

Viral Replication

I. Steps in Viral Replication

A. Attachment. This is the first step in viral replication. Surface proteins of the virus interact with specific receptors on the target cell surface. These may be specialized proteins with limited distribution or molecules that are more widely distributed on tissues throughout the body. The presence of a virus-specific receptor is necessary but not sufficient for viruses to infect cells and complete the replicative cycle.

B. Penetration. Enveloped viruses (e.g., HIV, influenza virus) penetrate cells through fusion of the viral envelope with the host cell membrane. Non-enveloped viruses penetrate cells by translocation of the virion across the host cell membrane or receptor mediated endocytosis of the virion in clathrin coated pits with accumulation of viruses in cytoplasmic vesicles.

C. Uncoating (disassembly). A complex process which differs by taxonomic class and is not fully understood for many agents. This process makes the nucleic acid available for transcription to permit multiplication of the virus.

D. Transcription and Translation. The key to understanding the genomic expression of viruses is noting the fact that viruses must use host cellular machinery to replicate and make functional and structural proteins. Strategies for genomic expression for different taxonomic groupings of viruses are described below (section II).

F. Assembly and Release. The process of virion assembly involves bringing together newly formed viral nucleic acid and the structural proteins to form the nucleocapsid of the virus. There are basically three strategies that viruses employ:

1. Nonenveloped viruses exhibit full maturation in the cytoplasm (e.g., picornaviruses) or the nucleus (e.g., adenoviruses) with disintegration of the cell and release of virions.
2. For enveloped viruses, including the (-) strand RNA viruses, the (+) strand togaviruses and the retroviruses, final maturation of the virion takes place as the virion exits the cell. Viral proteins are inserted into the host cell membrane. Nucleocapsids bind to the regions of the host cell membranes with these inserted proteins and bud into the extracellular space. Further cleavage and maturation of proteins may occur after viral

extrusion to impart full infectivity on the virion. Viruses in this group differ in their degree of cytolitic activity.

3. Herpesviruses, which are enveloped viruses, assemble their nucleocapsids in the nuclei of infected cells and mature at the inner lamella of the nuclear membrane. Virions accumulate in this region, in the endoplasmic reticulum and in vesicles protected from the cytoplasm. Release of virions from the cell surface is associated with cytolysis.

II. Strategies of Genomic Expression – RNA Viruses

A. Positive (+) Strand RNA Viruses Coding for One Genome-Sized mRNA [e.g., poliovirus, flaviviruses (including hepatitis C virus)].

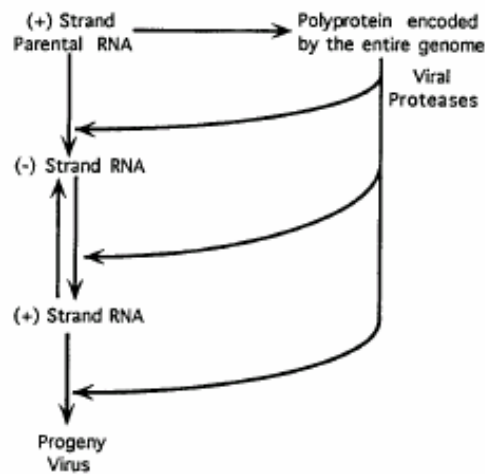


FIG. 2. Flow of events during the replication of positive strand RNA viruses that code for a single genome-sized (+) RNA. This RNA serves as their only mRNA species; it encodes a single polyprotein and is packaged into virions (e.g., picornaviruses, flaviviruses, hepatitis C viruses).

Figure 2, p. 105 from Fields Virology.

1. The genomic RNA binds to ribosomes and is translated into a polyprotein.
2. The polyprotein is cleaved.
3. Genomic RNA's then serve as templates for synthesis of complementary full length (-) strand RNA's by a viral polymerase.
4. (-) strand RNA serves as template for (+) strand RNA's. These (+) strands can then serve to produce more polyprotein, more (-) strand RNA's or as part of new virions which are forming.

