Lecture Series 6

DNA and Its Role in Heredity

Reading Assignments

- Read Chapter 5
  DNA and Chromosomes
- Read Chapter 6
  DNA Replication, Repair & Replication
  (skim pages 215 to 222 on Recombination)
- Read Chapter 10
  Pages 331 to 333 and 347 to 351
  (only sections on Sequencing and PCR)

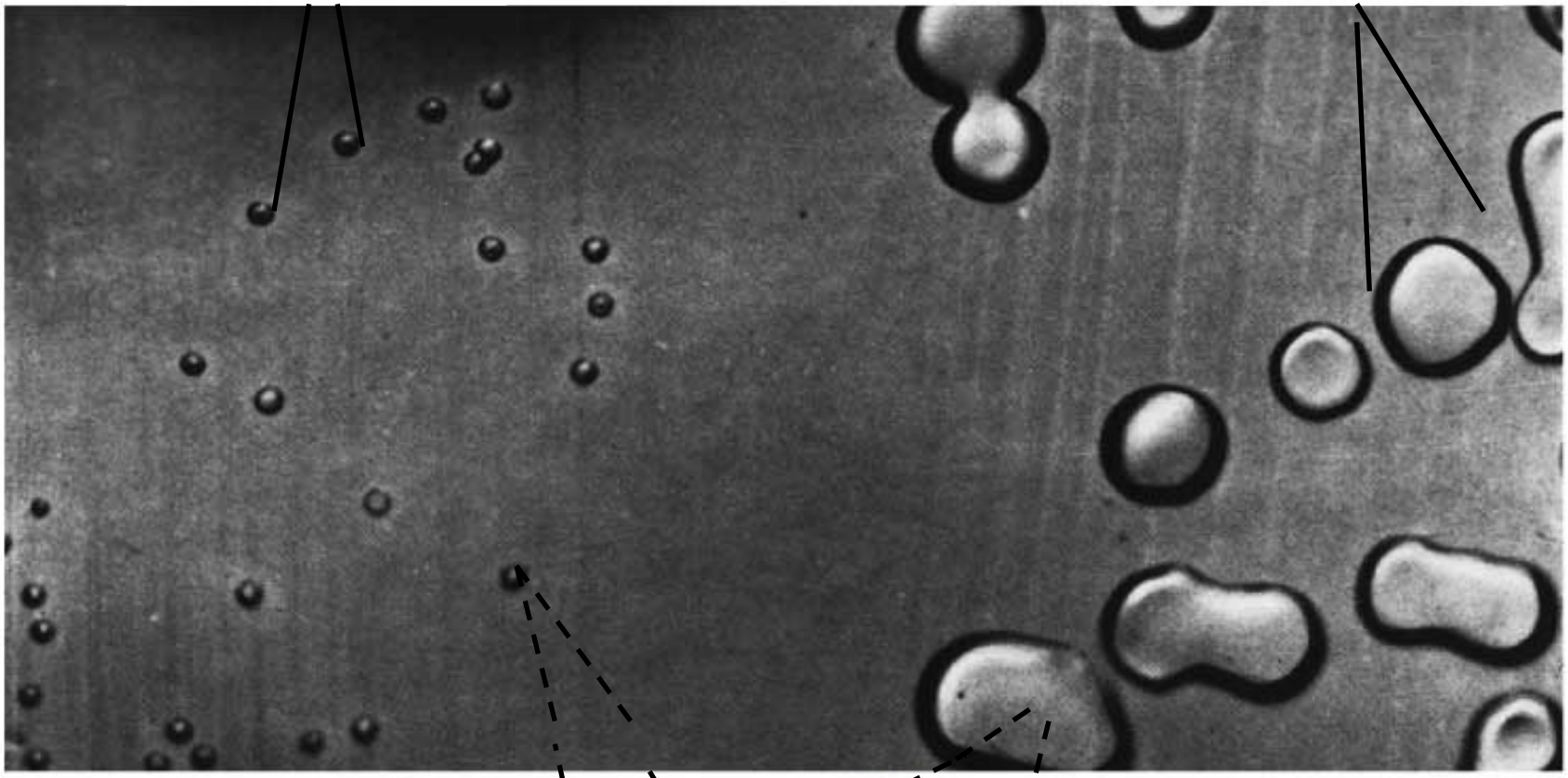
A. DNA: The Genetic Material

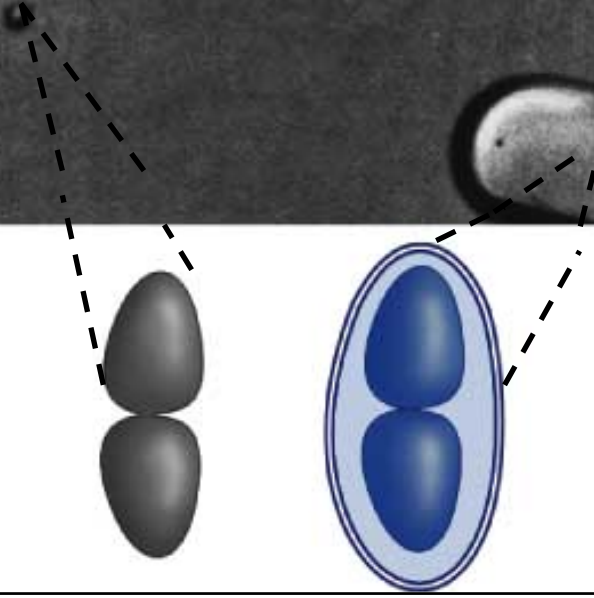
- In addition to circumstantial evidence, two key experiments demonstrated that DNA is the genetic material.
- In the first key experiment (Griffiths, 1928) showed that a virulent strain of *Streptococcus pneumoniae* genetically transformed nonvirulent *S. pneumoniae* into virulent bacteria.

There are two strains of *Streptococcus pneumoniae*.

