

# **RNA directed RNA synthesis**

Lecture 6

Biology 3310/4310

Virology

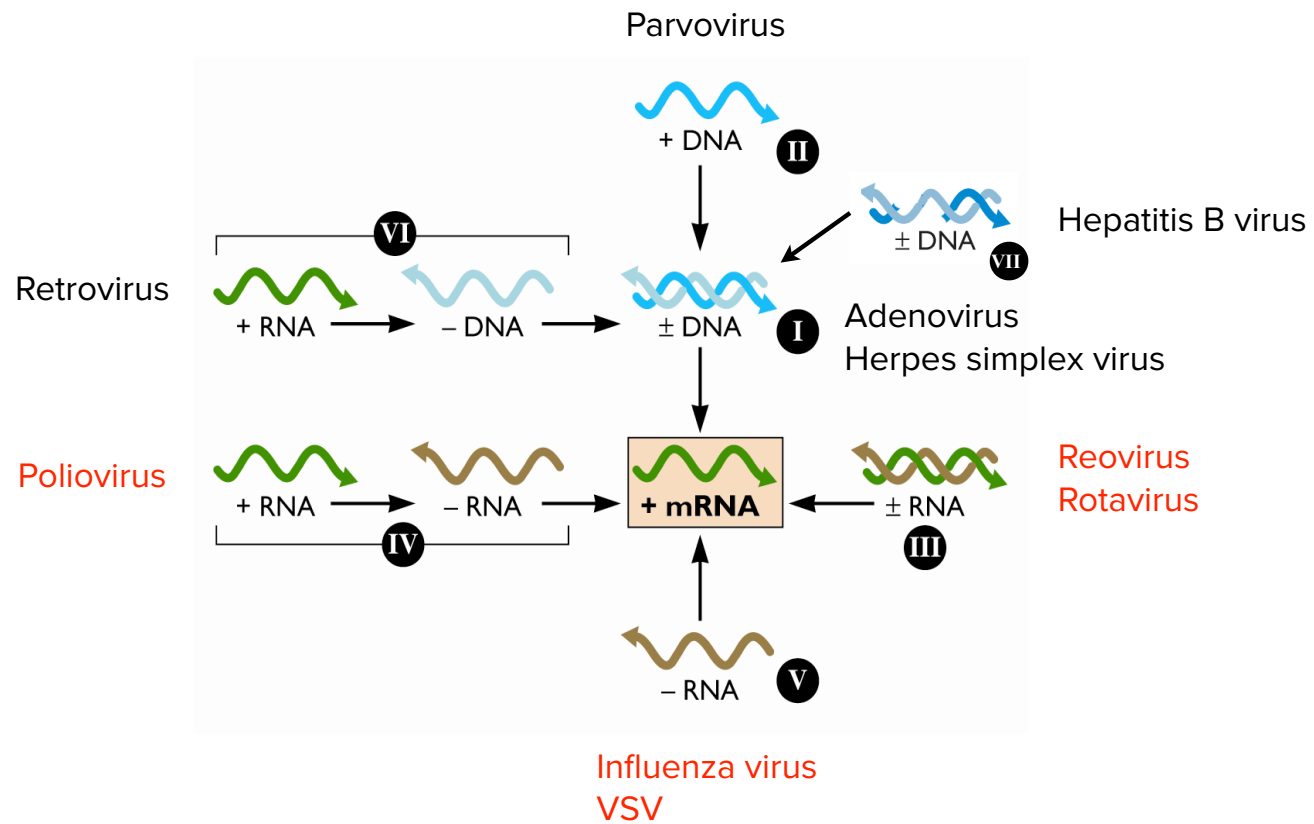
Spring 2017

*Truth is ever to be found in the  
simplicity, and not in the multiplicity and  
confusion of things*

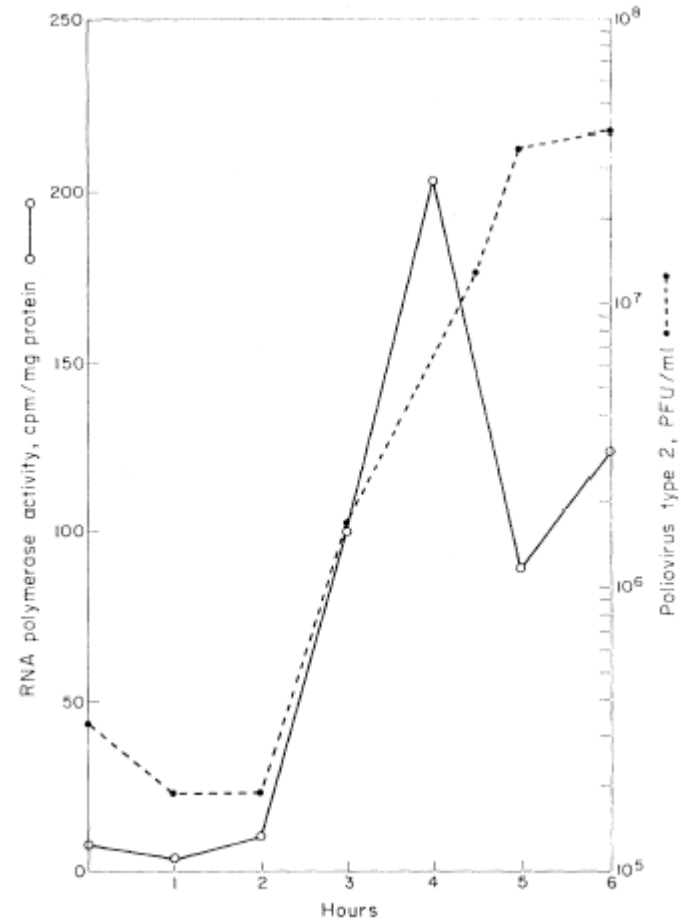
--SIR ISAAC NEWTON

## 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



# Identification of RNA polymerases

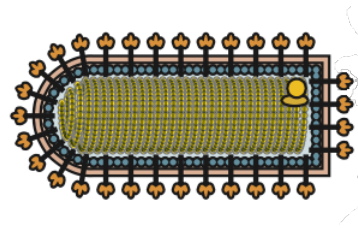


Assays: cell extracts incubated with NTPs



# Identification of RNA polymerases

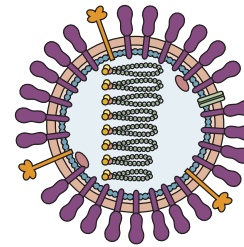
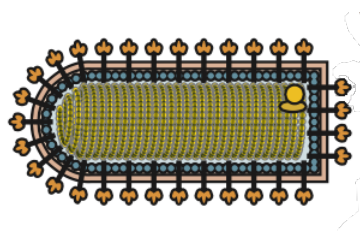
- Polymerase discovered in (-) strand virus particles



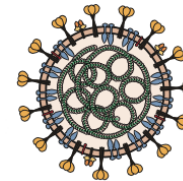
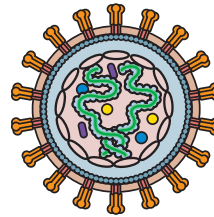
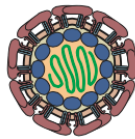
- Sequence alignments (GDD), synthesis of recombinant proteins
- Crystal structures

# RNA in the virus particle

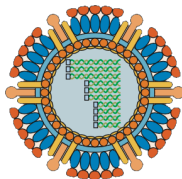
- (-) strand RNA genomes: coated with protein

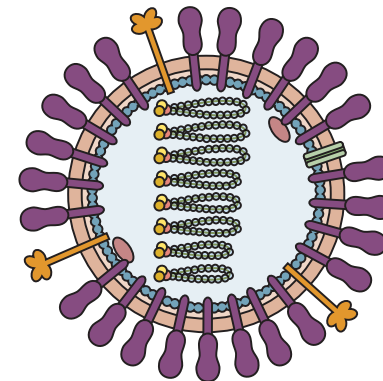
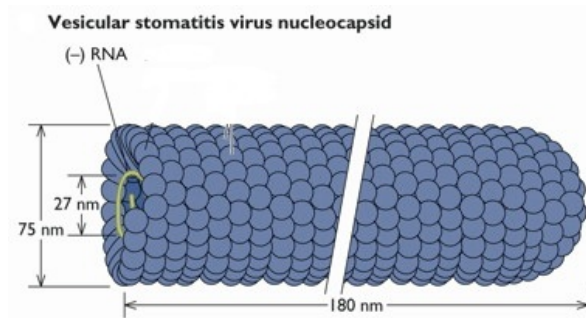
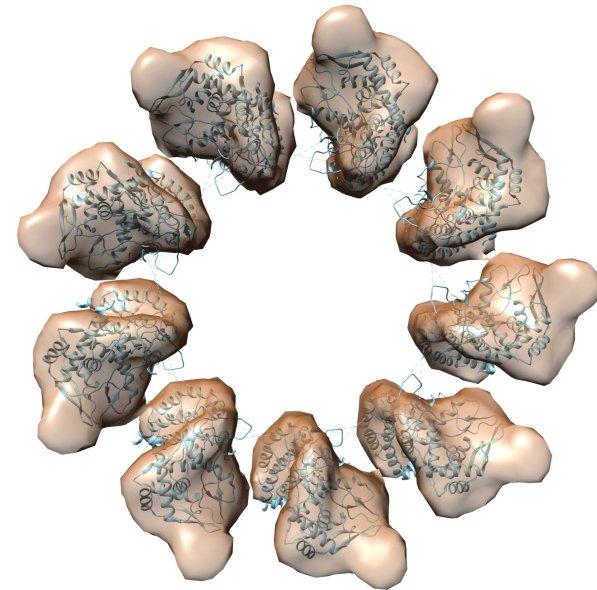
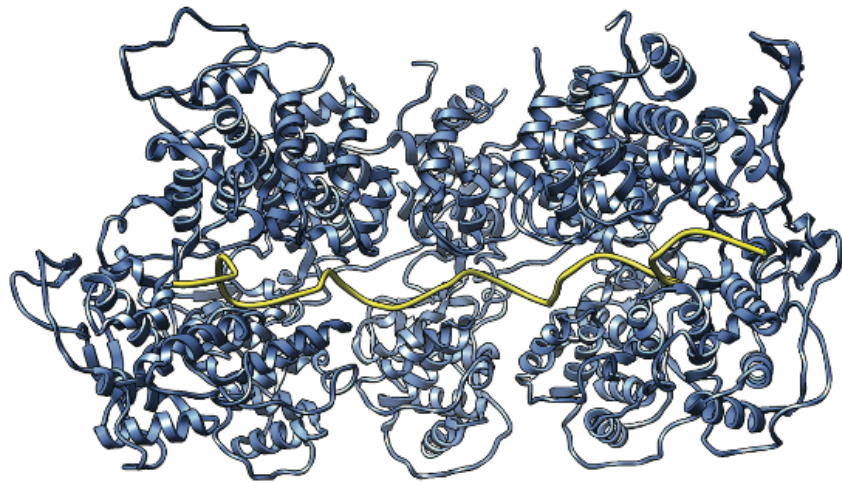


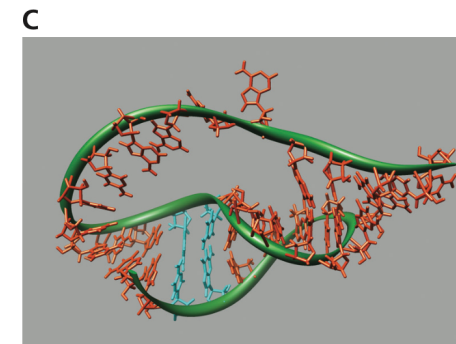
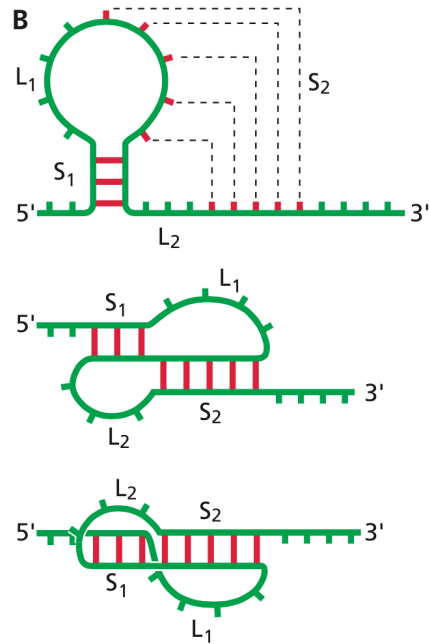
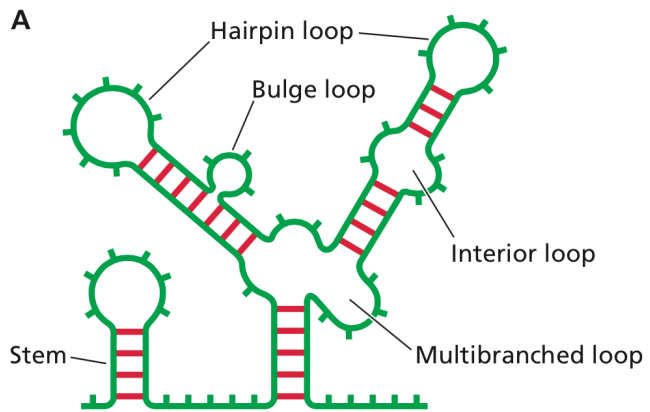
- (+) strand RNA genomes: naked (exceptions: retrovirus, coronavirus)



