REPLICATION OF VIRUS

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- Virus (Latin, poison)
- Viruses are sub-cellular, non-living, infectious entities which only become part of a living system when they have infected host cells, a form of borrowed life (van Regenmortel, 2000)

- Viruses are obligate intracellular parasites.
- They need the help of a host cell for their replication.
- All viruses have to penetrate, replicate & come out of a cell.
- Infection of a cell by virus may result in any one of the following types of infection.

Order ***Mononegavirales***

families **BFPPR**

Bornaviridae,

Filoviridae

Paramyxoviridae

Pneumoviridae

Rhabdoviridae

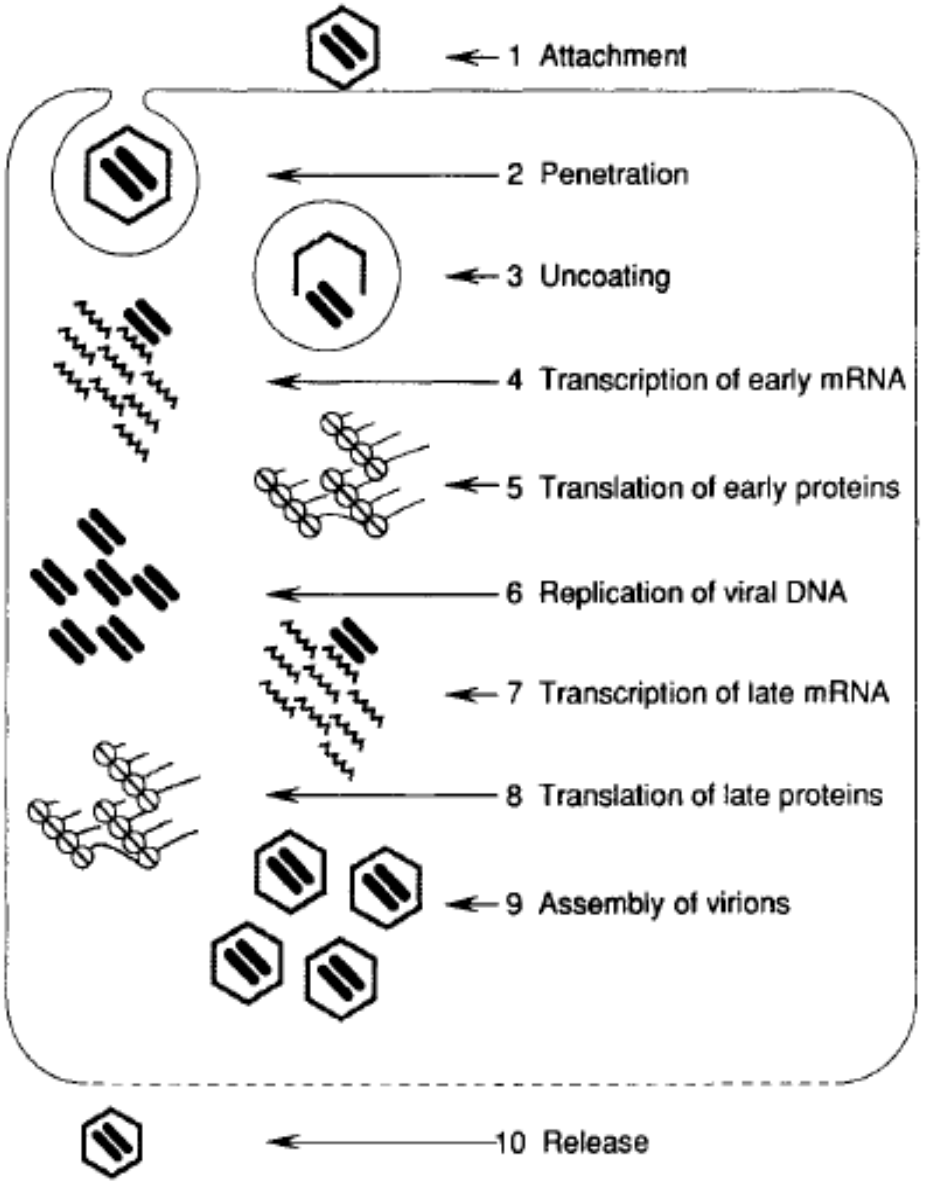
Genome is a linear, typically (but not always) nonsegmented, single-stranded, non-infectious RNA of negative polarity; possesses inverse-complementary 3' and 5' termini; and is not covalently linked to a protein.

Order: *Nidovirales*

Families include Arteriviridae, Coronaviridae, and Roniviridae.

The nidoviruses genome is an infectious, linear, positive sense RNA molecule, which is capped and polyadenylated.

Based on the genome size, they are divided into two groups large and small nidoviruses.



General features of the viral replication cycle, using adenovirus as a model. No topographic location for any step is implied. One step grades into the next such that, as the cycle progresses, several of these processes proceed simultaneously. Release occurs by cell lysis.

Stages of virus replication

Phase – I Initiation: This stage is characterized by introduction of genetic material of the virus into the cell

- Attachment
- Penetration
- Uncoating

Phase – II Replication: This stage is characterized by the genome size, composition and organization of viruses. There is no single pattern of replication. But all make proteins with 3 sets of functions to:

- Ensure replication of the genome
- Package the genome into virus particles
- Alter the metabolism of the infected cell so that viruses are produced.

•Genome synthesis
•RNA production
•Protein synthesis

Phase – III Assembly, Release, Maturation:

Phase I - Initiation

1. Attachment: Virus attaches to the cell surface.

•Attachment is via ionic interactions.

•Viral attachment proteins referred as **ligands** are present on the surface of viruses, which recognizes specific receptors on the cell surface.

The ligands in viruses are usually the fibres and spikes in the virus structures.

- **The receptors on cells are protein or carbohydrate or lipid components of the cell surface.**
- **The most common cellular receptors are glycoproteins.**
- **Some of the important cell receptors for viruses are CD4, ICAM etc. Cells without the appropriate receptors are not susceptible to the virus.**
- **Hence, a virus cannot produce infection in a host, which does not contain receptors for virus attachment.**
- **The joining of ligand to receptor on cell is also facilitated by co-receptors (Eg. gp120 of HIV)**
- **In some cases attachment leads to irreversible changes in the structure of the virion.**

2. Penetration:

- It is a process by which a virus enters into the cell.
- It is an energy dependant reaction and occurs quickly.
- It occurs as fusion, endocytois or translocation.
- It is different for enveloped and non-enveloped viruses.

Enveloped viruses –Two methods for enveloped viruses.

(A) Entry by fusing with the plasma membrane –

- Some enveloped viruses fuse directly with the plasma membrane.
- Thus, the internal components of the virion are immediately delivered to the cytoplasm of the cell.

B) ***Entry via endosomes at the cell surface*** –

Some enveloped viruses require an acid pH for fusion to occur & are unable to fuse directly with the plasma membrane.

These viruses are taken up by invagination of **clathrin coated pits** into endosomes.

As the endosomes become acidified, the fusion activity of the virus proteins becomes activated by the fall in pH and the virion membrane fuses with the endosome membrane.

This results in delivery of the internal components of the virus to the cytoplasm of the cell.

This endocytosis is also called **viropexis**.

Non-enveloped viruses - Non-enveloped viruses may cross the plasma membrane directly or may be taken up via clathrin-coated pits into endosomes.
They then cross the endosomal membrane

3.Uncoating: This is the general term applied to events after penetration, which allow the virus to express its genome.

- For successful viral infection, nucleic acid has to be sufficiently uncoated.
- The lysosomal enzymes play a major role in uncoating.

