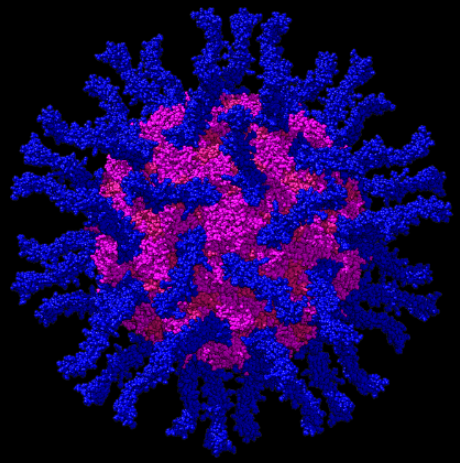


VIROLOGY LIVE

WITH VINCENT RACANIELLO

Viral DNA Replication

Lecture 8

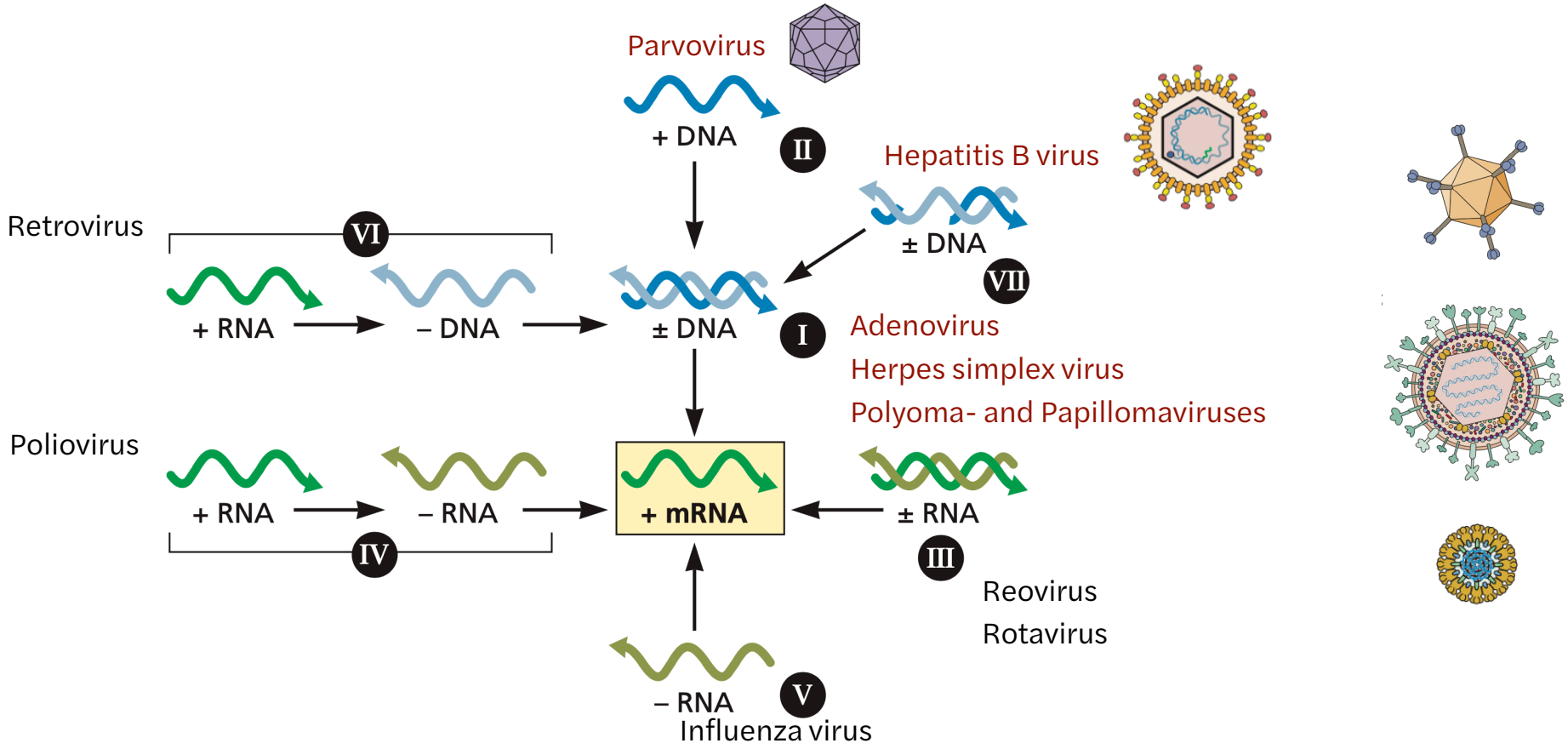
Virology Live

Fall 2021

The more the merrier

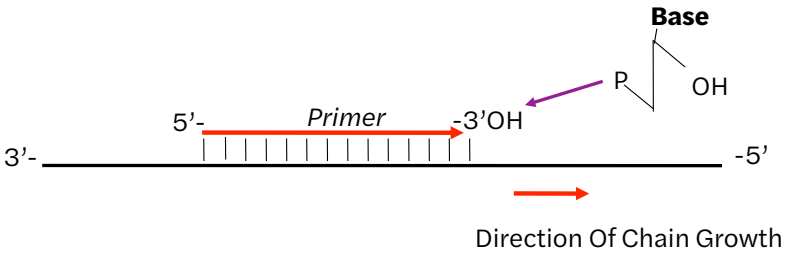
--ANONYMOUS

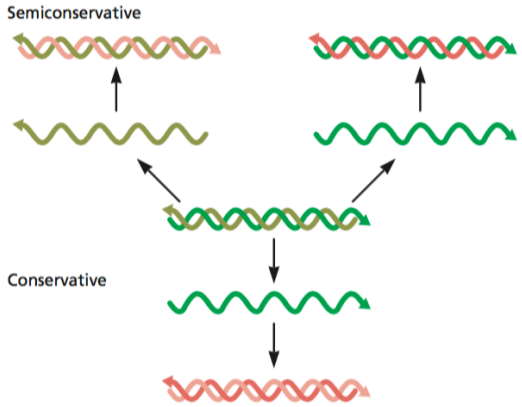
Viral DNA genomes must be replicated to make new progeny



Viral DNA replication is always delayed after infection!

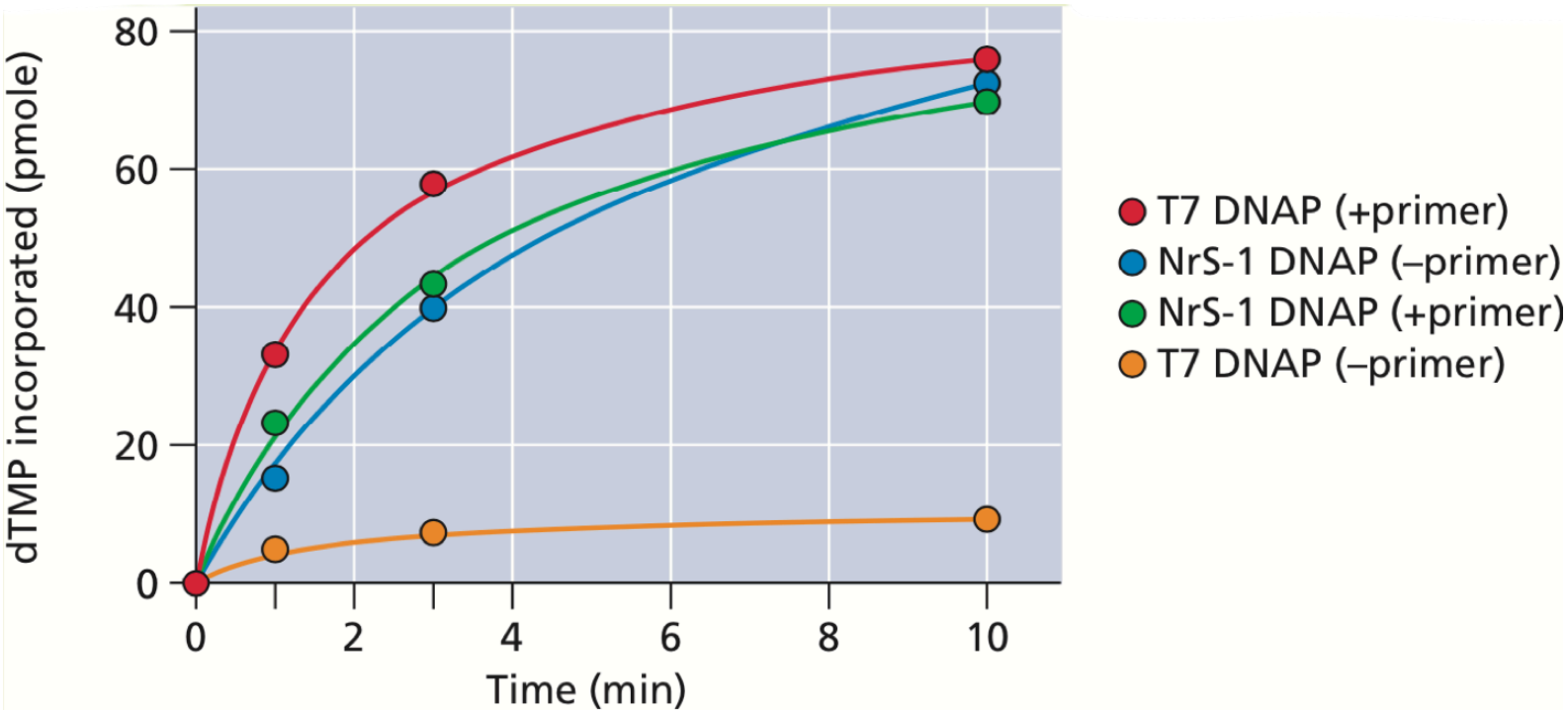
Universal rules of DNA replication



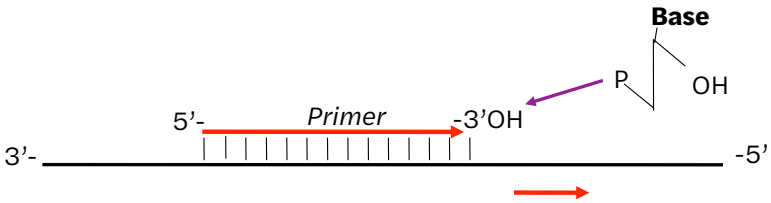


- DNA is synthesized by template-directed incorporation of dNMPs into 3'-OH of DNA chain
- DNA is always synthesized 5'-3' via semiconservative replication (two daughter strands)
- Replication initiates at specific sites on template called **origins**
- Catalyzed by DdDp + accessory proteins
- May be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

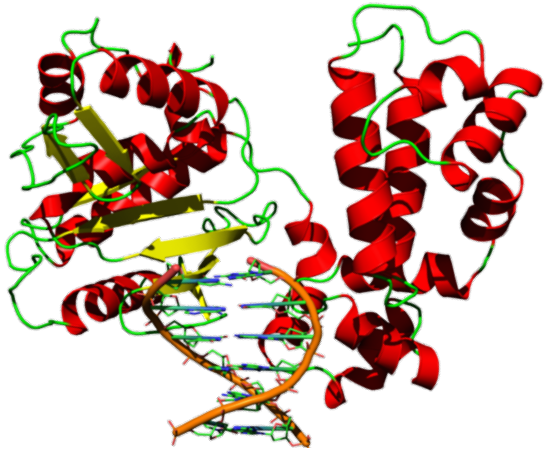
Primer-independent DNA polymerase: Dogma overturned

