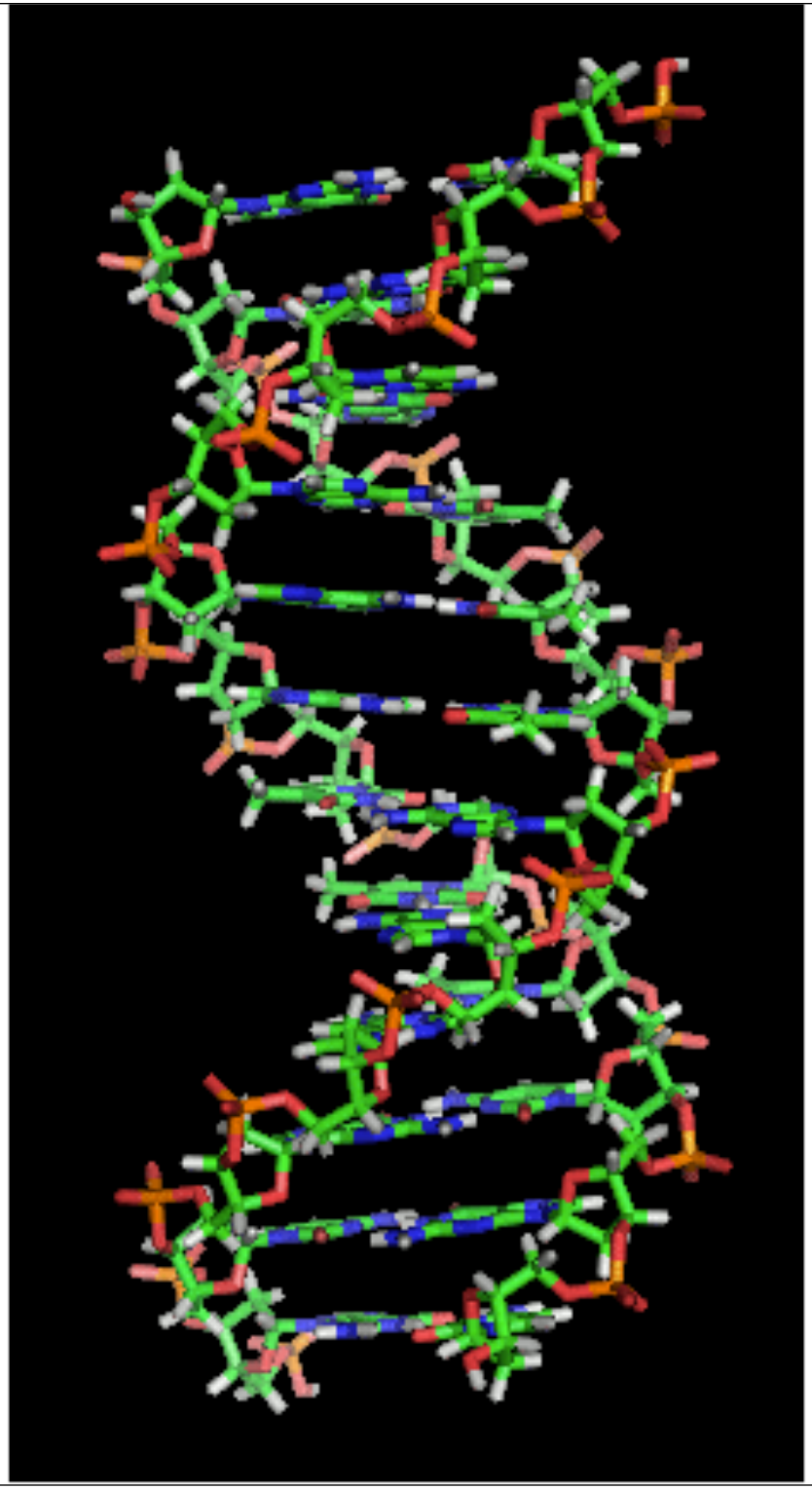
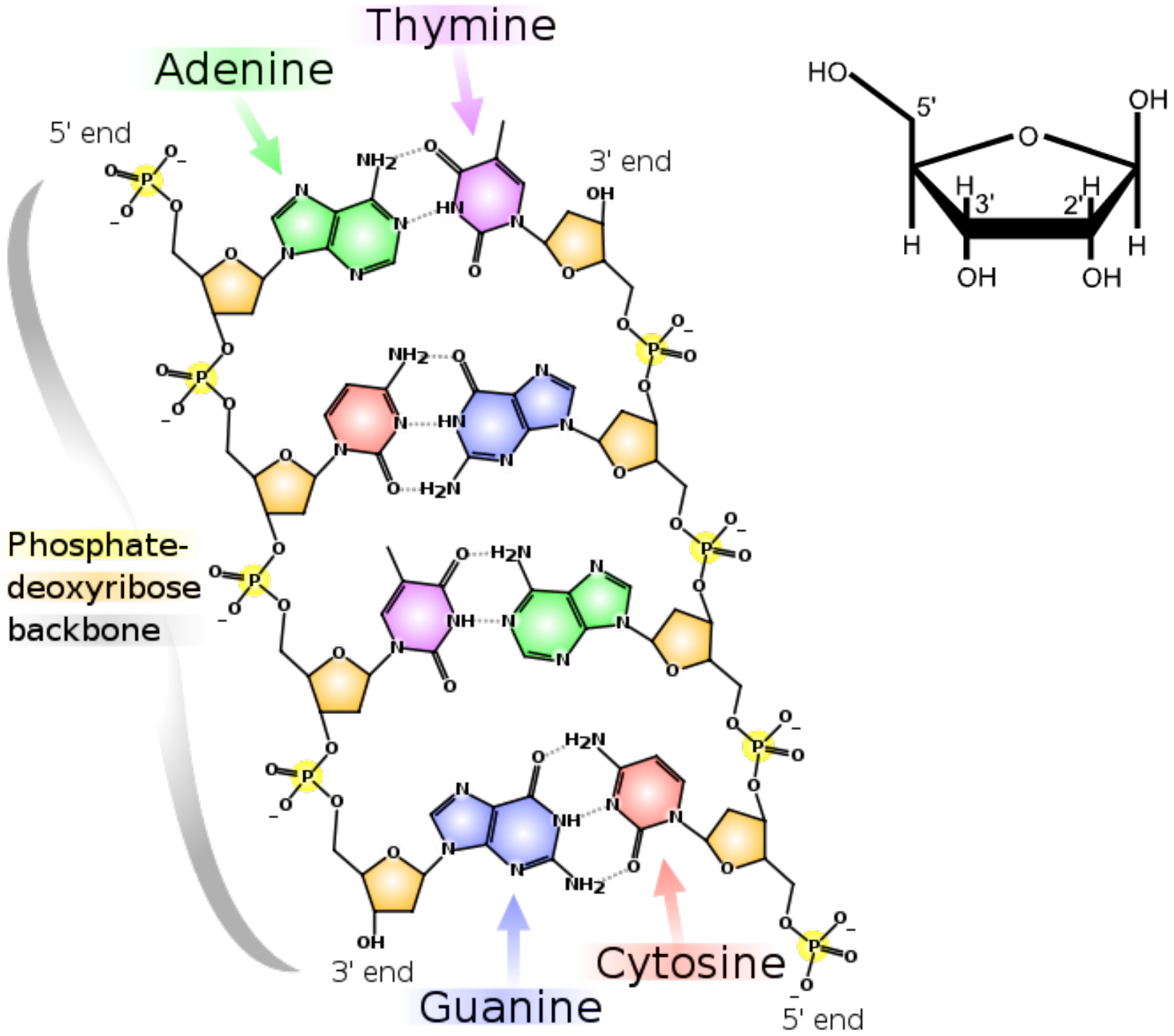
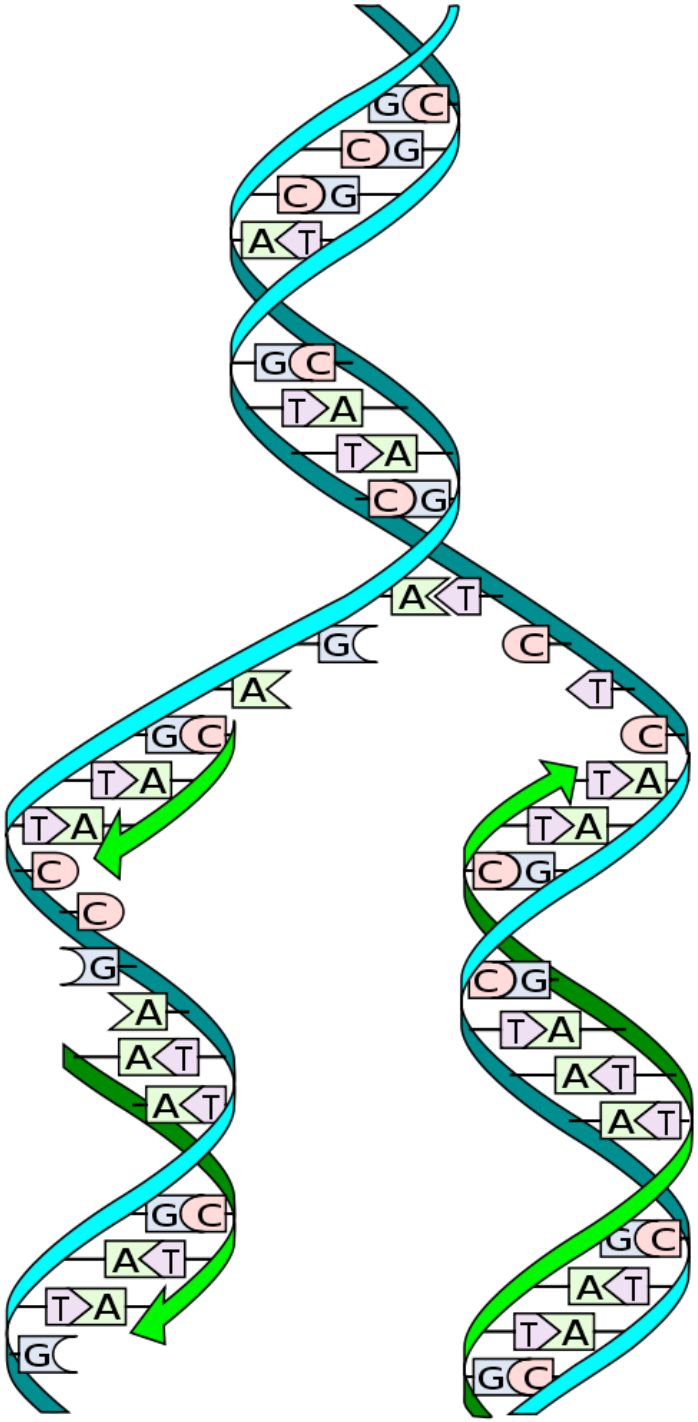


