. Virol. **8,** *478 (1971).]*

Papovaviridae. Little is known about the replication of papillomavirus DNA, but the polyomaviruses, especially SV40, have been studied in great detail. The SV40 genome, with its associated cellular histones, morphologically and functionally resembles cellular DNA and utilizes host cell enzymes, including DNA polymerase α, for its replication. An early viral protein, large-T, binds to three sites in the regulatory sequence of the viral DNA, thereby initiating DNA replication. Replication of this circular dsDNA commences from a unique palindromic sequence and proceeds simultaneously in both directions at the same rate (Fig. 4-7). As in the replication of mammalian DNA, both continuous and discontinuous DNA synthesis occurs (on leading and lagging strands, respectively) at the two growing forks. The discontinuous synthesis of the lagging strand involves repeated synthesis of short oligoribonucleotide primers, which in turn initiate short nascent strands of DNA (*Okazaki fragments*), which are then covalently joined to form one of the growing strands.

Adenoviridae. Adenovirus DNA is linear, the 5′ terminus of each strand being a mirror image of the other (terminally repeated inverted sequences), and each is covalently linked to a protein. The primer for adenoviral DNA synthesis is not, as is usual, another nucleic acid, but a precursor to this protein, referred to as adenovirus preterminal protein. DNA replication proceeds from both ends, continuously but asynchronously, in a 5′ to 3′ direction, using a virus-coded DNA polymerase. It does not require the synthesis of Okazaki fragments.

Herpesviridae. Unlike other DNA viruses that replicate in the nucleus, herpesviruses specify a large number of enzymes involved in DNA synthesis. Analysis of herpesvirus DNA replication is incomplete, but it appears that a rolling-circle mechanism operates, at least in the later stages. The replicating DNA initially consists of circles and linear forked forms, which are later replaced by large bodies of tangled DNA. There are three origins of replication, two on the S component and one on the L component (see Fig. 1-3), the latter being near the genes that specify the DNA polymerase and the major DNA-binding protein. Newly synthesized viral DNA appears to be cleaved to unit lengths during the process of packaging into newly formed capsids.

Poxviridae. The special features of poxvirus DNA replication are that it occurs in the cytoplasm and depends entirely on virus-coded proteins; it can occur in enucleated cells. Replication appears to begin at each end of the genome and involves a strand displacement mechanism, with the formation of small DNA fragments covalently linked to RNA primers. The discovery of the loop structure at the ends of the vaccinia virus

genome (see Fig. 1-3) suggested a model whereby nicks near the ends of the genome allow self-priming by the 3′ ends thus generated.

Parvoviridae. In the autonomous parvoviruses (genus *Parvovirus*), DNA replication occurs in close association with cellular chromatin and is dependent on cellular functions provided in the S phase of the cell cycle, i.e., when cellular DNA synthesis is occurring, a feature that is correlated with the pathogenic potential of these viruses (see Chapter 22). The virion (−) sense DNA is copied to give a dsDNA replicative form. Further DNA synthesis requires the binding of a virus-coded protein to the 5′ termini. Production of viral ssDNA appears to occur after nicks at the 5′ end and repeated rounds of synthesis.

Hepadnaviridae. Replication occurs in the nucleus by a unique process. The viral DNA polymerase converts the viral ss/dsDNA into a complete circular dsDNA. The (−) sense strand of this molecule is then transcribed by the cellular RNA polymerase to produce a full-length "pregenome" RNA. This (+) sense RNA is then encapsidated in viral cores together with newly synthesized DNA polymerase, which also carries reverse transcriptase activity. Minus-strand DNA is then synthesized by reverse transcription of the pregenome RNA; the template is degraded to leave a full-length (−) sense DNA strand. A small RNA fragment from the 5′ end of the pregenome is then used to prime the synthesis of the (+) sense DNA strand. Complete synthesis of this strand is not necessary for maturation of the virus, hence infectious particles contain dsDNA with a single-stranded region.

RNA Replication

The replication of RNA is a phenomenon restricted to viruses. Transcription of RNA from an RNA template requires an RNA-dependent RNA polymerase, a virus-coded enzyme not normally found in cells. It is not known whether the polymerase required to transcribe (+) sense RNA from (−) sense RNA is different from that needed to transcribe (−) sense RNA from (+) sense RNA. Both processes are essential because the replication of virion RNA requires first the synthesis of complementary RNA, which then serves as a template for making more virion RNA.

