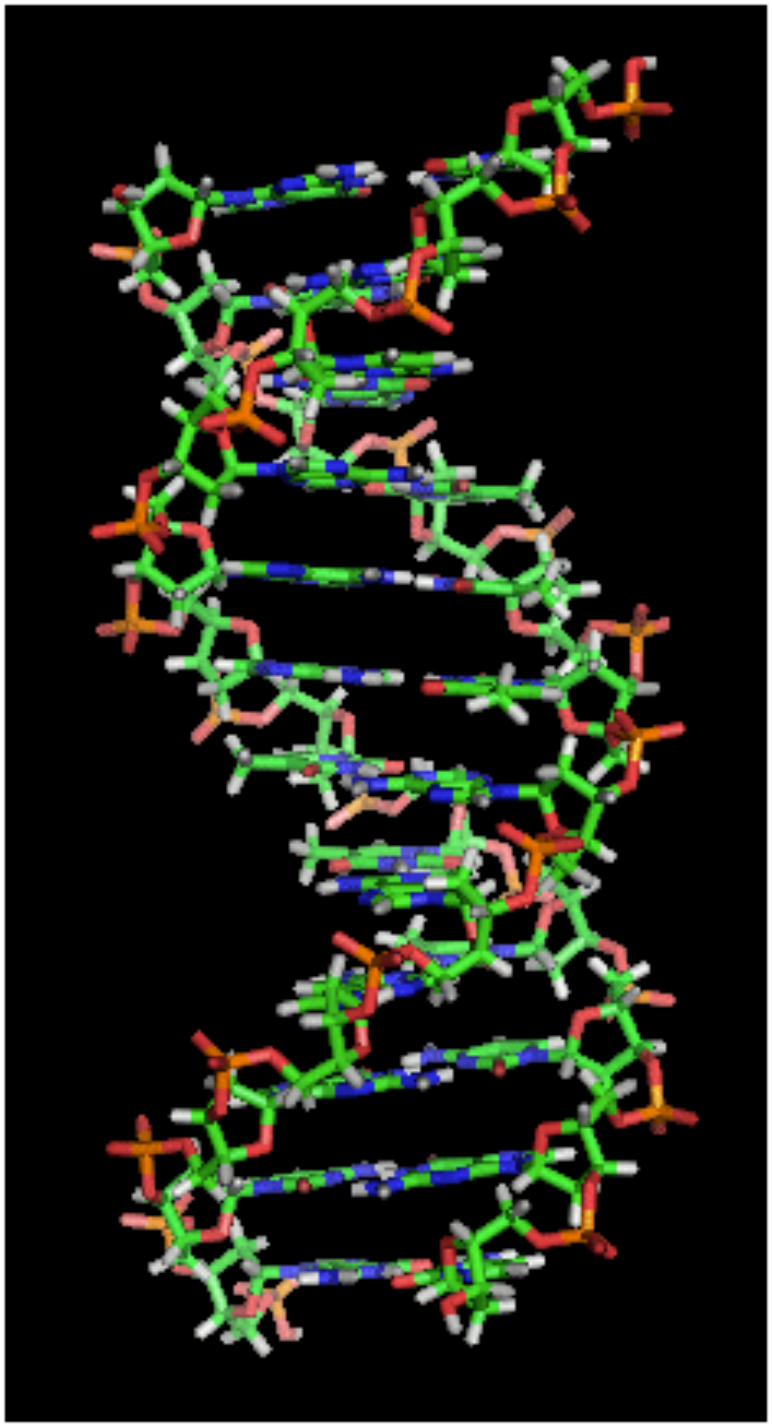
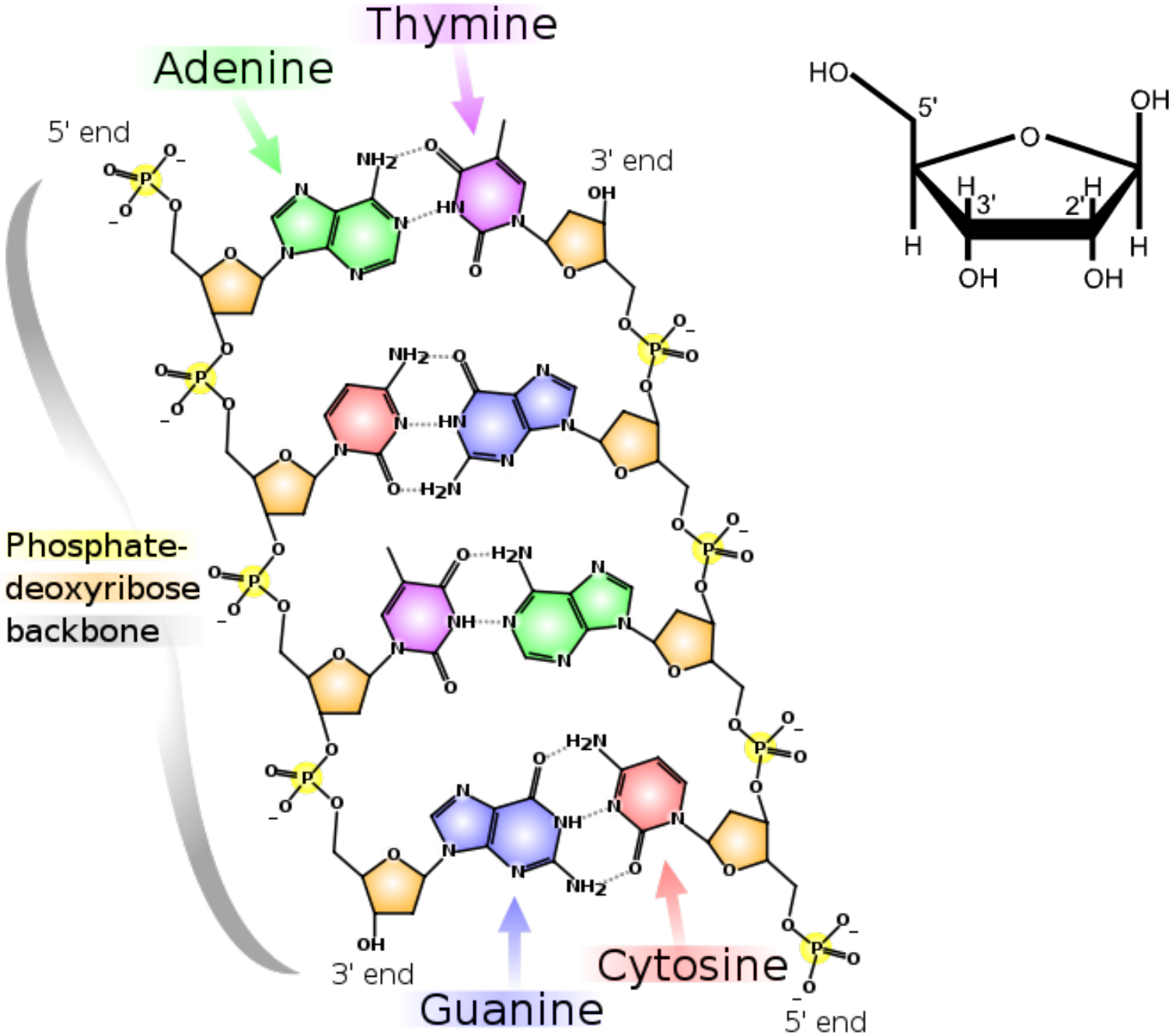
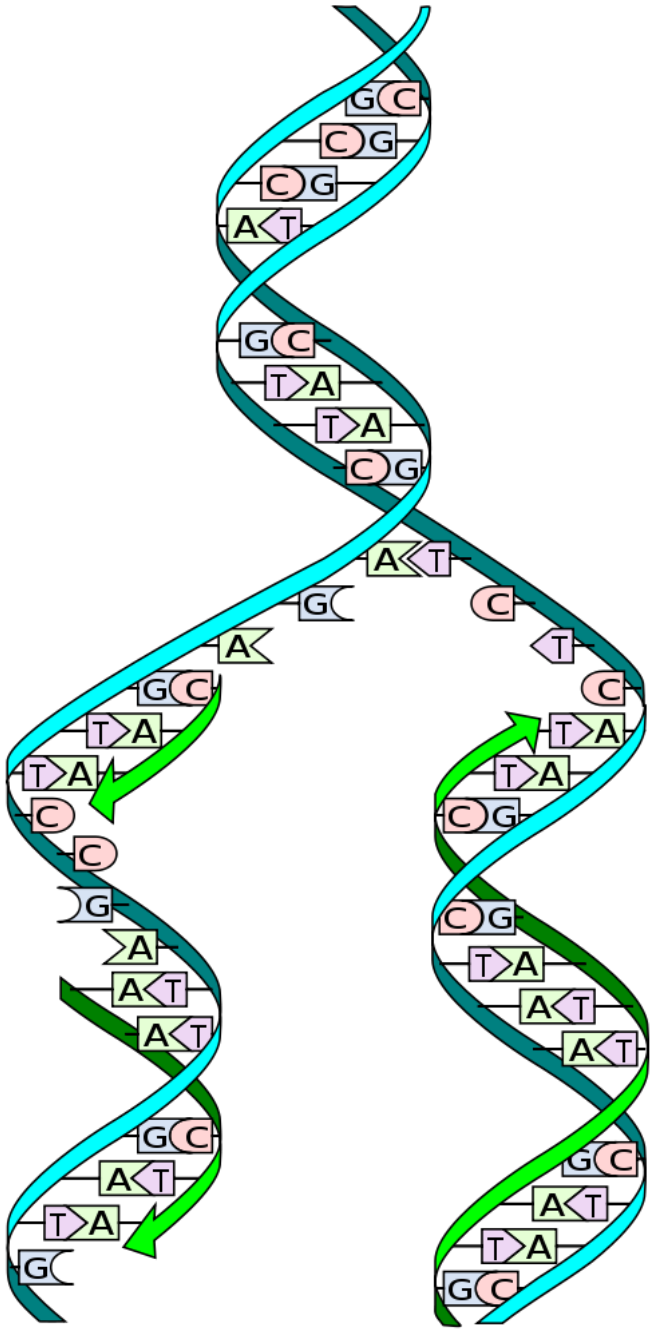



