

Structural Virology

Lecture 7

Pavel Plevka

Rhabdoviridae

Hosts: mammals

fishes

insects

plants

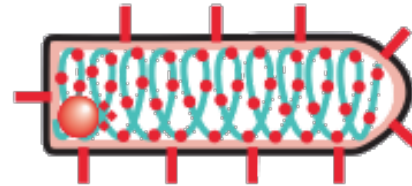
Diseases: rabies

vesicular stomatitis

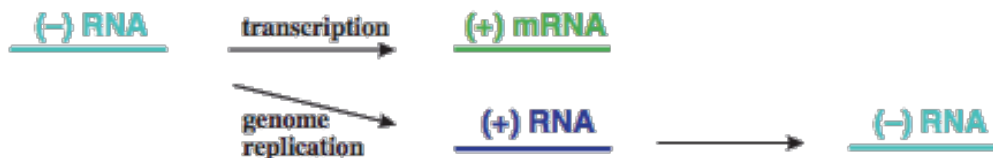
yellow dwarf of potato

Virion

- Enveloped
- Helical nucleocapsid
- Genome: single-stranded RNA
minus polarity
11–15 kb



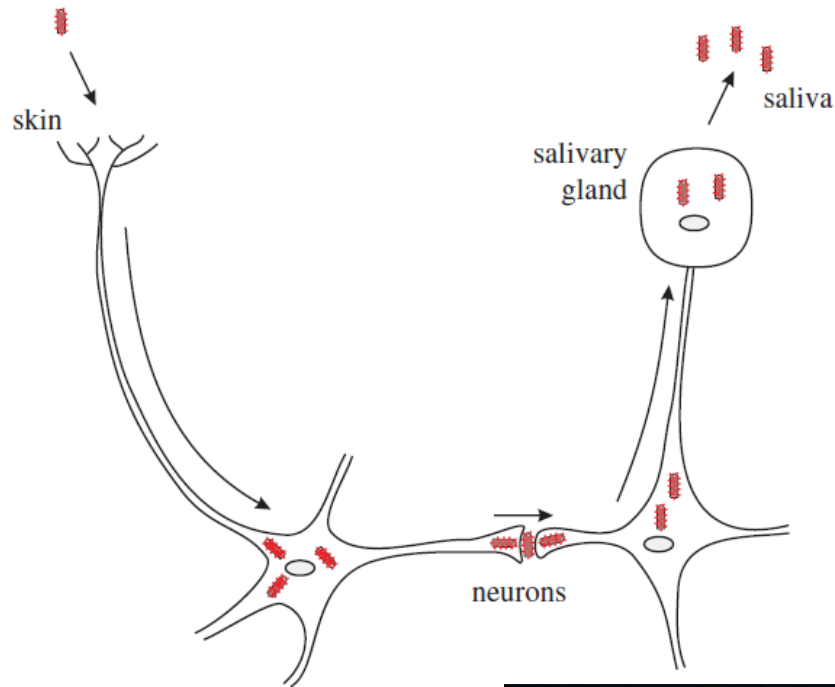
Virions are bullet shaped 180 nm long and 75 nm wide.



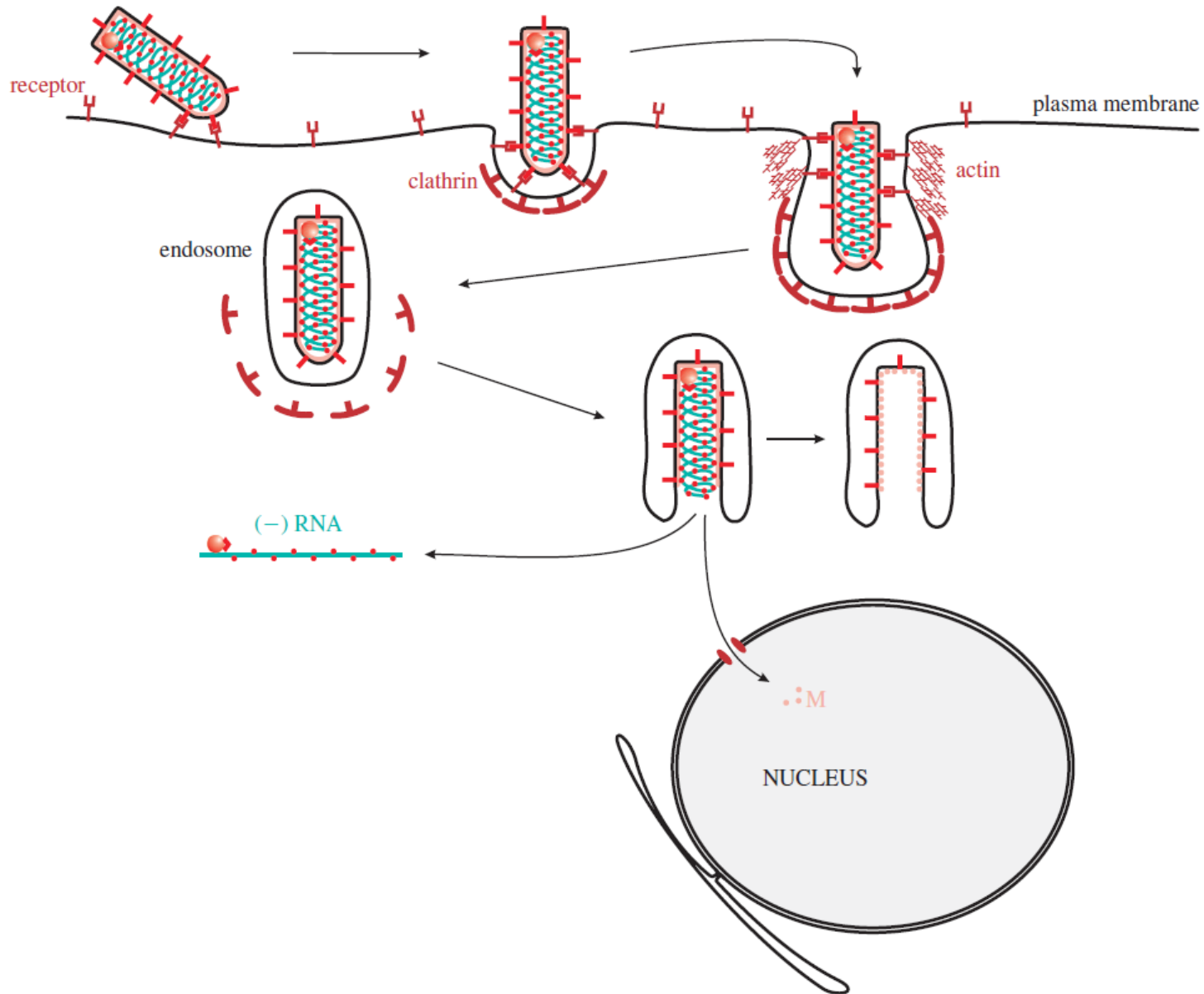
Vesicular stomatitis virus



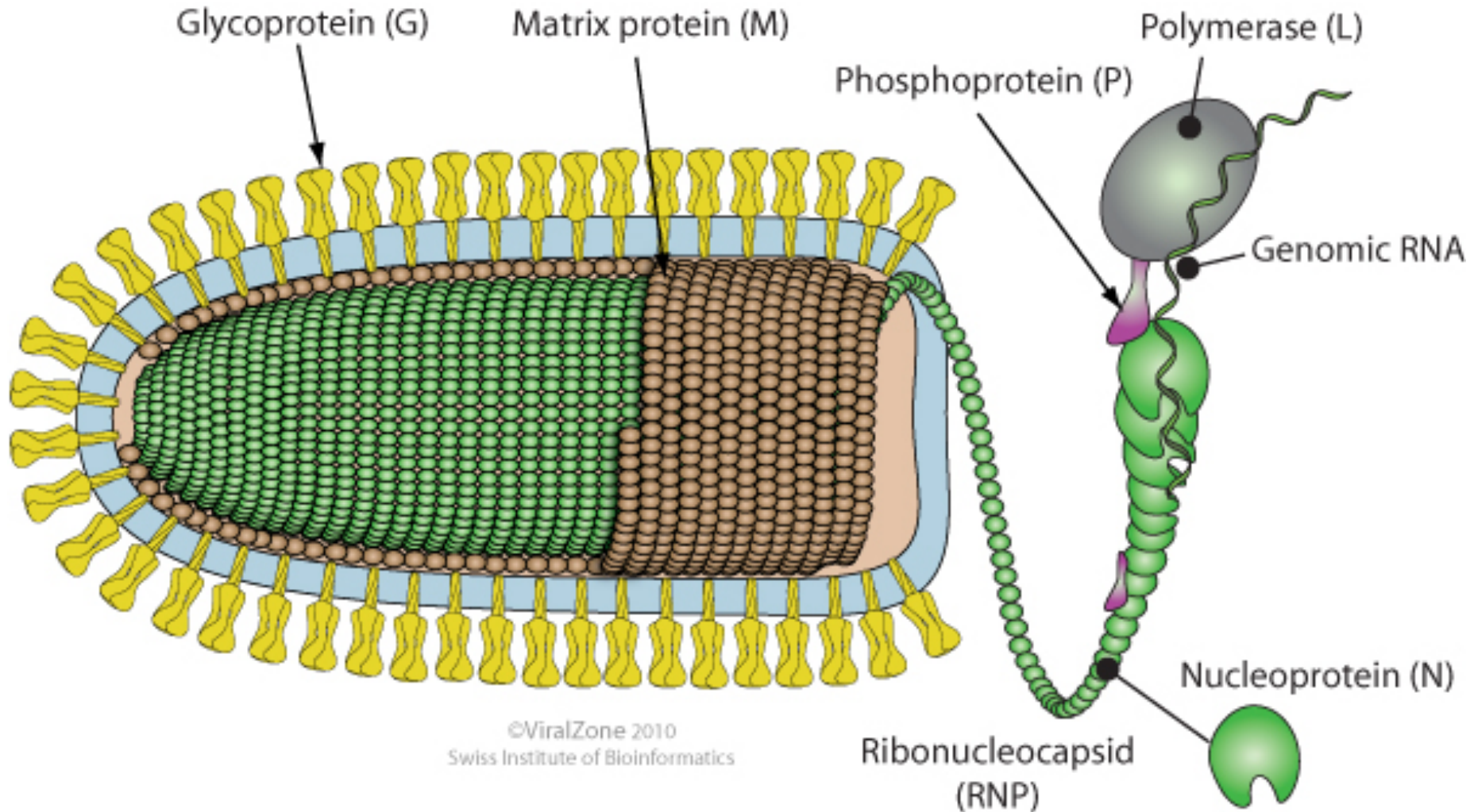
Rabies virus



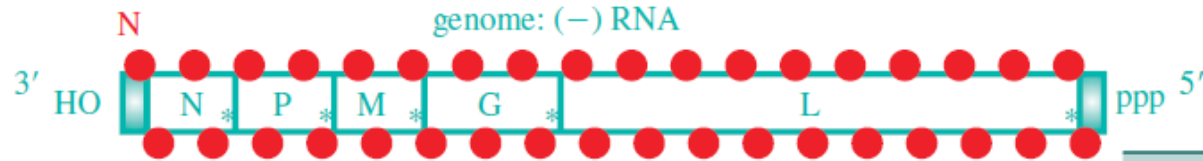
Attachment and cell entry



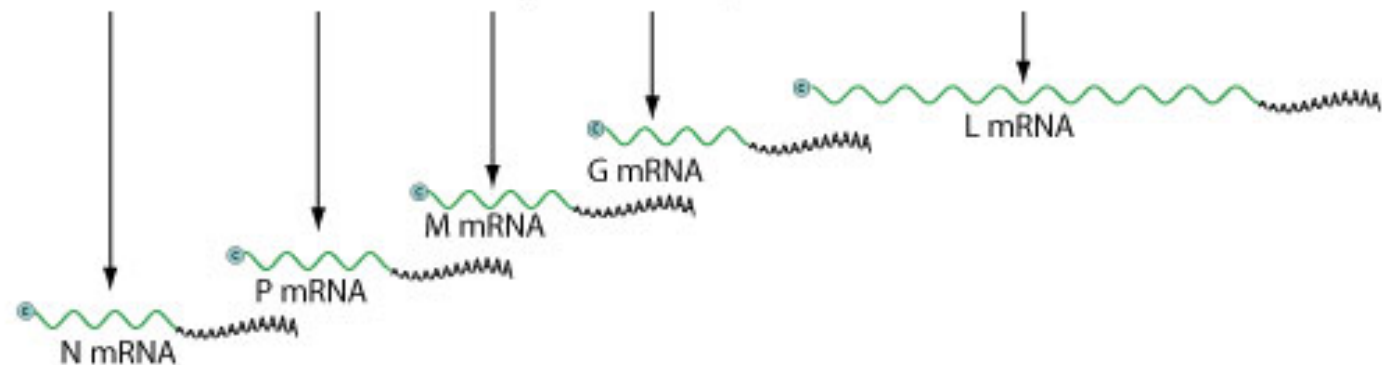
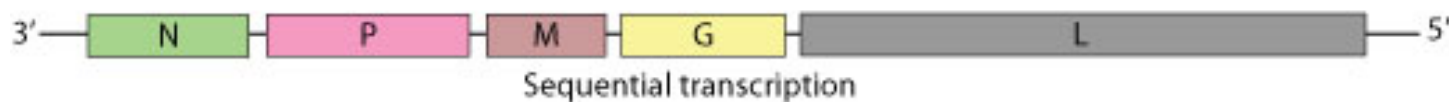
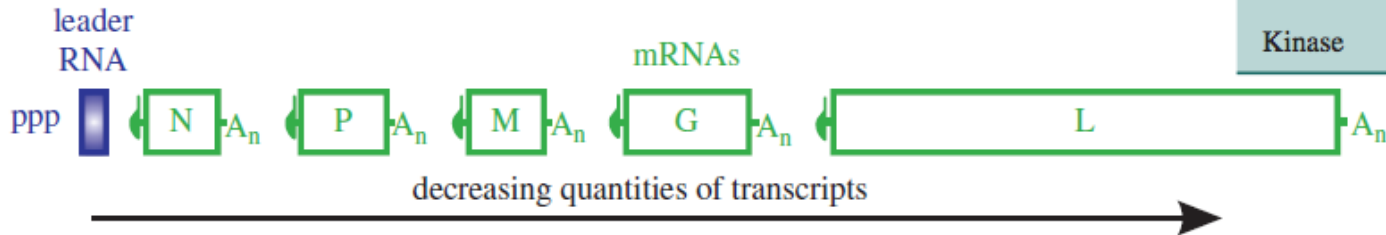
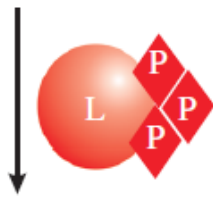
Virion organization



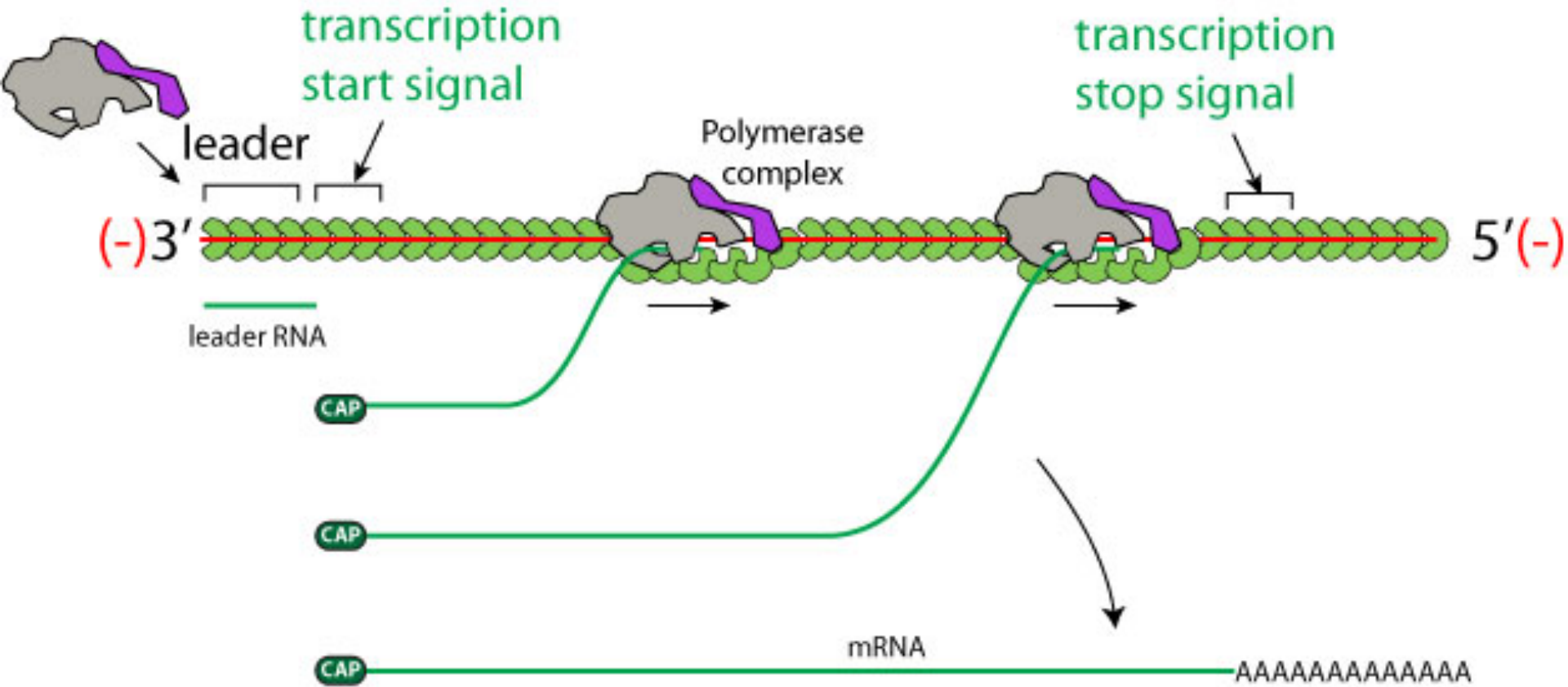
Genome organization and sequential transcription



Enzyme	Role
RNA-dependent RNA polymerase	RNA replication
Methyl transferase	Capping mRNAs
Guanylyl transferase	Capping mRNAs
Poly(A) polymerase	Polyadenylation of mRNAs
Kinase	Phosphorylation of P



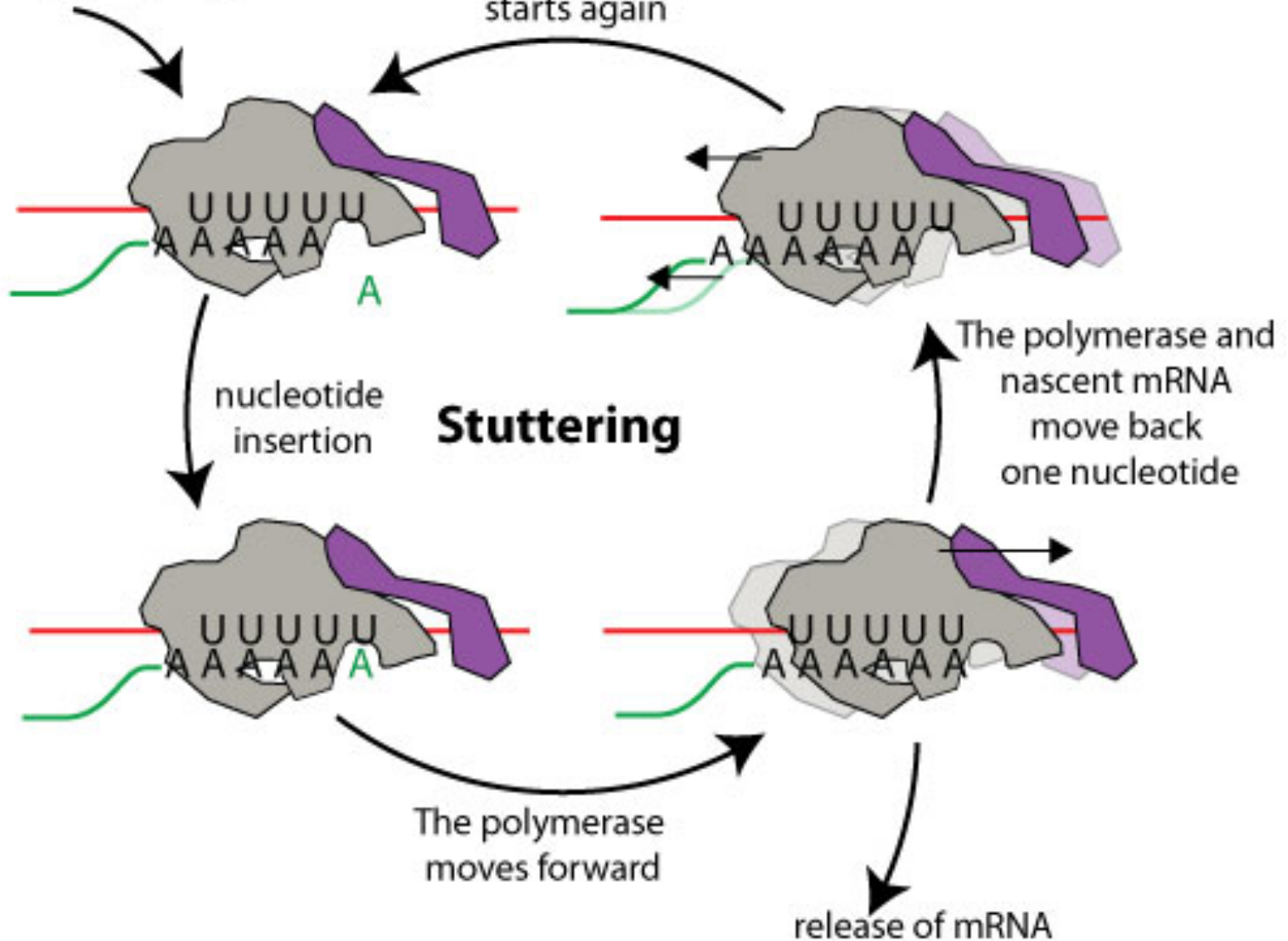
Sequential transcription



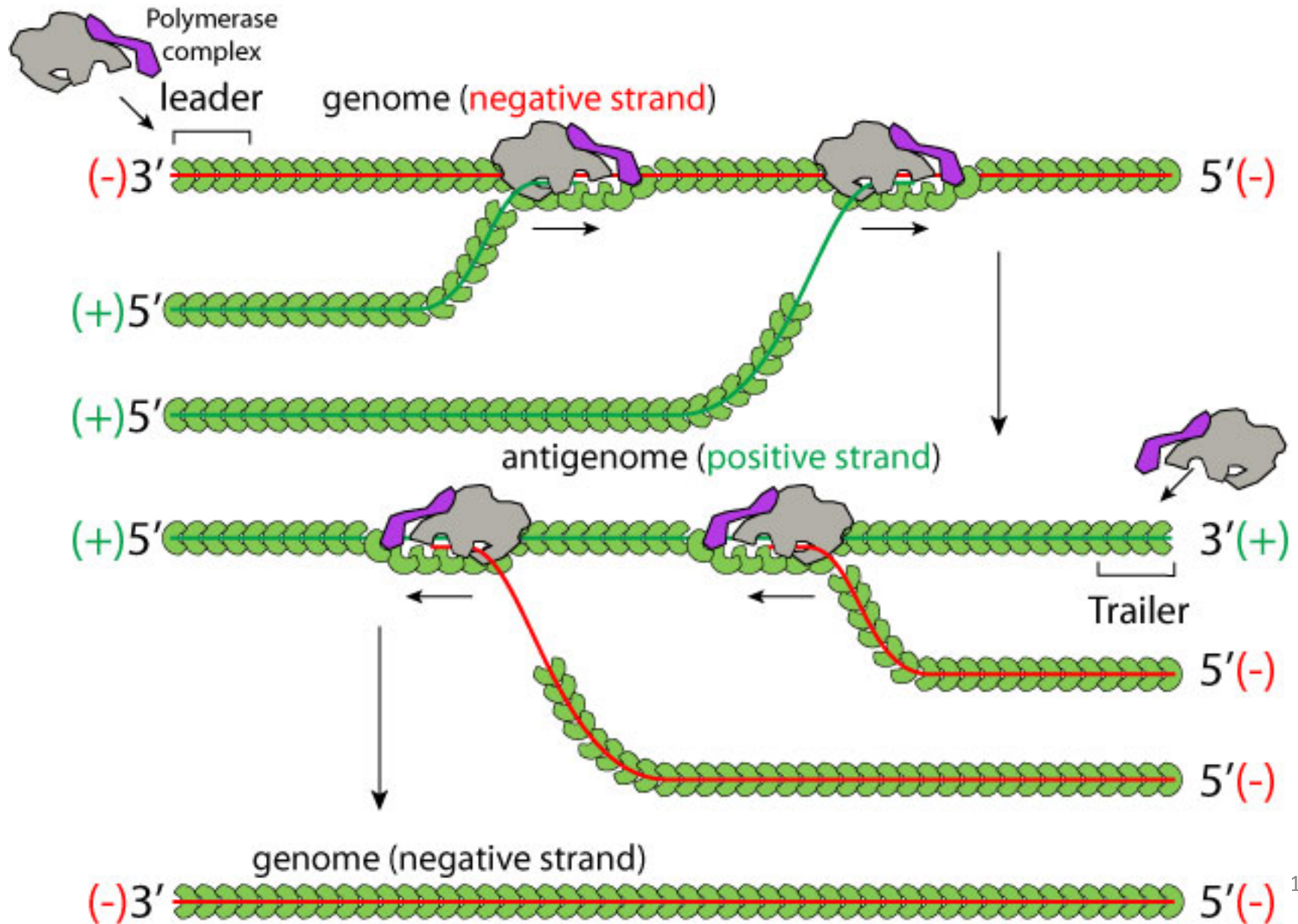
Negative-stranded RNA virus polymerase stuttering