Where virion RNA is of (−) sense the complementary RNA is of (+) sense and the RNA polymerase is the virion-associated transcriptase used for transcription of subgenomic RNAs. However, whereas the primary transcripts from such (−) sense virion RNA are subsequently cleaved (in most cases) to produce mRNAs, some must remain uncleaved to serve as a full-length template for virion RNA synthesis.

In the case of (+) sense virion RNA, the complementary RNA is of (−)

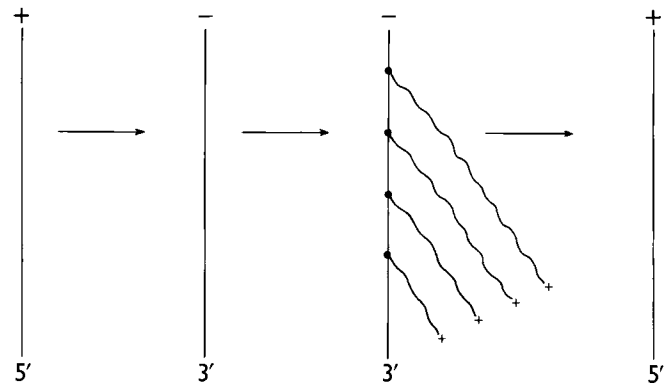

FIG. 4-8. *Mechanism of replication of ssRNA. The "replicative intermediate" (third from left) consists of several (+) sense strands being copied simultaneously from one (−) sense strand by separate molecules of RNA-dependent RNA polymerase (shown as dots).*

sense. Several RNA molecules can be transcribed simultaneously from a single complementary RNA template, each RNA transcript being the product of a separately bound polymerase molecule. The resulting structure, known as the *replicative intermediate,* is therefore partially double-stranded, with single-stranded tails (Fig. 4-8). Initiation of replication of picornavirus and calicivirus RNA, like that of adenovirus DNA, requires a protein, rather than a ribonucleoside triphosphate, as primer. This small protein, VPg, is covalently bound to the 5′ terminus of nascent (+) and (−) RNA strands, as well as virion RNA, but not to mRNA.

Replication of Retrovirus RNA. Retroviruses have a genome consisting of (+) sense ssRNA. Unlike other RNA viruses, they replicate via a DNA intermediate. A virion-associated RNA-dependent DNA polymerase (reverse transcriptase), using a tRNA molecule as a primer, makes a ssDNA copy. The reverse transcriptase, functioning as a ribonuclease, then removes the parental RNA molecule from the DNA–RNA hybrid. The free (−) sense ssDNA strand is then converted into linear dsDNA, which contains an additional sequence known as the *long terminal repeat* at each end. This linear dsDNA then migrates to the nucleus and becomes integrated into cellular DNA. Transcription of the viral RNA can then occur from this integrated (*proviral*) DNA (see Chapter 12).