ROUGH COLONY (R)

SMOOTH COLONY (S)





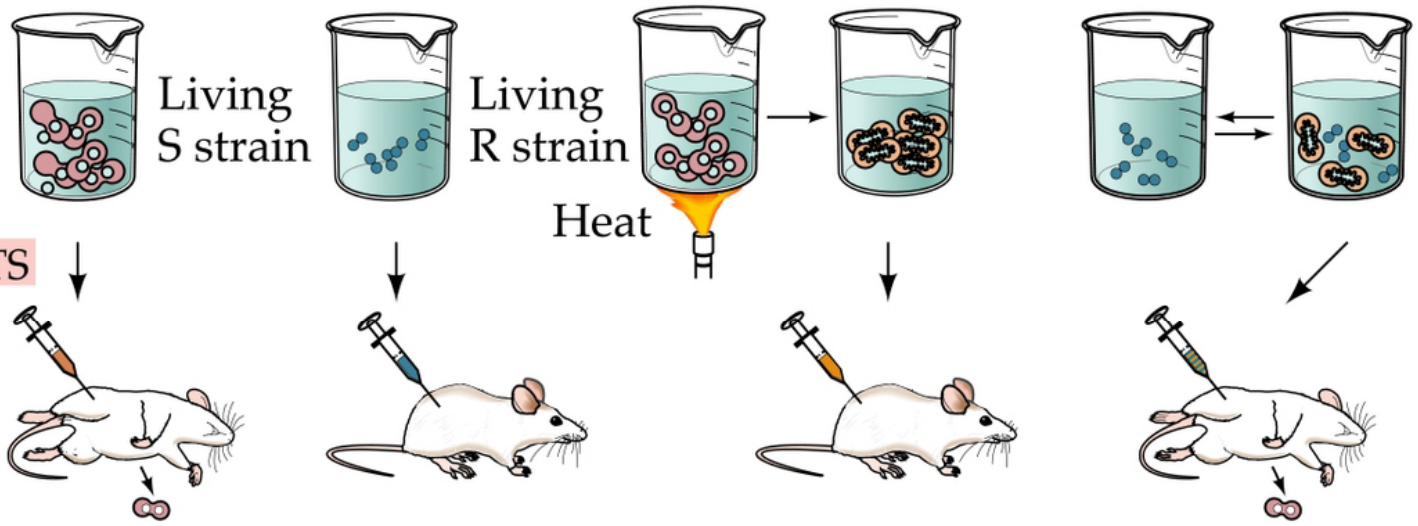
R strain is benign (Lacking a protective capsule, it is recognized and destroyed by host's immune system)

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



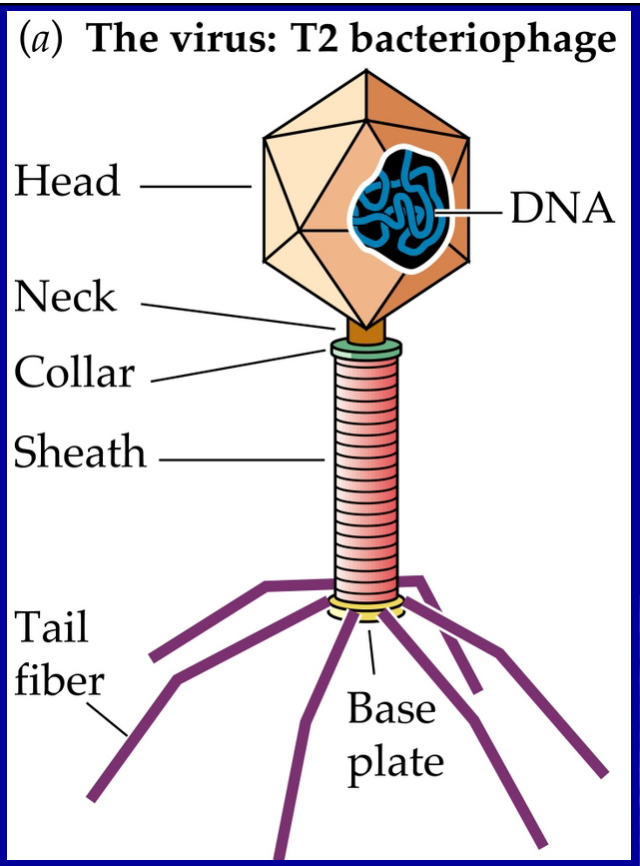
Mouse dies	Mouse healthy	Mouse healthy	Mouse dies
Living S strain cells isolated from heart	No bacterial cells found in heart	No bacterial cells found in heart	Living S strain cells isolated from heart

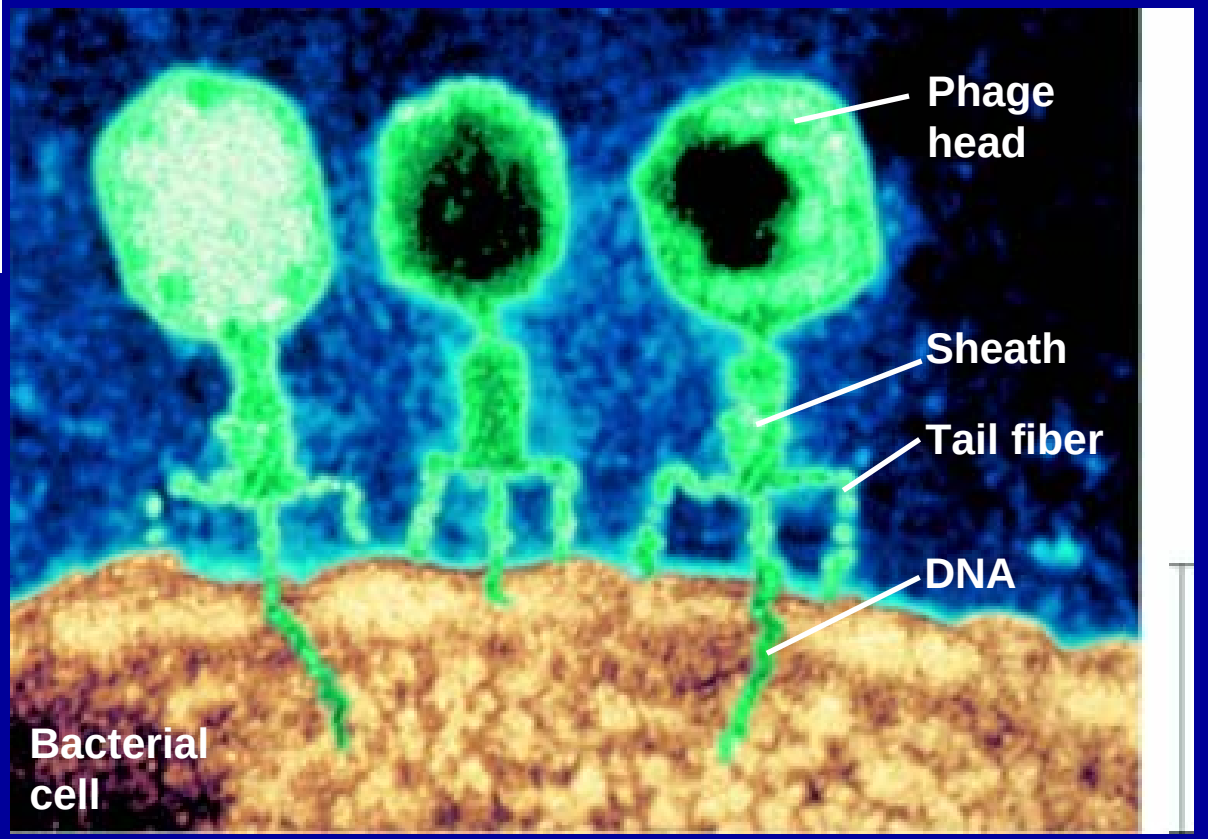
Conclusion: A chemical component from one cell is capable of genetically transforming another cell.