- dsRNA genomes







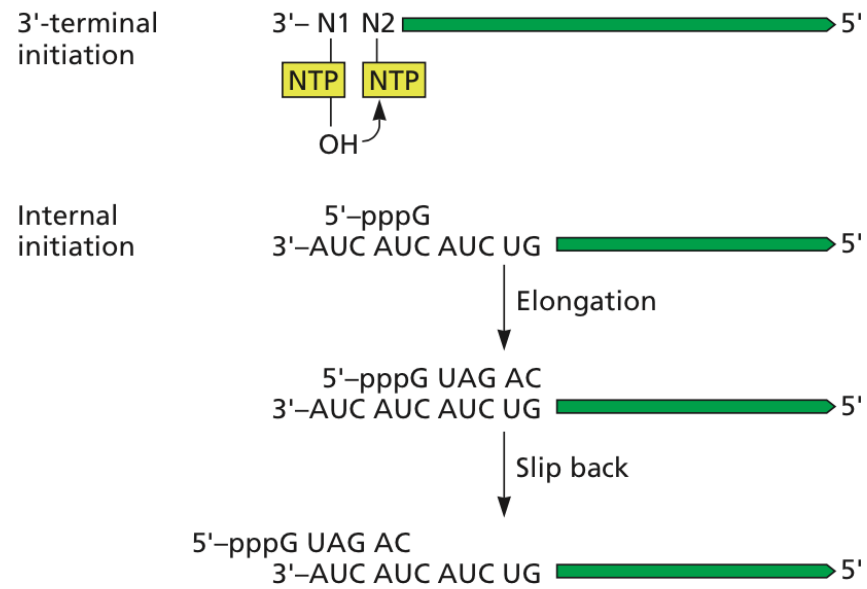


- RNA genome must be copied end to end with no loss of nucleotide sequence
- Production of viral mRNAs that can be efficiently translated by cellular protein synthesis machinery

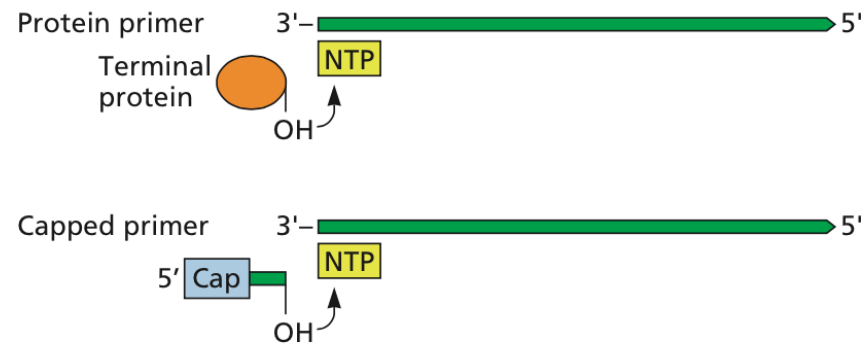
# Universal rules for RNA-directed RNA synthesis

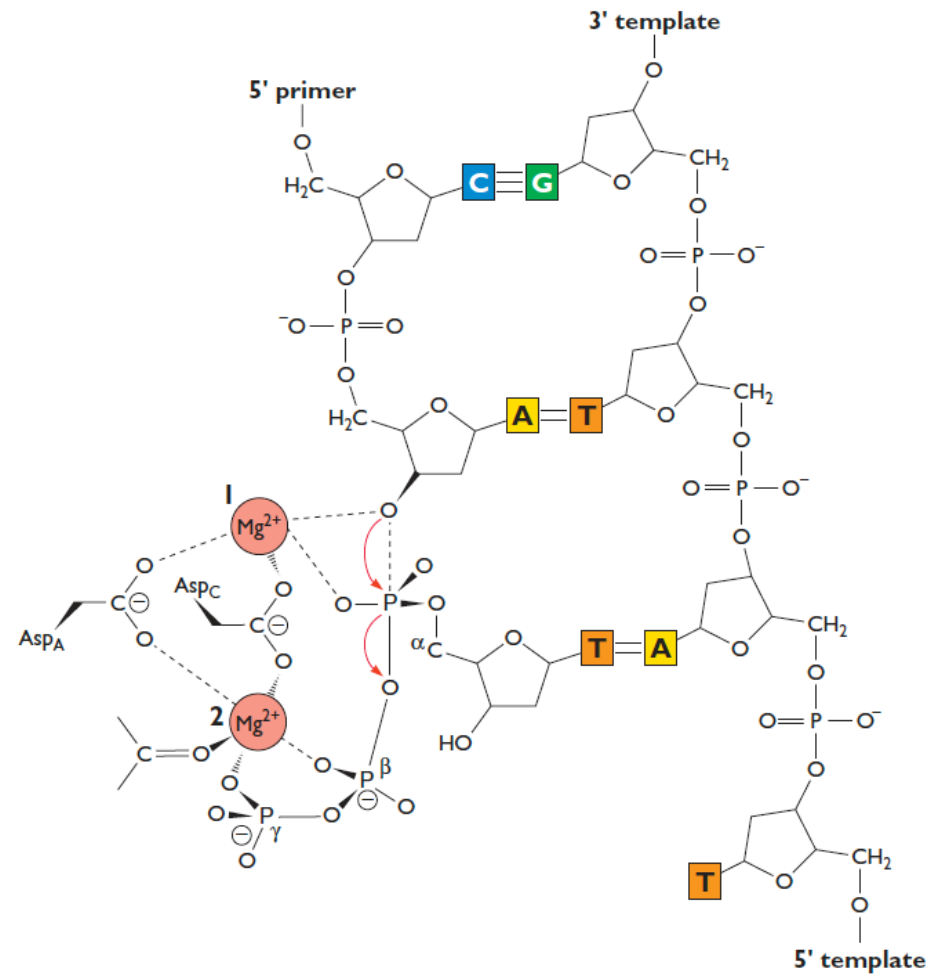
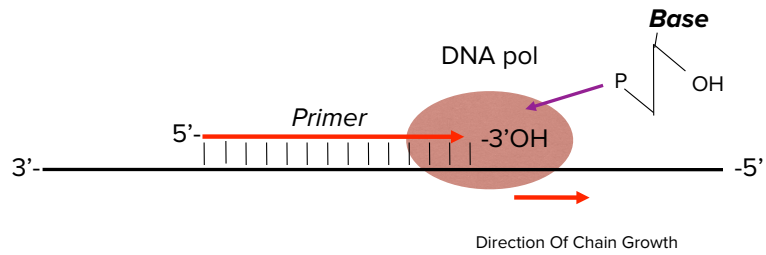
- RNA synthesis initiates and terminates at specific sites on the template
- RdRp may initiate synthesis *de novo* (like cellular DdRp) or require a primer
- Other viral and cell proteins may be required
- RNA is synthesized by template-directed stepwise incorporation of NTPs, elongated in 5'-3' direction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

### *De novo* initiation



### Primer-dependent initiation







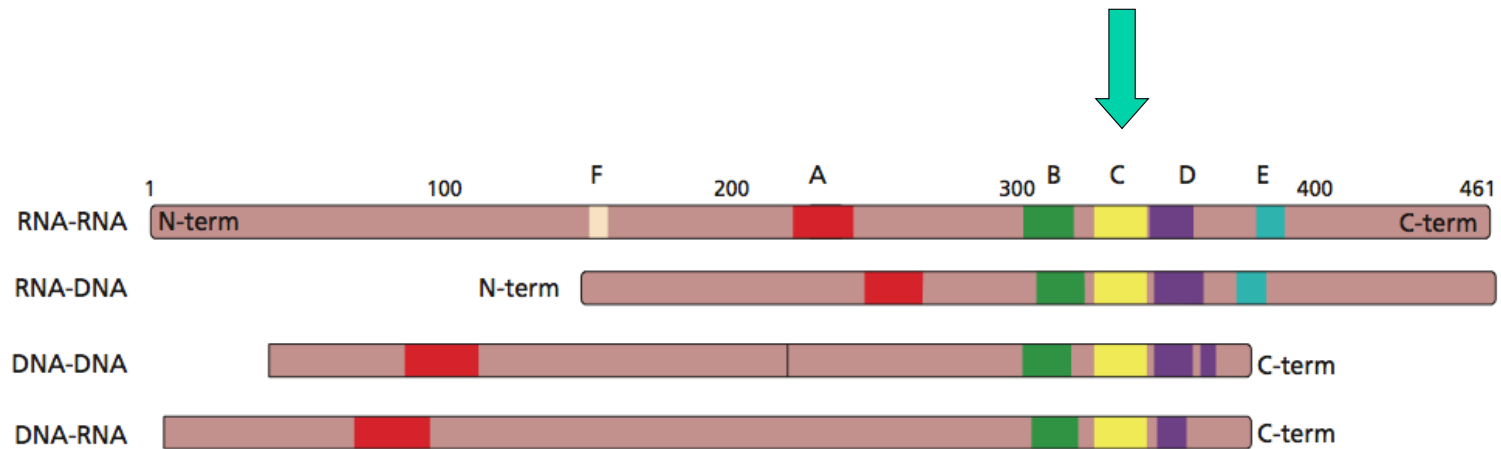
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room number: virus

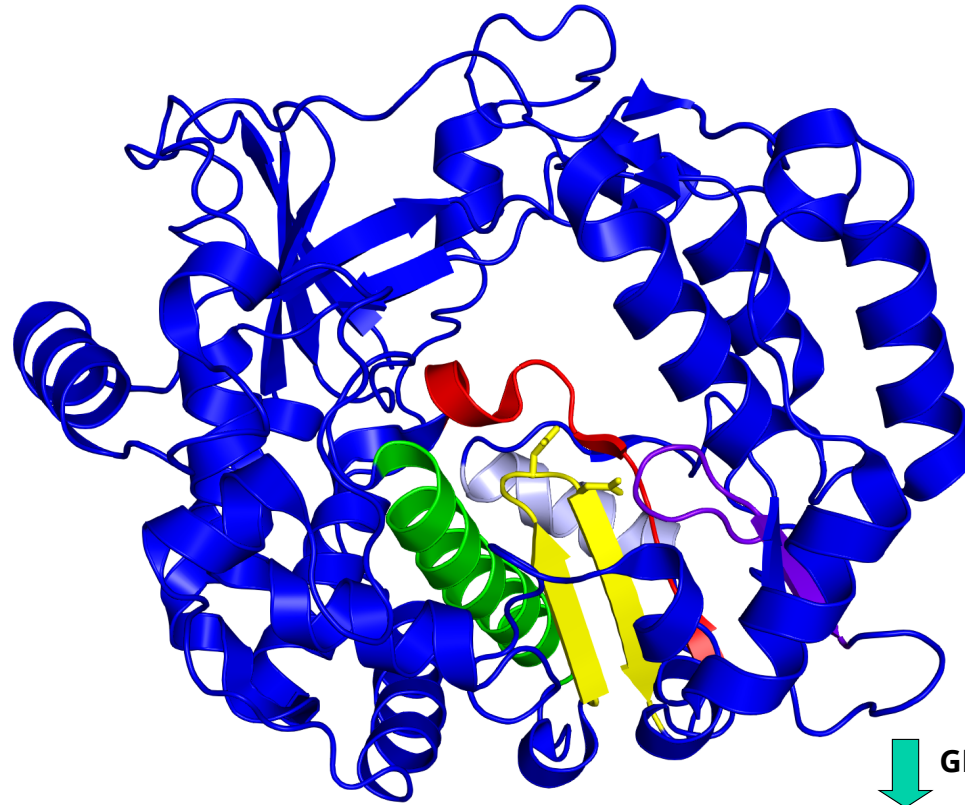
**Which is a universal rule about RNA directed RNA synthesis?**

- 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

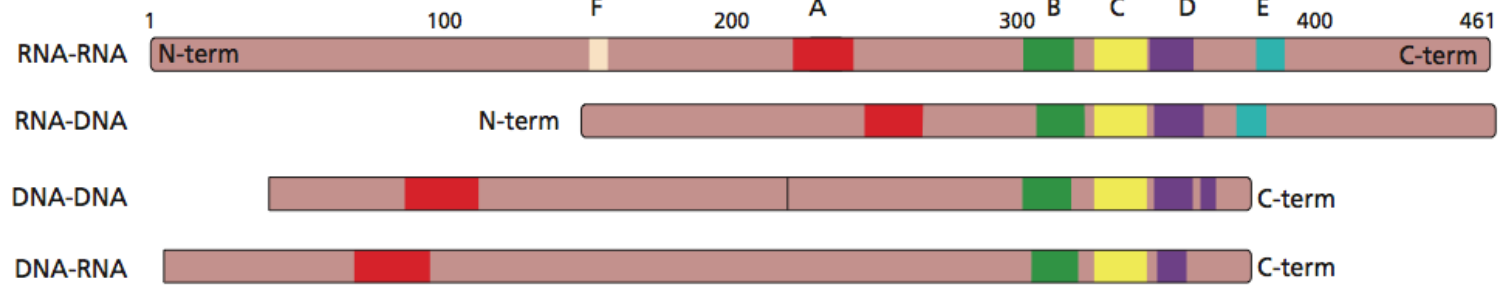
# Sequence relationships among polymerases