Transcriptase arrives
on stop/polyA signal

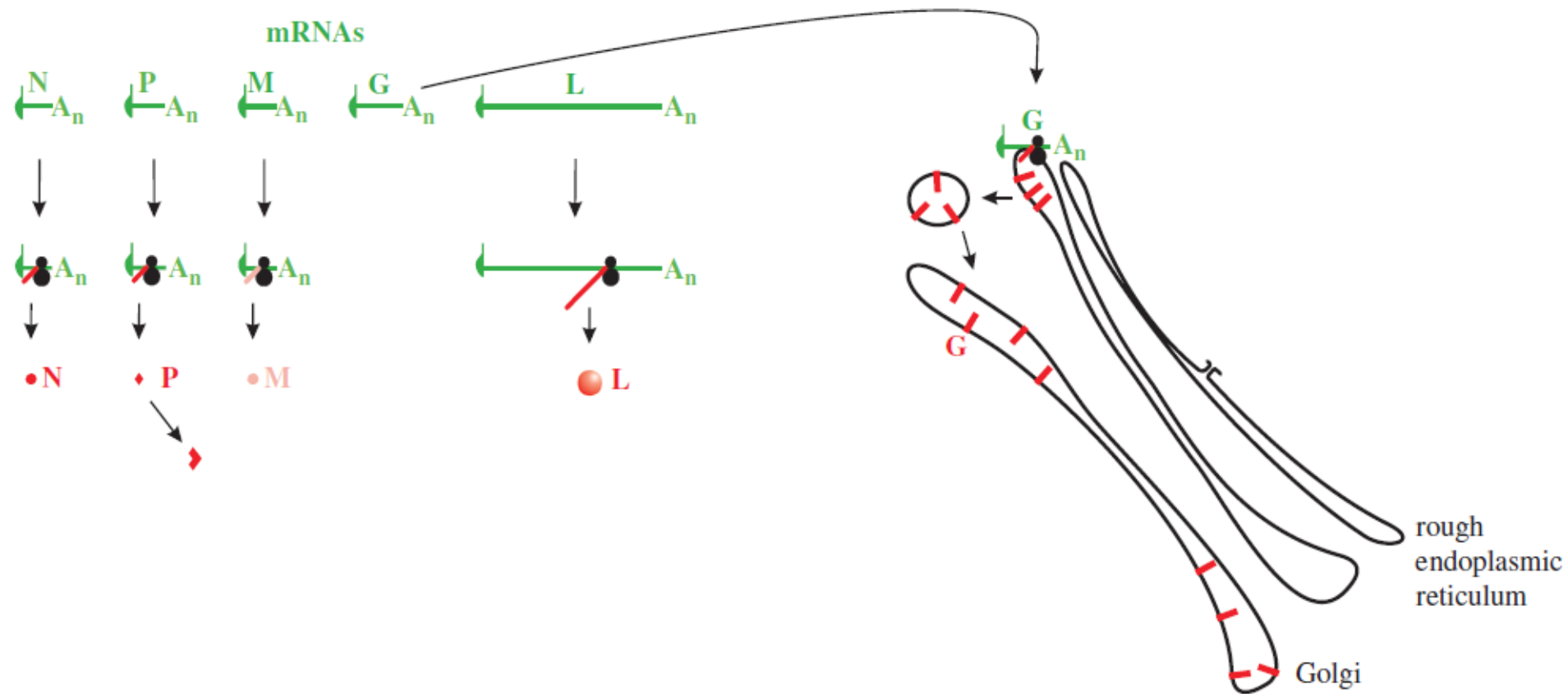
Transcription
starts again



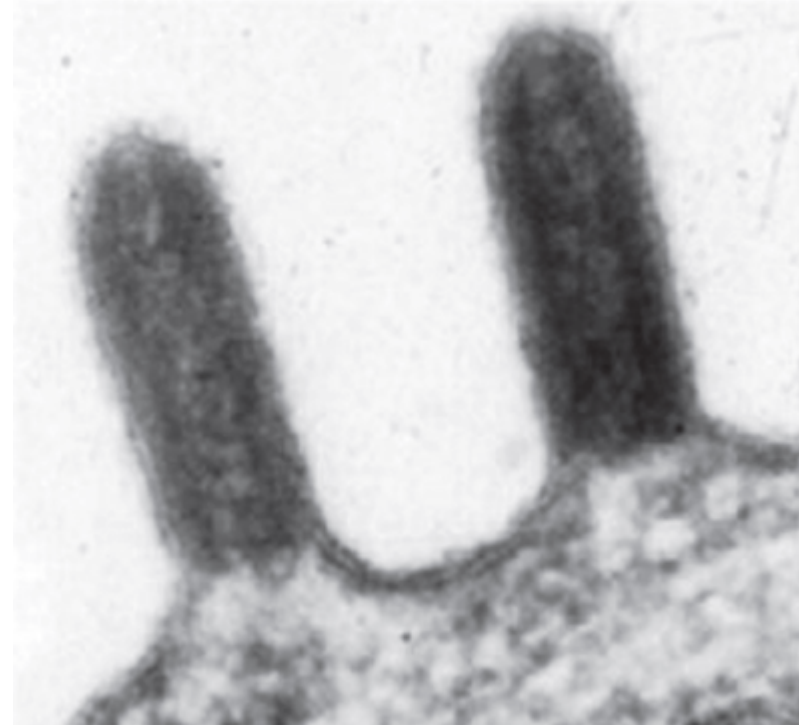
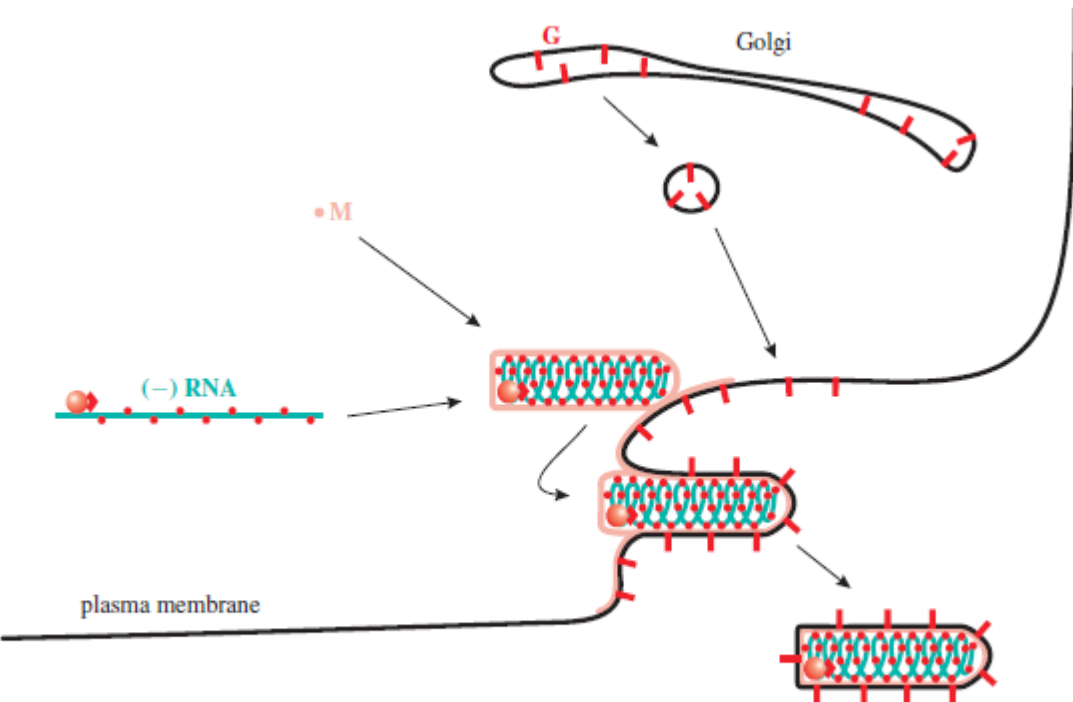
Negative-stranded RNA virus replication

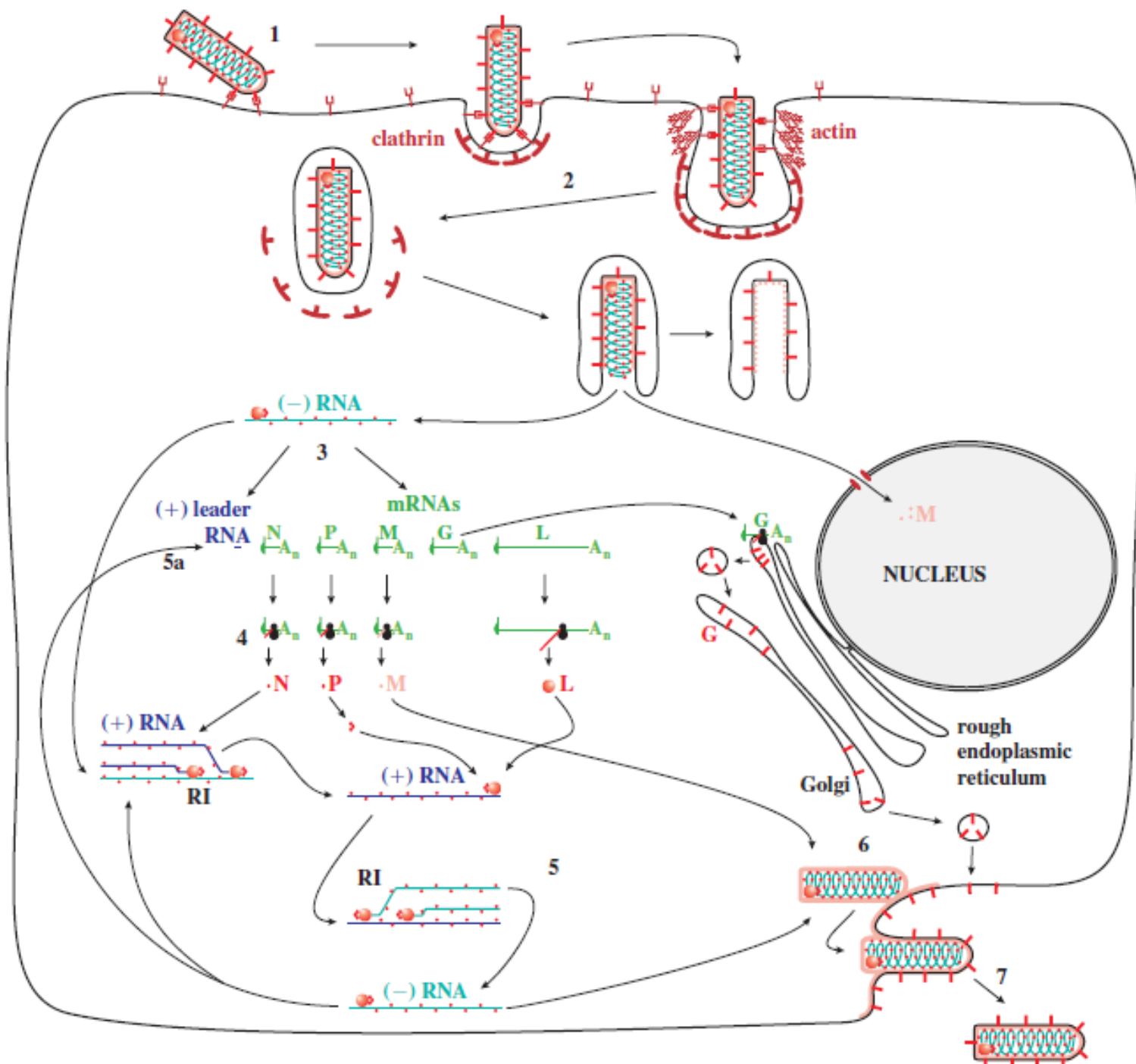


Translation and post-translational modifications

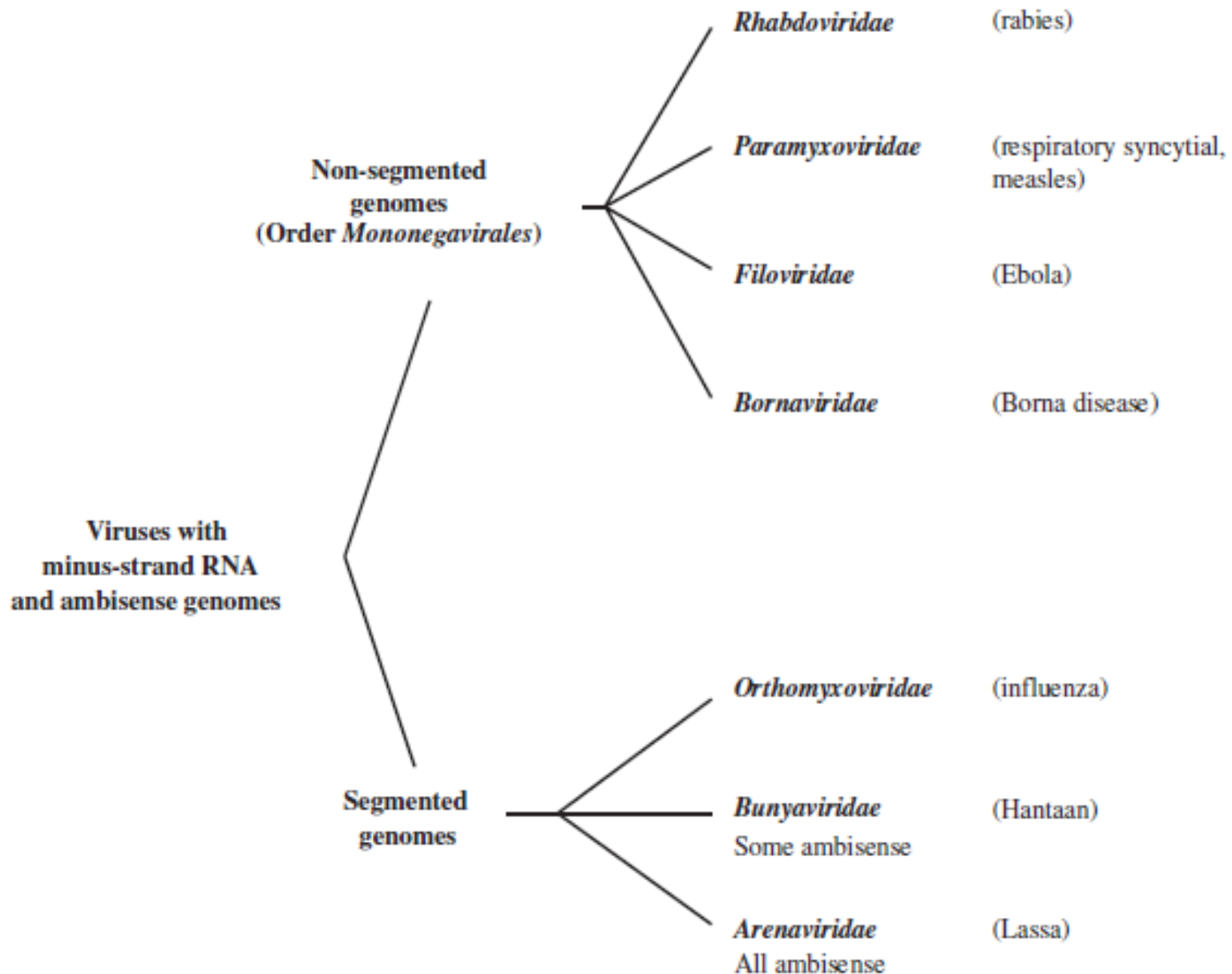


Budding

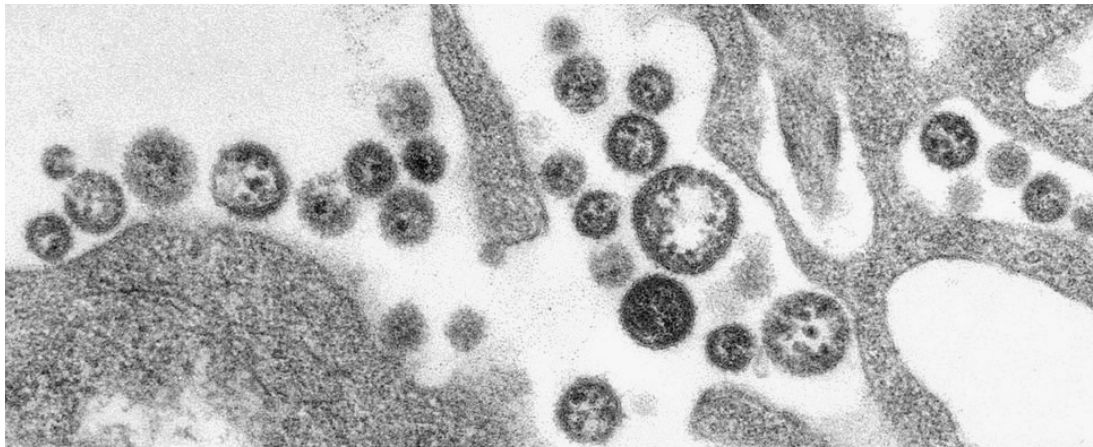
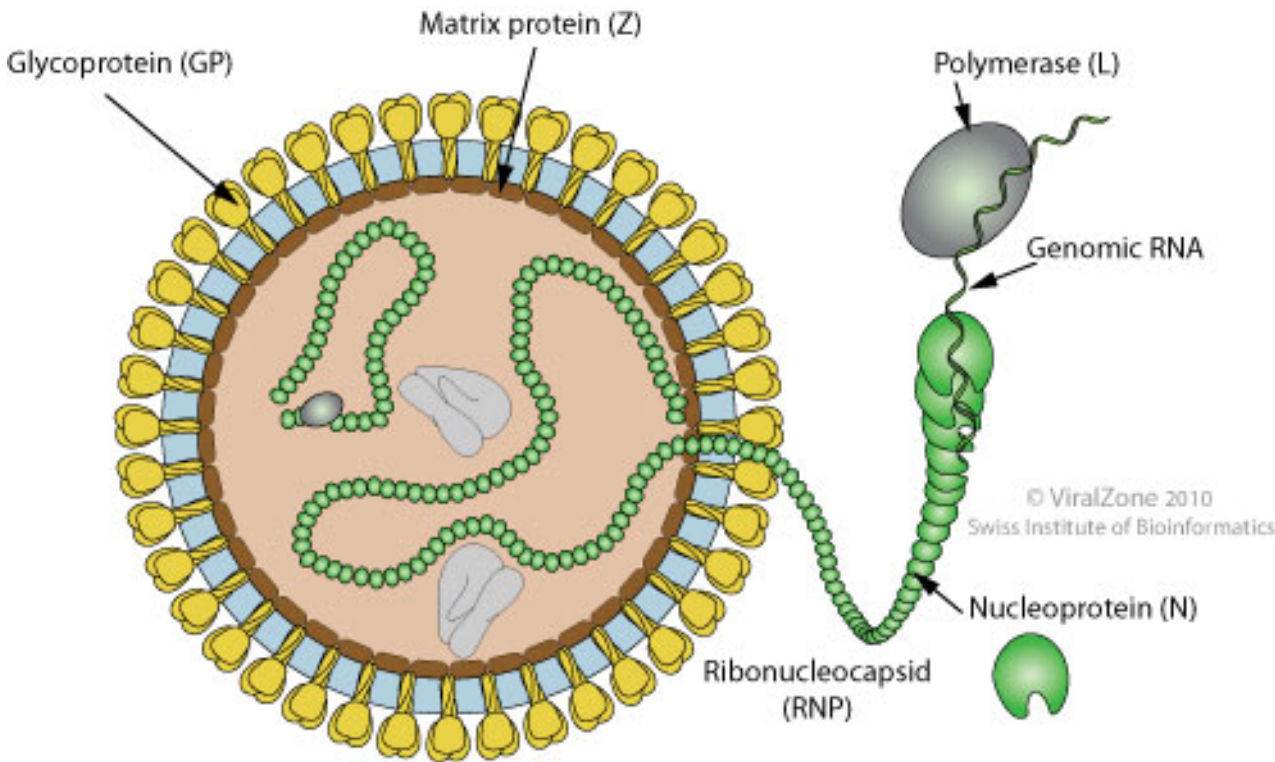




Viruses with minus or ambisense RNA genomes



Arenaviridae (Lassa fever)



Fever, facial swelling, muscle fatigue, conjunctivitis and mucosal bleeding.