Thymine
Adenine
5' end
3' end
HO
5'
OH
O
H
3'
2'
H
H
OH
OH
Phosphate-
deoxyribose
backbone
3' end
Cytosine
Guanine
5' end


G C
C G
C G
A T
G C
T A
T A
C G
A T
G
C
A
G C
T A
T A
C
C
G
A
A T
A T
G C
A T
T A
G
C
T
C
T A
T A
C G
C G
T A
A T
A T
G C
A T
T A
G C

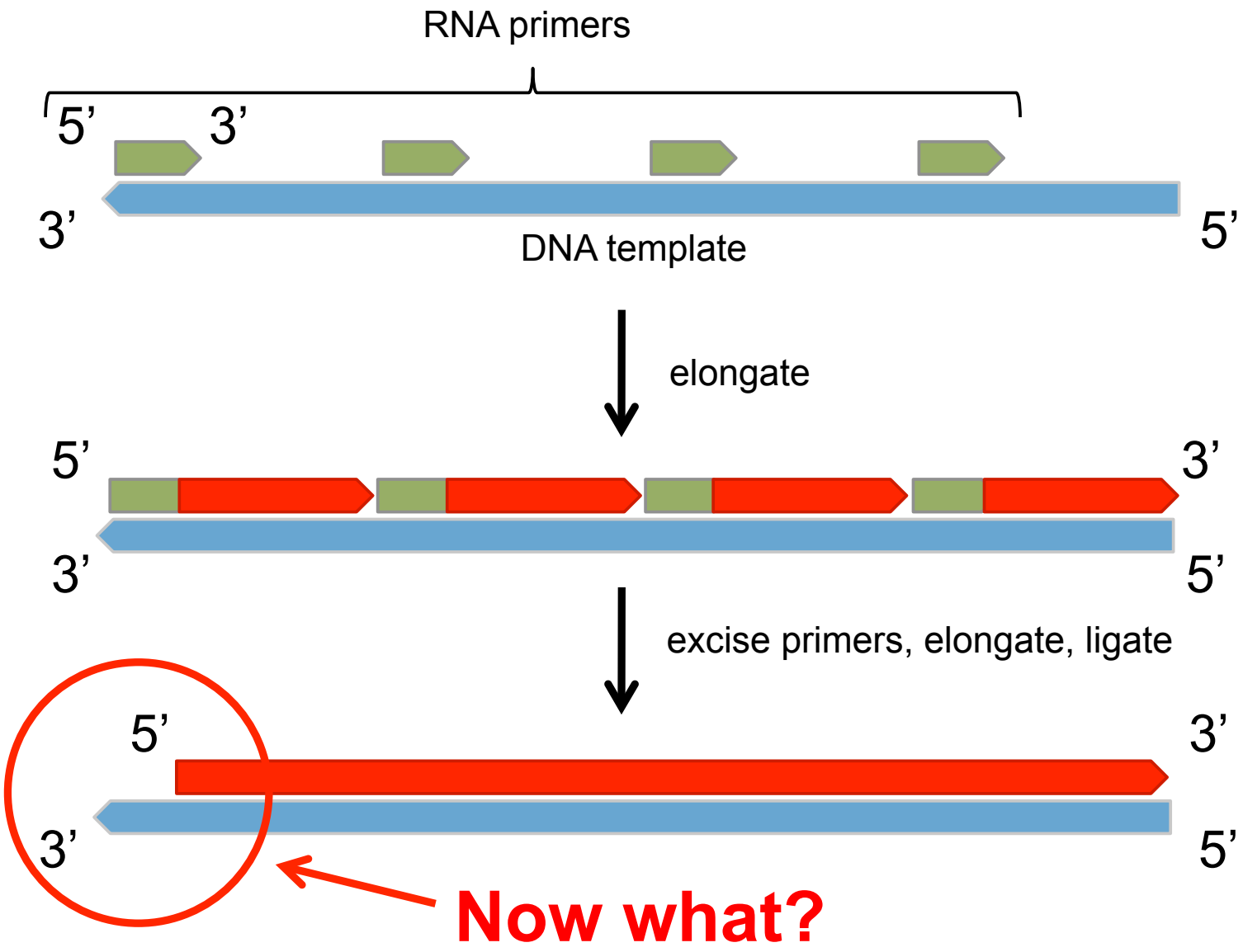
DNA Replication

- Viruses must replicate their genomes to make new progeny
- This always requires expression of at least one virus protein, sometimes many (hence always delayed after infection)
- DNA is always synthesized 5’ – 3’
- Replication initiates at a defined origin (Ori) using a primer
- The host provides other proteins