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Phase II:Replication of viral nucleic acid and protein synthesis

Once uncoating has taken place, synthesis of viral NA starts. This occurs as three different stages with differences between different families of the viruses.

Early transcription and translation: The proteins derived from this stage is mostly the enzymes required for virus replication.

Replication of Nucleic acid:

Late transcription and translation :The proteins produced during this stage are structural proteins.

- **The site of production of nucleic acid also varies between viruses.**
- **Most of the DNA viruses except Pox and Herpes replicate in nucleus.**
- **All RNA viruses replicate in cytoplasm except Orthomyxo, Borna and Retro,**
- **which for certain stages of replication get into the nucleus of the cell.**

Phase III

Assembly: This stage involves the assembly of all the components necessary for the formation of the mature virion at a particular site in the cell.

During this process, the basic structure of the virus is formed.

The site of assembly varies for different viruses, e.g:

•Picornaviruses, Poxviruses, Reoviruses - In the cytoplasm.

•Adenoviruses, Papovaviruses, Parvoviruses - In the nucleus.

•Retroviruses - On the inner surface of the cell membrane.

Release: For lytic viruses (**most non-enveloped viruses**), release is a simple process - the cell breaks open and releases the virus.

Enveloped viruses acquire the lipid membrane as the virus buds out through the cell membrane. Virion envelope proteins are picked up during this process as the virus is extruded. Budding may or may not kill the cell

Maturation:

- **At this stage of the lifecycle normally the virus becomes infectious.**
- **Usually it involves structural changes in the particle, often resulting from specific cleavage of capsid proteins to form the mature products, which frequently leads to a conformational change in the capsid, or the condensation of nucleoproteins with the genome.**
- **For some viruses, assembly and maturation are inseparable,**
- **whereas for others, maturation may occur after the virus particle has left the cell.**

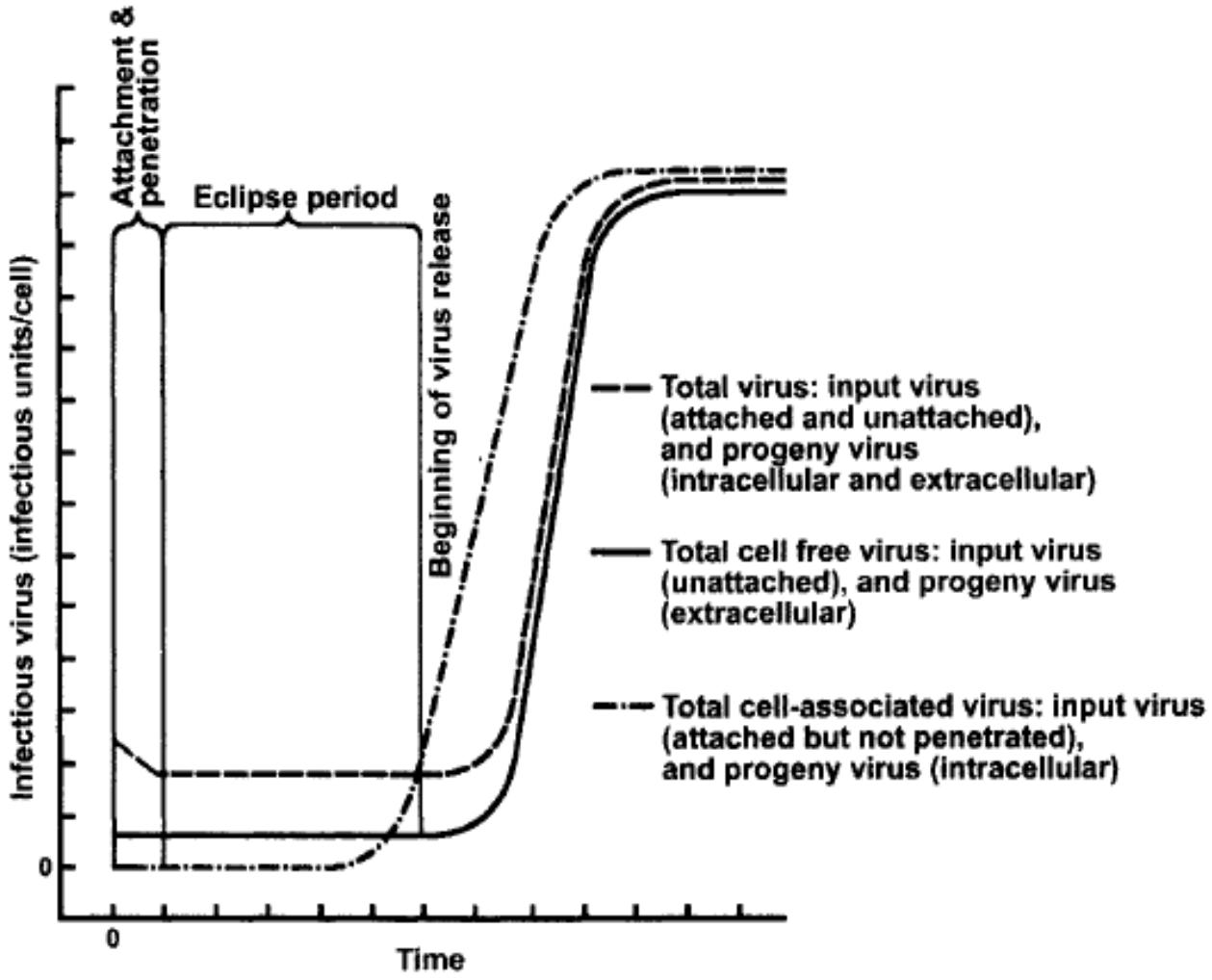
TABLE 2.2 Characteristics of Replication of Viruses of Different Families

Family	Uptake Route	Site of Nucleic Acid Replication	Site of Maturation (Budding)
Poxviridae	Variable	Cytoplasm	Cytoplasm
Asfaviridae	Clathrin-mediated endocytosis	Cytoplasm	(Plasma membrane)
Iridoviridae	Variable	Nucleus/cytoplasm	Cytoplasm
Herpesviridae	Variable	Nucleus	(Nuclear membrane)
Adenoviridae	Clathrin-mediated endocytosis	Nucleus	Nucleus
Polyomaviridae	Caveolar endocytosis	Nucleus	Nucleus
Papillomaviridae	Clathrin/caveolar endocytosis	Nucleus	Nucleus
Parvoviridae	Clathrin-mediated endocytosis	Nucleus	Nucleus
Hepadnaviridae	Clathrin-mediated endocytosis	Nucleus/cytoplasm	(Endoplasmic reticulum)

Retroviridae	Plasma membrane fusion or clathrin-mediated endocytosis	Nucleus	(Plasma membrane)
Reoviridae	Clathrin-mediated endocytosis	Cytoplasm	Cytoplasm
Paramyxoviridae	Plasma membrane fusion	Cytoplasm	(Plasma membrane)
Rhabdoviridae	Plasma membrane fusion	Cytoplasm	(Plasma membrane)
Filoviridae	Plasma membrane fusion	Cytoplasm	(Plasma membrane)
Bornaviridae	Clathrin-mediated endocytosis	Nucleus	(Plasma membrane)
Orthomyxoviridae	Clathrin-mediated endocytosis	Nucleus	(Plasma membrane)
Bunyaviridae	Clathrin-mediated endocytosis	Cytoplasm	(Golgi membrane)

Arenaviridae	Clathrin-mediated endocytosis	Cytoplasm	(Plasma membrane)
Coronaviridae	Clathrin-mediated endocytosis/plasma membrane fusion	Cytoplasm	(Endoplasmic reticulum)
Arteriviridae	Clathrin-mediated endocytosis	Cytoplasm	(Endoplasmic reticulum)
Picornaviridae	Caveolar endocytosis/plasma membrane insertion	Cytoplasm	Cytoplasm
Caliciviridae	Caveolar endocytosis/plasma membrane insertion?	Cytoplasm	Cytoplasm
Astroviridae	Caveolar endocytosis/plasma membrane insertion?	Cytoplasm	Cytoplasm
Togaviridae	Clathrin-mediated endocytosis	Cytoplasm	(Plasma membrane)
Flaviviridae	Clathrin-mediated endocytosis	Cytoplasm	(Endoplasmic reticulum)

FIGURE 3.2.