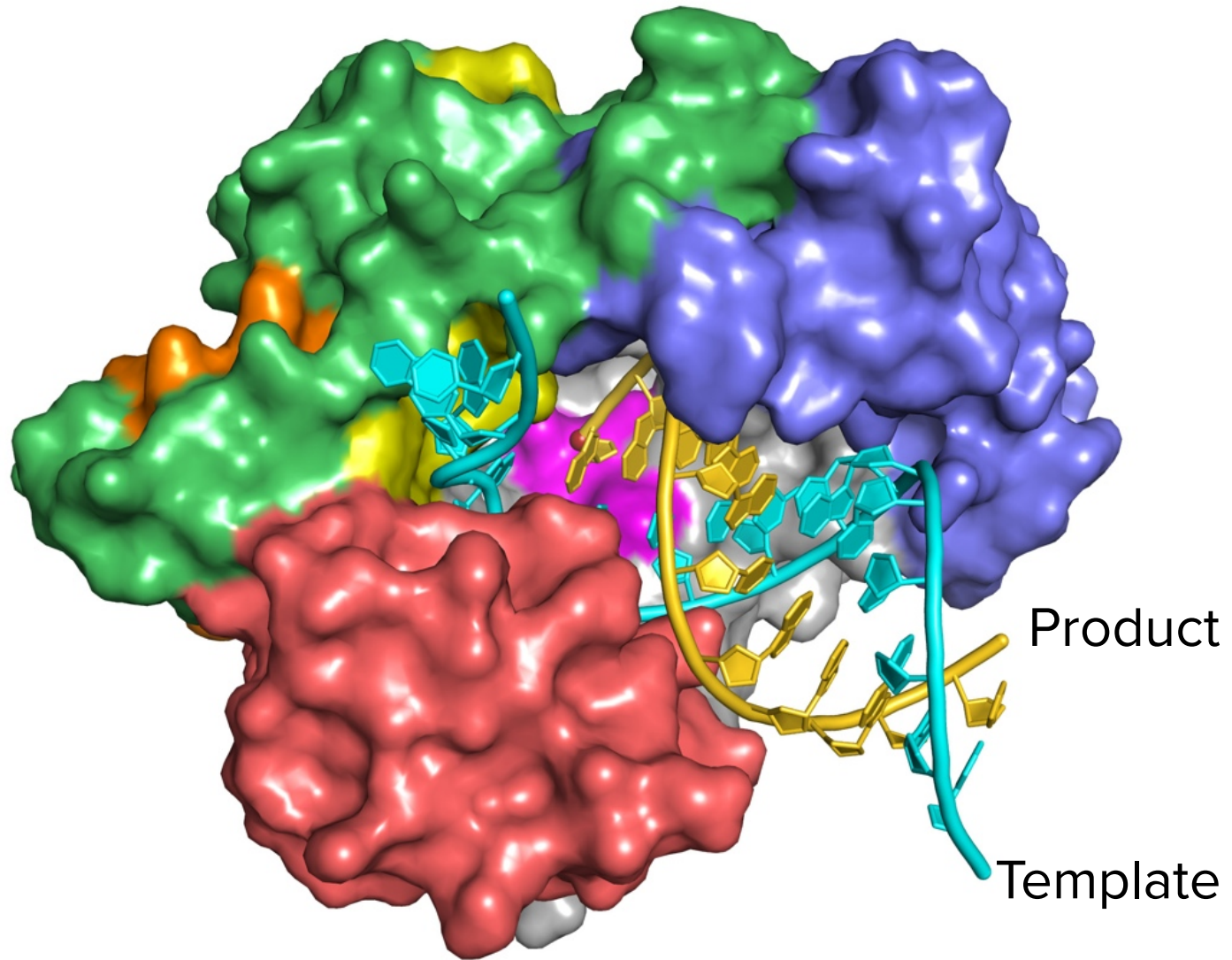


- ➡ • Gly-Asp-Asp in (+) strand RNA polymerases
- ➡ • Asp-Asp in RT, segmented (-) strand polymerases
- ➡ • Gly-Asp-Asn in nonsegmented (-) strand polymerases



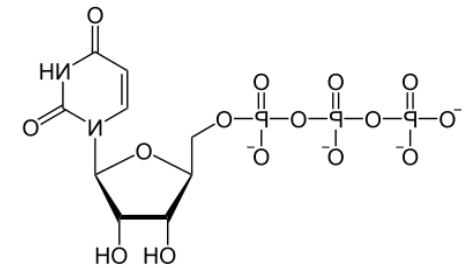
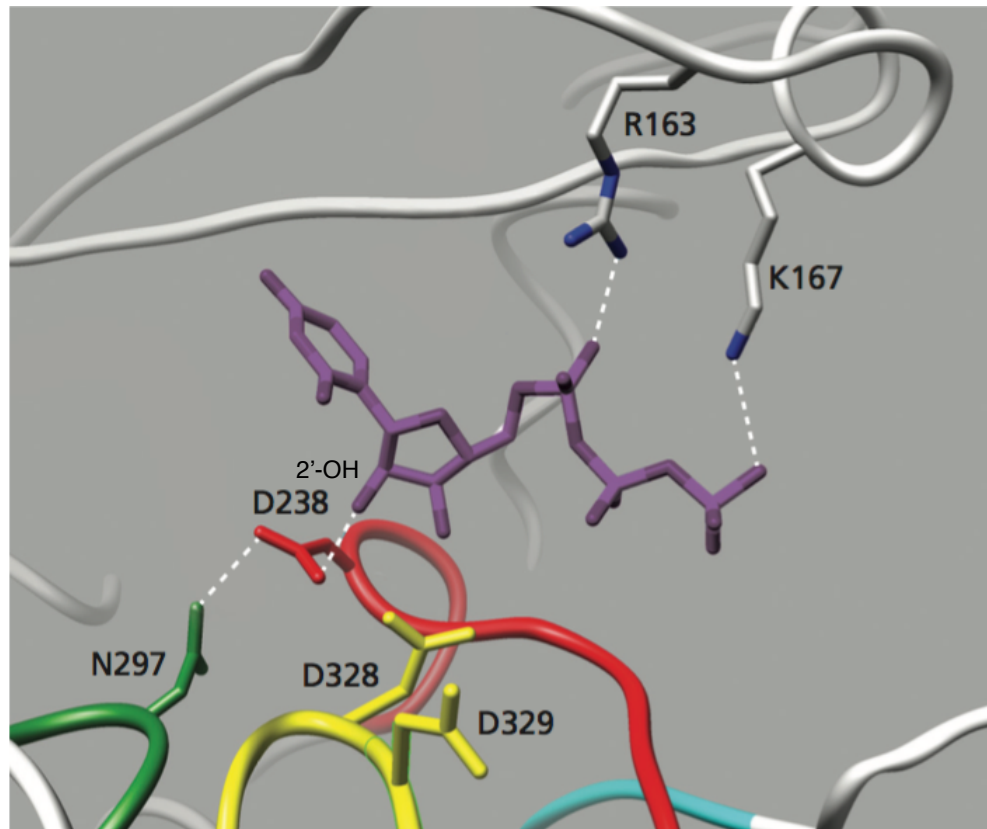
GDD







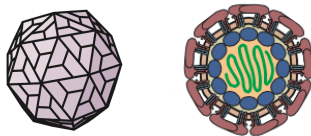
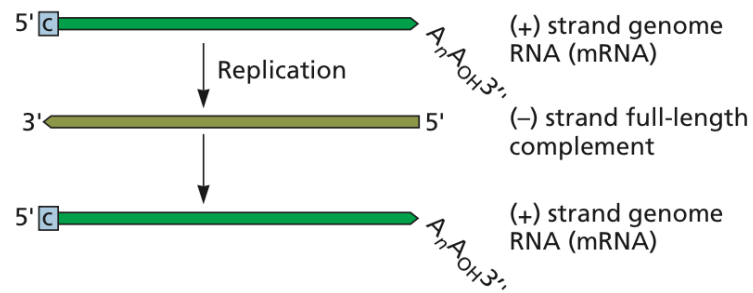
# Structure of UTP bound to poliovirus RdRp



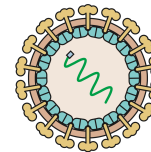
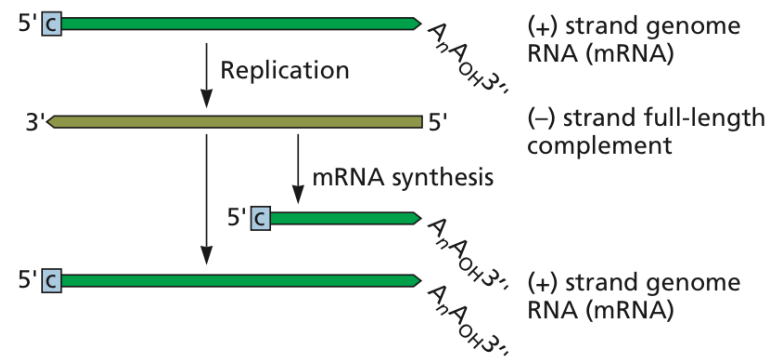
# (+) strand RNA viruses