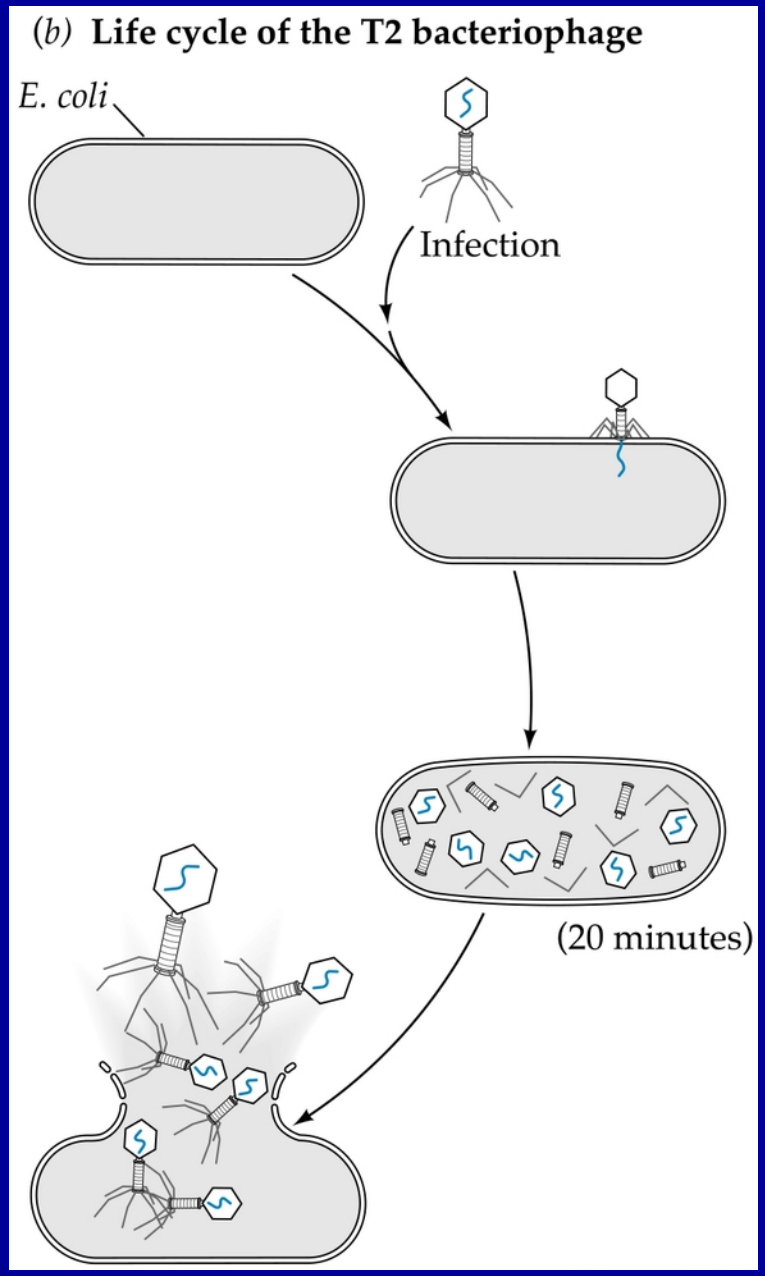
A. DNA: The Genetic Material

- In a prelude to 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 second 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.

