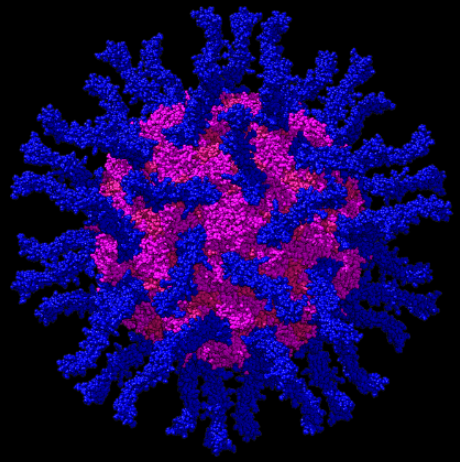


VIROLOGY LIVE

WITH VINCENT RACANIELLO

Synthesis of RNA from RNA

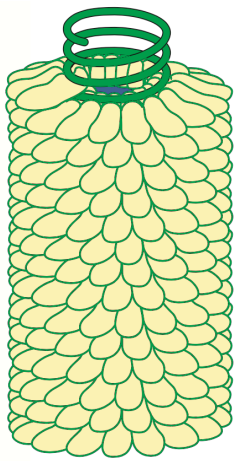
Session 6

Virology Live

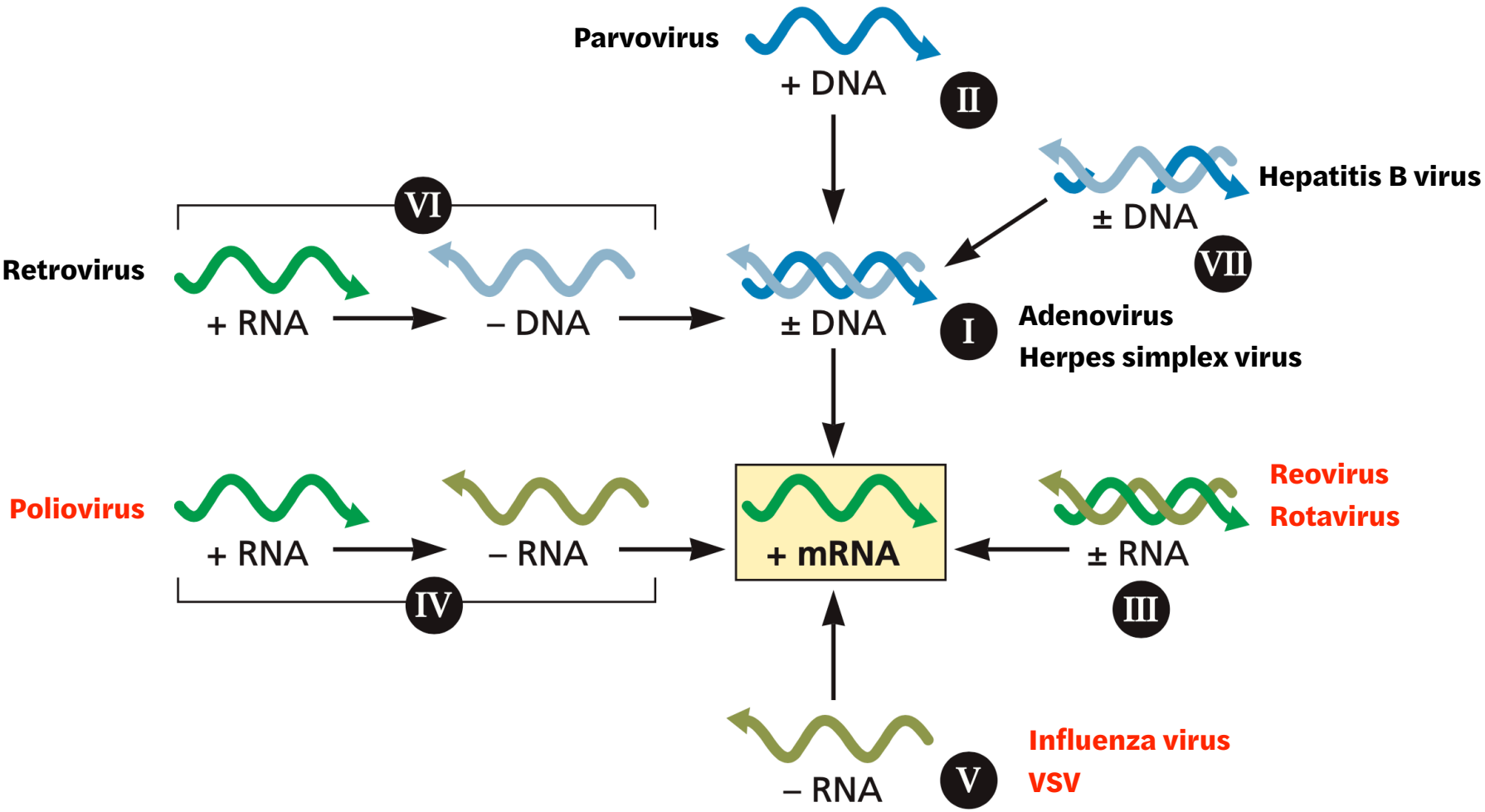
Fall 2021

Truth is ever to be found in the simplicity, and not in the multiplicity and confusion of things
--Sir Isaac Newton

Some RNA history



- 1935 - Stanley crystallizes TMV
- 1936 - TMV crystals contain 5% RNA
- 1944 - DNA is genetic material
- 1952 - Hershey-Chase experiment
- 1953 - Structure of DNA
- 1956 - Frankel-Conrat experiment, TMV RNA is genetic material
- By 1959, RNA was identified in many animal viruses
- 1960s - studies on viral RNA replication begin



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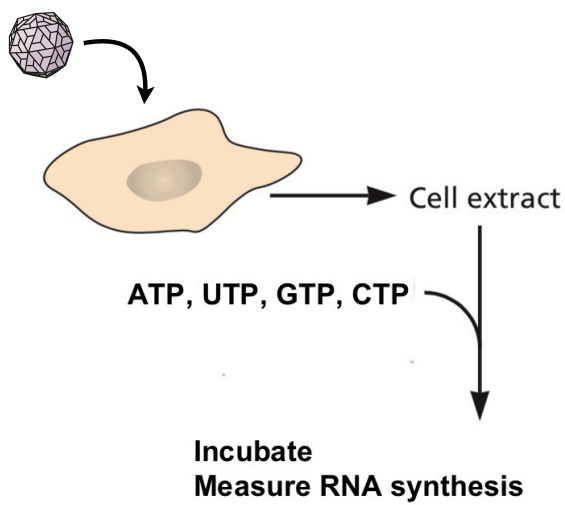
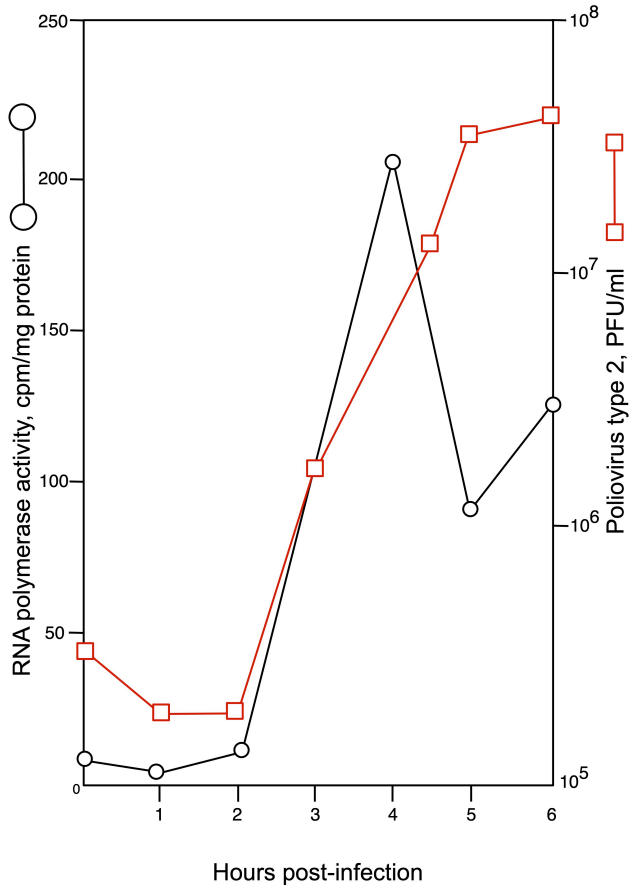
Identification of RNA polymerases

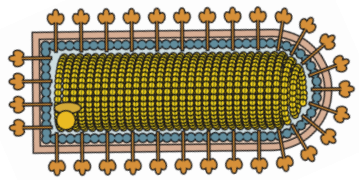


RNA polymerase activity in infected cells



Identification of RNA polymerases

- Polymerase discovered in (-) strand virus particles



- Sequence alignments (GDD), synthesis of recombinant proteins
- Crystal structures

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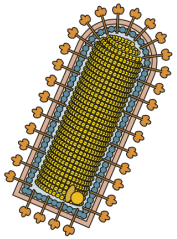
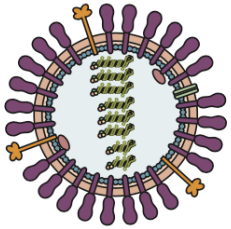
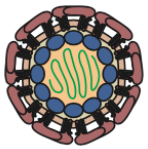
RNA and RdRp in the virus particle

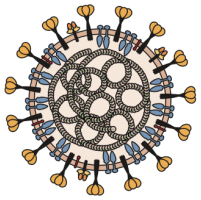
- (-) strand RNA genomes: RdRp, RNA coated with protein (nucleocapsid)



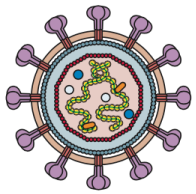


- (+) strand RNA genomes: no RdRp, naked (exceptions: retrovirus, coronavirus)



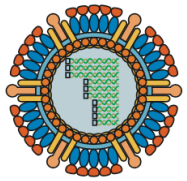


NP on RNA



RT in virus particle
NP on RNA

- dsRNA genomes: RdRp, naked RNA



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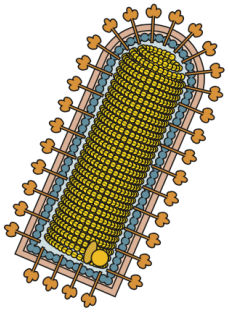
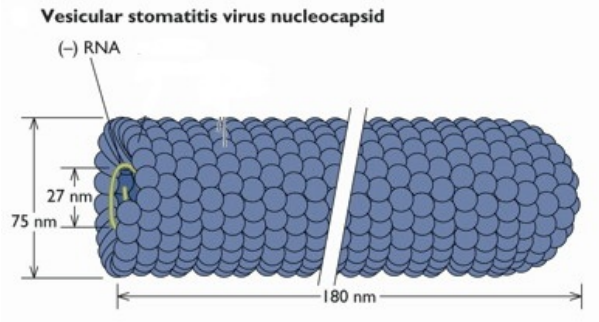
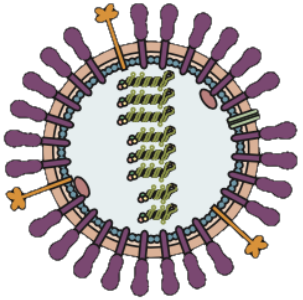
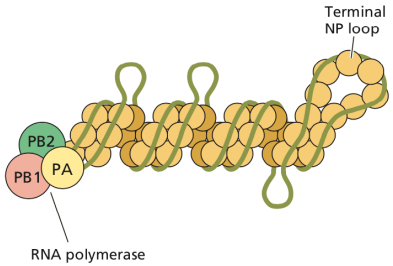
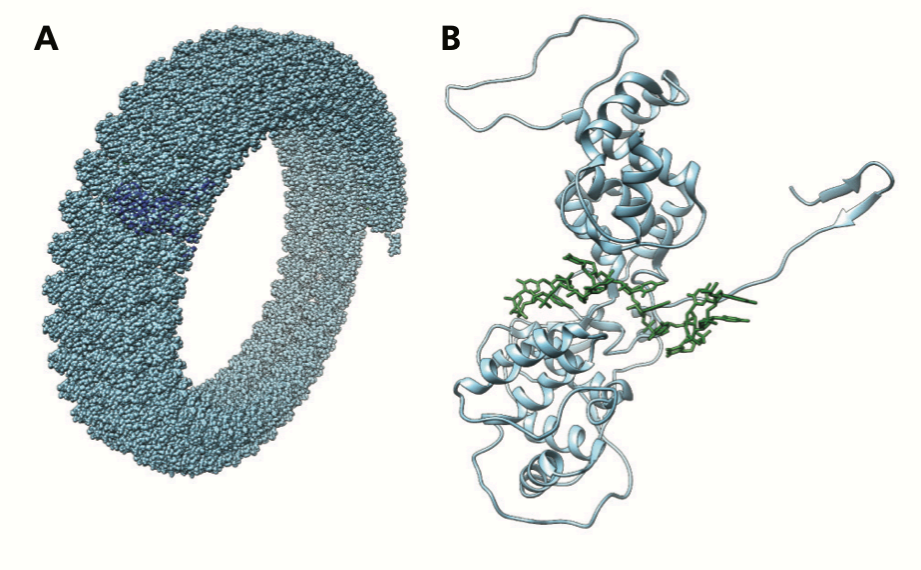
Nucleocapsids

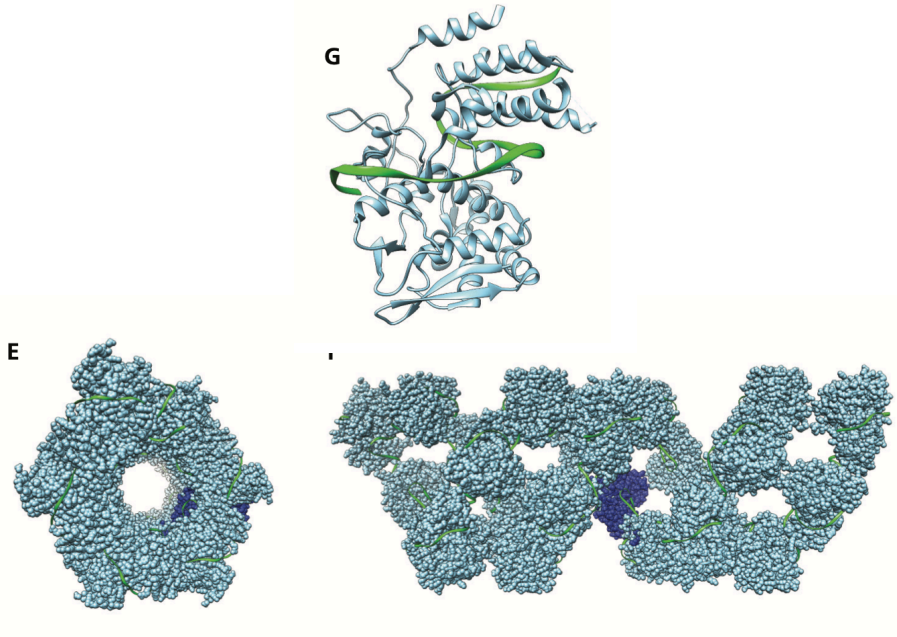












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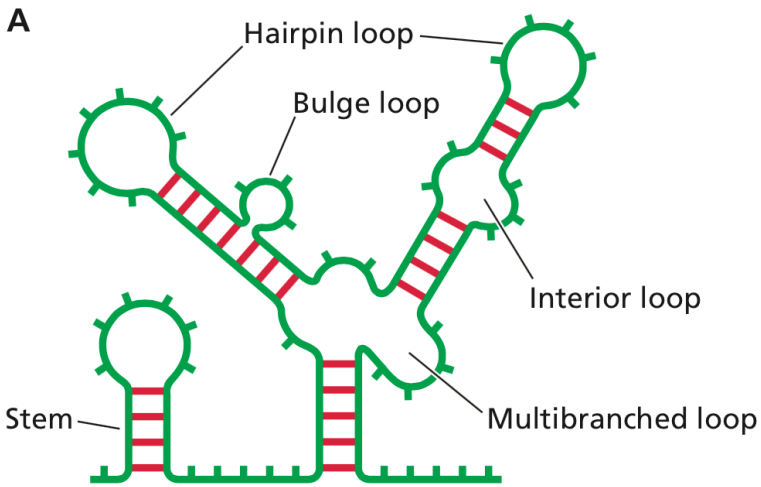
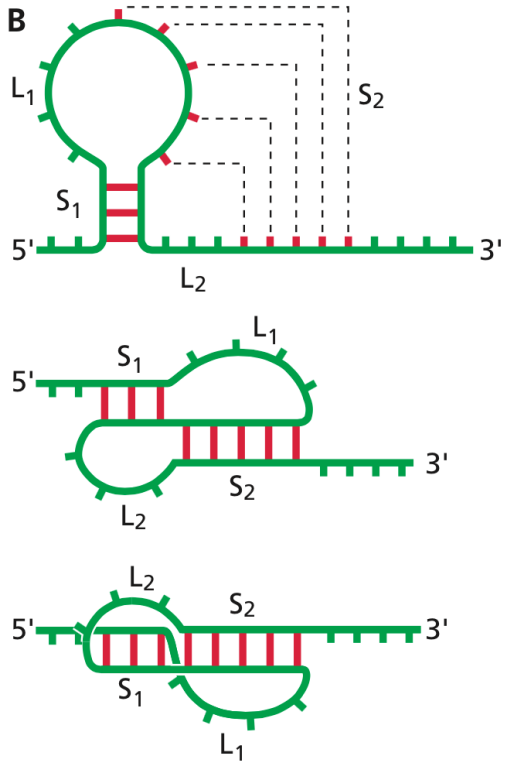
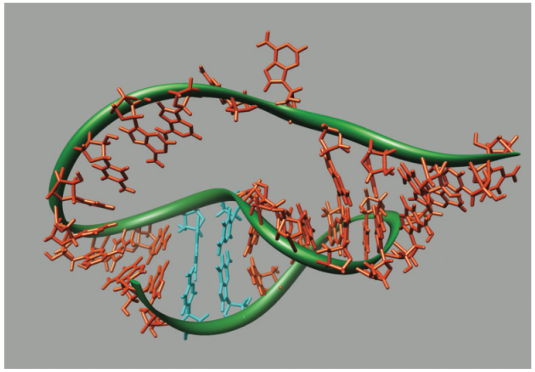
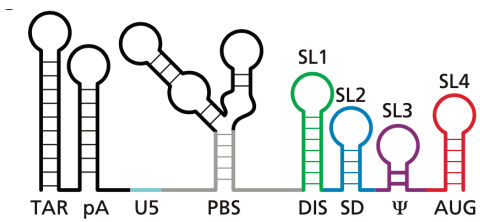
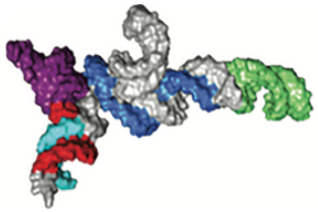
RNA structure











