# Viral DNA replication

Lecture 8

Biology 3310/4310

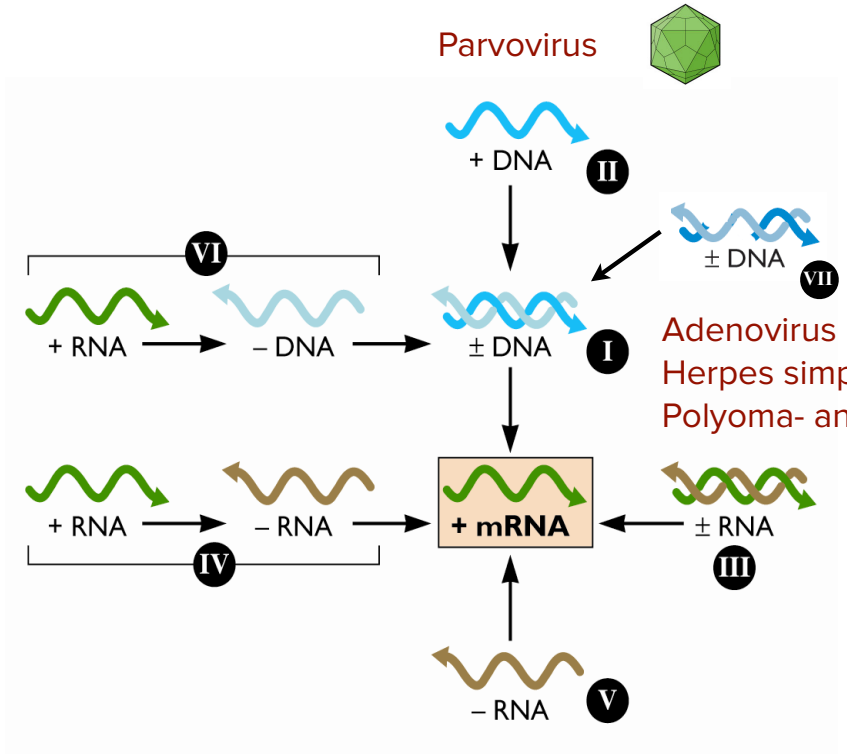
Virology

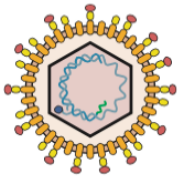
Spring 2017

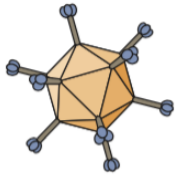
*The more the merrier*

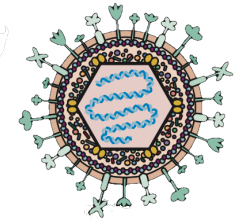
--ANONYMOUS

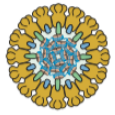
# Viral DNA genomes must be replicated to make new progeny



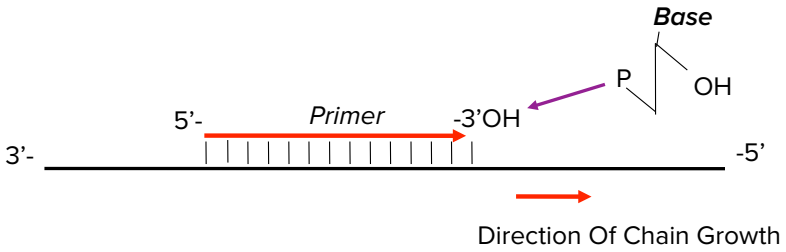





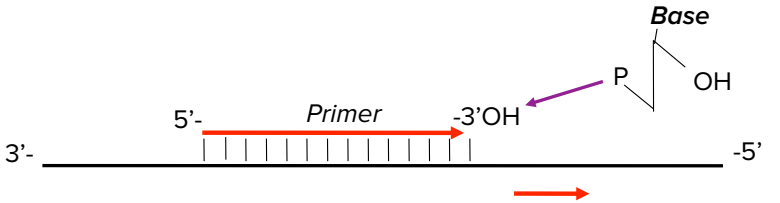


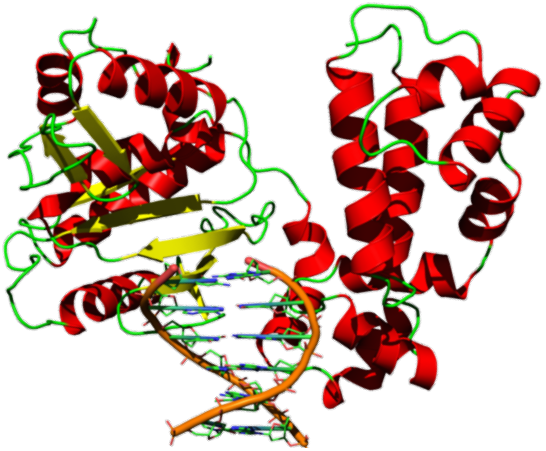


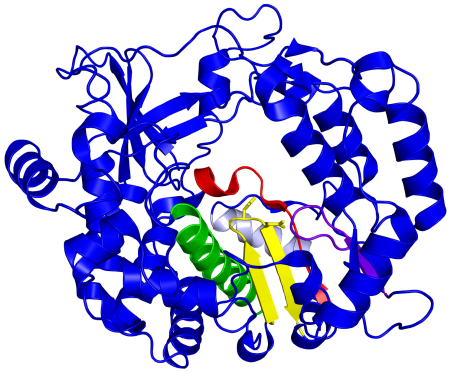
# Universal rules of DNA replication

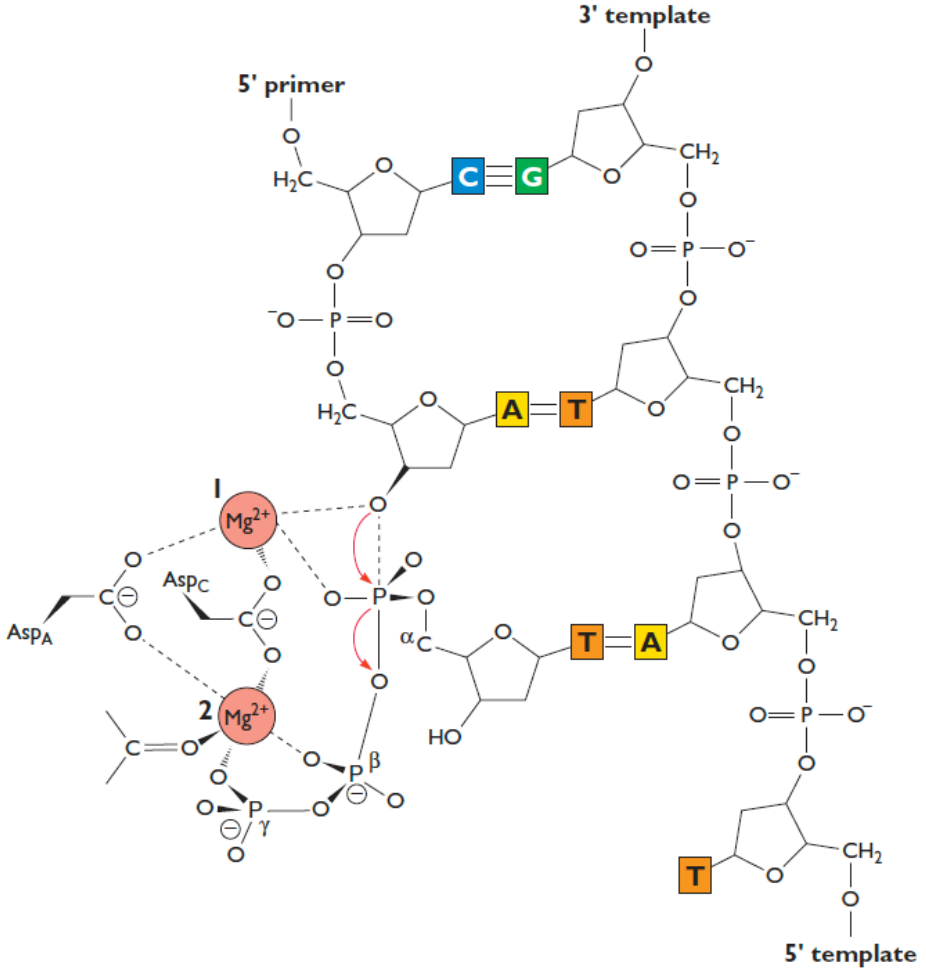



- DNA is synthesized by template-directed incorporation of dNMPs into 3'-OH of DNA chain
- DNA is always synthesized 5'-3' via semiconservative replication (two daughter strands)
- Replication initiates at specific sites on template called **origins**
- Catalyzed by DdDp + accessory proteins
- Always primer-dependent



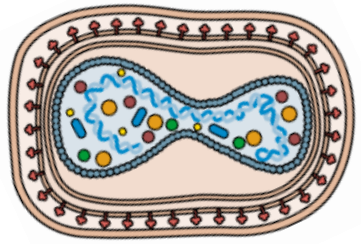







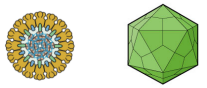
# What's the host for? Viruses can't do it themselves

- Viral DNA replication always requires synthesis of at least one viral protein, sometimes many (hence always delayed after infection)
- Simple viruses require more host proteins - genetic economy
- Complex viruses encode many, but not all proteins required for replication

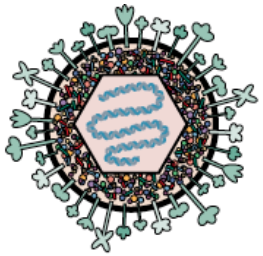


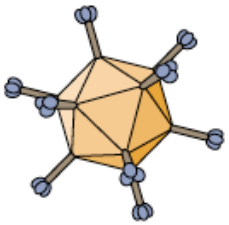
# Where does the polymerase come from?