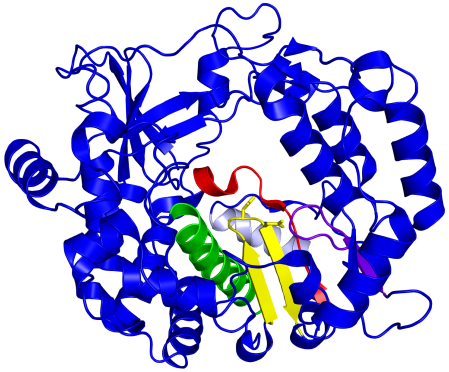


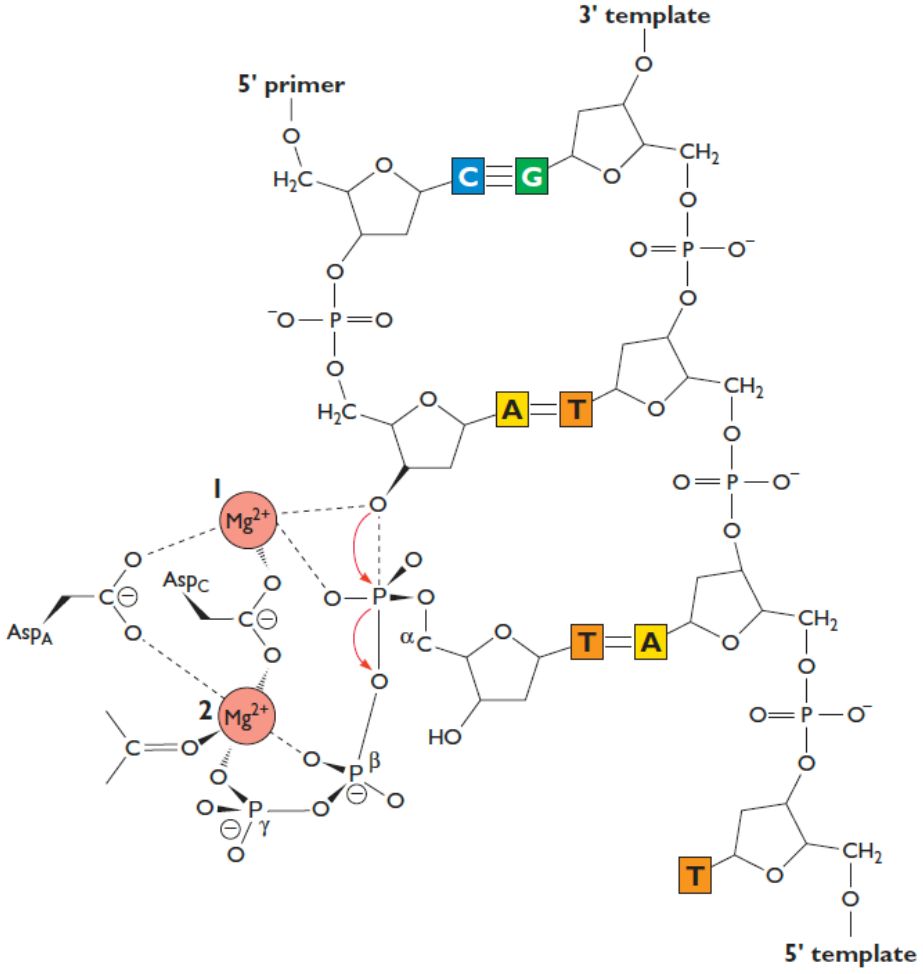
DNA polymerase encoded in genome of bacteriophage NrS-1
Infects bacteria that inhabit deep-sea vents

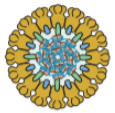


Direction Of Chain Growth

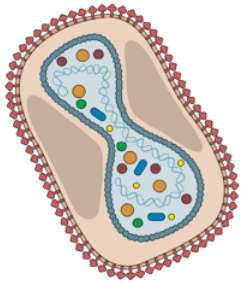








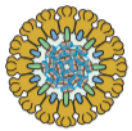
What does the host cell provide?



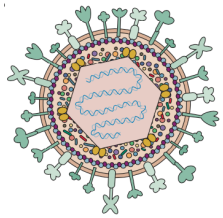
- Viral DNA replication always requires synthesis of at least one viral protein, sometimes many (hence delayed after infection)
- Viruses with small genomes require more host proteins - smaller genomes
- Viruses with larger genomes (poxviruses and mimiviruses) encode all proteins needed for DNA synthesis

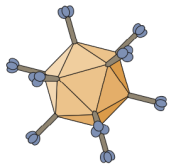
Where does the polymerase come from?

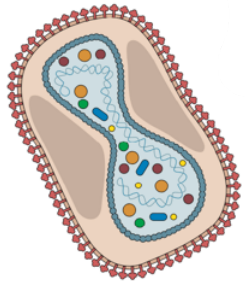
- Small DNA viruses do not encode an entire replication system
  - Encode proteins that orchestrate the host
  - *Papillomaviridae, Polyomaviridae, Parvoviridae*



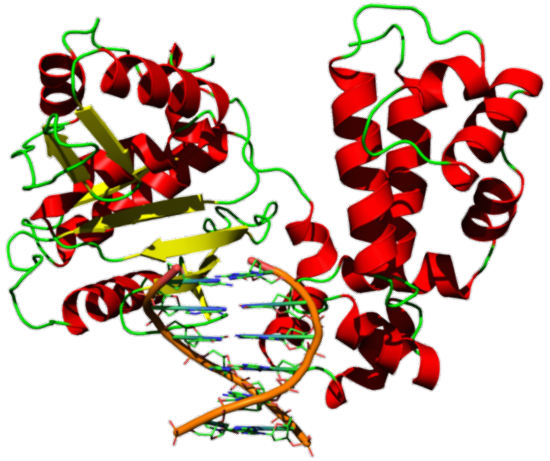
- Large DNA viruses encode most of their own replication systems
  - *Herpesviridae, Adenoviridae, Poxviridae*







Viral proteins involved in DNA replication



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

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room number: virus

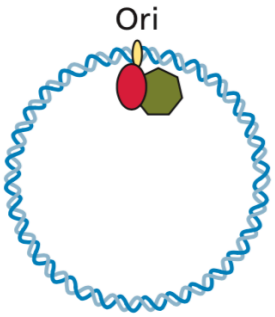
Which statement about viral DNA synthesis is NOT correct?

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 usually delayed after infection because it requires the synthesis of at least one viral protein
D. All DNA polymerases are primer-dependent

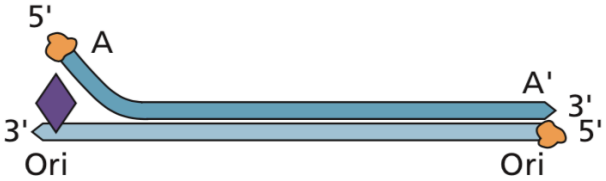
Diverse structures of viral DNAs

Adenovirus-associated virus type 2 (parvovirus), 4680 bp

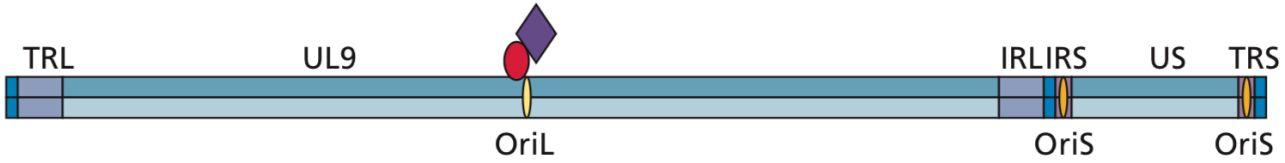
Simian virus 40 (polyomavirus), 5234 bp

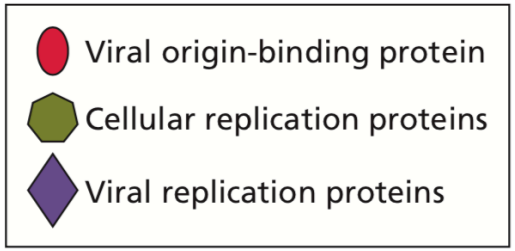


Human adenovirus type 5 , 35,937 bpb

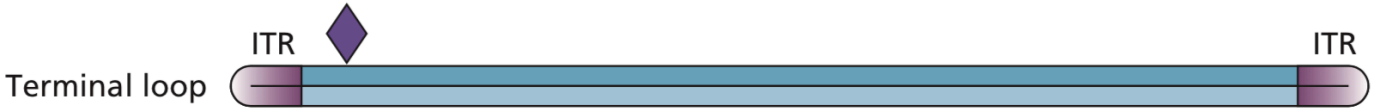


Herpes simplex virus type 1 (Herpesvirus), ~150 kbp



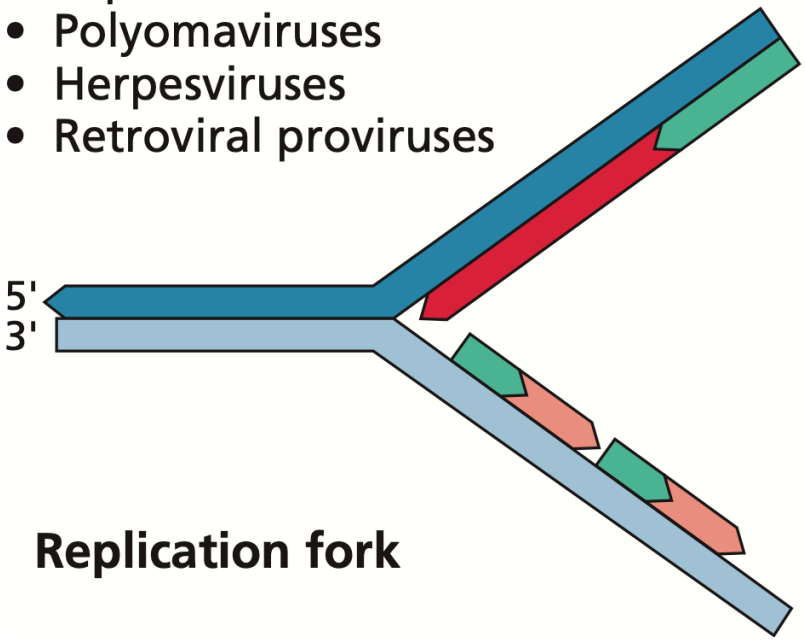


Vaccinia virus (poxvirus), ~200 kbp



Two mechanisms of dsDNA synthesis

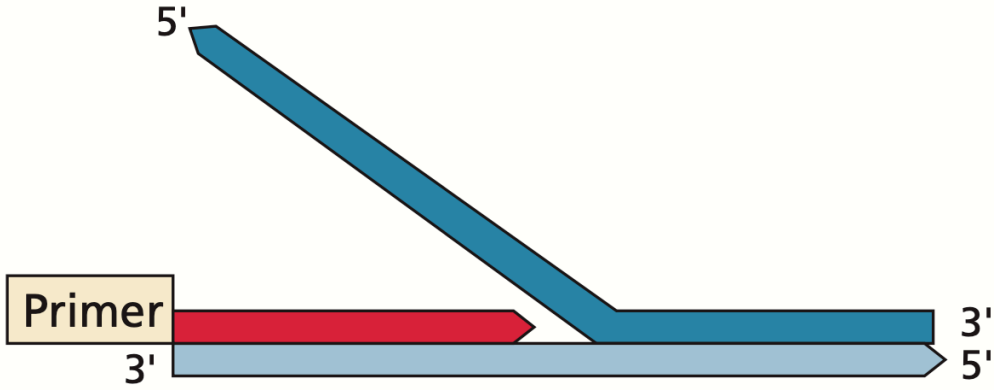
- Papillomaviruses
- Polyomaviruses
- Herpesviruses
- Retroviral proviruses



Replication fork

RNA primers

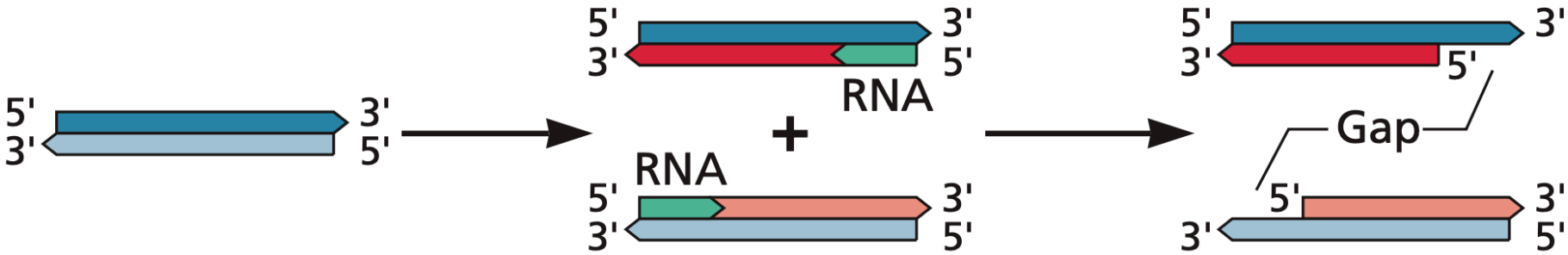
- Adenoviruses (protein)
- Parvoviruses (DNA hairpin)