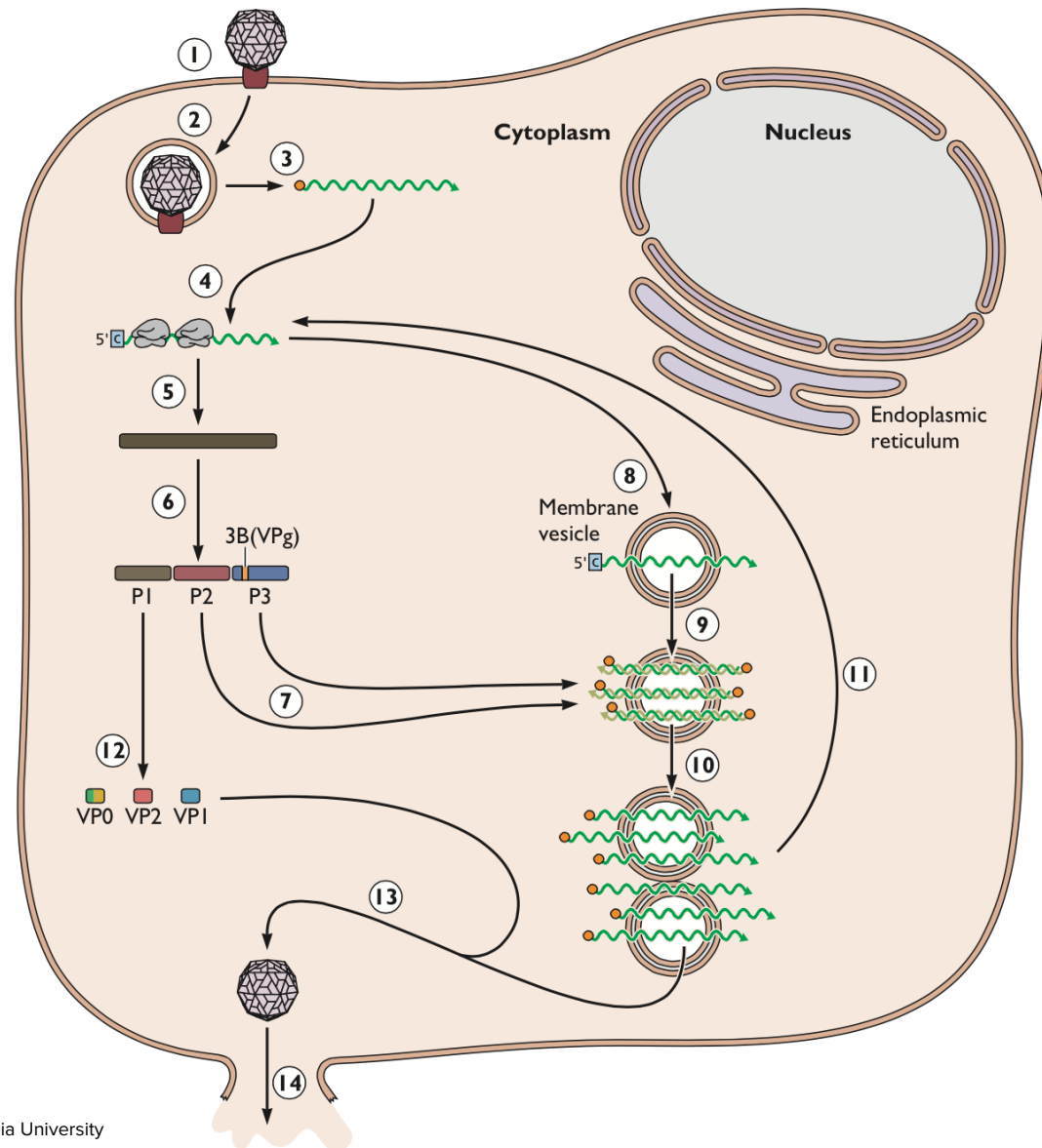
## (+) strand RNA viruses

Flavi- and picornaviruses



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



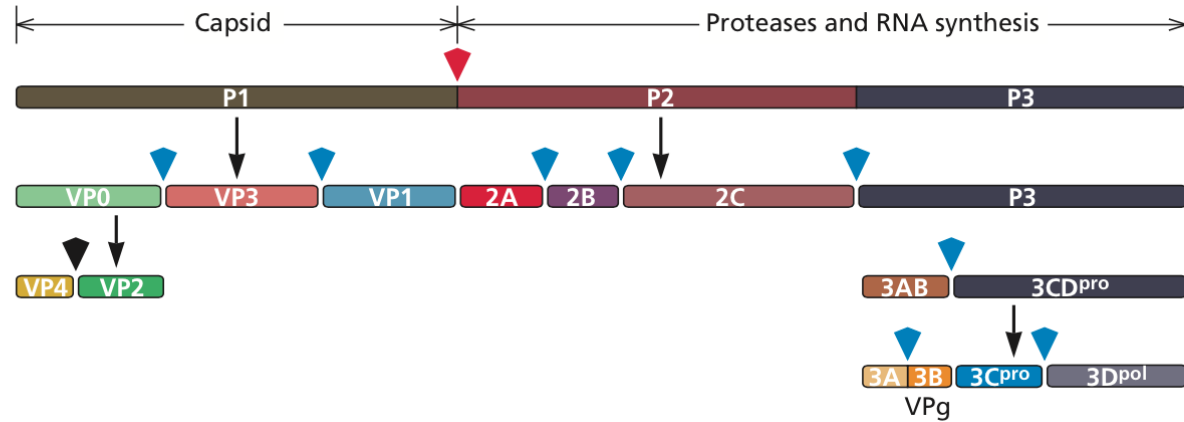




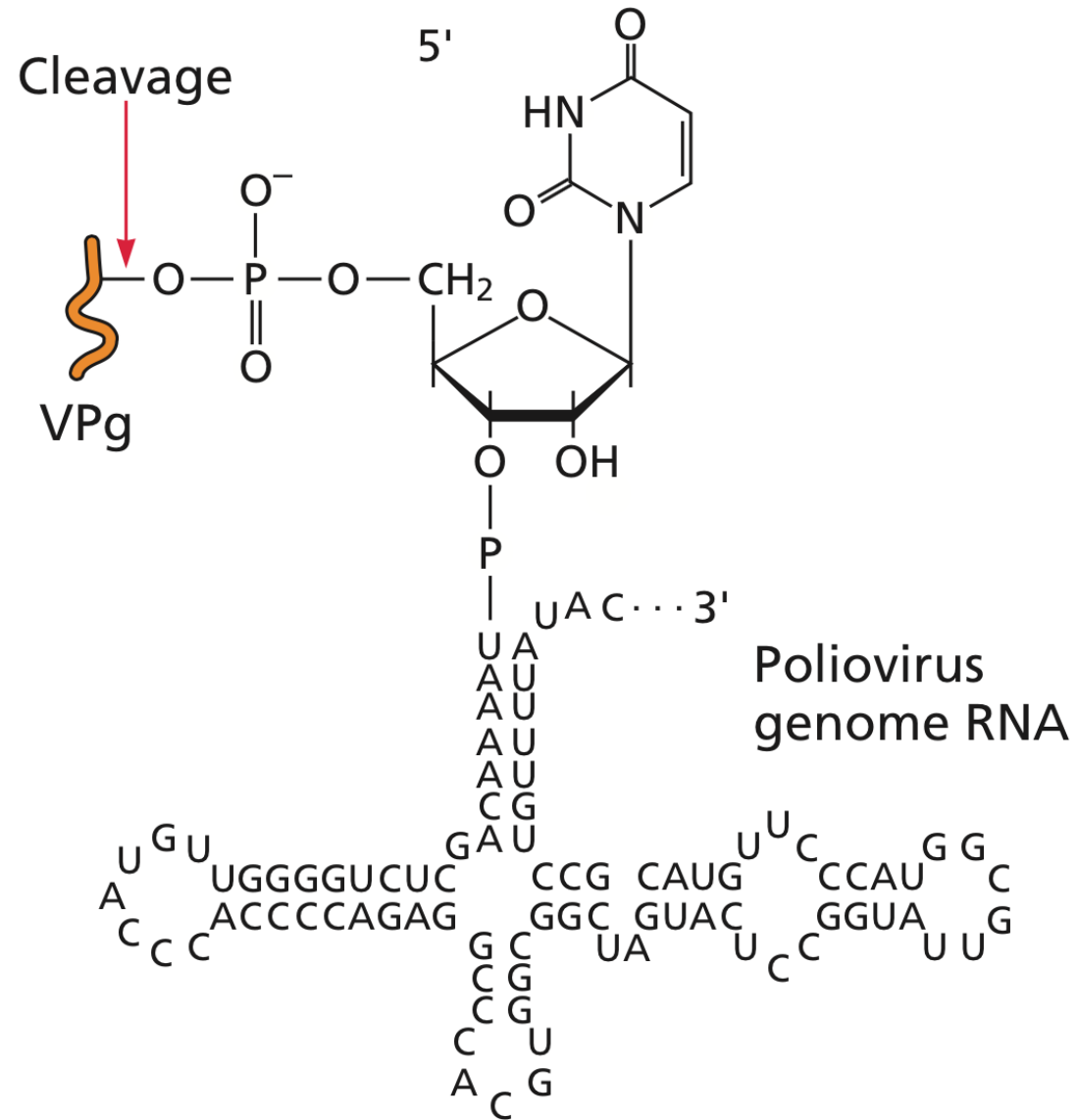
Viral (+) strand genome

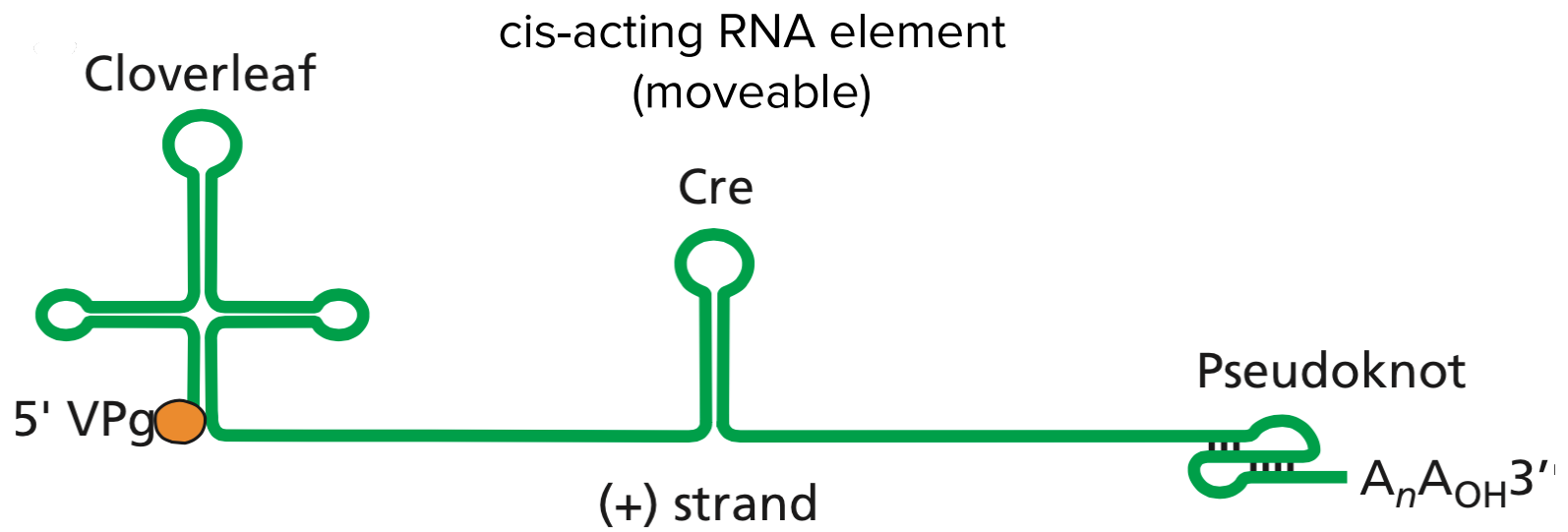


Translation, processing

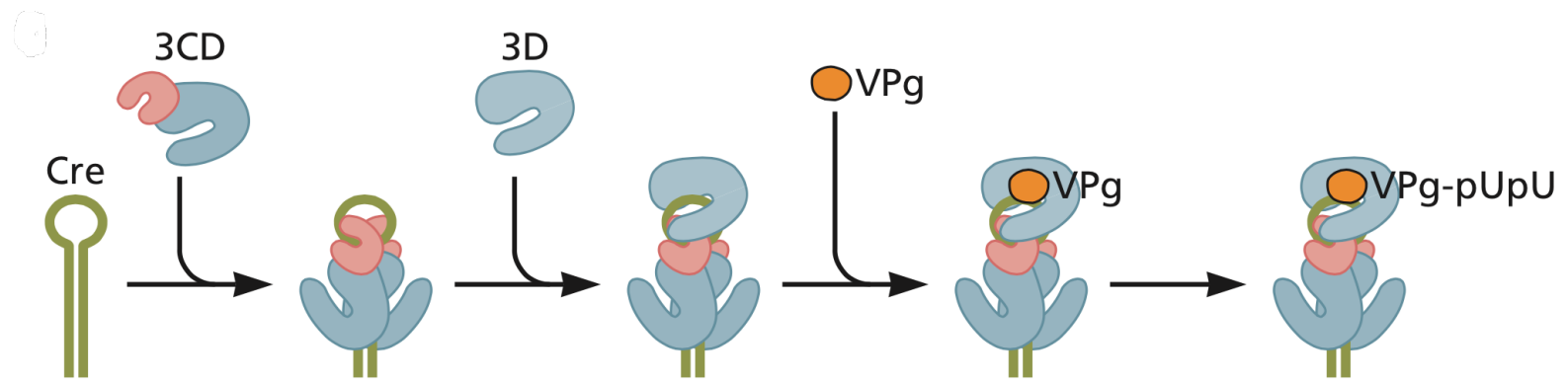


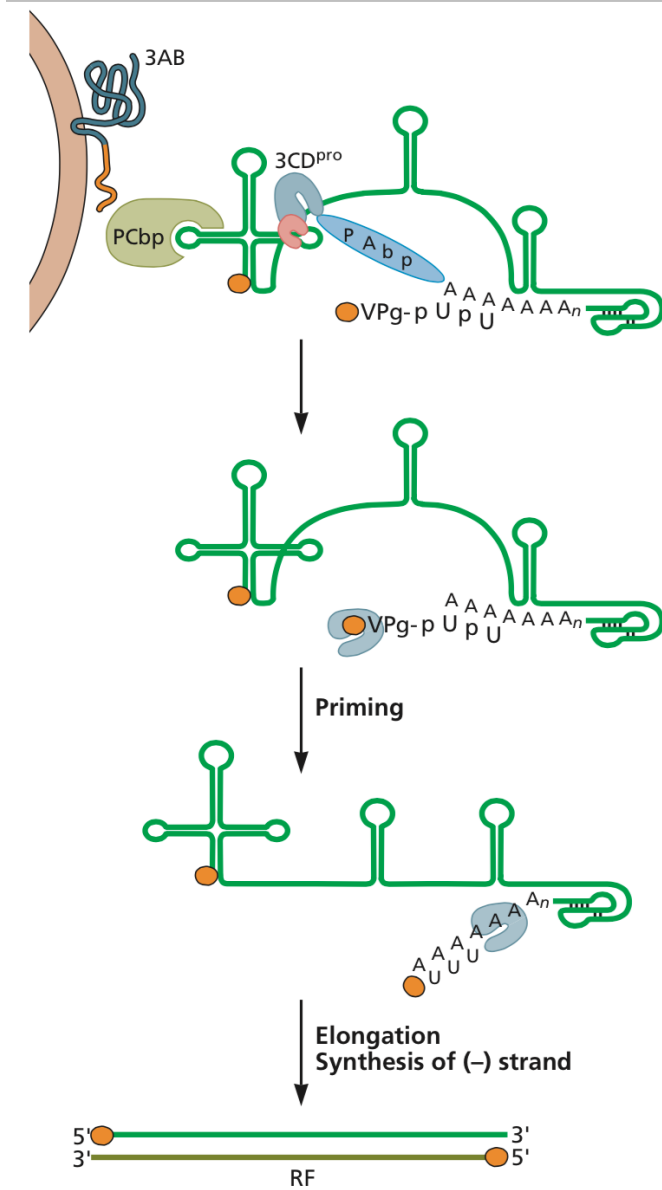




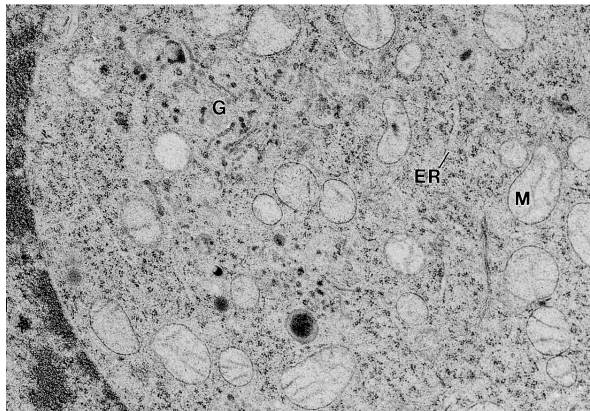


*Cellular polyadenylated RNAs not copied*

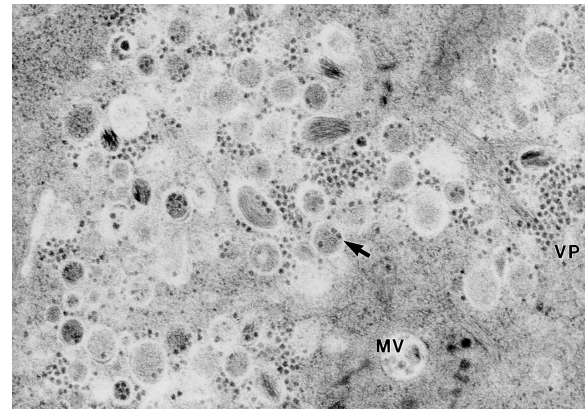




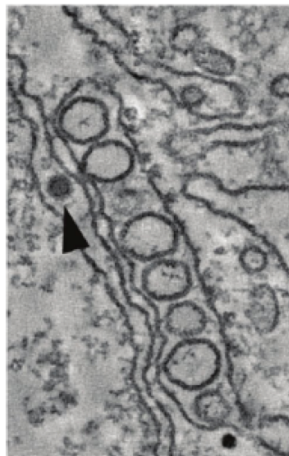
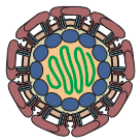
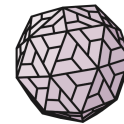
# Vesicle formation in virus-infected cells



