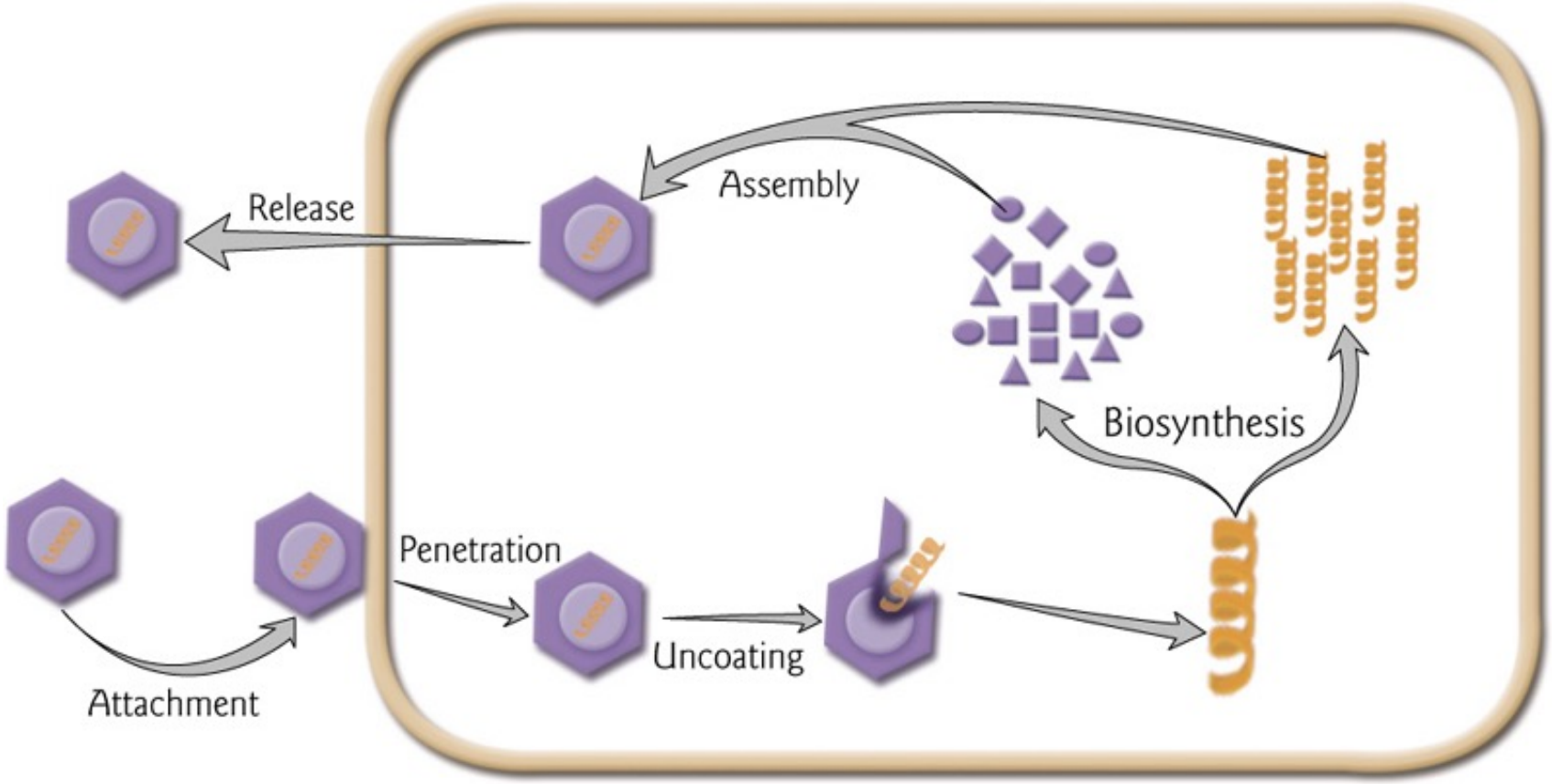
Viral genome drives the formation of new viral particles, which are the result of viral component assembly



From Dimmock NJ, Introduction to Modern Virology, Blackwell, 2008

What information is encoded in a viral genome?

Gene products and regulatory signals required for

- replication of the genome
- efficient expression of the genome
- assembly and packaging of the genome
- regulation and timing of the reproduction cycle
- modulation of host defences
- spread to other cells and hosts

Information not contained in viral genomes

• genes encoding a complete protein synthesis machinery (e.g., no ribosomal RNA and no ribosomal or translation proteins);
note: the genomes of some large DNA viruses contain genes for transfer RNAs (tRNAs), aminoacyl-tRNA synthetases, and enzymes that participate in sugar and lipid metabolism

• genes encoding proteins of energy metabolism or membrane biosynthesis

• telomeres (to maintain genomes) or centromeres (to ensure segregation of genomes)

The Baltimore scheme (replication classes)

*Baltimore classification system is based on the central role of translational machinery and places mRNA in the centre. It describes the pathways to form mRNA from DNA or RNA genomes. Viruses can replicate DNA and/or RNA, synthesize RNA from DNA or vice versa, but lack a complete system to make proteins, for which they must rely on host cell ribosomes. Host cells on the other hand can synthesize proteins only from +mRNA strands. Irrespective of the genomic nature of viruses, all viruses must synthesize viral + mRNAs to produce viral proteins "**no exception to date**".*

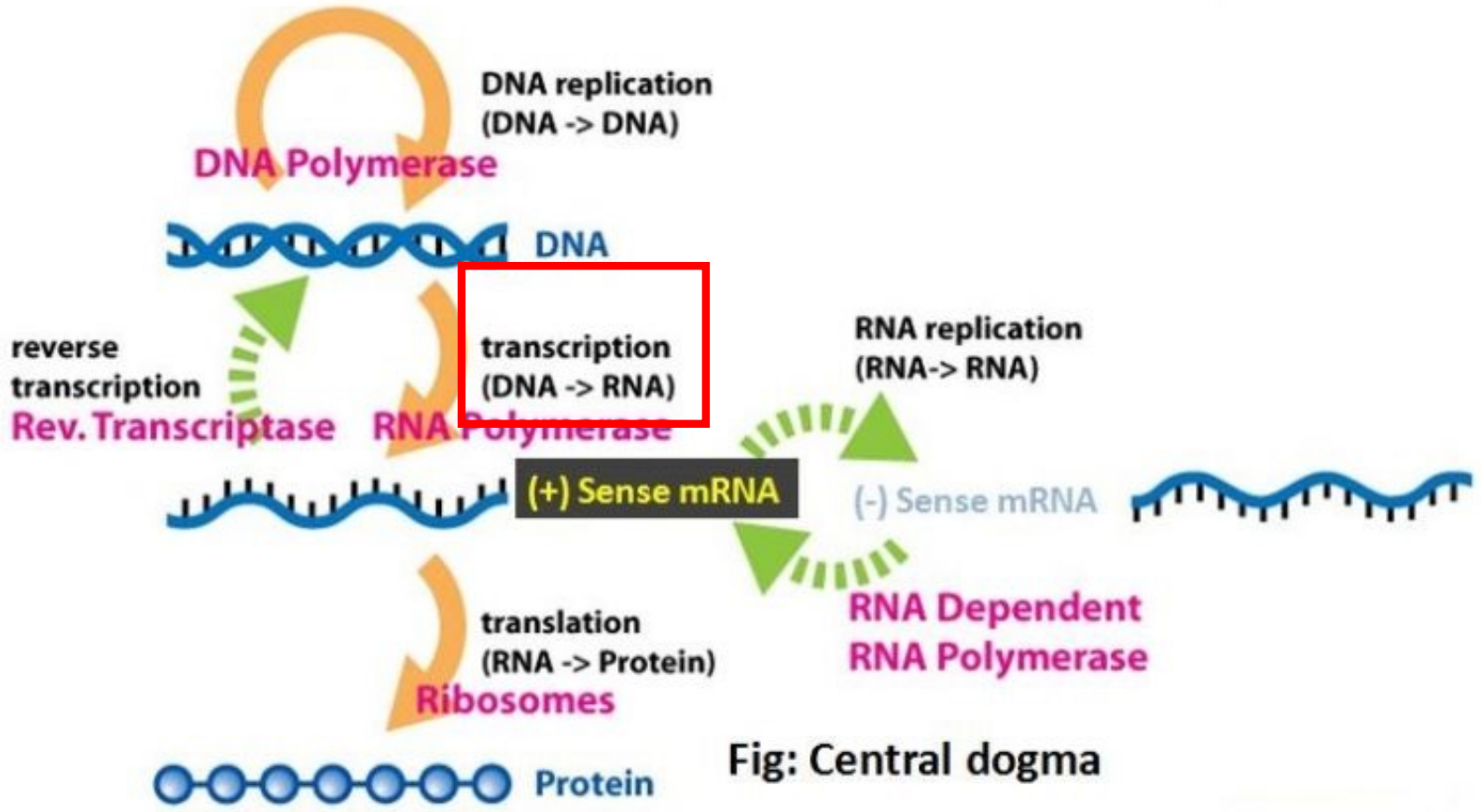
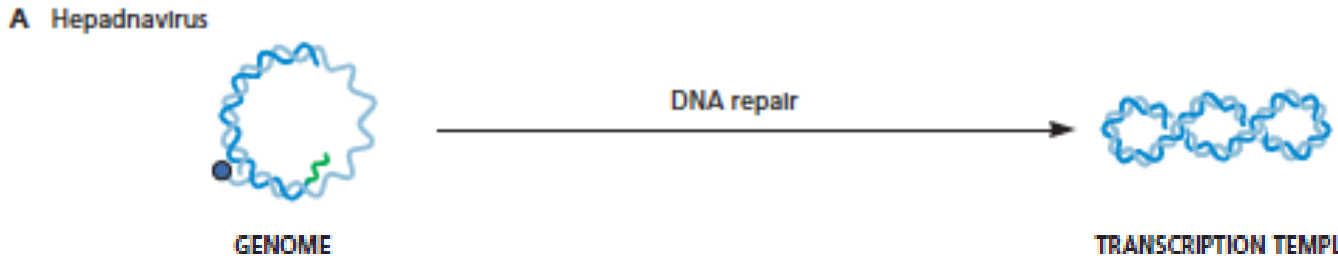


Fig: Central dogma

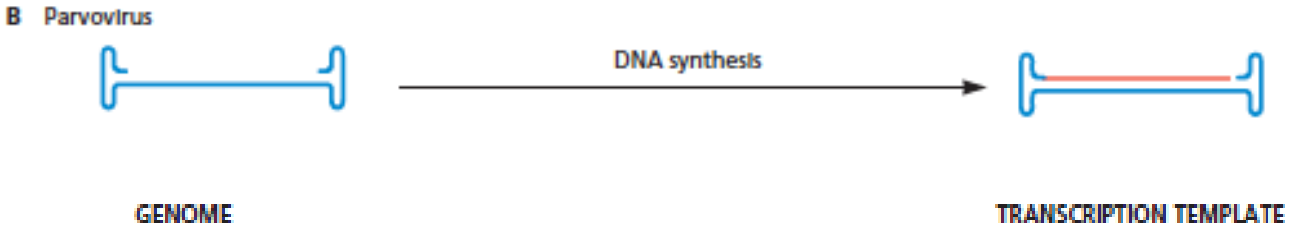
Transcription of viral genome by host RNApol II

Double-stranded DNA molecules can be transcribed as soon as they reach the nucleus.

Other viral DNA genomes must be converted from the form in which they enter the cell to double-stranded molecules that serve as transcriptional templates. The hepadnaviral genome is an incomplete circular DNA molecule with a large gap in one strand that is repaired by cellular enzymes to form a fully double-stranded DNA molecule.