B. Positive (+) Strand RNA Viruses Coding for Subgenomic RNA's (e.g., togaviruses).

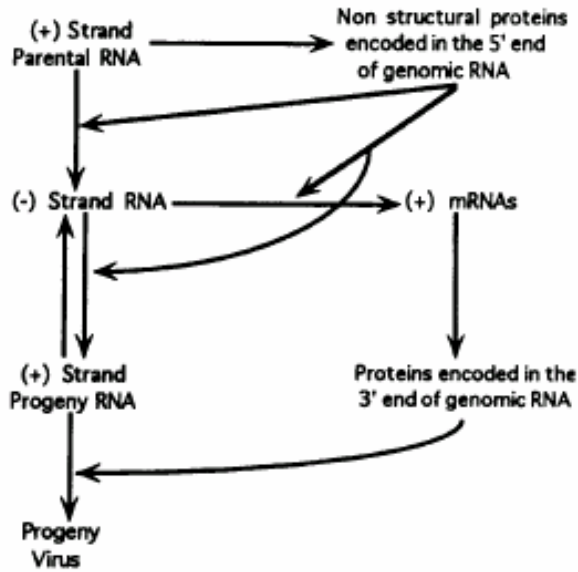


FIG. 3. Flow of events during the replication of positive strand RNA viruses that code for a genome-sized mRNA as well as for one or more subgenomic mRNAs (e.g., toga, corona, calici-, and hepatitis E viruses).

Figure 3, p105 from Fields Virology.

1. The genomic RNA binds to ribosomes but only a portion of the 5'-end is translated into non-structural proteins.
2. (-) strand RNA is synthesized. Different size classes of (+) RNA's are produced. One is translated into a polyprotein which is cleaved to form structural proteins. Another is full length and serves as genomic RNA for new virions which are forming.

C. Single Strand RNA Viruses with Two Identical Strands (e.g., retroviruses)

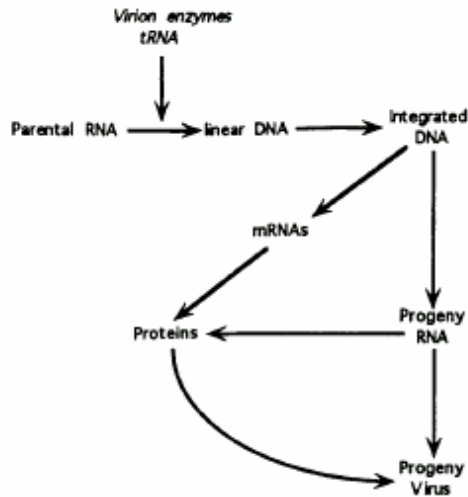


FIG. 4. Flow of events during the replication of retroviruses.

Figure 4, p 106 from Fields Virology.

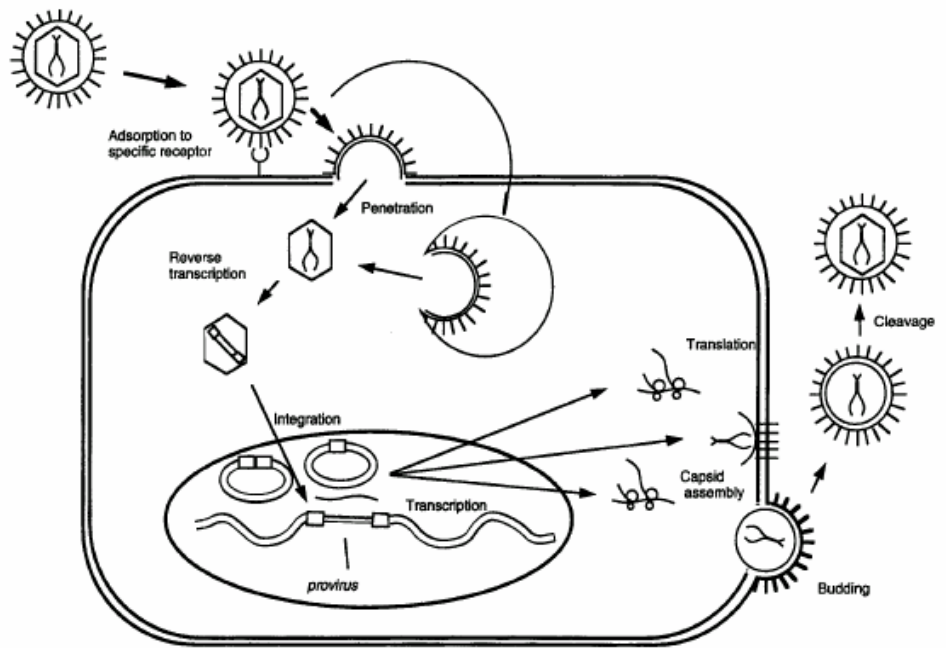


FIG. 12. Overview of retrovirus replication.