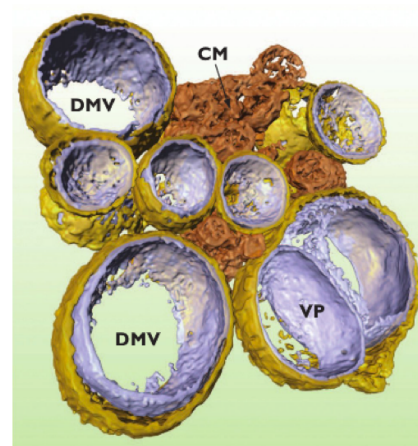
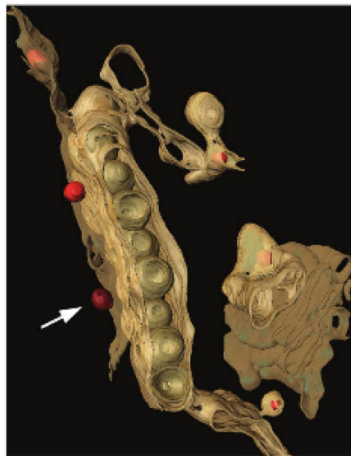
Uninfected HeLa cell



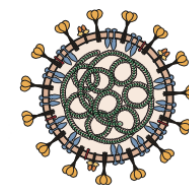
PV-infected HeLa cell



Flavivirus infected cell



Coronavirus-infected cell



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room number: virus

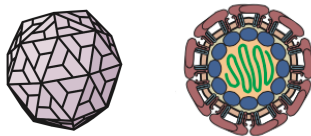
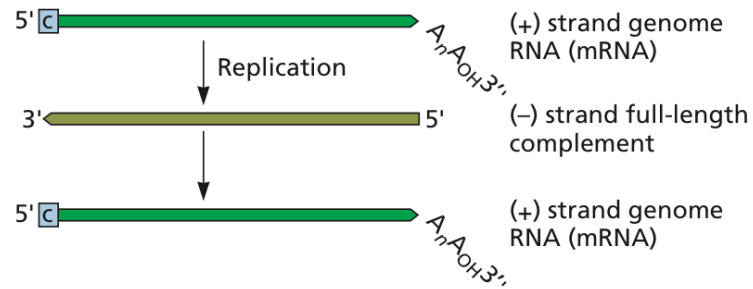
**Which is a part of the poliovirus replication strategy?**

- 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

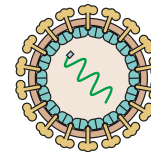
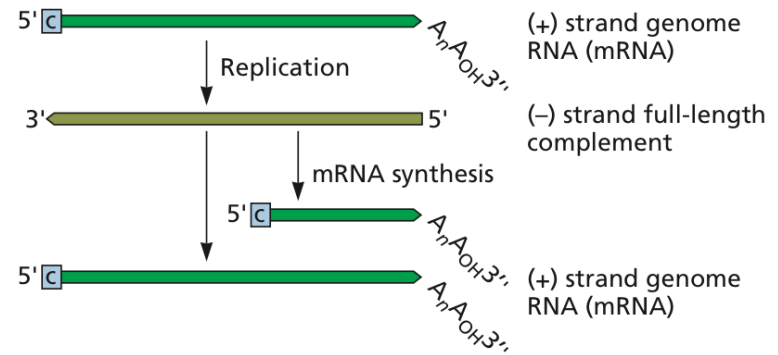
# (+) Strand RNA viruses

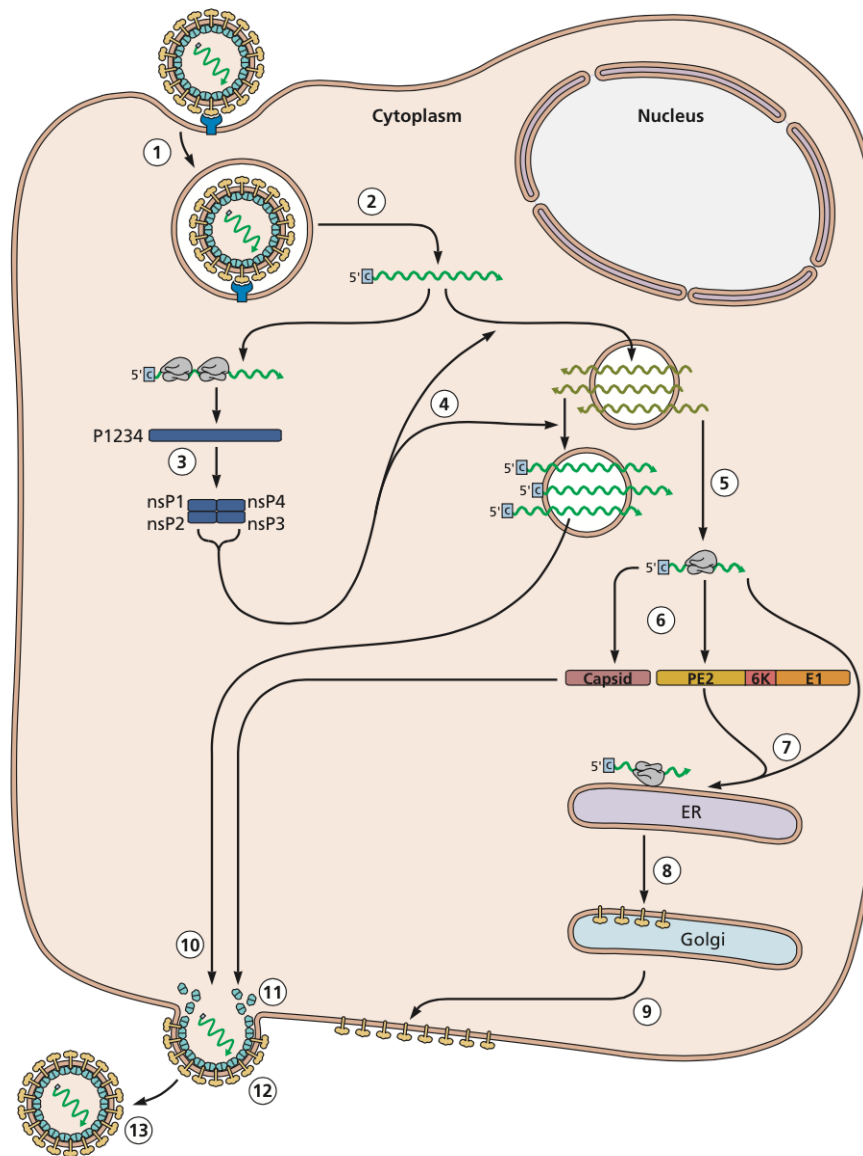
## (+) strand RNA viruses

Flavi- and picornaviruses

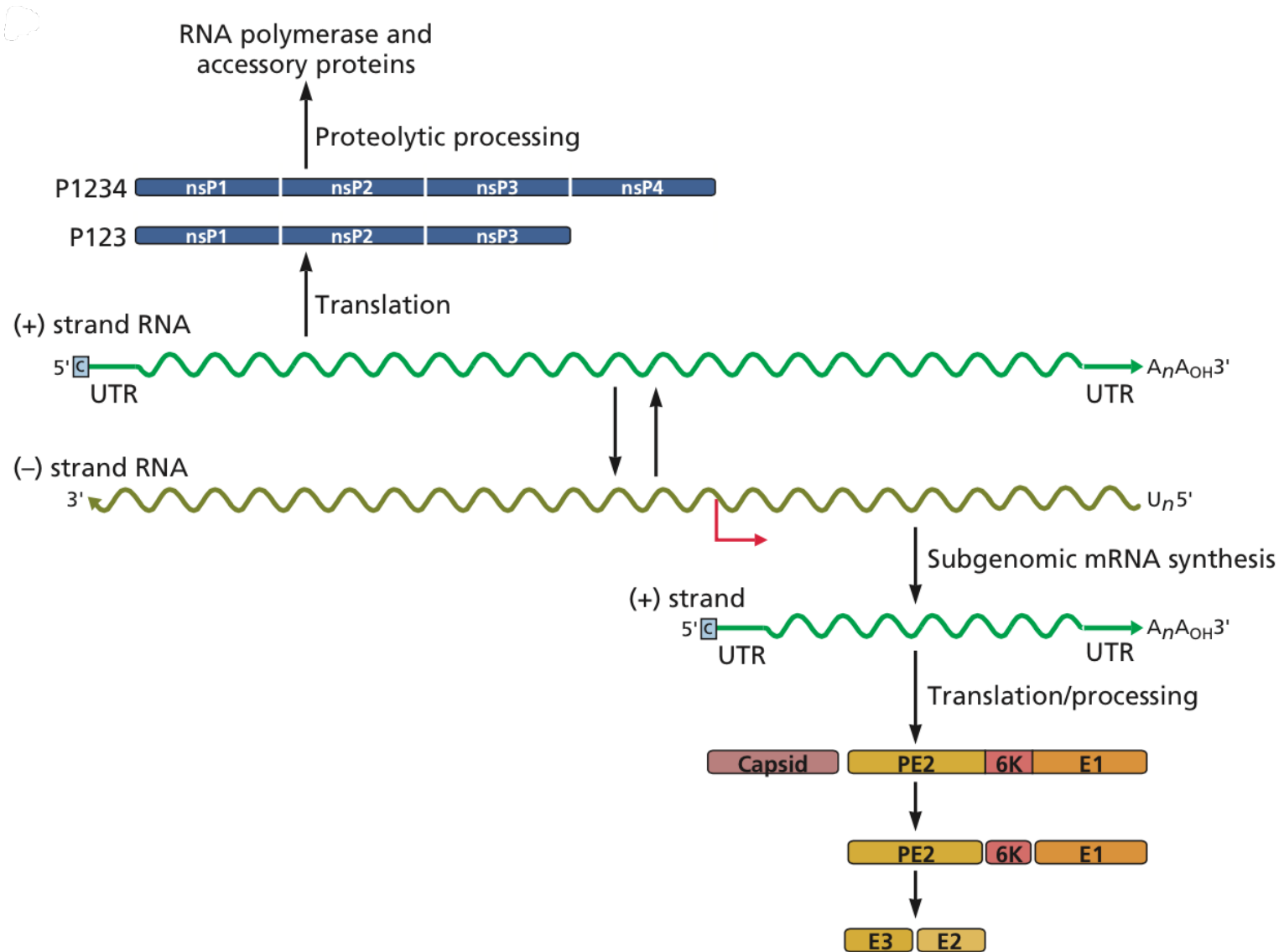
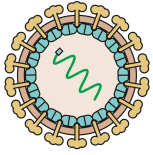


Alphaviruses (*Togaviridae* - Sindbis, SFV, Chik)





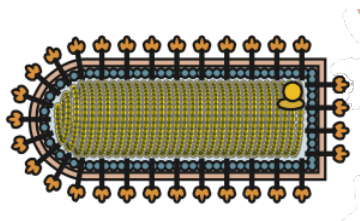
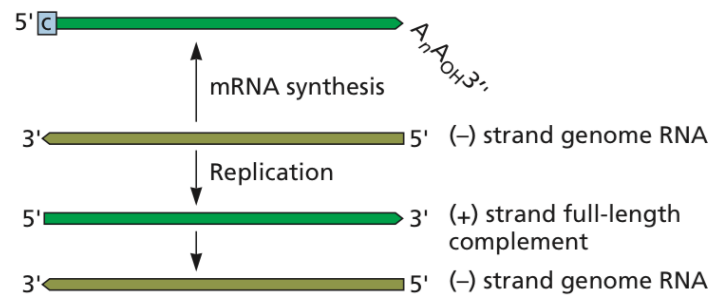




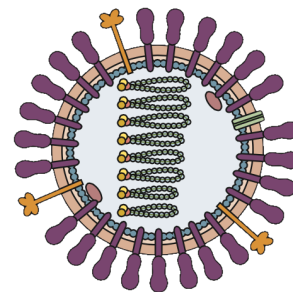
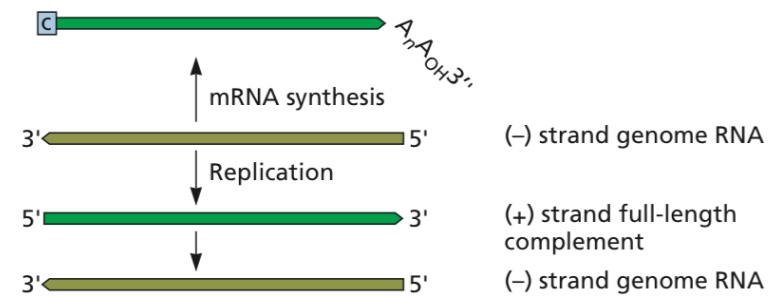
# (-) Strand RNA viruses