One-step growth curve of a nonenveloped virus. Attachment and penetration are followed by an eclipse period of 2 to 12 hours (see Table 3.1) during which cell-associated infectivity cannot be detected. This is followed by a period of several hours during which viral maturation occurs. Virions of nonenveloped viruses are often released late and incompletely, when the cell lyses. The release of enveloped virions occurs concurrently with maturation by budding from the plasma membrane.

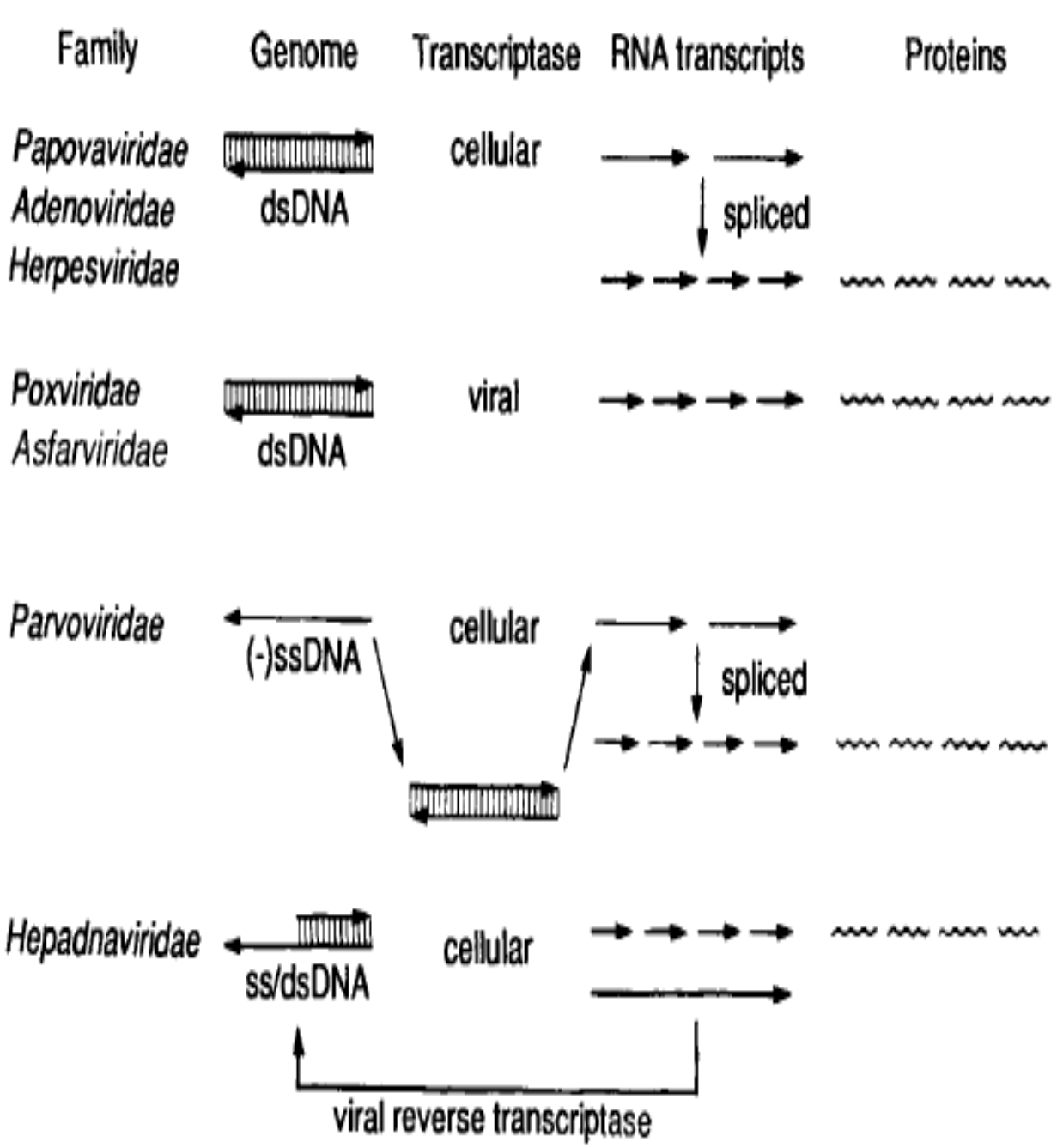
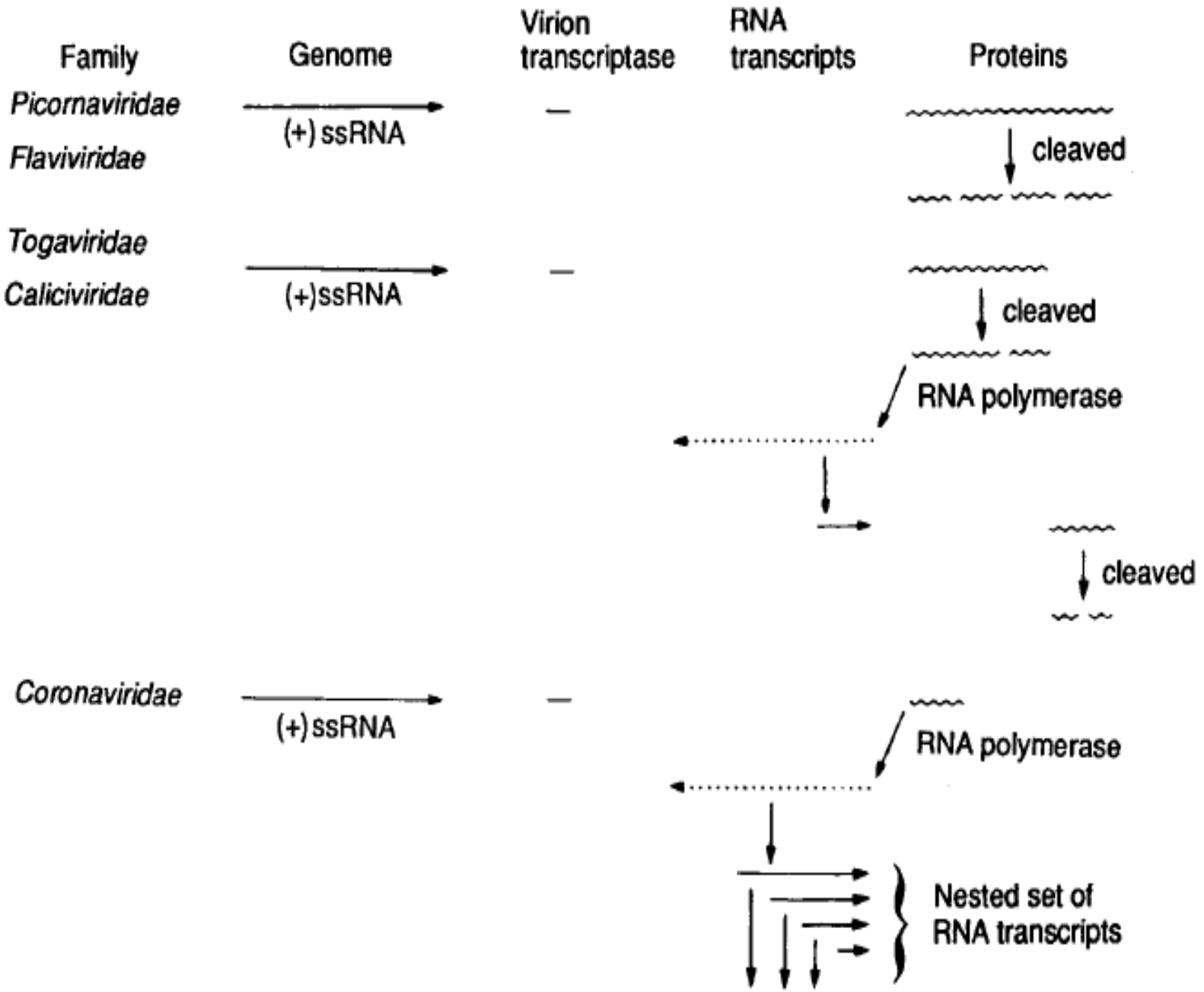
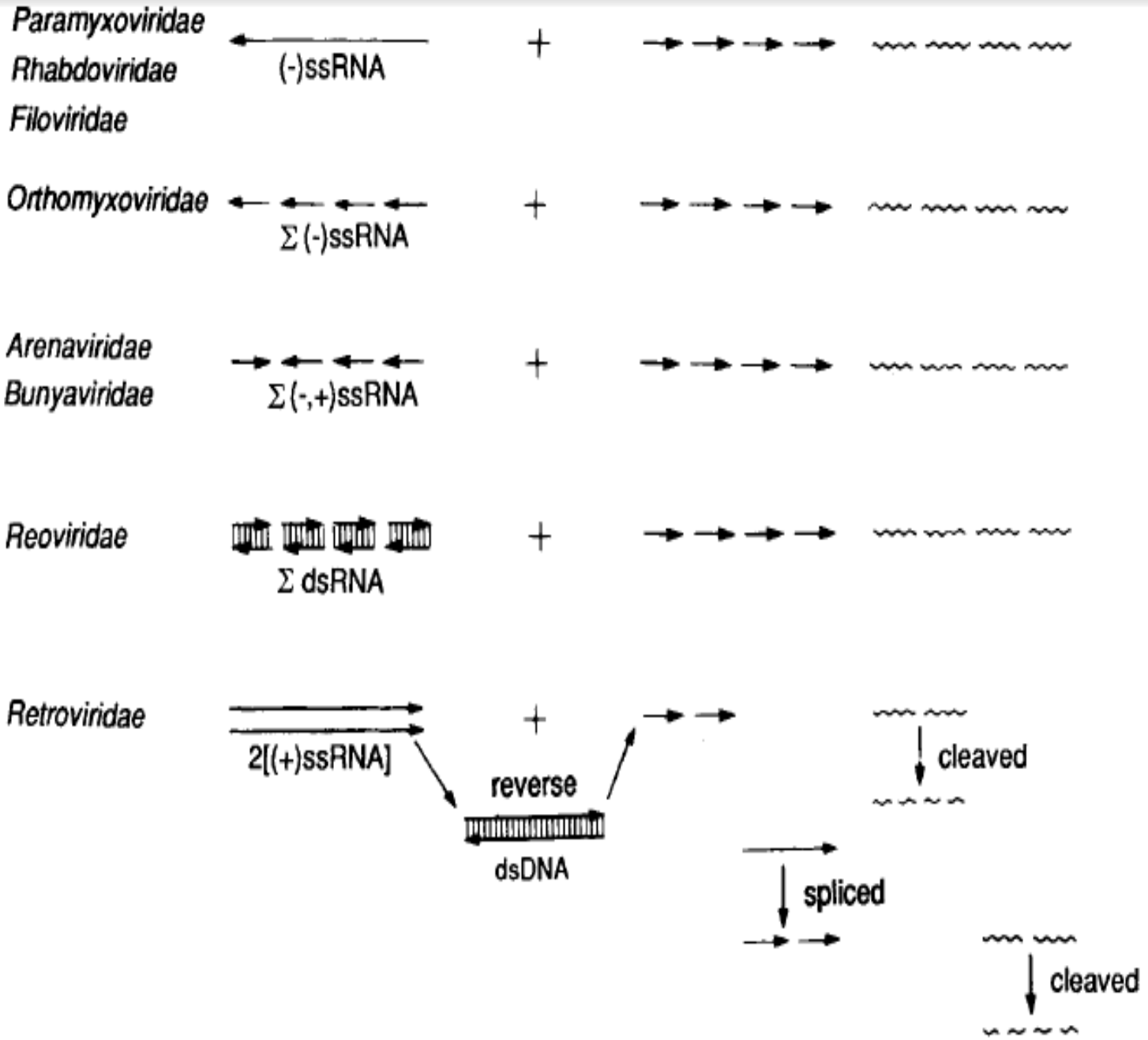

