

VIRAL REPLICATION AND GENETICS

Recommended general texts: Luria et al General Virology; 3rd Edition, Wiley, 1978
 Zinsser Microbiology (Joklik et al), 17th Edition, 1980
 Section IV, especially chapters 61, 65 & 67.

Introduction

Animal viruses exhibit a wide range of genetic and structural diversity, and this diversity is manifest in the several strategies utilized for their replication. The tasks of the replication process include: (i) duplication -- generally a multi-log amplification -- of the viral genome; (ii) expression of viral genetic information; and (iii) assembly of structural components of viruses into mature virions. For each virus class, these goals are achieved in ways that depend upon the chemical and physical structure of the viral genome, the coding potential and organization of the genome, and the physical properties of the virions. The strategies have been of great interest from at least two perspectives:

1. Viruses provide relatively "clean" preparations of comparatively simple genetic material (a few genes rather than the hundreds of thousands in eukaryotic cells); hence, they have served as useful models for replication and expression of eukaryotic genes.

2. Steps in the virus life cycle are often unique to the virus (i.e., not required for the viability of the host cells), and these steps constitute potential targets for specific anti-viral therapy. Unfortunately, the promise of this approach has yet to be fulfilled.

Methods for studying viral replication

Study of virus life cycles depends upon the ability to carry out synchronous infections of cultured cells at a high multiplicity of virus to cell. The course of the infection can be followed in a variety of ways:

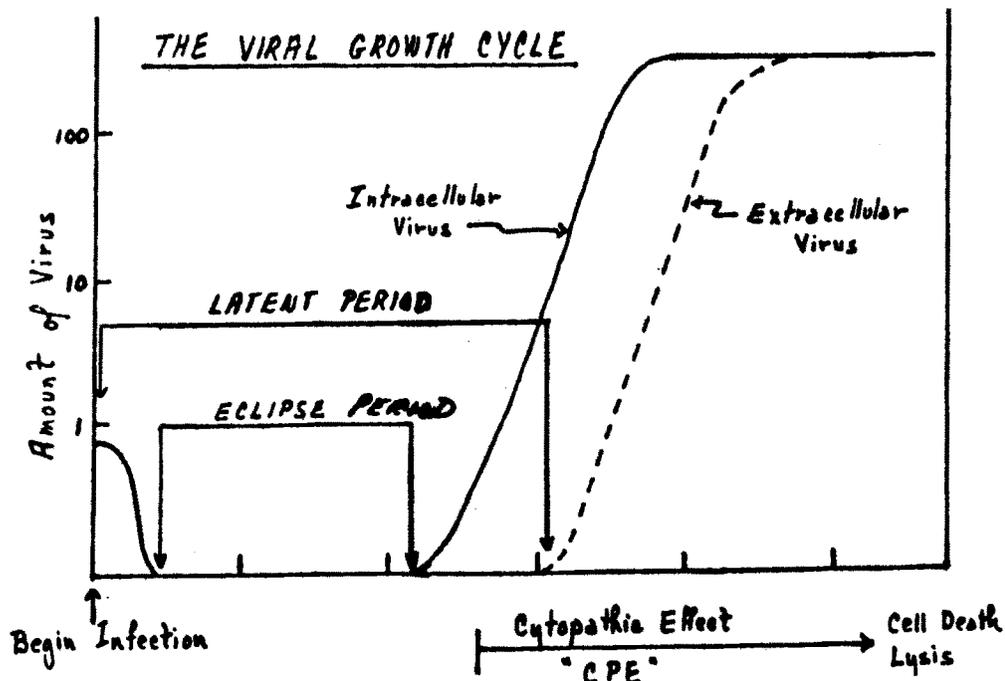
1. By infectivity tests (for virus [or infectious nucleic acid] in cell extracts or for virus in extracellular fluids)
2. Morphologically (e.g., electron microscopy to follow uptake of parental virus and subsequent appearance of progeny virions)
3. Biochemically (using chemical assays for virus-specific nucleic acids or immunological assays for viral proteins)

Specialized biochemical techniques particularly important for deciphering the strategies of viral gene expression include: determination of size and genetic content of viral genome and of viral messenger RNA's; translation of viral mRNA's in cell free systems; pulse-chase labeling of viral proteins to follow their synthesis and modification (cleavage) in infected cells; assays for virion-associated and virus-coded RNA and DNA polymerases. Recently, our understanding of the organization and function of viral genomes has been remarkably advanced by the determination of much or all of the nucleotide sequence of several prototypic viral genomes.

Overview of the life cycle

Regardless of specific strategy, all viruses exhibit a similar "macroscopic" life cycle and are obliged to traverse a number of common steps.

1. "Macroscopic" view of the life cycle. By determining the amount of infectious virus in extracellular fluids and in cell extracts at various times following infection, the following pattern is obtained:



Soon after infection, most of the "infectivity" disappears from the culture, suggesting viral components have been disassembled. After a short time (generally a few hours, dependent upon the virus and host cells in question), infectious virus can be detected in cell extracts, indicating new particles have been assembled within cells; this marks the end of the eclipse period. At some point thereafter (depending upon the nature of the release mechanism), infectious virus can be measured in the extracellular fluid, marking the end of the latent period. Cellular damage during these later events varies markedly among viruses, as discussed in later lectures, but generally cytopathic effect appears after the eclipse period, and the end of the latent period is often accompanied by cell death and lysis.

