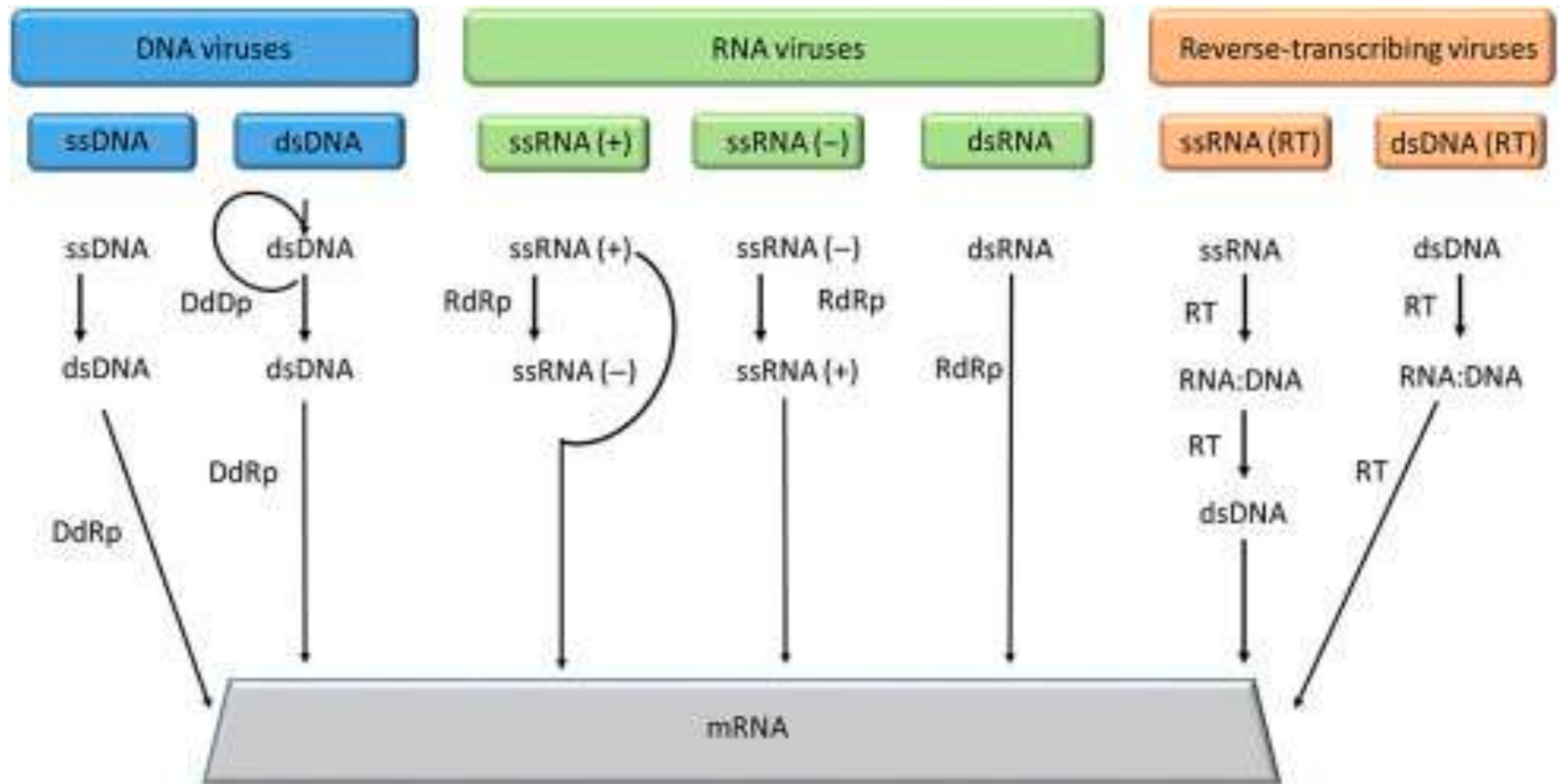


L04c

Recap

Summary of replication and transcription modes of different classes of viruses

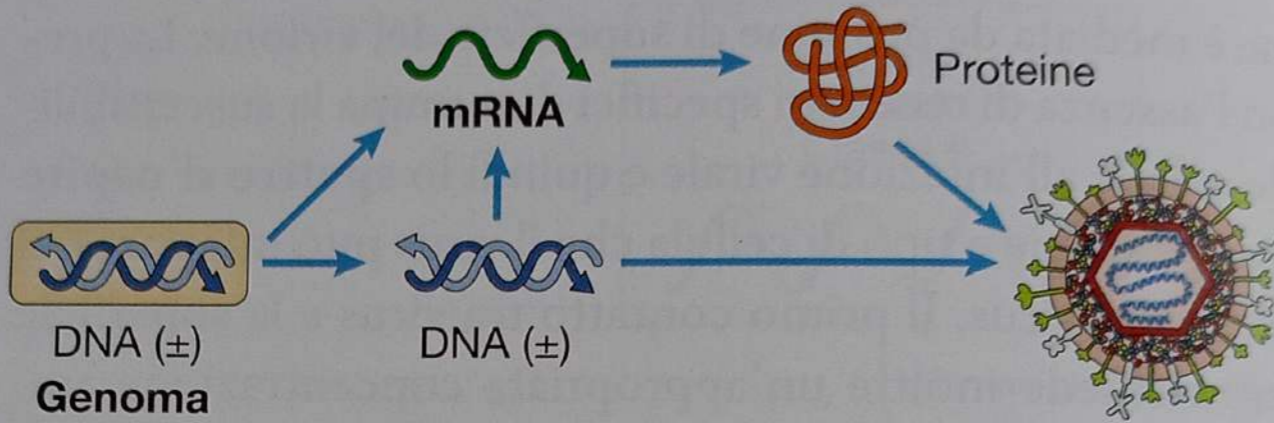


Rampersad & Tennants, 2018

DdDp, DNA-dependent DNA polymerase; DdRp, DNA-dependent RNA polymerase; RdRp, RNA-dependent RNA polymerase; RT, reverse transcriptase. The ssRNA(+) can serve as the template for translation and does not undergo any modification prior to translation.

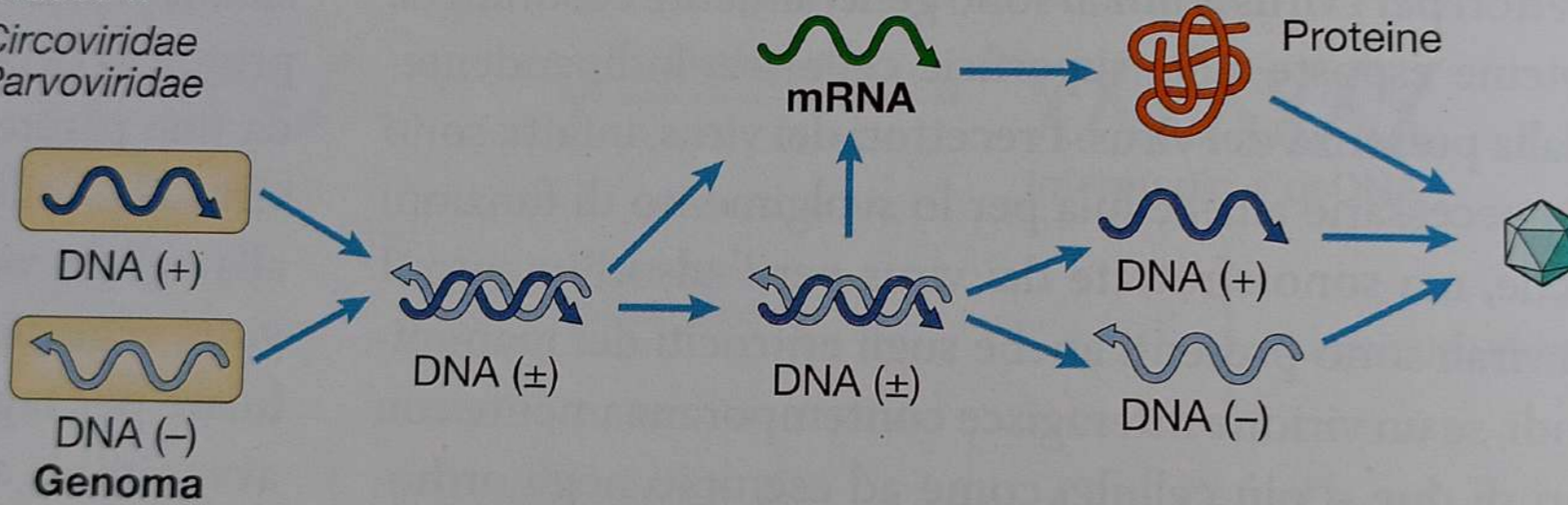
Classe I

Adenoviridae
Herpesviridae
Papillomaviridae
Polyomaviridae
Poxviridae



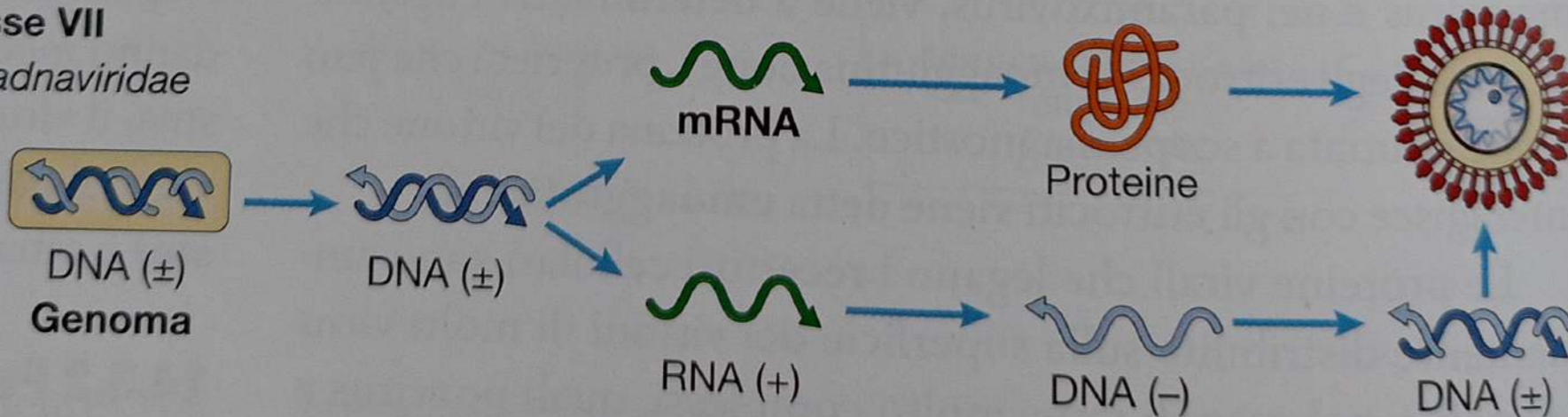
Classe II

Circoviridae
Parvoviridae

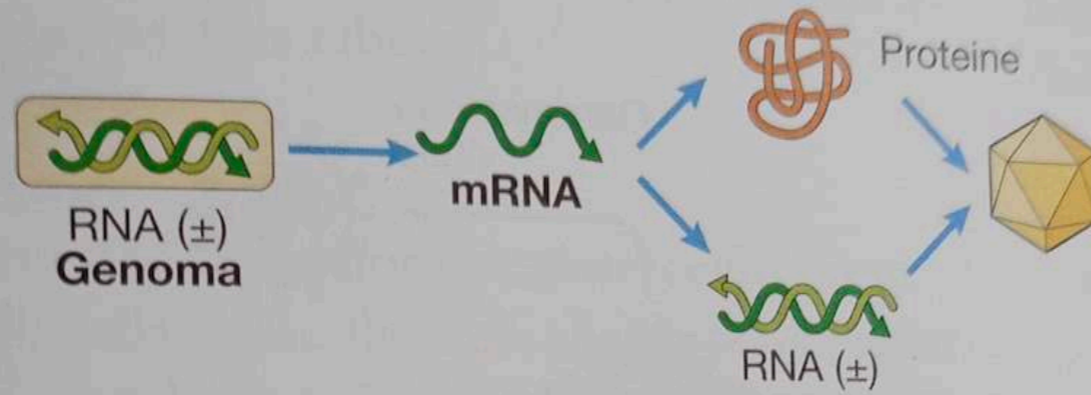


Classe VII

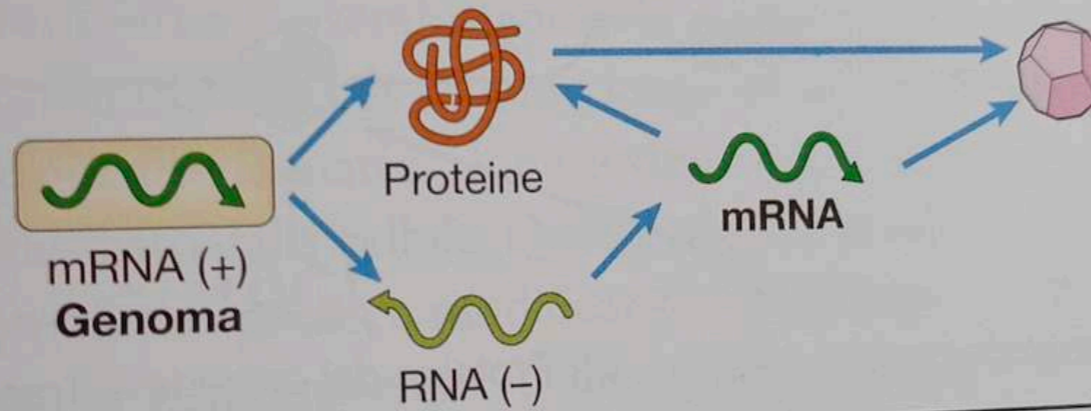
Hepadnaviridae



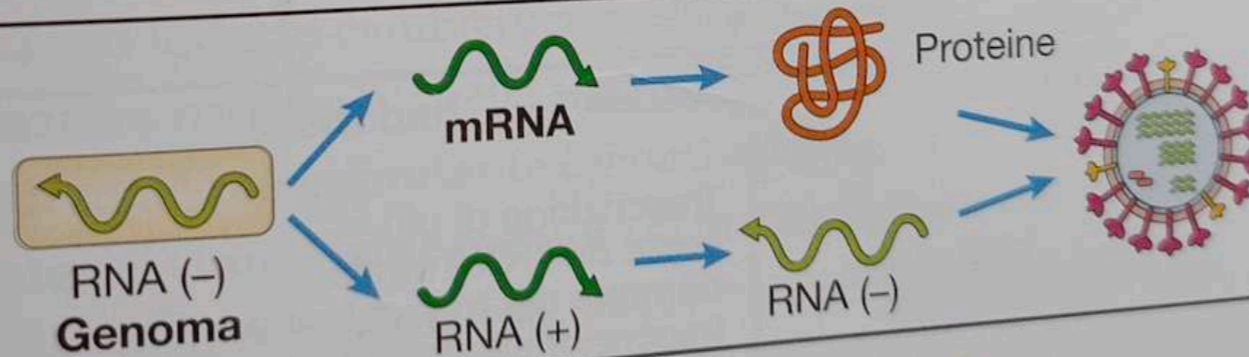
Classe III
Reoviridae



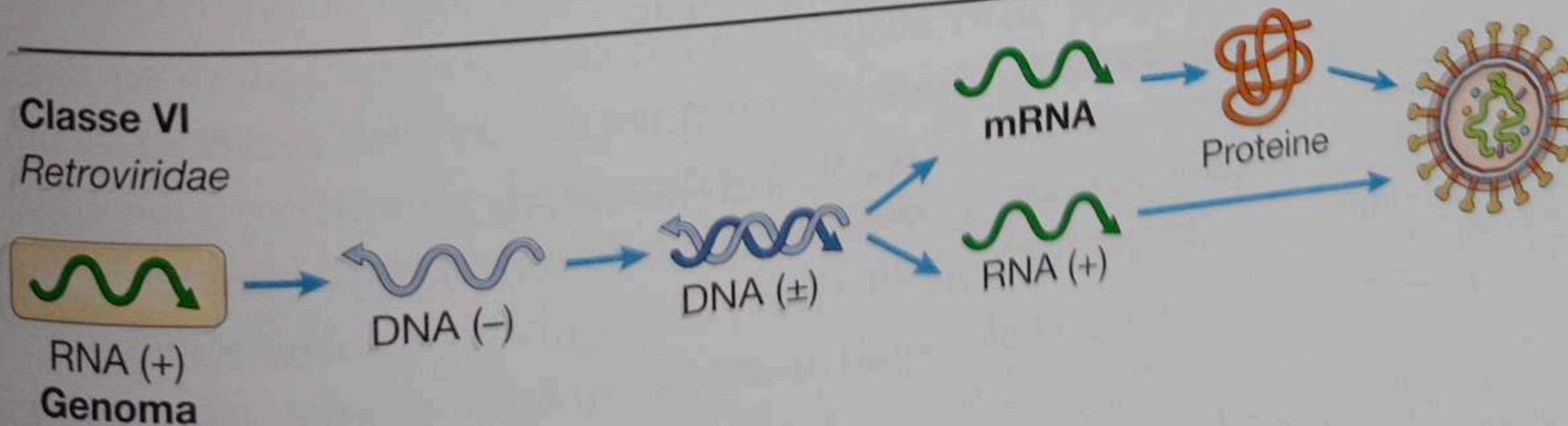
Classe IV
Picornaviridae
Togaviridae
Flaviviridae
Coronaviridae