Similarly, single-stranded genomes such as that of the adenovirus-associated virus, a parvovirus, are converted to double-stranded molecules by a cellular DNA polymerase



From Flint et al. *Principles of Virology* (2015), ASM Press

The Baltimore scheme (replication classes)

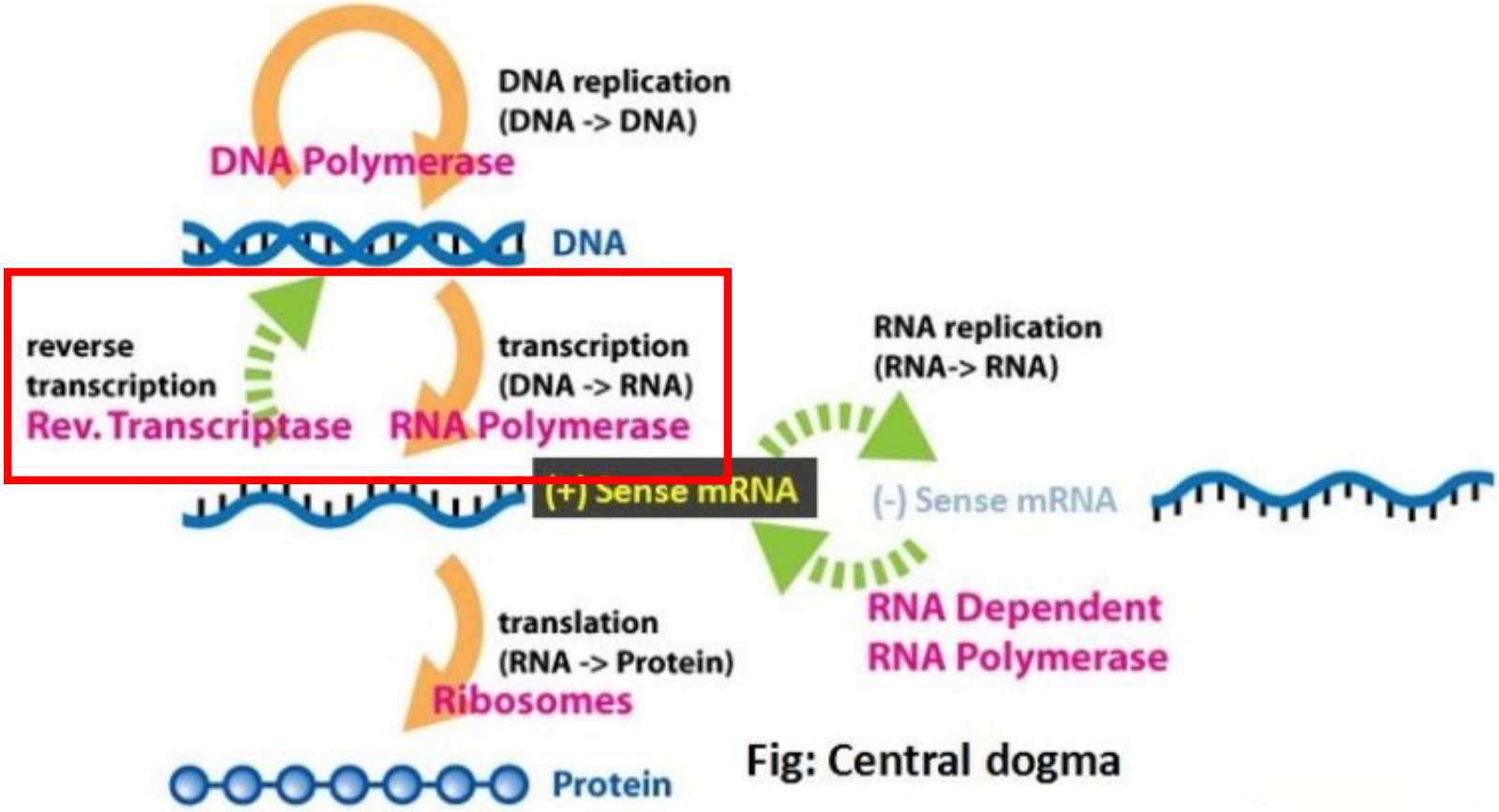
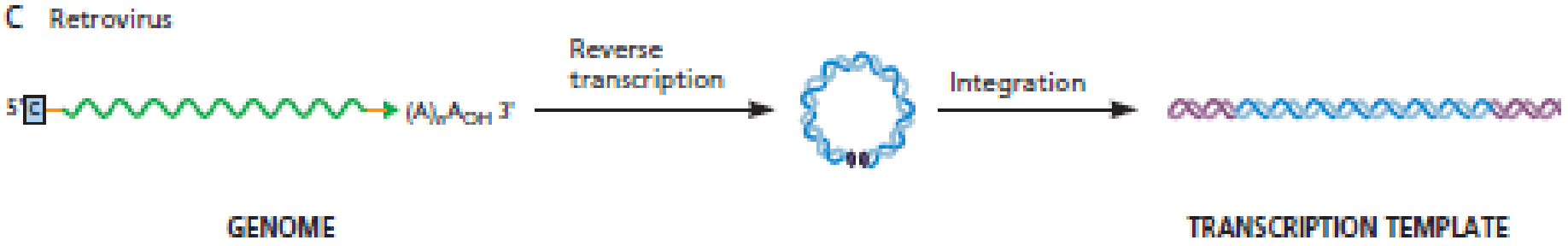


Fig: Central dogma

Transcription of viral genome by host RNApol II

The prerequisites for expression of retroviral genetic information are even more demanding, the (+) strand RNA genome must be both converted into viral DNA and integrated into the cellular genome. Reverse transcription creates an appropriate double-stranded DNA template that includes the signals needed for its recognition by components of the cellular transcriptional machinery



From Flint et al. *Principles of Virology* (2015), ASM Press

The Baltimore scheme (replication classes)

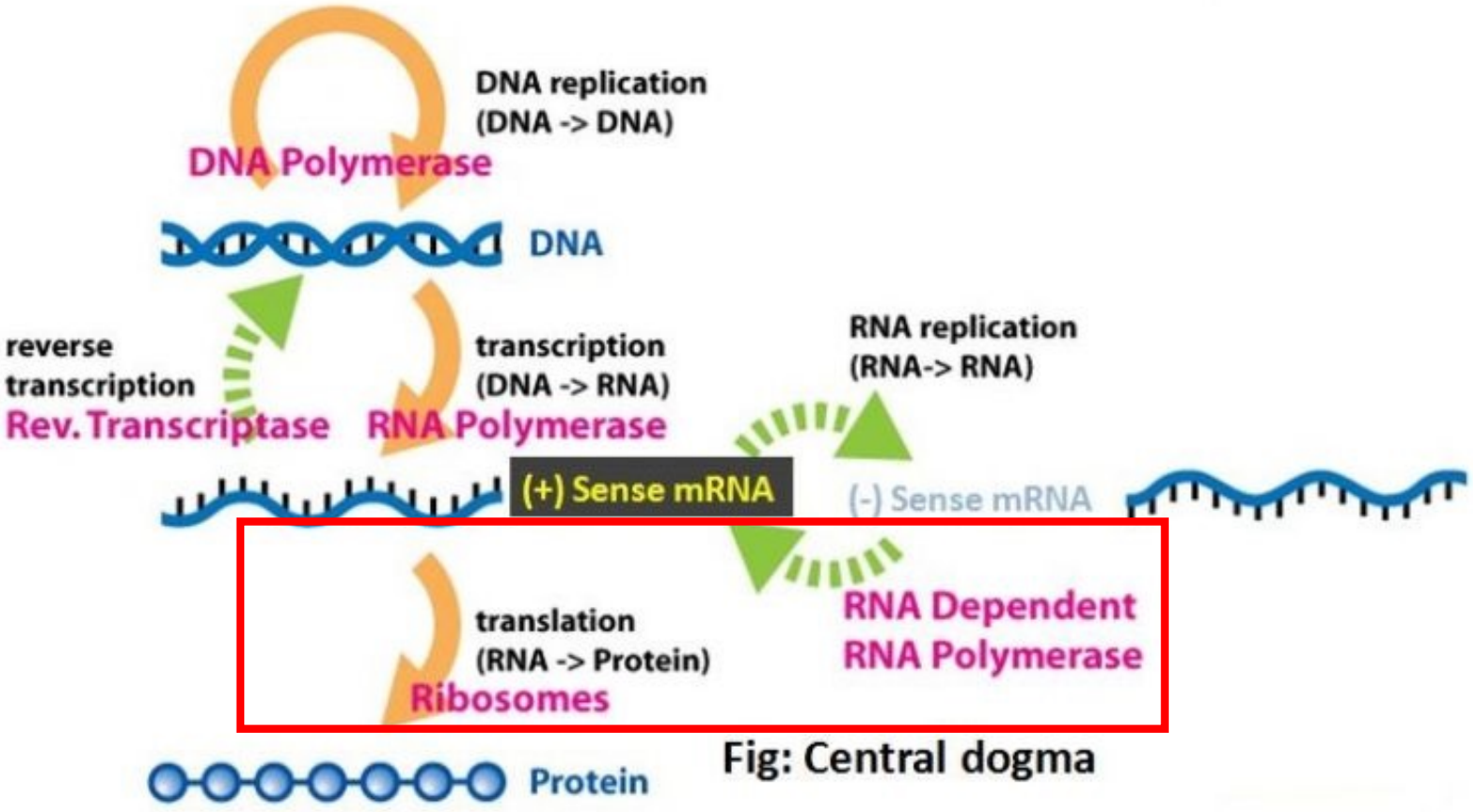
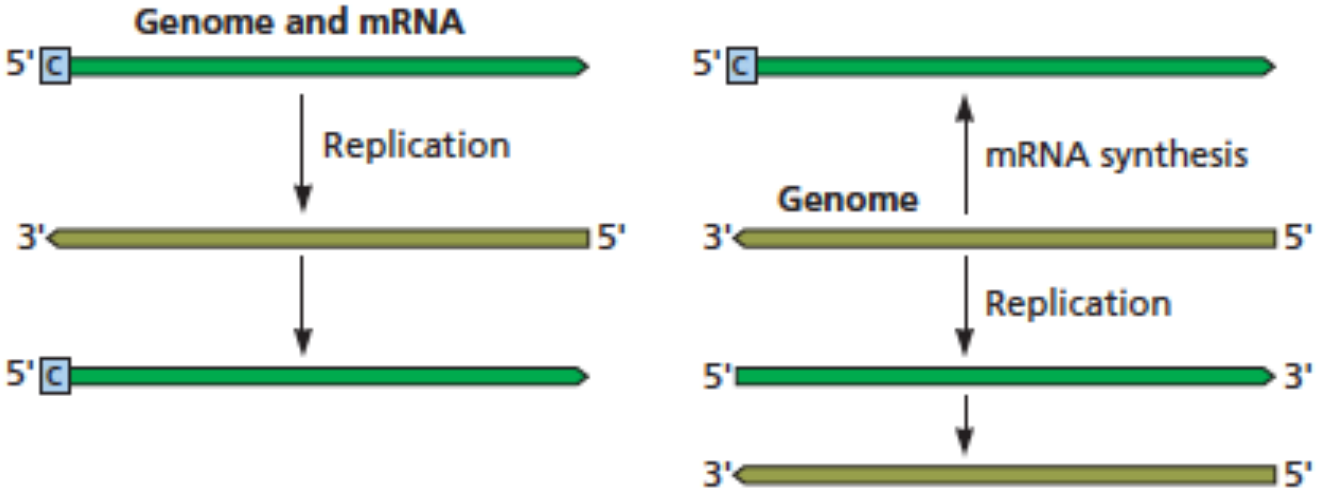


Fig: Central dogma

Synthesis of RNA from RNA Templates (III, IV and V classes)

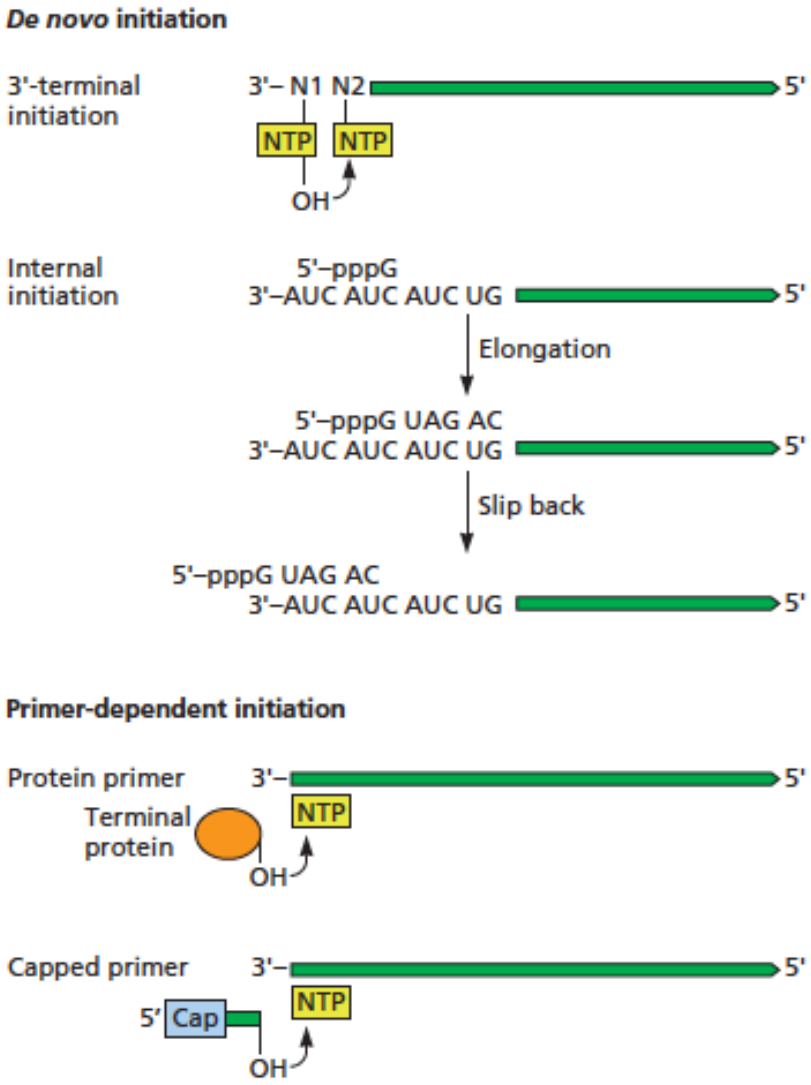
Viral RNA genomes must be copied to provide both mRNAs for the synthesis of viral proteins, and genomes for assembly into progeny virus particles. The synthesis of these RNA molecules is a unique process that has no parallel in the cell. The genomes of all RNA viruses except retroviruses encode an RNA-dependent RNA polymerase to catalyze the synthesis of mRNAs and new genomes.



RNA synthesis by RNA-dependent RNA polymerase is error prone, and this process, together with reassortment and recombination, yields diversity that is required for viral evolution.