2. Generalized replication pathway. Extensive biochemical analysis of infection by many viruses reveals a number of shared events:

a. Entry of the virus into the cell and conversion to a form suitable for subsequent events. This requires adsorption of virus particles to the cell membrane, often a non-specific process; penetration into the cell, either via specific viral receptors or by a process akin to phagocytosis; and sufficient uncoating to permit replication and expression of viral genes. In several situations, viral receptors and their viral ligands congregate in coated pits,

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which then form vesicles ("receptosomes") within the cytoplasm; entry into the cytoplasmic space can be induced by fusion of receptosomes with lysosomes, a mechanism used for internalization of some hormones and other substances that enter cells from outside.

Specific cellular receptors which mediate penetration by certain viruses (e.g., polioviruses, RNA tumor viruses, influenza viruses) are important determinants of the host range of these viruses, since their absence dramatically reduces the ability of the virus to infect. For some viruses (e.g., poliovirus), the distribution of receptors is narrowly confined to human and a few higher primate cells, but other viruses (e.g., rhabdoviruses) can usually enter a wide variety of host cells. The absence of receptors from certain species or from certain tissues within an animal often accounts for viral host range, but other factors affecting later stages in the growth cycle may operate in other cases. Moreover, ability of a virus to grow in a certain cell type does not necessarily determine the pathological consequences of infection.

Although thousands of receptors are present on the surface of susceptible cells, they become saturated with viral proteins during infection, rendering the cells resistant to superinfection by the same and closely related viruses. This type of viral interference, operating at the surface of infected cells, is clinically significant in a few instances (e.g., polio -- discussed later) and is frequently important in laboratory studies.

Although certain receptors appear to have a high degree of specificity for certain viruses, it is unknown why such receptors should exist in the first place; it would not seem to be of any obvious advantage to cells to harbor receptors for poliovirus, for example.

In general, little is known about the mechanisms of uncoating. In some cases, the genome appears to be completely stripped of surrounding viral protein but in other cases the virus particle may simply be sufficiently distorted to permit use of the genome as a template. No animal viruses appear to use the mechanism of injection of nucleic acid employed by certain bacteriophages (e.g., the T phages). However, when a naked viral genome is found to be infectious (see below), it is obvious that the completely uncoated genome carries full function. Infection with nucleic acid also bypasses these early steps and can occur in cells which lack receptors specific for viral proteins.

b. Replication of the viral genome. In all cases, the parental genome of a single infecting particle must be replicated (amplified) to permit a significant yield of virus in each replication cycle. For most viruses, 2-4 logs of amplification are achieved in each round of replication. The lack of host enzymes capable of copying an RNA template dictates that virus-coded polymerases replicate the genomes of RNA viruses; the replication of RNA genomes generally occurs in the cytoplasm. DNA viruses, on the other hand, can often use mainly or exclusively host enzymes normally involved in the replication of the animal cell genome; with the significant exception of pox viruses, replication of DNA genomes occurs in the cell nucleus. DNA viruses vary greatly in genetic complexity; as one might expect, the simple viruses (e.g., parvoviruses and papovaviruses) depend more heavily upon host machinery than do the viruses with complex genomes (adeno-, herpes-, and poxviruses).

? MPT later

Presumably viral proteins involved to facilitate binding to cell membrane proteins. Conclude to have receptors.

Regardless of mechanism or site of synthesis, replication is governed by the principle of complementarity. That is, to produce copies of single stranded genomes, whether of (+) or (-) polarity, it is first necessary to synthesize a strand complementary to the genome and then use the complementary strand as a template for production of new genomes. RNA tumor viruses represent a special case, since replication of the single stranded RNA genome proceeds via a DNA intermediate which is stably inserted into host chromosomes by covalent linkage and is called a "provirus". To synthesize double stranded genomes, either semi-conservative mechanisms similar to that used to replicate the host chromosomes may be used (e.g., for most DNA viruses) or (in the case of double stranded RNA viruses) one RNA strand may be synthesized initially, then used as a template to produce duplexes. One additional variation of this theme has recently been discovered with the hepatitis B viruses: (+) RNA synthesized from the double-stranded DNA genome is used as a template for making more dsDNA; this process is thus a permutation of the replication scheme for RNA tumor virus genomes.

REPLICATION OF VIRAL GENOMES: PRINCIPLE OF COMPLEMENTARITY

<u>Parental genome</u>	<u>Intermediate</u>	<u>Progeny genome</u>	<u>Example(s)</u>
ssRNA(+)	→ ssRNA(-)	→ ssRNA(+)	Poliovirus, togaviruses
ssRNA(-)	→ ssRNA(+)	→ ssRNA(-)	Rhabdoviruses, myxoviruses Paramyxoviruses
dsRNA(+)	→ ssRNA(+)	→ dsRNA(+)	Reoviruses
ssDNA(+)	→ dsDNA(+)	→ ssDNA(+)	Certain phage or parvoviruses
dsDNA(+)	→ dsDNA(+)	→ dsDNA(+)	Papovaviruses, adenoviruses, herpesviruses, poxviruses
ssRNA(+)	→ dsDNA(+)	→ ssRNA(+)	RNA tumor viruses (retroviruses)
dsDNA(+)	→ ssRNA(+)	→ dsDNA(+)	Hepatitis B viruses

c. Synthesis of viral gene products. Regardless of the specific strategy used for viral gene expression, there is a primary requirement for viral messenger RNA (mRNA). As discussed in more detail below, in some cases an incoming RNA genome can serve as mRNA; in some cases virus-coded polymerases are needed to generate mRNA; and in some cases cellular polymerases are responsible for synthesis of viral mRNA. The viral mRNA's are frequently subject to the same modifications as found in cellular mRNA's -- splicing (a process which joins non-contiguous regions of precursor RNA and was discovered during study of adenovirus mRNA's); polyadenylation; and "capping" (the addition of an inverted, methylated nucleotide at the 5' end of mRNA's). In all cases, viral mRNA's are translated by the host cell apparatus for protein synthesis, often at the expense of cellular proteins. Each mRNA directs the synthesis of one (and only one) polypeptide. However, in some cases these polypeptides contain multiple functional units, which are subsequently separated by proteolytic cleavage; in other cases, each polypeptide represents a single functional unit and little or no subsequent modification is necessary. Many viruses, particularly enveloped viruses, encode glycoproteins which are synthesized on membrane-bound polyribosomes, inserted into the plasma membrane, and glycosylated in apparently the same manner as host membrane glycoproteins. Viral gene expression is often subject to temporal regulation within the virus life cycle. Most commonly, there are two recognizable phases, that which occurs prior to replication of the

