RNA directed RNA synthesis

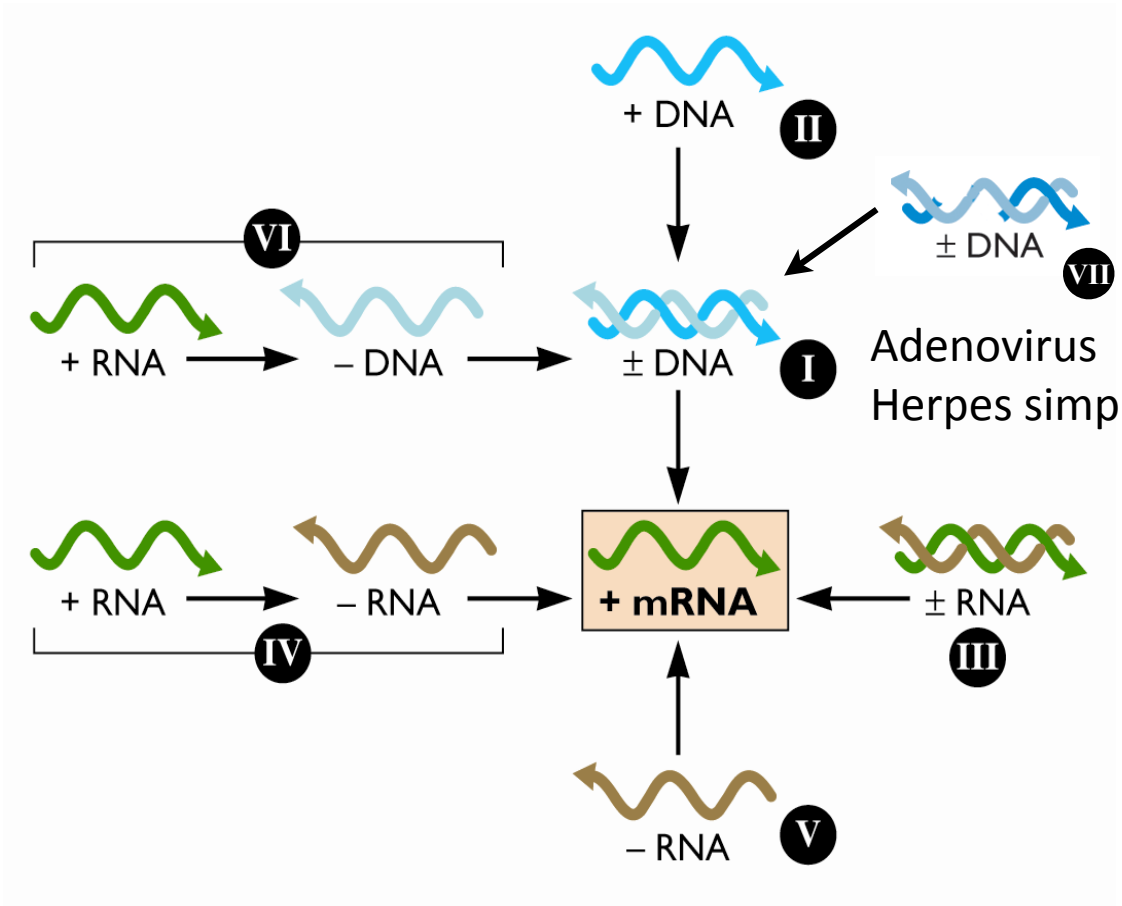
Lecture 6
Biology W3310/4310
Virology
Spring 2016

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 - TMV nucleic acid is infectious; first demonstration that RNA can be genetic material
- By 1959, RNA was identified in many animal viruses
- 1960s - studies on viral RNA replication begin

Parvovirus



Hepatitis B virus

Retrovirus

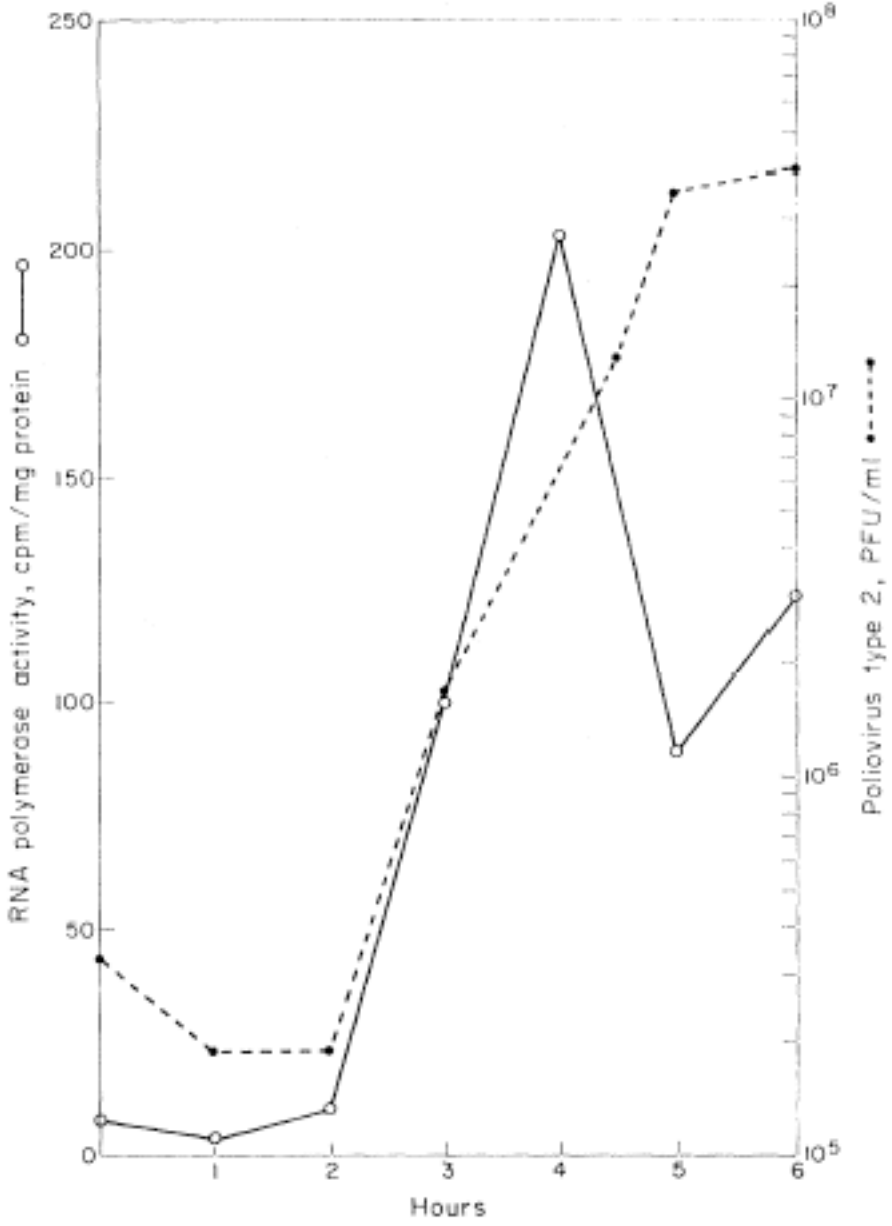
Adenovirus
Herpes simplex virus

Poliovirus

Reovirus
Rotavirus

Influenza virus
VSV

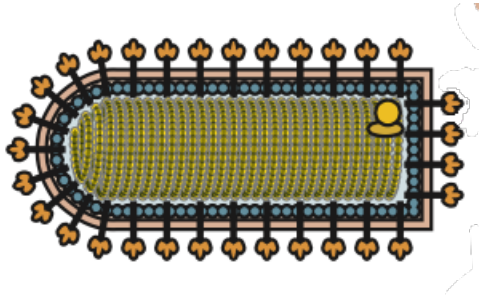
Identification of RNA polymerases



Assays: cell extracts incubated with NTPs

Identification of RNA polymerases

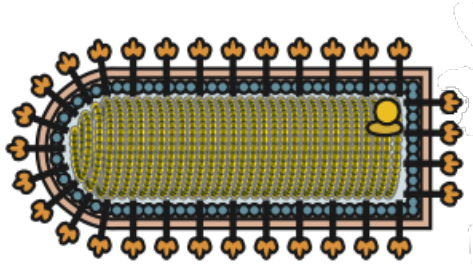
- Polymerase discovered in (-) strand virus particles

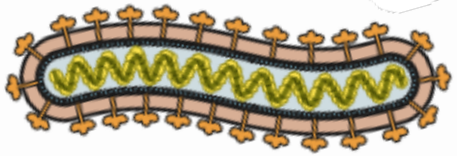


- Sequence alignments (GDD), synthesis of recombinant proteins
- Crystal structures

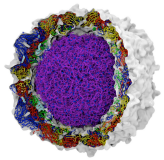
RNA in the virus particle

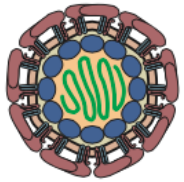
- (-) strand RNA genomes: coated with protein

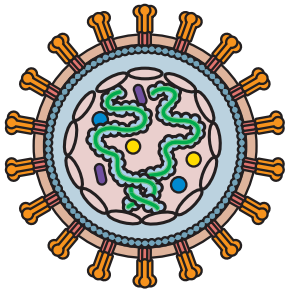


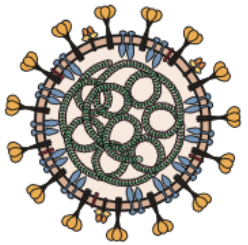


- (+) strand RNA genomes: naked (exceptions: retrovirus, coronavirus)