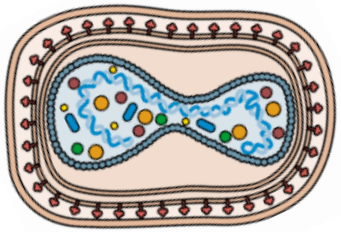
- Small DNA viruses do not encode an entire replication system
  - Encode proteins that orchestrate the host
  - *Papillomaviridae, Polyomaviridae, Parvoviridae*



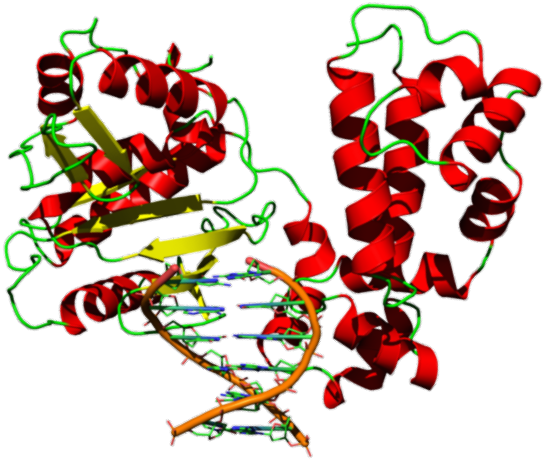
- Large DNA viruses encode most of their own replication systems
  - *Herpesviridae, Adenoviridae, Poxviridae*







# Viral proteins



- DNA polymerase and accessory proteins
- Origin binding protein, helicases
- Exonucleases
- Enzymes of nucleic acid metabolism (thymidine kinase, ribonucleotide reductase, dUTPase)

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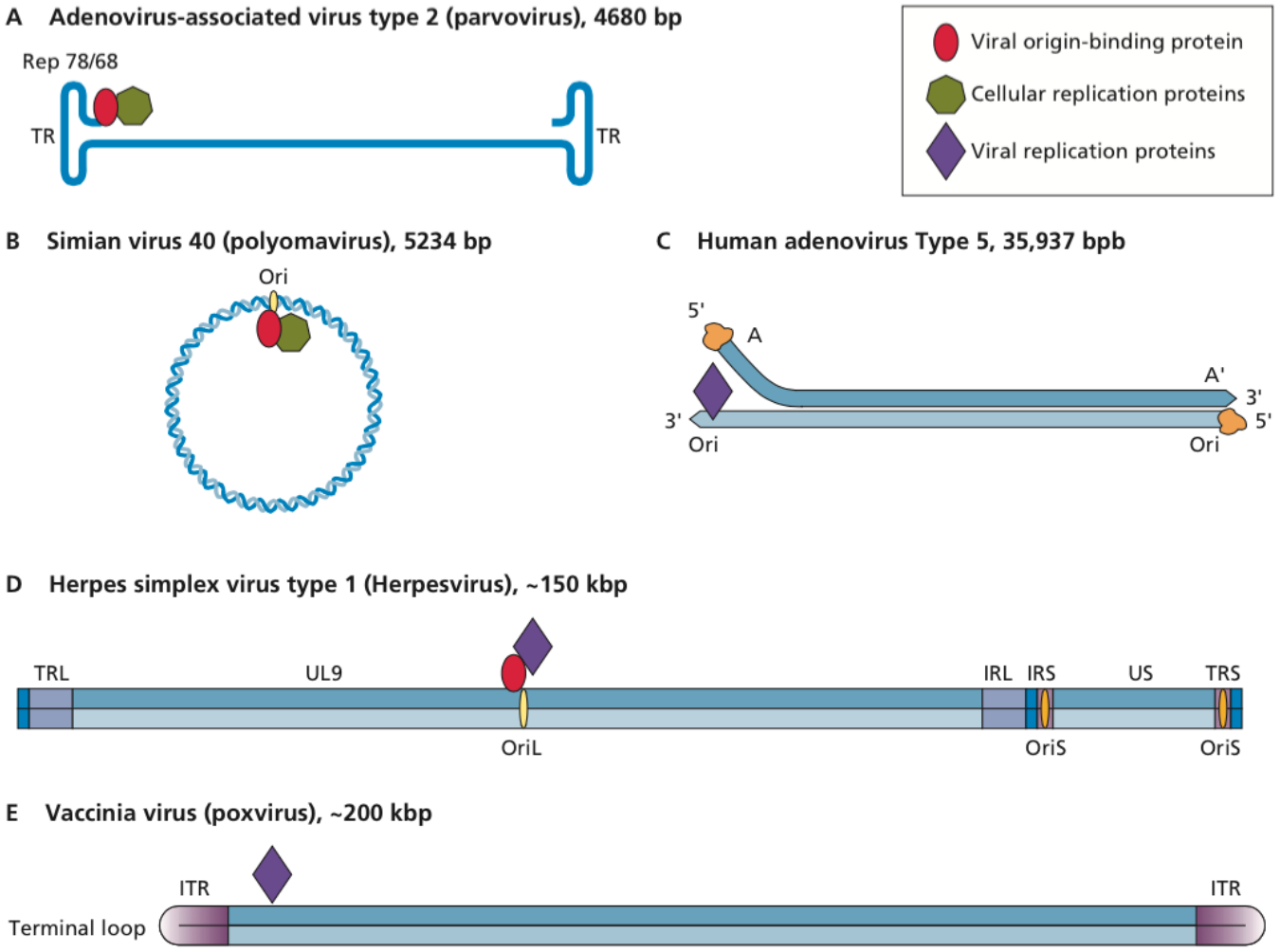
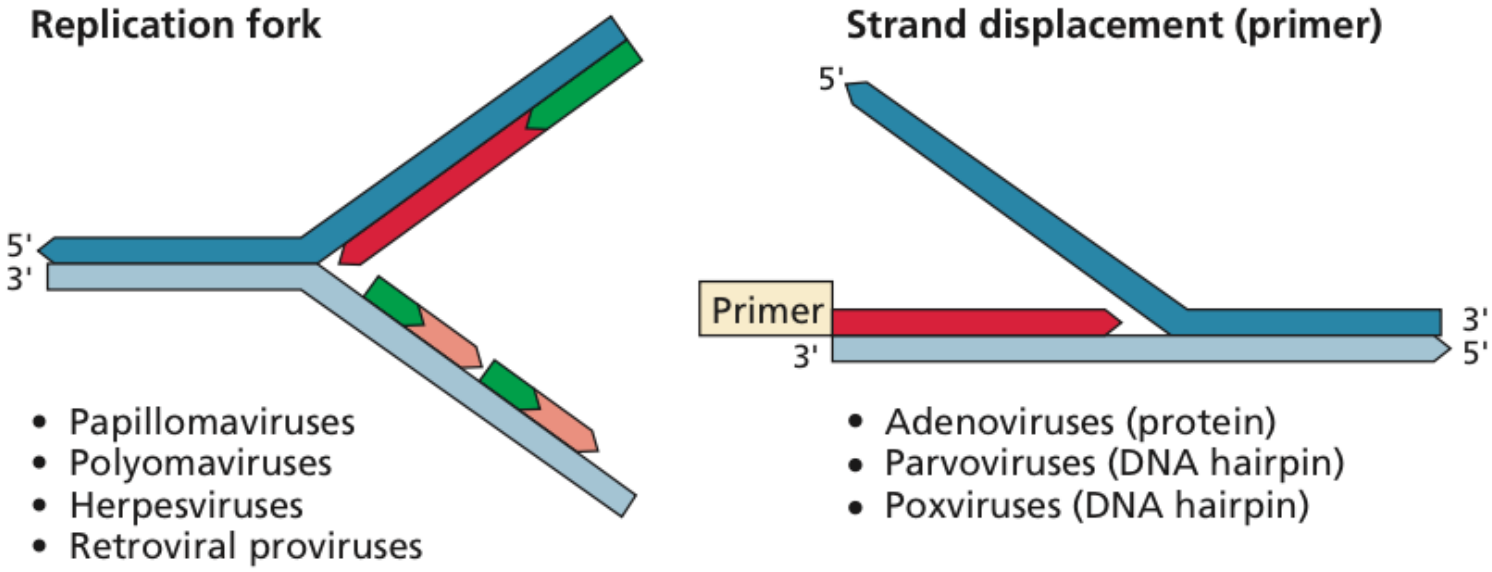
**Which statement about viral DNA synthesis is NOT correct?**

A. Large DNA viruses encode many proteins involved in DNA synthesis
B. Small DNA viruses encode at least one protein involved in DNA synthesis
C. Viral DNA replication is always delayed after infection because it requires the synthesis of at least one viral protein
D. Some viruses encode all proteins needed for DNA replication