? regulatory needs:
 enhancers
 divergent promoters
 94
 trans activation

viral genome (early) and that which occurs during and after replication of the genome (late). As one might predict, the viral gene products made early in the life cycle are frequently so-called nonstructural proteins, e.g., enzymes involved in the replication of the genome but not packaged in progeny particles, whereas late products are generally structural proteins of the virus particles.

Varieties of strategies used for viral gene expression. Animal viruses exhibit an astounding degree of ingenuity in the schemes that have evolved for expression of their genes.

i.e. viruses
 - note if its genes are not expressed

1. mRNA. To establish infection by any virus, RNA representing the "sense" strand [(+) polarity] must be made available for translation.

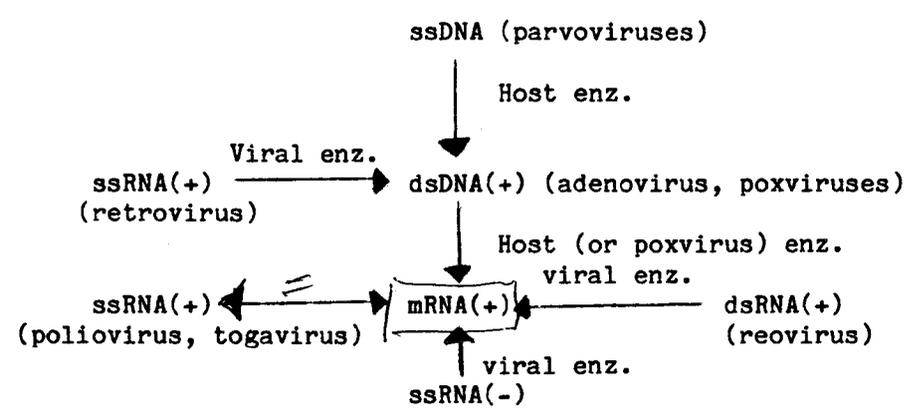
a. Some RNA genomes are functionally equivalent to mRNA's (e.g., poliovirus) and hence can initiate infection without further synthesis of nucleic acids; these genomes are thus infectious in the naked form. Subsequent replication of these genomes under the influence of primary gene products (RNA dependent RNA polymerases) leads to an amplified source of mRNA late in infection.

b. Some RNA genomes (those with (-) polarity, duplex RNA's, and RNA tumor virus genomes) must be copied by virion-associated, virus-coded polymerases in order to initiate infection; thus the naked genomes are not infectious.

c. DNA genomes must be transcribed into RNA before viral proteins can be synthesized, but this function is generally performed by host DNA dependent RNA polymerase; thus the naked genomes can initiate infection in most cases. An important exception is provided by poxviruses which carry their own DNA dependent RNA polymerase in their virions; unlike other DNA viruses, pox viruses synthesize mRNA in the cytoplasm.

A summary of these relationships of genome to mRNA is provided in the following charts:

SYNTHESIS OF VIRAL mRNA



(Influenza, rabies, parainfluenza)

*Arms
murray*

<u>Class & Prototype Virus</u>	<u>Genome</u>	<u>Polarity</u>	<u>Virion Polymerase</u>	<u>Genome Infectious</u>
Picornavirus/togavirus (polio/yellow fever)	ssRNA	+	No	Yes
Paramyxovirus/rhabdovirus (measles, mumps, rabies)	ssRNA	-	(-)RNA → (+)RNA	No
<i>Spice</i> Orthomyxovirus (influenza)	ssRNA	-	(-)RNA → (+)RNA	No
Reovirus	dsRNA	±	dsRNA → (+)RNA	No
Retrovirus	ssRNA	+	(+)RNA → dsDNA*	No**
Parvovirus	ssDNA	+ or +/-	No*	Yes
Papovavirus	dsDNA	±	No*	Yes
Hepatitis B virus	partly-dsDNA	±	***	?***
Adeno/Herpes virus	dsDNA	±	No*	Yes
Poxvirus	dsDNA	±	dsDNA → (+)RNA	No

* In these cases, host RNA polymerase II mainly responsible for synthesis of mRNA.

** The viral DNA intermediates (e.g. proviral DNA) are infectious.

*** In the case of hepatitis B virus, it is not known whether the virion polymerase is needed to initiate an infection. During the life cycle, the enzyme probably copies viral RNA into partly dsDNA within viral cores in the cytoplasm; presence of the enzyme in the mature virion may not have functional significance, although it can convert partially ds to fully dsDNA in vitro, in an assay that is sometimes used to detect HBV particles. The infectivity of the partially dsDNA has not been tested, but fully dsDNA is infectious.