Classe V
Orthomyxoviridae
Paramyxoviridae
Rhabdoviridae

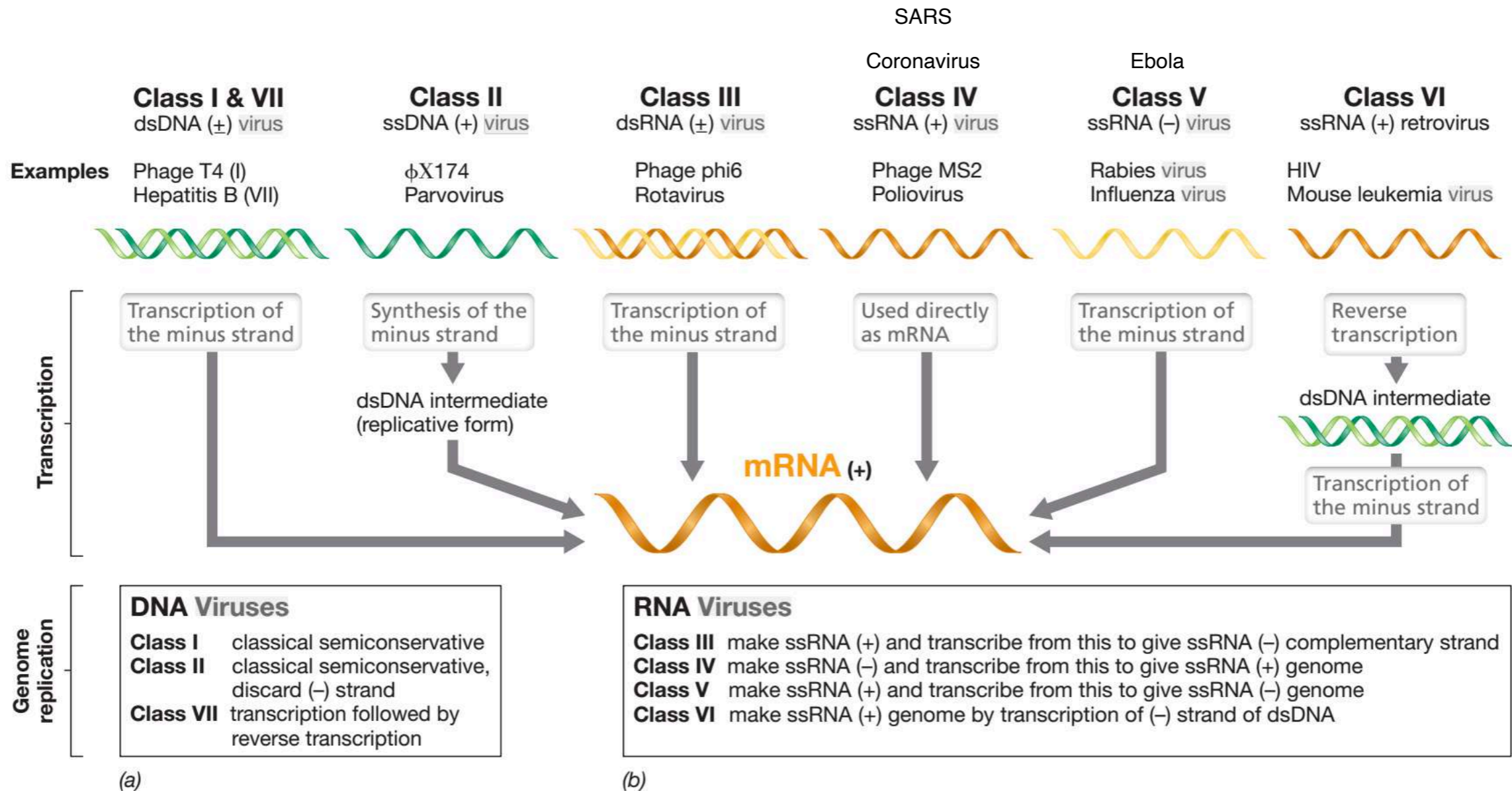


Classe VI
Retroviridae



Nucleic acid content

"The Baltimore classification of viral genome"

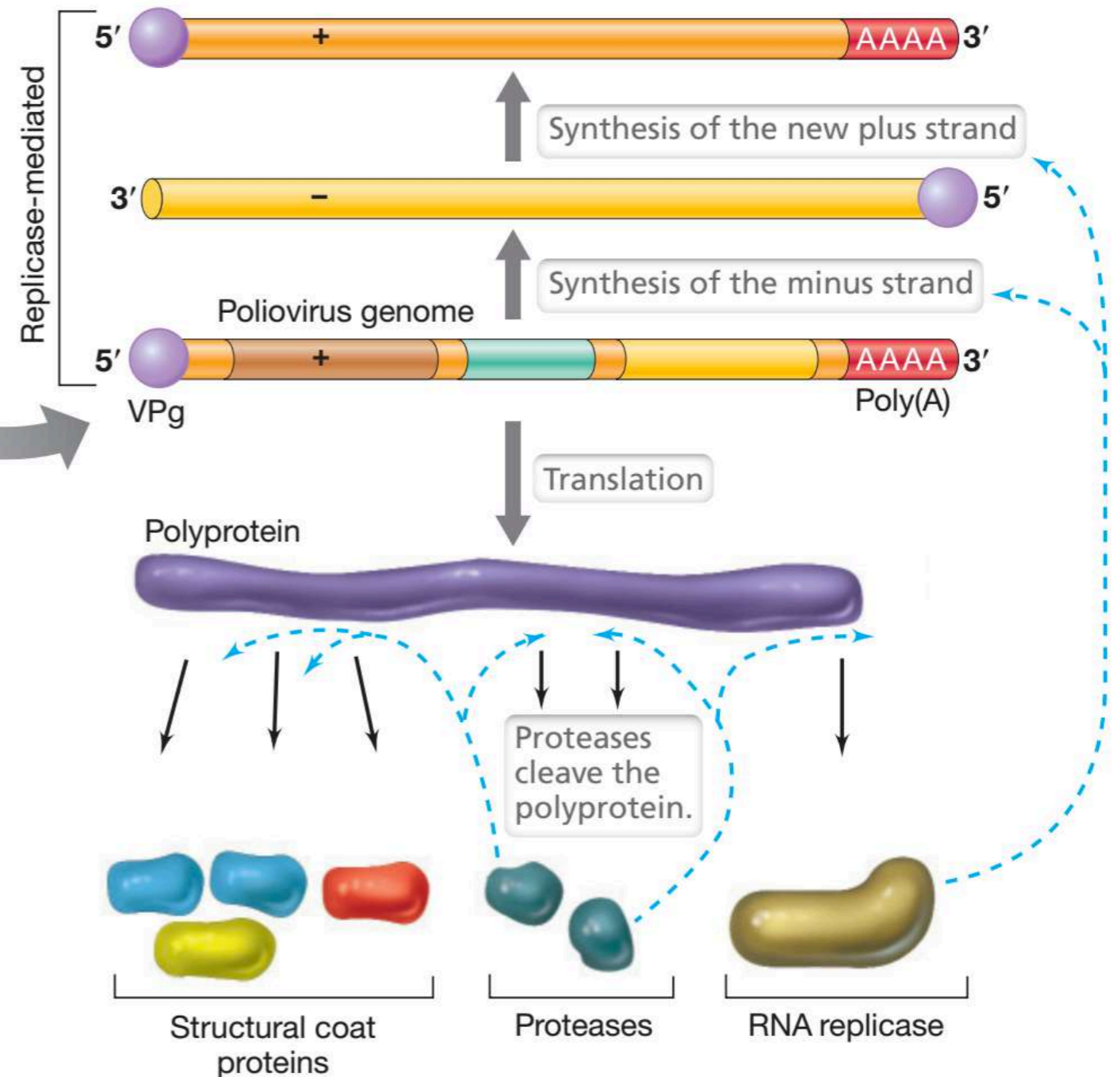


Madigan et al. 2020

- Linear, circular & single stranded genomes
- Plus sense, minus sense in terms of their base sequence
- + configuration have = base sequence as host mRNA \rightarrow translation to form viral proteins
- - configuration are complementary in base sequence to viral mRNA

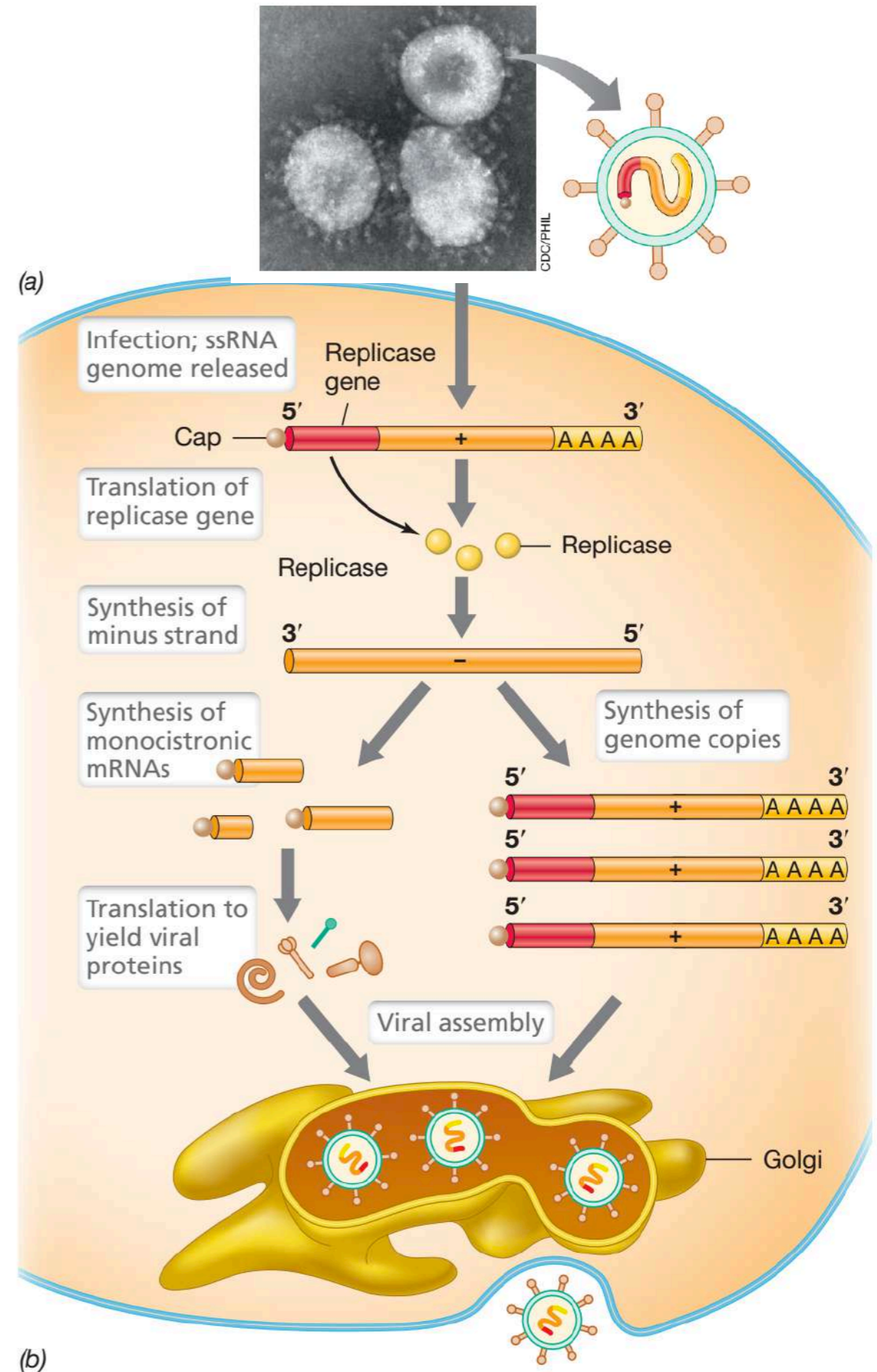
Poliovirus

- Positive ssRNA
- 7.4 kb
- At 5' terminus of viral RNA is a protein, called the **VPg protein**
- VPg protein is **attached covalently** to genomic RNA
- At 3' terminus is a **poly(A) tail**, a sign of Euk mRNA
- VPg facilitate **binding to host ribosome tightly**
- Translation yields a **polyprotein, a single protein that self-cleaves into several smaller proteins**
- **RNA replicase synthesizes RNA- and RNA+**
- 5 hr lytic cycle
- In cytoplasm
- Hotspots in person's throat and intestines
- Spinal cord infection —> paralysis



Coronavirus

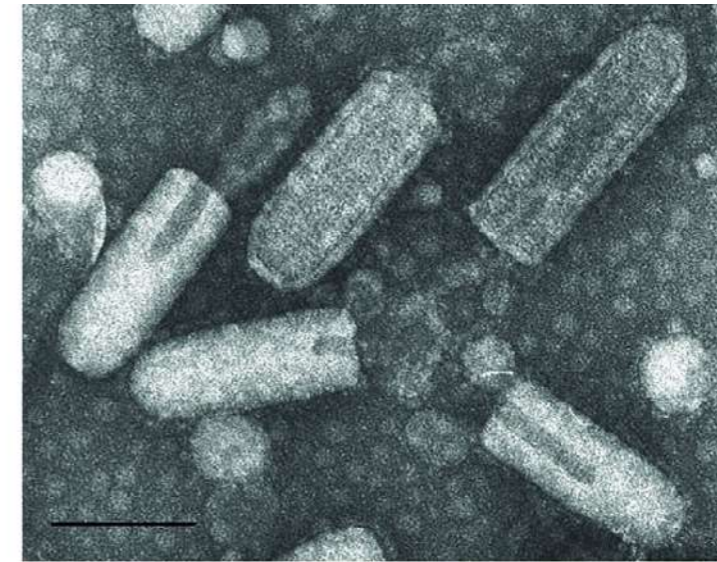
- Coronaviruses single-stranded plus RNA viruses
- ssRNA+ \rightarrow translate only a few genes: **replicase**
- **Replicase produces - strands for translation of other genes**
- **Replicate in cytoplasm**
- Club-shaped glycoprotein spikes on their surfaces \rightarrow crown
- Largest of any known RNA viruses, about **30 kb**
- **Plus sense**, coronavirus genome can function directly in cell as mRNA
- **Virions assembled in Golgi complex**



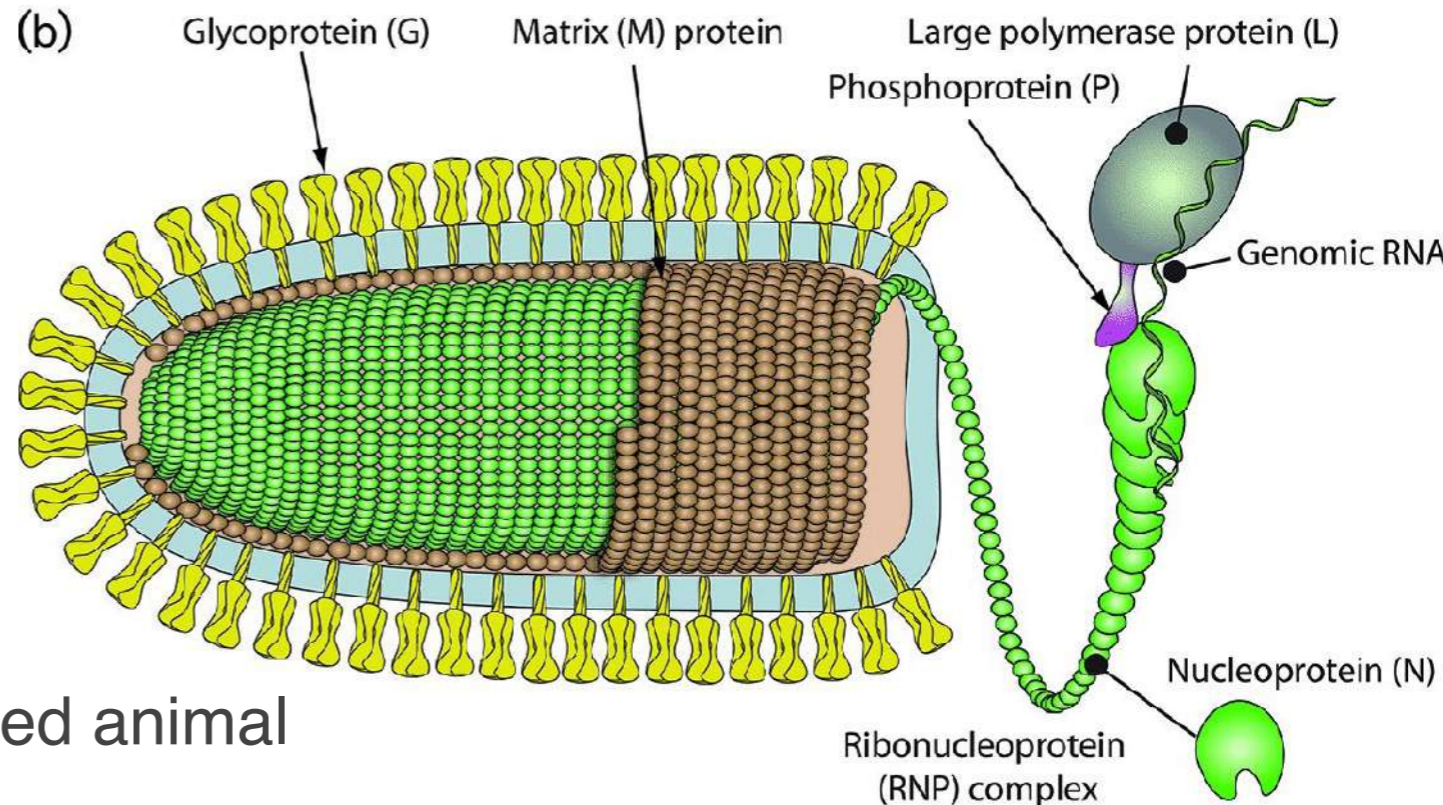
Rhabdovirus

- Enveloped virus
- ssRNA -
- Bullet-shape
- Complex structure
- Rabies is a zoonotic disease
- Transmitted via the saliva of an infected animal
- Dogs are the most important reservoir for rabies viruses,
- Dog bites account for >99% of human cases
- Virus first infects peripheral motor neurons, and symptoms occur after the virus reaches the central nervous system

(a)