Family
Genome
Transcriptase
RNA transcripts
Proteins
Papovaviridae
Adenoviridae
Herpesviridae
dsDNA
cellular
spliced
Poxviridae
Asfarviridae
dsDNA
viral
Parvoviridae
(-)ssDNA
cellular
spliced
Hepadnaviridae
ss/dsDNA
cellular
viral reverse transcriptase

FIGURE 3.6.


Family
Genome
Virion transcriptase
RNA transcripts
Proteins
Picornaviridae
Flaviviridae
(+) ssRNA
–
cleaved
Togaviridae
Caliciviridae
(+)ssRNA
–
cleaved
RNA polymerase
cleaved
Coronaviridae
(+) ssRNA
–
RNA polymerase
Nested set of RNA transcripts


Paramyxoviridae
Rhabdoviridae
Filoviridae
(-)ssRNA
+
Orthomyxoviridae
Σ (-)ssRNA
+
Arenaviridae
Bunyaviridae
Σ (-,+)ssRNA
+
Reoviridae
Σ dsRNA
+
Retroviridae
2[(+)ssRNA]
+
reverse
dsDNA
cleaved
spliced
cleaved

DNA VIRUS REPLICATION

- The replication of DNA virus is a straight-forward one since the mechanism available for viral DNA replication is readily available in the cell.
- DNA viruses are able to use the host cell's replication machinery to transcribe their genome into mRNA immediately.
- DNA viruses generally replicate within the host cell nucleus.
- Eg. Papovaviruses, papillomaviruses, polyomaviruses, adenoviruses and herpesviruses.
- Poxviruses replicate in the cytoplasm of the host cell and also carry their own RNA polymerase (transcriptase).
- A replicating DNA virus has to satisfy the following;

- **The virus needs to make mRNAs that can be translated into protein by the host cell translation machinery.**
- **The virus needs to replicate its genome.**
- **Host enzymes for mRNA synthesis and DNA replication are available in nucleus hence, it needs to enter the nucleus.**
- **Transcription and translation: The gene expression is divided into early and late phases.**
- **The early genes encode enzymes and regulatory proteins needed to start viral replication processes Late genes encode structural proteins, proteins needed for assembly of the mature virus.**

DNA Replication

Semi-conservative method: DNA replication uses host cell DNA polymerase. Replication is bi-directional. (There are two replication forks per circular DNA genome and replication involves leading/lagging strands, Okazaki fragments, DNA ligase, etc.). This process of DNA replication is very similar to that which occurs in the host cell (Eg. Papova viruses).