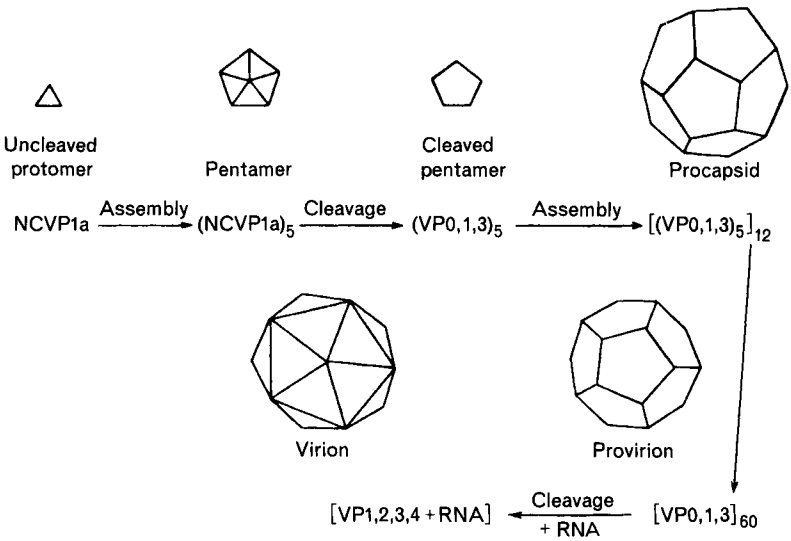

FIG. 4-9. *Assembly of picornavirions.*

MATURATION OF THE VIRION

Icosahedral Viruses

Structural proteins of nonenveloped icosahedral viruses associate spontaneously to form capsomers, which self-assemble to form empty procapsids, into which viral nucleic acid is packaged. Completion of the virion often involves proteolytic cleavage of one or more species of capsid protein. The best-studied examples among animal viruses are the picornaviruses (Fig. 4-9). The capsomer precursor protein (noncapsid viral protein, NCVP1a) aggregates to form pentamers; each of the 5 NCVP1a molecules is then cleaved by virus-specific proteases into VP0, VP1, and VP3. Twelve such pentamers aggregate to form a procapsid. A final proteolytic event, which cleaves the VP0 molecule into VP2 and VP4, is required for RNA incorporation. The mature virion is a dodecahedron with 60 capsomers, each of which is made up of one molecule each of VP1, 2, 3, and 4. There are also one or two uncleaved molecules of VP0 in the virion. X-Ray crystallography shows that the assembling units are not just rigid preformed "bricks"; they have extensions that reach across adjacent units to form second- and third-nearest neighbor

relationships. Such studies have also shown that there is no fixed way in which RNA interacts with ordered parts of the protein.

The mechanism of packaging viral nucleic acid into a preassembled empty procapsid has been elucidated for adenovirus. One terminus of the viral DNA is characterized by a nucleotide sequence referred to as the packaging sequence, which enables the DNA to enter the procapsid bound to basic core proteins, after which some of the capsid proteins are cleaved to make the mature virion.

Enveloped Viruses

All mammalian viruses with helical nucleocapsids, as well as some with icosahedral nucleocapsids, acquire an envelope by budding through cellular membranes. Since such envelopes always contain viral glycoproteins, we begin by discussing the mechanism of glycosylation of these proteins.

Glycosylation of Envelope Proteins. Much of our understanding of the glycosylation of viral proteins comes from studies with vesicular stomatitis virus (a rhabdovirus), Semliki Forest virus (a togavirus), and the orthomyxoviruses and paramyxoviruses. The essential steps appear much the same for all enveloped viruses, hence a general overview is presented (Fig. 4-10). Viruses exploit existing cellular pathways normally used for the synthesis of membrane-inserted and exported secretory glycoproteins.

The amino-terminus of viral envelope proteins initially contains a sequence of 15 to 30 hydrophobic amino acids, known as the *signal sequence,* which characterizes the protein as one destined for insertion into membrane and/or export from the cell. The hydrophobicity of the signal