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Rules for viral RNA synthesis

- RNA genome must be copied end to end with no loss of nucleotide sequence
- Viral mRNAs must be produced that can be efficiently translated by cellular protein synthesis machinery

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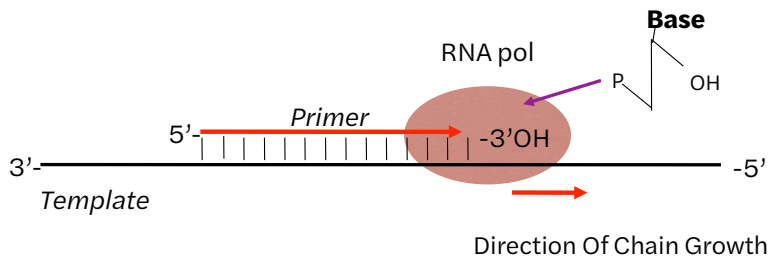
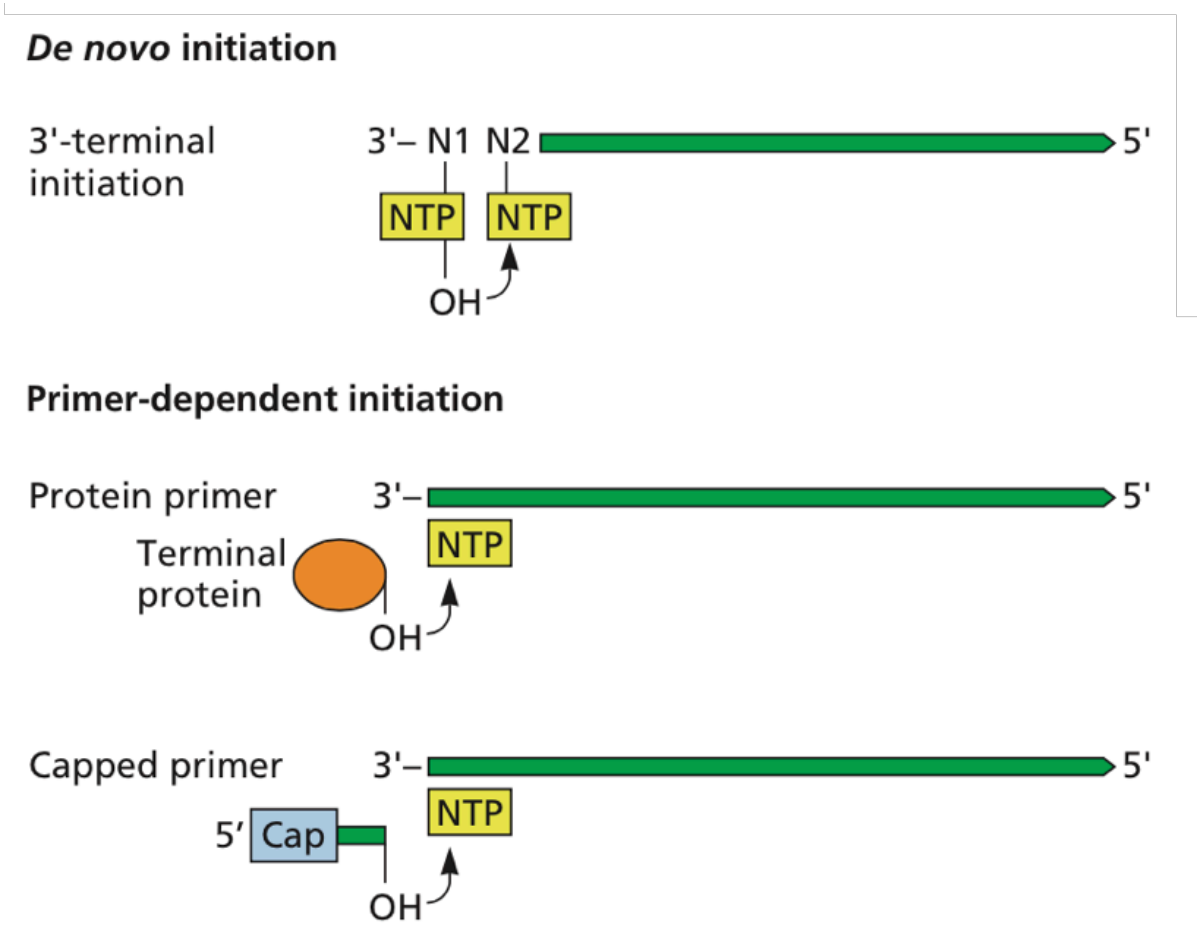
Universal rules for RNA-directed RNA synthesis



- RNA synthesis initiates and terminates at specific sites on the template
- RdRp may initiate synthesis *de novo* (like cellular DdRp) or require a primer
- Other viral and cell proteins may be required
- RNA is synthesized by template-directed stepwise incorporation of NTPs, elongated in 5'-3' direction
- There is some non-templated synthesis

Two modes of initiation of RNA synthesis



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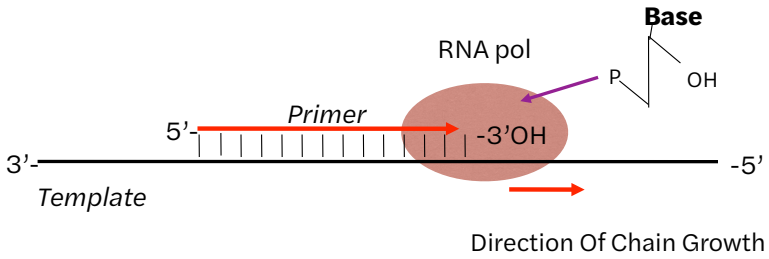
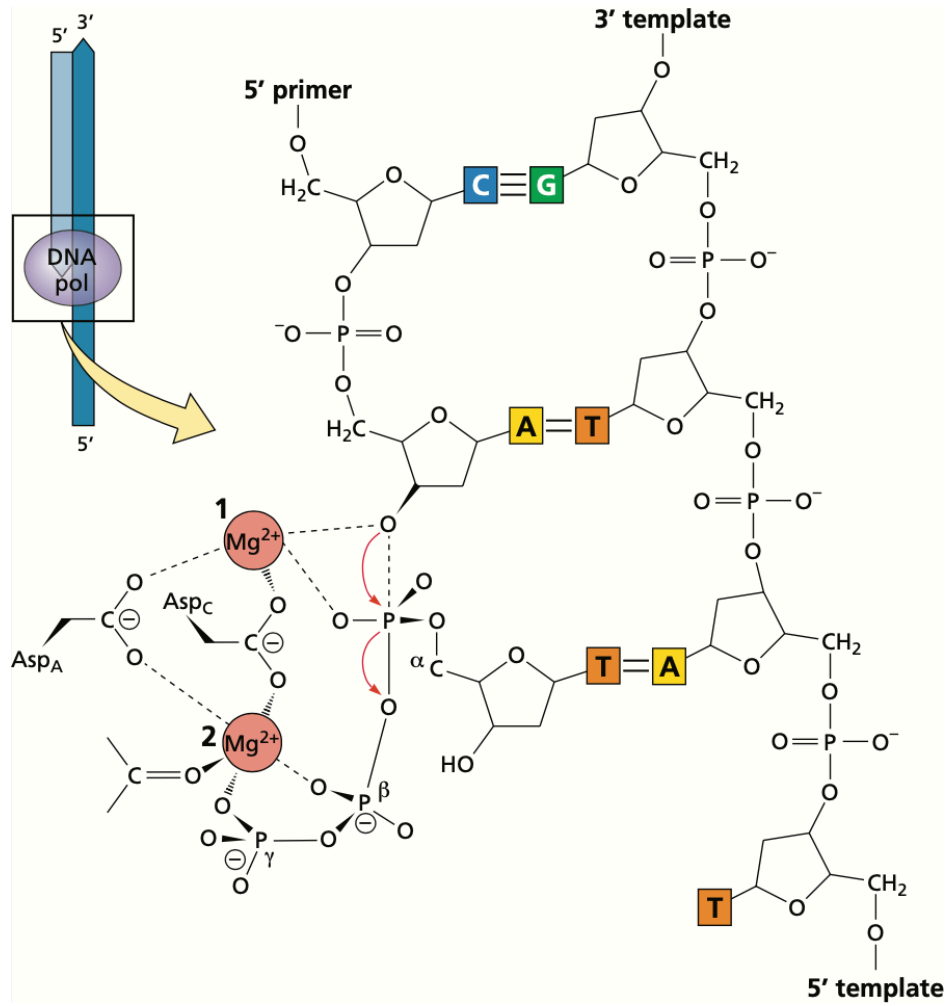


Two-metal mechanism of polymerase catalysis



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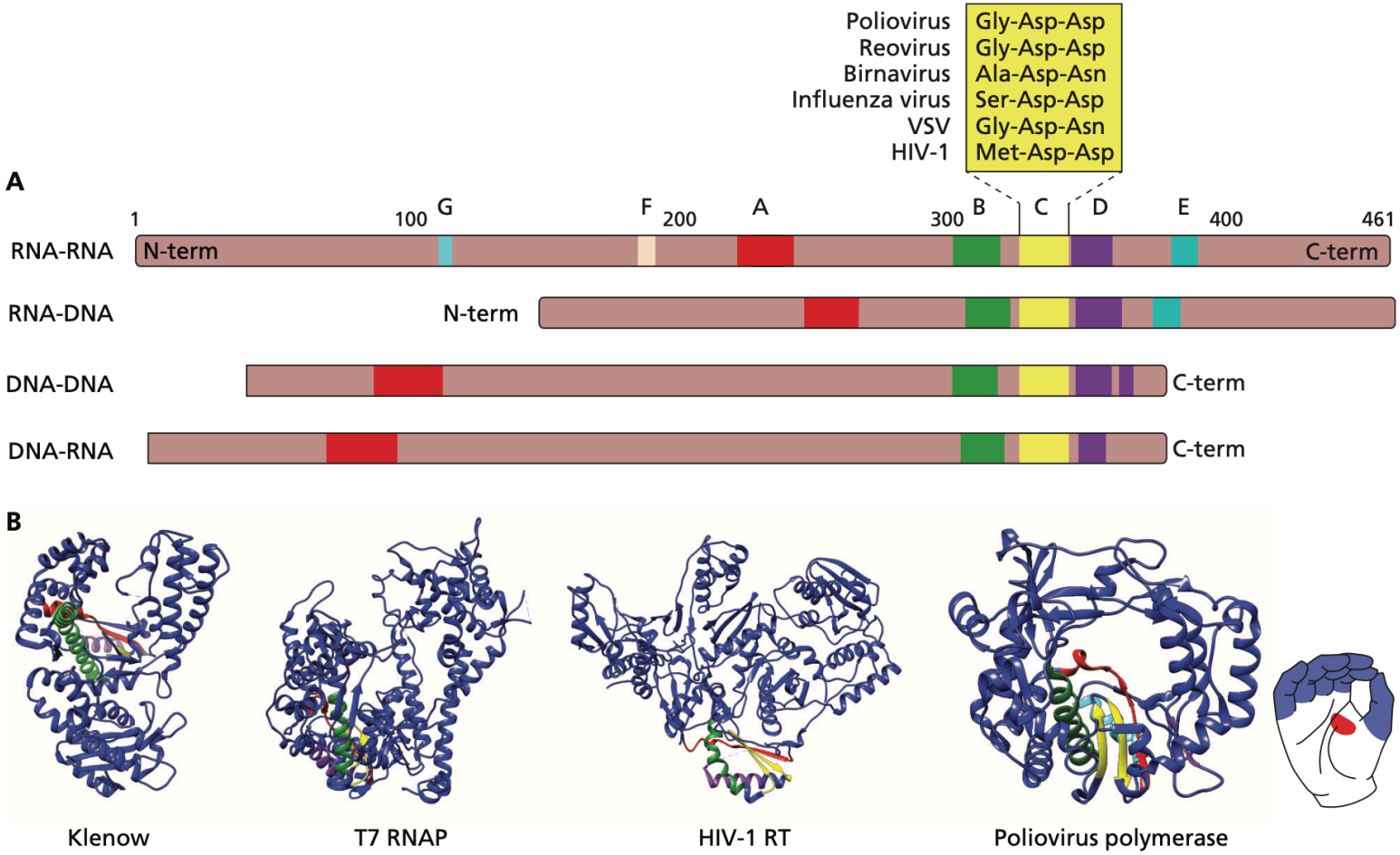
Which is a universal rule about RNA directed RNA synthesis?

A. RdRp may initiate *de novo* or require a primer
B. RNA synthesis initiates randomly on the RNA template
C. RNA is synthesized in a 3'-5' direction
D. RNA synthesis is always template-directed

Sequence relationships among polymerases



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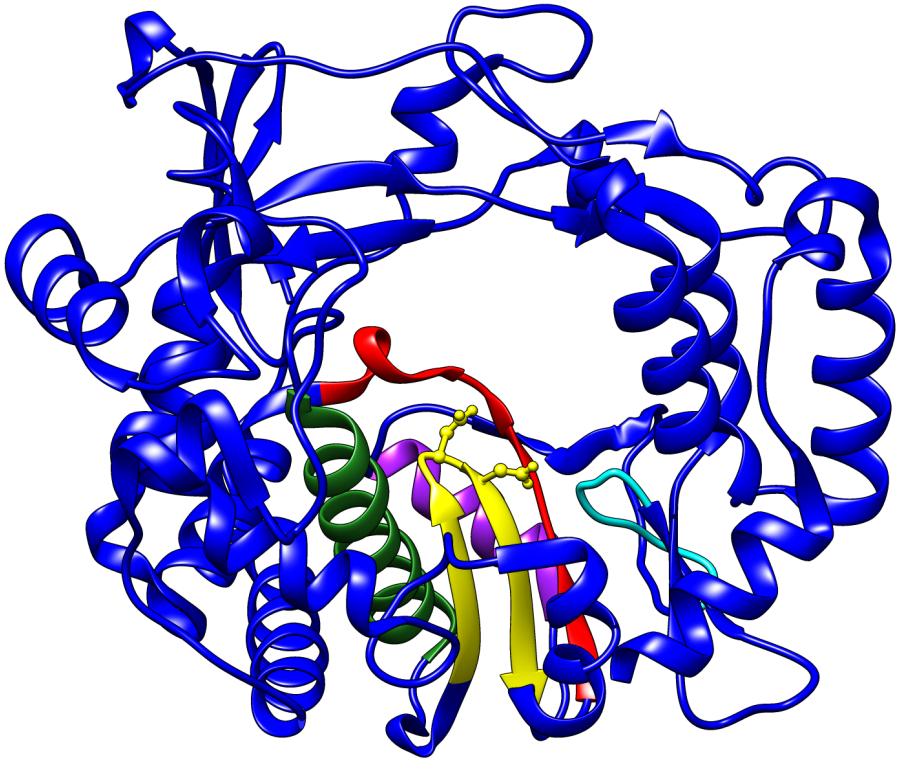
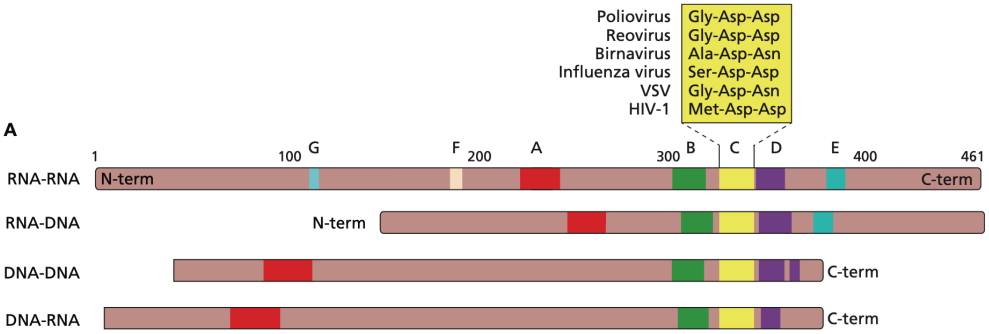
Poliovirus RdRp





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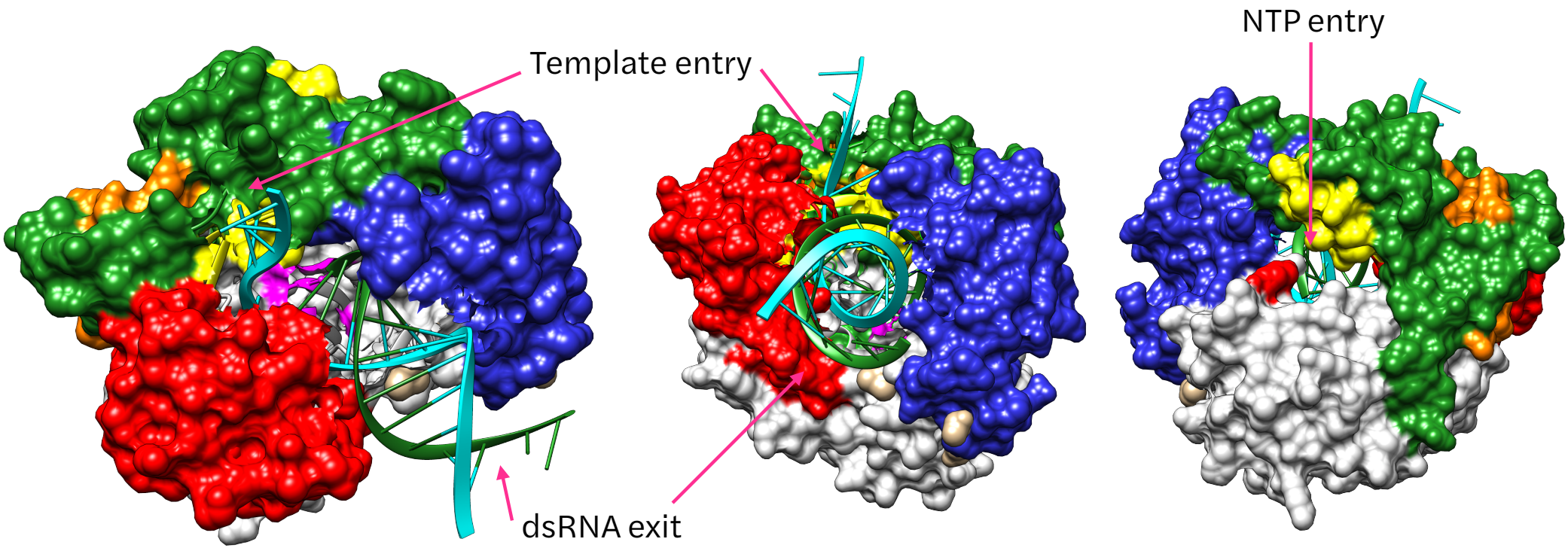
Poliovirus RdRp