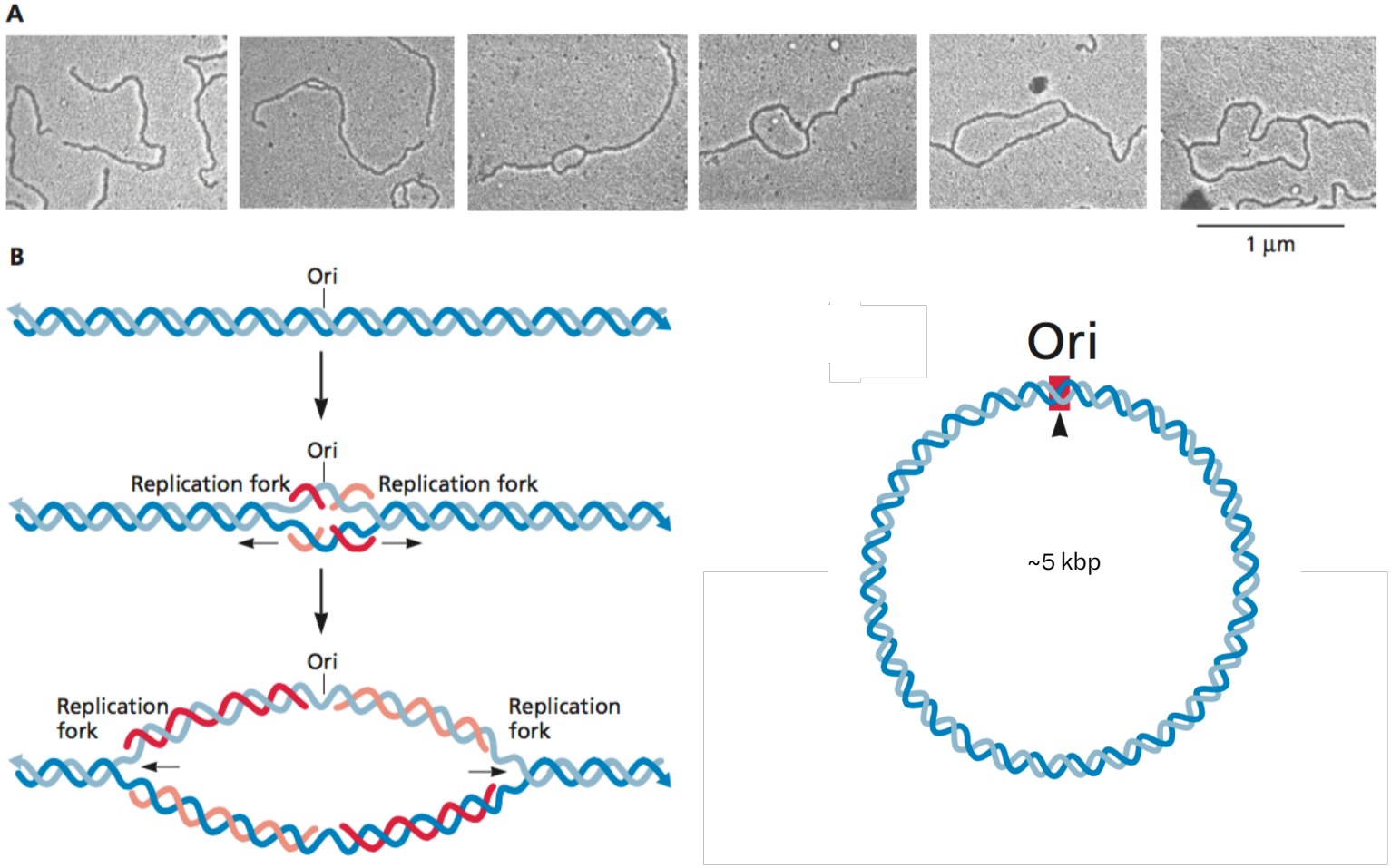
Strand displacement (primer)

Never RNA primed
(protein or DNA primer)

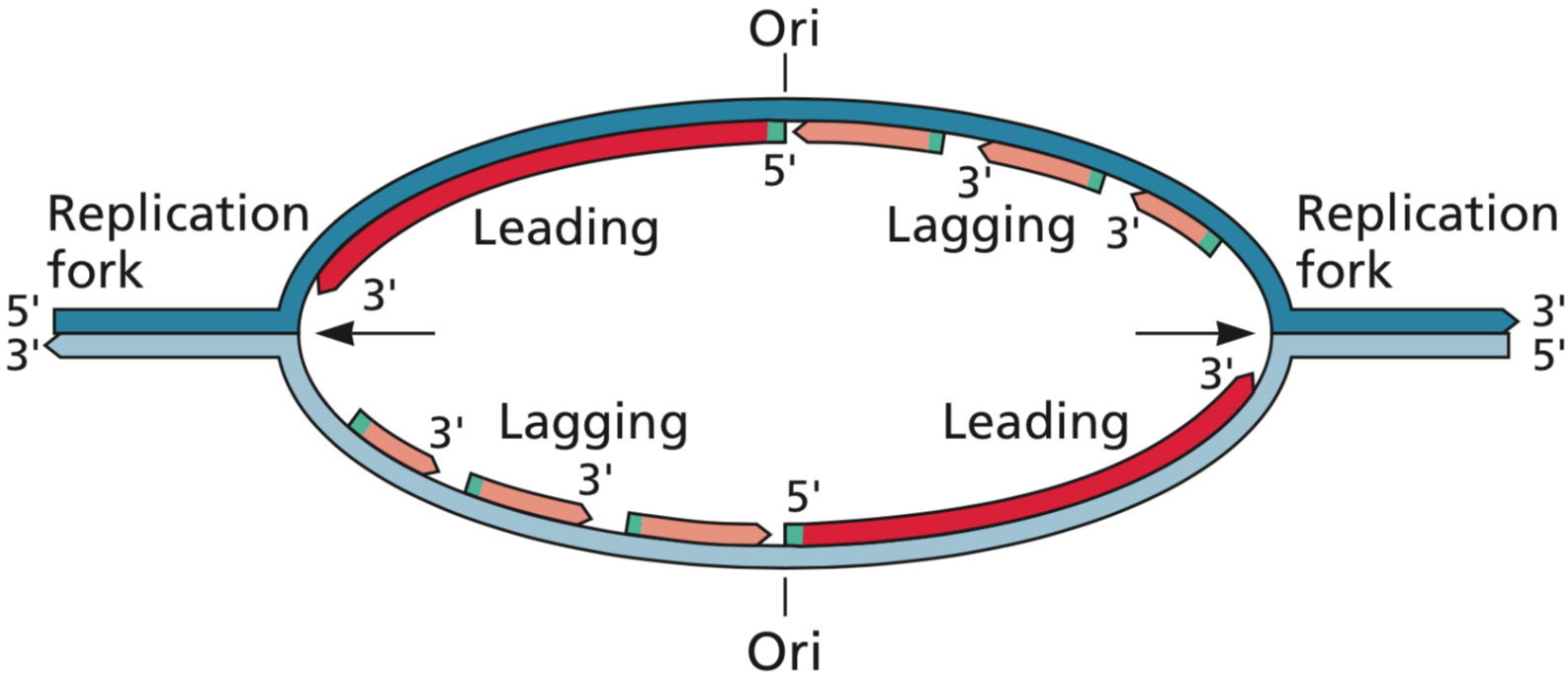
The 5’-end problem



Lessons from SV40

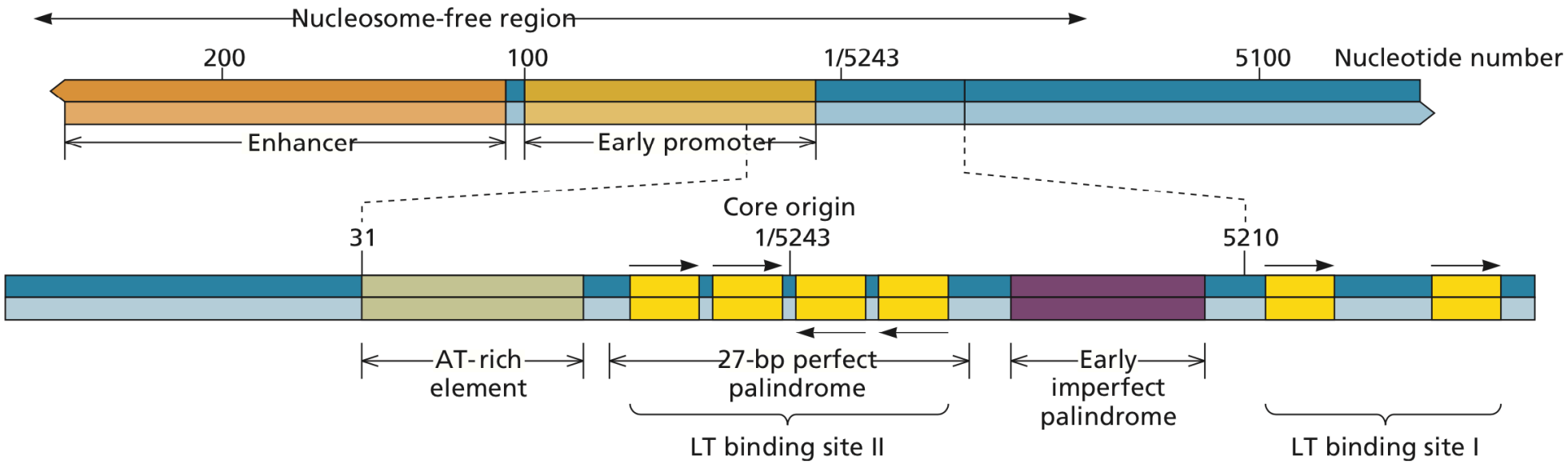


Semi-discontinuous DNA synthesis from a bidirectional origin

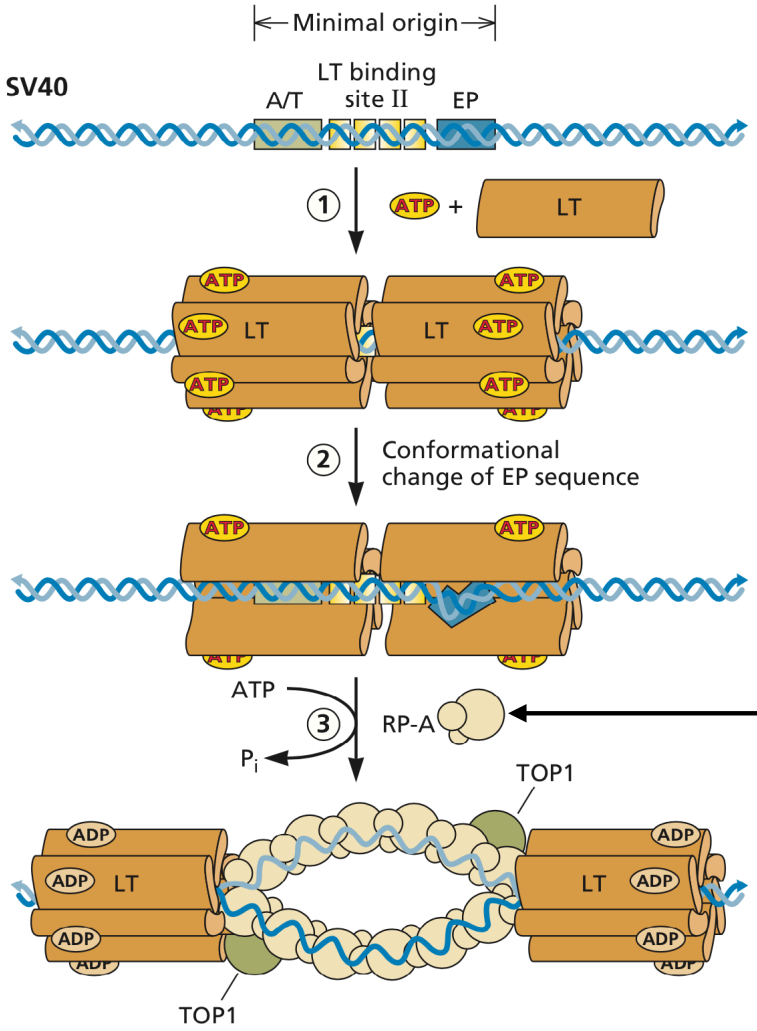


No end problem!

Origin of SV40 DNA replication



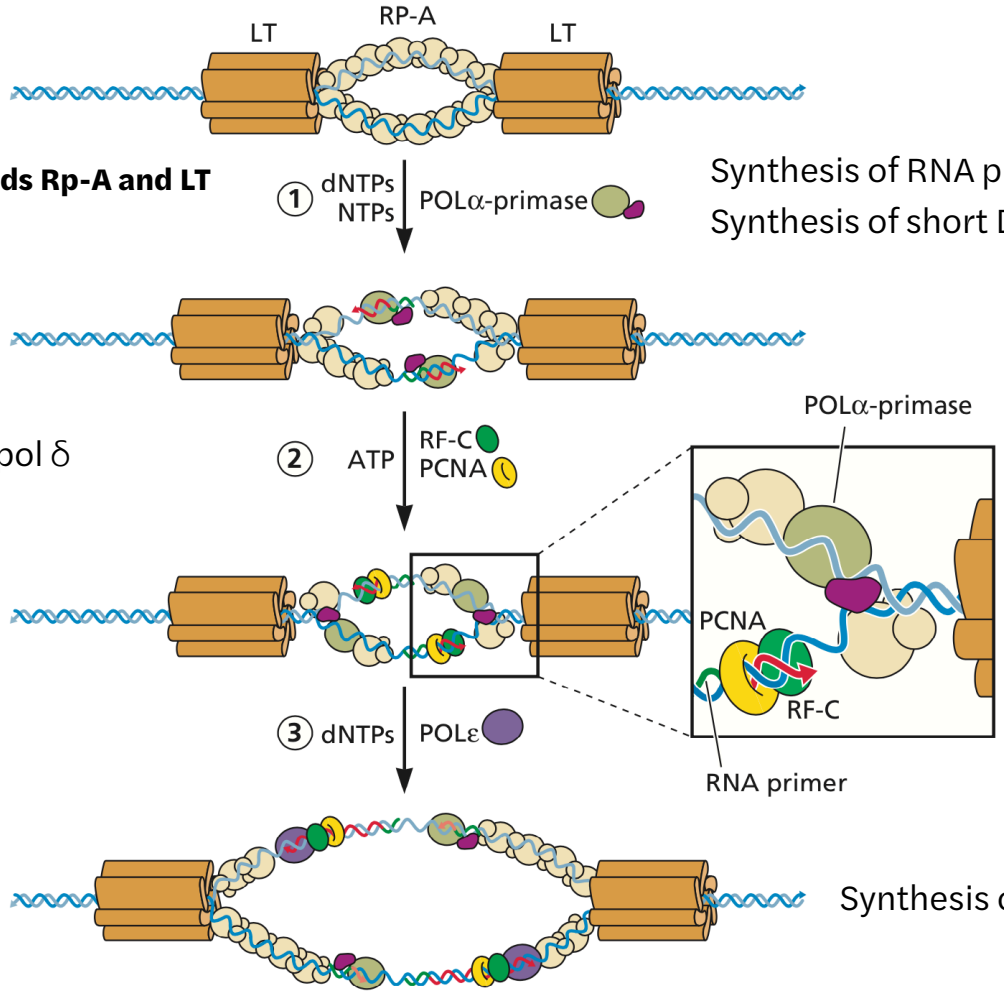
Recognition and unwinding of SV40 origin



Rp-A binds LT!