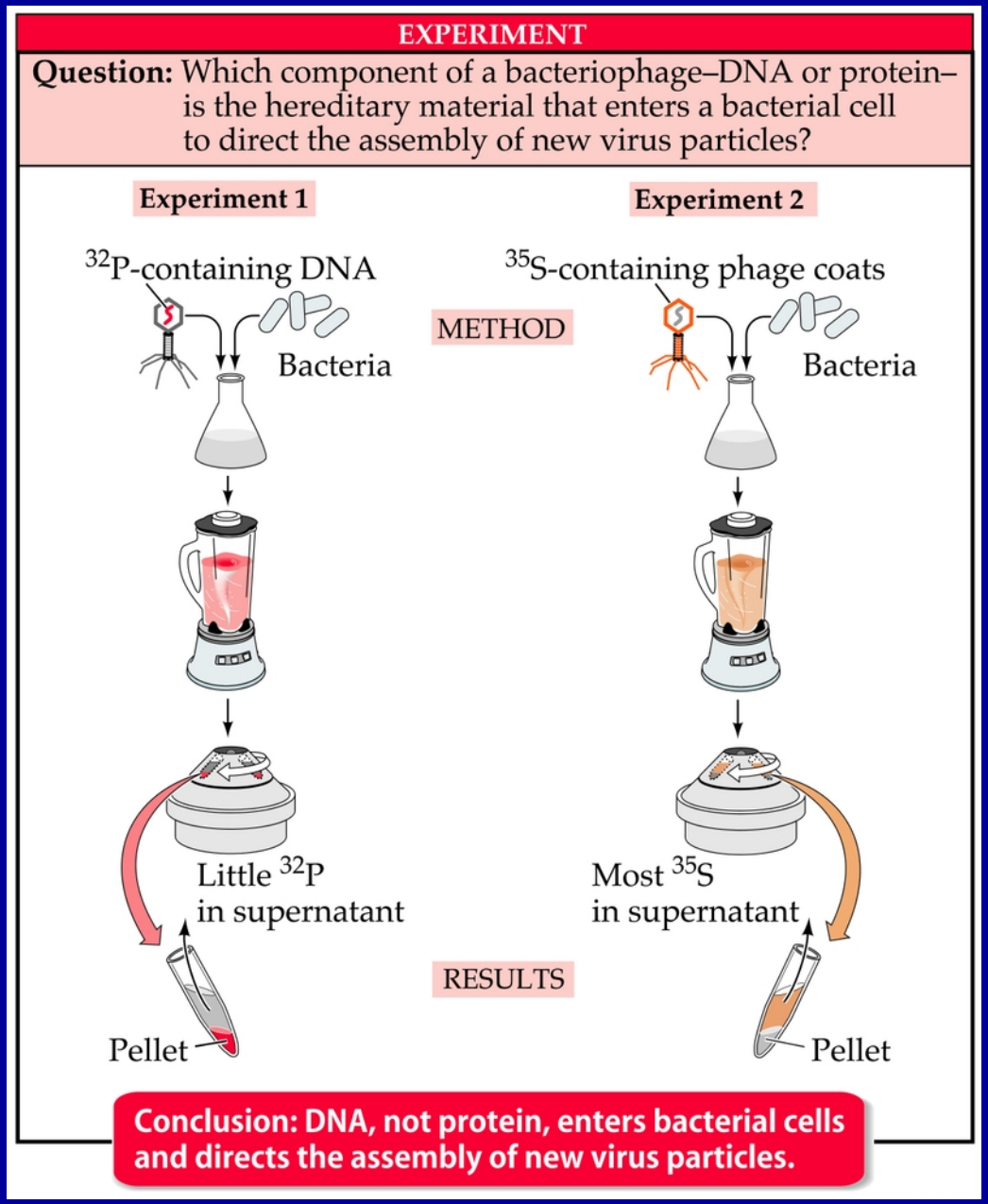

(a) The virus: T2 bacteriophage
Head
DNA
Neck
Collar
Sheath
Tail fiber
Base plate