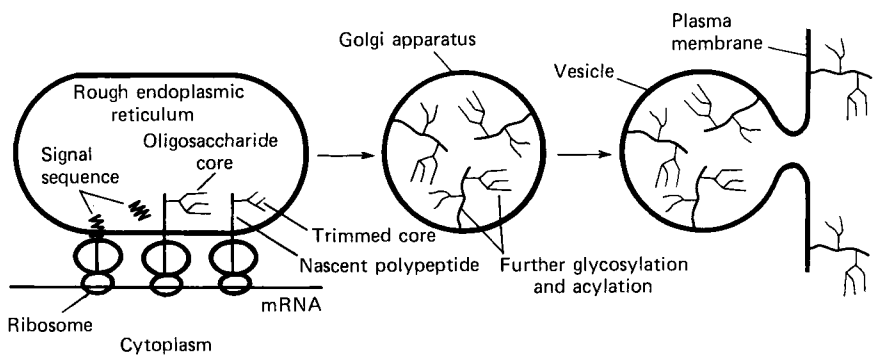

FIG. 4-10. *Glycosylation of viral protein.*

sequence facilitates binding of the growing polypeptide chain to a receptor site on the cytoplasmic side of the rough endoplasmic reticulum and its passage through the lipid bilayer to the luminal side. A signal peptidase then removes the signal sequence. Oligosaccharides are added to asparagine residues of the nascent polypeptide in the lumen of the rough endoplasmic reticulum by *en bloc* transfer of a mannose-rich core of preformed oligosaccharides from a lipid-linked intermediate, an oligosaccharide pyrophosphoryldolichol. Glucose residues are then removed by glycosidases, a process known as "trimming" of the core. The viral glycoprotein is then transported from the rough endoplasmic reticulum to the Golgi complex, probably within a coated vesicle. Here the core carbohydrate is further modified by the removal of several mannose residues and the addition of further *N*-acetylglucosamine, galactose, and the terminal sugars, sialic acid and fucose. The completed side chains are a mixture of simple ("high-mannose") and complex oligosaccharides. While in the Golgi complex the glycoprotein may become acylated, by the covalent attachment of fatty acids such as methyl palmitate to the hydrophobic membrane attachment end of the molecule. Another coated vesicle then transports the completed glycoprotein to the cellular membrane from which the particular virus buds.

Transport of Glycoproteins. Different viruses bud from different sites in the plasma membrane (orthomyxoviruses, paramyxoviruses, rhabdoviruses, arenaviruses, togaviruses, and retroviruses), some from the apical and others from the basolateral surface. Others bud from intracytoplasmic smooth endoplasmic reticulum (flaviviruses, bunyaviruses, coronaviruses) or from the nuclear membrane (herpesviruses). Presumably some structural feature of the glycoprotein serves as the "zip code" ensuring delivery to the correct location in the cell.

Cleavage of Envelope Proteins. With the orthomyxoviruses and paramyxoviruses, which bud through the plasma membrane, a cellular protease cleaves the envelope protein at the time of its insertion into the membrane into two polypeptide chains, which remain covalently linked by disulfide bonds. Cleavage is not required for viral release and does not occur in certain types of host cells, but it is essential for the production of infectious virions in the orthomyxoviruses (cleavage of the hemagglutinin) and paramyxoviruses (cleavage of both the hemagglutinin-neuraminidase and the fusion protein). Following fusion of the coated vesicle with the plasma membrane, the hydrophilic N-terminus of the glycoprotein projects from the external surface of the membrane, while the hydrophobic domain, which is near the C-terminus, remains anchored in the lipid bilayer.

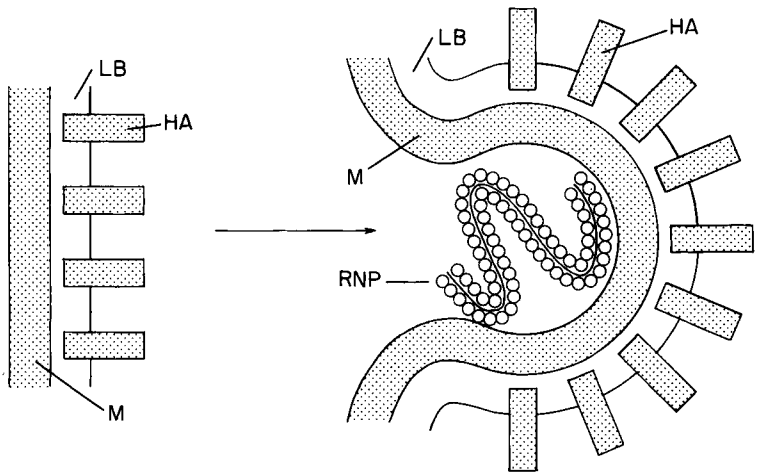

FIG. 4-11. *Budding of virus from plasma membrane. LB, Lipid bilayer; HA, hemagglutinin; M, matrix protein; RNP, ribonucleoprotein.*

Budding. Budding may be regarded as a nonphysiological form of exocytosis (Fig. 4-11). The process begins with the insertion of the completed viral glycoprotein into the appropriate cellular membrane. Because proteins are free to move laterally in the "sea of lipid" that constitutes the lipid bilayer of the plasma membrane, cellular proteins are displaced from the patch of membrane into which viral glycoproteins are inserted. It is not known whether there is selection of particular lipids for incorporation into the viral envelope, but the ratio of phospholipids to glycolipids and cholesterol is essentially the same as that of the membrane of the particular host cell.

