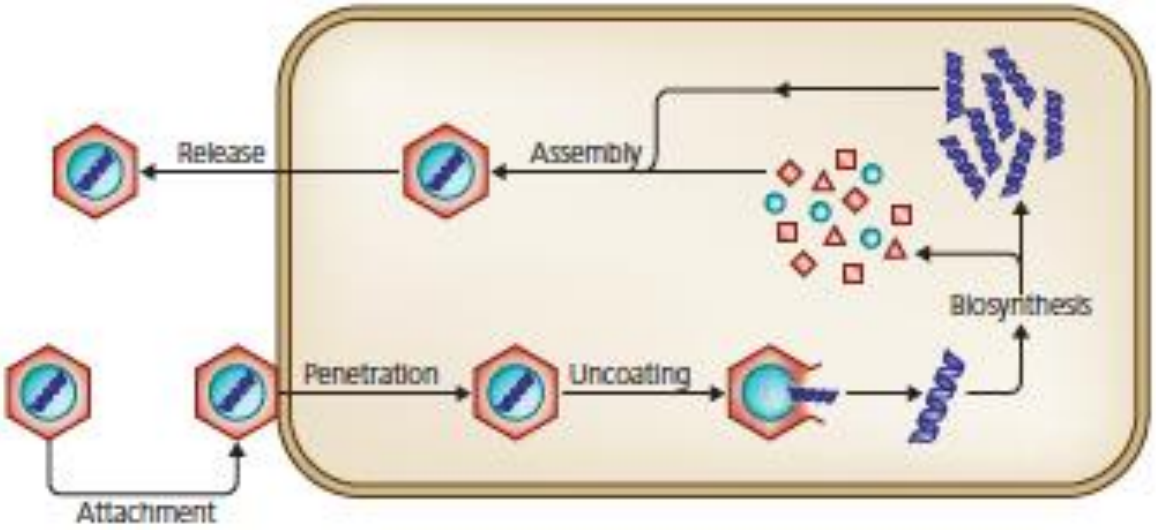
Virus Multiplication

Viruses multiply by assembling many progeny particles from a pool of virus specified components, whereas cells multiply by binary fission.



Introduction to Modern Virology, Seventh Edition. N. J. Dimmock, A. J. Easton and K. N. Leppard.
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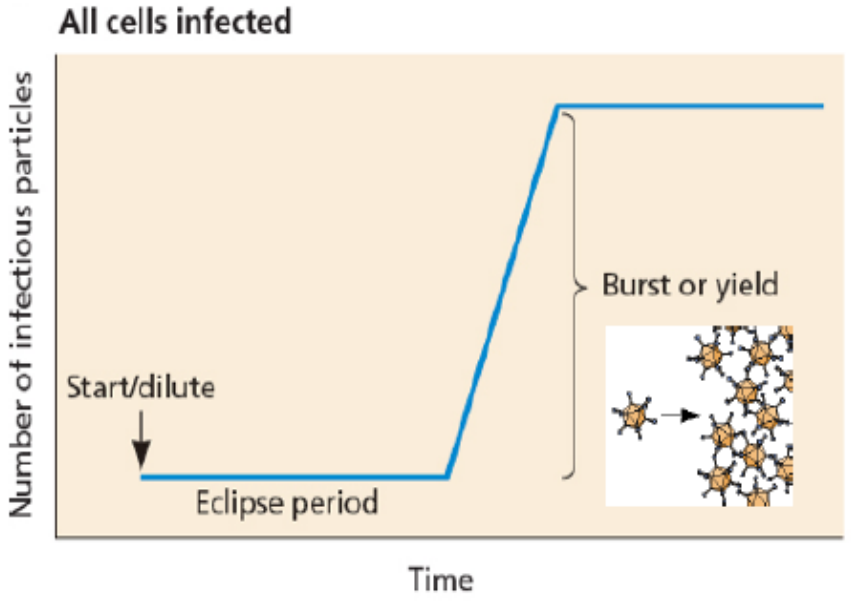
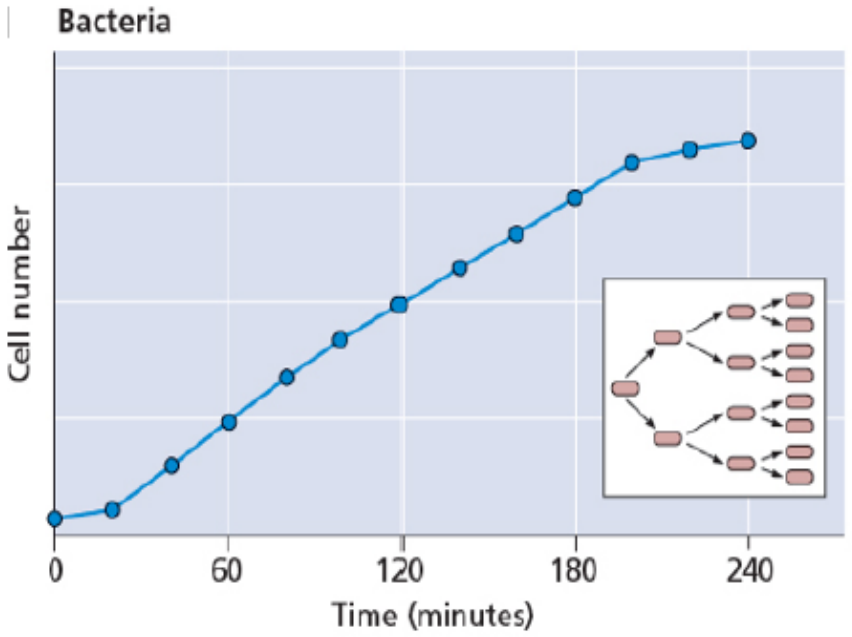
Viruses replicate by assembly of pre-formed components into many particles

Make the parts, assemble the final product

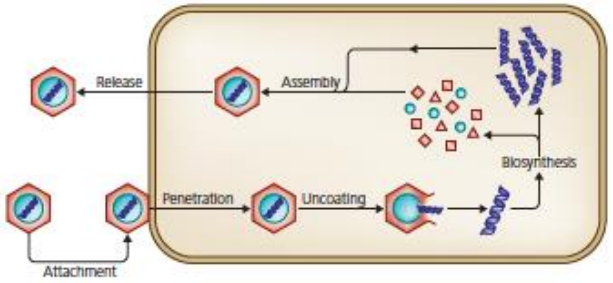
Not binary fission like cells





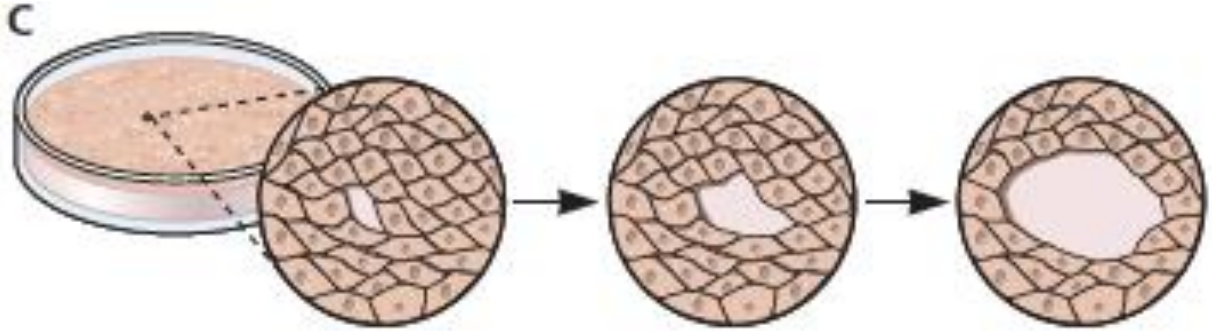
Counting virus infectious particles by the plaque assay

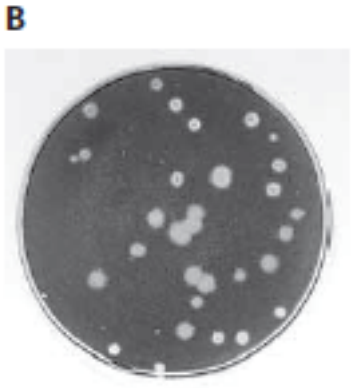
Many, many new particles ←



How do I «count» the viral particles ?

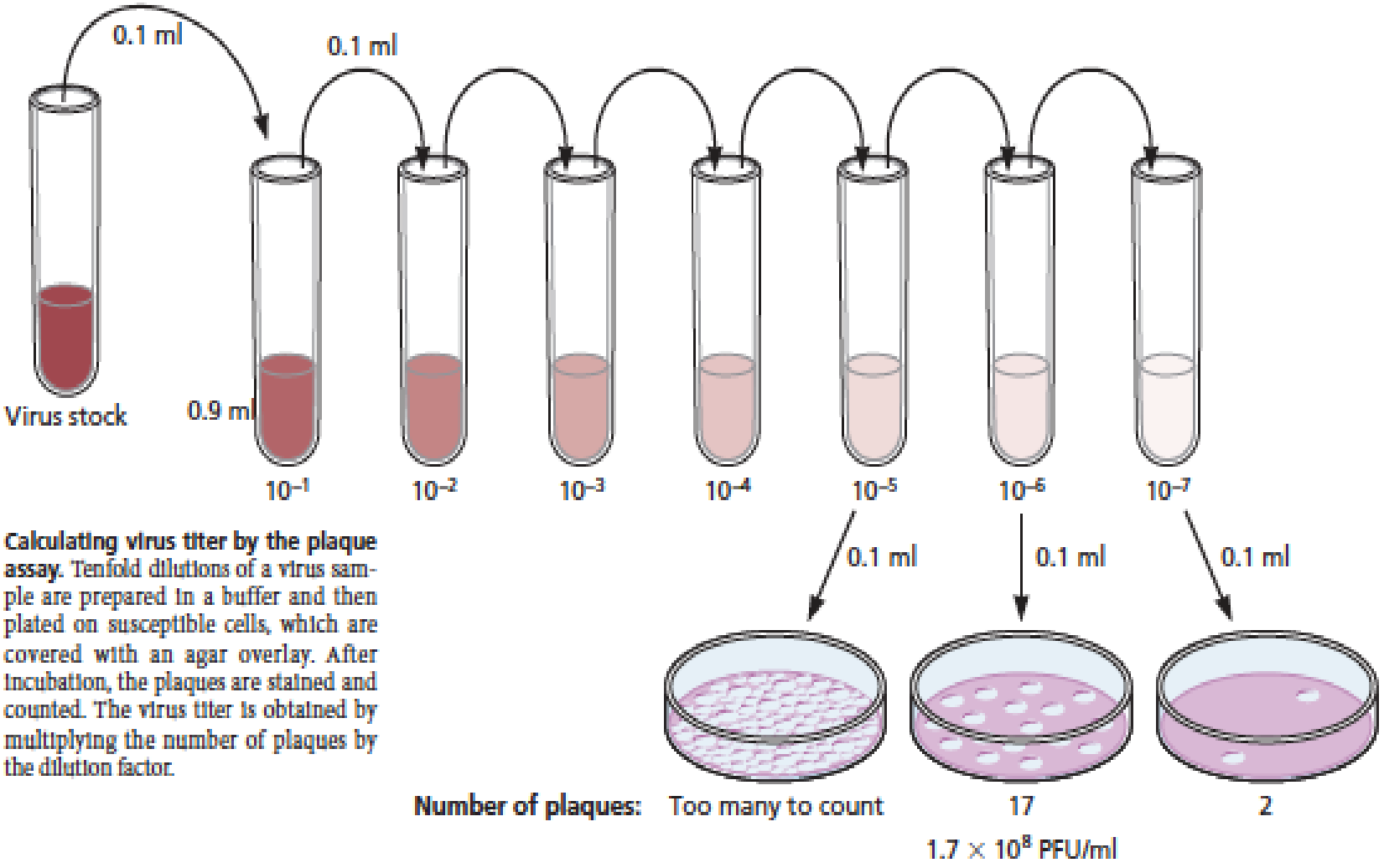
How do I «count» the infectious particles ?





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

Calculating virus titre by the plaque assay



Calculating virus titer by the plaque assay. Tenfold dilutions of a virus sample are prepared in a buffer and then plated on susceptible cells, which are covered with an agar overlay. After incubation, the plaques are stained and counted. The virus titer is obtained by multiplying the number of plaques by the dilution factor.

Plaque Forming Units=PFU

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

Titolazione virale

La titolazione virale è un metodo per quantificare le particelle virali infettanti: metodo delle placche

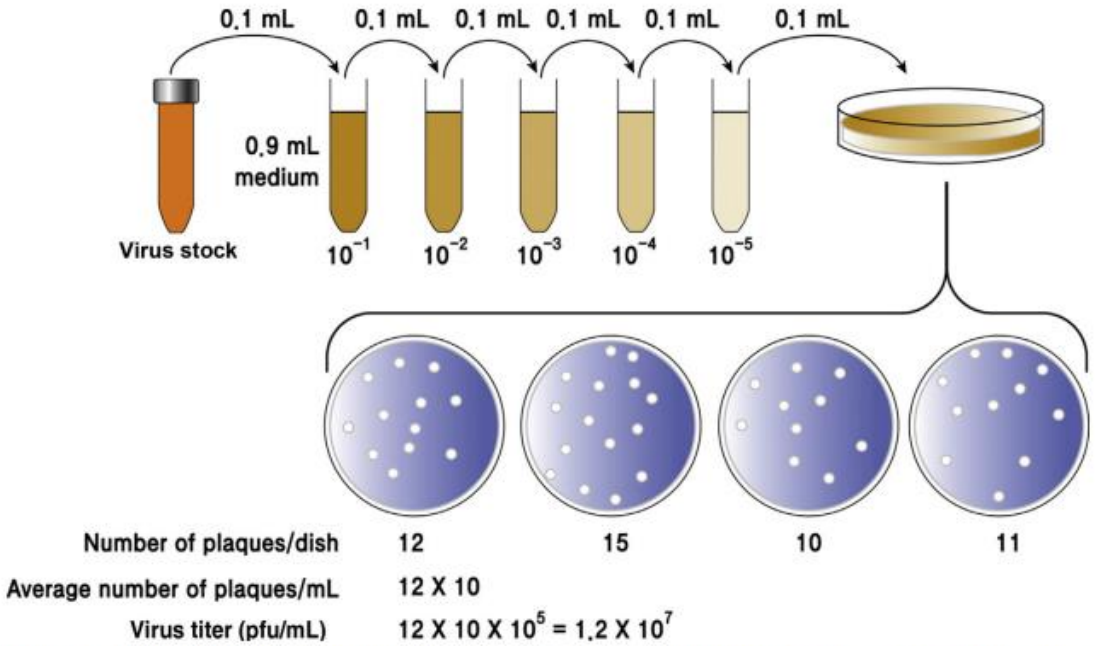


FIGURE 4.10 Virus quantification by plaque assay. This assay is based on a microbiological method conducted in a plate. Specifically, a confluent monolayer of host cells is infected with the virus at varying dilutions and covered with a semisolid medium, such as agar, to prevent the virus infection from spreading indiscriminately. Plaque formation can take about 10–14 days, depending on the virus being analyzed. Plaques are generally counted manually and the results, in combination with the dilution factor used to prepare the plate, can be used to calculate the number of pfu per sample unit volume (pfu/mL).

Pfu/ml=plaque forming unit/ml

TCID50/ml=Tissue culture infectious unit/ml

One-step growth curve

THE GROWTH OF BACTERIOPHAGE

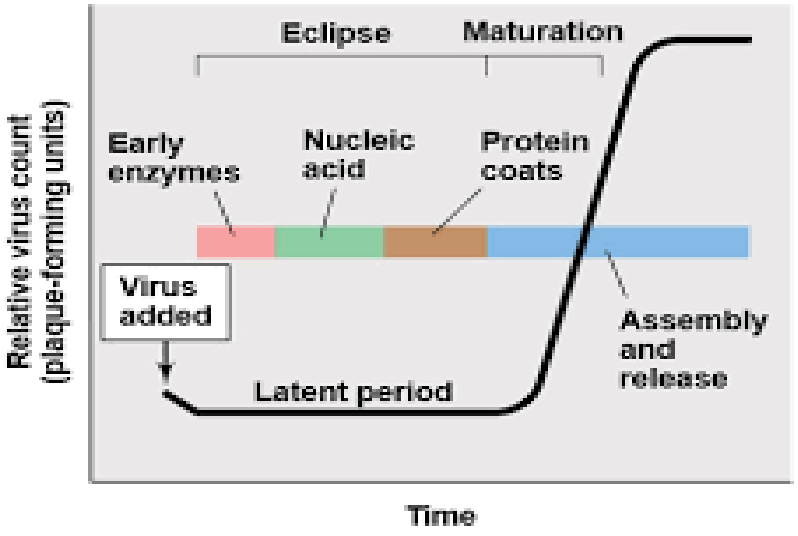
By EMORY L. ELLIS and MAX DELBRÜCK*

(From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena)

(Accepted for publication, September 7, 1938)

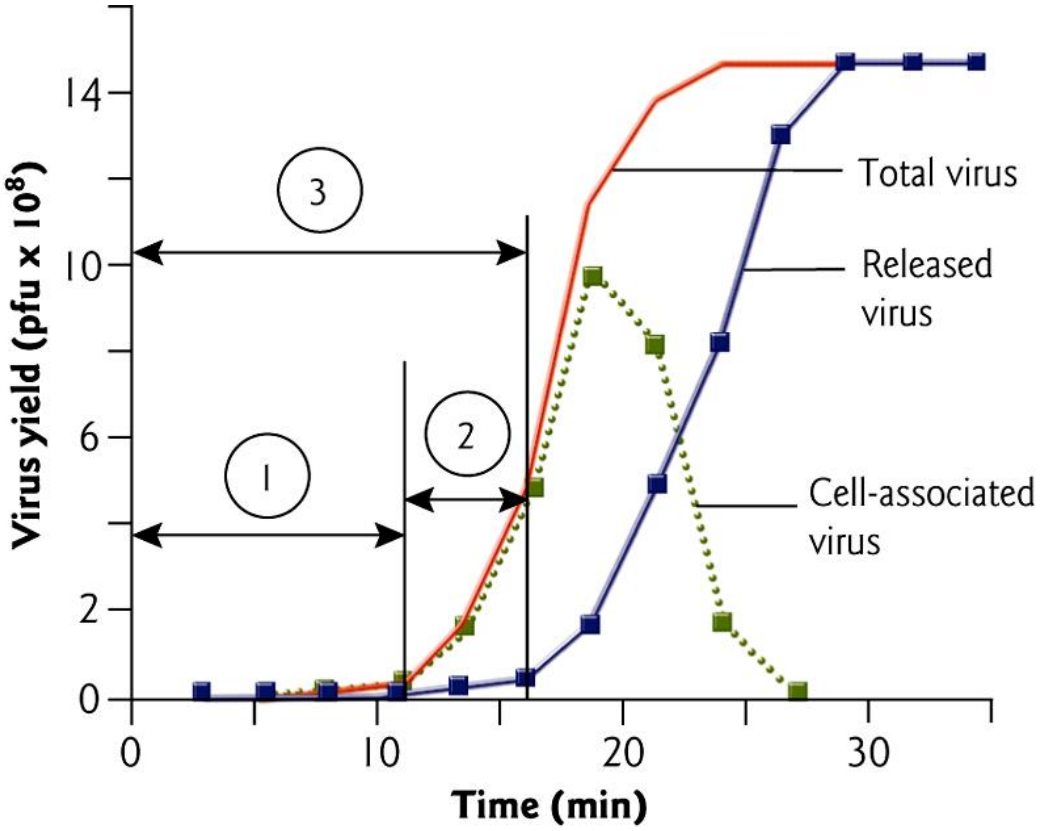
INTRODUCTION

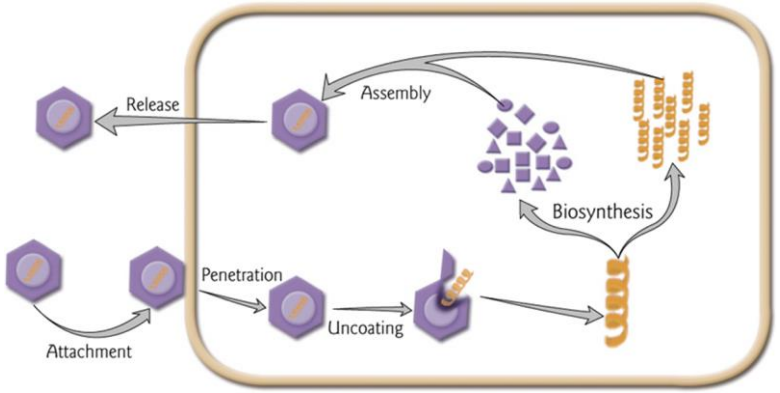
Certain large protein molecules (viruses) possess the property of multiplying within living organisms. This process, which is at once so foreign to chemistry and so fundamental to biology, is exemplified in the multiplication of bacteriophage in the presence of susceptible bacteria.



- A phage preparation is mixed with bacteria at a multiplicity of infection (m.o.i) of 10 infectious phages per cells, ensuring that all the cells are infected.
- At regular intervals cells and medium are separated and tested for infectious phages
- During the eclipse phase no infectious particles (the virus is uncoating)
- During the maturation the virus is assembled inside the cells
- The virus is then released (burst)

Ellis and Delbruck (1939)
The 'Single-Burst' Experiment
or 'One-Step Growth Curve'





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

Replicative Cycle

Attachment (adsorption) of the virion to a susceptible host cell and interaction with the specific receptor.

Penetration of the virion or its genome into the cell.

Uncoating of the viral genome and its exposure to the genetic/biosynthetic machinery of the host cell.

Early phase of viral genome expression, during which the biosynthetic machinery of the host cell is modified as a prelude to the synthesis of viral nucleic acids. In this phase, virus-specific enzymes are produced—the first wave of viral gene expression.

Replication of the viral genome.

Synthesis of virion components, including the capsid protein subunits and the proteins associated with the pericapsid envelope

Assembly of the protein subunits (and membrane components in the case of enveloped viruses) and packaging of the genome into the newly formed viral particles.

Release of mature virions from the cell.