# Diverse viral genome structures



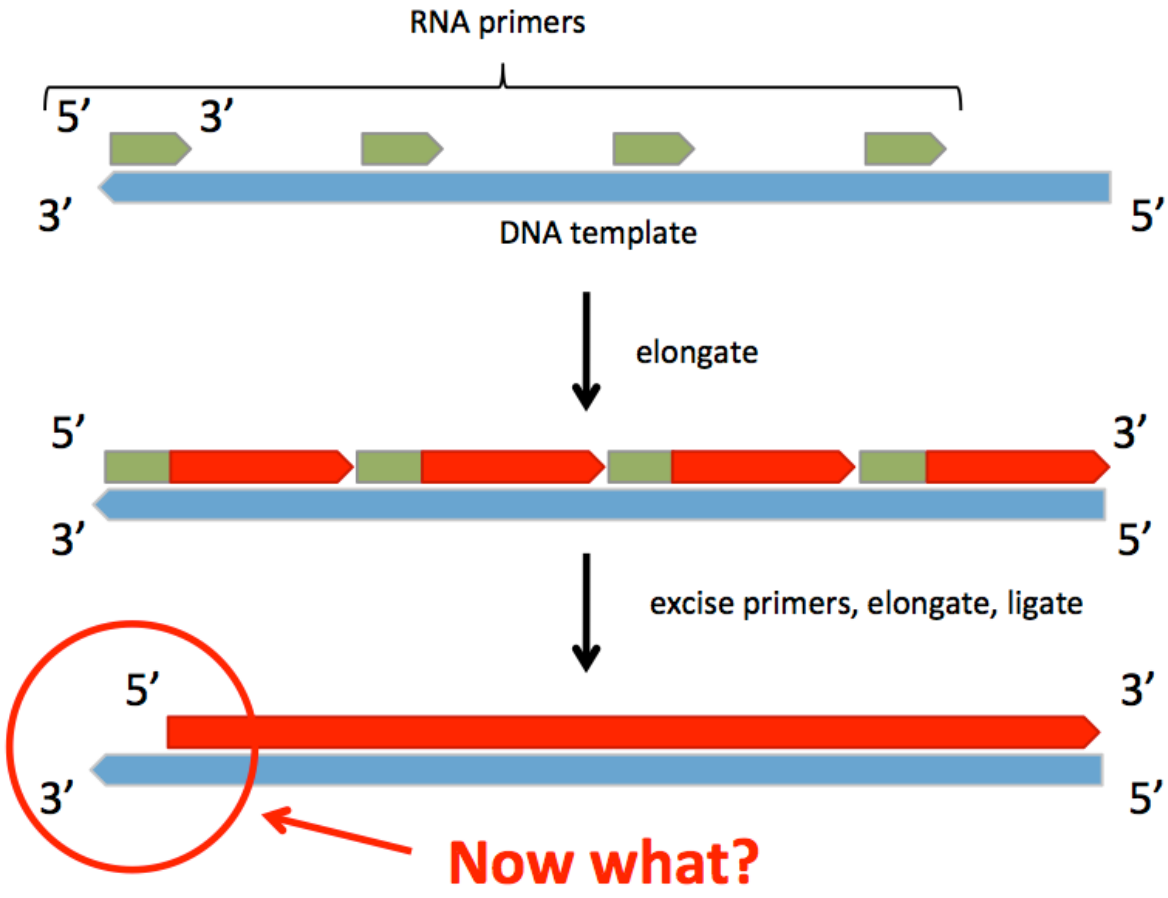
# Two mechanisms of dsDNA synthesis



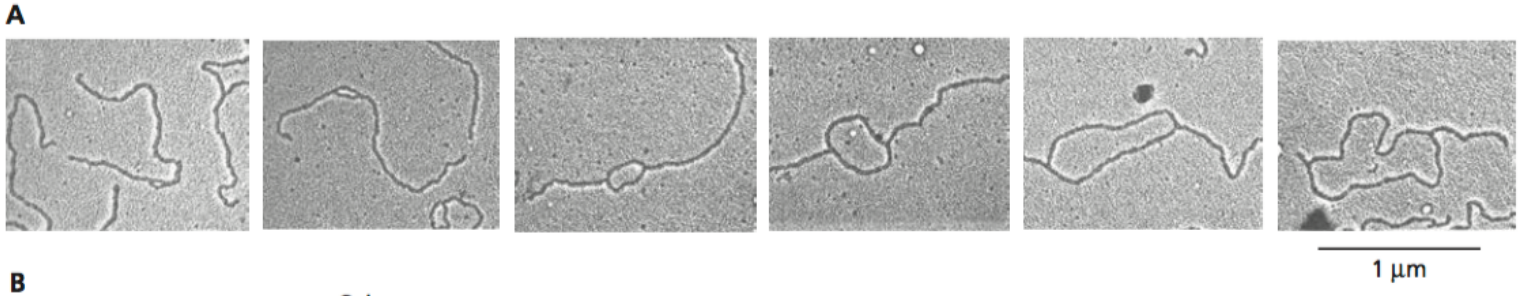

RNA primers

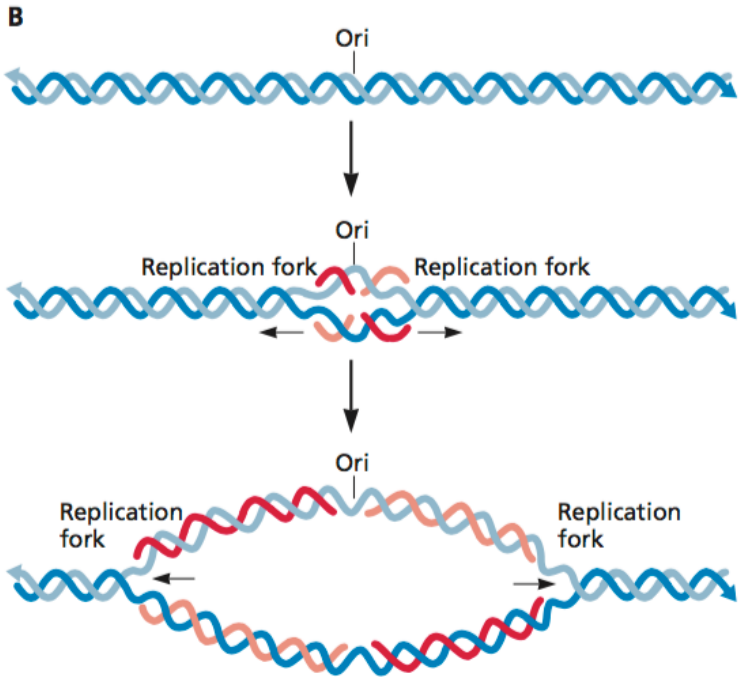
Never RNA primed

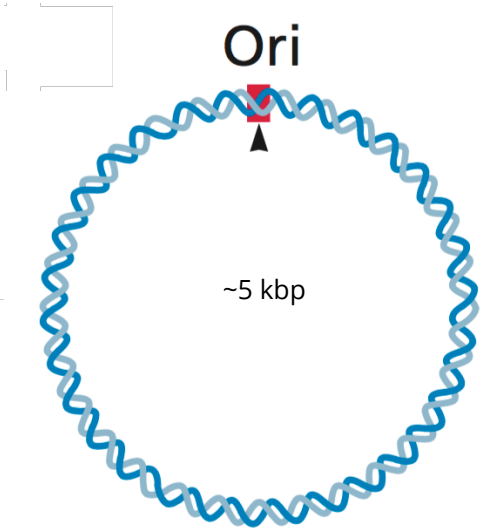
# The 5’-end problem



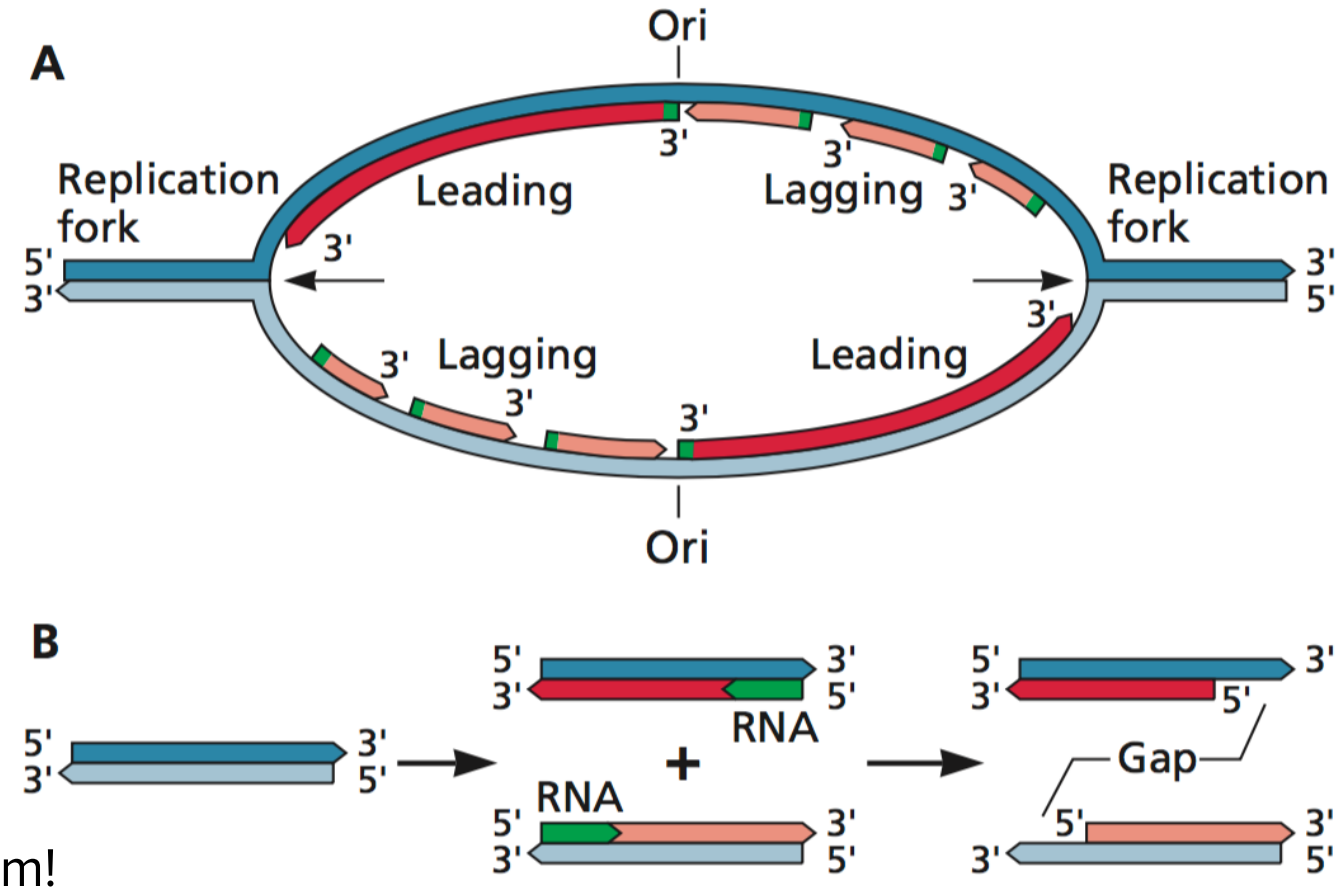
# Lessons from SV40



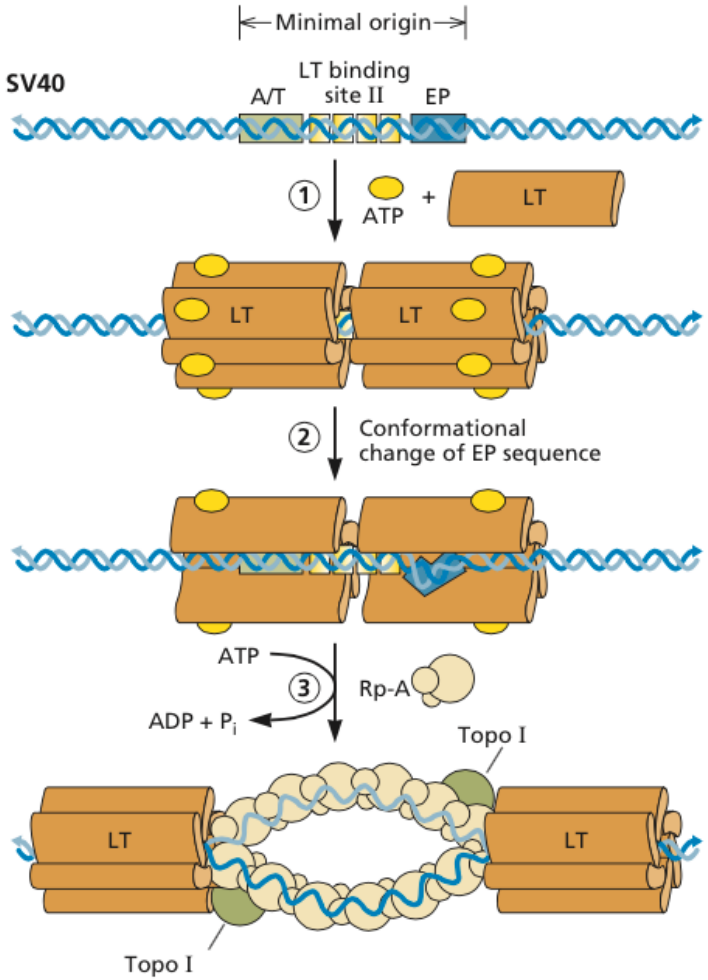



# Semi-discontinuous DNA synthesis from a bidirectional origin




No end problem!