The monomeric, cleaved viral glycoprotein molecules associate into oligomers, to form the typical rod-shaped peplomer with a prominent hydrophilic domain projecting from the external surface of the membrane; the hydrophobic domain near the C-terminus spans the membrane and a short hydrophilic domain at the C-terminus projects slightly into the cytoplasm. In the icosahedral togaviruses (Plate 4-3A), each protein molecule of the nucleocapsid binds directly to the C-terminus of a glycoprotein oligomer of the envelope. Multivalent attachment of numerous peplomers, each to an underlying molecule on the surface of the icosahedron, molds the envelope around the nucleocapsid, forcing it to bulge progressively outward until finally the nucleocapsid is completely enclosed in a tightly fitting envelope and the new virion buds off. The capsid proteins of most enveloped viruses with helical nucleocapsids do

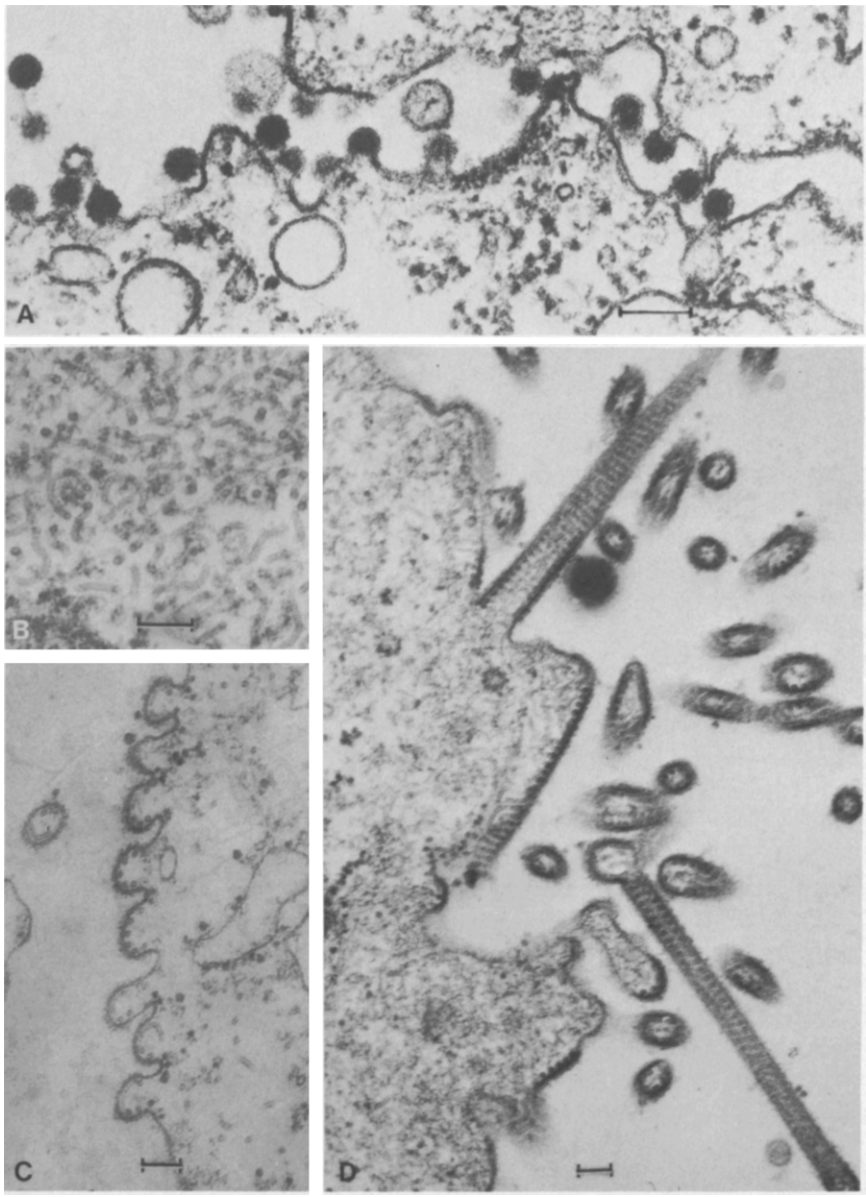

PLATE 4-3. *Viruses budding from the plasma membrane. (A) Togavirus. (B) Accumulation of paramyxovirus SV5 nucleocapsids. (C, D) Budding of SV5 from the plasma membrane, with some filamentous forms (bars = 100 nm). [A, courtesy Dr. A. Helenius; B, C, and D, from R. W. Compans* et al., Virology **30,** *411 (1966), courtesy Dr. P. W. Choppin.]*