Phage head
Sheath
Tail fiber
DNA
Bacterial cell
100 nm


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

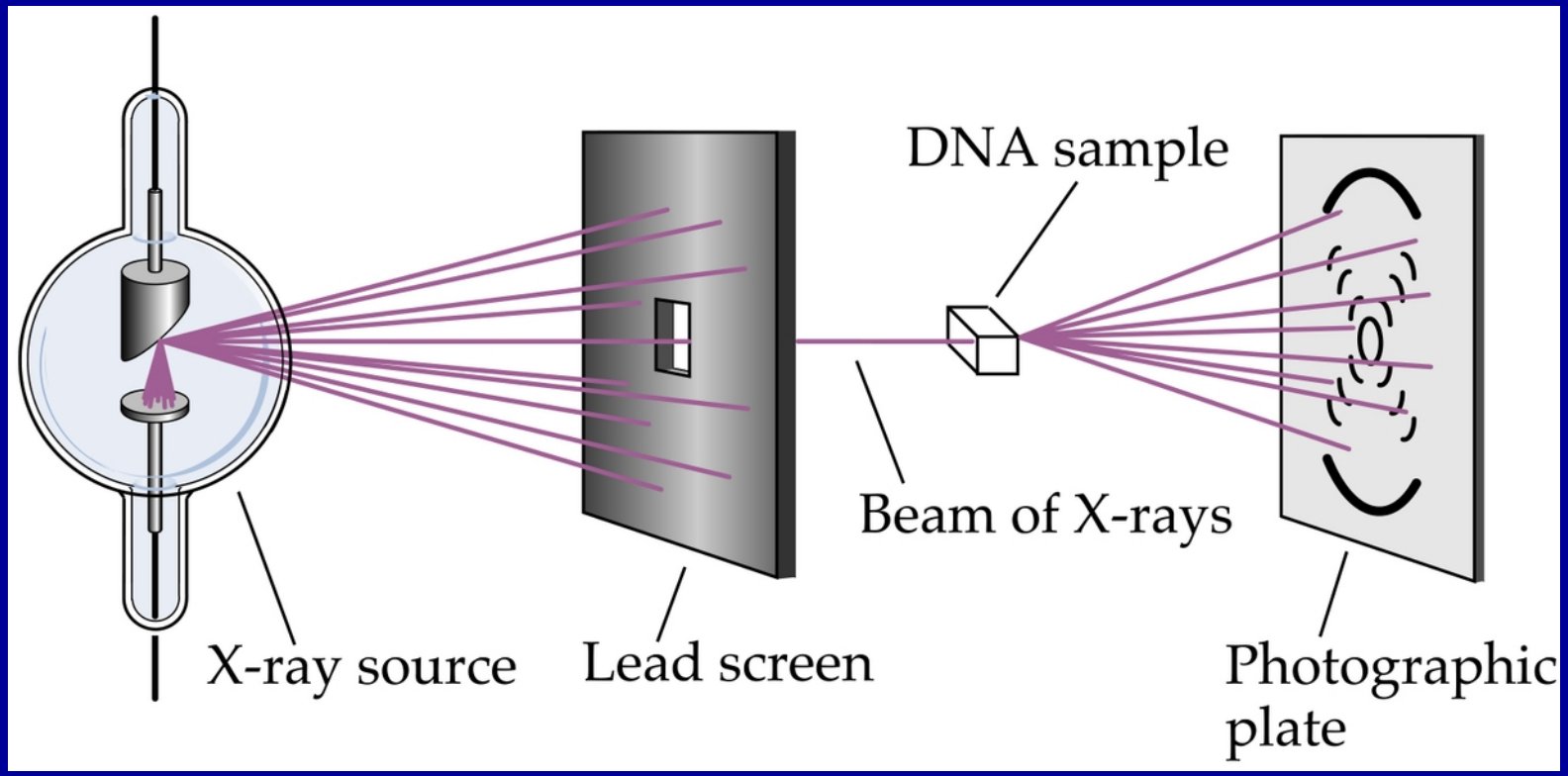
Lytic Cycle

The Hershey-Chase Blender Experiment

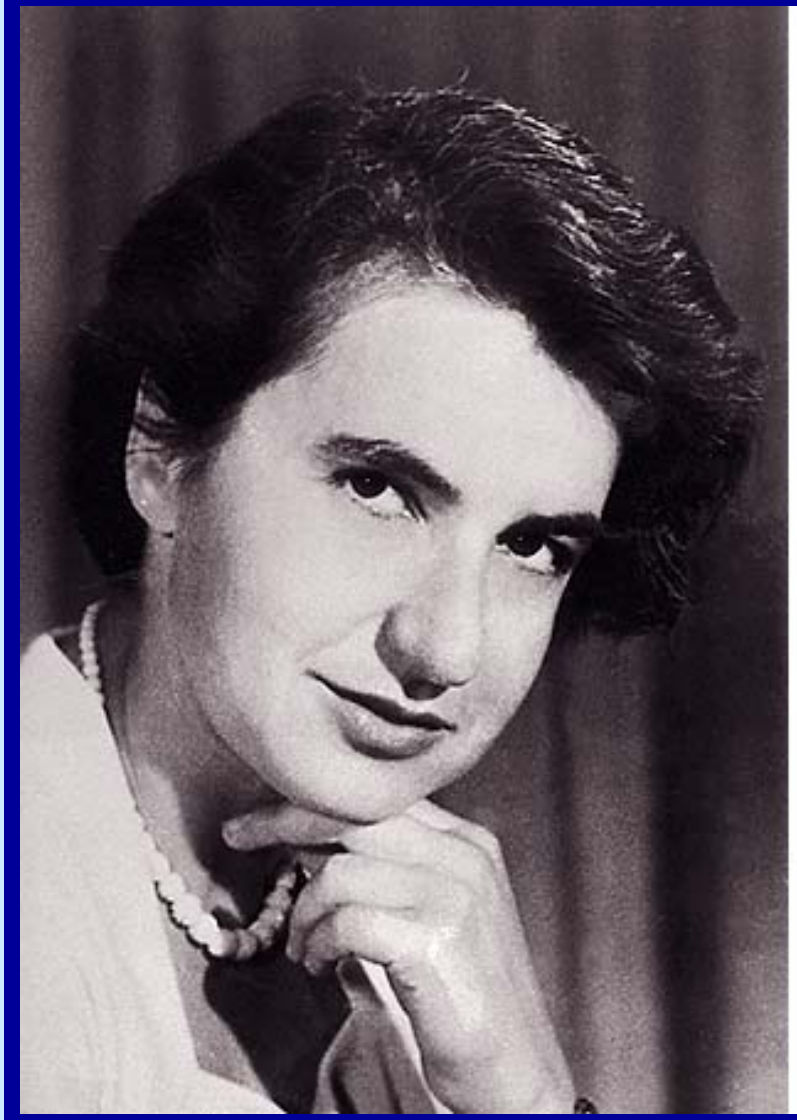


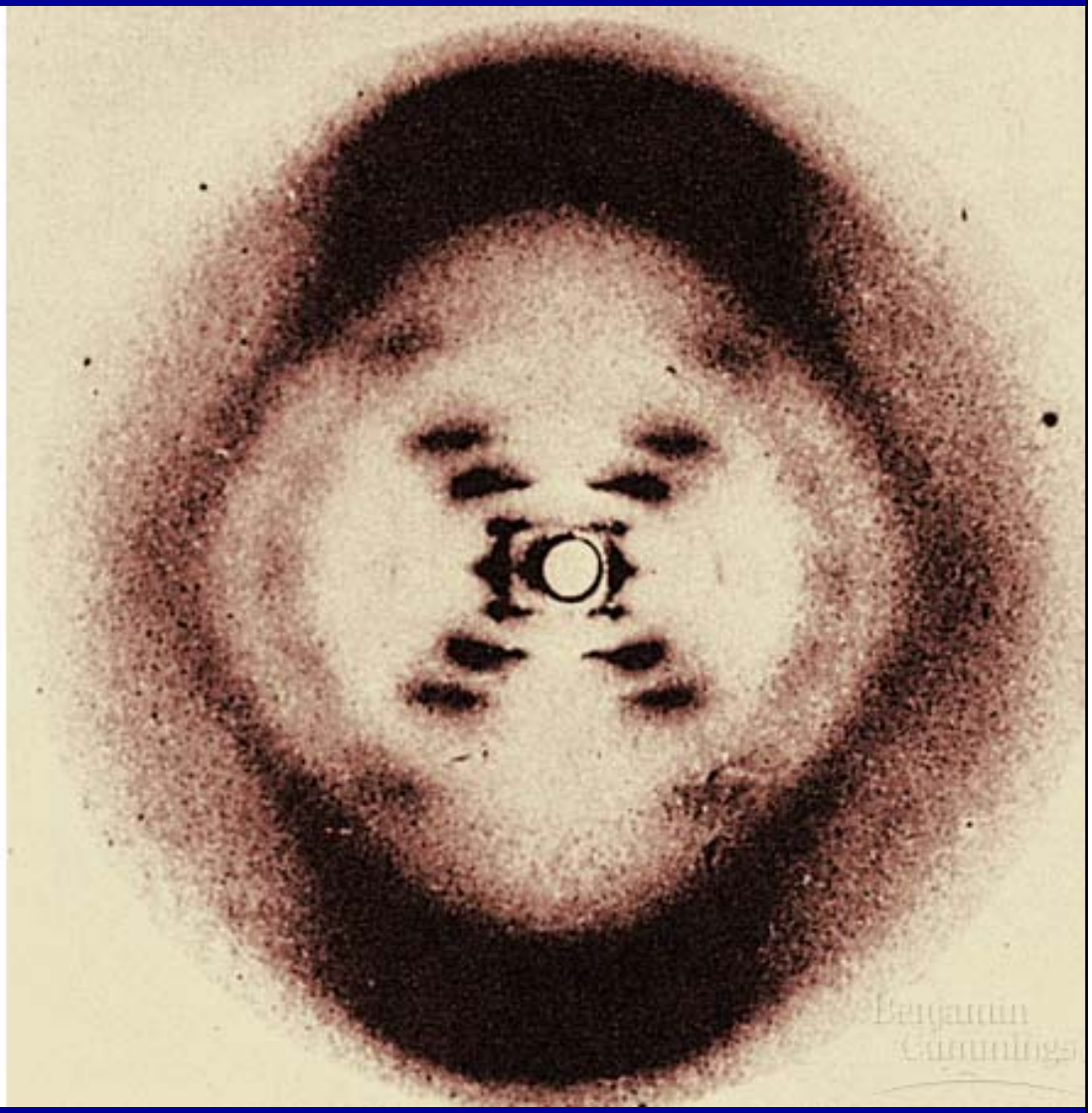