From Flint et al. *Principles of Virology* (2015), ASM Press

Synthesis of RNA from RNA Templates (III, IV and V classes)



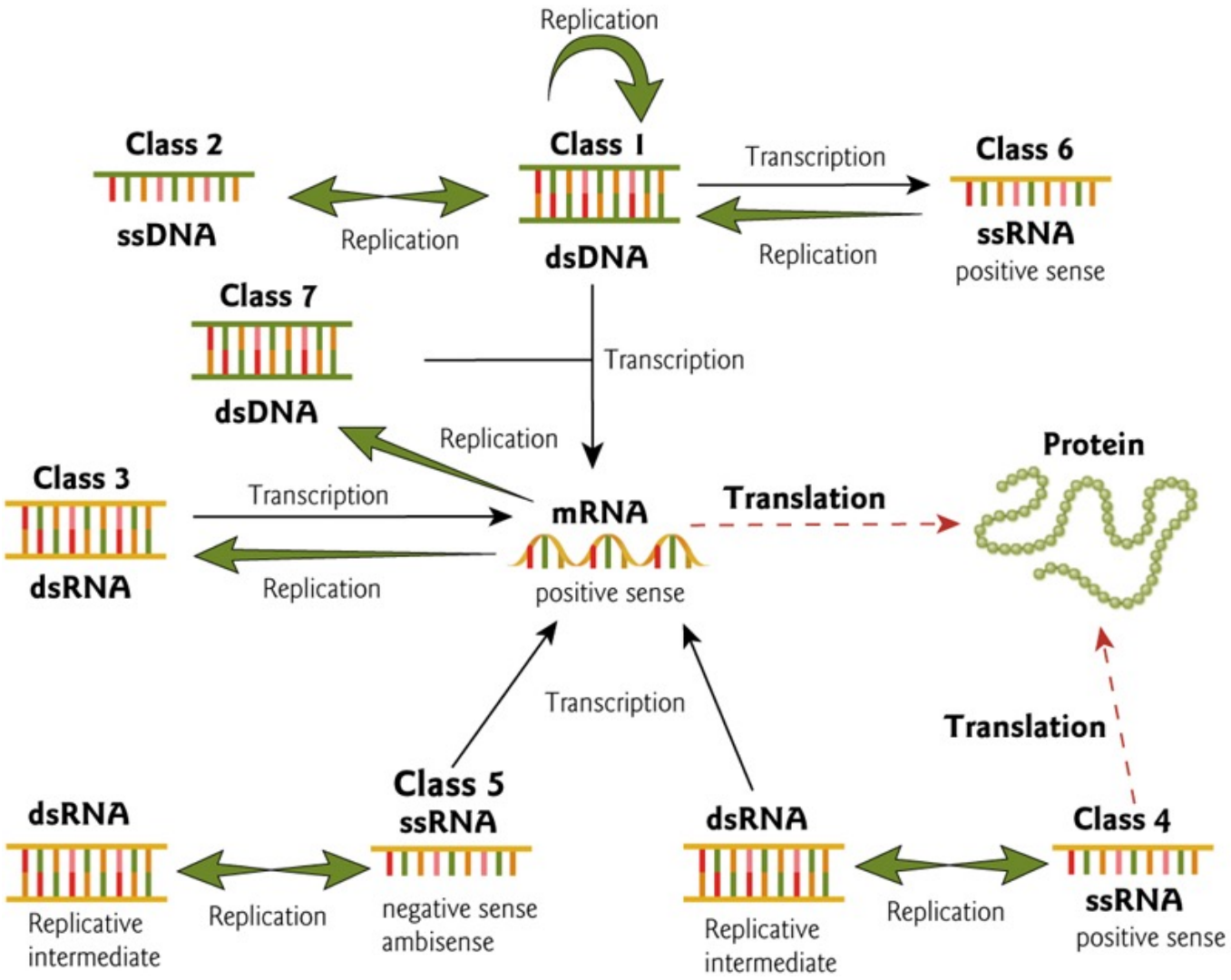
Mechanisms of initiation of RNA synthesis.

De novo initiation may occur at the 3' end of the viral RNA or from an internal base.

When a primer is required, it may be a capped or protein-linked oligonucleotide.

From Flint et al. *Principles of Virology* (2015), ASM Press

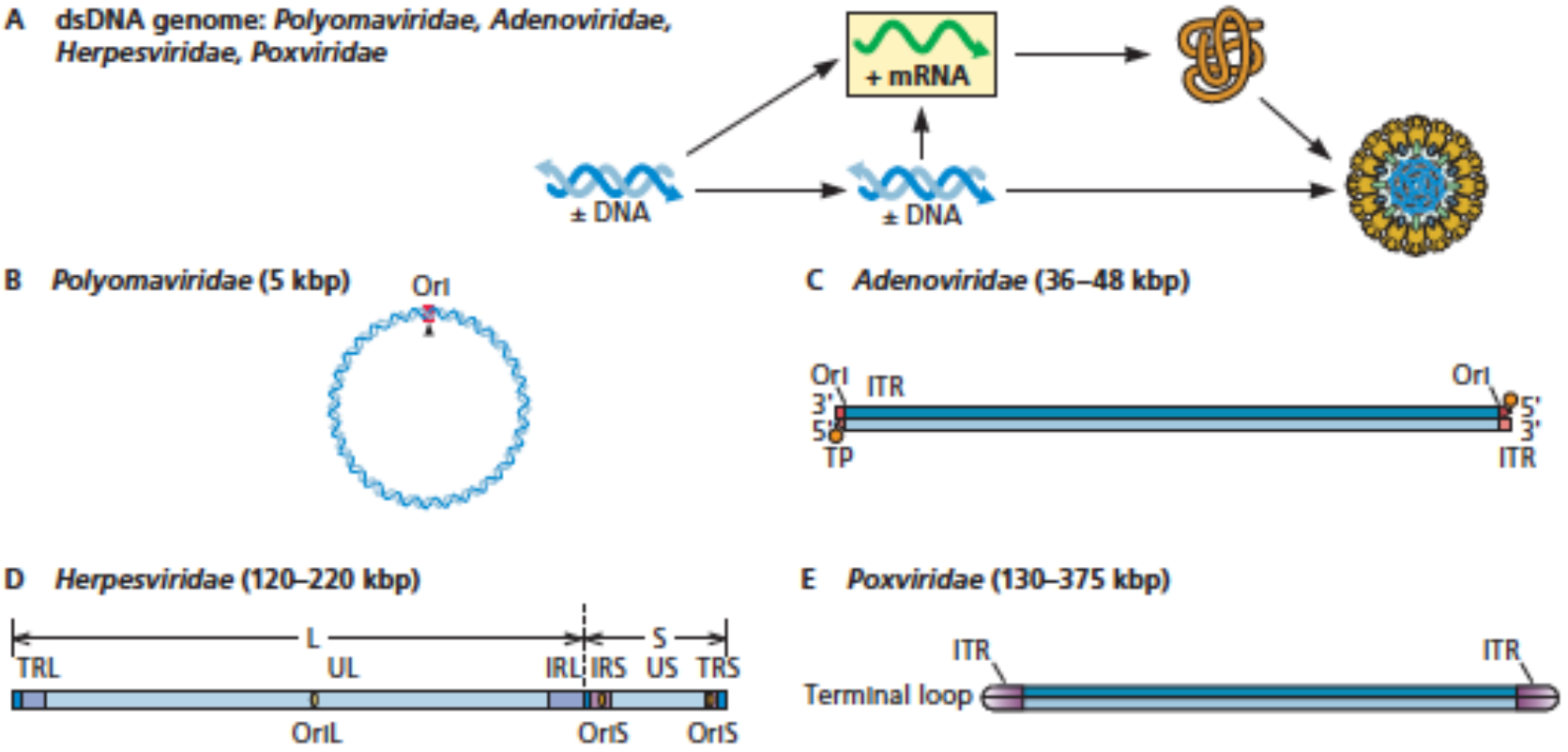
The Baltimore scheme (replication classes)



Class I: dsDNA, Expression-Replication

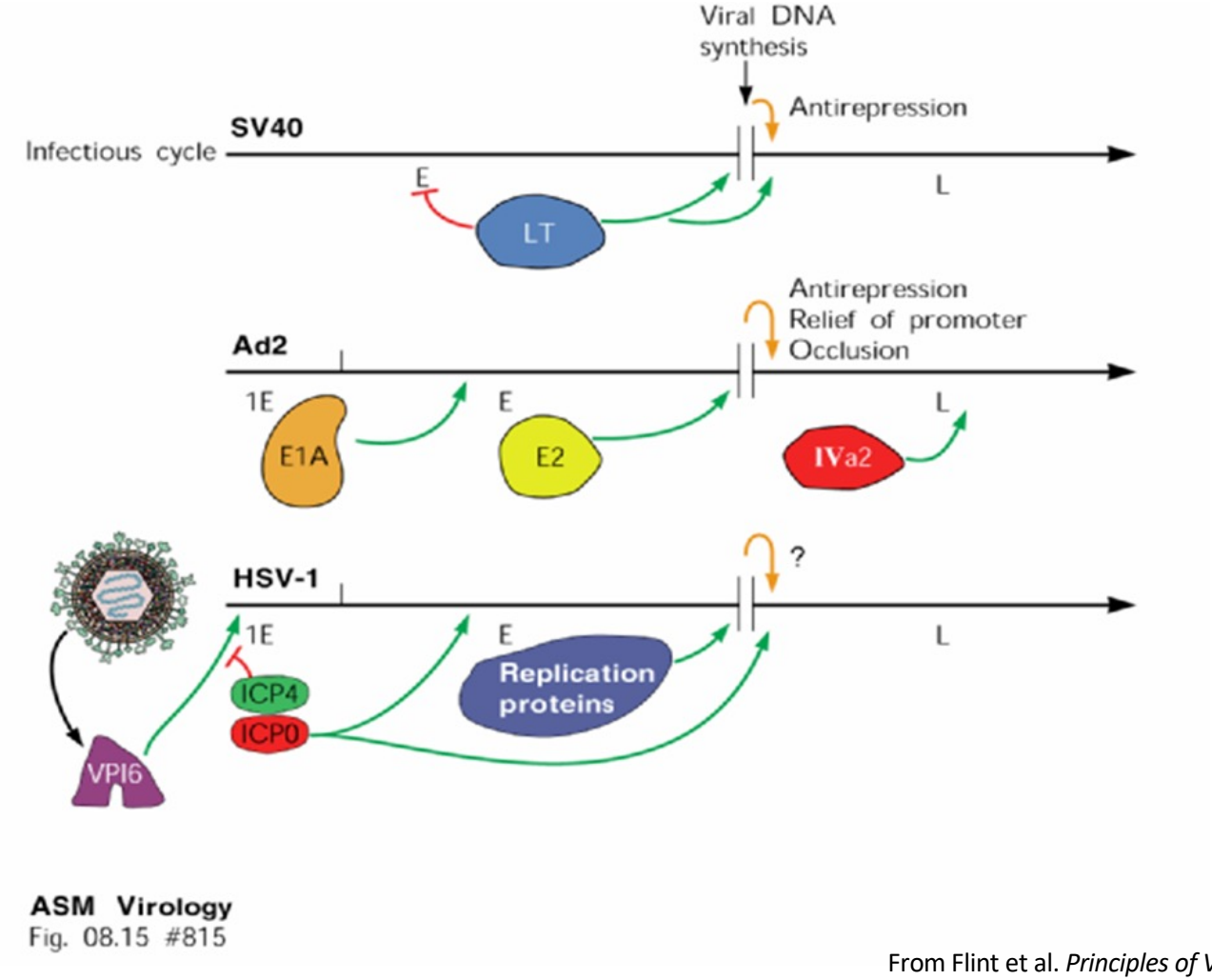
This class can be divided into two further groups

- Replication is exclusively nuclear. The replication of these viruses is relatively dependent on cellular factors. (Most of class I viruses)
- Replication occurs in cytoplasm (*Poxviridae*). These viruses have evolved all the necessary factors for transcription and replication of their genome and are therefore largely independent of the cellular machinery