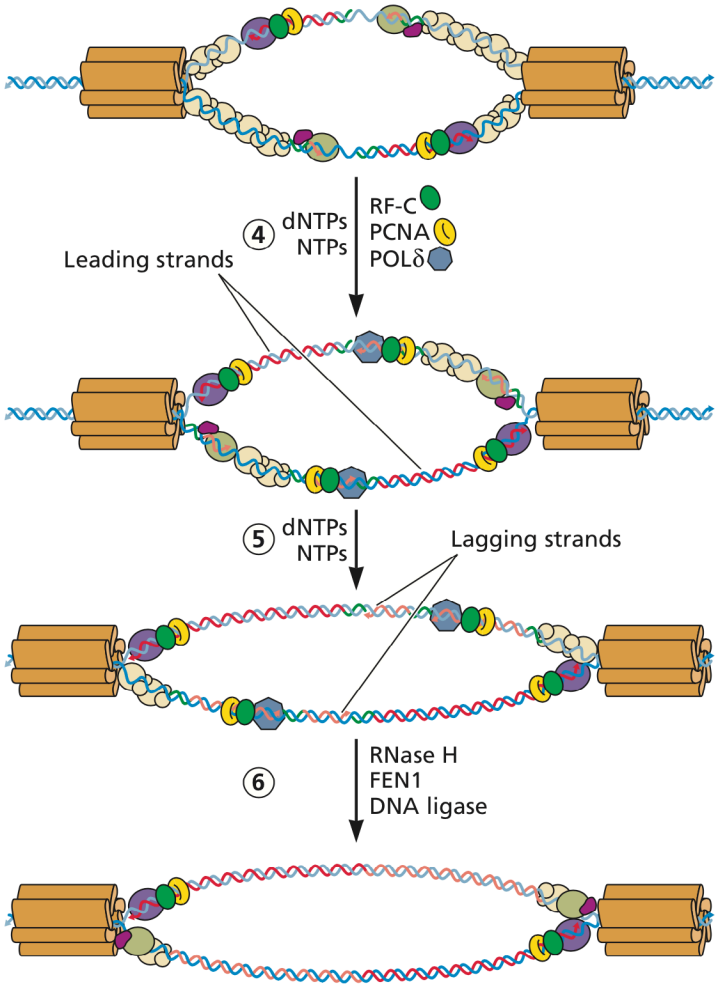
T has 3’-5’ helicase activity

Synthesis of leading and lagging strands

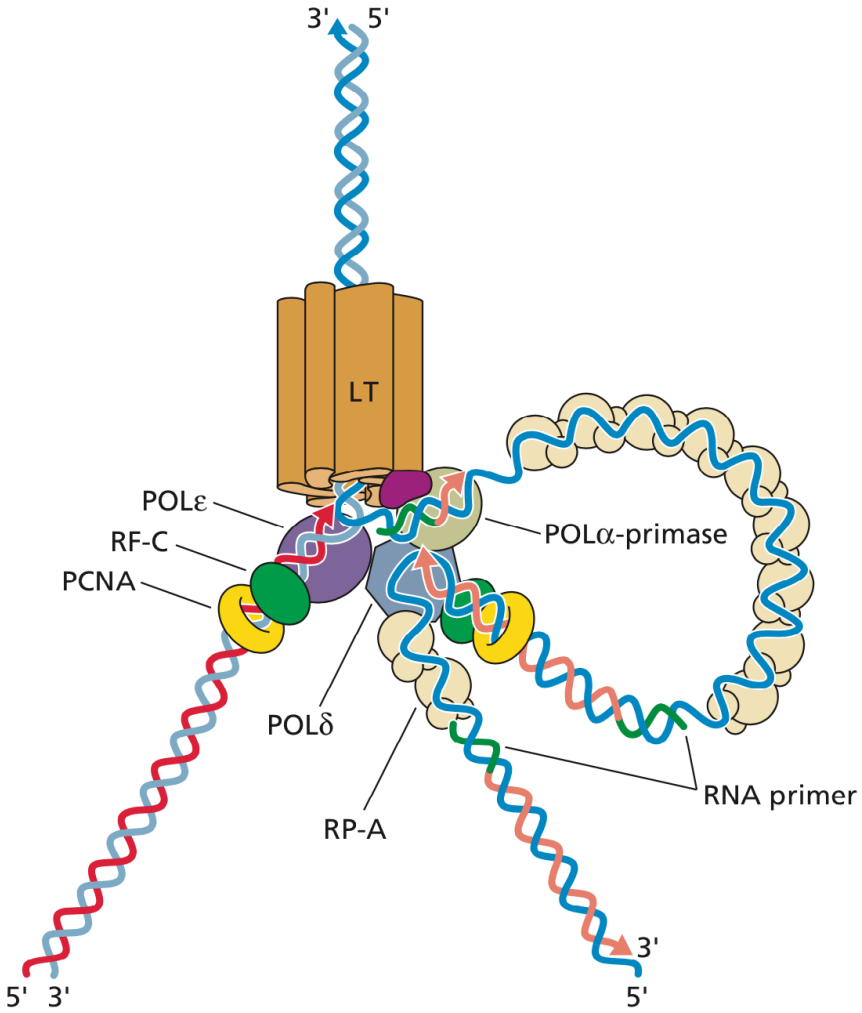


Rf-C binds 3'OH along with PCNA and pol δ
—RF-C a clamp loading protein
—Allows entry of PCNA on DNA
—Causes release of pol α
Form sliding clamps along DNA

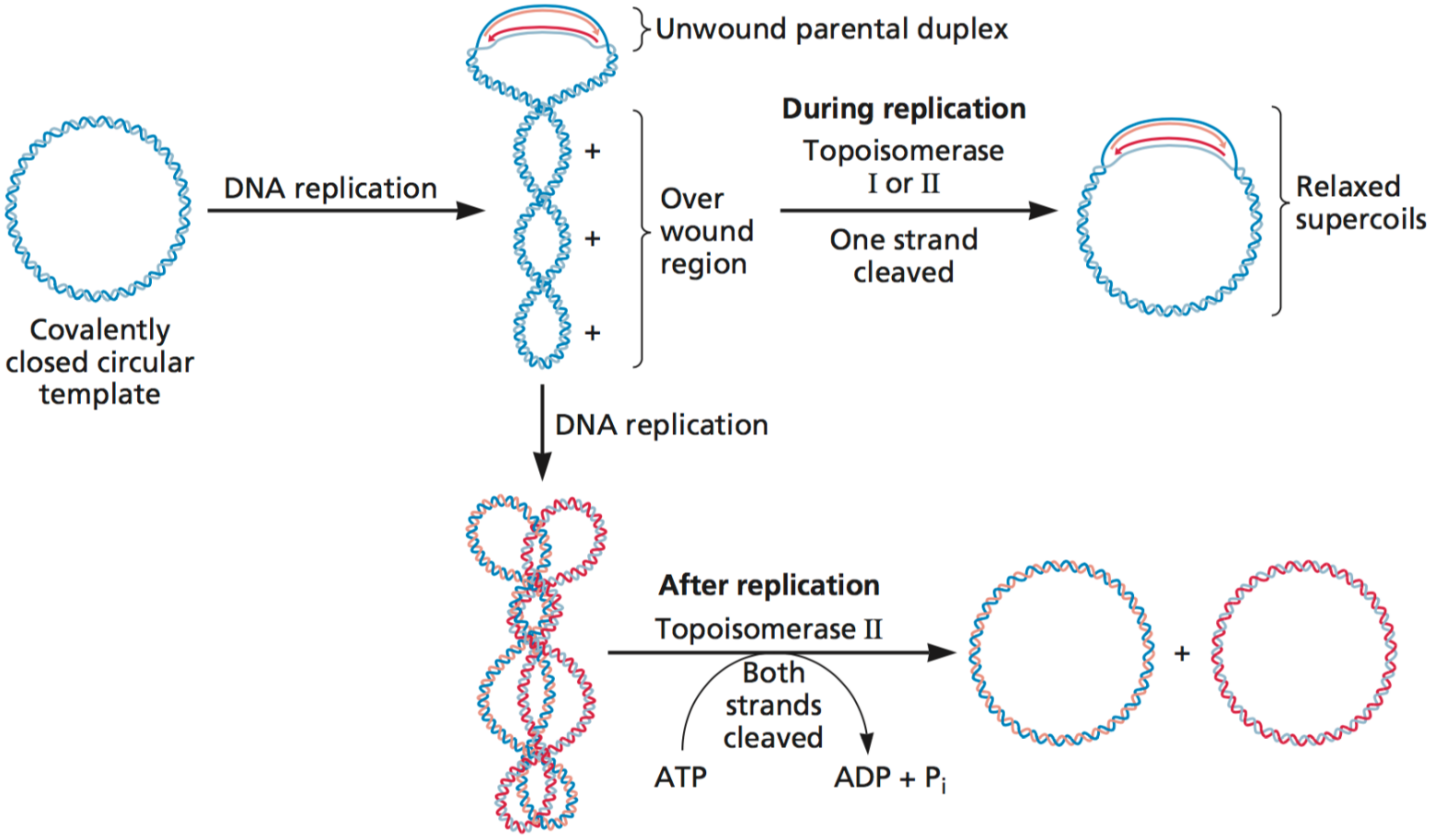
Synthesis of leading and lagging strands



SV40 DNA replication machine



Function of topoisomerases



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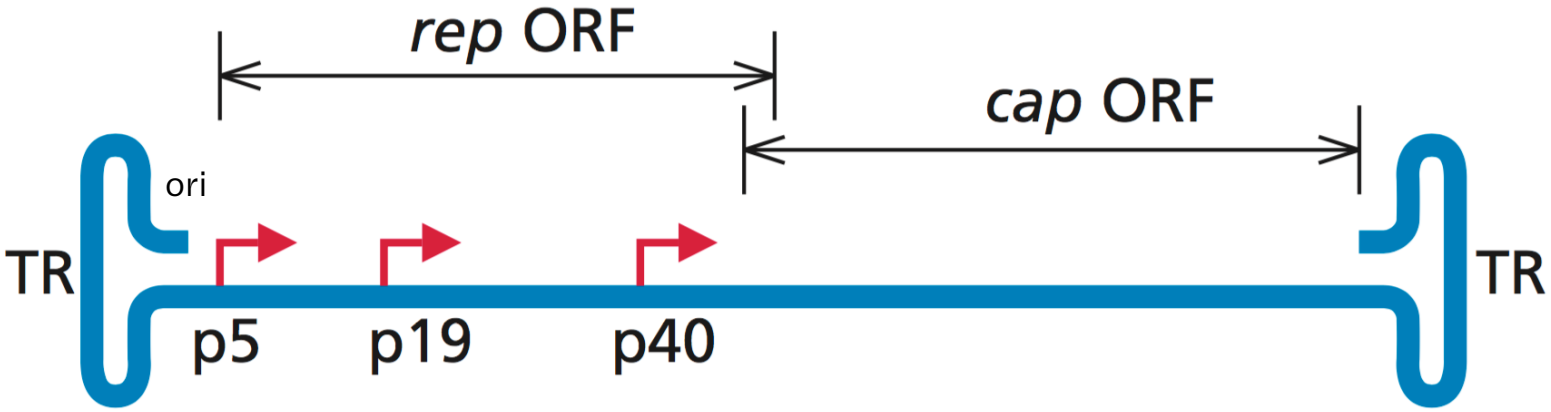
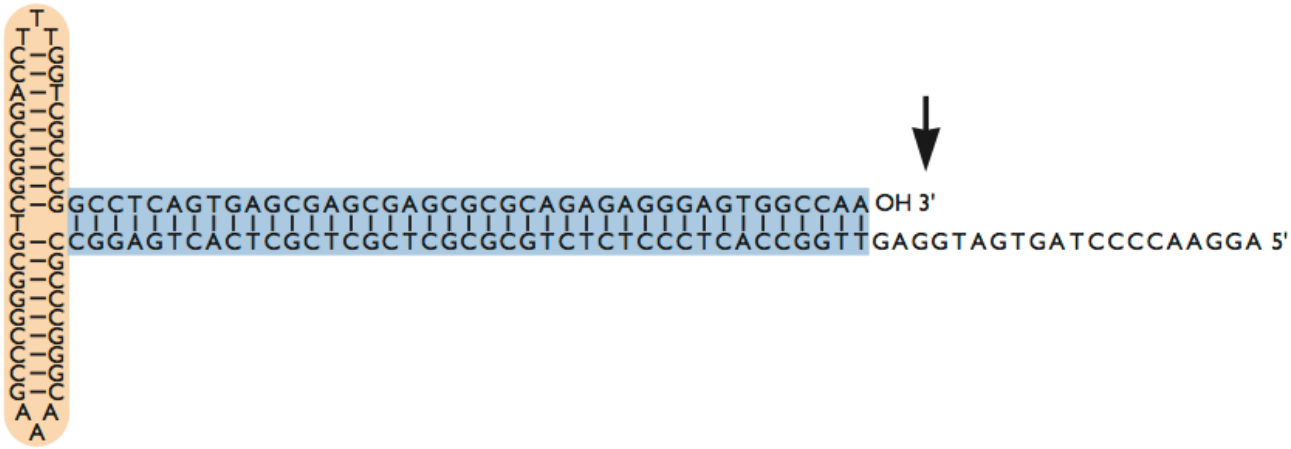
room number: virus