Gastrointestinal tract

- Nausea
- Vomiting (bloody)
- Diarrhea (bloody)
- Stomach ache
- Constipation
- Dysphagia (difficulty swallowing)
- Hepatitis

Cardiovascular system

- Pericarditis
- Hypertension
- Hypotension
- Tachycardia

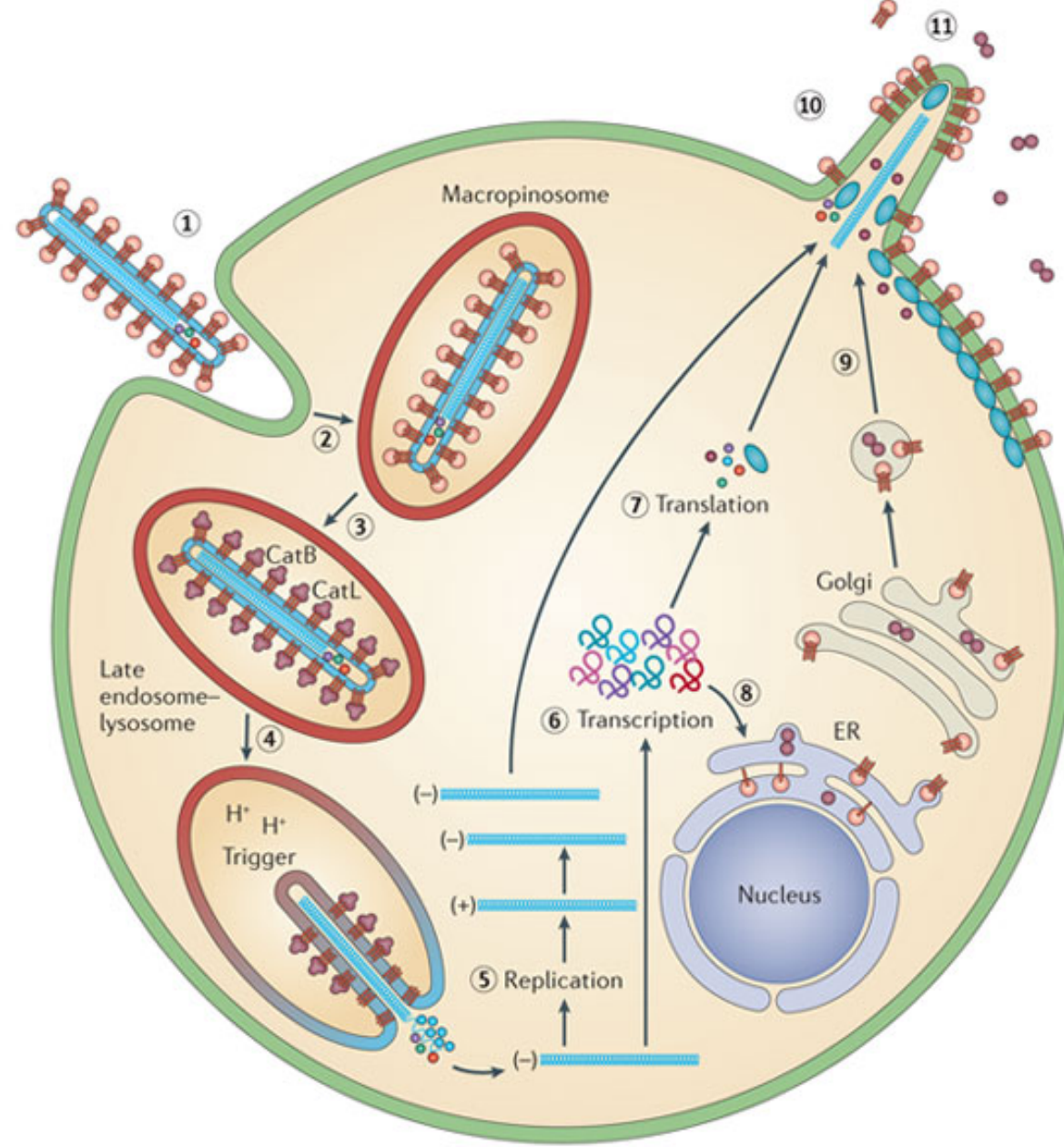
Respiratory tract

- Cough
- Chest pain
- Dyspnoea
- Pharyngitis
- Pleuritis

Nervous system

- Encephalitis
- Meningitis
- Hearing deficit
- Seizures

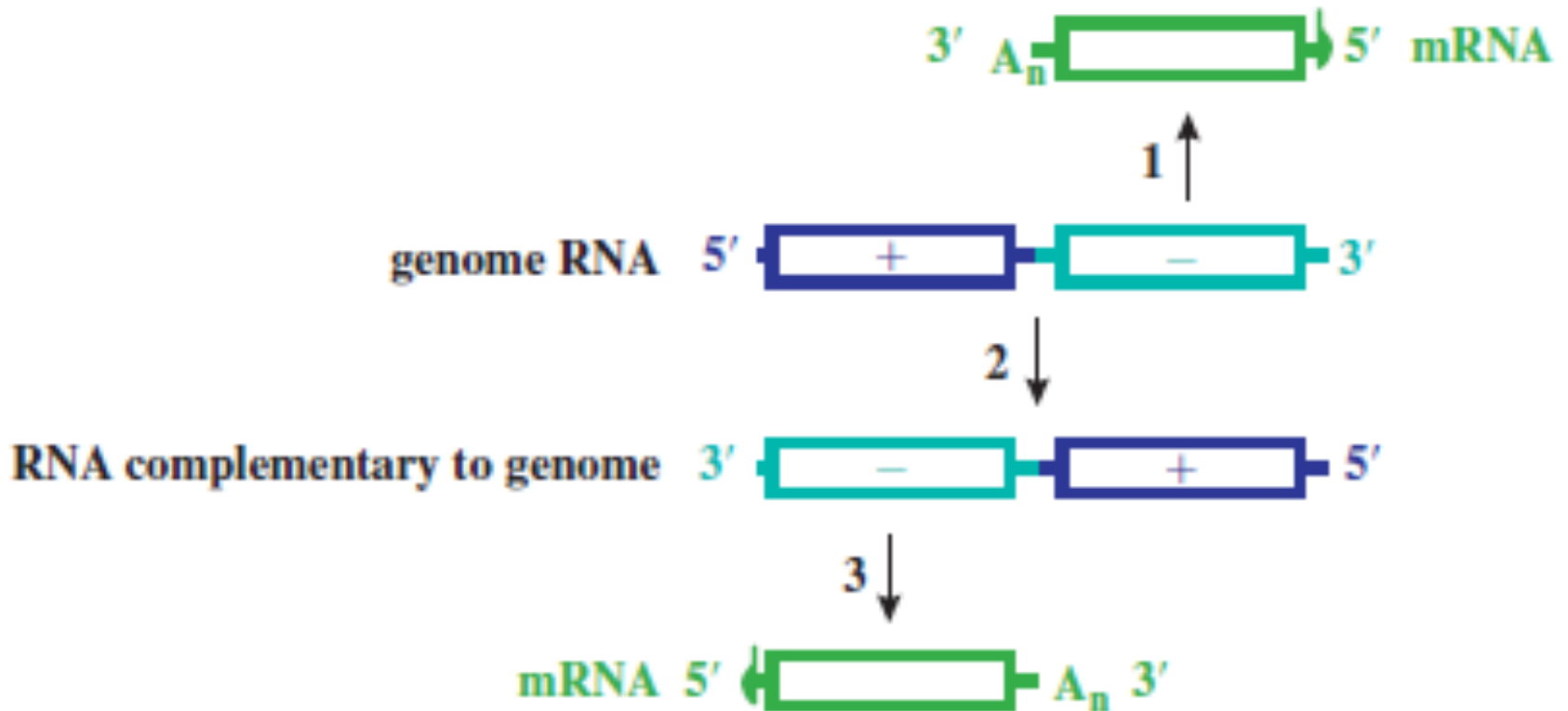
Ebola virus



Symptoms

- Fever
- Severe headache
- Muscle pain
- Weakness
- Fatigue
- Diarrhea
- Vomiting
- Abdominal (stomach) pain
- Unexplained hemorrhage (bleeding or bruising)

Ambisense virus translation

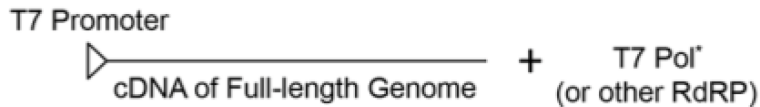


POSITIVE-STRAND RNA VIRUSES

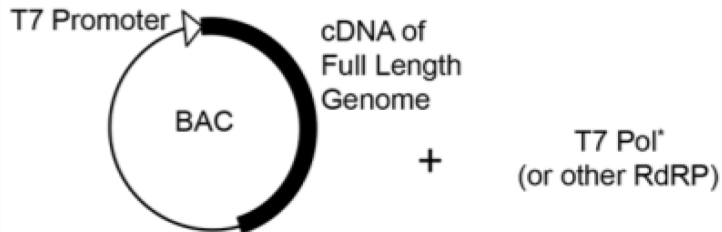
(1) Full-length Genomic RNA Transcripts



(2) Full-length cDNA / RdRp

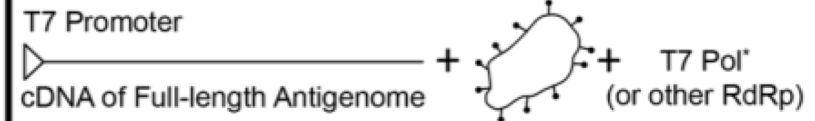


(3) Bacterial Artificial Chromosome / RdRp

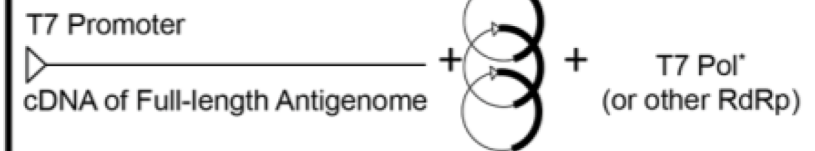


NEGATIVE-STRAND RNA VIRUSES

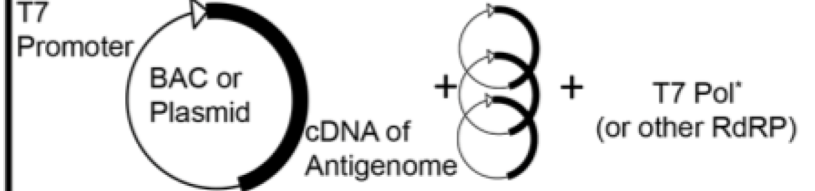
(1) Antigenomic cDNA / Helper Virus / RdRp



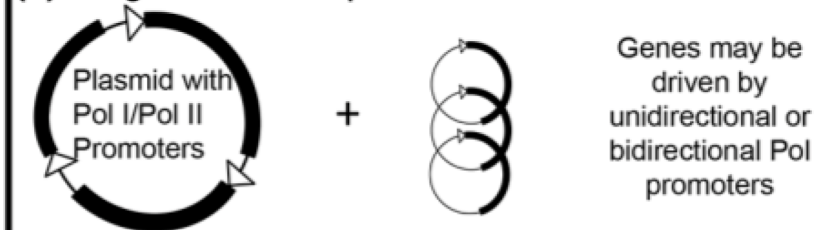
(2) Antigenomic cDNA / Helper Plasmids / RdRp



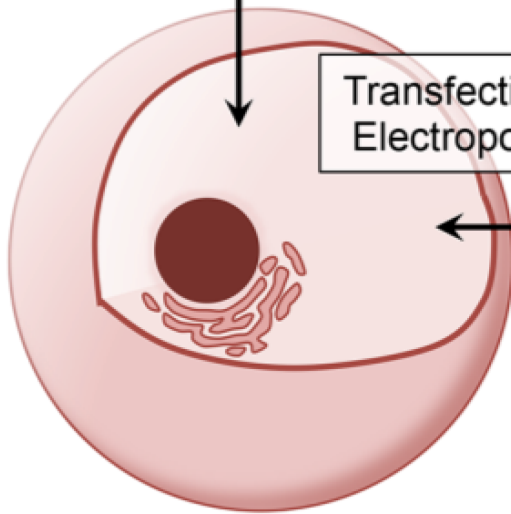
(3) Antigenomic cDNA BAC / Helper Plasmids / RdRp



(4) Antigenomic & Helper Plasmids / Pol I/II Promoters



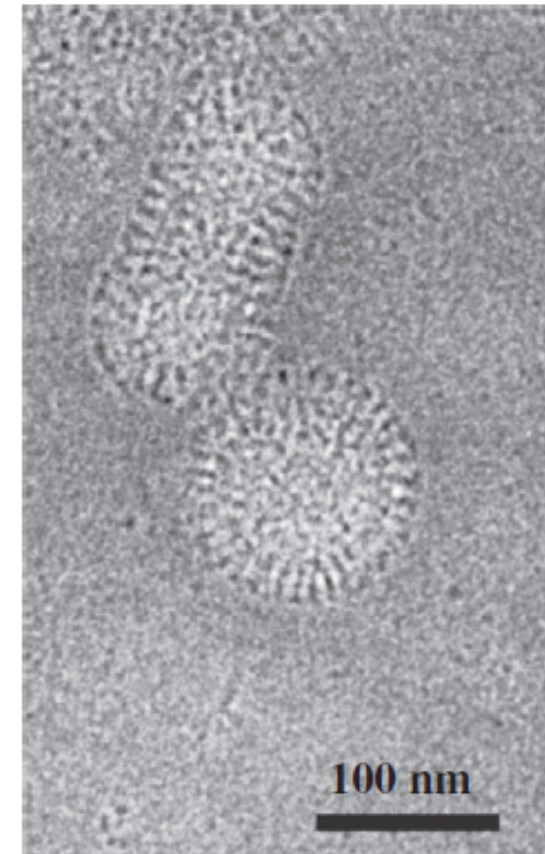
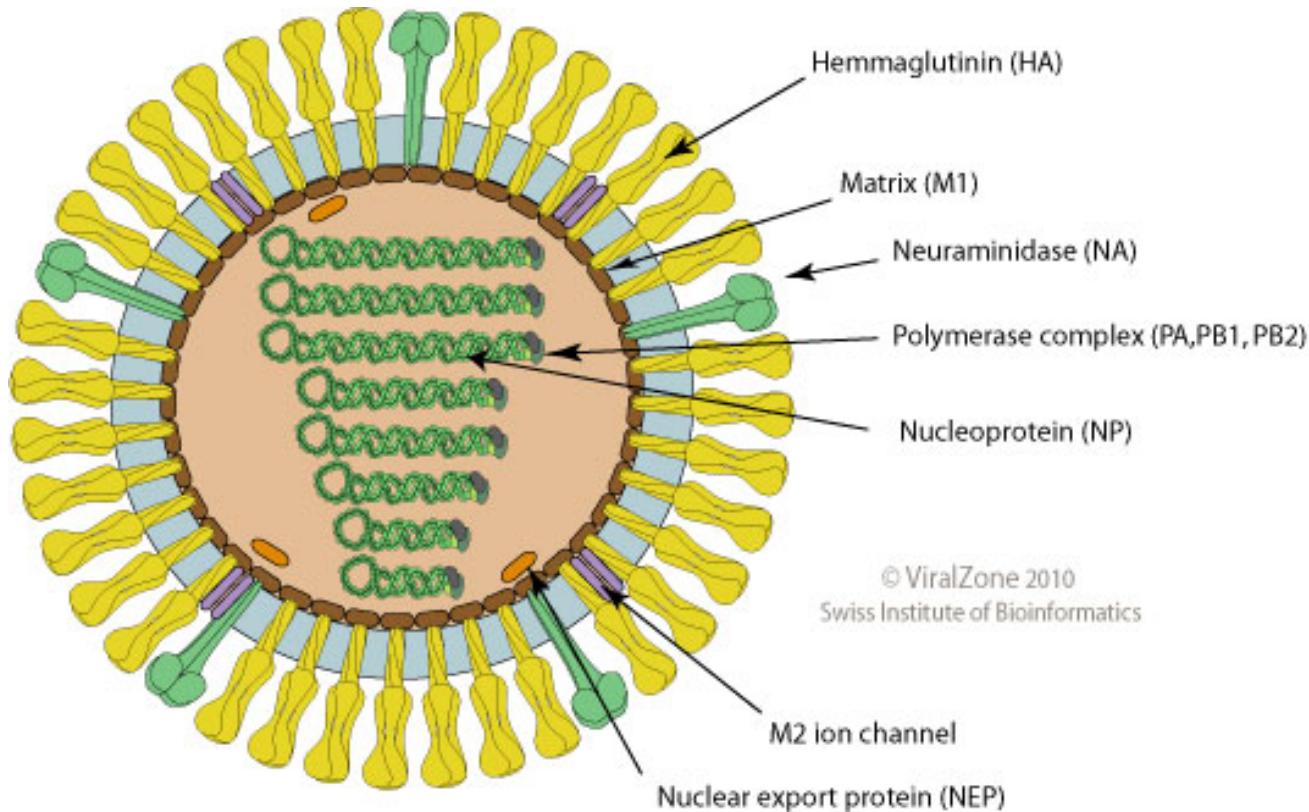
Transfection OR Electroporation

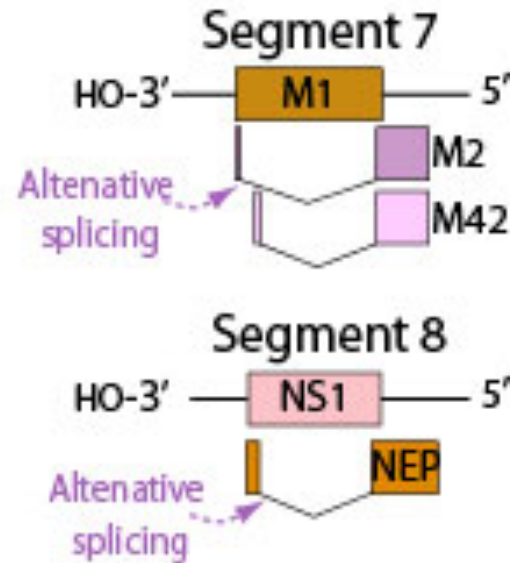
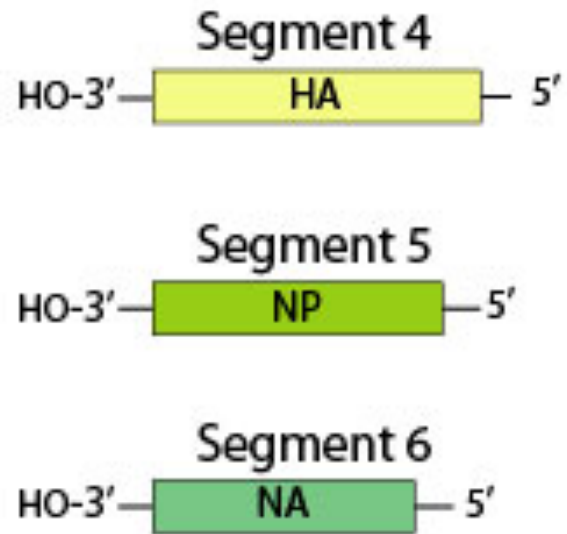
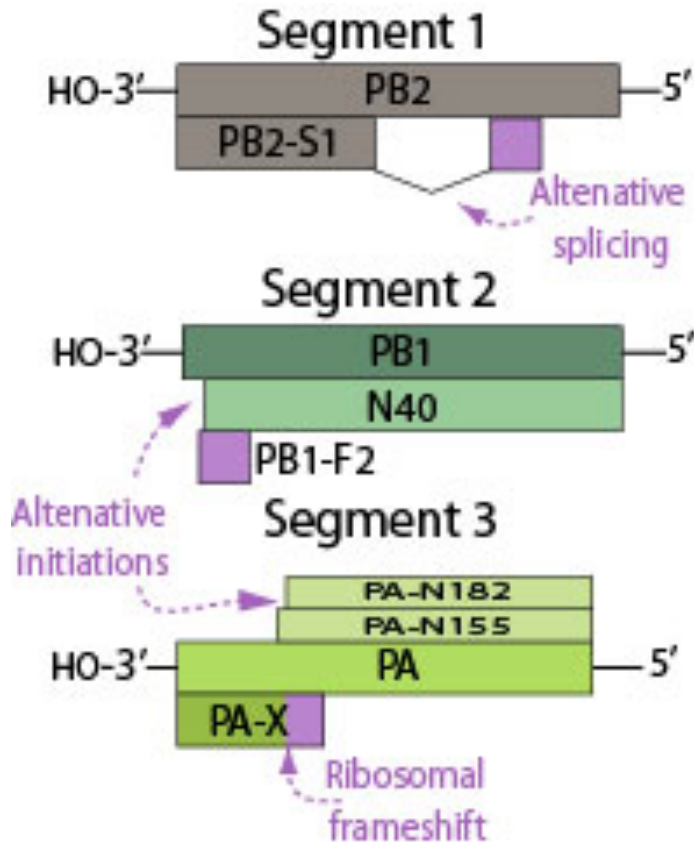


Learning outcomes

- discuss the importance of rabies virus, vesicular stomatitis virus and other minus-strand RNA viruses
- describe the rhabdovirus virion
- outline the main characteristics of the rhabdovirus genome
- discuss the replication cycle of rhabdoviruses
- explain the term ‘ambisense genome’
- discuss the development of reverse genetics procedures for minus-strand RNA viruses

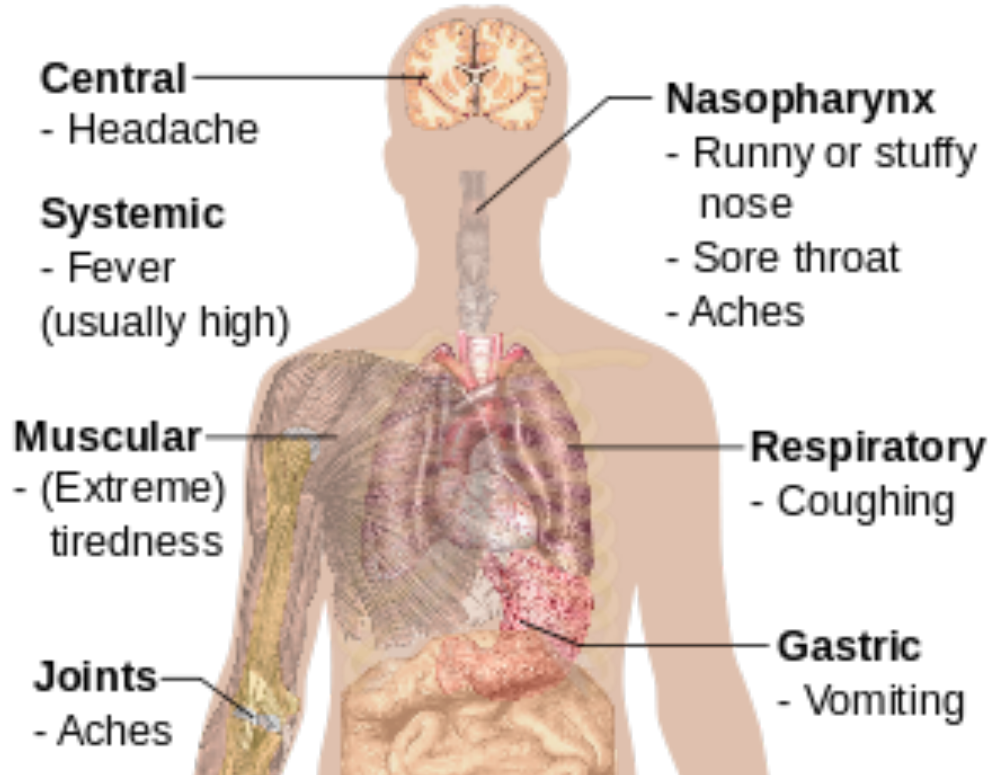
Orthomyxoviridae - Influenza





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SIB Swiss Institute of Bioinformatics