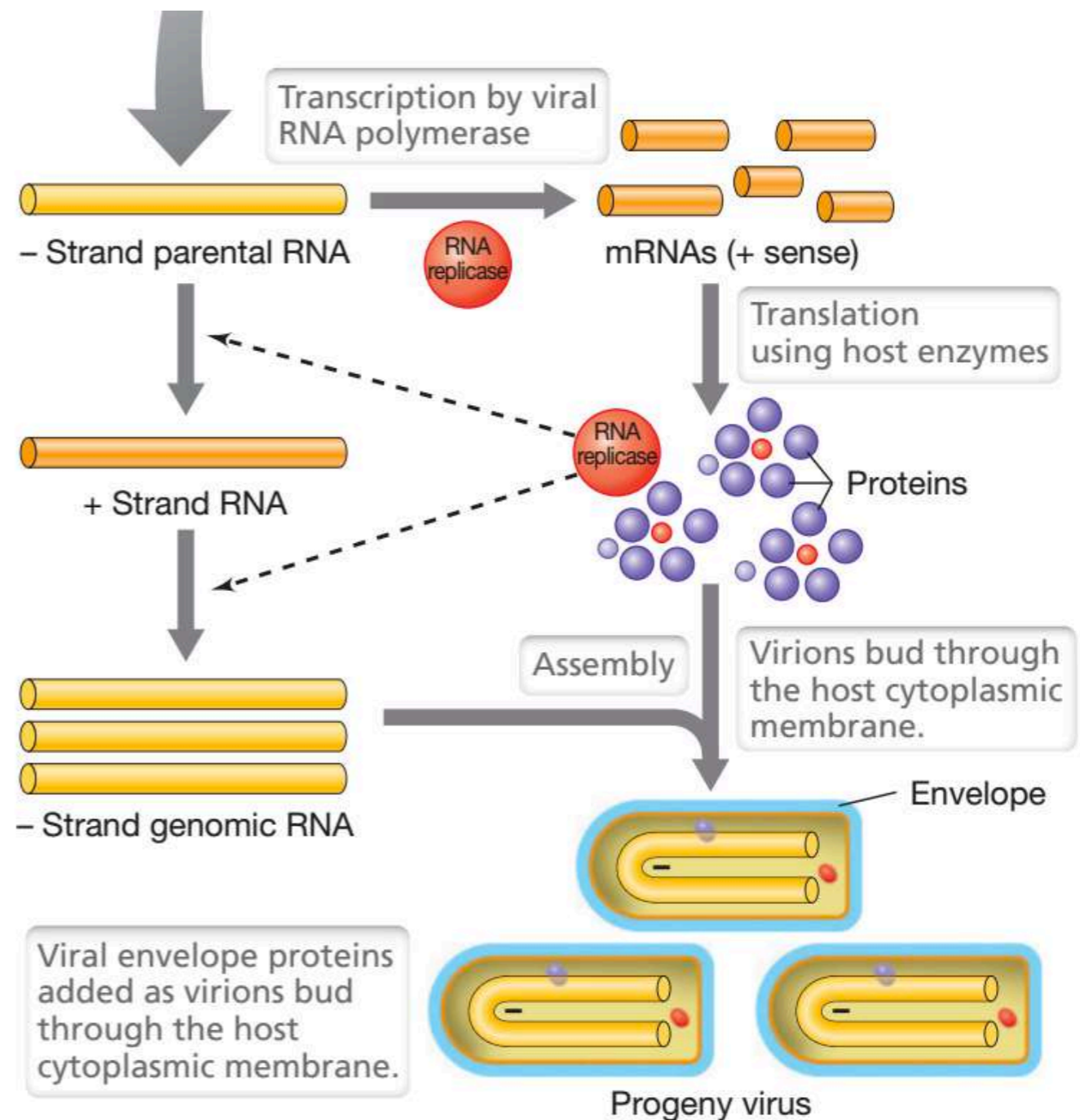


(b)



Rhabdovirus

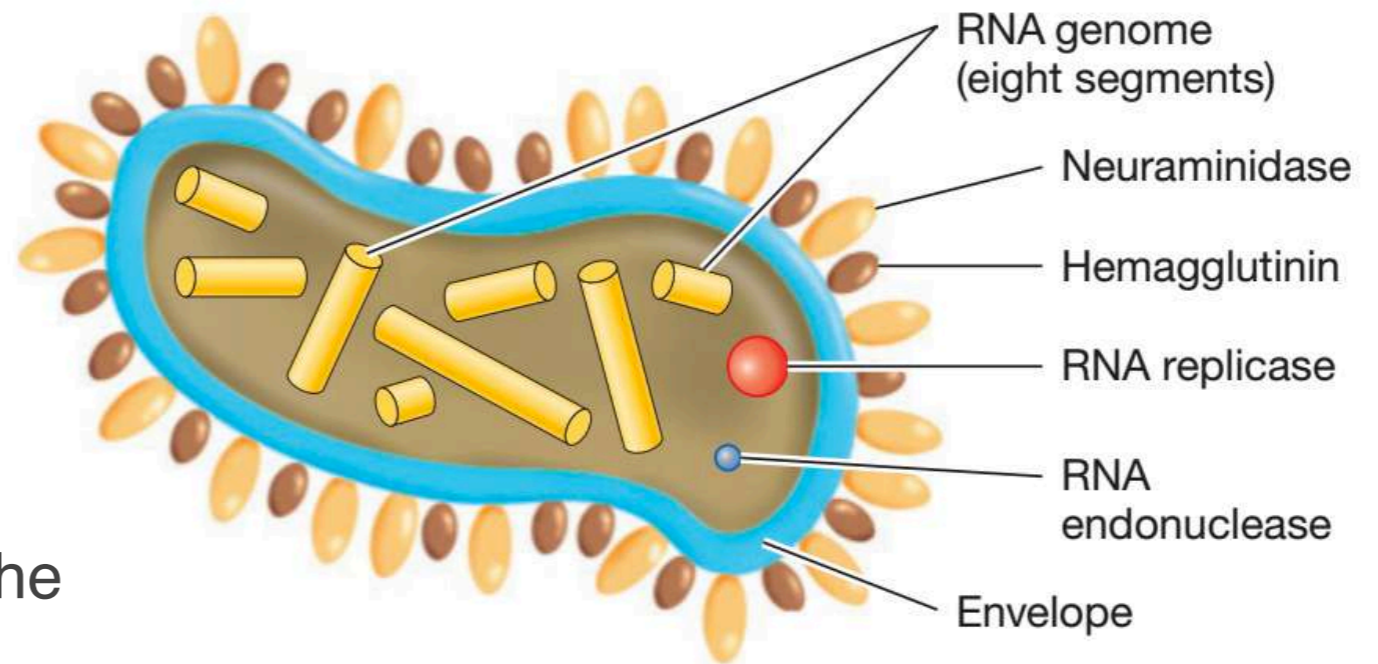
- Negative-single strand RNA viruses are complementary in base sequence to the mRNA that is formed
- **Genome is transcribed by the Replicase**
- Transcription in cytoplasm and generates **two classes of RNAs**
- The **first** is a series of mRNAs encoding each of **viral proteins**
- The **second** is a complementary copy of the entire viral genome (+) → **functions as a template for synthesis ssRNA (-)**
- **Budding virions from host cytoplasmic membrane**

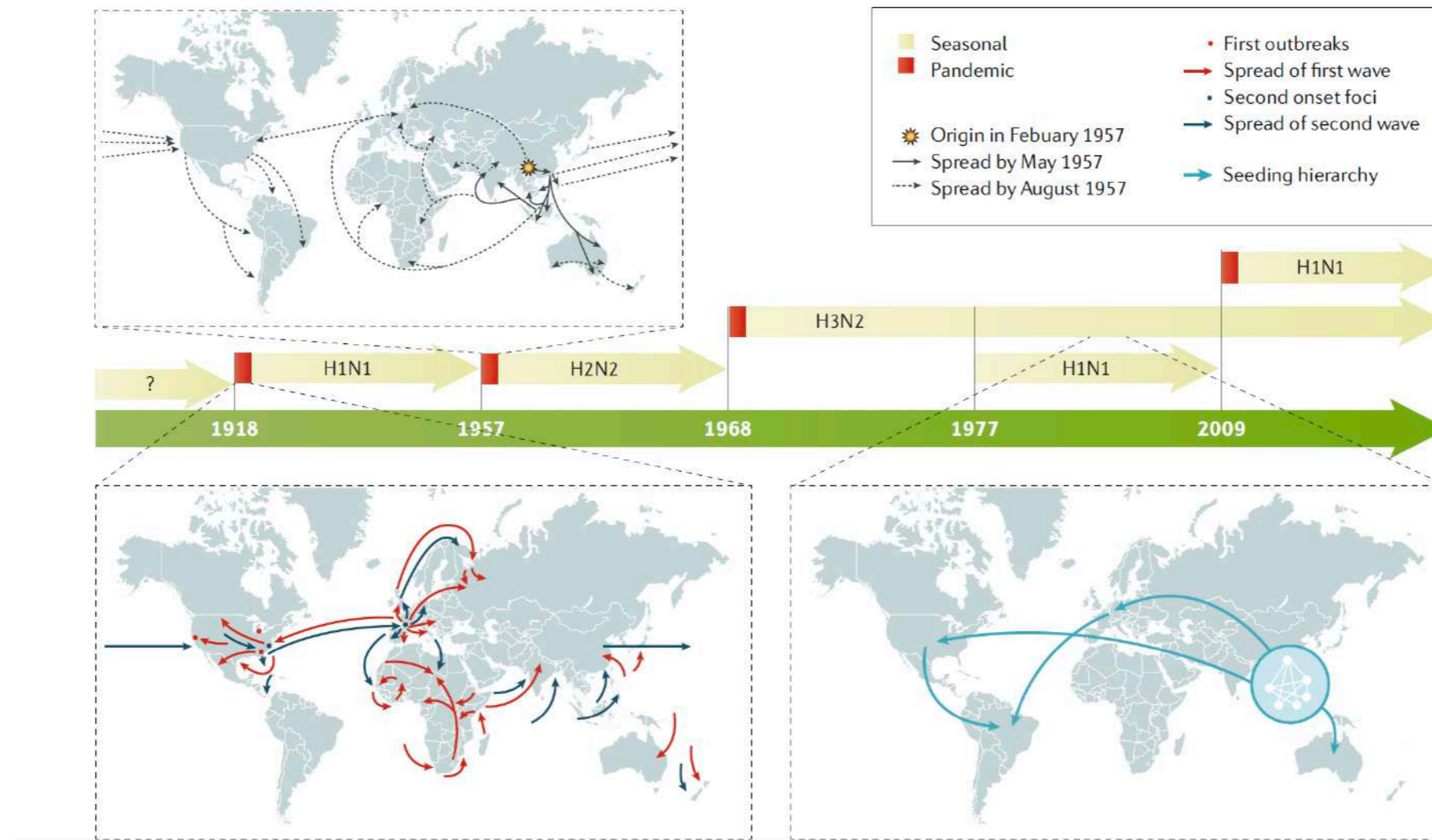


(b)

Influenza Virus

- **Enveloped virus**
- **Negative-strand RNA viruses** are complementary in base sequence to the mRNA that is formed
- **Segmented genome (13.5 kb)**
- **8 linear single-stranded molecules** ranging 890 to 2341 nucleotides
- **Influenza virus exhibits antigenic shift in which segments of the RNA genome from two different viral strains infecting the same cell are reassorted**





Influenza viruses are capable of evading the antibody mediated immunity induced during previous infections or vaccinations by gradually accumulating mutations in HA and NA

Each influenza virus isolate receives a unique name according to a set of rules.

First, the name denotes the type of influenza virus (A, B, C or D), followed by the host species from which the virus was isolated (if not specified, the isolate is considered human), the geographical location at which the virus was isolated, the isolate number and the year of isolation.

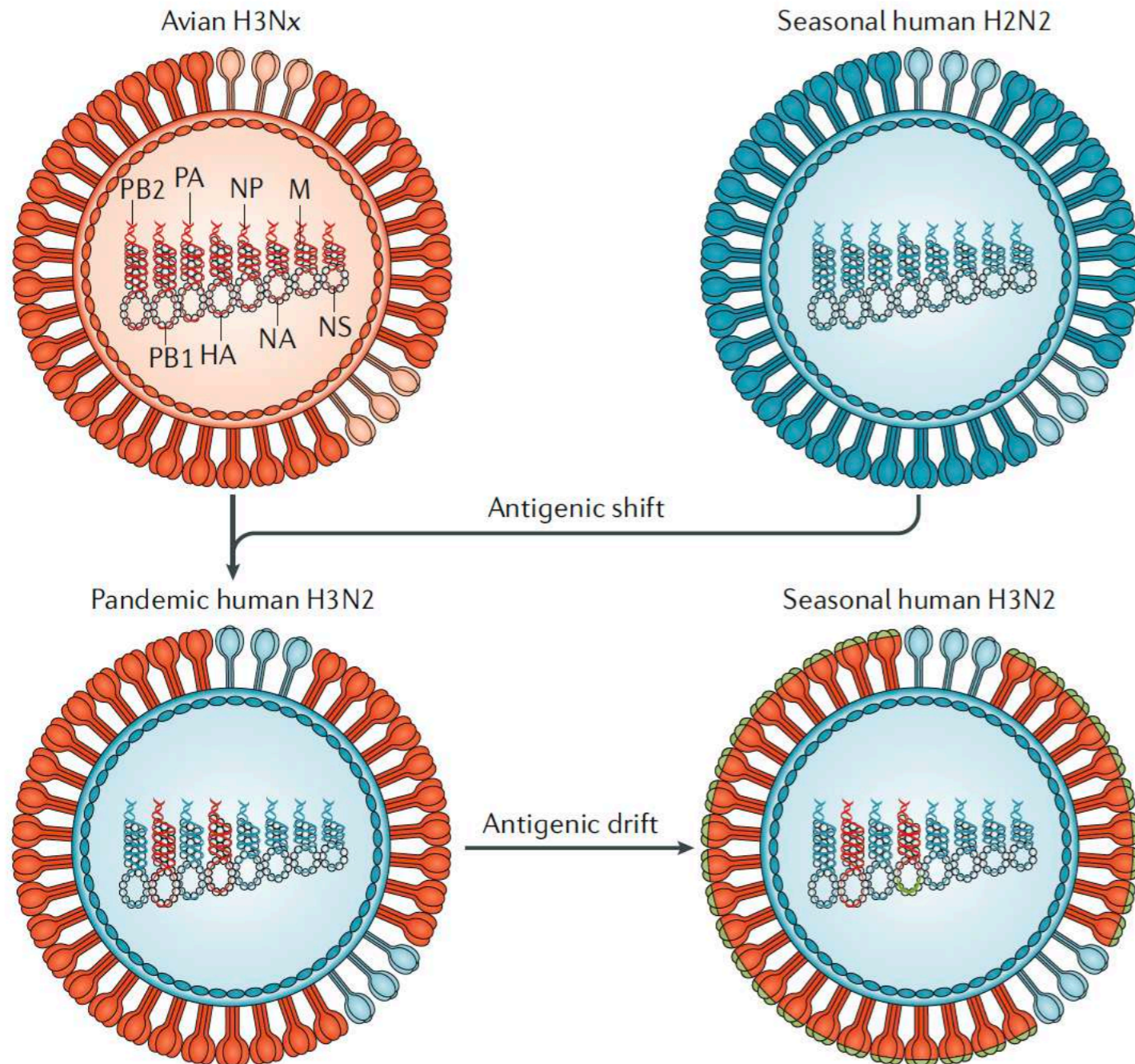
In the case of influenza A viruses, the haemagglutinin (HA) and neuraminidase (NA) subtype is also usually indicated after the viral isolate name.

For example, influenza A/Turkey/Ontario/6118/1968 (H8N4) virus is an influenza A virus isolated from a turkey in Ontario in 1968, isolate number 6118; the virus isolate has an HA from the HA antigenic subtype 8 and an NA from the NA antigenic subtype 4.