Strand displacement method: There are no Okazaki fragments, both strands are synthesized in a continuous fashion.

ds DNA (±) virus
Class I
Class VII

ss DNA (+)
virus
Class II

Synthesis of other strand

ds DNA intermediate

– Transcription —
of minus strand

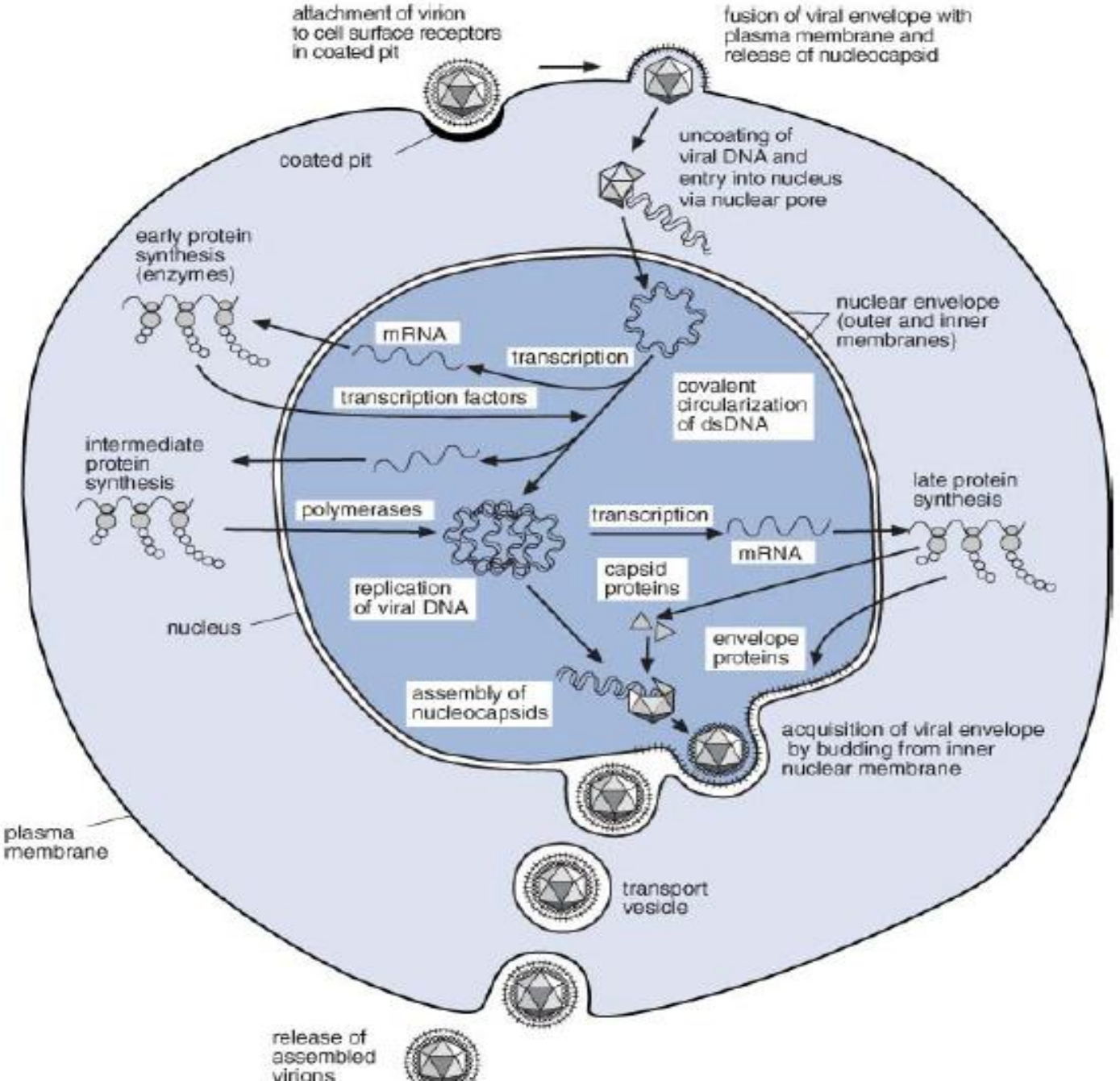
mRNA (+)

Genome replication:
Class I, classical semiconservative
Class II, classical semiconservative, discard (–) strand
Class VII, transcription followed by reverse transcription

DNA Viruses

Figure 9-11a Brock Biology of Microorganisms 11/e

Herpes Virus



Adeno Virus

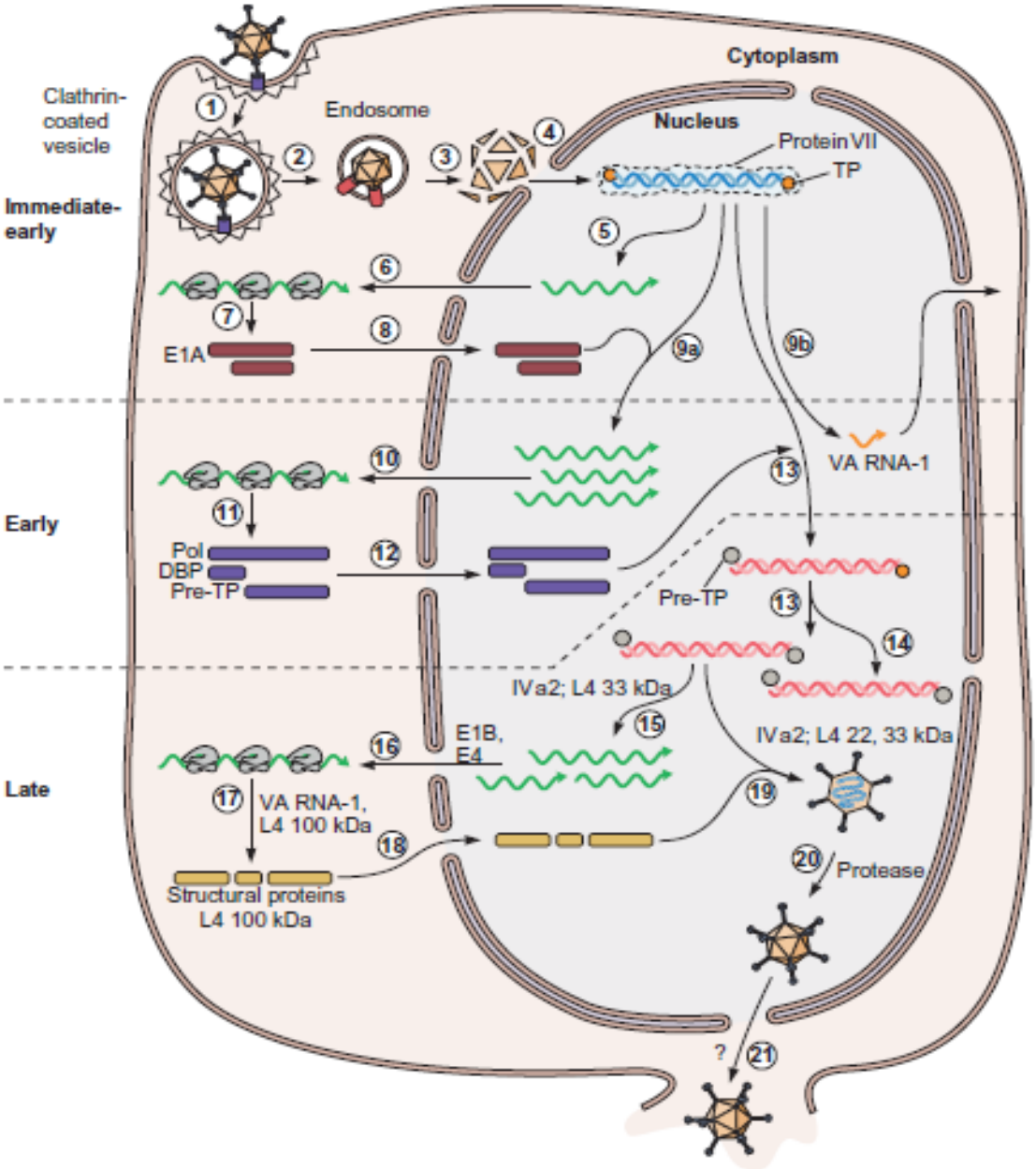


FIGURE 2.10 Single-cell reproductive cycle of human adenovirus type 2. The virus attaches to a permissive human cell via interaction between the fiber and (with most serotypes) the coxsackie-adenovirus receptor on the cell surface. The virus enters the cell via endocytosis (1 and 2), a step that

RNA virus replication:

- RNA viruses have a relatively small genome.
- RNA viruses code for only a few proteins.
- These normally include the enzyme Polymerase, which can copy RNA into a complementary nucleic acid and a viral attachment protein.