Steps in DNA Replication

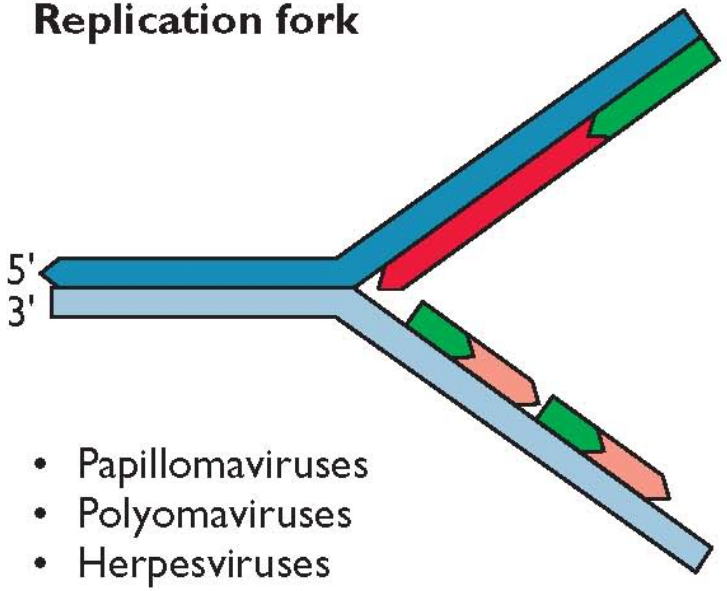
- Origin (Ori) recognition for initiation
- Priming of DNA synthesis
- Elongation
- Termination

The 5’ end problem



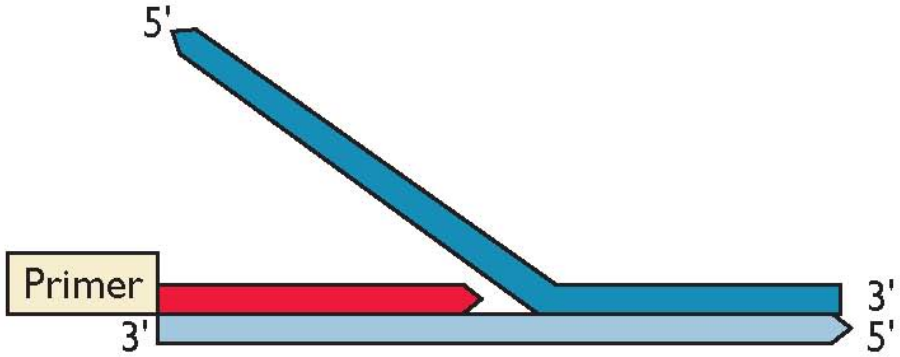
Two Basic Modes of Replication

Replication fork



- Papillomaviruses
- Polyomaviruses
- Herpesviruses
- Retroviral proviruses

Strand displacement (primer)



- Adenoviruses (protein)
- Parvoviruses (DNA hairpin)
- Poxviruses (DNA hairpin)

Where Does the DNA Polymerase Come From?

- Small DNA viruses do not encode an entire genome 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

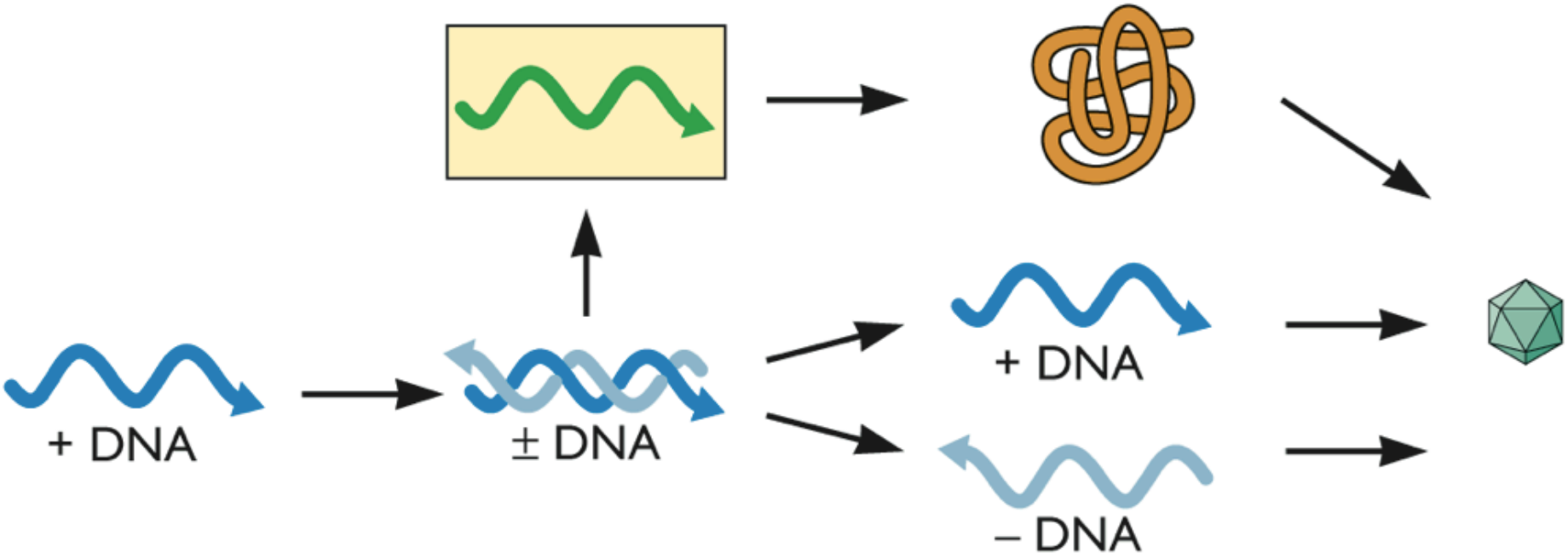
- DNA polymerase and accessory proteins
- Origin binding protein, helicases
- Exonucleases
- Enzymes of nucleic acid metabolism (thymidine kinase, RR, dUTPase)