B. The Structure of DNA

- 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

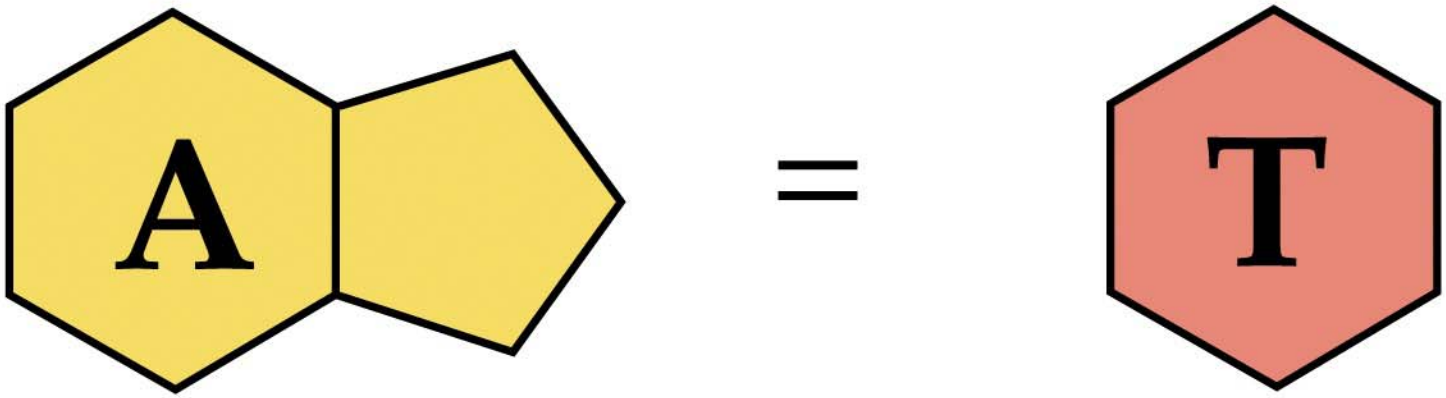


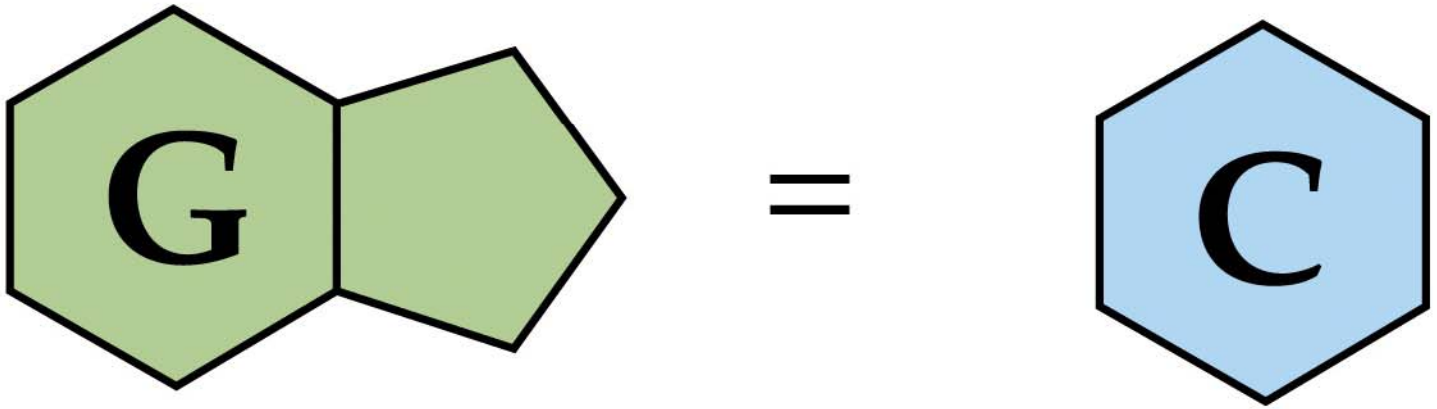


B. The Structure of DNA

- 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).