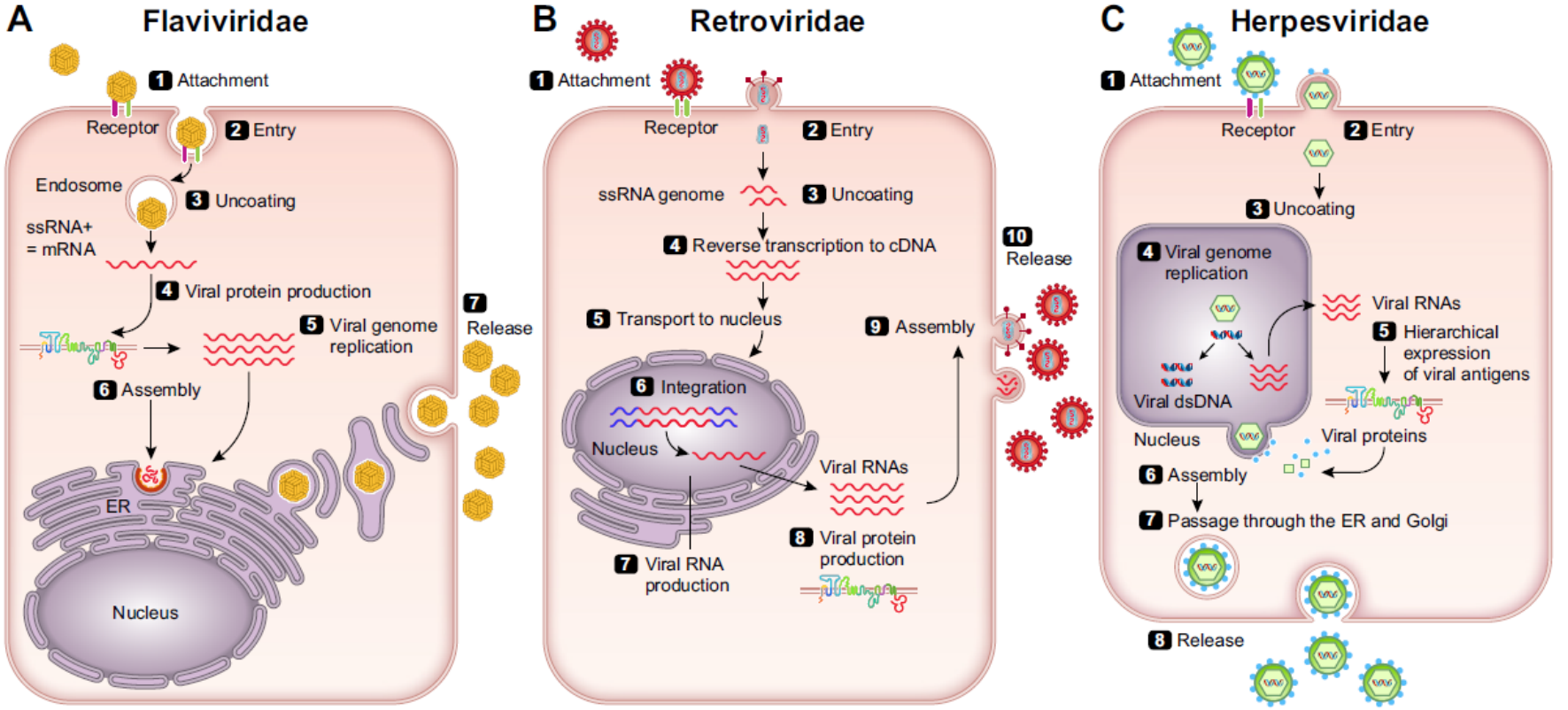
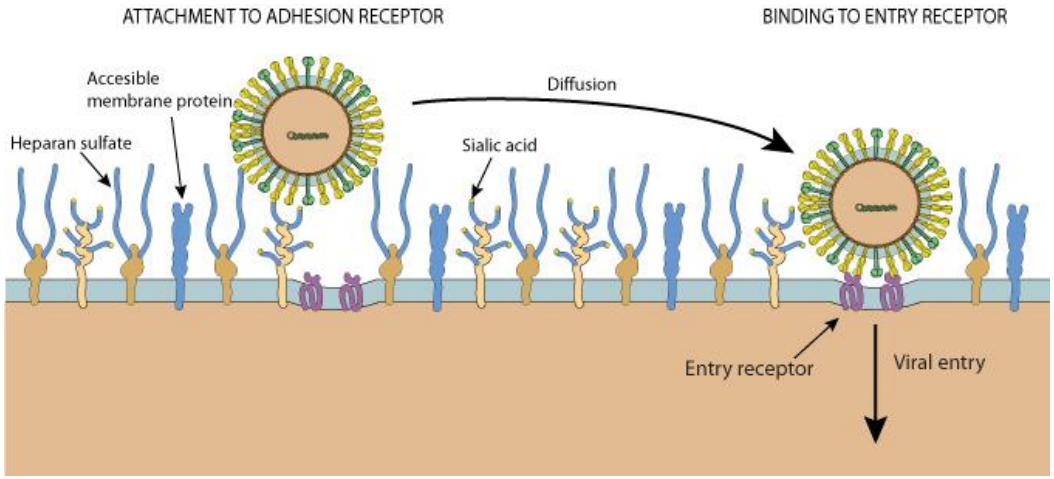


FIGURE 1. Life cycle of three representative viral families that infect the male genital tract. *A*: Flaviviridae (e.g., ZIKV, HCV). *B*: Retroviridae (e.g., HIV-1/2, HTLV-1/2). *C*: Herpesviridae (e.g., HSV, CMV). At the host cell level, the main common steps of the life cycle of a virus consists of attachment to the cell surface through receptor recognition, cell entry through either endocytosis/pinocytosis (e.g., Flaviviridae) or a fusion process (e.g., Retroviridae, Herpesviridae), uncoating followed by the release of the nucleic acid in the cytoplasm (Flaviviridae, Retroviriae) or in the nucleus (Herpesviridae), expression and replication of viral genome, assembly of progeny viral particles, and release of newly formed viral particles (virions) through budding or cell lysis. In a cell that is susceptible (i.e., in which the virus may enter through receptor binding), the virus may establish productive infection (cell is fully permissive to viral replication and viral progeny is released, which may lead to cell death resulting in cytolytic infection), restrictive infection (cell is only transiently permissive), latent infection (viral progeny is not produced until active replication is triggered by specific stimuli, as may happen for HSV, VZV, CMV, EBV, and HIV), or abortive infection (replication cannot be completed due to a nonpermissive host or cell, or because the virus is defective). ER, endoplasmic reticulum.

Attacco/Adsorbimento del virus alla cellula ospite



Adhesion receptors – they allow the virus to bind reversibly to target organs/cells. They are not strictly required for viral entry and, on their own, do not enable the virus to enter the cell. However, interaction with these receptors greatly increases viral infectivity by increasing its local concentration near its specific entry receptor or by facilitating attachment to an organ where its target cells are present.

Entry receptors – they trigger viral entry through endocytosis or by inducing fusion/penetration. Binding to these receptors is irreversible. Access to these receptors is often difficult for the virus, which overcomes this obstacle by first binding to adhesion receptors, thereby increasing the probability of interacting with the specific entry receptor.

Attachment/Binding

Host cell recognition requires the interaction between specific surface structures of the virion (antireceptors) and receptors on the cytoplasmic membrane of the cell.

The specificity of this binding determines viral tropism, that is, the ability of viruses to infect predominantly (or even exclusively) certain animal species (or specific tissues and organs).

Many antireceptors have been identified and characterized: they are generally glycoproteins in enveloped viruses and simple proteins in non-enveloped (naked) viruses.

Receptors are structures that normally serve a well-defined physiological function in the cell (which the virus has evolutionarily adapted to use for its own “purposes”). Adsorption requires only passive participation from the cell.

Penetration

Entry of the virus into the host cell can occur in the following ways

1) **Fusion** The lipoproteic envelope of the virus fuses with the cytoplasmic membrane. The nucleocapsid is then released into the cytoplasm. In some viruses (Paramyxoviruses, Herpesviruses, HIV) this process is facilitated by fusion proteins (enveloped viruses).

2) **Endocytosis** The adhesion of the virus to the membrane induces its invagination, which transports the virus into the cytoplasm enclosed within a phagocytic vacuole (non-enveloped viruses, viruses with a pericapsid).

3) Translocation The viral genome crosses the cytoplasmic membrane and enters the cytoplasm directly in its naked form (non-enveloped viruses).

Uncoating

Mechanism that allows the disaggregation of the proteins that enclose the nucleic acid (or are otherwise associated with it) and can occur through various modalities.

The mechanism is not well understood, and it is possible that the same virus may follow different pathways depending on the circumstances and the type of host cell.For many viruses, viral proteins disaggregate spontaneously or with the intervention of lysosomal enzymes, which are released inside phagocytic vacuoles (phagolysosomes or virosomes).

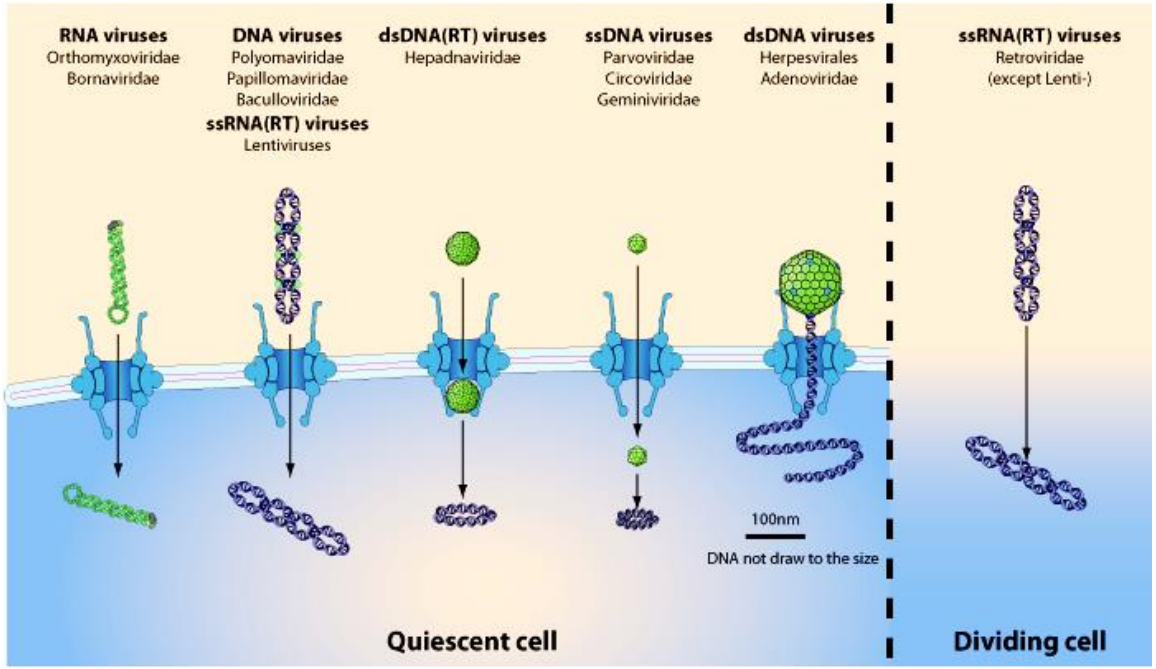
Post entry

After uncoating, the genome may:

1) remain in the cytoplasm (typical of RNA viruses and of DNA viruses such as poxviruses)

2) be transported into the nucleus (typical of DNA viruses and of some RNA viruses, such as retroviruses and influenza virus)

Viral penetration into host nucleus



Most DNA and few RNA viruses target their genome to the host nucleus. The crossing of nuclear membrane occurs in several ways:

-RNA virus, dsDNA virus and lentivirus genomes enter via the nuclear pore complex (NPC) through the cellular Importin transport.

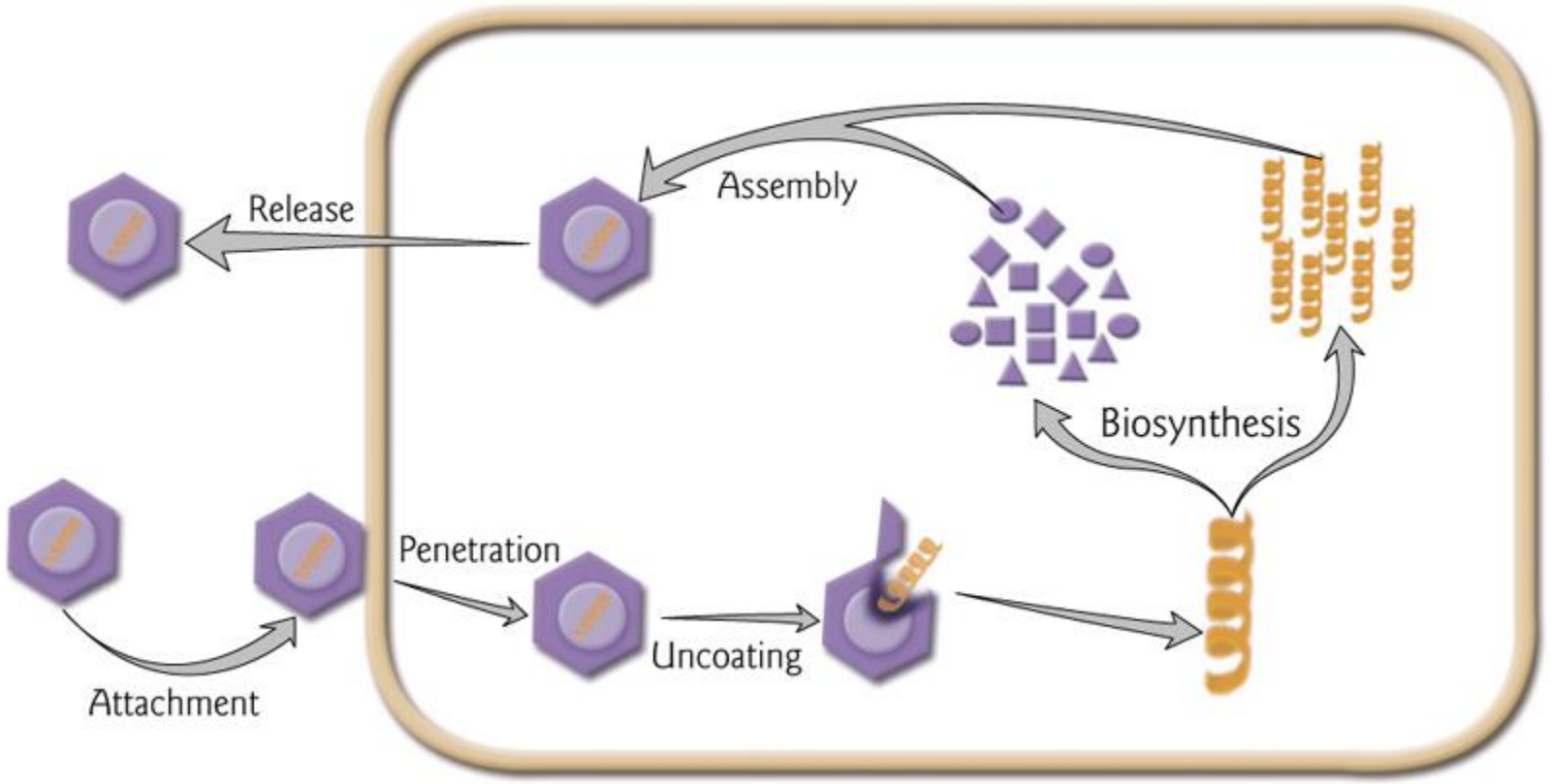
-ssDNA virus capsid seems to be small enough to cross the NPC and enter the nucleus as an intact capsid.

-Hepadnaviridae capsid would enter the NPC pore, but remains attached to it and releases the viral genomic DNA into the nucleoplasm.

-Herpesvirales capsid is too large to enter the NPC pore, the viral genome is directly injected through the NPC on which the capsid docks.

-All retroviridae except lentivirus would enter the nucleus during mitosis, when the nuclear membrane temporarily disintegrates.

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

Maturation

Maturation is the stage of the replicative cycle during which the virus becomes infectious.

It includes structural modifications of the virion, which may result from specific processing events involving capsid proteins or from conformational changes occurring during assembly.

Viral proteases are often involved in the maturation process, although in some cases both viral and cellular enzymes may participate.

As with the early stages of the replicative cycle, it is not always possible to clearly distinguish assembly, maturation, and release as separate steps.

Release

Lytic viruses (most non-enveloped viruses) leave the cell following its lysis.

Enveloped viruses can exit the infected cell through

1) budding directly from the outer plasma membrane, which will form the pericapsid envelope

2) transport within intracellular vesicles, after budding from internal cellular membranes

During the budding process, viral envelope proteins are incorporated along with the lipid membrane.

Virus Multiplication

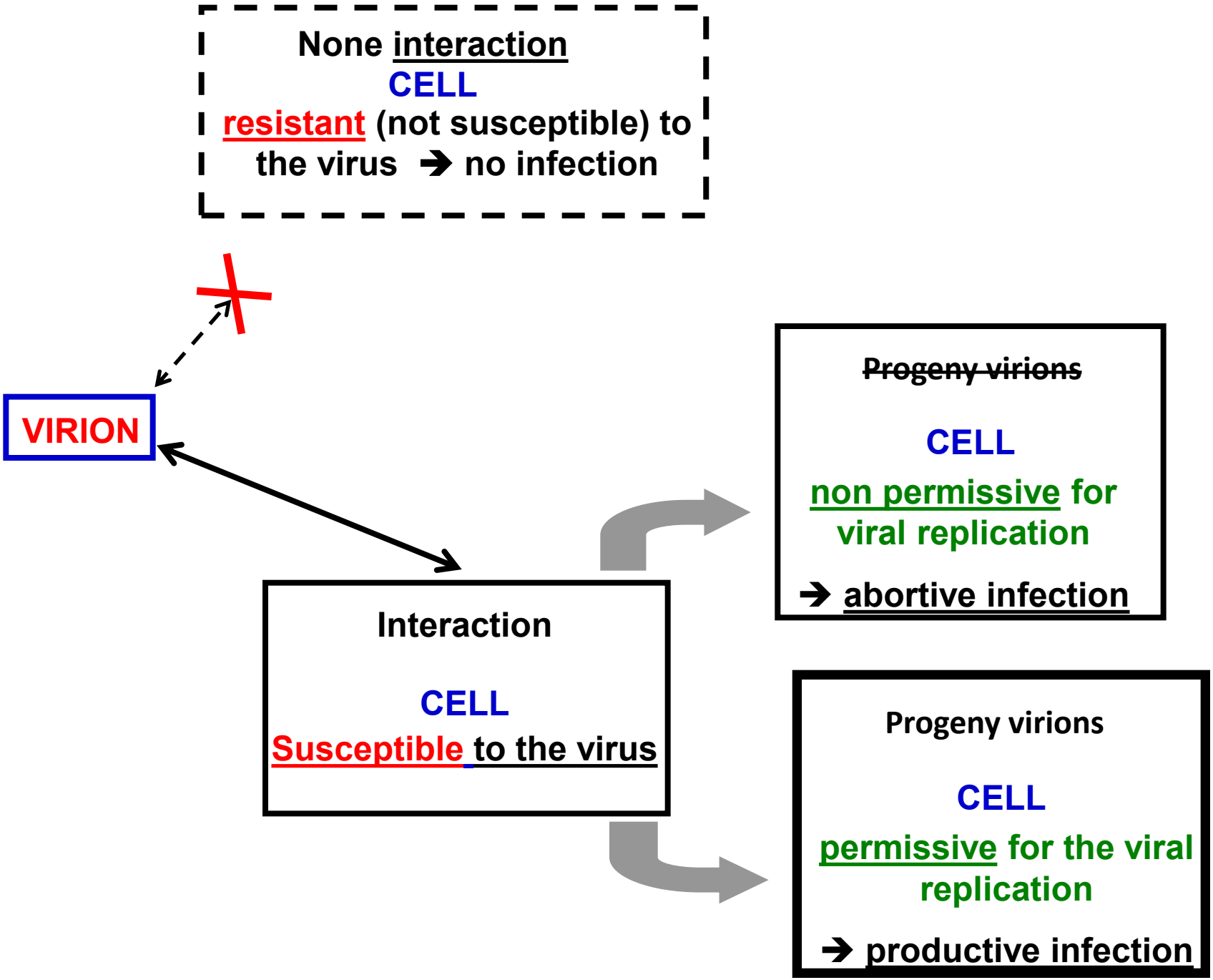
A causa della natura di **PARASSITA INTRACELLULARE OBBLIGATO**, il virus può, eventualmente anche sopravvivere all'esterno delle cellule, ma compiere il suo ciclo replicativo solo all'interno di una **CELLULA OSPITE.**

La cellula ospite, per consentire la replicazione, deve essere:

Sensibile

+

Permissiva


None interaction
CELL
resistant (not susceptible) to the virus ➔ no infection
VIRION
Progeny virions
CELL
non permissive for viral replication
➔ abortive infection
Interaction
CELL
Susceptible to the virus
Progeny virions
CELL
permissive for the viral replication
➔ productive infection

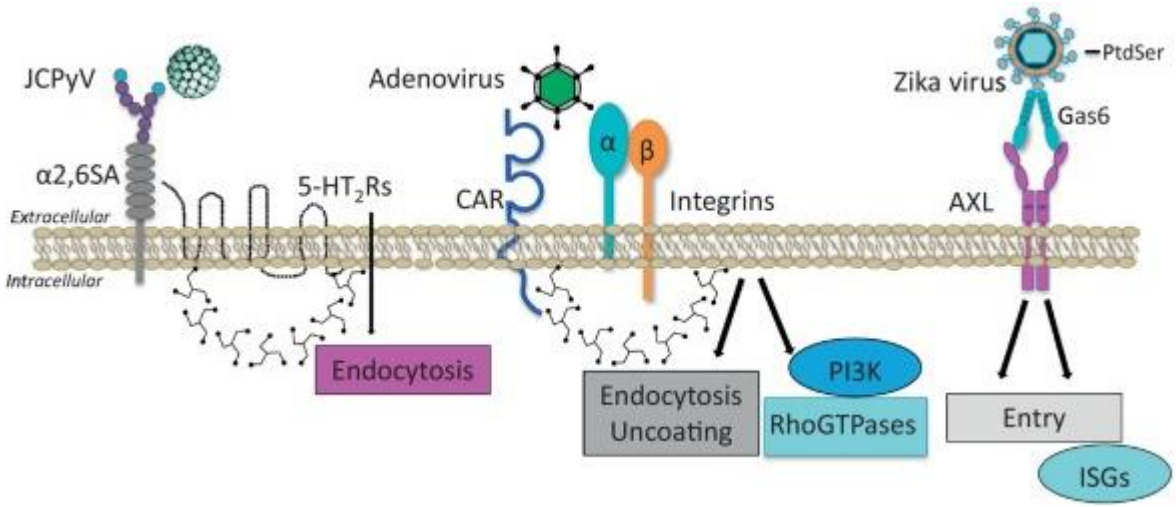
Susceptibility or Resistance to Viral Infection

Determined by the possibility of a specific extracellular interaction between:

- **A viral ligand** : viral envelope protein for enveloped viruses
  or viral capsid protein for naked viruses

and

- **A specific cell surface receptor** on the plasma membrane of the host cell



Permissivity or Non Permissivity to viral replication

Conditioned by : the expression of cellular genes essential for the replication of the virus

the expression of the viral genome (damaged or defective virus…)

the expression of antiviral defense mechanisms

are associated the notions of:

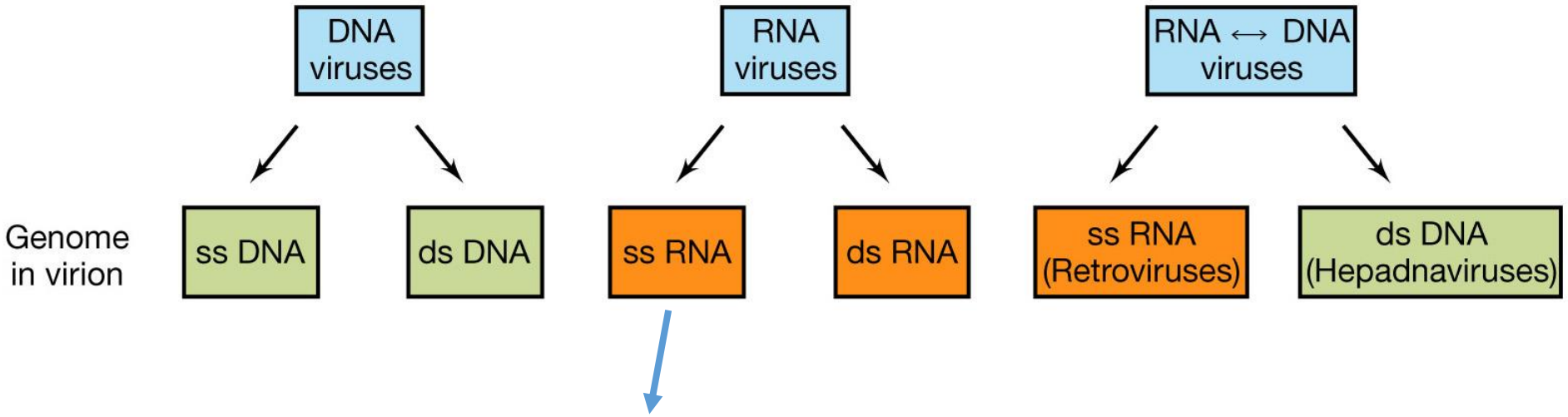
HOST RANGE

- **The range of host species that a virus is capable to infect and in which it can multiply** ➔ for most viruses, host range restricted to one animal family or species