Viral Origins of DNA Replication

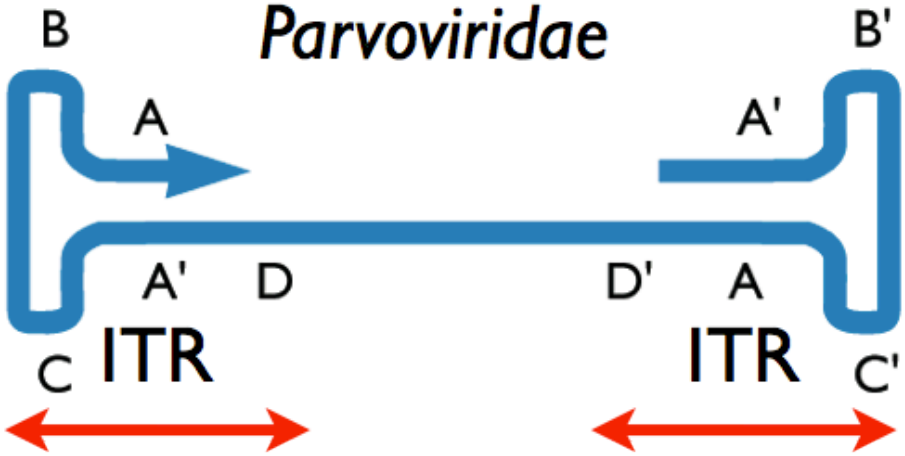
- AT-rich DNA segments recognized by viral origin recognition proteins
- Some viruses have one Ori; others up to three, used for different purposes

Requirements for DNA Replication

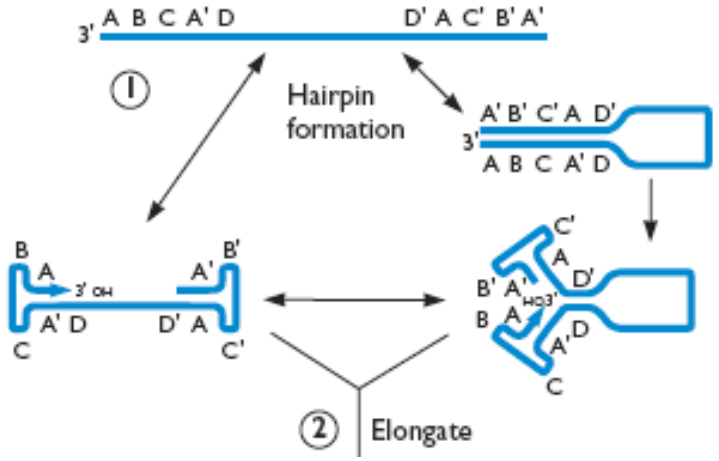
- Viruses don’t replicate well in quiescent cells
- Induction of host replication enzymes and cell cycle regulators
- Activation of cellular DNA synthesis machinery

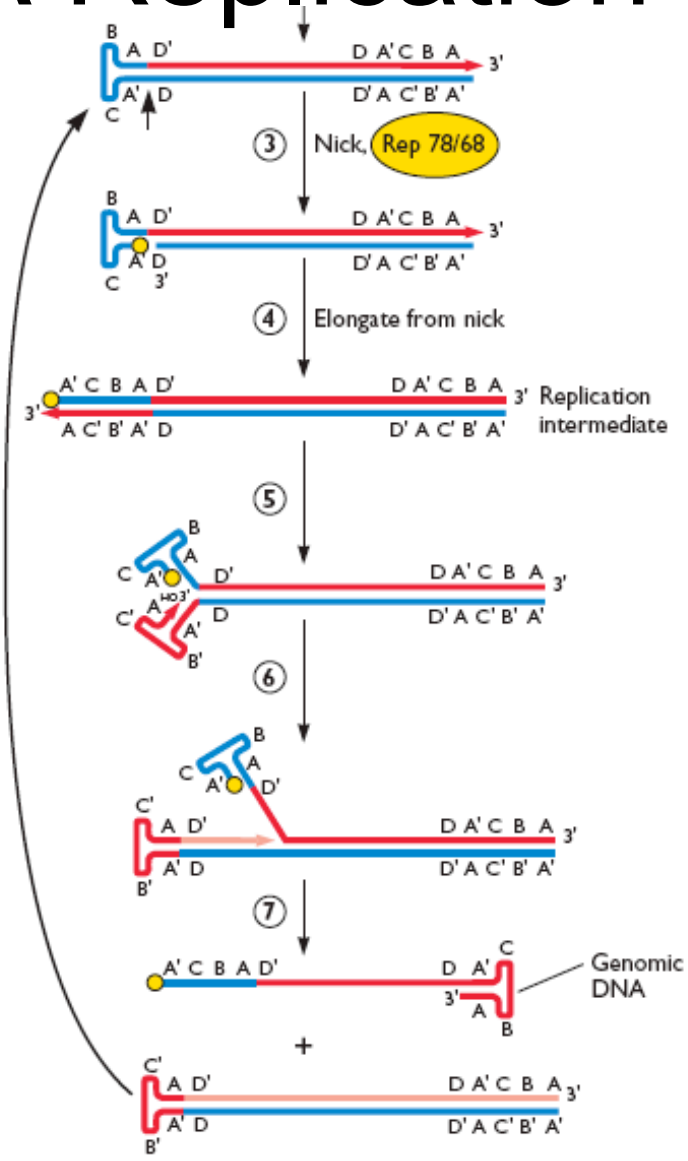

+ DNA
± DNA
+ DNA
– DNA


Circoviridae


Parvoviridae
B
B'
A
A'
A'
D
D'
A
C
ITR
ITR
C'