From Flint et al. *Principles of Virology* (2015), ASM Press

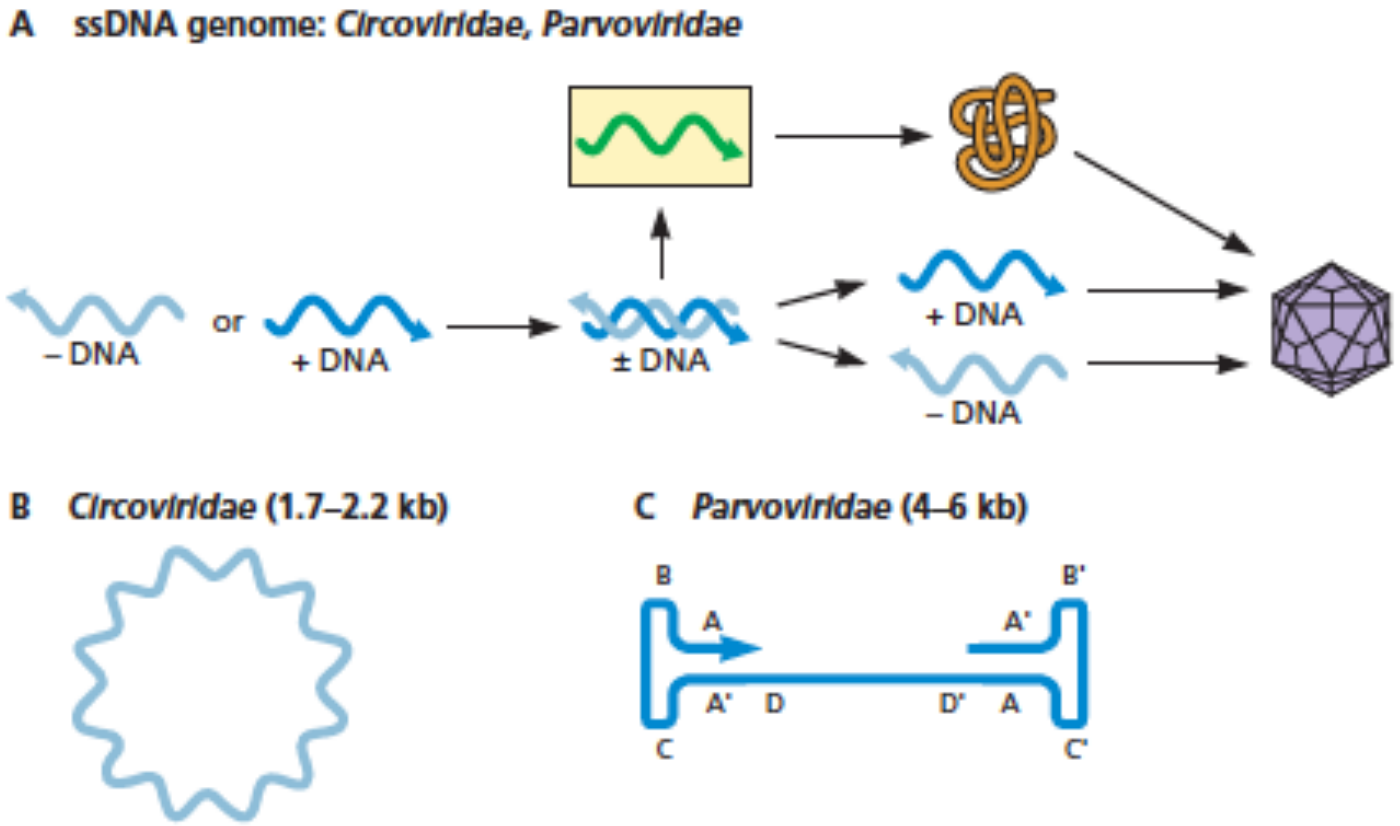
Class I viruses impose a temporal phasing on their genome expression



From Flint et al. *Principles of Virology* (2015), ASM Press

Class II: ssDNA, Expression-Replication

Replication occurs in the nucleus and involves the formation of a double-stranded intermediate which serves as a template for the mRNA transcription and for the synthesis of new viral genomes



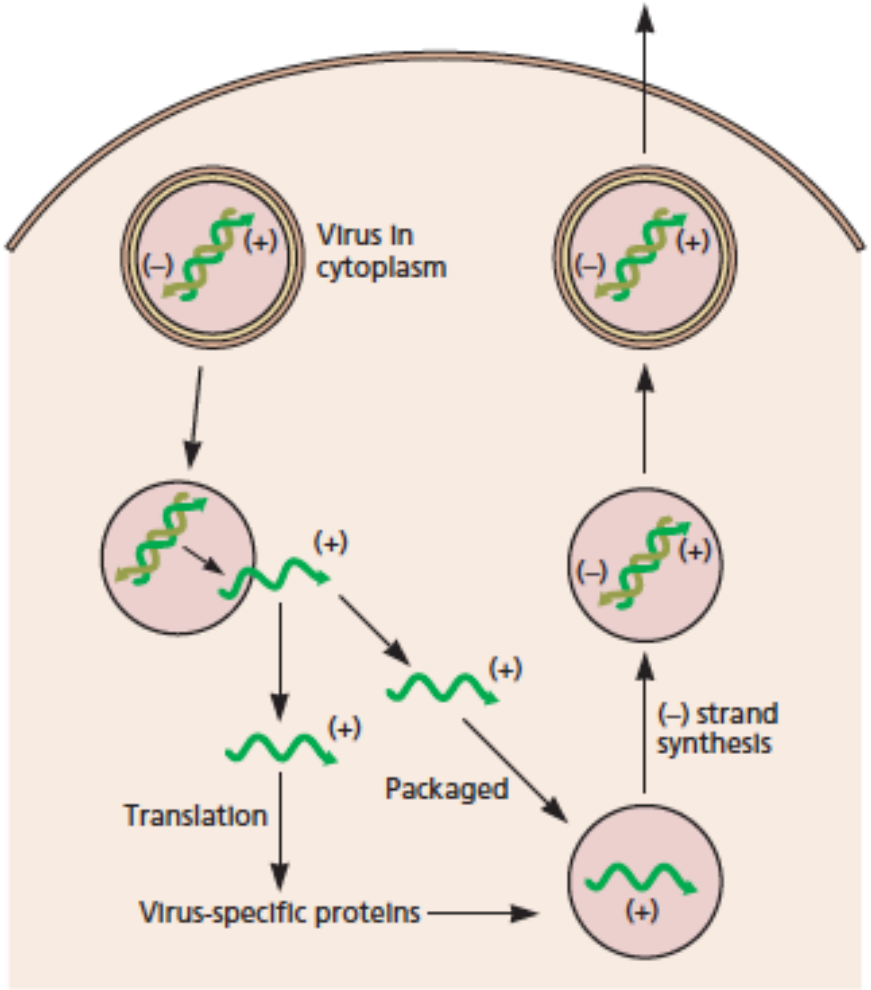
From Flint et al. *Principles of Virology* (2015), ASM Press

Class III: dsRNA, Expression-Replication

Reoviridae (Rotavirus)

These viruses have segmented genome. Each segment is transcribed separately to produce individual monocistronic mRNAs

These processes occur in subviral particles containing the RNA templates and necessary enzymes. During cell entry, the virion passes through the lysosomal compartment, and proteolysis of viral capsid proteins activates the RNA synthetic machinery. Single-stranded (+) viral mRNAs, which are synthesized in parental subviral particles, are extruded into the cytoplasm, where they serve either as mRNAs or as templates for the synthesis of (-) RNA strands. In the latter case, viral mRNAs are first packaged into newly assembled subviral particles in which the synthesis of (-) RNAs to produce double-stranded RNAs occurs. These subviral particles become infectious particles. Only 1 of the 10 to 12 double-stranded RNA segments of the reoviral genome is shown.



From Flint et al. *Principles of Virology* (2015), ASM Press

RNA genome replication of Class IV and V viruses

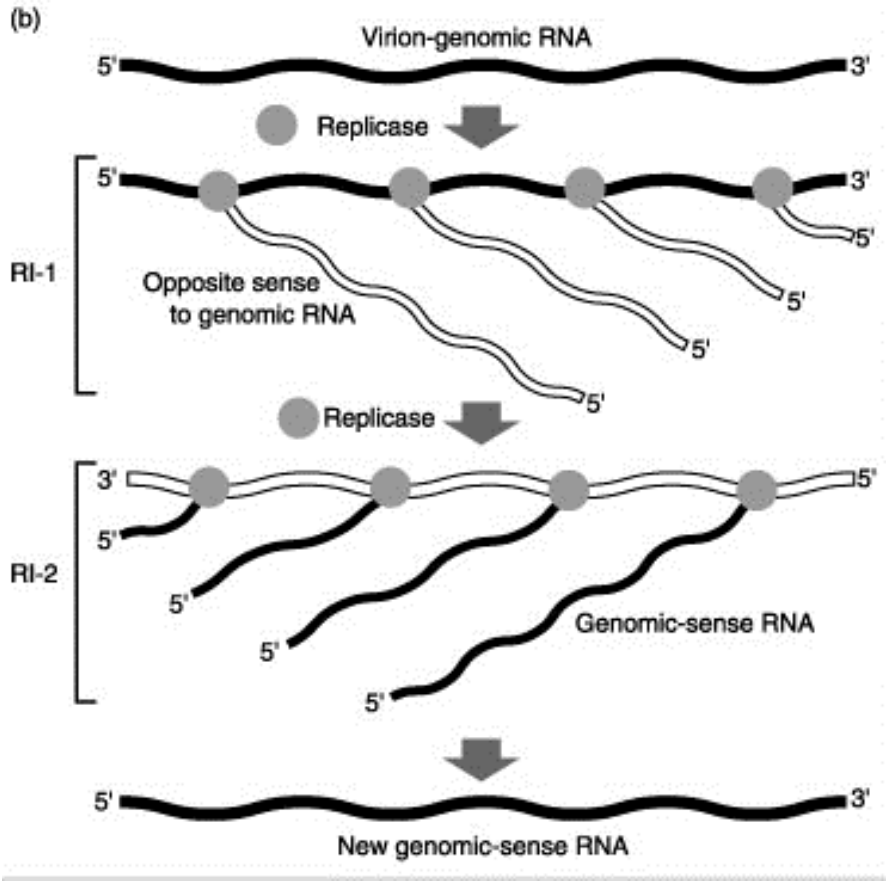
vRNA is the templare in RI-1

In RI-1, the molecule of opposite polarity to vRNA (replication intermediate) is produced

The RNA complementary to the vRNA (or antigenome) is the template in RI-2

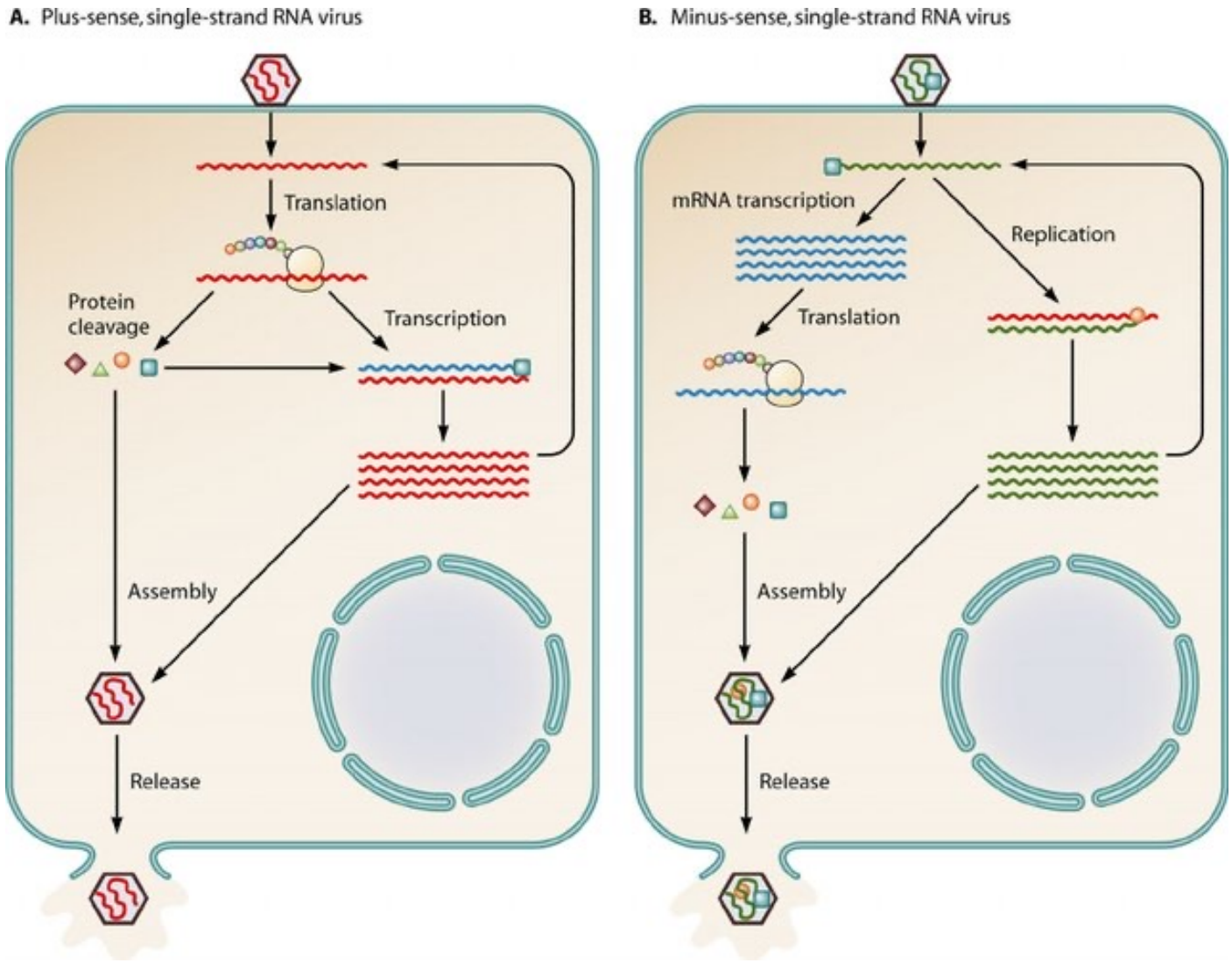
In RI-2, RNA of the same polarity as that present in the virion (vRNA) is synthesized