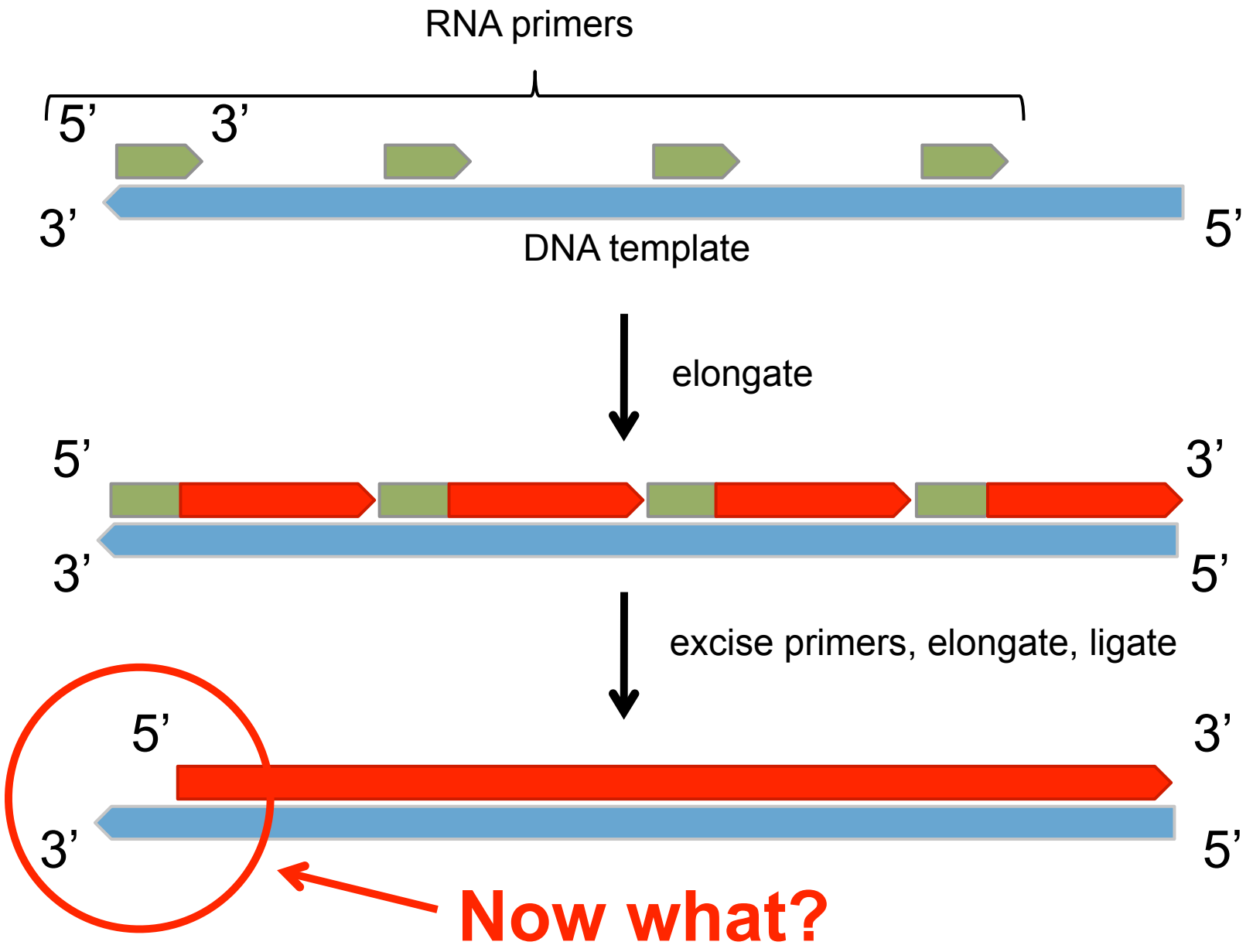

Thymine
Adenine
5' end
3' end
Phosphate-
deoxyribose
backbone
3' end
Guanine
Cytosine
5' end
HO
5'
OH
O
H
3'
2'
H
H
OH
OH
H


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
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

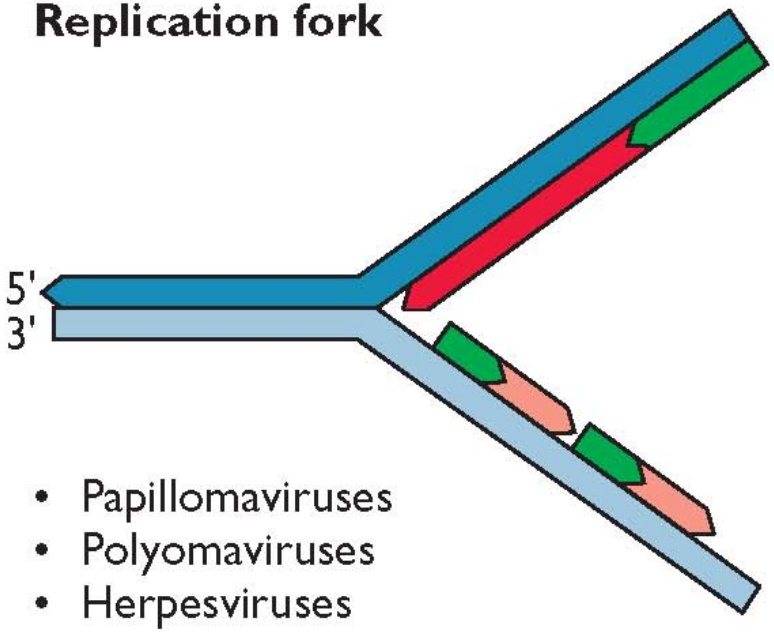
- Viruses must replicate their genomes to make new progeny
- This always requires expression of at least one virus protein, sometimes many (hence always delayed after infection)
- DNA is always synthesized 5’ – 3’
- Replication initiates at a defined origin (Ori) using a primer
- The host provides other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