Problems associated with RNA virus replication:

- Prokaryotic and eukaryotic cells do not carry RNA dependant RNA Polymerase enzyme,
- which is needed for nascent RNA strand synthesis from a RNA template. Hence, the negative strand RNA viruses carry this enzyme and positive strand RNA viruses have is as a gene.

•Eucaryotic host cell translation protein synthesis machinery in general uses monocistronic mRNAs and so there is a problem in making more than one type of protein from a single mRNA.

•RNA viruses have several solutions to this problem:

•The virus makes multiple monocistronic mRNAs

•The virus makes primary transcripts, which are processed by the host splicing machinery to give more than one monocistronic RNA

•The viral mRNA acts as a monocistronic transcript.

- **A large polypeptide (called a polyprotein) is made which is then cleaved into separate proteins –**

- **Thus, one initial translation product is processed to give rise to multiple proteins.**
- **Eg.Picornaviruses**

- **The RNA virus replication is described here as different strategies:**

1.RNA VIRUSES THAT DO NOT COPY THEIR RNA IN TO DNA:

As described earlier, these viruses need an RNA-dependent RNA-polymerase to replicate their RNA.

a. Plus-strand RNA viruses –

The virion (genomic) RNA is the same sense as mRNA and so functions as mRNA.

This mRNA can be translated immediately upon infection of the host cell.

Eg. FMD virus (picornavirus); togaviruses; flaviviruses

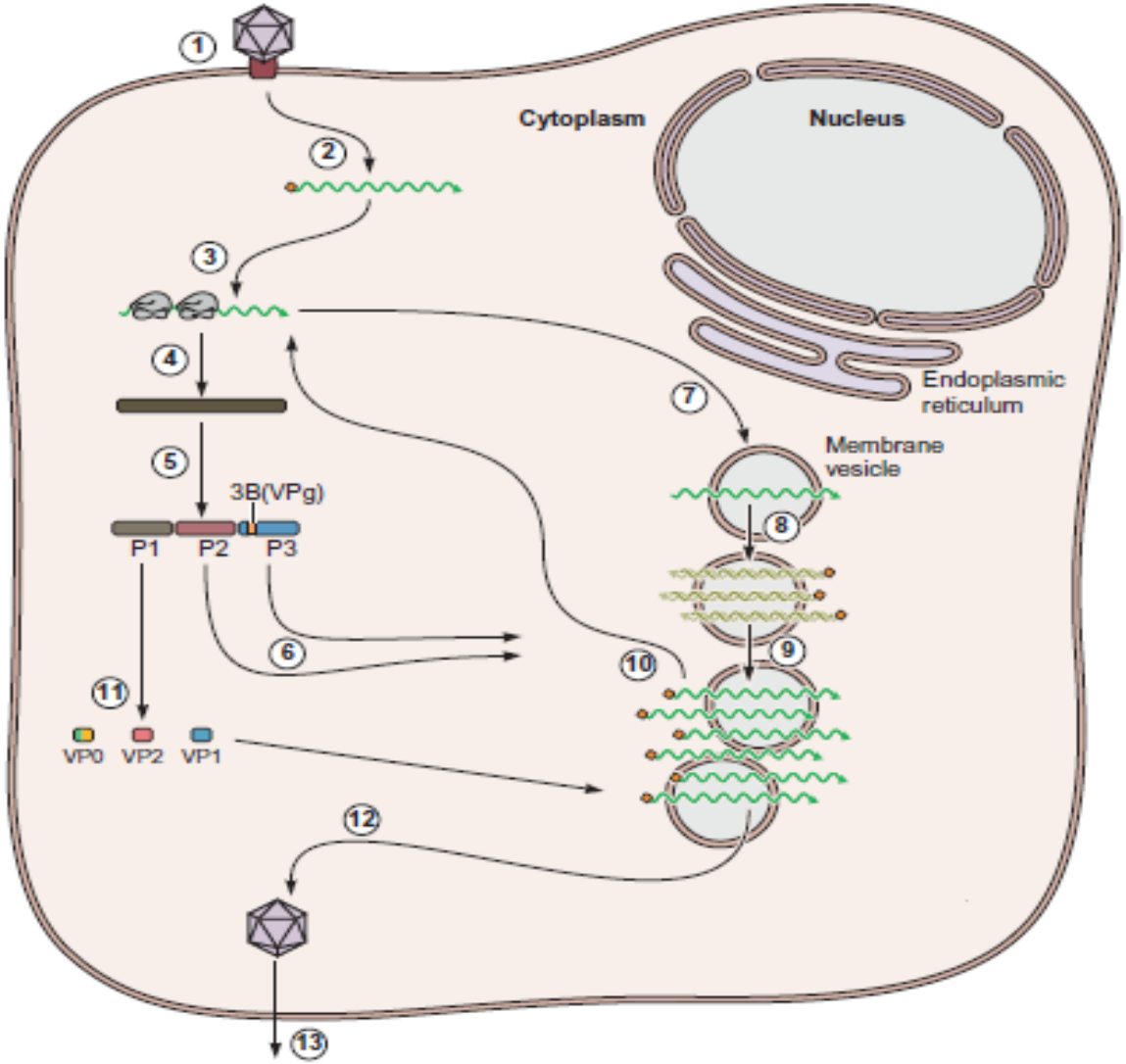


FIGURE 2.7 Single-cell reproductive cycle of a picornavirus, in this case poliovirus. The virion binds to a cellular receptor (1); release of the poliovirus genome occurs from within early endosomes located close (within 100 to 200 nm) to the plasma membrane (2). The VPg protein, depicted as a small orange circle at the 5′ end of the virion RNA, is removed, and the RNA associates with ribosomes (3). Translation is initiated at an internal site 741 nucleotides from the 5′ end of the viral mRNA, and a polyprotein precursor is synthesized (4). The polyprotein is cleaved during and after its synthesis to yield the individual viral proteins (5). Only the initial cleavages are shown here. The proteins that participate in viral RNA synthesis are transported to membrane vesicles (6). RNA synthesis occurs on the surfaces of these infected-cell-specific membrane vesicles. The (+) strand RNA is transported to these membrane vesicles (7), where it is copied into double-stranded RNAs (8). Newly synthesized (−) strands serve as templates for the synthesis of (+) strand genomic RNAs (9). Some of the newly synthesized (+) strand RNA molecules are translated after the removal of VPg (10). Structural proteins formed by partial cleavage of the PI precursor (11) associate with (+) strand RNA molecules that retain VPg to form progeny virions (12), which are released from the cell upon lysis (13). *[From* Principles of Virology, *S. J. Flint, L. W. Enquist, V. R. Racaniello, A. M. Skalka, 3rd ed., vol. 1, p. 519. Copyright © 2008 Wiley, with permission.]*