RI= Replication intermediate



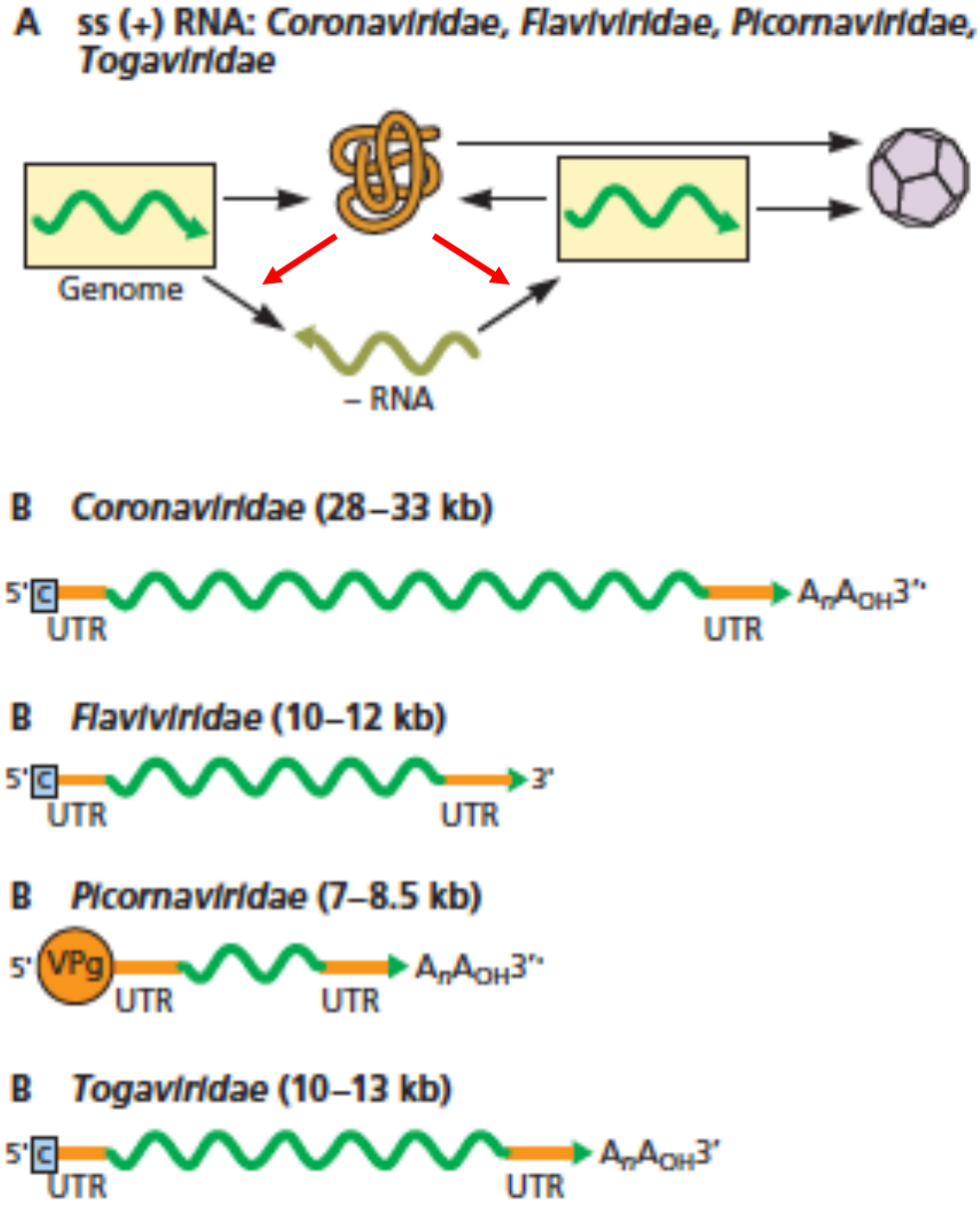
From Wagner and Hewlett *Basic virology* (2003) Blackwell Science Press

Replication strategies of plus- and minus-strand RNA viruses.



Class IV: ssRNA(+), Expression-Replication

First step in multiplication is translation



From Flint et al. *Principles of Virology* (2015), ASM Press

Class IV: ssRNA(+), Expression-Replication

First step in replication is translation

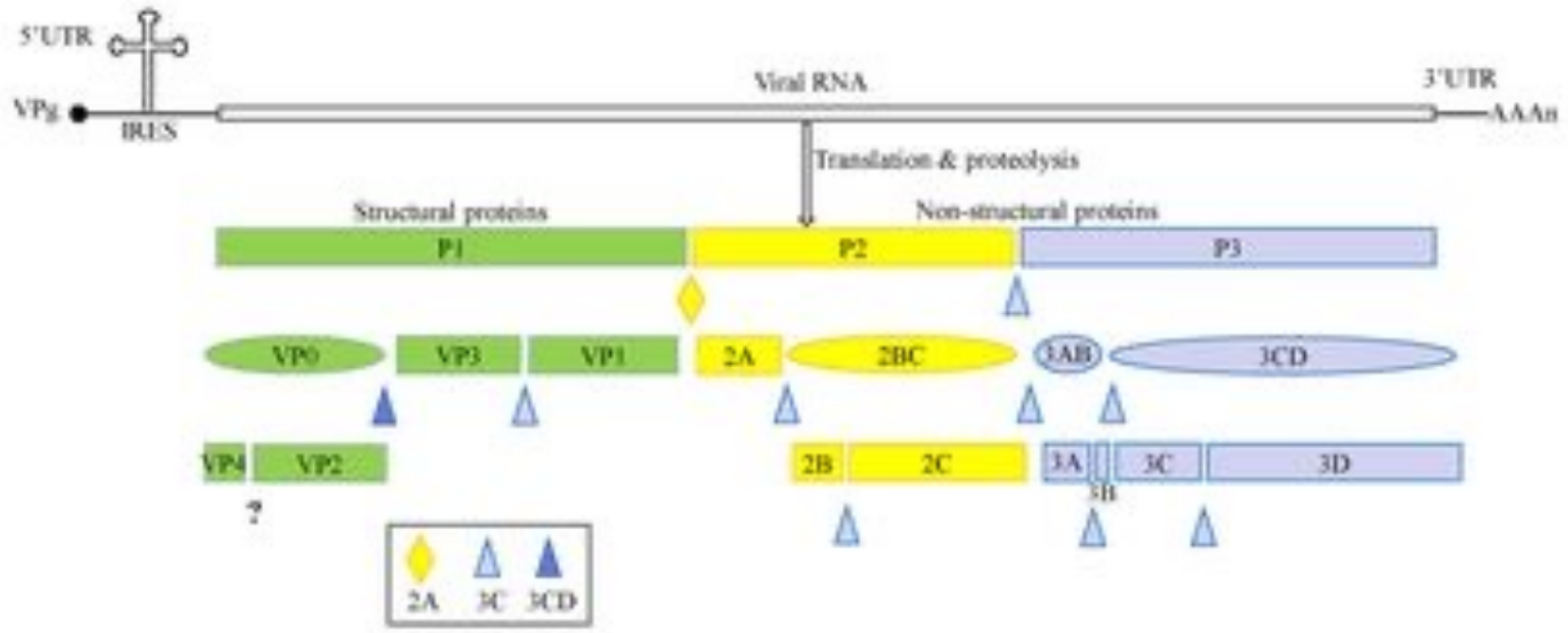
Can be subdivided into two groups

A. vRNA is translated to form a single polyprotein that is subsequently cleaved to give the mature products (polioviruses are an example)

B. vRNA contains more than one ORF. Two rounds of translation (Togaviridae, Coronaviridae) and production of subgenomic mRNA

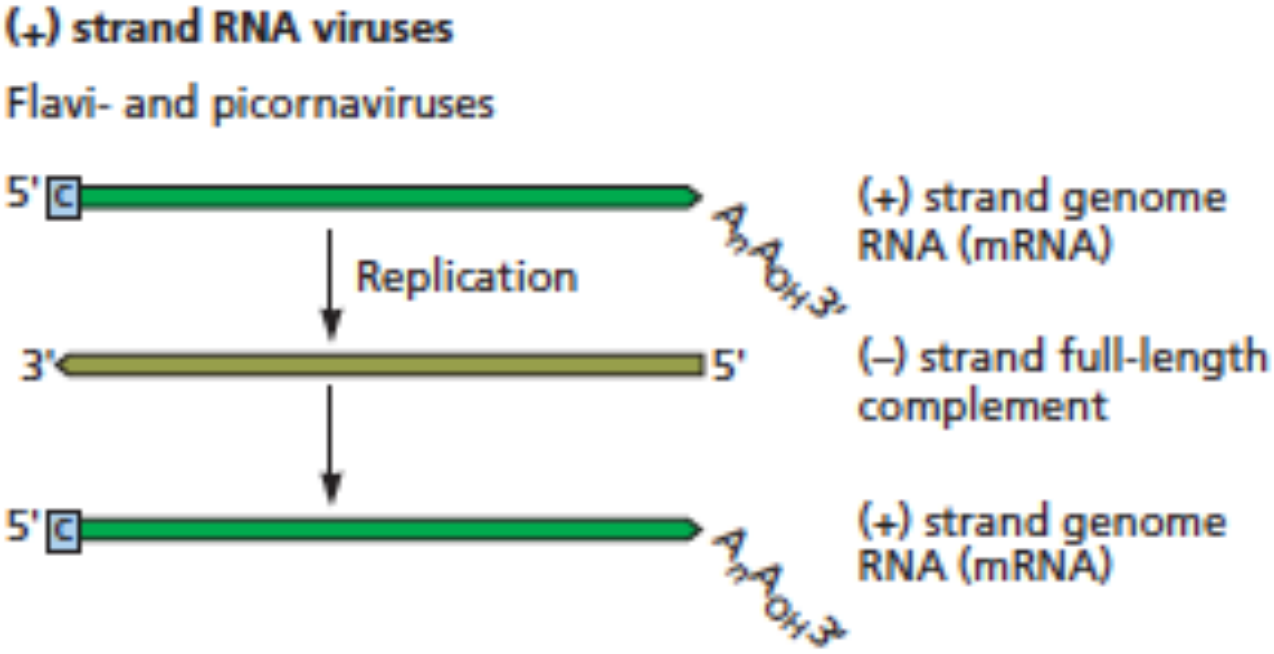
vRNA is translated to form a polyprotein that is subsequently cleaved to give the mature products (polioviruses are an example)

Poliovirus Expression (class IV)



Monopartite, linear ssRNA(+) genome of 7.1-8.9 kb, polyadenylated, composed of a single ORF encoding a polyprotein. Viral genomic RNA has a viral protein (VPg) at its 5' end instead of a methylated nucleotide cap structure. The long UTR at the 5' end contains an internal ribosome entry site (IRES). The P1 region encodes the structural polypeptides. The P2 and P3 regions encode the nonstructural proteins associated with replication. The shorter 3' UTR is important in (-)strand synthesis.

Class IV
(events after primary translation, Poliovirus, Flavivirus)



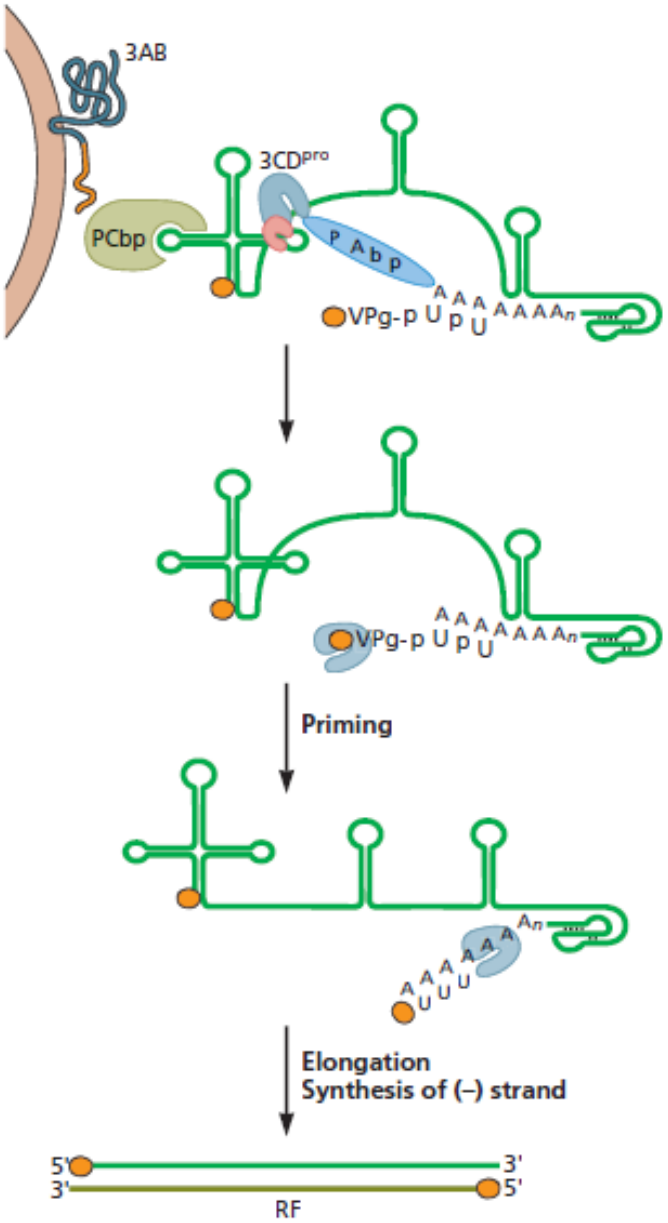
Replicazione del genoma a RNA dei picornavirus, priming

Uridilazione della VPg e sintesi RNA (-).