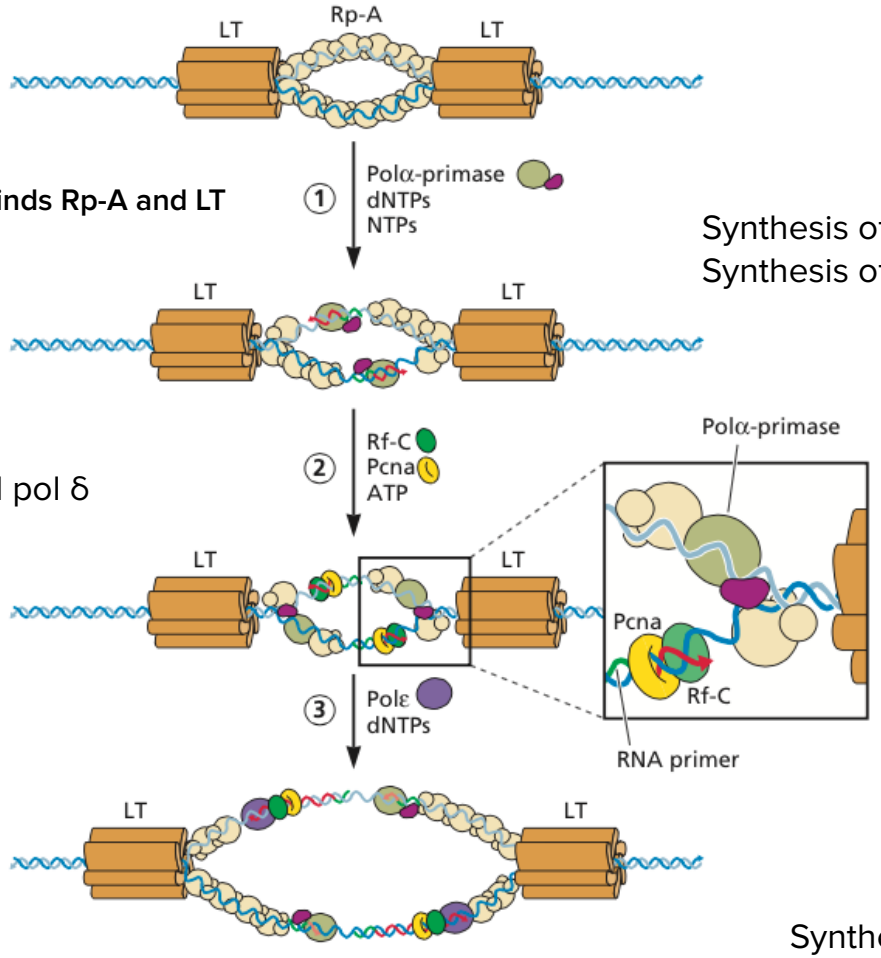
# Recognition and unwinding of SV40 origin




**Rp-A binds LT!**

T has 3'-5' helicase activity

# Synthesis of leading and lagging strands

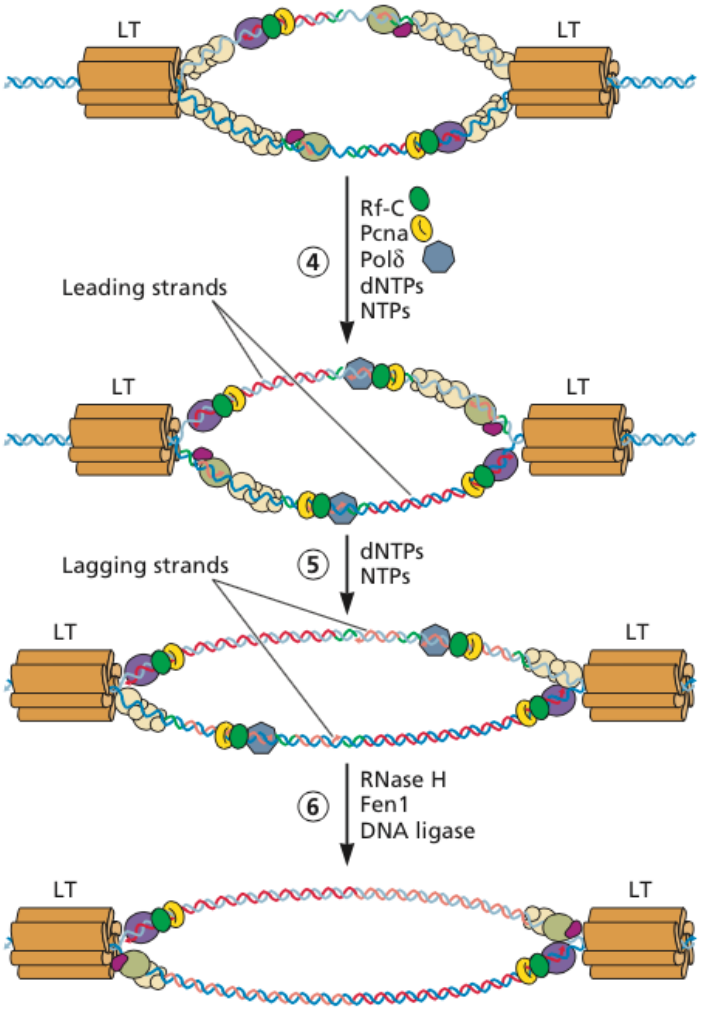



Synthesis of RNA primers
Synthesis of short DNA fragments

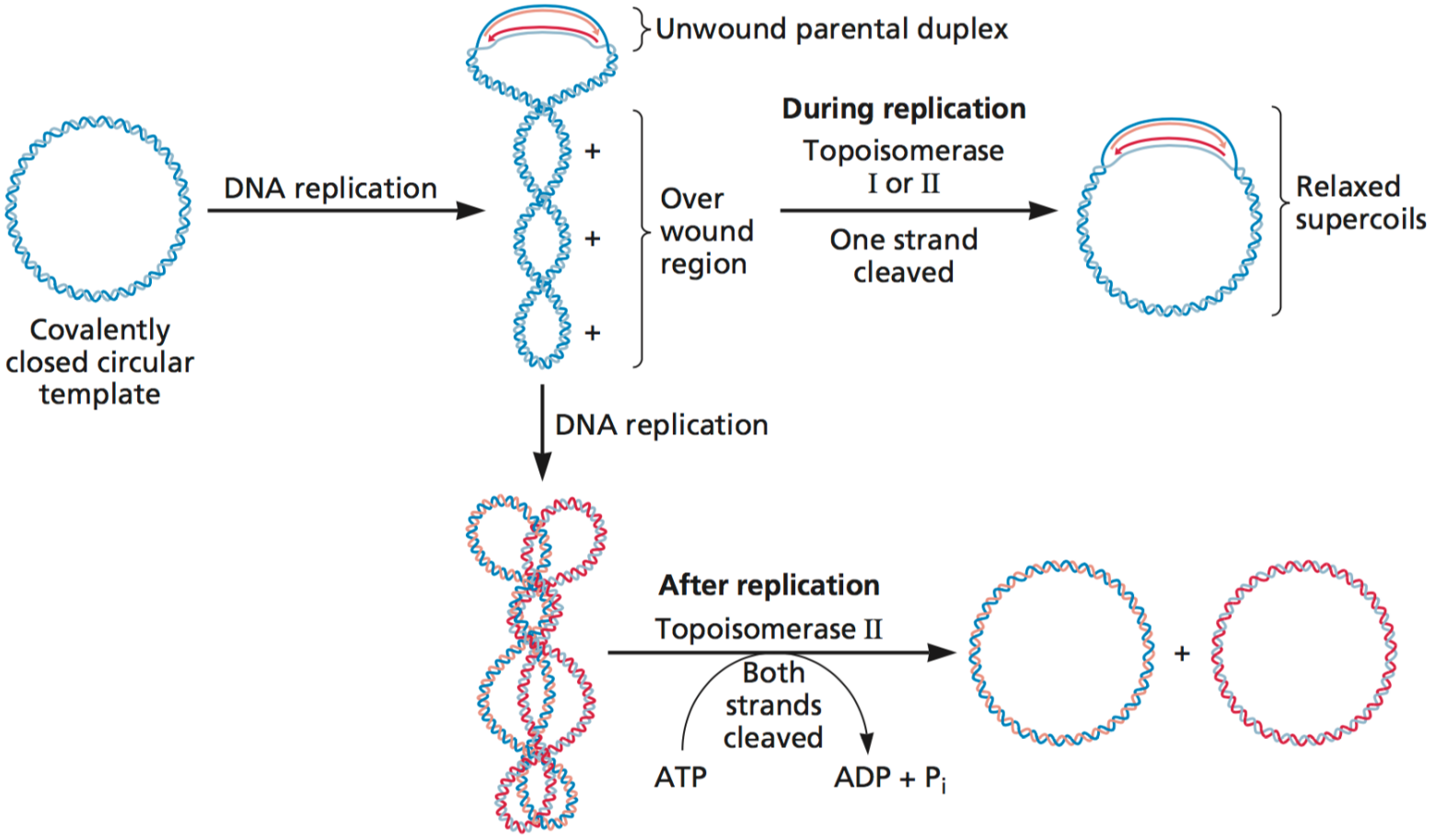
Rf-C binds 3'OH along with PCNA and pol δ
—RF-C a clamp loading protein
—Allows entry of PCNA on DNA
—Causes release of pol α
Form sliding clamps along DNA

Synthesis of long DNA

# Synthesis of leading and lagging strands



# Function of topoisomerases



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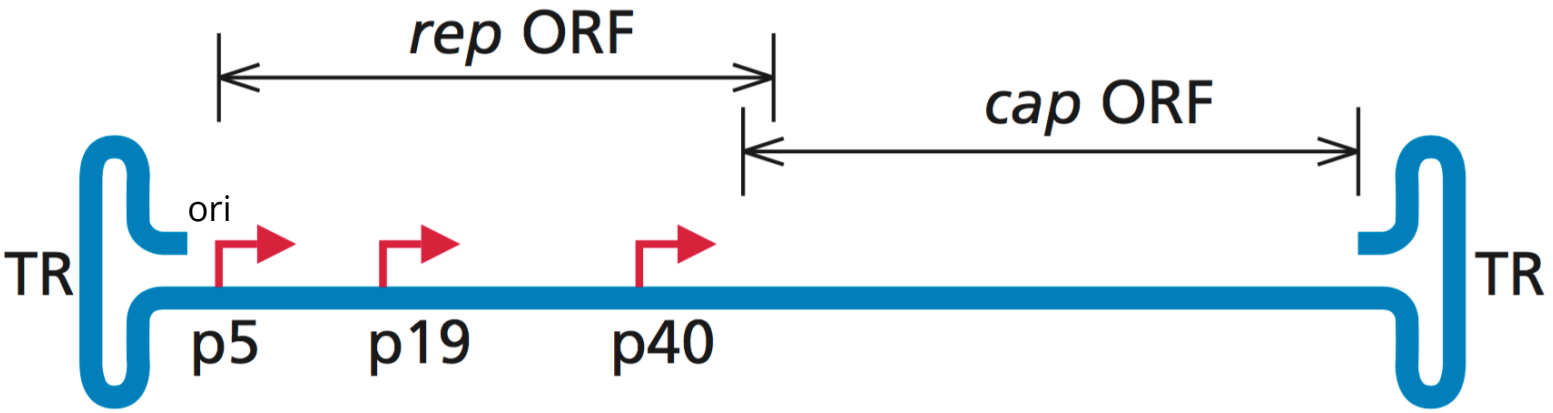
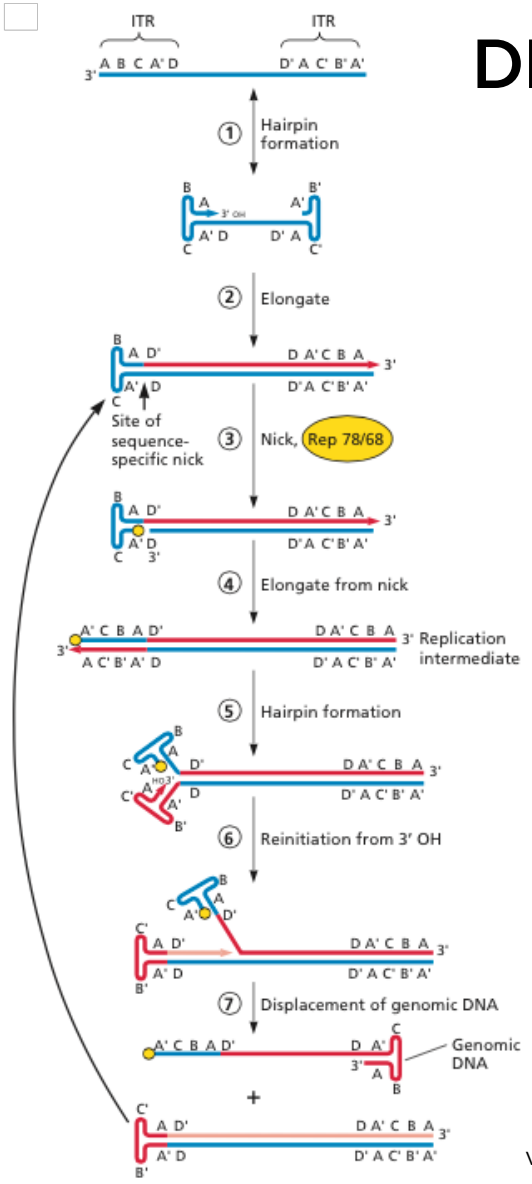
**The SV40 genome is a circular dsDNA. Which statement about its replication is correct?**