The 5’ end problem



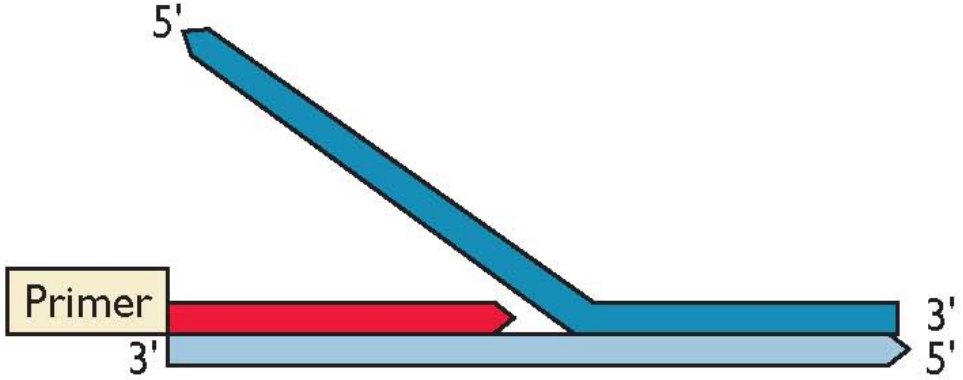
Two Basic Modes of Replication

Replication fork



- Papillomaviruses
- Polyomaviruses
- Herpesviruses
- Retroviral proviruses

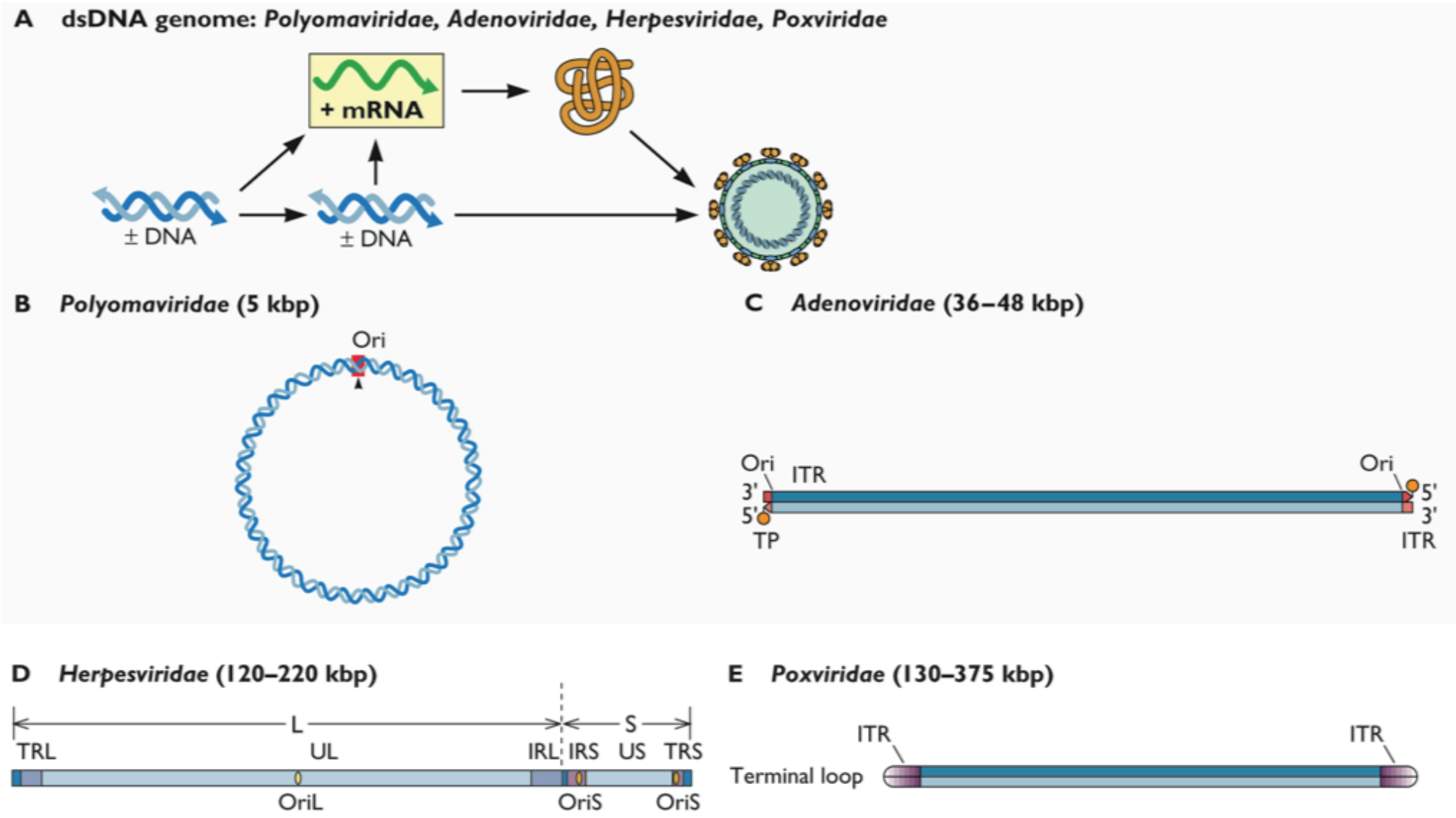
Strand displacement (primer)



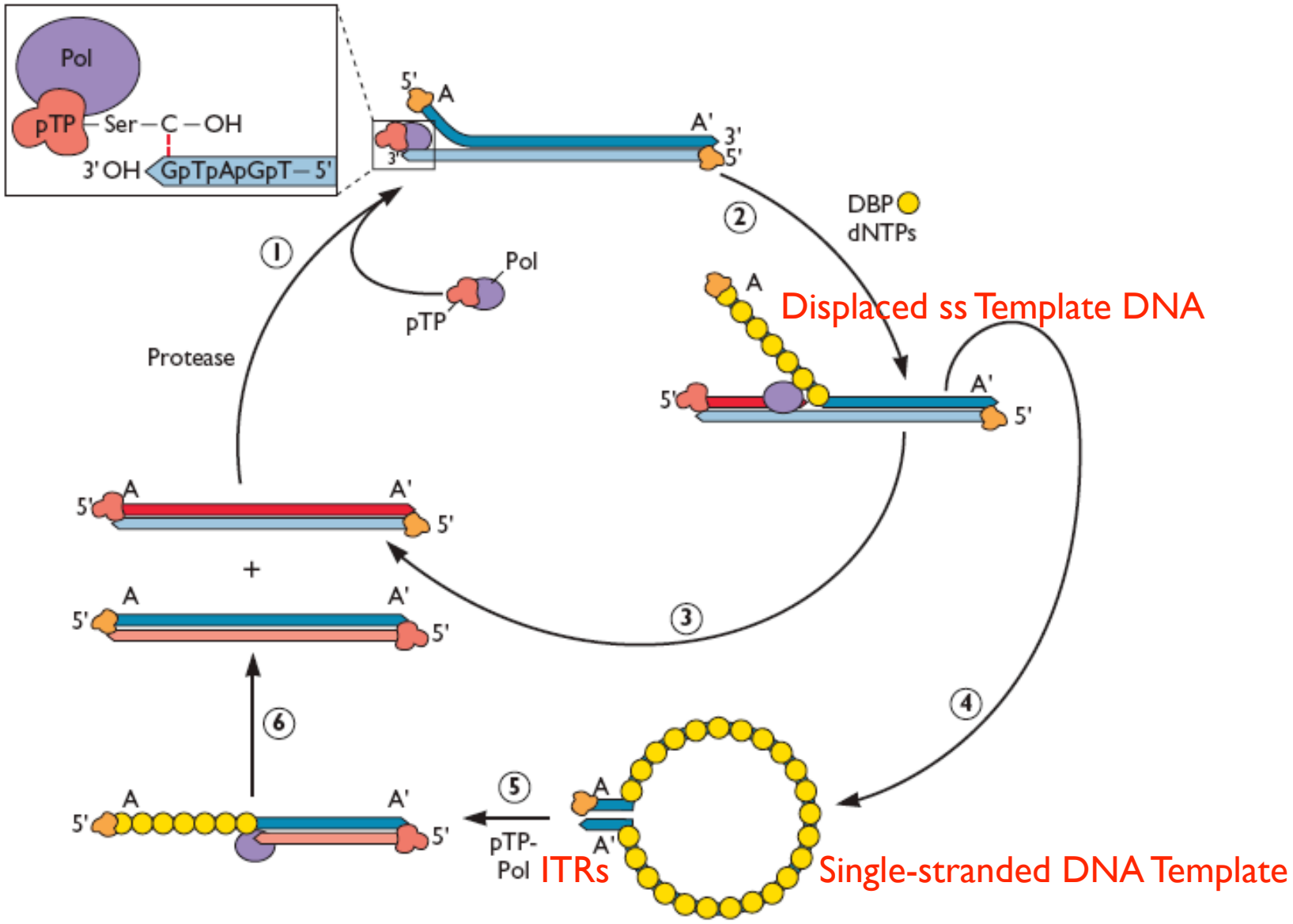
- Adenoviruses (protein)
- Parvoviruses (DNA hairpin)
- Poxviruses (DNA hairpin)

Where Does the DNA Polymerase Come From?