2. Punctuation. The production of a single polypeptide from a single mRNA is a general property of both cellular and viral mRNA's in eukaryotic cells. To permit the production of multiple proteins from a single viral genome, the genes can be "punctuated" in a variety of ways: (a) the genome itself may be segmented, with each segment encoding one (or more) proteins; (b) multiple mRNA's of different genetic composition may be generated from a single genome (each mRNA will direct synthesis of the protein encoded closest to its 5' terminus); and (c) multiple proteins may be generated from a single polypeptide by proteolytic cleavage. Each virus uses one or more of these mechanisms for punctuation as summarized in the following chart and diagrams. DNA viruses frequently exploit the coding potential of their genomes to the fullest by producing multiple different mRNA's from overlapping regions of the genome; these mRNA's may differ by their sites of initiation or by their processing (splicing and/or termination). In some cases, more than one reading frame in a region is used for the synthesis of viral proteins. In addition, different strands may be coding in different regions of DNA genomes. For example, half of a papovavirus genome is expressed at early times from one strand, and the other half is expressed at late times (after DNA replication) from the other strand.

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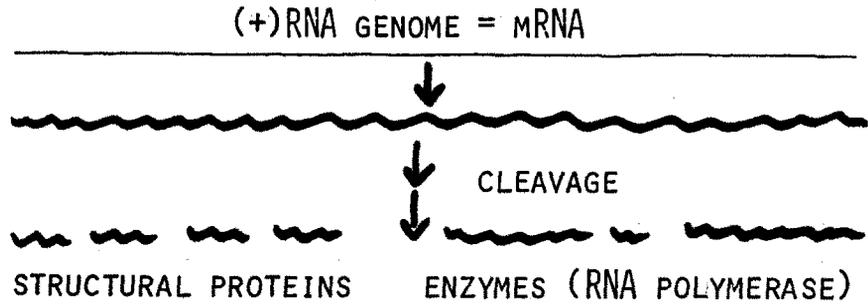
Or, the several adenovirus mRNA's made early in infection are derived from different regions of both strands; most of the late mRNA's are initiated at a single site but contain different regions from the same strand after processing.

<u>Genome</u>	<u>mRNA</u>	<u>Protein</u>	<u>Example</u>
Polygenic, nonsegmented ssRNA(+)	→ Polygenic	→ Polygenic → [*] Monogenic	Polio
Polygenic, nonsegmented	→ Monogenic	→ Monogenic	Rabies
Monogenic*, segmented ss(-)RNA	→ Monogenic	→ Monogenic	Influenza
Monogenic*, segmented ds(+) ₂ RNA	→ Monogenic	→ Monogenic	Reovirus
Polygenic nonsegmented ds(+) ₂ DNA	→ [*] Monogenic	→ Monogenic	Papova, adeno, herpesviruses
Polygenic nonsegmented ss(+) ₂ RNA	→ (dsDNA) → [*] Monogenic	→ Monogenic	Retrovirus
		+ Polygenic → [*] Monogenic + Polygenic → [*] Monogenic	

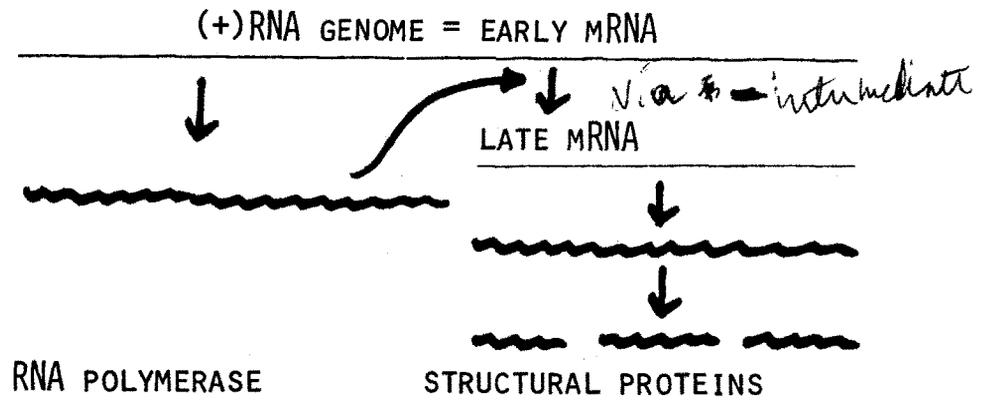
*Step at which major punctuation occurs

17. Assembly and release of virus particles. Viruses appear to be efficiently organized for self-assembly, though the determinants of this process are poorly understood. Assembly of genome and core components of nucleocapsids may occur in the nucleus, free in the cytoplasm, or adjacent to defined regions of the cell membrane, depending upon the virus in question. Envelope components are generally added when an enveloped virus exits from the cell by "budding" through a restricted region of the cell membrane into which viral glycoproteins have been previously inserted. Naked nucleocapsid viruses are most commonly released after the host cell is killed and has "lysed"; in the interim, large aggregates of viral particles may accumulate at the sites of assembly (seen as "inclusion bodies"). In the few cases examined in detail, the assembly process appears to have a strict order for addition and modification (e.g., cleavage) of components, perhaps determined by recognition signals for protein-protein or protein-nucleic acid interactions. These events may serve as models for development of cellular organelles.