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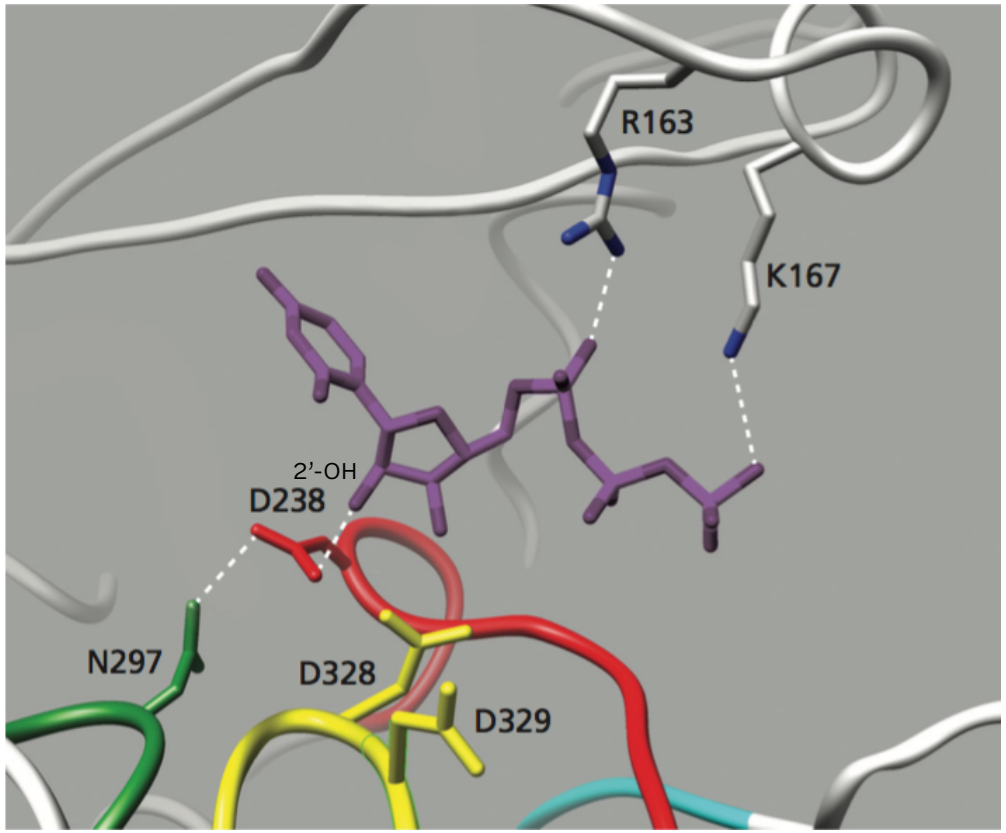
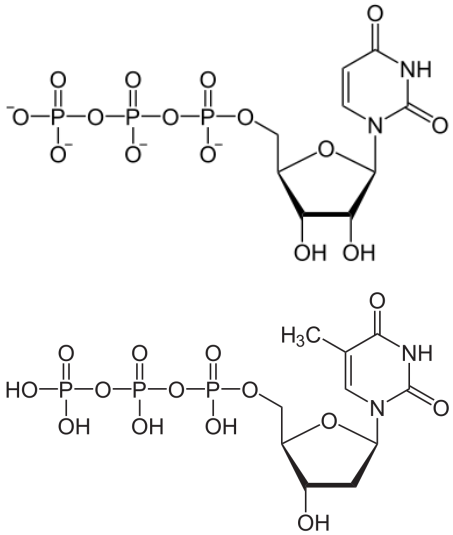
Front

Back

Structure of UTP bound to poliovirus RdRp





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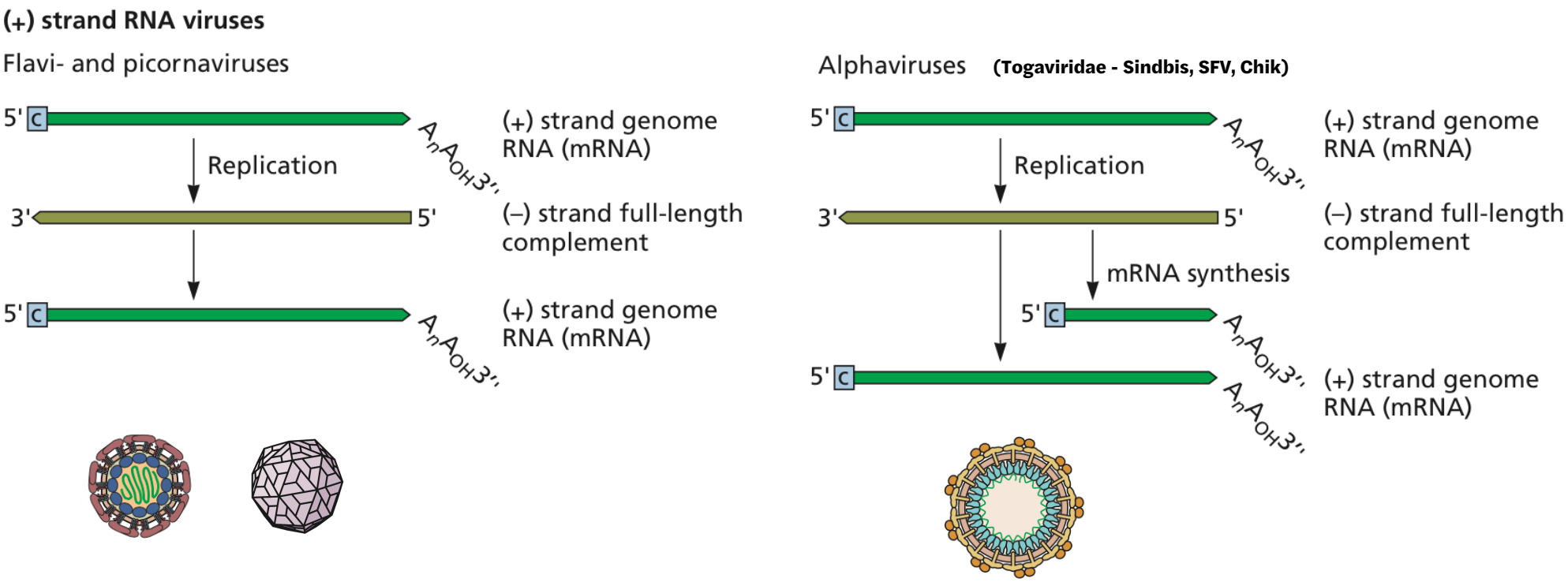
(+) strand RNA viruses



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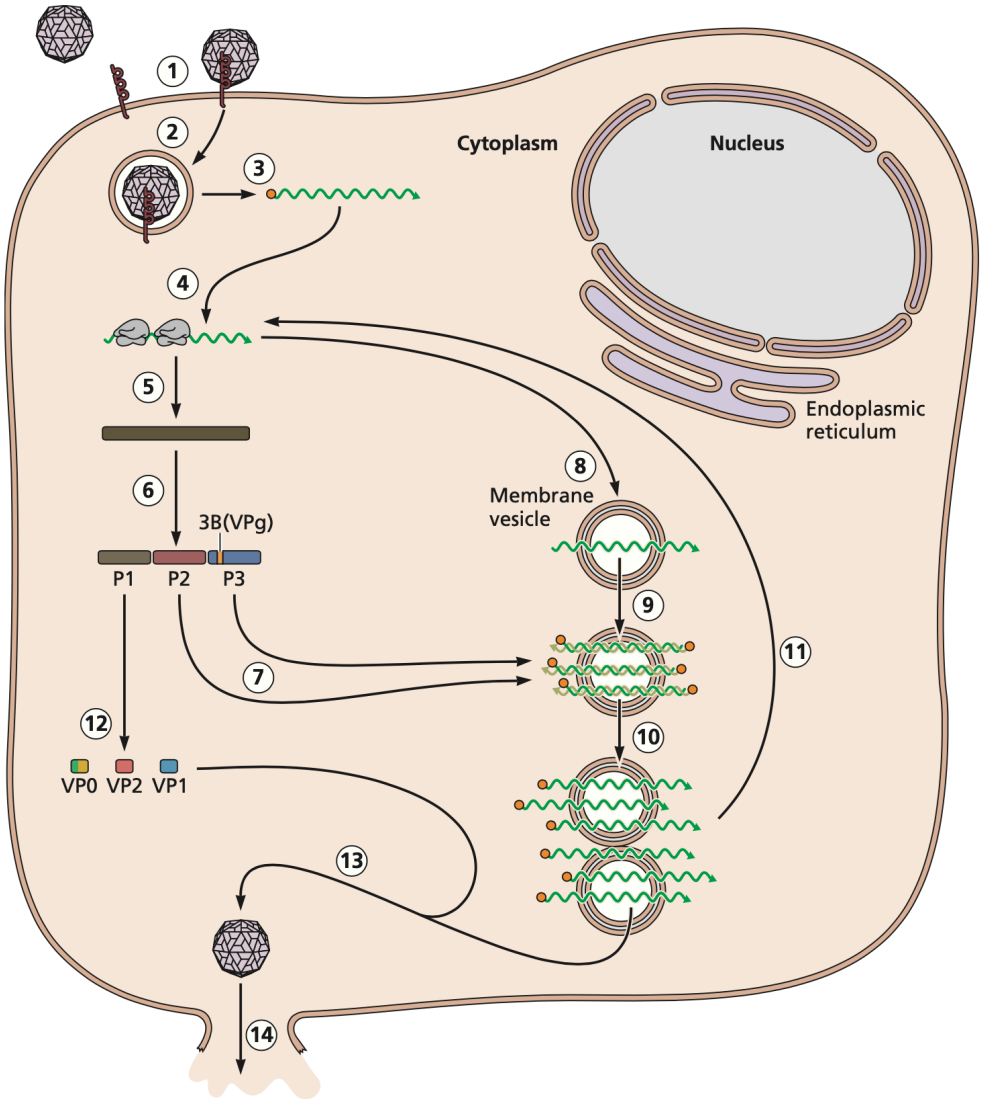
Poliovirus
viral genome = mRNA



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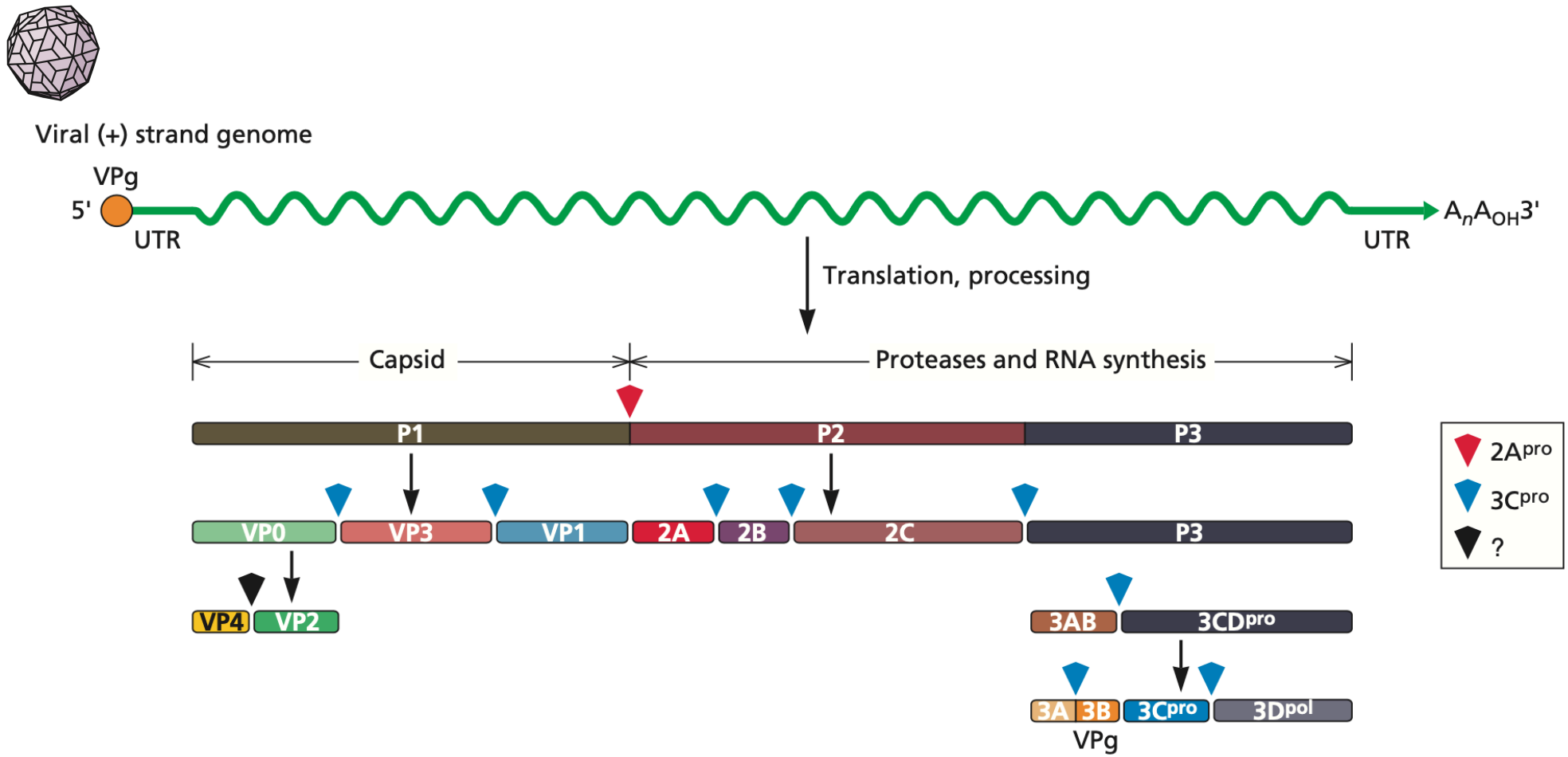

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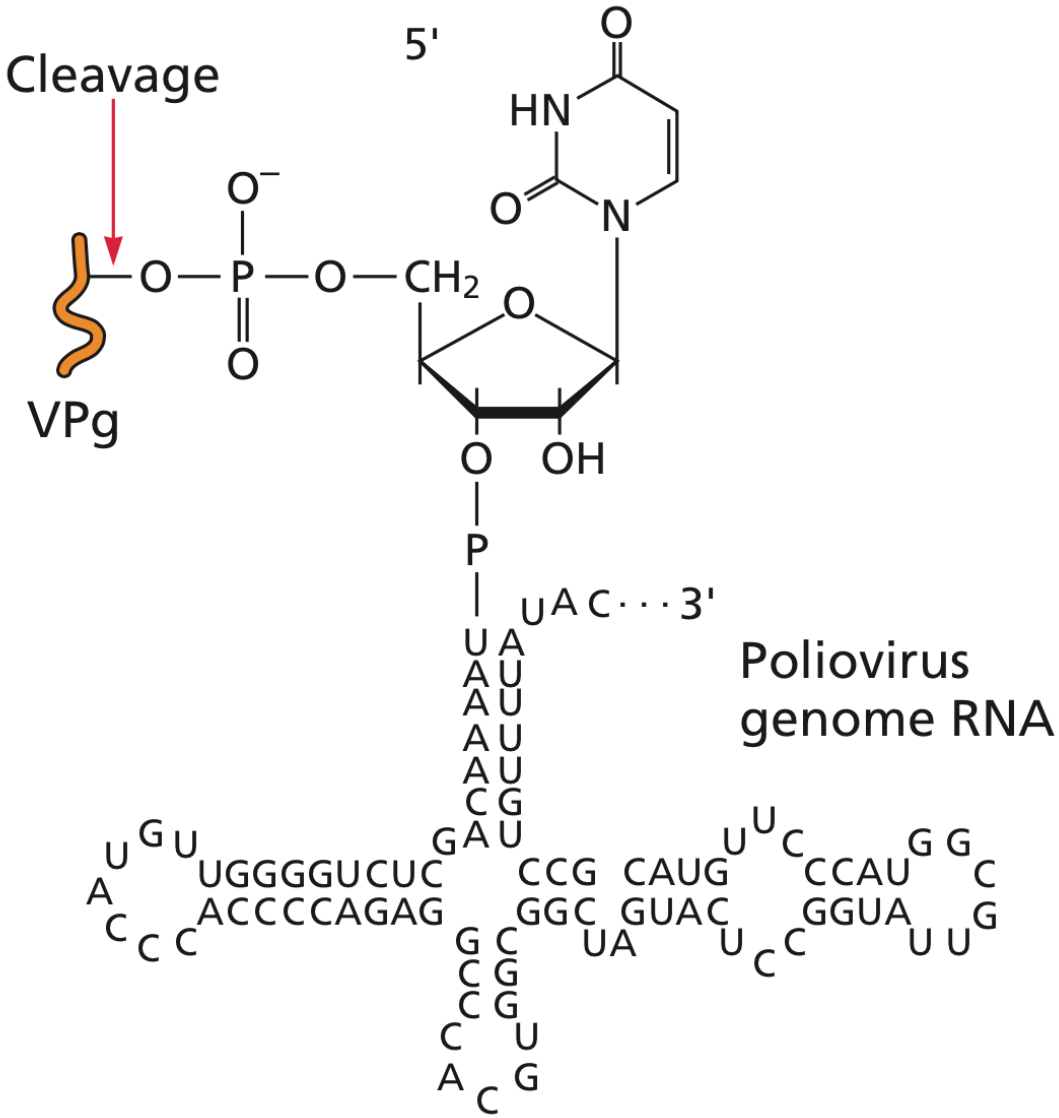