A. Viral T antigen binds and unwinds the ori
B. Replication is bidirectional from a single ori
C. The 5'-end problem is solved
D. Has leading and lagging strand synthesis
E. All of the above

# DNA priming: Parvoviruses



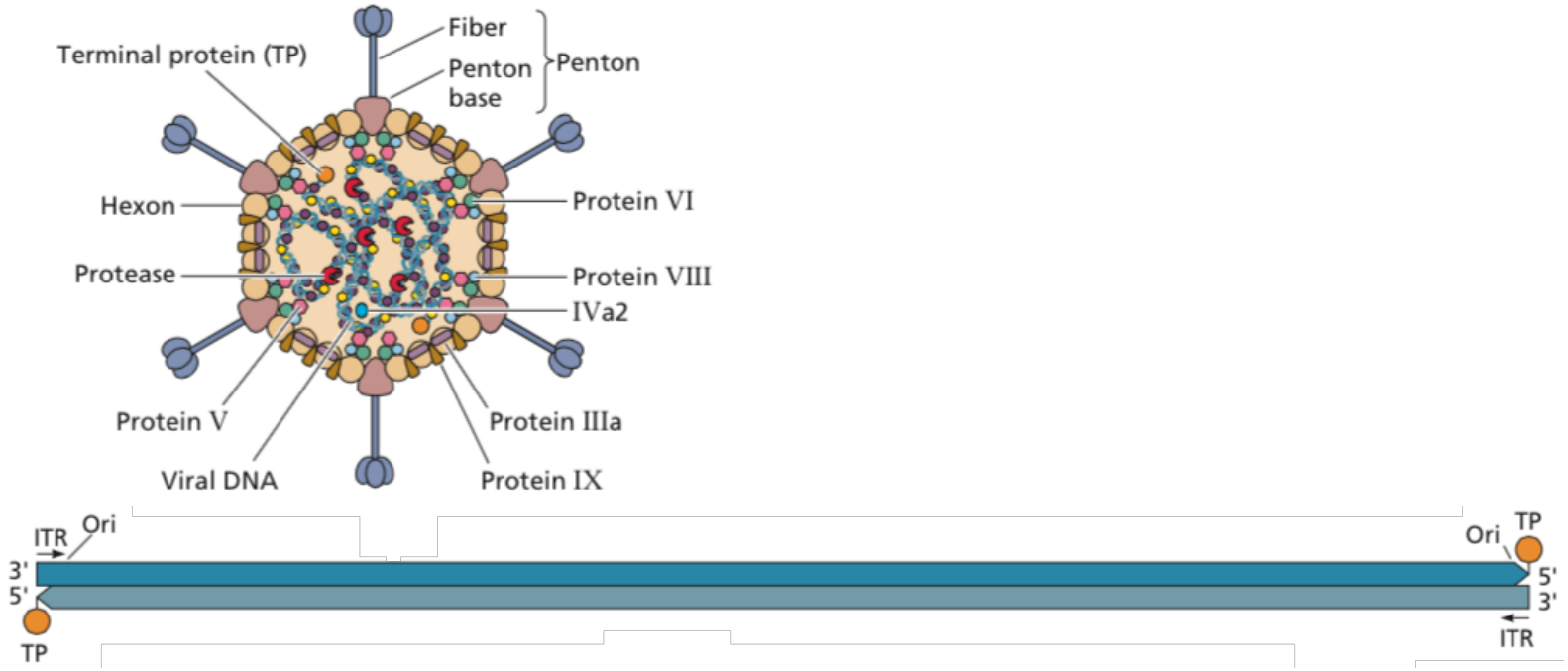

# DNA priming: Parvoviruses



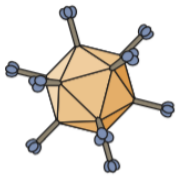

- Replication is continuous
- No pol α, uses ITR to self-prime
- Requires pol δ, RF-C and PCNA
- Rep78/68 proteins are required for initiation and resolution: endonuclease, helicase, binds 5'-terminus
- No replication fork, strand displacement

No end problem!

# Protein priming: Adenovirus



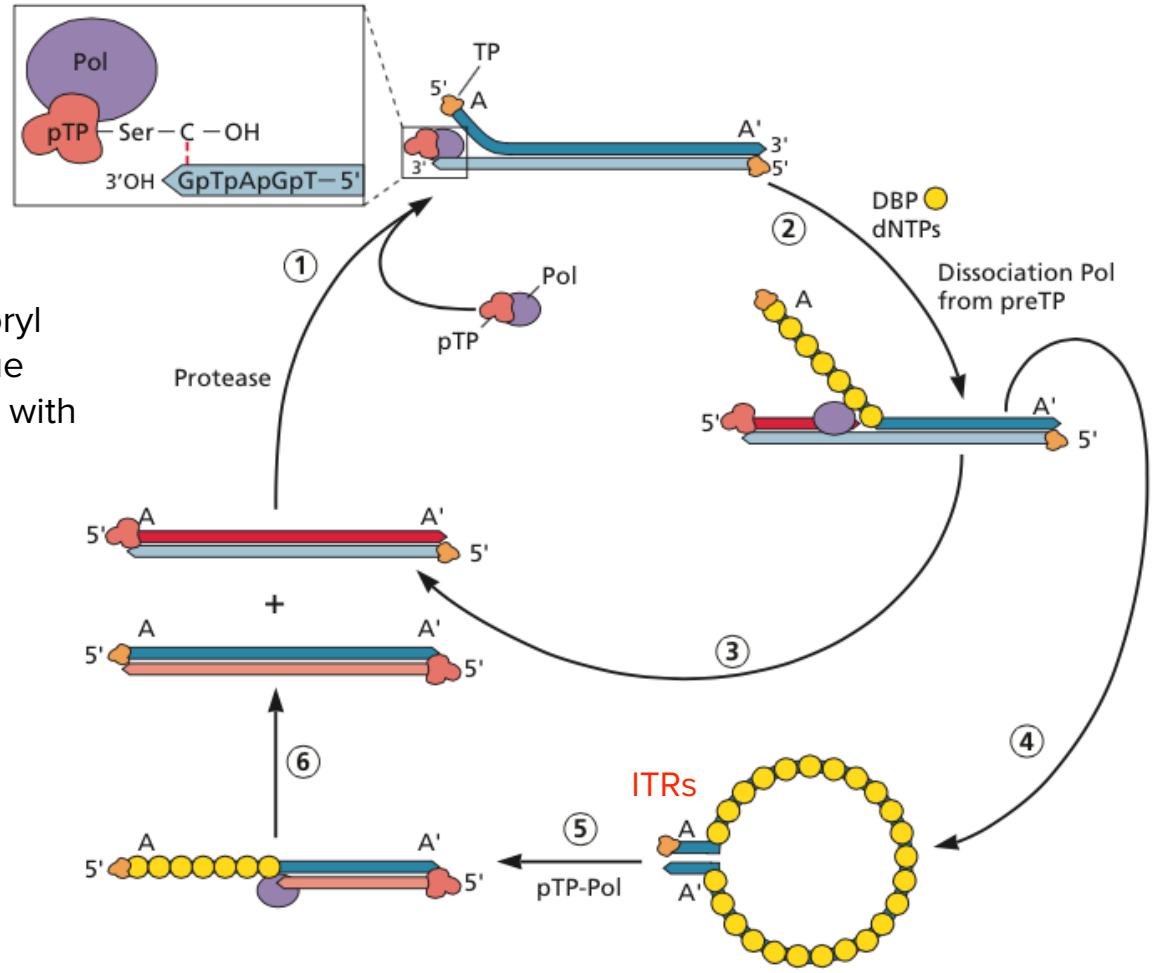

- Origins at both ends
- Strand displacement synthesis
- Semiconservative DNA replication