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

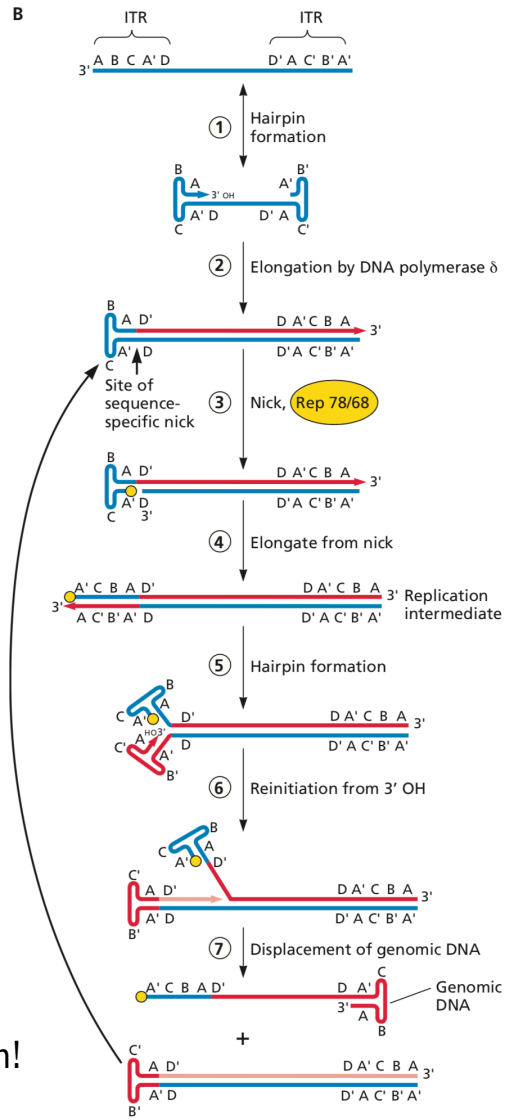
A. Viral T antigen binds and unwinds the ori
B. Replication is bidirectional from a single ori
C. The 5'-end problem is solved
D. Has leading and lagging strand synthesis
E. All of the above

DNA priming: Parvoviruses





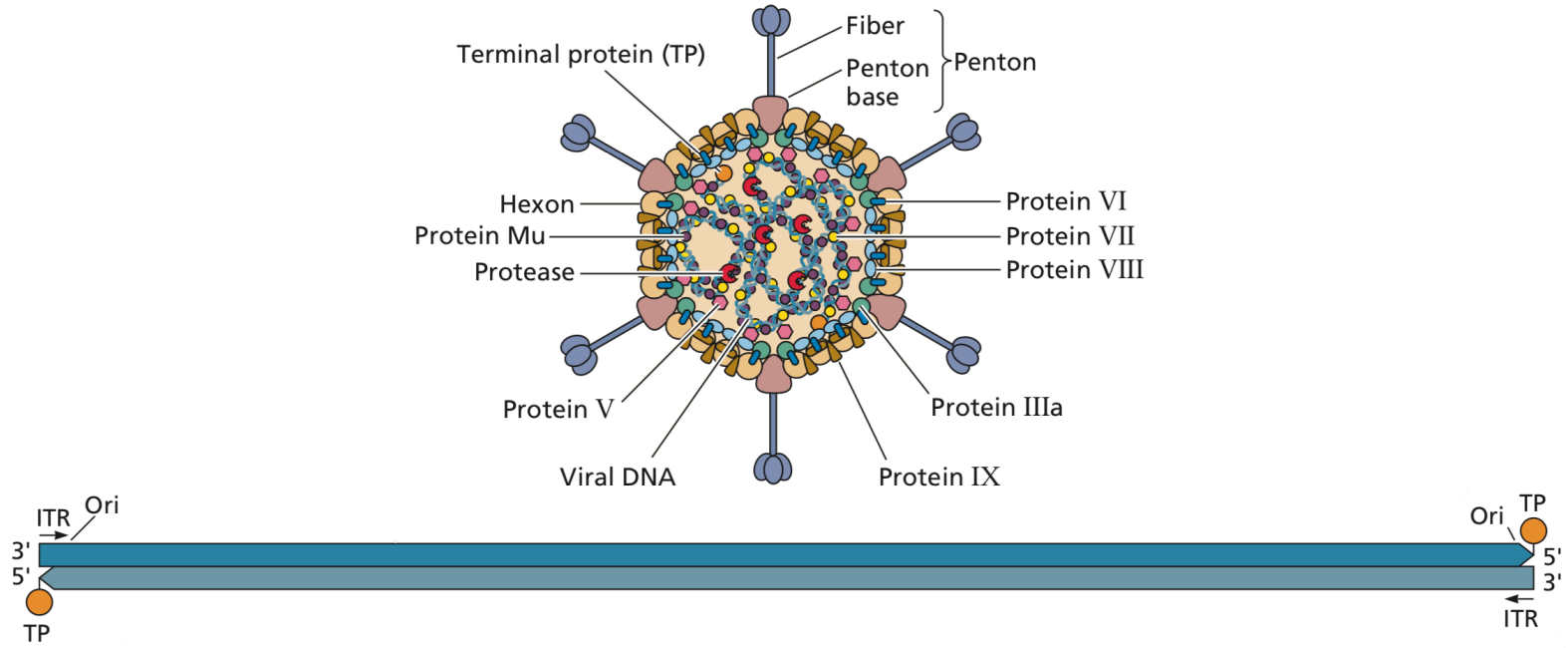
DNA priming: Parvoviruses



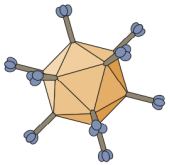
- Replication is continuous
- No pol α, uses ITR to self-prime
- Requires pol δ, RF-C and PCNA
- Rep78/68 proteins are required for initiation and resolution: endonuclease, helicase, binds 5’-terminus
- No replication fork, strand displacement

No end problem!

Protein priming: Adenovirus



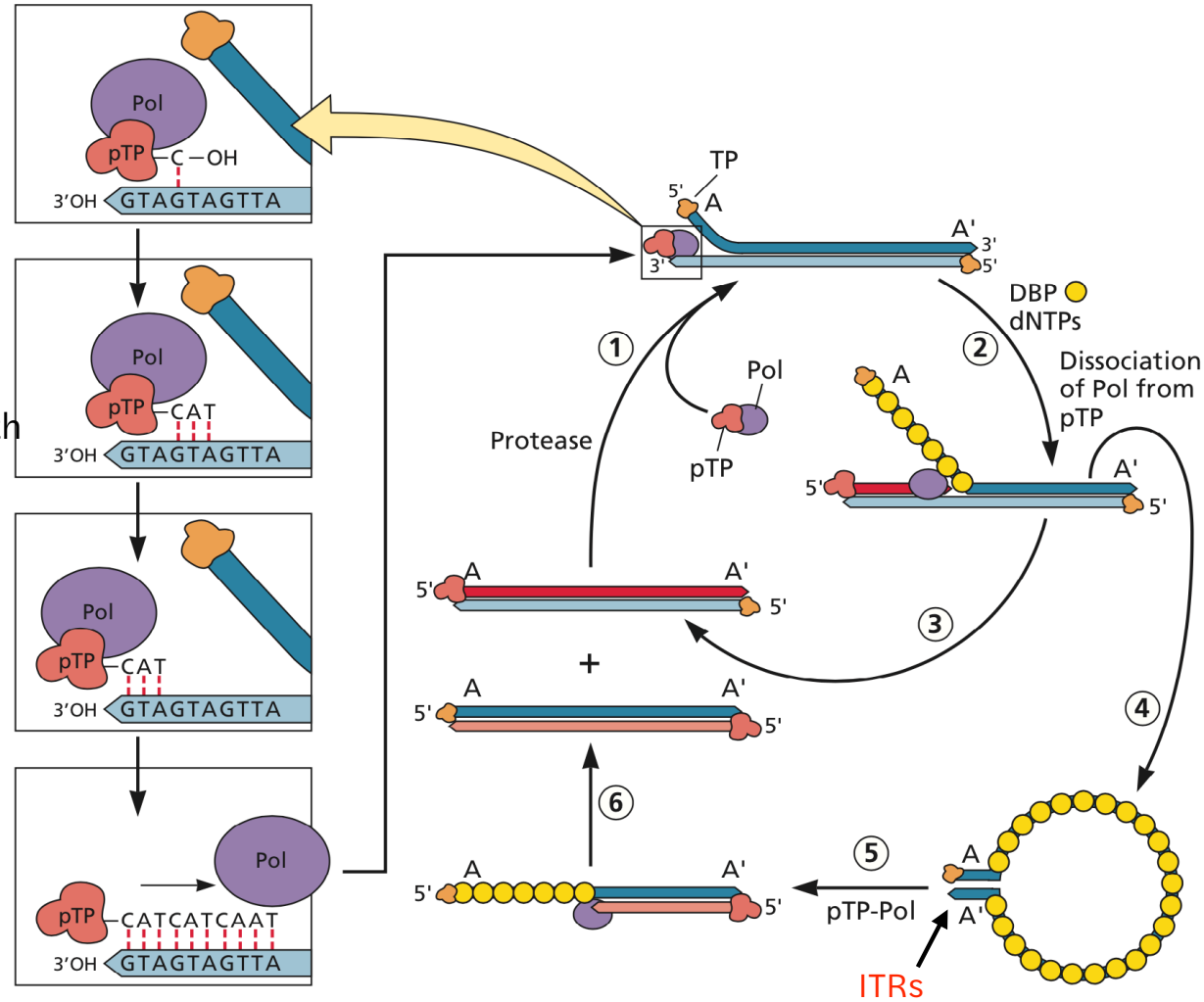
- Origins at both ends
- Strand displacement synthesis



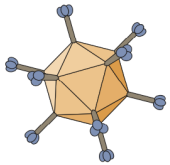
Protein priming: Adenovirus

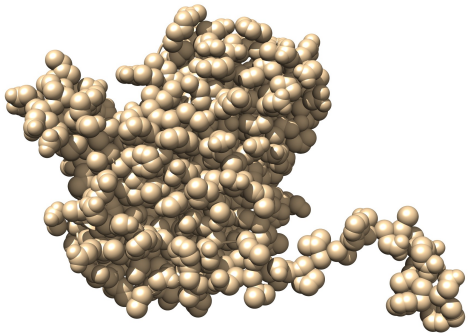
Ad DNA pol links α-phosphoryl of dCMP to OH of Ser residue only when pTP is assembled with DNA pol into preinitiation complex at ori