Parvovirus DNA Replication

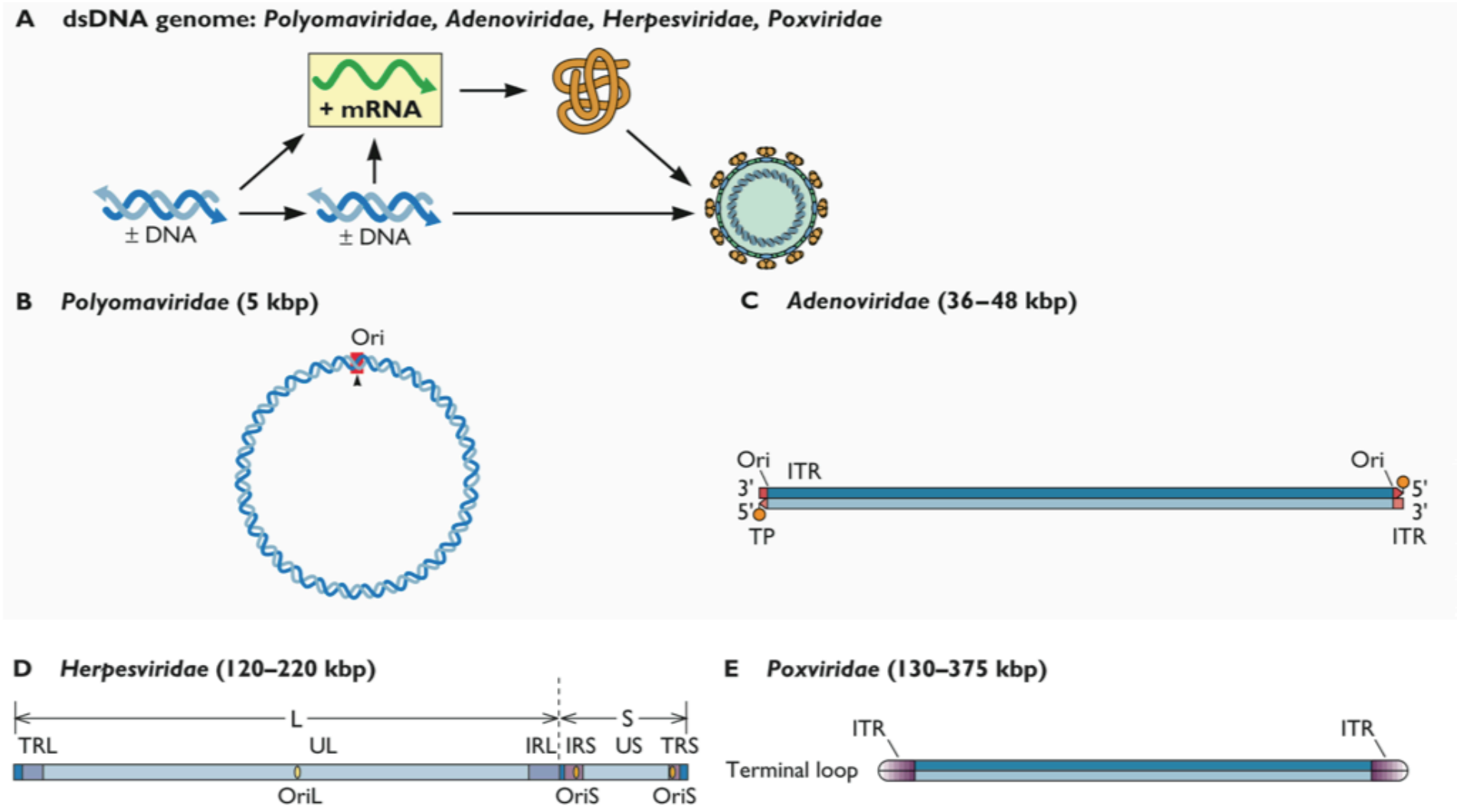




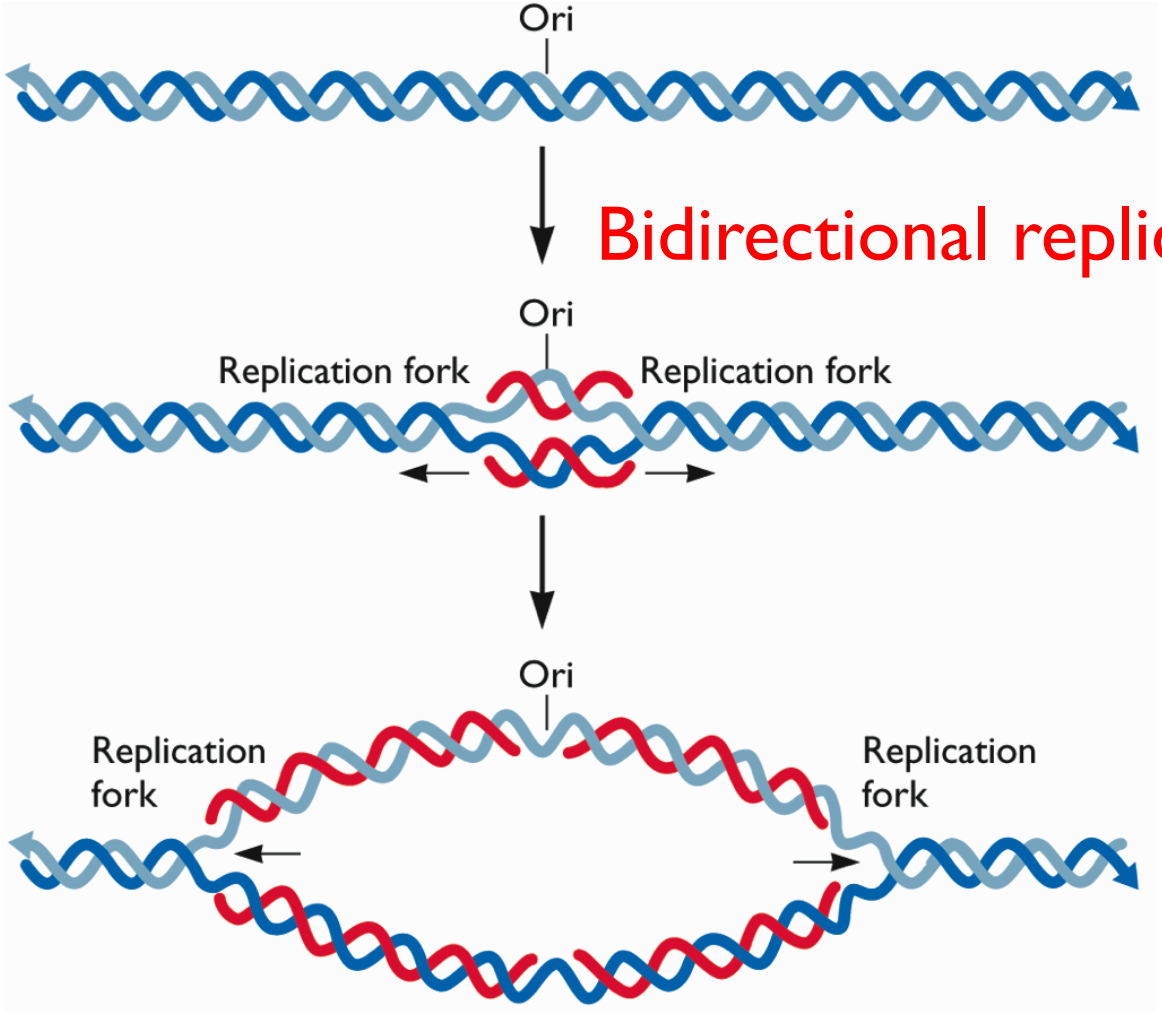
Rep78/68 a site & strand-specific endonuclease

Parvovirus DNA Priming

- Parvoviruses self prime, form a template primer
- Only one viral protein, Rep 78/68, needed
- Infection has no effect on cellular DNA synthesis