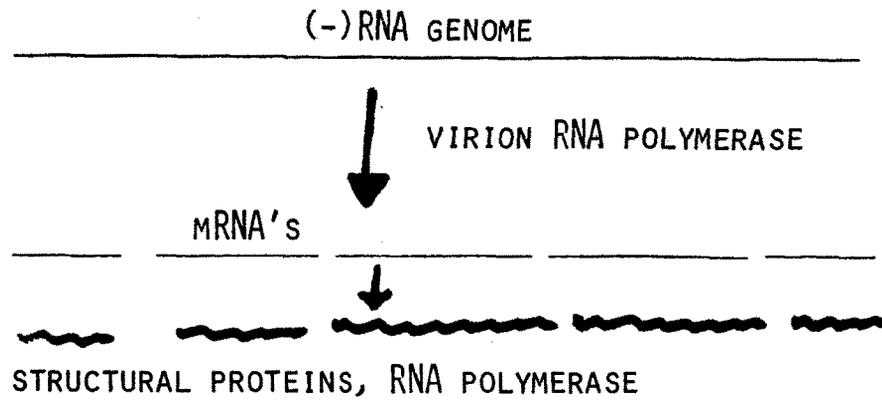
PICORNAVIRUS



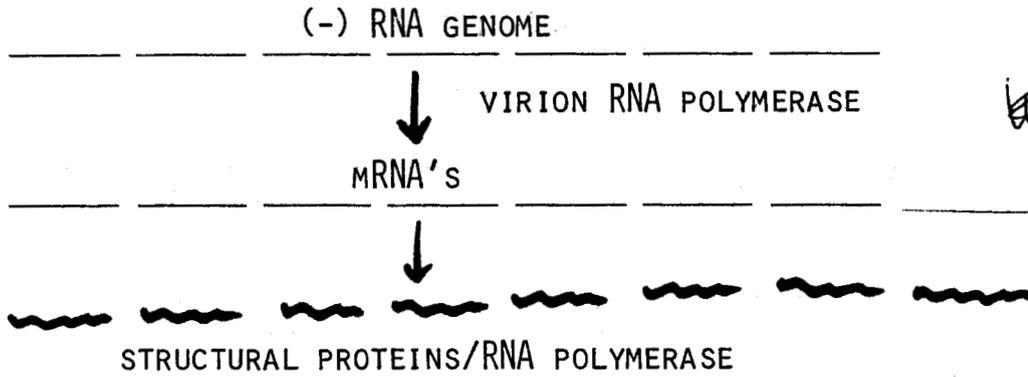
TOGAVIRUS



RHABDOVIRUS/PARAMYXOVIRUS

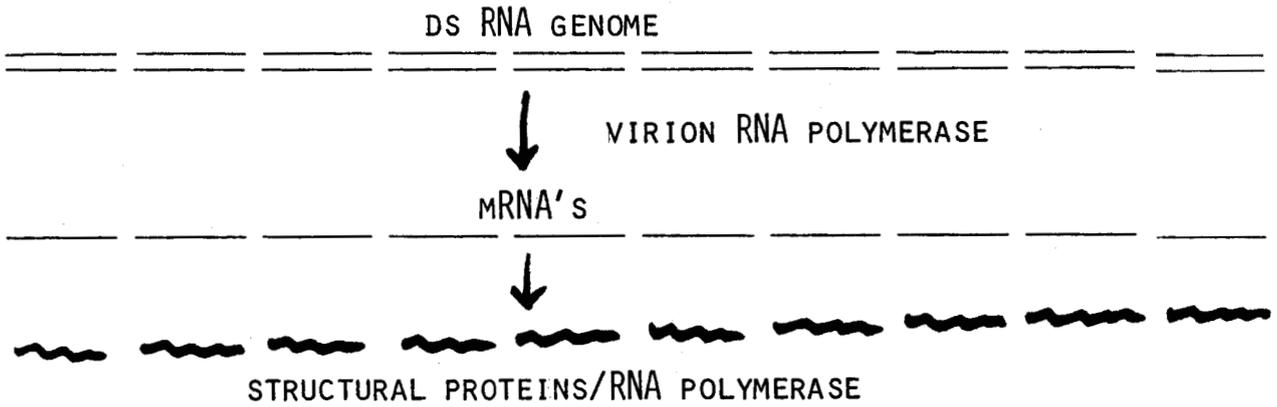