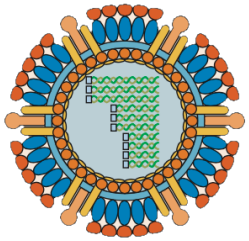


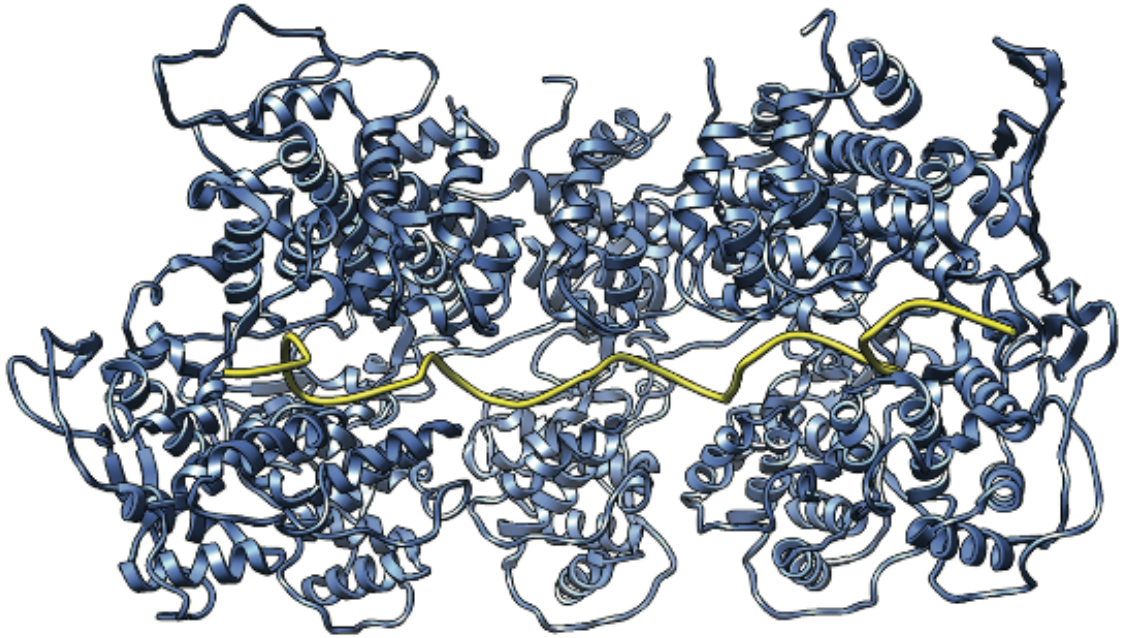


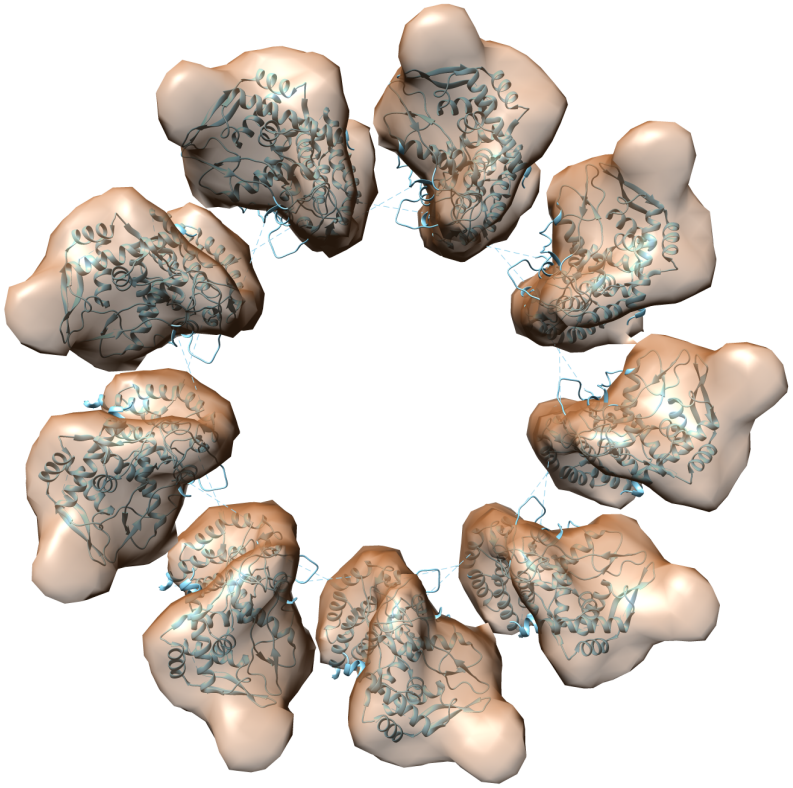


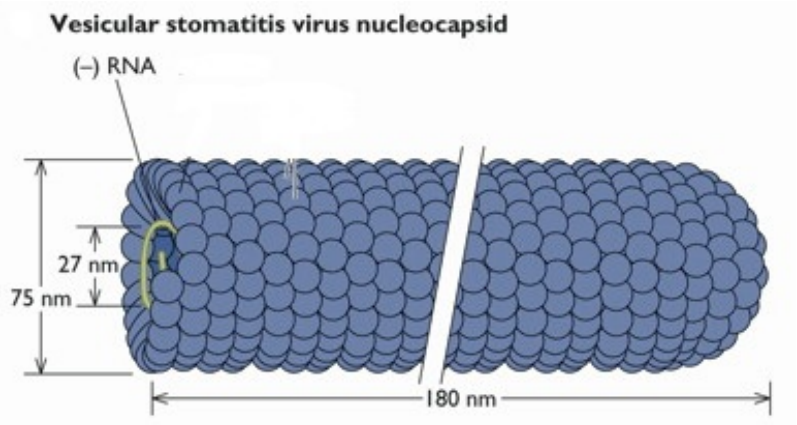


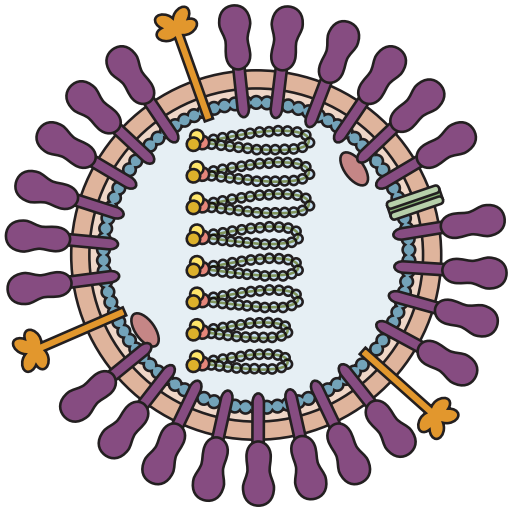
- dsRNA genomes

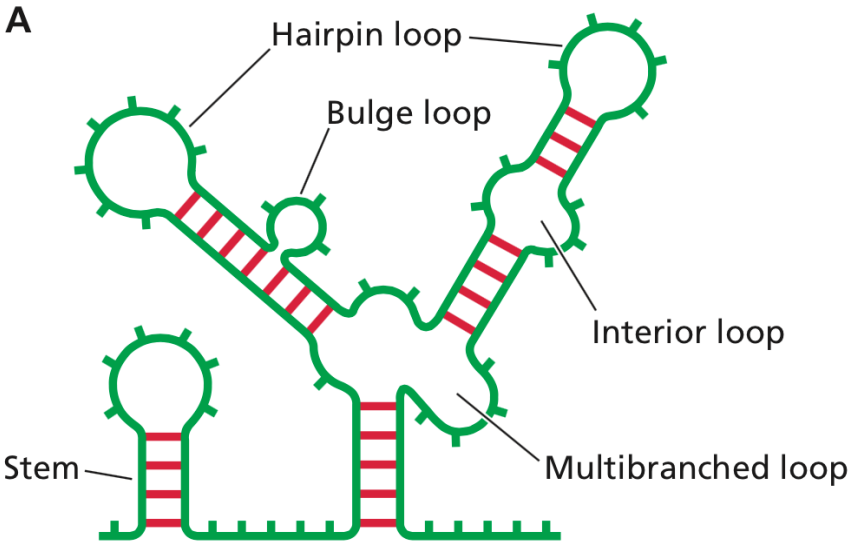


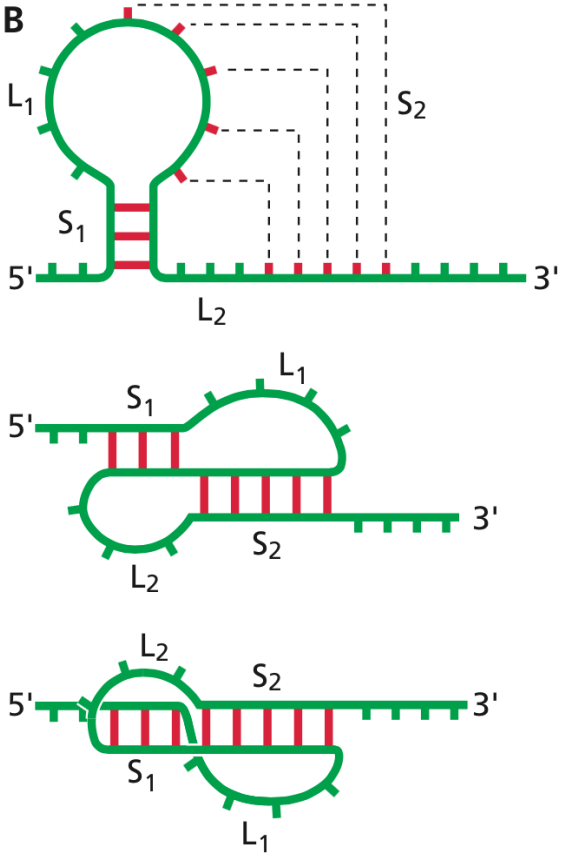


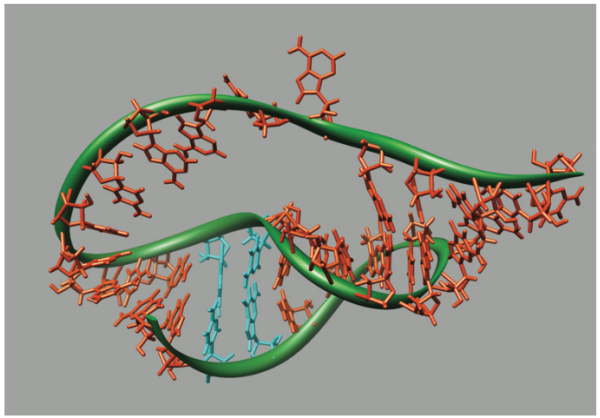










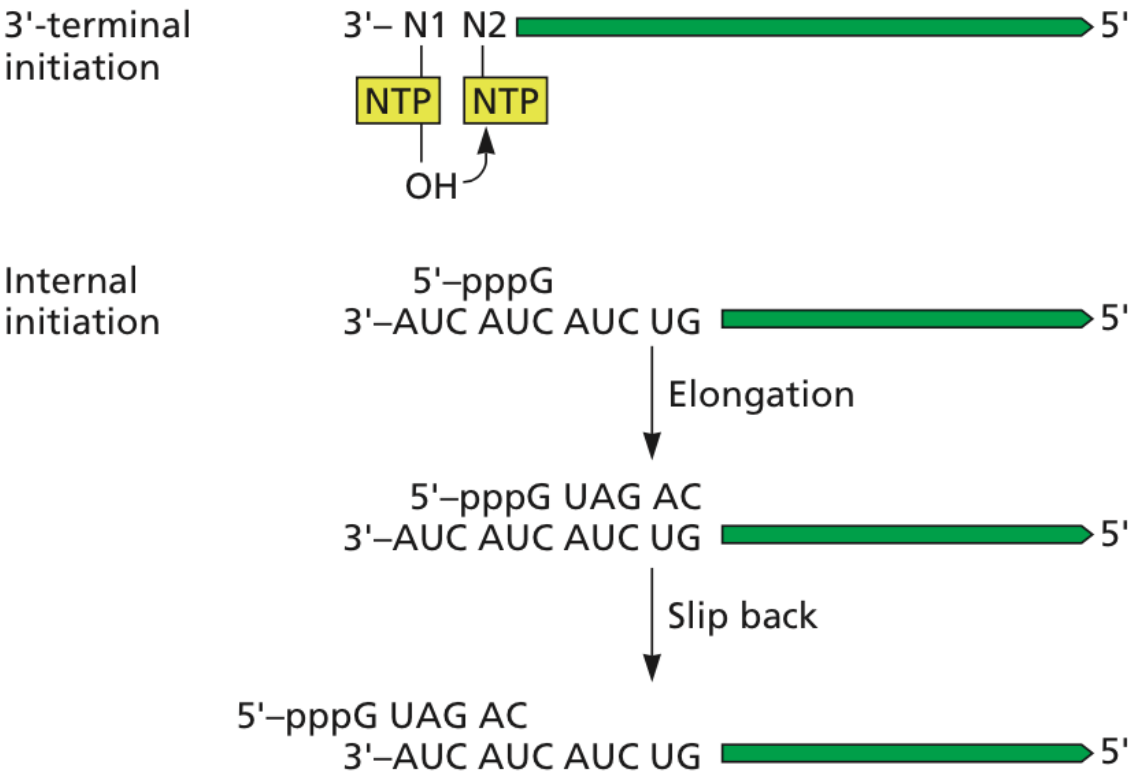


- RNA genome must be copied end to end with no loss of nucleotide sequence
- Production of viral mRNAs that can be efficiently translated by cellular protein synthesis machinery

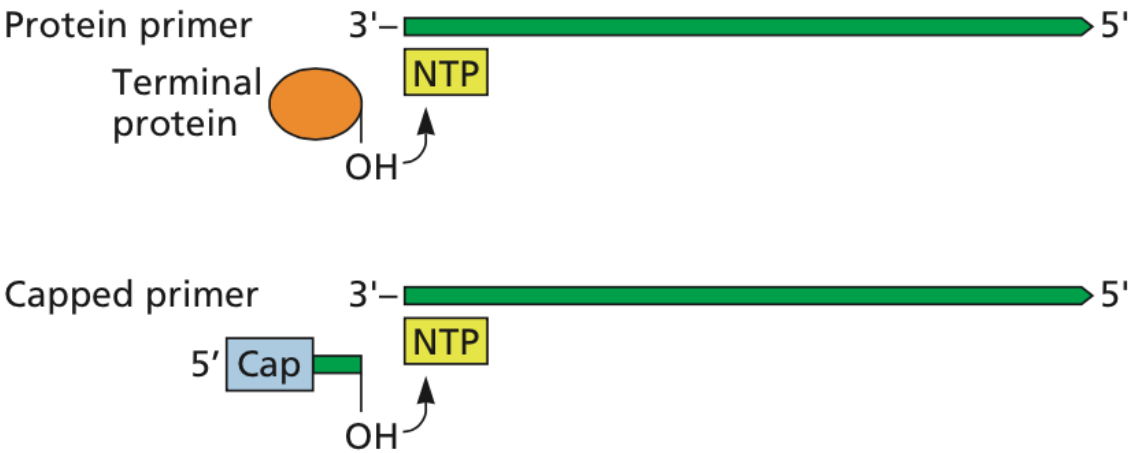
Universal rules for RNA-directed RNA synthesis

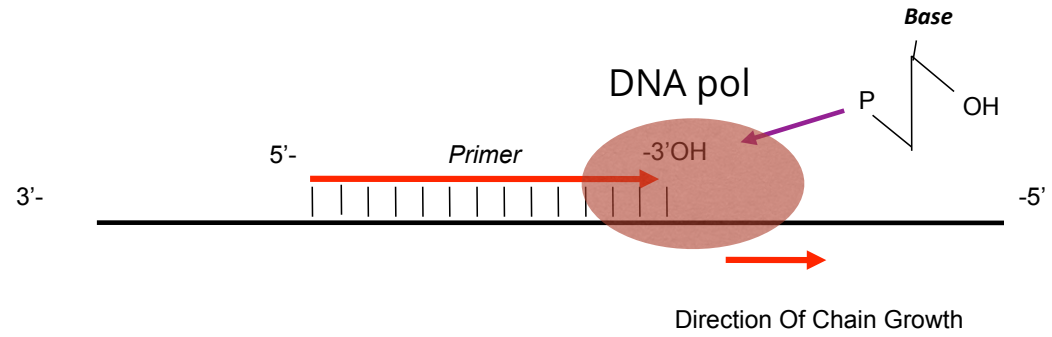
- RNA synthesis initiates and terminates at specific sites on the template
- RdRp may initiate synthesis *de novo* (like cellular DdRp) or require a primer
- Other viral and cell proteins may be required
- RNA is synthesized by template-directed stepwise incorporation of NTPs, elongated in 5’-3’ direction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

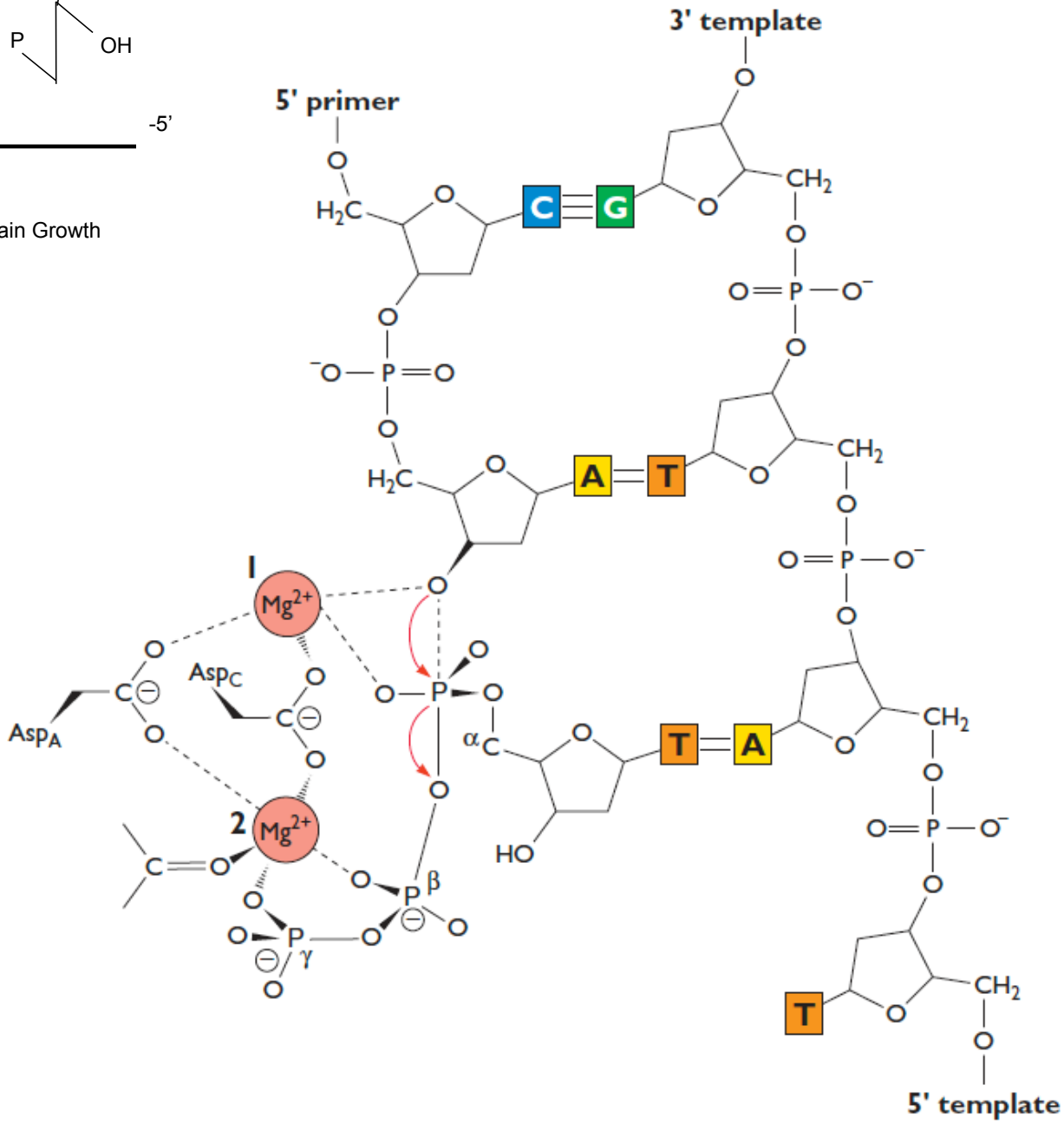
***De novo* initiation**



Primer-dependent initiation







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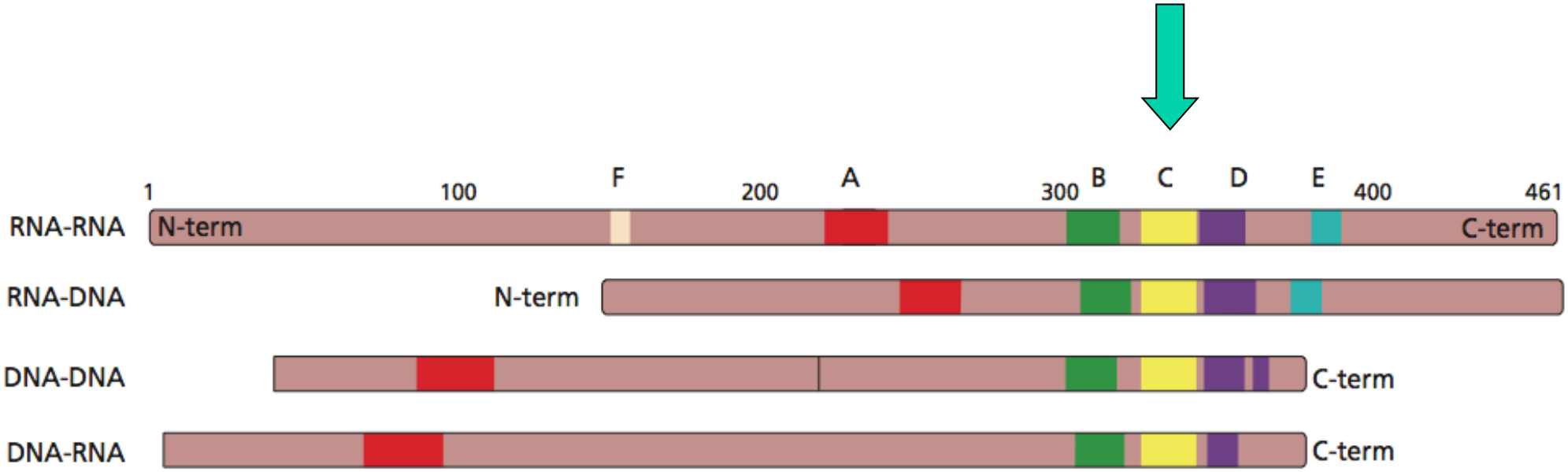
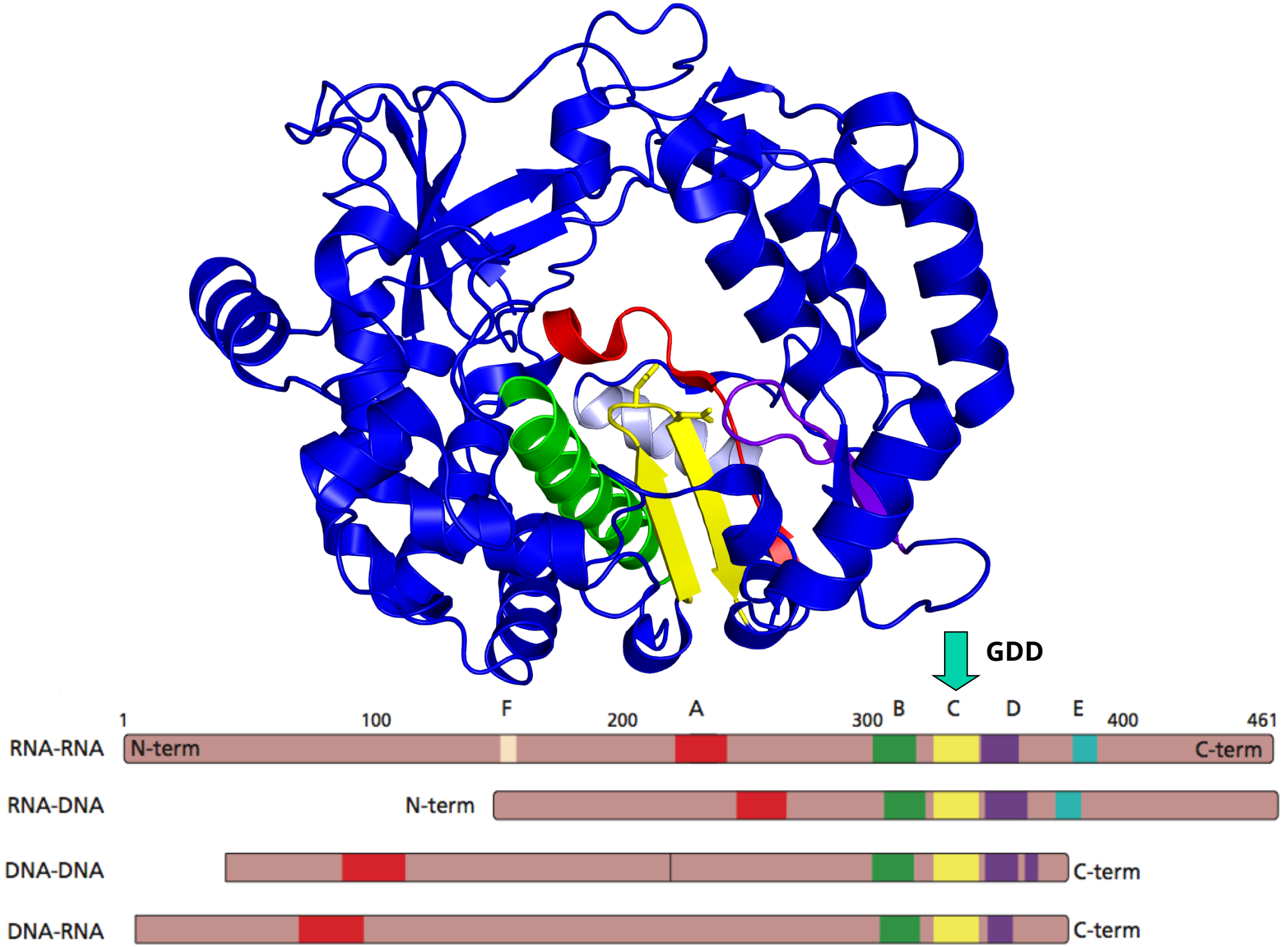
Which is a universal rule about RNA directed RNA synthesis?

