Lecture Series 6

DNA and Its Role in Heredity

Reading Assignments

- Read Chapter 5
  DNA and Chromosomes
- Read Chapter 6
  DNA Replication, Repair & Replication
- Read Chapter 10
  pages 331 to 333 & 347 to 351 2nd Editon
  pages 340 to 343 & 345 to 347 3rd Edition
  (Sections on Sequencing and PCR only)

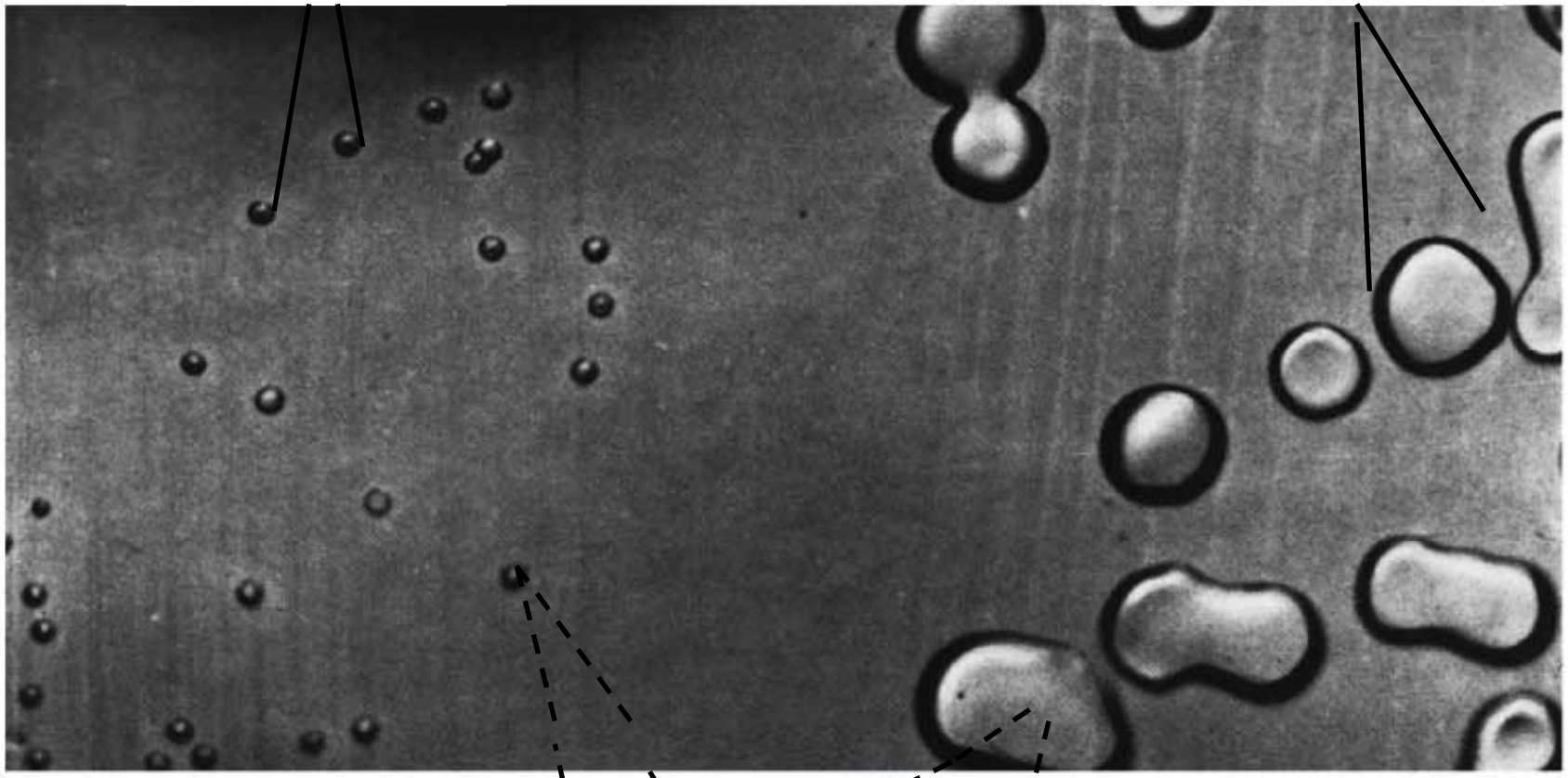
A. DNA: The Genetic Material

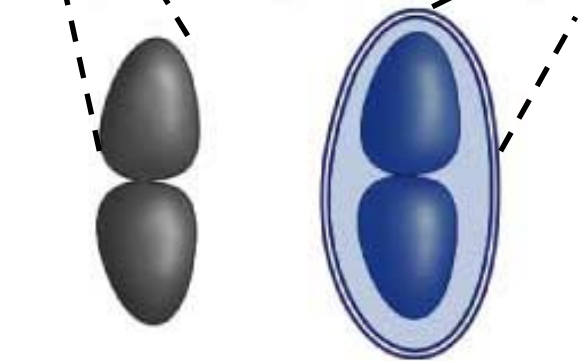
- In addition to circumstantial evidence, three key experiments demonstrated that DNA is the genetic material.
- In the first key experiment (Griffiths, 1928) showed that a virulent strain of *Streptococcus pneumoniae* genetically transformed nonvirulent *S. pneumoniae* into virulent bacteria. Showed principle of "**transformation**".

There are two strains of *Streptococcus pneumoniae*.

ROUGH COLONY (R)

SMOOTH COLONY (S)





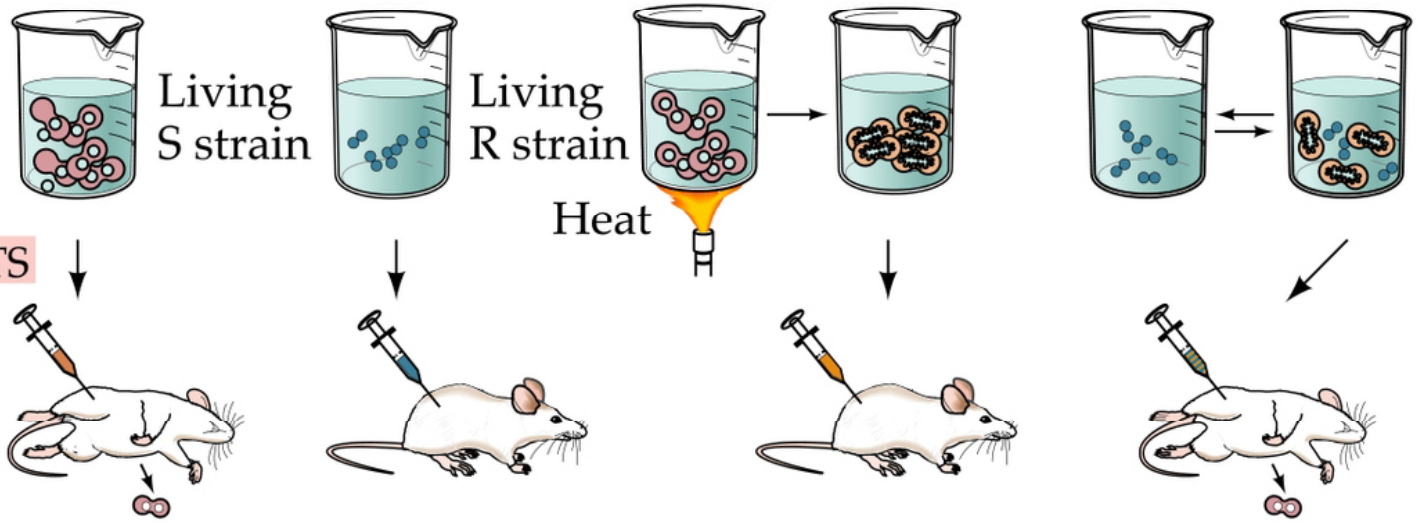
R strain is benign (Lacking a protective capsule, it is recognized and destroyed by host's immune system)

S strain is virulent (Polysaccharide capsule prevents detection by host's immune system)

EXPERIMENT

Question: Can an extract from dead bacterial cells genetically transform living bacterial cells?

METHOD



RESULTS

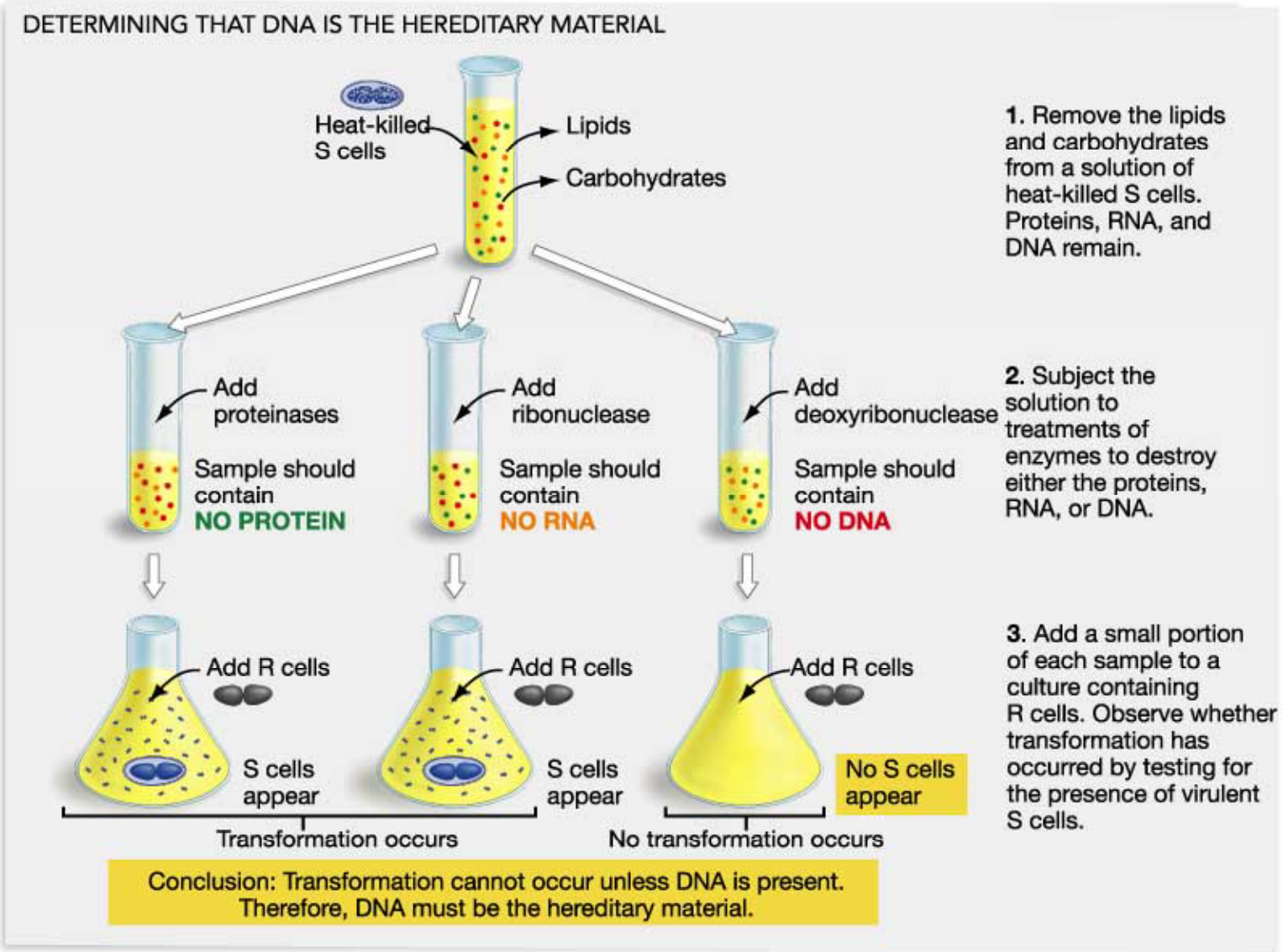
Mouse dies	Mouse healthy	Mouse healthy	Mouse dies
Living S strain cells isolated from heart	No bacterial cells found in heart	No bacterial cells found in heart	Living S strain cells isolated from heart

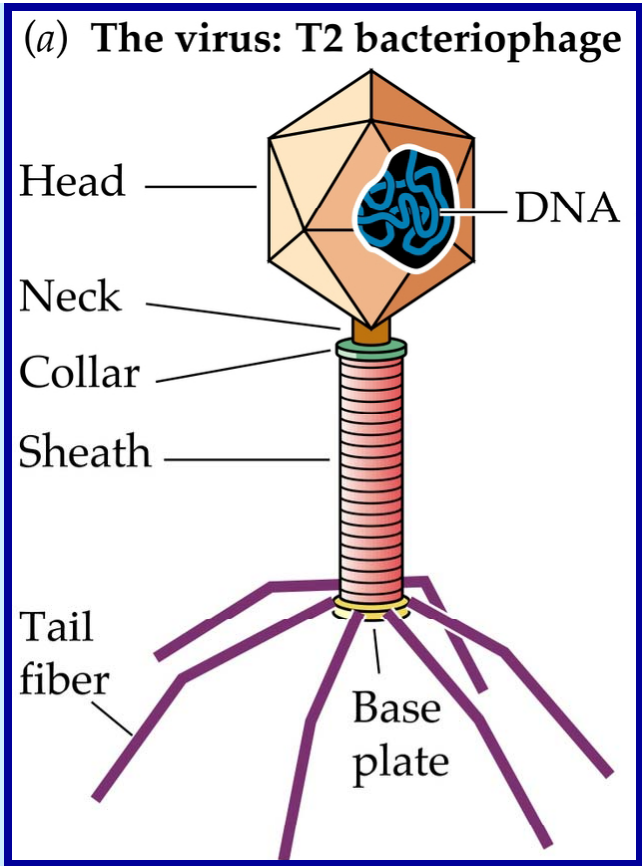
Conclusion: A chemical component from one cell is capable of genetically transforming another cell.

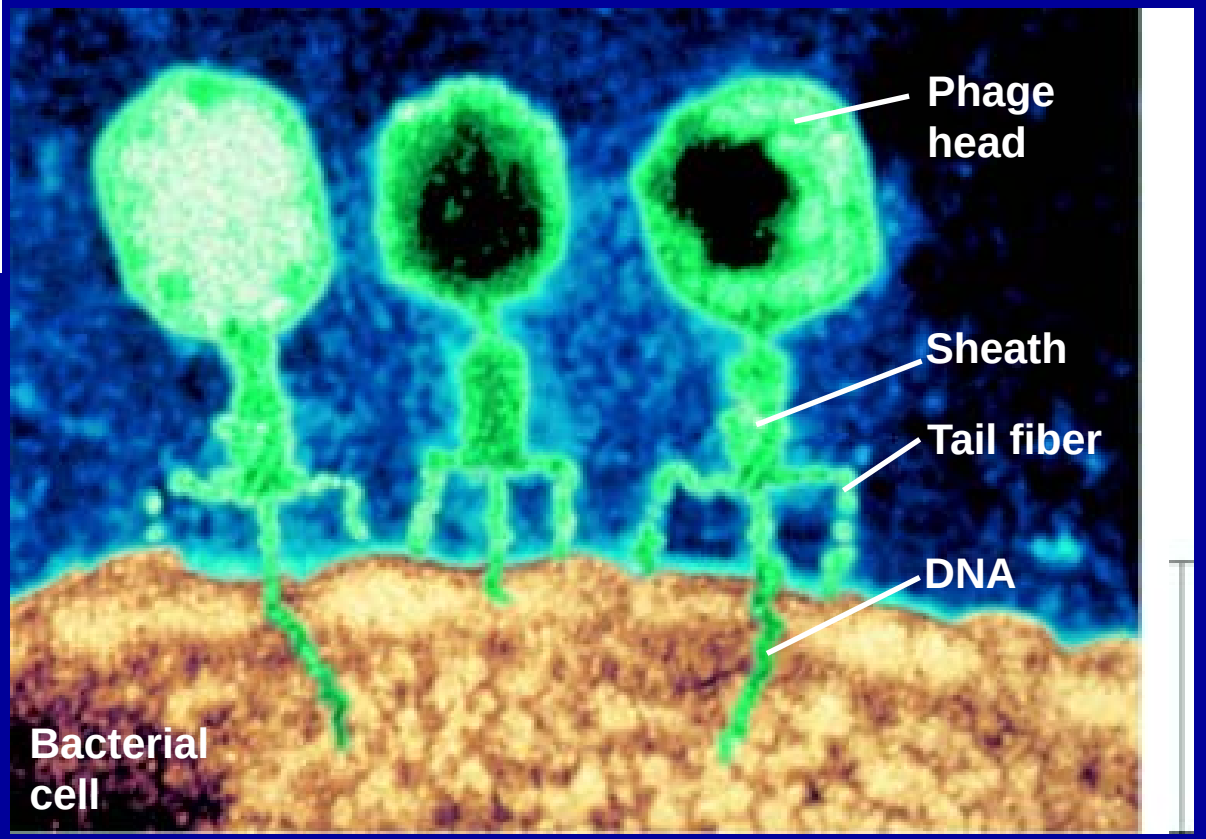
A. DNA: The Genetic Material

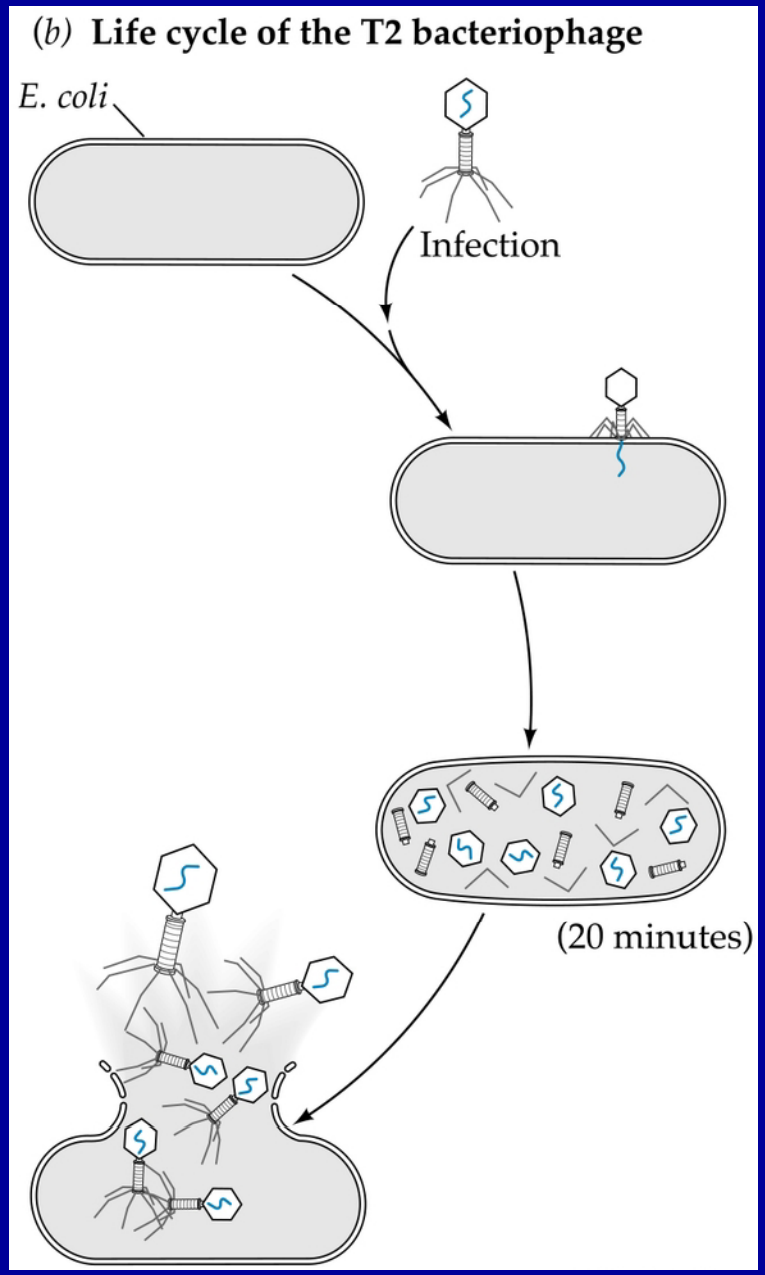
- The second key experiment (Avery, 1944) showed that "**DNA**" was the transforming agent through studies of T-even bacteriophage and their treatment with hydrolytic enzymes.
- The third key experiment (Hershey & Chase, 1952) showed that labeled viruses were incubated with host bacteria. Labeled viral DNA entered host cells, producing many label-bearing viruses. This confirmed DNA instead of proteins.