Symptoms of Influenza

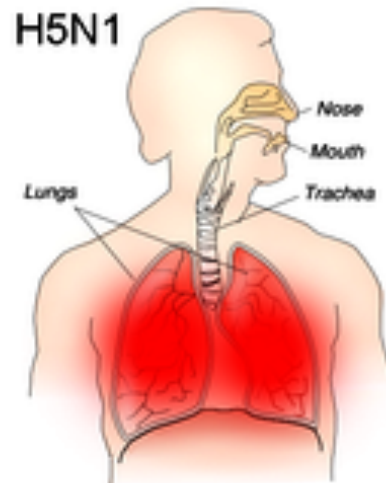


H1N1



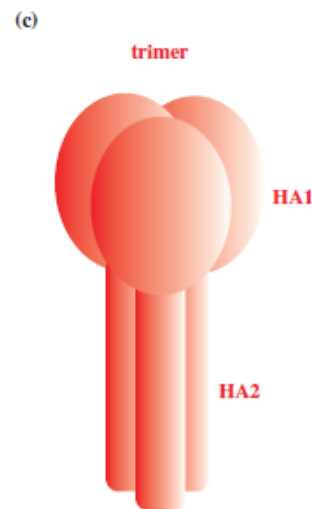
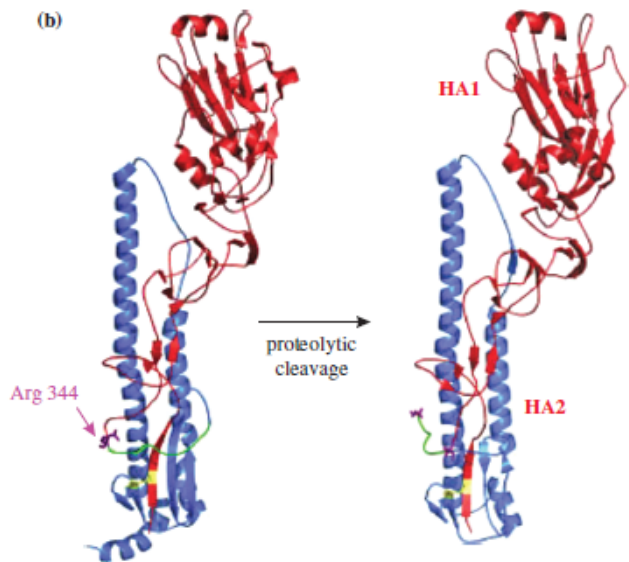
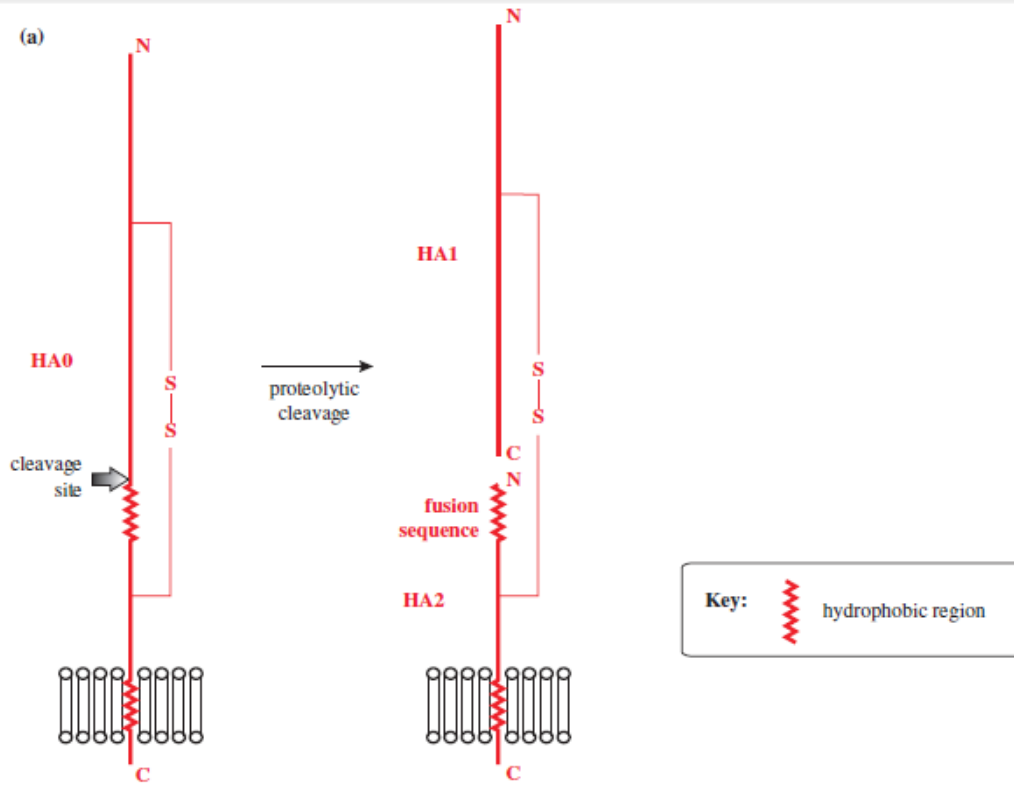
Easily spread
Rarely fatal

H5N1

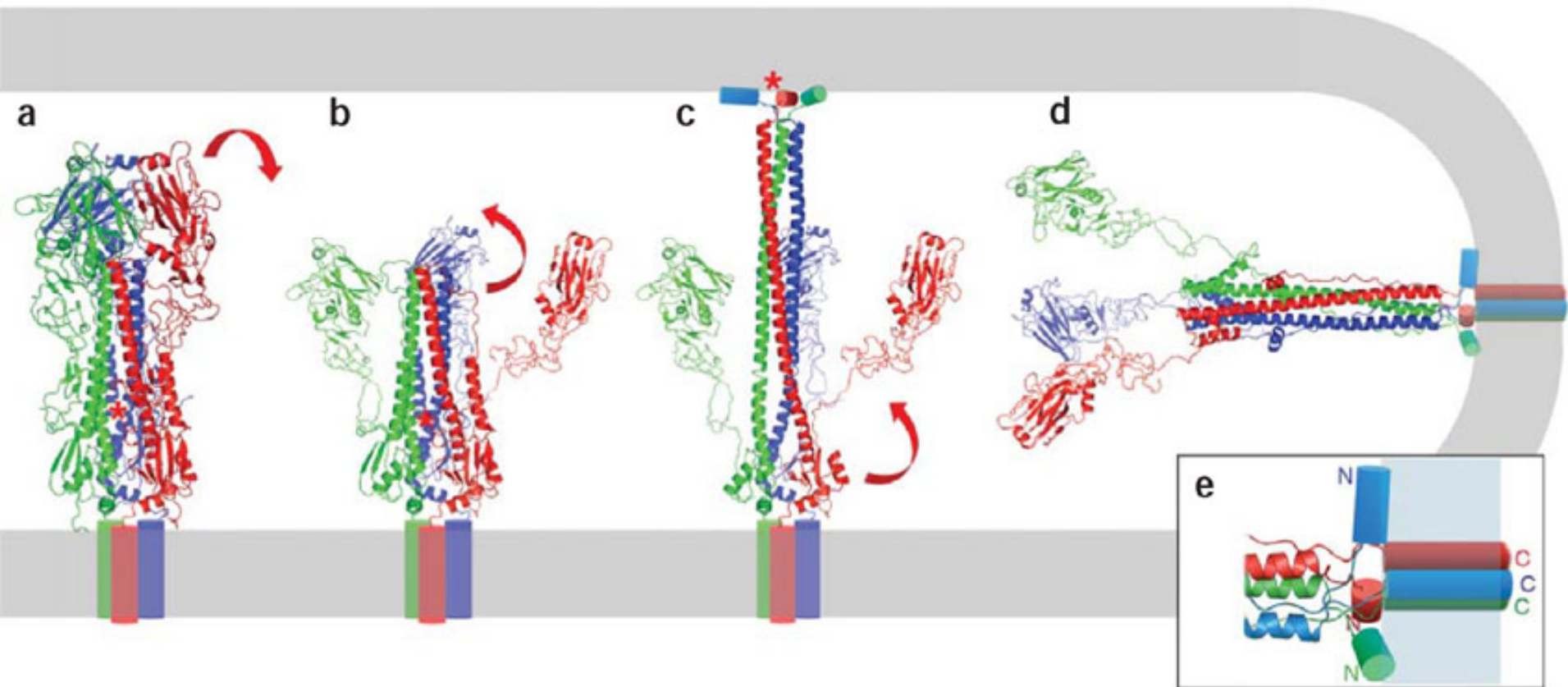


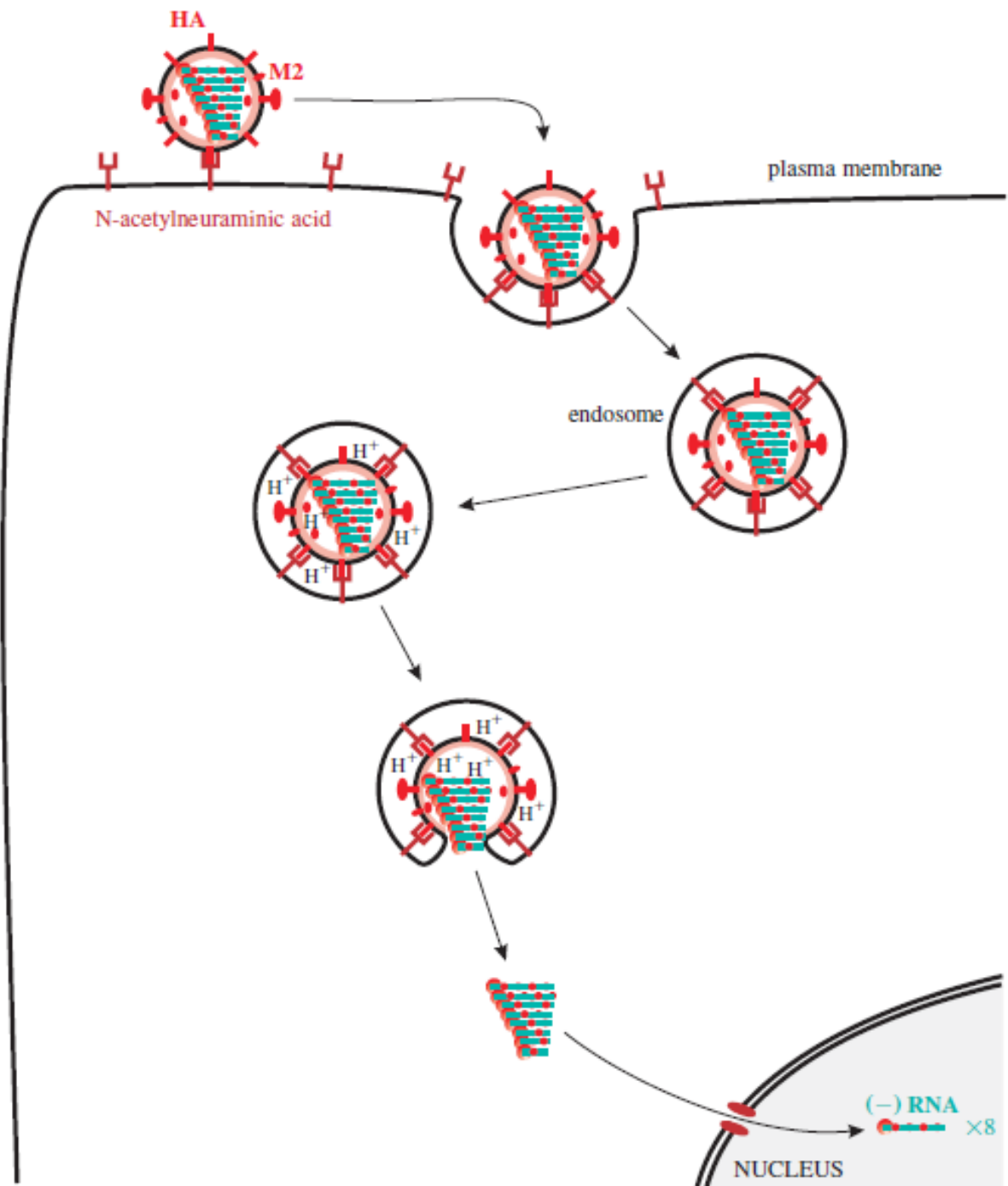
Spreads slowly
Often fatal

Hemagglutinin

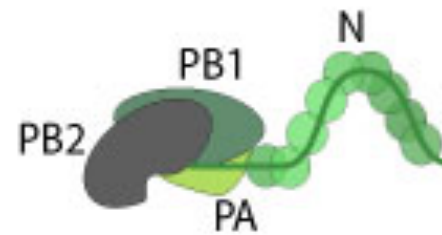
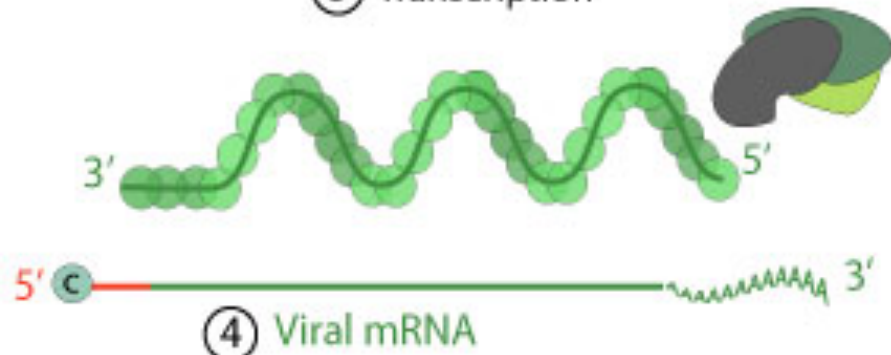
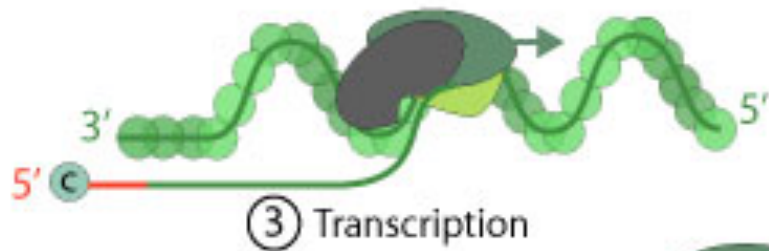
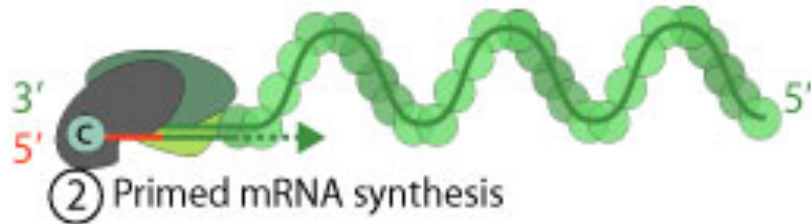
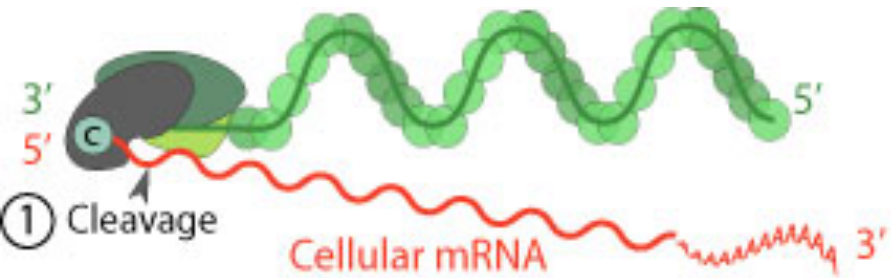


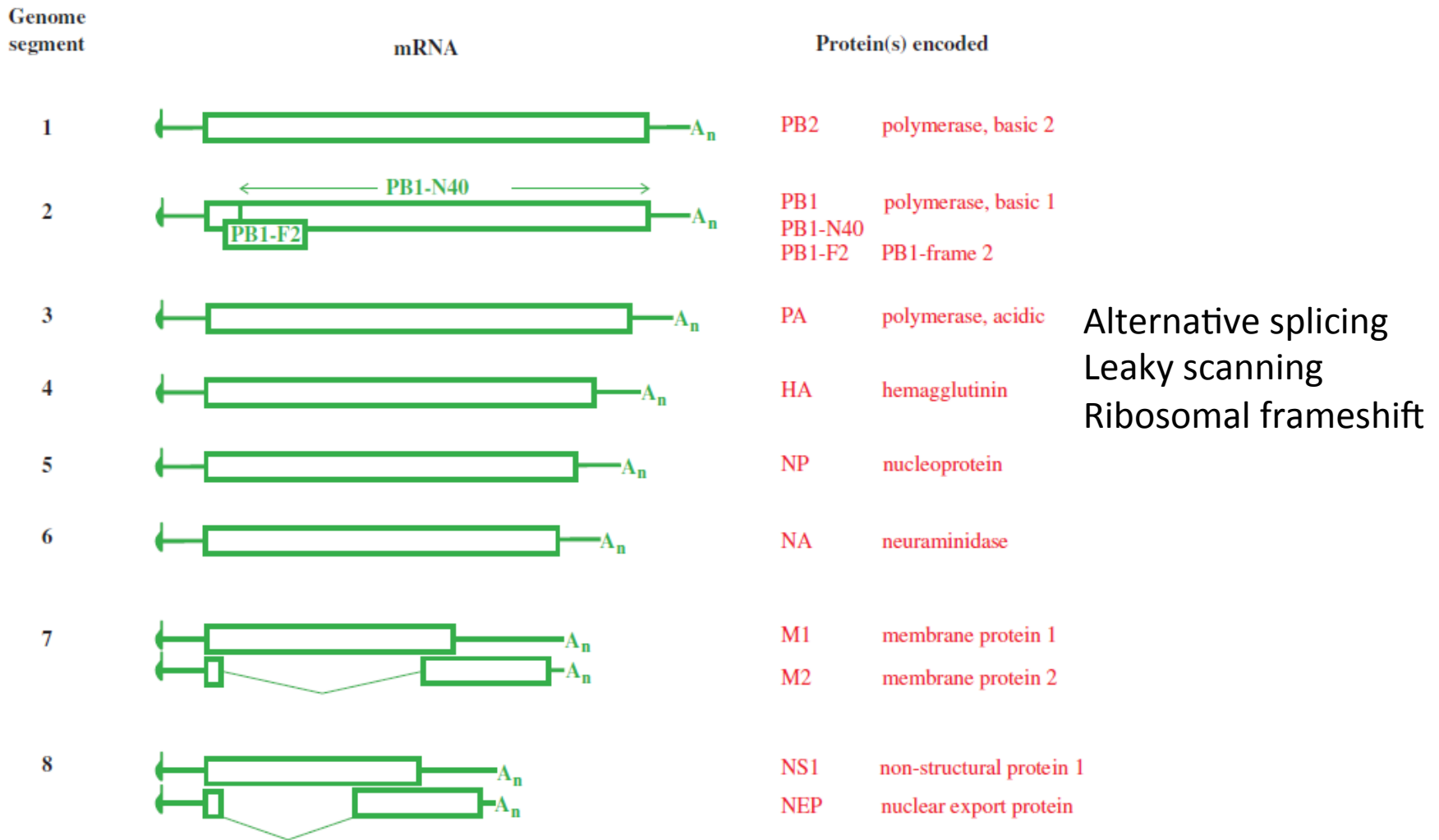
Membrane fusion





Cap snatching

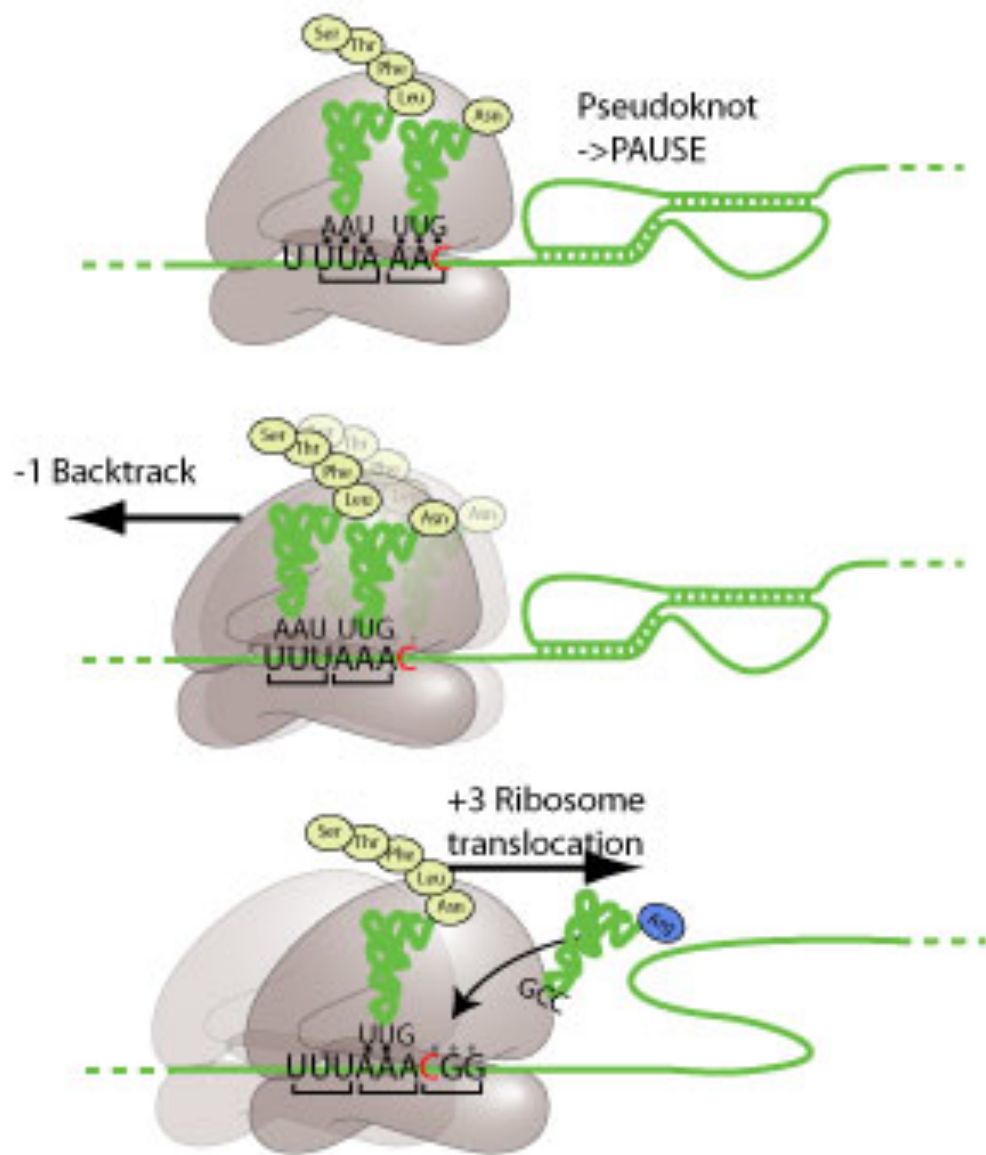




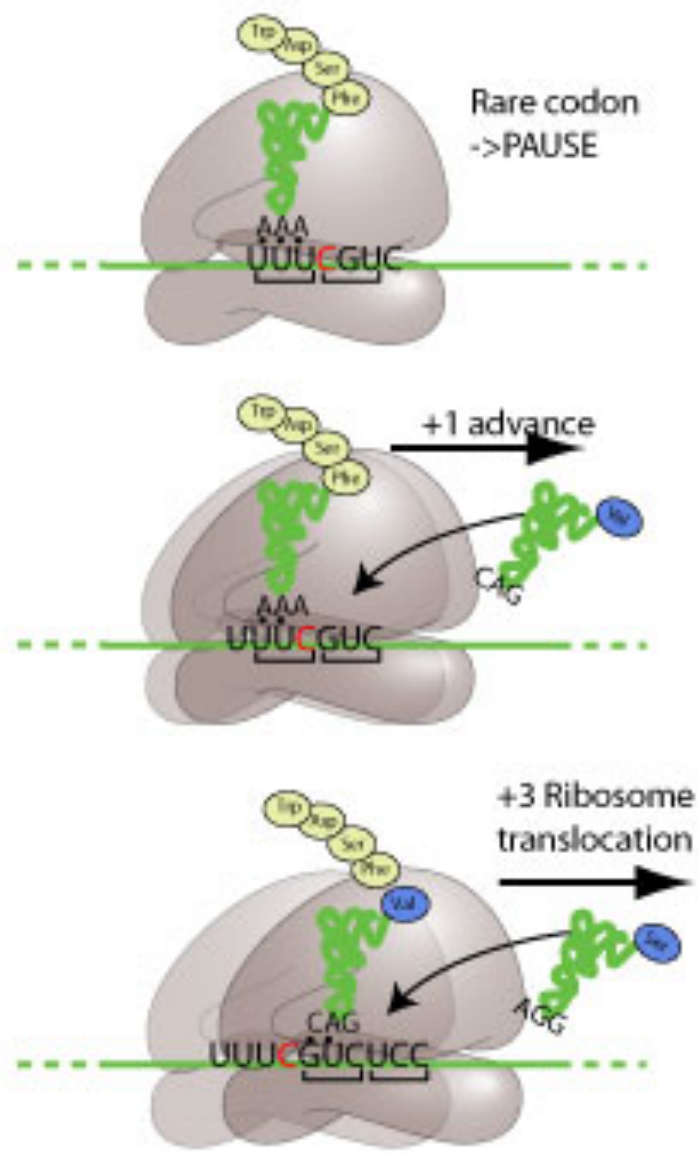
Alternative splicing
Leaky scanning
Ribosomal frameshift

Figure 16.5 Influenza A virus transcripts and the proteins they encode. Codon 40 of the PB1 ORF is the start codon for PB1-N40. The PB1-F2 ORF is within the PB1 ORF, in the +1 reading frame. M2 and NEP are encoded by spliced mRNAs.