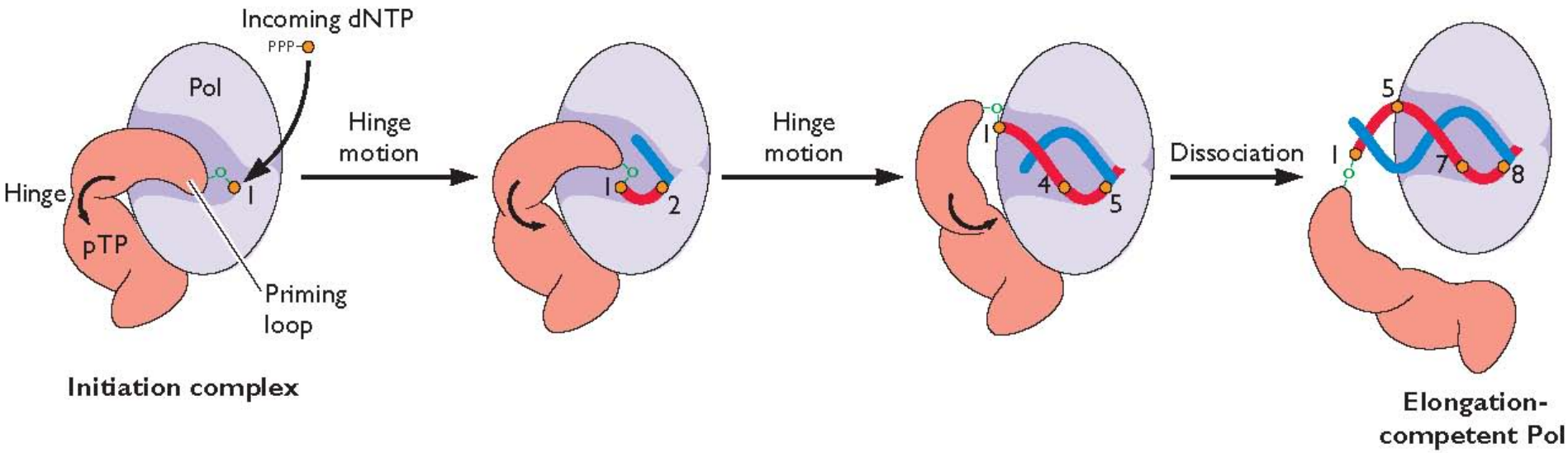
- Small DNA viruses do not encode an entire genome replication system
  -encode proteins that orchestrate the host
  -*Papillomaviridae*, *Polyomaviridae*, *Parvoviridae*
- Large DNA viruses encode most of their own replication systems
  -*Herpesviridae*, *Adenoviridae*, *Poxviridae*