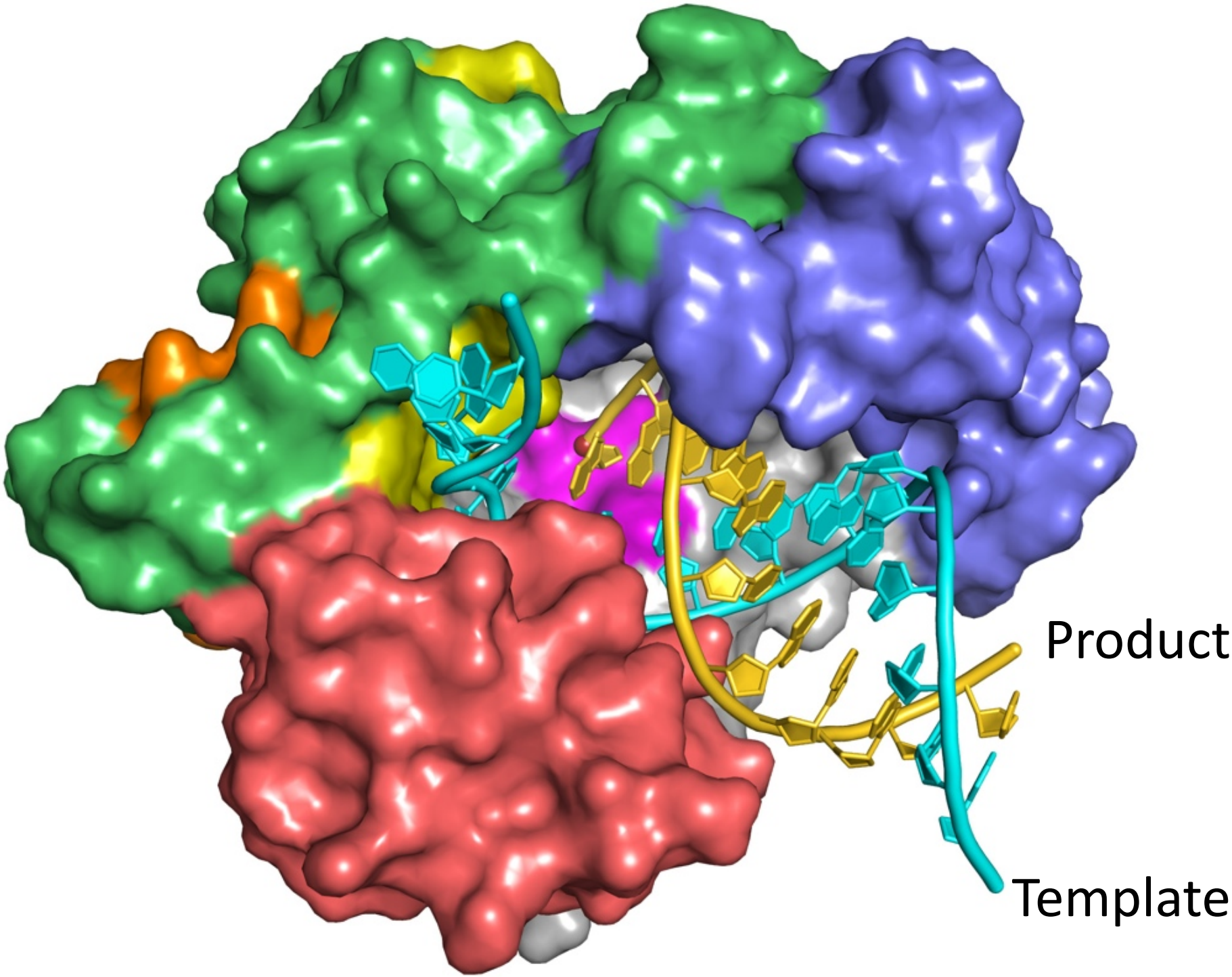
1. RdRp may initiate *de novo* or require a primer
2. RNA synthesis initiates randomly on the RNA template
3. RNA is synthesized in a 3’-5’ direction
4. RNA synthesis is always template-directed

Sequence relationships among polymerases

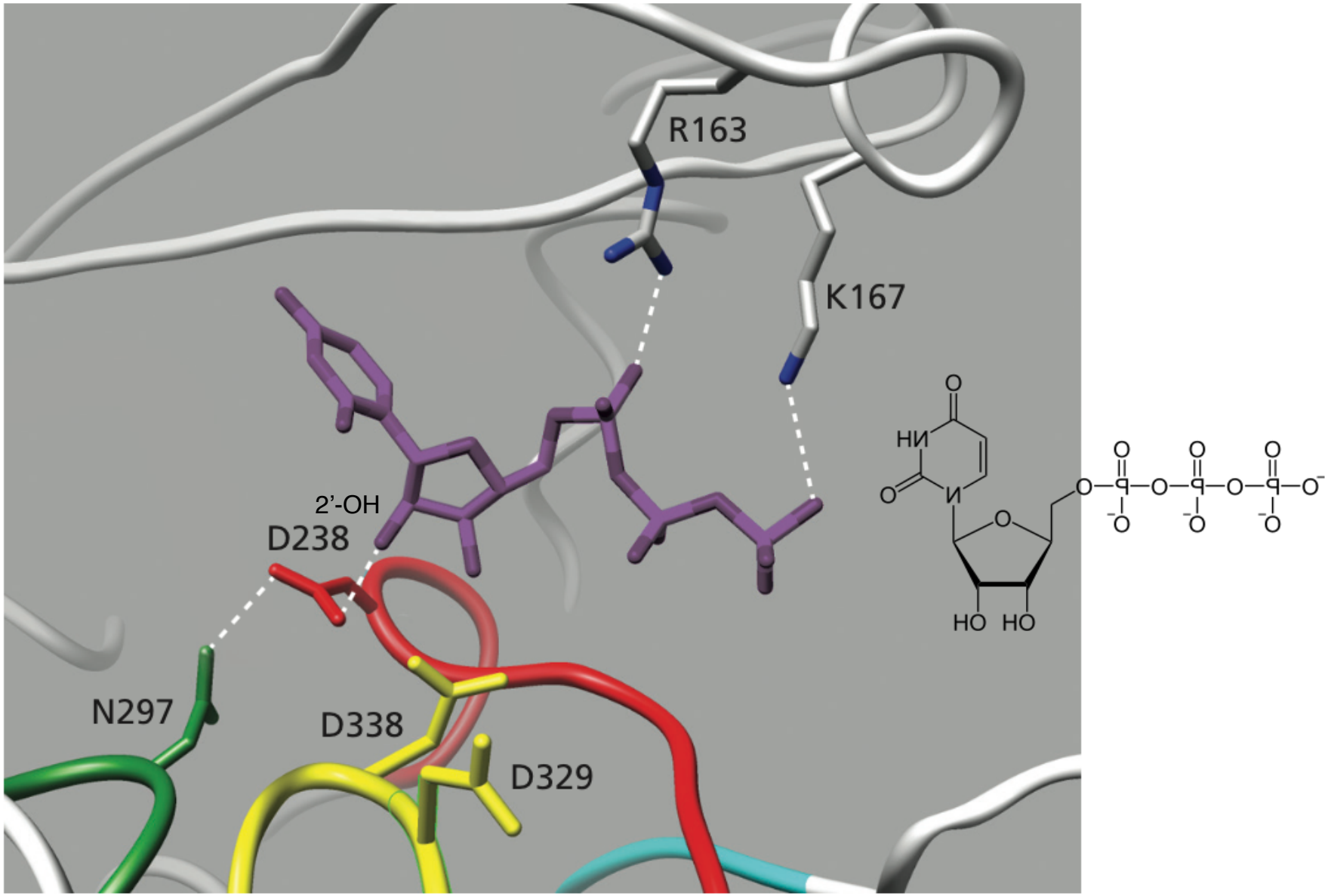


- Gly-Asp-Asp in (+) strand RNA polymerases
- Asp-Asp in RT, segmented (-) strand polymerases
- Gly-Asp-Asn in nonsegmented (-) strand polymerases