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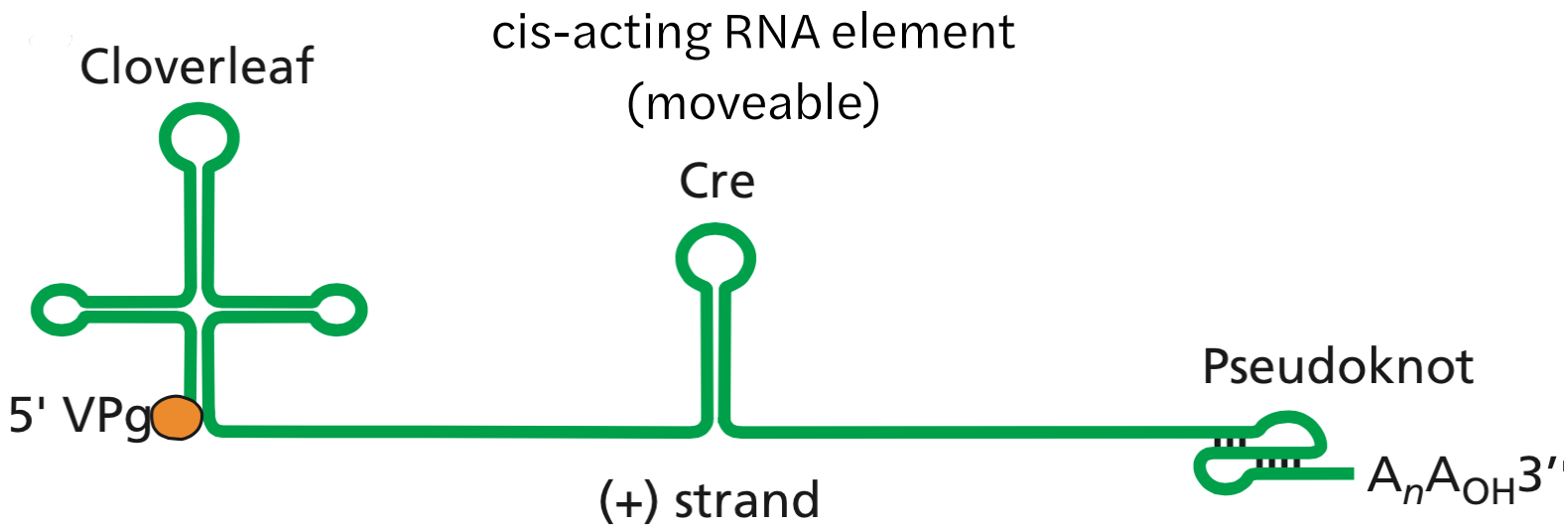

Cellular polyadenylated RNAs not copied

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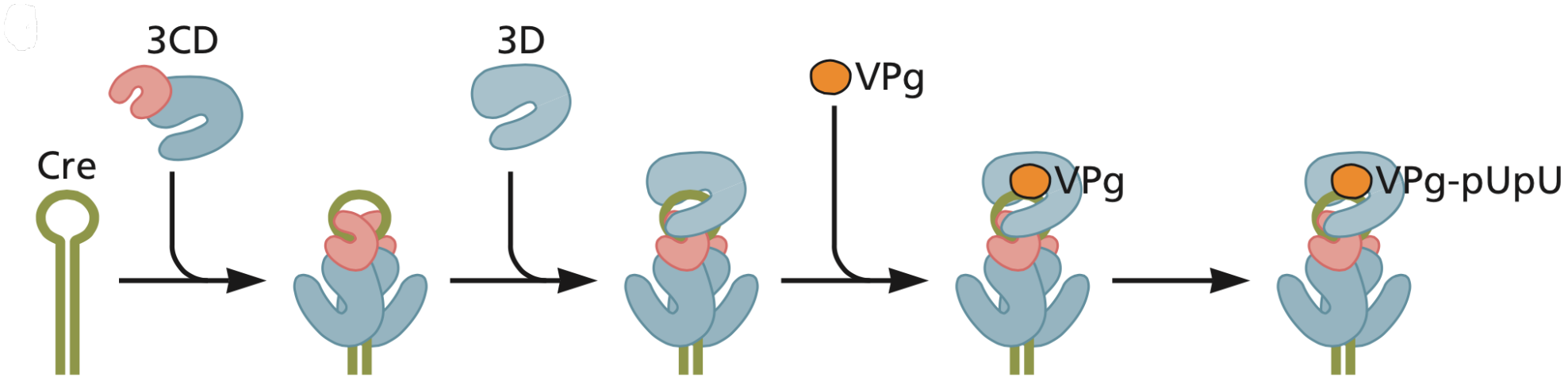

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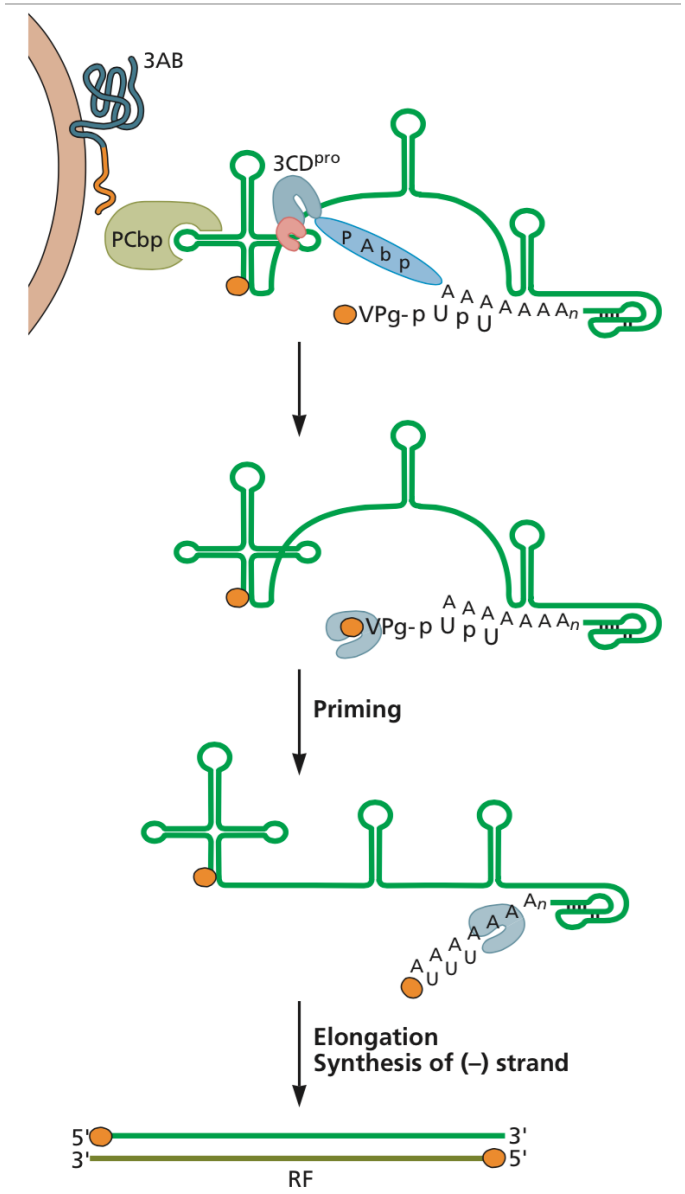

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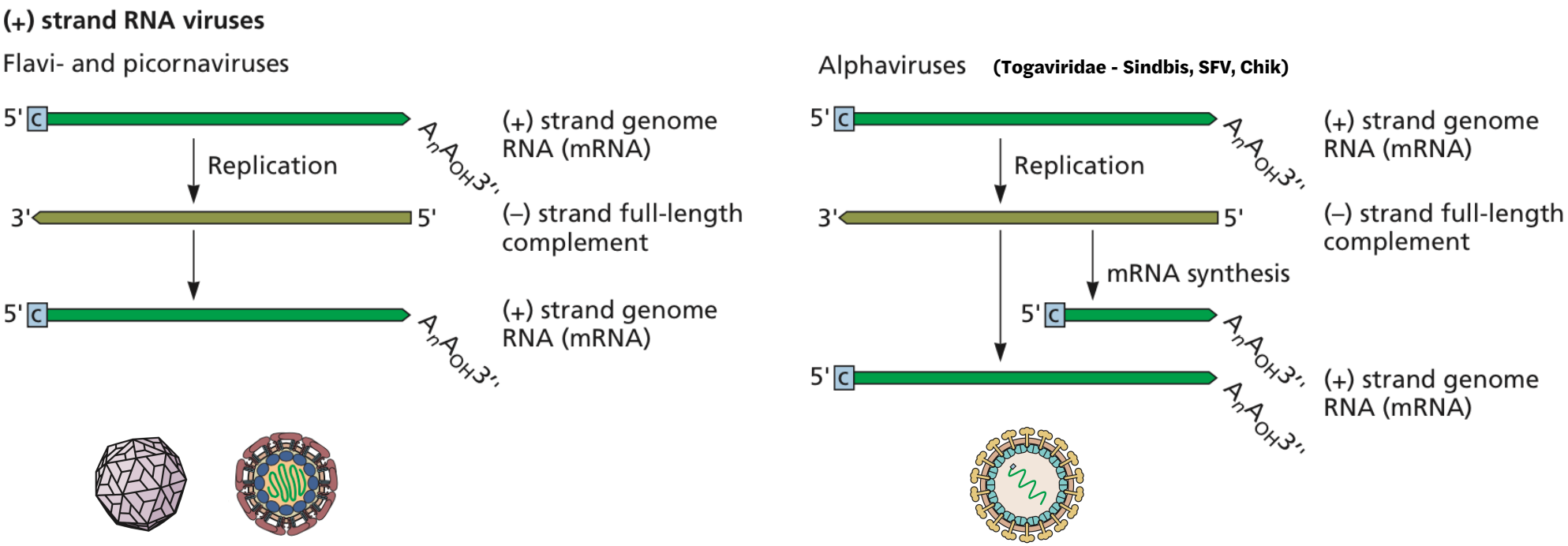
Which is a part of the poliovirus replication strategy?

A. The production of subgenomic mRNAs
B. *De novo* (without primer) initiation of RNA synthesis
C. Circularization of template for initiation of RNA synthesis
D. All of the above

(+) strand RNA viruses



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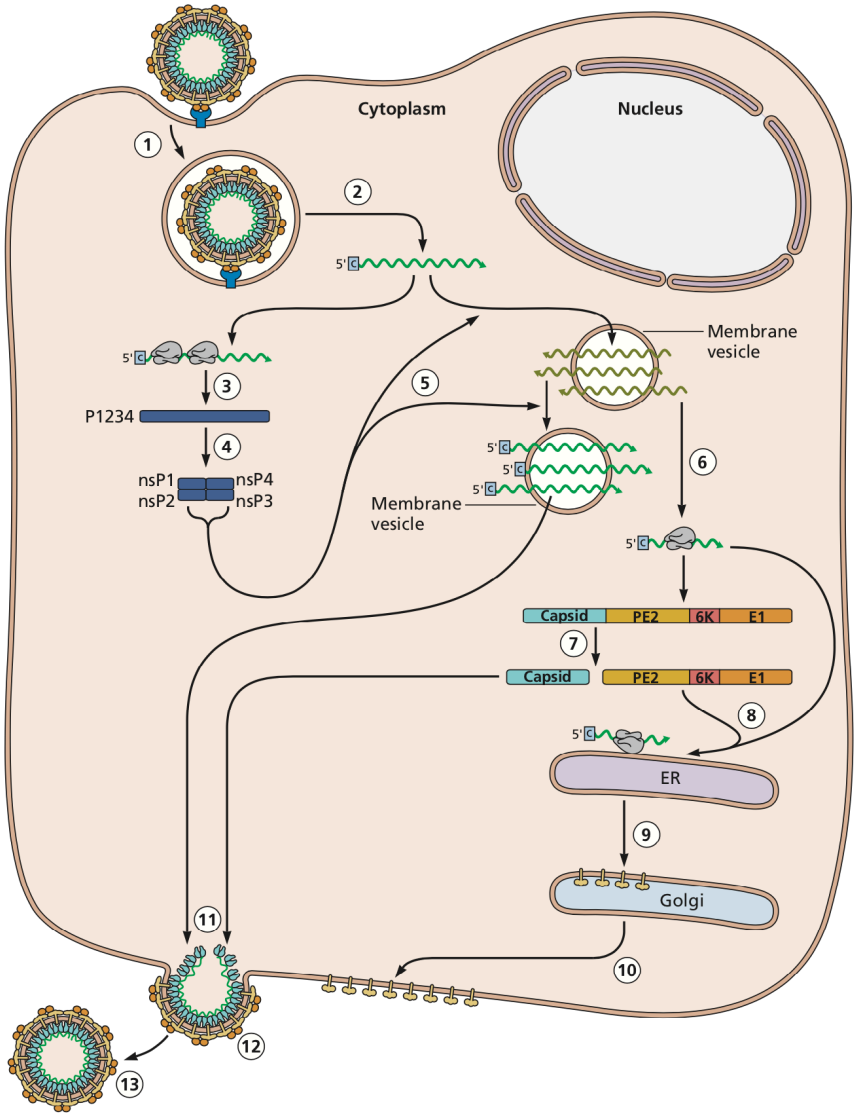
Togaviridae

viral genome = mRNA

But not all of it is translated!



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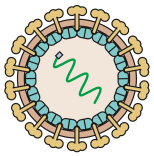