Figure 12, p 1786 from Fields Virology.

1. Genomic RNA serves as template for production of DNA copy. This is produced by an RNA-dependent DNA polymerase (reverse transcriptase) contained in the virion.

2. Digestion of genomic RNA and synthesis of second, complementary DNA strands then proceeds.
3. Double-stranded DNA migrates to the nucleus and integrates into the host cell genome.
4. Integrated DNA may remain silent or be transcribed into genomic, full-length RNA or shorter, spliced RNA's. The latter code for accessory and structural proteins. Full-length RNA transcripts are packaged into forming virions.

D. Negative (-) Strand Nonsegmented RNA Viruses (e.g., paramyxoviruses, rhabdoviruses, filoviruses)

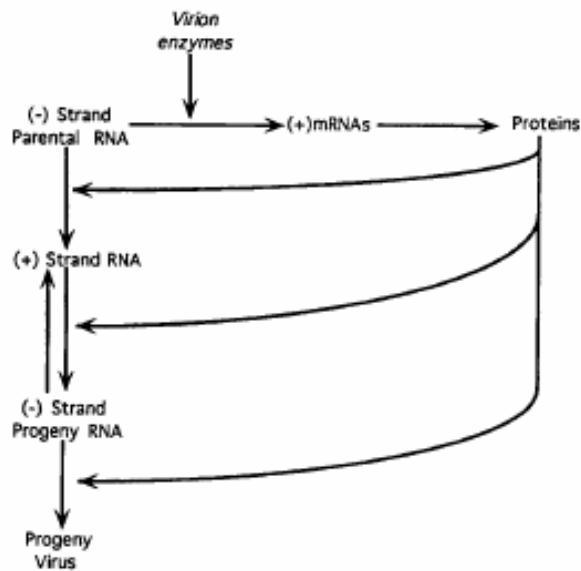


FIG. 5. Flow of events during the replication of nonsegmented negative strand RNA viruses (e.g., paramyxoviruses, rhabdoviruses, filoviruses).

Figure 5, p106 from Fields Virology.

1. Transcription of (-) strand occurs after entry and is mediated by virion packaged transcriptase.
2. (+) strand RNA's are produced; proteins are synthesized.
3. Full-length (-) strand RNA's are produced and packaged into newly forming virions.
4. Transcription and translation take place entirely in the cytoplasm of infected cells.

E. Negative (-) Strand Segmented RNA Viruses (e.g., bunyaviruses and orthomyxoviruses)

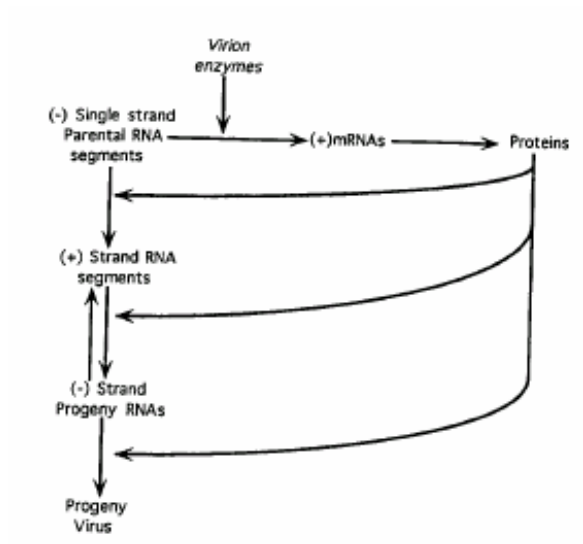


FIG. 6. Flow of events during the replication of segmented negative strand RNA viruses (e.g., orthomyxoviruses and bunyaviruses).

Figure 6, p107 from Fields Virology.

1. mRNA's are synthesized from each segment.
2. Viral proteins are synthesized.
3. (+) strand RNA's are synthesized and serve as templates for (-) strand genomic RNA's.