b. Negative-strand RNA viruses –

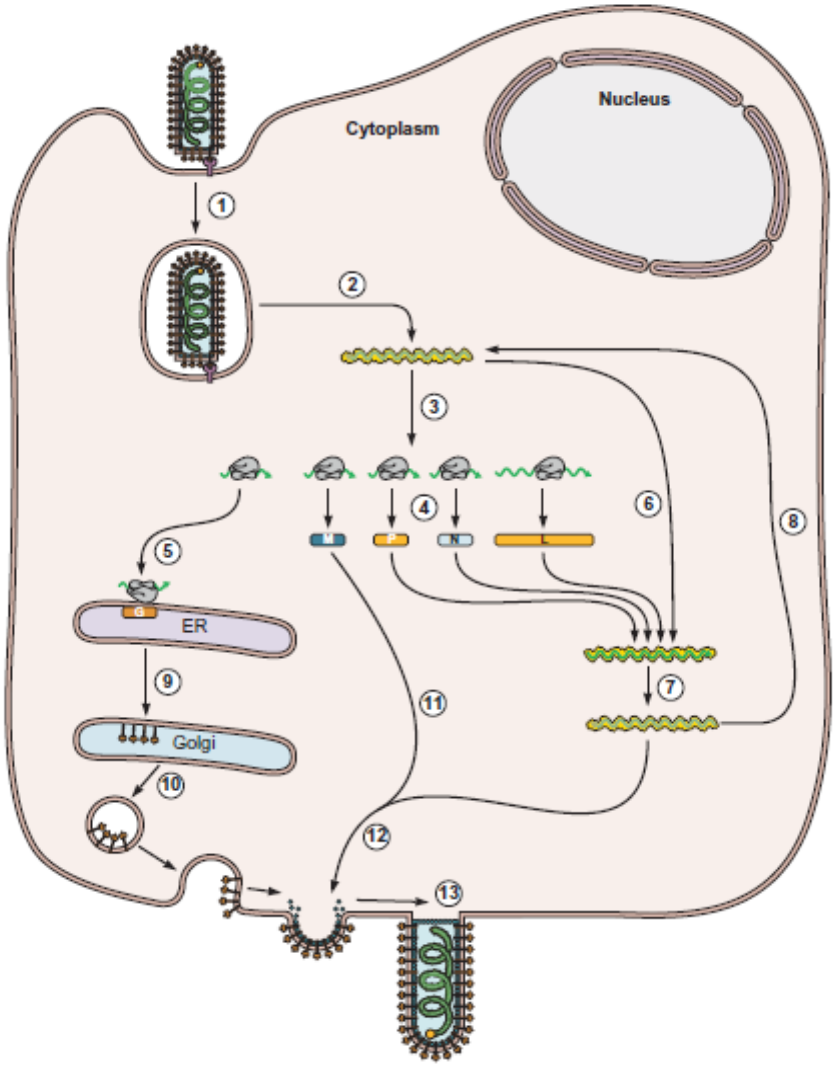
The virion RNA is negative sense (complementary to mRNA) and must therefore be copied into the complementary plus-sense mRNA before proteins can be made.

Eg. Newcastle disease virus (paramyxoviruses) rabies virus (rhabdovirus)

c. Double-strand RNA viruses –

The virion (genomic) RNA is double stranded and so cannot function as mRNA.

These viruses also carry the enzyme RNA polymerase as negative strand RNA viruses.


Cytoplasm
Nucleus
1
2
3
4
5
6
7
8
9
10
11
12
13
G
ER
Golgi

PLUS STRAND RNA VIRUSES

The plus strand viruses are also known as positive strand viruses and in this category of viruses the genomic RNA can act as mRNA. Eg. Picornaviruses; togaviruses; flaviviruses

Transcription and Translation: Poliovirus virion RNA functions as an mRNA and it is translated into a single polypeptide (polyprotein) immediately after entering into a cell.

These polyprotein is later cleaved into number of small proteins.

The cleavages are carried out by virus- coded proteases.

The products of cleavage include RNA polymerase (replicase) needed for RNA synthesis, structural components of the virion and proteases.

- **RNA replication:**

- **RNA replication is the process by, which new copies of genome-length RNAs are made.**
- **The products of cleavage of protein support the replication of genomic RNA.**
- **The Viral RNA polymerase copies plus-sense genomic RNA into complementary minus-sense RNA.**
- **The new minus sense strands serve as template for new plus sense strands.**
- **The new plus strand RNA serve as a template for more minus strands.**

NEGATIVE STRAND RNA VIRUSES – NON-SEGMENTED

The negative strand viruses those viruses wherein the genomic RNA cannot act as mRNA and mRNA has to be synthesized using genomic RNA as template.

Hence, these viruses always carry the enzyme RNA dependant RNA polymerase.

Eg. Rhabdoviruses, Paramyxoviruses

NEGATIVE STRAND RNA VIRUSES – NON-SEGMENTED

Transcription and translation:

The RNA polymerase enzyme copy virion RNA into a mRNA.

Messenger RNAs are translated on host ribosomes and viral proteins are made. There is no distinction between early and late functions.

RNA replication: RNA replication occurs in the cytoplasm and is carried out by the viral RNA polymerase. The new positive strand (mRNA) is copied into full length minus strand.

New negative strands may be used as templates for the synthesis of more full length plus strands (mRNA).

SEGMENTED NEGATIVE STRAND VIRUSES

Negative-sense, single stranded segmented genome.

Virions contain RNA polymerase packaged within the virus particle. Eg. Orthomyxoviridae; Influenza virus.

Transcription and translation:

mRNA synthesis and replication of viral RNA occurs in the nucleus. This is very unusual for an RNA virus. Influenza virus has an unusual mechanism for acquiring a methylated capped 5' end to its mRNAs referred as cap snatching in which, the virion mRNA go to nucleus to obtain the cap from host mRNA.

The viral RNA polymerase (transcriptase) copies the template into complementary plus sense mRNA.

mRNAs are translated in the cytoplasm.

RNA replication:

RNA replication occurs in the nucleus using a virus-coded enzyme.

A full length, exact complementary copy of virion RNA is made.

Full length plus strand RNA is then used as a template for full-length minus strand synthesis.

New minus strands can be used as templates for replication, mRNA synthesis, or packaged.

DOUBLE STRANDED RNA VIRUSES

The members under this category are peculiar in that the RNA exists in the form of double strands.

Transcription and translation:

Double stranded RNA does not function as an mRNA and so the initial step is to make mRNA (transcription).

The mRNAs are made by virion-packaged RNA polymerase.