Genome rearrangement in human hosts

In poultry, the viral strains are transmitted both by aerosol and faecal contamination and cause systemic haemorrhagic disease and death

Kramer et al., 2018



- Antigenic shift is thought to trigger major outbreaks of influenza because immunity to the new forms of the virus is essentially absent from the population
- Reassortment generates hybrid influenza virions that express unique surface proteins unrecognized by the immune system
- Once the virus becomes established in humans, the virus begins to drift, as is the case with all other human seasonal influenza viruses.
- During drift, small antigenic changes in the HA protein generated by mutation are selected to increase immune evasion, although not as dramatically as during shift

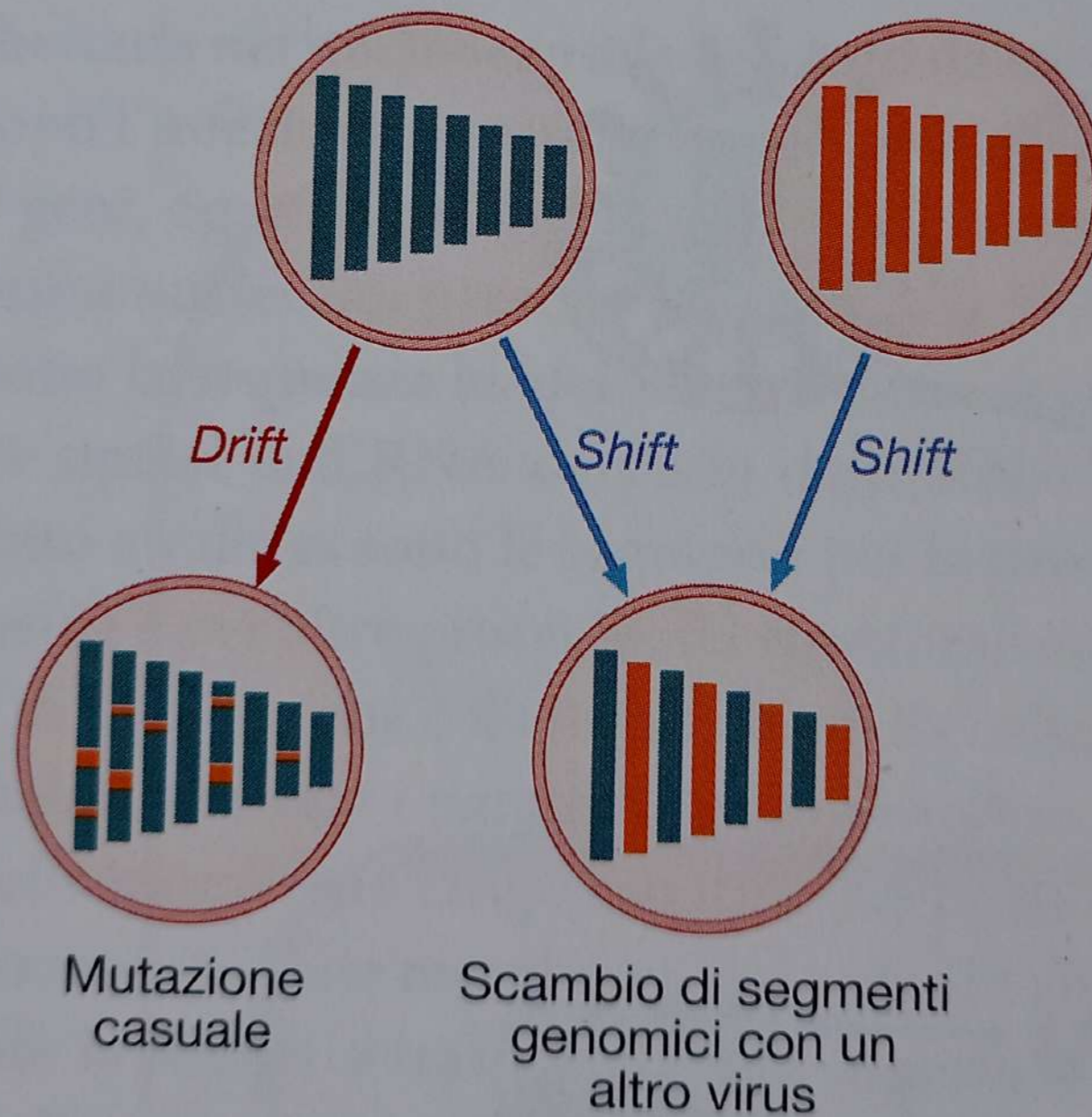
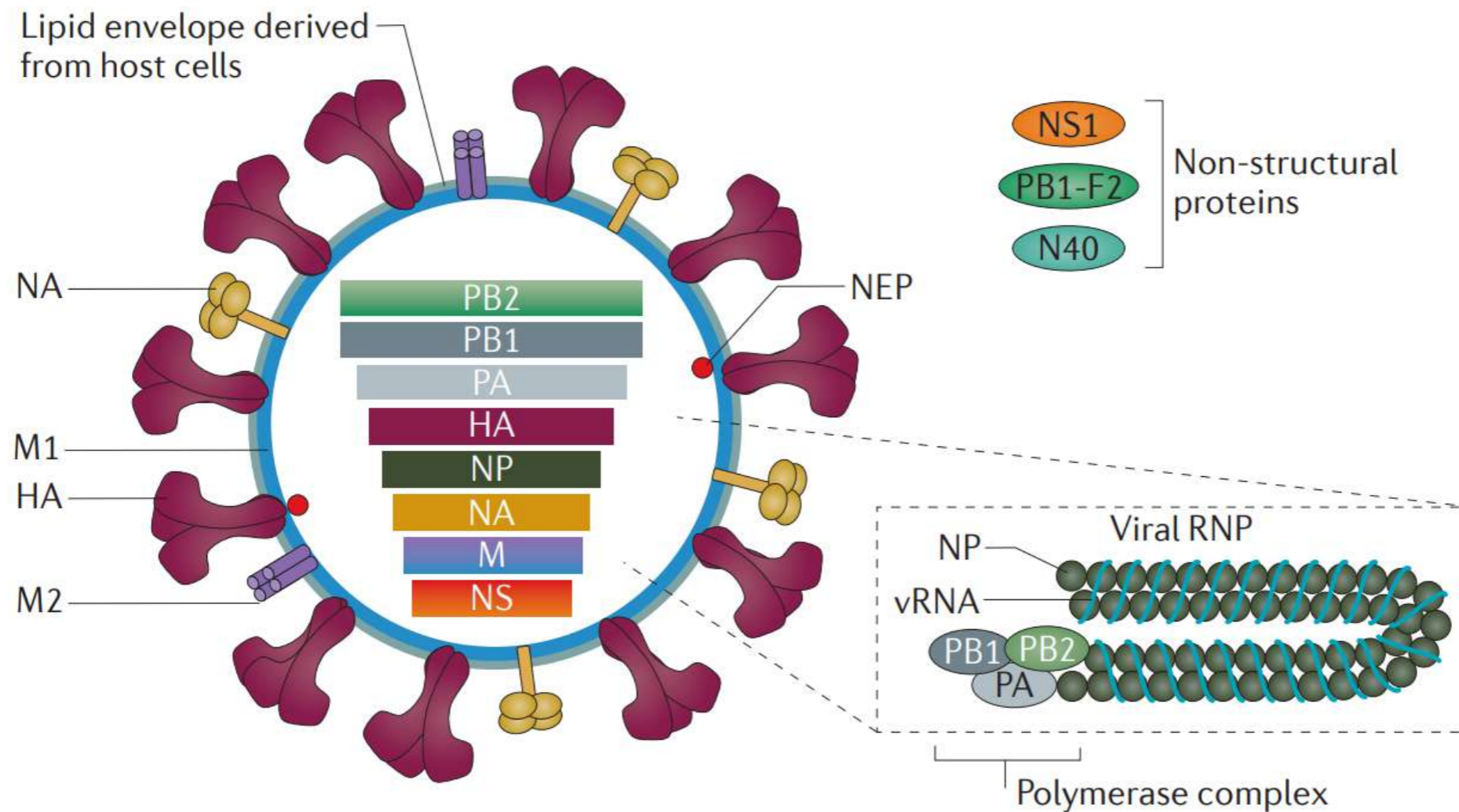


Figura 14.41 DERIVA ANTIGENICA (ANTIGENIC DRIFT) E RIASSORTIMENTO ANTIGENICO (ANTIGENIC SHIFT) NEL VIRUS DELL'INFLUENZA.

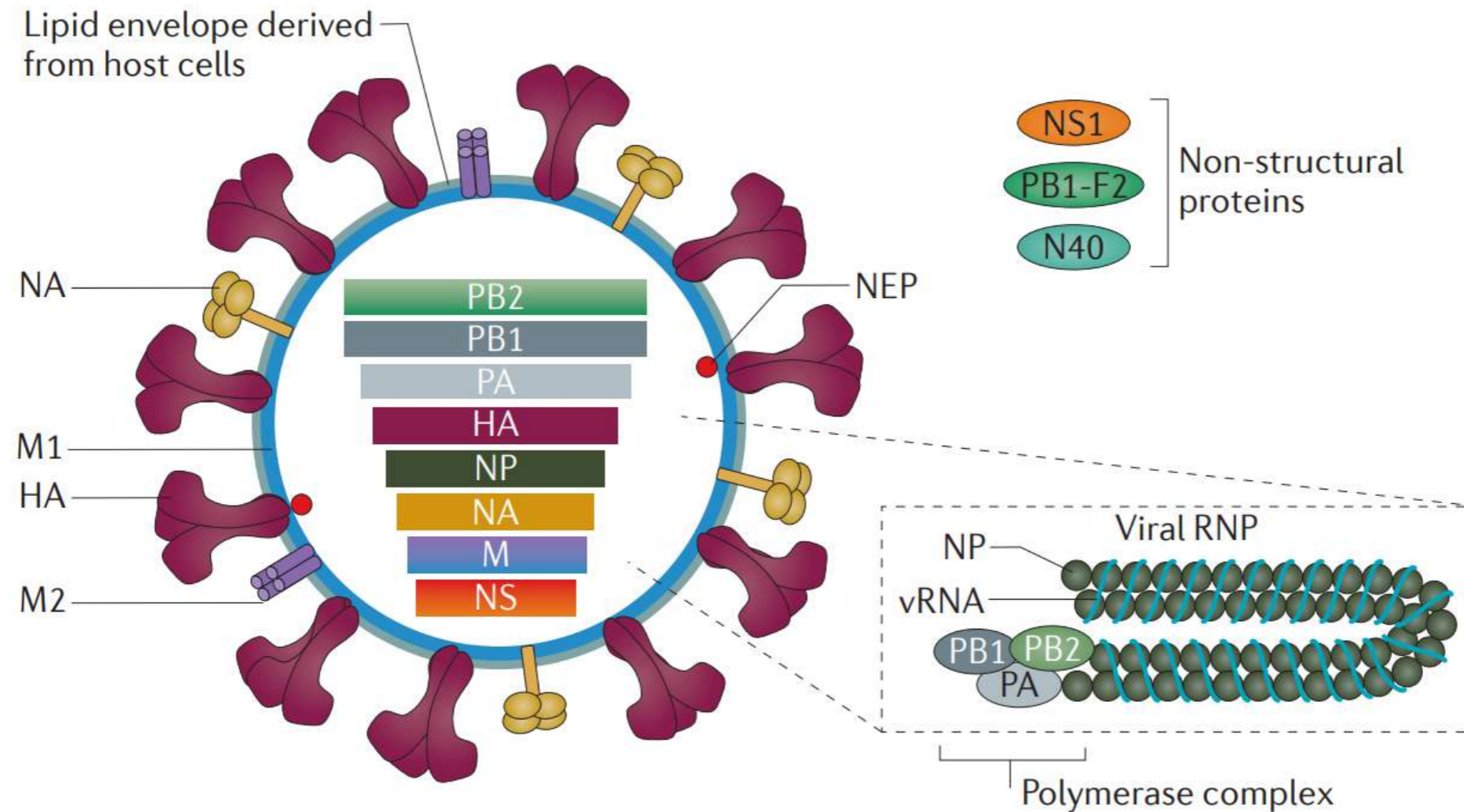


8 segment genome, I



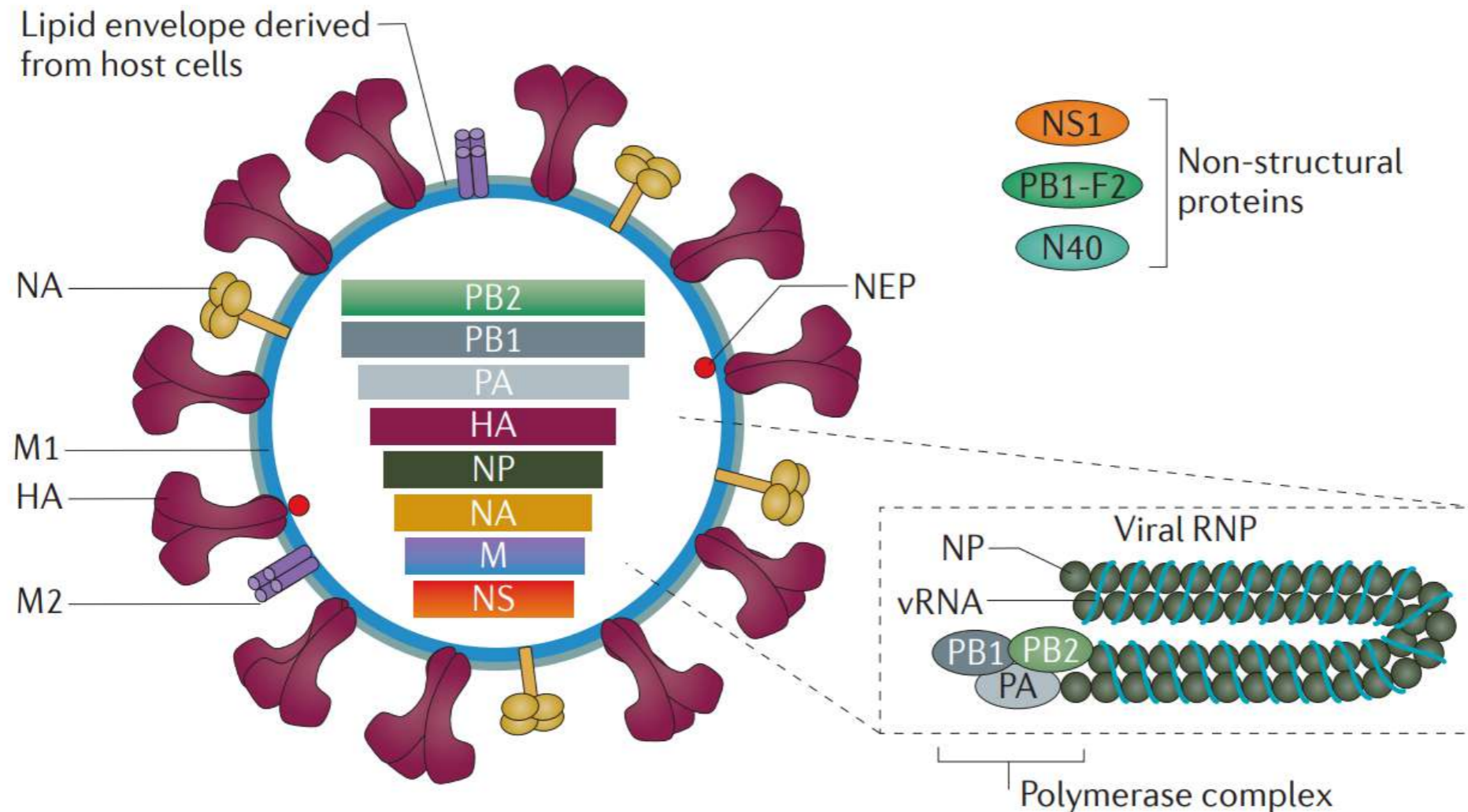
- **NS segment encodes nuclear export protein (NEP) & host antiviral response antagonist non-structural protein 1 (NS1)**
- **M segment encodes matrix protein M1 & the ion channel M2**
- **HA segment the receptor-binding protein haemagglutinin (HA)**

8 segment genome, II



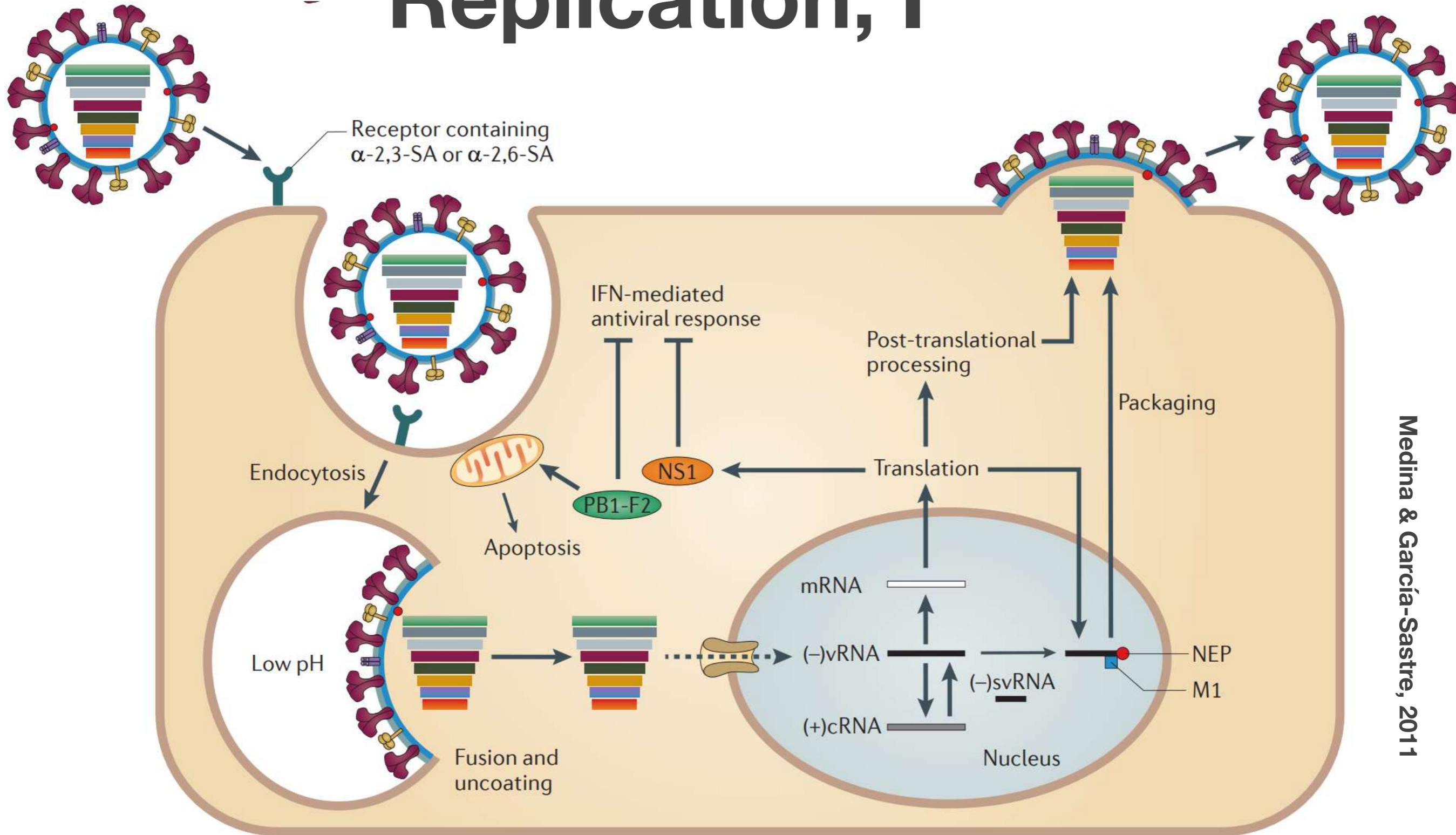
- **NA segment the silica acid-destroying enzyme neuraminidase (NA)**
- **NP segment nucleoprotein (NP)**
- **RNA-dependent RNA polymerase complex from PB1, PB2 and PA segments**

8 segment genome, III



Within the virion, each of the eight viral segments forms a viral ribonucleoprotein (RNP) complex: viral RNA is wrapped around NP, and this structure is then bound to the viral polymerase complex