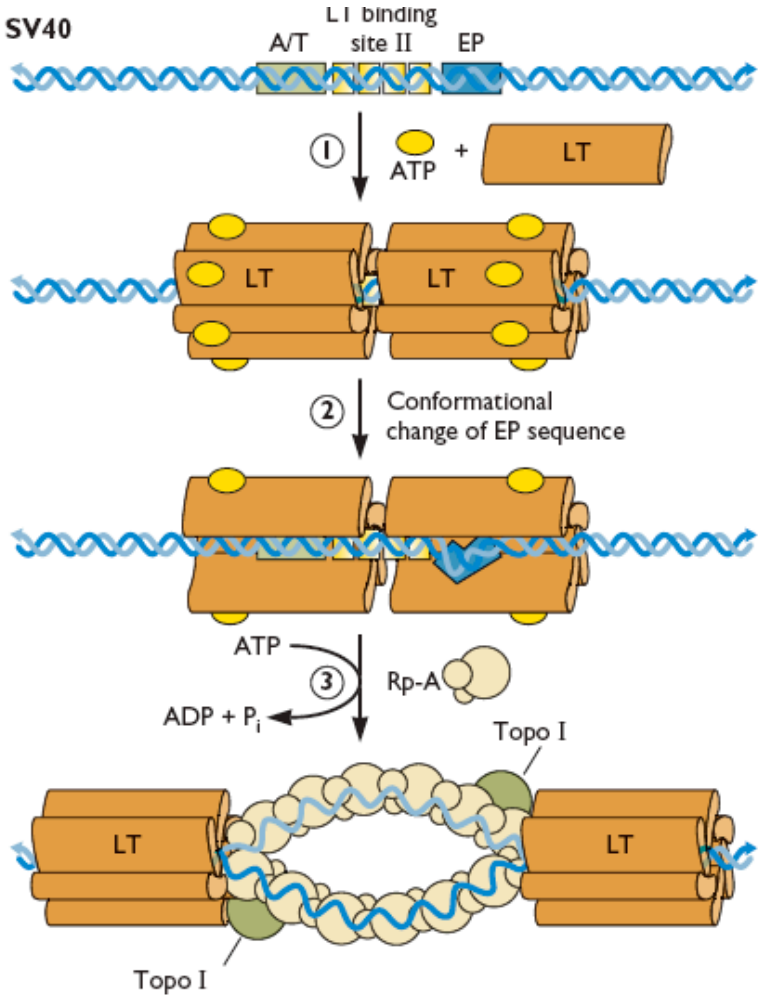

A dsDNA genome: Polyomaviridae, Adenoviridae, Herpesviridae, Poxviridae
+ mRNA
± DNA
± DNA
B Polyomaviridae (5 kbp)
Ori
C Adenoviridae (36–48 kbp)
Ori
ITR
3'
5'
TP
Ori
5'
3'
ITR
D Herpesviridae (120–220 kbp)
L
S
TRL
UL
IRL
IRS
US
TRS
OriL
OriS
OriS
E Poxviridae (130–375 kbp)
ITR
ITR
Terminal loop