TROPISM

- **All cell types or tissues in which a virus can multiply:** often restricted to one or a very limited number of cell types

Genome architecture

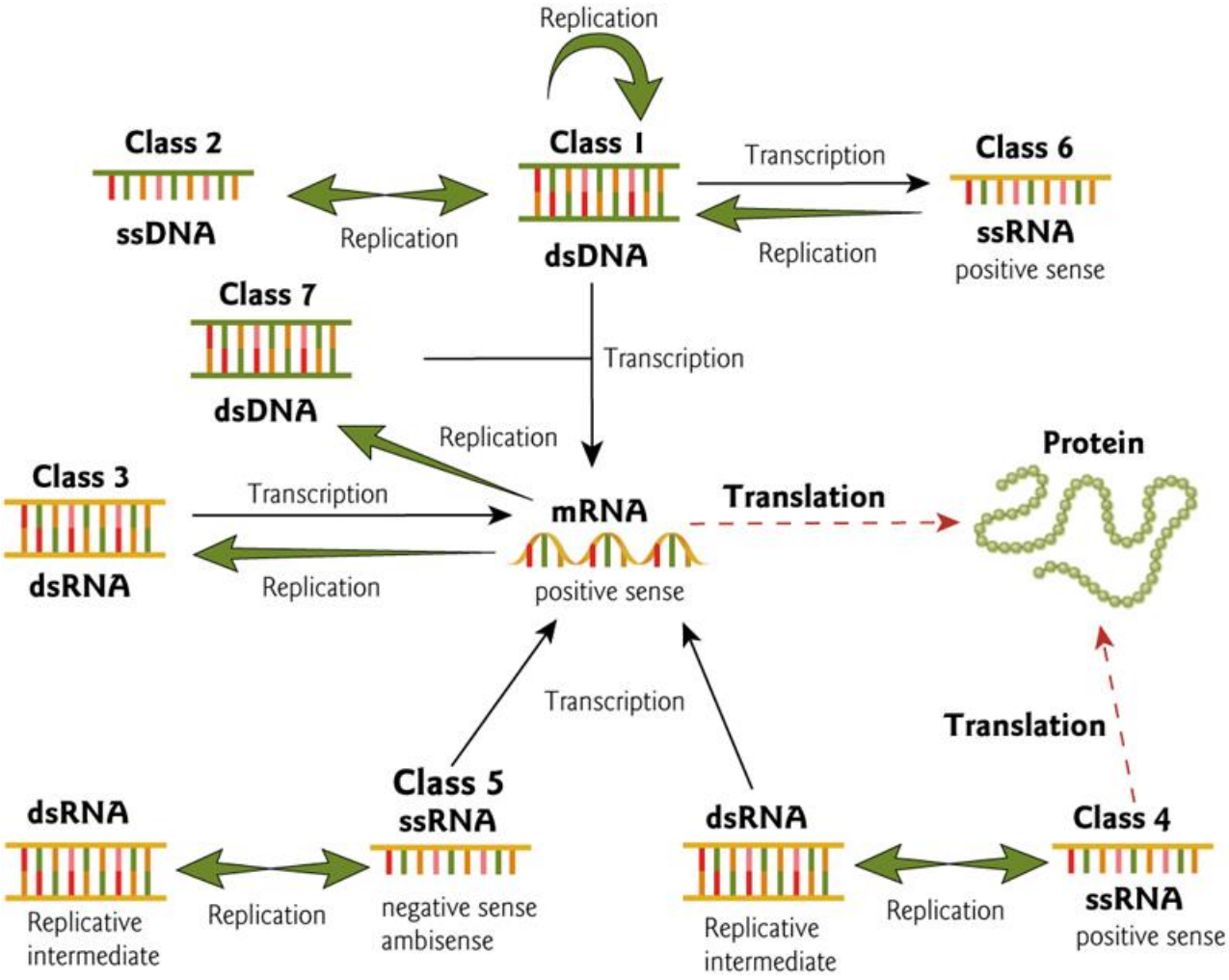


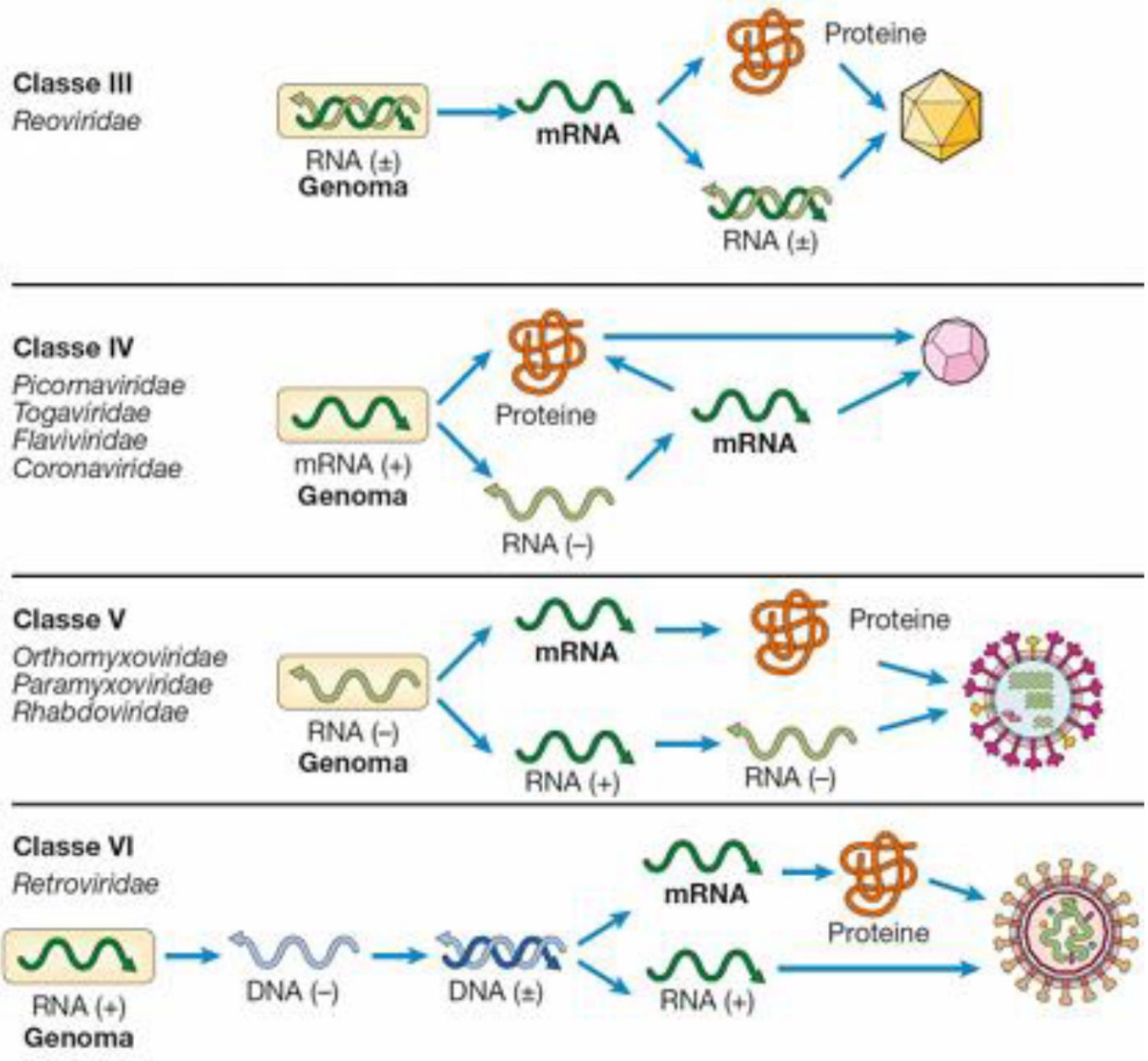
Virus con RNA a **polarità positiva (+)** che può fungere nella cellula direttamente da RNA messaggero.

Virus con RNA a **polarità negativa (-)** che funge da stampo per la sintesi dell'RNA messaggero- questi virus hanno l'enzima RNA polimerasi RNA dipendente associato al virione.

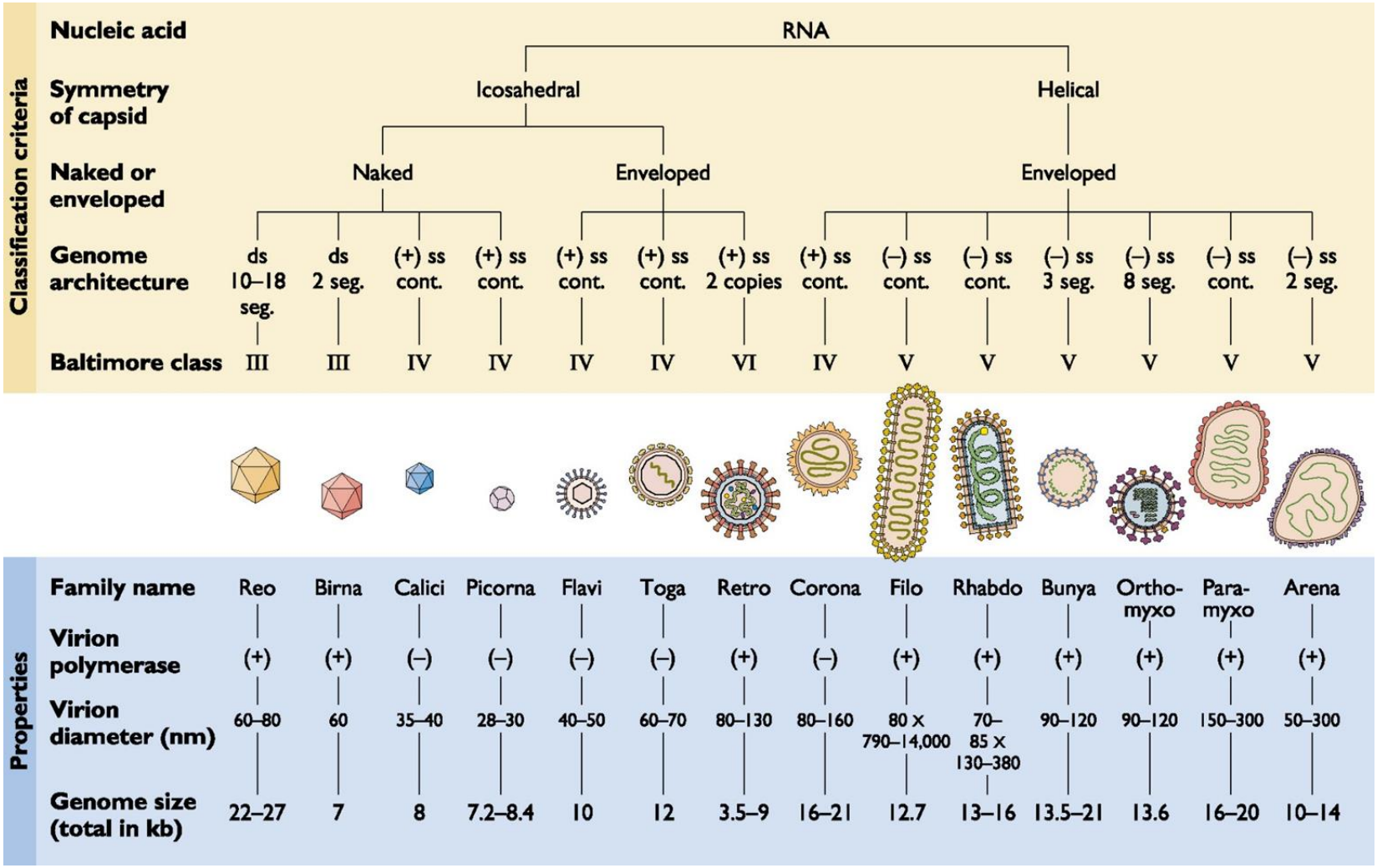
I retrovirus hanno due molecole di RNA (+): sono diploidi

The Baltimore scheme (replication classes)




Classe III
Reoviridae
RNA (±)
Genoma
mRNA
Proteine
RNA (±)
Classe IV
Picornaviridae
Togaviridae
Flaviviridae
Coronaviridae
mRNA (+)
Genoma
Proteine
mRNA
RNA (–)
Classe V
Orthomyxoviridae
Paramyxoviridae
Rhabdoviridae
RNA (–)
Genoma
mRNA
Proteine
RNA (+)
RNA (–)
Classe VI
Retroviridae
RNA (+)
Genoma
DNA (–)
DNA (±)
mRNA
Proteine
RNA (+)

RNA viruses



From Flint et al. *Principles of Virology* (2000), ASM Press

Human RNA virus families

FAMILY	EXAMPLES	VIRION	CAPSID	NUCLEIC ACID*
Reoviridae	Reovirus, Rotavirus	Naked	Icosahedral	DS,
Picornaviridae	Enterovirus, Poliovirus	Naked	Icosahedral	SS, positive
Caliciviridae	Norwalk	Naked	Icosahedral	SS, positive
Togaviridae	Rubella	Enveloped	Icosahedral	SS, positive
Arenaviridae	LCM, Lassa, Junin	Enveloped	Helicoidal	SS, negative
Flaviviridae	Hepatitis C, Yellow fever	Enveloped	Icosahedral	SS, positive
Orthomyxoviridae	Influenza	Enveloped	Helicoidal	SS, negative
Paramyxoviridae	Measles, Mumps	Enveloped	Helicoidal	SS, negative
Bunyaviridae	Hantavirus	Enveloped	Helicoidal	SS, negative
Rhabdoviridae	Rabies	Enveloped	Helicoidal	SS, negative
Filoviridae	Ebola, Marburg	Enveloped	Helicoidal	SS, negative
Coronaviridae	SARS-CoVs, MERS	Enveloped	Helicoidal	SS, positive
Astroviridae	Astrovirus	Naked	Icosahedral	SS, positive
Bornaviridae	Borna	Enveloped	Helicoidal	SS, negative
Hepeviridae	Hepatitis E	Naked	Icosahedral	SS, positive

*SS, single-stranded; DS, double-stranded. All human RNA viruses have linear genome except for the Delta satellite virus

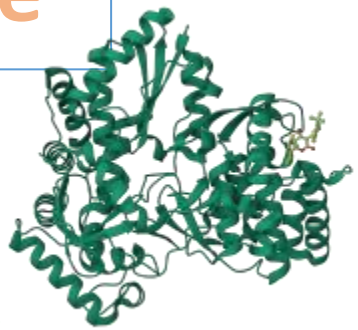
Cellular and Viral Polymerases

Type	Template → Product	Function
DNA-dependent DNA-Polymerase	DNA → DNA	Replication
DNA-dependent RNA-Polymerase	DNA → RNA	Transcription
RNA-dependent RNA-Polymerase	RNA → RNA	Replication and transcription of RNA-viruses
RNA-dependent DNA-Polymerase	RNA → DNA	Reverse transcription

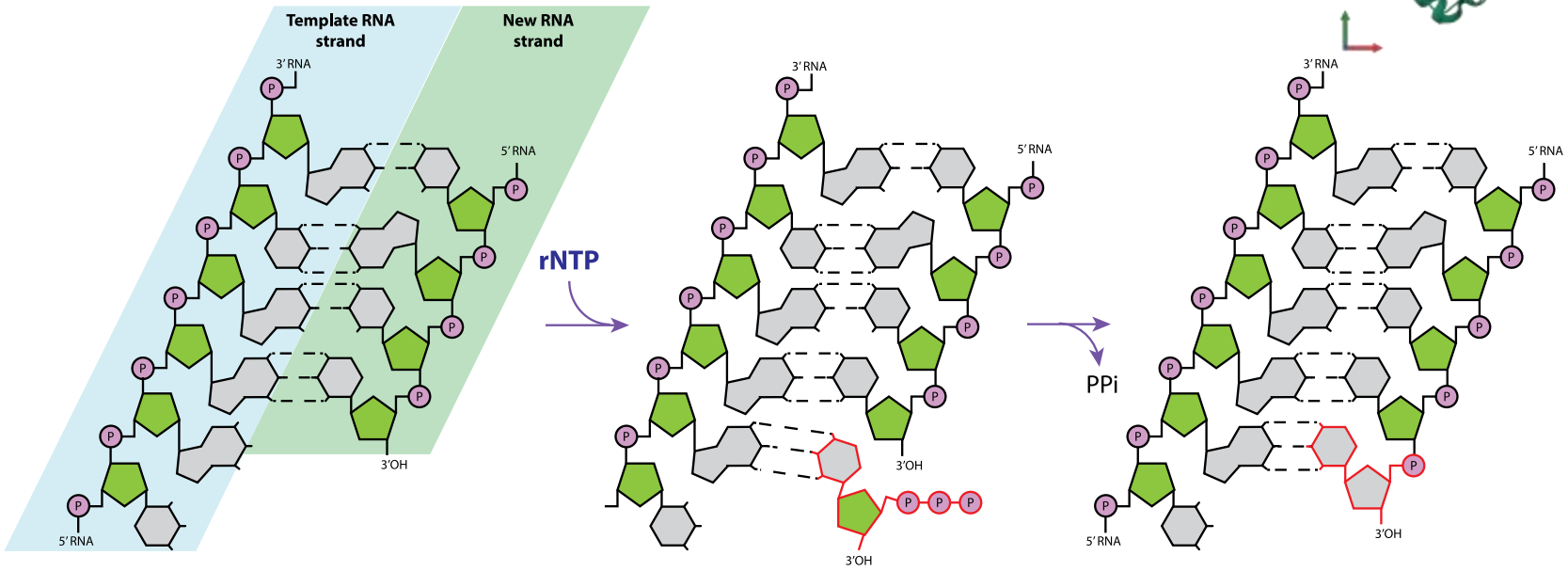
RNA viruses from class III, IV, V use RNA dependent RNA polymerases

RNA viruses from class VI use RNA/DNA-dependent DNA-Polymerase activities and the RNApol II of the host

RNA dependent RNA polymerase



RNA-dependent RNA polymerase

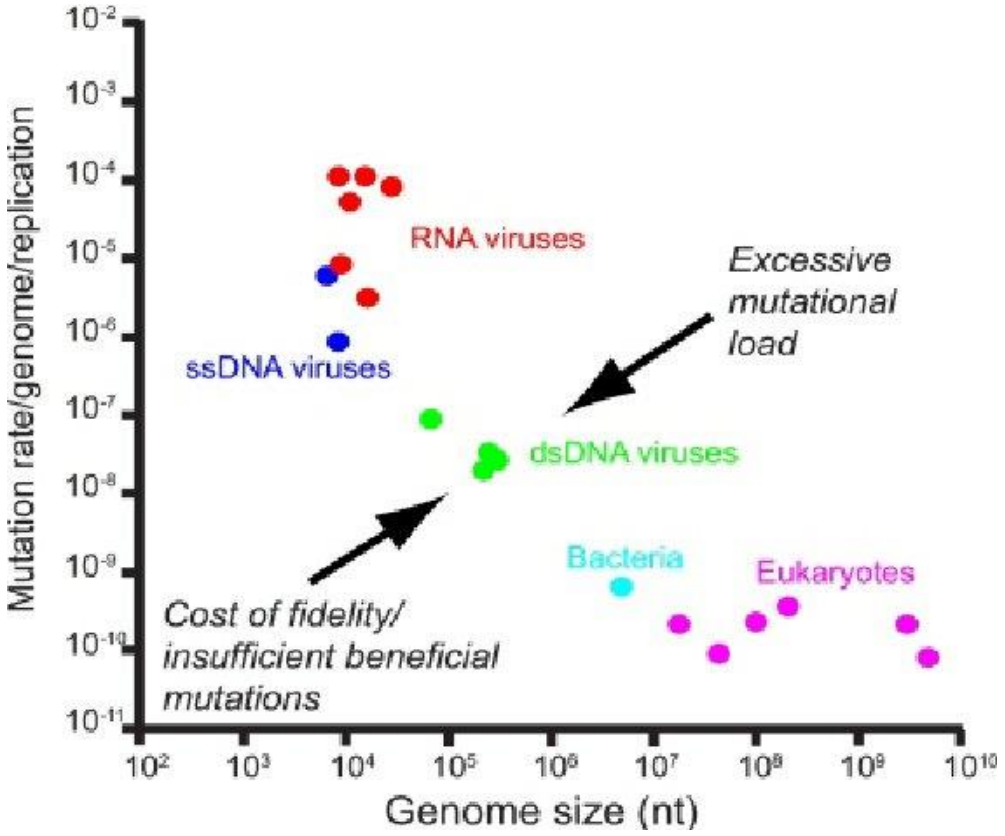


Le RNApolRNAdip mancano di attività di correzione di bozze (proofread) ⇒ tassi di mutazione più elevati rispetto ai virus a DNA

https://viralzone.expasy.org/10557

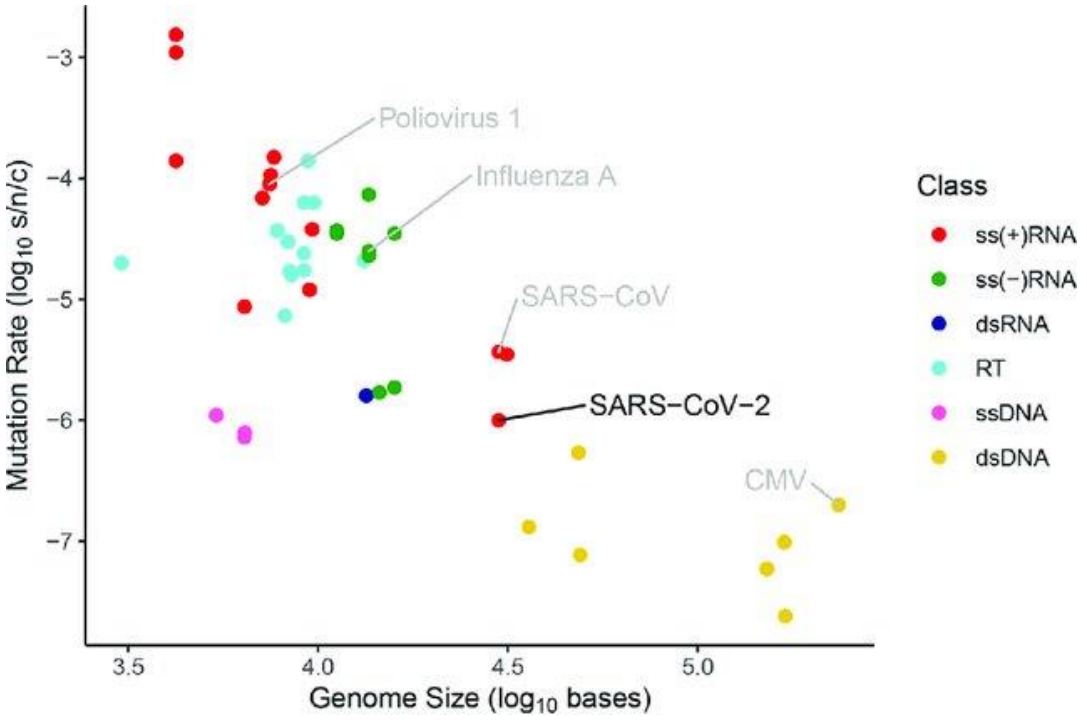
RdRp=RNA dependent RNA polymerase

Genetic variability and mutation rate



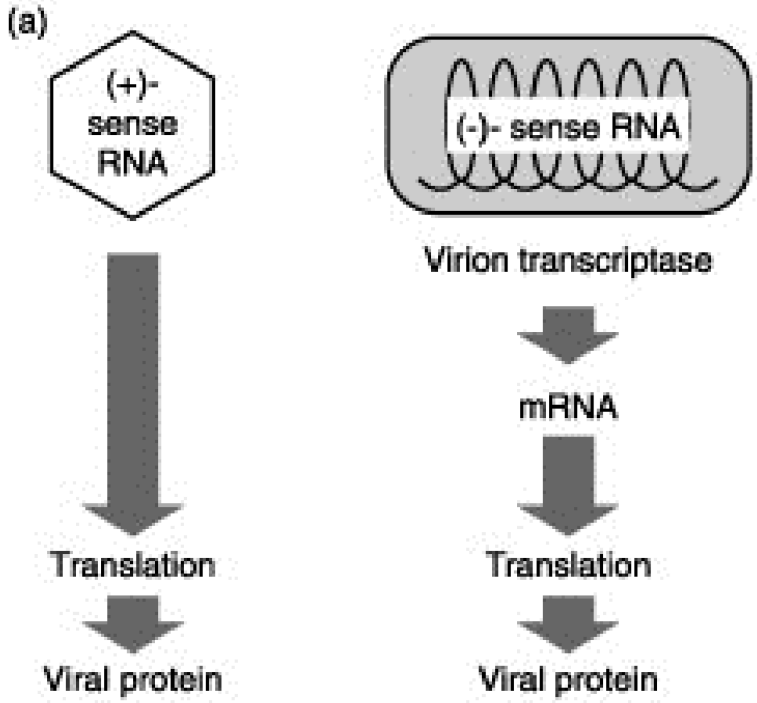
In alcuni virus la frequenza di mutazioni è molto elevata e può raggiungere 10^{-3} 10^{-4} per nucleotide incorporato (es. virus con genoma ad RNA), mentre in altri i valori sono simili a quelli riscontrati per il DNA cellulare, da 10^{-8} a 10^{-11} (es. herpesvirus e altri virus con genoma a dsDNA). Queste differenze sono associate alle modalità di replicazione del genoma, infatti, la frequenza di errori della trascrittasi inversa e delle RNA polimerasi RNA-dipendenti è più alta di quella della DNA polimerasi DNA-dipendente