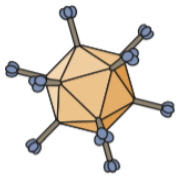


# Protein priming: Adenovirus

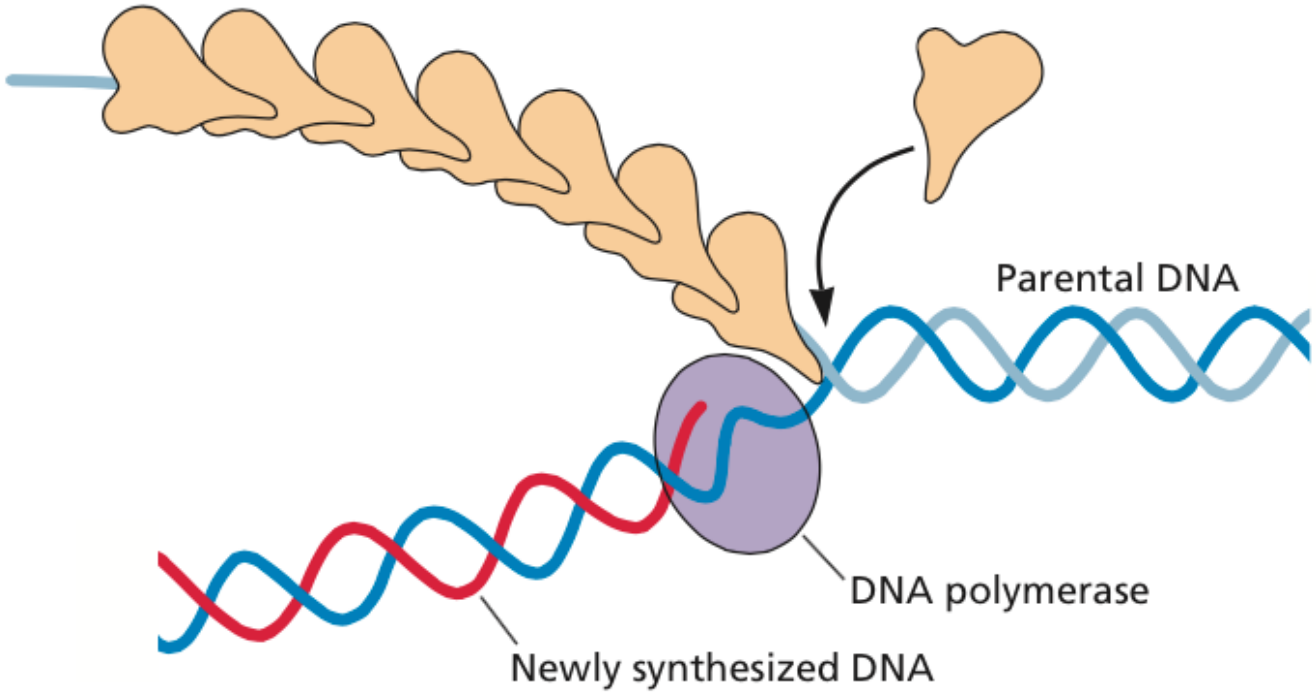
Ad DNA pol links α-phosphoryl of dCMP to OH of Ser residue only when pTP is assembled with DNA pol into preinitiation complex at ori

No end problem!





# Adenoviral ssDNA binding protein



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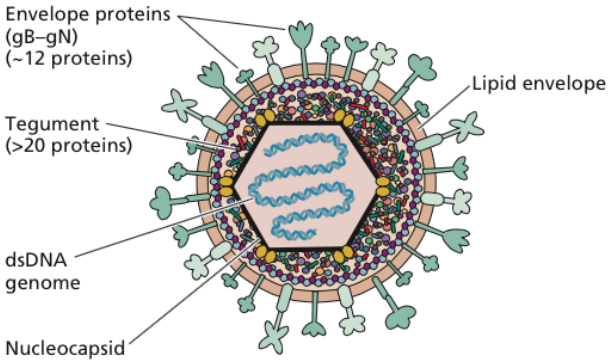
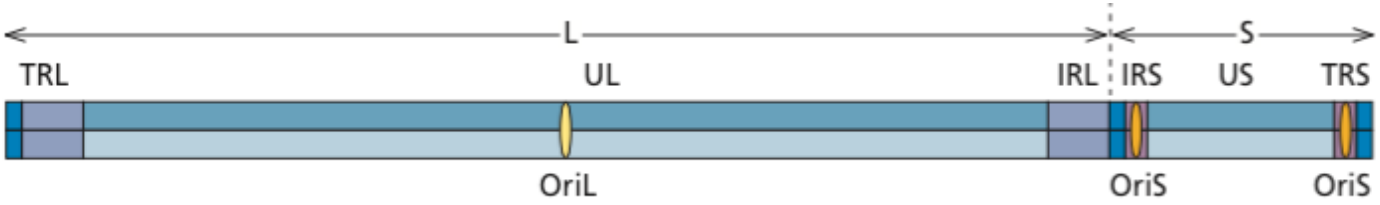
**How is DNA replication of parvovirus and adenovirus similar?**

A. They both require protein-linked primers
B. Replication occurs by strand displacement
C. DNA synthesis occurs in the cytoplasm
D. A replication fork occurs in both
E. None of the above

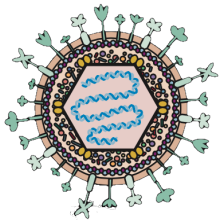



# Herpes simplex virus

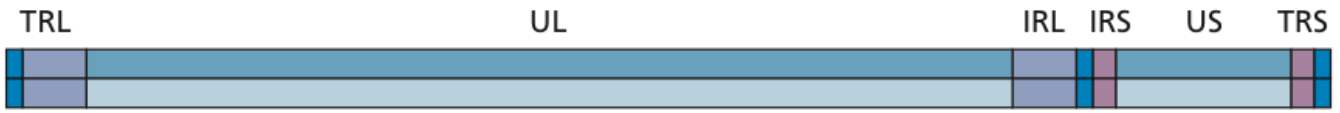
- UL5, 8 and 53 - primase
- UL42 - processivity protein
- UL9 - origin binding protein
- UL29 - ssDNA binding protein
- UL30 - DNA polymerase

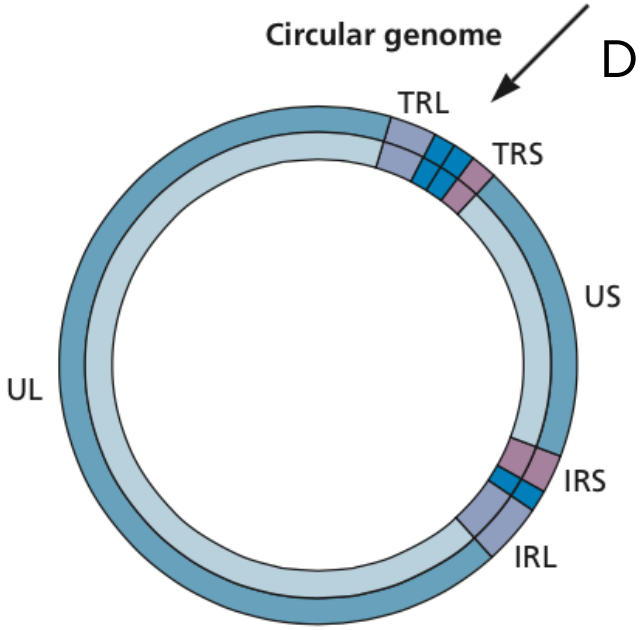



- 2 oriS and a unique oriL sequence
- DNA enters as a linear molecule and converts to circle
- Replicates as rolling circle



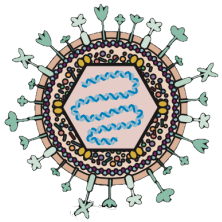
# Initiation of herpesvirus DNA replication



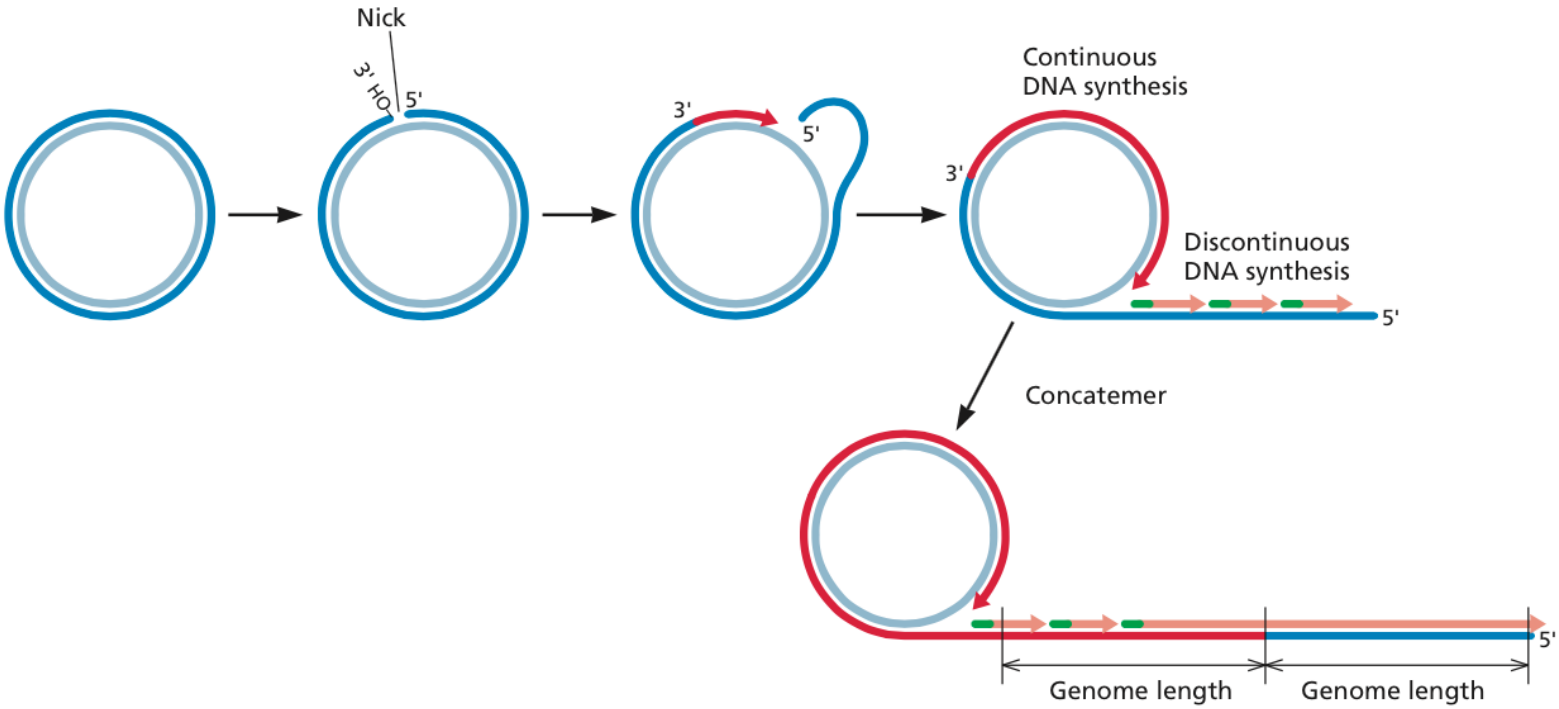




DNA ligase IV/XRCC4

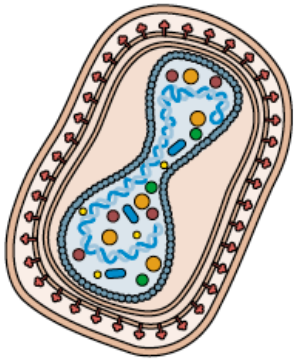
Host proteins are responsible for circularization



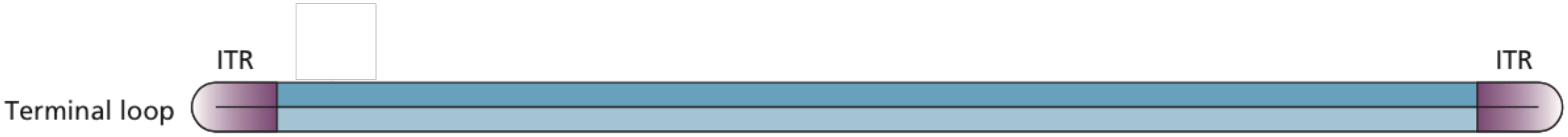
# Rolling circle replication




No end problem!