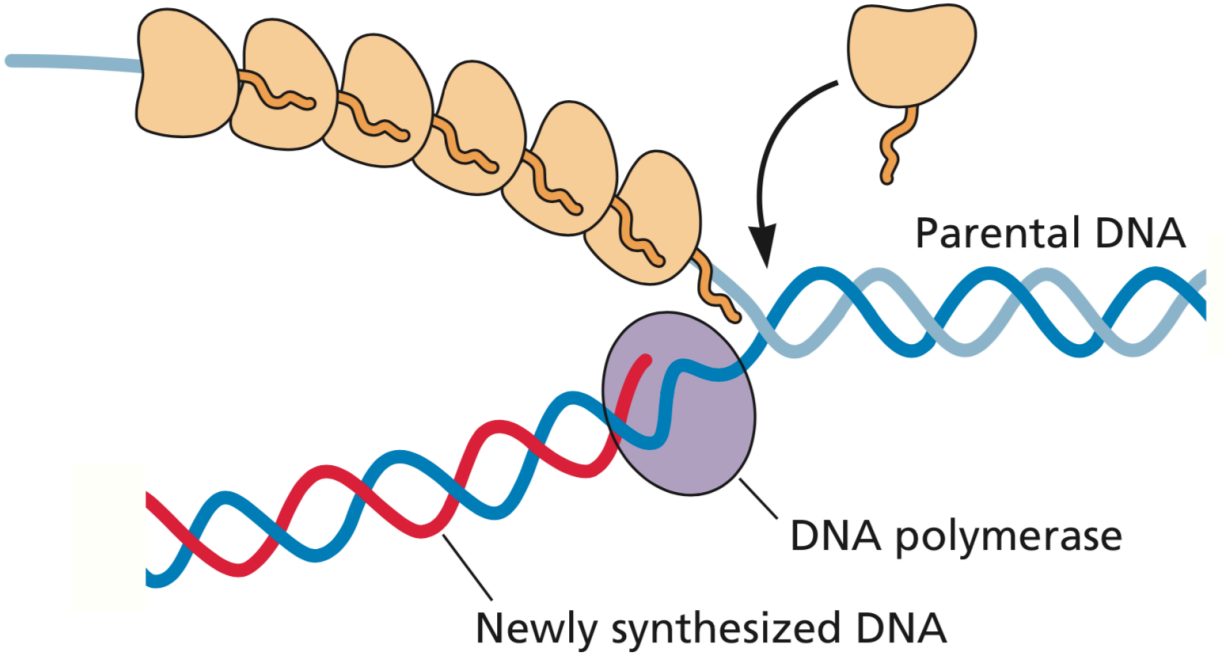
No end problem!



Adenoviral ssDNA binding protein







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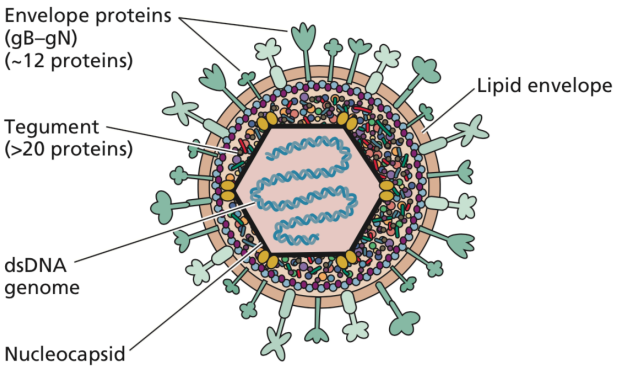
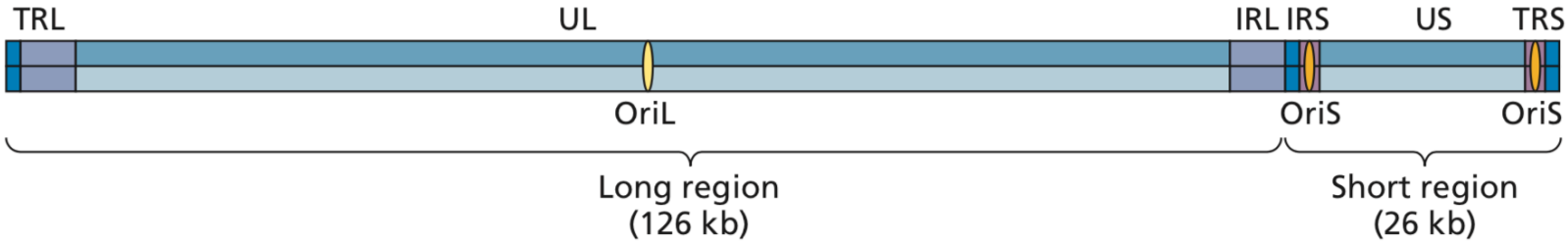
How is DNA replication of parvovirus and adenovirus similar?

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

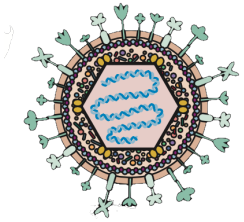
Herpes simplex virus



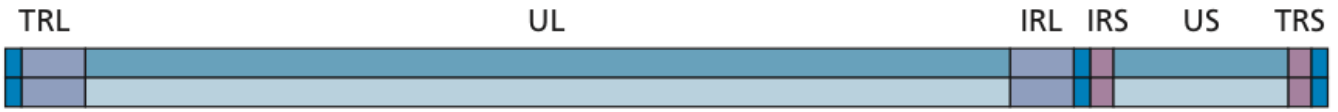
- UL5, 8 and 53 - primase
- UL42 - processivity protein
- UL9 - origin binding protein
- UL29 - ssDNA binding protein
- UL30 - DNA polymerase

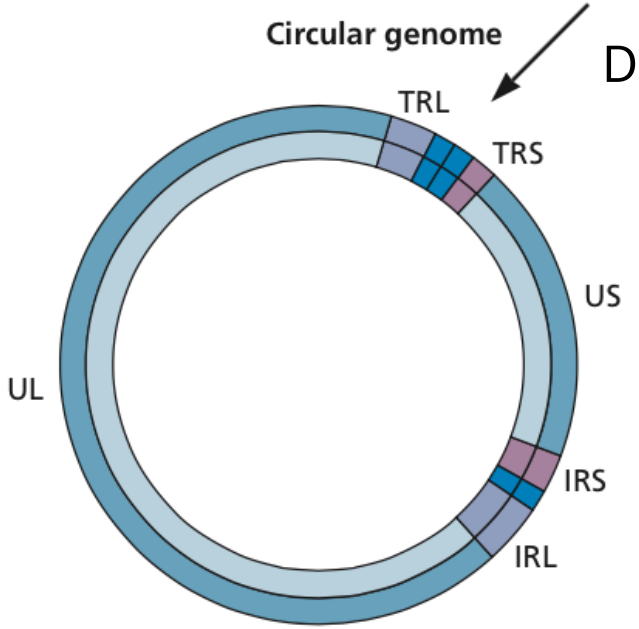


- 2 oriS and a unique oriL sequence
- DNA enters as a linear molecule and converts to circle
- Replicates as rolling circle



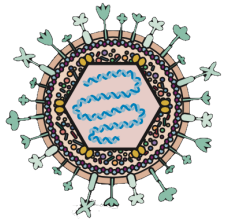
Initiation of herpesvirus DNA replication



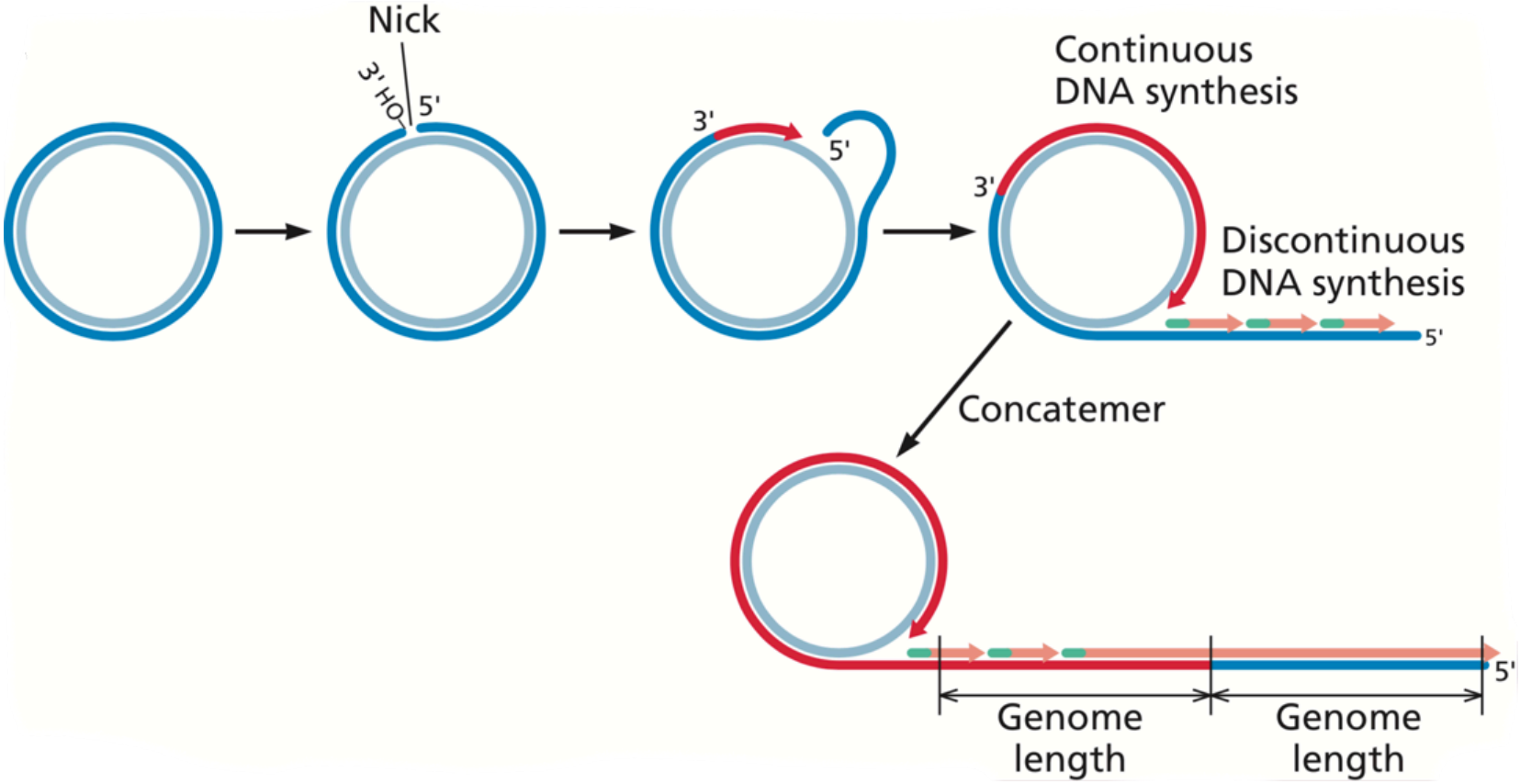


DNA ligase IV/XRCC4

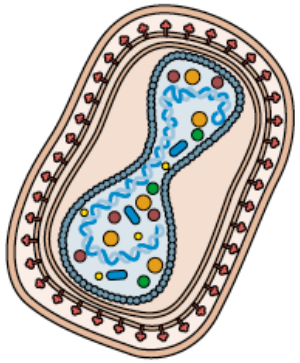
Host proteins are responsible for circularization



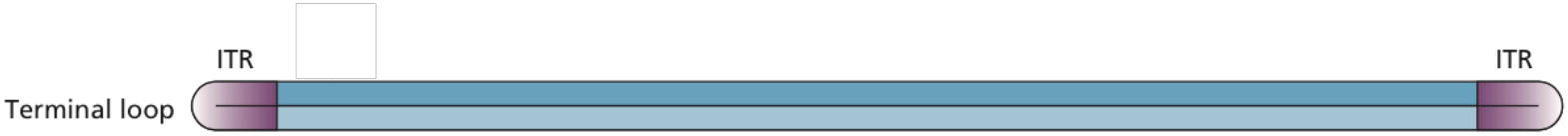
Rolling circle replication



No end problem!