Variabilità genetica: mutazioni



Queste differenze sono associate alle modalità di replicazione del genoma, infatti, la frequenza di errori della trascrittasi inversa e delle RNA polimerasi RNA-dipendenti è più alta di quella della DNA polimerasi DNA-dipendente

Class IV and V: ssRNA viruses



Caratteristiche comuni: genomi lineari, RNAdependent RNA polymerase (RdRP), intermedi dsRNA

Le RNApolRNAdip sono o nel virione (-) o prodotte in fase precoce (+)

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

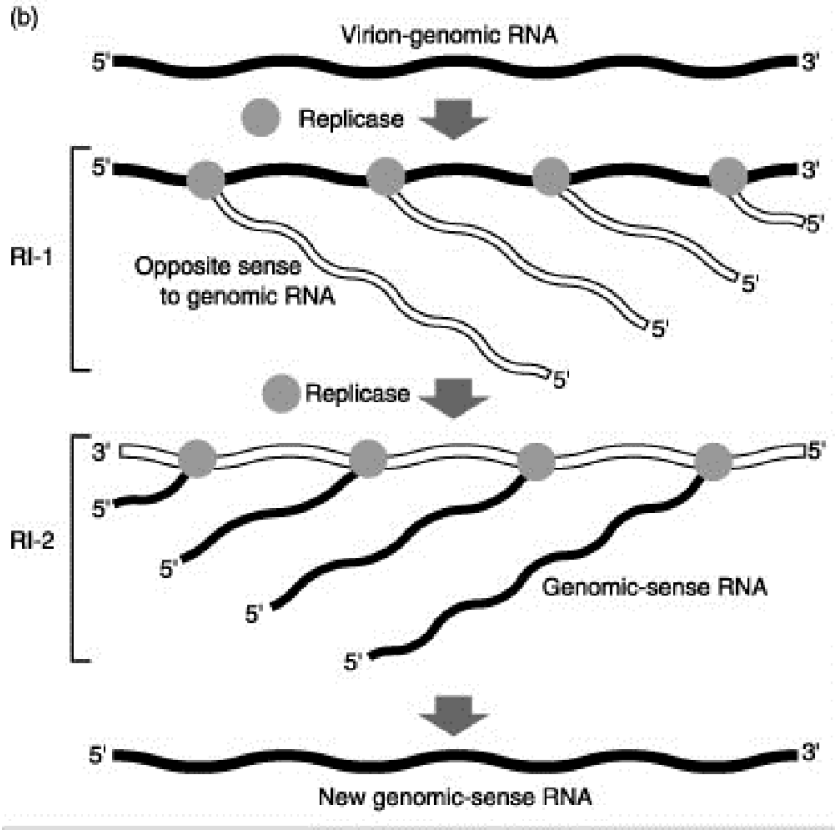
Class IV and V: ssRNA viruses

Per la replicazione, indipendentemente dalla polarità del genoma ad RNA del virus, la RdRp genera, a partire dall' RNA virionico un filamento complementare (antigenoma) della stessa lunghezza del genoma.

In RI-1 viene prodotto lo stampo di polarità opposta al vRNA (intermedio di replicazione)

L'RNA complementare al vRNA (o antigenoma) è lo stampo in RI-2

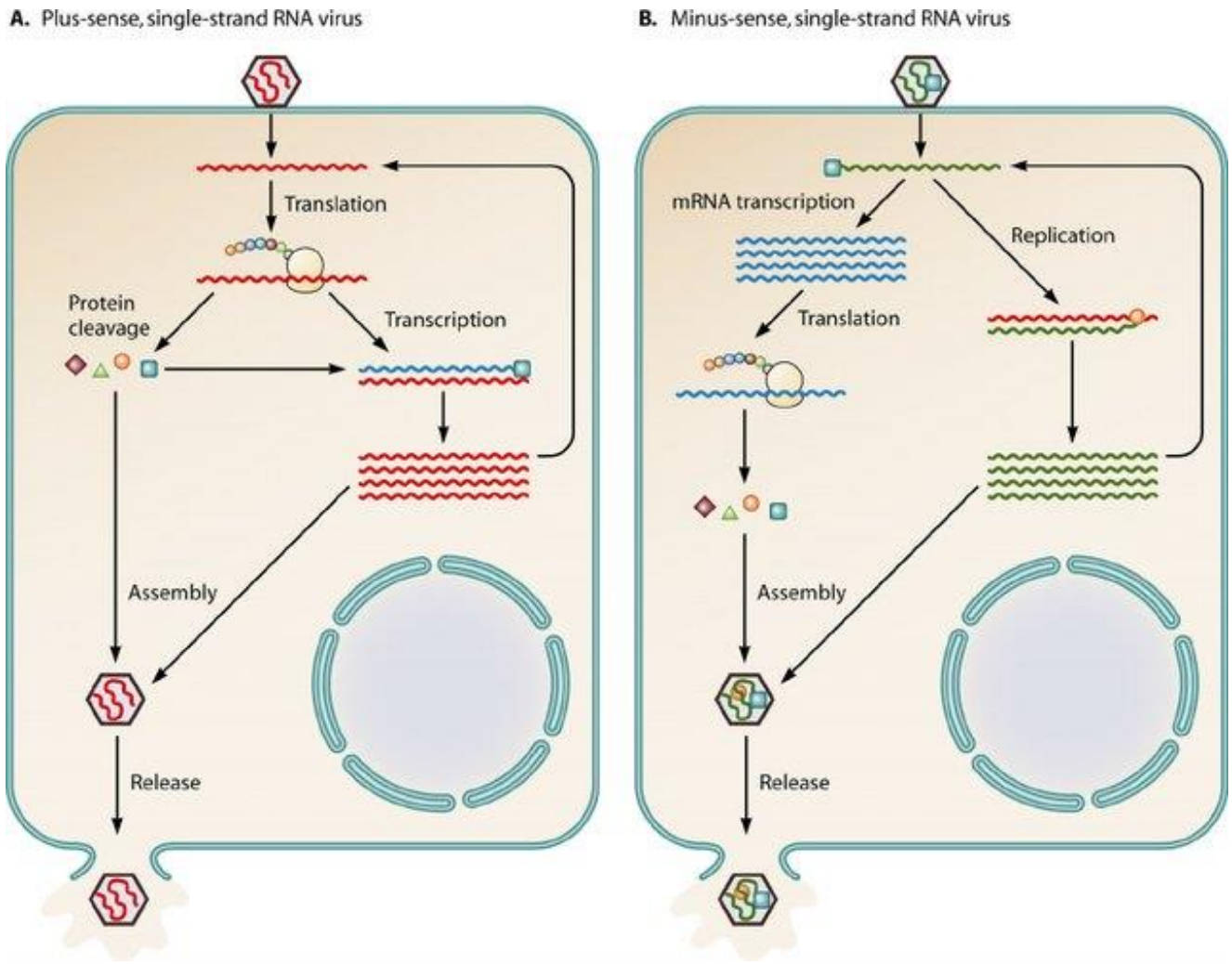
In RI-2 viene sintetizzato l'RNA della stessa polarità di quello presente nel virione (vRNA)



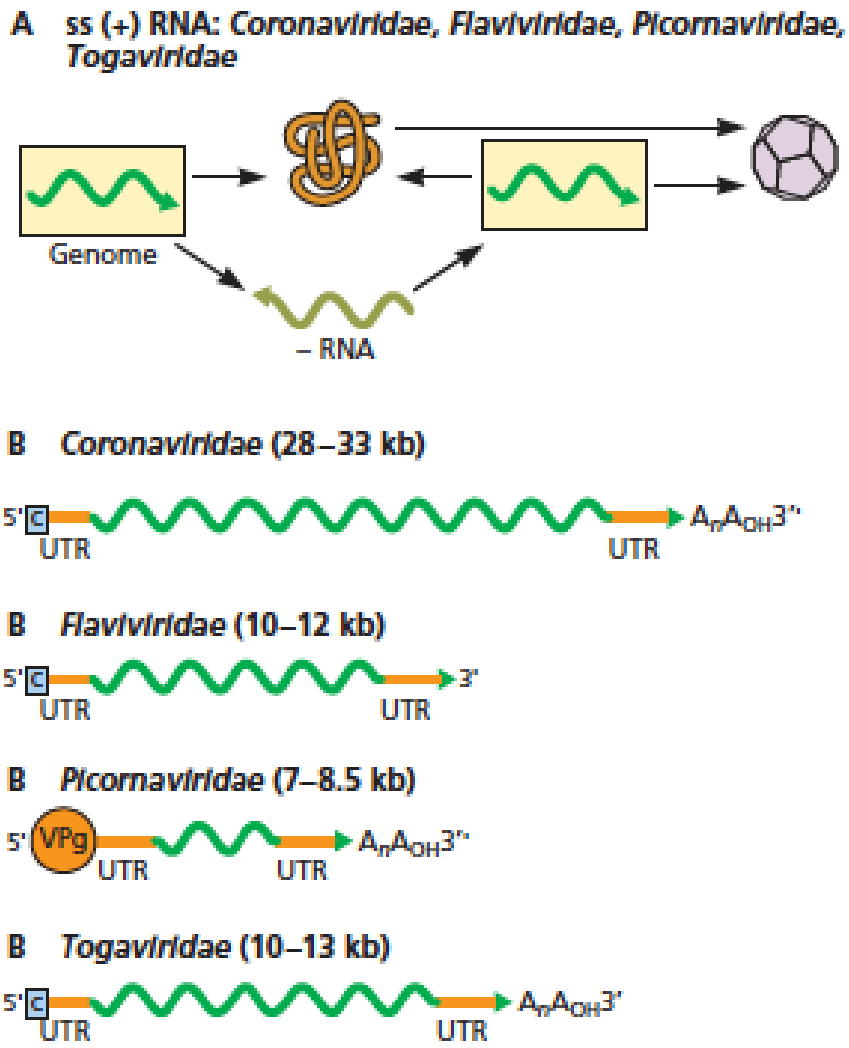
RI= Intermedio di Replicazione

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

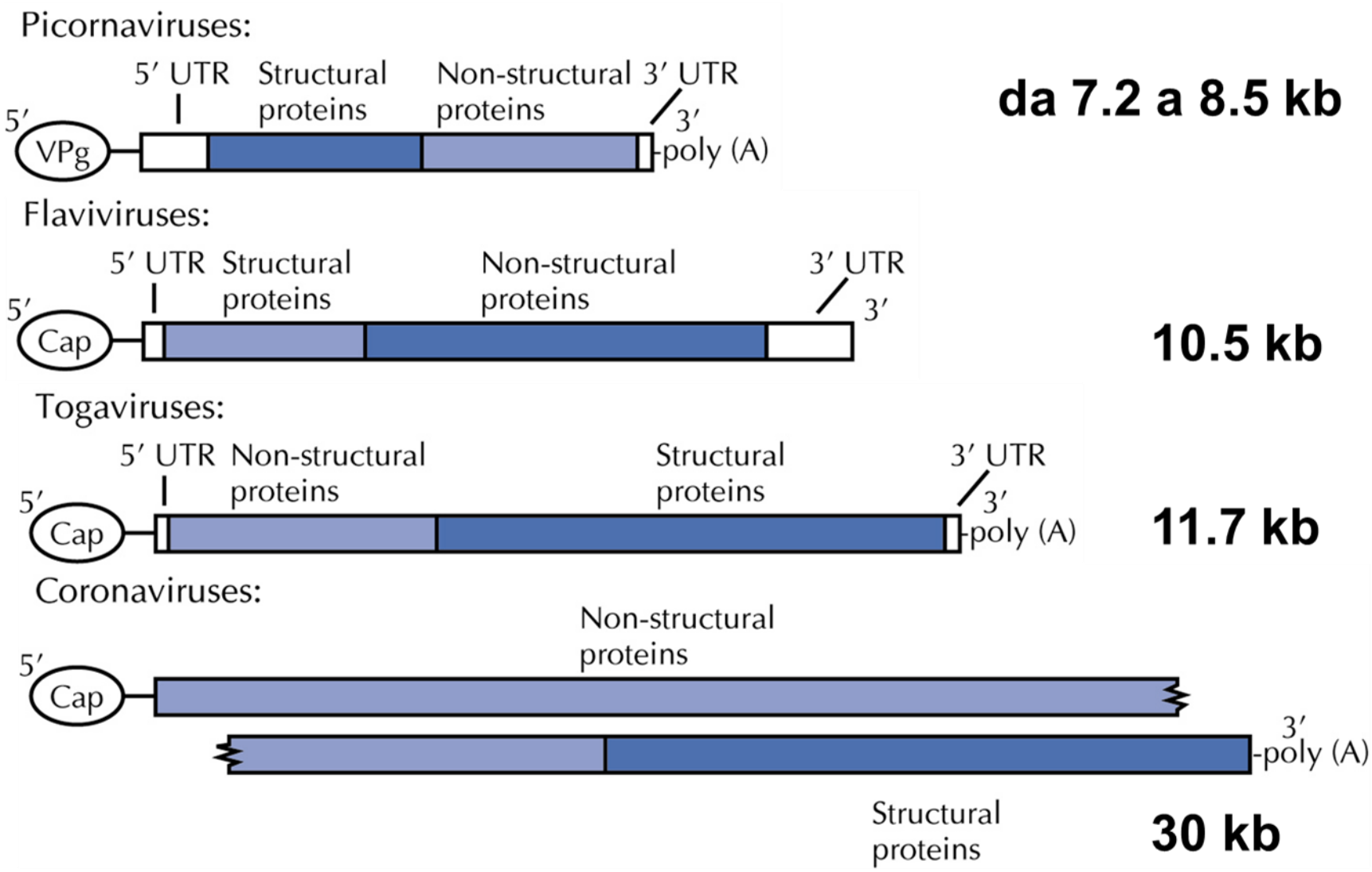
From Flint et al. *Principles of Virology* (2015), ASM Press

Class IV: ssRNA(+), Expression-Replication

Two groups

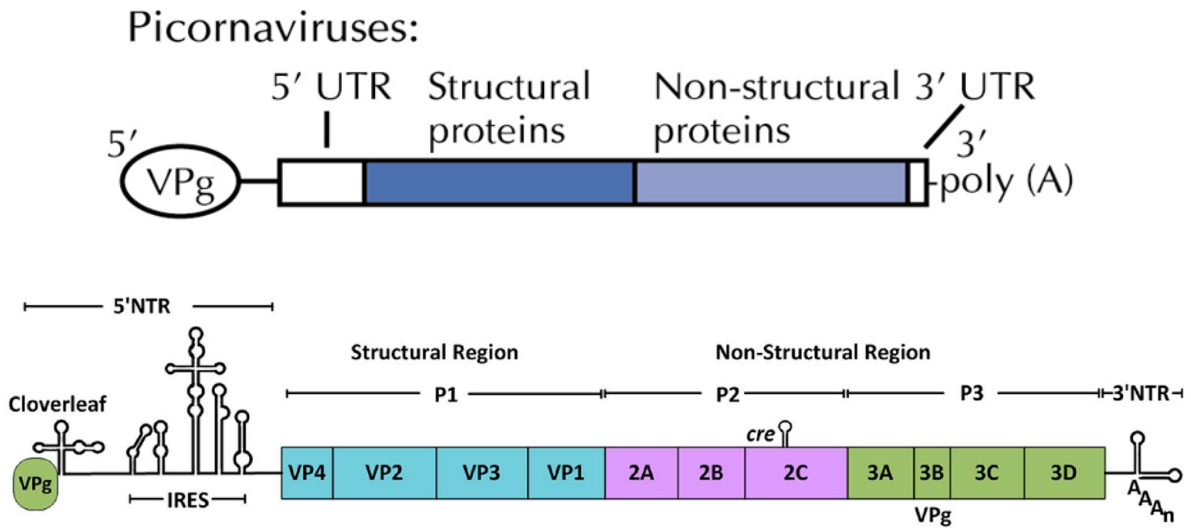
A. vRNA is translated to form a single polyprotein that is subsequently cleaved to give the mature products (polioviruses and flaviviruses are examples) (1 ORF)

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


Picornaviruses:
5′ UTR
Structural proteins
Non-structural proteins
3′ UTR
5′
VPg
3′
poly (A)
da 7.2 a 8.5 kb
Flaviviruses:
5′ UTR
Structural proteins
Non-structural proteins
3′ UTR
5′
Cap
3′
10.5 kb
Togaviruses:
5′ UTR
Non-structural proteins
Structural proteins
3′ UTR
5′
Cap
3′
poly (A)
11.7 kb
Coronaviruses:
Non-structural proteins
5′
Cap
3′
poly (A)
Structural proteins
30 kb

Class IV: Picornaviridae (Polivirus)

Genome size ranges from 7.2 to 8.5 kb. It contains a long 5' UTR (600–1200 nt) important for translation, including IRES (Internal Ribosome Entry Site) sequences; the 3' UTR is shorter (50–100 nt) and is important for the synthesis of the (–) strand.Both ends of the genome are modified: a VPg protein at the 5' end and a polyadenylated tail at the 3' end.

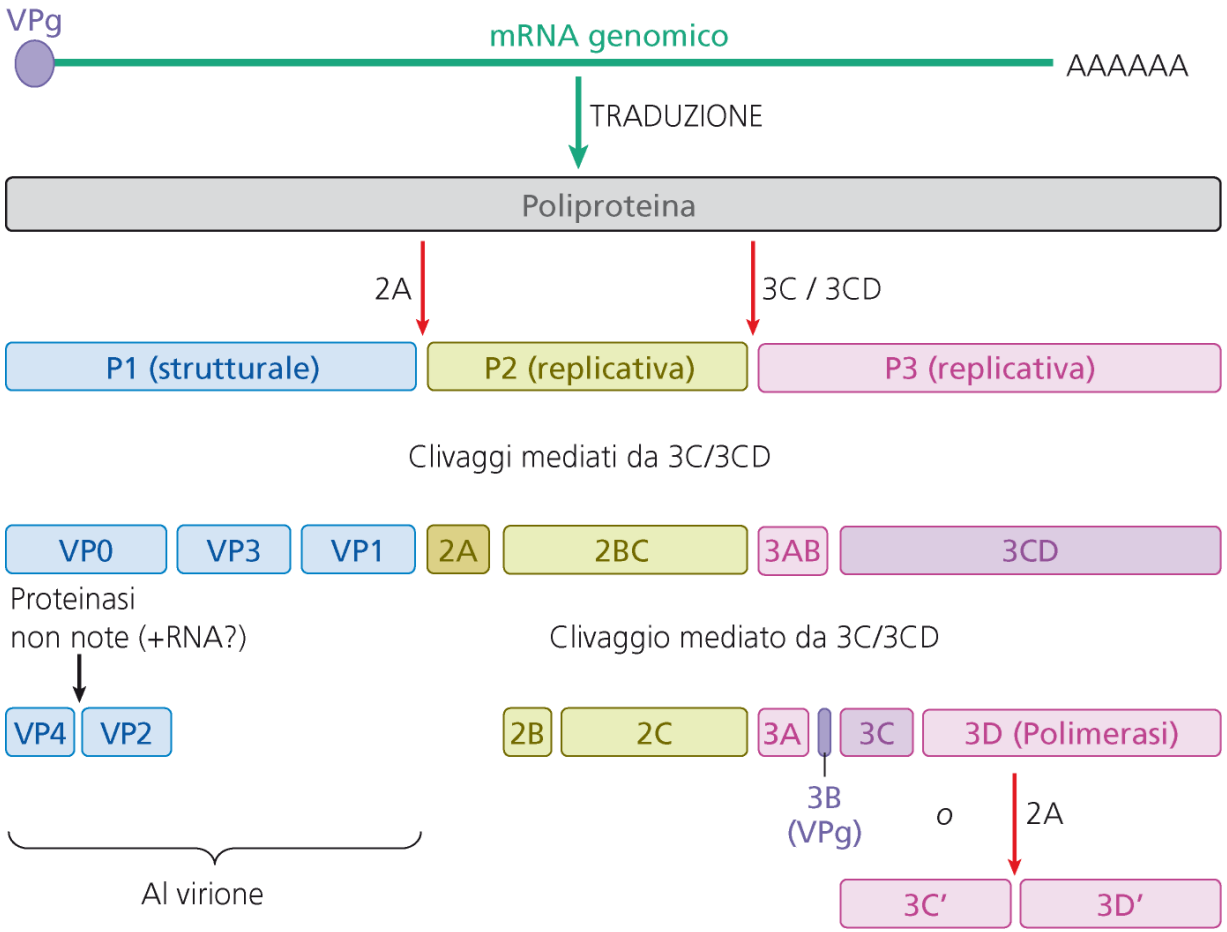


It encodes a single polyprotein.The structural proteins are located at the 5' end, while the non-structural proteins are at the 3' end, and they are all translated simultaneously.