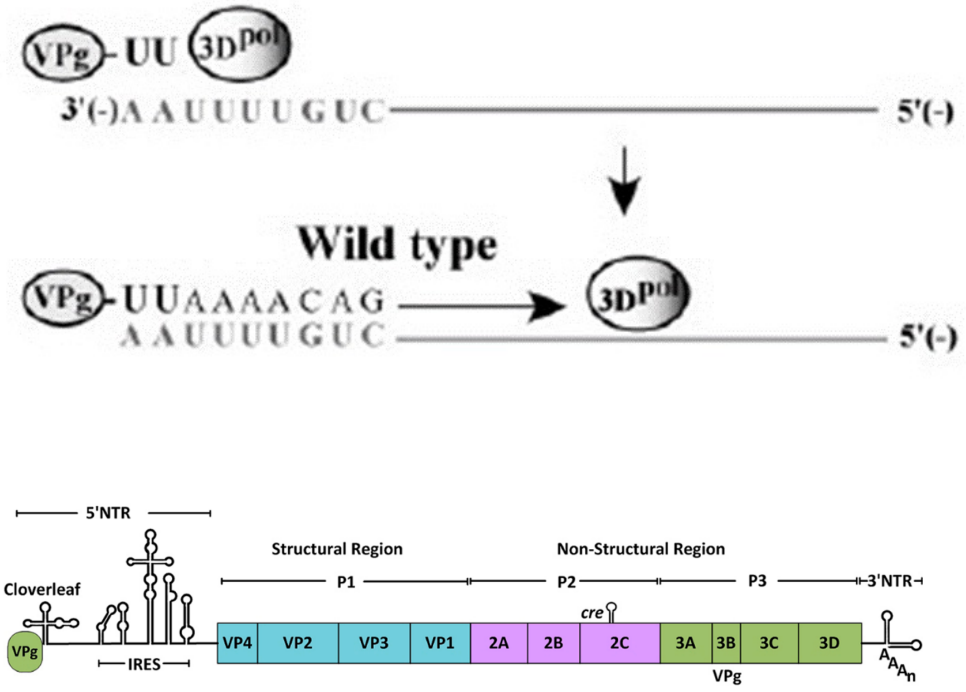
1 Sintesi RNA(-)

2 Sintesi RNA(+)

1



2

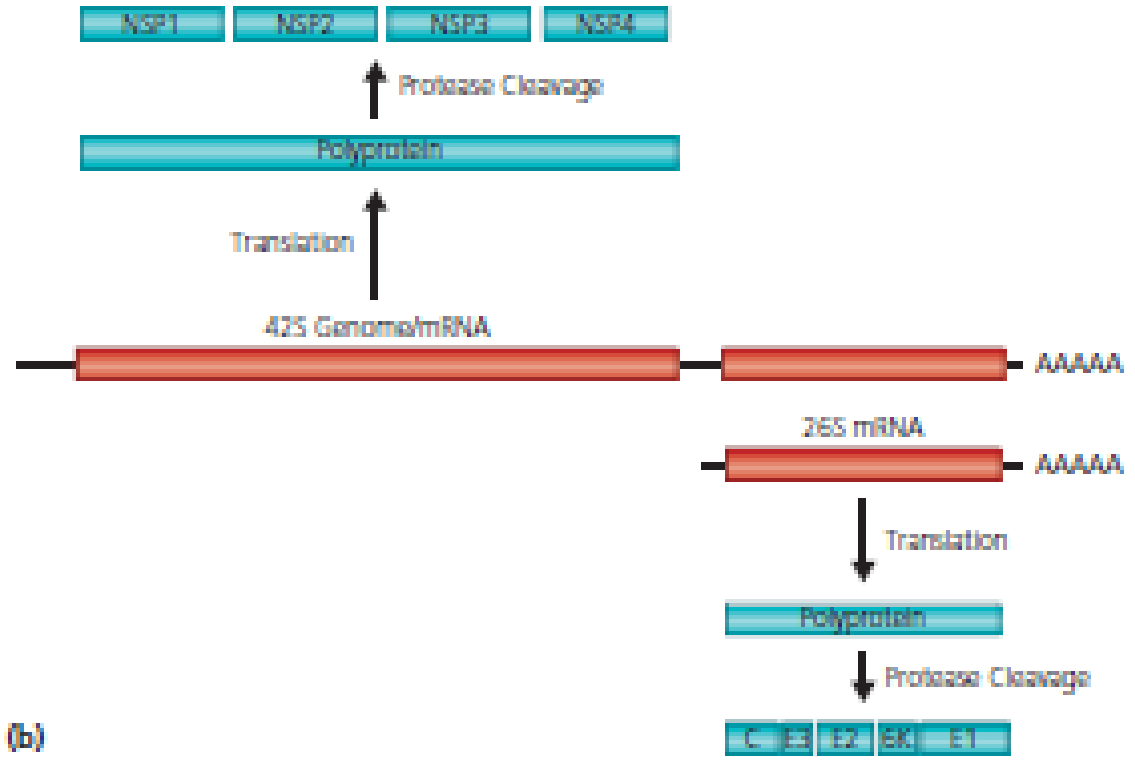


RNA genome

From Flint et al. *Principles of Virology* (2015), ASM Press

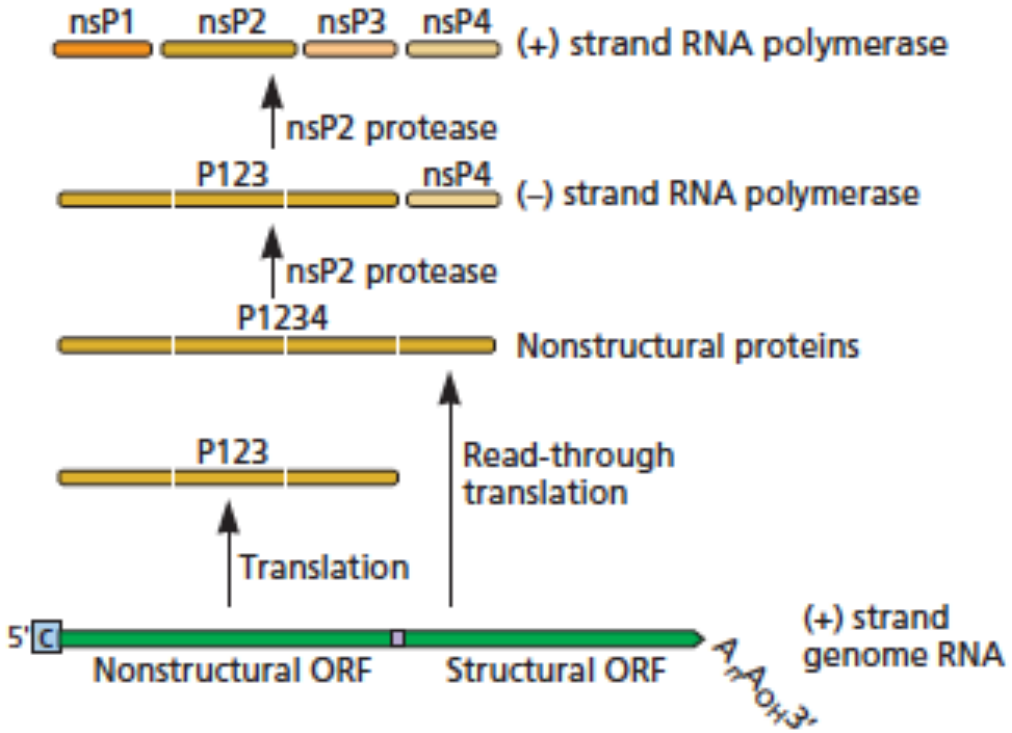
Class IV: ssRNA(+), Two rounds of translation (Togaviridae, Coronaviridae) and production of subgenomic mRNAs

Togaviridae



Nigel J. Dimmock, Andrew J. Easton, Keith N. Leppard
Introduzione alla Virologia moderna *Casa Editrice Ambrosiana. 2017*

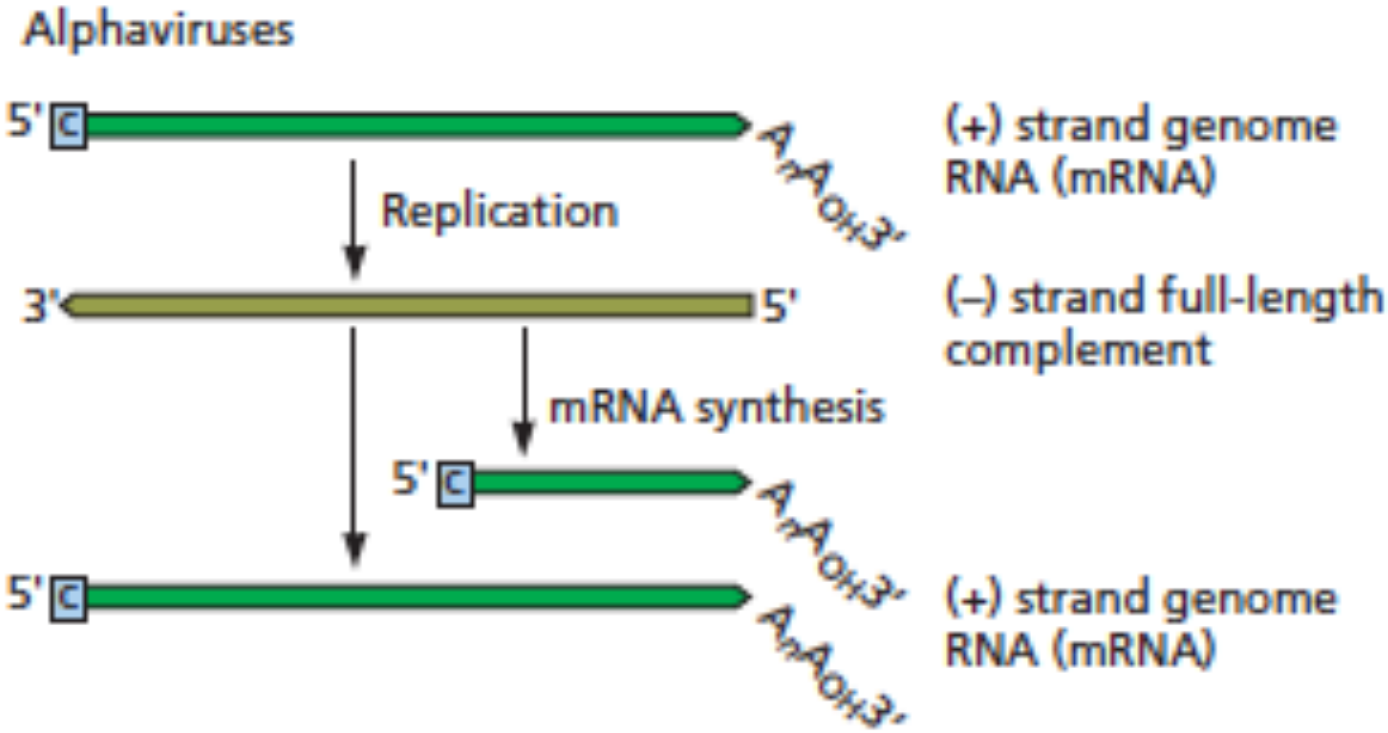
RNA (+) Togaviridae



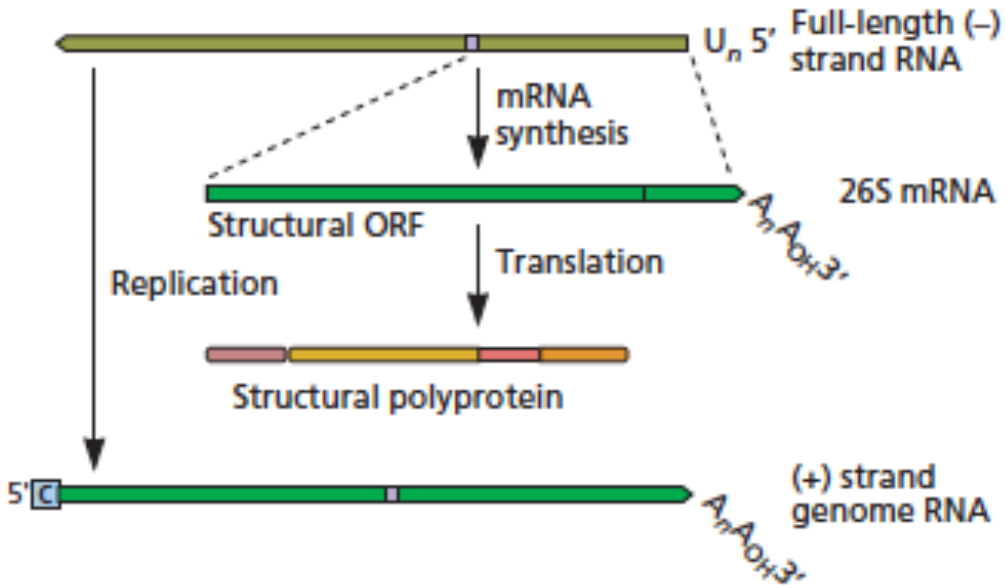
The 11,703-nucleotide Sindbis virus genome contains a 5'-terminal cap structure and a 3-poly(A) tail. A conserved RNA secondary structure at the 3 end of (+) strand genomic RNA is thought to control the initiation of (-) strand RNA synthesis. At early times after infection, the 5' region of the genomic RNA (nonstructural open reading frame [ORF]) is translated to produce two nonstructural polyproteins: P123, whose synthesis is terminated at the first translational stop codon (indicated by the box), and P1234, produced by an occasional (15%) readthrough of this stop codon.