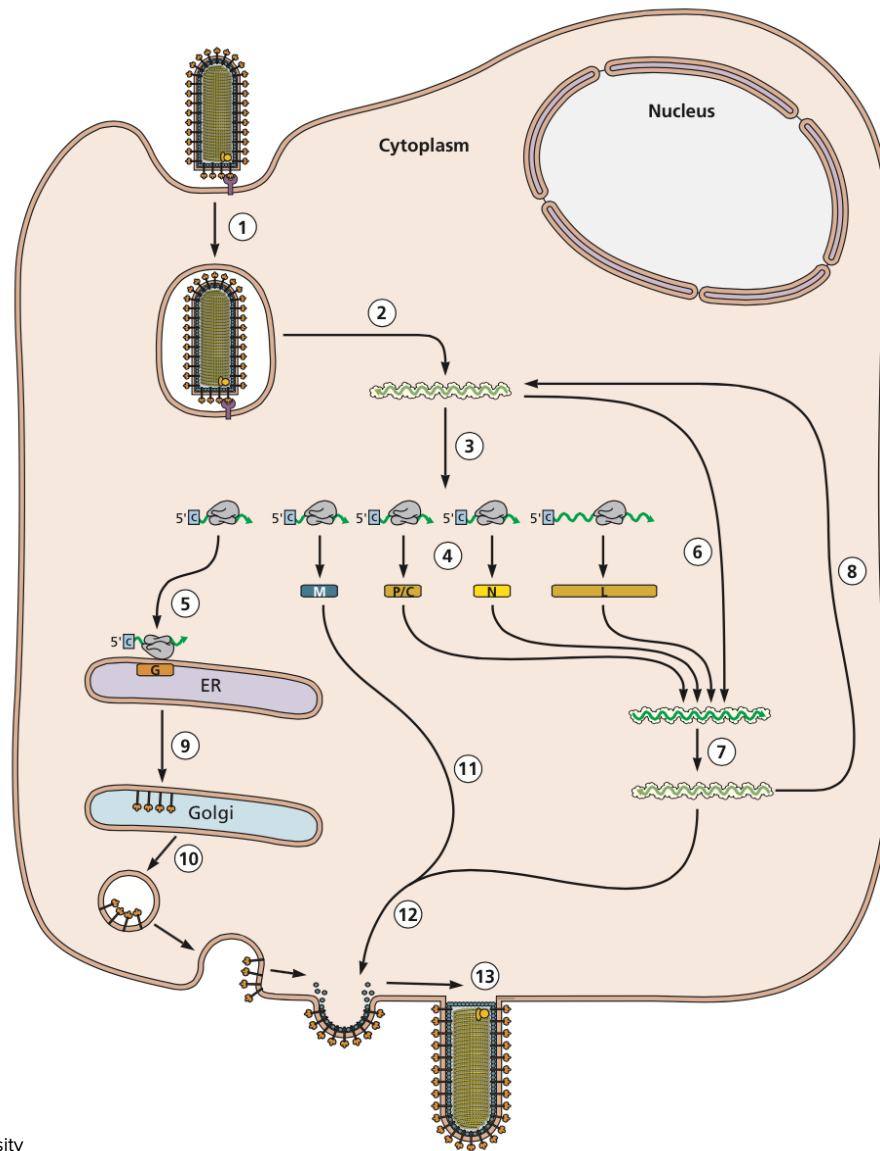
## (-) strand RNA viruses

Unimolecular

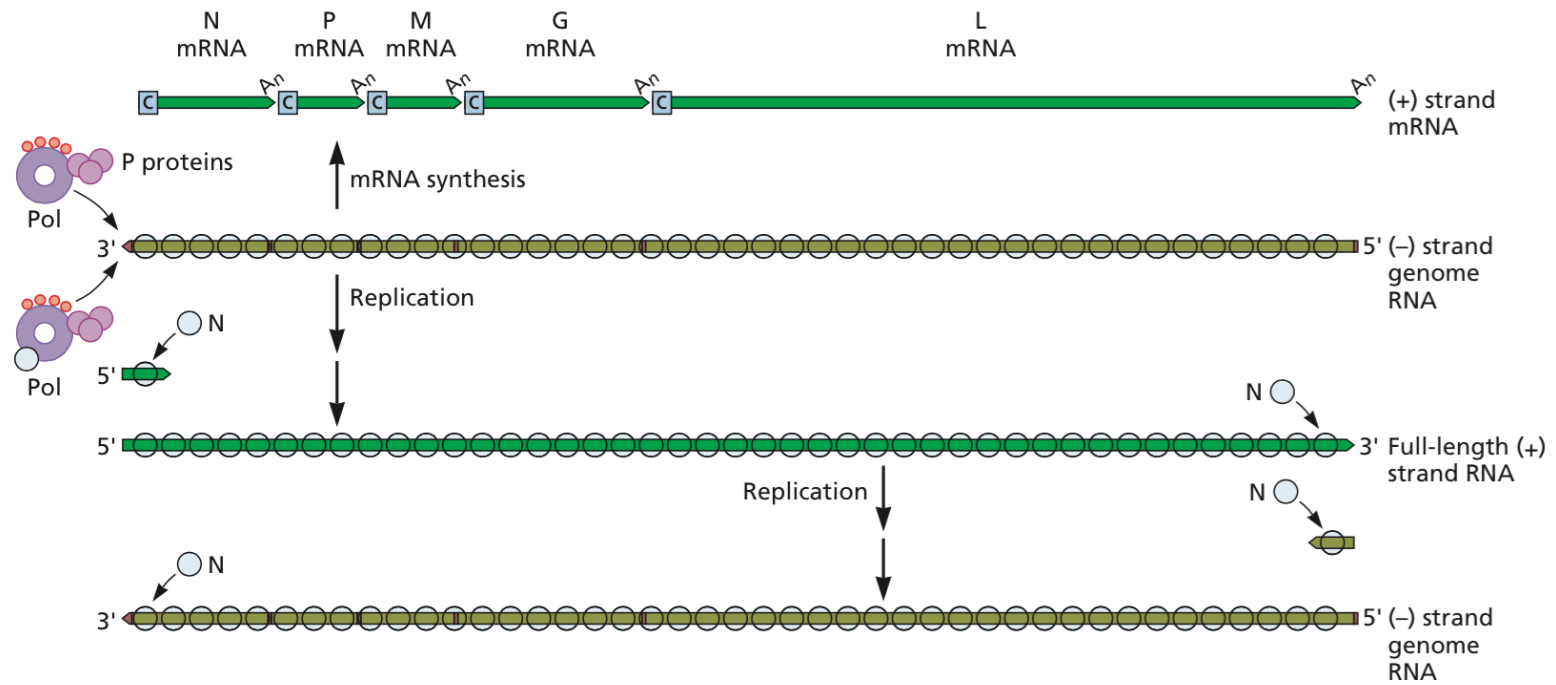
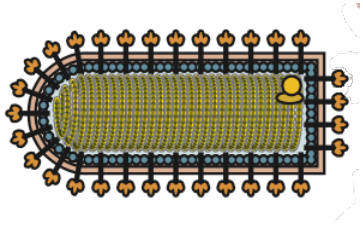


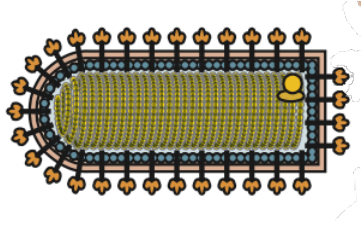
Segmented



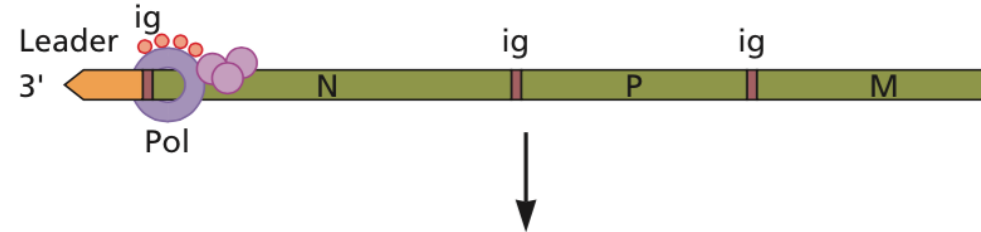




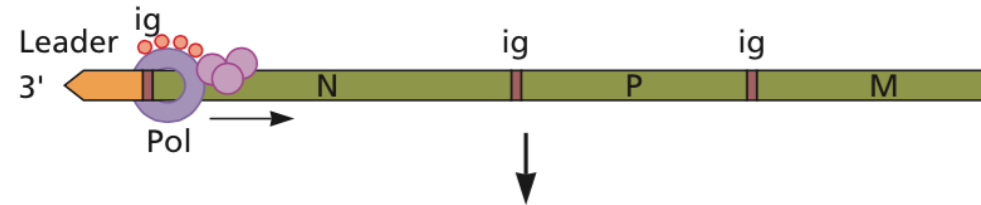




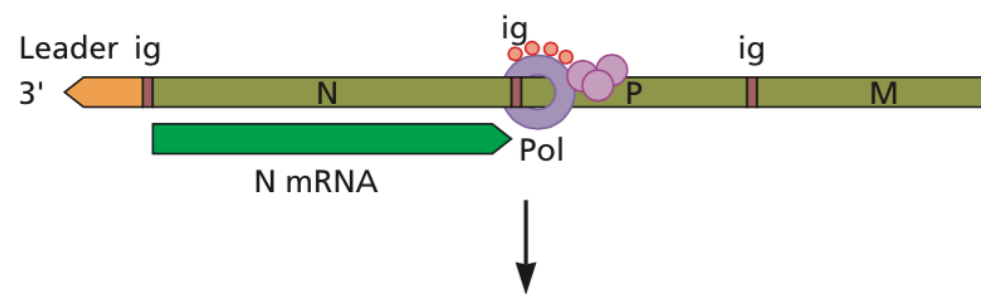
**RNA polymerase binds at 3' end of N gene**



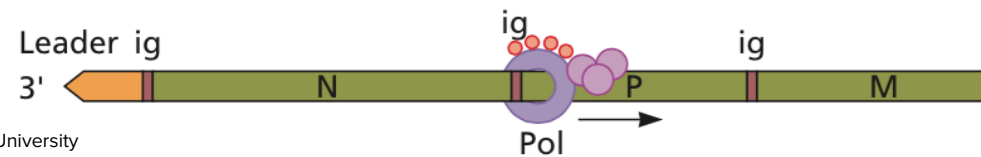
**Initiation of mRNA synthesis at 3' end of N gene**

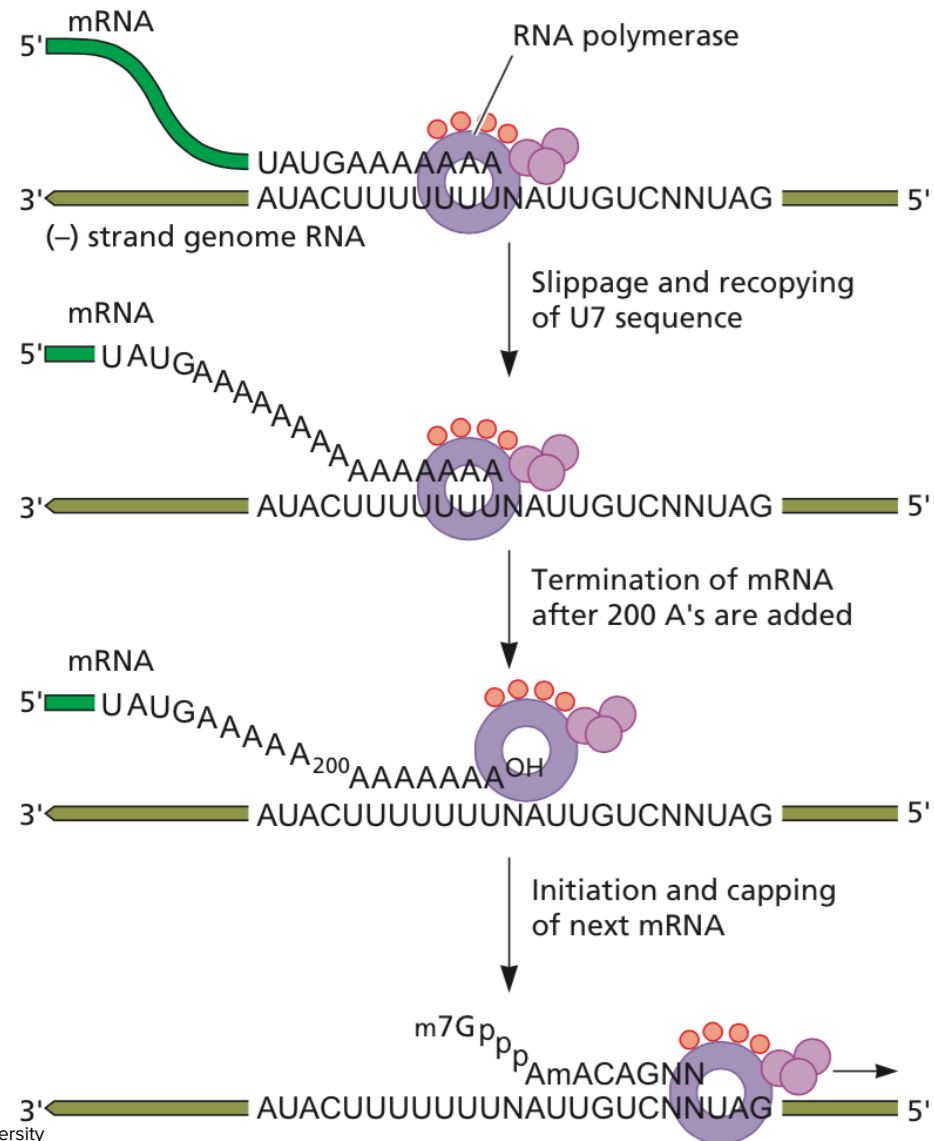
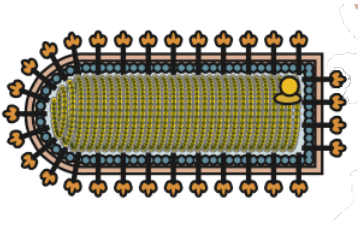