The transforming principle is DNA



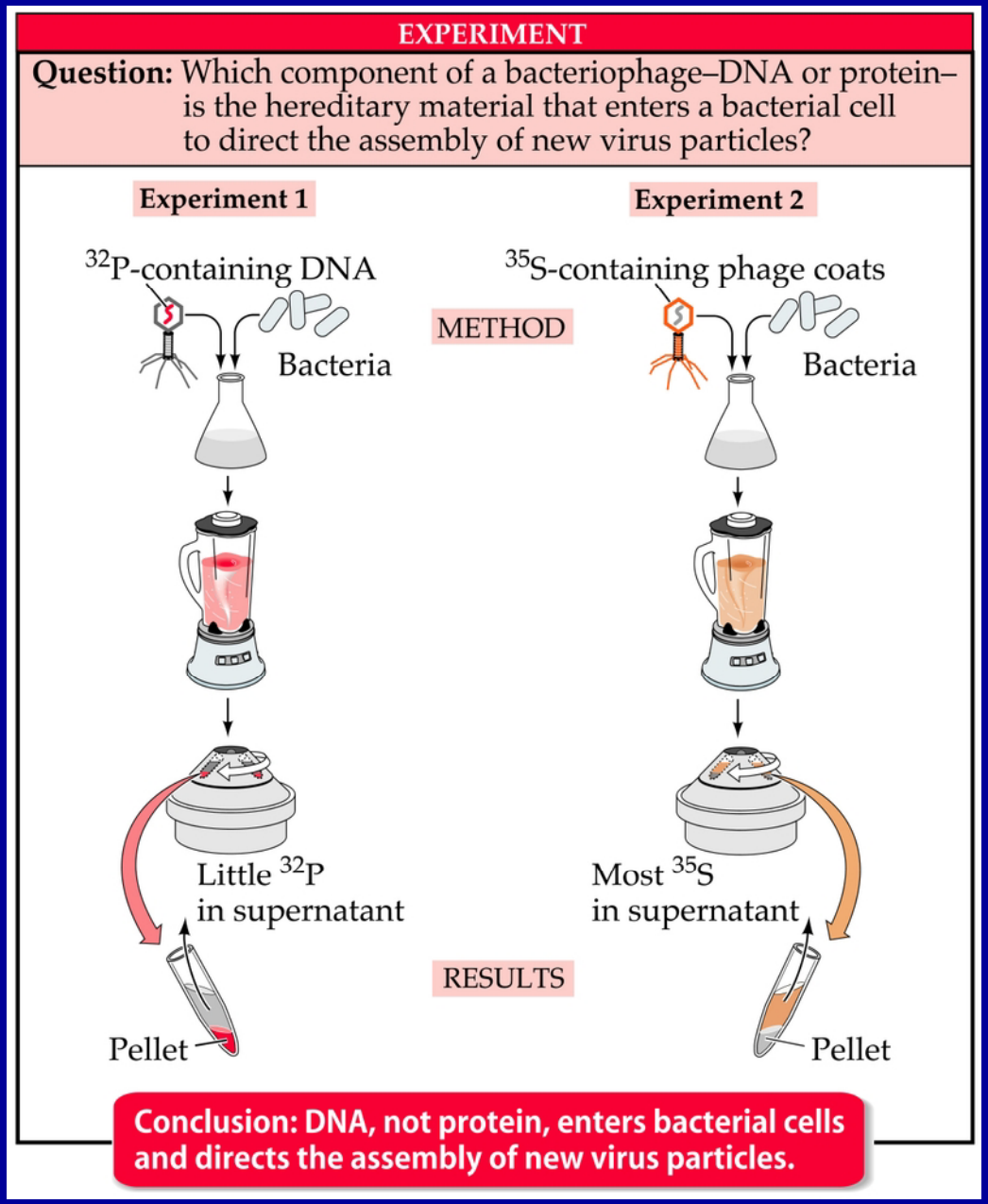

(a) The virus: T2 bacteriophage
Head
DNA
Neck
Collar
Sheath
Tail fiber
Base plate


Phage head
Sheath
Tail fiber
DNA
Bacterial cell
100 nm


(b) Life cycle of the T2 bacteriophage
E. coli
Infection
(20 minutes)

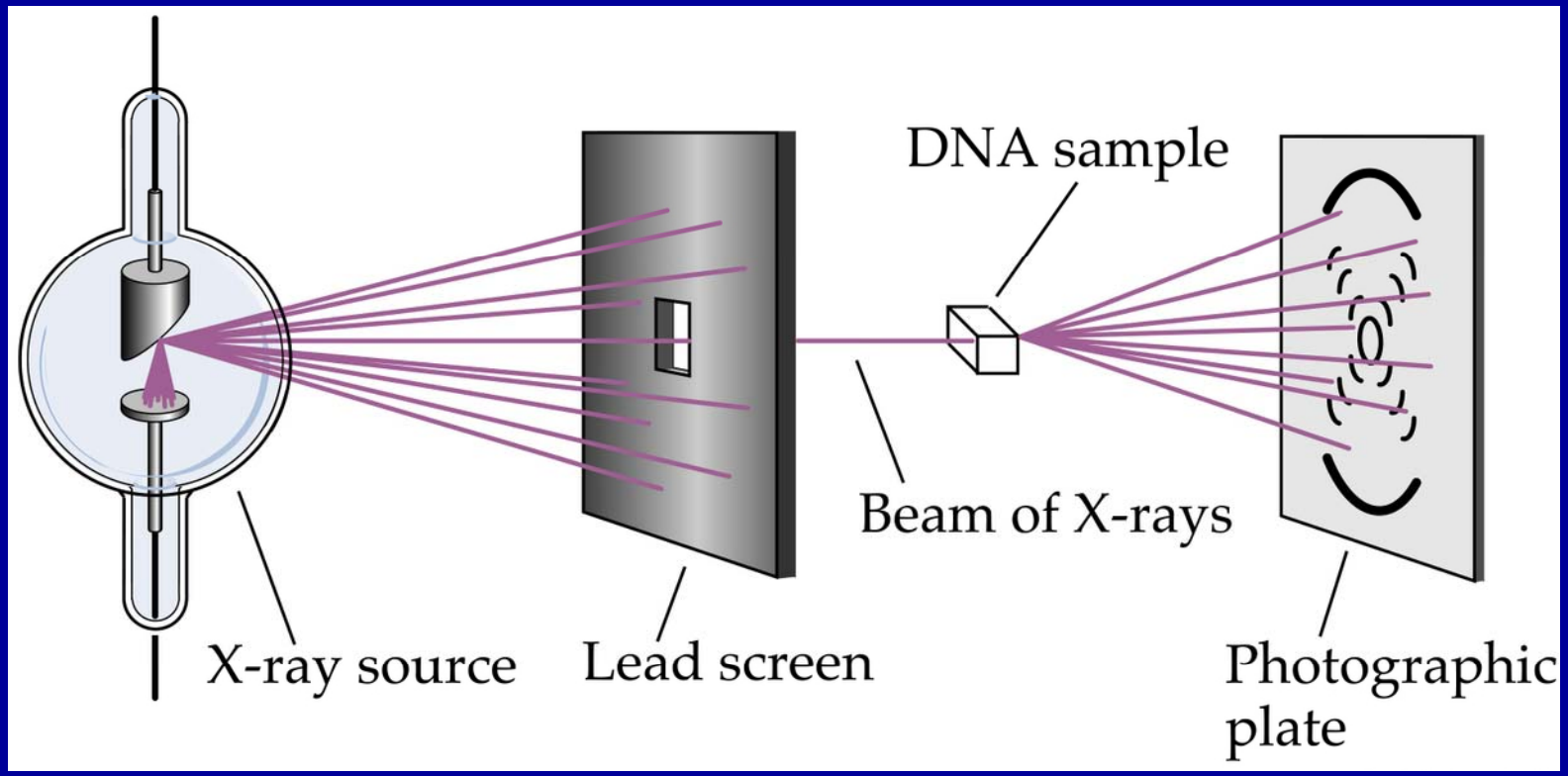
Lytic Cycle

The Hershey-Chase Blender Experiment

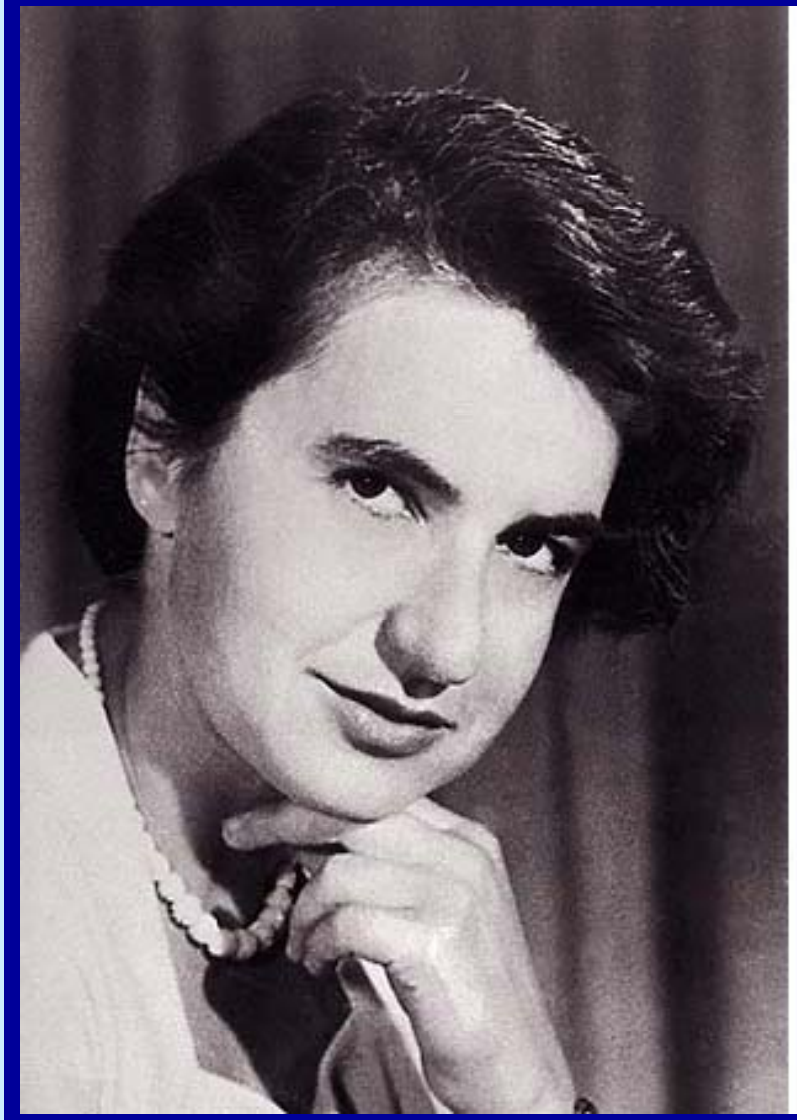


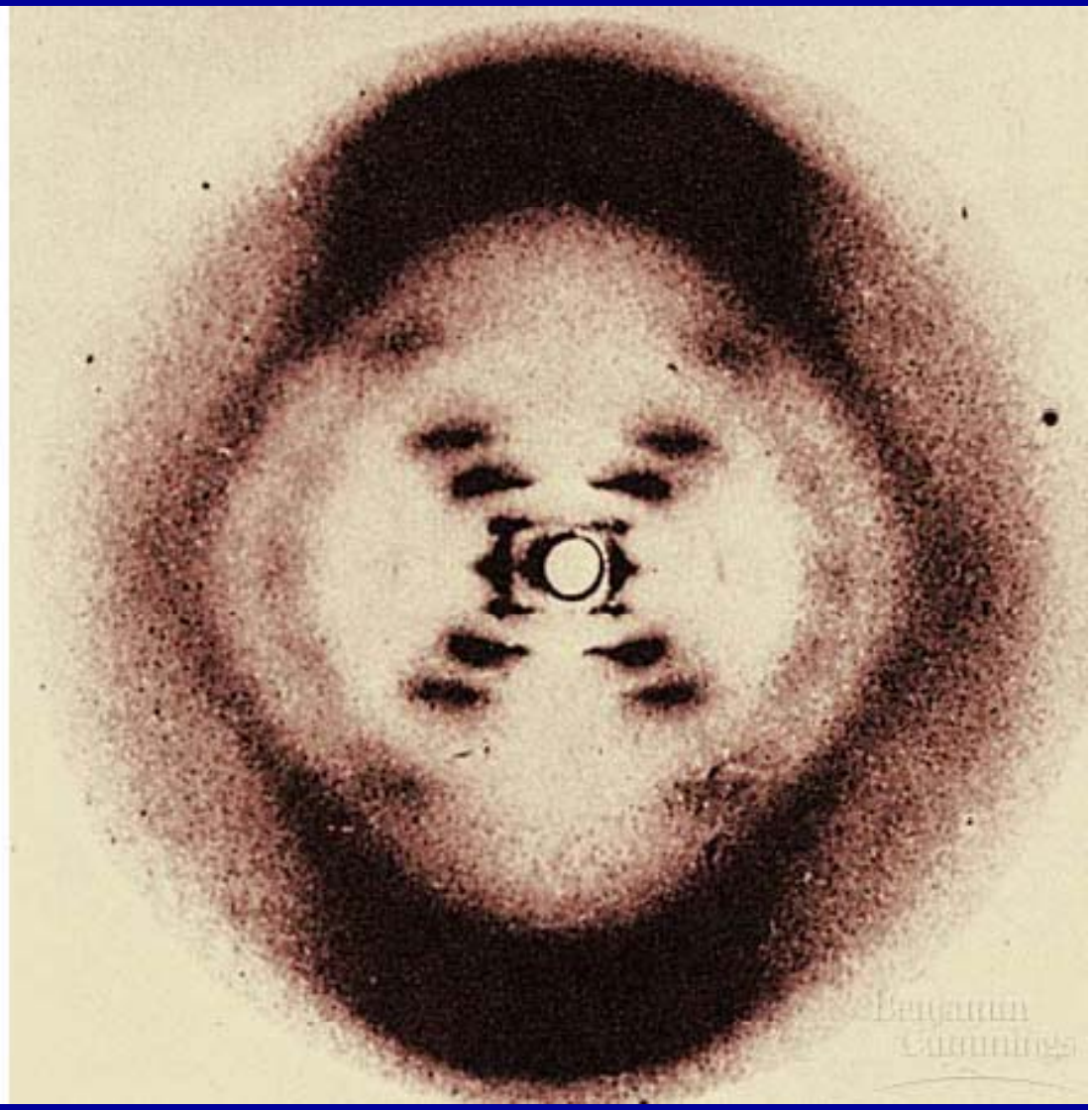
B. The Structure of DNA

- X-ray crystallography showed that the DNA molecule is a helix.


DNA sample
Beam of X-rays
X-ray source
Lead screen
Photographic plate

Rosalind Franklin and her X-ray diffraction photo of DNA

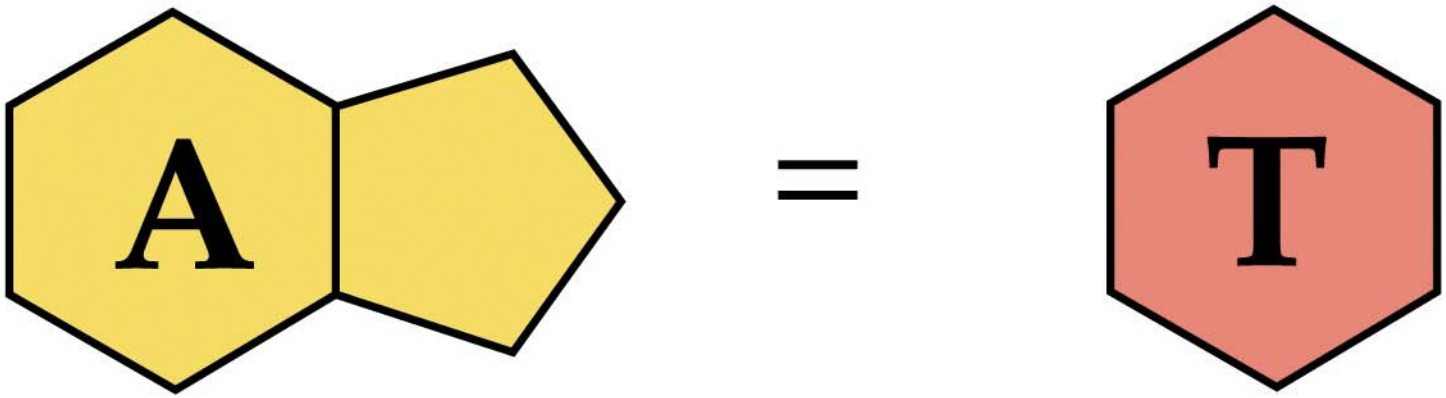


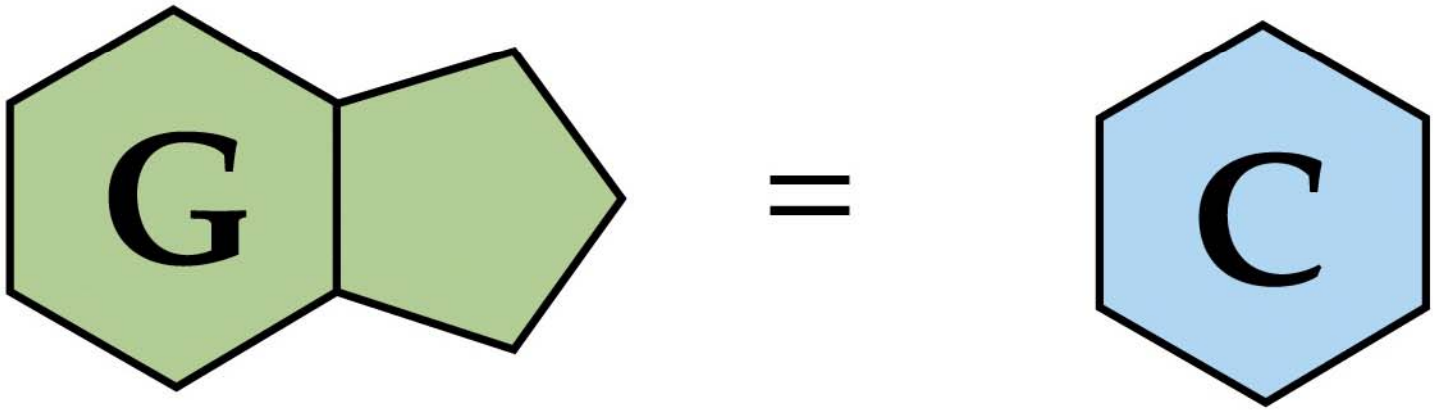


B. The Structure of DNA

- DNA is composed of nucleotides, each containing adenine, cytosine, thymine, or guanine.

- There are equal amounts of adenine and thymine and equal amounts of guanine and cytosine. This is known as Chargaff's Rule (1950, using paper TLC).



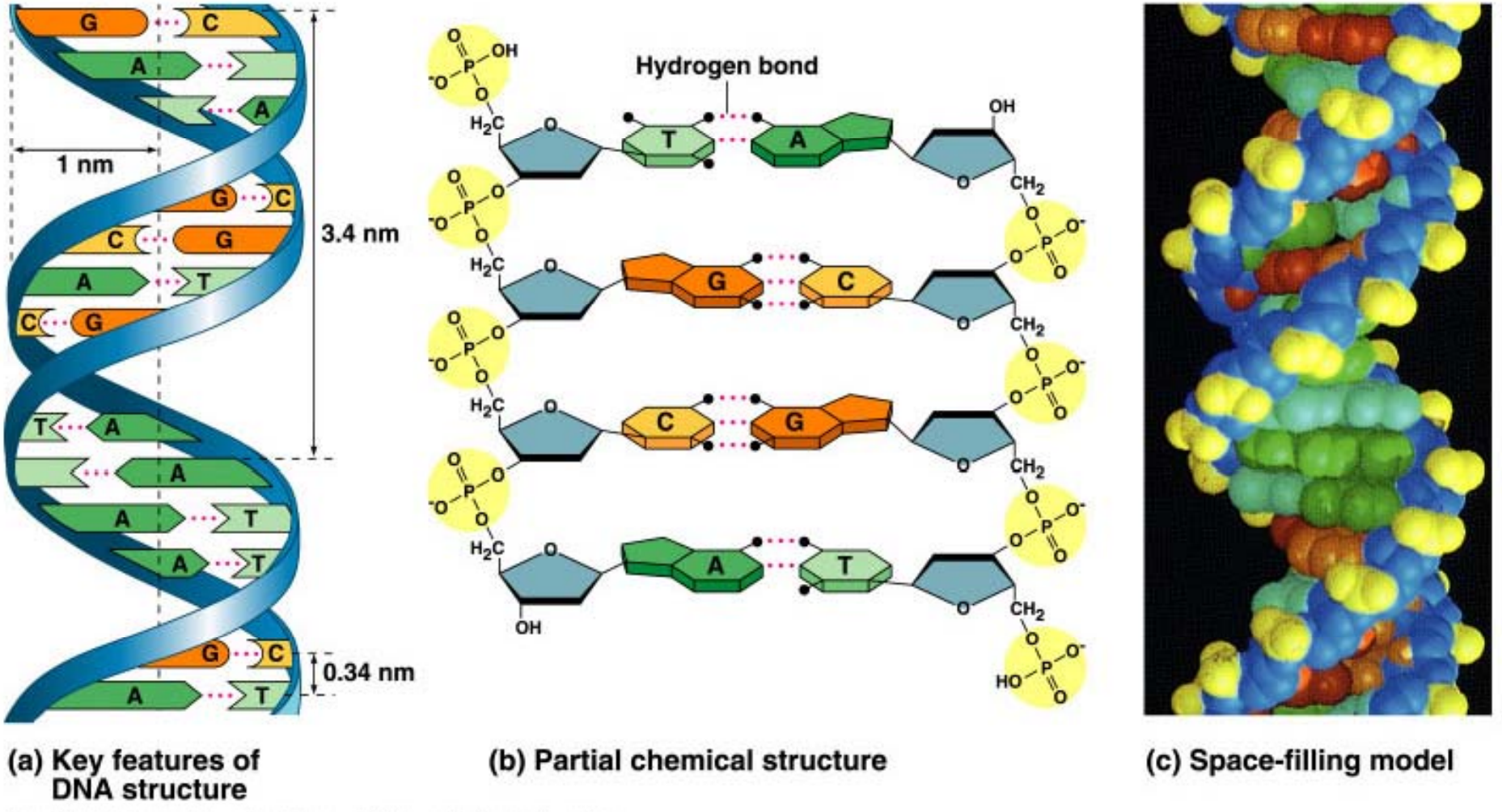


Purines = Pyrimidines

B. The Structure of DNA

- Watson and Crick (1953) proposed that DNA is a double-stranded helix with antiparallel strands, and with bases linked by hydrogen bonding.
- Their model accounts for genetic information, mutation, and replication functions of DNA.