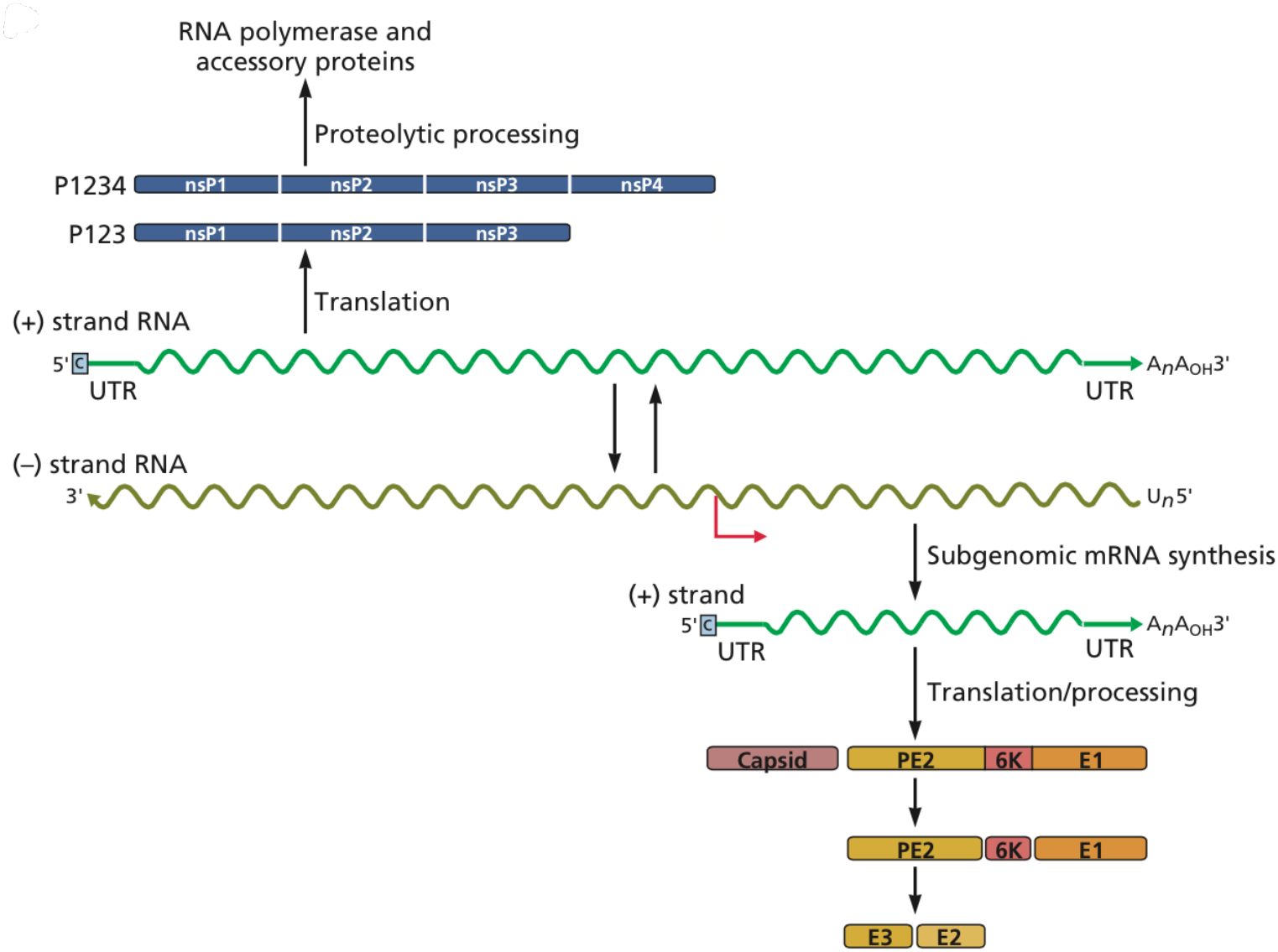

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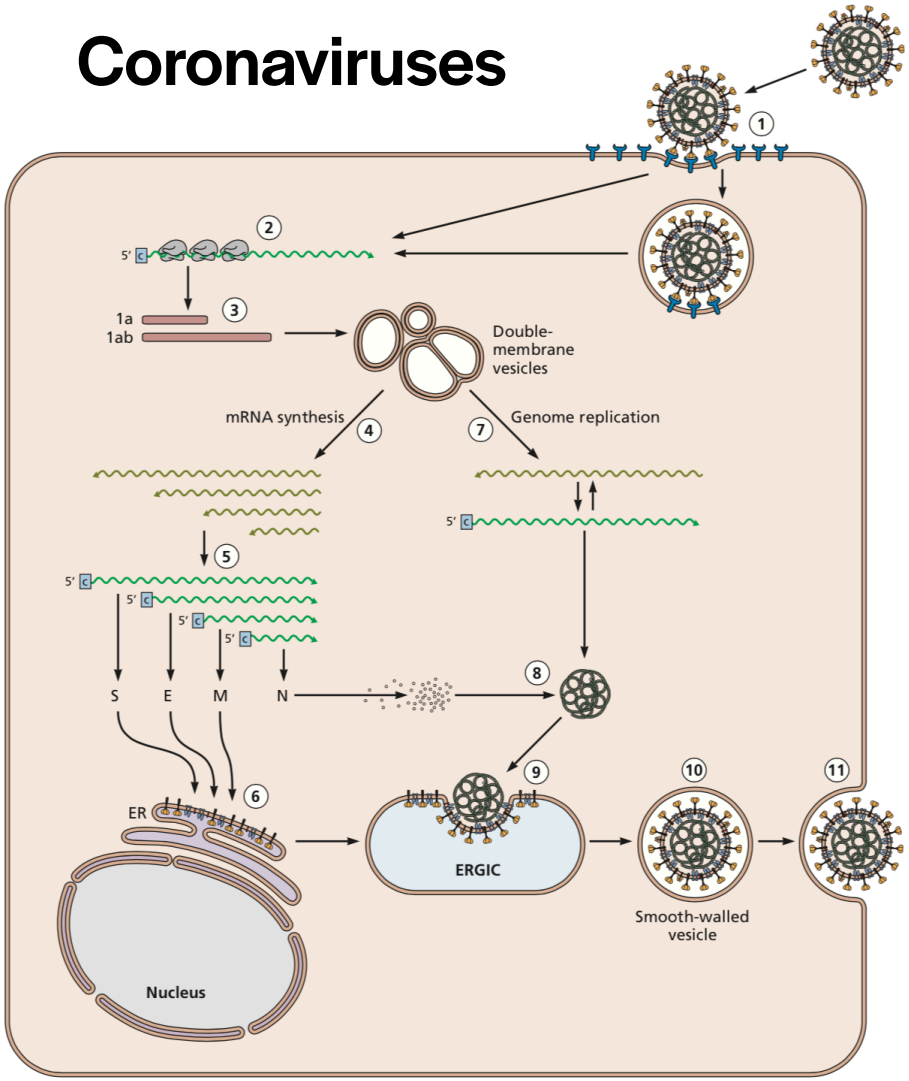
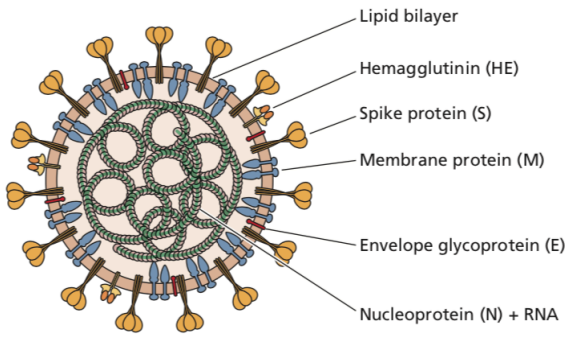
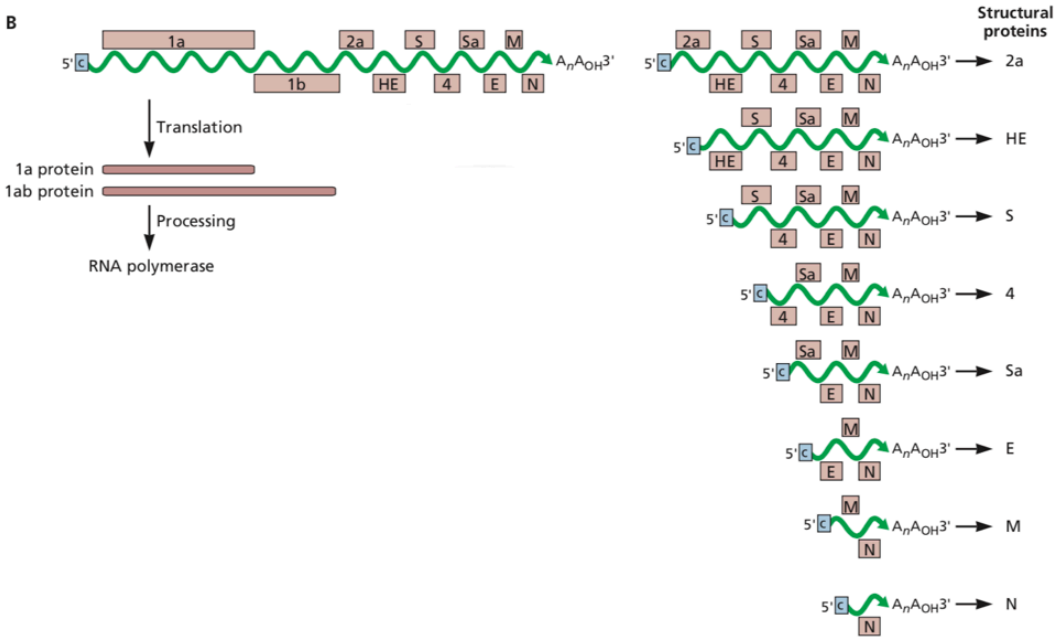
Coronaviruses







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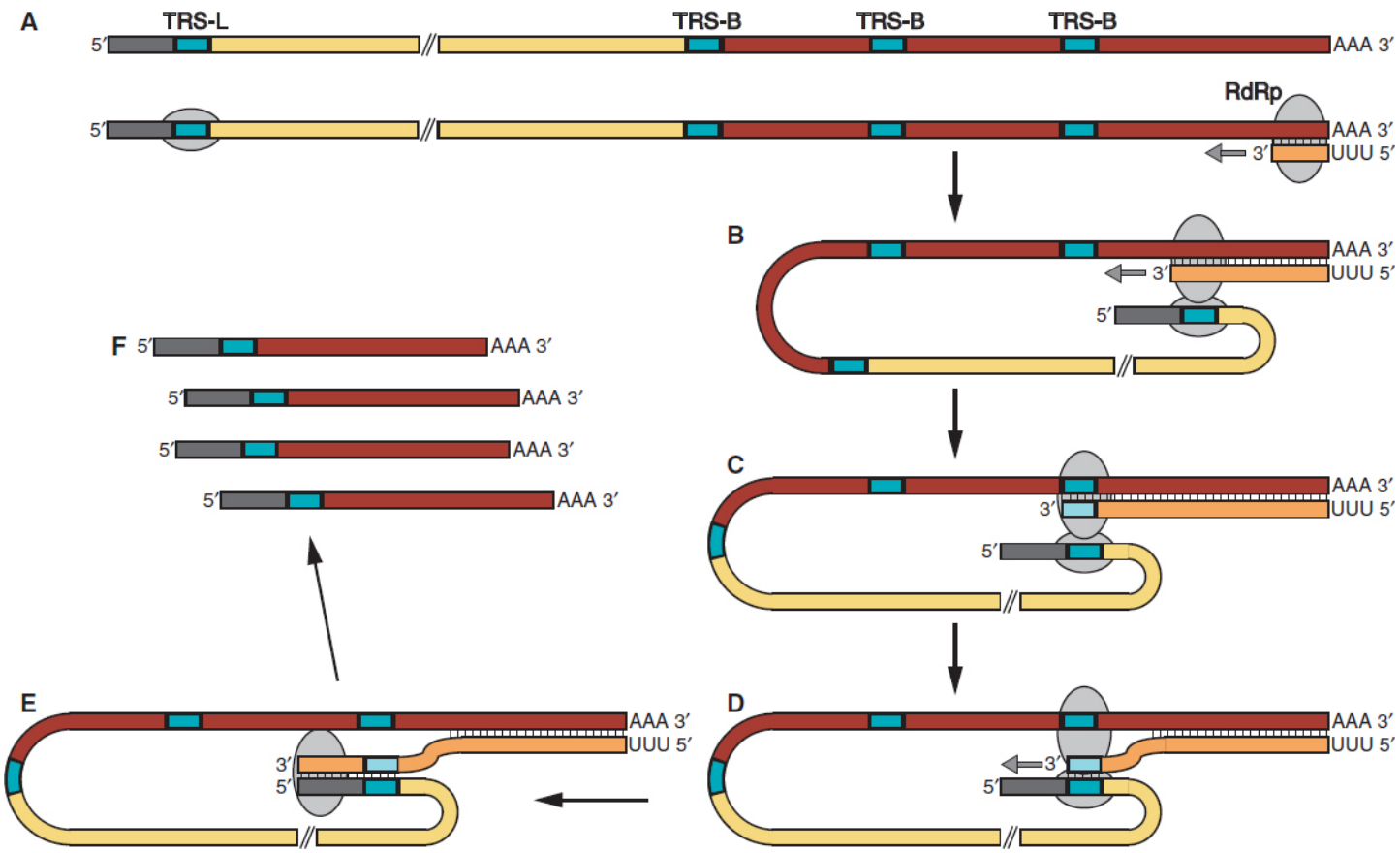
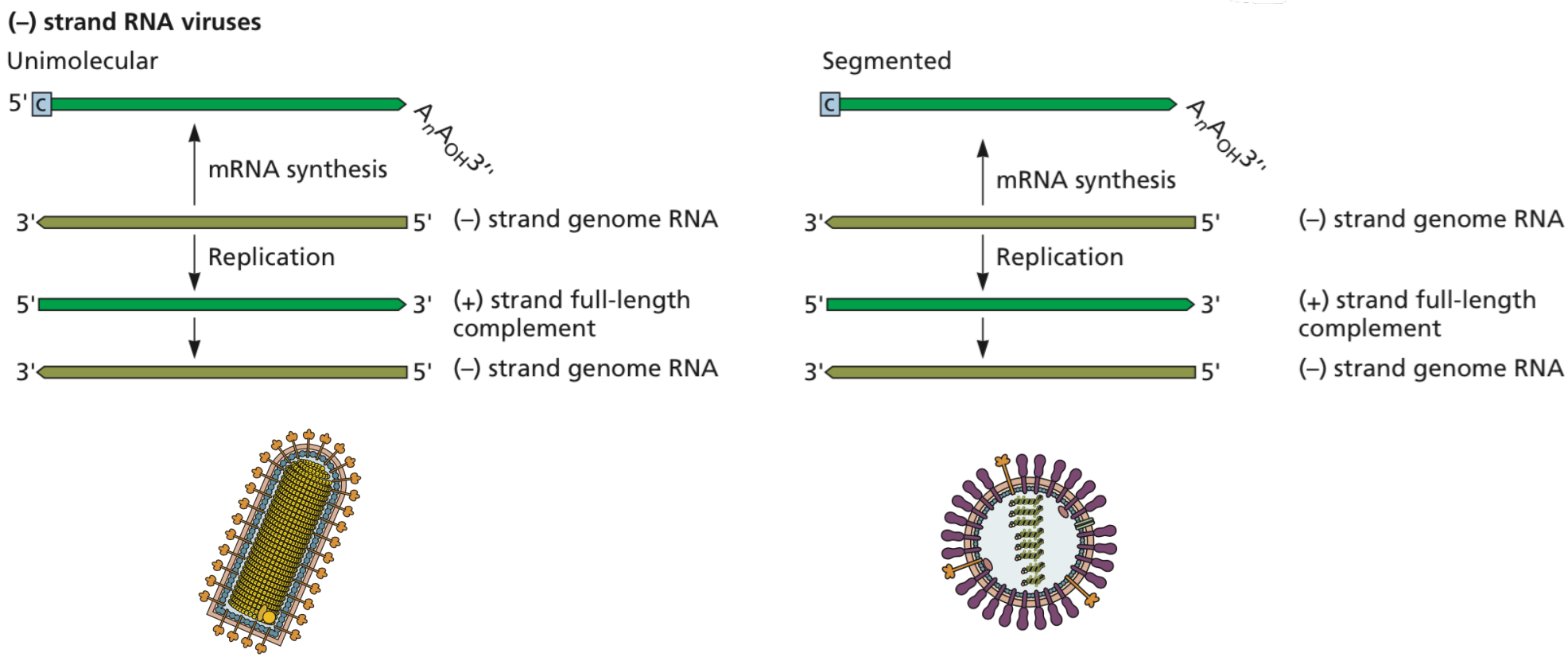
CoV RNA synthesis



This mechanism of mRNA synthesis allows for high rates of recombination among CoVs

(-) Strand RNA viruses



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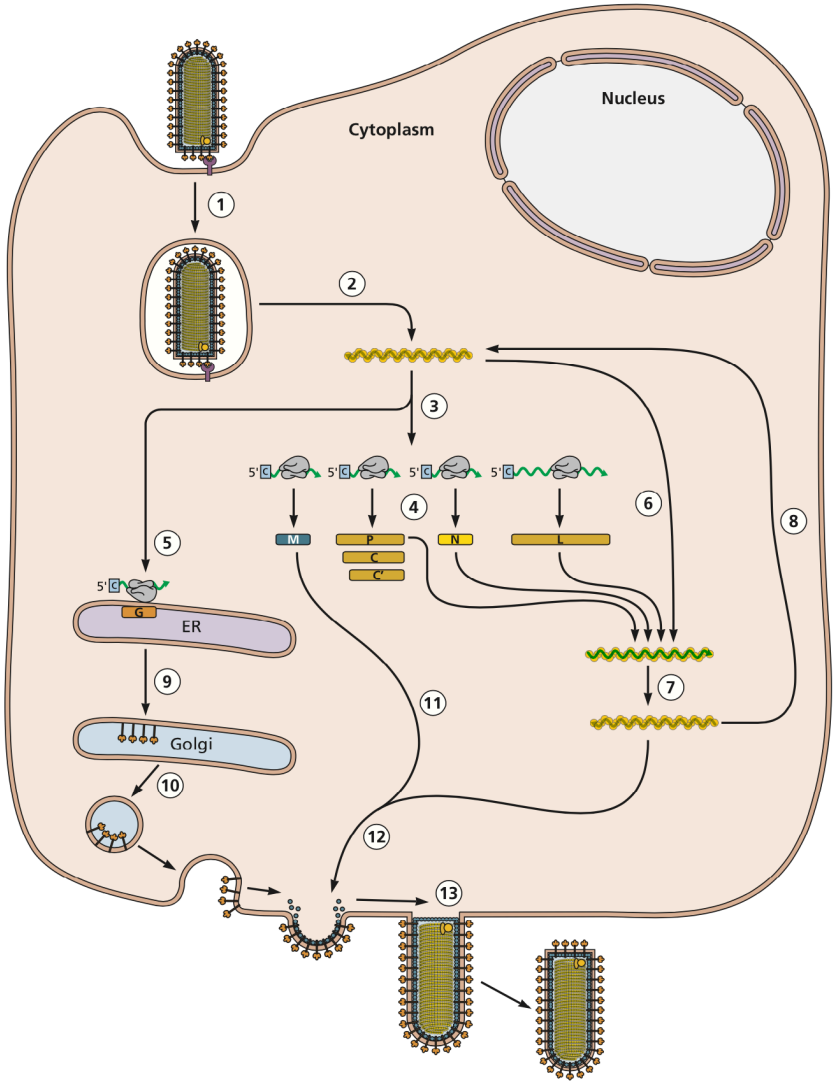
VSV

viral genome is not mRNA



When the viral genome is NOT mRNA, there must be a switch from mRNA to genome RNA synthesis

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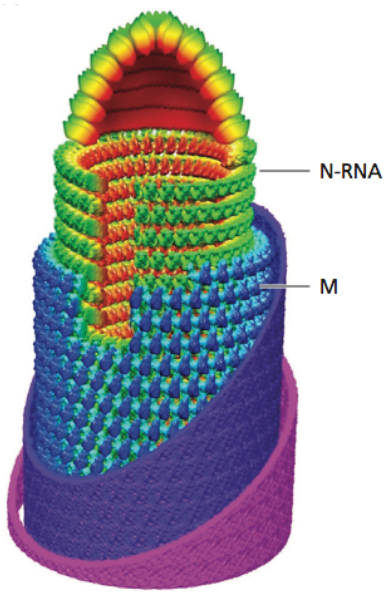
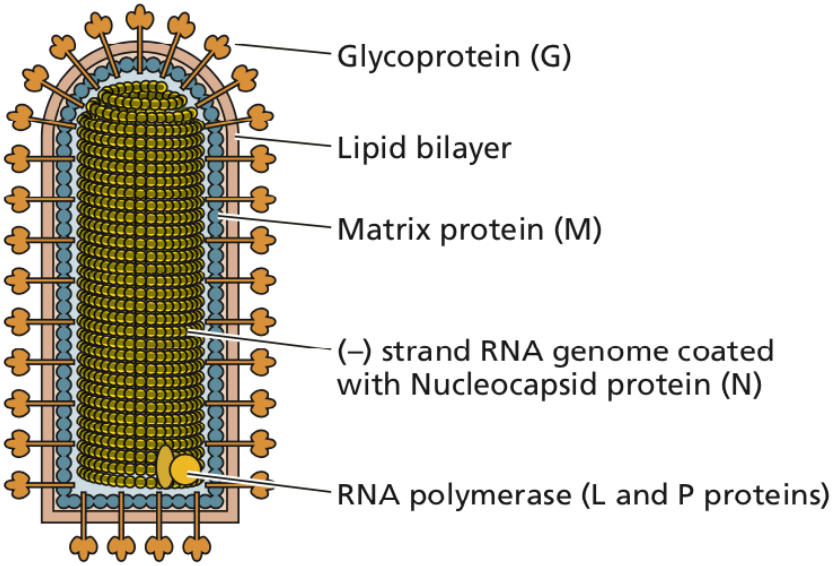
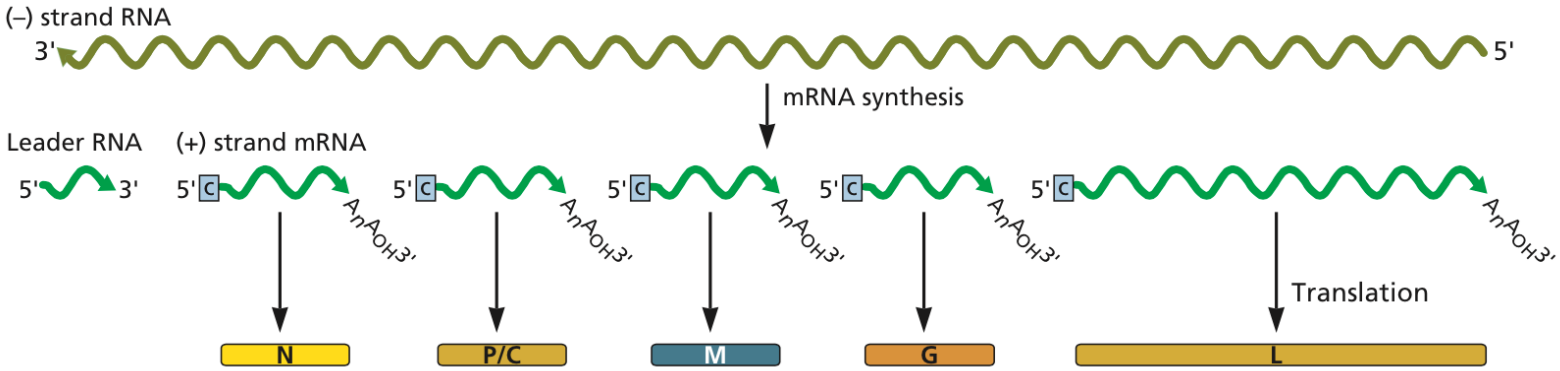