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

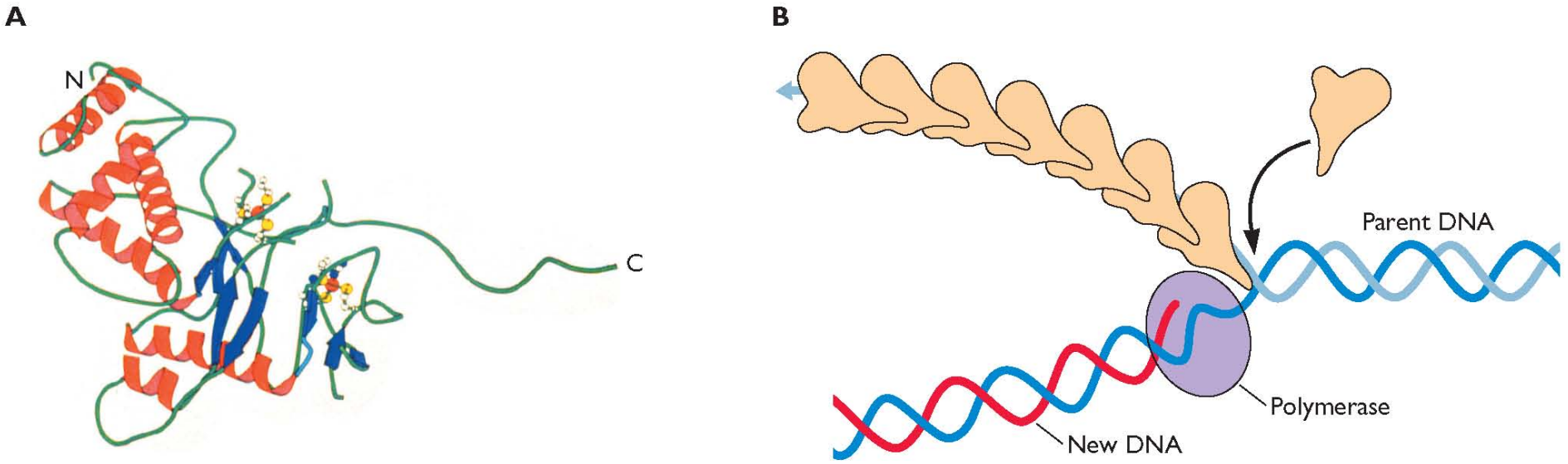
Protein Priming



Lessons from bacteriophage ϕ29

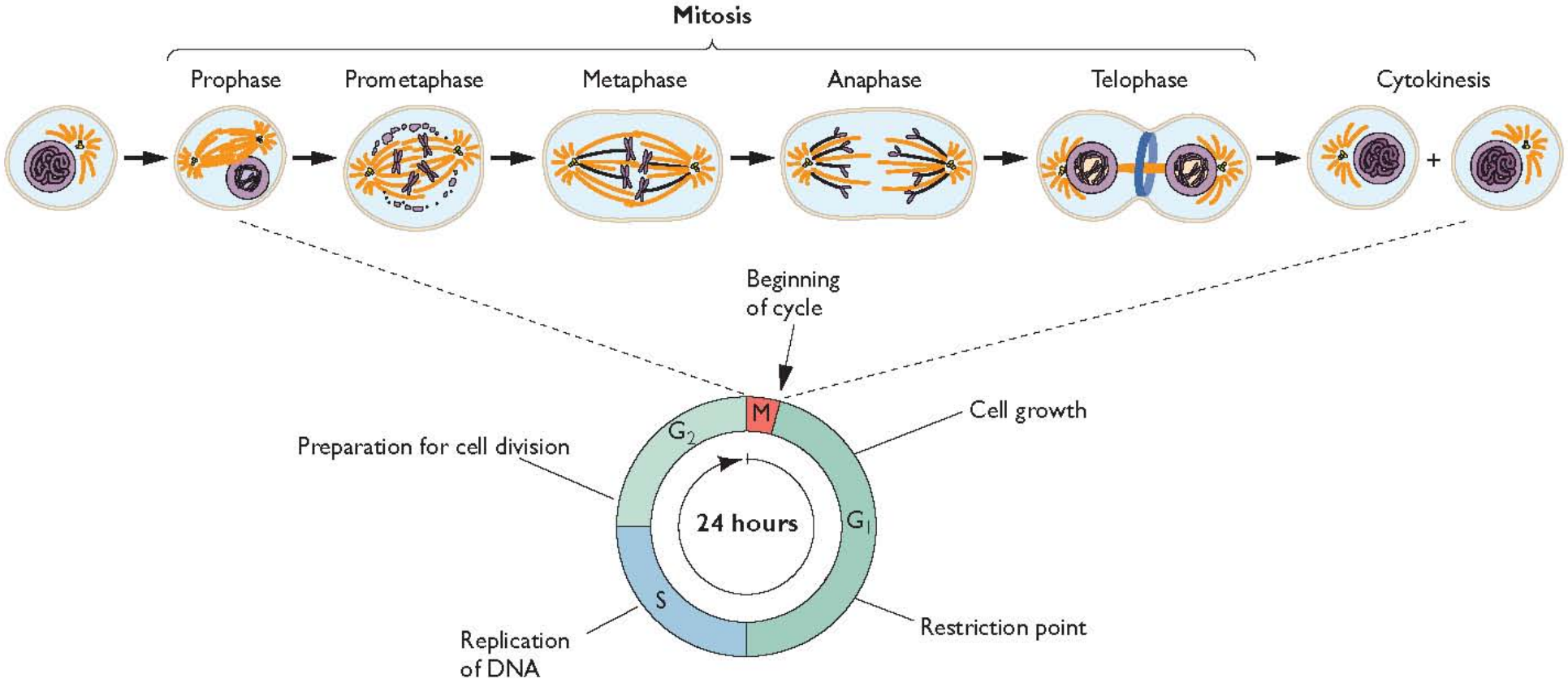


Adenoviral DBP

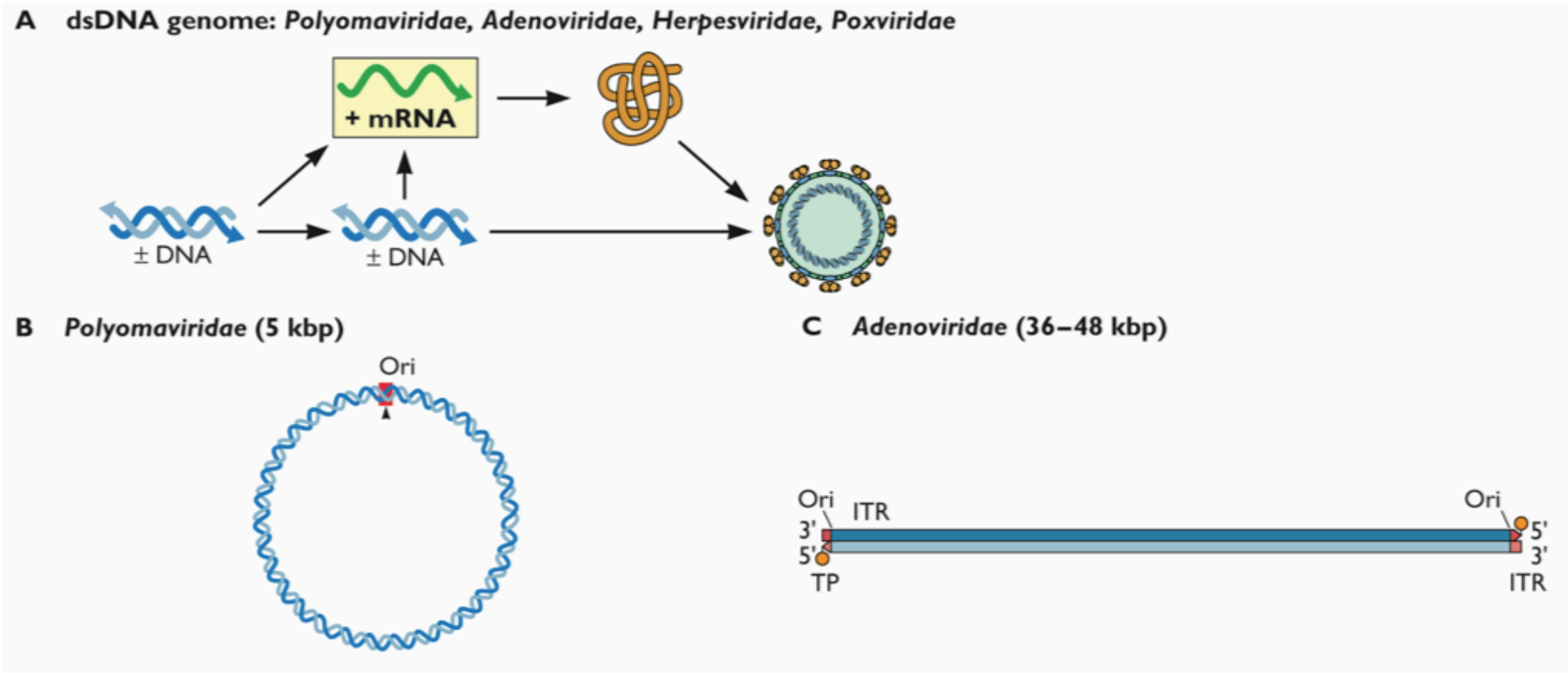


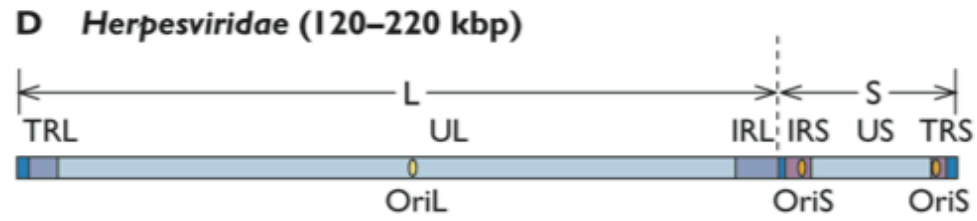
Replication of adenovirus genome

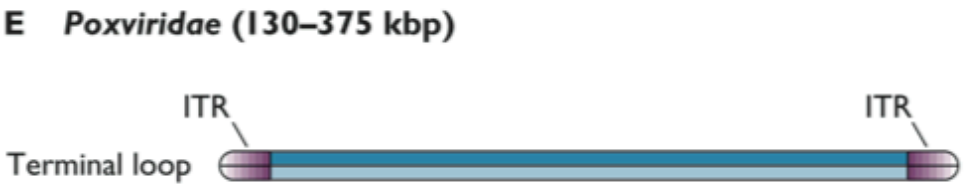
- An example of strand displacement synthesis
- Utilizes a protein primer
- Origin is at both ends
- DNA polymerase is viral
- Other viral proteins involved: terminal protein, ssDNA binding protein
- Viral early proteins (E1a) induce quiescent cells into S phase



Adenovirus DNA replication requires cellular proteins

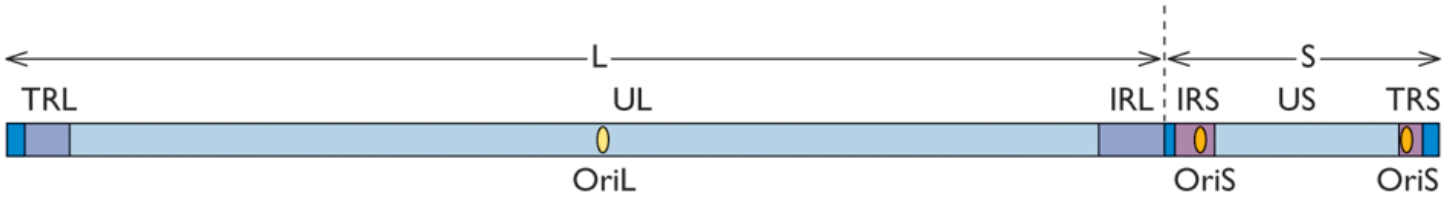

A dsDNA genome: Polyomaviridae, Adenoviridae, Herpesviridae, Poxviridae
+ mRNA
± DNA
± DNA
B Polyomaviridae (5 kbp)
Ori
C Adenoviridae (36–48 kbp)
Ori
ITR
3'
5'
TP
Ori
5'
3'
ITR

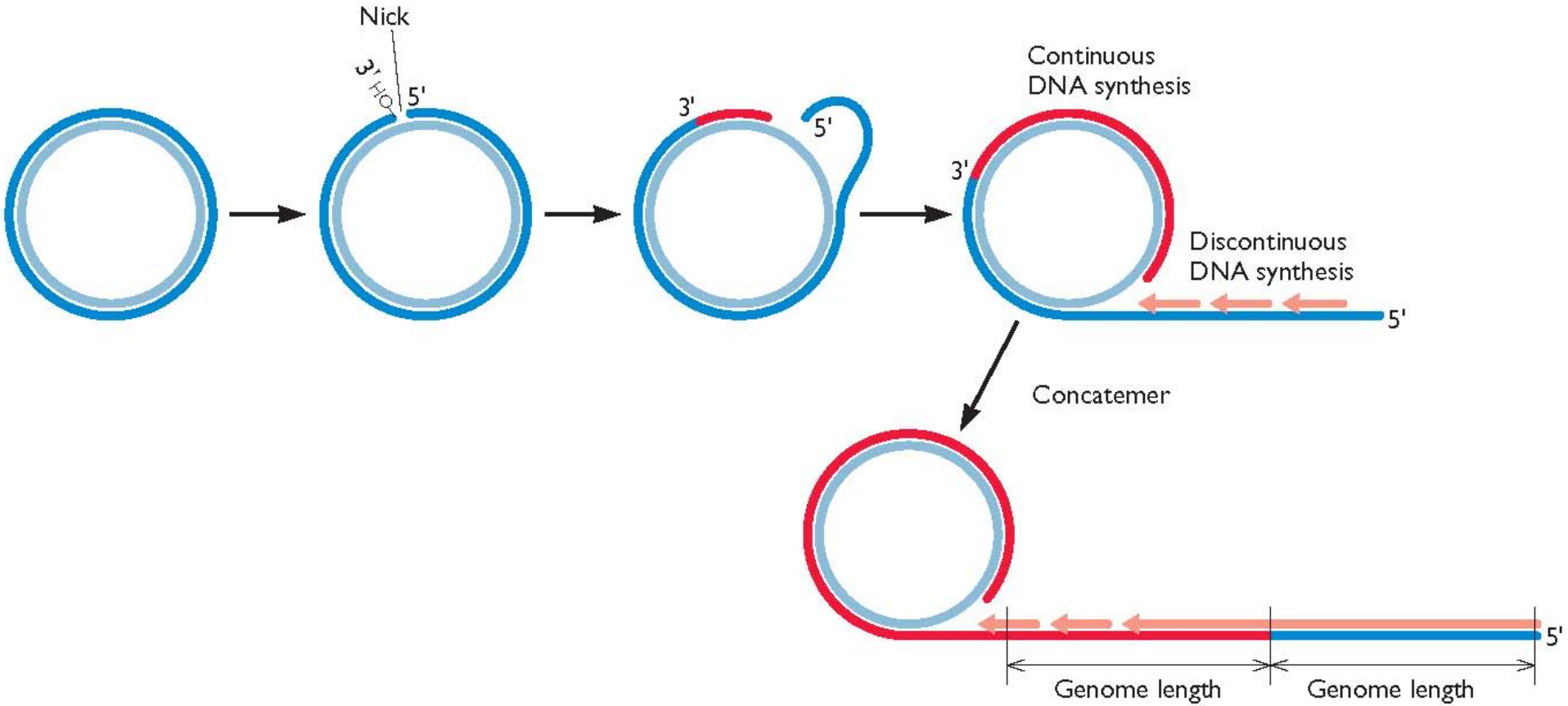

D Herpesviridae (120–220 kbp)
L
S
TRL
UL
IRL
IRS
US
TRS
OriL
OriS
OriS


E Poxviridae (130–375 kbp)
ITR
ITR
Terminal loop

Herpes simplex virus

- HSV has 2 identical oriS and a unique oriL that is active in terminally differentiated neurons – role in transition from latent to productive infection?
- DNA enters as linear molecule converts to circle
- Replicates as a rolling circle