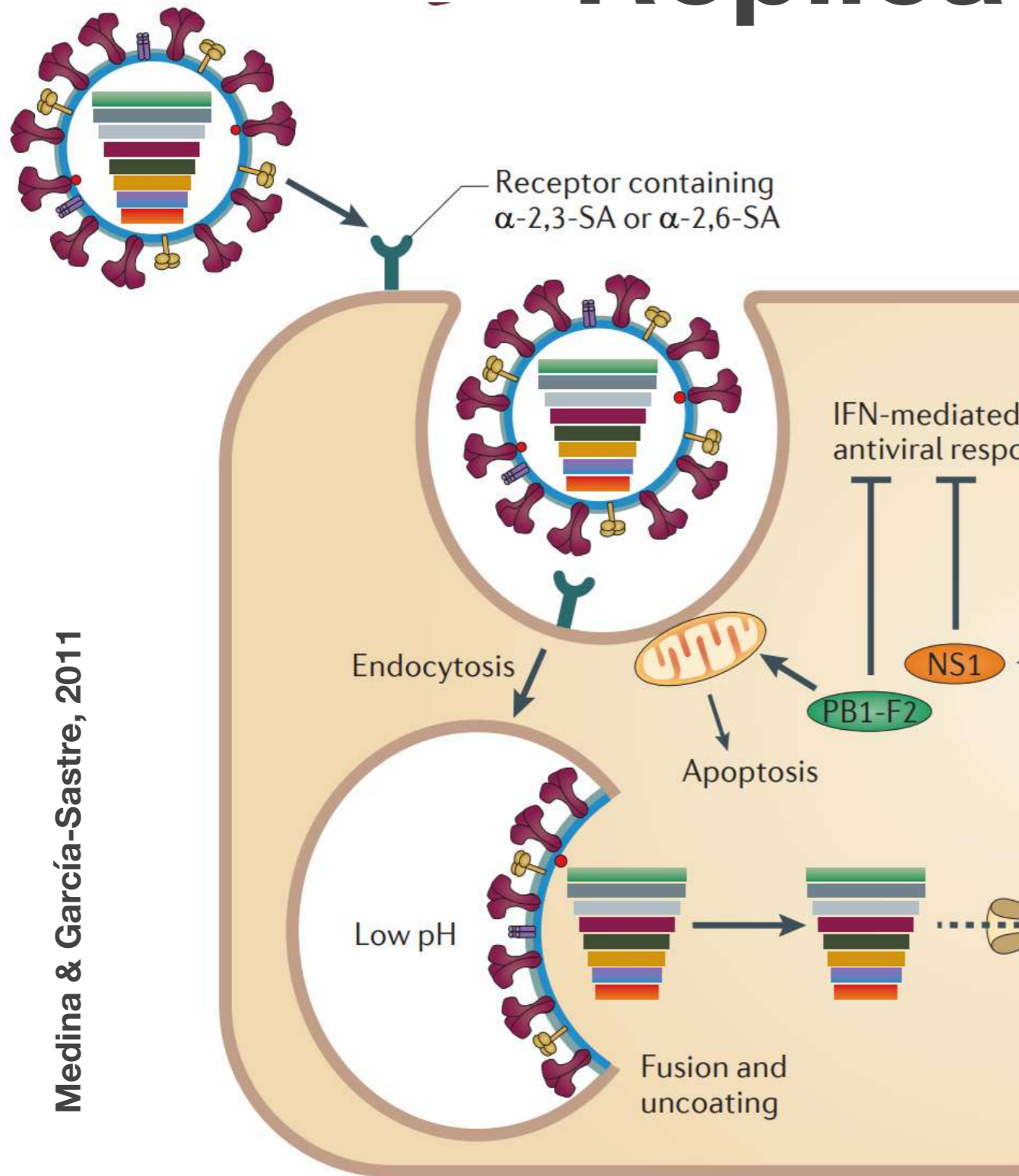
Replication, I



Medina & García-Sastre, 2011

Virus enters the cell by receptor-mediated endocytosis

Replication, II



HA cleavage by cellular proteases is required to expose HA peptide that is responsible for fusion between viral envelope and endosomal membrane

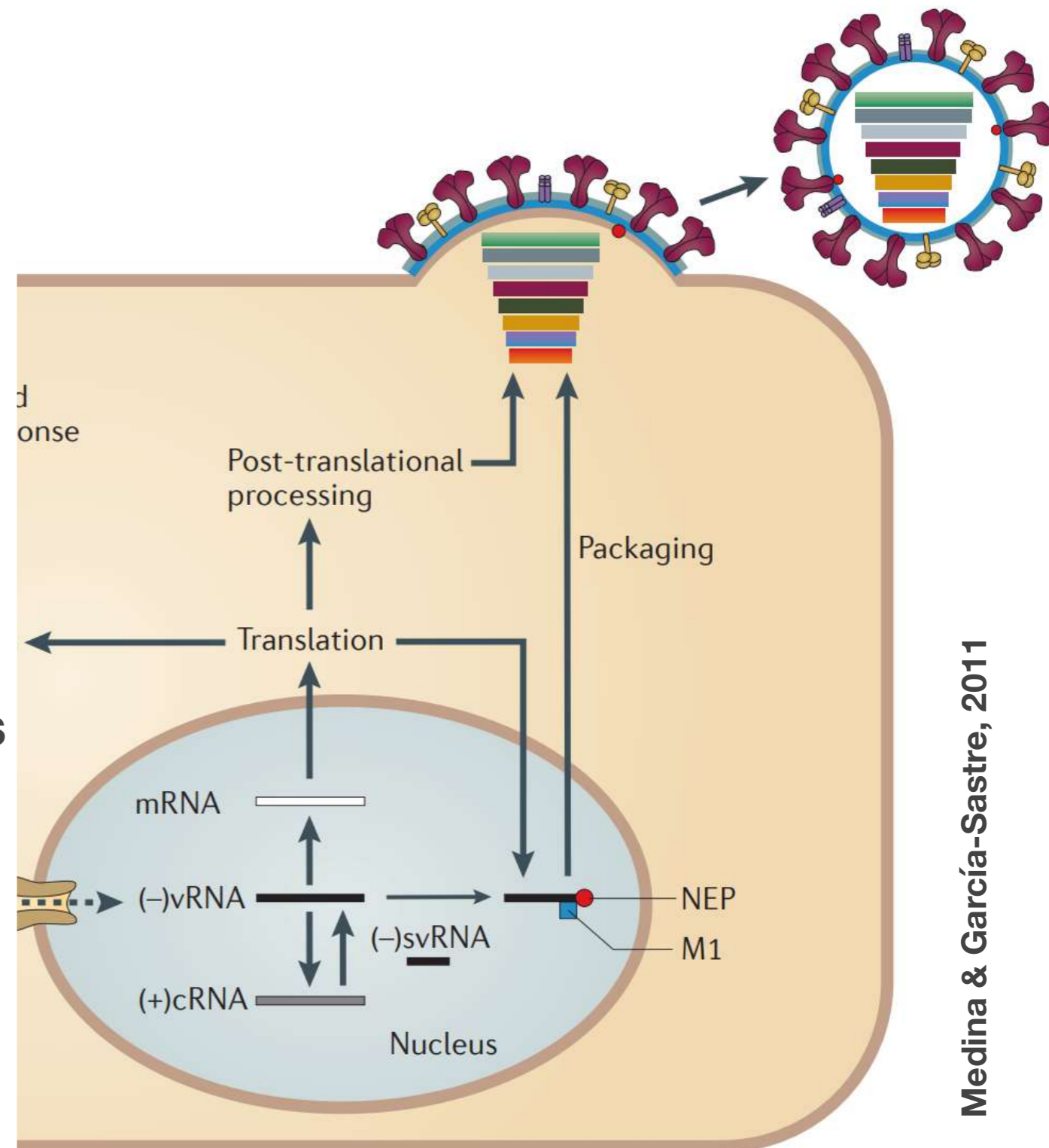
Acidification of endocytic vesicle opens M2 ion channel, resulting in acidification of the inside of virion, a process that is required for proper uncoating of RNP complexes that contain viral genome

Acidification of endosome also triggers pH-dependent fusion step that is mediated by HA and results in cytoplasmic release of RNP complexes

Replication, III

RNA-dependent RNA polymerase transcribes and replicates negative-sense viral RNA ((-) vRNA), giving rise to three types of RNA molecules:

1. **Complementary positive-sense RNA ((+)cRNA), which it uses as a template to generate more vRNA**
2. **Negative-sense small viral RNAs (svRNAs), which are thought to regulate switch from transcription to replication**
3. **Viral mRNAs, which are exported to cytoplasm for translation**

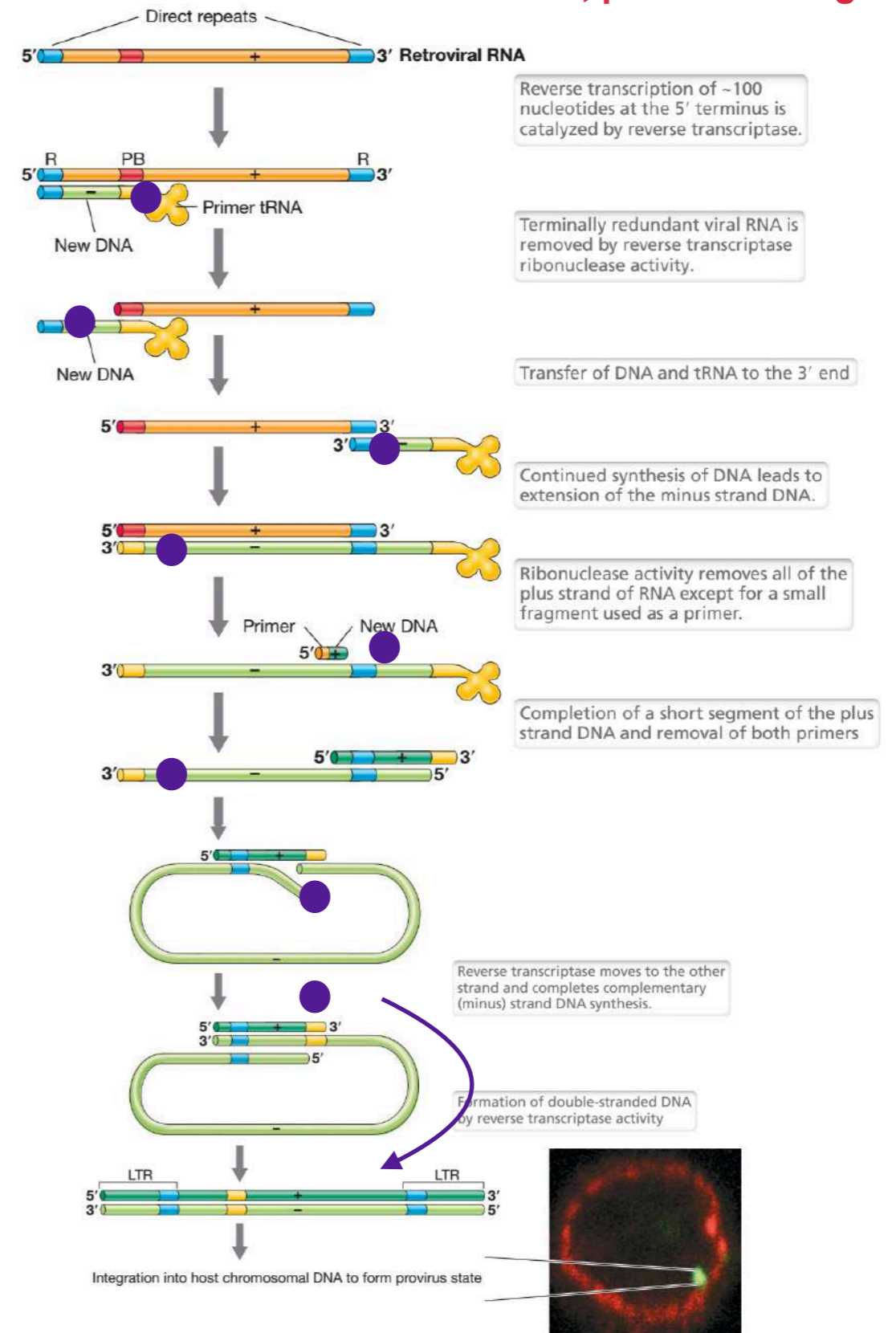


Medina & García-Sastre, 2011

Retrovirus, I

- ssRNA genomes (+)
- **Reverse transcriptase (RT)** has 3 enzymatic activities:
 1. **Reverse transcription** (to synthesize DNA from an RNA template), $3' \rightarrow 5'$
 2. **Ribonuclease activity** (to degrade RNA strand of an RNA:DNA hybrid)
 3. **DNA polymerase** (to make double-stranded DNA from single-stranded DNA)
- RT needs a **primer for DNA synthesis: the viral tRNA**

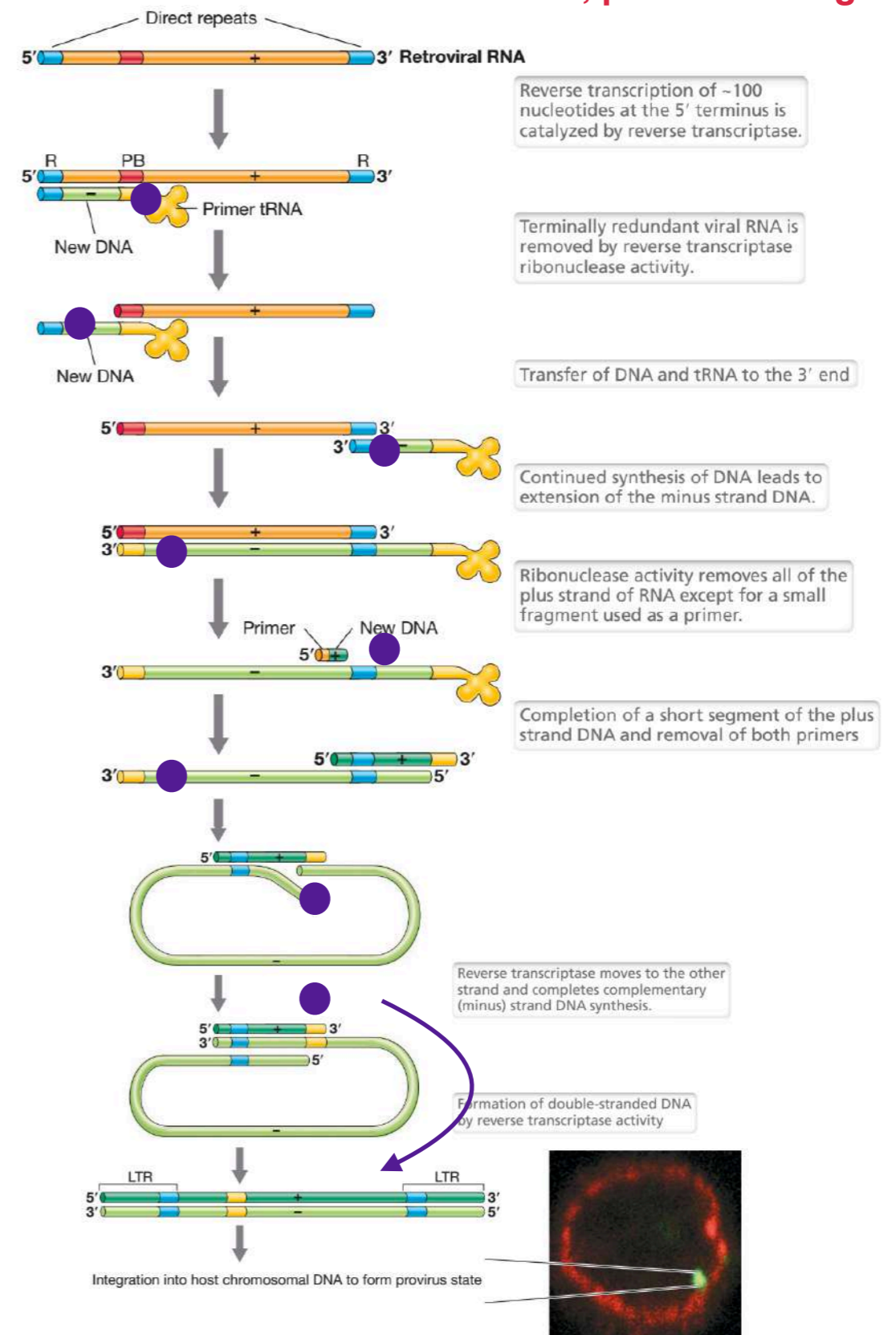
● RT
PB, primer binding site



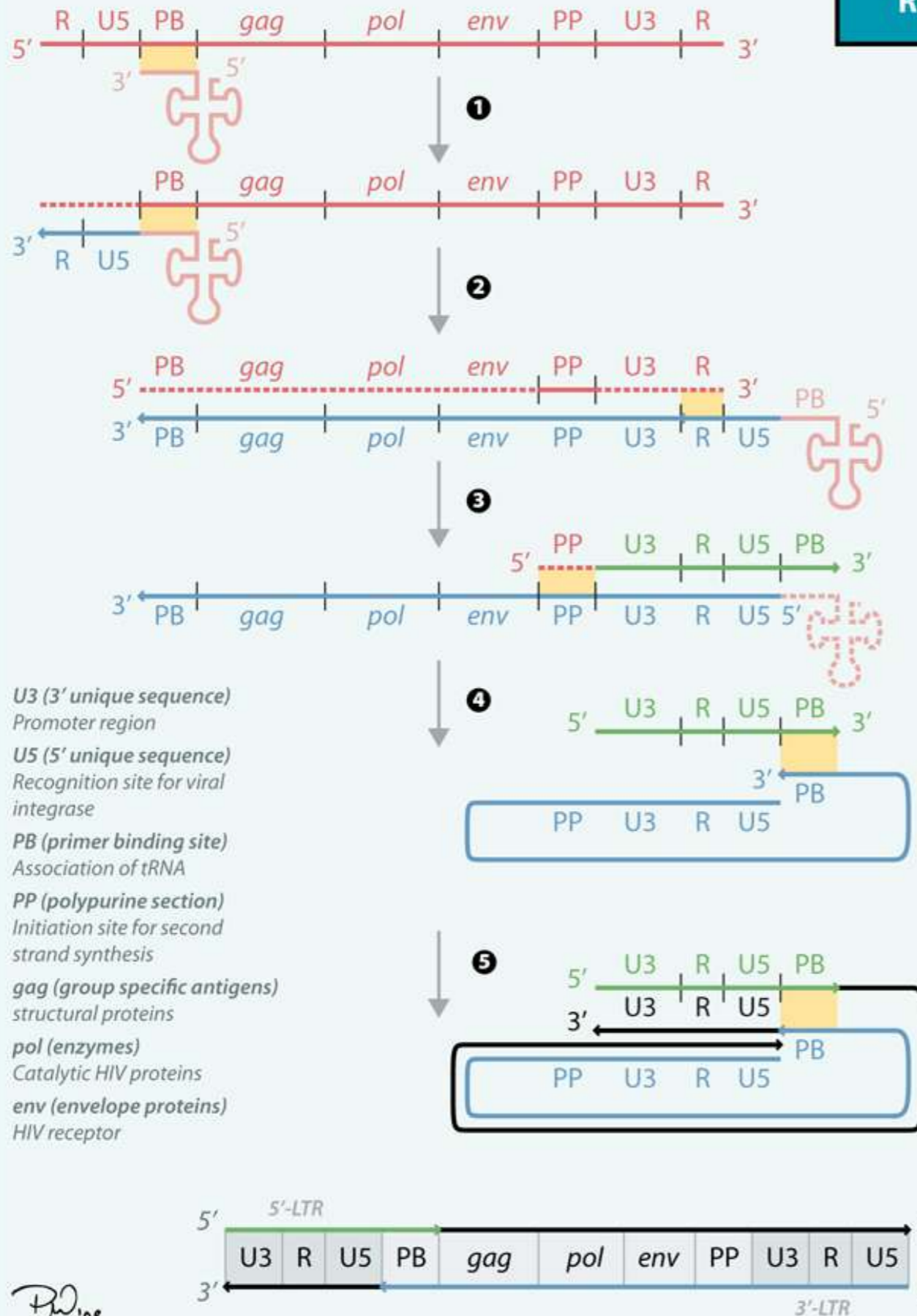
Retrovirus, II

- Using this primer, nucleotides near the 5' terminus of RNA are reverse-transcribed into DNA
- Once reverse transcription reaches 5' end of RNA, process stops
- Terminally redundant RNA sequences at 5' end are removed by RT
- Formation of a small, ssDNA complementary to RNA segment at 3' of viral RNA
- This short, ssDNA hybridizes with the other end of viral RNA molecule, where synthesis of DNA begins once again

● RT
PB, primer binding site



Reverse transcription of HIV-RNA into dsDNA



1 During transport to the nucleus, the viral ssRNA genome (red) is reverse transcribed into double strand DNA by the viral RT. Reverse transcription takes always place in 3'→5' direction. The tRNA (rose), which hybridizes to the PB site, provides a hydroxyl-group for initiation of reverse transcription.