**Synthesize N mRNA and terminate at intergenic region (ig)**



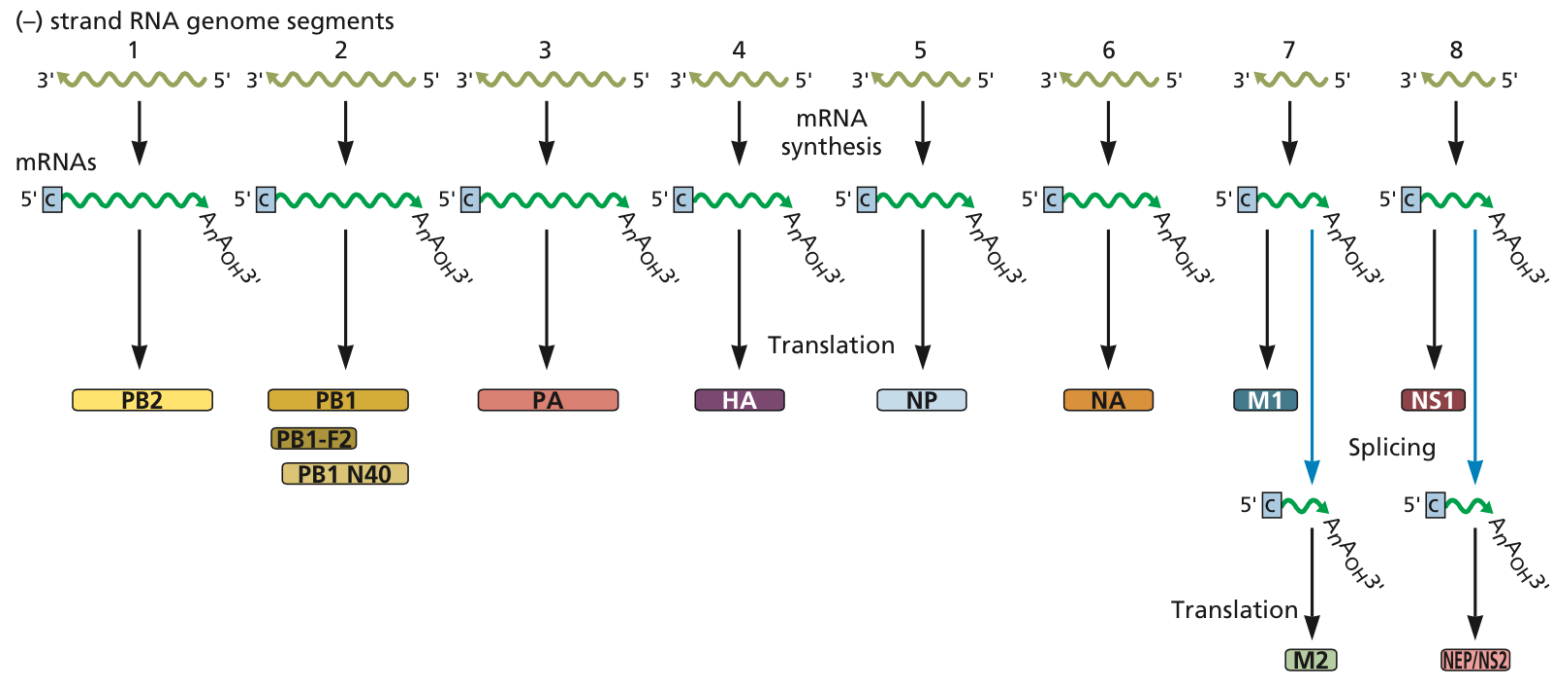
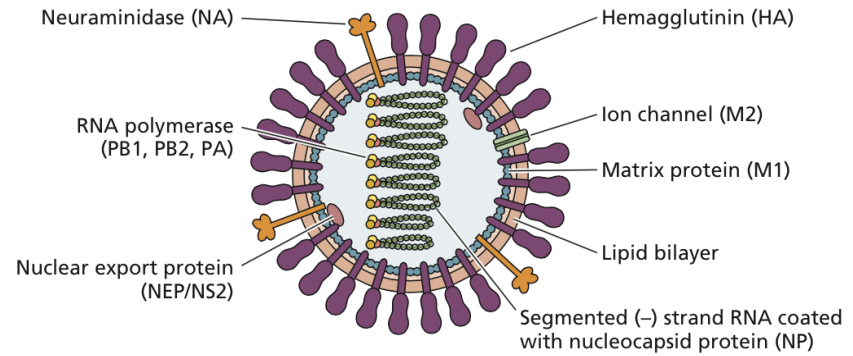
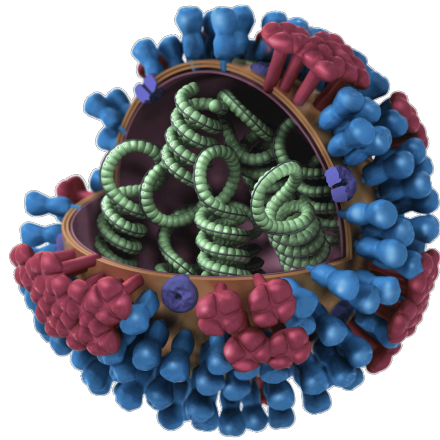
**Reinitiate at 3' end of P gene**

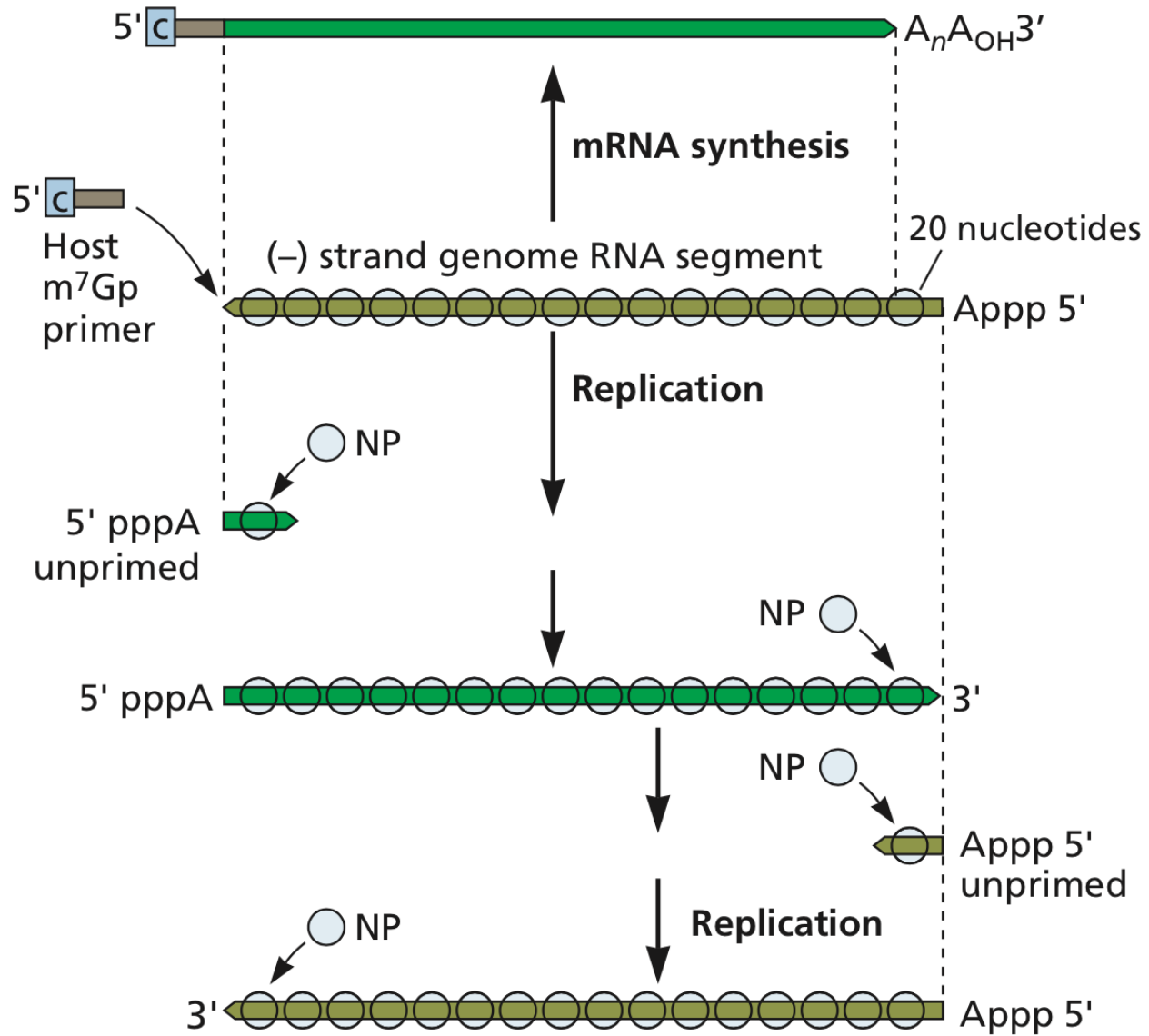
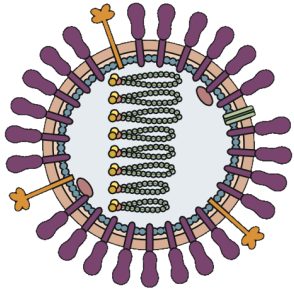


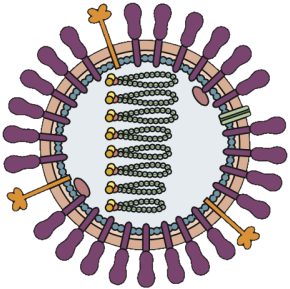












m<sup>7</sup>Gpppm<sup>6</sup>AmpC(m)pAp.....UpUpGpApCp...

**Cleavage** 13

(-) strand RNA

UpCpCpUpUpUpUpCp...

**Initiation**

m<sup>7</sup>Gpppm<sup>6</sup>AmpC(m)pAp.....UpUpG

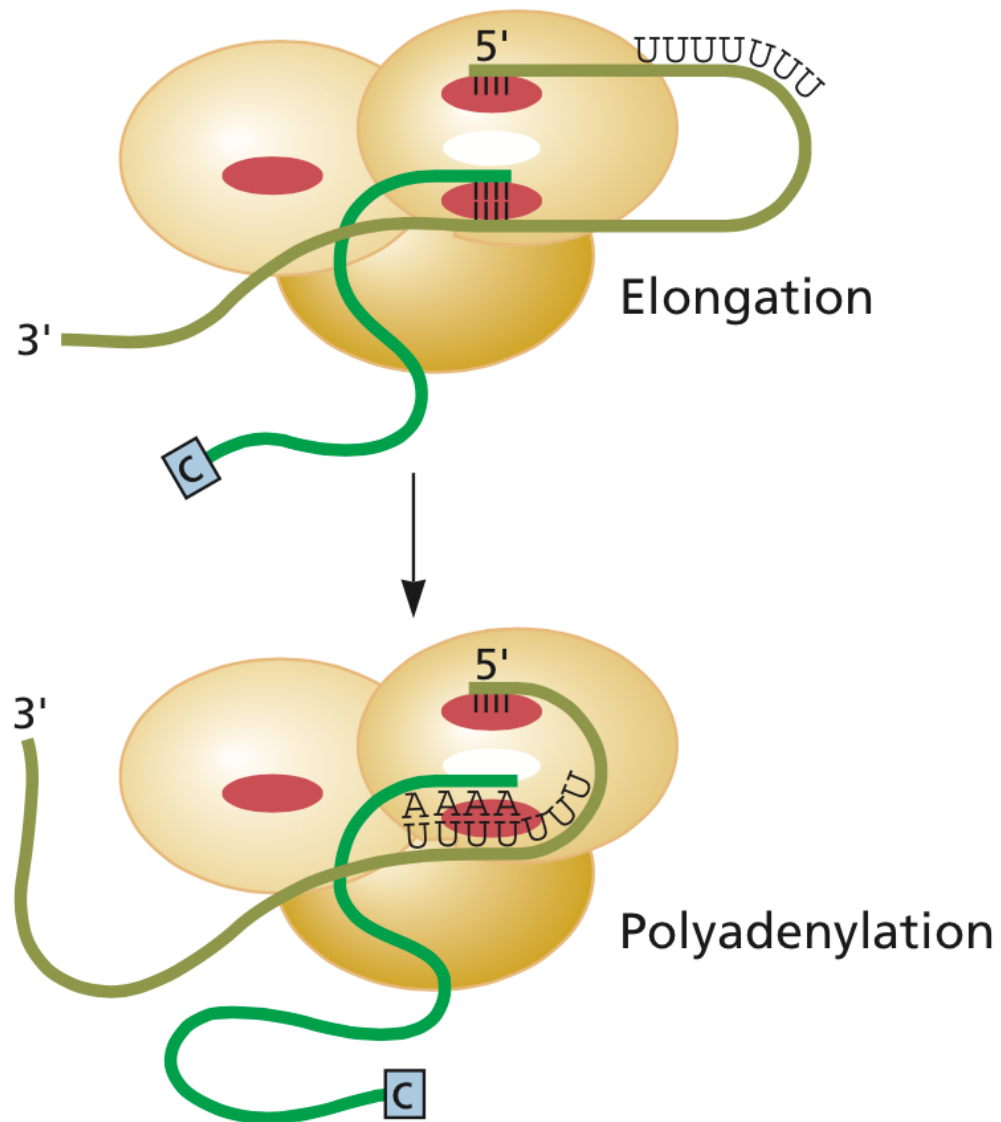
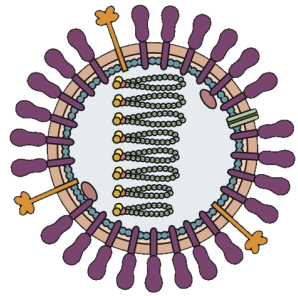
13 p p pG

**Elongation**

UpCpCpUpUpUpUpCp...

m<sup>7</sup>Gpppm<sup>6</sup>AmpC(m)pAp.....UpUpGpGPCpApApApApGp...