Unimolecular



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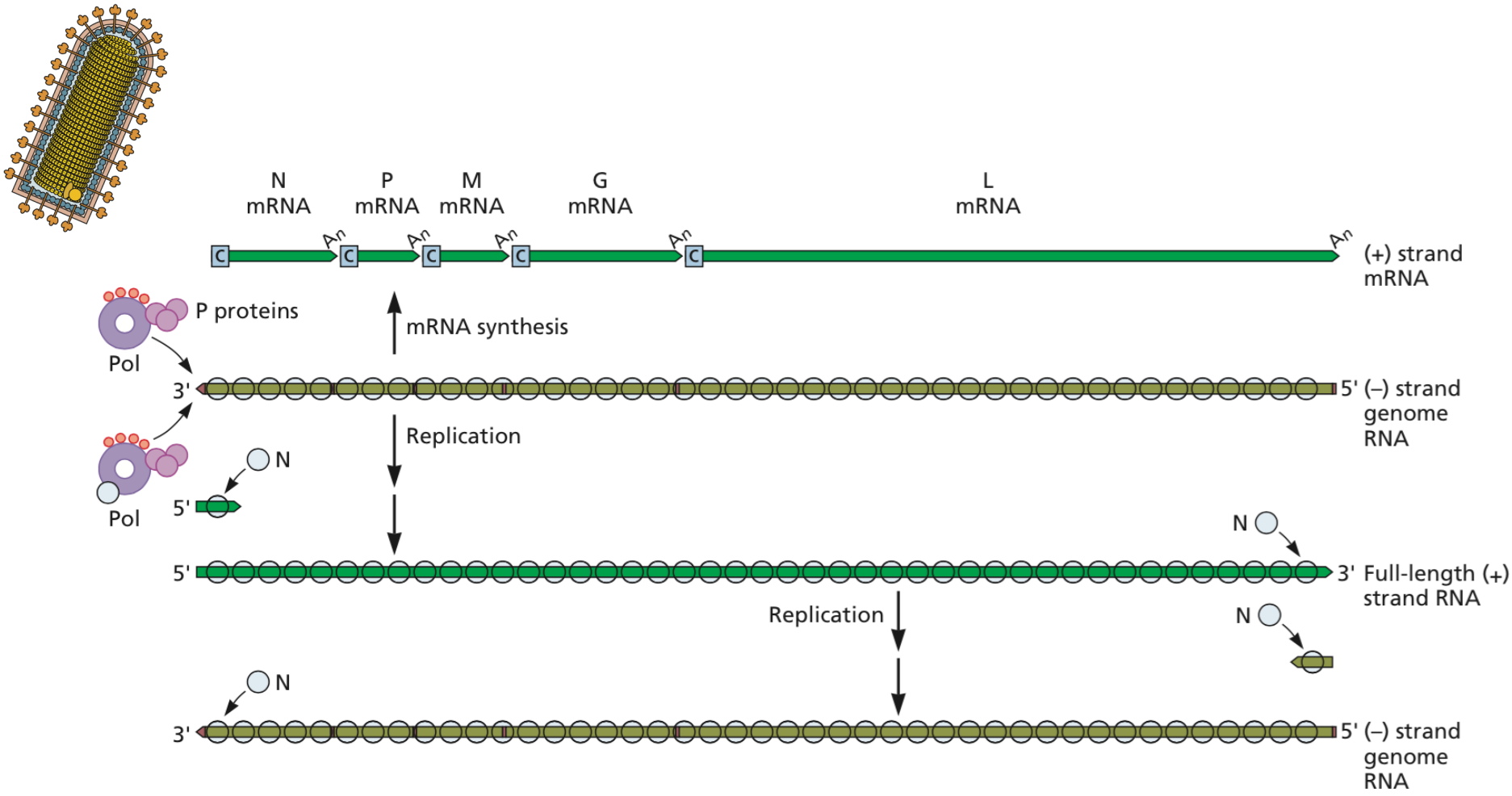

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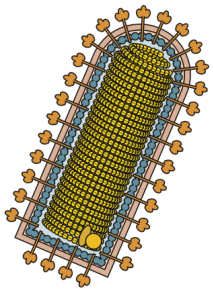
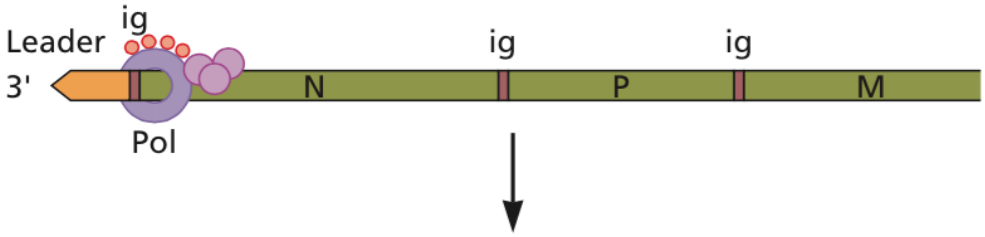
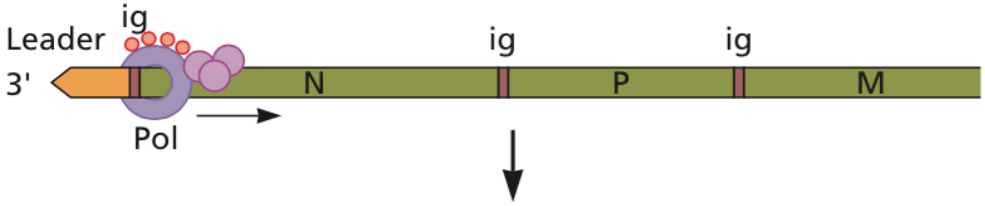


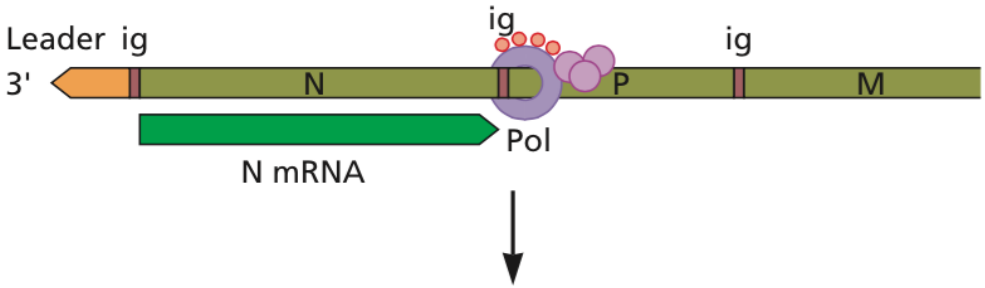
RNA polymerase binds at 3' end of N gene



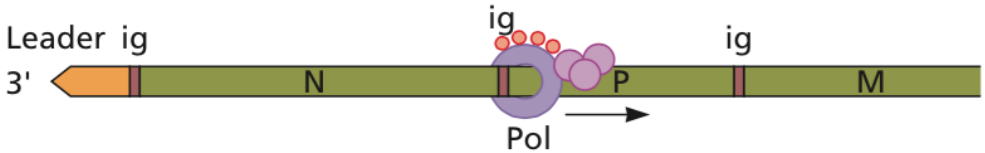
Initiation of mRNA synthesis at 3' end of N gene



Synthesize N mRNA and terminate at intergenic region (ig)



Reinitiate at 3' end of P gene



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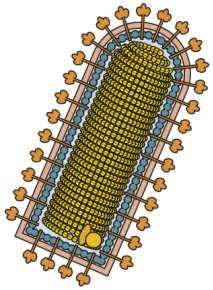

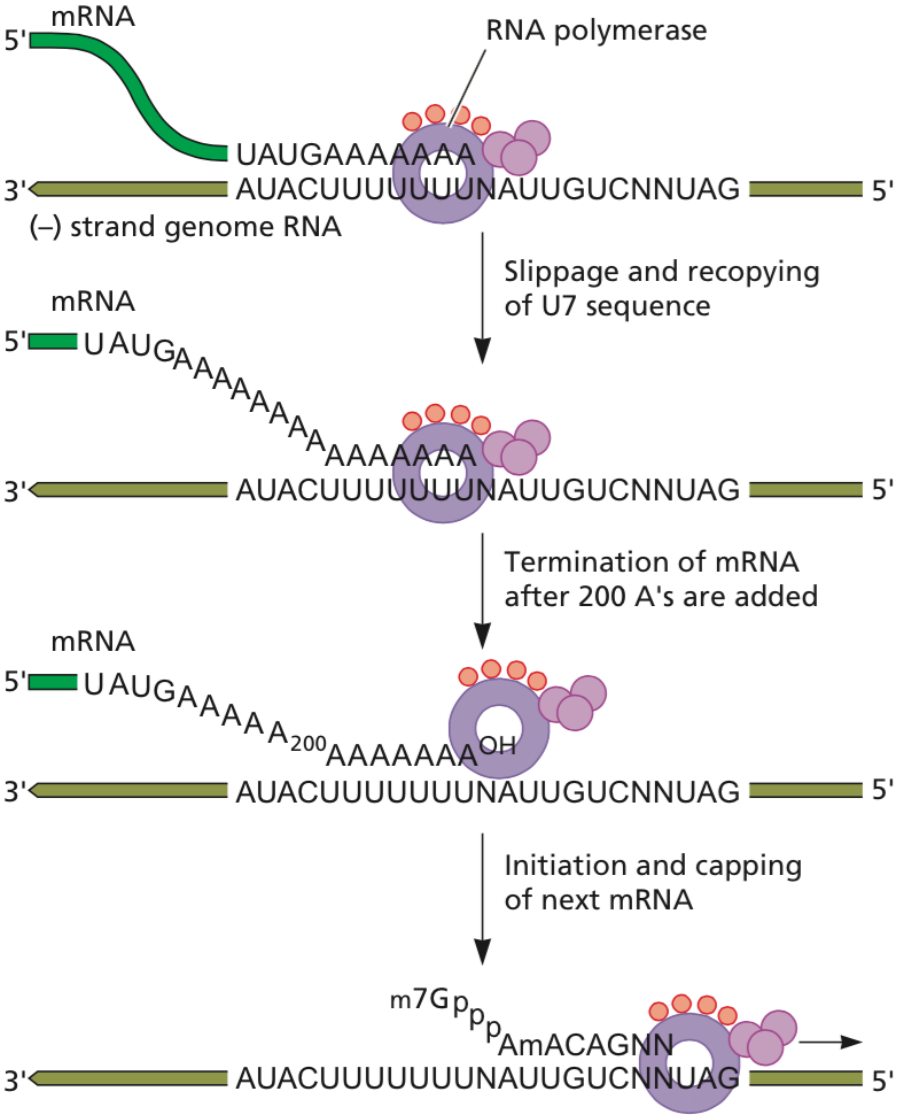

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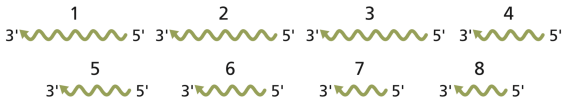
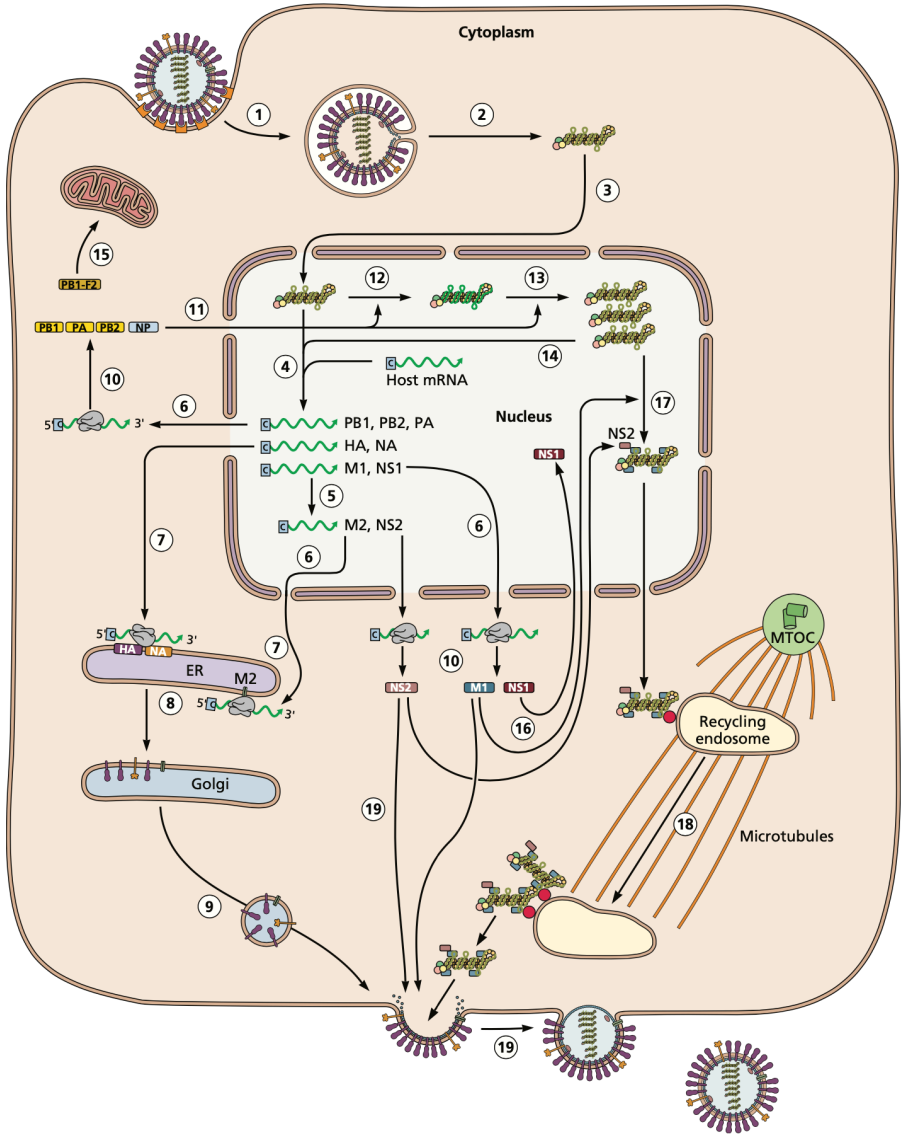
Influenza virus
viral genome is not mRNA





When the viral genome is NOT mRNA, there must be a switch from mRNA to genome RNA synthesis

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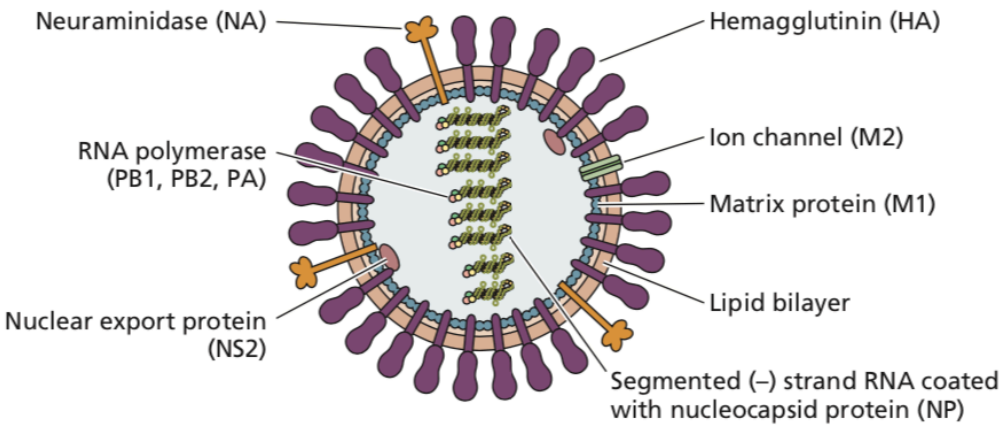