Polyomaviruses



Initiation from a single ori, requires expression of T

Initiation of DNA Synthesis

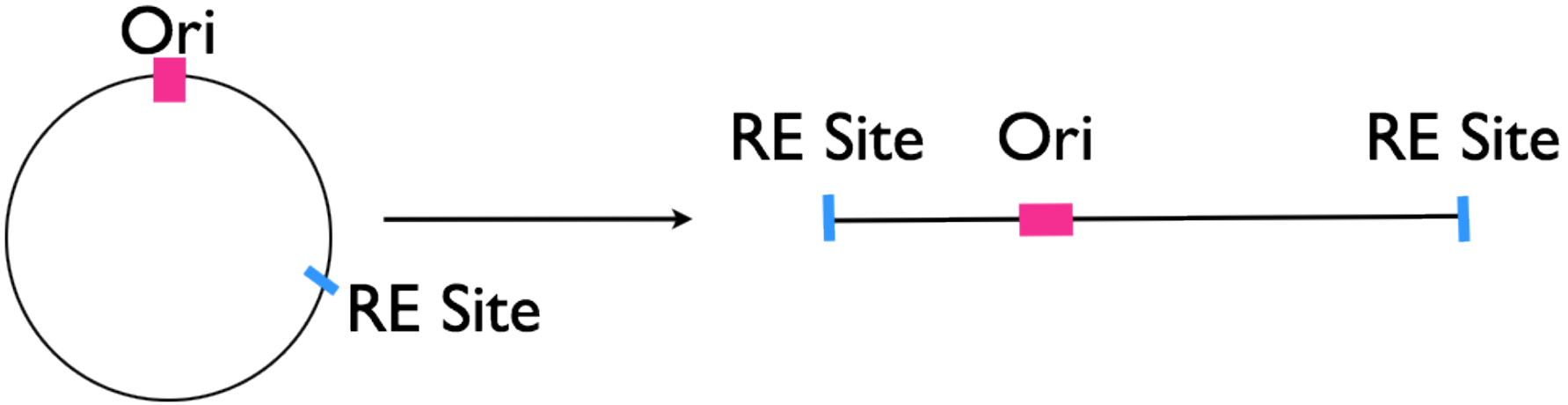


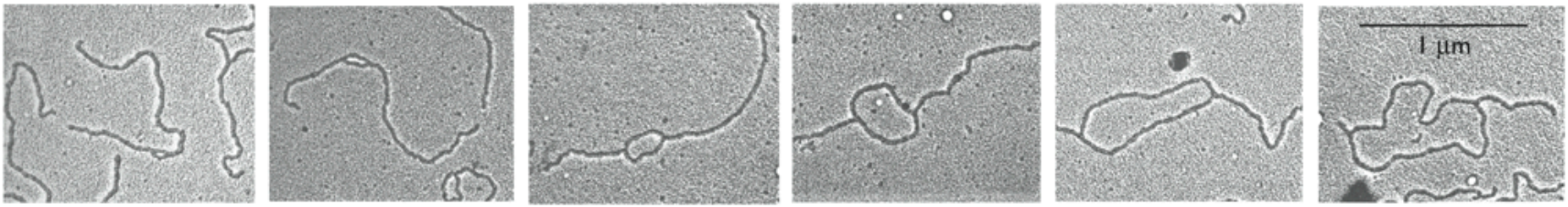
Two LT hexamers bind

Binding distorts early palindrome unwinding origin

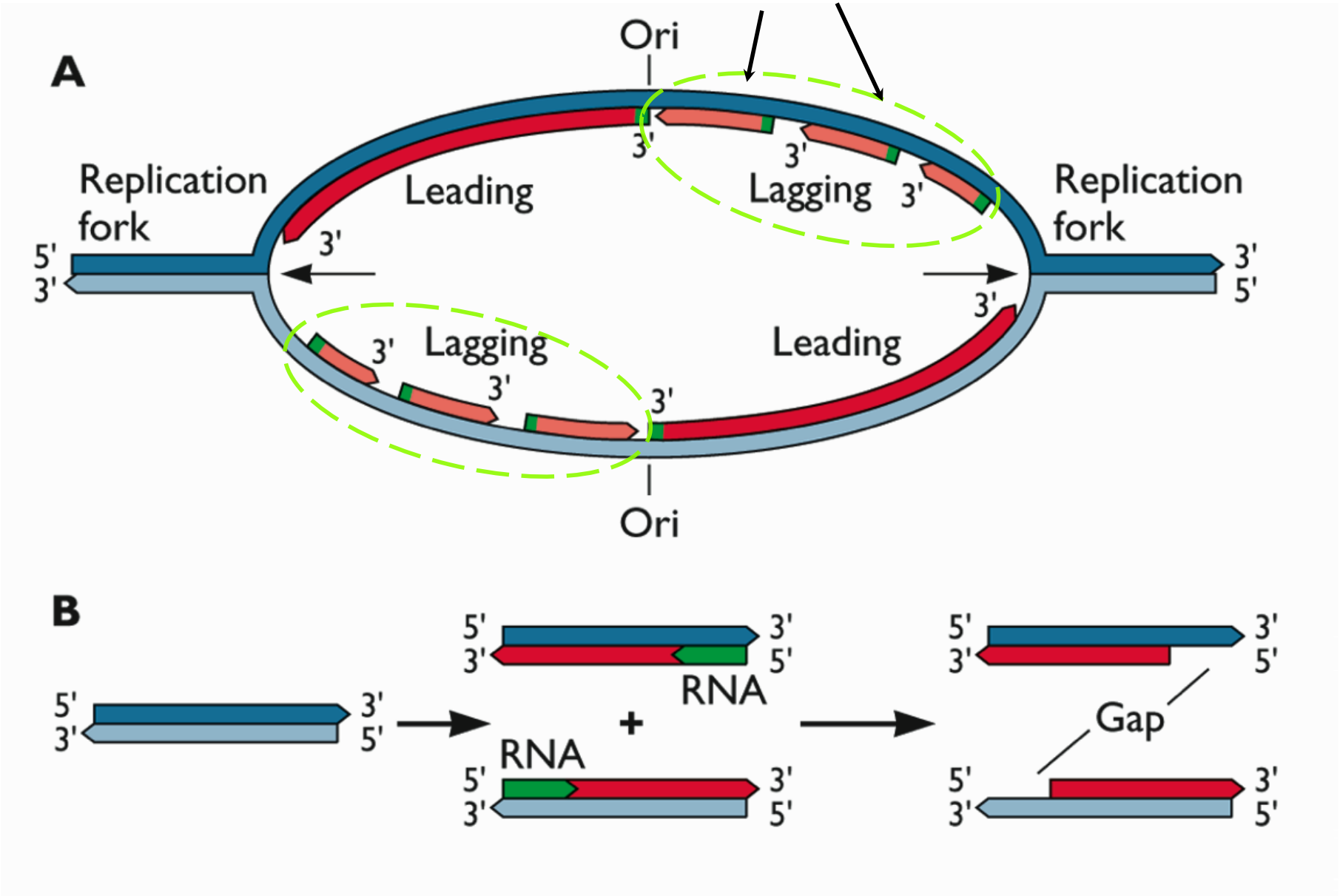
Binding of Rpa occurs

Where does it start?