not bind directly to envelope glycoprotein but to a *matrix protein* which is bound to the cytoplasmic side of the plasma membrane beneath patches of viral glycoprotein (Fig. 4-11).

Coronaviruses and bunyaviruses bud from rough endoplasmic reticulum and the Golgi complex; orthopoxviruses may acquire an envelope in the Golgi, but enveloped forms are released from the plasma membrane. The envelope of the herpesviruses is acquired by budding through the inner lamella of the nuclear membrane; the enveloped virions then pass directly from the space between the two lamellae of the nuclear membrane to the exterior of the cell via the cisternae of the endoplasmic reticulum.

RELEASE

There are basically two mechanisms for the release of mature virions from the infected cell. With most nonenveloped viruses that accumulate within the cytoplasm or nucleus, release occurs only when the cell lyses. This may occur shortly after the completion of viral replication; e.g., cells infected with picornaviruses lyse as soon as assembly of virions is completed, with immediate release of the progeny virions. On the other hand, parvoviruses accumulate within the cell nucleus and are not released until the cell slowly degenerates and dies. Most enveloped viruses, on the other hand, are released by budding, a process which can occur over a prolonged period without much damage to the cell, hence many such viruses (e.g., arenaviruses, retroviruses) are noncytopathogenic and are associated with persistent infections. However, some enveloped viruses that are released by budding are cytolytic, e.g., the alphaherpesviruses. Orthopoxviruses may be released as enveloped forms by budding from the plasma membrane or as nonenveloped forms, by cell lysis; both forms are infectious.

INHIBITION OF VIRAL REPLICATION: ANTIVIRAL CHEMOTHERAPY

If this had been a book about bacterial diseases of domestic animals, there would have been at least one chapter on antibacterial chemotherapy. However, of the hundreds of antibiotics and other antibacterial compounds now available, not one has the slightest effect on any virus, and there are no specifically antiviral chemotherapeutic agents in common use. The reason is that viruses are absolutely dependent on the metabolic pathways of the host cell for their replication, hence most

agents that interfere with viral replication are toxic to the cell. Increased knowledge of the biochemistry of viral replication has led to a more rational approach to the search for antiviral chemotherapeutic agents.

Strategy for Development of Antiviral Agents

Several steps in the viral replication cycle represent potential targets for selective attack. Theoretically, all virus-coded enzymes are vulnerable, as are all processes (enzymatic or nonenzymatic) that are more essential to the replication of the virus than to the survival of the cell. Obvious examples include: (1) transcription of viral mRNA (or copy DNA, in the case of the retroviruses) by the viral transcriptase, (2) replication of viral DNA or RNA by the virus-coded DNA polymerase or RNA-dependent RNA polymerase, (3) posttranslational cleavage of protein(s) by (virus-coded) protease(s). Less obvious at first sight, but proven points of action of currently known antiviral agents are: (4) penetration/uncoating, (5) polyadenylation, methylation, or capping of viral mRNA, (6) translation of viral mRNA into protein, and (7) assembly/maturation of the virion.

A logical approach to the discovery of new antiviral chemotherapeutic agents is to isolate or synthesize substances that might be predicted to serve as an inhibitor of a known virus-coded enzyme. Analogs (congeners) of this prototype are then synthesized with a view to enhancing activity and/or selectivity. The discovery of a class of nucleoside analogs which selectively inhibit herpesvirus DNA synthesis has led to a realization that virus-coded enzymes with a broader (or different) substrate specificity than their cellular counterparts can be exploited to convert an inactive precursor ("prodrug") to an active antiviral agent. Because the viral enzyme occurs only in infected cells, such drugs are nontoxic for uninfected cells. Exploitation of this principle may revolutionize antiviral chemotherapy.