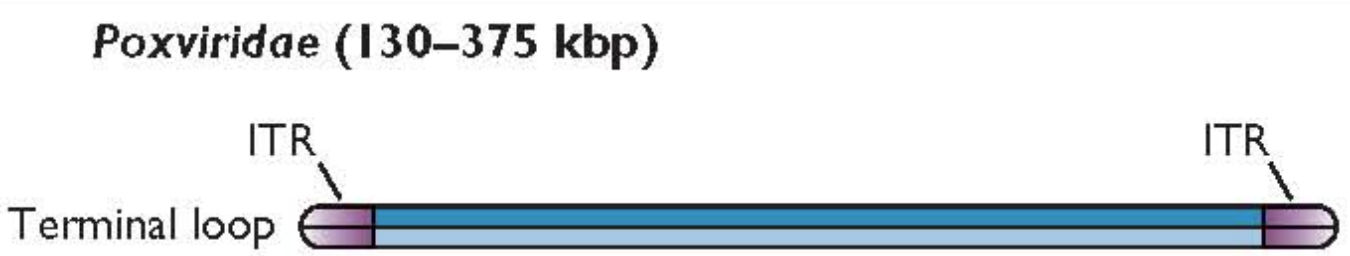

Nick
3'
HO
5'
3'
5'
Continuous
DNA synthesis
3'
Discontinuous
DNA synthesis
5'
Concatemer
5'
Genome length
Genome length

Viral proteins for Herpes simplex virus genome replication

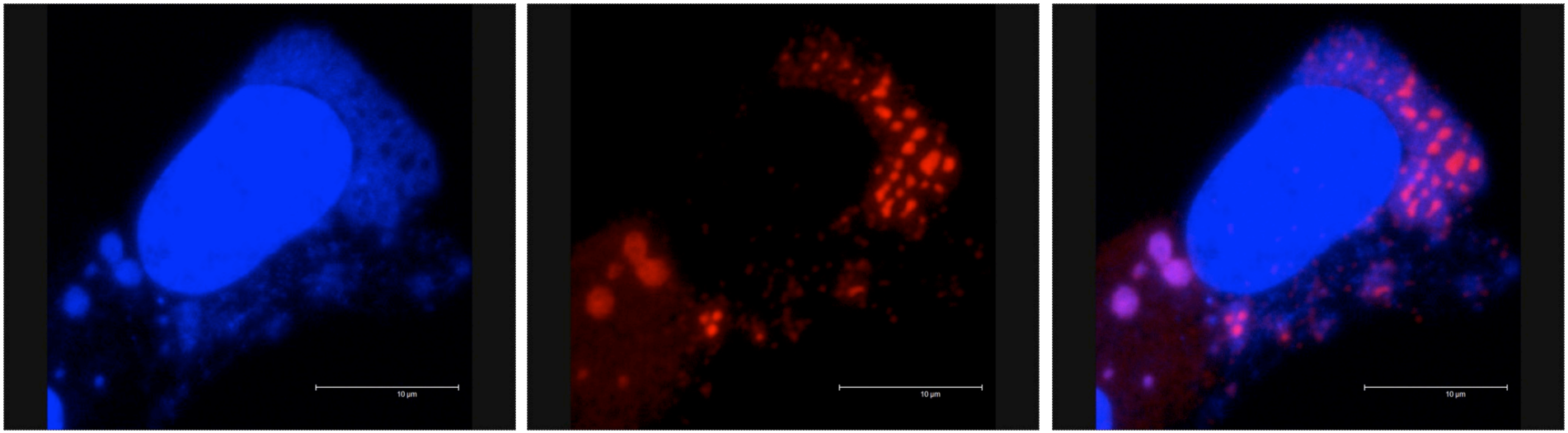
- UL5, 8 and 52 - form primase, helicase
- UL42 - a processivity protein
- UL9 - Origin Binding Protein
- UL29 - ssDNA Binding Protein
- UL30 - DNA polymerase
- 5 enzymes of nucleic acid metabolism, such as TK
- Necessary but not sufficient!

Poxviruses

- All viruses discussed replicate in nucleus
- Poxvirus - cytoplasmic factories - DNA synthesis is independent of cellular proteins



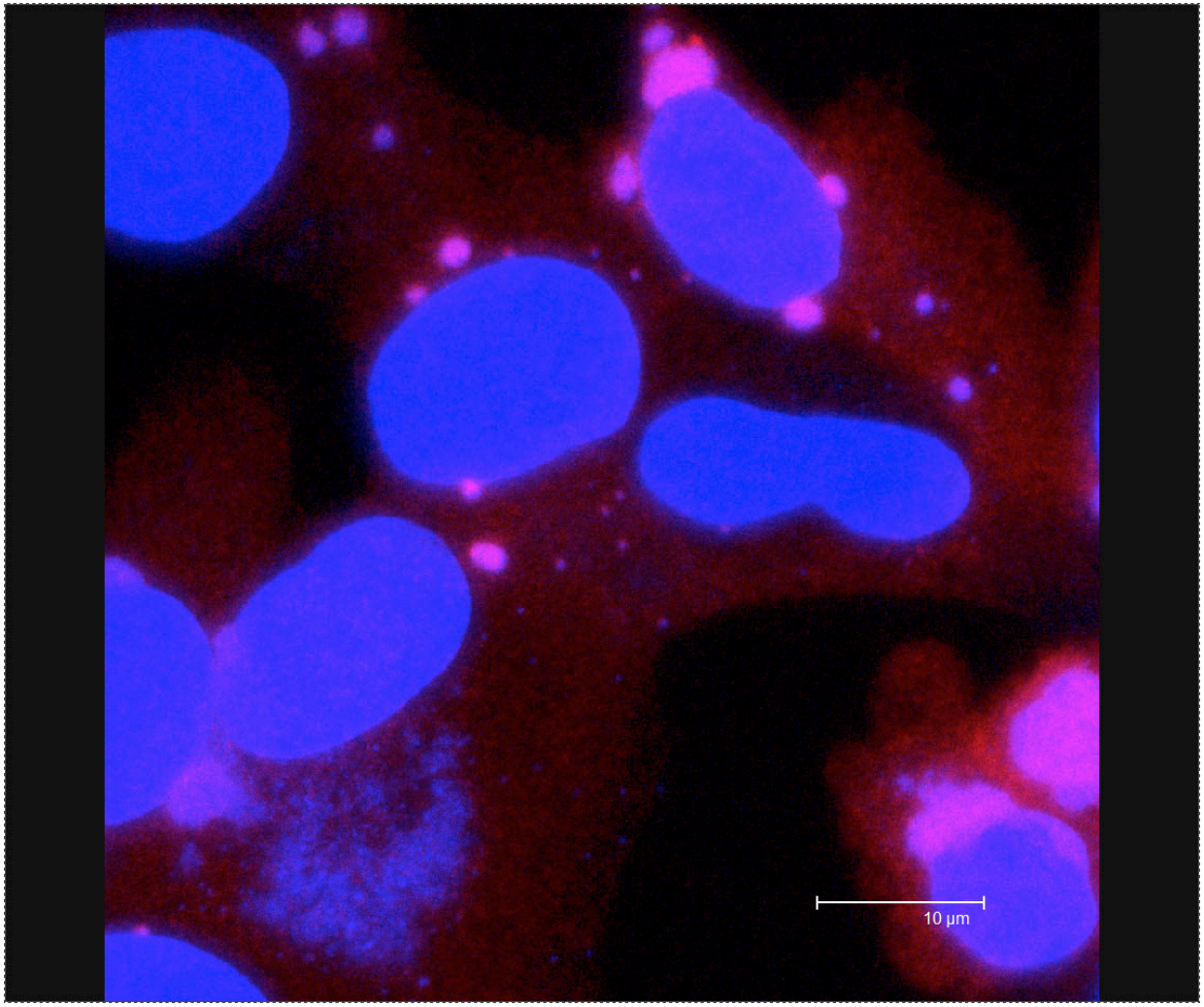
Vaccinia DNA factories



DNA only I3 only merge

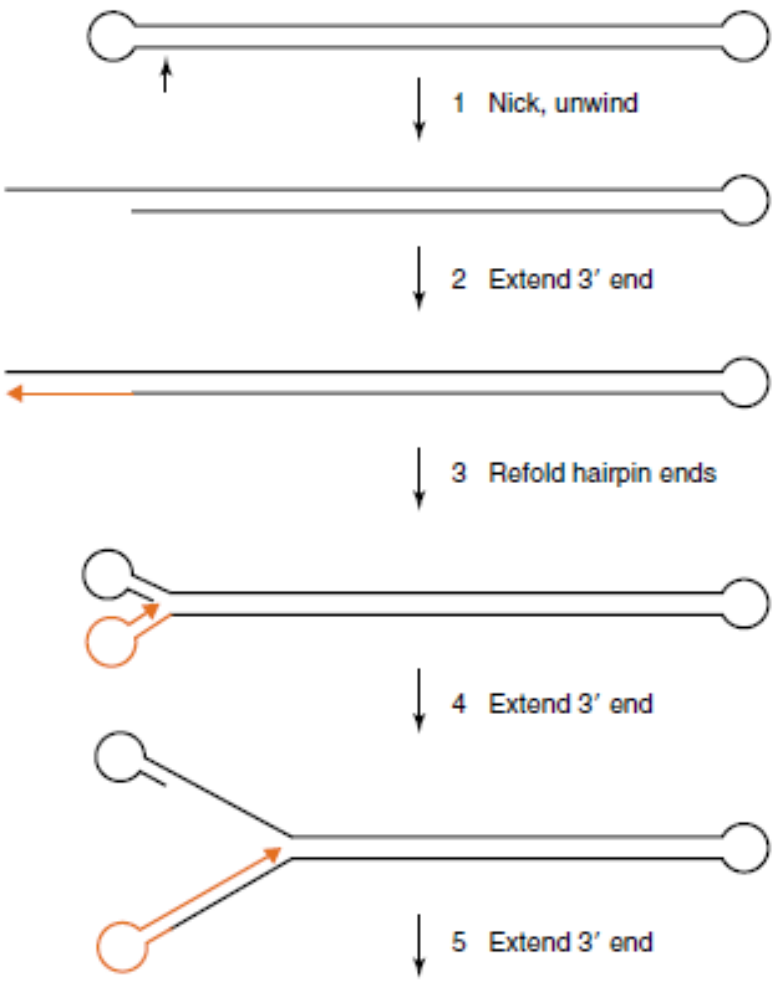
DNA stained blue; viral DNA binding protein (I3) stained red