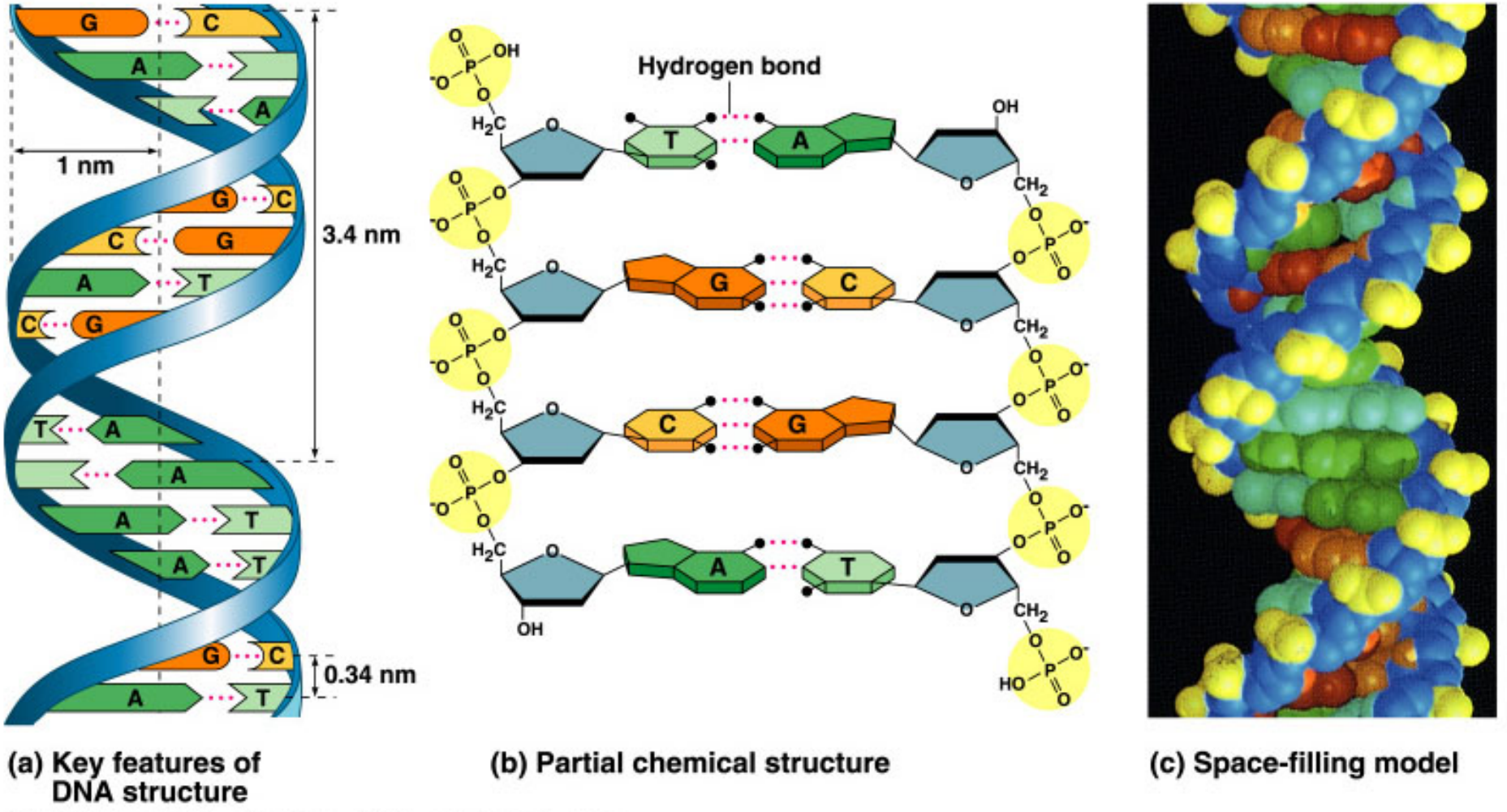


Purines = Pyrimidines

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

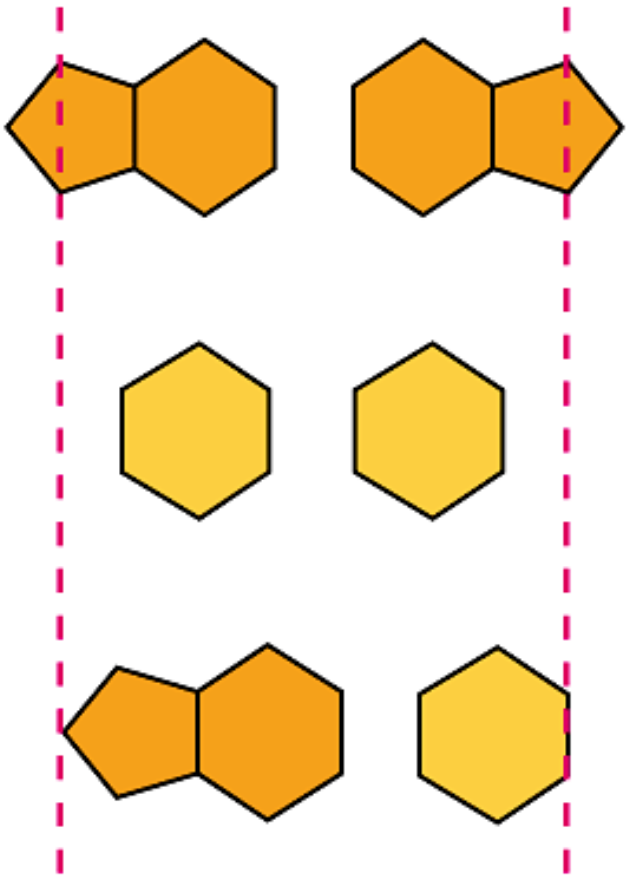


Purine and Pyridimine Fit

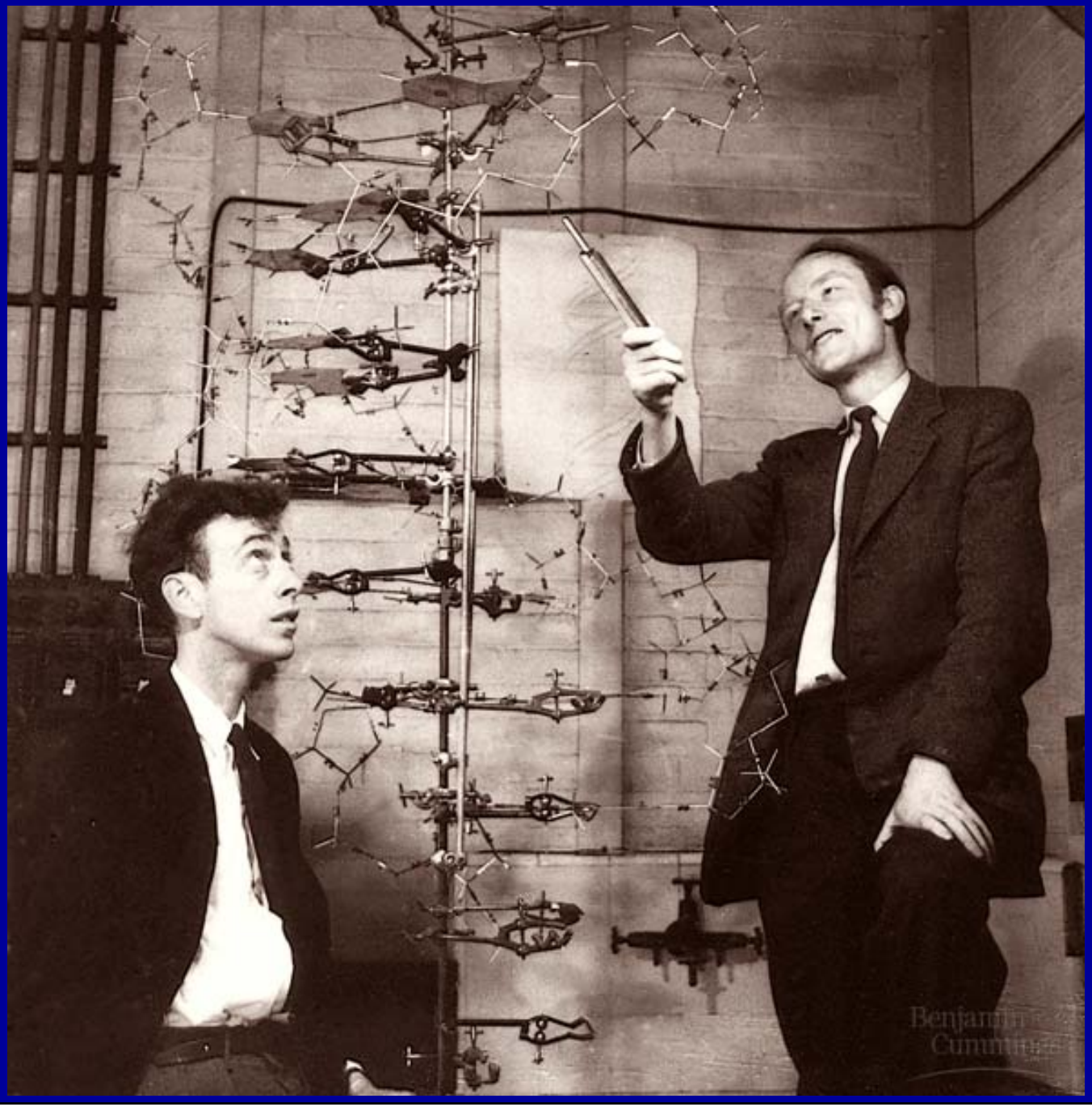