From Flint et al. *Principles of Virology* (2015), ASM Press

Class IV
(events after primary translation, Alphaviruses)



RNA (+) Togaviridae

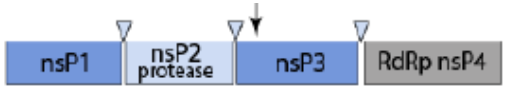


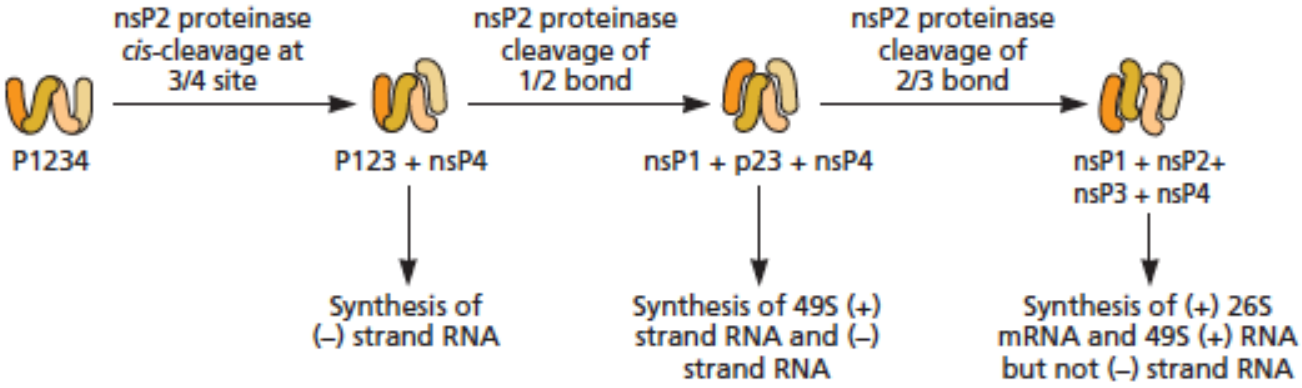
The P1234 polyprotein is proteolytically cleaved to produce the enzymes that catalyze the various steps in genomic RNA replication: the synthesis of a full-length (-) strand RNA, which serves as the template for (+) strand synthesis, and either full length genomic RNA or subgenomic 26S mRNA. The 26S mRNA, shown in expanded form, is translated into a structural polyprotein (p130) that undergoes proteolytic cleavage to produce the virion structural proteins. The 26S RNA is not copied into a (-) strand because a functional initiation site fails to form at the 3' end.

From Flint et al. *Principles of Virology* (2015), ASM Press

RNA (+) Togaviridae

Three RNA polymerase complexes with distinct specificities in alphavirus-infected cells.

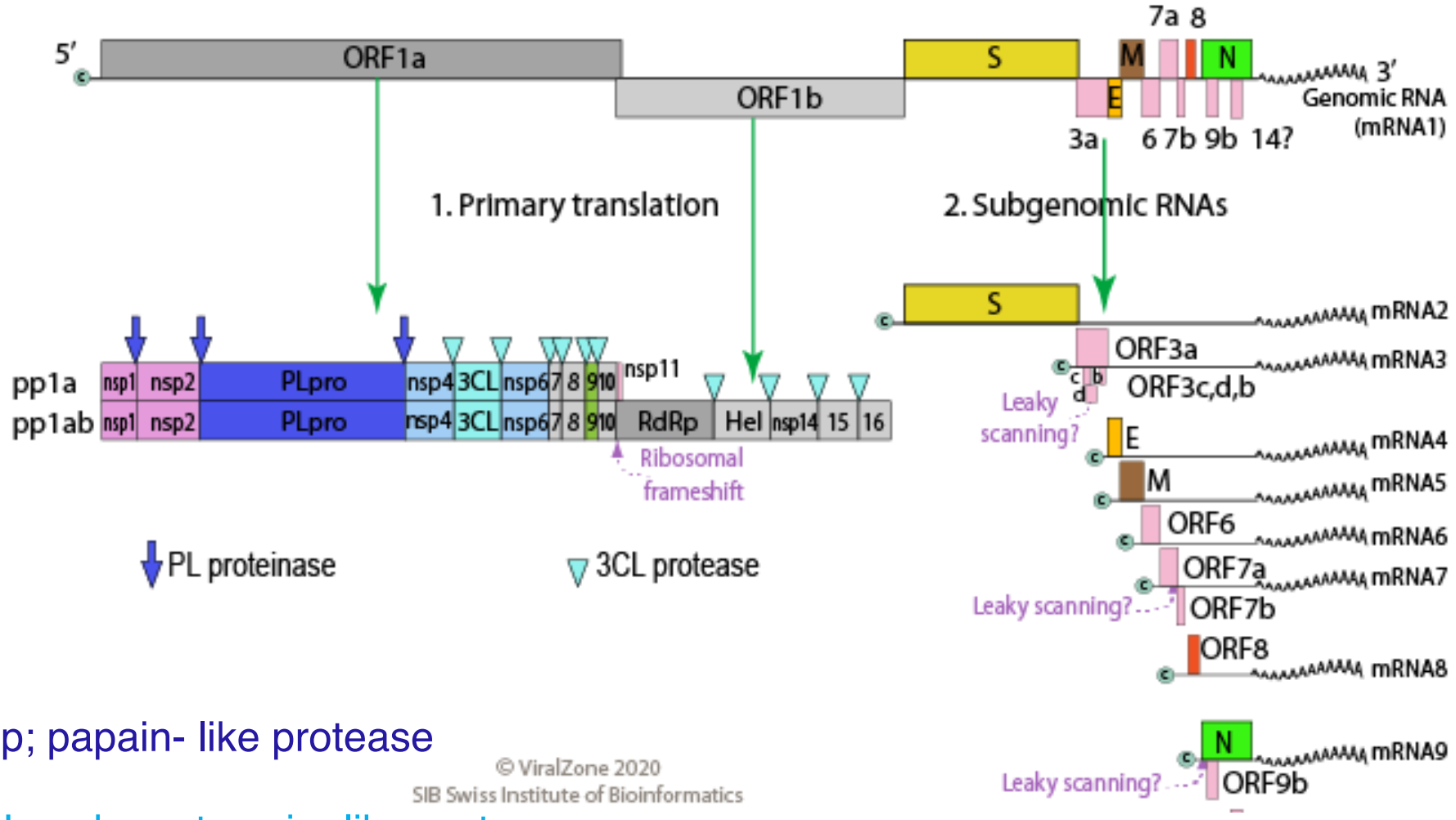




Alphaviral genome and mRNA synthesis is regulated by the sequential production of three RNA polymerase complexes with different template specificities. All three complexes are derived from the nonstructural polyprotein P1234 and contain the complete amino acid sequence of this precursor. The covalent connections among the segments of the polyprotein are successively broken, with ensuing alterations in the specificity of the polymerase. It seems likely that each proteolytic cleavage induces a conformational change in the polymerase that alters its template specificity.