The Double Helix

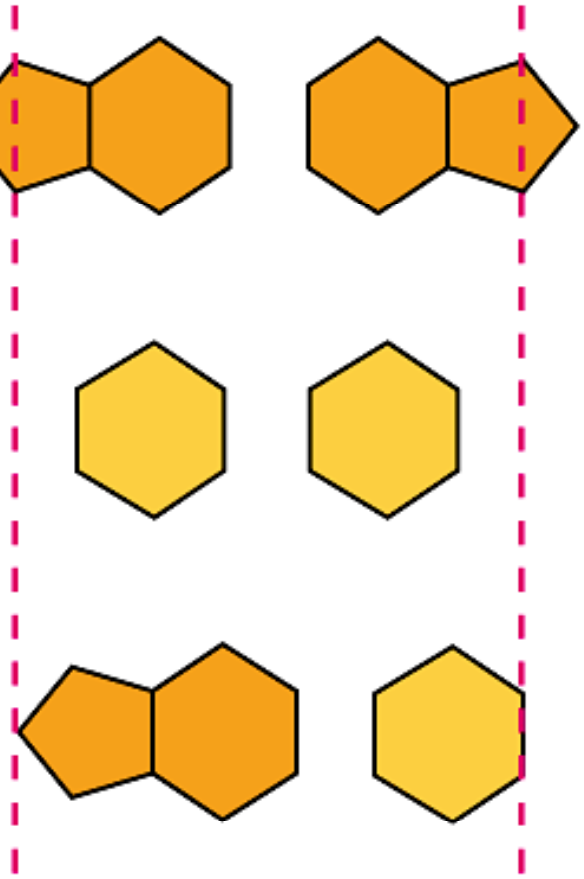


Purine and Pyridimine Fit

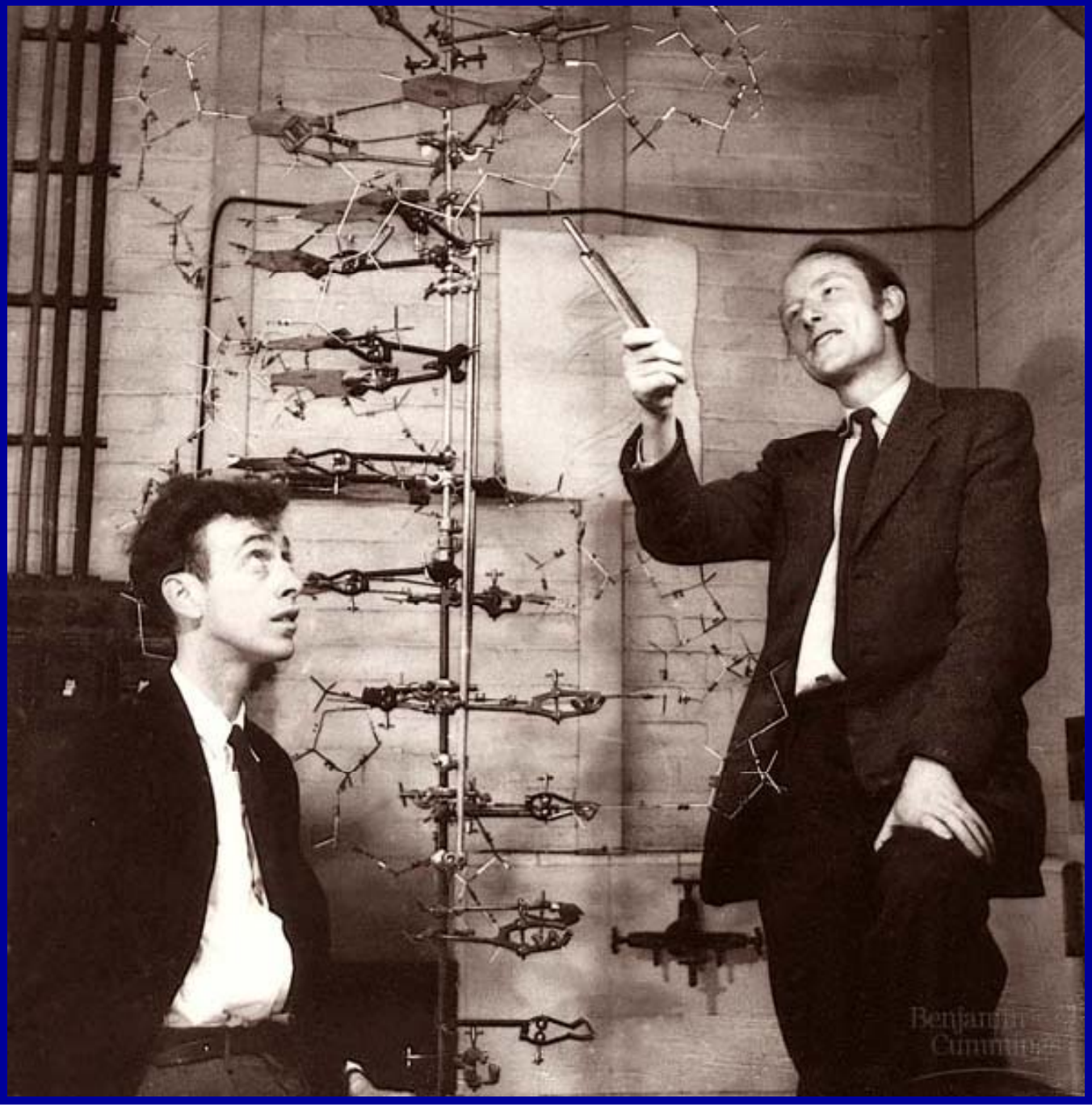
Purine + purine: too wide

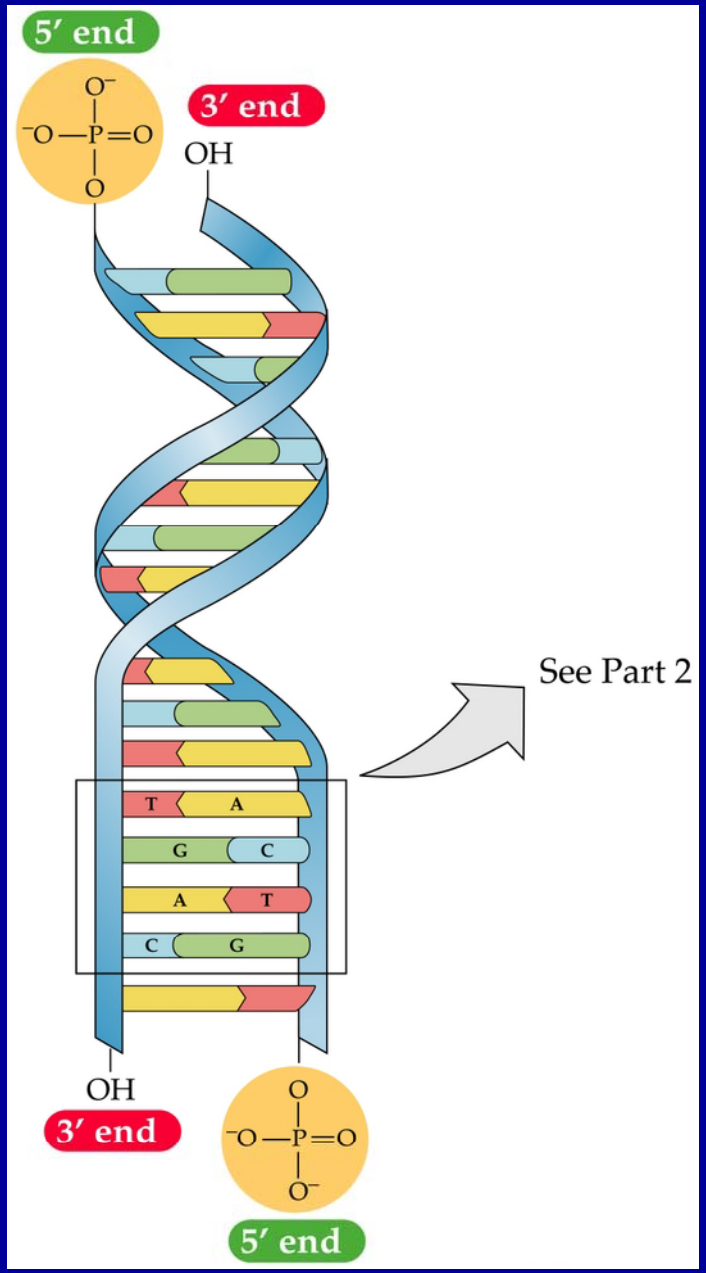
Pyrimidine + pyrimidine: too narrow

Purine + pyrimidine: width consistent with X-ray data

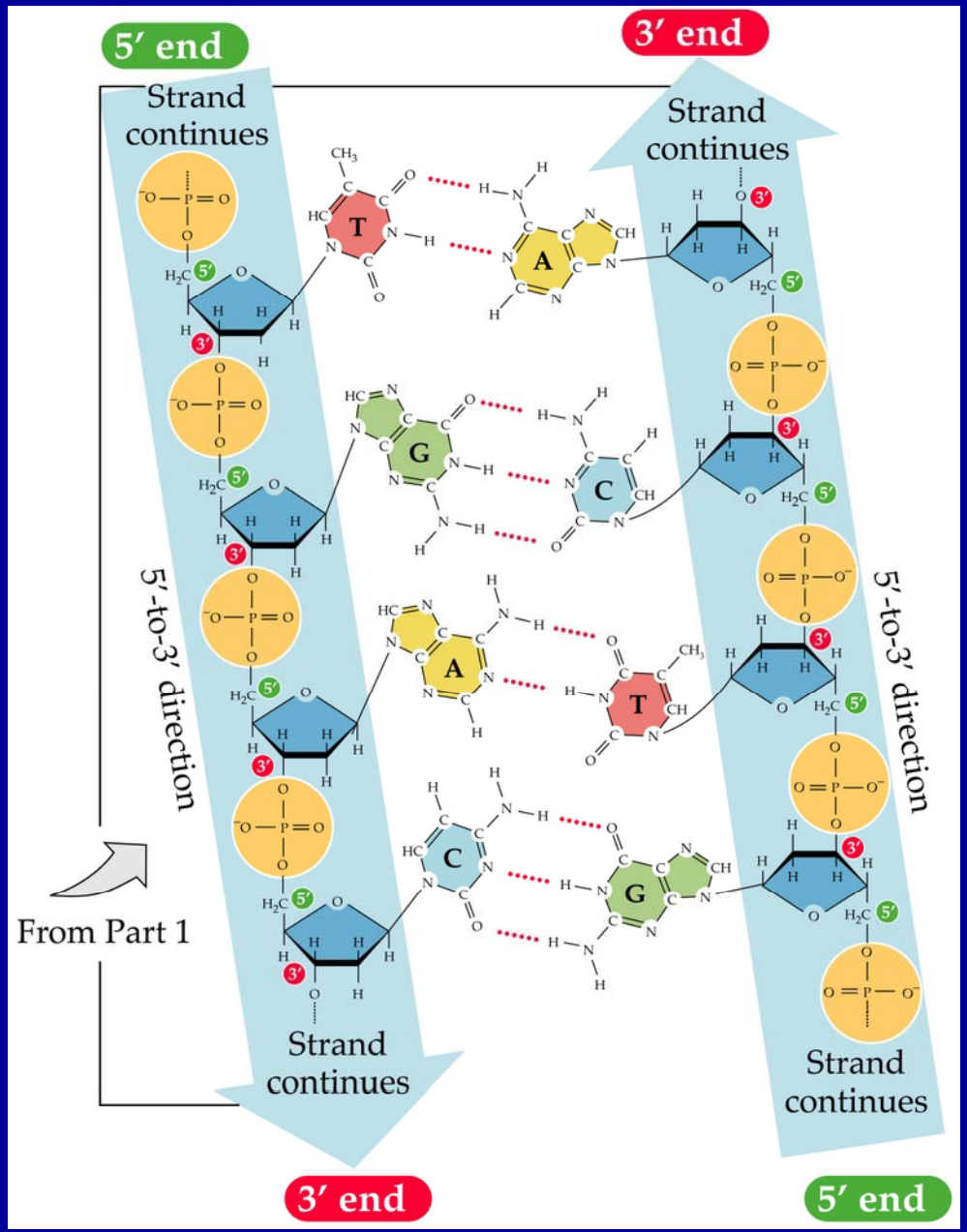


Watson and Crick and their Model



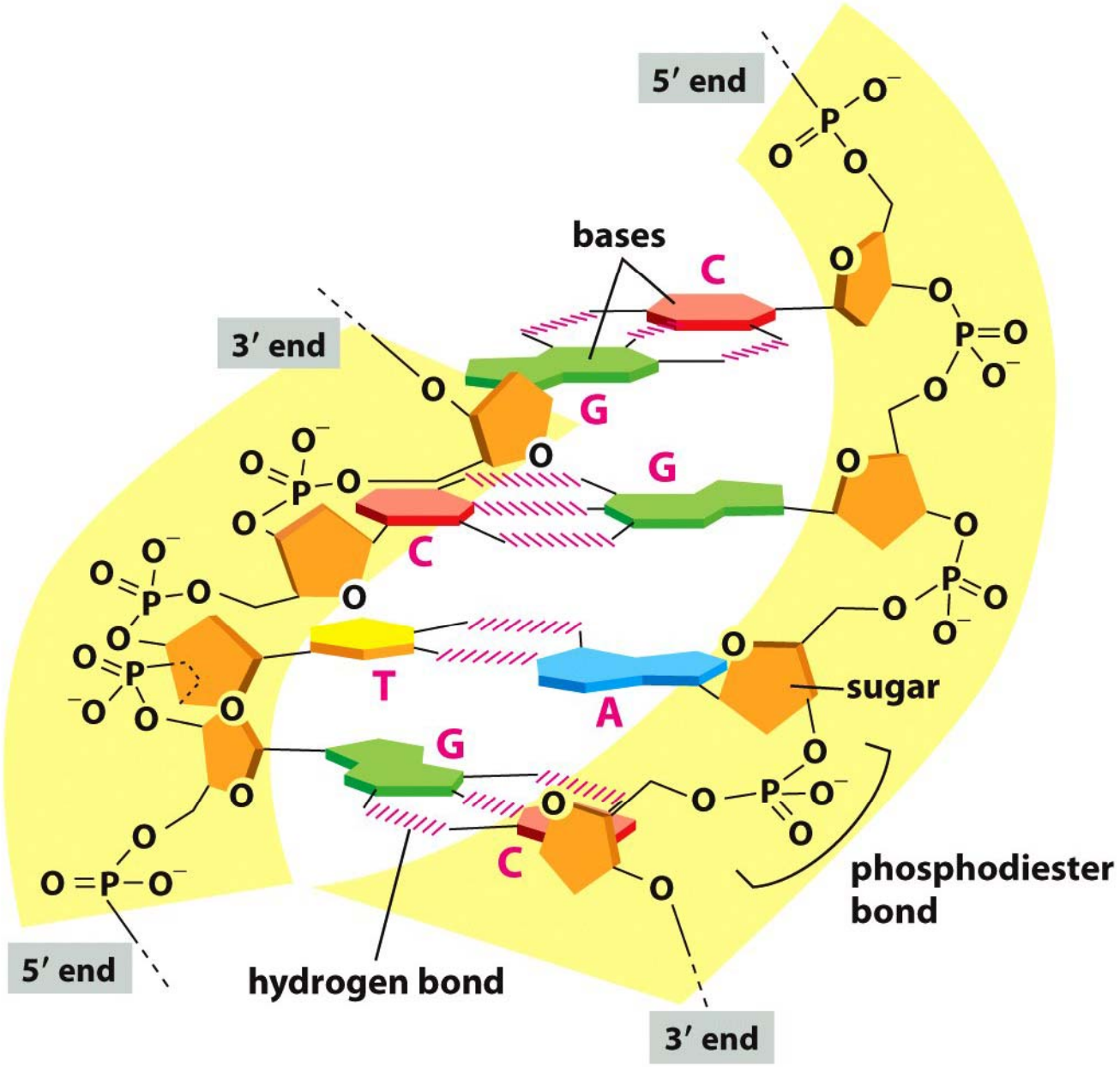


1) DS Helix
2) Uniform Diameter
3) RT handed twist
4) Anti-parallel



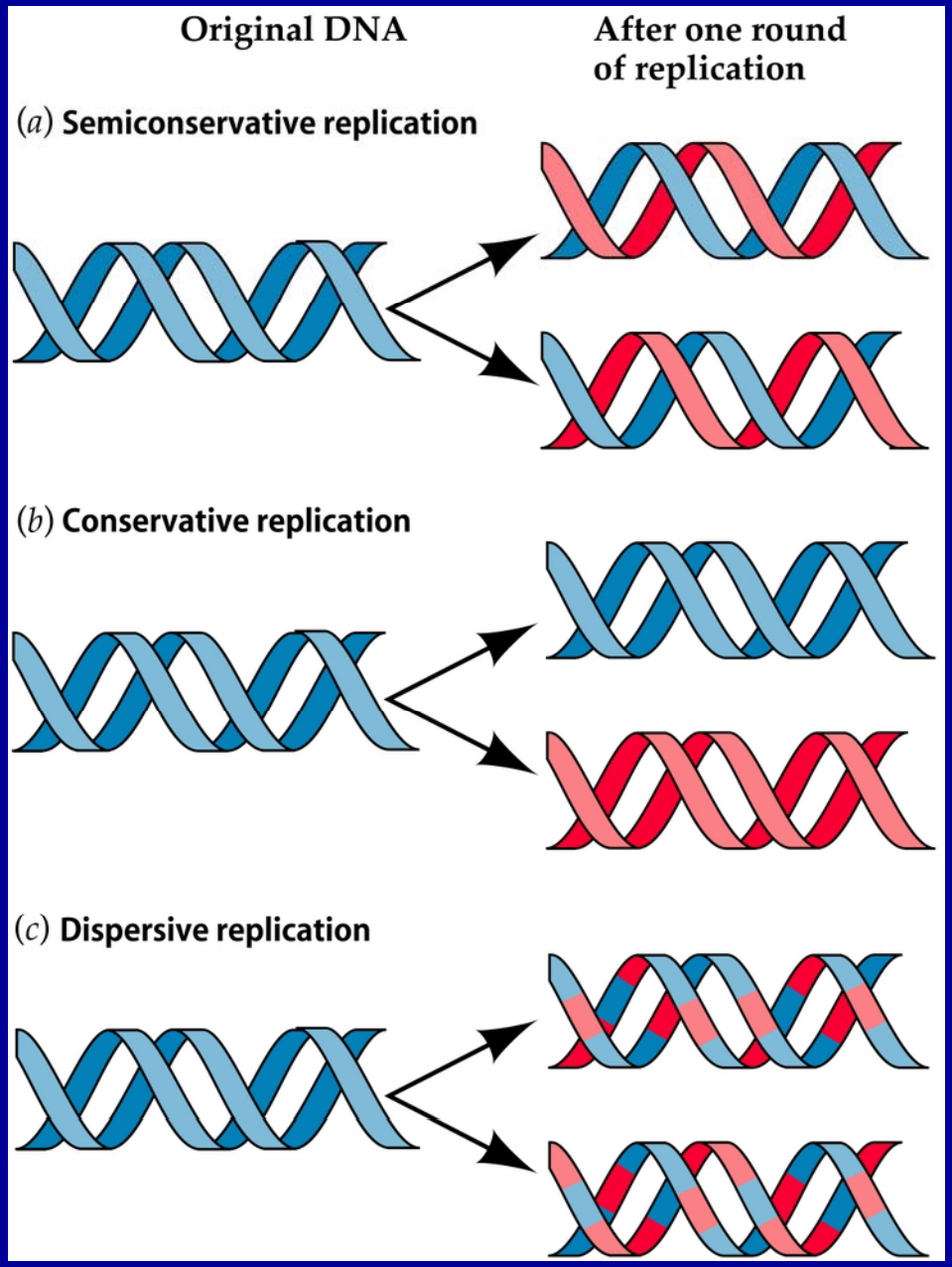
5) Complementary Base pairing

6) Double Helix is Essential to DNA's Function


5′ end
bases
C
3′ end
G
G
C
T
A
sugar
G
C
phosphodiester bond
5′ end
hydrogen bond
3′ end

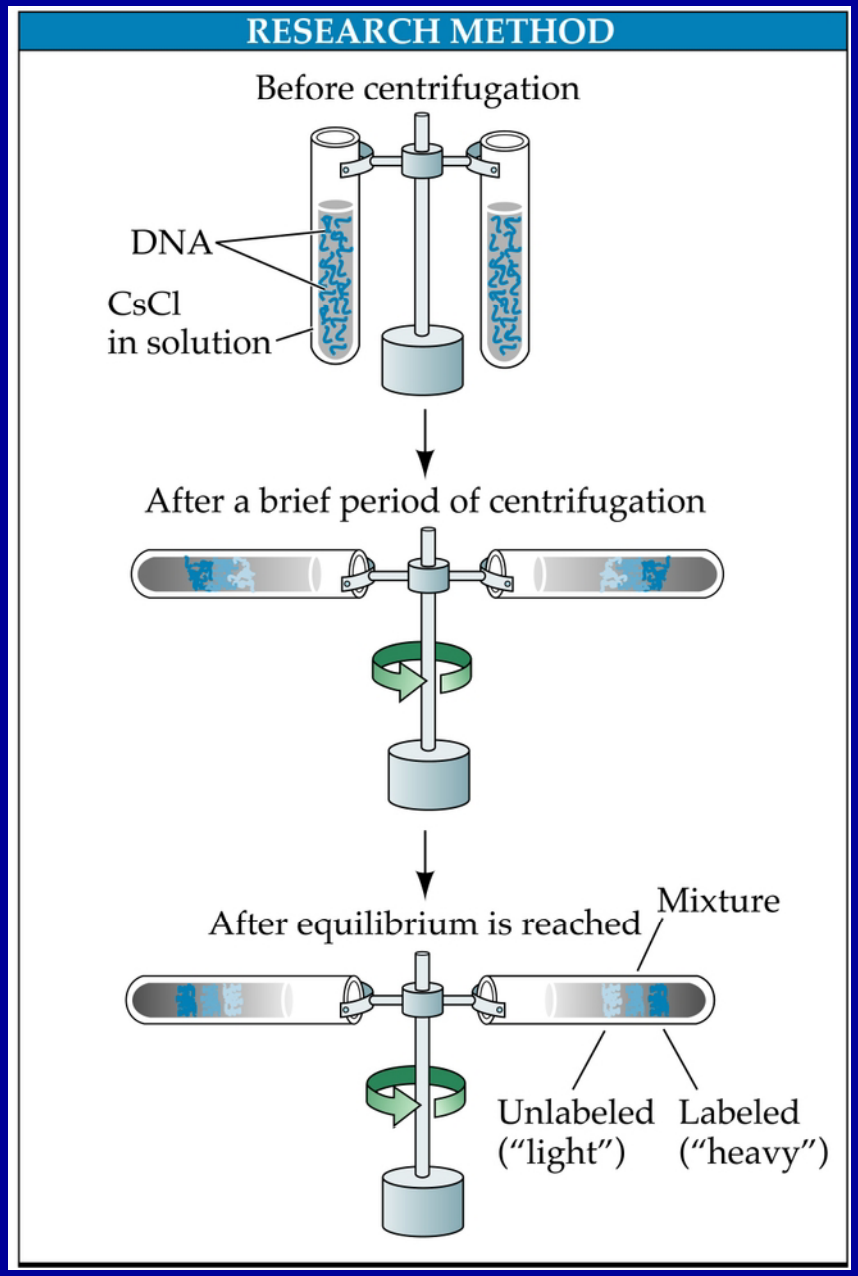
C. DNA Replication

- Semiconservative, conservative, and dispersive models for DNA replication were hypothesized.
- Each obeyed base-pairing rules.


Original DNA
After one round of replication
(a) Semiconservative replication
(b) Conservative replication
(c) Dispersive replication

C. DNA Replication

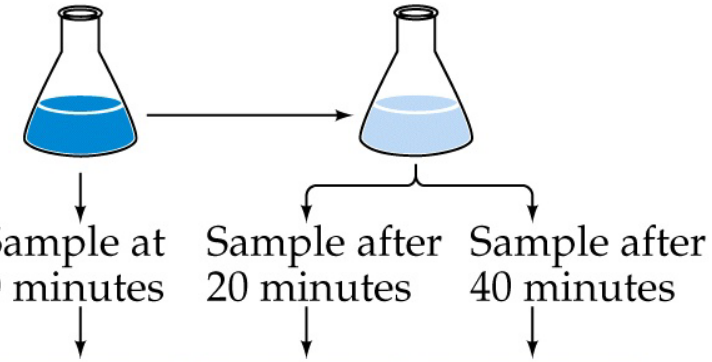
- Kornberg (1956) demonstrated *in vitro* that DNA served as its own template during replication.
- Meselson and Stahl's experiment (1957) proved replication of DNA to be semiconservative. A parent strand is a template for synthesis of a new strand. Two replicated DNA helices contain one parent strand and one synthesized strand each.


RESEARCH METHOD
Before centrifugation
DNA
CsCl
in solution
After a brief period of centrifugation
After equilibrium is reached
Mixture
Unlabeled
("light")
Labeled
("heavy")

EXPERIMENT

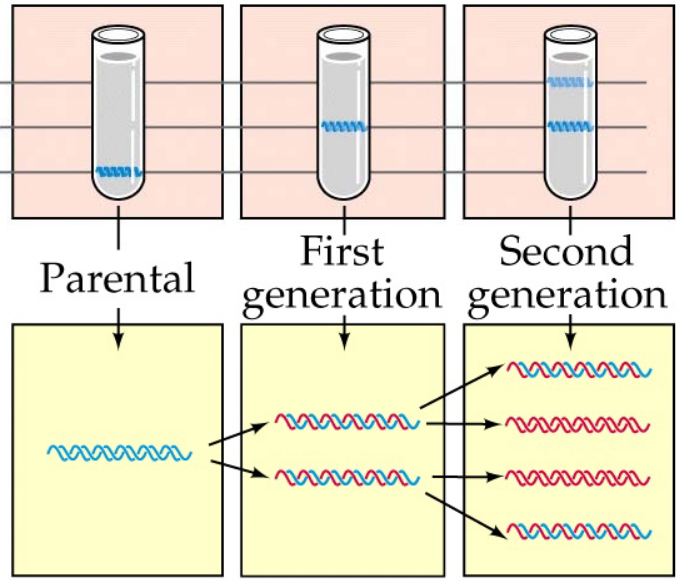
Question: Does DNA replicate semiconservatively, or by some other mechanism?