While a ssDNA (blue) sequence is synthesized, the complementary ssRNA is degraded by the RNase H function of RT.

2 The DNA-tRNA hybrid molecule is then transferred to the 3'-end of the template and is used for first strand synthesis. Afterwards, the ssRNA is degraded except for the PP site, which serves as a new primer.

3 The initial second strand synthesis of ssDNA (green) starts from the 3'-end of PP, which will be finally degraded. The tRNA makes it possible to synthesize the complementary PB site.

4 After the tRNA is degraded, the first and second DNA strand hybridize at their PB sites, which they harbor on their ends.

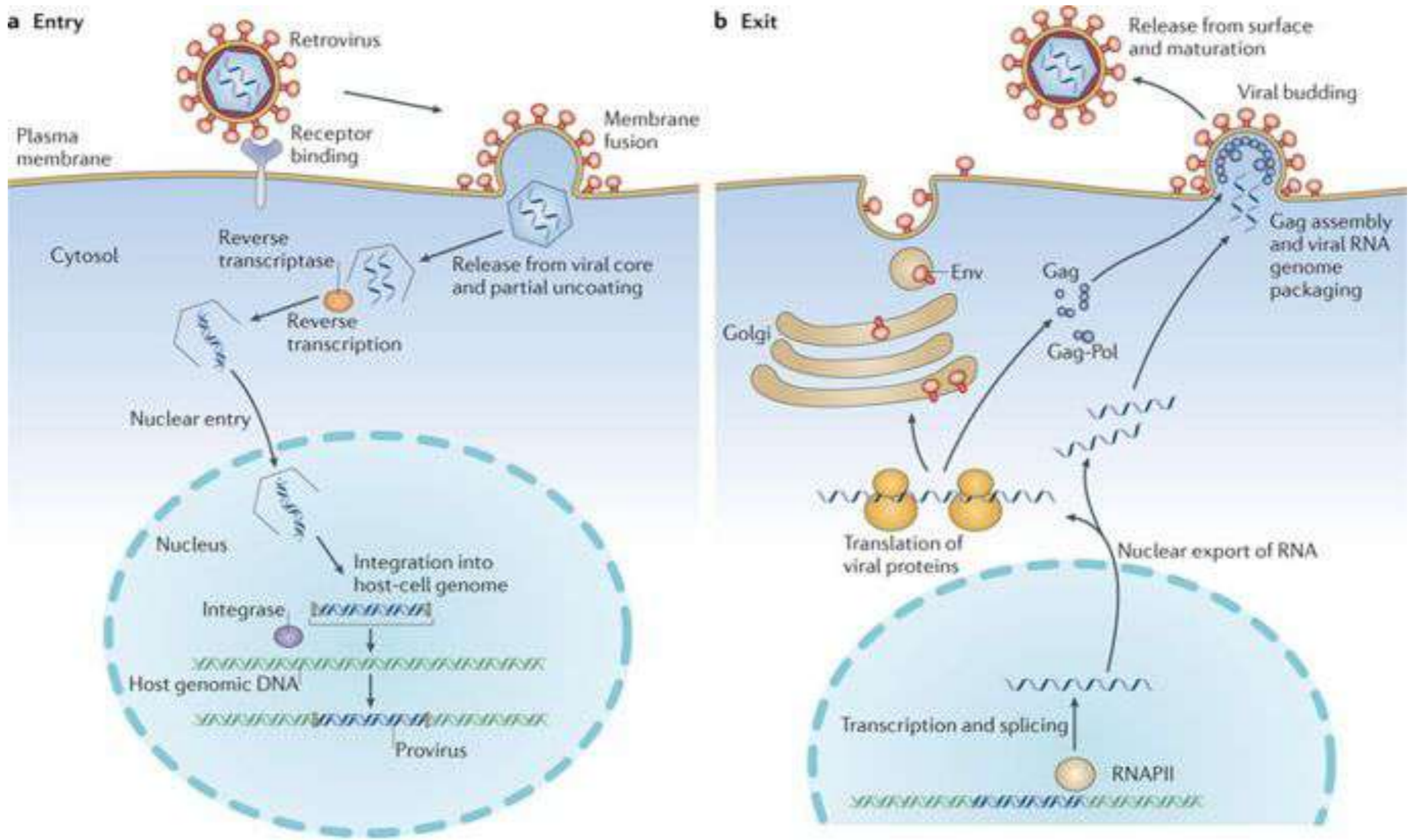
5 Both strands will be completed by the DNAP function of RT. Compared to the ssRNA, both dsDNA ends now have a U3–R–U5 sequence that is also called long terminal repeat (LTR).

Colors: RNA (red), transfer RNA (rose), first DNA strand (blue), second DNA strand (green), DNA completions (black), site with complementarity (yellow; not shown for all sequences).

RNase H: Ribonuclease H; **tRNA:** transfer RNA; **ssDNA:** single strand DNA; **ssRNA:** single strand RNA; **dsDNA:** double strand DNA; **RT:** Reverse transcriptase; **DNAP:** DNA polymerase.

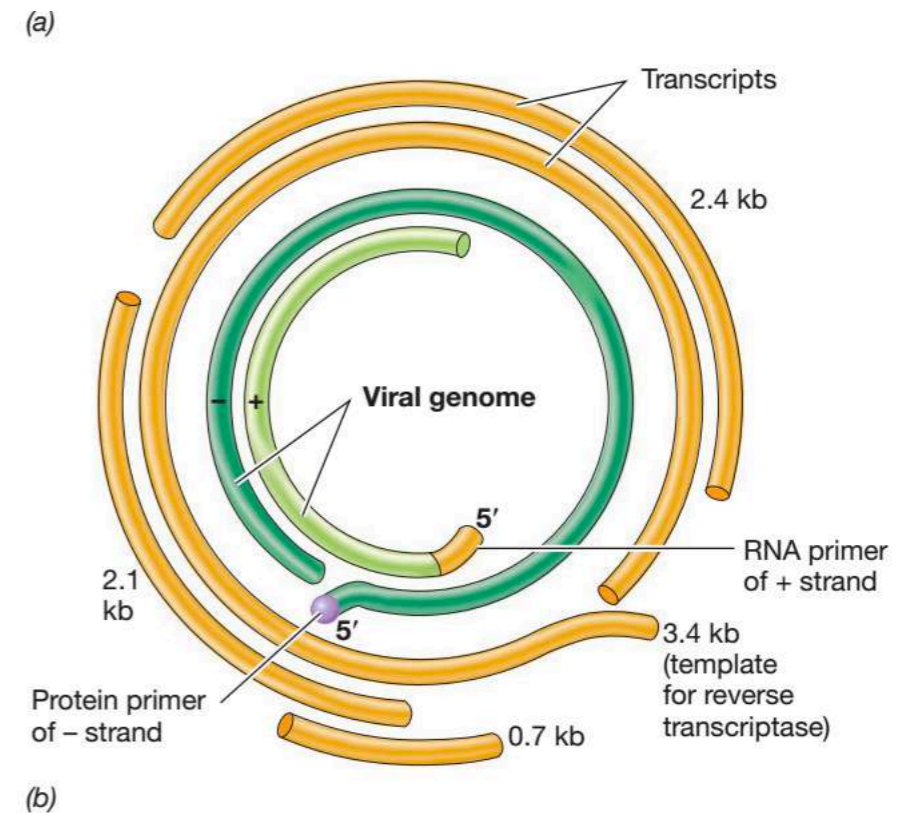
Not drawn to scale! Enzymatic reactions, interacting proteins, splicing sites, and binding site ψ are omitted for clarity. In style of Mudrow S, Falke D, Truyen U (2003). *Molekulare Virologie*, 2. Aufl. (engl.: *Molecular Virology*, 2nd ed.) Spektr Akad Verl. Heidelberg, Berlin.

Retrovirus



Hepadnavirus, I

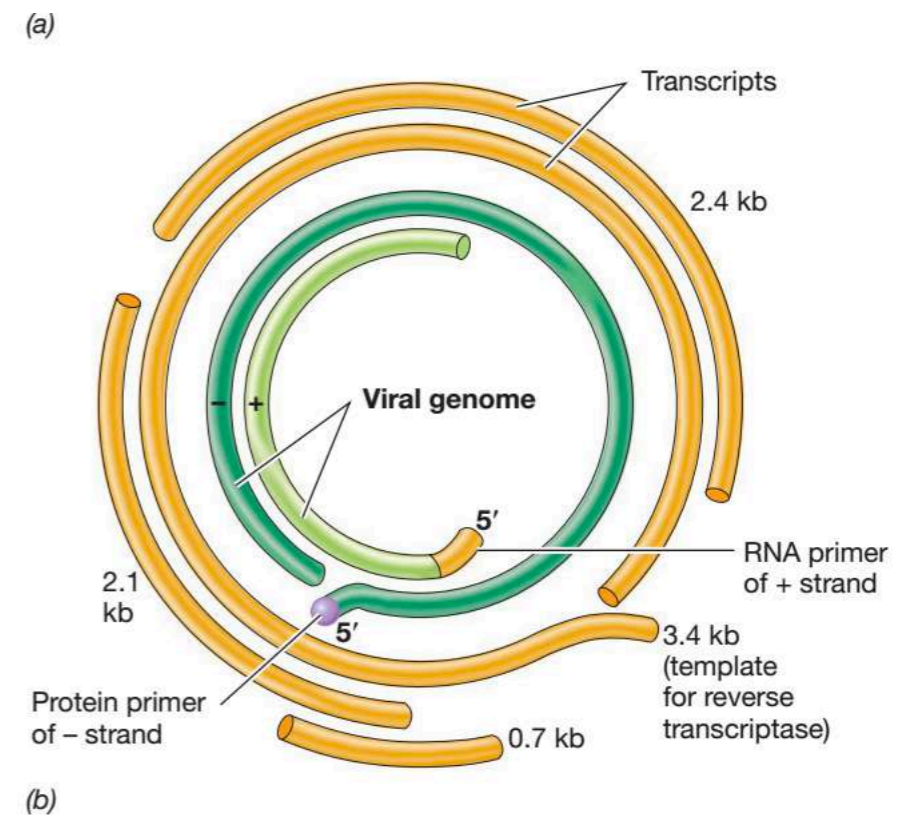
- Hepatitis B, HBV
- **Partially ds DNA**
- 3-4 kb pairs genome
- Hepadnavirus genomes encode several proteins by employing **overlapping genes**
- Hepadnaviral **reverse transcriptase (RT)** functions as a protein primer for synthesis of **one DNA strand**
- **DNA genome is replicated via an RNA intermediate**

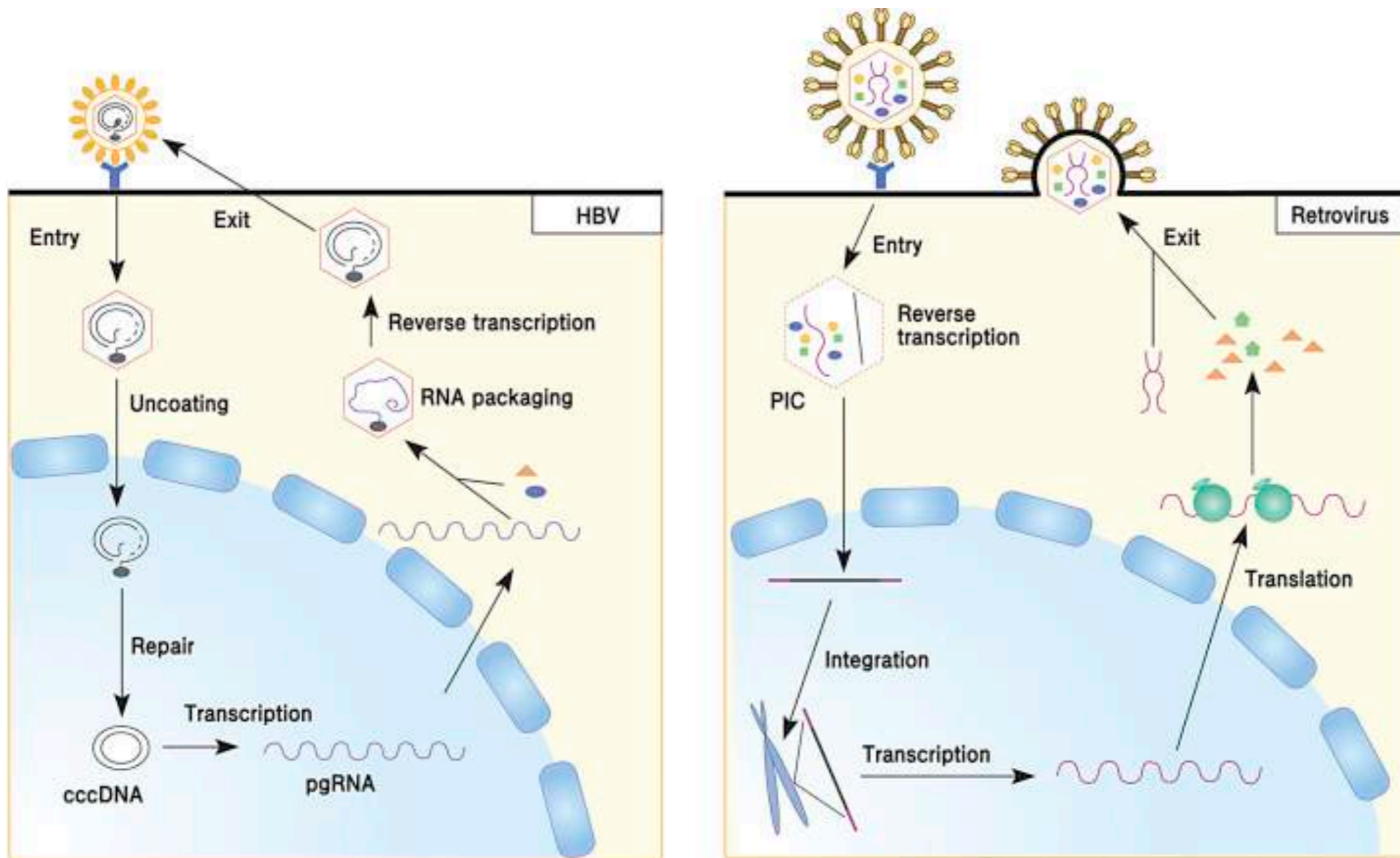


- *In retroviruses, the RNA genome is replicated through a DNA intermediate*

Hepadnavirus, II

- Nucleocapsid enters host nucleus
- Partial genomic DNA strand is completed by **viral polymerase to form an entire dsDNA**
- **Transcription by host RNA polymerase yields four size classes of viral mRNAs** translated to proteins
- The **largest** of these transcripts is $>$ the viral genome and, with RT, associates with viral proteins in host cytoplasm to form genomes for new virions
- RT forms single-stranded DNA off of this large transcript inside virion to form the (-)strand of DNA genome and then uses this as a template to form a portion of (+) strand, \rightarrow partially ds genome