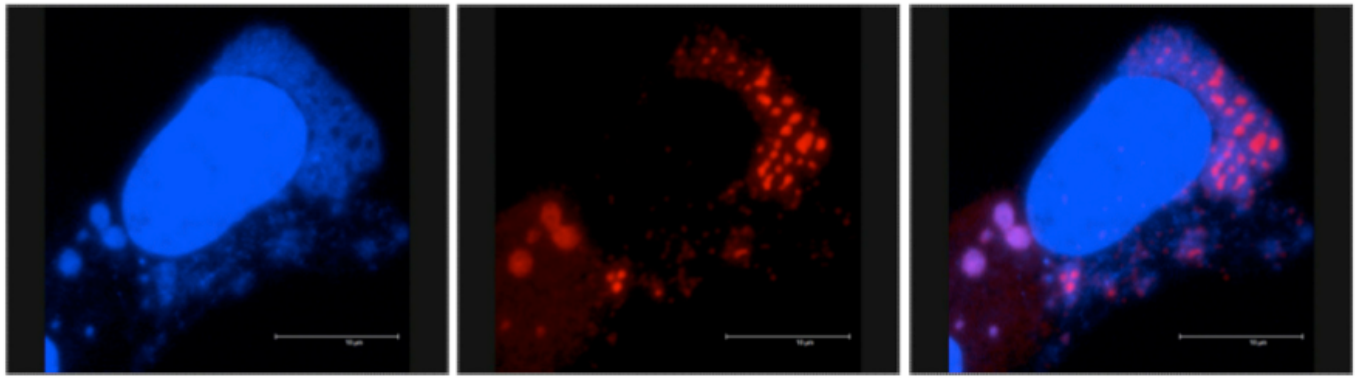


Poxvirus

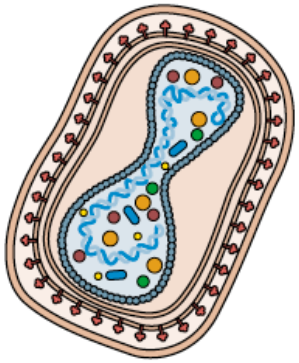


- All viruses discussed replicate in nucleus
- Poxviruses replicate in cytoplasm
- Encode all proteins needed for DNA replication

Poxvirus DNA factories

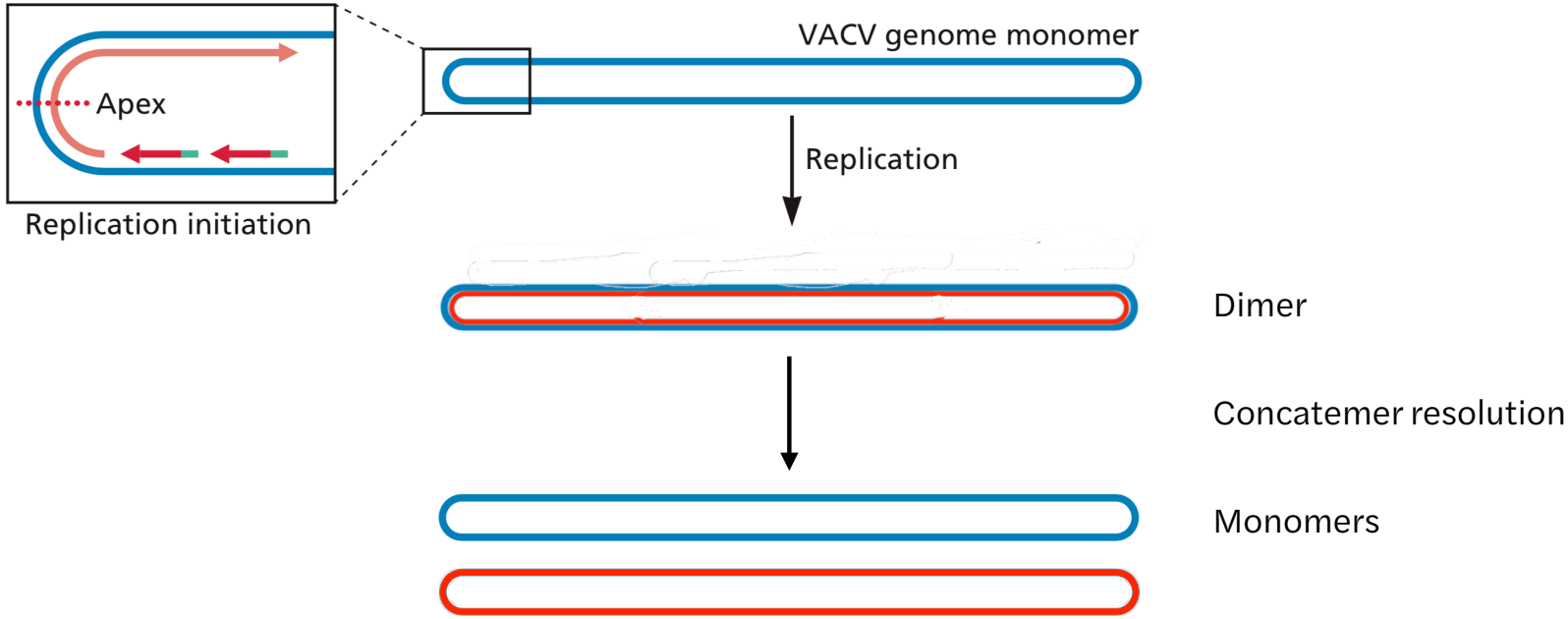


DNA | DNA binding protein | merge



Poxvirus DNA replication

At least 15 viral proteins involved in viral DNA synthesis



No end problem!

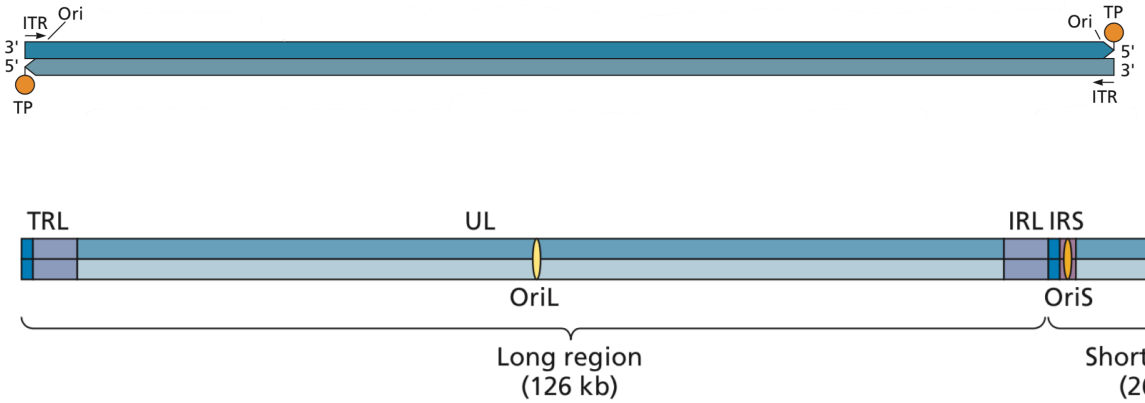
Go to:

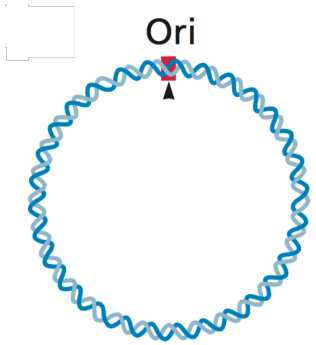
b.socrative.com/login/student
room number: virus

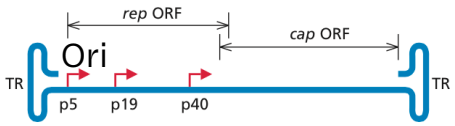
What makes poxvirus DNA replication different from all of the other viruses we discussed today?

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

Viral origins

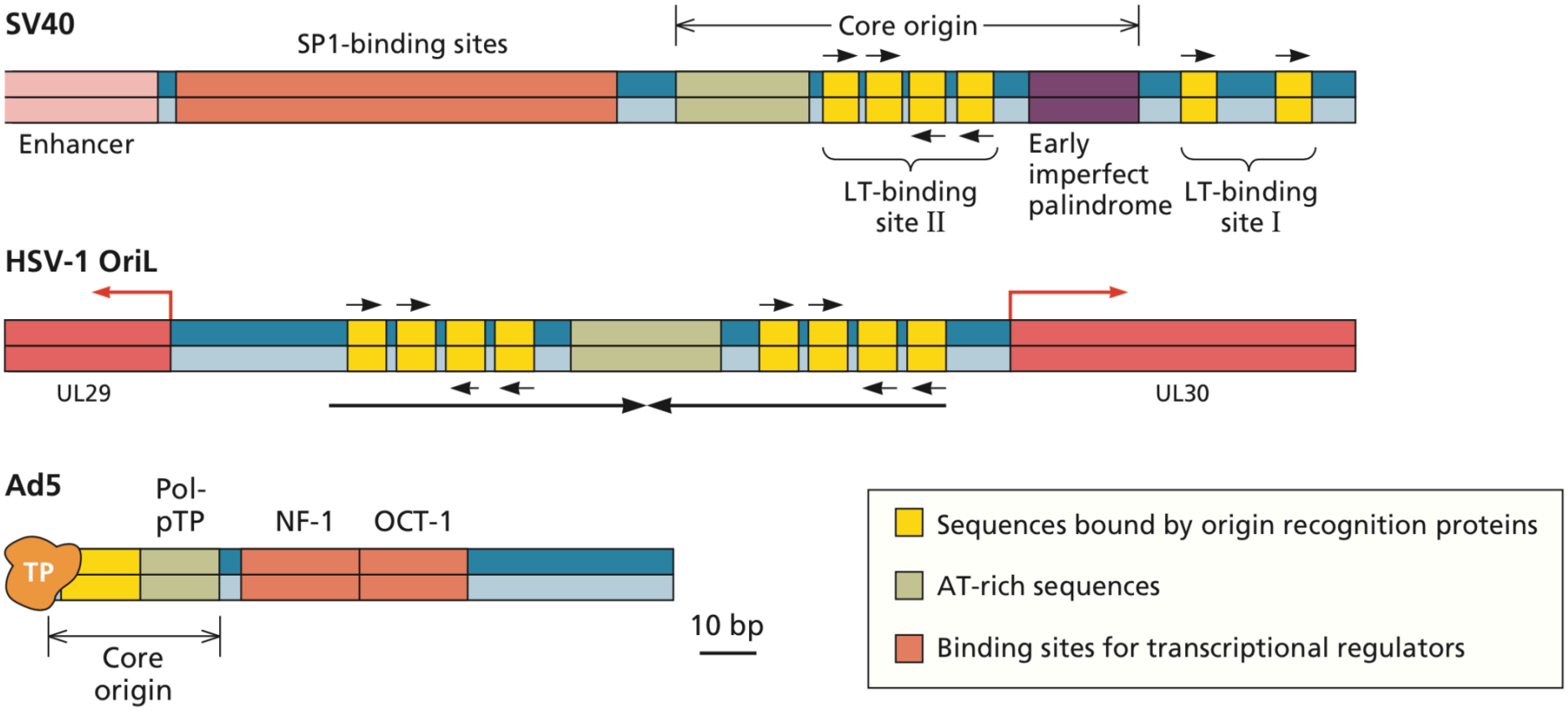






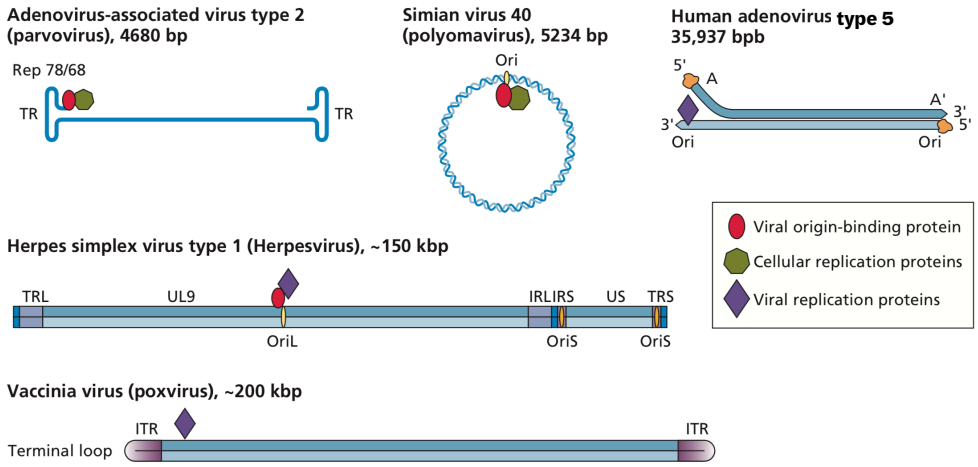
- AT-rich segments recognized by viral origin recognition proteins
- Assembly points for multi-protein DNA replication machines
- Some viral genomes have one ori; others up to 3