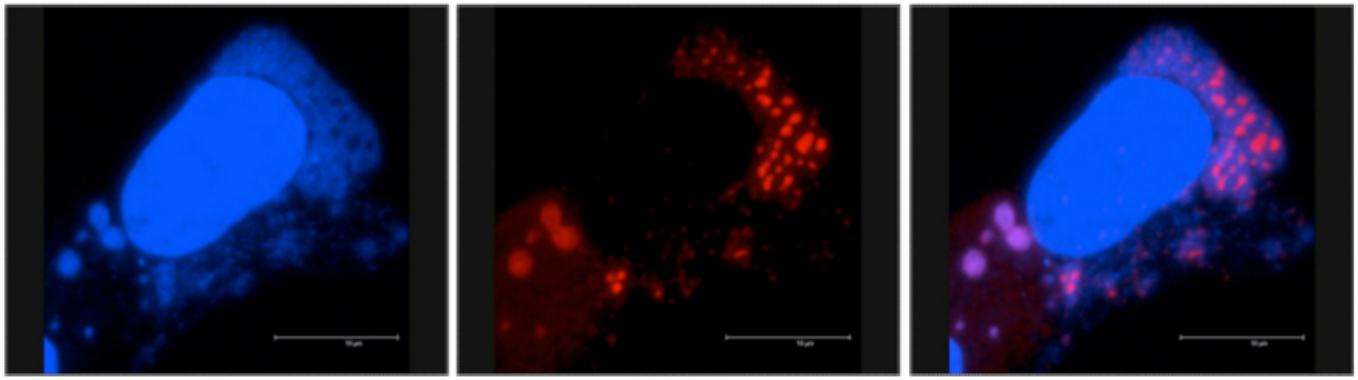


# Poxvirus



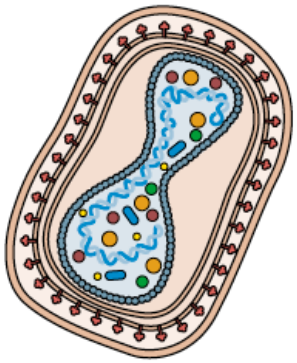

- All viruses discussed replicate in nucleus
- Poxviruses replicate in cytoplasm

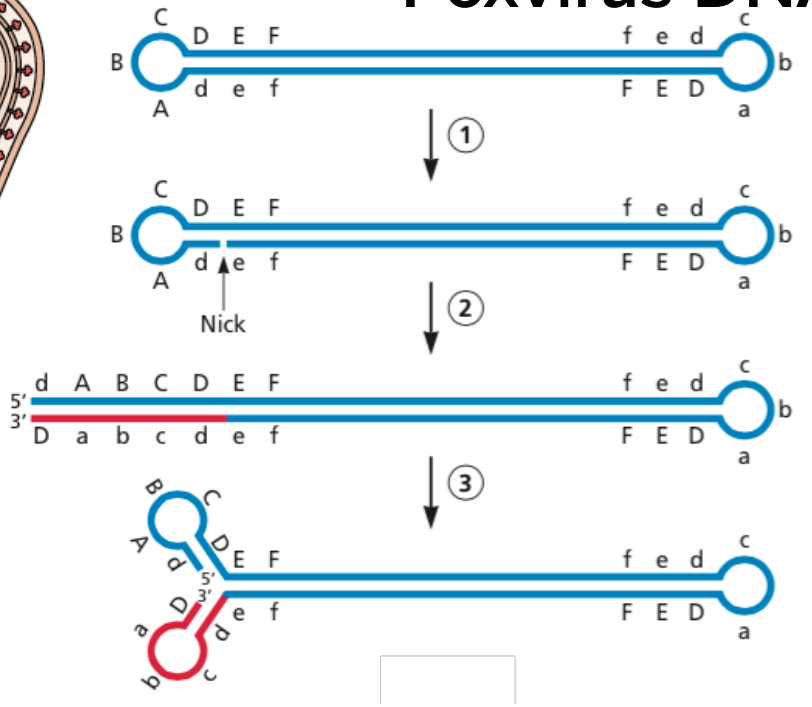
# Poxvirus DNA factories

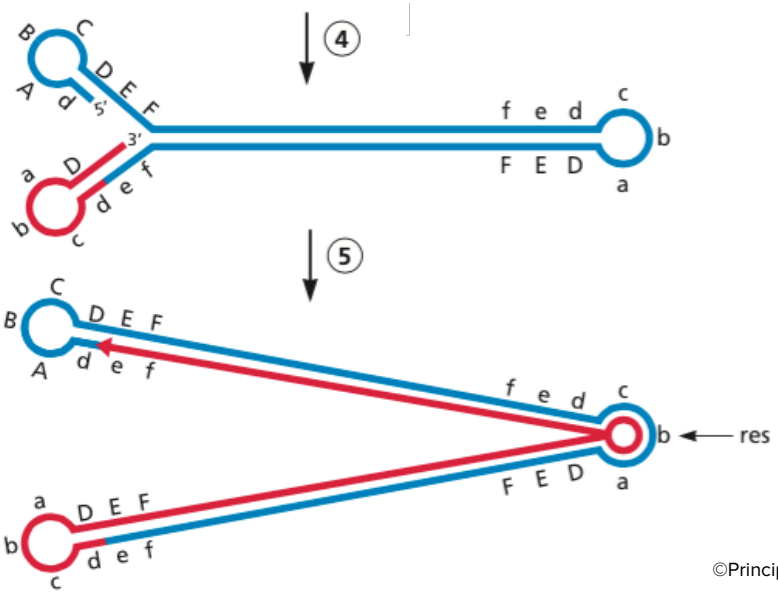


DNA DNA binding protein merge

# Poxvirus DNA replication






*At least 15 viral proteins involved in viral DNA synthesis*

No end problem!

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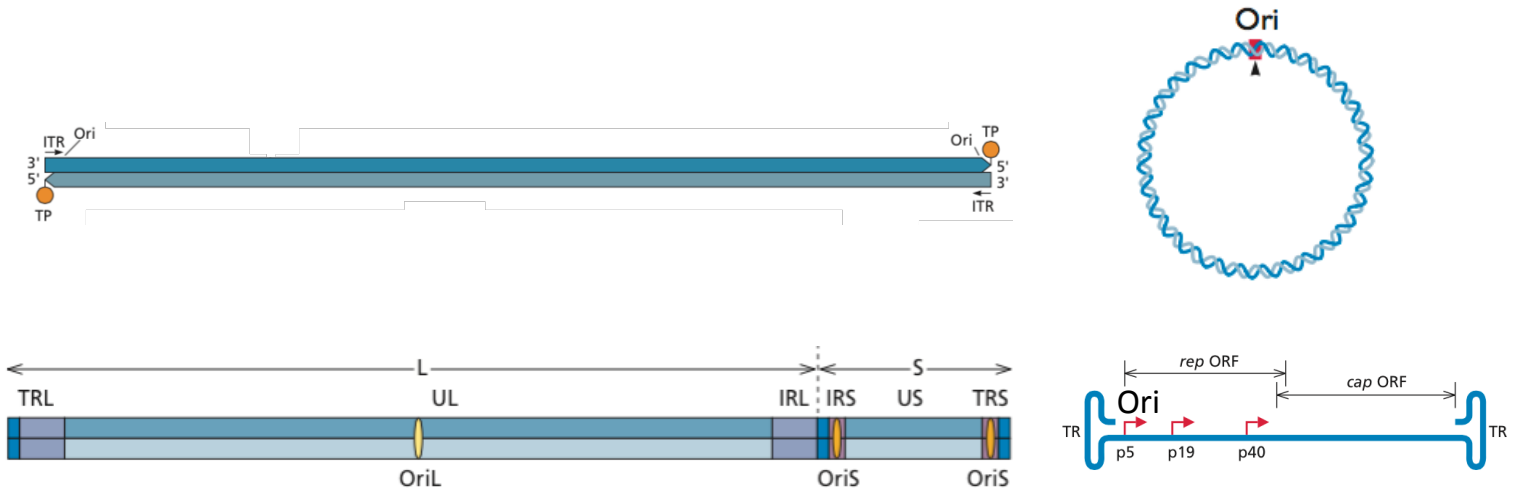
**What makes poxvirus DNA replication different from all of the other viruses we discussed today?**

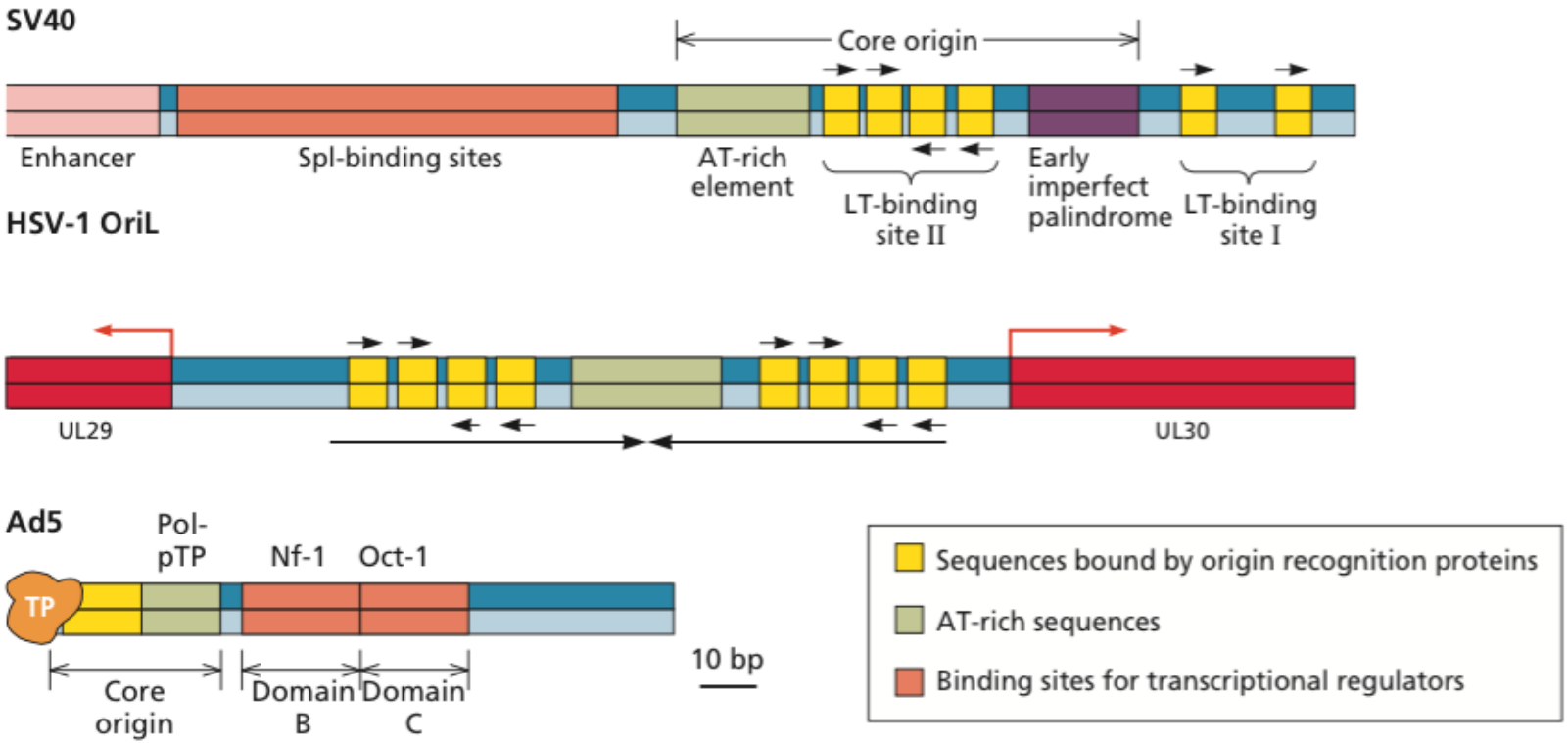
A. The complete replication machinery is encoded by the viral genome
B. DNA synthesis occurs in the nucleus
C. DNA synthesis occurs by strand displacement
D. None of the above