Vaccinia DNA factories



DNA stained blue; viral DNA polymerase (E9) stained red

Poxvirus DNA replication



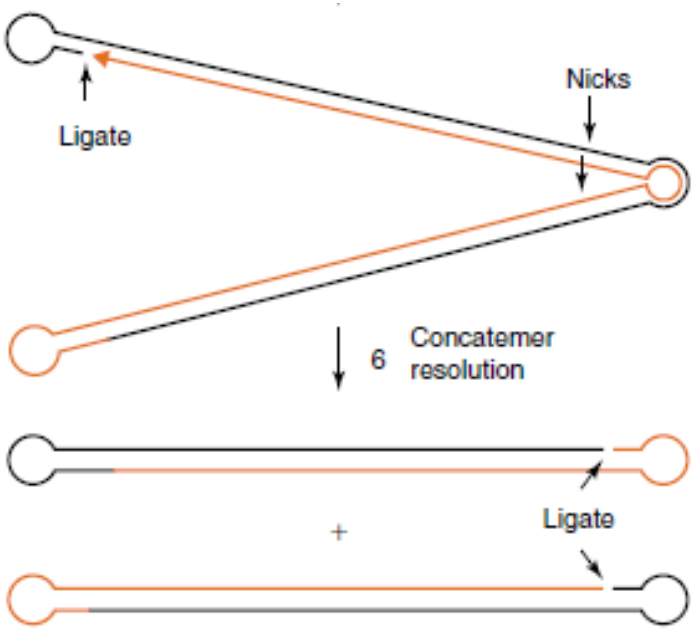


FIGURE 15.5 **A model for vaccinia virus DNA replication.** See text for details. Newly synthesized DNA is shown in orange.

From "Fundamentals of Molecular Virology"
by Nicholas H. Acheson, John Wiley & sons, 2007

Poxvirus DNA replication enzymes

Function	Protein
DNA replication, repair, recombination	DNA polymerase*
	DNA pol. processivity factor
	DNA primase*
	Topoisomerase I
	ssDNA binding protein
	DNA ligase*
	Holliday junction resolvase
	Protein kinase (BAF antagonist)*
	Multifunctional "scaffold" protein*
	Uracil DNA glycosylase*
	dUTPase
	dsDNA break repair
Nucleotide metabolism	Thymidine kinase
	Thymidylate kinase
	Ribonucleotide reductase

* Genes coding for these proteins have been shown to be required for carrying out viral DNA replication.

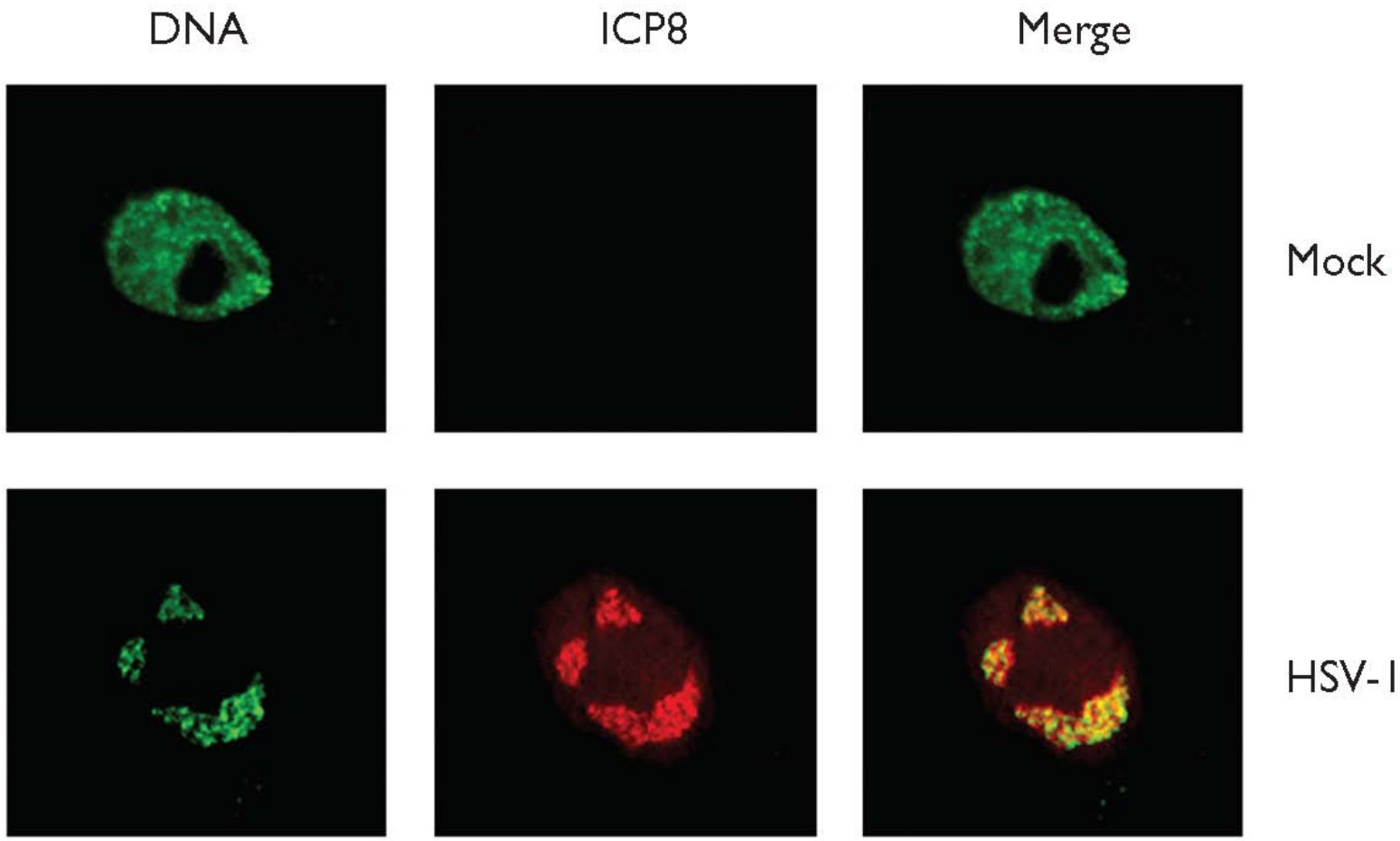
Delayed synthesis of virion structural proteins prevents

- When new viral DNAs are encapsidated they cannot be used as templates for additional DNA synthesis
- Consequently genome packaging into particles is delayed – transcriptional control