From Flint et al. *Principles of Virology* (2015), ASM Press

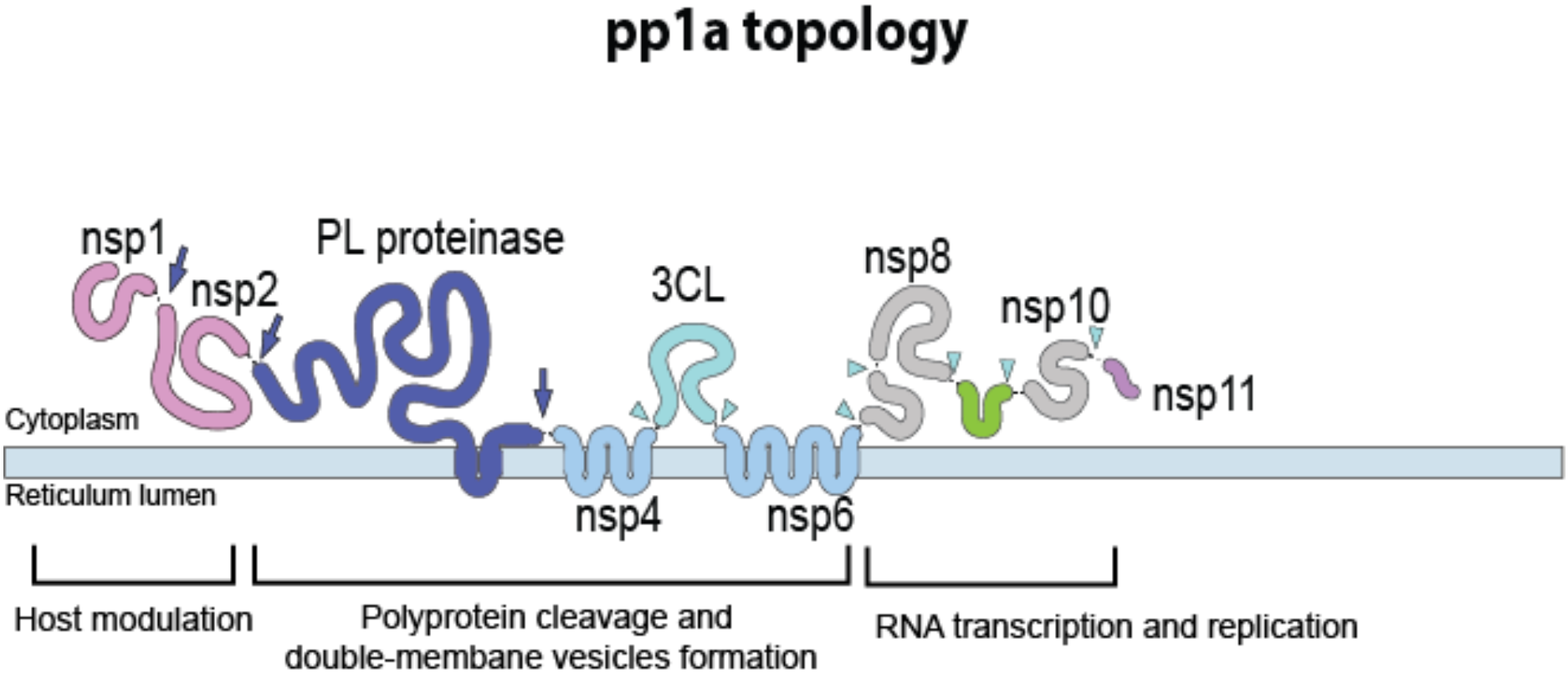
SARS-CoV-2 genome



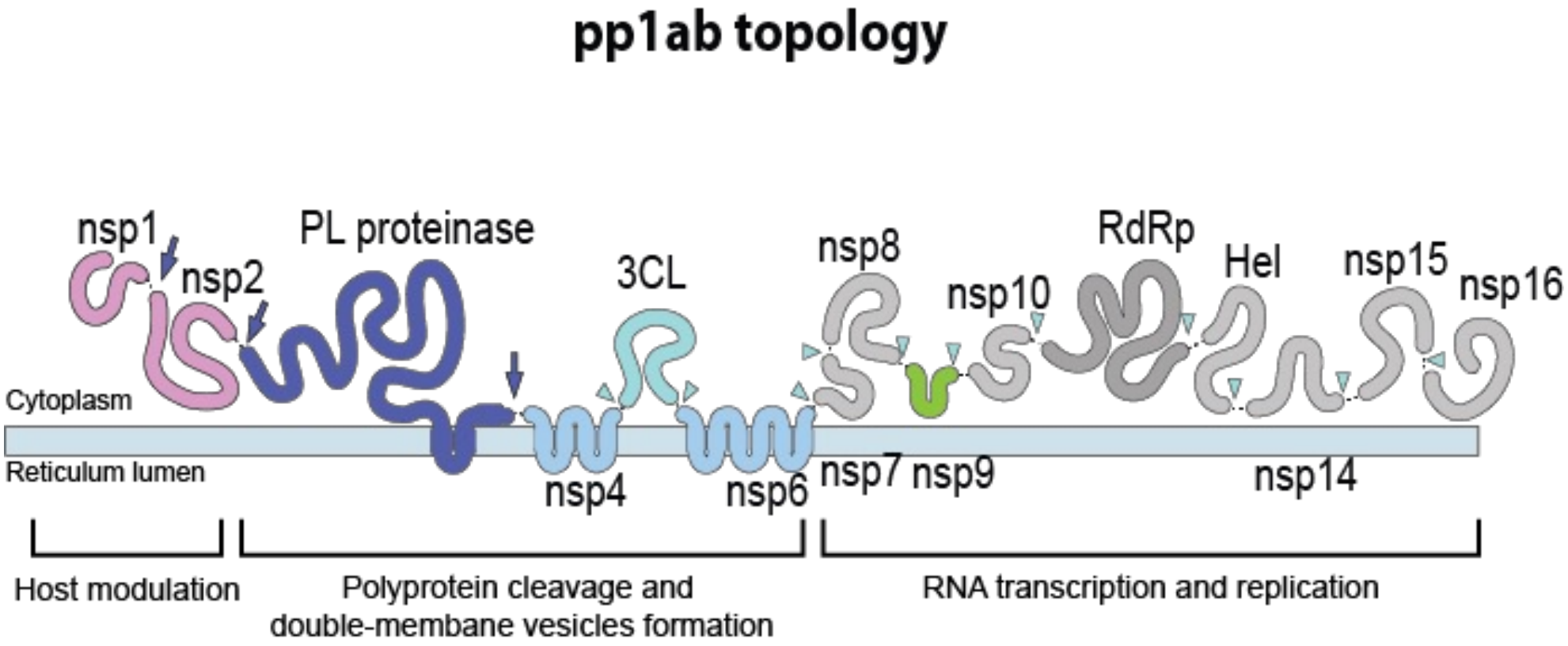
PLp; papain- like protease

3CLp; chymotrypsin- like protease
or Major (M^{pro}) protease

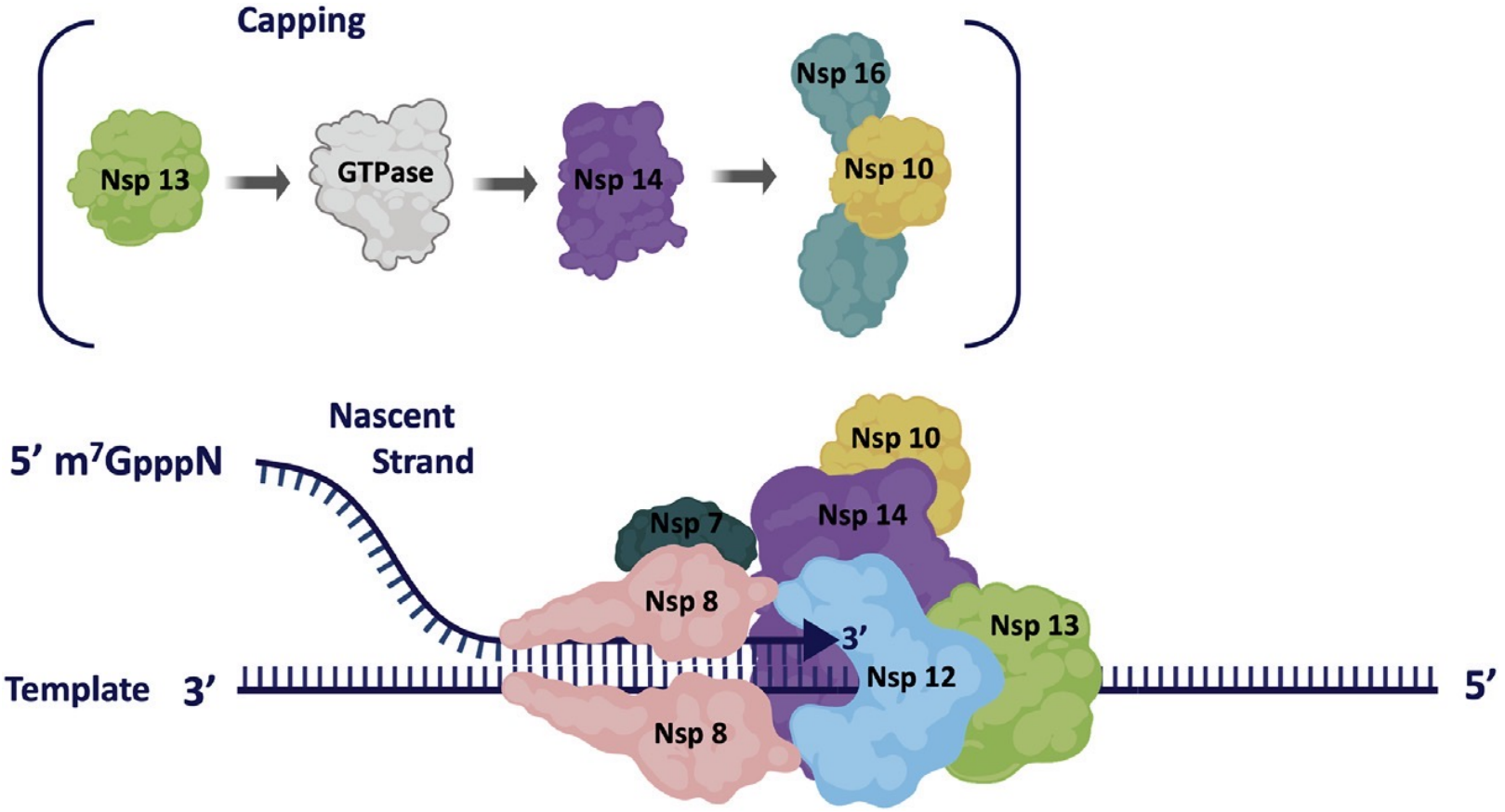
Polyprotein product expression



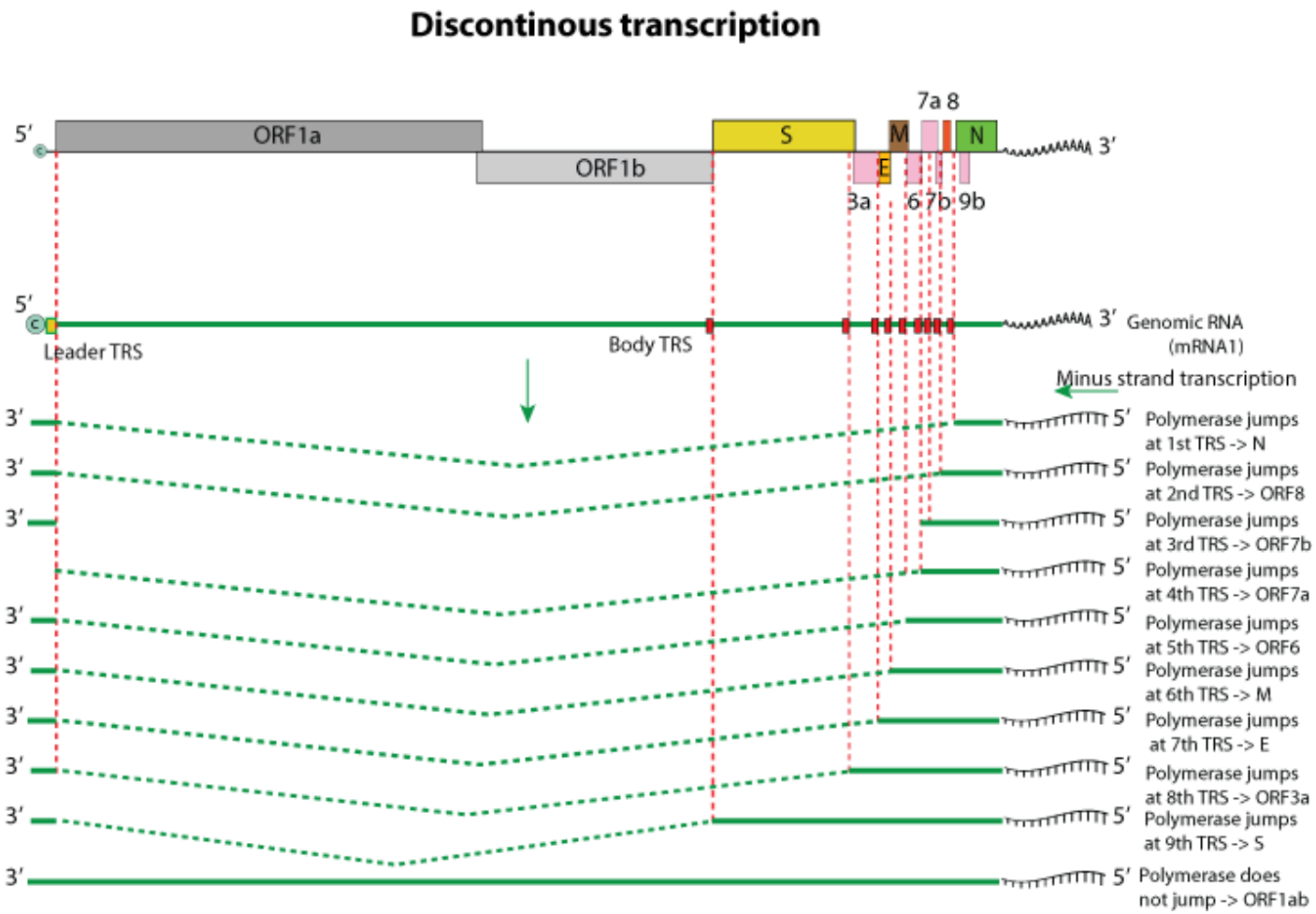
Polyprotein product expression



Model of the Core Replication and Proofreading Complex of SARS-CoV Nsp12-RdRp replicates and transcribes the genome and sgmRNAs. Nsp7/nsp8 proteins confer processivity to the polymerase. Nsp13 unwinds dsRNA ahead of the polymerase. Nsp14-ExoN complexed with its co-factor nsp10 proofreads the nascent RNA strand and excises misincorporated nucleotides. Nsp13, an unknown GTPase, Nsp14- N7-methyltransferase, and the Nsp16-20-O-methyltransferase/Nsp10 complex are involved in the capping mechanism.

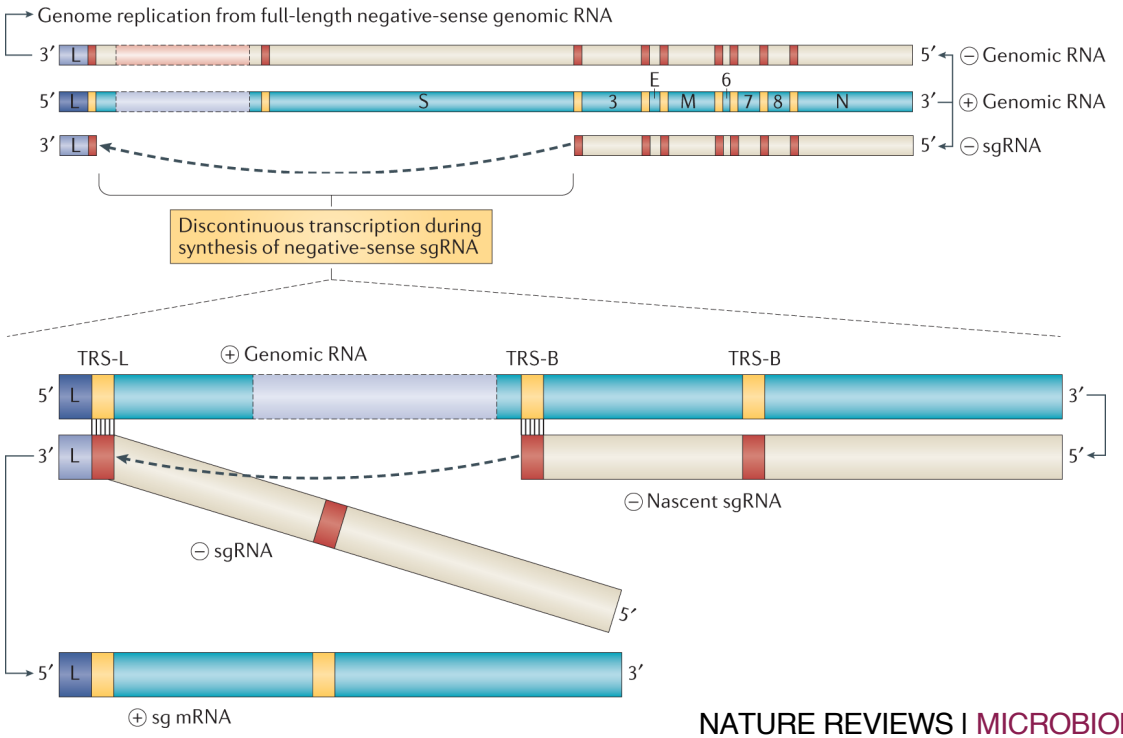


Molecular Cell *79*, September 3, 2020



Subgenomic RNAs (sgRNAs) are created by discontinous transcription. During synthesis of minus strand RNA, the polymerase have chances to pause on **transcription-regulating sequences (TRS)** and jump to leader TRS, thereby creating a major deletion. This creates a set of 9 (-)RNAs that are subsequently transcribed and translated. sgRNAs allow translation of all the structural proteins. The figure illustrate the discontinous transcription leading into 10 different RNAs. Only mRNA1 is encapsided and assembled in virions.

Coronavirus replication and discontinuous transcription.



NATURE REVIEWS | MICROBIOLOGY Ottobre 2020

Full- length positive- sense genomic RNA is used as a template to produce both full- length negative-sense copies for genome replication and subgenomic negative- sense RNAs (–sgRNA) to produce the subgenomic mRNAs (sg mRNA). The negative strand RNA synthesis involving a template switch from a body transcription regulatory sequence (TRS-B) to the leader TRS (TRS- L) is illustrated to produce one sg mRNA. This process can take place at any TRS- B and will collectively result in the production of the characteristic nested set of coronaviral mRNAs.

See also: *Structures and functions of coronavirus replication–transcription complexes and their relevance for SARS-CoV-2 drug design* NATURE REVIEWS | MOLECULAR CELL BIOLOGY REVIEWS VOLUME 23 | JANUARY 2022

Coronavirus subgenomig mRNA products

Subgenomic mRNA are translated into four structural proteins: S, E, M and nucleocapsid (N) proteins and accessory proteins.

S (spike glycoprotein) is responsible for host cell receptor recognition and binding, and for fusion of virion envelope with endosomal membrane

E proteins are small integral membrane proteins with roles in virus morphogenesis, assembly and budding. In the absence of E proteins, virus release is inhibited completely or partially. The E protein also possesses ion channel activity, which is required for optimal virus replication.

M protein is the most abundant protein in the coronavirus virion. It is a multipass transmembrane protein. Homotypic interaction between M protein provides the scaffold for virion assembly, while heterotypic interaction recruits other structural protein and genomic RNA to the assembly site.

N protein is important for encapsidation of viral RNA and acts as an interferon (IFN) antagonist.

Accessory proteins are not required for virus replication in cultured cells. However, they are conserved in virus species isolated at different times and locales (for example, for SARS-CoV), which suggests that these proteins have an important role in replication in the natural host. Several accessory proteins are virion-associated, although whether these proteins are truly structural is controversial.