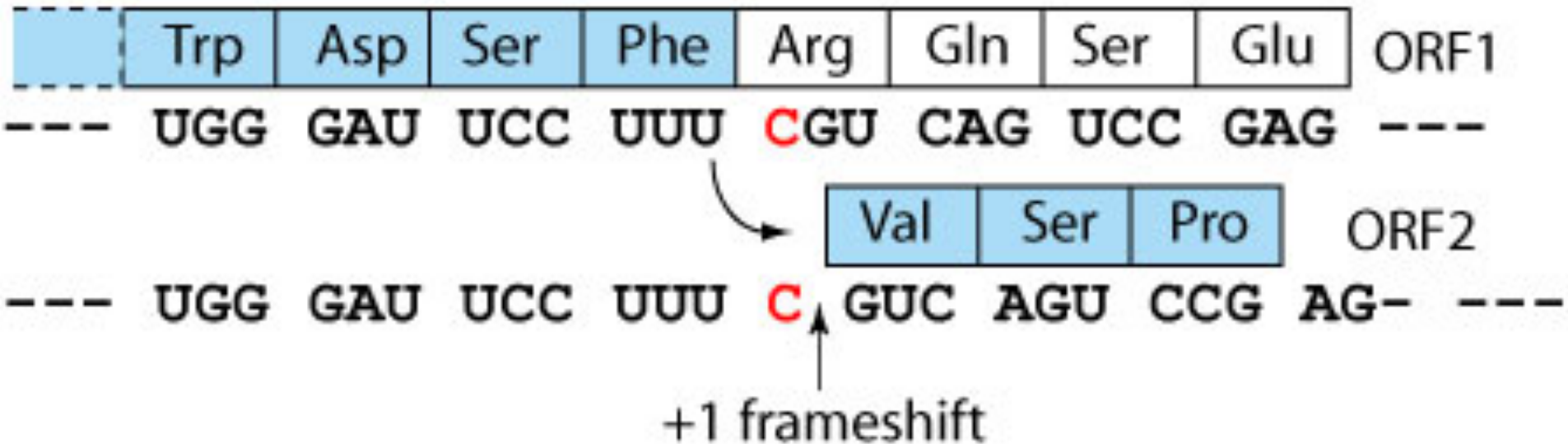
-1 Ribosomal frameshift

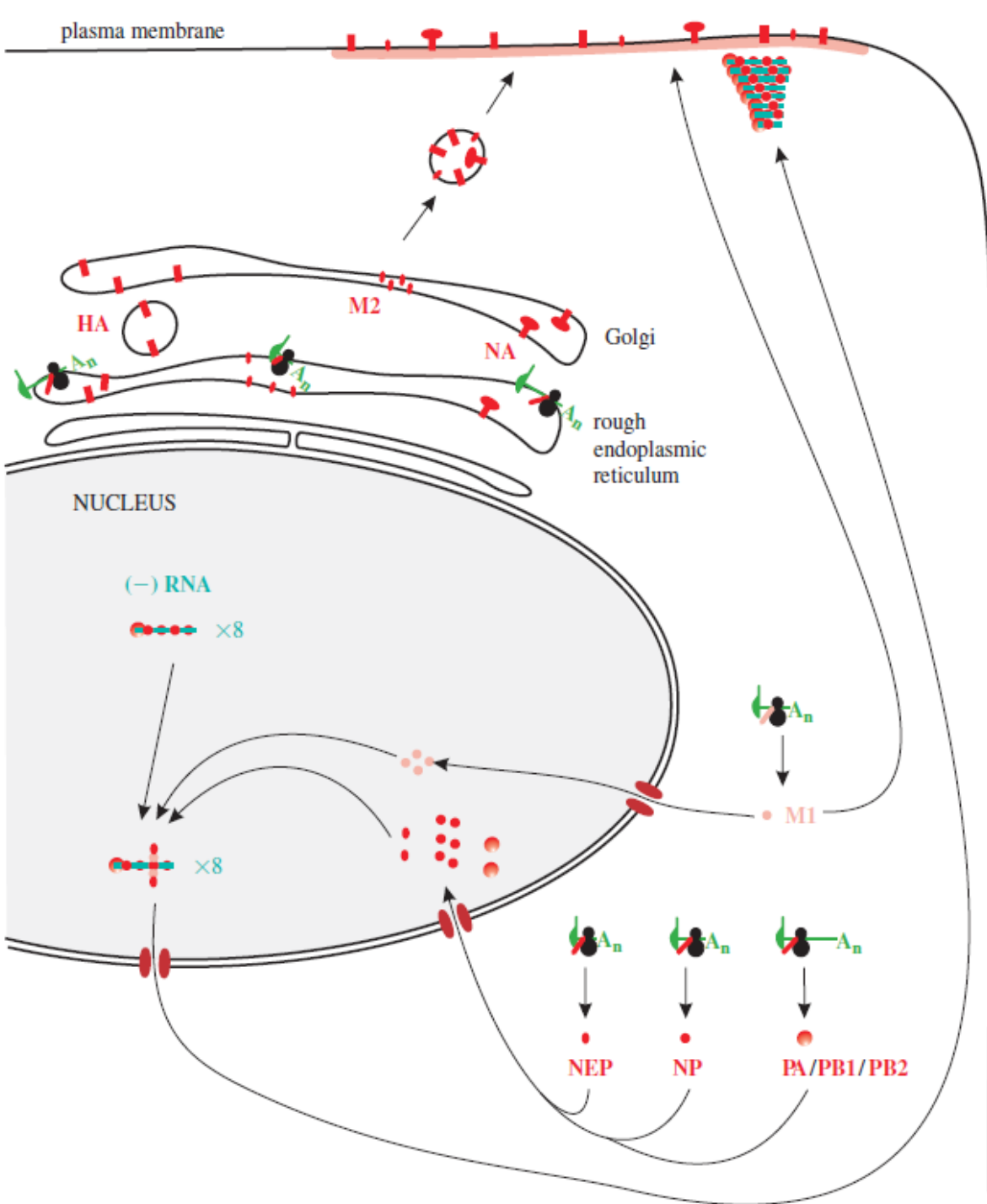


+1 Ribosomal frameshift

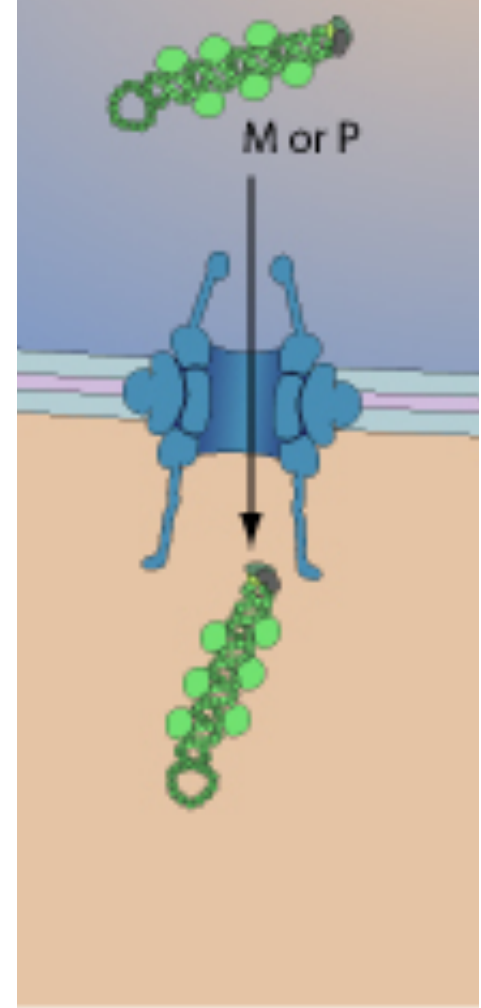


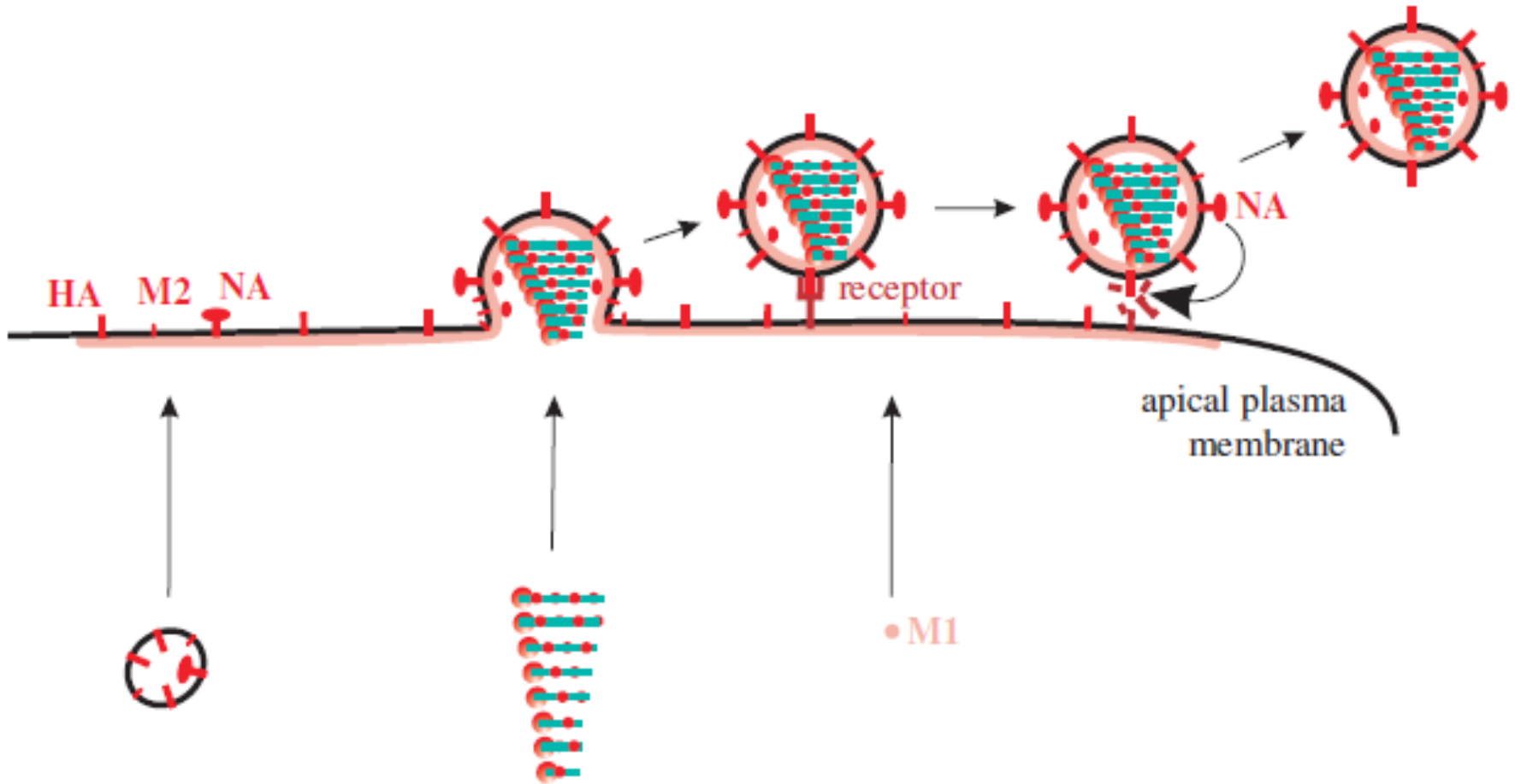
Influenza A virus +1 frameshift

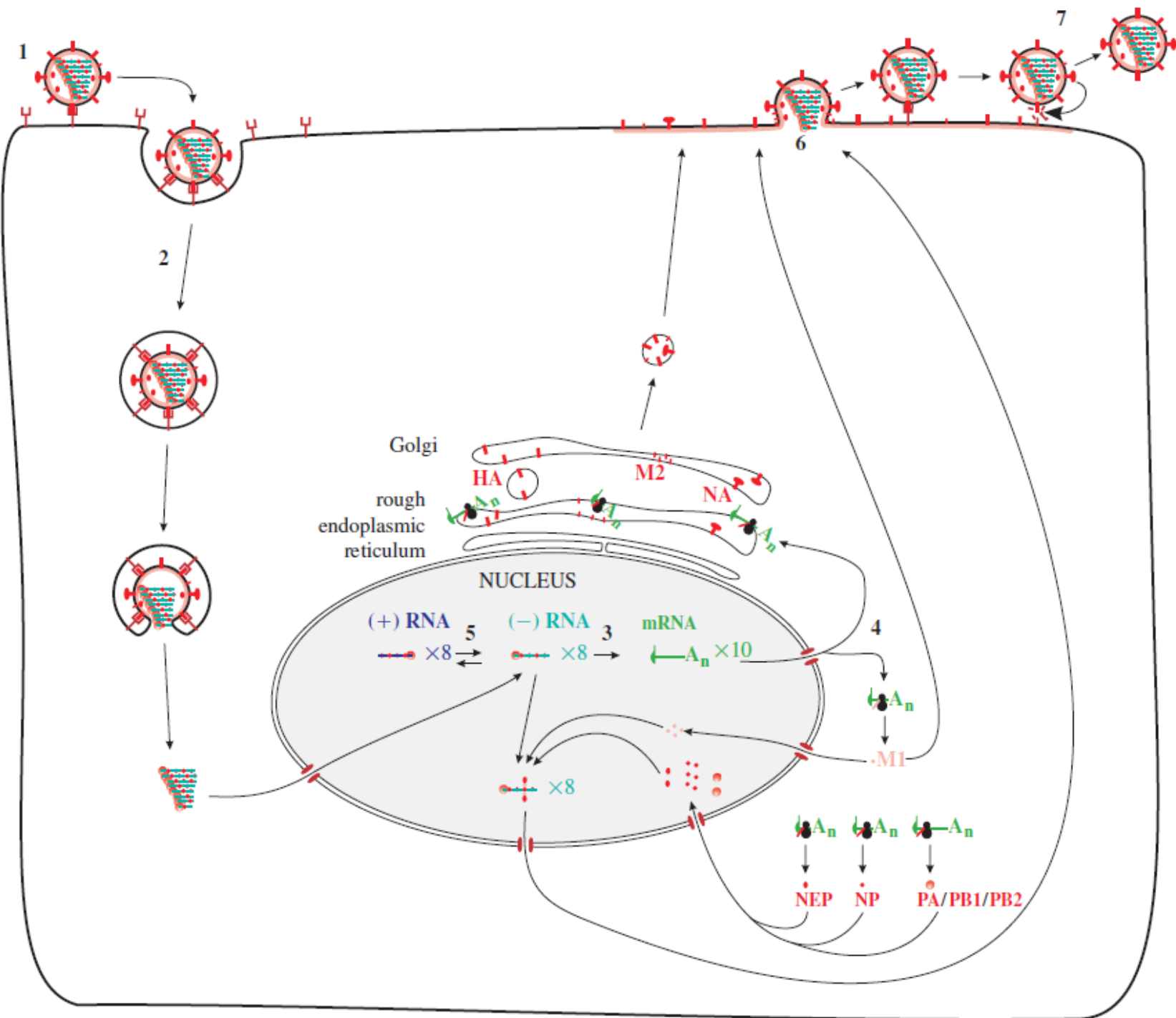




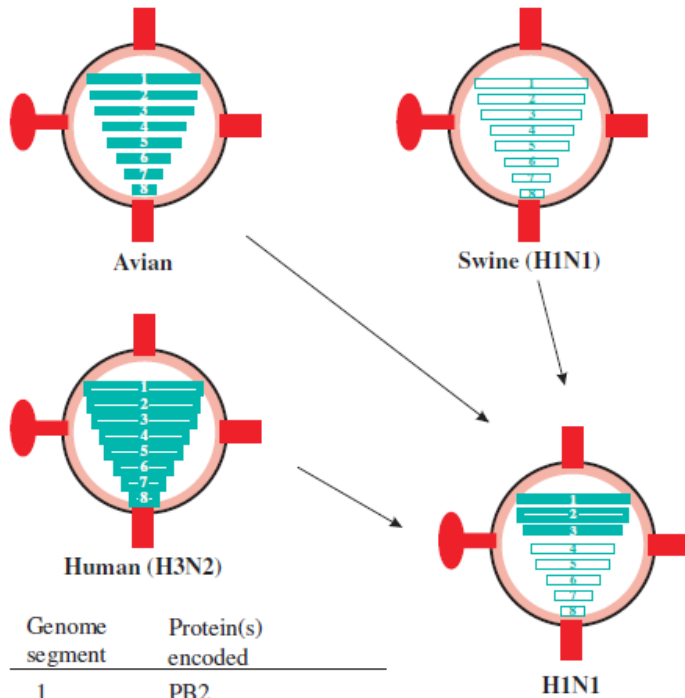
RNA viruses
 Orthomyxoviridae
 Bornaviridae







Antigenic drift x antigenic shift



Genome segment	Protein(s) encoded
1	PB2
2	PB1 + PB1-N40 + PB1-F2
3	PA
4	HA
5	NP
6	NA
7	M1 + M2
8	NS1 + NEP

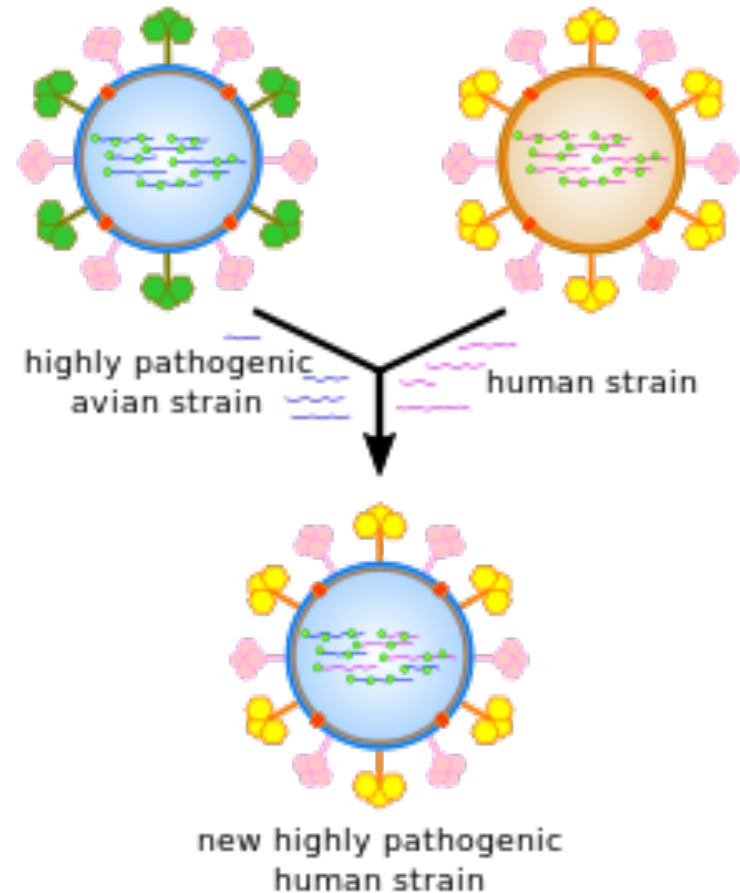


Figure 16.9 Origin of the 2009 “swine flu” virus. The new H1N1 virus was a triple reassortant, with genes derived from swine, human, and avian viruses.

Learning outcomes

- Describe structure of Influenza A virion
- Describe the Influenza A genome
- Describe Influenza A replication cycle
- Explain antigenic drift and shift
- Explain membrane fusion mechanism

Retroviridae

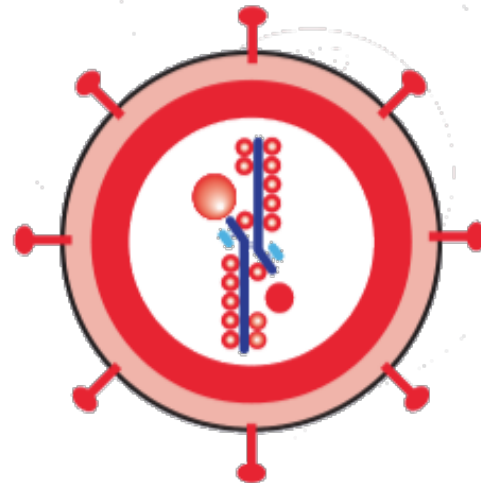
retro (Latin) = backwards

Hosts: mammals
birds
other vertebrate animals

Diseases: immunodeficiency diseases
leukaemias
solid tumours

Virion

- Enveloped
- 80–110 nm diameter
- Genome: single-stranded RNA
plus polarity
9–10 kb
- Contains reverse transcriptase



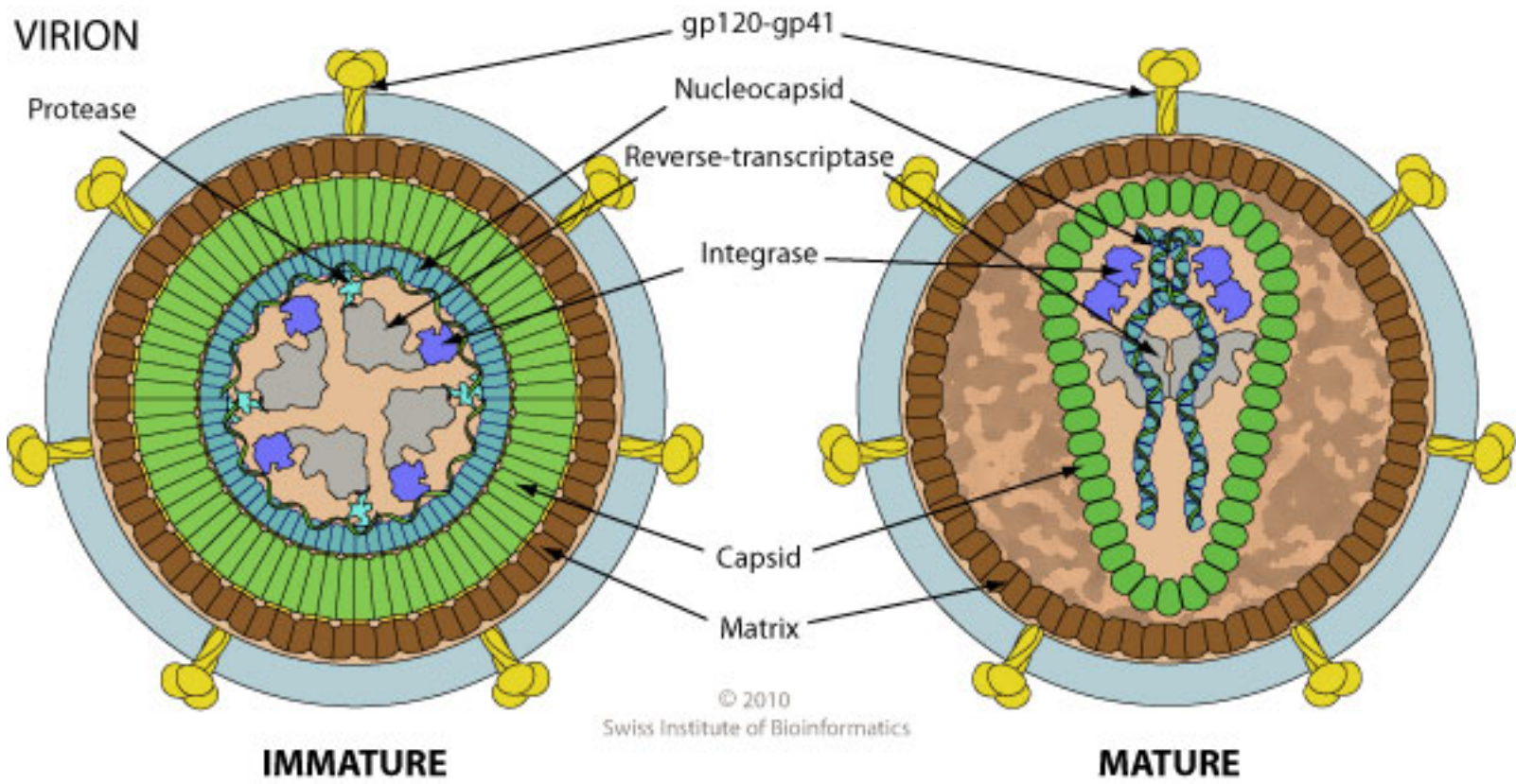
Reverse transcriptases



Murine leukemia virus (MLV)