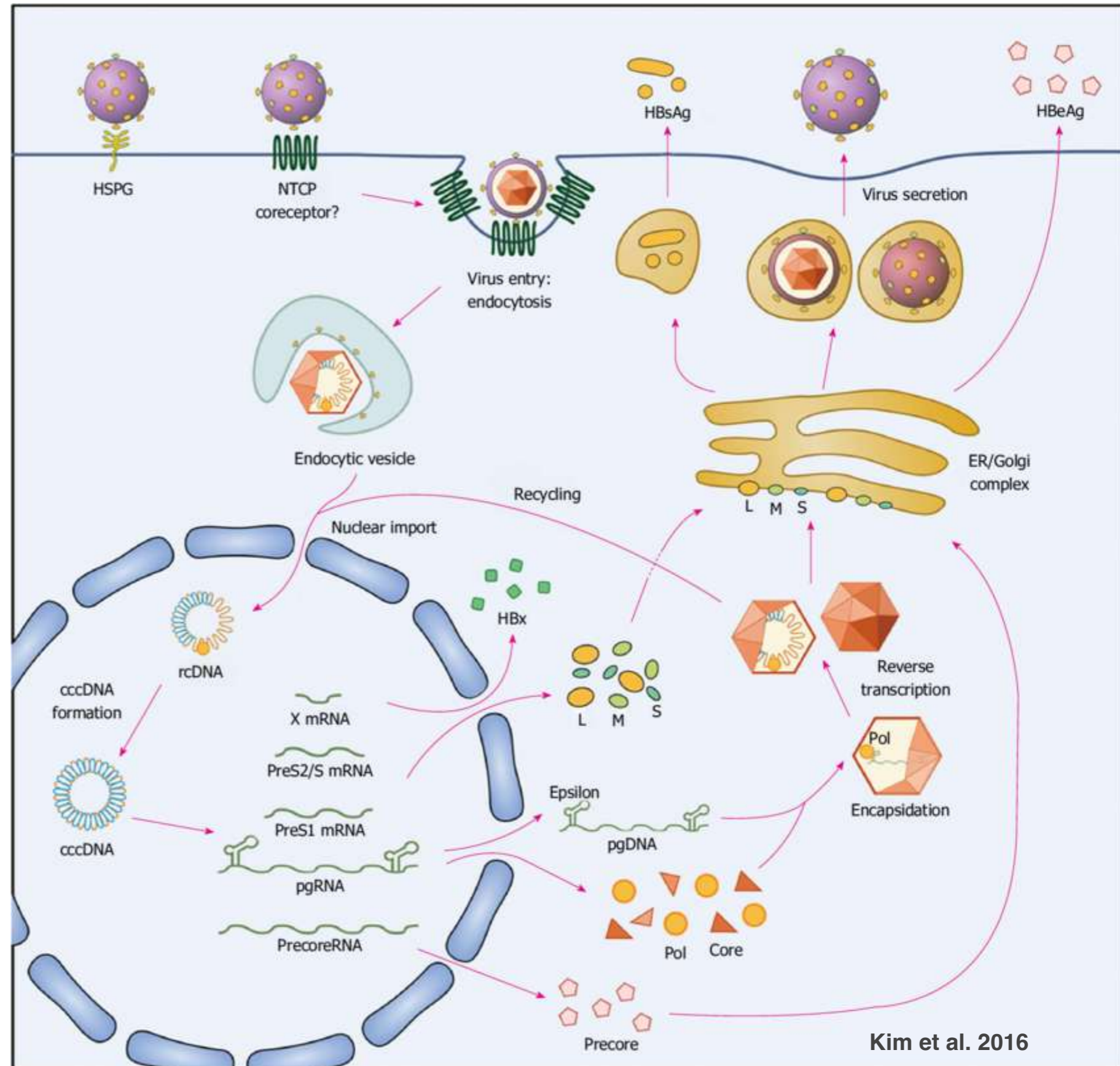




- Both retrovirus and HBV replicate via reverse transcription, RT
- A **retrovirus** has an **RNA molecule** inside the virion particles, whereas **HBV** has a **DNA molecule**
- The step during the virus life cycle at which the reverse transcription step occurs is different
- The **retroviral capsids** exit the cells without reverse transcription, RT during viral entry
- **HBV capsids** exit the cell following viral reverse transcription

Sophisticated changes in viron informative architecture

- Relaxed circular DNA genome, not complete
- Covalently closed circular DNA (cccDNA)
- Reverse transcription of a pregenomic RNA (pgRNA) in core particles leading to synthesis of the relaxed circular DNA (rcDNA)
- cccDNA, the template for viral RNA transcription

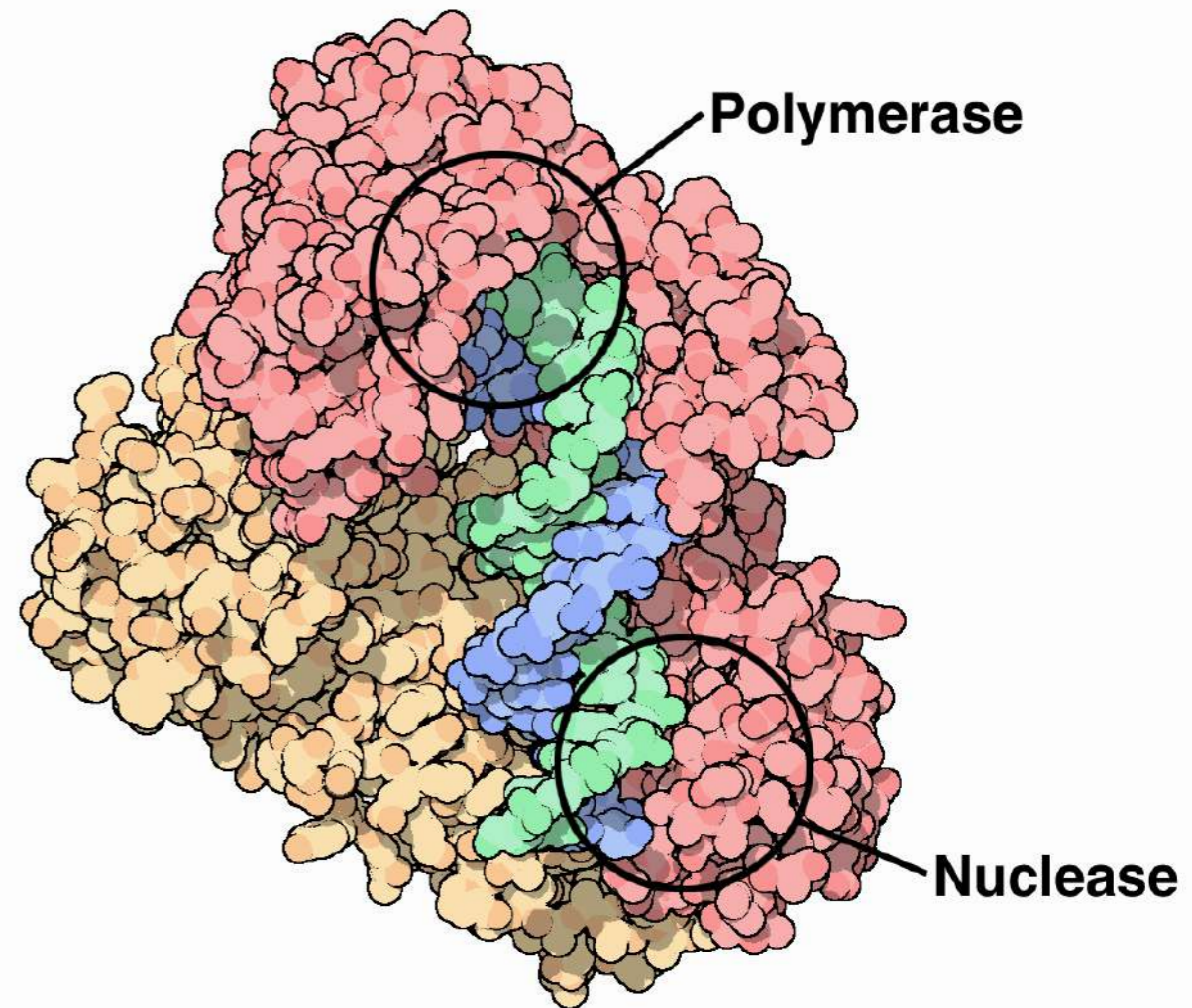


Hu & Seeger, 2015

Kim et al. 2016

Reverse transcriptase (RT)

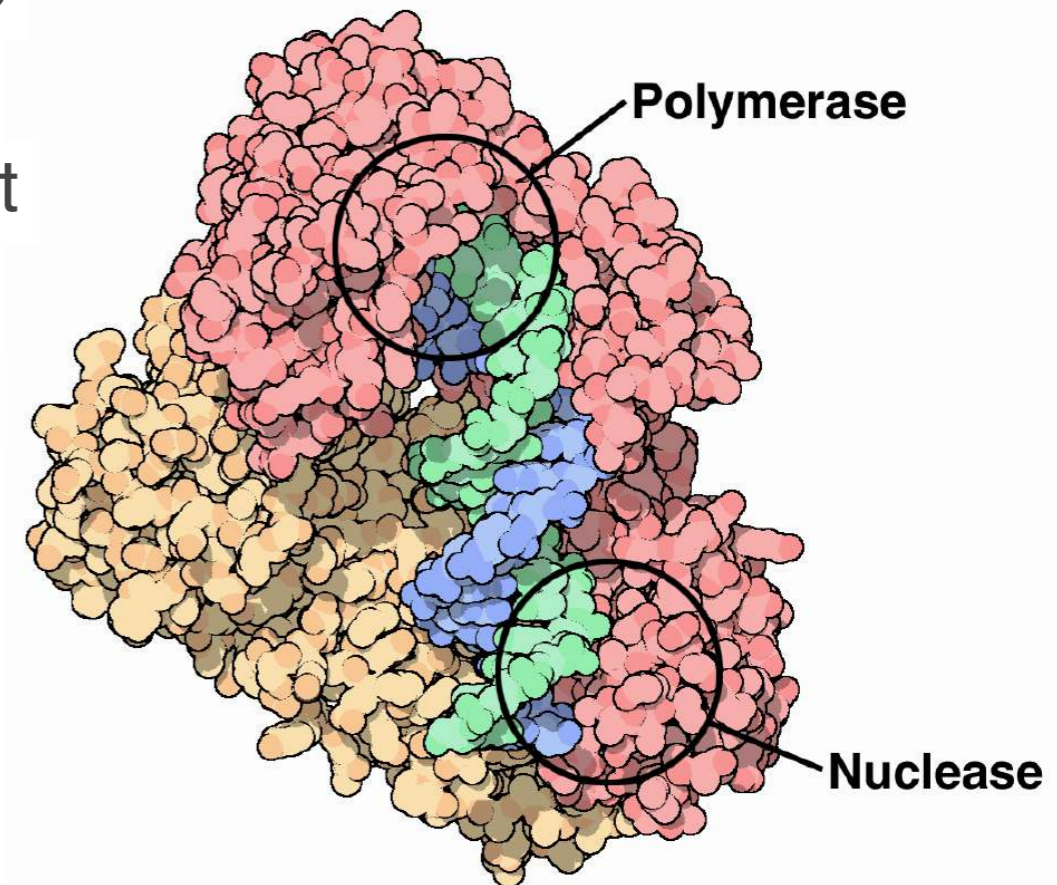
- In 1970 Temin, Mizutan, and Baltimore, working independently, reported the discovery of an **enzyme that could synthesize proviral DNA <math>\leftarrow</math> – RNA viral genome**
- In 1975 Temin, Baltimore, and Dulbecco (who mentored both Temin and Baltimore) were awarded the Nobel Prize for Physiology or Medicine “for their discoveries concerning the interaction between tumour viruses and the genetic material of the cell



Reverse transcriptase (RT)

Retroviruses have ssRNA genomes (+)
Hepadnaviruses have dsDNA genomes

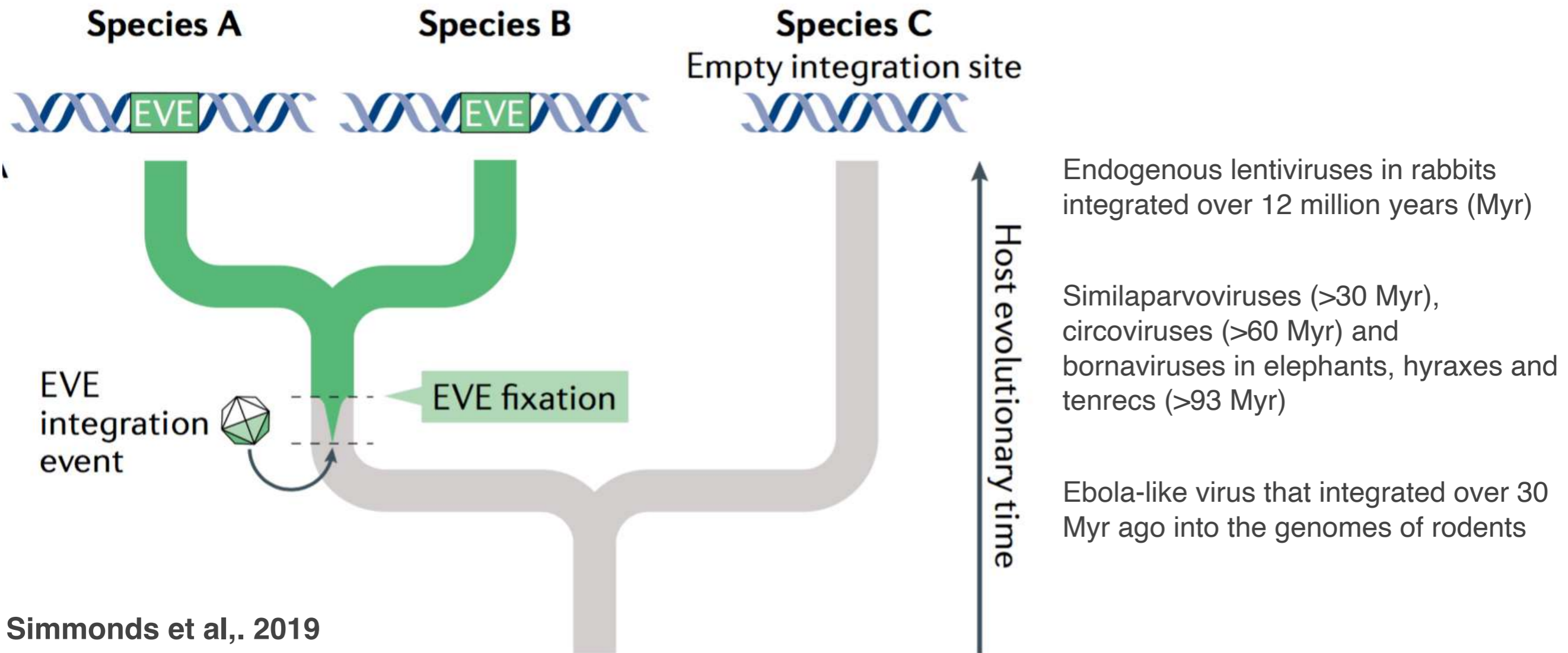
- **RT is composed of 2 different subunits, encoded by the same gene with diverse 3D arrangement**
- After the protein is made, one of the subunits is clipped to a smaller size (yellow) so that it can form the proper mate with one full-sized subunit (red)
- **RT makes lots of mistakes, up to about one in every 2,000 bases that it copies**
- **The errors allow HIV to mutate rapidly, finding drug resistant strains in a matter of weeks after treatment begins**



Endogenous retroviruses

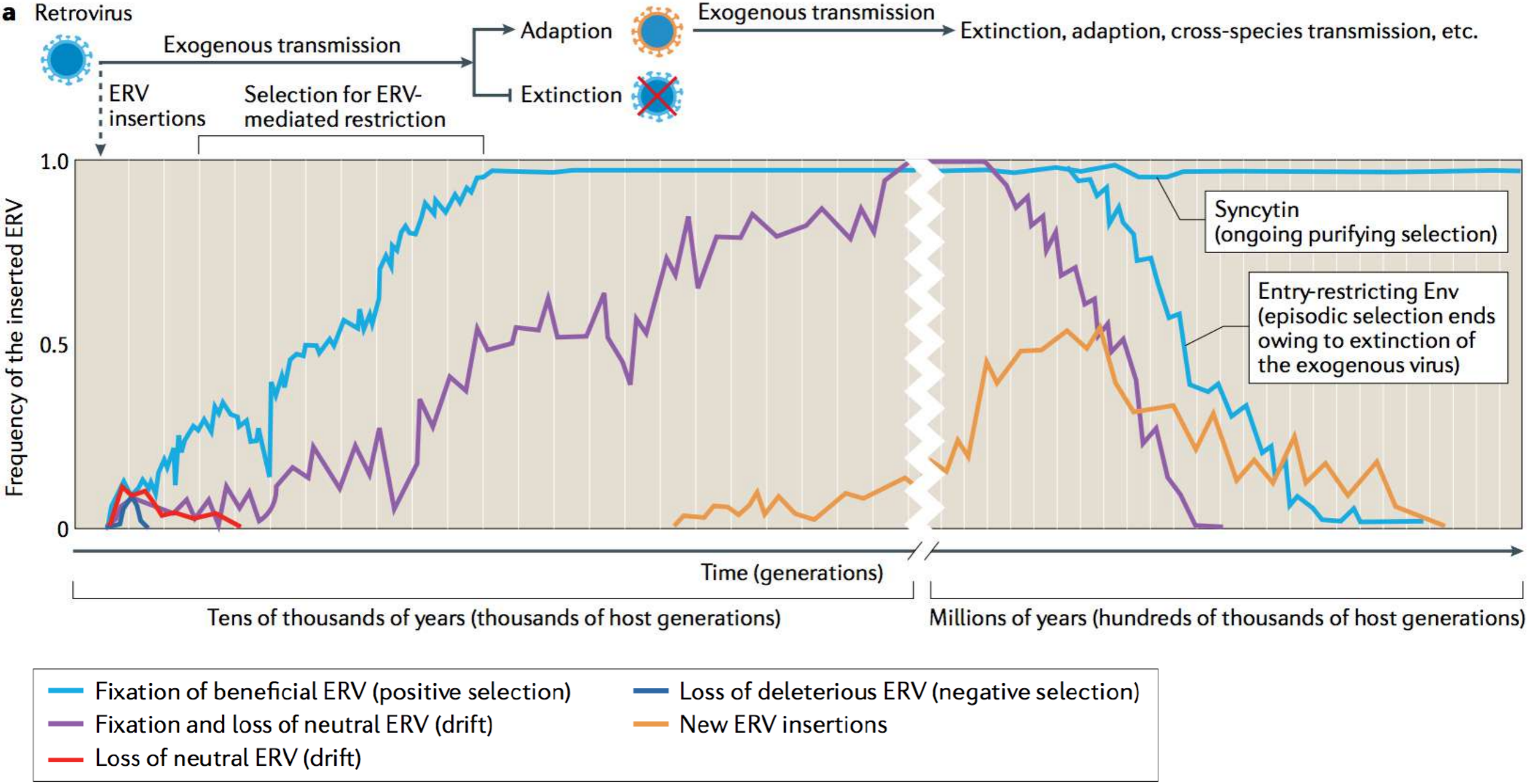
- The **presence of viruses was positively selected** because they promoted cellular variation and functional diversification
- **Endogenous retroviruses (ERVs), which are ancient traces of past infections** found as viral footprints in the genome of different species
- About 8% of the human genome is made of endogenous retroviral-like elements
- These viral footprints are virus-associated sequences that closely **resemble present-day retroviral** (e.g., HIV) elements, including the 5' and 3' long terminal repeats (LTR), and the coding genes gag, pro-pol, and env
- Long-time persistence in the host genome —> accumulation of **mutations** as well as insertions and deletions, which have generally **affected their capacity to produce infectious virions**