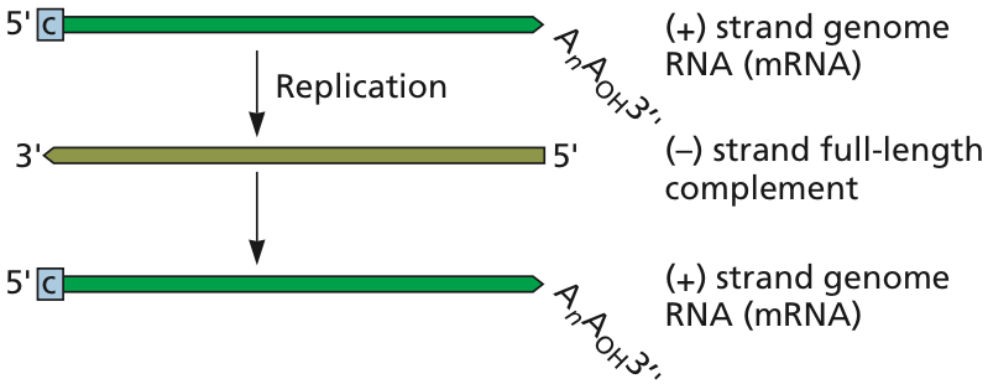

Product
Template

Structure of UTP bound to poliovirus RdRp

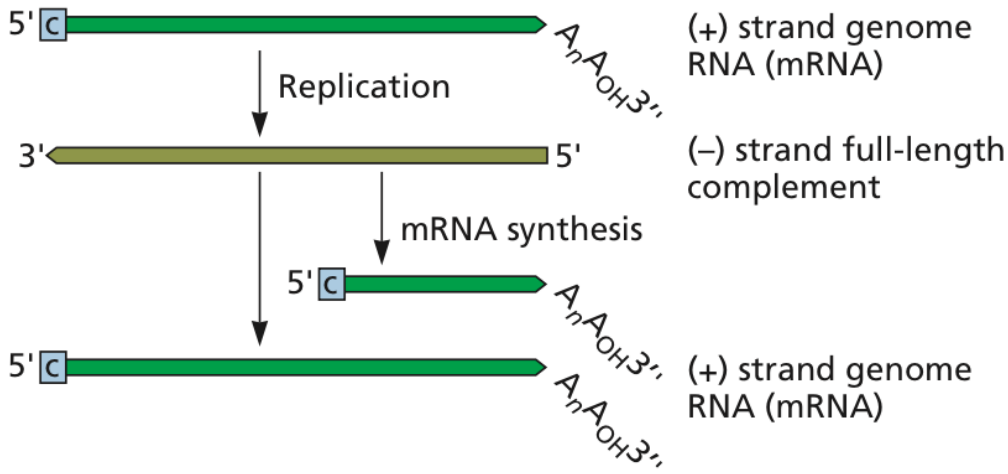


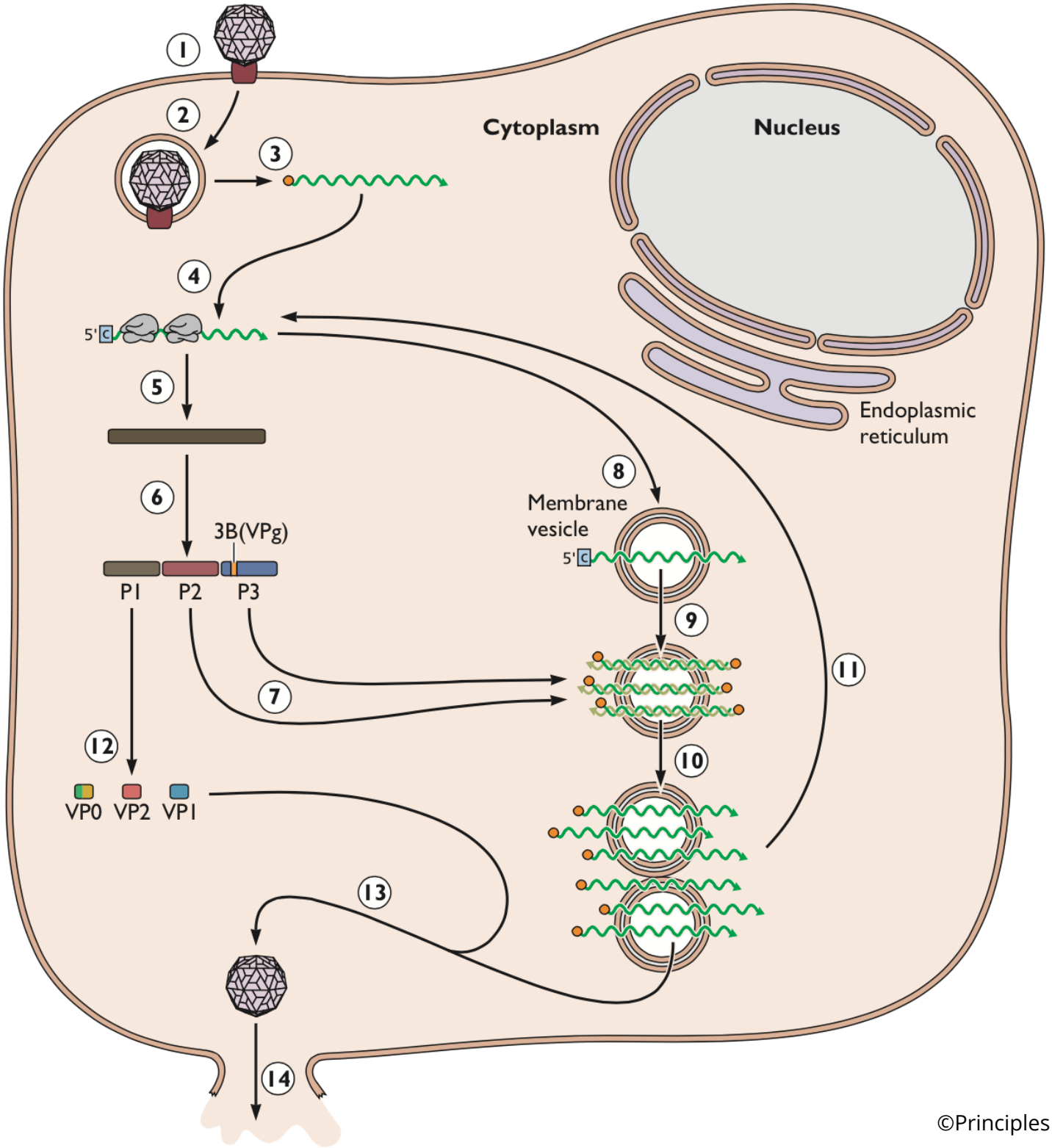
(+) strand RNA viruses

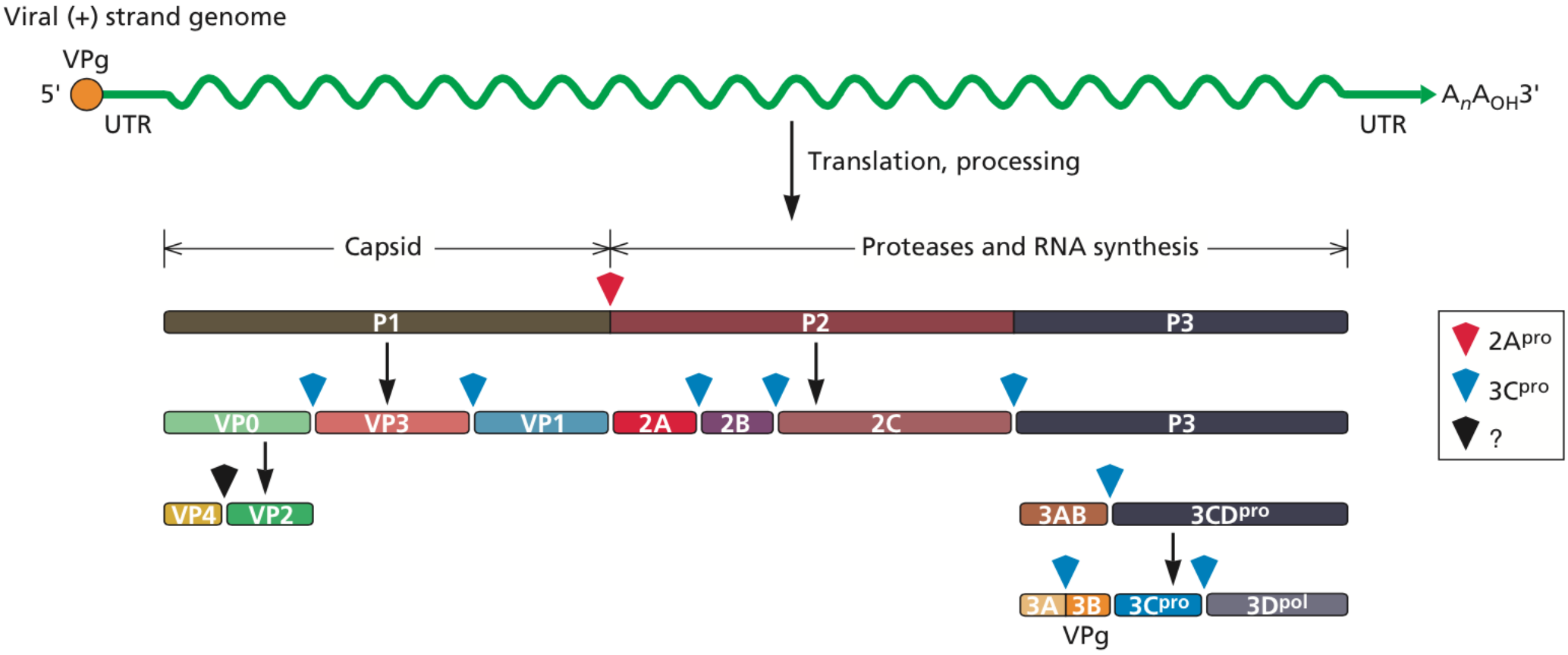
Flavi- and picornaviruses

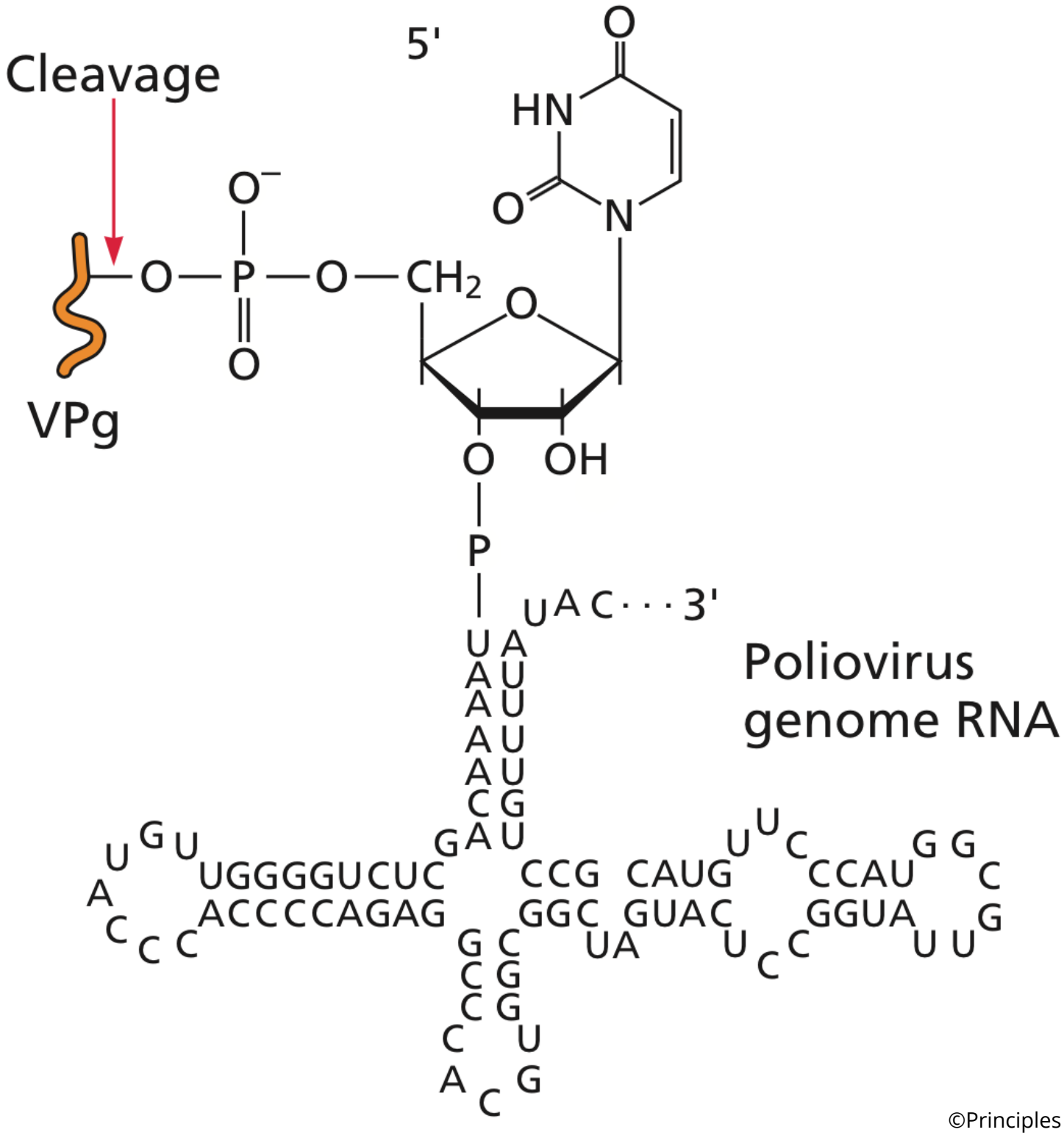


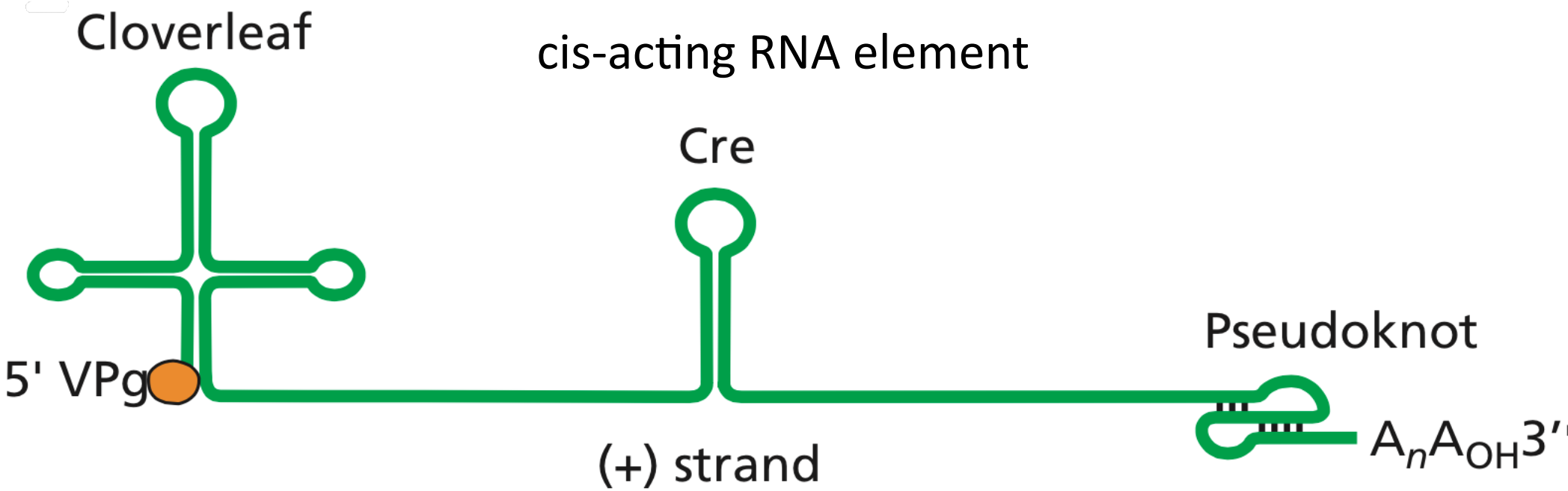
Alphaviruses *(Togaviridae* - Sindbis, SFV, Chik*)*



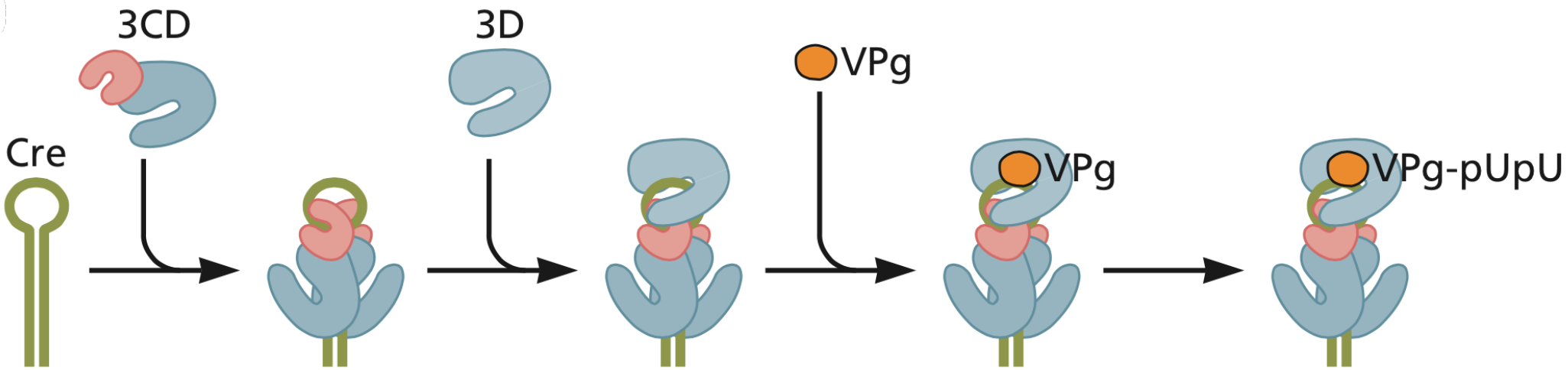


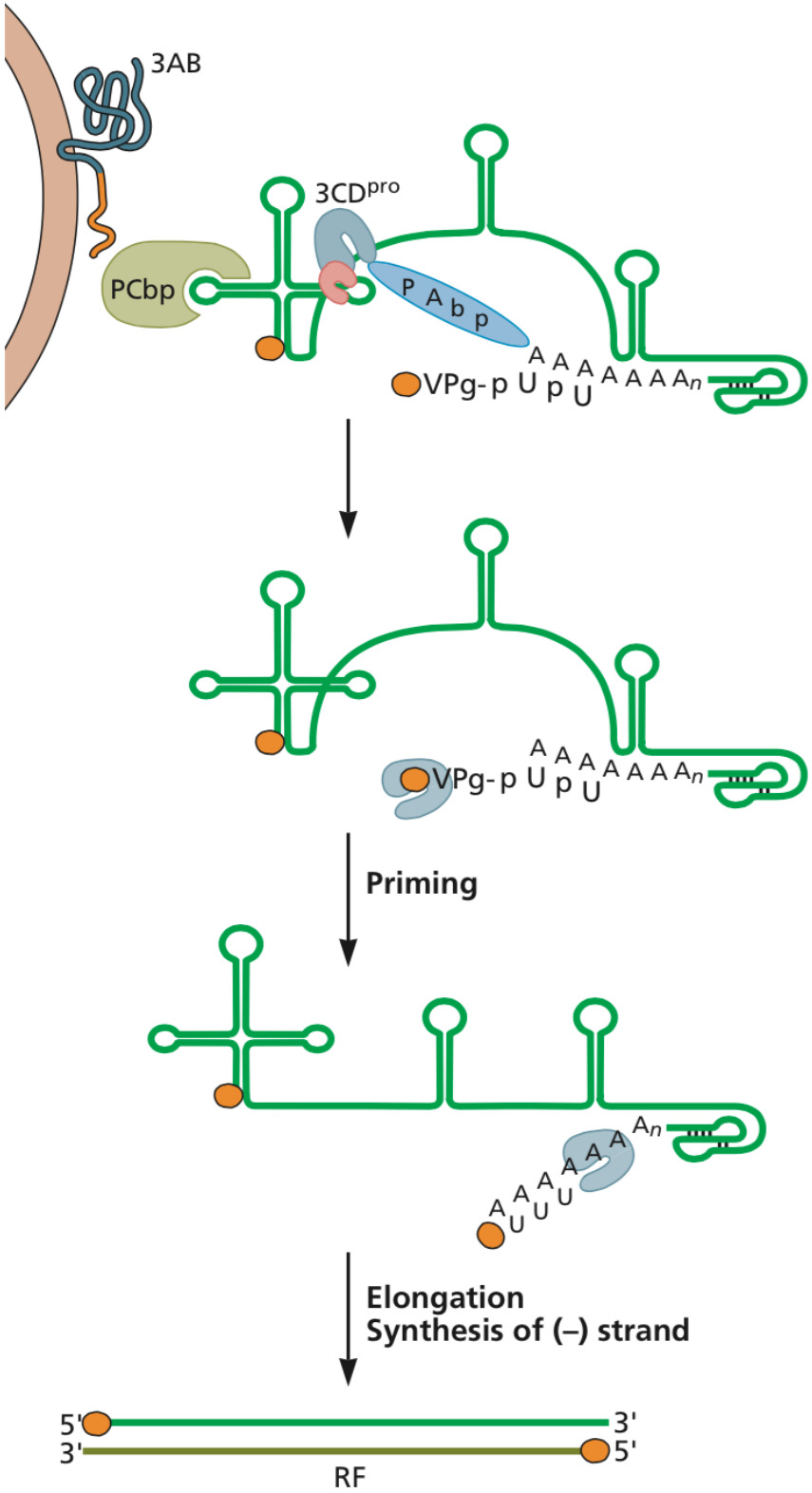




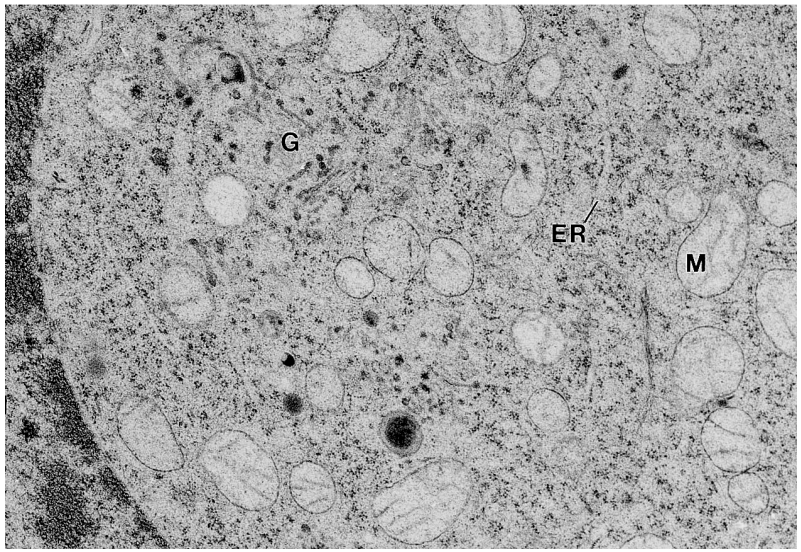


cellular polyadenylated RNAs not copied

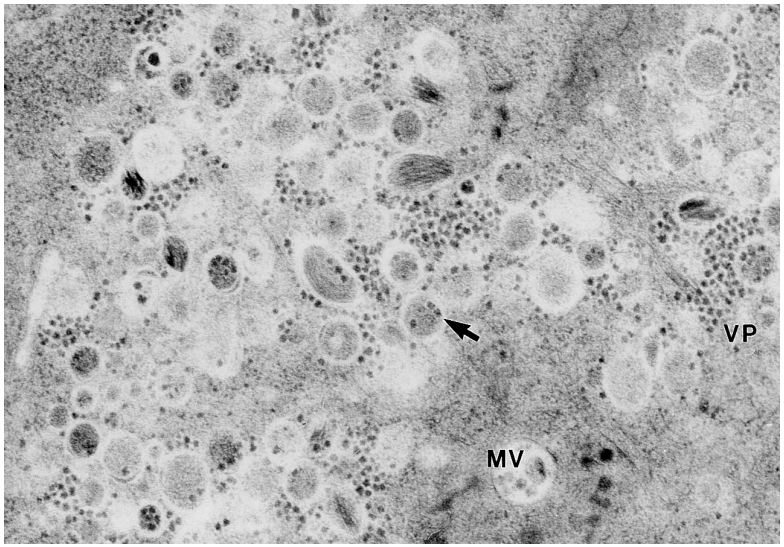




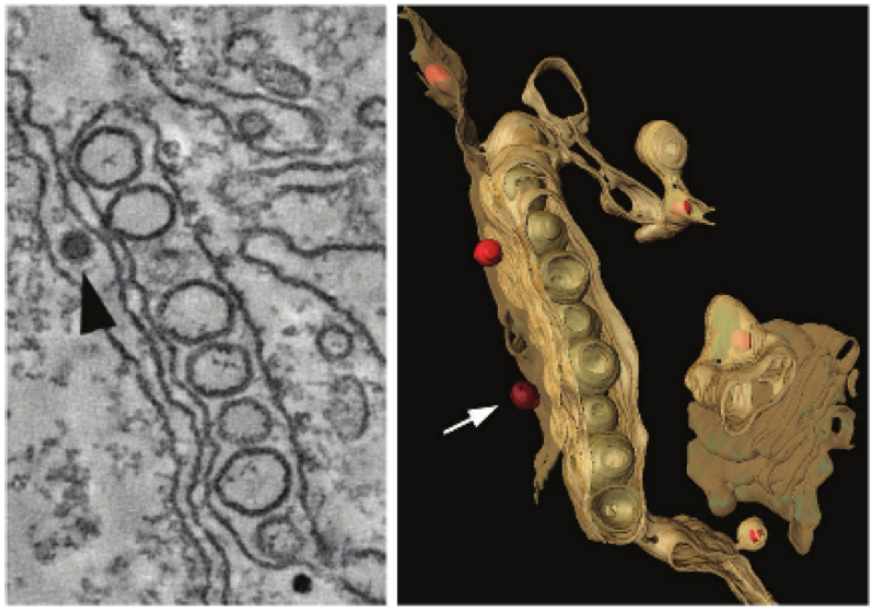
Vesicle formation in virus-infected cells



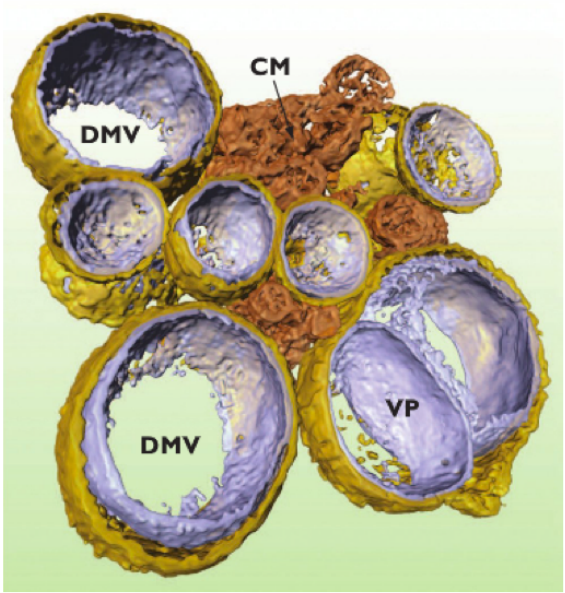
Uninfected HeLa cell



PV-infected HeLa cell



Flavivirus infected cell



Coronavirus-infected cell

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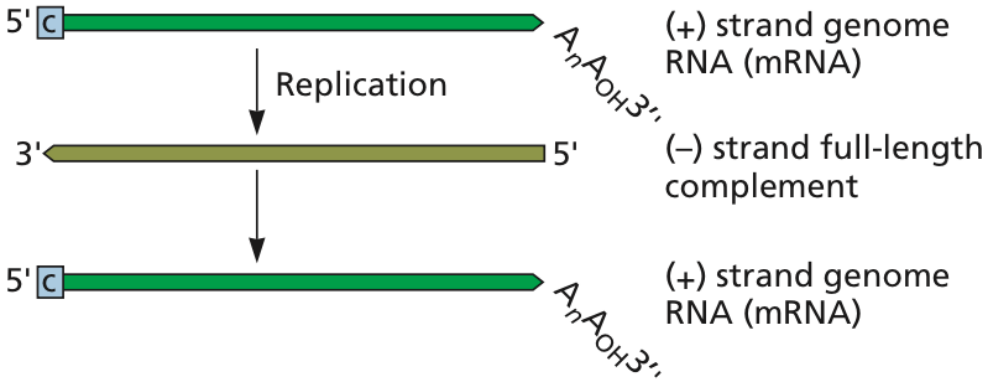
Which is a part of the poliovirus replication strategy?