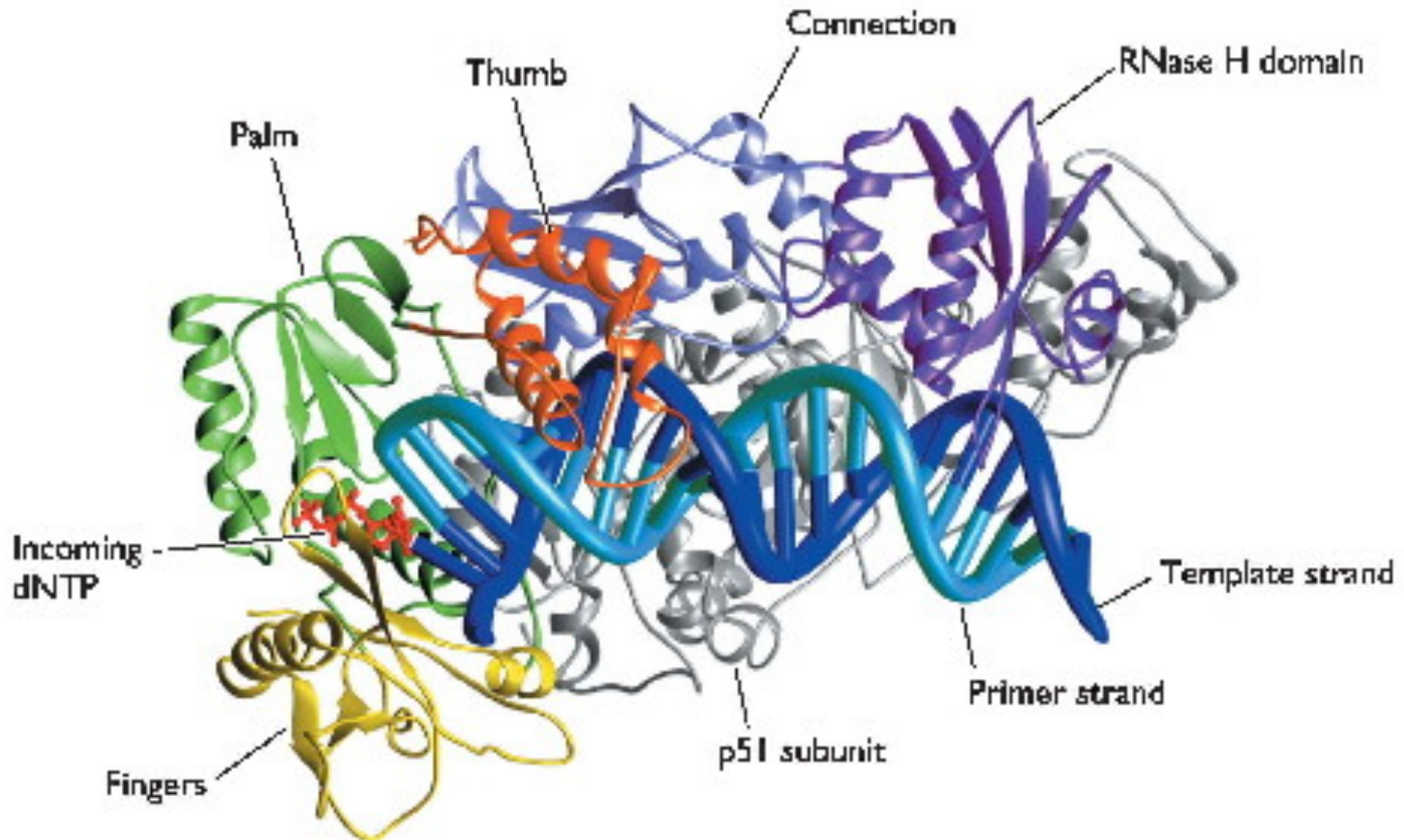


VIRION



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Reverse transcriptase



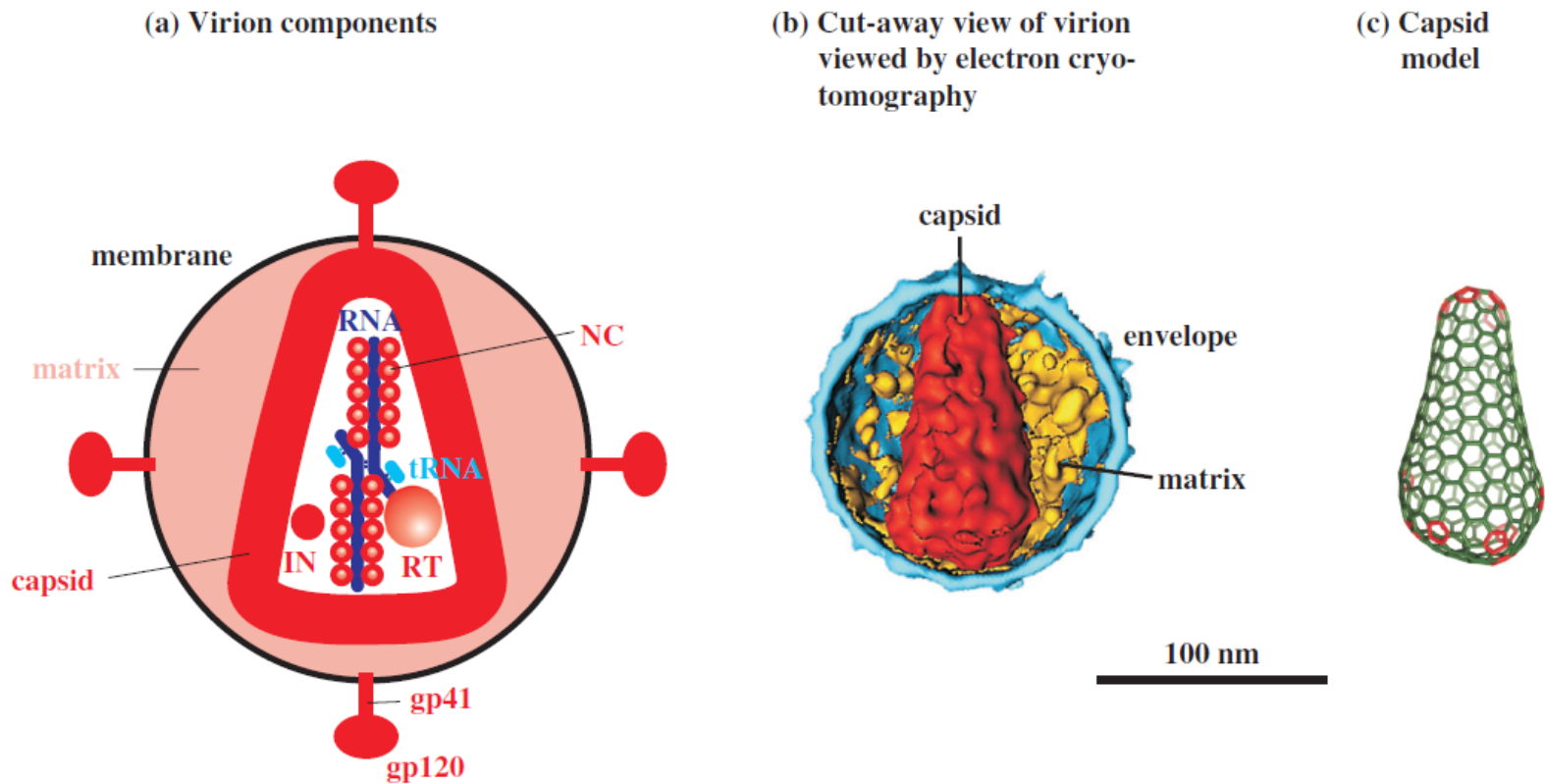
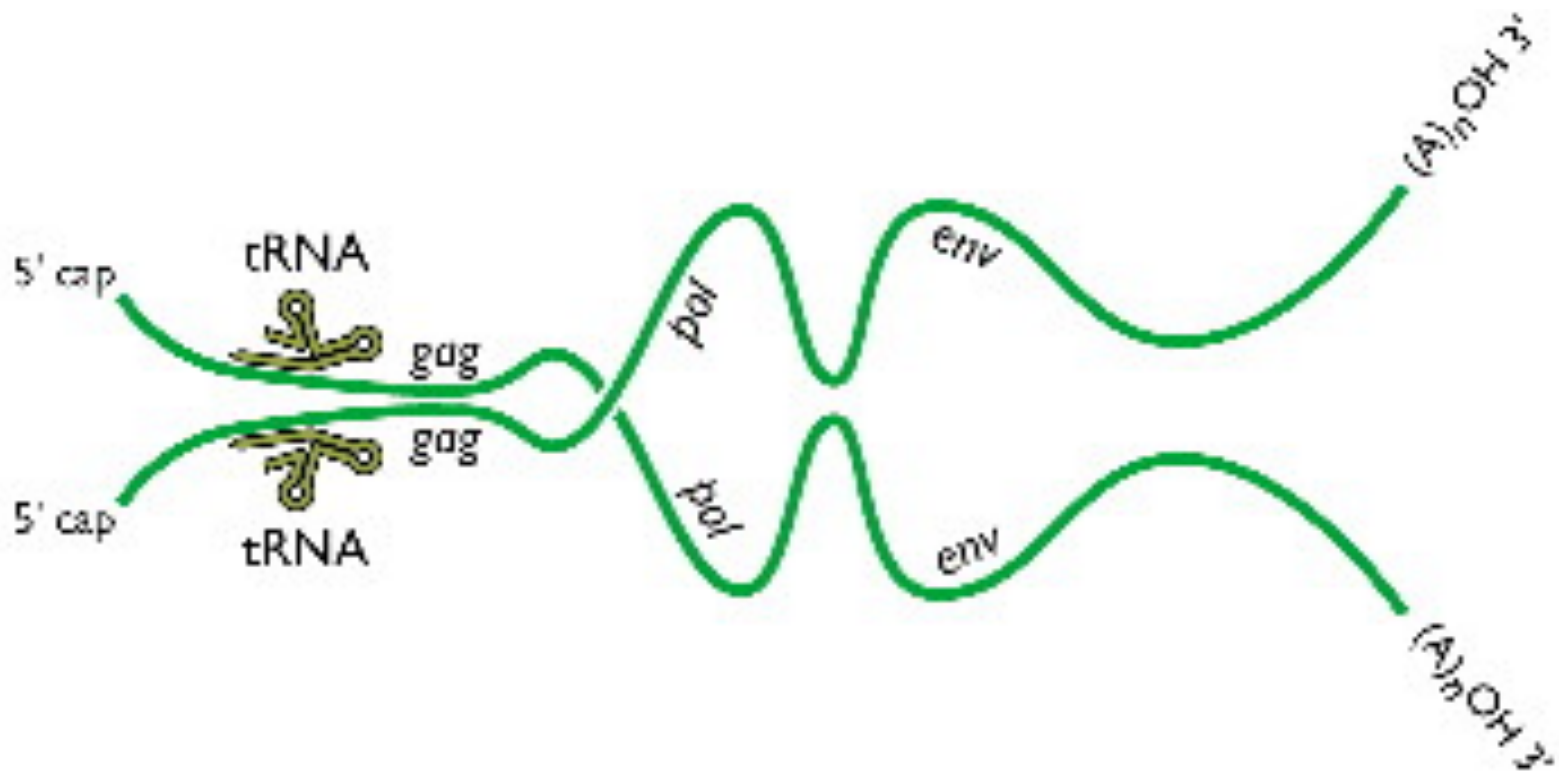
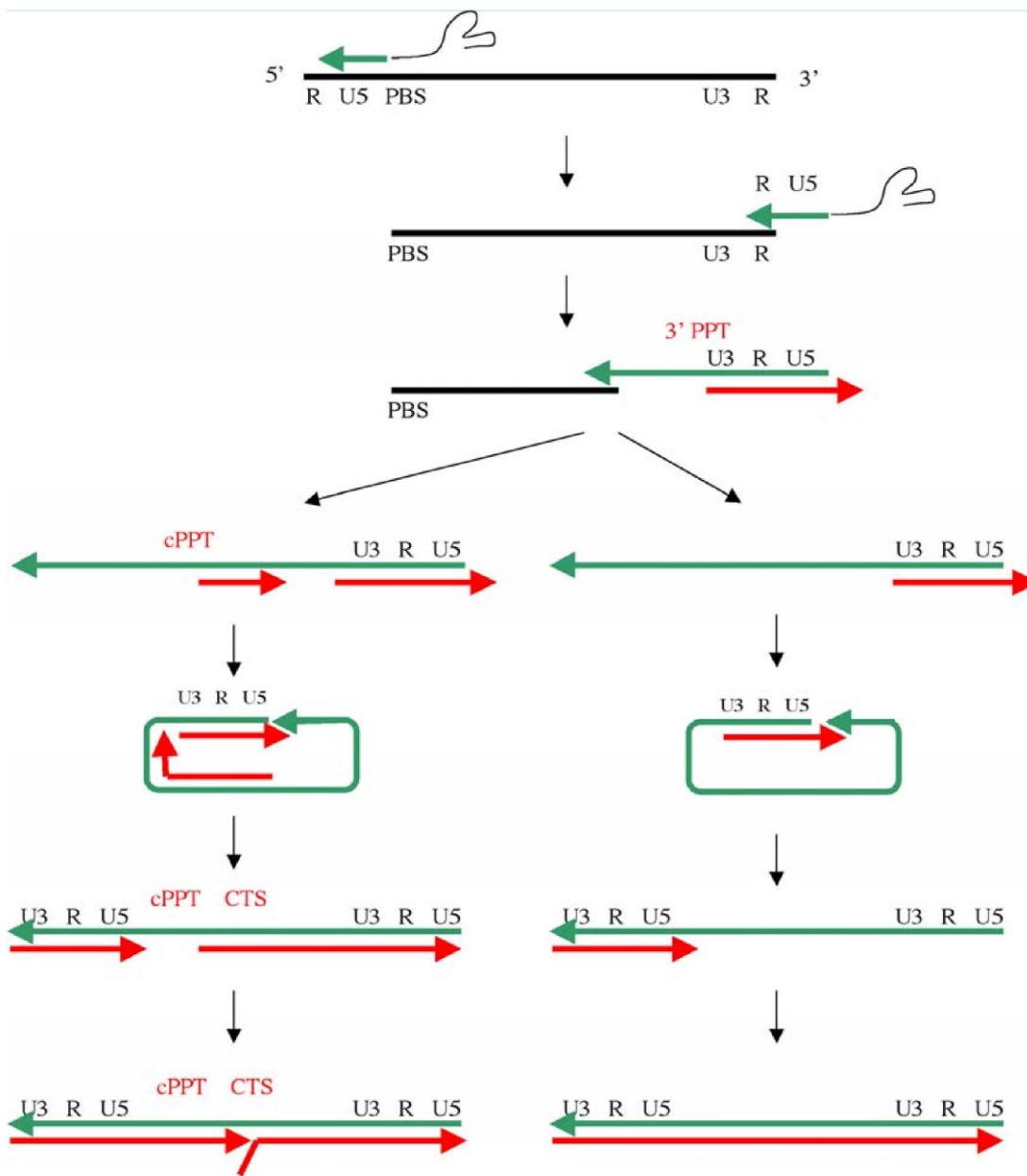


Figure 18.1 HIV virion. (a) Virion components. IN: integrase. NC: nucleocapsid protein. RT: reverse transcriptase. The TM and SU glycoproteins indicated are those of HIV-1 (gp41 and gp120). (c) Capsid model, showing protein hexamers in green and pentamers in red.

Sources: (b) Grünewald and Cyrklaff (2006) *Current Opinion in Microbiology*, 9, 437. (c) Ganser-Pornillos, Yeager, and Sundquist (2008) *Current Opinion in Structural Biology*, 18, 203. (b) and (c) reproduced by permission of Elsevier Limited and the authors





Lentiviruses

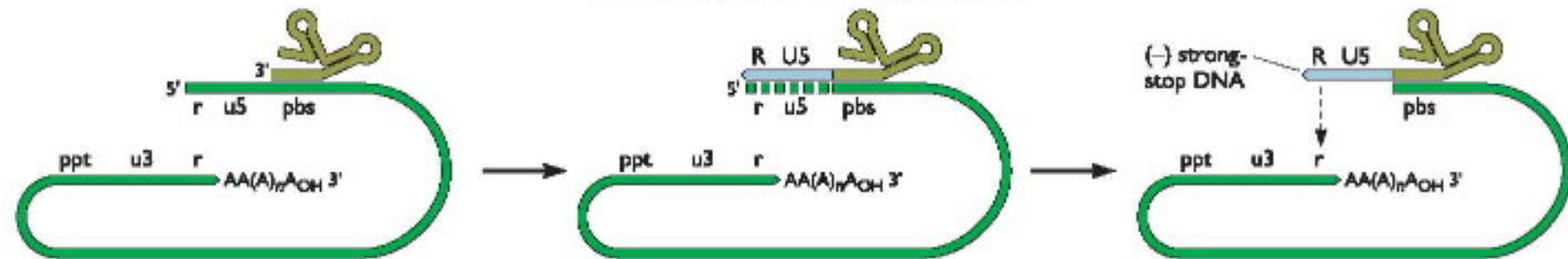
HIV-1

Other orthoretroviruses

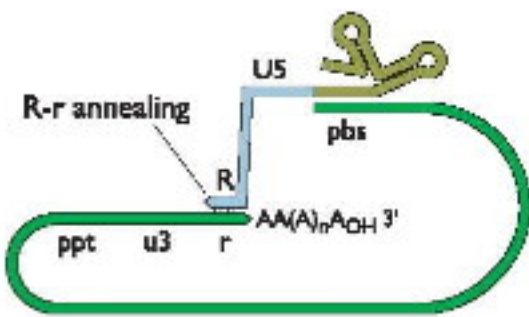
HIV-1 cPPT-

Initiation of (-) strand DNA synthesis

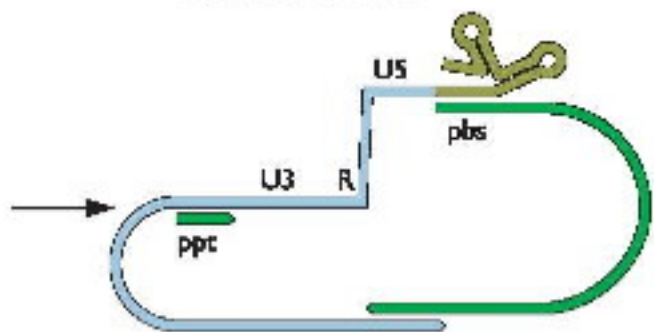
The 5' end of the viral RNA genome is degraded by the RNase H activity of RT as the (-) strand DNA is synthesized.



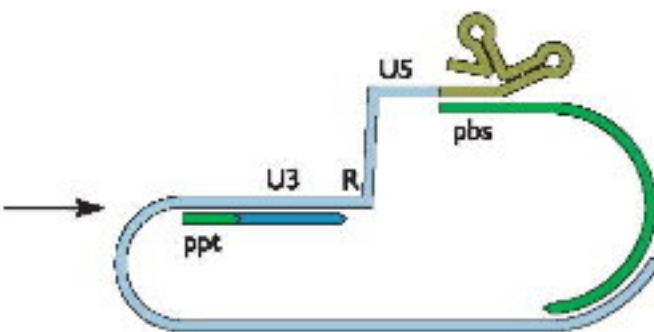
First template exchange



The RNA genome continues to be degraded as (-) strand DNA is synthesized

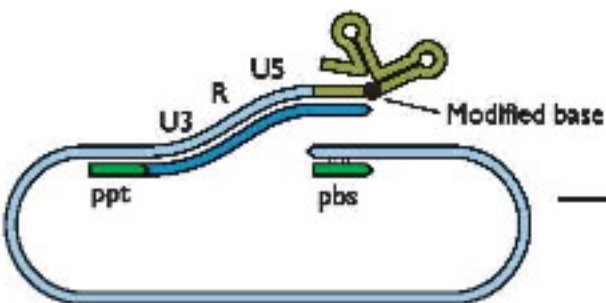


(+) strand DNA synthesis begins, primed by the ppt RNA

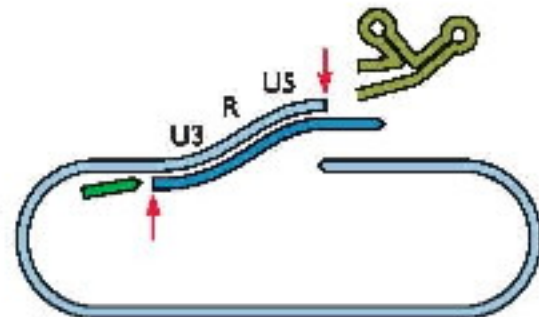


(+) strand DNA synthesis

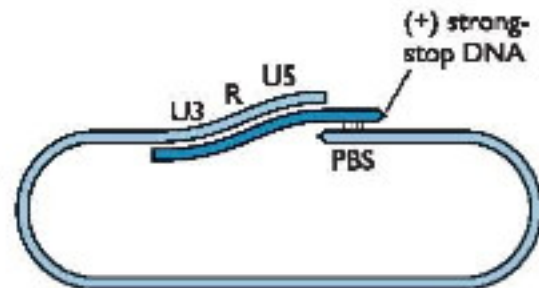
The pbs sequence is copied twice:
• once from the RNA genome
• once from the tRNA primer