METHOD



RESULTS

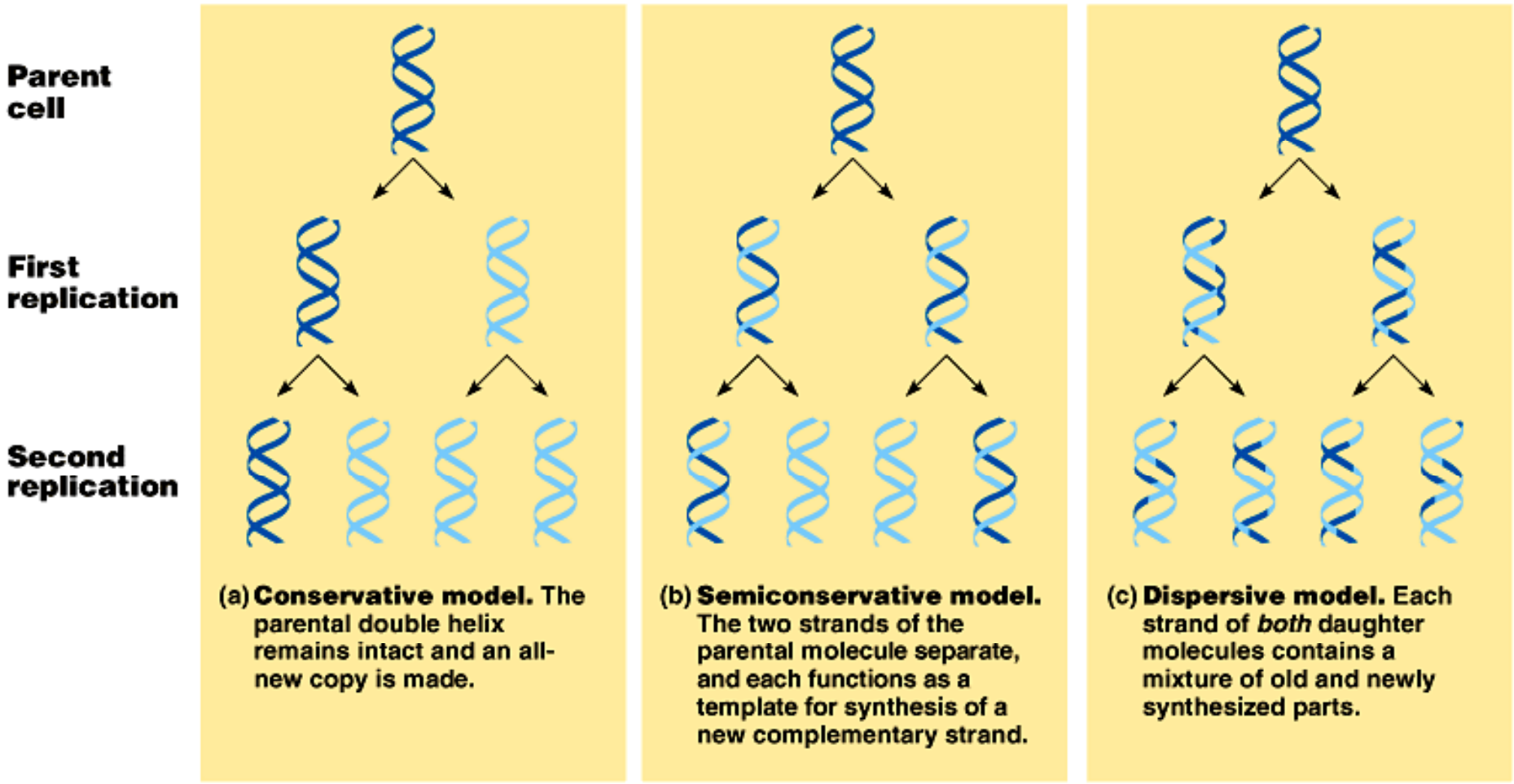
$^{14}N/^{14}N$ (light) DNA
$^{14}N/^{15}N$ (intermediate) DNA
$^{15}N/^{15}N$ (heavy) DNA



INTERPRETATION

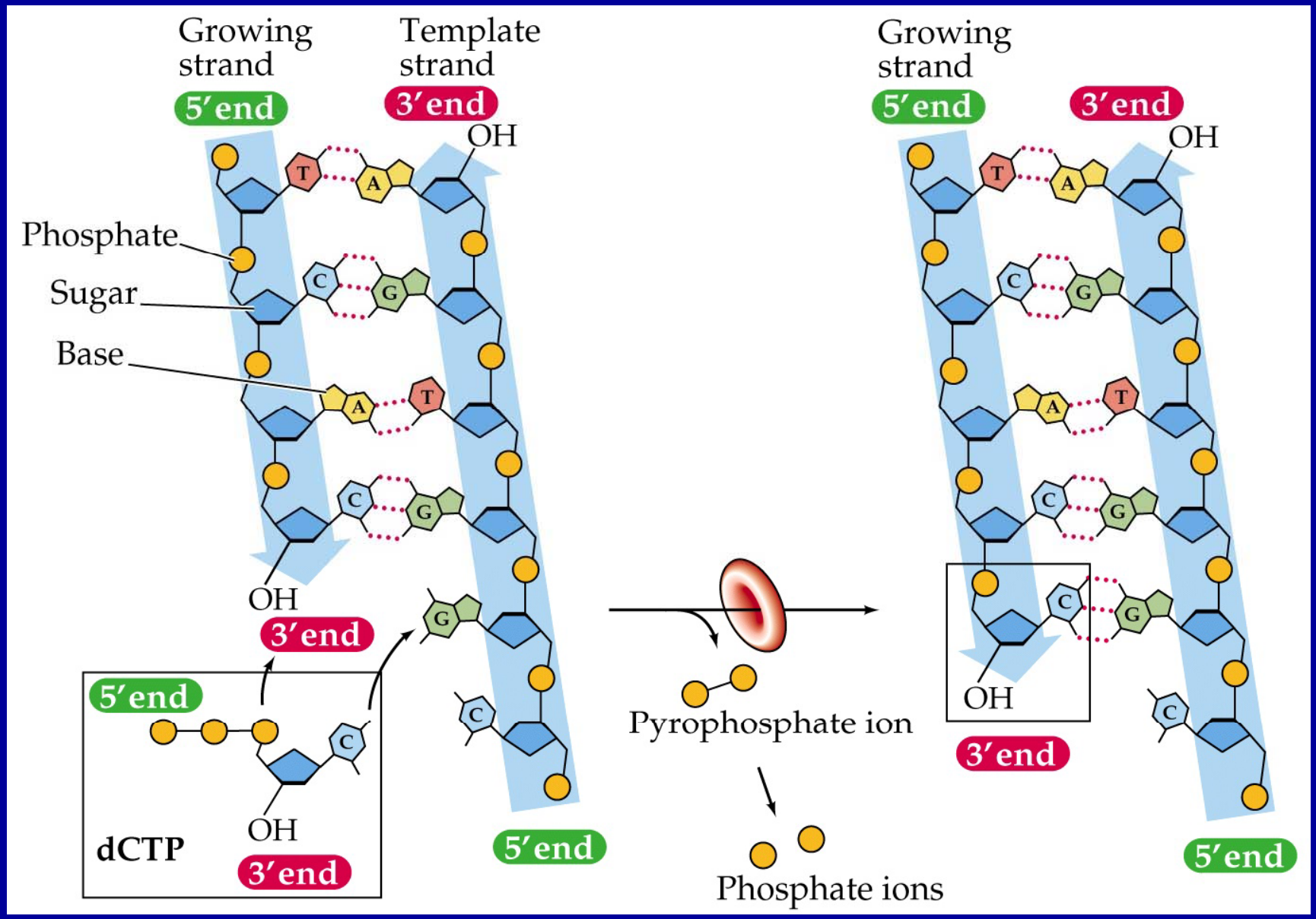
Conclusion: DNA replication is semiconservative.

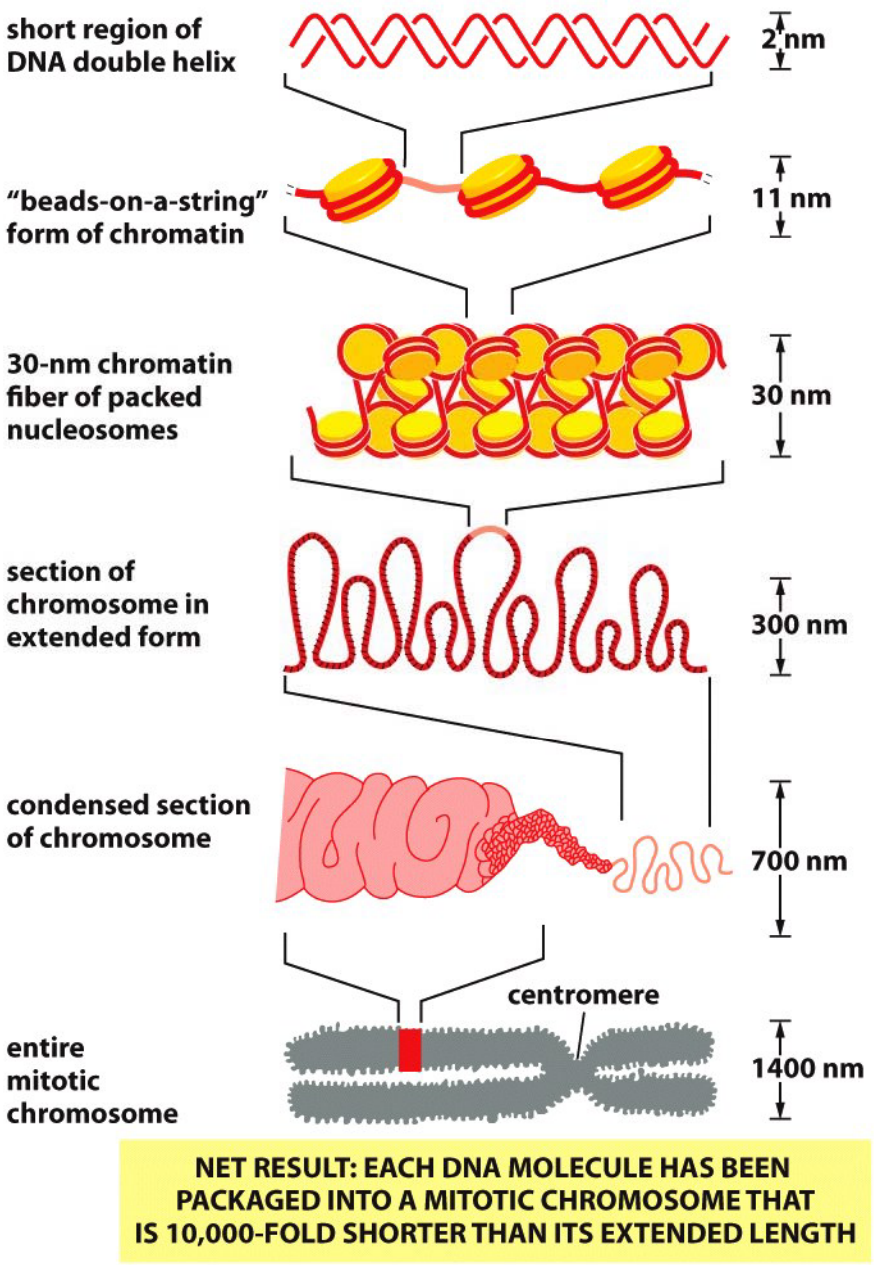
Three alternative models of DNA replication



D. The Mechanism of DNA Replication

- DNA polymerase catalyzes nucleotides from the 5′ to the 3′ end.
- Nucleotides are added by complementary base pairing with the template strand.
- The substrates, deoxyribonucleoside triphosphates, are hydrolyzed as added, releasing energy for DNA synthesis.

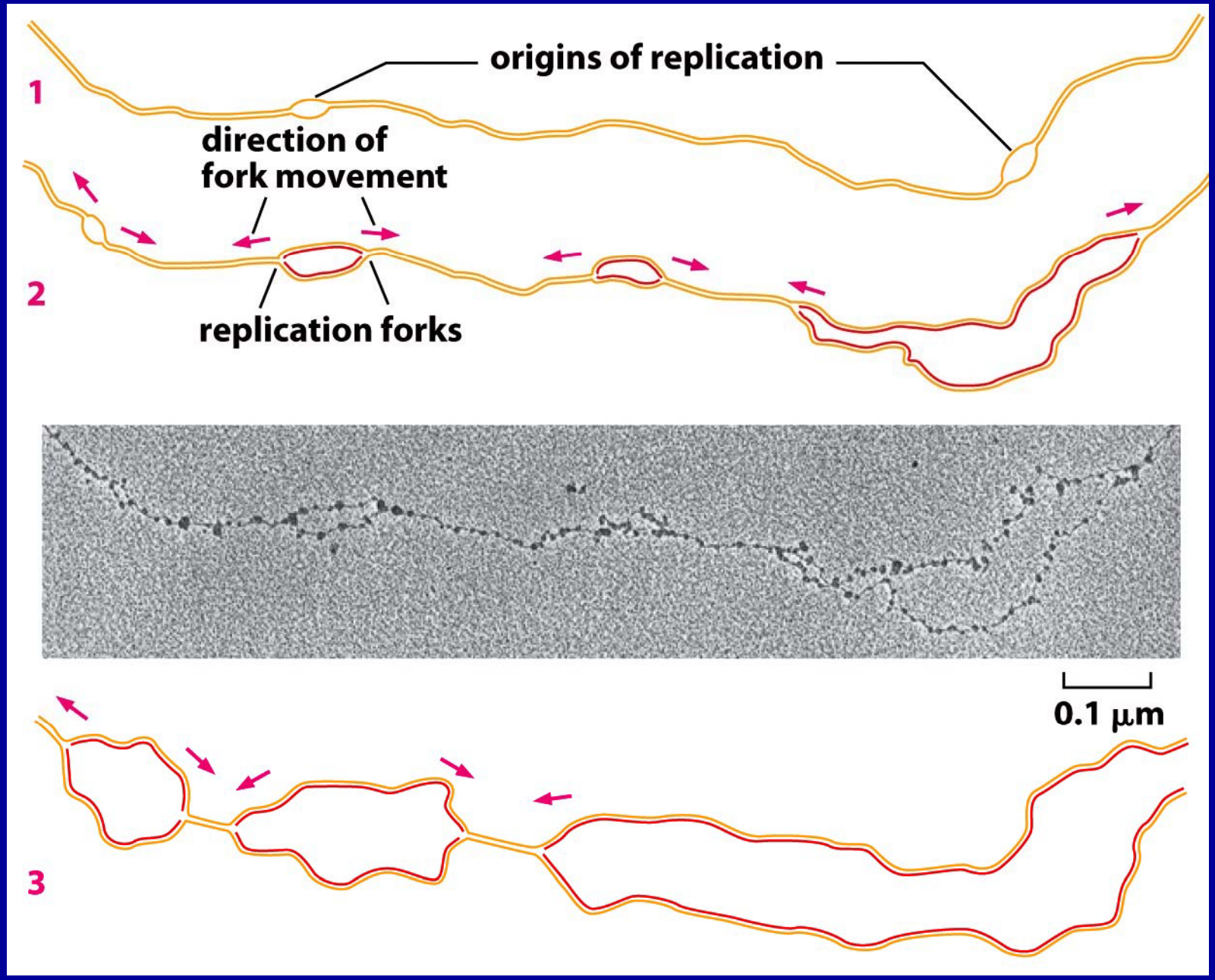

Growing strand
Template strand
5′ end
3′ end
OH
Phosphate
Sugar
Base
T
A
C
G
OH
3′ end
5′ end
dCTP
OH
3′ end
5′ end
Pyrophosphate ion
Phosphate ions
Growing strand
5′ end
3′ end
OH
OH
3′ end
5′ end

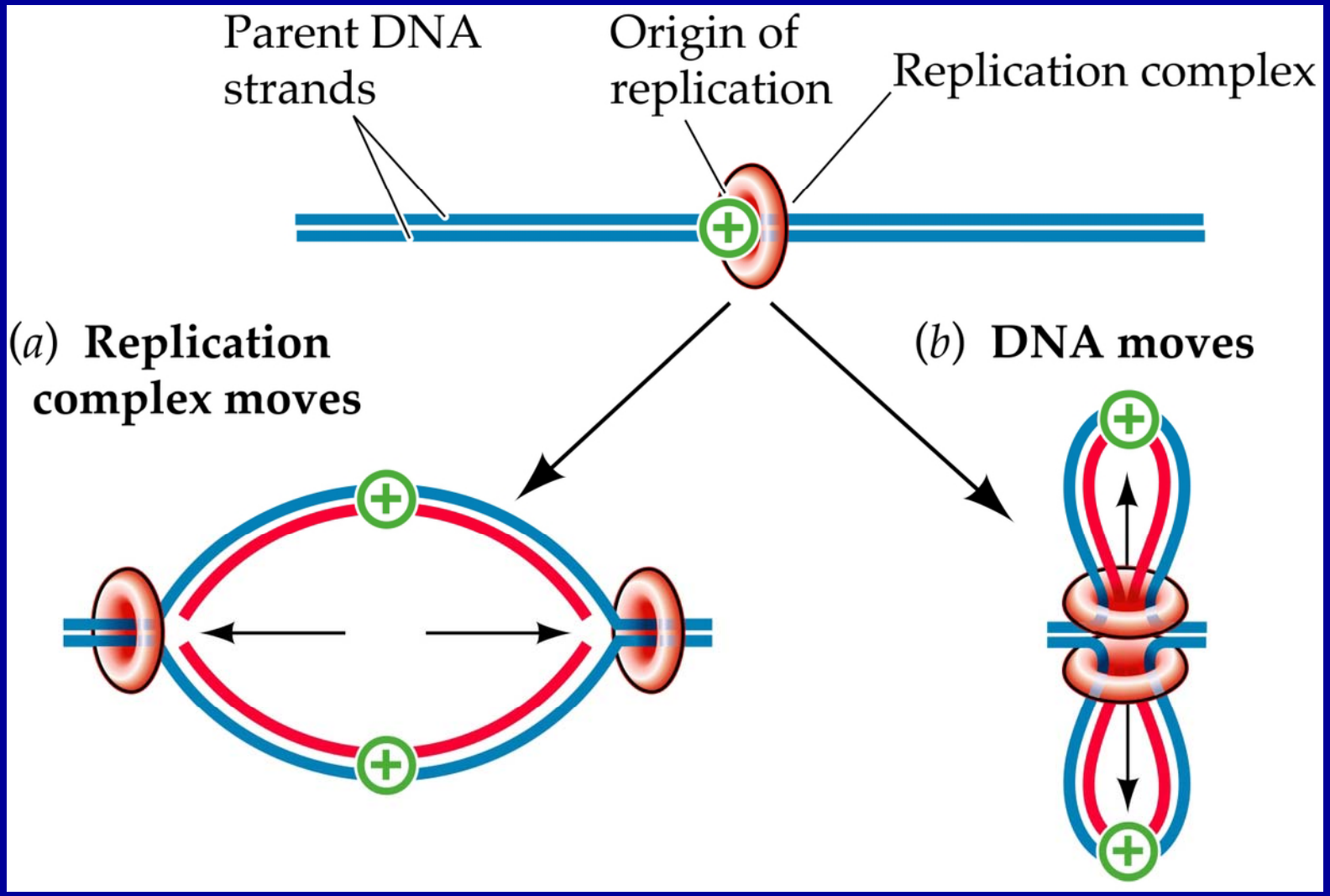


Rem: Chromatin packaging, a hierarchy of scale

D. The Mechanism of DNA Replication

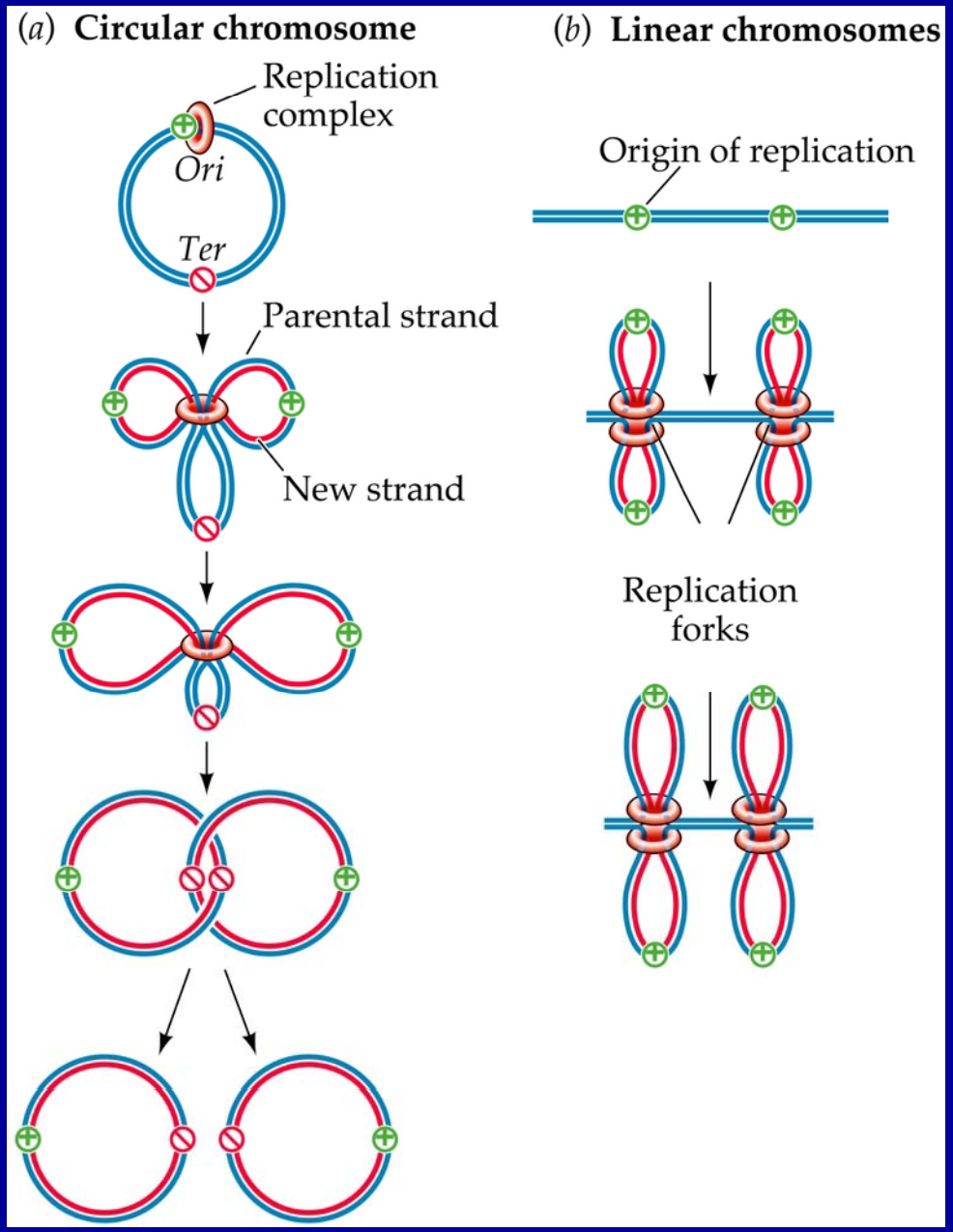
- News Flash: The DNA replication complex is in a fixed location and DNA is threaded through it for replication.
- Old idea was via moving replication forks.