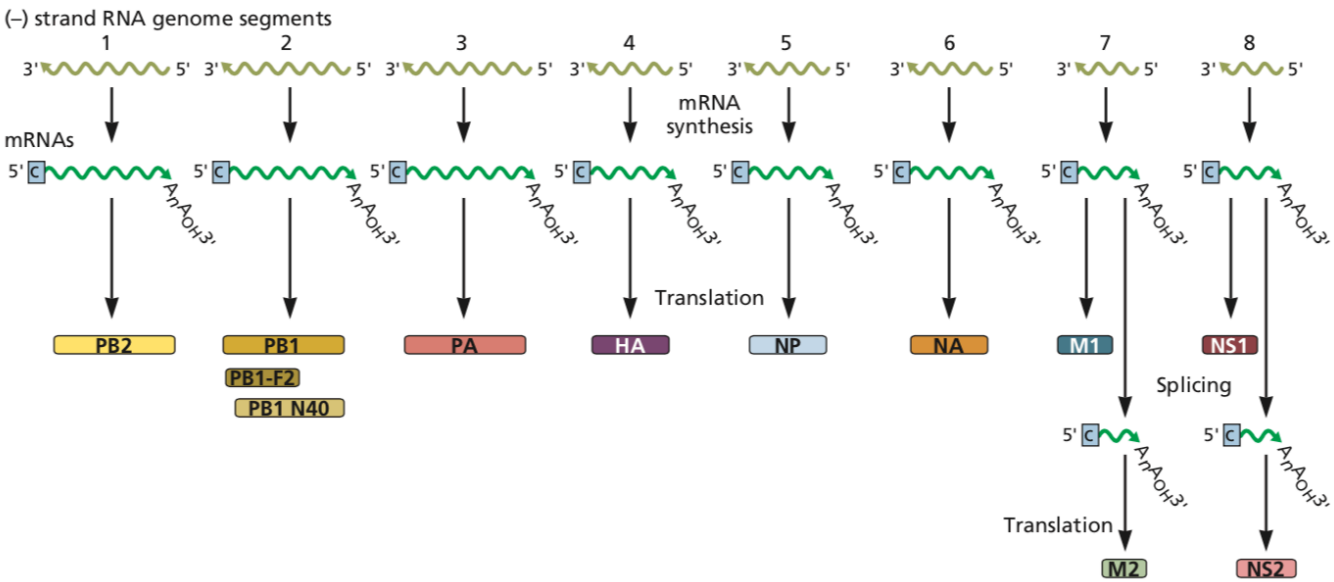

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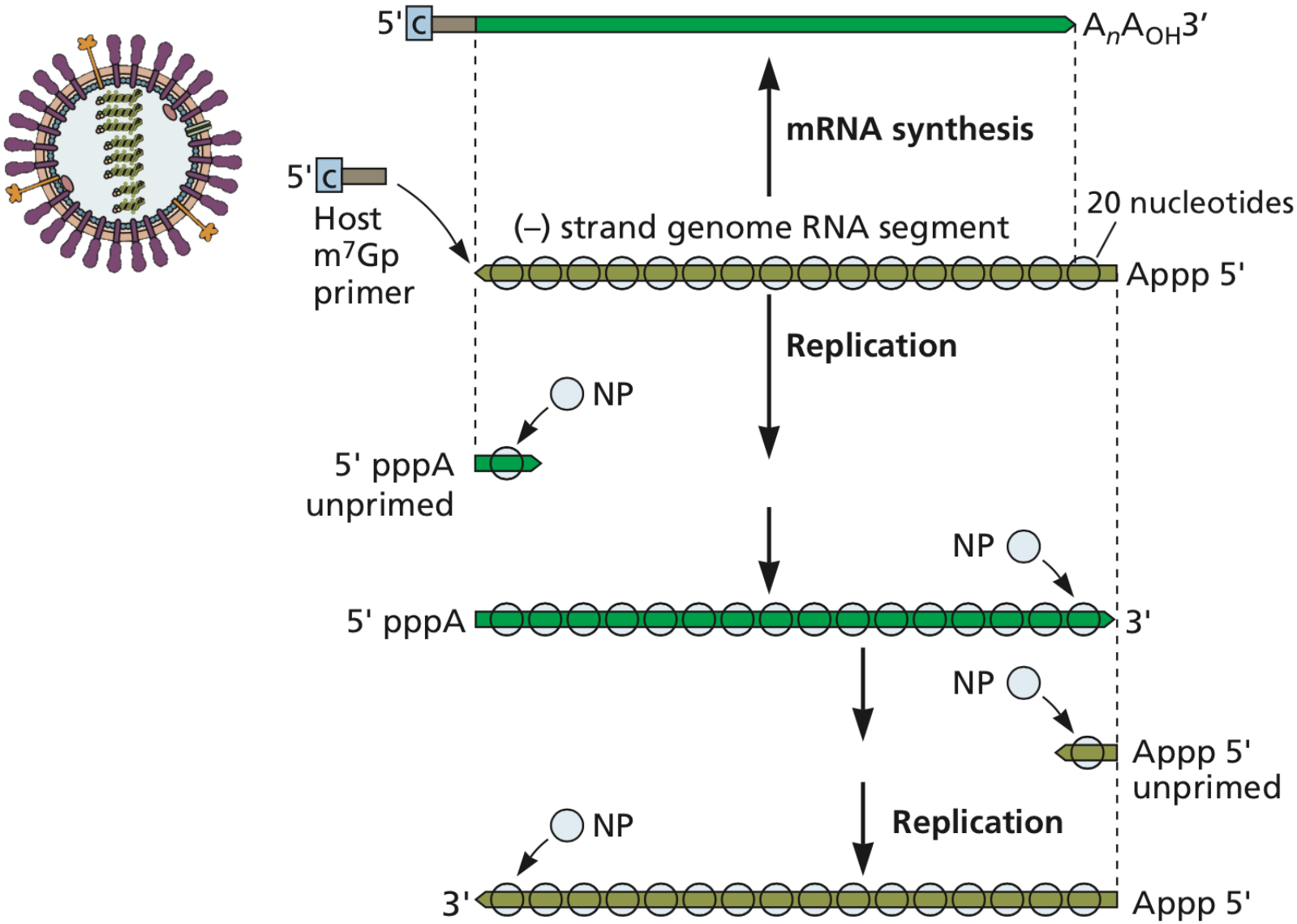

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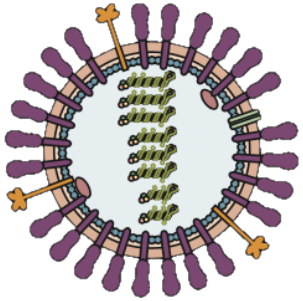

$m^7Gpppm^6AmpC(m)pAp\ldots\ldots UpUpG\,pApCp\ldots$

Cleavage 13

(–) strand RNA

UpCpCpUpUpUpUpCp…

Initiation

$m^7Gpppm^6AmpC(m)pAp\ldots\ldots UpUpG$

13 p p pG

Up CpCpUpUpUpUpCp…

Elongation

$m^7Gpppm^6AmpC(m)pAp\ldots\ldots UpUpG\,pGPCpApApApApGp\ldots$

13

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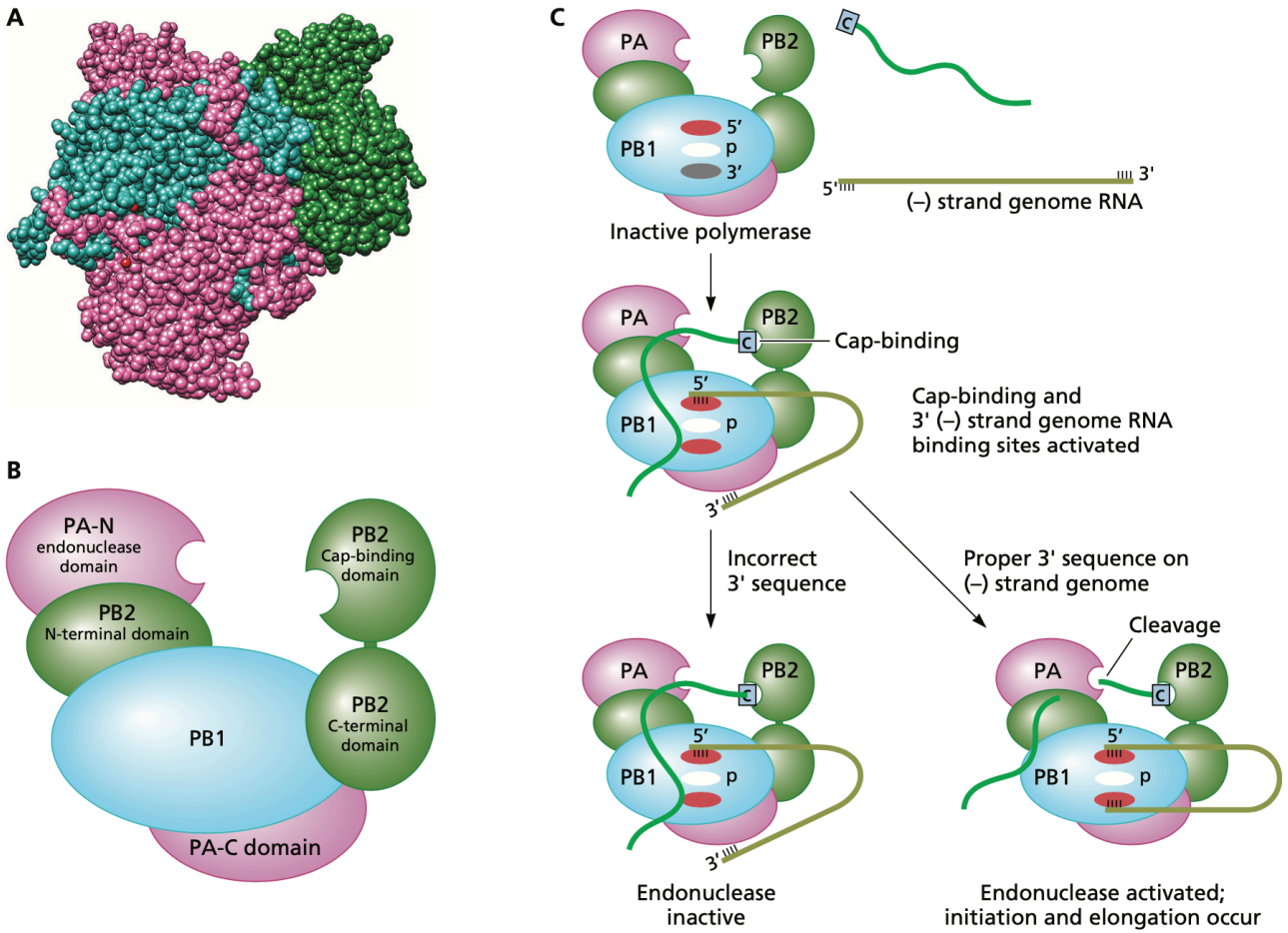
Activation of influenza virus RNA polymerase



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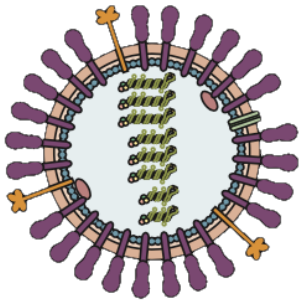

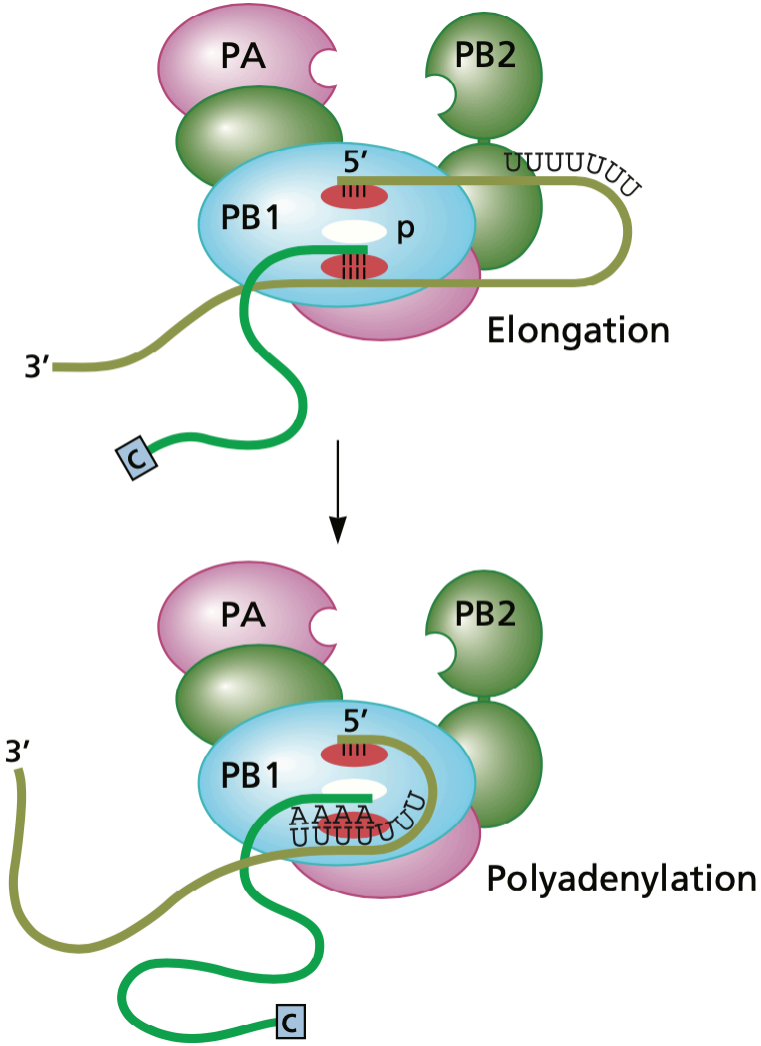

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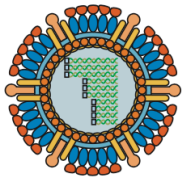
Go to:

b.socrative.com/login/student
room number: virus

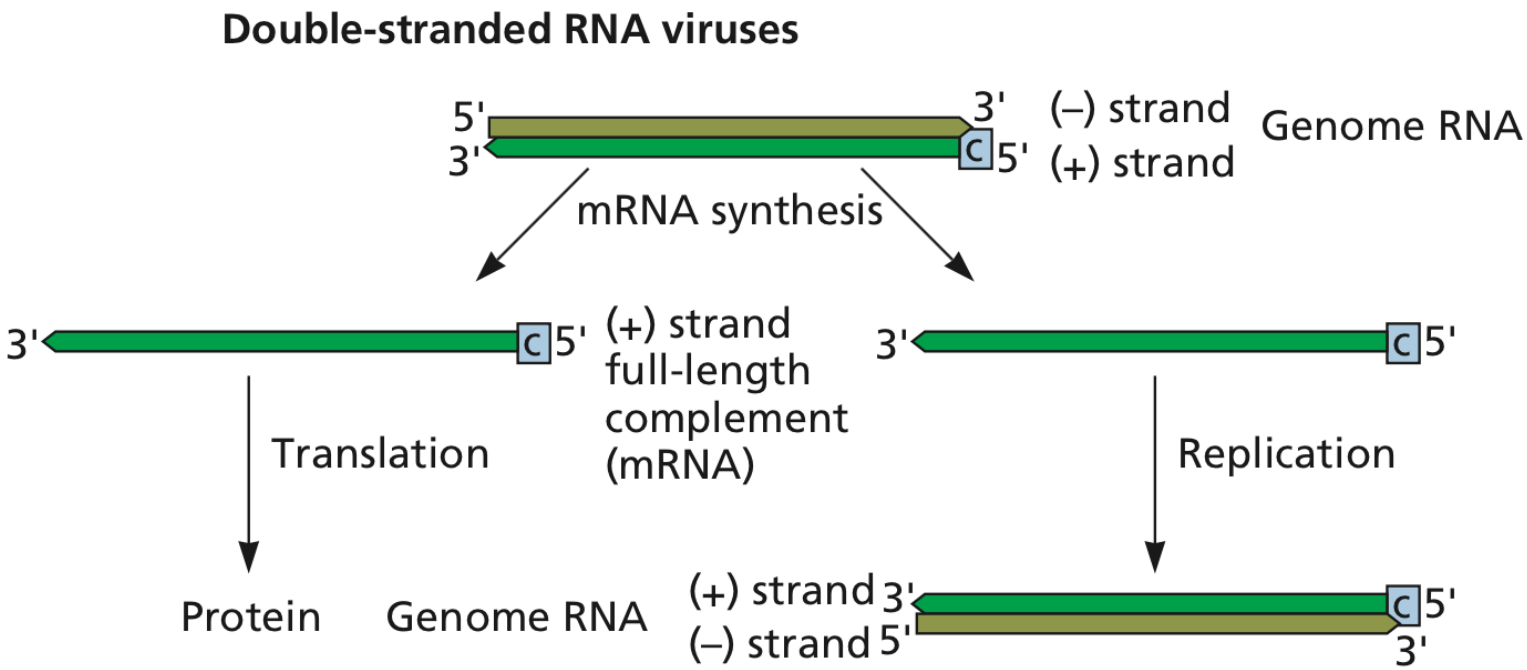
How are influenza virus and VSV RNA synthesis similar?

A. The switch from mRNA to genome RNA synthesis is controlled by an RNA binding protein
B. Polyadenylation occurs at a short stretch of U residues
C. Viral mRNAs are shorter than (-) genome RNA
D. All of the above



dsRNA viruses

Reoviridae: reovirus, rotavirus



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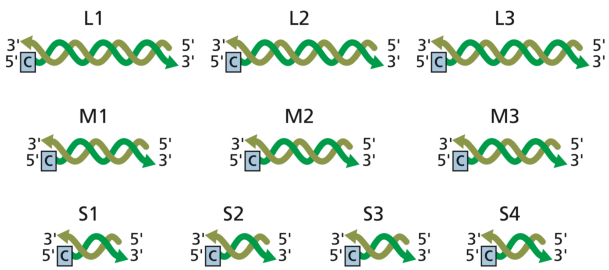
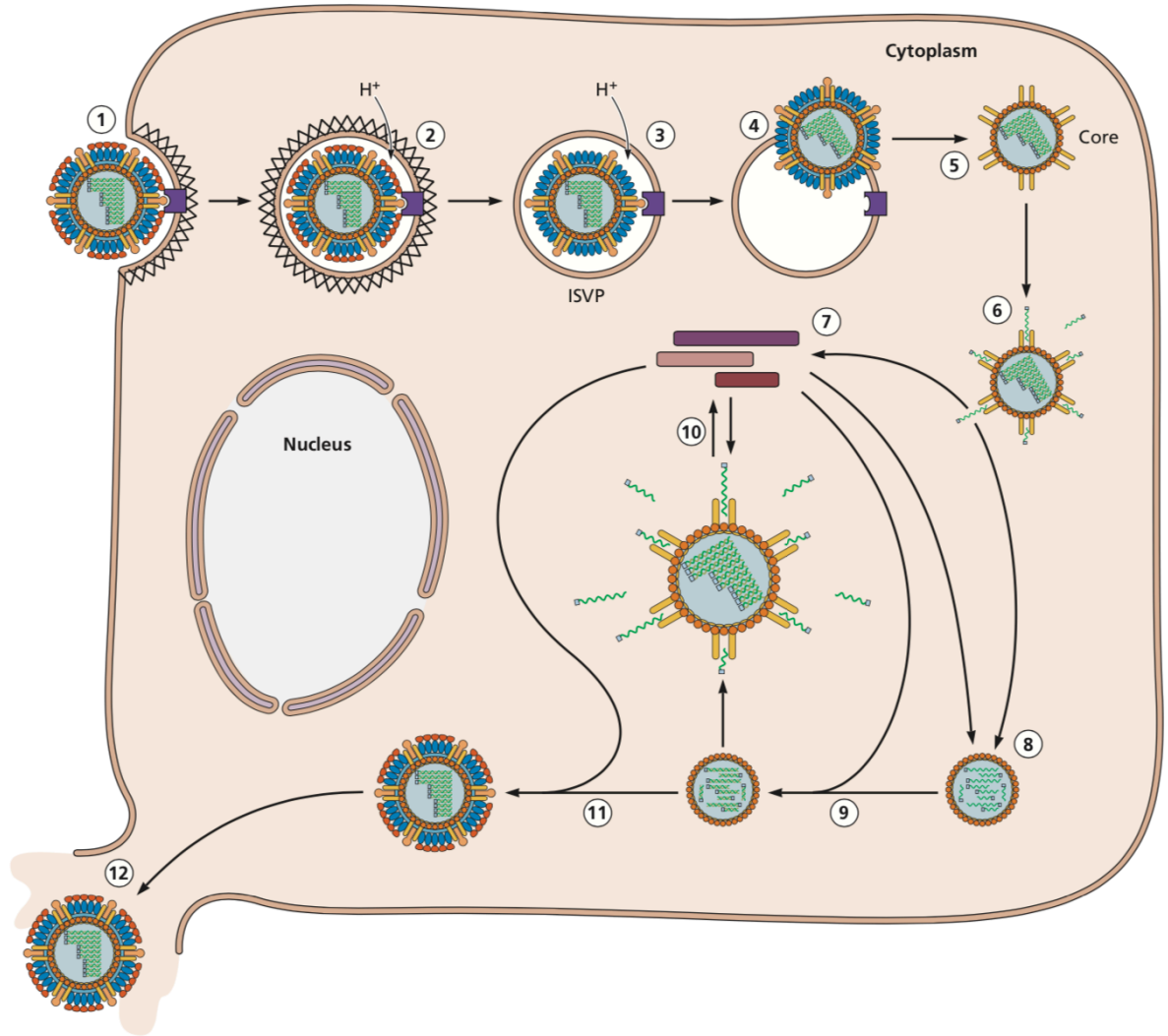
Reovirus
(+) strand not accessible by ribosomes!