MYXOVIRUS

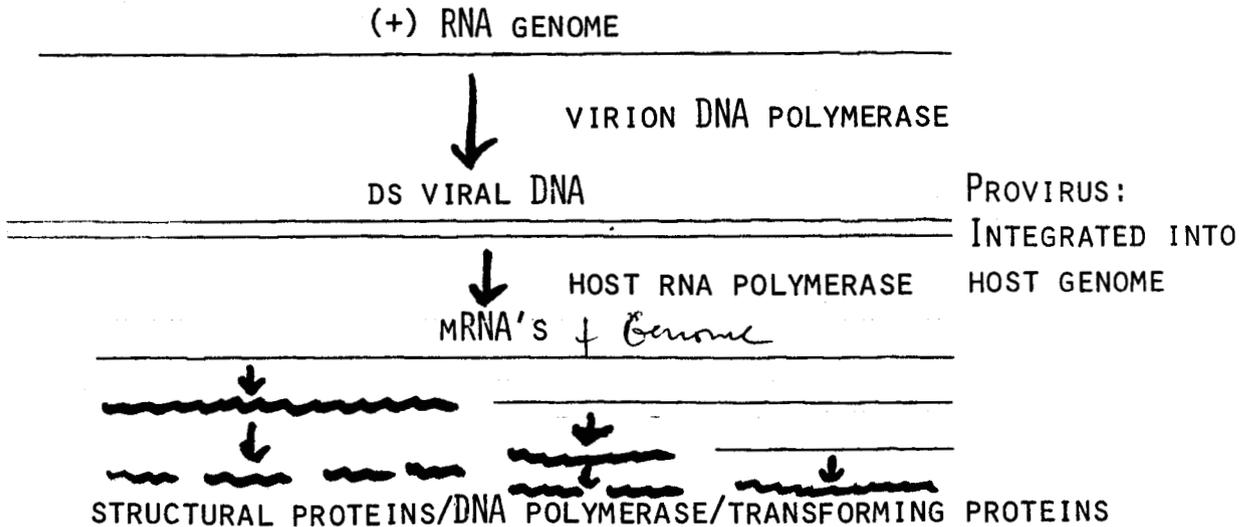


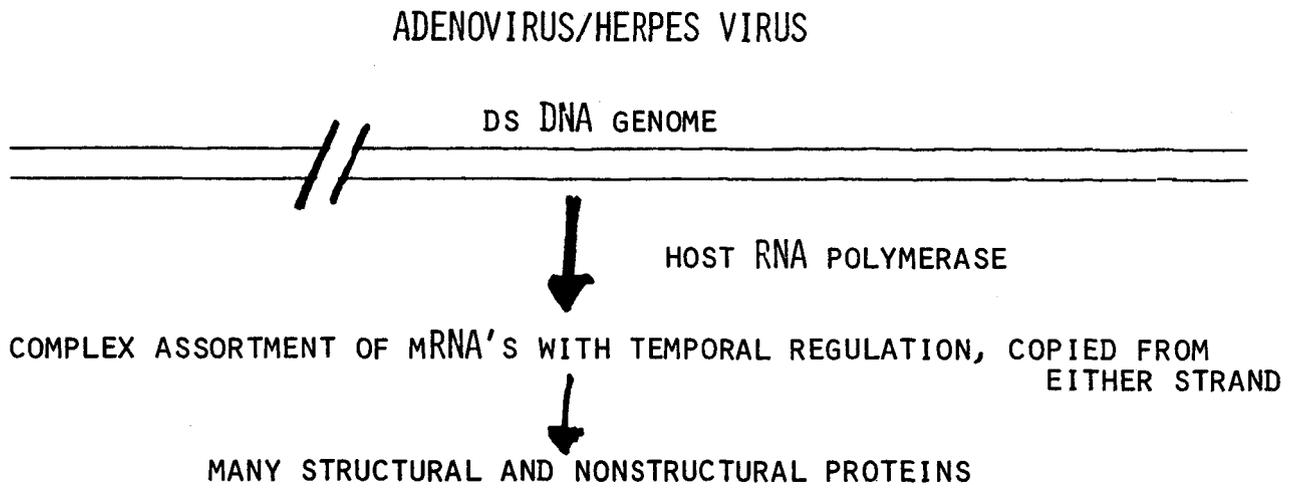
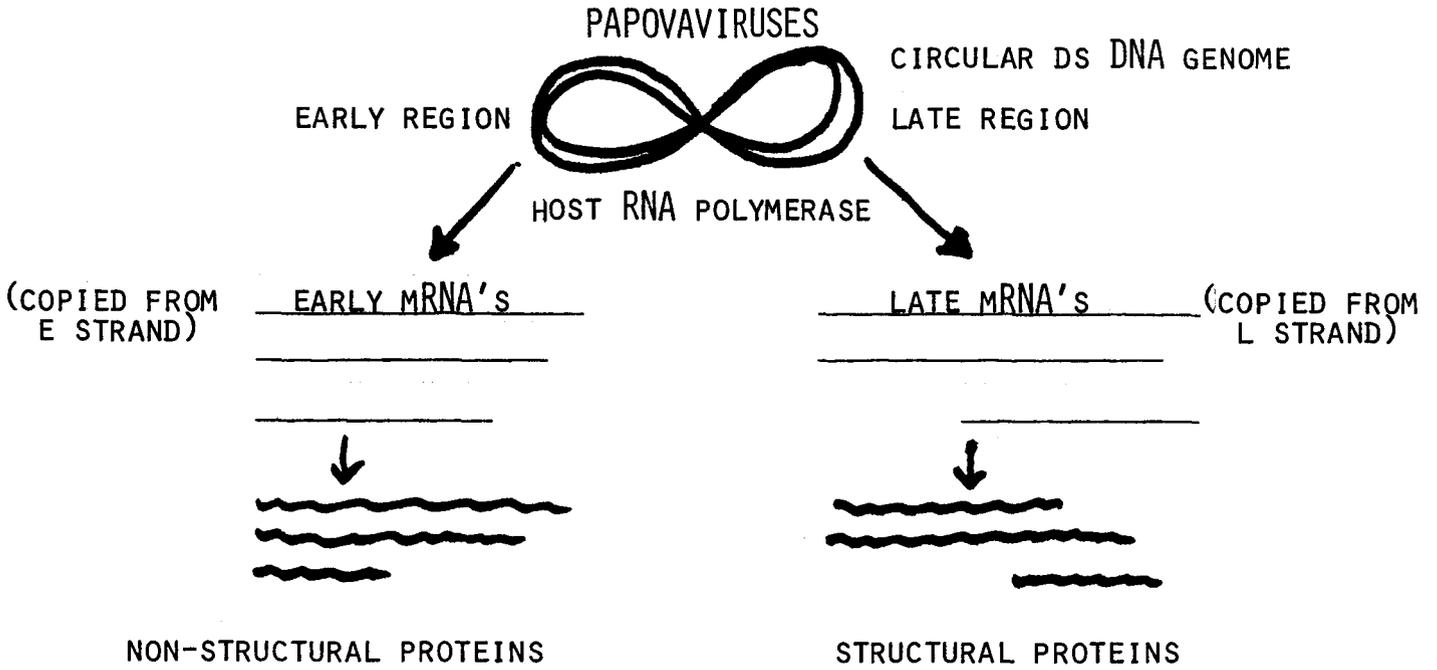
splicing