13

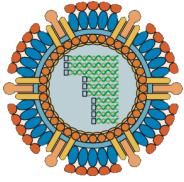


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**How are influenza virus and VSV RNA synthesis similar?**

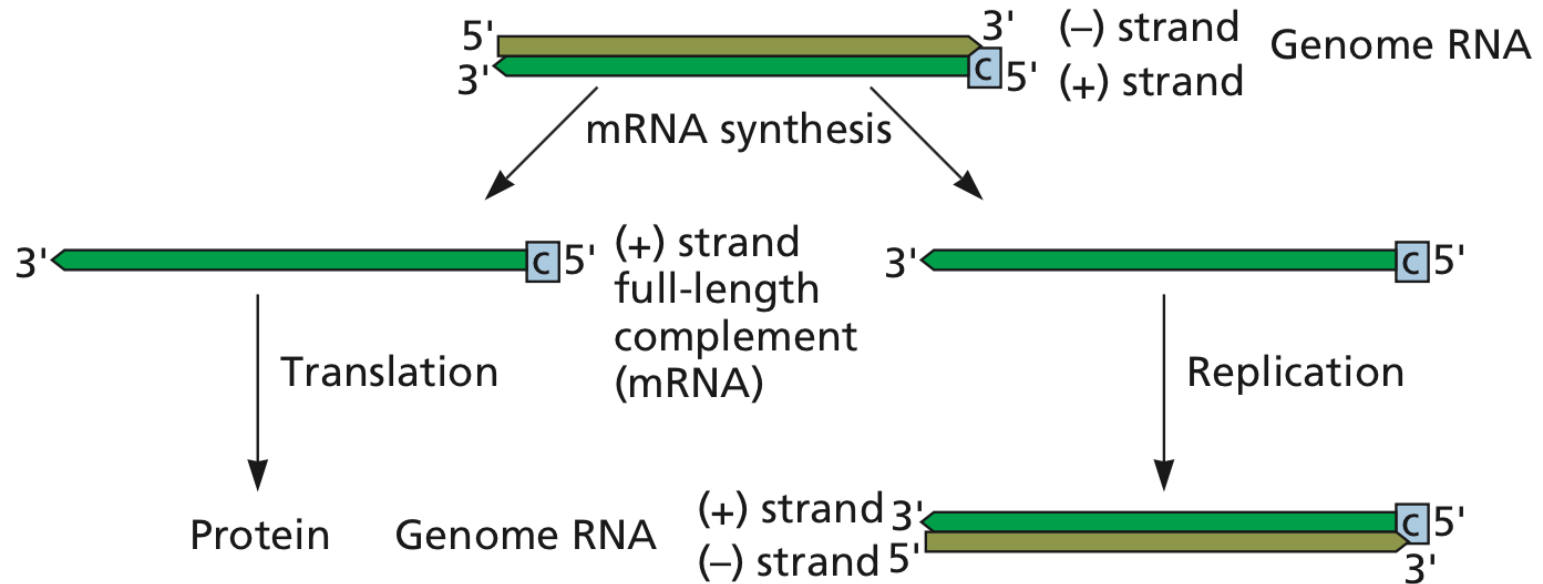
- A. The switch from mRNA to genome RNA synthesis is controlled by an RNA binding protein
- B. Polyadenylation occurs at a short stretch of U residues
- C. Viral mRNAs are shorter than (-) genome RNA
- D. All of the above

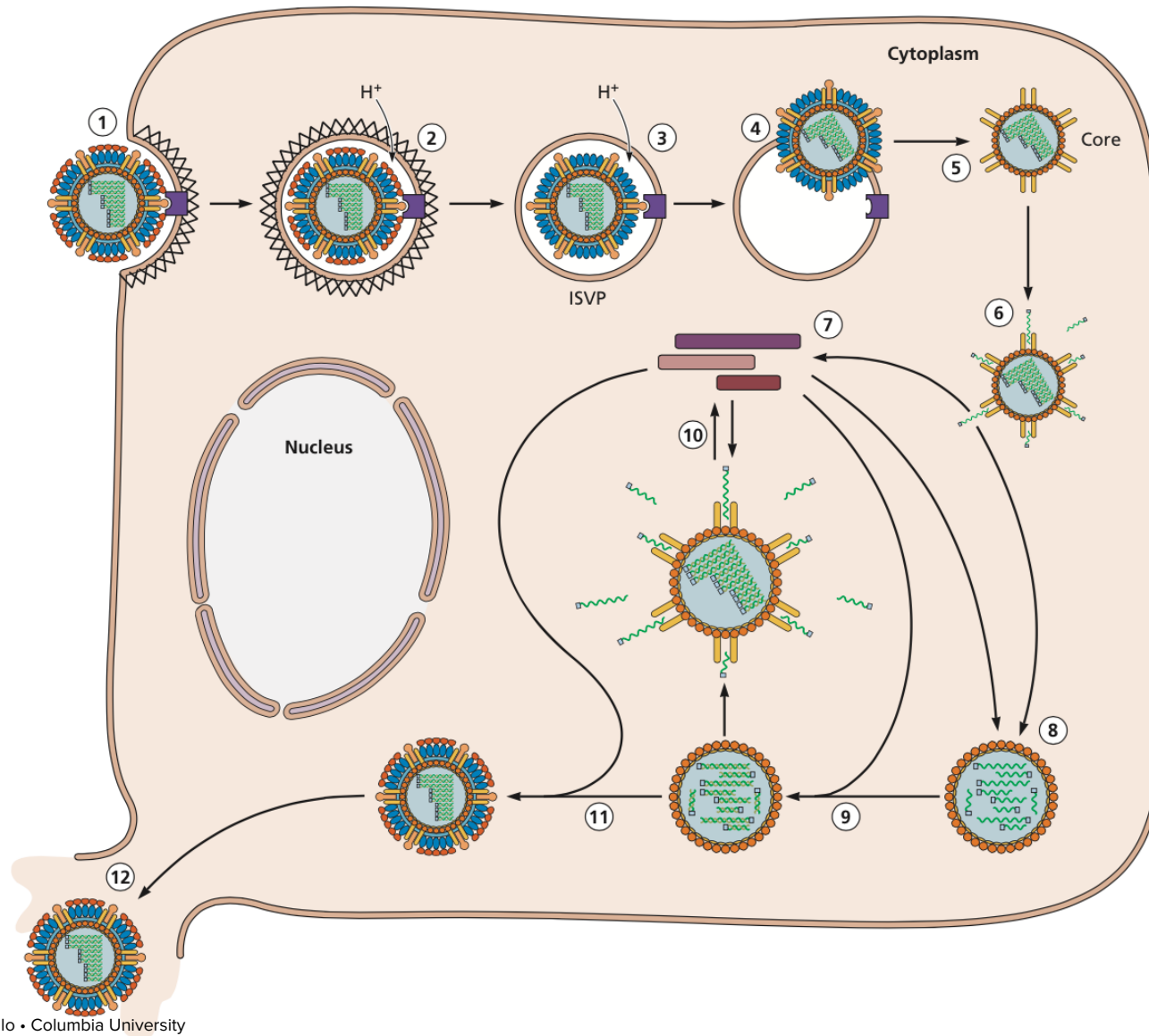


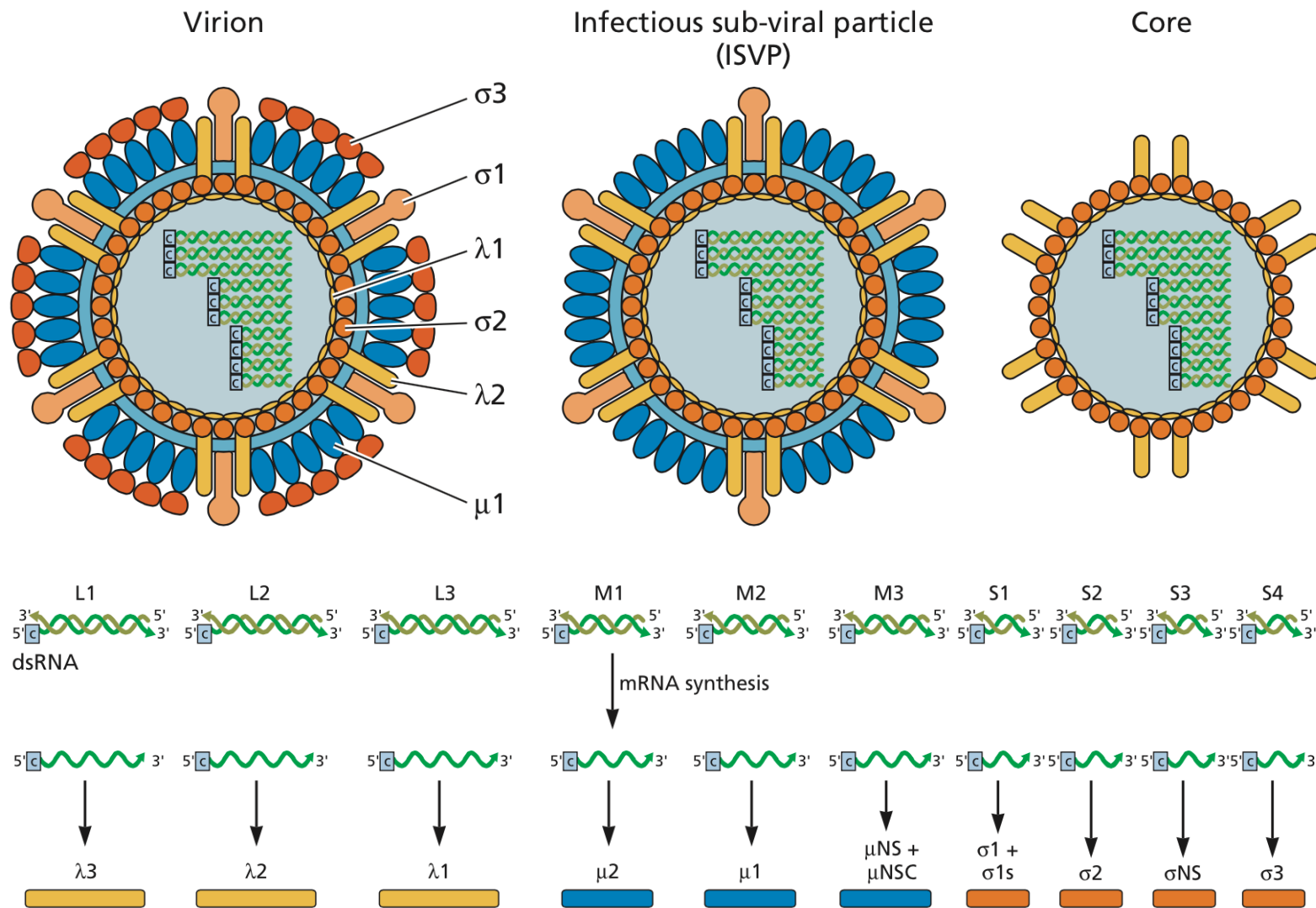
# dsRNA viruses

*Reoviridae*: reovirus, rotavirus

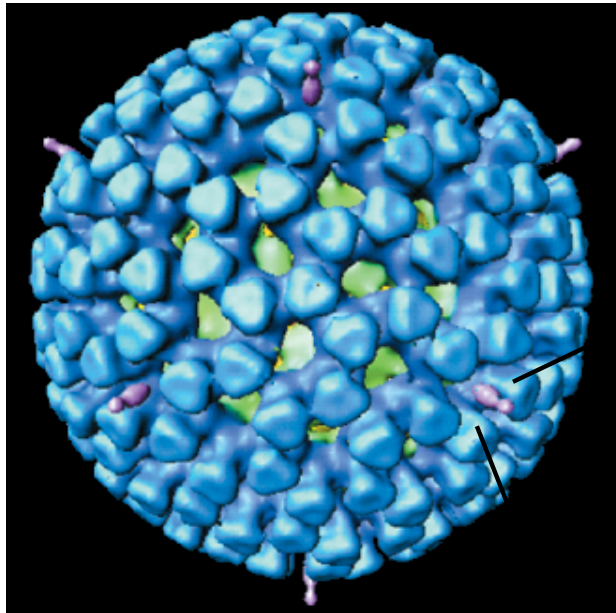
## Double-stranded RNA viruses



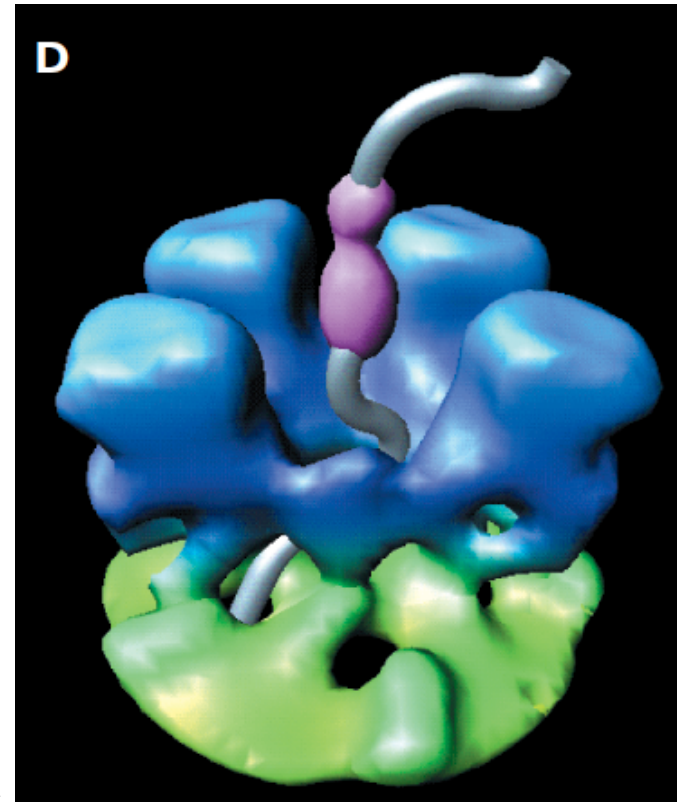








Each dsRNA segment is attached to RdRp via the 5'-cap



# RNA directed RNA synthesis

- (+) RNA, (-) RNA, dsRNA
- Polymerase basics
- Site of RNA synthesis
- Genome replication
- mRNA synthesis
- poly(A) addition