1. The production of subgenomic mRNAs
2. *De novo* (without primer) initiation of RNA synthesis
3. Circularization of template for initiation of RNA synthesis
4. All of the above

(+) strand RNA viruses

Flavi- and picornaviruses



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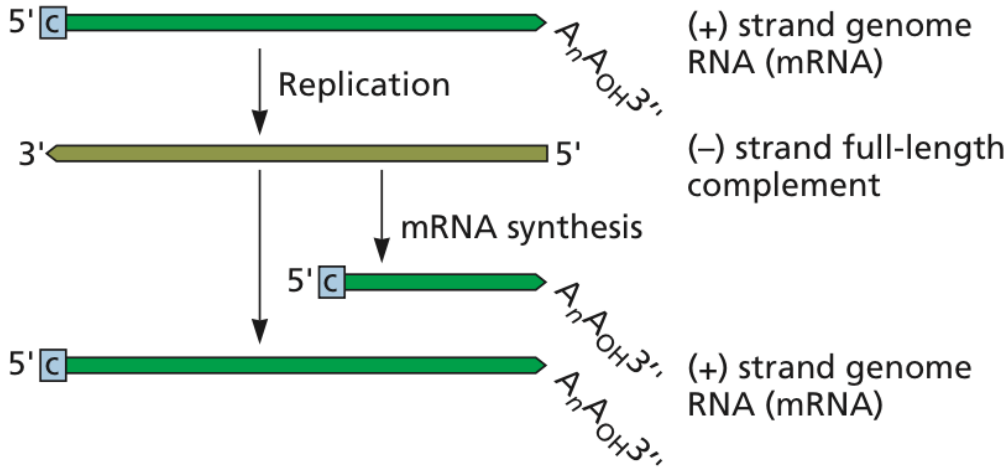
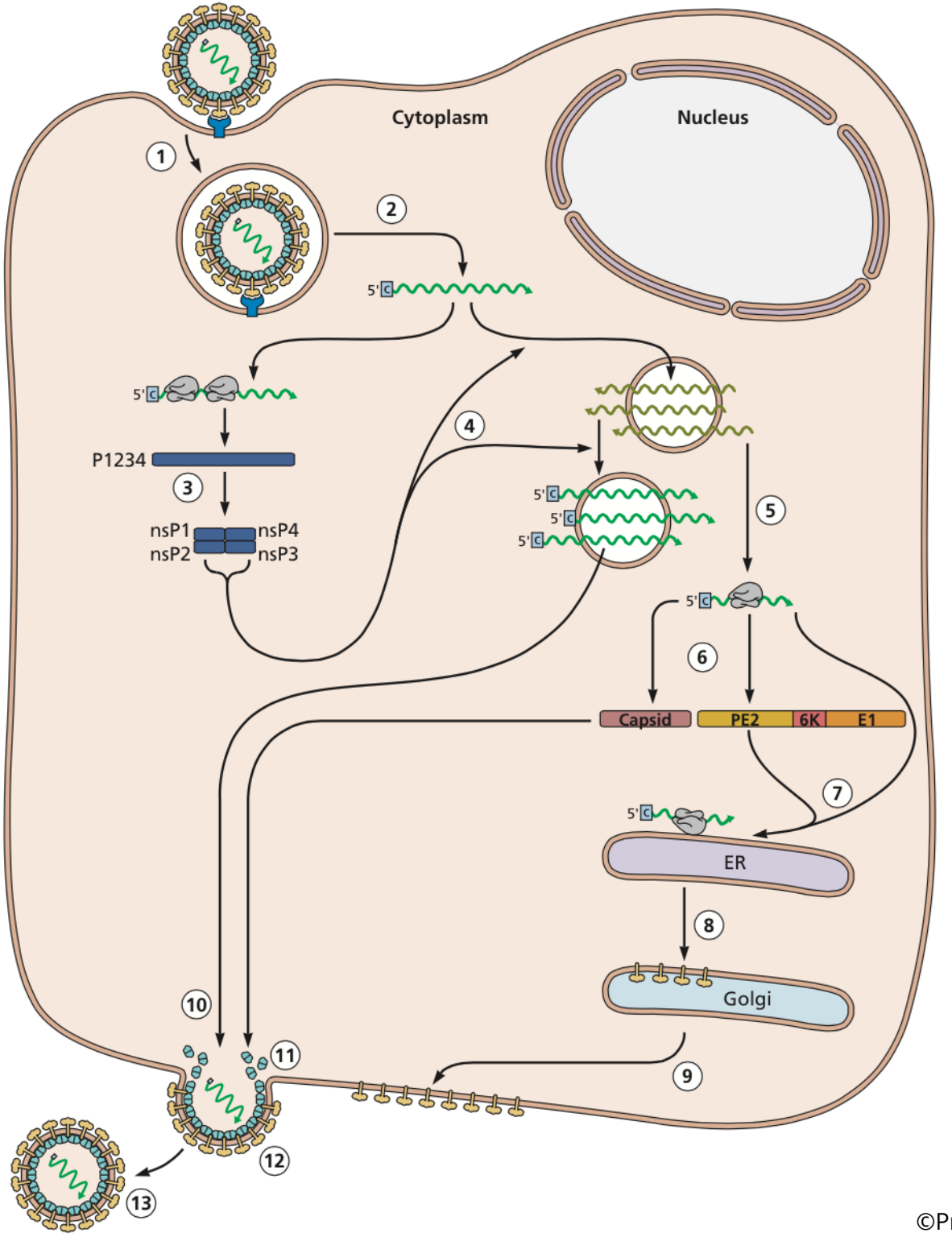
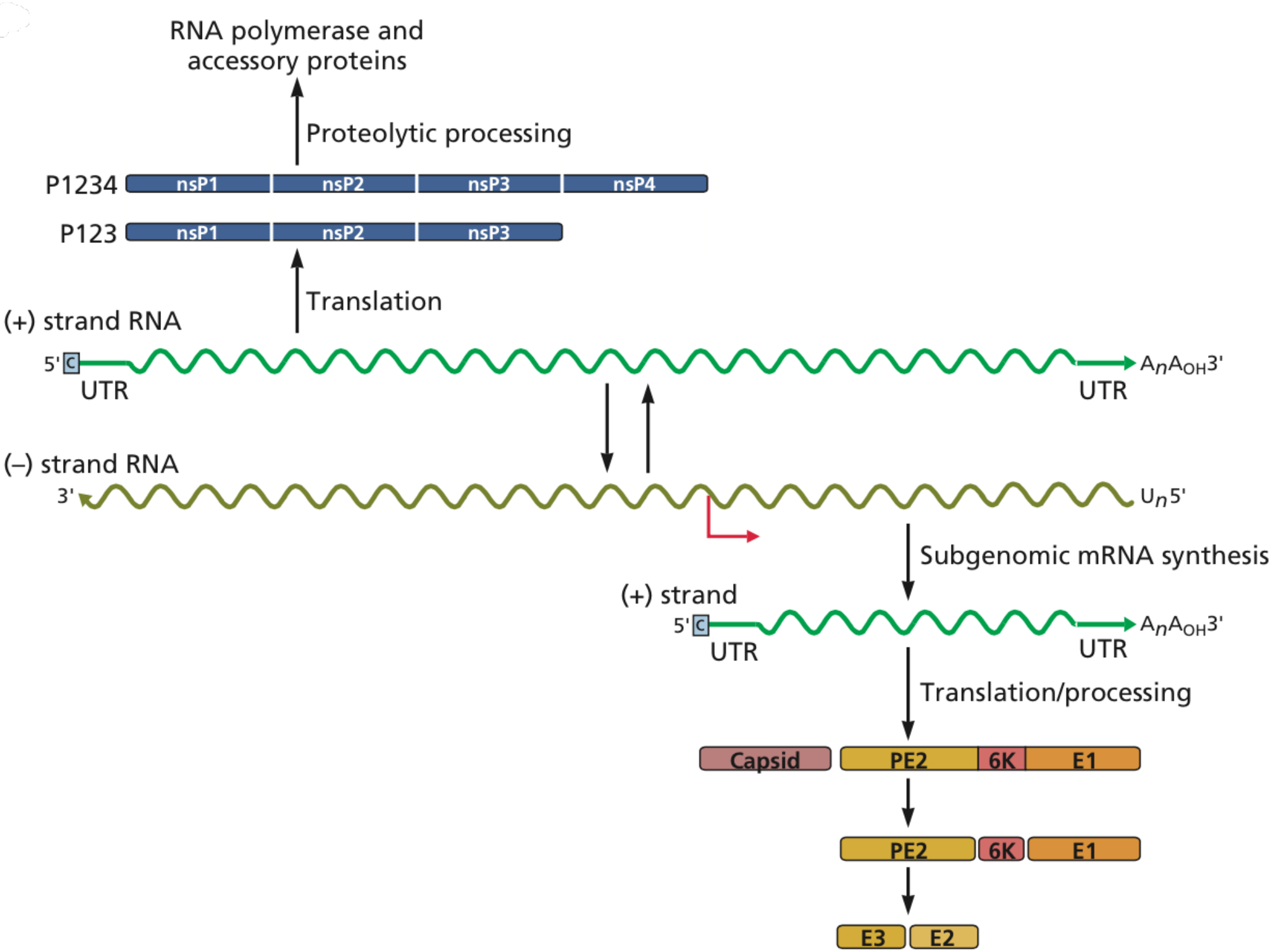
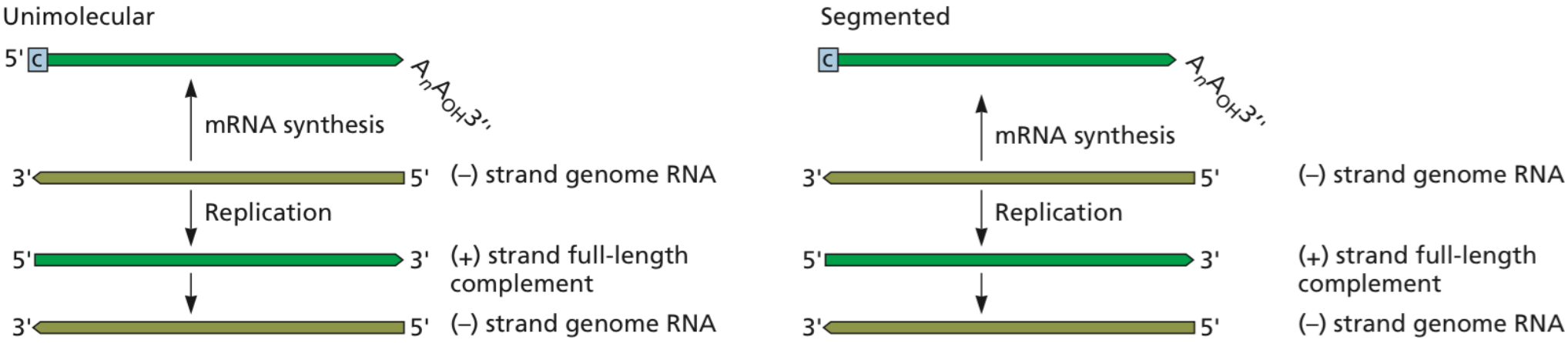
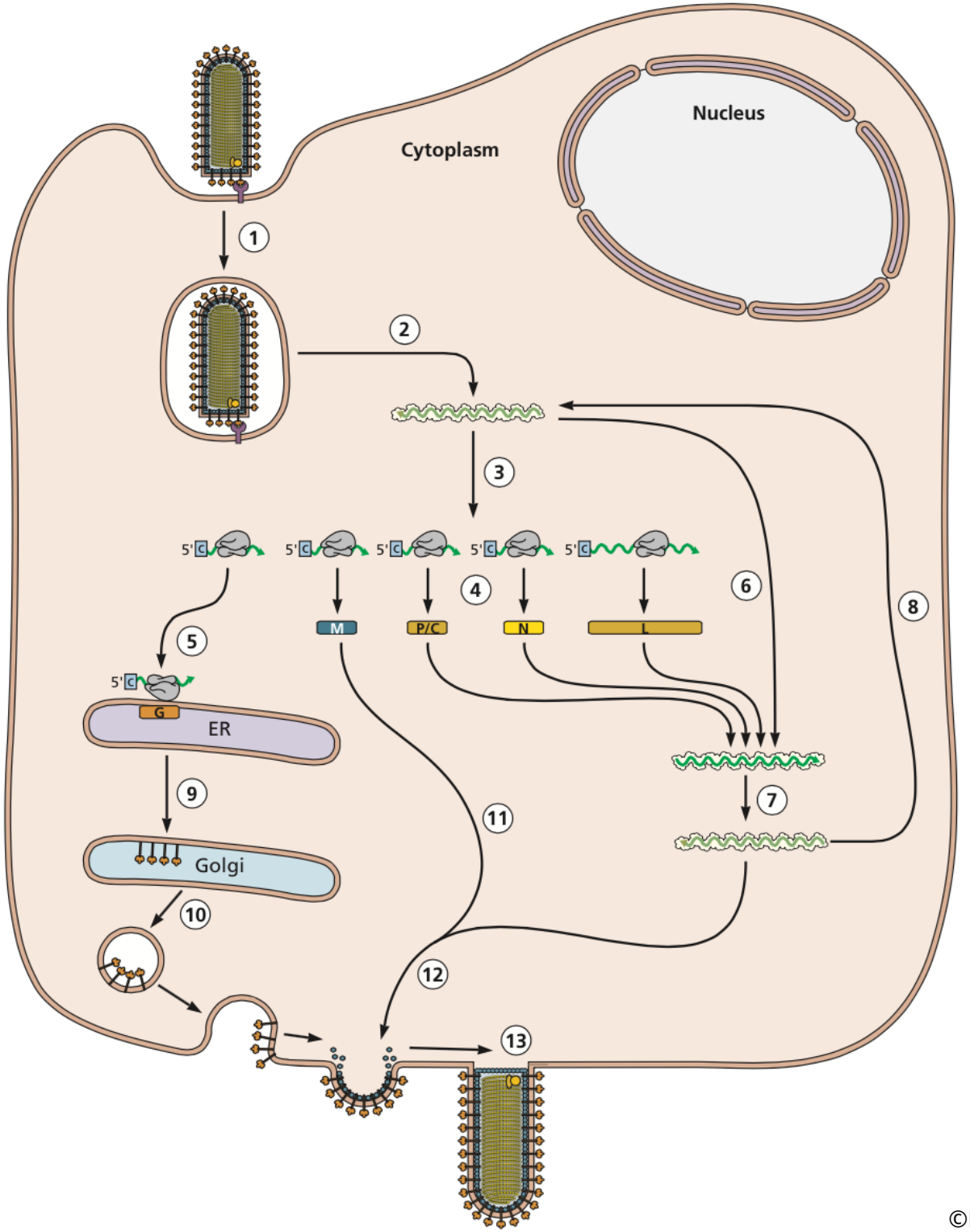