Viruses 2016, 8, 82

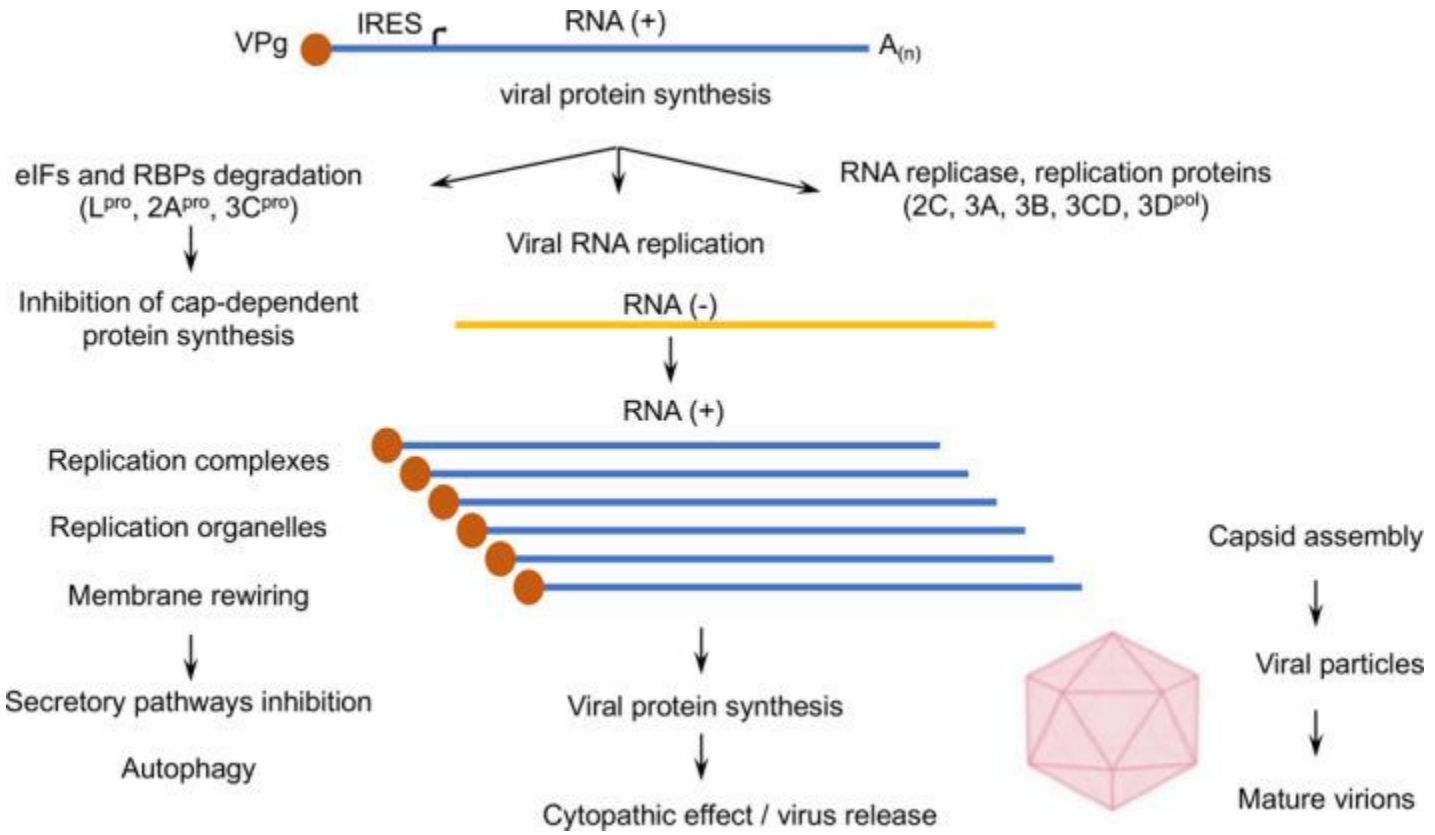
Picornaviridae

Formation of a polyprotein which, following an intramolecular reaction, gives rise to three polypeptides that subsequently undergo further maturation through proteolytic cleavages. Polyprotein processing is co-translational and is carried out by viral autocatalytic activities associated with the 2A protease and the 3C protease.

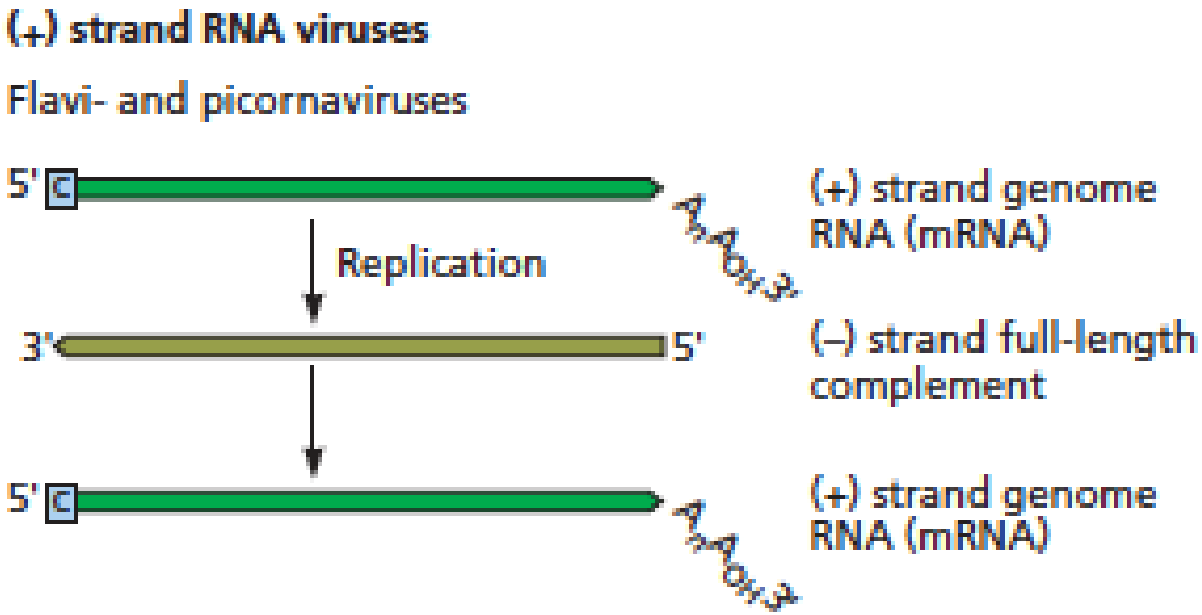


Picornaviridae

The translation of cellular mRNAs is cap-dependent, whereas that of picornavirus mRNAs is IRES-dependent. These viruses are able to block the translation of cellular mRNAs through the proteolytic degradation of components of the cap-binding complex (eIF4GI, eIF4GII).



Class IV: events after primary translation



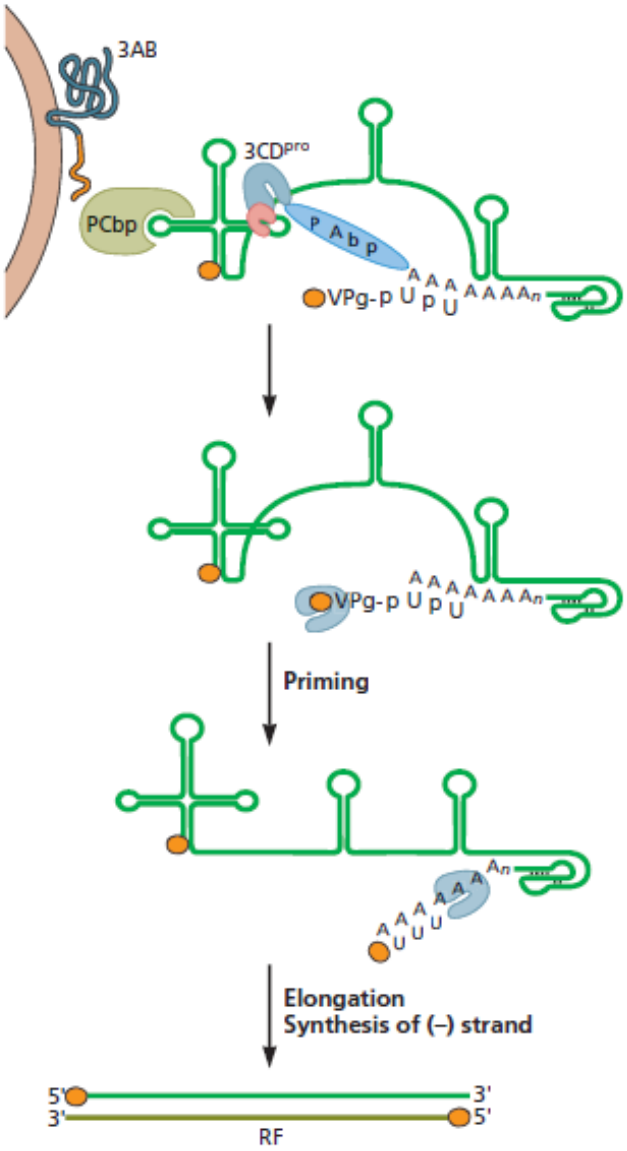
Replication of Picornaviridae: priming

Uridilazione della VPg e sintesi RNA (-).

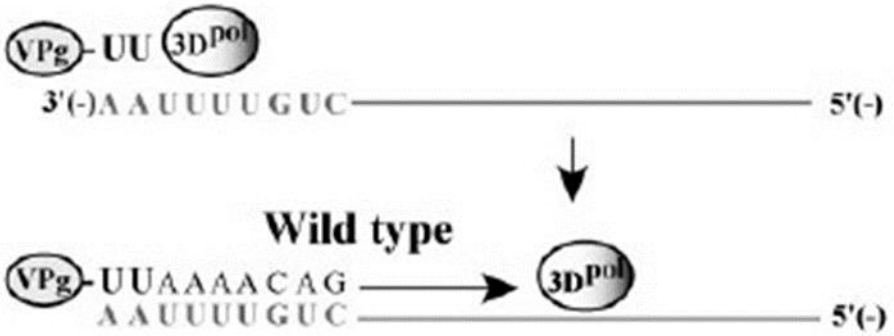
1 Sintesi RNA(-)

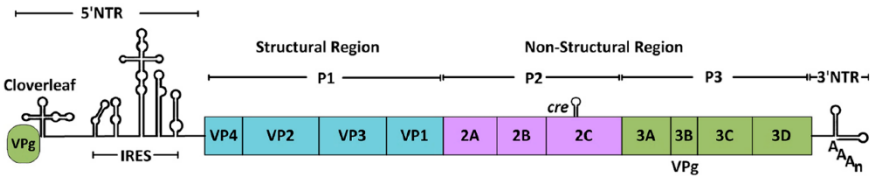
2 Sintesi RNA(+)

1



2



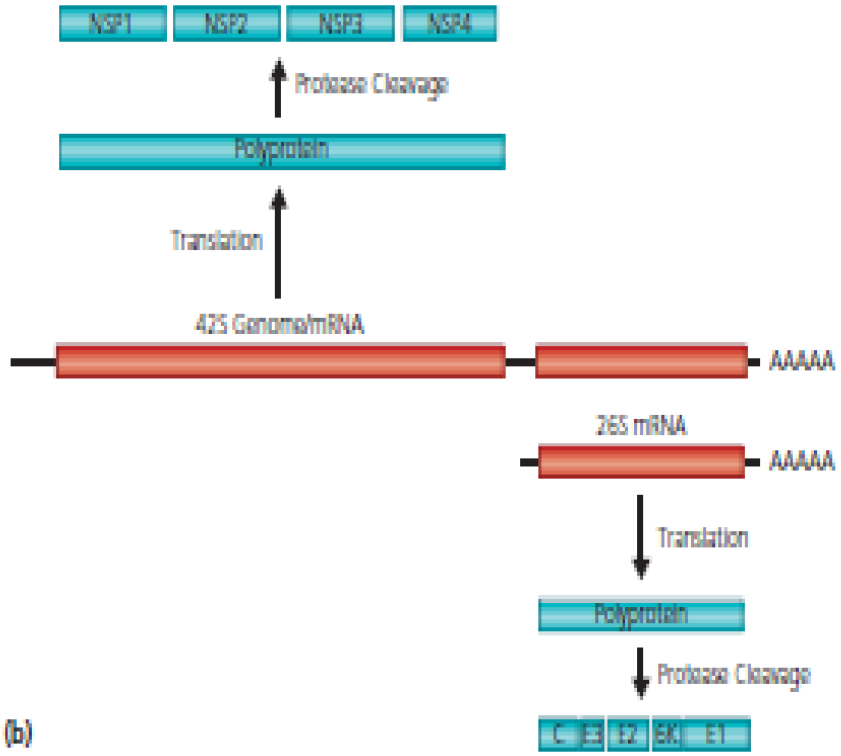


RNA genome

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

Class IV: Togaviridae

Il genoma contiene due ORF. Soltanto quella al 5' è tradotta all'inizio dell'infezione. La seconda ORF è invece tradotta da un mRNA subgenomico



Genoma:11.4 kb coefficiente di sedimentazione 42S

Nella cellula troviamo due mRNA:42S e 26S cap-RNA-polyA, i prodotti di entrambi processati da proteasi

RNA (–): solo 42S
Internal initiation site per il 26S

mRNA26S prodotto solo a seguito di replicazione (regolazione temporale)
Ed in maniera molto efficiente (molte copie delle proteine strutturali)

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

Class IV: Togaviridae

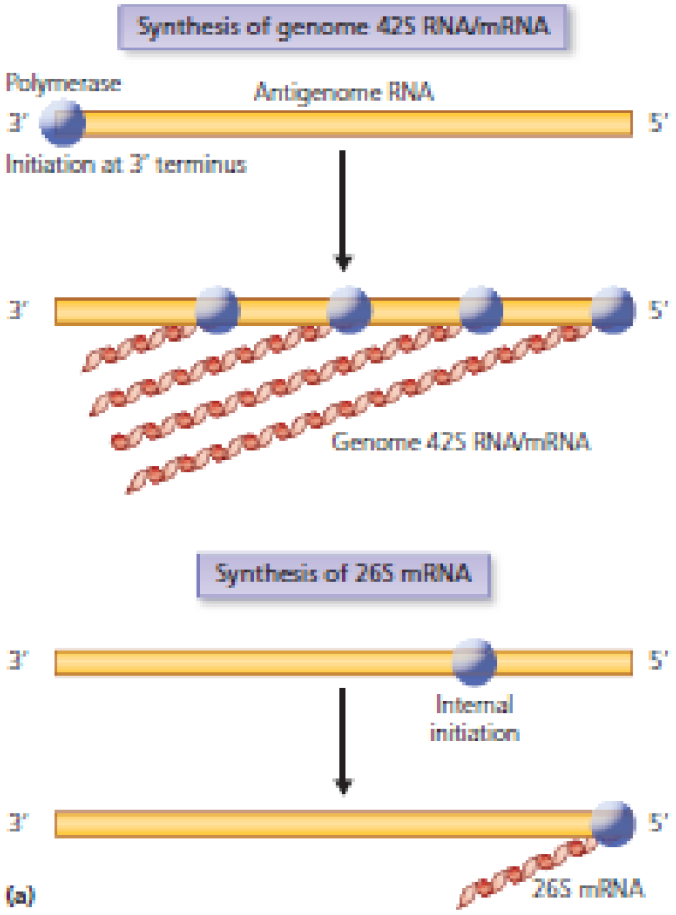
Sintesi dell'RNA genomico e dell'RNA subgenomico

Il prodotto della **traduzione primaria** è una poliproteina che viene maturata dalle attività autocatalitiche proteasiche virali e poi maturata ulteriormente da altre proteasi.

Tra le proteine non strutturali viene prodotta l'RNA polimerasi RNA dipendente la quale, utilizzando come stampo il genoma liberato nel citoplasma, sintetizza l'**antigenoma** (filamento a polarità –).

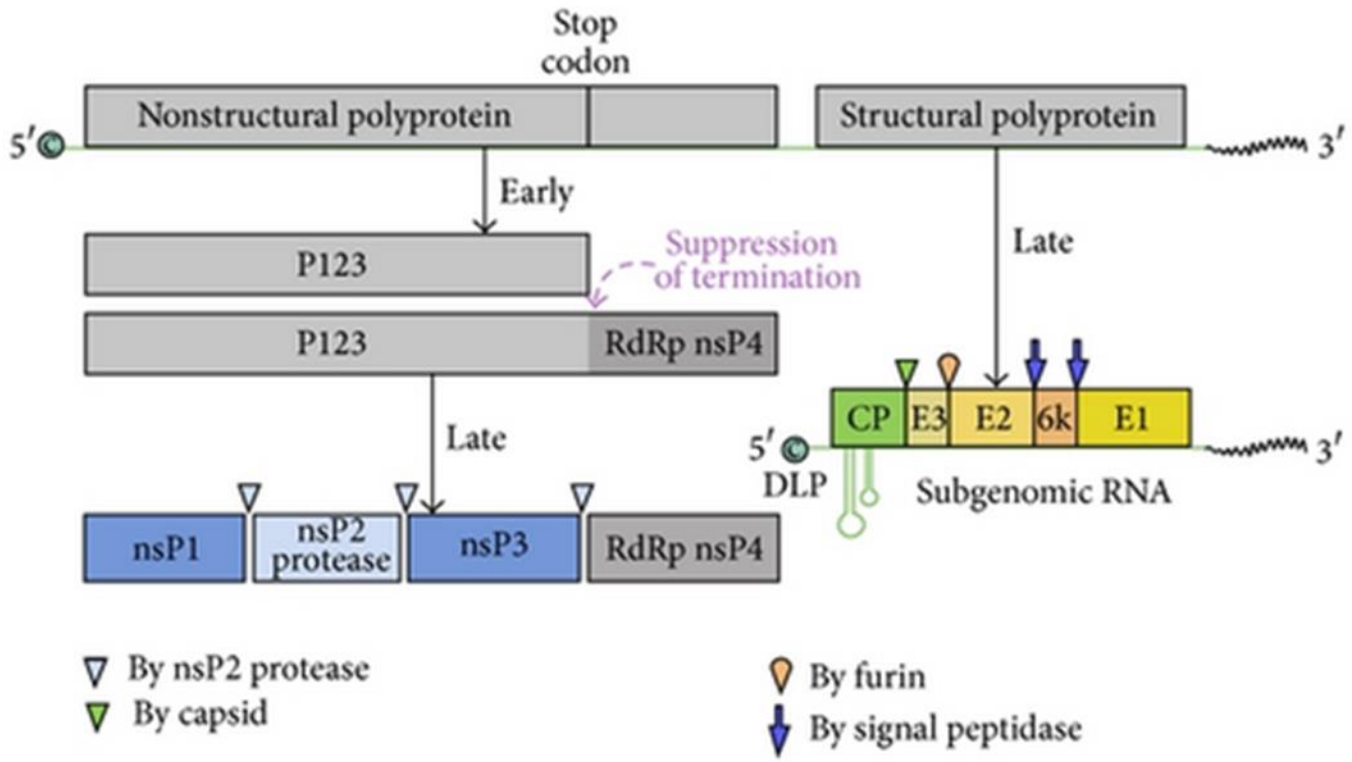
L'antigenoma verrà utilizzato come stampo per sintetizzare nuovi genomi virali (42S RNA).

Tra le proteine non strutturali vengono prodotti fattori che portano alla formazione di complessi polimerasici con specificità diverse per quanto riguarda l'inizio della trascrizione in grado di sintetizzare l'mRNA 26S ⇒ traduzione delle proteine strutturali



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

Class IV: Togaviridae

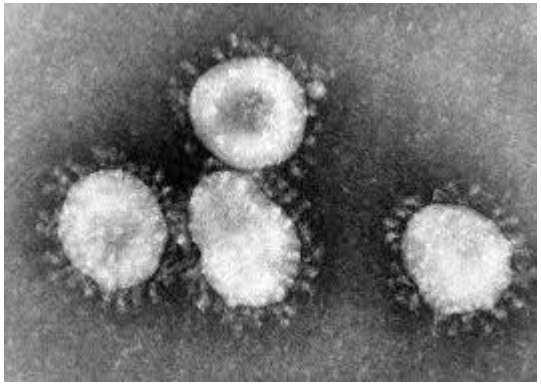


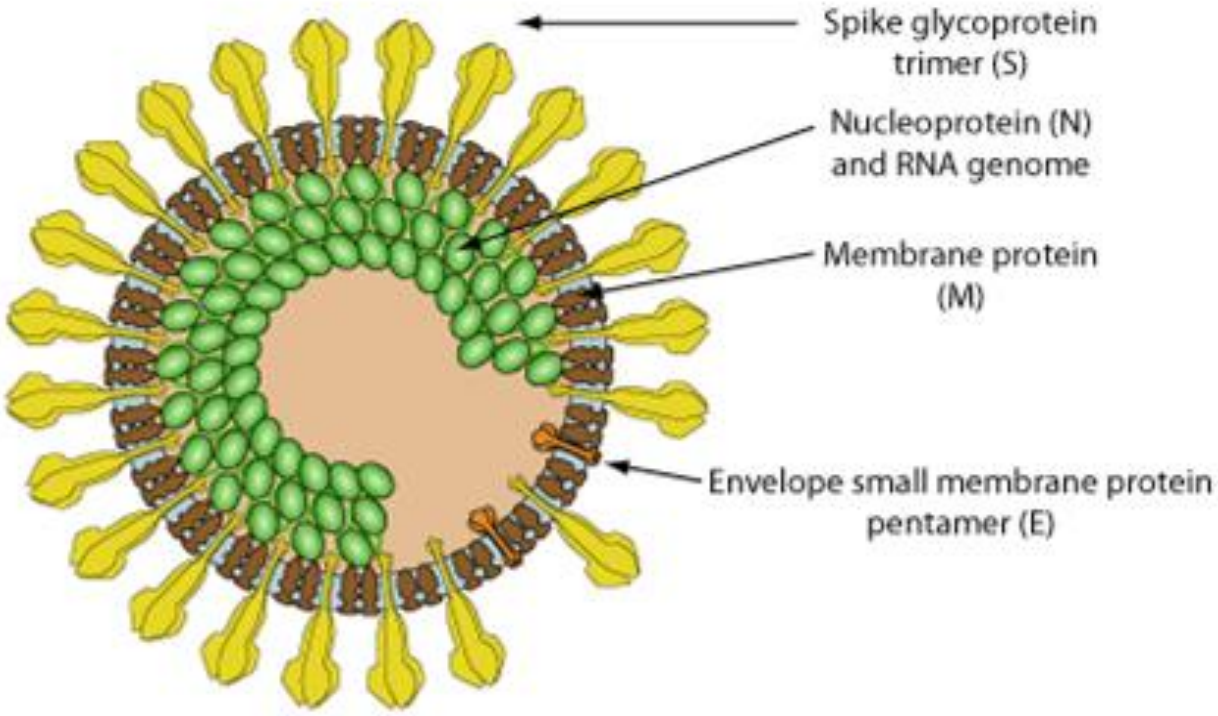
The virion RNA is infectious and serves as both genome and viral messenger RNA. The whole genome is translated into a non-structural polyprotein which is processed by host and viral proteases. RdRp is expressed by suppression of termination at the end of 10% of nsP polyproteins.