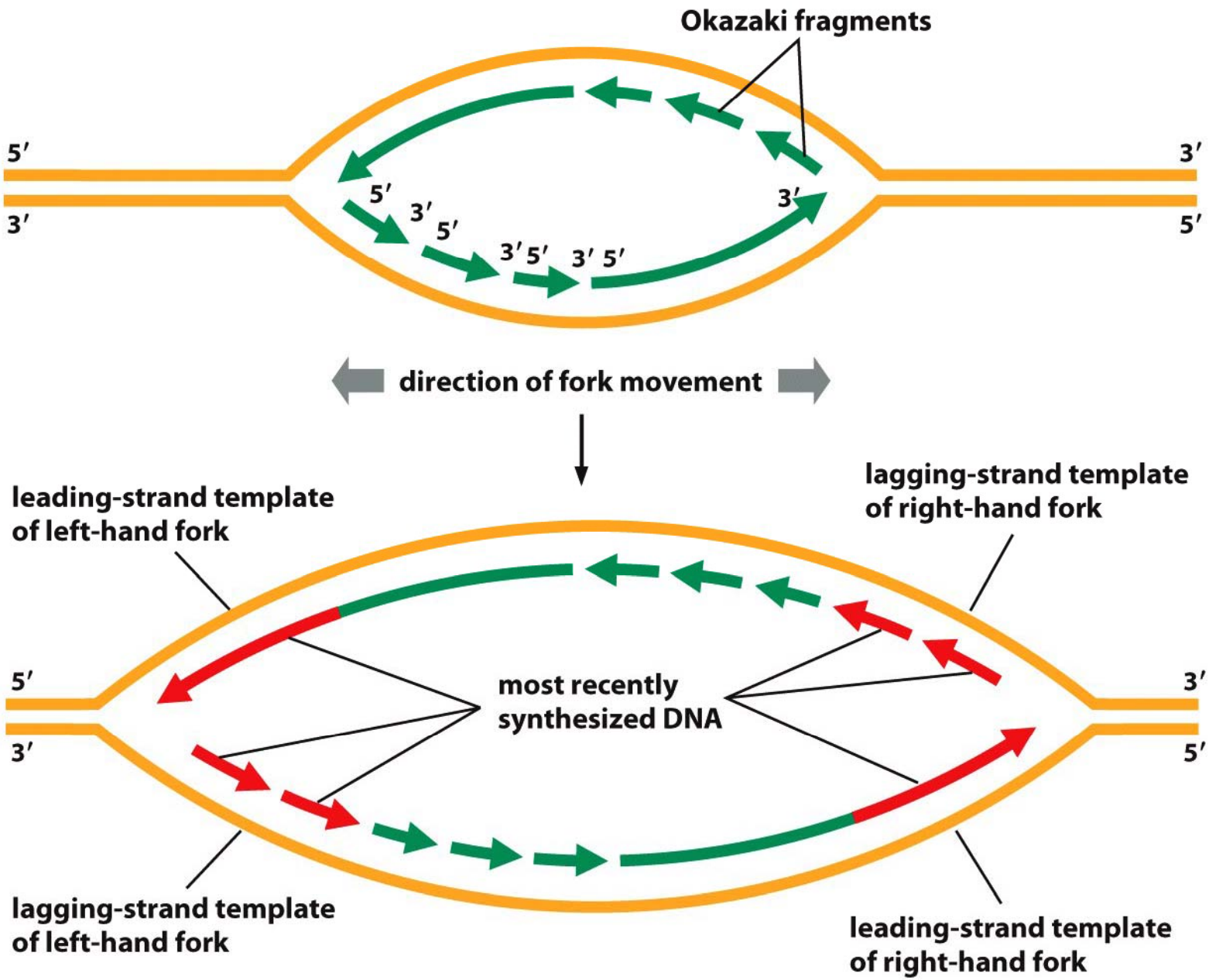

origins of replication
1
direction of
fork movement
2
replication forks
0.1 μm
3


Parent DNA strands
Origin of replication
Replication complex
(a) Replication complex moves
(b) DNA moves

D. The Mechanism of DNA Replication

- Bacteria have a single origin of replication; eucaryotes have many (10^2 to 10^3).
- Replication for each proceeds in both directions from an origin of replication.


(a) Circular chromosome
Replication complex
Ori
Ter
Parental strand
New strand
(b) Linear chromosomes
Origin of replication
Replication forks

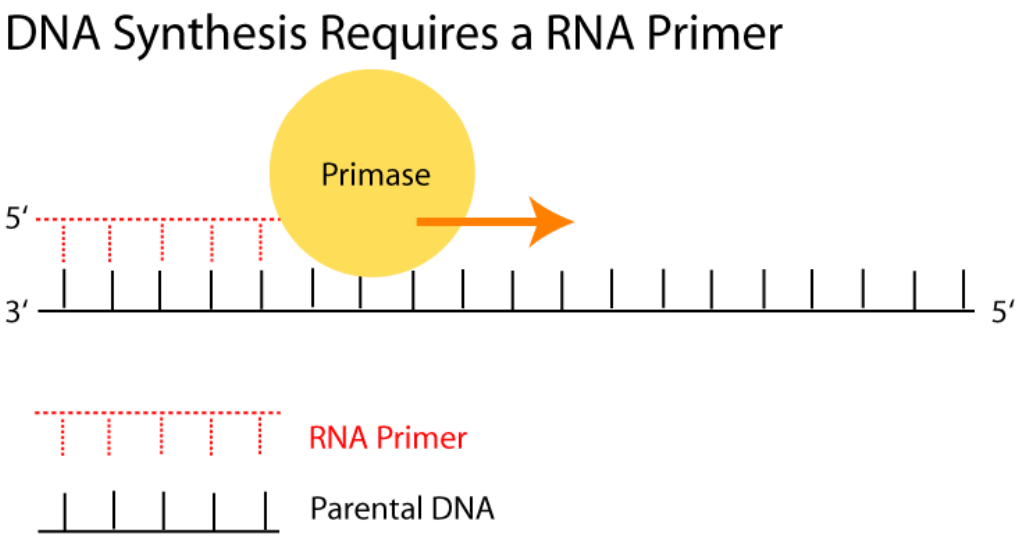

Okazaki fragments
5′
3′
3′
5′
5′
3′
3′ 5′
3′ 5′
3′
5′
direction of fork movement
leading-strand template
of left-hand fork
lagging-strand template
of right-hand fork
5′
3′
most recently
synthesized DNA
3′
5′
lagging-strand template
of left-hand fork
leading-strand template
of right-hand fork

D. The Mechanism of DNA Replication

- Many proteins assist in DNA replication. DNA helicases unwind the double helix, the template strands are stabilized by single-stranded binding proteins.

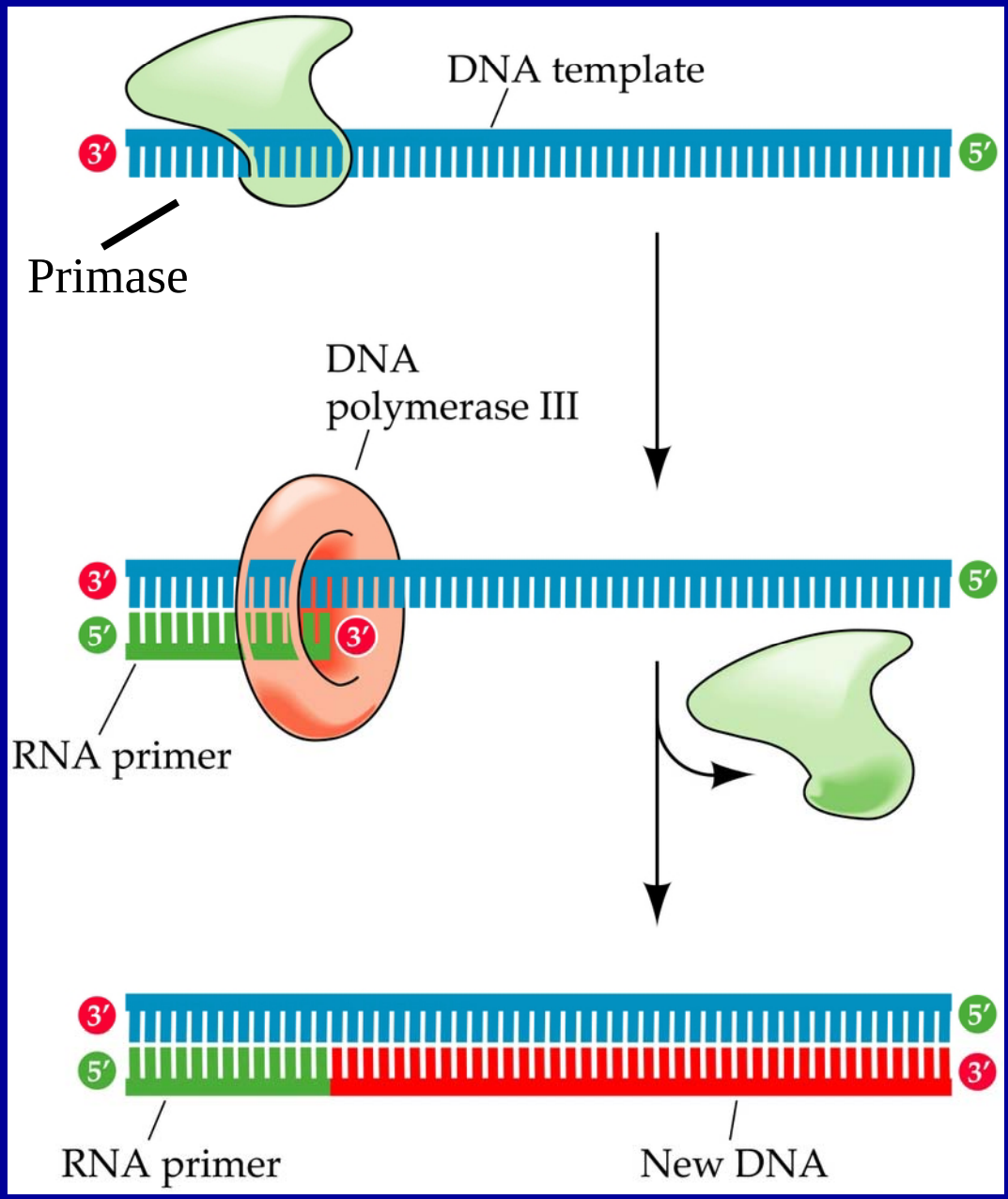
- An RNA primase catalyzes the synthesis of short RNA primers, and to which nucleotides are added.

Enzymes for DNA replication: Primase



- Primase: provides a short, complementary strand of RNA that is required for DNA synthesis from a naked DNA template.
- There is no known DNA polymerase that can initiate synthesis of a DNA strand – they can only add nucleotides to a pre-existing strand.

No DNA forms without an RNA Primer



D. The Mechanism of DNA Replication

- DNA polymerase III action causes the leading strand to grow in the 5'-to-3' direction until replication of that section of DNA is complete.

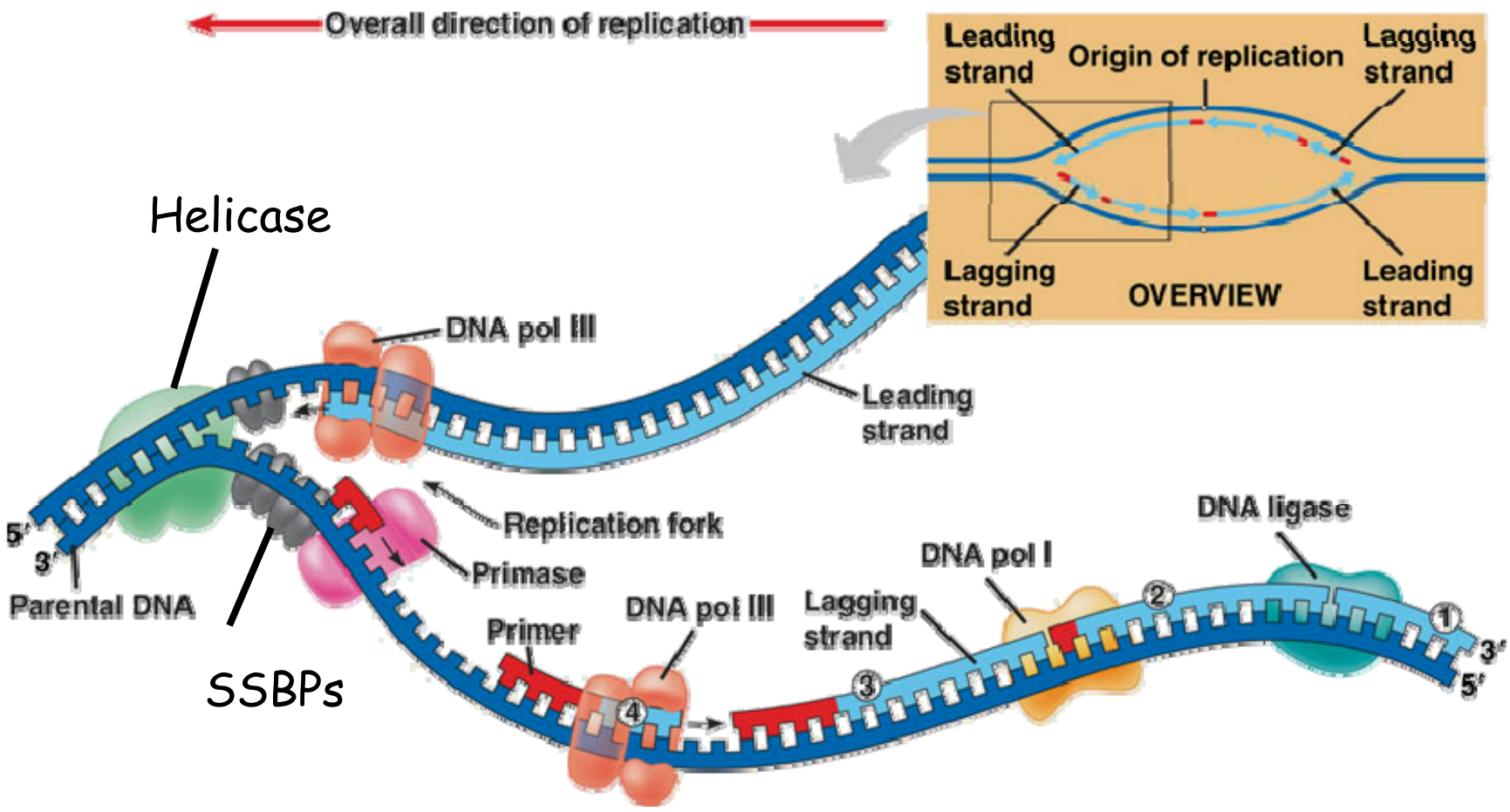
- RNA primer is degraded and DNA is replaced by DNA polymerase I.

D. The Mechanism of DNA Replication

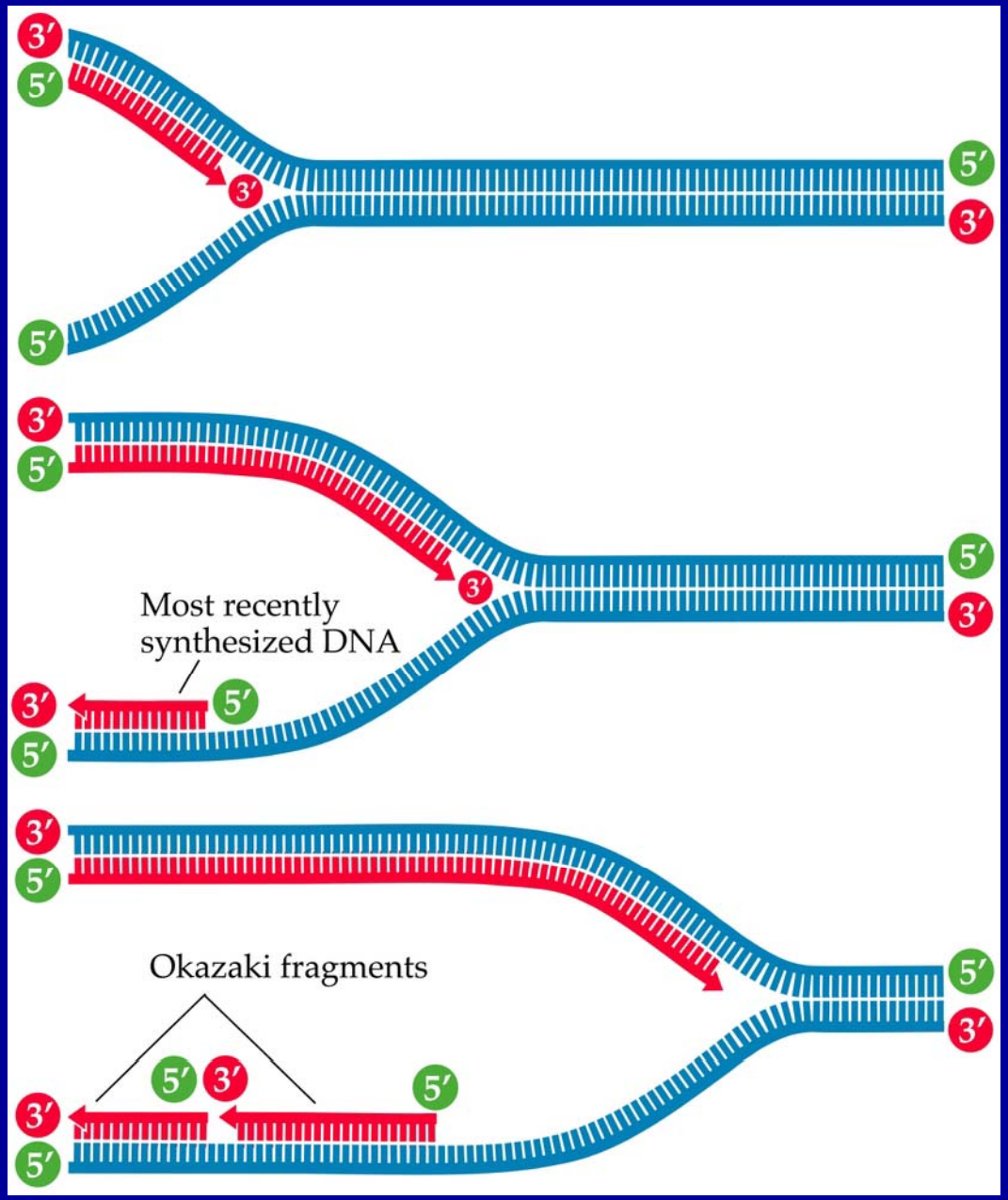
- On the lagging strand, growing in the other direction, DNA is made in the 5′-to-3′ direction but synthesis is discontinuous: DNA is added as short Okazaki fragments to primers, then DNA polymerase III skips past the 5′ end to make the next fragment.

- DNA polymerase I and Ligase are required to make lagging strand "continuous".

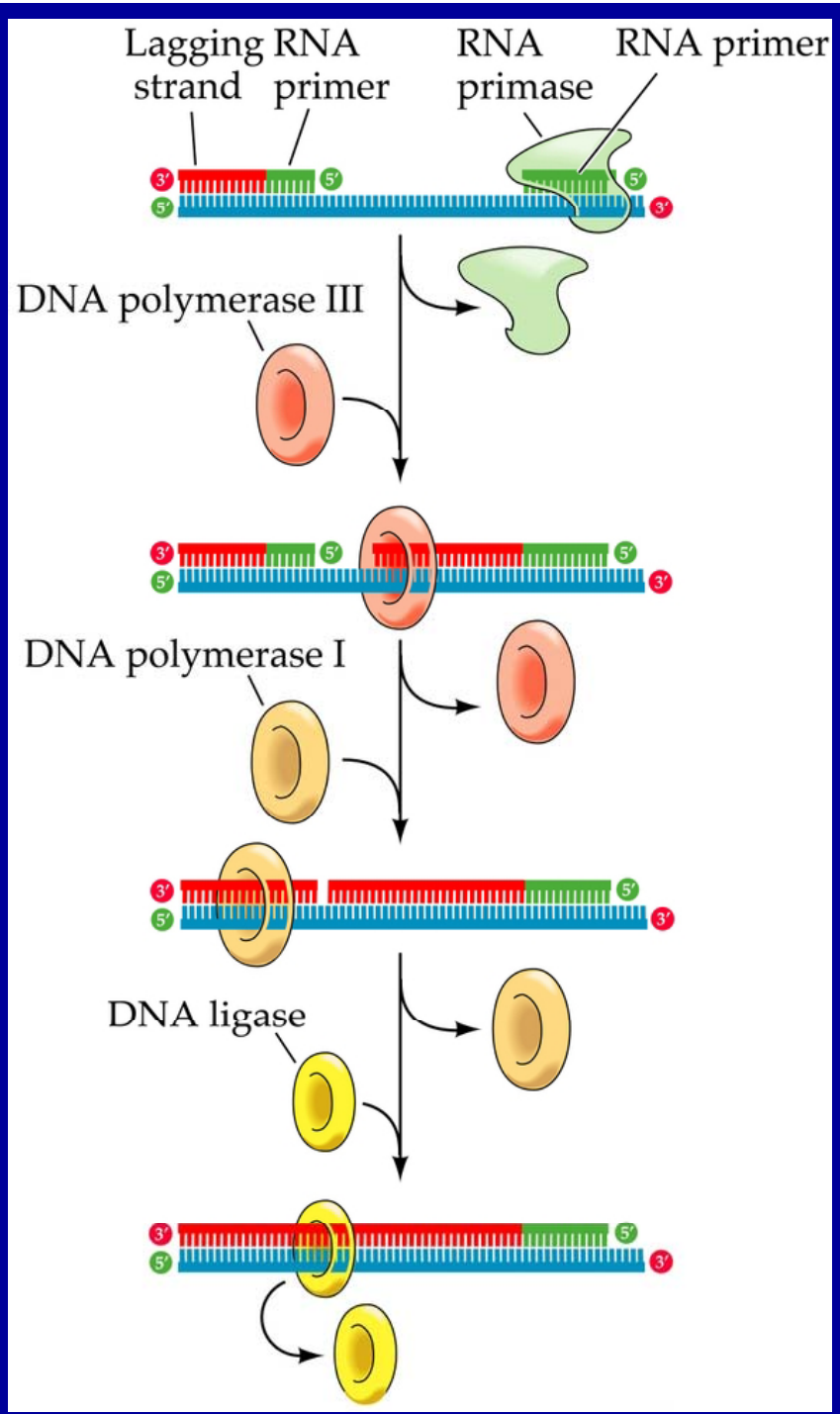
Many Proteins Collaborate at the Replication Fork