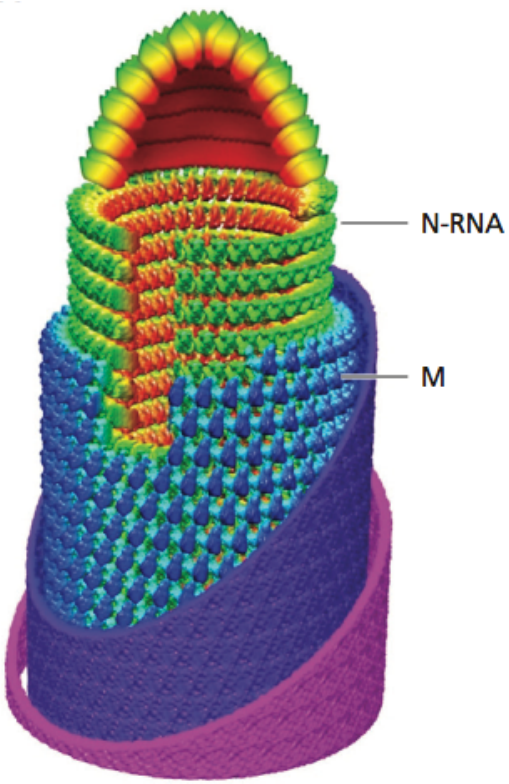


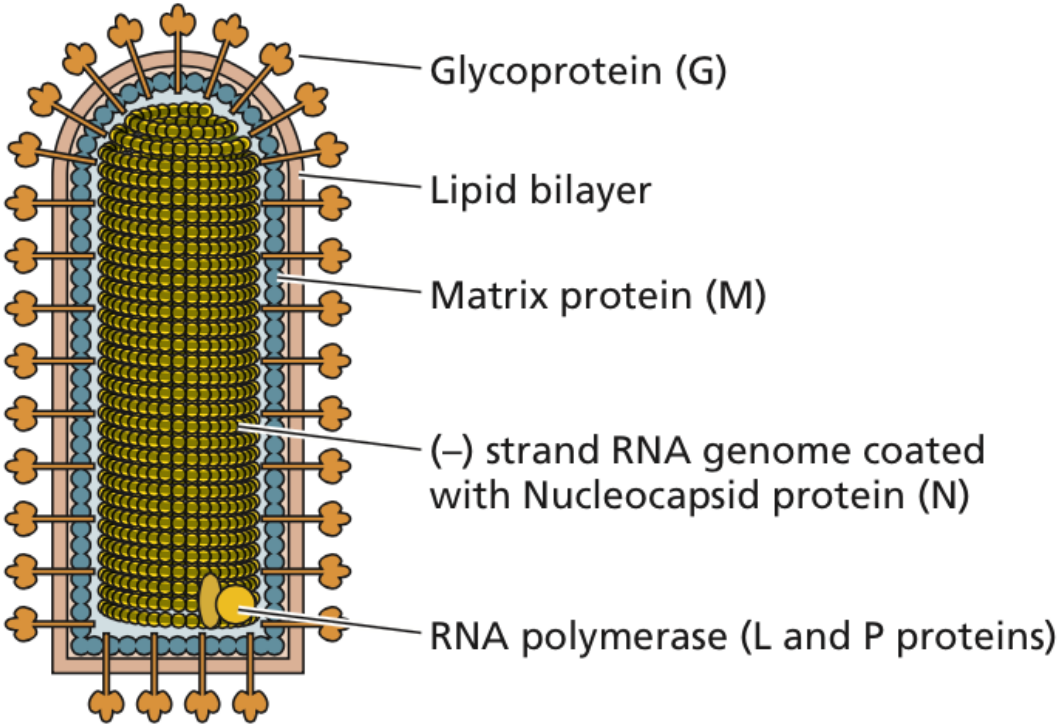


(–) strand RNA viruses

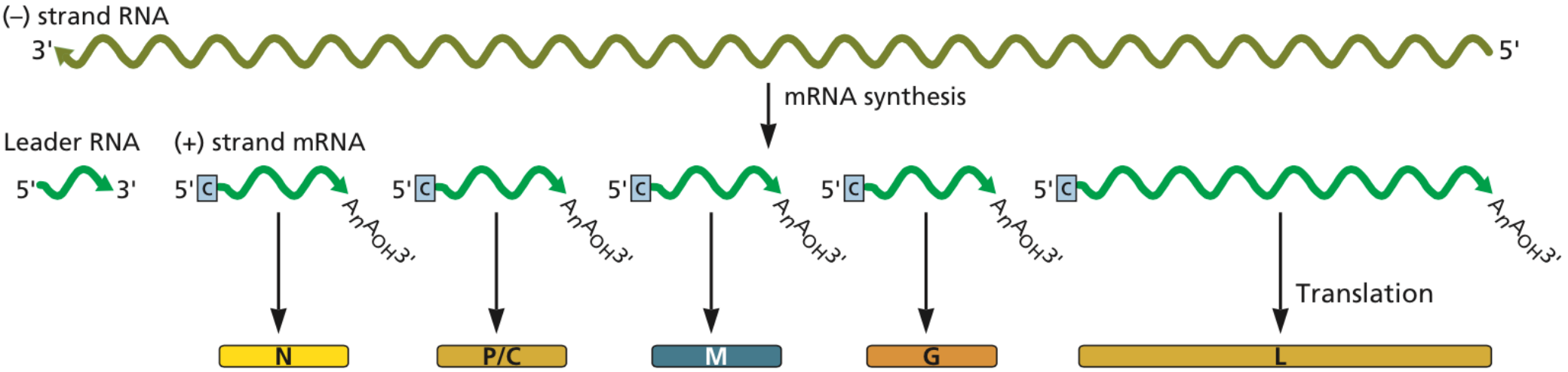


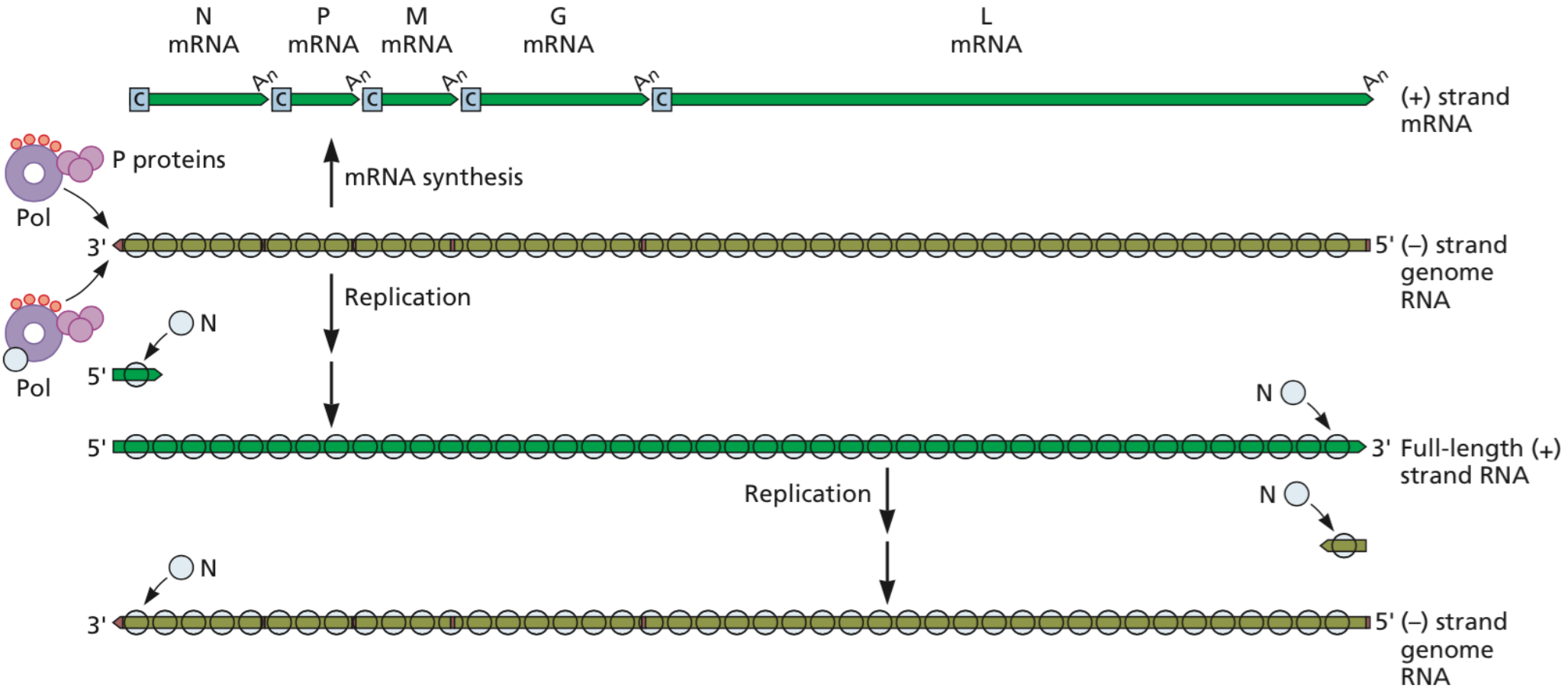


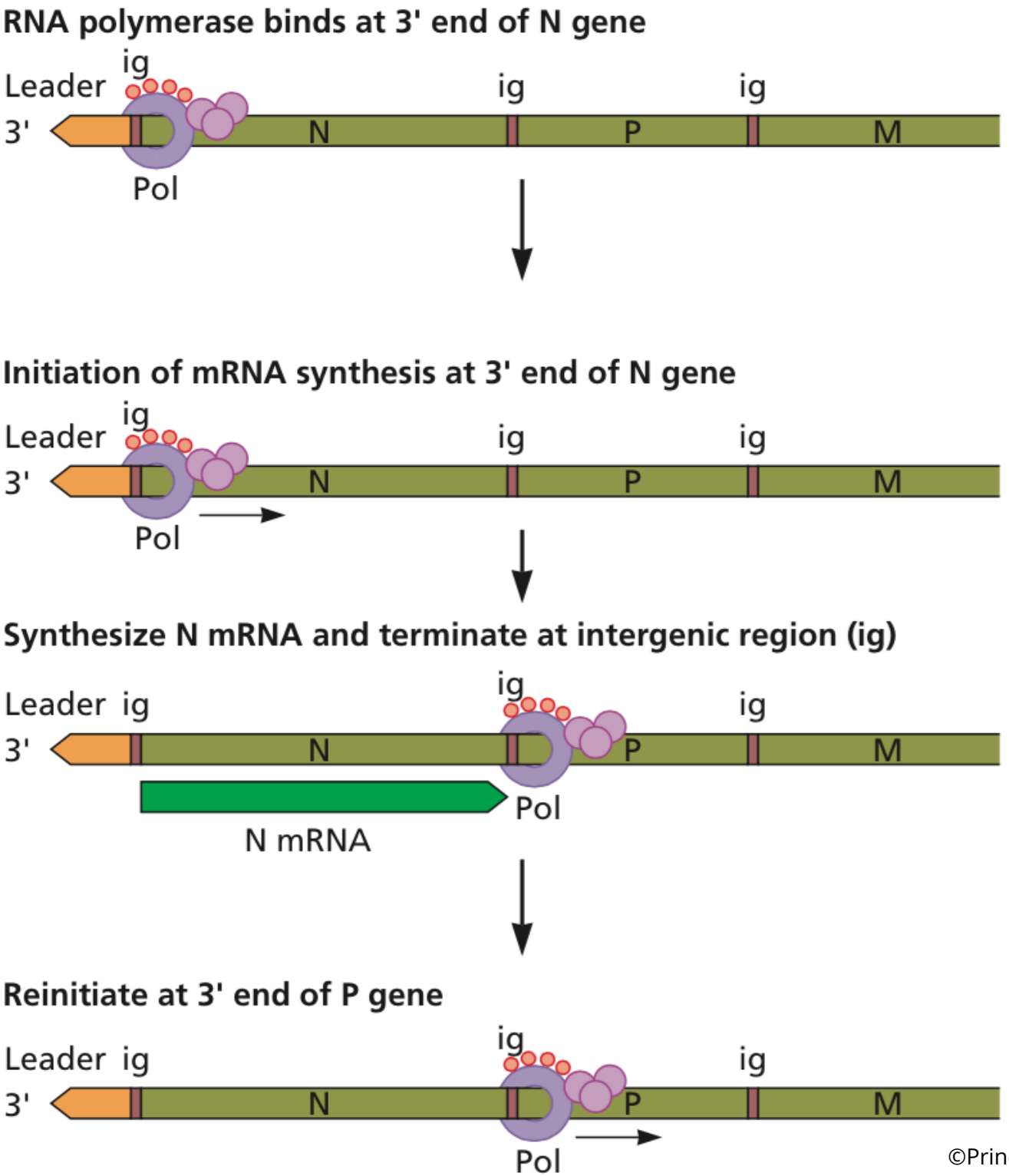


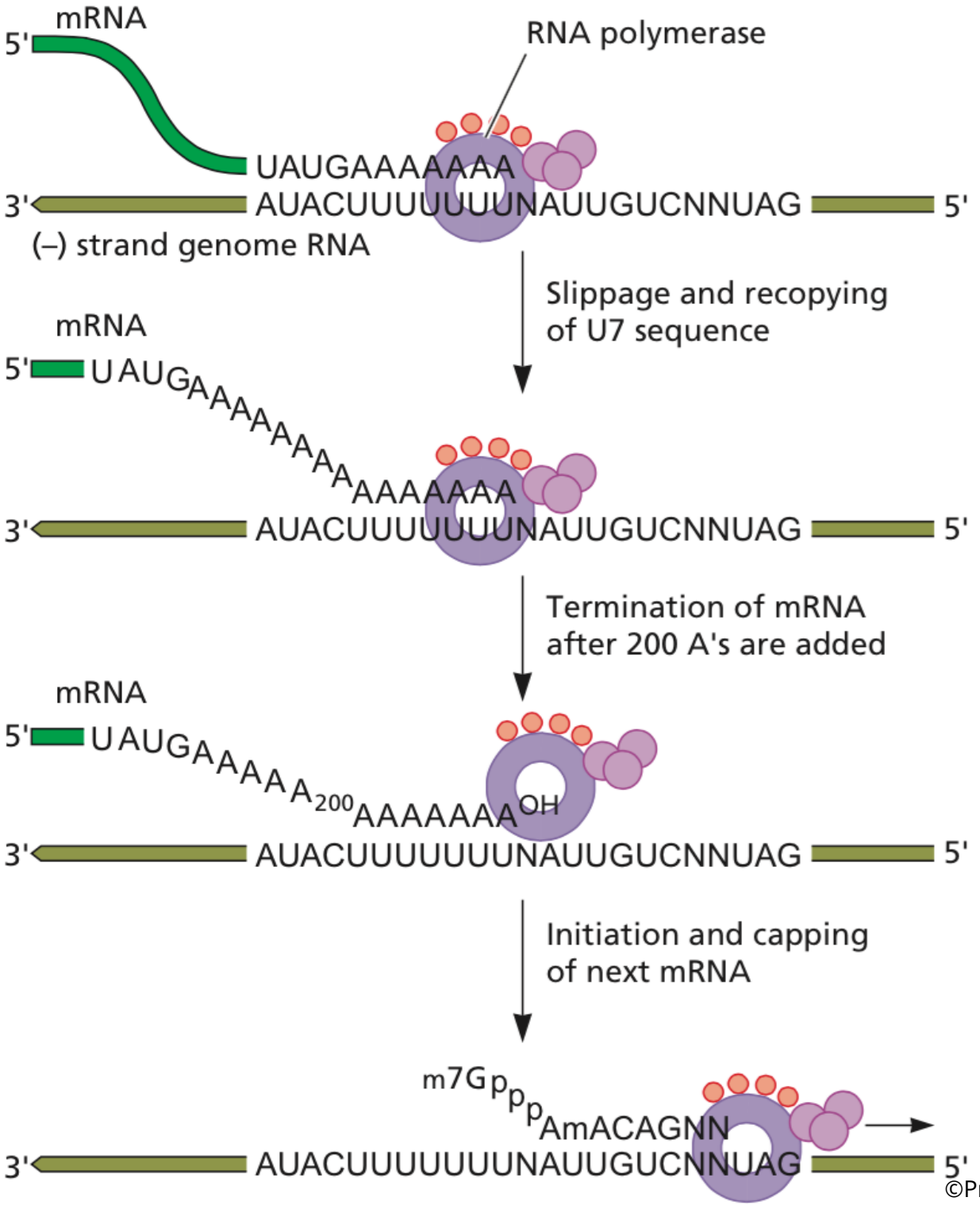


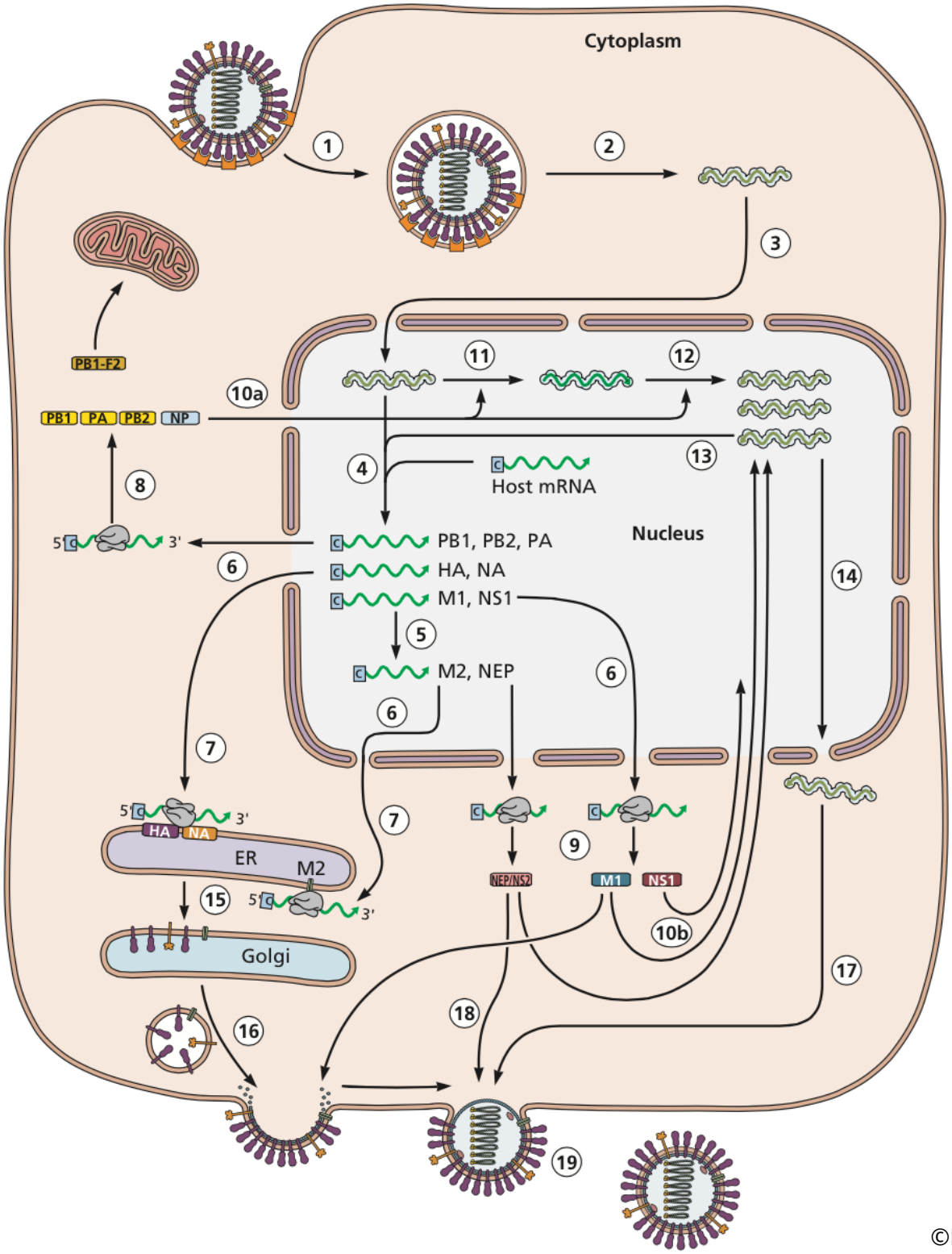
Unimolecular

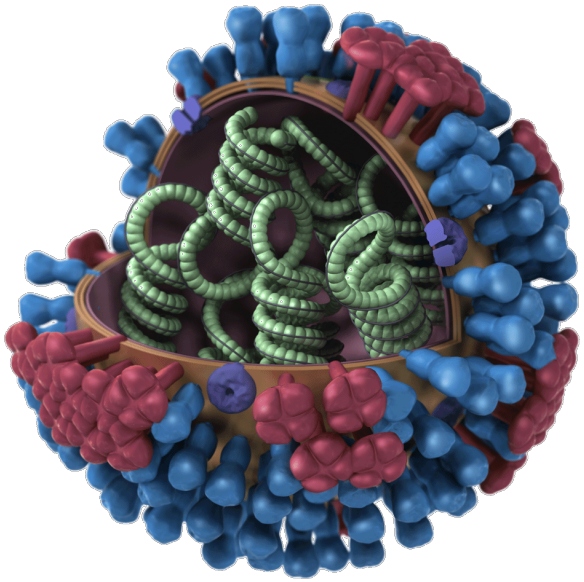


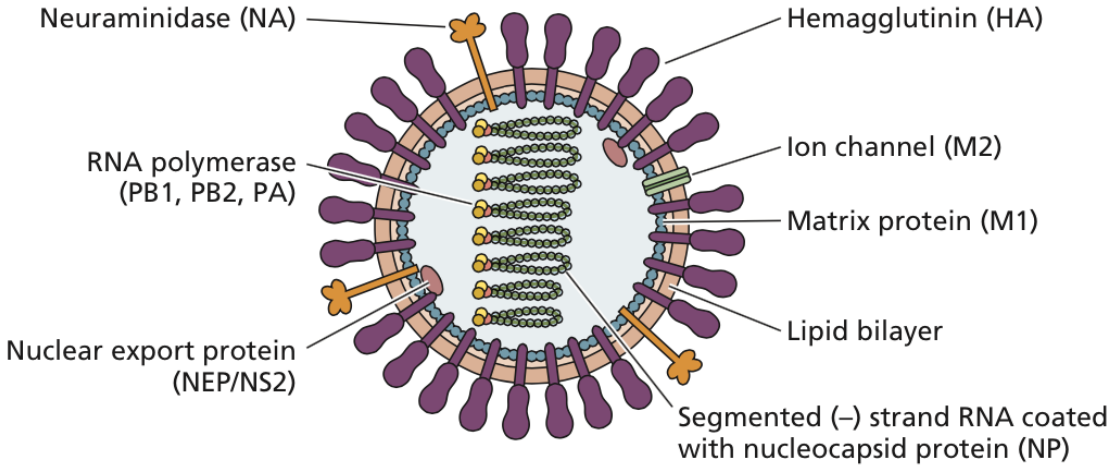


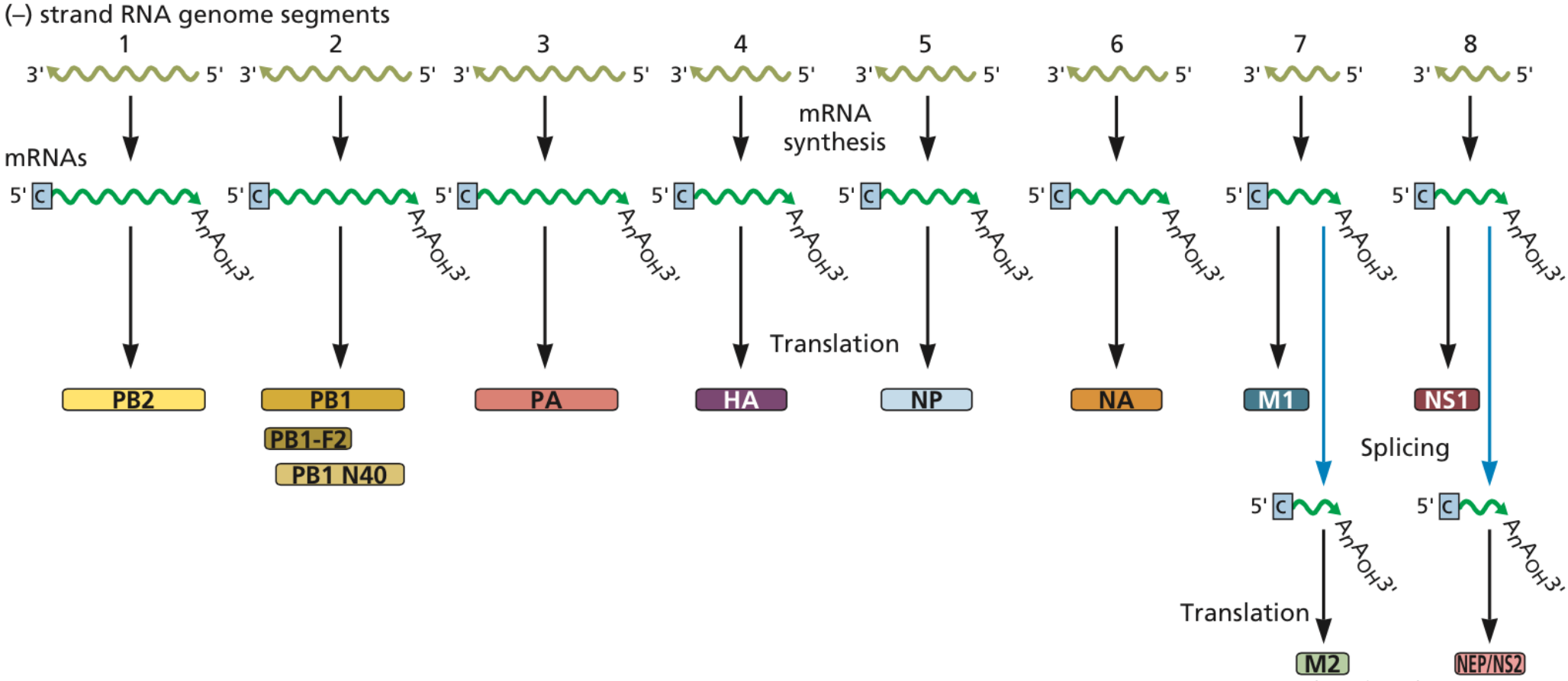