In late phase of infection, structural polyprotein is expressed through a subgenomic mRNA. The mRNA contains a Downstream Hairpin Loop (DLP)to avoid the translation shutoff induced by the host PKR in the late phase of infection.

ssRNA (+) viruses: Coronaviridae

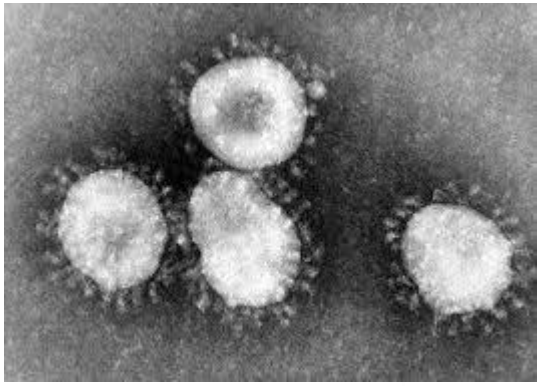
Coronavirus virion

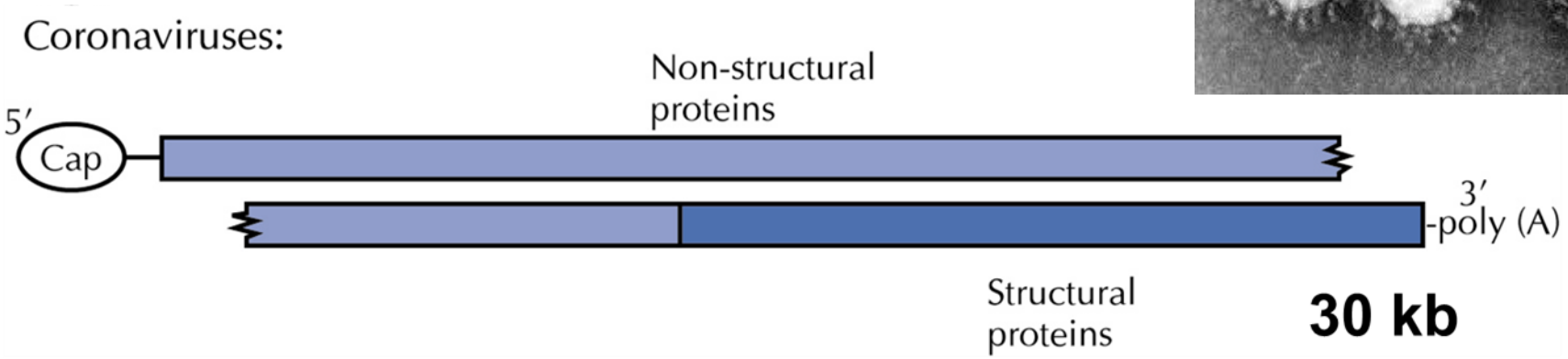




Proteina S maggiore bersaglio di anticorpi neutralizzanti, responsabile del legame al recettore cellulare

ssRNA (+) viruses: Coronaviridae

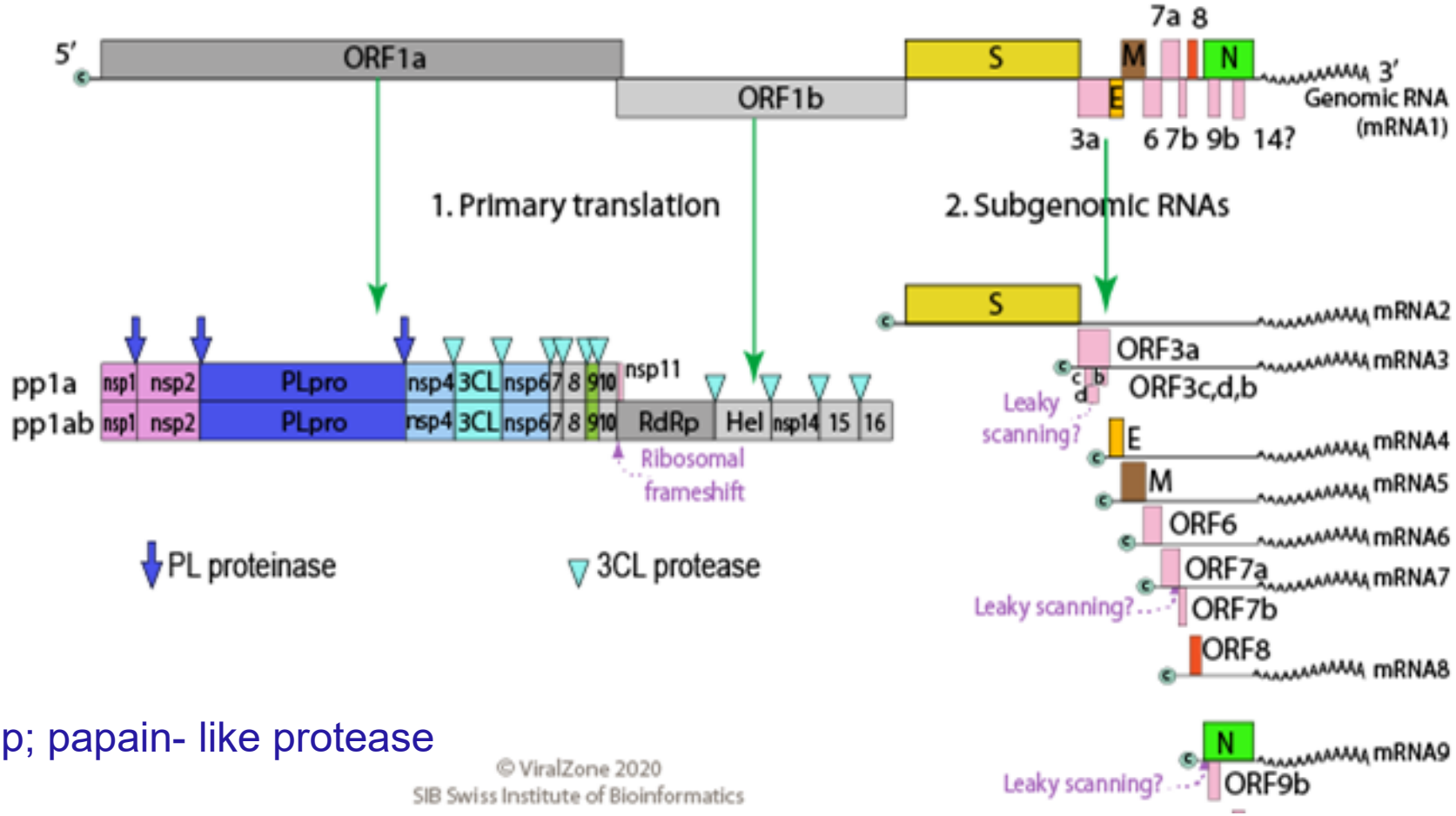




La temporalità della produzione delle proteine virali è simile a quanto osservato per i Togaviridae.

Nei Coronaviridae è presente una lunga porzione al 5’ che viene tradotta durante la traduzione primaria e che porta alla produzione di due poliproteine che verranno maturate dall’azione di due proteasi virali e formeranno fino a 16 proteine non strutturali

SARS-CoV-2 genome



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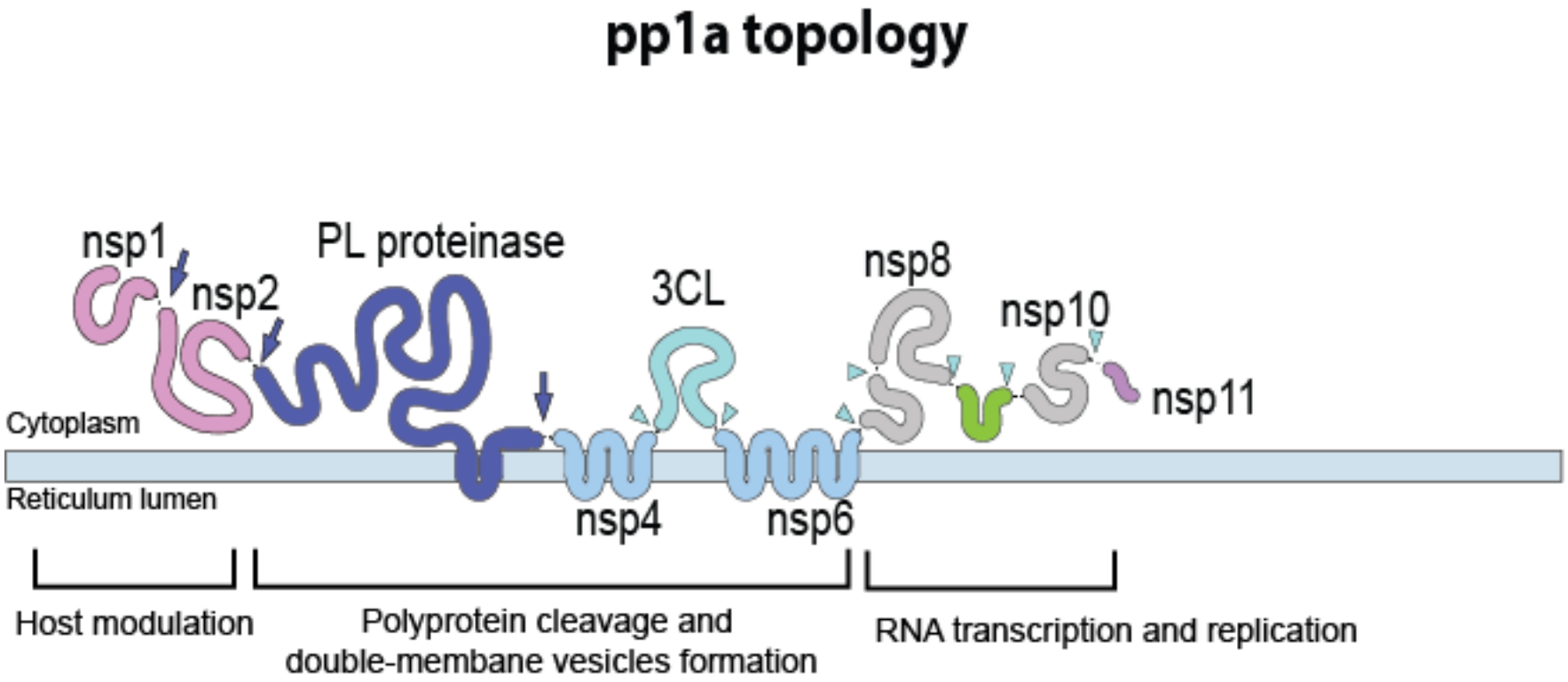
SIB Swiss Institute of Bioinformatics

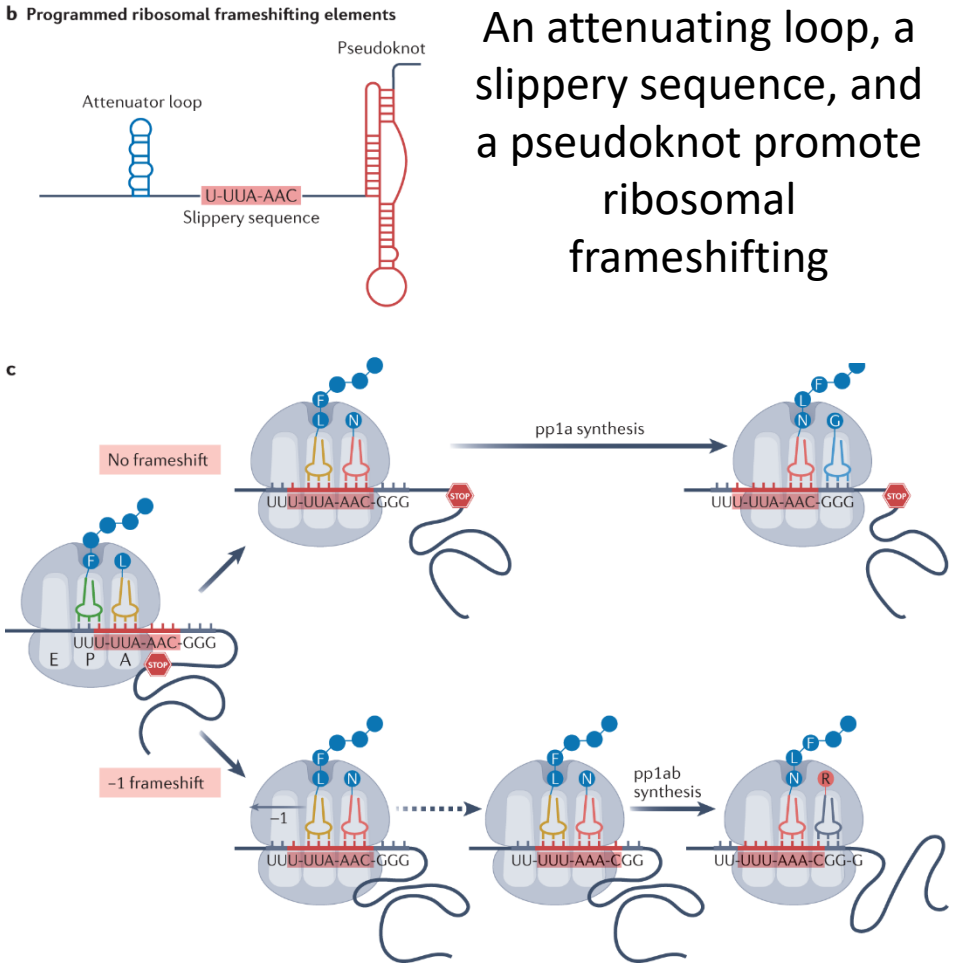
PLp; papain- like protease

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

Polyprotein product expression



Ribosomal frameshift

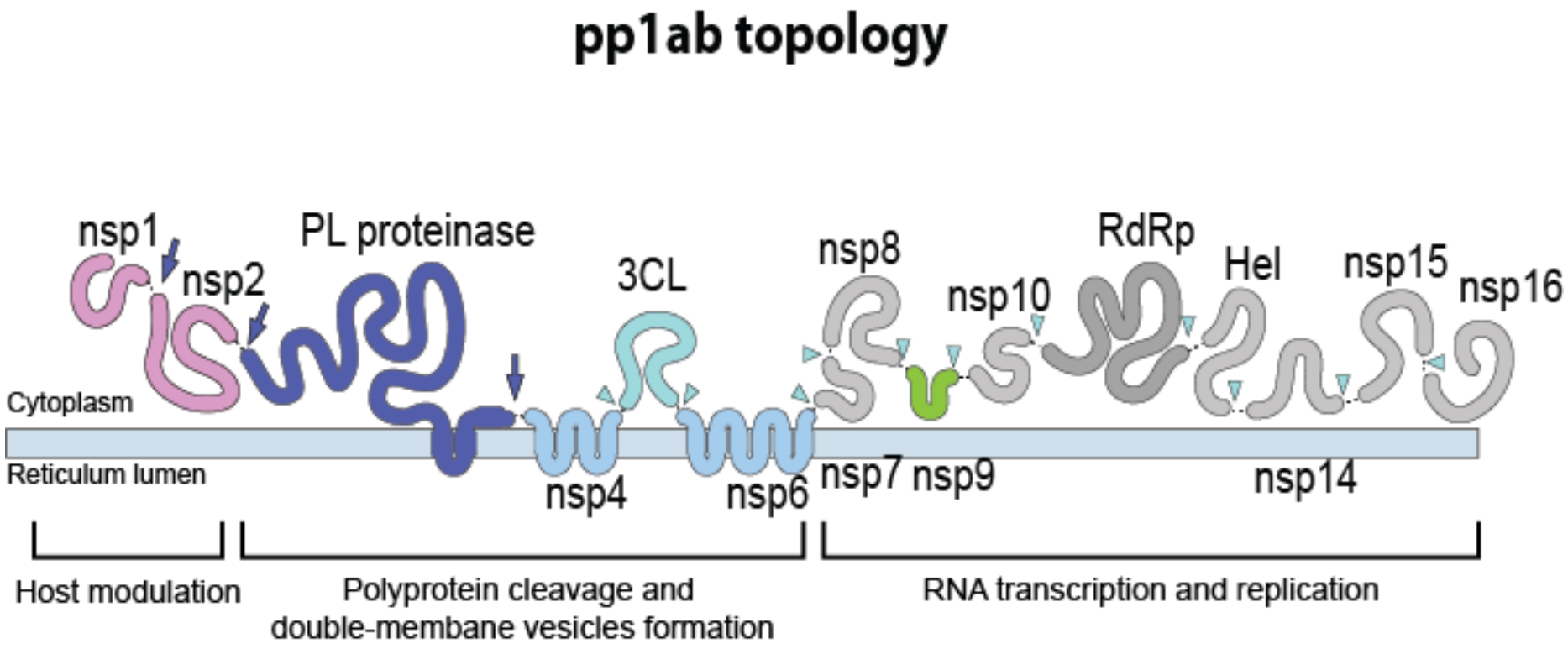


An attenuating loop, a slippery sequence, and a pseudoknot promote ribosomal frameshifting

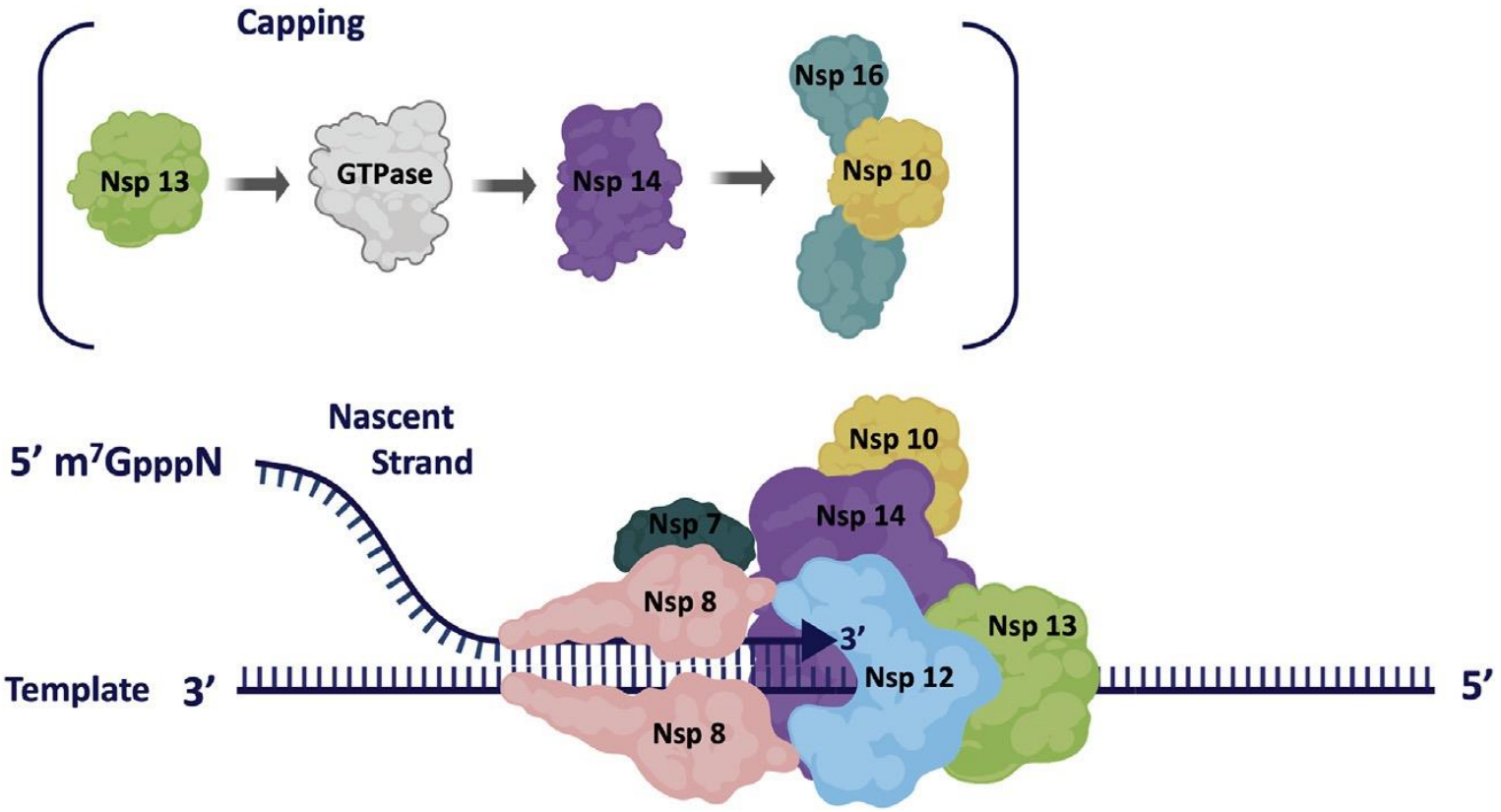
b | RNA motif and structures that promote a frameshift from ORF1a to ORF1b, thereby controlling the synthesis of pp1ab. The key programmed ribosomal frameshifting (PRF)-stimulating RNA structure — a pseudoknot — interacts with the ribosome and induces its pausing, which generates tension in the gRNA template. As a result, ribosomes can slip one nucleotide backwards on the 'slippery sequence' (−1 PRF). An attenuating RNA loop located upstream of the slippery sequence also contributes to modulating PRF frequency.

c | Model of −1 PRF at the ORF1a–ORF1b junction, showing the regulatory RNA elements inducing a simultaneous −1 shift of the tRNAs bound to the A and P sites of the ribosome, which can then translate ORF1b. The one-letter code for amino acids (circles) is used. A stop sign represents the ORF1a stop codon.

Polyprotein product expression

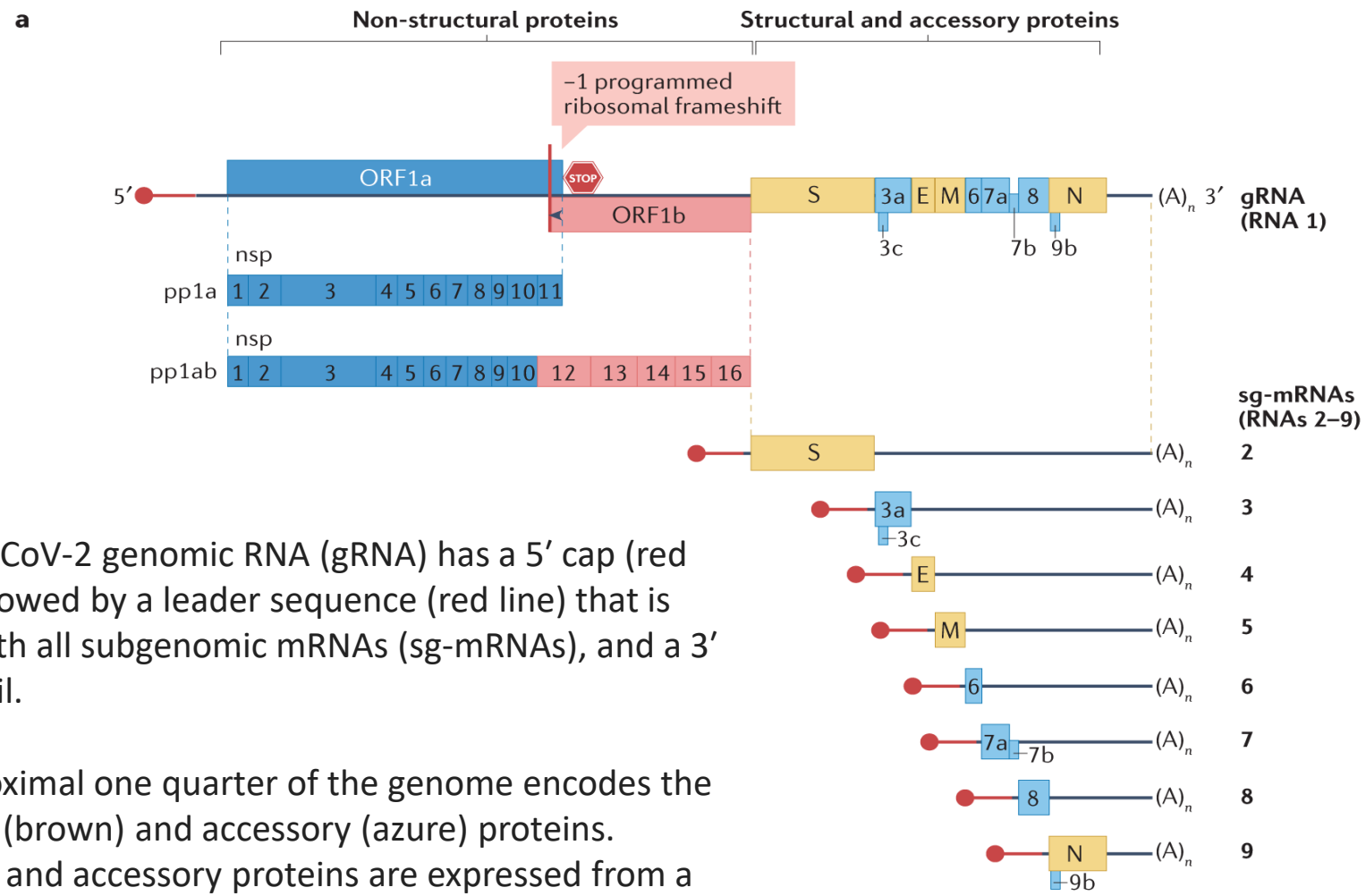


Model of the Core Replication and Proofreading Complex of SARS-CoV Nsp12-RdRp replicates and transcribes the genome and sgmRNAs. Nsp7/nsp8 proteins confer processivity to the polymerase. Nsp13 unwinds dsRNA ahead of the polymerase. Nsp14-ExoN complexed with its co-factor nsp10 proofreads the nascent 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