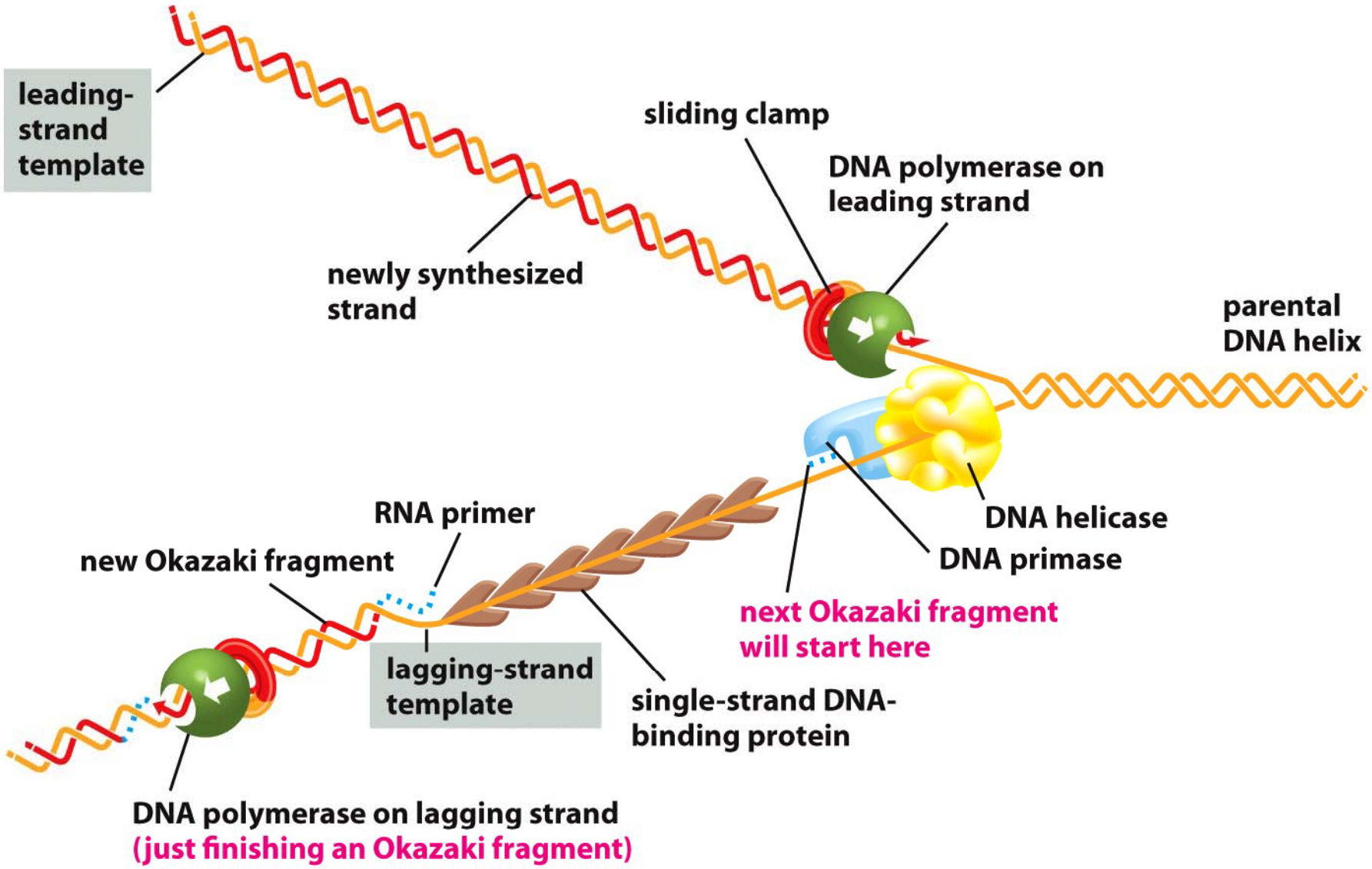
Two Daughter Stands form Different Ways

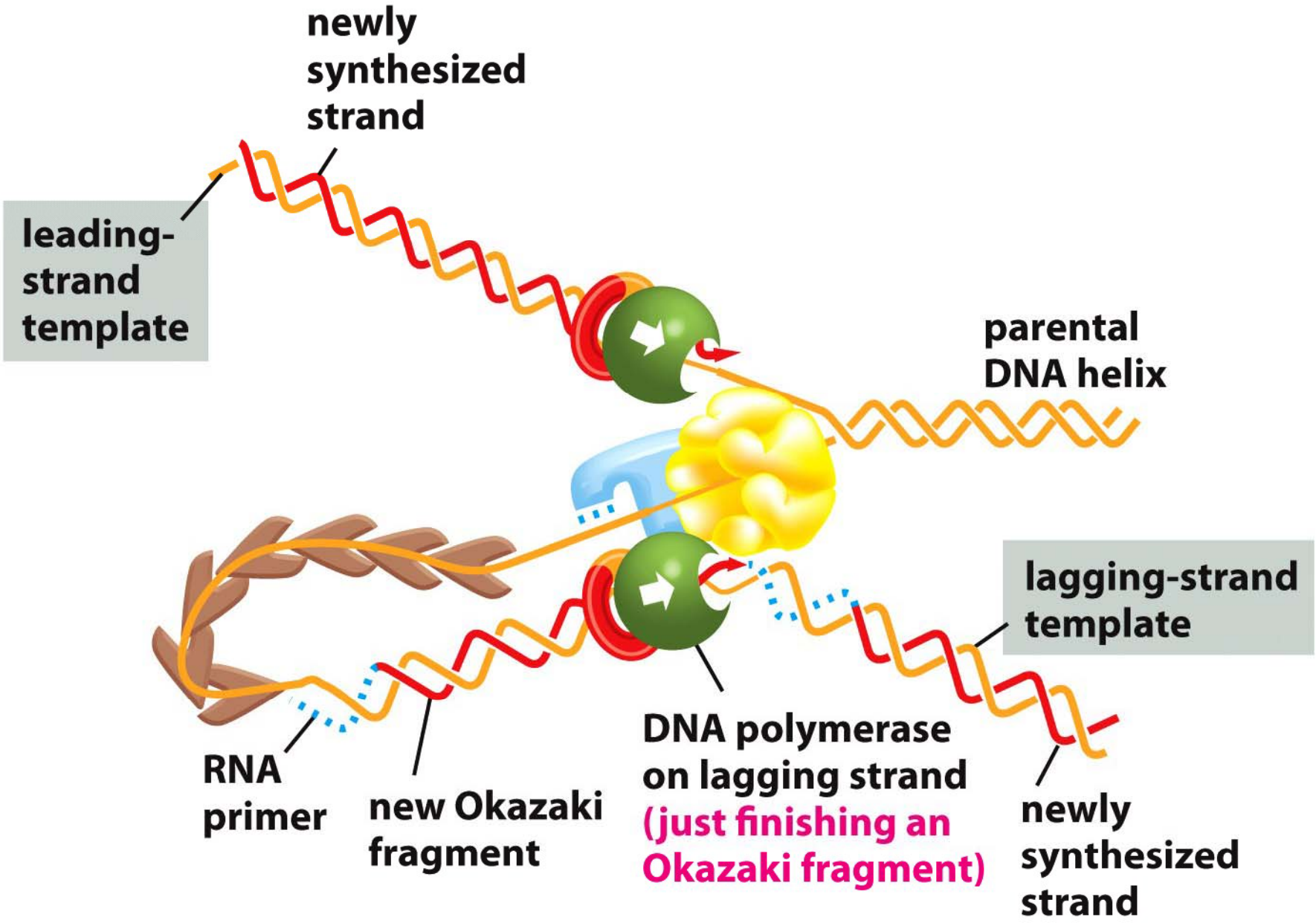


Continuous vs. Discontinuous!



Finishing touches on the discontinuous or lagging strand


leading-strand template
newly synthesized strand
sliding clamp
DNA polymerase on leading strand
parental DNA helix
DNA helicase
DNA primase
RNA primer
new Okazaki fragment
lagging-strand template
single-strand DNA-binding protein
next Okazaki fragment will start here
DNA polymerase on lagging strand
(just finishing an Okazaki fragment)

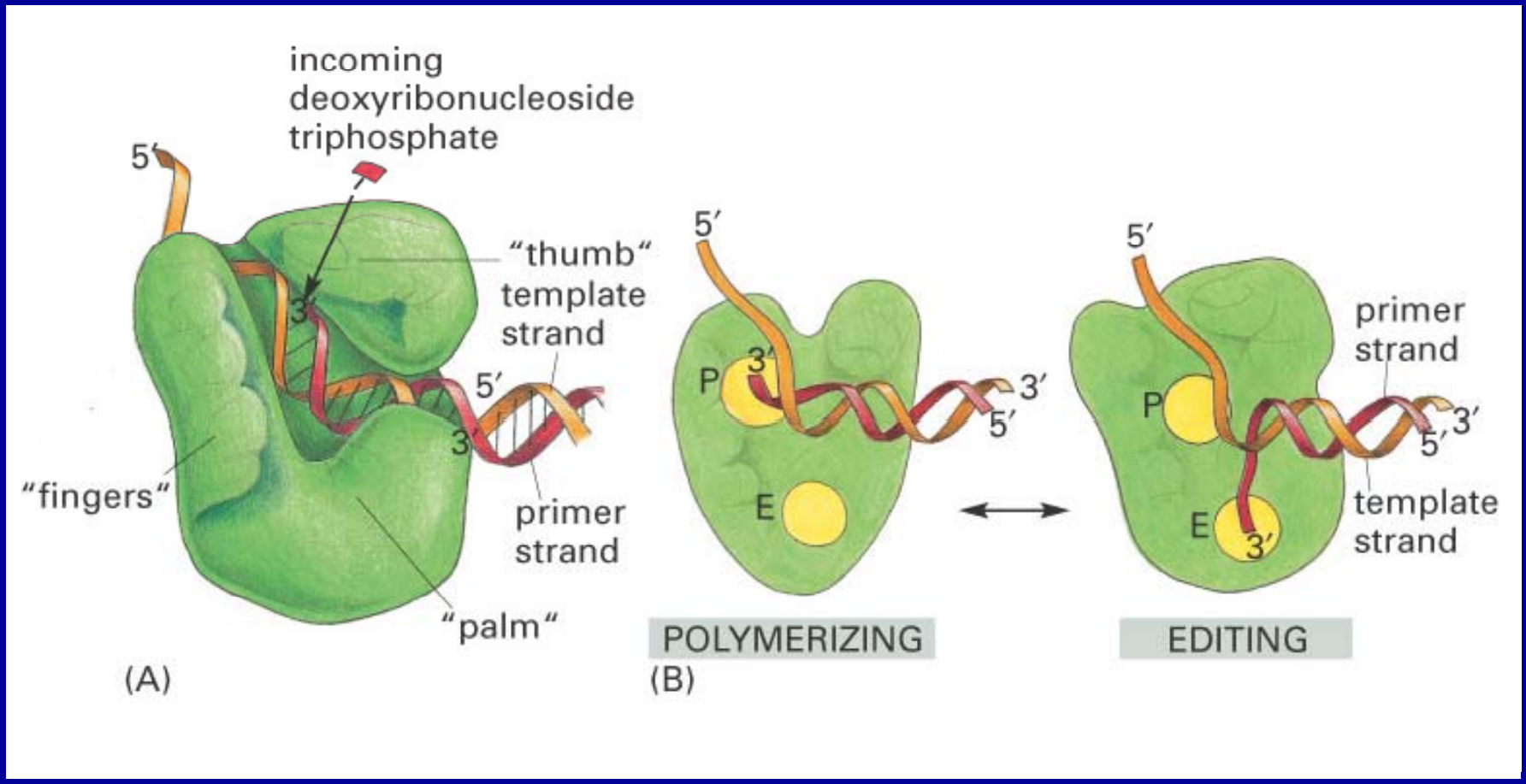

newly synthesized strand
leading-strand template
parental DNA helix
lagging-strand template
RNA primer
new Okazaki fragment
DNA polymerase on lagging strand (just finishing an Okazaki fragment)
newly synthesized strand

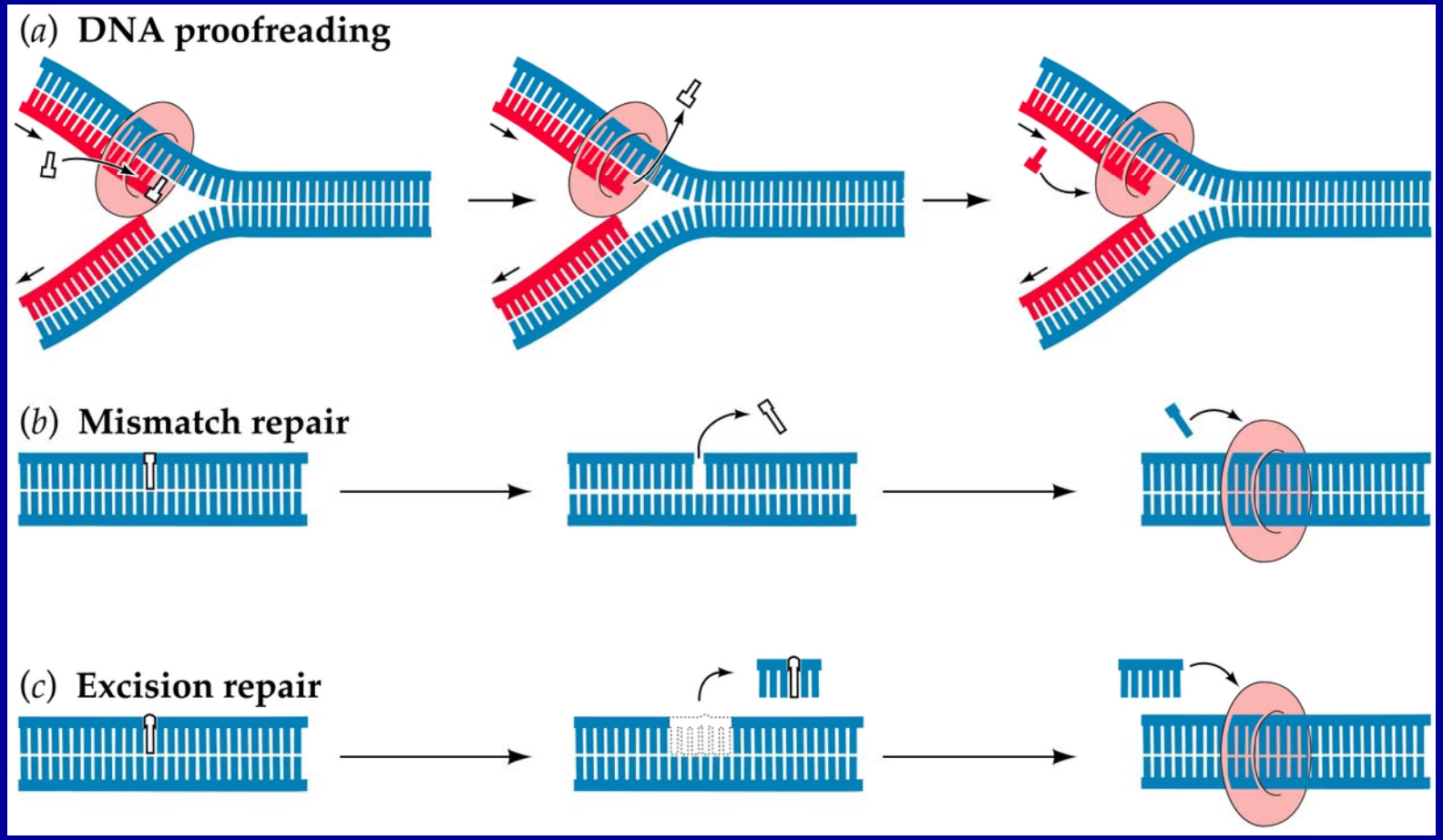
E. DNA Proofreading and Repair

- There is about about one error in 10^7 nucleotides bases added in DNA replication, repaired by: proofreading, mismatch repair, and excision repair.
- DNA repair mechanisms lower the error rate to about one base in 10^9.

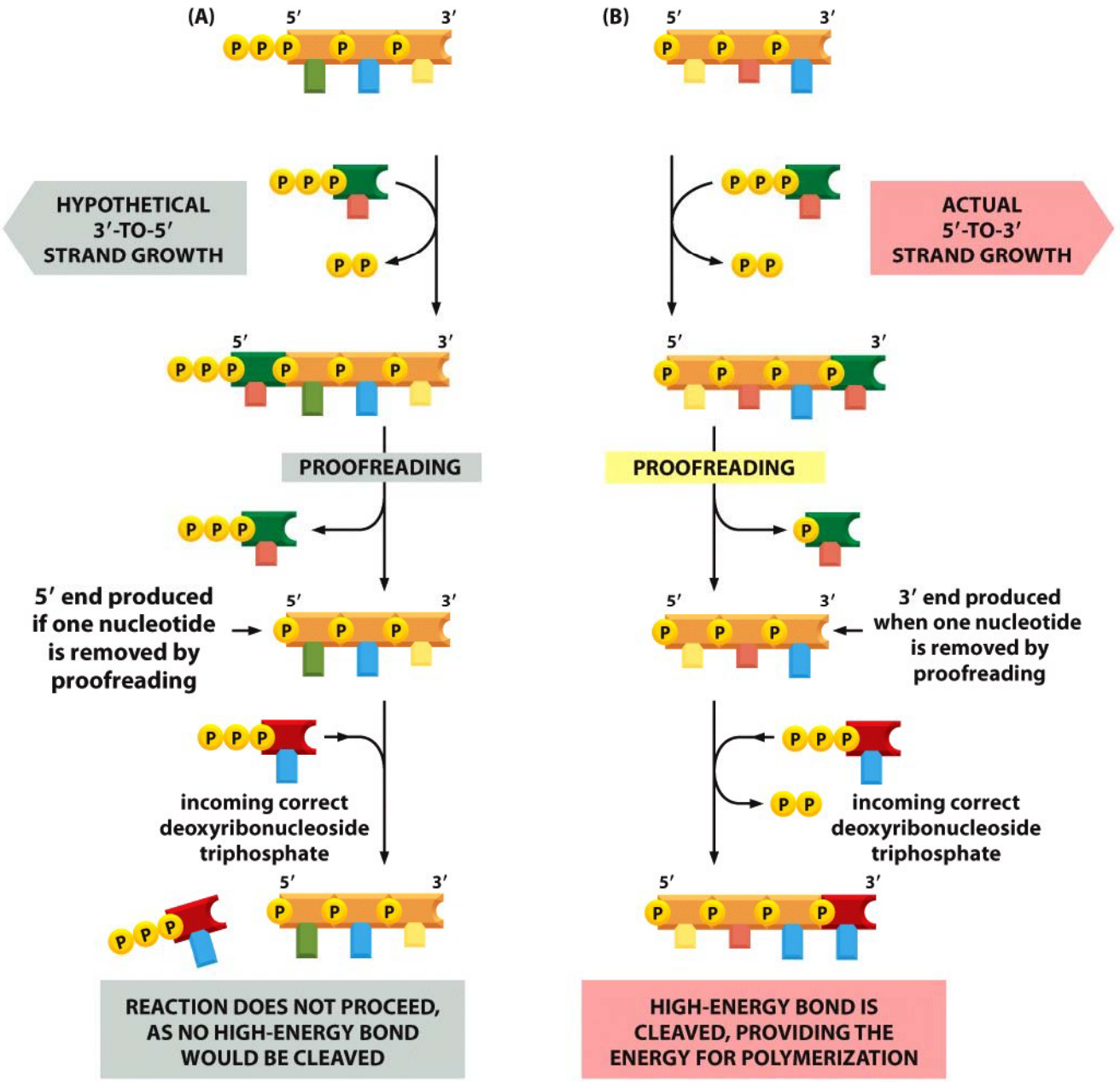
TABLE 6–1 ERROR RATES	
US Postal Service on-time delivery of local first-class mail	13 late deliveries per 100 parcels
Airline luggage system	1 lost bag per 200
A professional typist typing at 120 words per minute	1 mistake per 250 characters
Driving a car in the United States	1 death per 10^4 people per year
DNA replication (without mismatch repair)	1 mistake per 10^7 nucleotides copied
DNA replication (including mismatch repair)	1 mistake per 10^9 nucleotides copied

Proofreading: 3′ to 5′



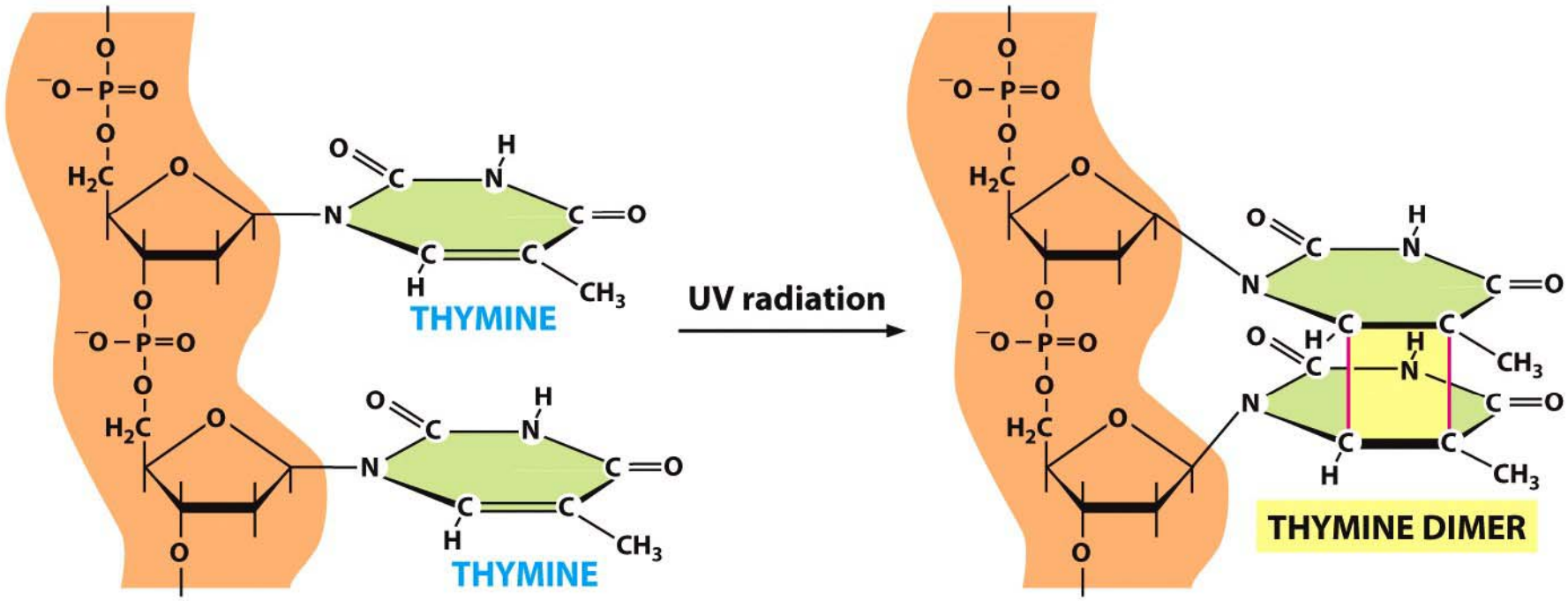

(a) DNA proofreading
(b) Mismatch repair
(c) Excision repair

Proof-reading by the DNA polymerase: 5′ to 3′…always!