Viral origins of DNA replication

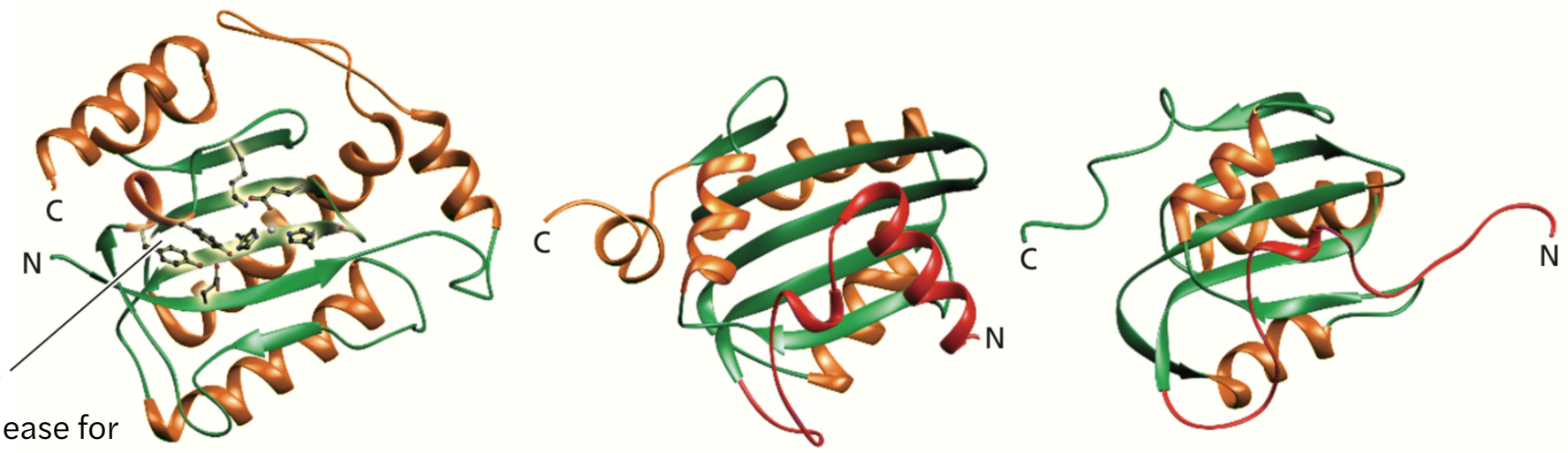


Viral origin recognition proteins

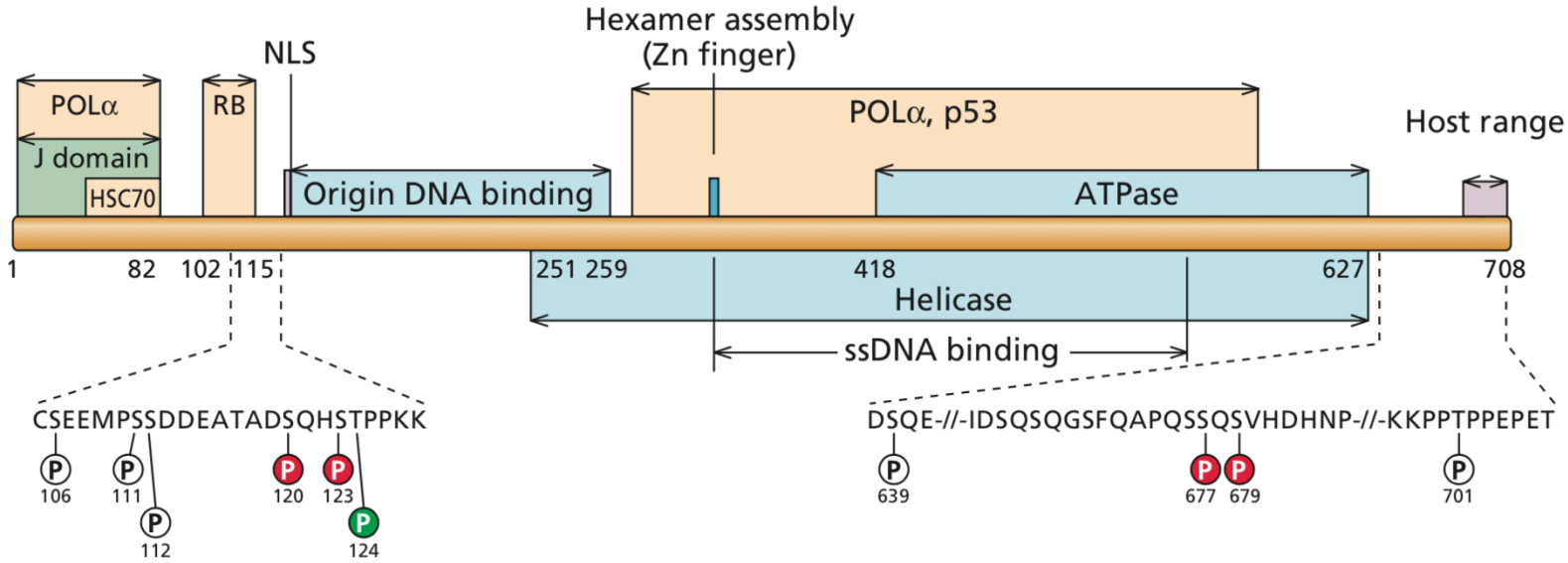
- Polyomavirus T binds specifically to DNA
- Parvovirus Rep68/78 binds at ends and unwinds DNA, also involved in terminal resolution
- Adenovirus pTP binds at terminus and recruits DNA pol
- Herpesvirus UL9 protein recruits viral proteins to AT-rich ori and then unwinds DNA



Structural homology among DNA binding domains of viral origin recognition proteins



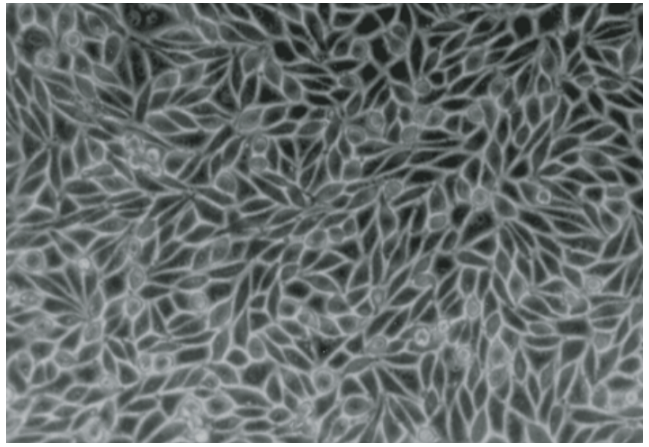
SV40 large T

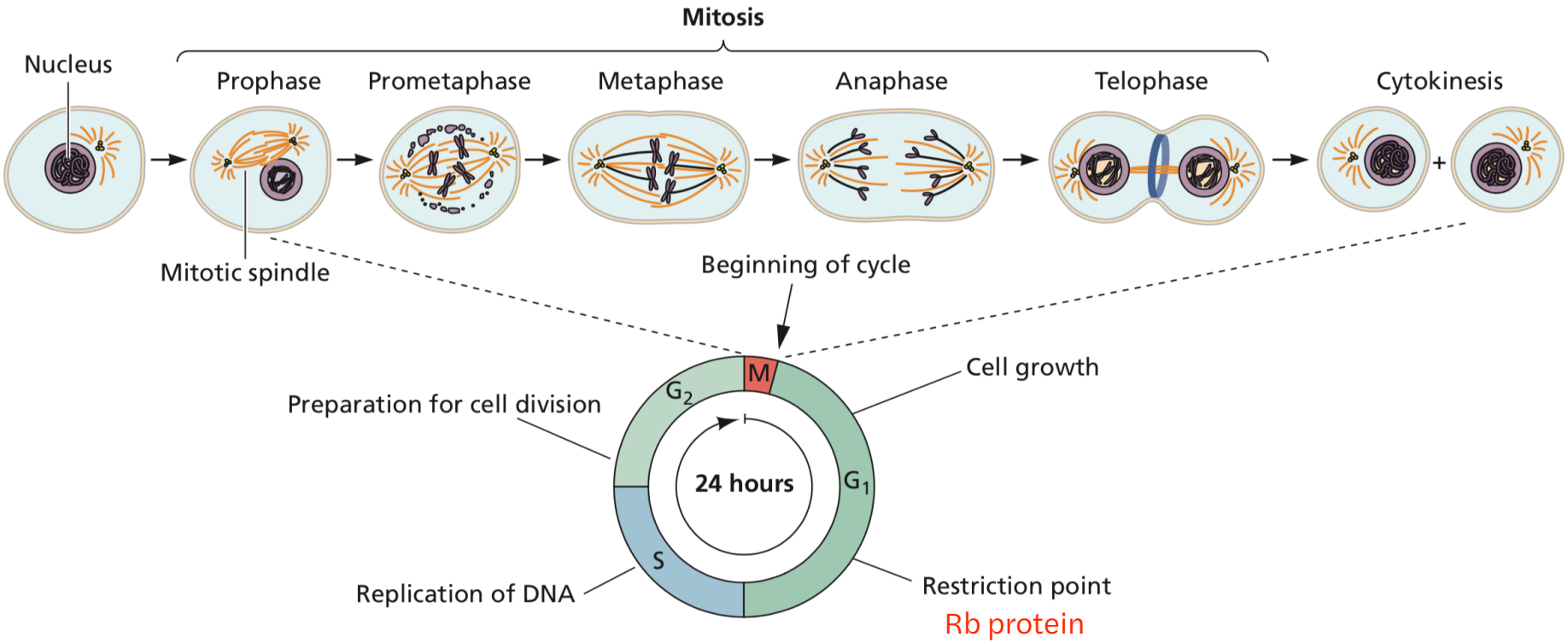


- T is a species-specific DBP/OBP
  - Pre-initiation complexes do not form in the wrong species
  - Failure to interact with DNA pol α - primase
- Binds and sequesters cell cycle regulators
  - Causes cells to enter S phase

Regulation of DNA synthesis

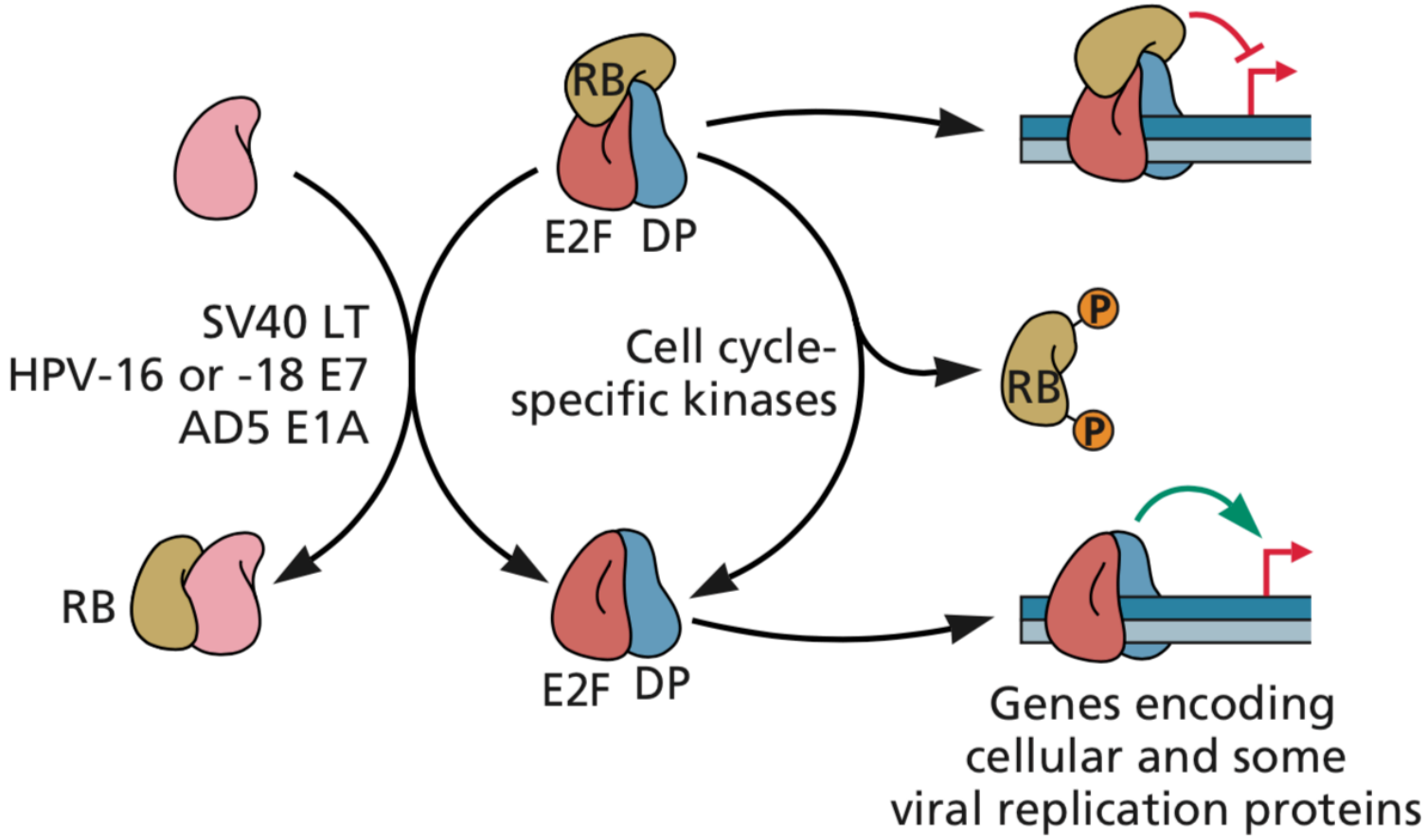
- Most of our cells do not divide or do so rarely
- Viruses do not replicate well in quiescent cells
- Viruses must induce host replication proteins
- Done by virus encoded early gene products





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

Abrogation of Rb by viral proteins



(needed for DNA synthesis, and to pass through cell cycle)

Next time: Reverse transcription and integration