# Viral origins



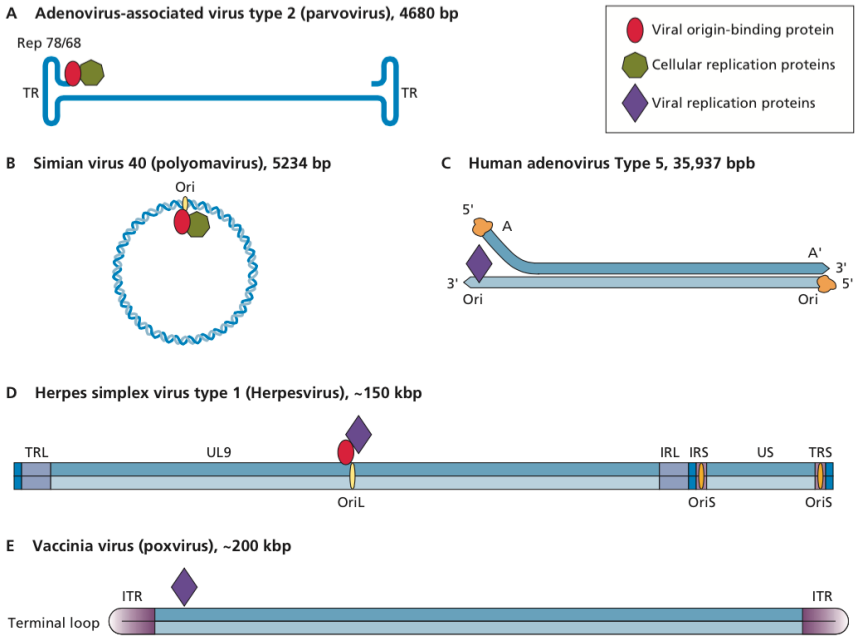

- AT-rich segments recognized by viral origin recognition proteins
- Assembly points for multi-protein DNA replication machines
- Some viral genomes have one ori; others up to 3

# Viral origins of DNA replication

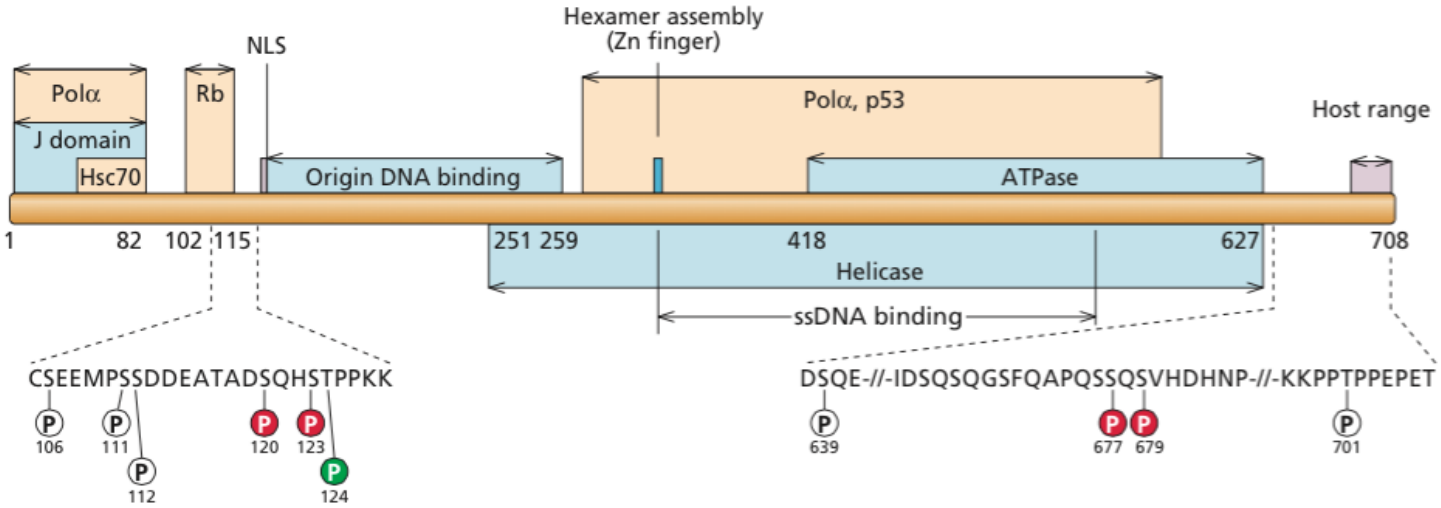


# Viral origin recognition proteins

- Polyomavirus T binds specifically to DNA
- Papillomavirus E1 binds ori in presence of E2
- Parvovirus Rep68/78 binds at ends and unwinds DNA, also involved in terminal resolution
- Adenovirus pTP binds at terminus and recruits DNA pol
- Herpesvirus UL9 protein recruits viral proteins to AT-rich ori and then unwinds DNA



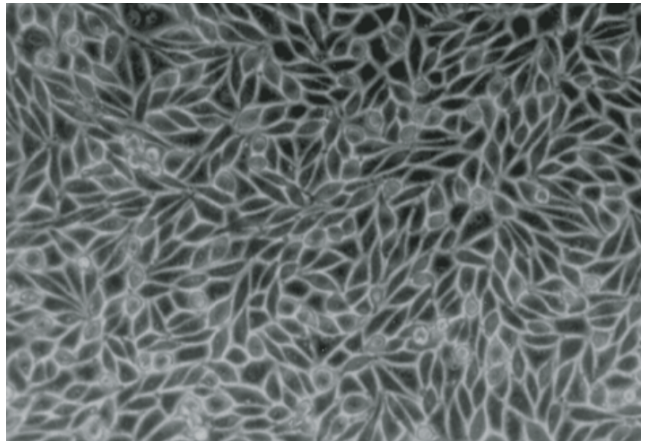
# SV40 large T

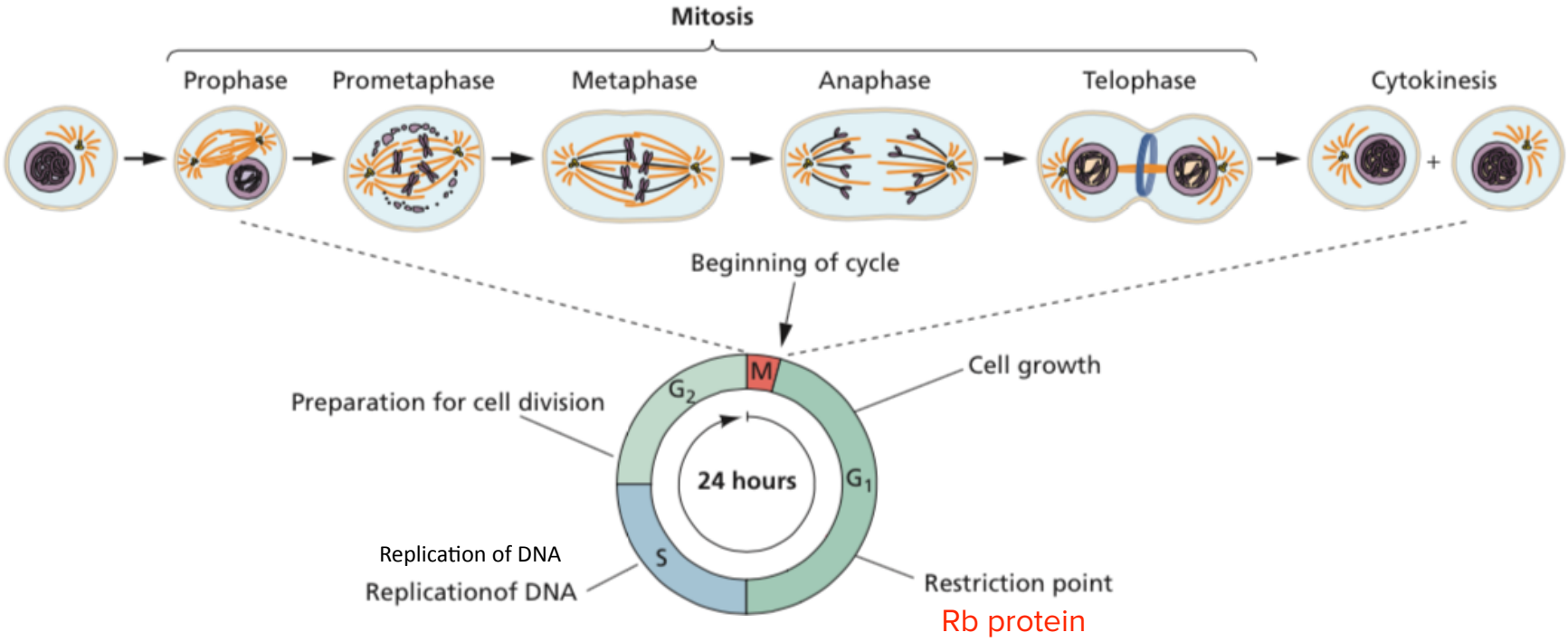



- T is a species-specific DBP/OBP
  - Pre-initiation complexes do not form in the wrong species
  - Failure to interact with DNA pol α - primase
- Binds and sequesters cell cycle regulators
  - Causes cells to enter S phase

# Regulation of DNA synthesis

- Most of our cells do not divide or do so rarely
- Viruses do not replicate well in quiescent cells
- Viruses must induce host replication proteins
- Done by virus encoded early gene products






- Cellular retinoblastoma (rb) gene
- Rb protein controls entry into S
- Rb loss associated with tumors = tumor suppressor gene

# Abrogation of Rb by viral proteins