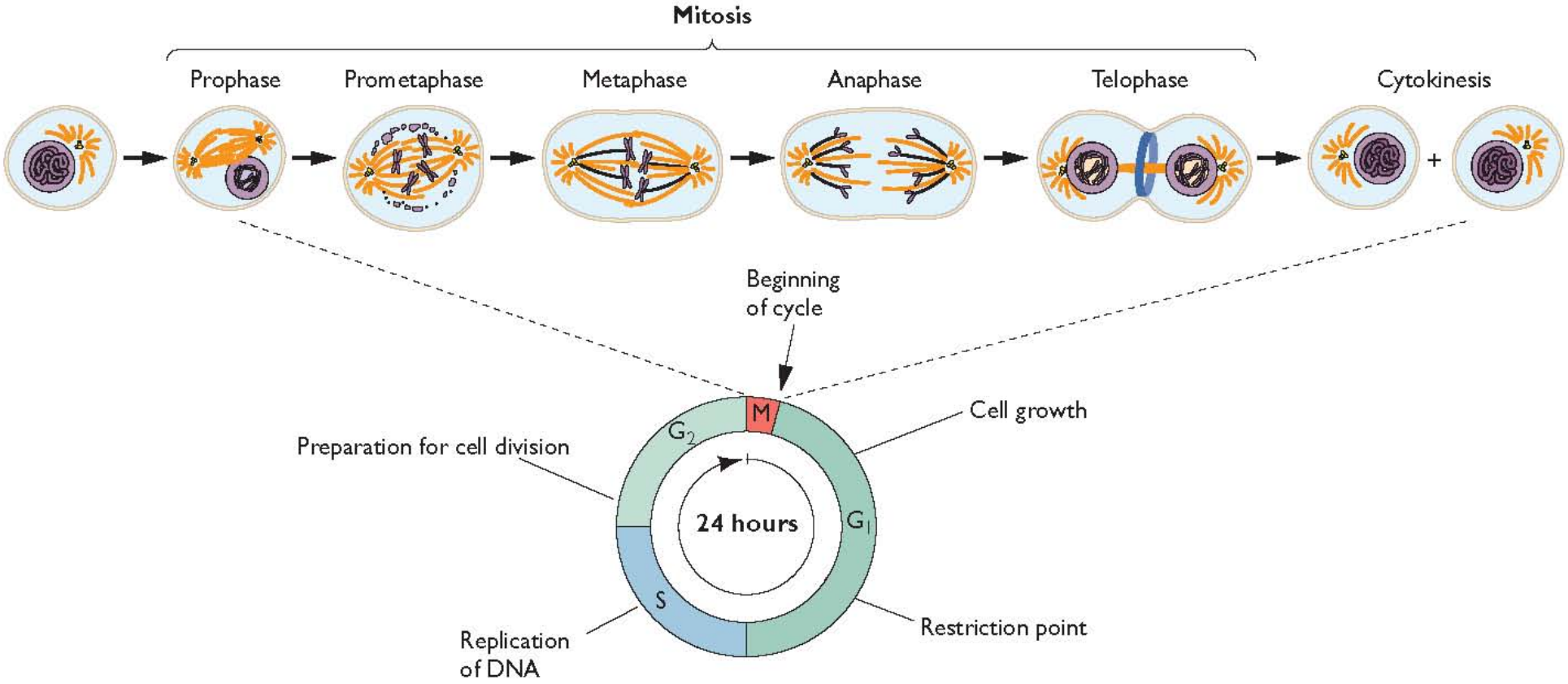
Inhibition of cellular DNA synthesis

- When viral DNA replication is carried out mostly by viral proteins, cell DNA synthesis is often inhibited
- Increases availability of substrates
- Adenovirus, herpesvirus, poxvirus
- Mechanisms not understood

Viral DNAs are synthesized in specialized intracellular

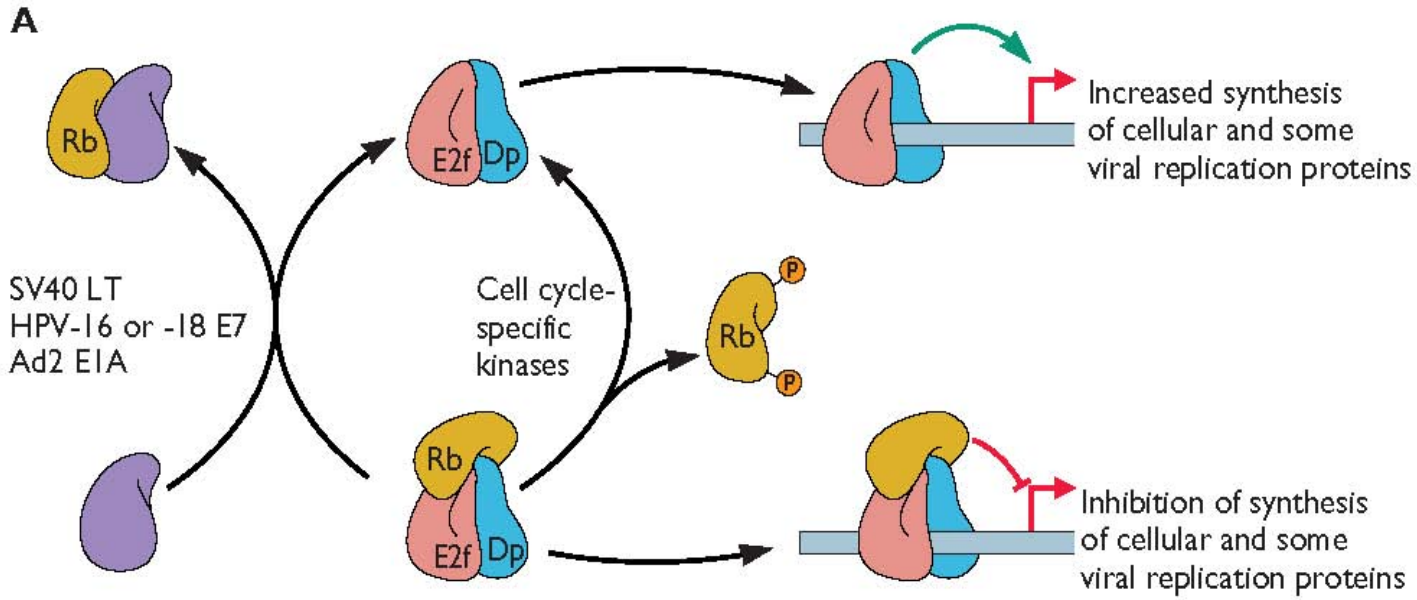


Mechanism of exponential viral DNA replication



Cellular retinoblastoma (*rb*) gene:
Rb protein controls entry into S from G1
Loss is associated with tumors = tumor suppressor gene

Adenovirus E1A protein binds Rb

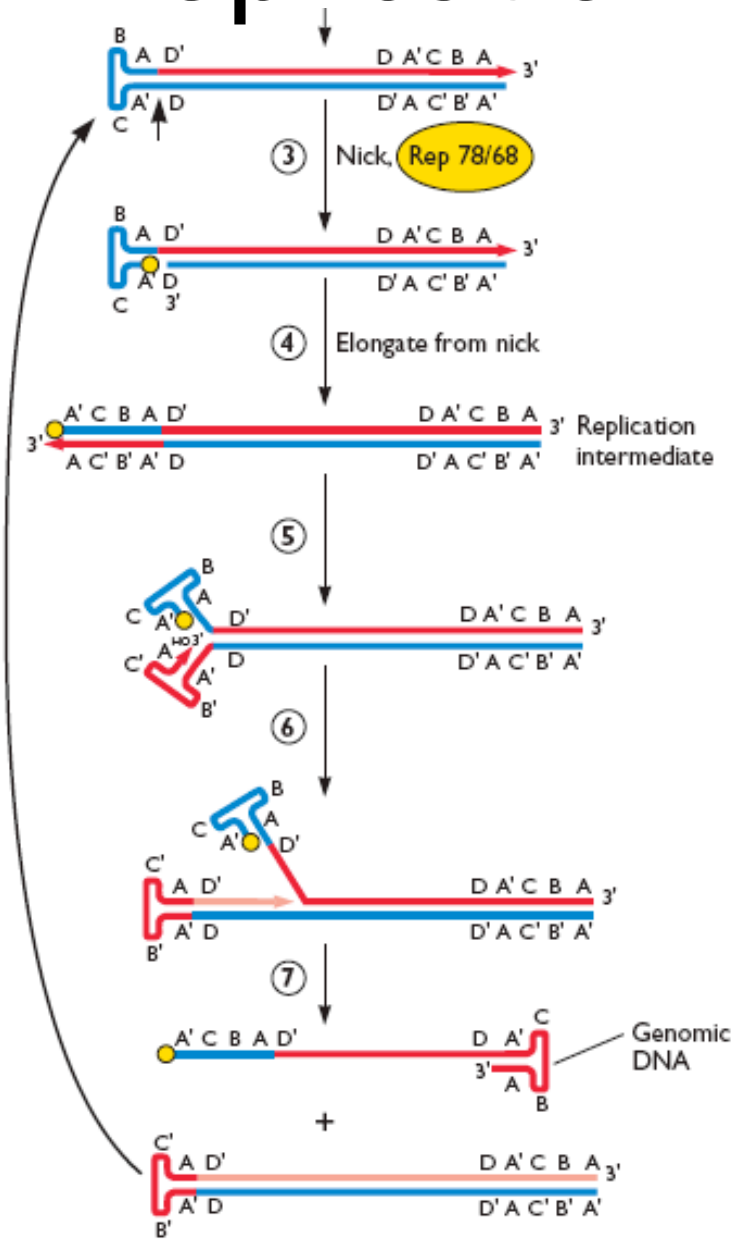


Limited replication of viral DNA

- Most DNA viruses: exponential replication of genomes
- Some establish long-term relationships with cells, number of genomes is limited
- Various mechanisms effect genome copy number

Parvovirus DNA Replication

Rep78/68 a site & strand-specific endonuclease



Parvovirus DNA replication

- DNA replicates only in cells coinfected with helper adenovirus
- Adenoviral helper proteins allow synthesis of large quantities of Rep 78/68

Parvovirus DNA replication

- When no helper adenovirus is present, Rep 78/68 level is low
- Little viral DNA synthesis occurs
- Genome integrates into host cell DNA

Papillomaviruses: Controlled and exponential replication from a single Ori