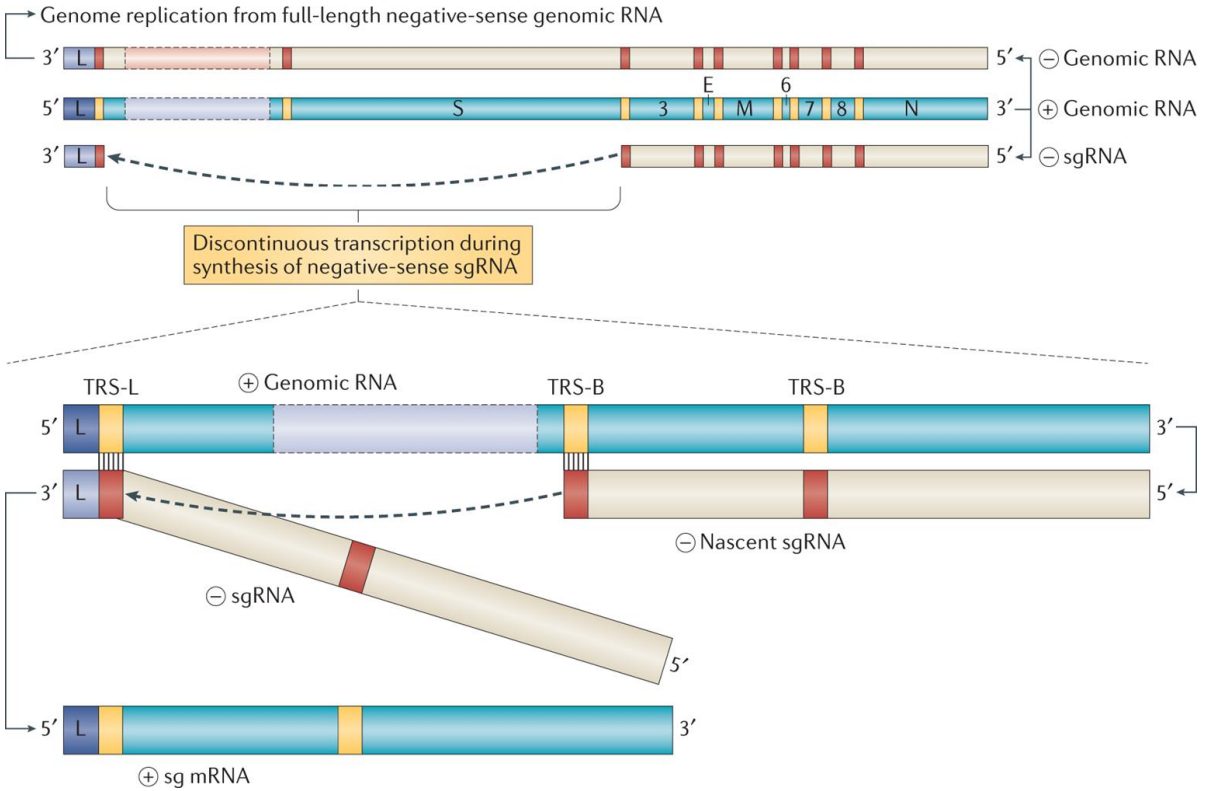
Subgenomic mRNAs



The SARS-CoV-2 genomic RNA (gRNA) has a 5′ cap (red circle) followed by a leader sequence (red line) that is shared with all subgenomic mRNAs (sg-mRNAs), and a 3′ poly(A) tail.

The 3′-proximal one quarter of the genome encodes the structural (brown) and accessory (azure) proteins. Structural and accessory proteins are expressed from a nested set of sg-mRNAs, *with ORF3c, ORF7b and ORF9b being expressed via ribosomal 'leaky scanning'.*

Coronavirus discontinuous transcription.



Full- length +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.

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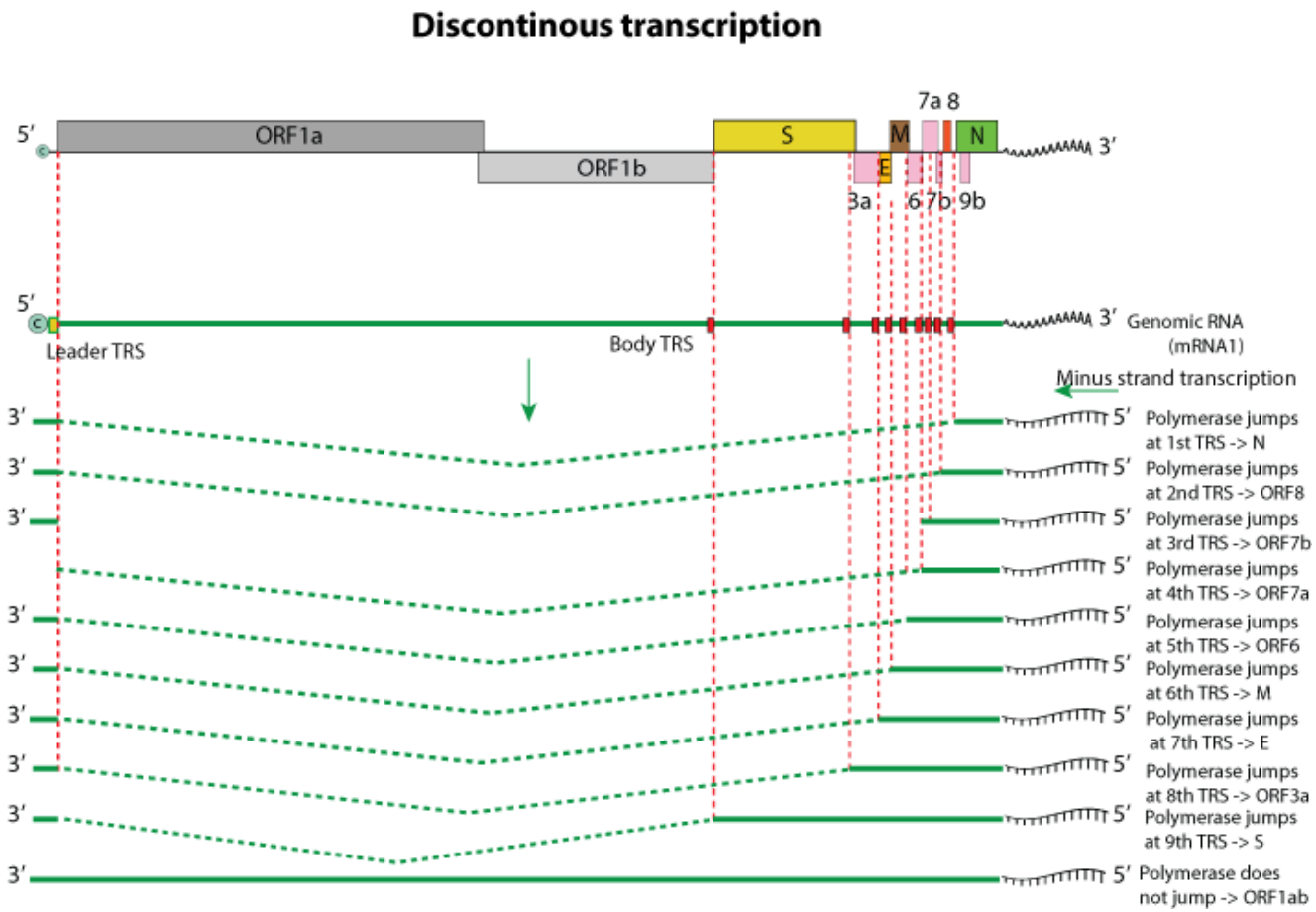


Subgenomic RNAs (sgRNAs) are created by discontinous transcription. During transcription of minus strand RNA, the polymerase have chances to pause on **transcription-regulating sequences (TRS)** and jump to leader TRS, thereby creating a major deletion. This creates a set of 9 (-)RNAs that are subsequently replicated 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 subgenomig mRNA products

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

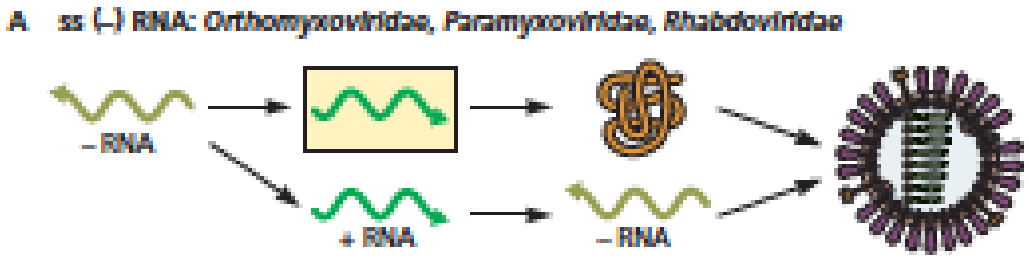
S (spike glycoprotein) is responsible for host cell receptor recognition and binding, and for fusion of virion envelope with endosomal membrane

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.

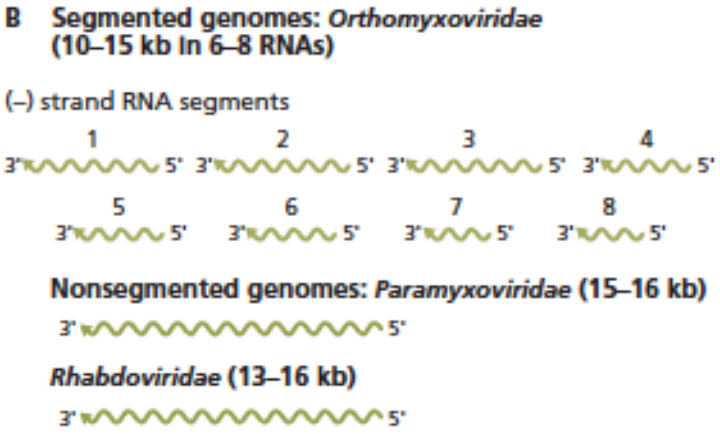
N protein is important for encapsidation of viral RNA and acts as an interferon (IFN) antagonist.

Class V: ssRNA (-)



The genome of these viruses can be divided into two types

- Non-segmented genome (order *Mononegavirales*)
- Segmented genome (for example *Orthomyxoviridae*)



Negative-strand RNA viruses have genomes with a polarity opposite to that of messenger RNA.

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

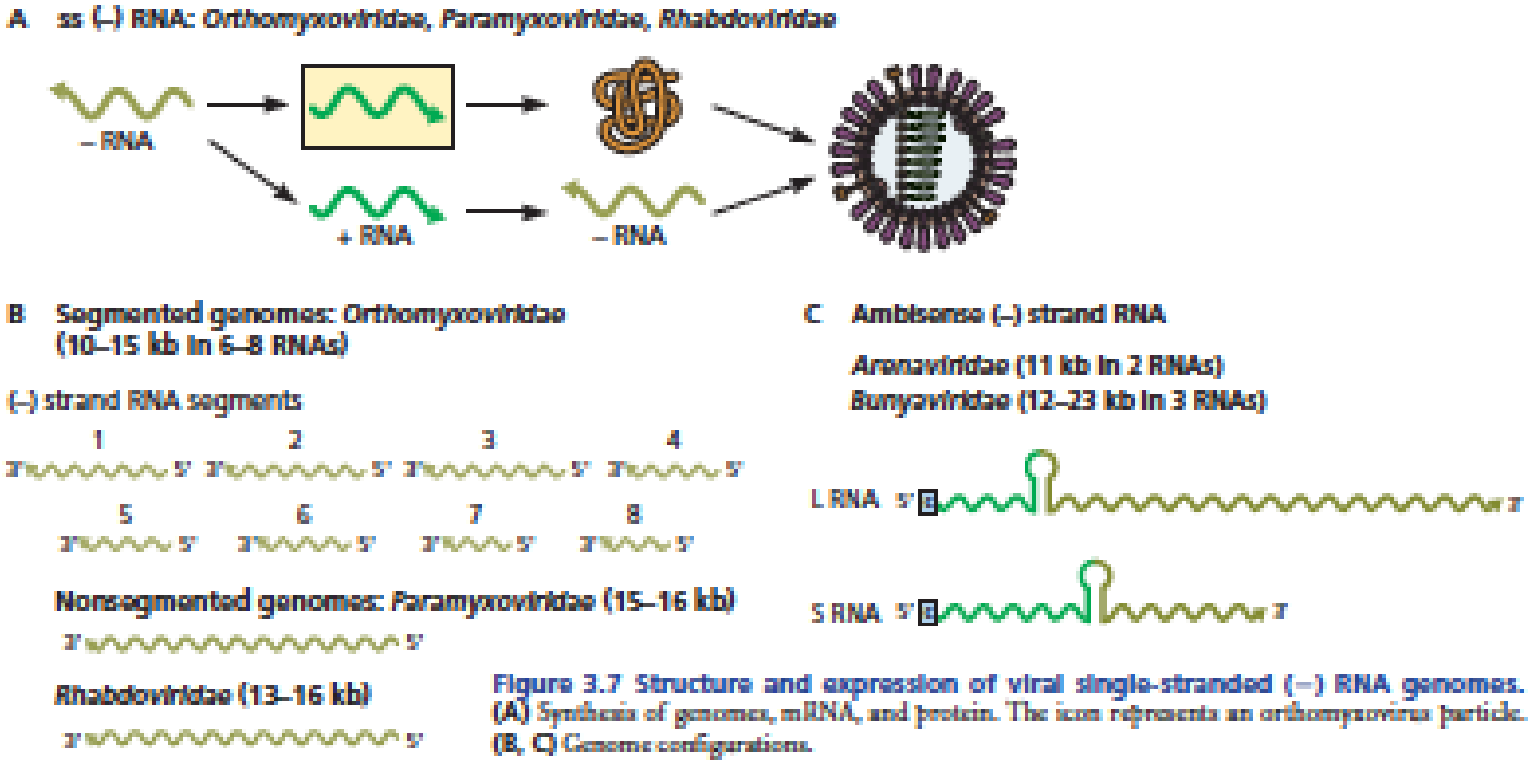


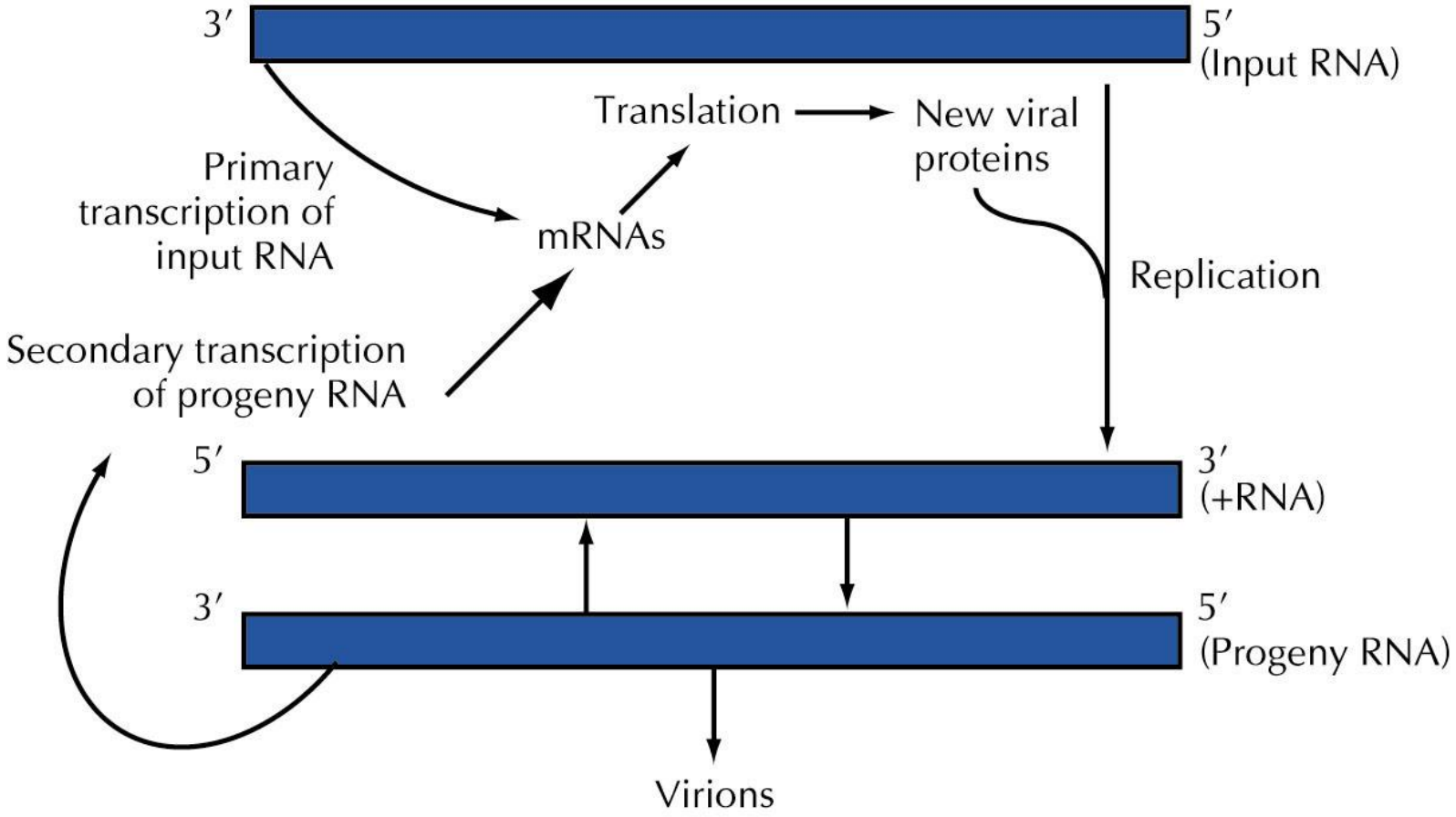
Figure 3.7 Structure and expression of viral single-stranded (–) RNA genomes. (A) Synthesis of genomes, mRNA, and protein. The icon represents an orthomyxovirus particle. (B, C) Genome configurations.

Some families have a monopartite genome, such as those belonging to the order Mononegavirales; others have a segmented genome (Orthomyxoviridae with 8 or 7 segments, Arenaviridae with 2 segments, and Bunyaviridae with 3 segments).

The latter two families are unique because they possess a genome defined as ambisense (i.e., a genome containing RNA regions of both positive and negative polarity).

Class V: ssRNA (-), Expression-Replication

First step in virus multiplication is transcription by the virion RNA-dependent RNA polymerase

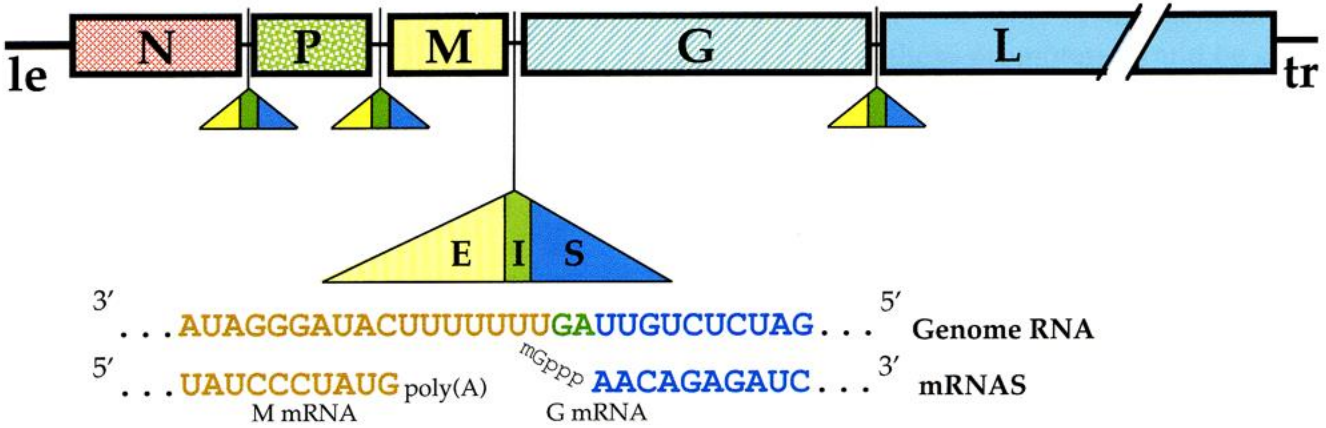


From Cann *Principles of molecular virology* (2001). Academic Press

ssRNA (-), Rhabdoviridae (VSV)

Genome organization

A. Location of intergenic sequences of VSV (a rhabdovirus), and detailed view of the M/G intergenic region

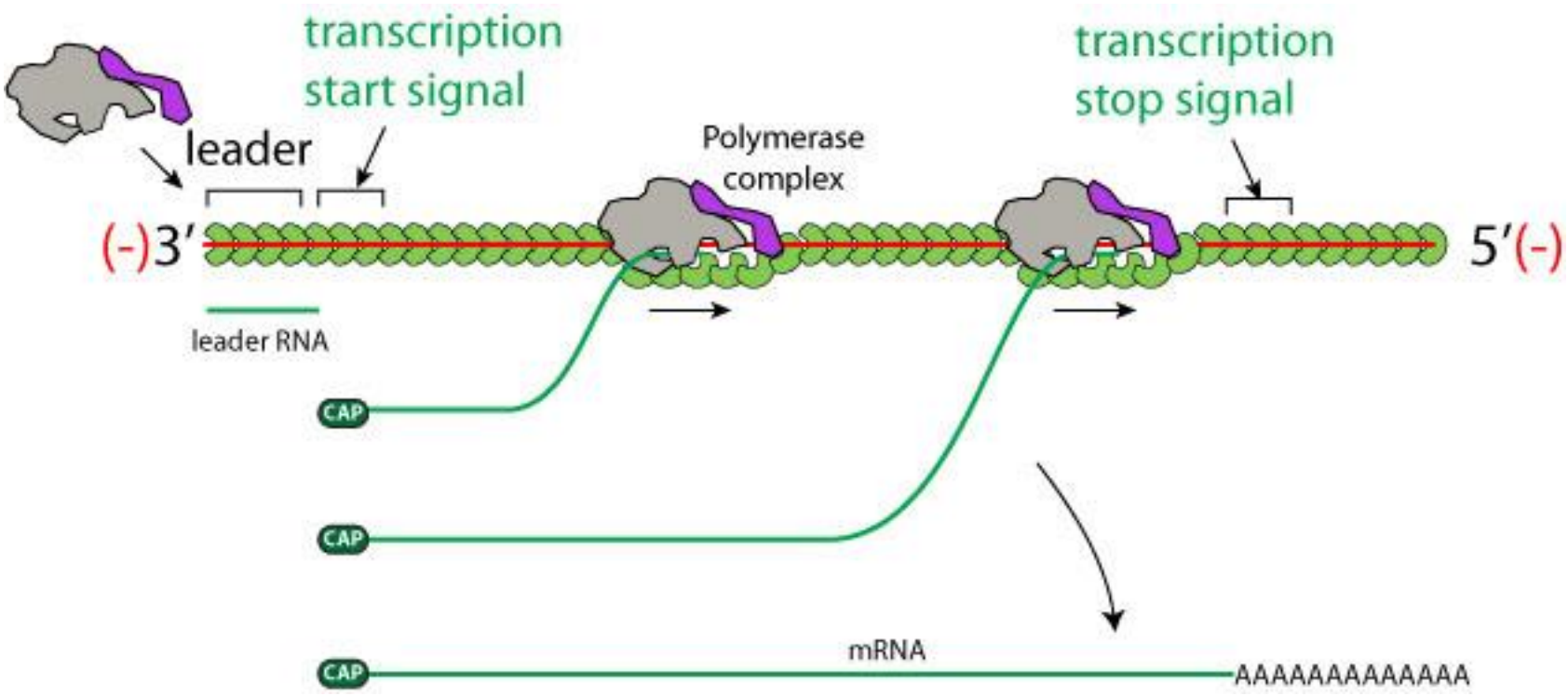


B. Genomic sequences at other intergenic regions in the VSV genome

	3′	5′
N/P	. . . CGAUGUAUACUUUUUUUGAUUGUCUAUAG . . .	
P/M	. . . CAUCUGAUACUUUUUUUCAUUGUCUAUAG . . .	
G/L	. . . UUAAAAAUACUUUUUUUGAUUGUCGUUAG . . .	