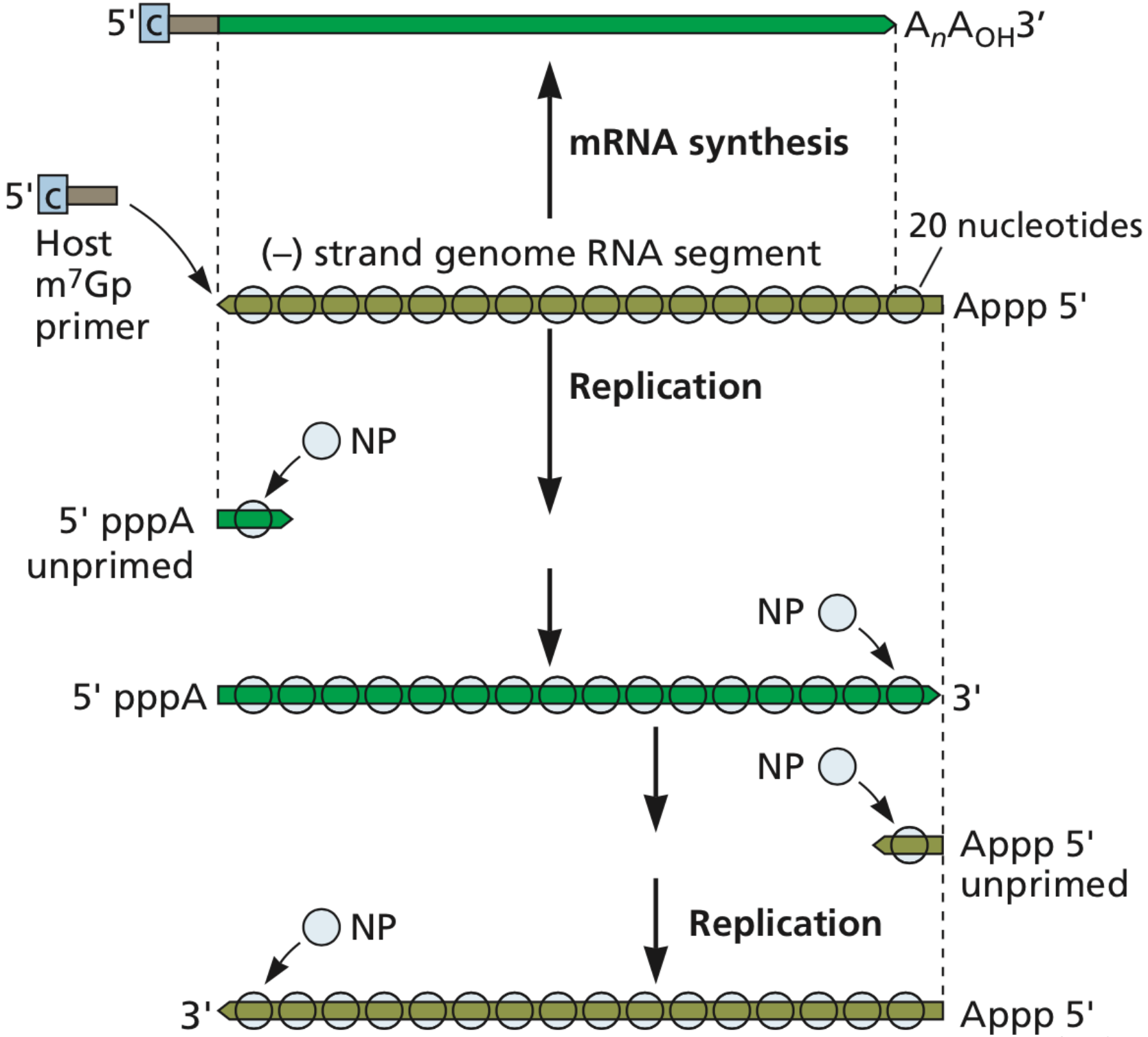


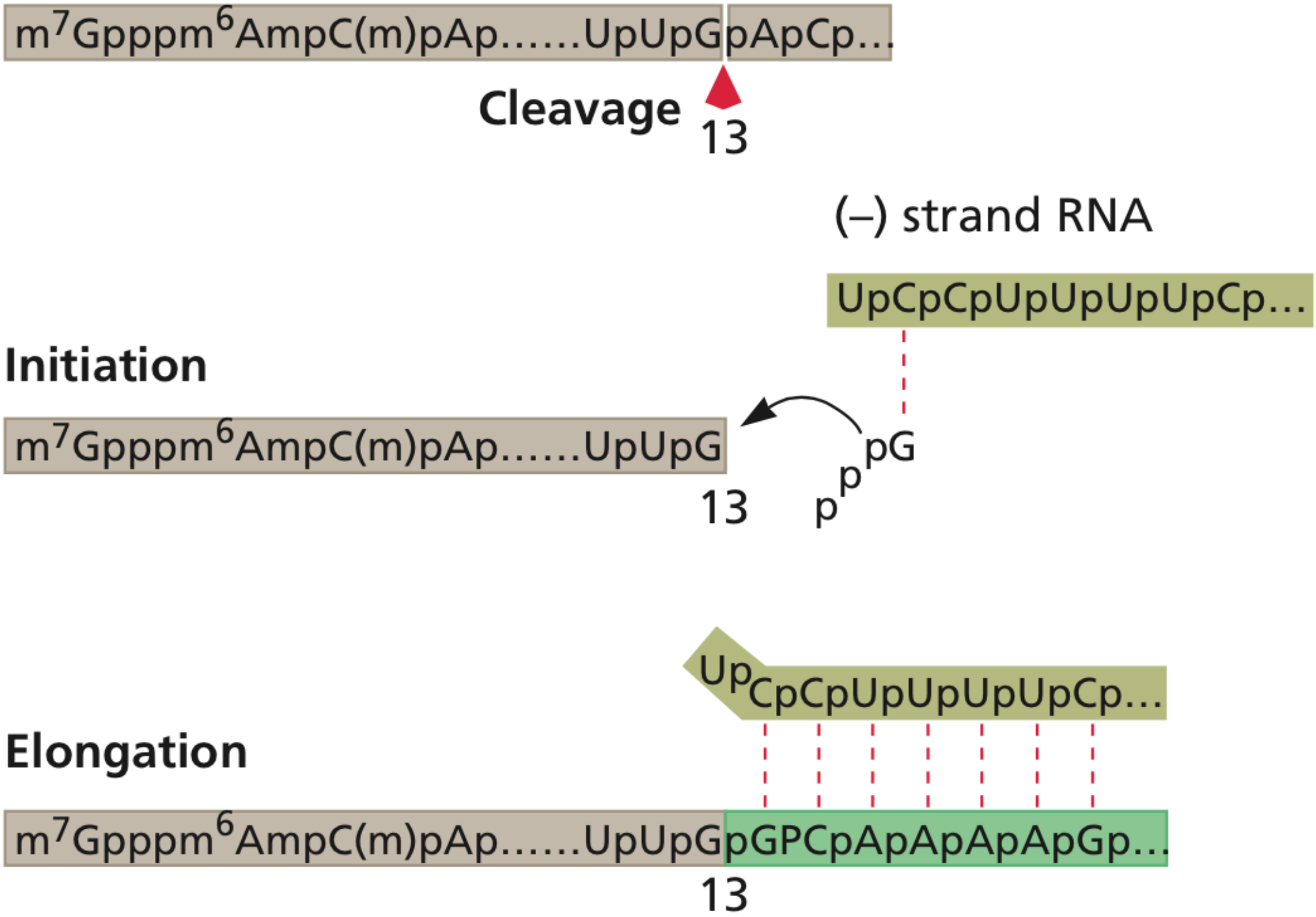


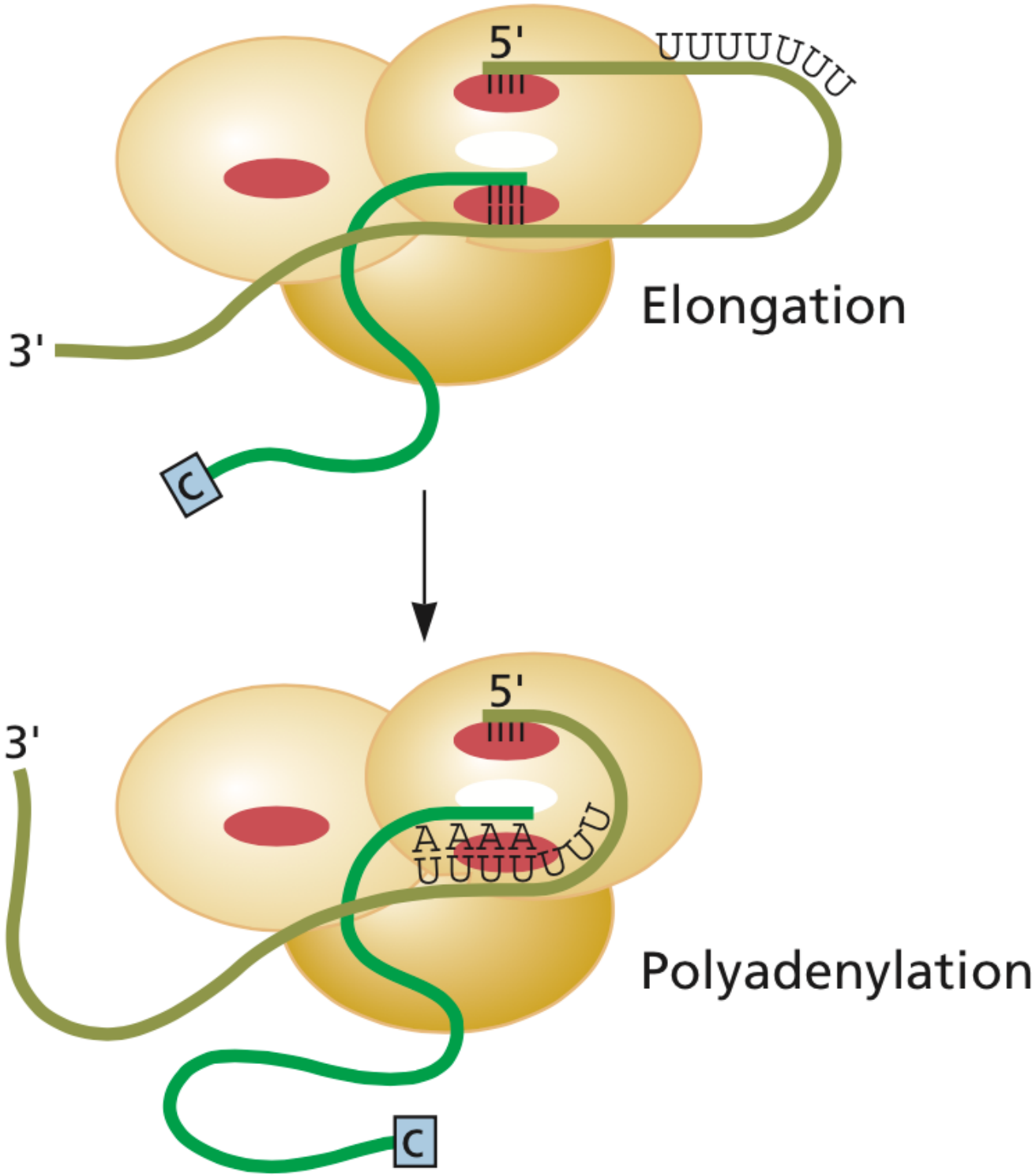












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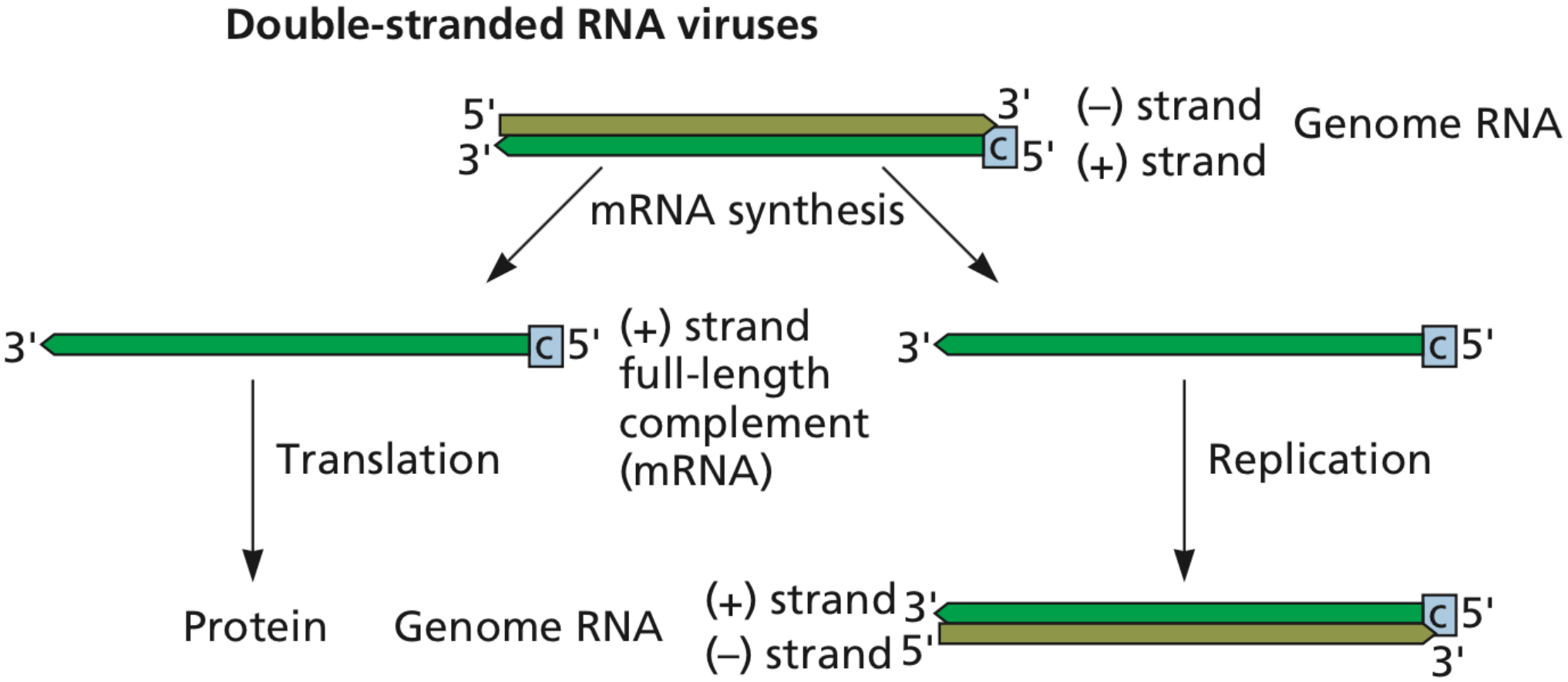
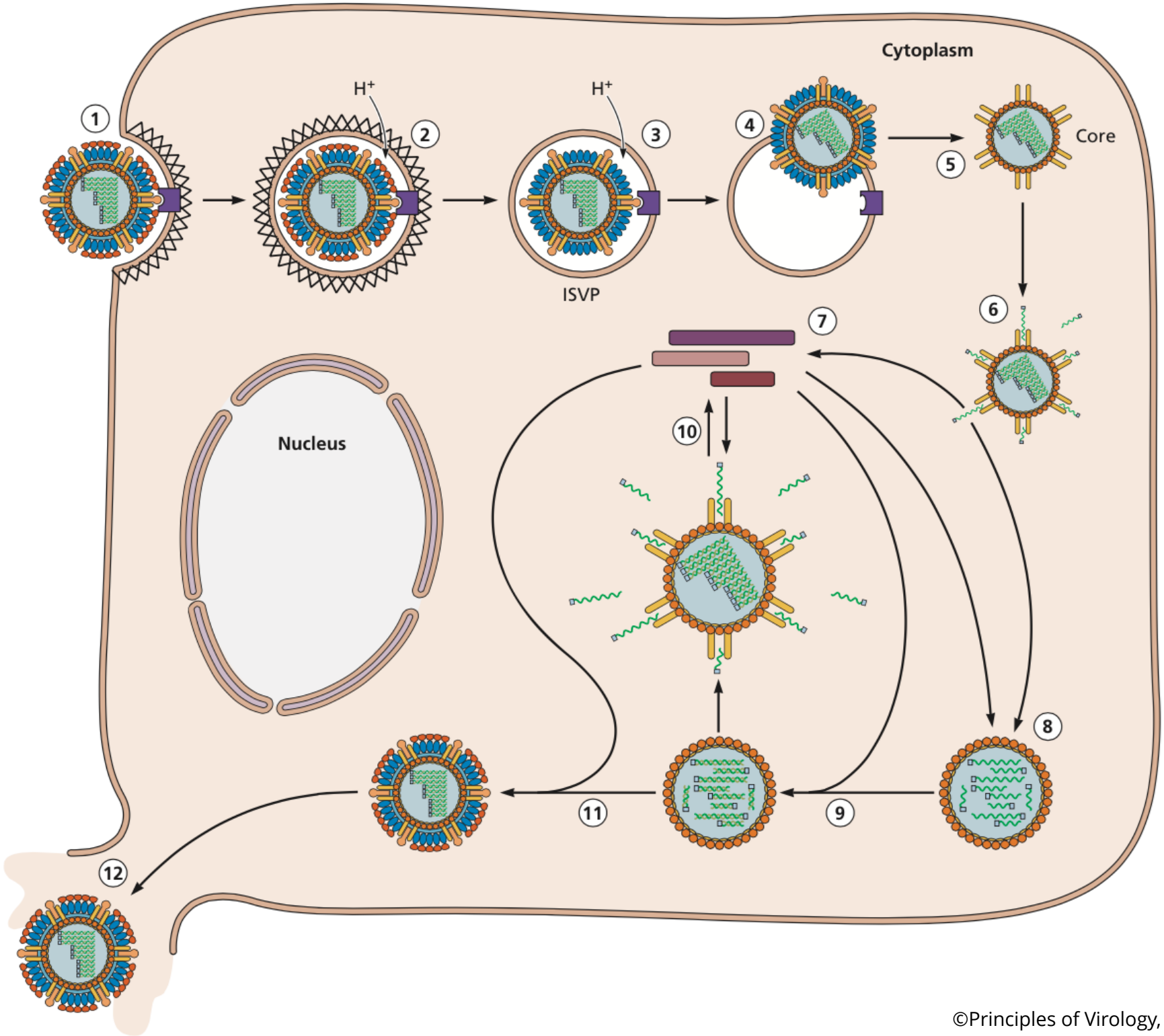
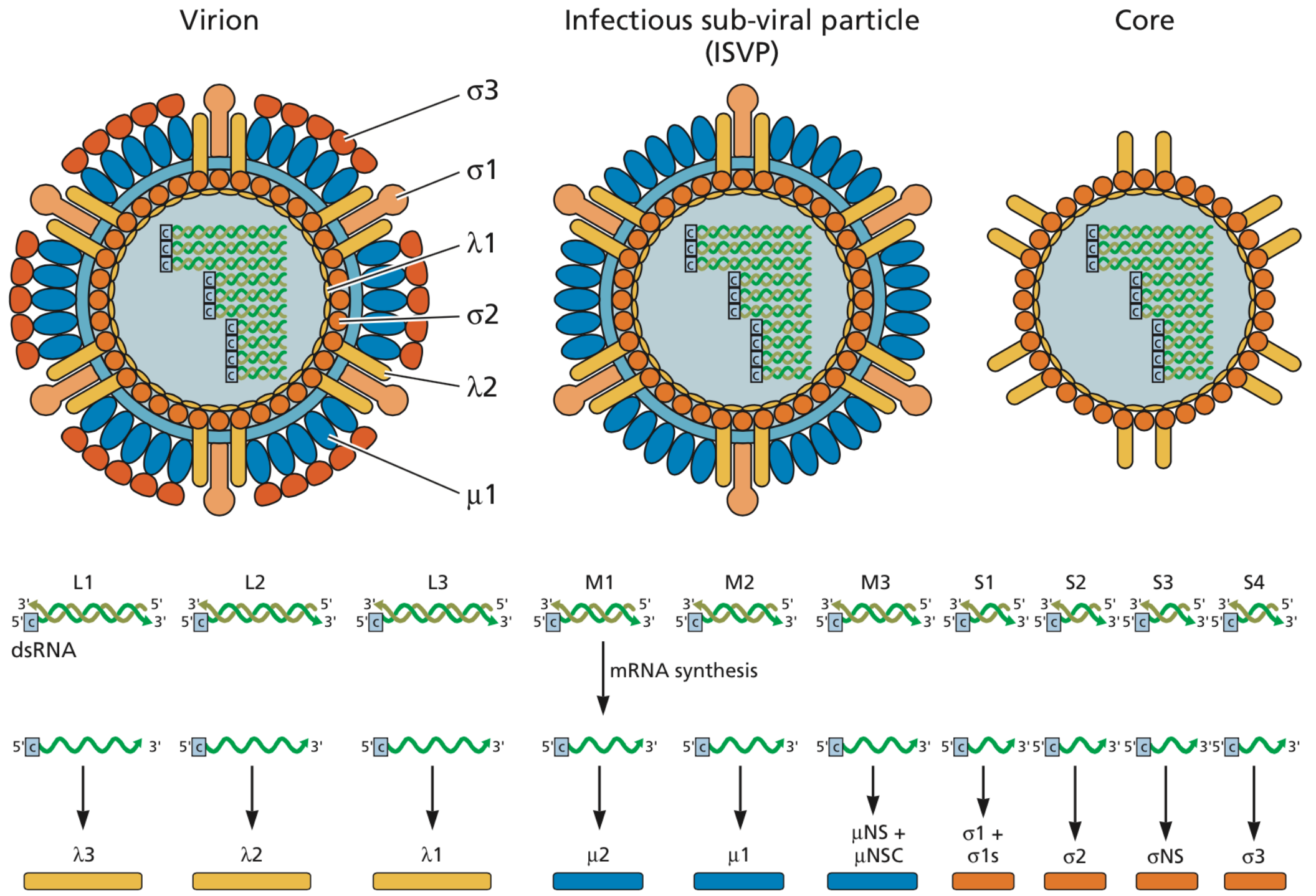
How are influenza virus and VSV RNA synthesis similar?

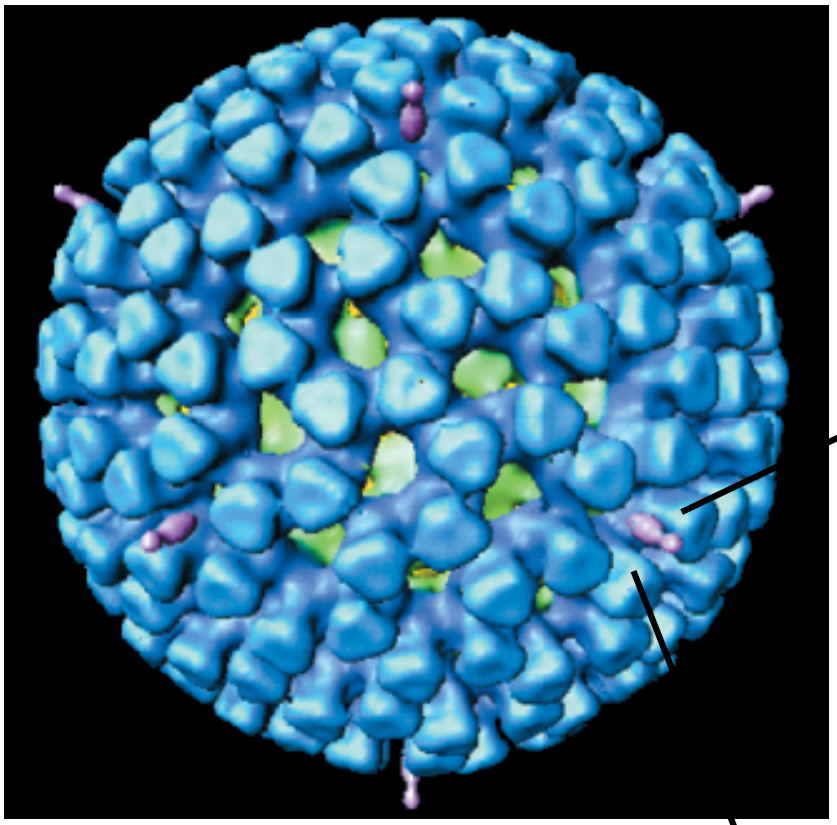