REOVIRUS

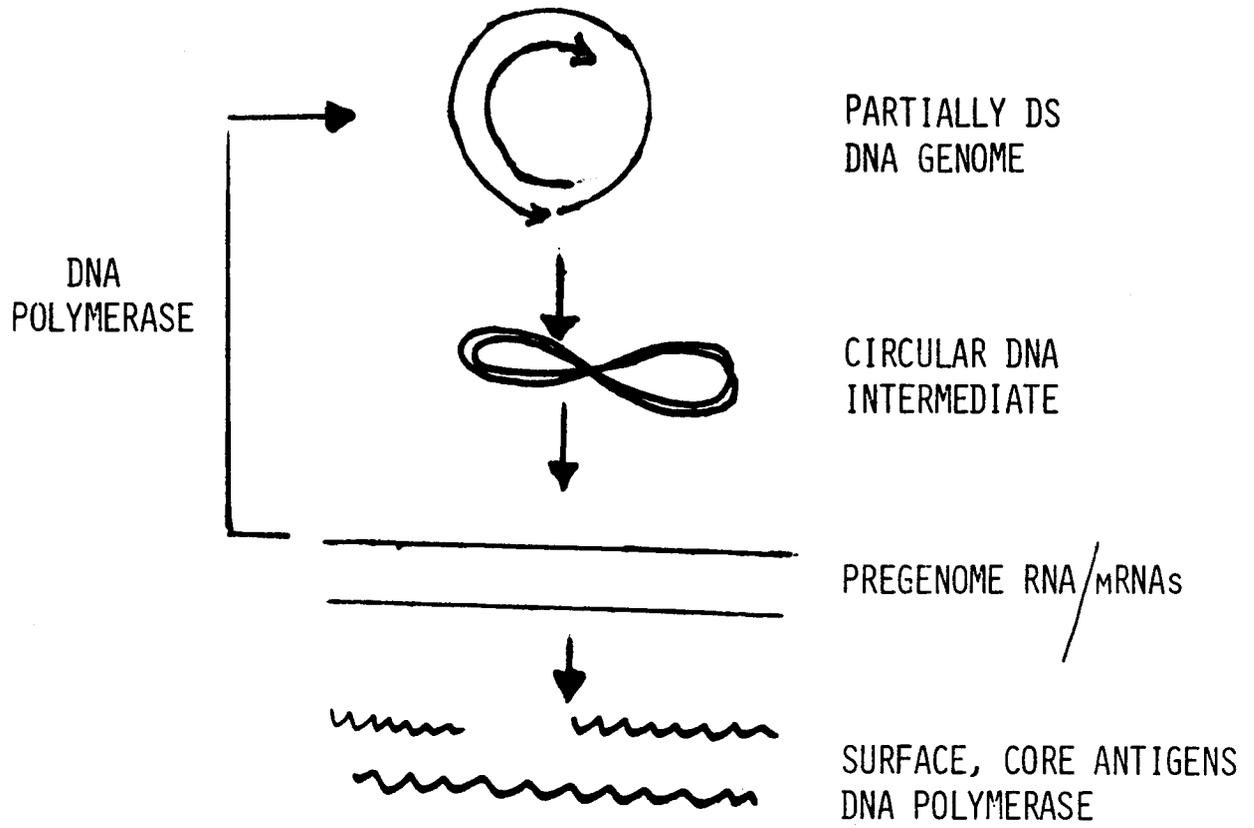


RETROVIRUS



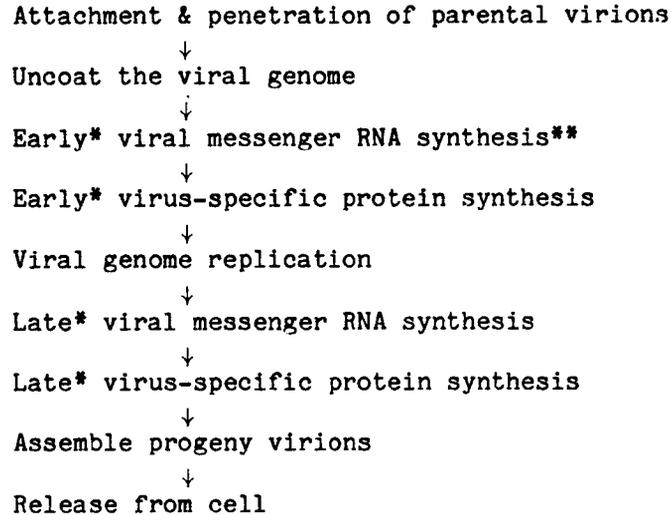


HEPATITIS B VIRUSES



This general picture of the virus life cycle can be summarized in the following flow chart:

THE VIRAL GROWTH CYCLE



* Not all animal viruses exhibit a distinction between early and late functions

**In some cases, the viral genome is functionally equivalent to mRNA; thus early mRNA need not be synthesized.

Host "shut off". During the course of the life cycle of many viruses, major aspects of host cell metabolism (e.g., synthesis of host RNA or host proteins) may be turned off, generally by mechanisms as yet poorly defined. In some cases, viral reagents appear to be used preferentially by host cell machinery (e.g., preferential transport of viral rather than host RNA from nucleus to cytoplasm, or preferential translation of viral rather than cellular mRNA by host polyribosomes); alternatively, viral gene products specifically toxic for host cells may be made. Not all viruses exhibit this sort of behavior, however. Some viruses (e.g., retroviruses) may replicate without obvious deleterious effect upon their hosts, and some (e.g., papovaviruses) may stimulate host cell metabolism (e.g., host DNA synthesis) at some point in their life cycle.

Viral Genetics

A brief survey of the genetics of animal viruses is relevant to several issues discussed in these introductory lectures and in the later, more clinically-oriented part of this course. (i) Viral mutants have facilitated fundamental insights into the virus life cycle. (ii) Mutants have helped define viral genes involved in pathogenesis, providing a basis for understanding virulence and for planning alterations that might attenuate virus strains. (iii) The mechanism of genetic changes which determine antigenic variation of certain viruses (such as influenza virus) informs efforts to control viral diseases. (iv) Certain kinds of viral mutants (e.g., defective, interfering particles, see below) may contribute to mechanisms of pathogenicity and

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persistence.