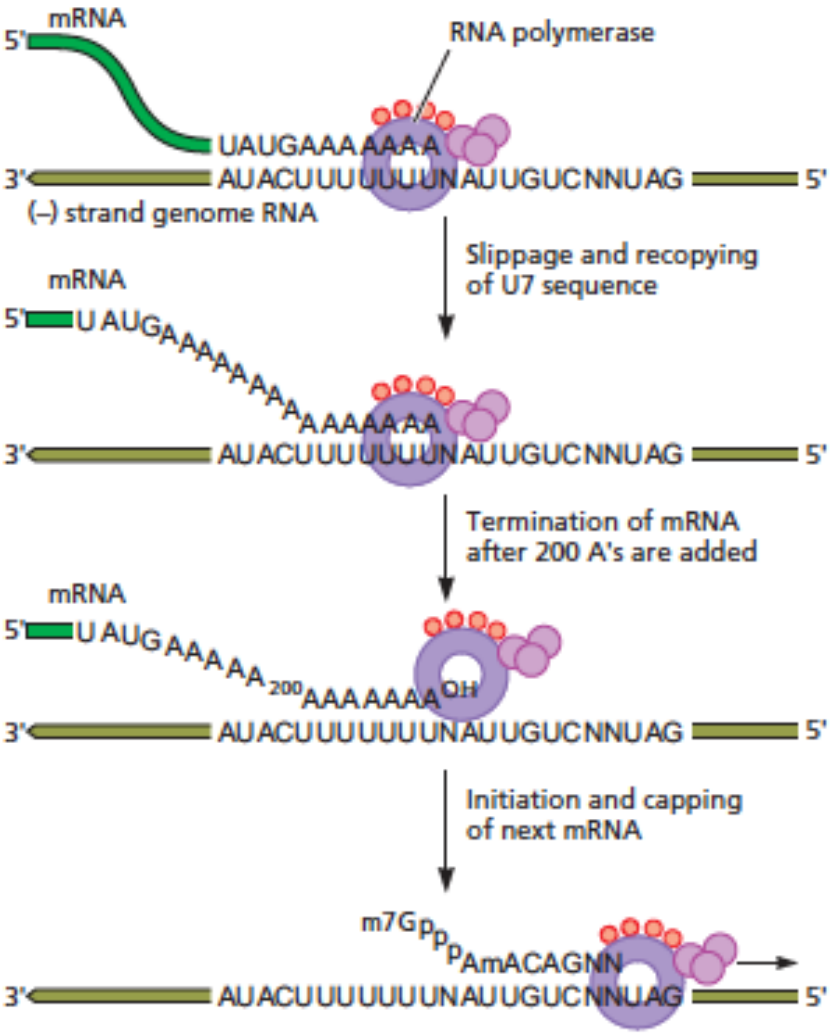
E=end
I=intergenic
S=start

ssRNA (-), Rhabdoviridae (VSV)



The RNA dependent RNA polymerase (RdRp)complex initiates transcription by binding to the leader sequence in 3' of the genomic negative strand RNA. The RdRp transcribes a 5' triphosphate-leader RNA, then stop and restart on the transcription initiation signal of the N gene. The RNA initiated on this signal is capped.

ssRNA (-): Rhabdoviridae (VSV)



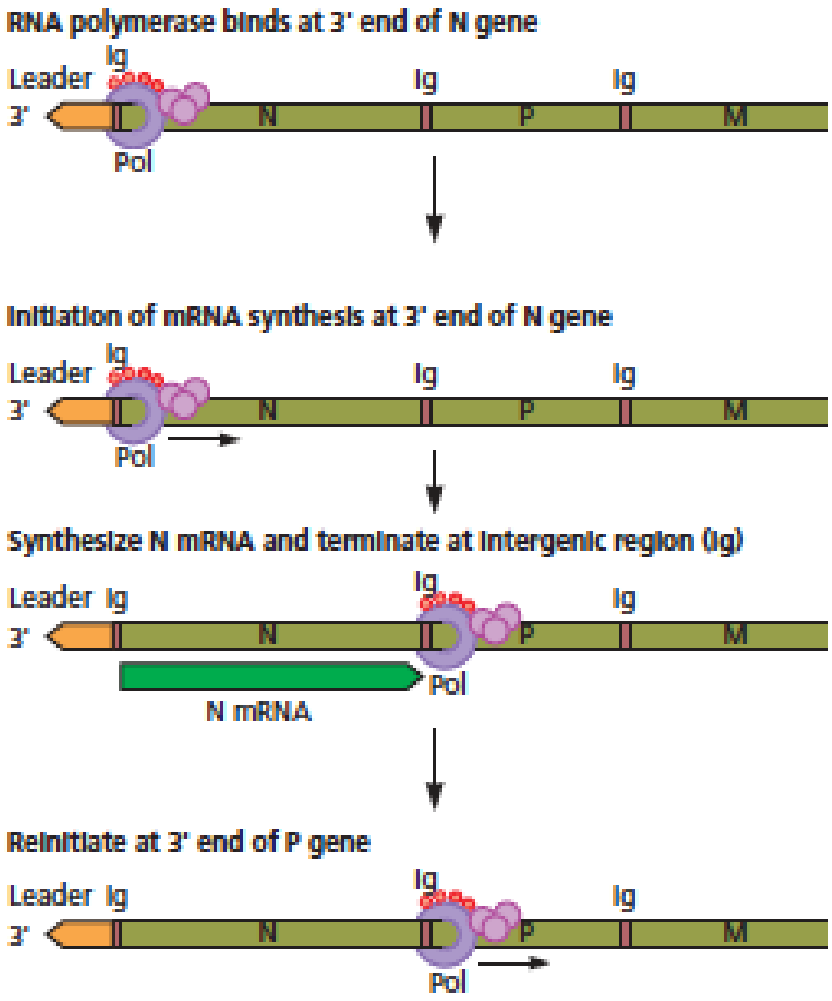
Poly(A) addition and termination at an intergenic region during vesicular stomatitis virus mRNA synthesis.

Copying of the last seven U residues of an mRNA-encoding sequence is followed by slipping of the resulting seven A residues in the mRNA off the genomic sequence, which is then recopied. This process continues until approximately 150 A residues are added to the 3’ end of the mRNA.

Termination then occurs, followed by initiation and capping of the next mRNA.

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

Stop-start model of VSV mRNA synthesis



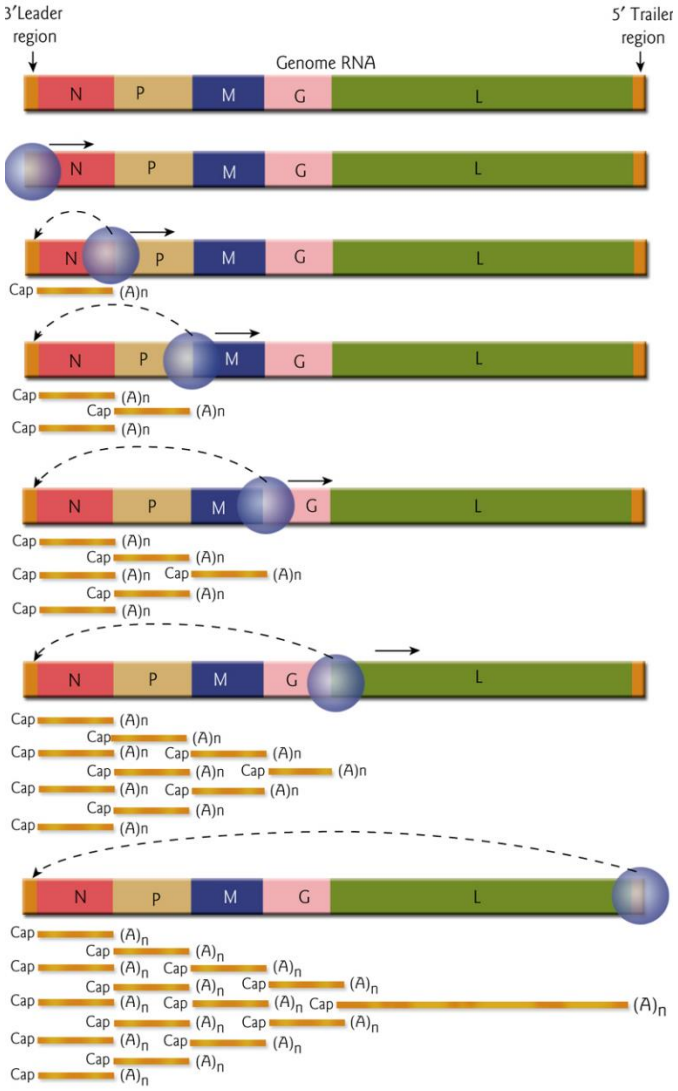
After synthesis of the N mRNA, RNA synthesis terminates at the intergenic region, followed by reinitiation at the 3' end of the P gene.

This process continues until all five mRNAs are synthesized.
Reinitiation does not occur after the last mRNA (the L mRNA) is synthesized, and, as a consequence, the 595-terminal nucleotides of the vesicular stomatitis virus genomic RNA are not copied.

Only a fraction of the polymerase molecules successfully make the transition (70% of the time) from termination to reinitiation of mRNA synthesis at each intergenic region.

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

ssRNA (-): Rhabdoviridae (VSV)

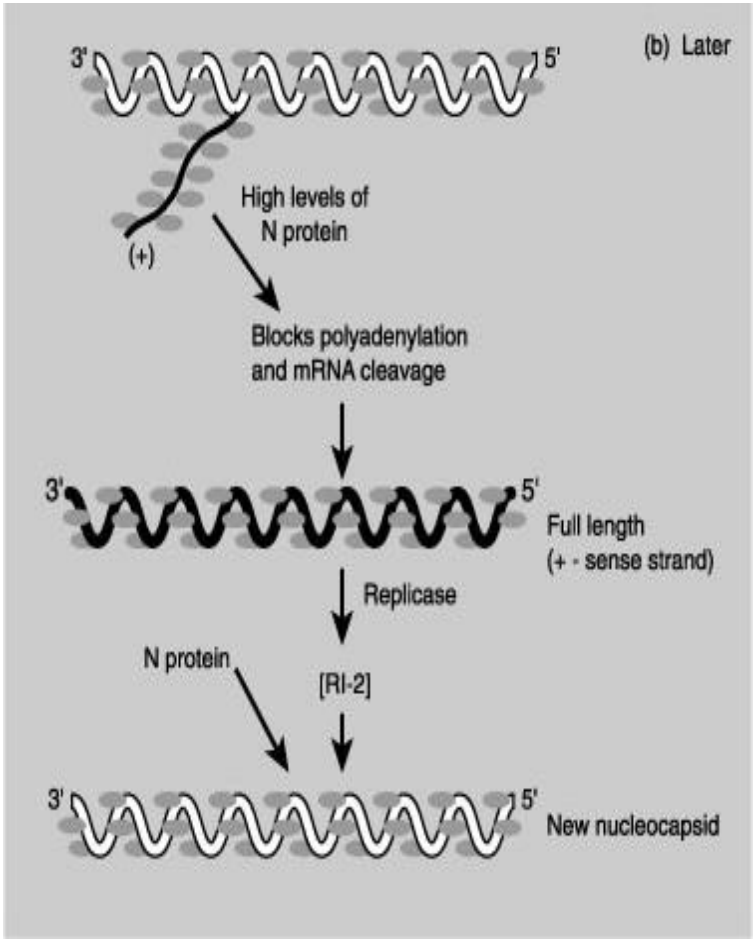


Genome is transcribed following a "stop and start" mechanism
mRNAs are synthesized sequentially in decreasing proportions as the polymerase moves toward the 5' terminus of the genome.

The relative abundances of the proteins reflect those of the mRNAs

From Dimmock NJ, Introduction to Modern Virology, Blackwell, 2016

VSV from transcription to genome replication



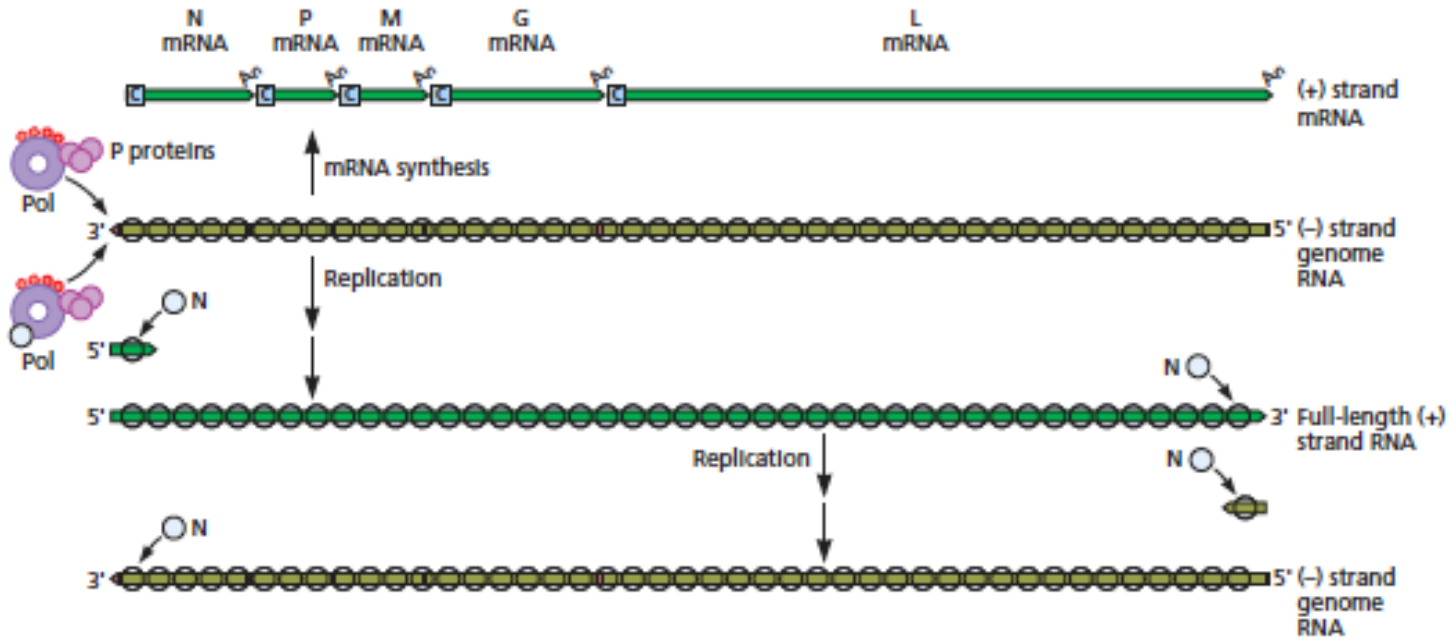
The abundance of the neosynthesized N and P proteins regulate the transition from transcription to the genome replication phase.
To produce a full-length (+) strand RNA, the stop-start reactions at intergenic regions must be suppressed, a process that depends on the N and P proteins.

N-P assemblies bind to leader RNA and cause Antitermination.

Additional N protein molecules then associate with the (+) strand RNA as it is elongated, and eventually bind to the seven A bases in the intergenic region. This interaction blocks reiterative copying of the seven U bases.
Consequently, RNA synthesis continues through the intergenic regions. The number of N-P protein complexes in infected cells therefore regulates the relative efficiencies of mRNA synthesis and genome RNA replication.

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

VSV from transcription to genome replication



Synthesis of the full-length (+) strand begins at the exact 3' end of the viral genome and is carried out by the RNA polymerase. The (+) strand RNA is bound by the viral nucleocapsid (N) protein, which is associated with the P protein in a 1:1 molar ratio. The N-P complexes bind to the nascent (+) strand RNA, allowing the RNA polymerase to read through the intergenic junctions at which polyadenylation and termination take place during mRNA synthesis.

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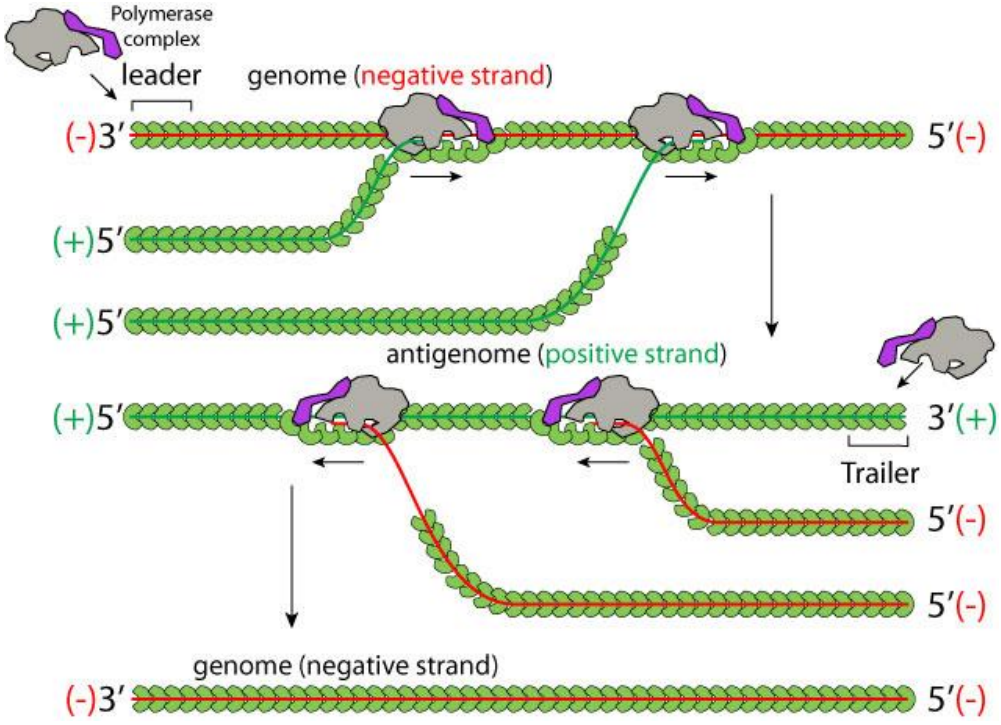


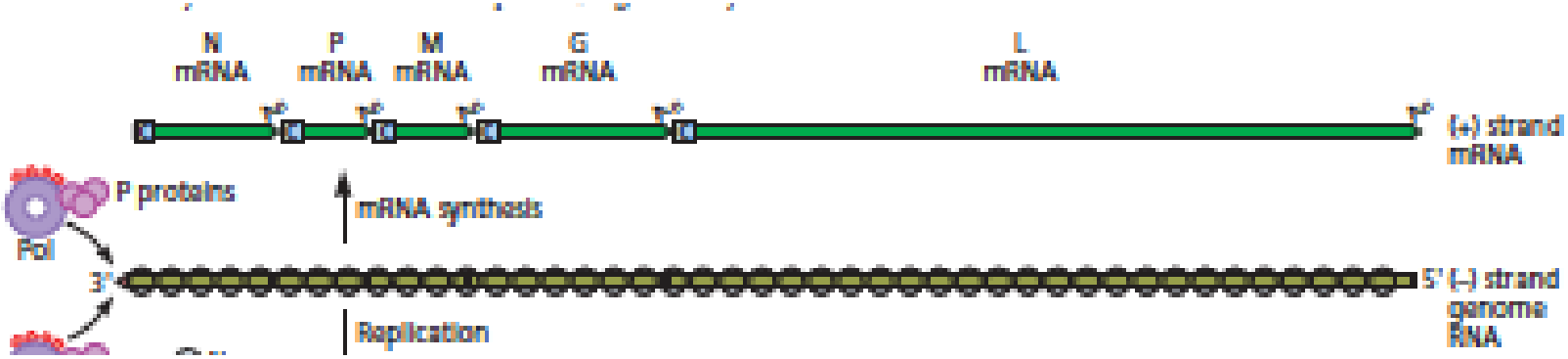
The RdRp complex binds to the leader sequence on the RNA genome, and starts replication. The antigenome is concomitantly encapsidated during replication.

The RdRp complex ignores all transcription signals when in replication mode.

The antigenome is then replicated (thus new genomes synthetised) under the same process, the viral polymerase complex binding first to the trailer sequence.

The genome/antigenome ratio is about 10 for many viruses. Presumably the trailer sequence is a stronger replication promoter that the leader sequence.

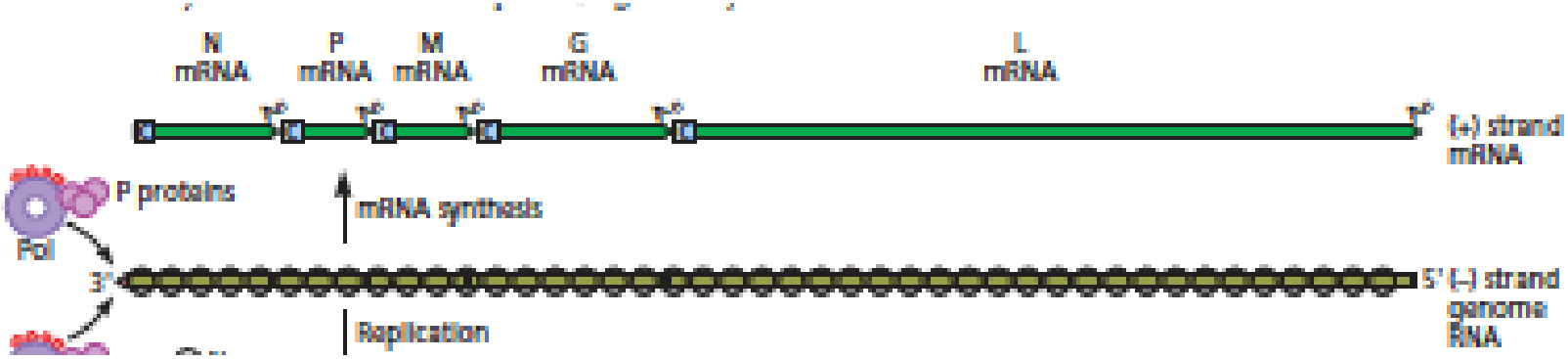
ssRNA (-), Rhabdoviridae (VSV)



Vesicular stomatitis viral RNA synthesis. Viral (-) strand genomes are templates for the production of either subgenomic mRNAs or full-length (+) strand RNAs. Once the nucleocapsid is released into the cytoplasm, the RNA genome is repetitively transcribed (primary transcription) by the virion transcriptase. N removal does not occur since the transcriptase only recognizes the RNA-N protein complex as template. The switch from mRNA synthesis to genomic RNA replication is mediated by two RNA polymerase complexes and by the N protein.

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

ssRNA (-), Rhabdoviridae (VSV)



mRNA synthesis initiates at the beginning of the N gene, near the 3' end of the viral genome. Poly(A) addition is a result of reiterative copying of a sequence of seven U residues present in each intergenic region.
Chain termination and release occur after approximately 150 A residues have been added to the mRNA. The RNA polymerase then initiates synthesis of the next mRNA at the conserved start site 3'UUGUC . . . 5'. This process is repeated for all five viral genes.

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