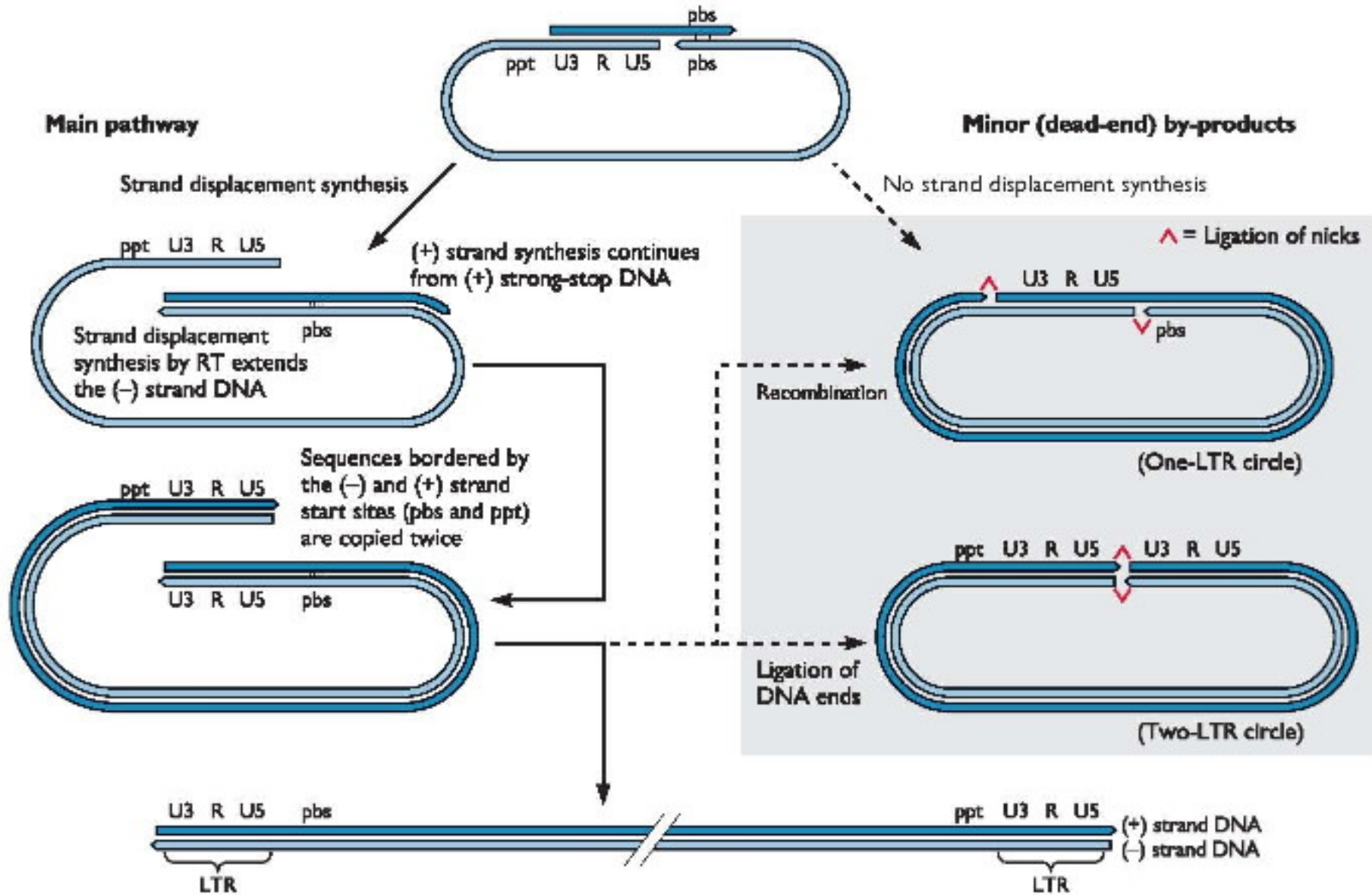
RNase H endonuclease activity of RT removes both primer RNAs

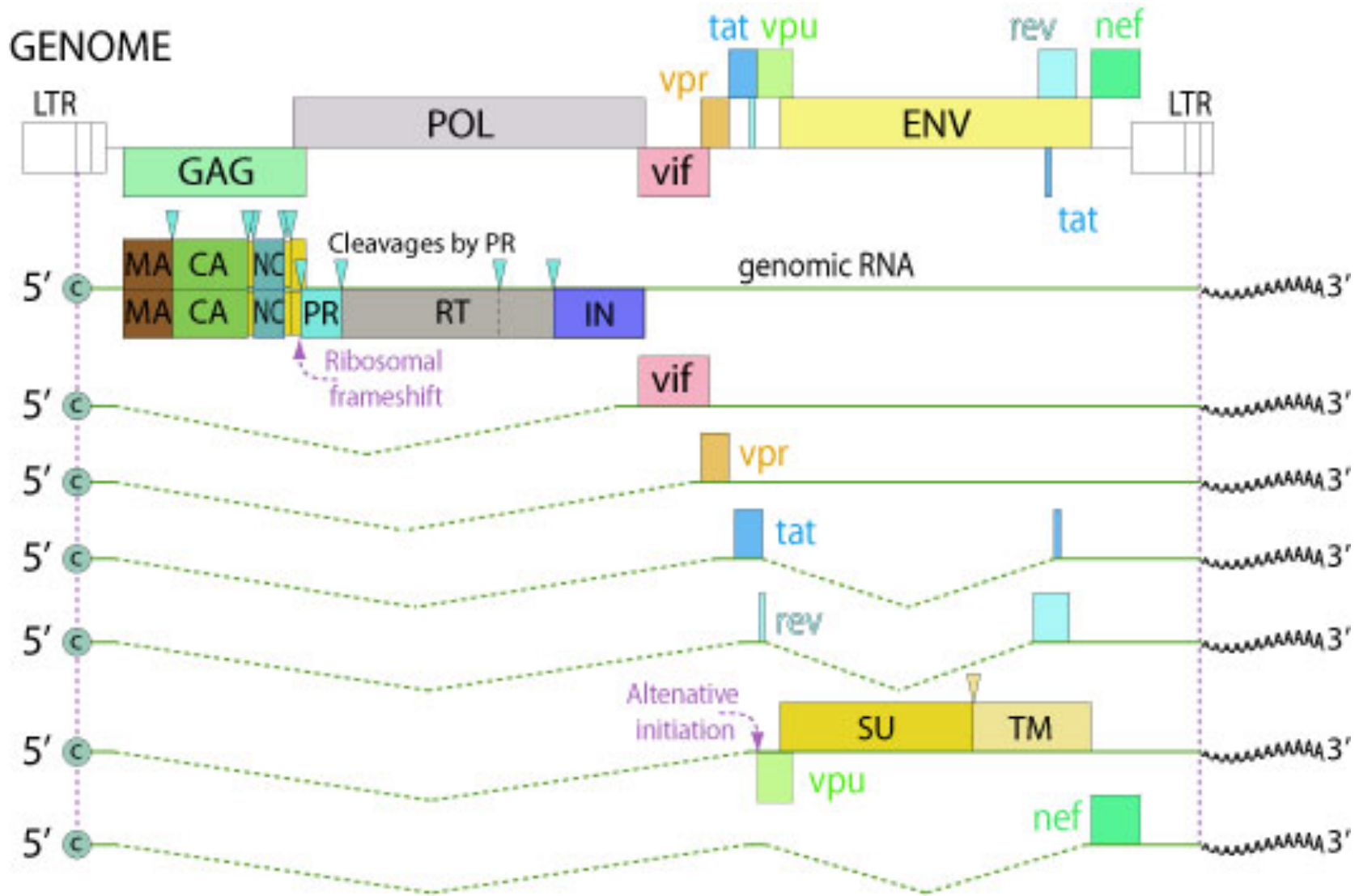


DNA ends are juxtaposed by annealing at complementary PBS sequences



Second template exchange is facilitated by annealing of PBS sequences

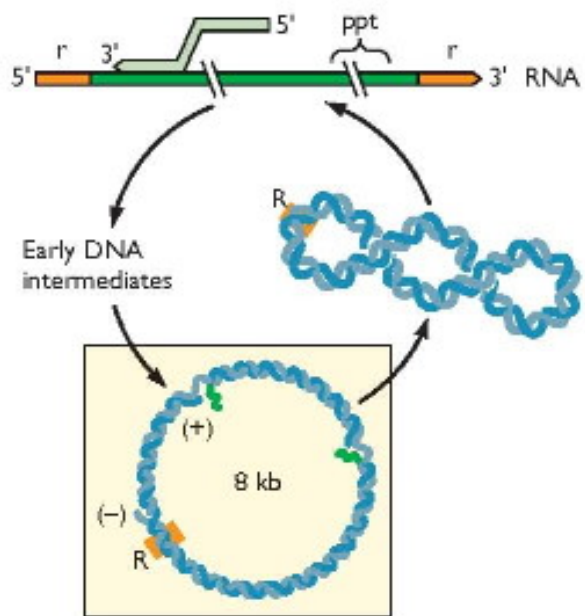




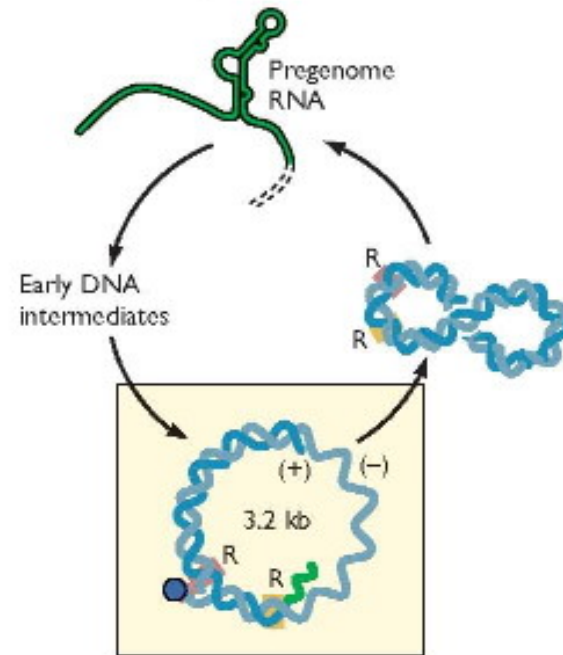
Characteristics of retroelements resident in eukaryotic genomes

	Designation	Characteristic	Example	Copy no.
<p>Endogenous retrovirus</p>	Endogenous retroviruses	RT, LTR (internal Pol II promoter), and <i>env</i>	HERVs (human)	$1-10^2$
<p>Retrotransposons</p>	Retrotransposons	RT, LTR (internal Pol II promoter)	Ty3 (yeast)	10^2-10^4
<p>LINEs</p>	Retroposons (LINEs)	RT, internal Pol III promoter, A-rich sequence at end	LINE 1 (human)	10^4-10^5
<p>SINEs</p>	Retrosequences (SINEs)	A-rich sequence at end, internal Pol III promoter, but no RT	<i>Alu</i> (human)	10^5-10^6
<p>Processed pseudogenes</p>	Processed pseudogenes	A-rich sequence at end, no internal promoter, no RT	β -Tubulin (human)	$1-10^2$

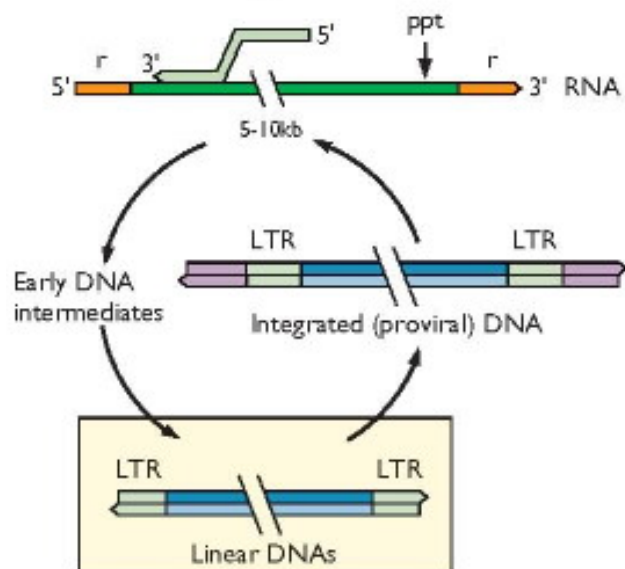
Cauliflower mosaic virus



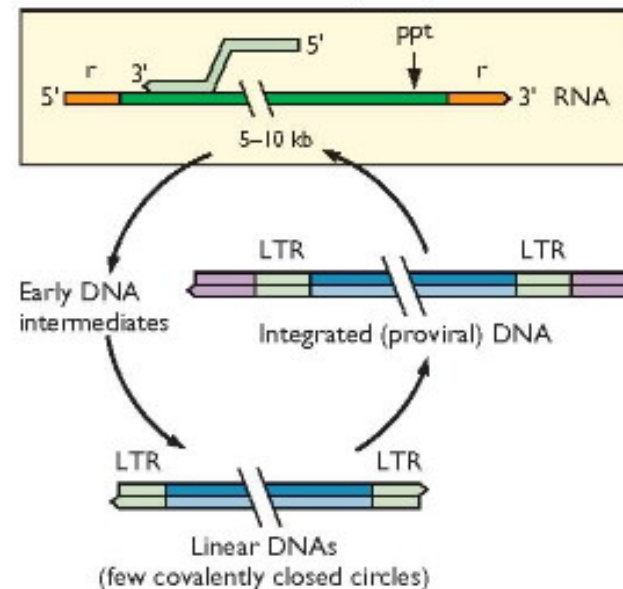
Hepadnaviruses



Foamy retroviruses



Retroviruses (most)



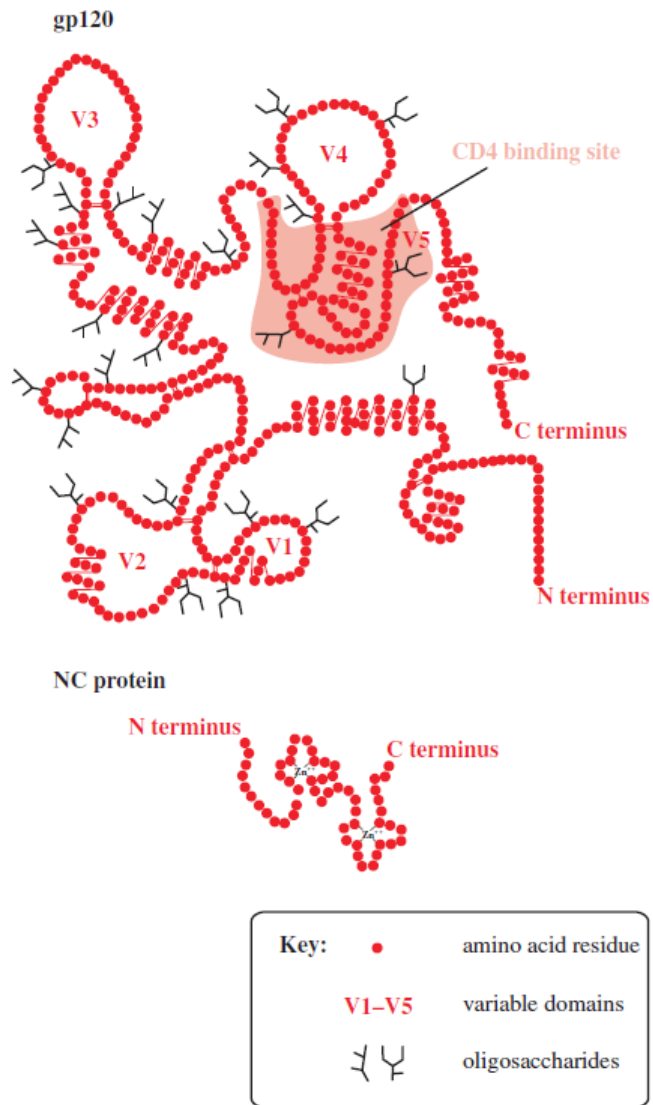


Figure 18.2 Structures of HIV-1 gp120 and NC protein. gp120 has five domains that are highly variable (V1-V5). The NC protein has two zinc fingers.

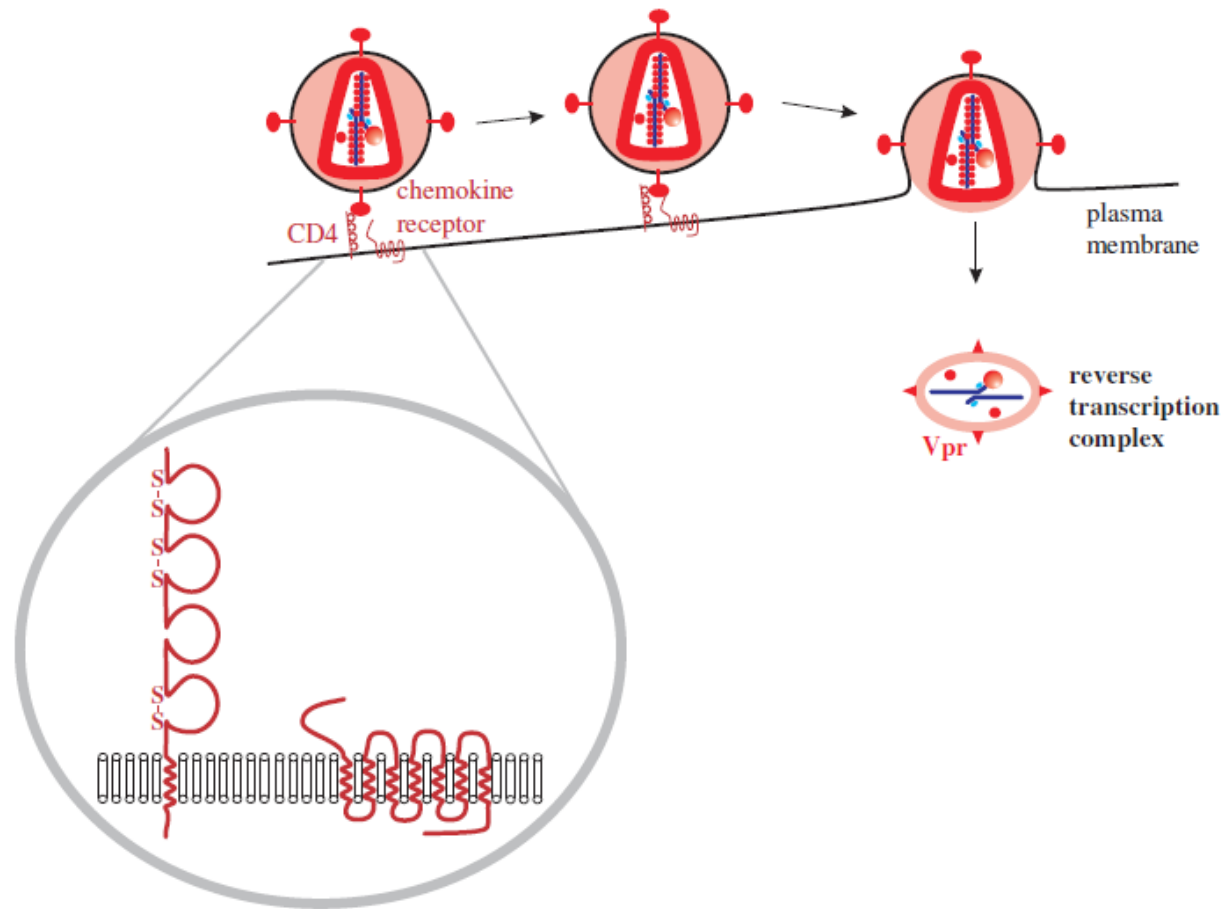


Figure 18.4 HIV-1 attachment and entry. The receptor is CD4; each of the loops represents an immunoglobulin-like domain, three of which are stabilized by disulfide bonds. The co-receptor is a chemokine receptor. Fusion of the virion and cell membranes releases a structure that forms a reverse transcription complex, consisting of the virus genome, tRNA, and several proteins, including Vpr.

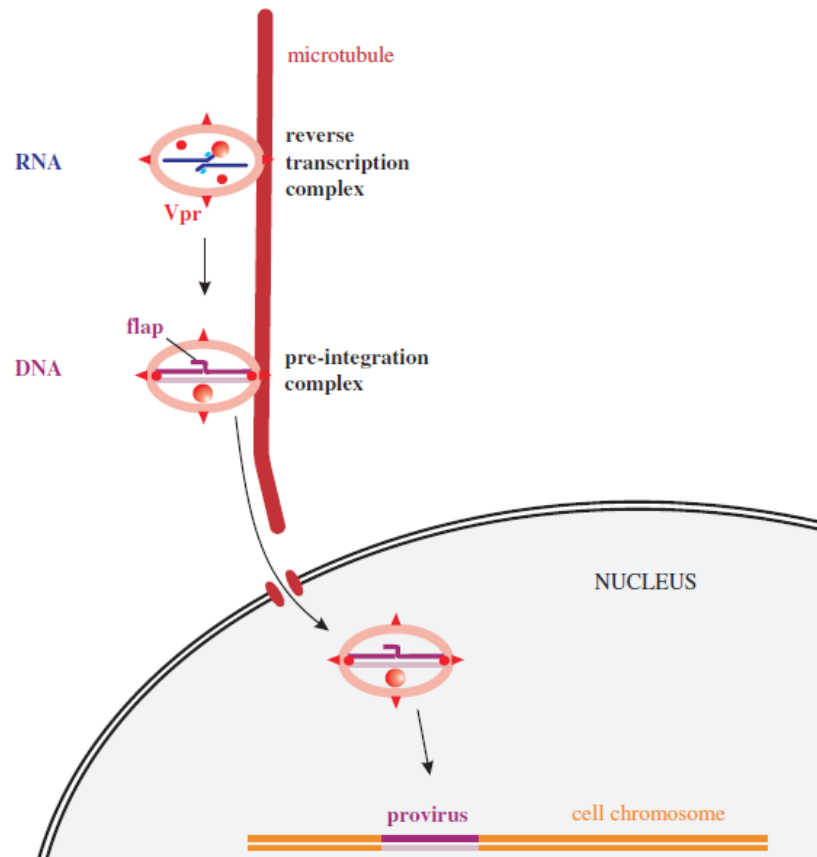


Figure 18.5 HIV-1 reverse transcription and integration of the provirus. A DNA flap is formed during reverse transcription. There is evidence that the pre-integration complex is transported toward the nucleus via the microtubule network.

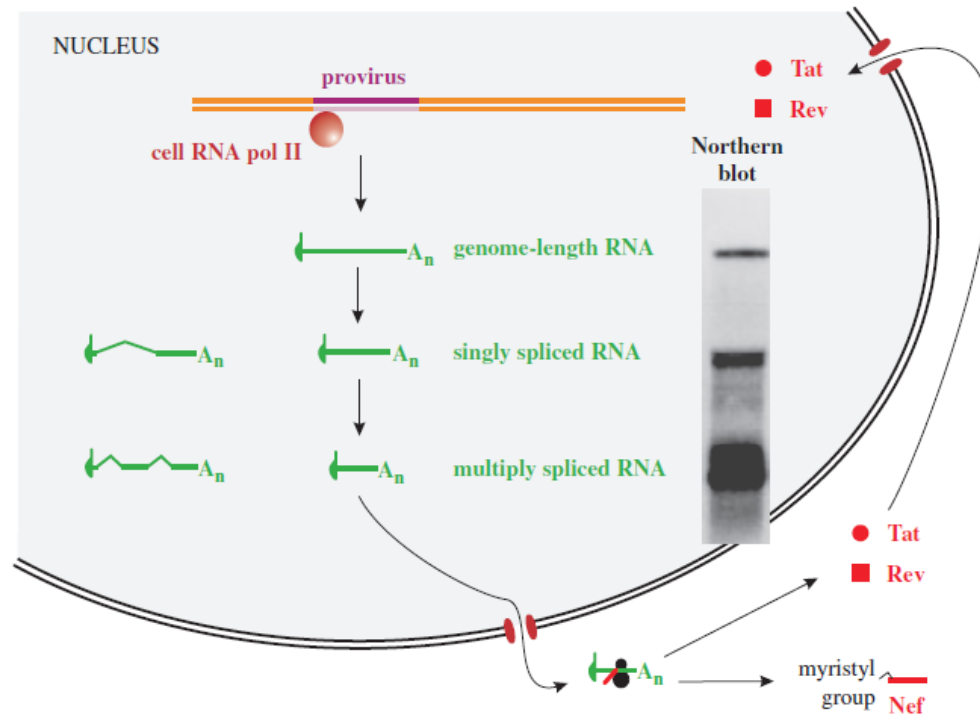


Figure 18.6 HIV-1 early gene expression. Genome-length RNA is transcribed then much of it is spliced, giving rise to two further size classes of RNA that can be detected in northern blots of RNA from infected cells. Early in infection most of the RNA is multiply spliced and is transported to the cytoplasm, where the Tat, Rev, and Nef proteins are translated. Nef is myristylated and performs a number of roles in the cytoplasm, while Tat and Rev are transported to the nucleus.
 Source: Northern blot from Malim *et al.* (1990) *Cell*, 60, 675; reproduced by permission of Elsevier Limited and the authors.