1. The switch from mRNA to genome RNA synthesis is controlled by an RNA binding protein
2. Polyadenylation occurs at a short stretch of U residues
3. Viral mRNAs are shorter than (-) genome RNA
4. All of the above

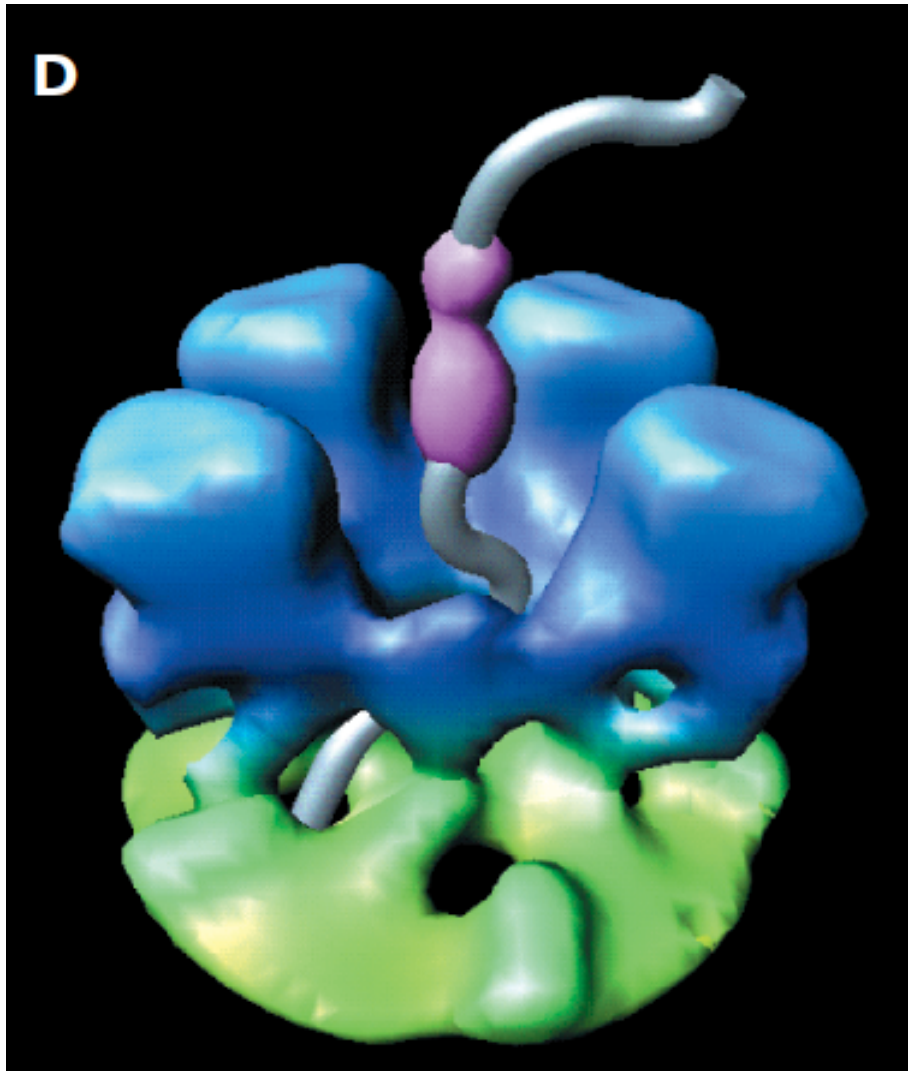


Reoviridae: reovirus, rotavirus



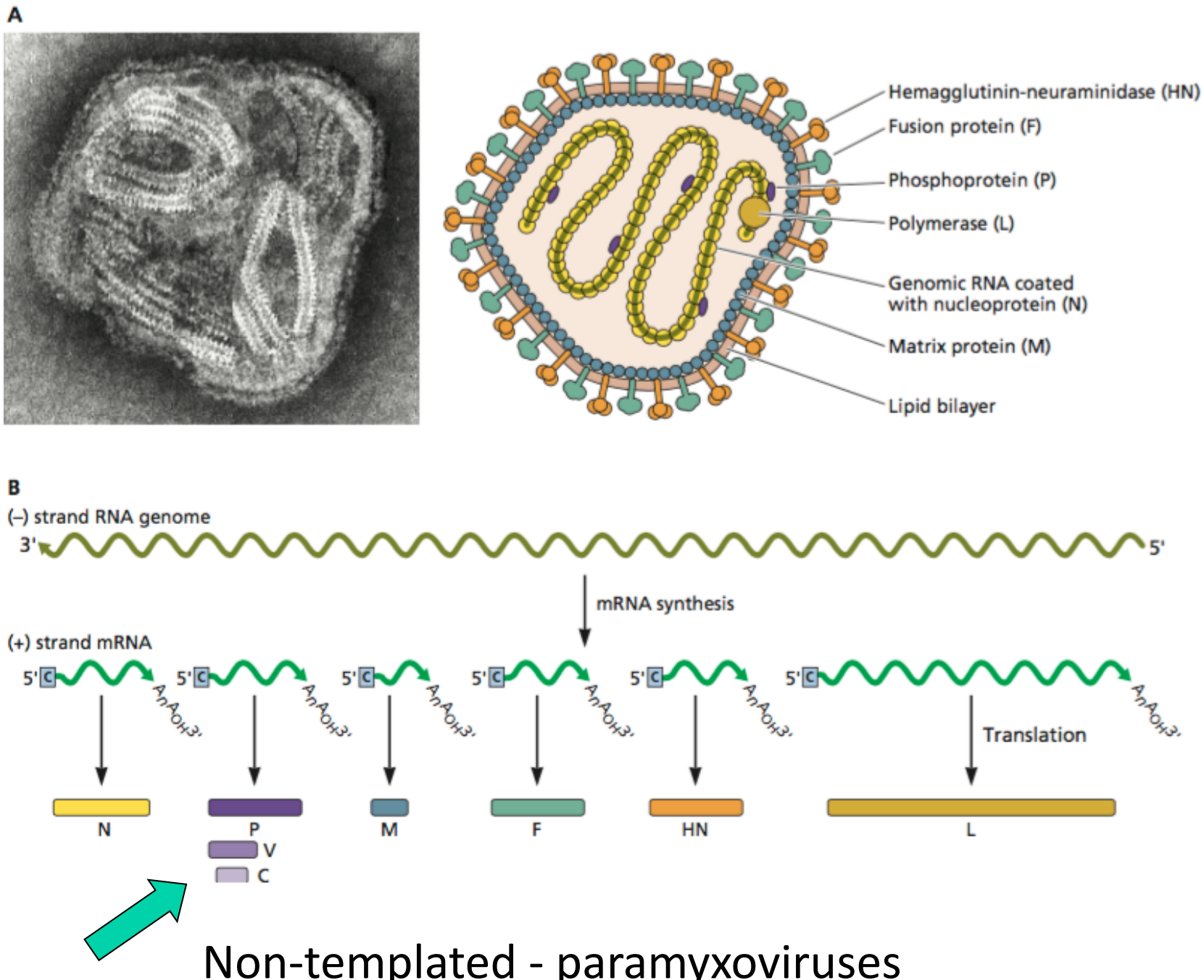






Each dsRNA segment is attached to RdRp via the 5’-cap

RNA editing



Non-templated - paramyxoviruses

RNA editing produces mRNA for Ebolavirus glycoprotein