F. Double Stranded RNA Viruses (e.g., reoviruses)

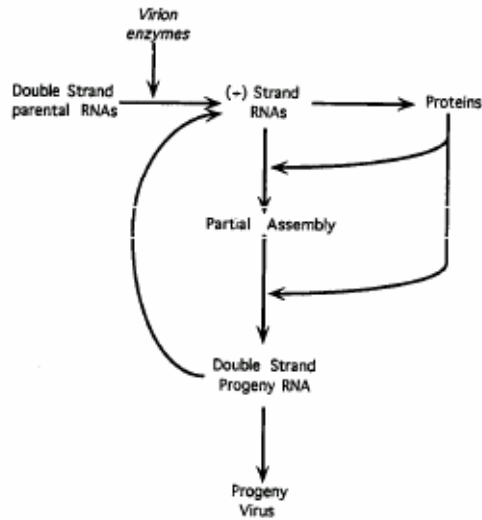


FIG. 8. Flow of events during the replication of double-strand RNA viruses (e.g., reoviruses, orbiviruses).

Figure 8, p 108 from Fields Virology.

1. Genome is transcribed by virion packaged polymerase.
2. mRNA's are translated to viral proteins or transcribed to complementary strands to yield double stranded RNA genomes for new virion formation.

III. Strategies of Genomic Expression – DNA Viruses

A. Double Stranded DNA Viruses Which Replicate in Nucleus (e.g., papovaviruses, papillomaviruses, adenoviruses, herpesviruses)

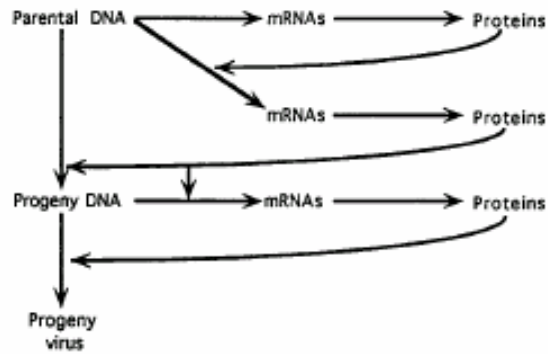


FIG. 10. Flow of events during the replication of herpesviruses (herpes simplex virus).

Figure 10, p 109 from Fields Virology.

1. Sequential, ordered rounds of mRNA and protein production regulate replication.
2. Structural proteins produced during last cycle of transcription.