The viral genome is not mRNA
Where is the switch to genome synthesis?

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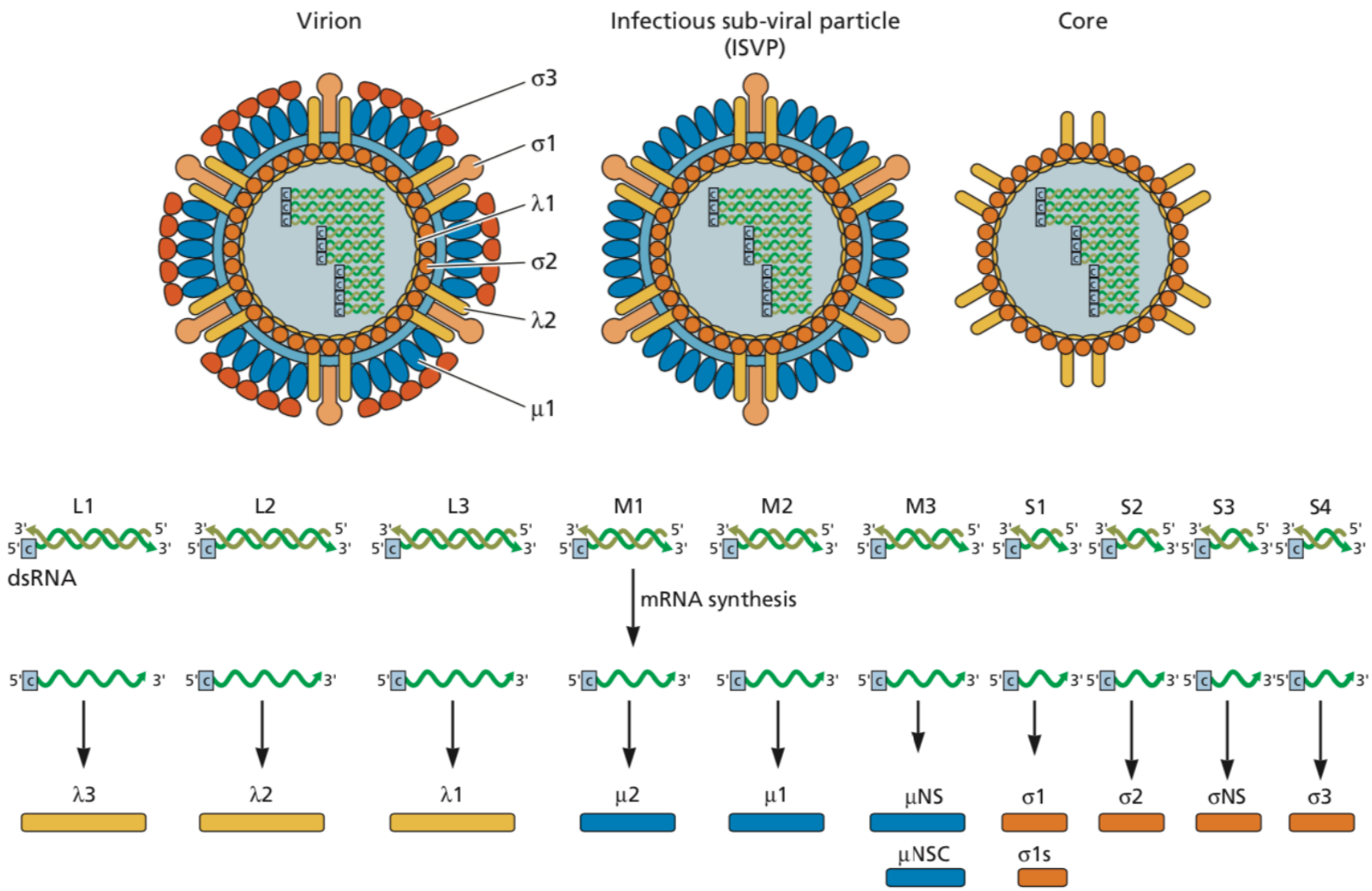

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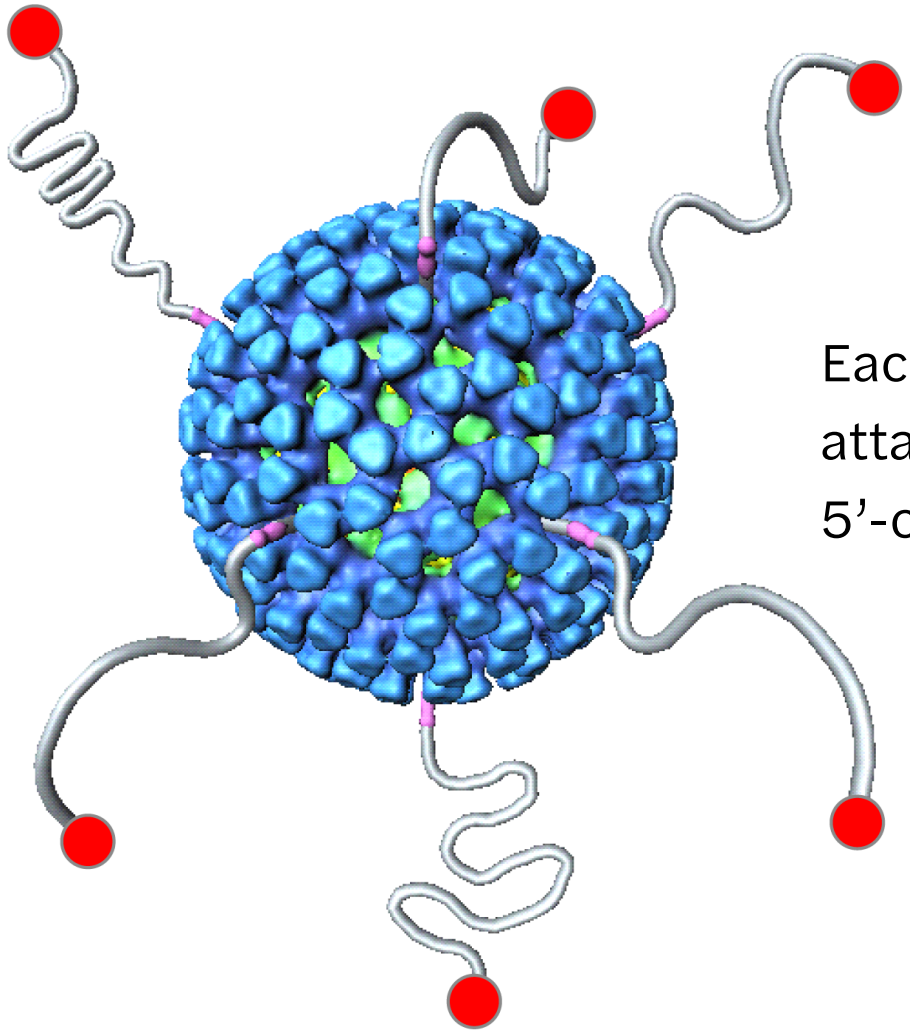
Release of mRNA from rotavirus particles



Each dsRNA segment is attached to RdRp via the 5’-cap

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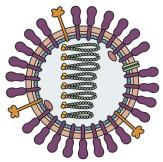
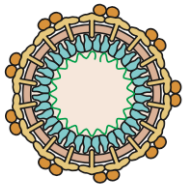
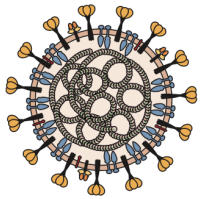
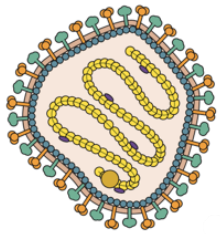
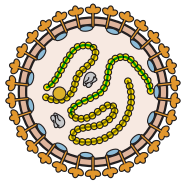
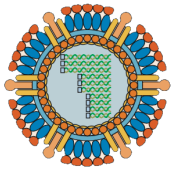
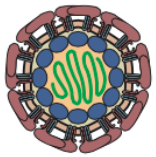
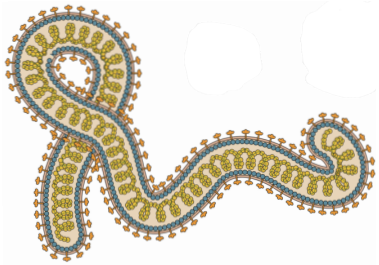
Origins of diversity among RNA viruses

- Misincorporation of nucleotides
  - Lack of proofreading activity in RNA dependent RNA polymerase: high error frequencies (1 misincorporation / 10^3 - 10^4 nt polymerized)
  - Average error frequency: 1 in 10^4 or 10^5 nucleotides polymerized
  - In a 10 kb RNA virus genome, a mutation frequency of 1 in 10^4 results in about 1 mutation per genome



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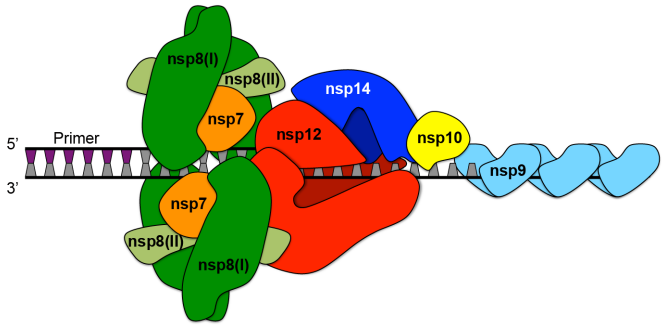
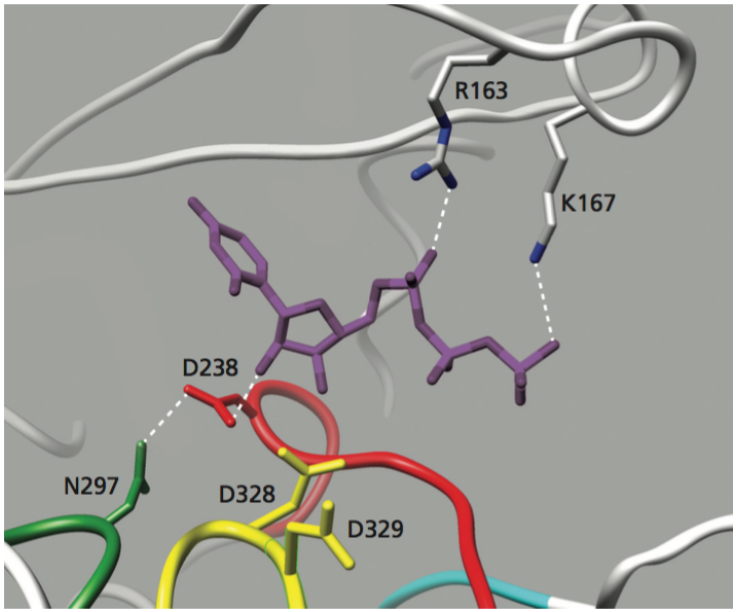
Nidovirales are an exception

- exoN protein is a 3’-5’ exonuclease that corrects RNA polymerase errors
- Removal of exoN produces a virus with 15-20 fold increase in mutation rate
- ExoN may allow faithful replication of large (up to 41 kb) RNA genomes



Major fidelity checkpoint for poliovirus RdRp

- Determined by how template, primer, NTP interact at active site
- NTP first binds in a way that does not allow ribose to interact with Asp-Asn amino acids
- If NTP is correctly base paired, conformational change in enzyme occurs which reorients triphosphate, allows phosphoryltransfer to occur



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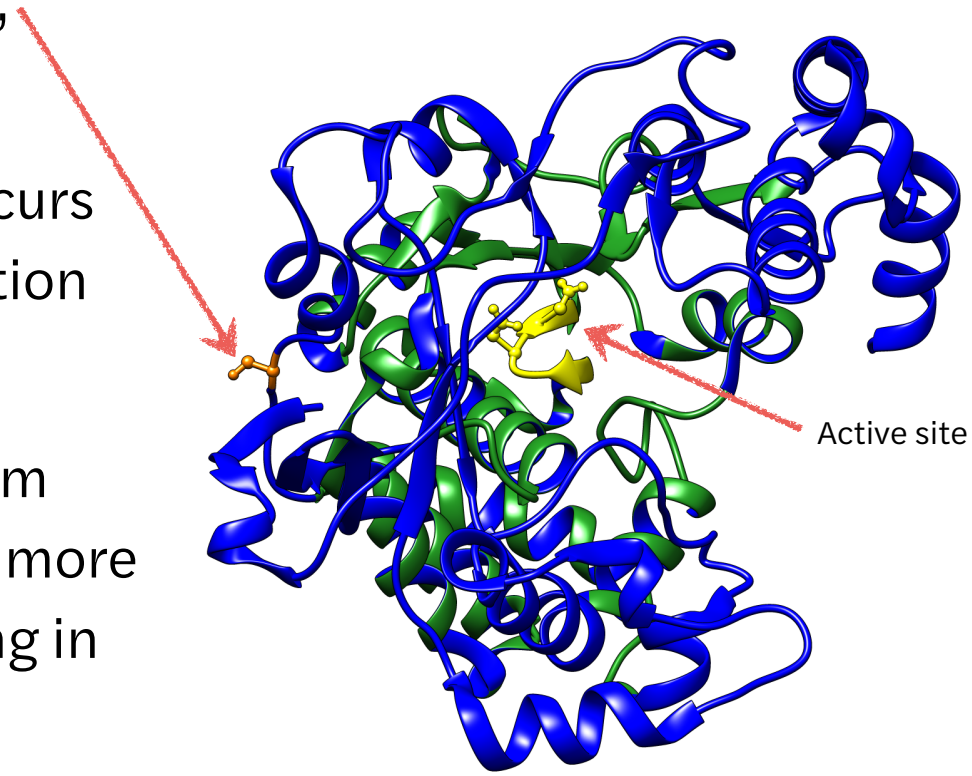
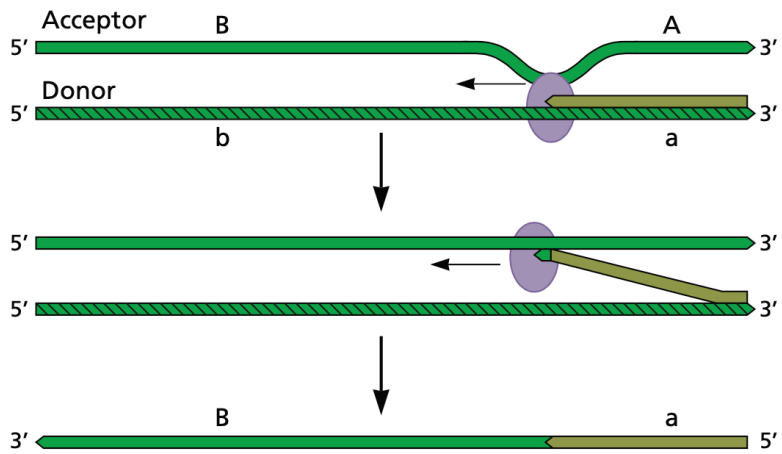
Fidelity control by poliovirus RdRp

- $3D^{pol}$ single amino acid change, G64S, makes fewer errors
- Slows conformational change that occurs on NTP base pairing, reducing elongation rate
- AA 64 is in fingers domain, remote from active site but change makes enzyme more dependent on correct NTP base pairing in active site, increasing fidelity
- Mechanism likely conserved among RdRps



RNA recombination



- Exchange of nucleotide sequences among different genomic RNA molecules (distinct from reassortment)
- Shapes RNA virus world by rearranging genomes and creating new one
- Can be relatively high: 10-20% of poliovirus RNA molecules recombine in a single replication cycle

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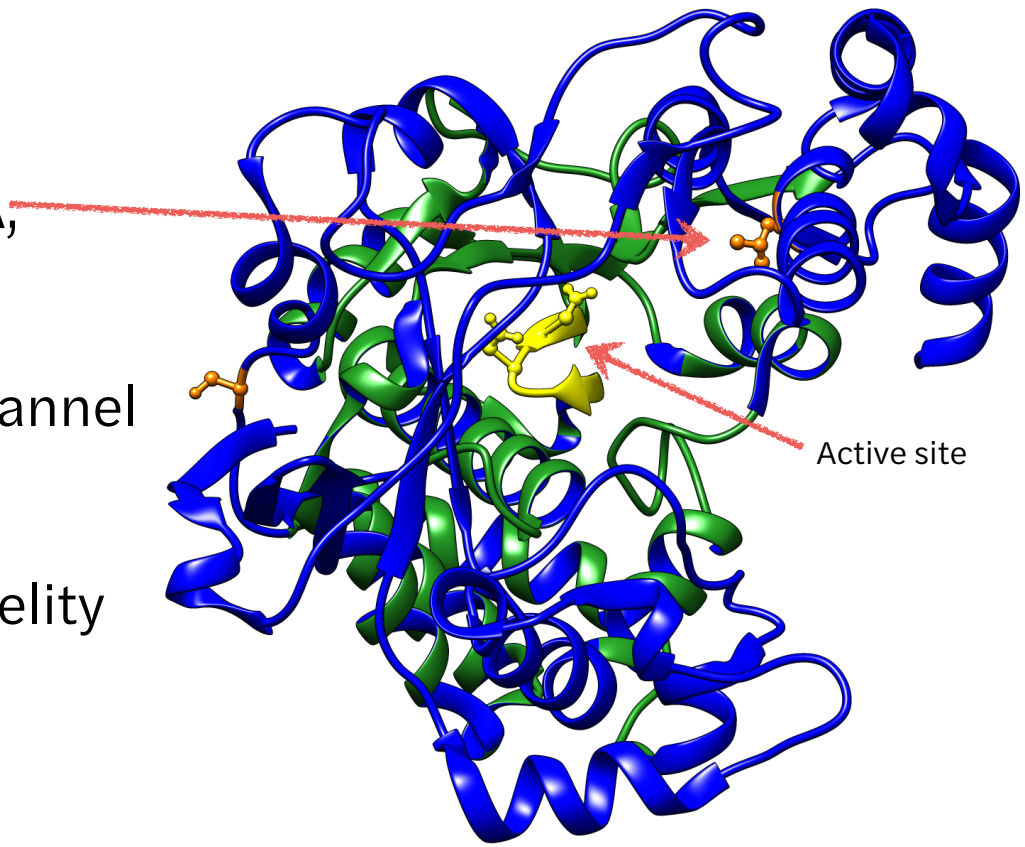
Recombination control by poliovirus RdRp

- $3D^{pol}$ single amino acid change, L420A, reduced recombination frequency
- Located in thumb domain, RNA exit channel
- Reduces initiation rate and stability of elongation complexes, no affect on fidelity



Next time: Transcription and RNA processing