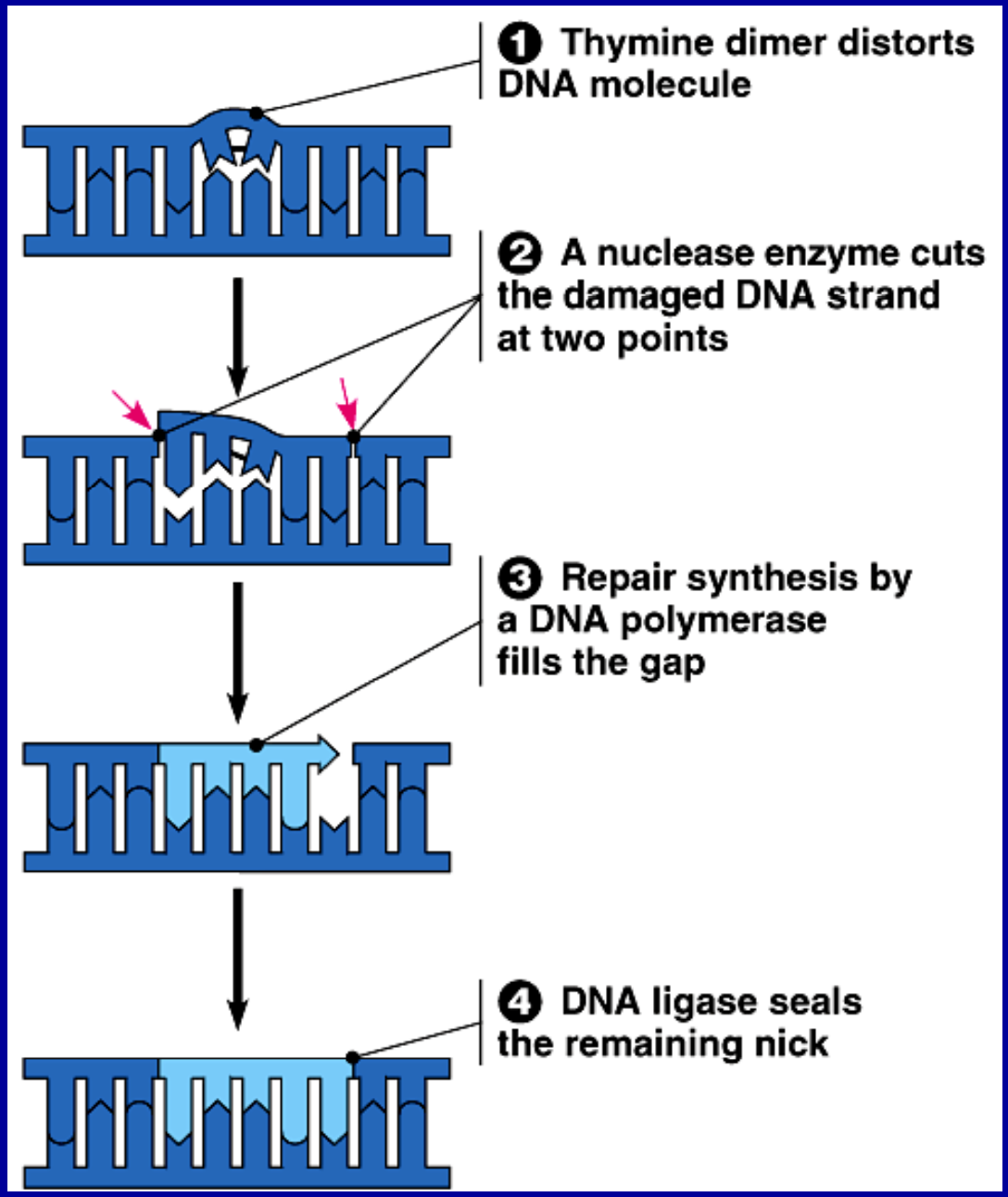


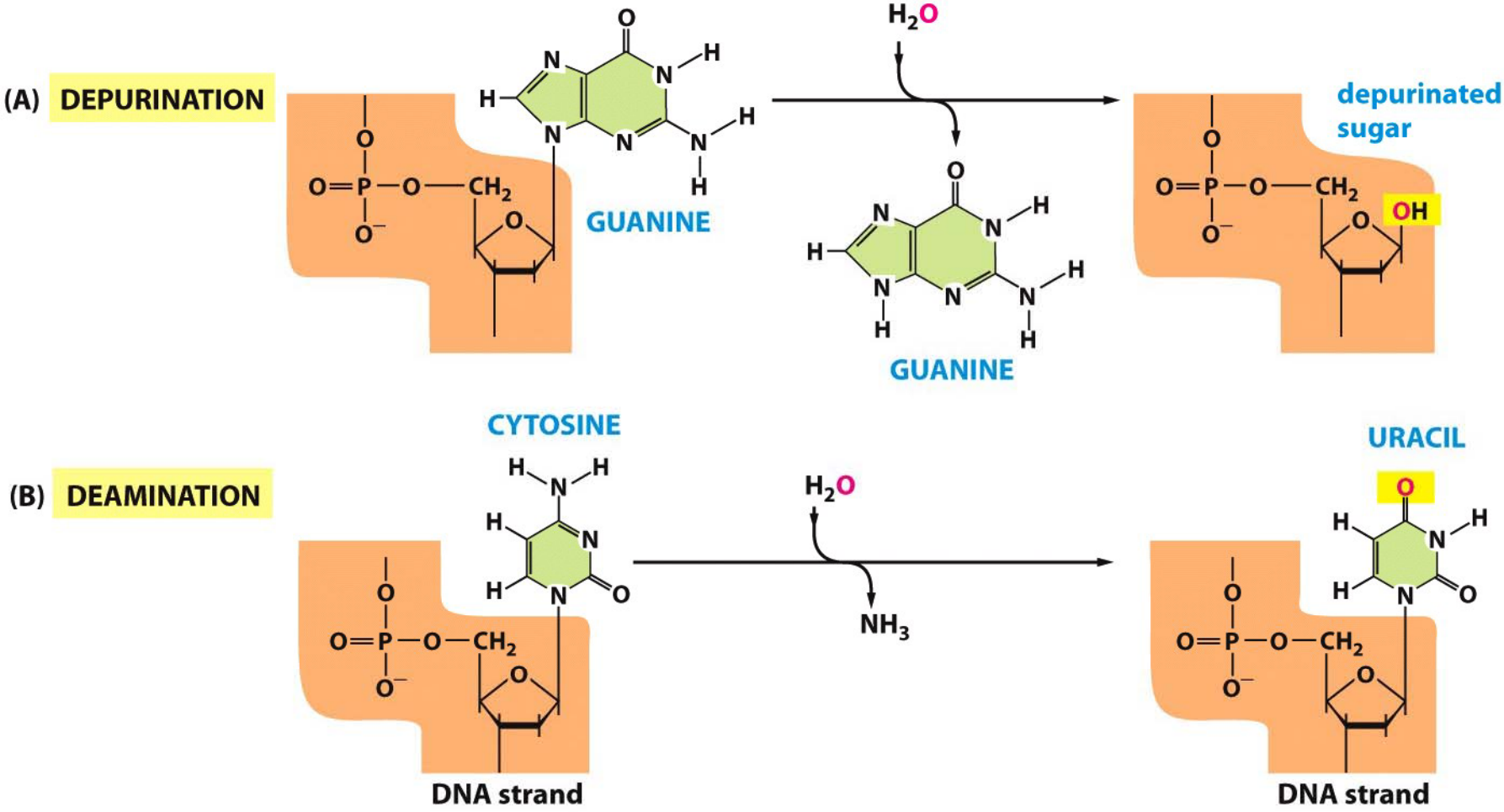
E. DNA Proofreading and Repair

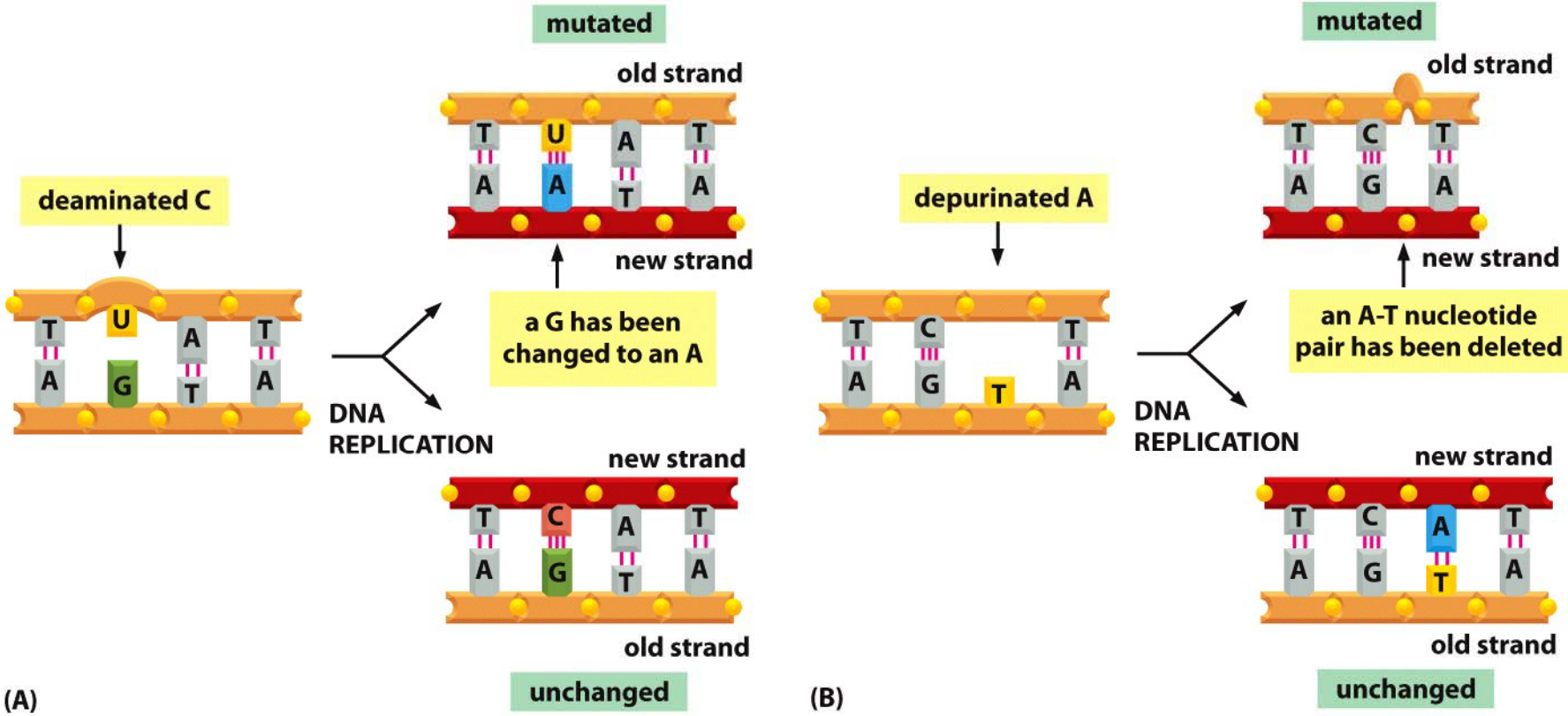
- Although energetically costly and somewhat redundant, DNA repair is crucial to the survival of the cell.


THYMINE
THYMINE
UV radiation
THYMINE DIMER

Nucleotide excision repair of DNA damage



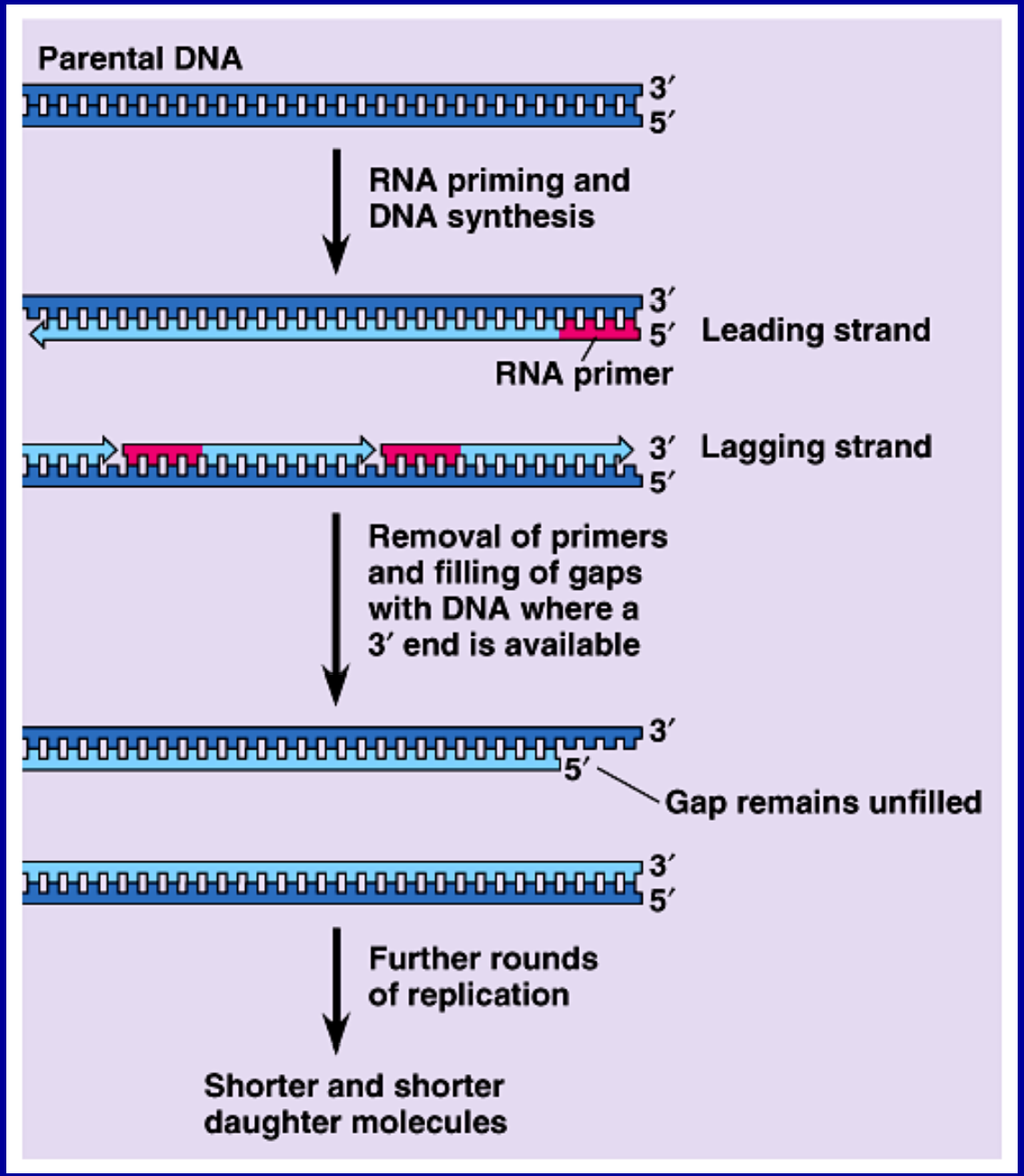

(A) DEPURINATION
GUANINE
H_2O
GUANINE
depurinated sugar
OH
CYTOSINE
(B) DEAMINATION
H_2O
NH_3
URACIL
DNA strand
DNA strand


mutated
old strand
deaminated C
new strand
a G has been changed to an A
DNA REPLICATION
new strand
old strand
unchanged
(A)
mutated
old strand
depurinated A
new strand
an A-T nucleotide pair has been deleted
DNA REPLICATION
new strand
old strand
unchanged
(B)

E. DNA Proofreading and Repair

- Some moderately repetitive DNA sequences, such as telomeric DNA is found at the ends of chromosomes. Some may be lost during each DNA replication, leading to chromosome instability and cell death.
- Telomerase catalyzes the restoration of lost telomeric DNA.
- Most somatic cells lack telomerase and thus have limited life spans.

The linear end-replication problem

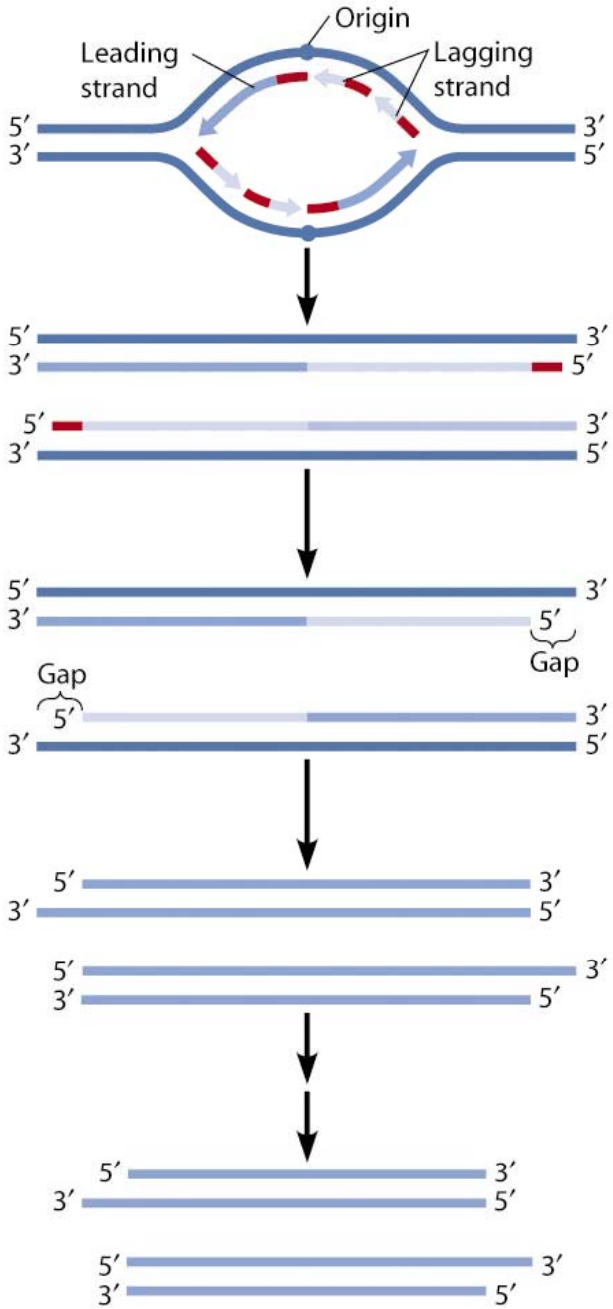


1. DNA replication is initiated at the origin; the replication bubble grows as the two replication forks move in opposite directions.

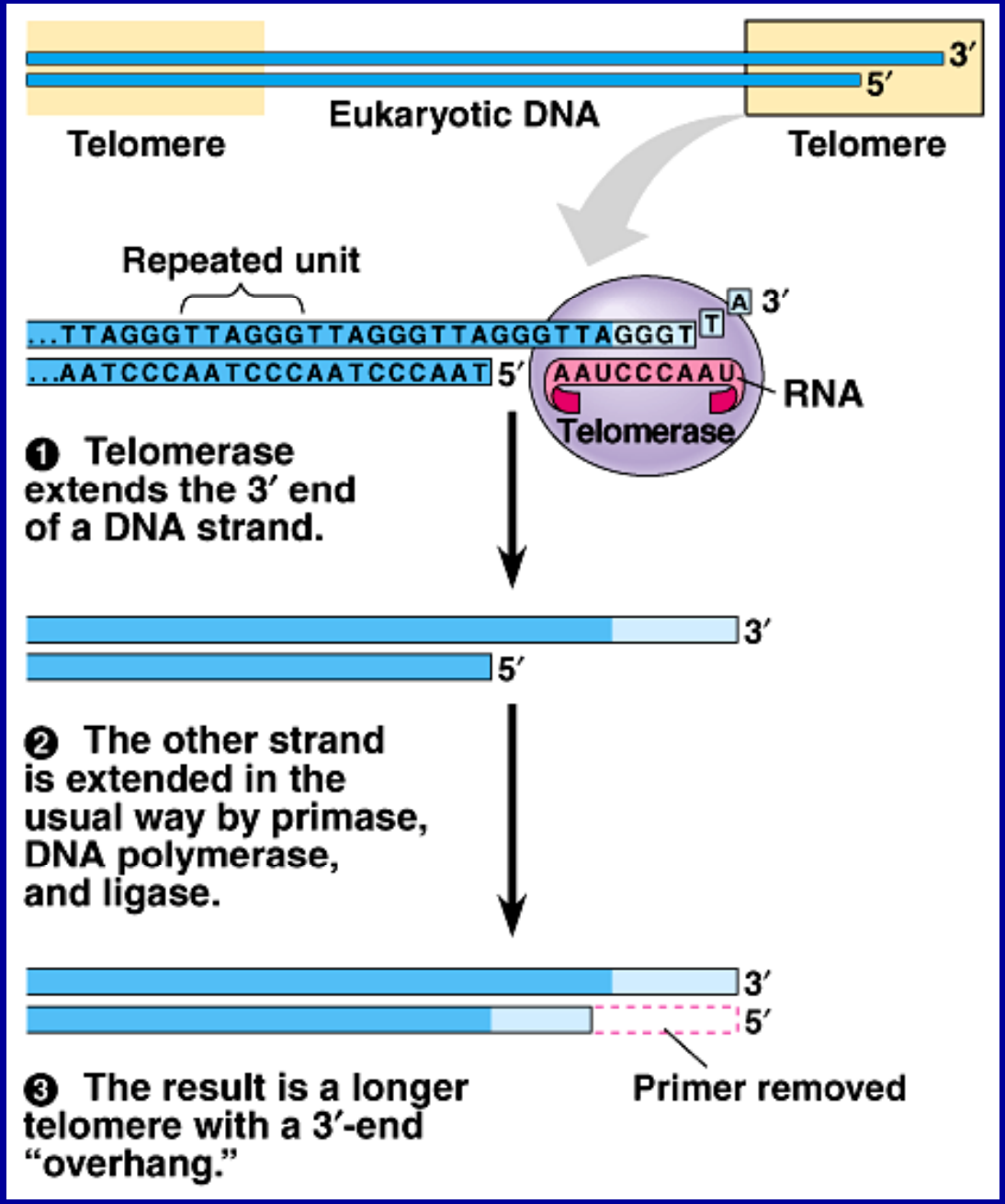
2. Finally only one primer (red) remains on each daughter DNA molecule.

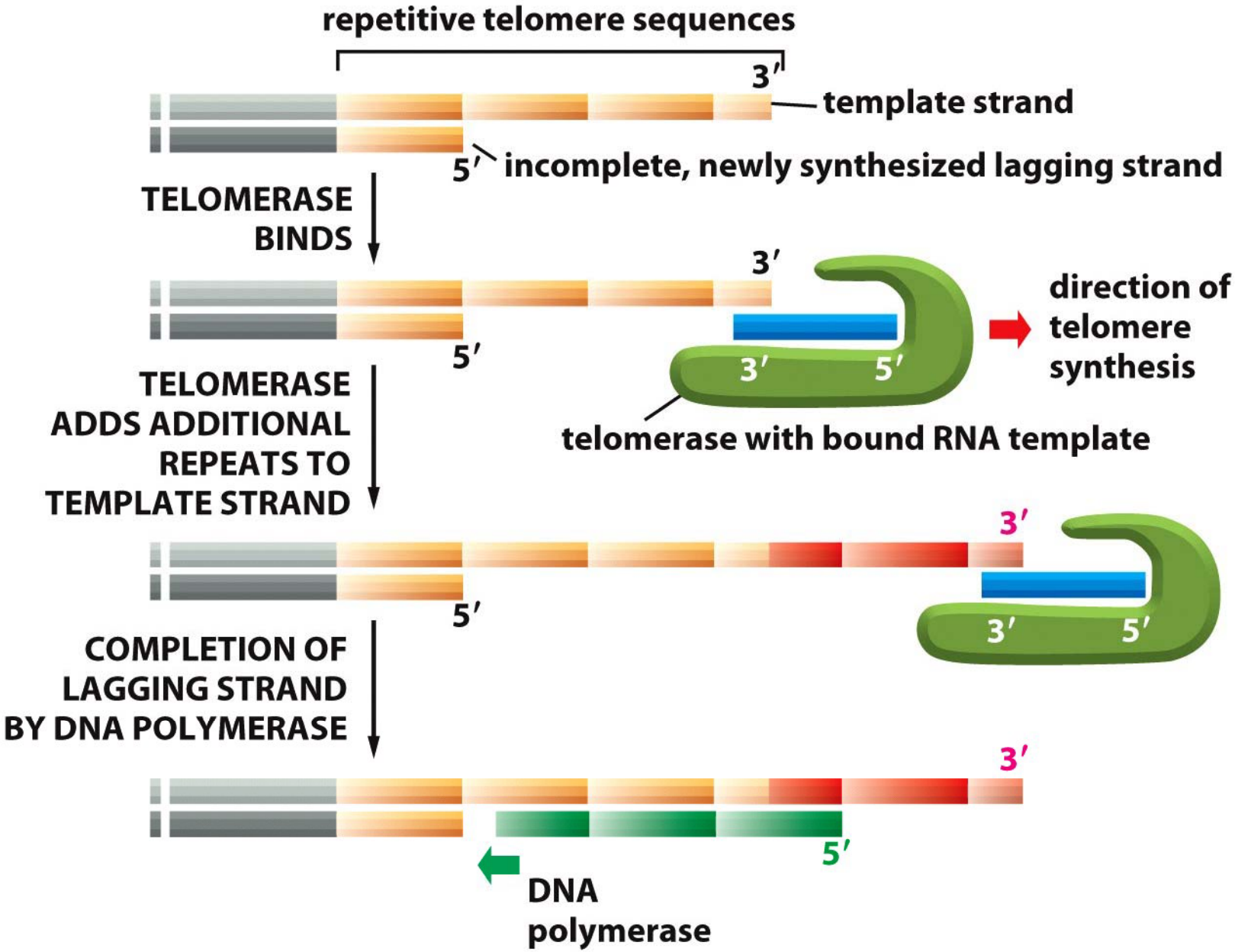
3. The last primers are removed by a 5′→3′ exonuclease, but no DNA polymerase can fill the resulting gaps because there is no 3′ OH available to which a nucleotide can be added.