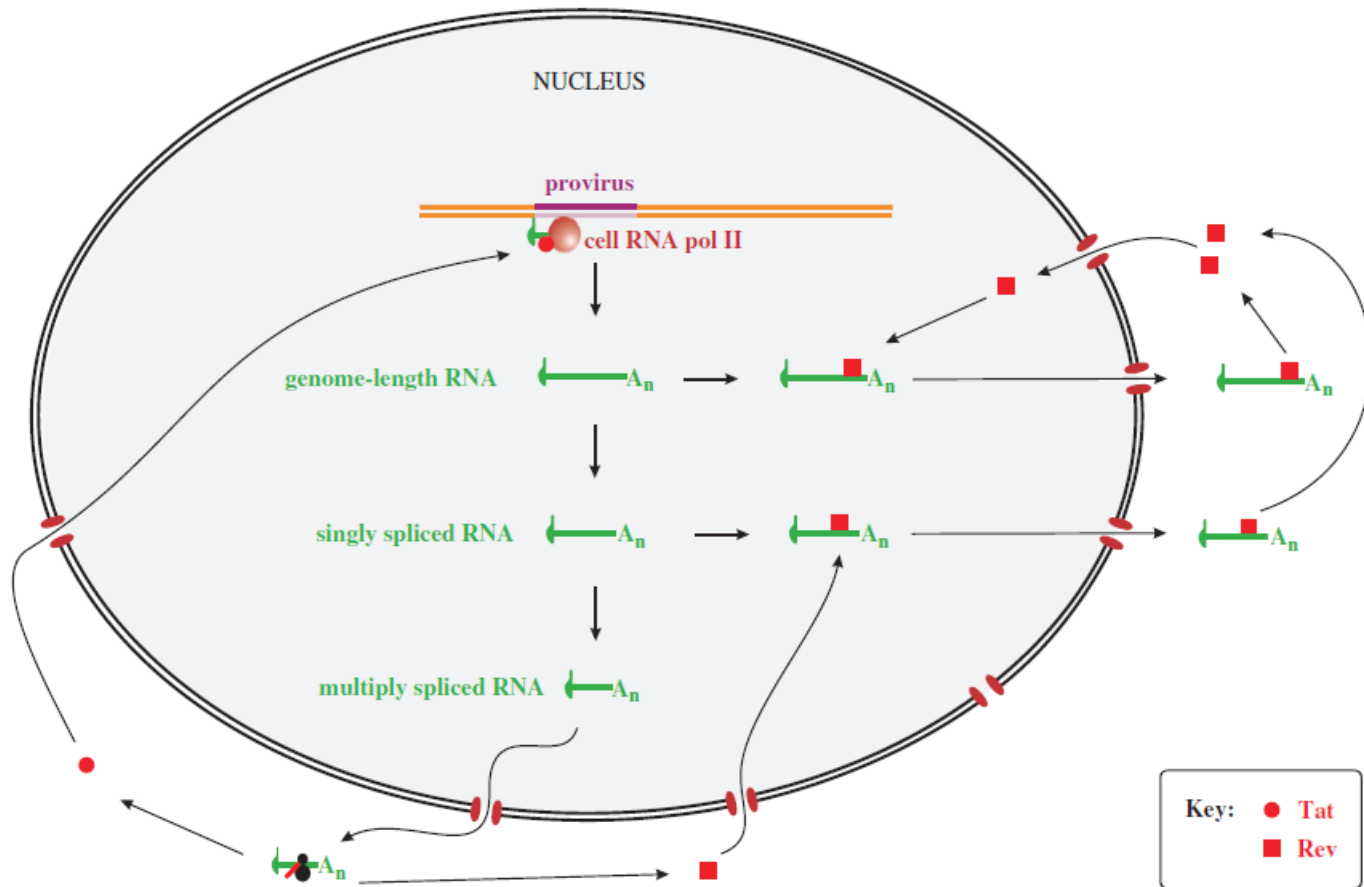


Figure 18.7 Roles of Tat and Rev. Tat binds to nascent transcripts and helps to ensure that the entire virus genome is transcribed. Rev binds to genome-length RNA and singly spliced RNA and aids their transport to the cytoplasm, where the late proteins are translated. Rev is recycled to the nucleus.

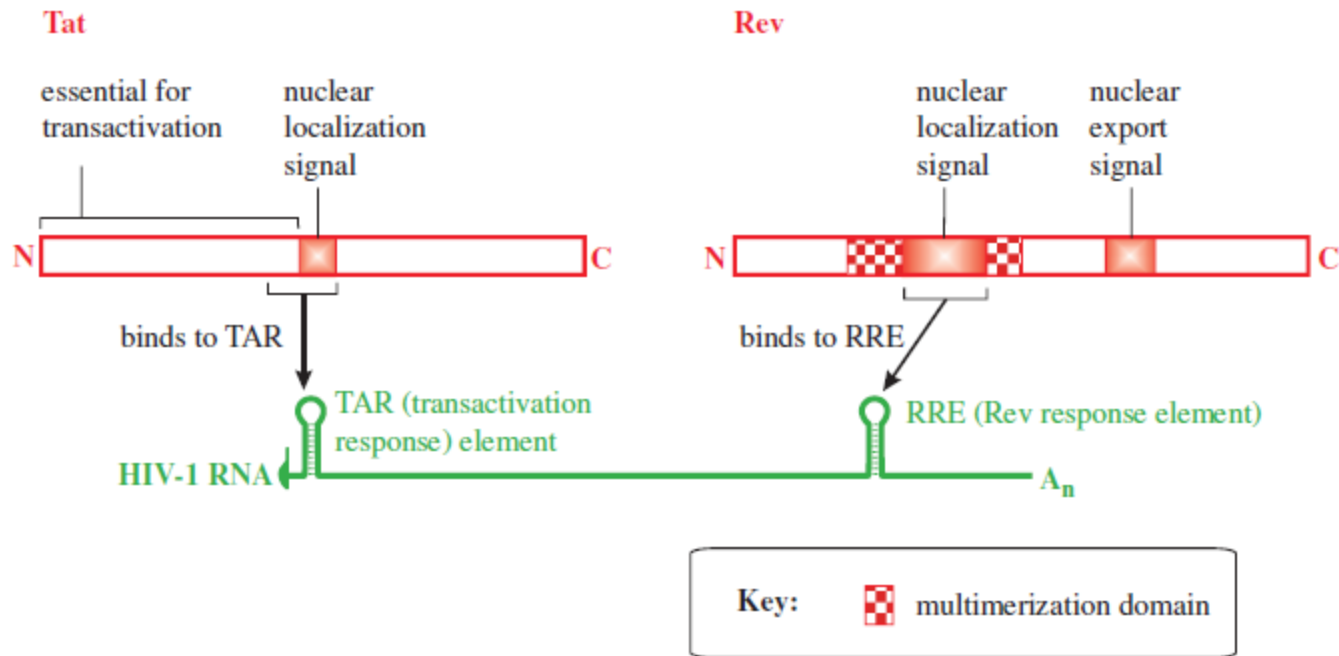


Figure 18.8 HIV-1 Tat and Rev proteins and their binding sites in the virus RNA. The TAR and RRE regions of the RNA have complex secondary structures. The RRE is present in genome-length RNA and the singly spliced RNAs, but it is absent from the multiply spliced RNAs.

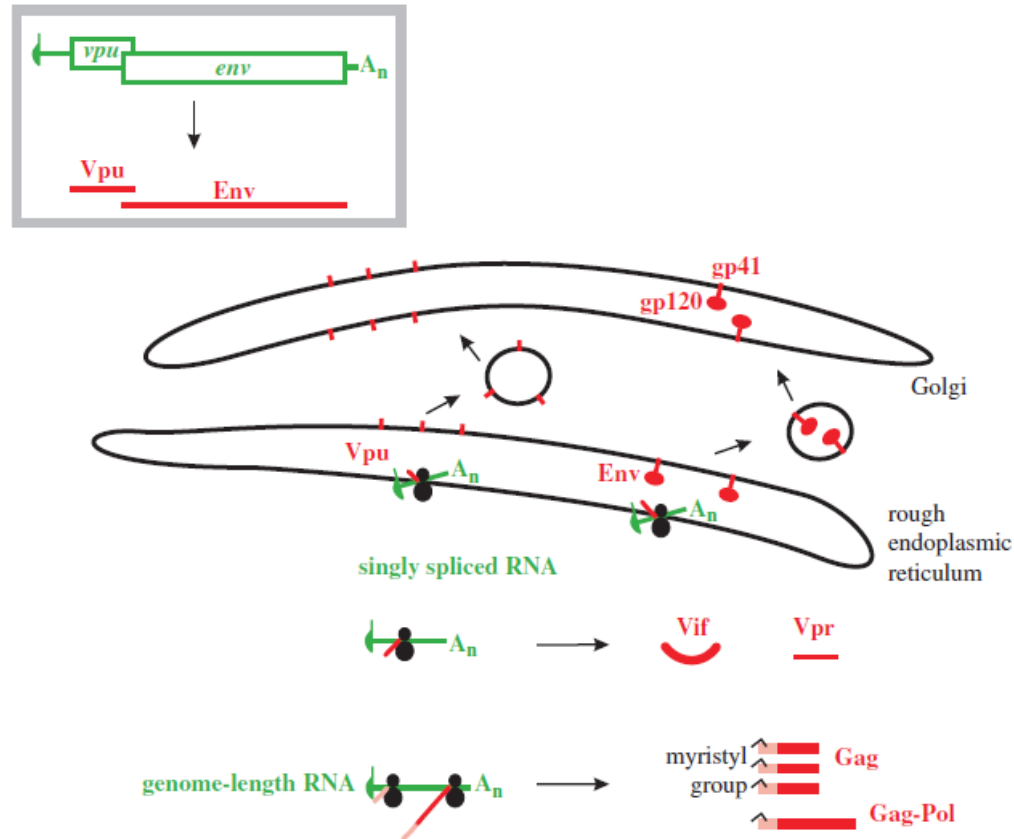


Figure 18.9 HIV-1 late gene expression. Vpu and Env are translated from singly spliced RNAs in the rough endoplasmic reticulum. The inset shows translation of Vpu and Env from a bicistronic mRNA. Env is synthesized when the *vpu* start codon is bypassed during leaky scanning. The remaining proteins are translated on free ribosomes: Vif and Vpr from singly spliced RNAs, and Gag and Gag-Pol from genome-length RNAs.

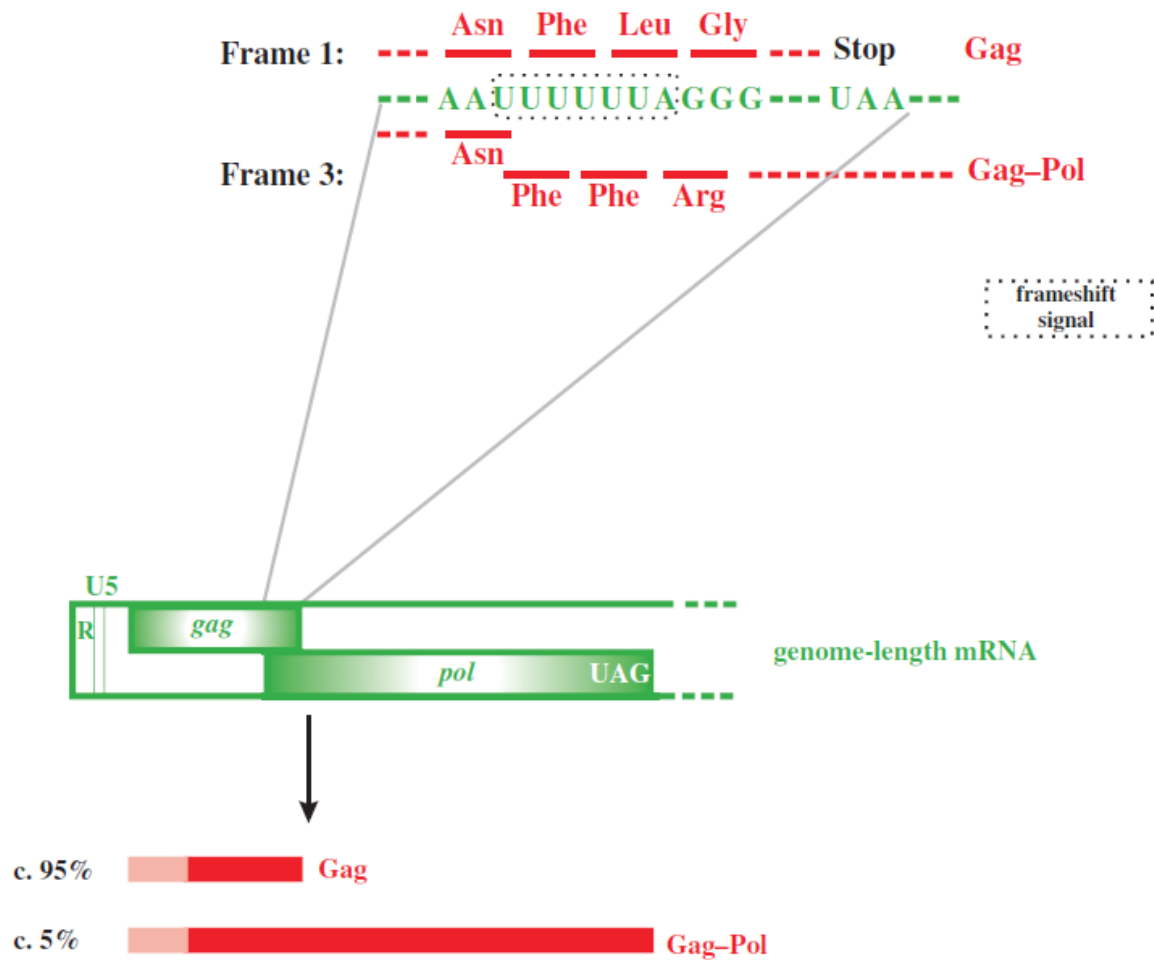


Figure 18.10 Expression of HIV-1 Gag-Pol by ribosomal frameshifting. A ribosome reading in frame 1 shifts at the slippery sequence UUUUUUA to reading in frame 3.

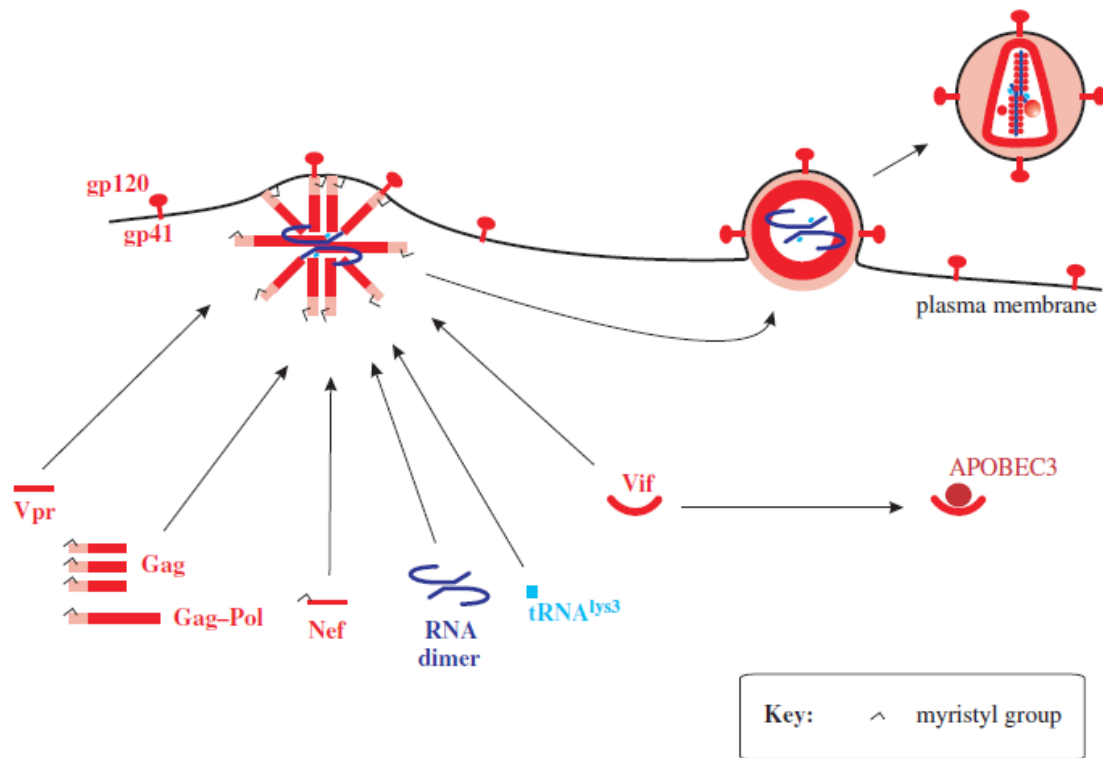


Figure 18.11 Assembly of HIV-1 virions. All of the virus proteins in the diagram are incorporated into new virions, along with two copies of the virus genome and cell tRNA^{lys3}. Vif binds to APOBEC3 proteins and prevents their incorporation into virions.

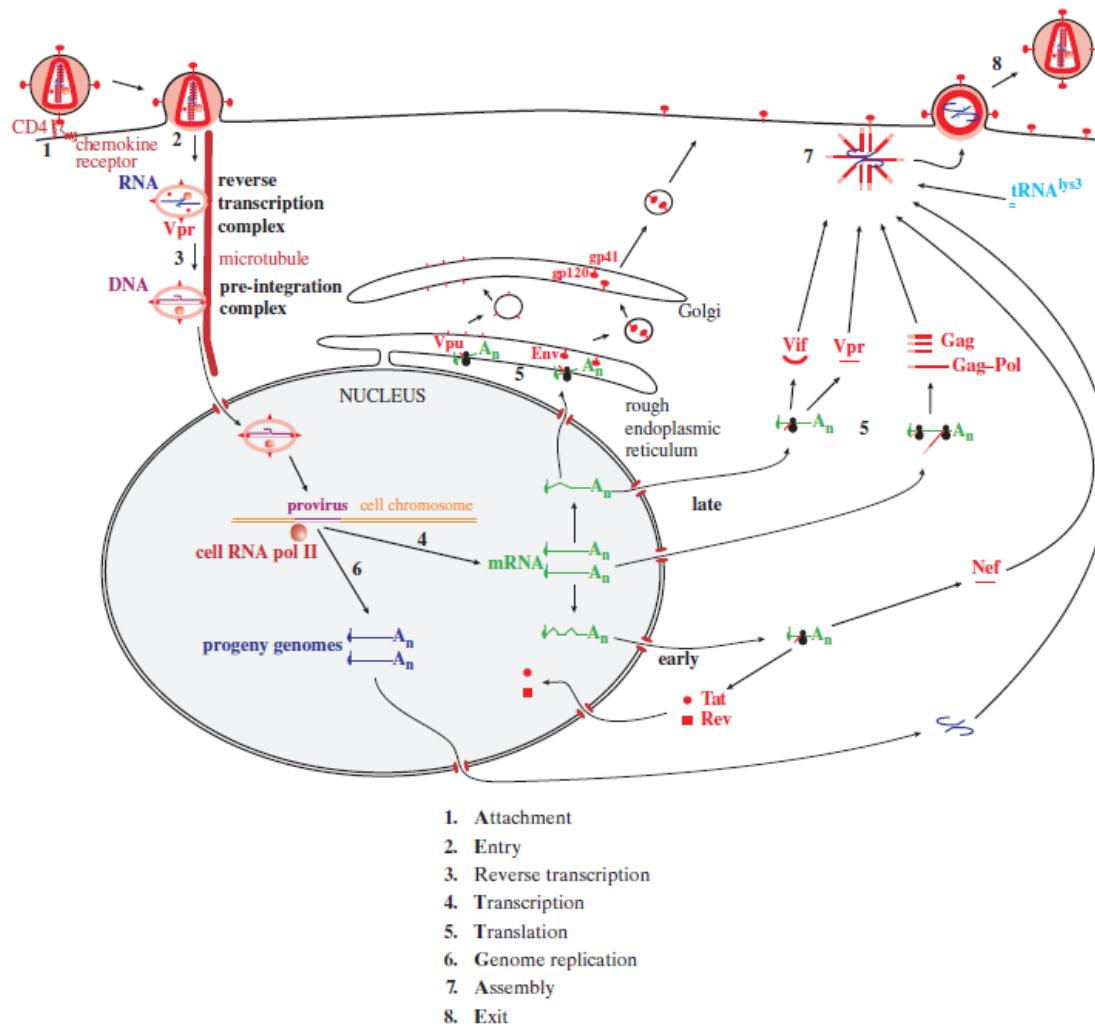


Figure 18.12 The HIV-1 replication cycle. Note the additional step in retrovirus replication: reverse transcription, which takes place between entry and transcription.

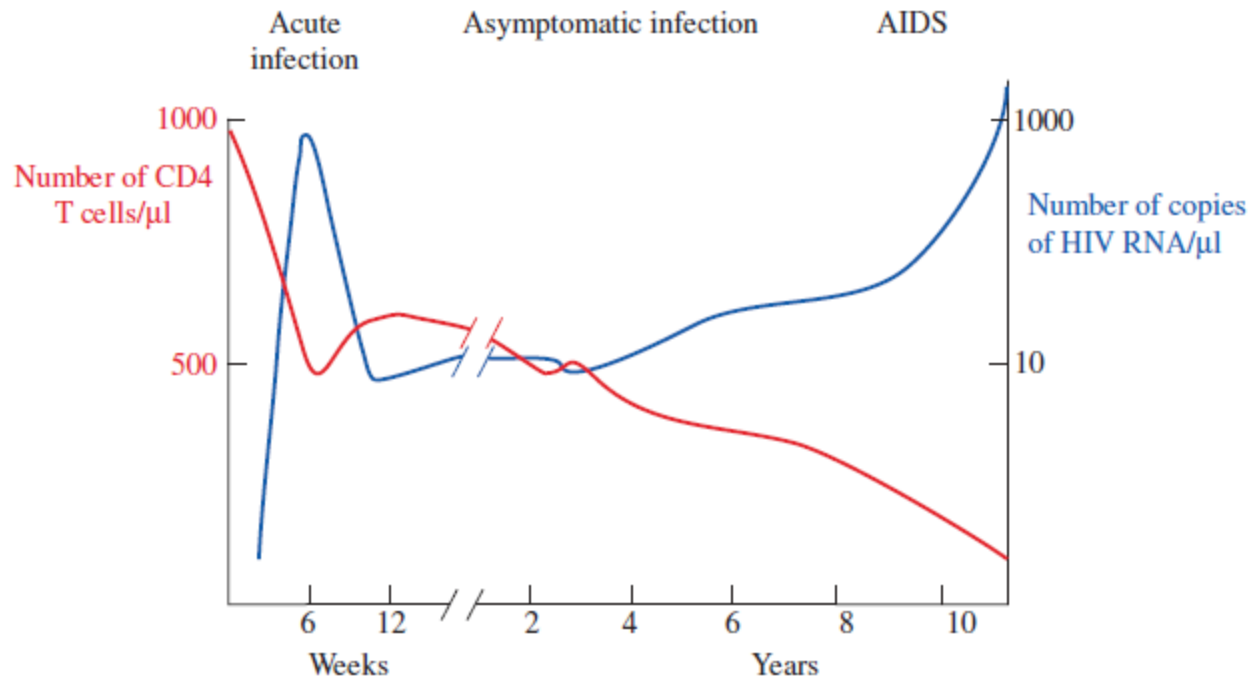


Figure 18.13 Levels of CD4 T cells and HIV RNA in blood during HIV infection. The concentration of HIV RNA is a measure of viremia. Shortly after infection viremia rises, then it falls off and relatively low levels are detectable throughout the asymptomatic period. A rise in viremia heralds the onset of AIDS.

Learning outcomes

genome RNA (-) ×8

3' UCGUUUUCGUCCCAC-// -UUUUUUGUGGGGAACAAAGAUGA 5'

Transcription


RNA replication


mRNA ×10

antigenome RNA (+) ×8

5'  N₍₉₋₁₅₎AGCAAAGCAGGGUG-// -A_n 3'

5' AGCAAAGCAGGGUG-// -AAAAACACCCUUGUUUCUACU 3'

Key:  RNA polymerase (PA, PB1, PB2)

 N₍₉₋₁₅₎ cap + 9-15 nucleotides from cell mRNA