At least three kinds of genetic behavior need to be considered: changes that occur within the genome of a single virus during its propagation; interactions that occur between two viruses, usually two identifiable strains of the same virus type, during co-propagation; and genetic interactions that occur between viruses and their host cells. (The last topic will be the subject of a separate lecture, Viruses as Vehicles of Genetic Change.)

1. Changes within the genome of a single virus. Identified mutations are due to base substitutions, deletions, and rearrangements (inversions, duplications, etc.) in the viral genome. Phenotypic consequences (e.g., failure to grow, to cause CPE, etc.) may be conditional (e.g., present only at elevated temperatures or in certain host cells) or non-conditional. Mutations can be assigned to genetic units by complementation tests in which two mutants are tested for their capacity to assist each other in a mixed infection; if they can, their lesions are assumed to lie in different genes (see illustration on next page).

Single base changes may affect the clinical significance of virus isolates, altering their antigenic properties or affecting virulence. For example, such mutations account for minor changes in the antigenicity of influenza virus (antigenic "drift") and have been induced in efforts to produce conditionally or non-conditionally attenuated strains of several viruses for use in live vaccines.

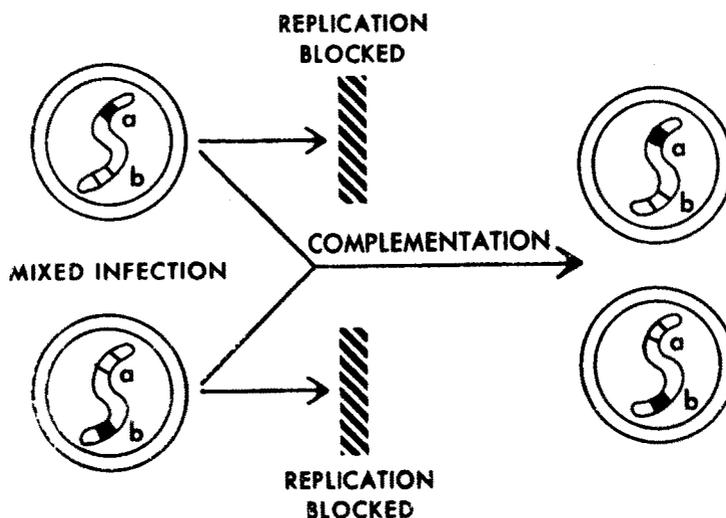
An interesting class of deletion mutants has been associated with a large number of both DNA and RNA viruses. These mutants are called defective, interfering (or DI) particles. They are unable to replicate on their own, but can be replicated in the presence of wild type virus, which acts as "helper virus," supplying missing functions; however, the DI particles also interfere with the replication of the wild type virus, e.g., by competing for polymerases during genome replication or for nucleocapsid proteins during assembly. Thus DI's can diminish the amount of virus produced, affect the time course and severity of infections, and contribute to the establishment of persistent infections of both cultured cells and animals. It has been proposed that they might have contributory roles in certain chronic viral diseases, but these claims have yet to be confirmed.

There is increasing medical interest in minor differences in base sequence between genomes; these often produce alterations in biochemical tests of genomes (e.g., patterns of fragments generated by digestion of DNA genomes with restriction endonucleases) without any phenotypic alterations. Such biochemical tests are gaining increasing use in tracing the transmission of certain viruses (e.g., herpes viruses) in minor outbreaks.

2. Interactions between viruses.

a. Complementation. As noted above, two mutants bearing lesions in different genes and infecting the same cell can supply missing functions to each other without actual exchange of genetic information. An example is diagrammed below:

*part of
the sample
is pol*



b. Phenotypic mixing. Mixed infection by two viruses with distinguishable but related structural proteins (e.g., envelope glycoproteins) often results in production of progeny in which the genome of one virus is carried in particles containing proteins partly (or even completely) supplied by the other virus. Such events can drastically alter the host range of a virus or its capacity to be neutralized by antisera against the intermixing proteins. This is not a stable genetic change, since infection of a new cell by a single particle of the phenotypically mixed virus will result in production of proteins encoded only by the single genome. Phenotypic mixing occurs most often between two viruses of the same group (e.g., two retroviruses with different envelope glycoproteins) but can occur between viruses of different types (e.g., a retrovirus can supply the envelope proteins for a rhabdovirus genome). For an extreme example, see discussion of the delta agent in the hepatitis virus lectures.

c. Recombination. During mixed infection with closely related viruses (sufficiently close to have homologous regions of their genomes), the genomes may recombine to form what is, in effect, a new virus strain. This is a stable change, heritable during subsequent growth of the recombinant virus particle. Many, if not all, viruses can undergo recombination, but the frequency varies enormously from very rare (poliovirus) to very common (retroviruses).

d. Heterozygosis. Occasionally more than one genome may be packaged within the nucleocapsids of certain viruses; when this happens during a mixed infection, the resulting particle may be a heterozygote, capable of producing both strains of virus during a subsequent round of infection. This is not, however, a stable genetic change, and its general significance is not known.

e. Genetic reassortment. During mixed infection with viruses containing segmented genomes (e.g., influenza viruses or reoviruses), both parents may contribute segments to single progeny. Such reassortment occurs at an extremely high frequency and produces a stable genetic change indistinguishable on functional grounds from recombination. This mechanism accounts for the sudden appearance of new antigenic properties of influenza viruses (antigenic "shift").

The foregoing interactions are diagrammed on the next page.