Acycloguanosine (Acyclovir) and Homologs

Acycloguanosine, now commonly known as acyclovir, is a guanine derivative with an acyclic side chain, the full chemical name being 9-(2-hydroxyethoxymethyl)guanine (Fig. 4-12). Its unique advantage over earlier nucleoside derivatives is that it requires the herpesvirus-specified enzyme, deoxythymidine-deoxycytidine kinase, to phosphorylate it intracellularly to acycloguanosine monophosphate; a cellular GMP kinase then completes the phosphorylation to the active agent, acycloguanosine triphosphate (Fig. 4-12). Acycloguanosine triphosphate inhibits the herpesvirus-specified DNA polymerase. Since activation of the prodrug

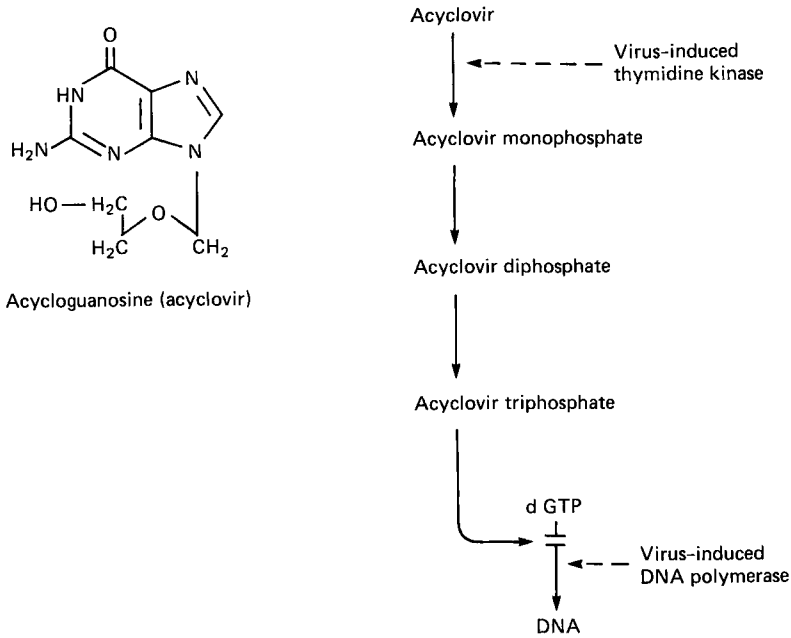

FIG. 4-12. *Structure of acyclovir and its mode of action, which is dependent on the presence in the cell of herpesvirus thymidine kinase.*

needs the viral thymidine kinase, acyclovir is essentially nontoxic to uninfected cells but is powerfully inhibitory to viral DNA synthesis in infected cells.

Acyclovir and various derivatives, as well as other nucleoside analogs dependent on viral enzymes for conversion to the active form, are beginning to be used in human medicine for the treatment of herpesvirus infections. It is a small start, but it does demonstrate that antiviral chemotherapy may have a future. Such drugs find limited use in veterinary medicine, e.g., for treatment of feline herpesvirus 1 corneal ulcers.

Other Antiviral Agents

A few other antiviral agents are in use in human medicine. For example, rimantadine and amantadine can prevent the uncoating of influenza virus, and several compounds known to inhibit reverse transcriptase are undergoing clinical trials against AIDS.

Interferons

In theory at least, interferons are the ideal antiviral antibiotics. They are naturally occurring, relatively nontoxic, and display a broad spectrum of activity against essentially all viruses (see Chapters 6 and 8). However, clinical trials in humans have been disappointing. Currently, it appears that they have a demonstrable effect on infections with papillomaviruses, herpesviruses, and rhinoviruses. It is now possible to produce large amounts of various human and other interferons using cloned interferon genes, but it is still uncertain whether they will be of clinical value in humans. Their use for therapy in viral diseases of domestic animals is even further away.

Perspective

Overall, in spite of decades of effort and massive expenditure by the pharmaceutical industry, the yield of useful antiviral drugs has been meager. Only a handful of marginally effective agents have found a place in human medicine, and very few are used in veterinary practice. Nevertheless, it is important to be aware of the continuing research in this field, for antiviral chemotherapy may one day come to constitute an integral part of veterinary medicine.

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