4. Each round of replication generates shorter and shorter DNA molecules.

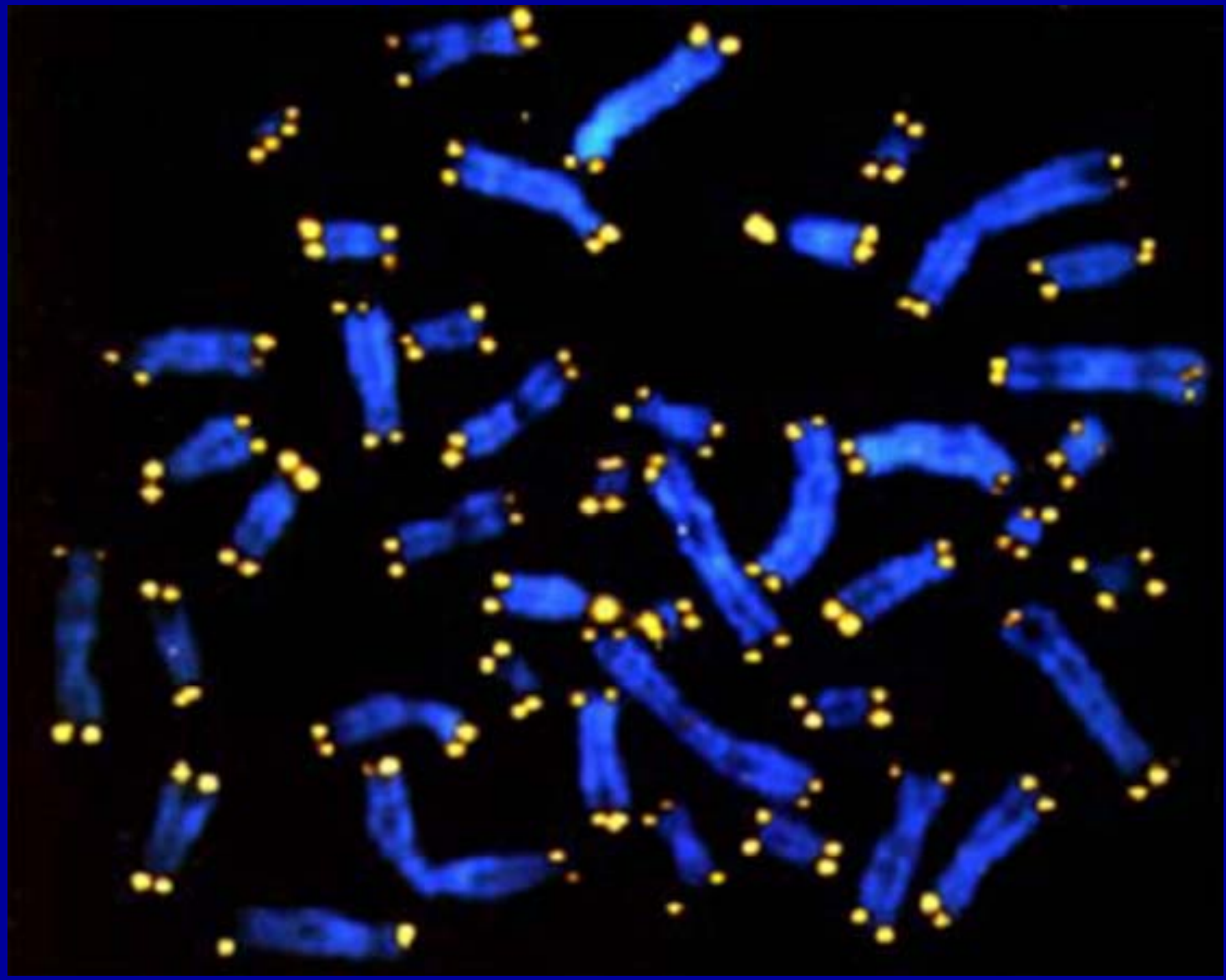


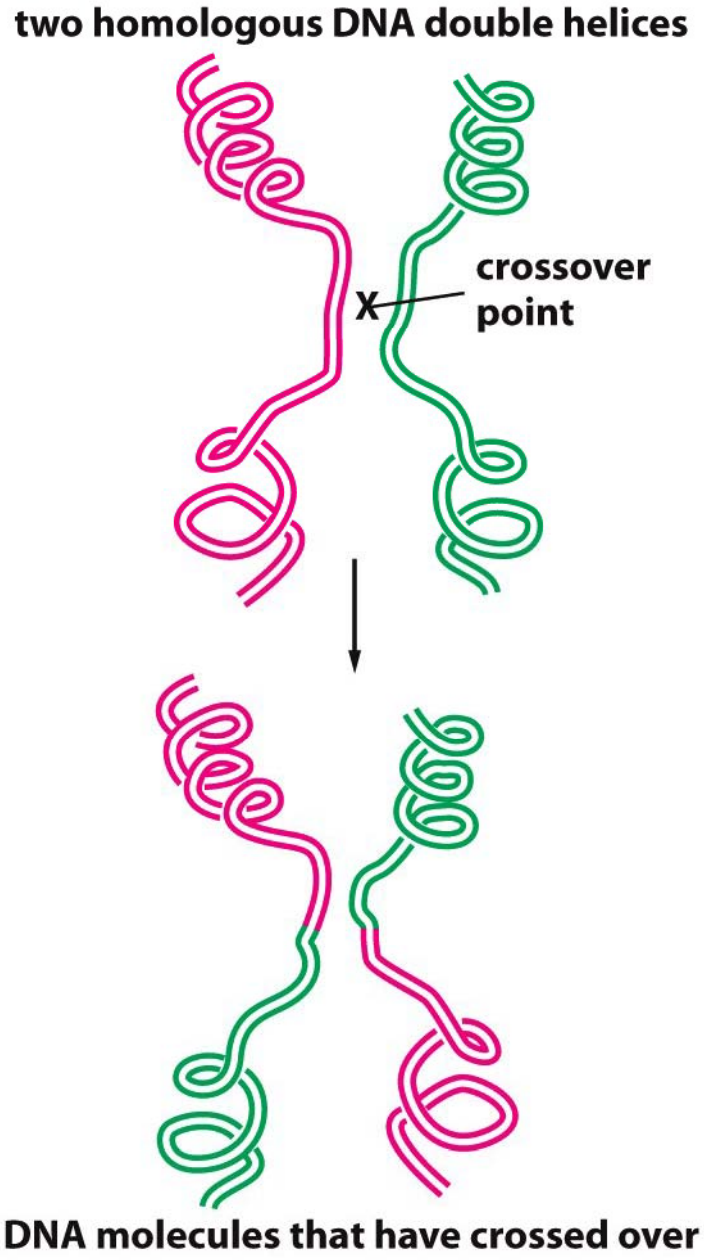
Telomeres and telomerase



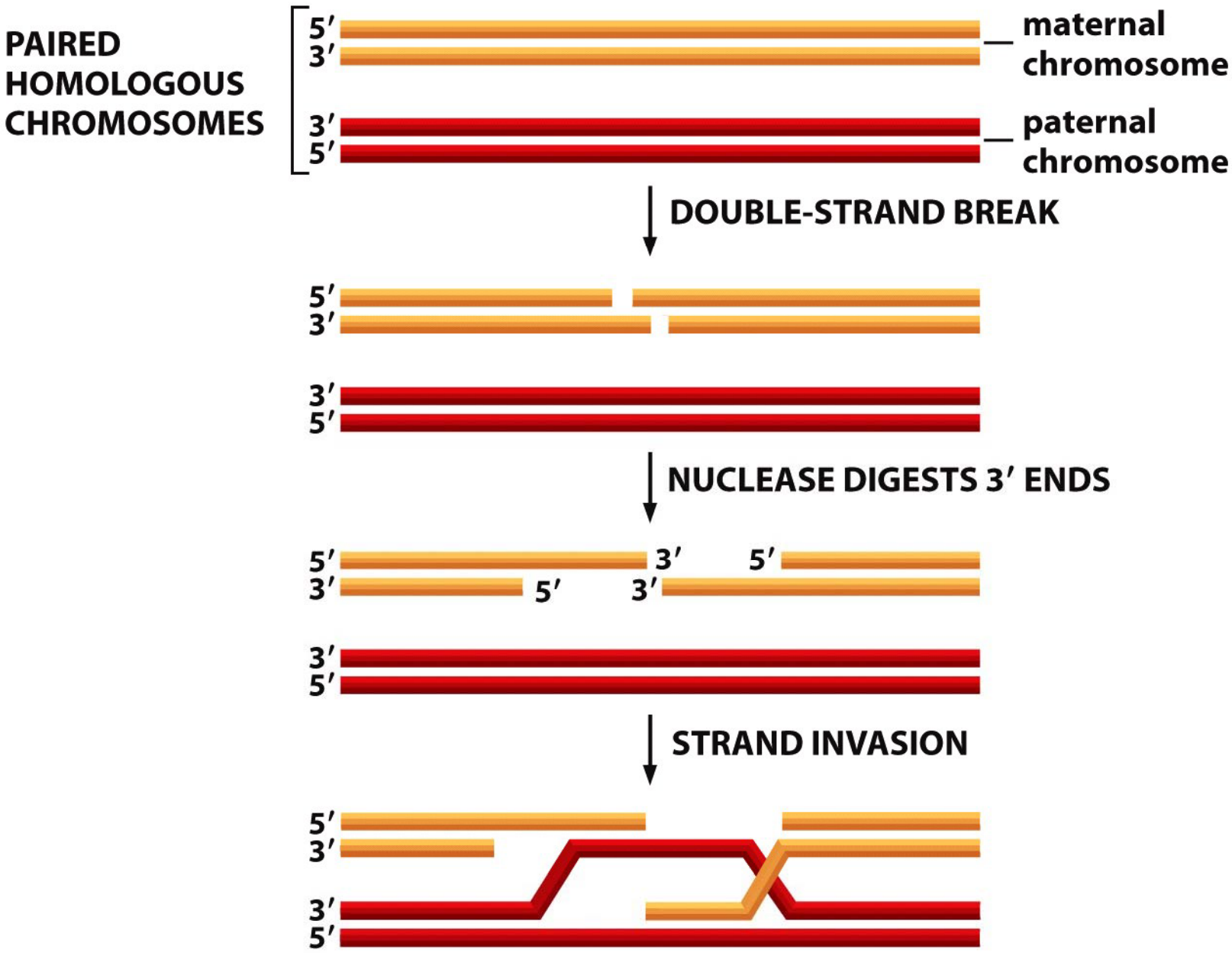

repetitive telomere sequences
3′
template strand
5′
incomplete, newly synthesized lagging strand
TELOMERASE BINDS
3′
5′
3′
5′
direction of telomere synthesis
telomerase with bound RNA template
TELOMERASE ADDS ADDITIONAL REPEATS TO TEMPLATE STRAND
3′
5′
3′
5′
COMPLETION OF LAGGING STRAND BY DNA POLYMERASE
3′
5′
DNA polymerase

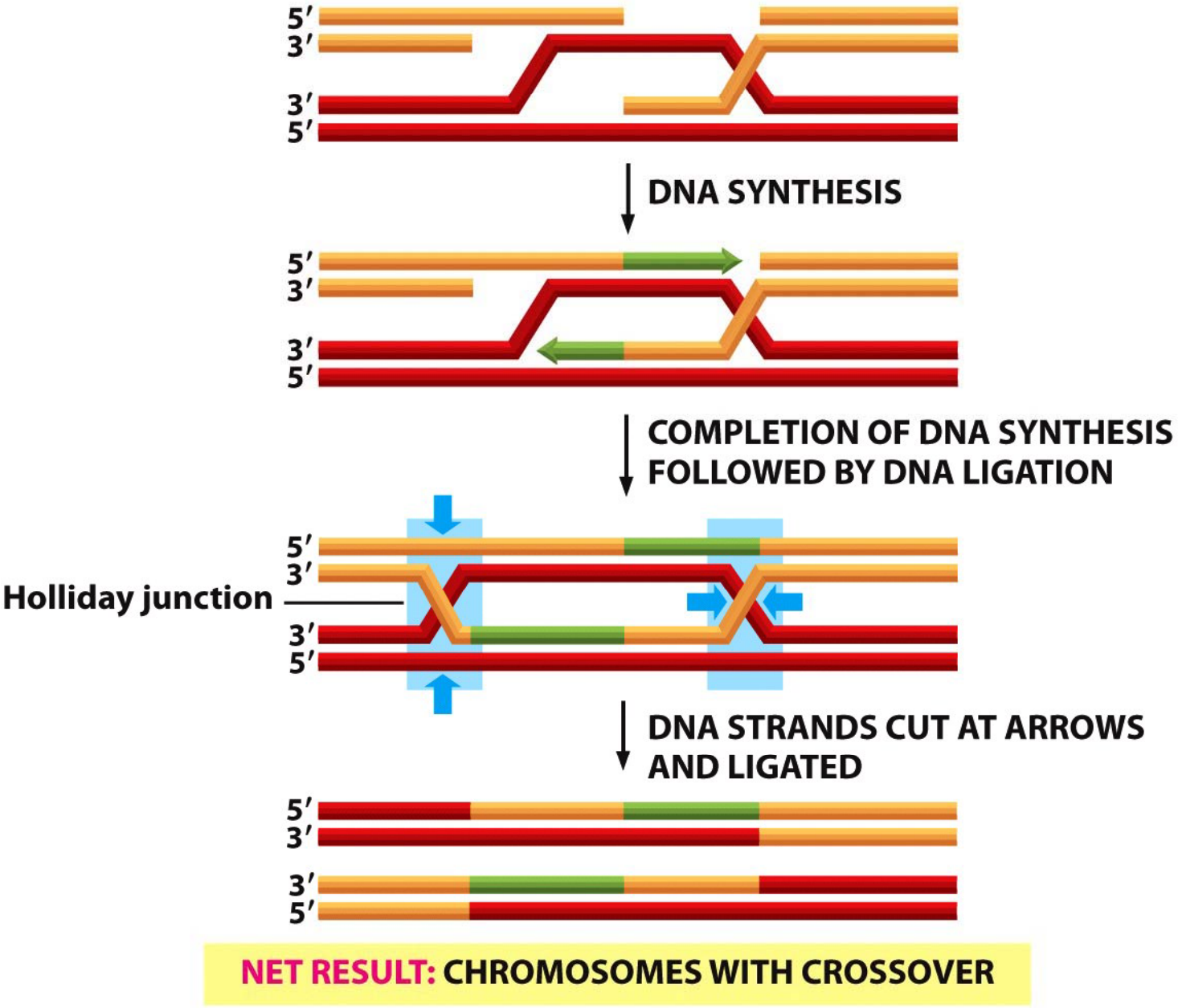
Telomeres (aka telomeric DNA)





Rem:
"Crossing Over"
in meiosis

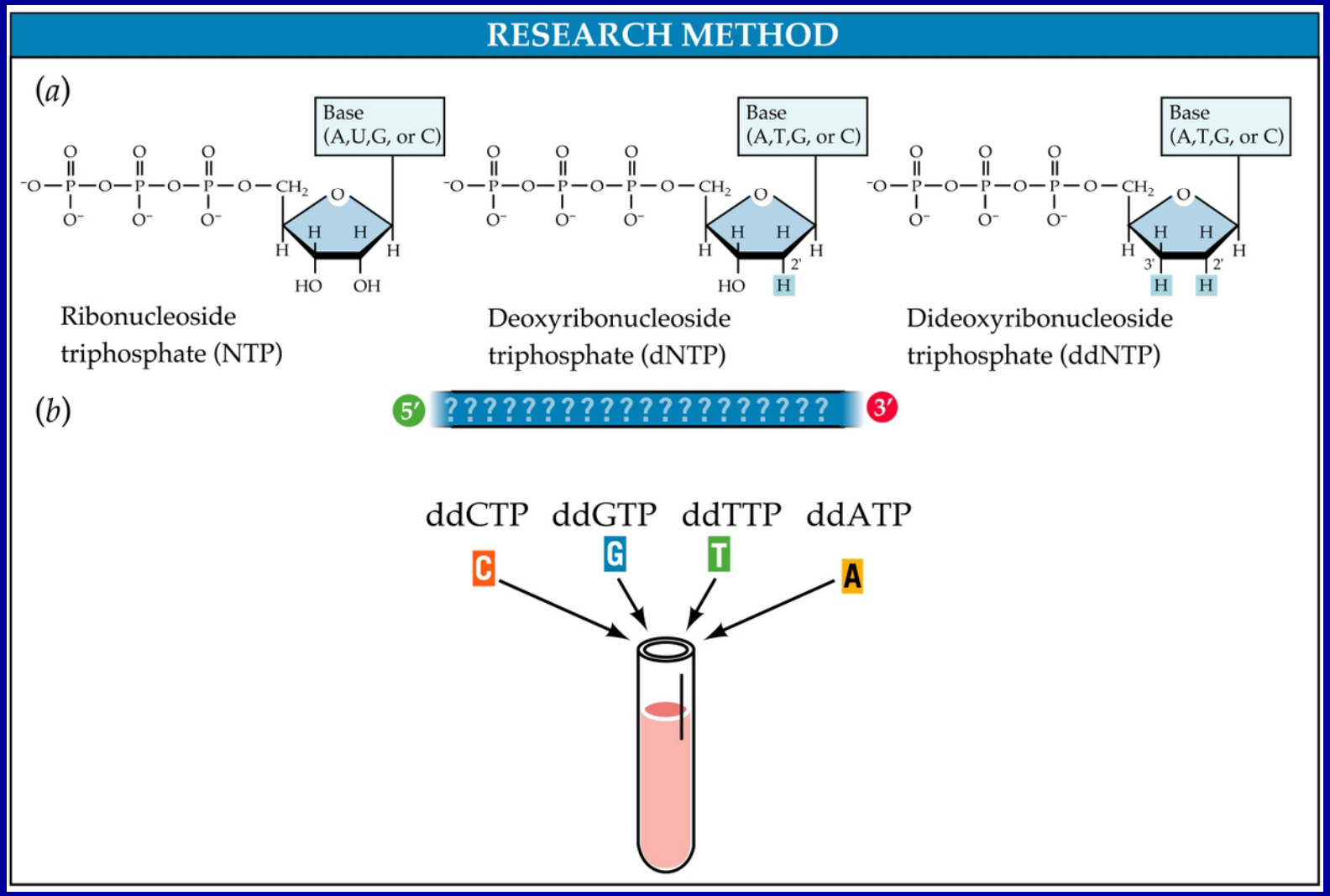

PAIRED HOMOLOGOUS CHROMOSOMES
5′
3′
maternal chromosome
3′
5′
paternal chromosome
DOUBLE-STRAND BREAK
NUCLEASE DIGESTS 3′ ENDS
STRAND INVASION

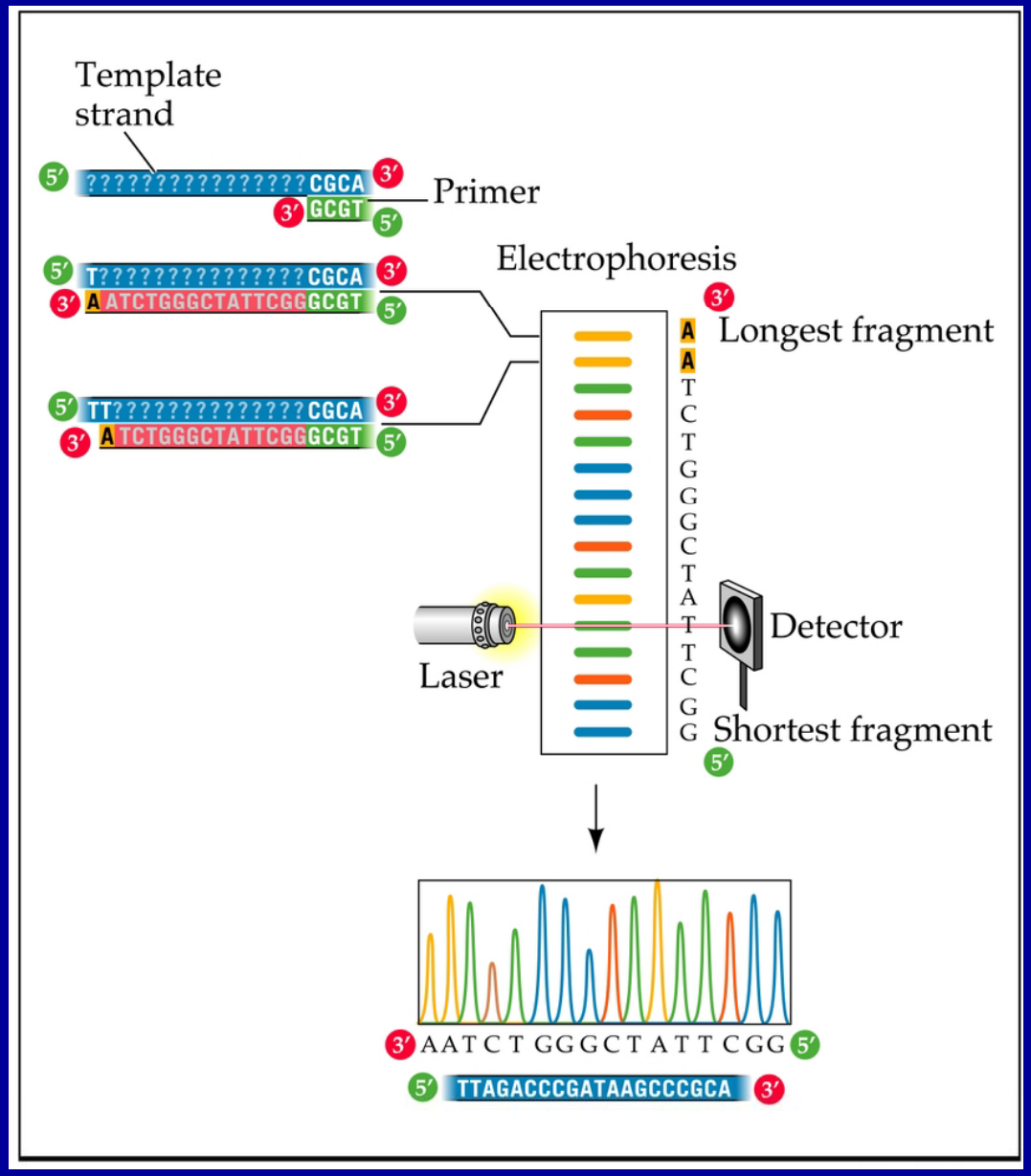

5′
3′
3′
5′
DNA SYNTHESIS
5′
3′
3′
5′
COMPLETION OF DNA SYNTHESIS
FOLLOWED BY DNA LIGATION
Holliday junction
5′
3′
3′
5′
DNA STRANDS CUT AT ARROWS
AND LIGATED
5′
3′
3′
5′
NET RESULT: CHROMOSOMES WITH CROSSOVER

F. Practical Applications of DNA Replication

- The principles of DNA replication can be used to determine the nucleotide sequence of DNA.
- The polymerase chain reaction technique uses DNA polymerases to repeatedly replicate DNA in the test tube.

DNA Sequencing




Template strand
5′ ??????????????CGCA 3′
3′ GCGT 5′
Primer
Electrophoresis
5′ T?????????????CGCA 3′
3′ AATCTGGGCTATTCGGGCGT 5′
5′ TT????????????CGCA 3′
3′ ATCTGGGCTATTCGGGCGT 5′
3′
Longest fragment
A A T C T G G G C T A T T C G G
5′
Shortest fragment
Laser
Detector
3′ AATCTGGGCTATTCGG 5′
5′ TTAGACCCGATAAGCCCGCA 3′

Polymerase Chain Reaction: PCR