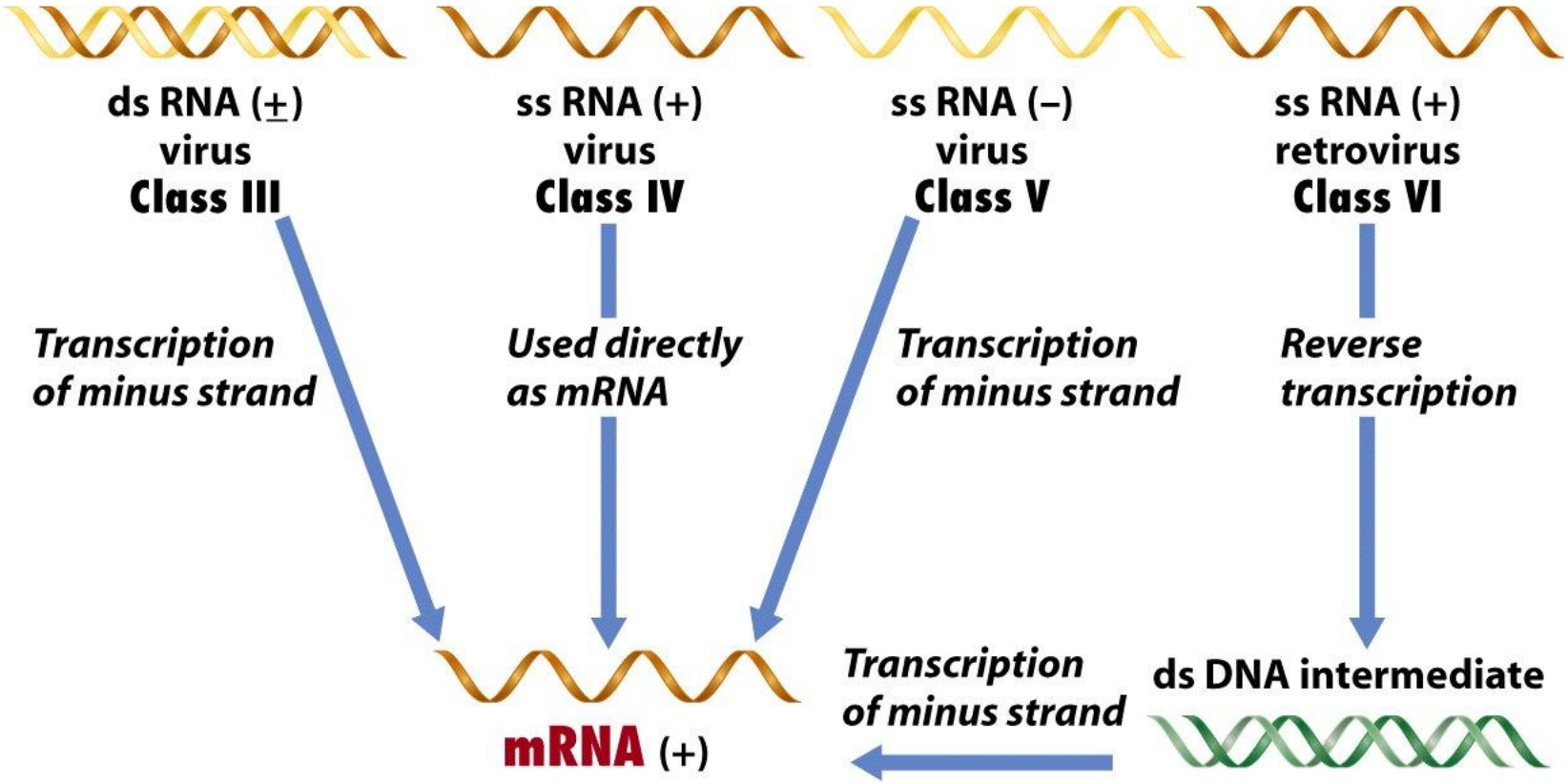
REVERSE TRANSCRIPTASE RNA VIRUS

Retroviruses are unique in that their genomes are transcribed into DNA and not RNA.

They contain two identical ssRNA of +ve polarity, with a poly A tail at the 3' end and a cap at the 5' end.

Each is transcribed into DNA by reverse transcriptase that then integrates into the cellular DNA as provirus.

Transcription of the provirus by the cellular transcriptase yields the viral molecules that end up in virions.



Genome replication:

Class III, classical semiconservative replication, but of RNA not DNA
Class IV, make ss RNA (–) and transcribe from this to give ss RNA (+) genome
Class V, make ss RNA (+) and transcribe from this to give ss RNA (–) genome
Class VI, make ss RNA (+) genome by transcription off of (–) strand of ds DNA

RNA Viruses

Figure 9-11b Brock Biology of Microorganisms 11/e

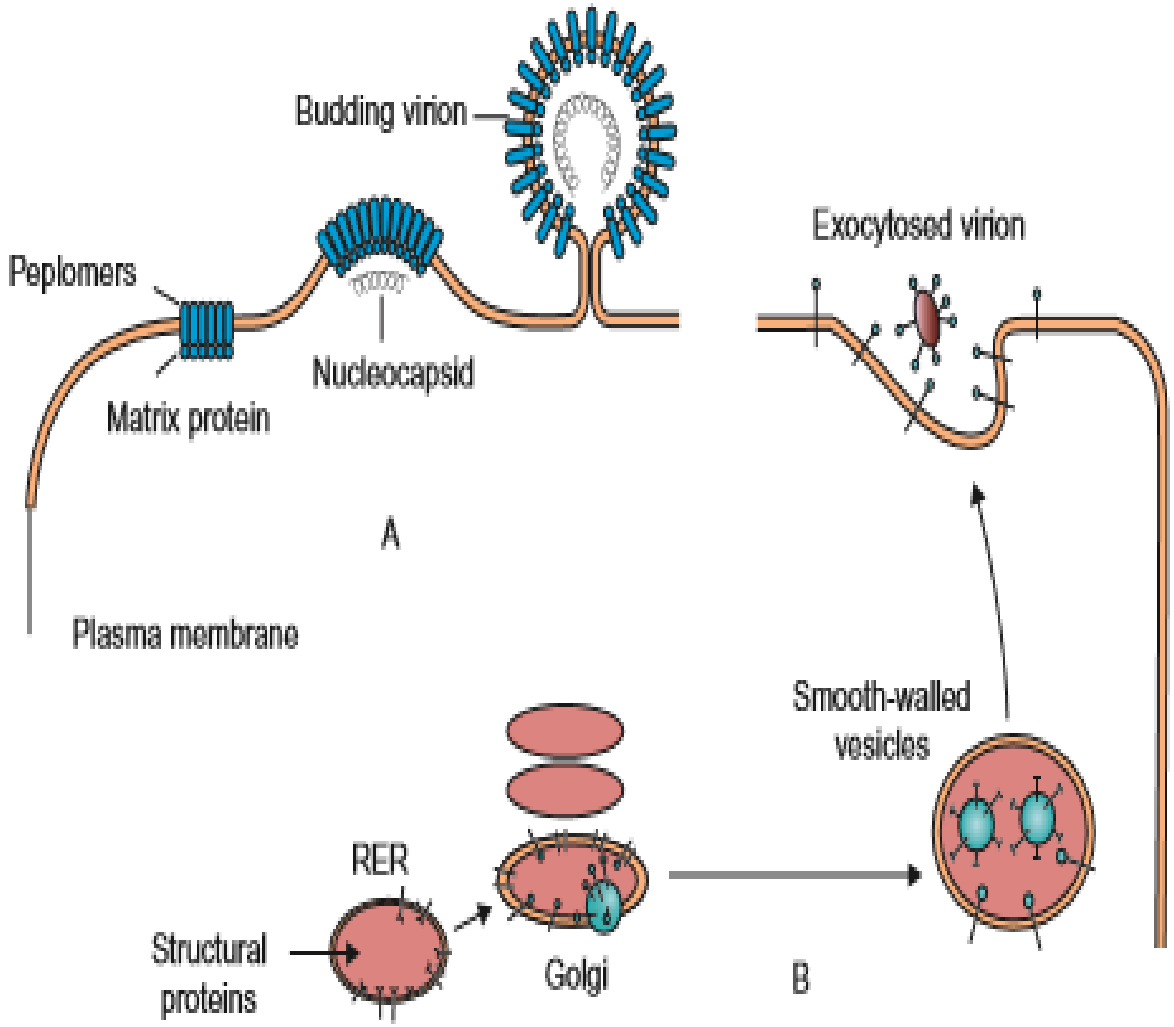


FIGURE 2.12 Maturation of enveloped viruses. (A) Viruses with a matrix protein (and some viruses without a matrix protein) bud through a patch of the plasma membrane in which glycoprotein spikes (peplomers) have accumulated over matrix protein molecules. (B) Most enveloped viruses that do not have a matrix protein bud into cytoplasmic vesicles [rough endoplasmic reticulum (RER) or Golgi], then pass through the cytoplasm in smooth vesicles and are released by exocytosis.