B. Double Stranded DNA Viruses Which Replicate in the Cytoplasm (e.g., poxviruses)

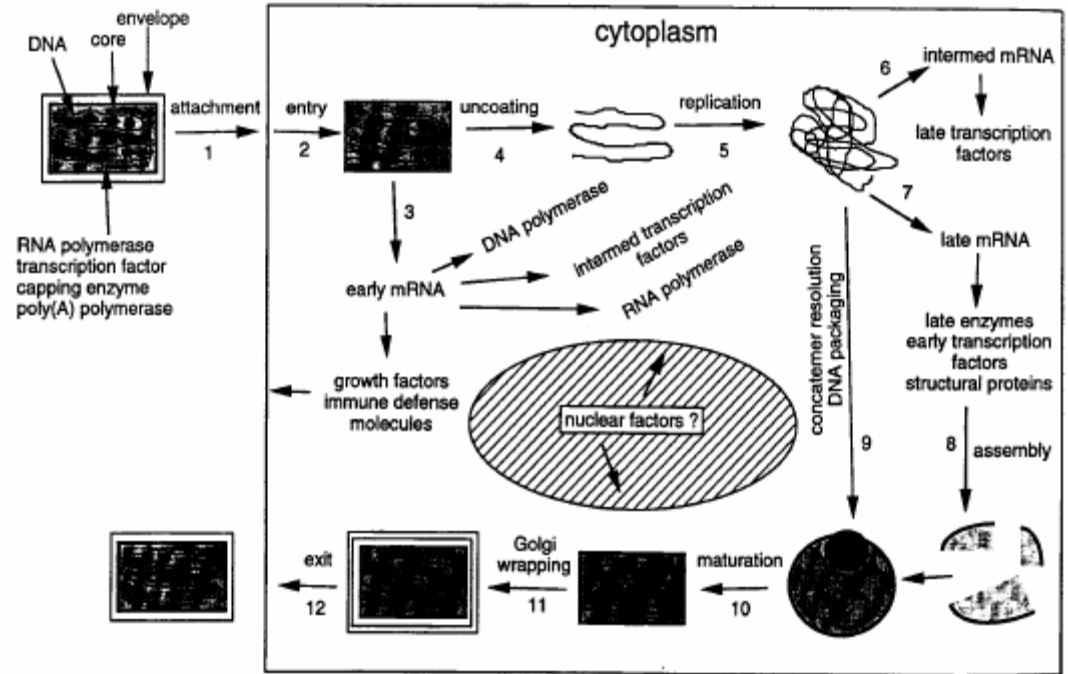


FIG. 1. The replication cycle of vaccinia virus. Virions, containing a double-stranded DNA genome, enzymes and transcription factors, attach to cells [1] and fuse with the cell membrane, releasing cores into the cytoplasm [2]. The cores synthesize early mRNAs that are translated into a variety of proteins, including growth factors, immune defense molecules, enzymes, and factors for DNA replication and intermediate transcription [3]. Uncoating occurs [4] and the DNA is replicated to form concatemeric molecules [5]. Intermediate genes in the progeny DNA are transcribed and the mRNAs are translated to form late transcription factors [6]. The late genes are transcribed and the mRNAs are translated to form virion structural proteins, enzymes, and early transcription factors [7]. Assembly begins with the formation of discrete membrane structures [8]. The concatemeric DNA intermediates are resolved into unit genomes and packaged in immature virions [9]. Maturation proceeds to the formation of infectious intracellular mature virions [10]. The virions are wrapped by modified Golgi membranes and transported to the periphery of the cell [11]. Fusion of the wrapped virions with the plasma membrane results in release of extracellular enveloped virus [12]. Although replication occurs entirely in the cytoplasm, nuclear factors may be involved in transcription and assembly.

Figure 1, p 2638 from Fields Virology.

1. Initial transcription takes place in core of virion.
2. Proteins produced release genome from virion core.

C. Single-Strand DNA Viruses (e.g., parvoviruses)

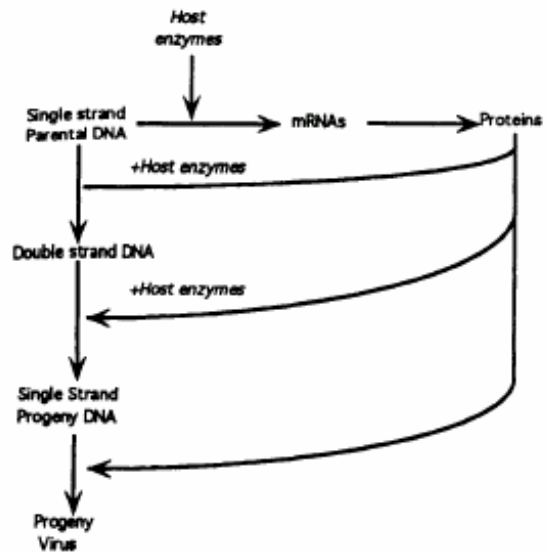


FIG. 11. Flow of events during the replication of autonomous parvoviruses.

Figure 11, p 110 from *Fields Virology*.

1. Complementary DNA strand synthesized in nucleus.
2. Transcription of the genome ensues.

D. Partially Double Stranded DNA Viruses (hepadnaviruses)

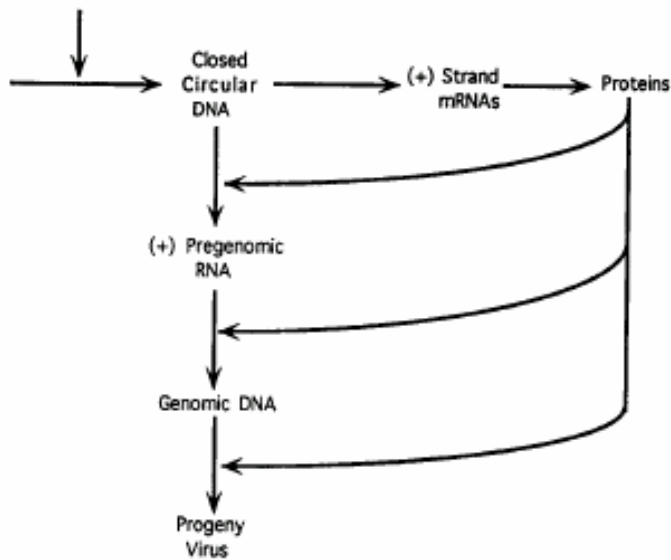


Figure 12, p 110 from *Fields Virology*.

1. Genome of hepatitis B is circular and partially double stranded; it is replicated in the nucleus.
2. Genome is converted to a closed circular molecule by a DNA polymerase which is virion packaged.
3. Two classes of RNA species are produced: one that codes for viral proteins and one that serves to produce genomic DNA by a virally encoded reverse transcriptase.