Endogenous viral elements

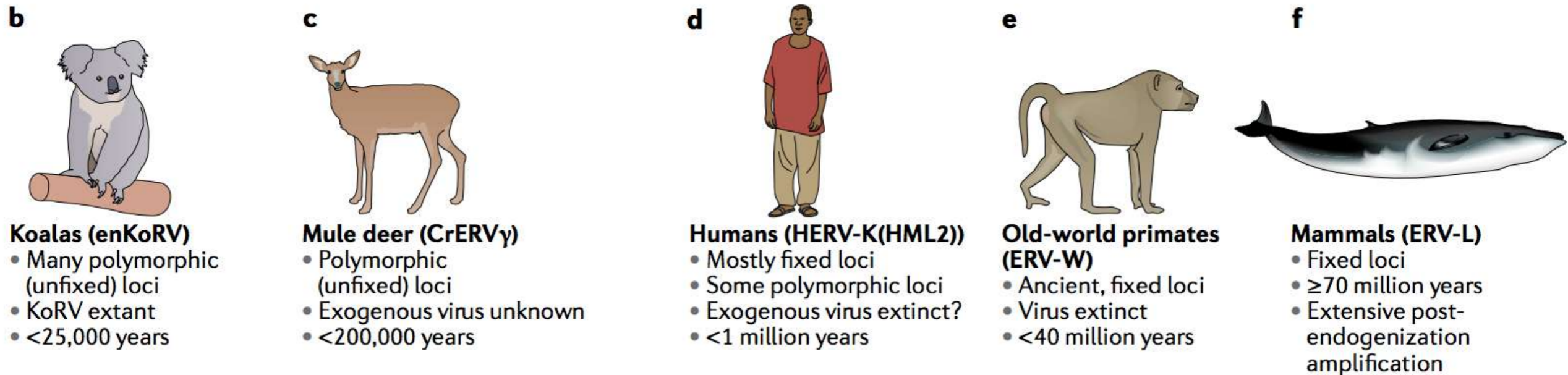


As part of host genomes, endogenous viral elements (EVEs) are inherited, vertically passing from parents to offspring to create a genomic fossil record stretching back millions of years

Hypothetical evolutionary stages of endogenous retrovirus (ERV) loci



Examples of ERVs at different stages in the evolutionary process in natural populations



- Retroviruses have been infecting vertebrates for over 450 million years
- In retrovirus lifecycle is **retrotransposition**, in which the RNA-based virus genome is reverse transcribed and integrated into the DNA of the host cell
- Occasional integrations into the **germline genomes** of egg or sperm cells have the potential to become **endogenous retroviruses (ERVs)** that become fixtures in the host genome

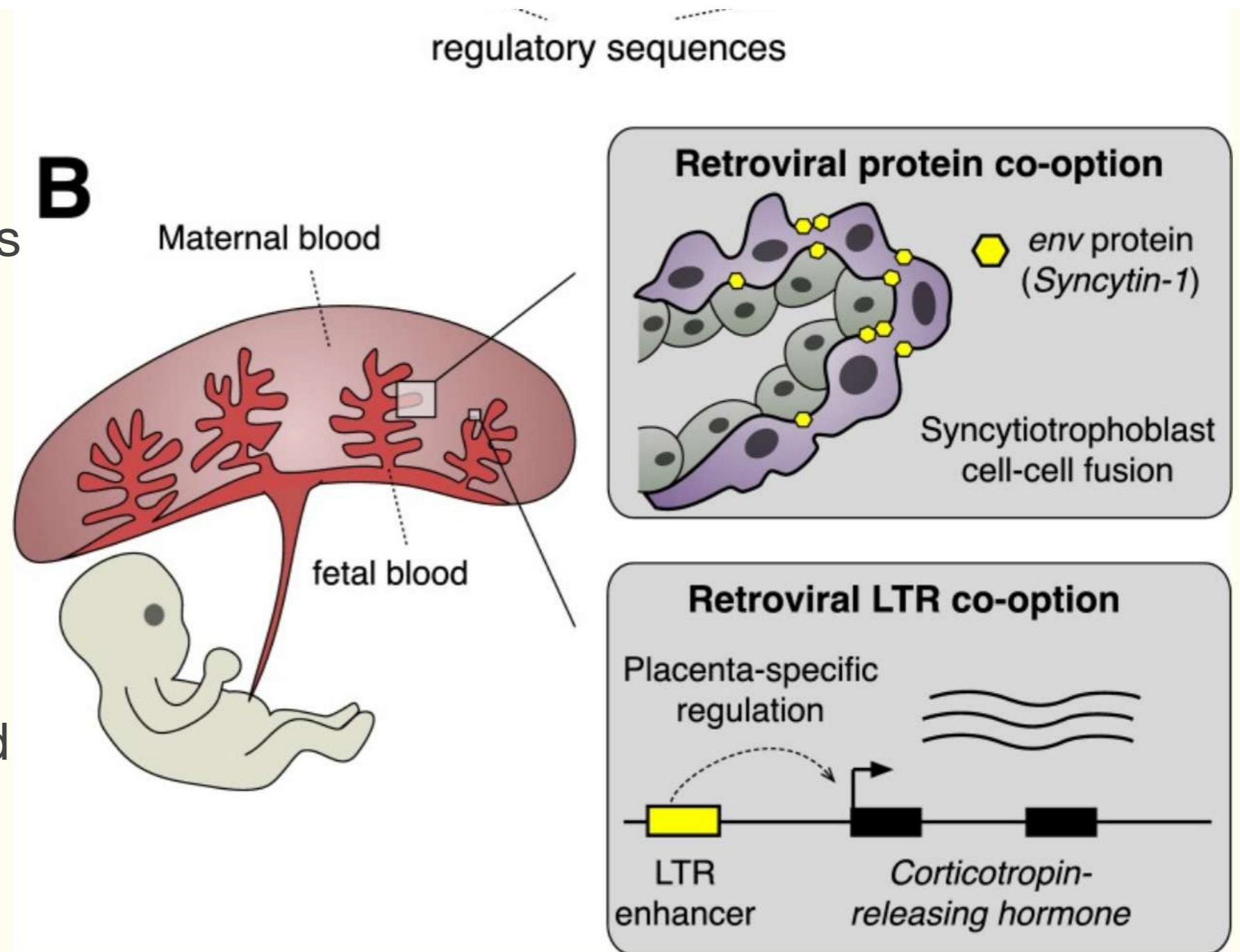
Endogenous retroviruses, II

- Present-day human endogenous retroviruses probably contribute to **pluripotency of human cells**, and **genome regulation and placenta fusion, brain development**
- ERVs: RNA transcribed from HERV-K provide stem cells in embryos **pluriplotency** but when expressed in adults —> cancers of the testes
- LINE-1, has a viral origin 6000 letter DNA —> reversetranscriptase —-> 500K copies a grand total of **17% in human genome brain development in mouse brain embryo** —> are actively operate as retrotrasposons
- ERVs can function as genomic regulators of transcription
- ERVs can trigger inflammation response
- ERVs are unregulated in some kind of cancer

The placenta goes viral: Retroviruses control gene expression in pregnancy

ERVs → genetic novelties → 10 lineages in mammals → placenta cells are working together → fusion for merging syncytin-1

The fusion of cells from the placenta is mediated by syncytin, a protein in HERV-W endogenous retroviruses



Functional properties of antiviral compounds

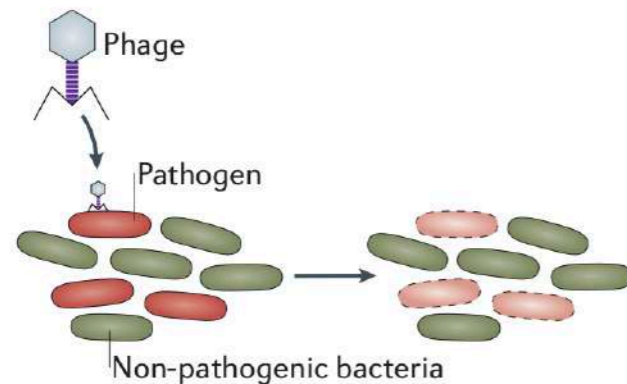
TABLE 28.6 Antiviral compounds

<i>Examples</i>	<i>Mechanism of action</i>	<i>Virus affected</i>
Enfuvirtide	Blocks fusion of HIV with T lymphocyte membrane	HIV (human immunodeficiency virus)
α , β , γ -Interferon	Induces proteins that inhibit viral replication	Broad spectrum (host-specific)
Oseltamivir (Tamiflu [®]) and zanamivir (Relenza [®])	Block active site of influenza neuraminidase	Influenza A and B
Nevirapine	Reverse transcriptase inhibitor	HIV
Acyclovir (↻ Figure 30.42)	Viral polymerase inhibitor	Herpes viruses, <i>Varicella zoster</i>
Zidovudine (AZT) (↻ Figure 30.48a)	Reverse transcriptase inhibitor	HIV
Ribavirin	Blocks capping of viral RNA	Respiratory syncytial virus, influenza A and B, Lassa fever
Cidofovir	Viral polymerase inhibitor	Cytomegalovirus, herpesviruses
Tenofovir (TDF)	Reverse transcriptase inhibitor	HIV
Indinavir, saquinavir (Figure 28.35)	Viral protease inhibitors	HIV

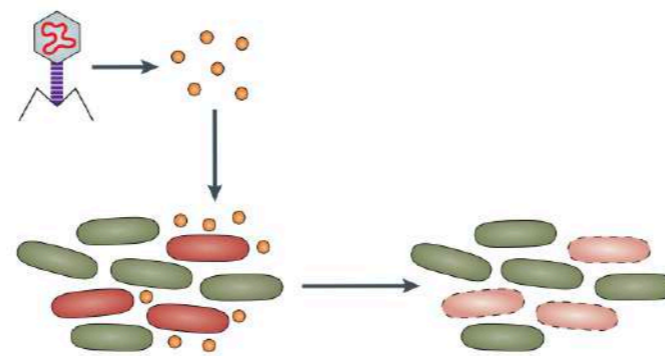
Phage therapy

Bacteriophages were discovered independently in 1915 by Frederick Twort, a British pathologist, and in 1917 by Félix d'Hérelle, a French–Canadian microbiologist

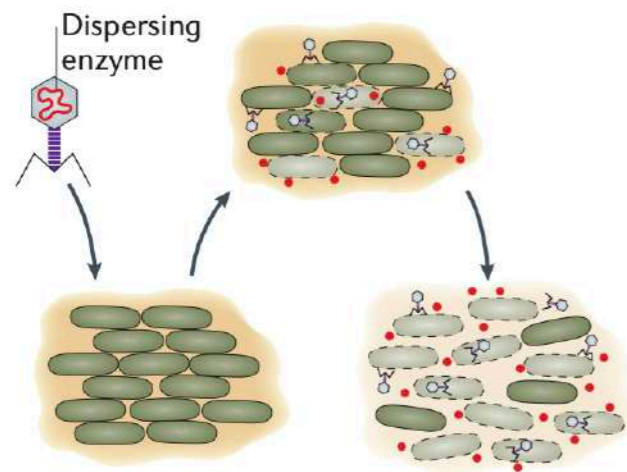
a Phage therapy



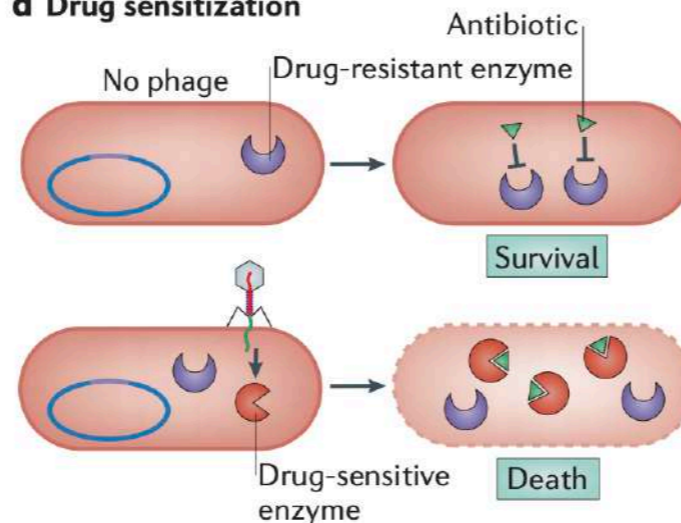
b Phage enzymes



c Biofilm dispersal



d Drug sensitization



a | The specificity of phages can be explored for phage therapy, by which phages target particular bacterial pathogens.

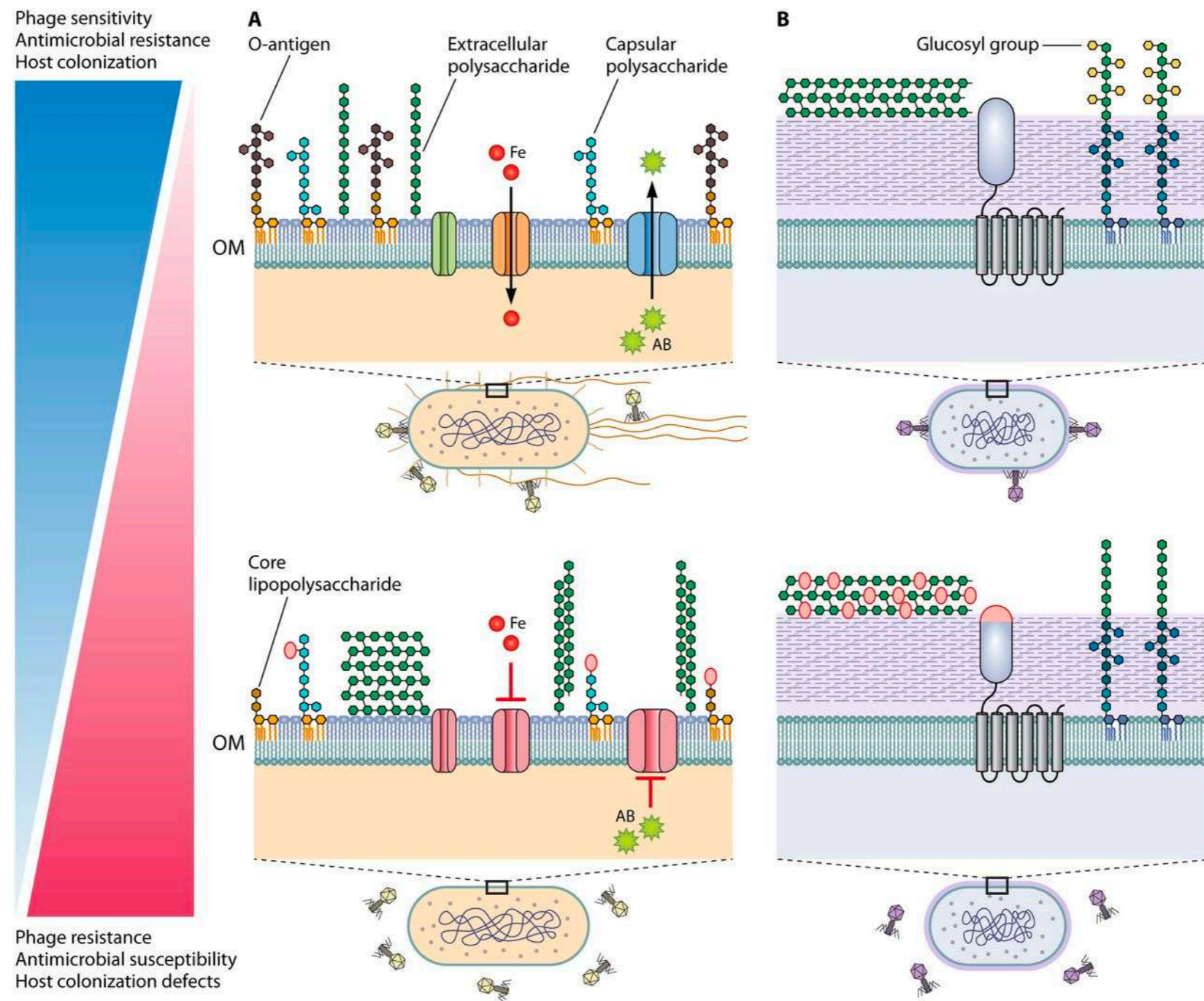
b | Phage products, such as enzymes, can be used to target specific bacteria, including pathogens.

c | Phages can be used to disrupt biofilms, by targeting bacteria embedded in these structures, and can be engineered to release specific enzymes that degrade the biofilm matrix.

d | Phages can be used to sensitize antibiotic-resistant bacteria. For example, phages can introduce antibiotic-sensitive genes into drug-resistant hosts, and this strategy can be combined with antibiotic treatment.

Fitness Trade-Offs Resulting from Bacteriophage Resistance Potentiate Synergistic Antibacterial Strategies

Mangalea & Duerkop, 2020



Bacterial cell surface mutations and modifications produce fitness trade-offs between bacteriophage resistance, antimicrobial susceptibility, and host colonization.