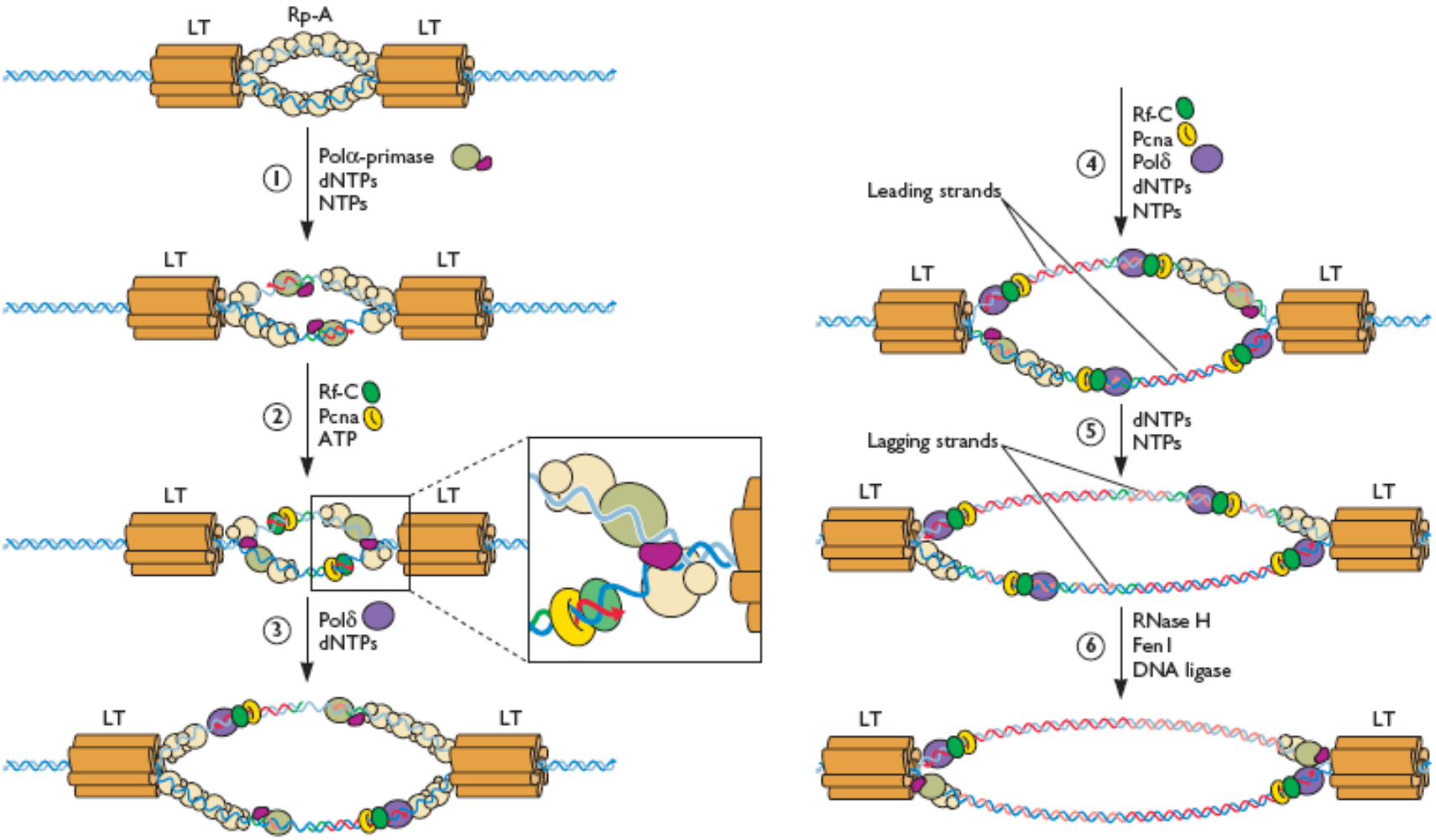




How to connect the Okazaki fragments

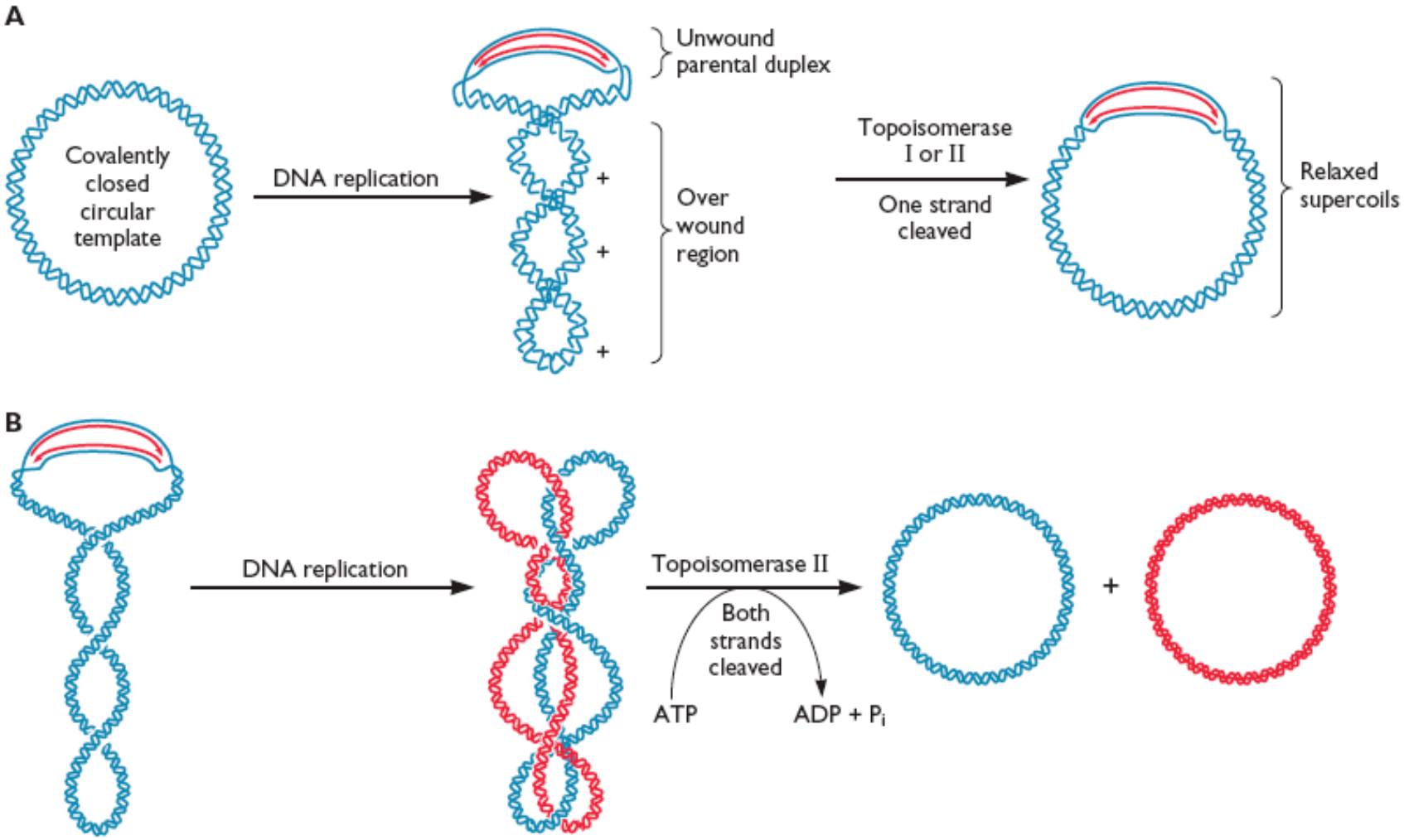


DNA Synthesis by *Polyomaviridae*



Remove RNA, fill gaps, seal

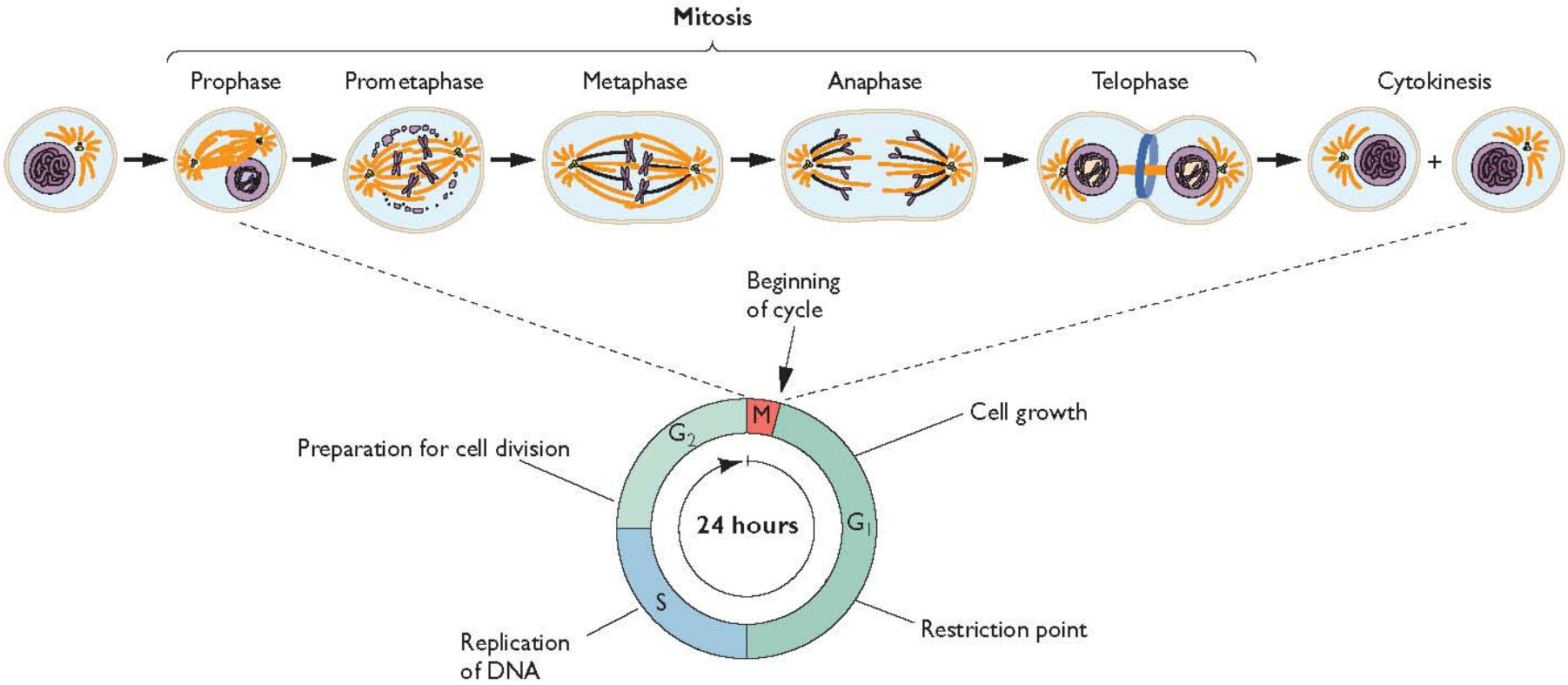
Resolution



Catenated molecules

Polyomavirus T

- Binds and sequesters cell cycle regulators, causes cells to enter S phase, induces DNA synthesis - WHY?


Mitosis
Prophase
Prometaphase
Metaphase
Anaphase
Telophase
Cytokinesis
+
Beginning of cycle
M
G2
Cell growth
Preparation for cell division
24 hours
G1
S
Replication of DNA
Restriction point