Purine + purine: too wide

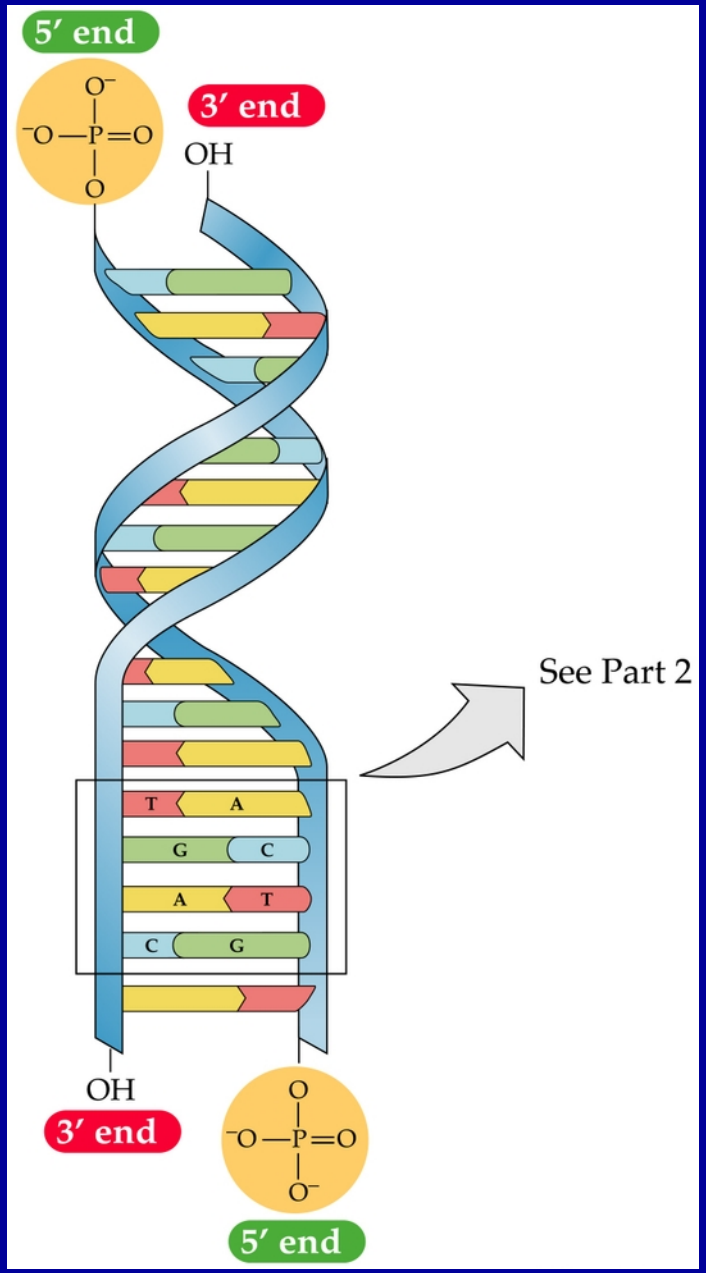
Pyrimidine + pyrimidine: too narrow

Purine + pyrimidine: width consistent with X-ray data

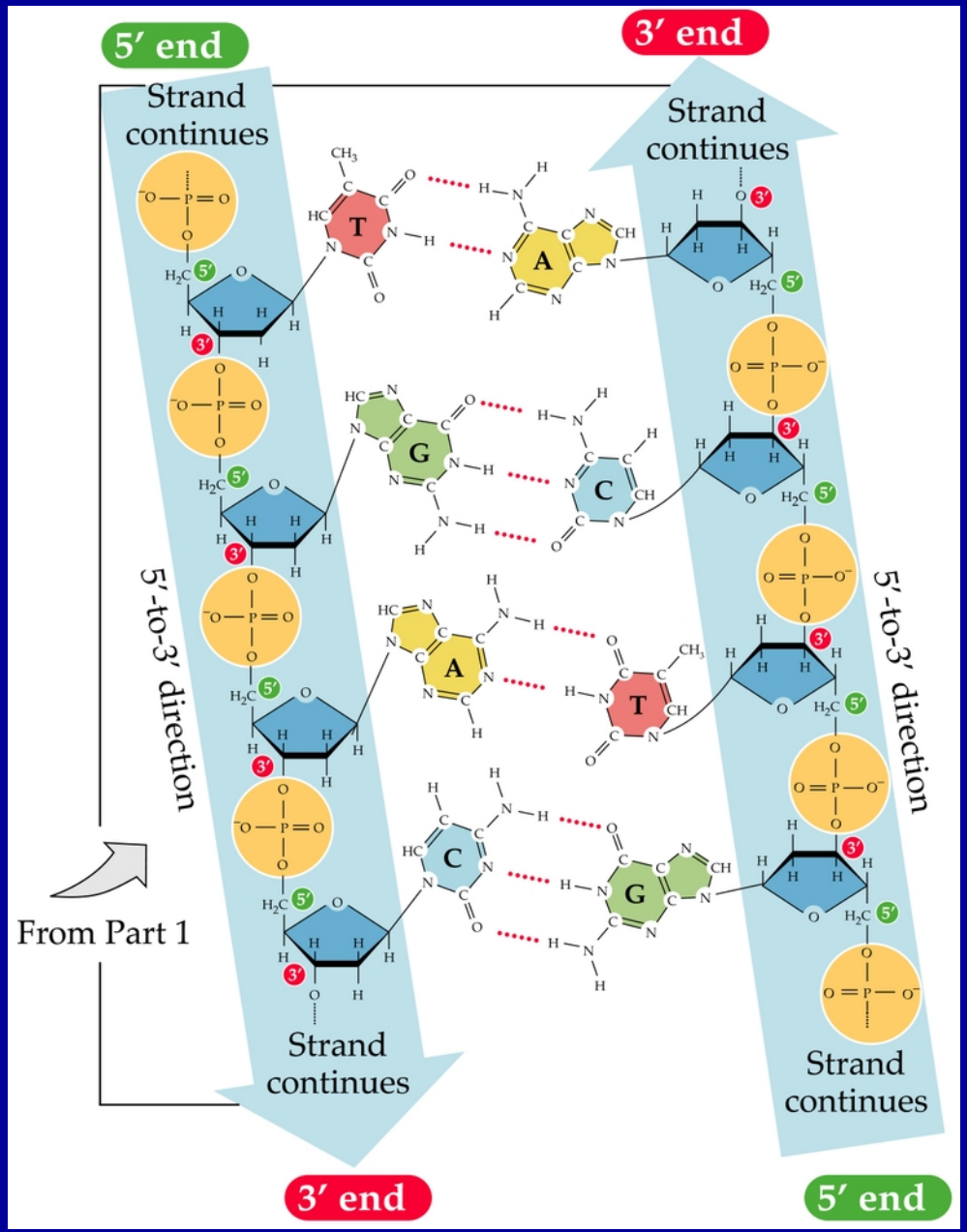


Watson and Crick and their Model



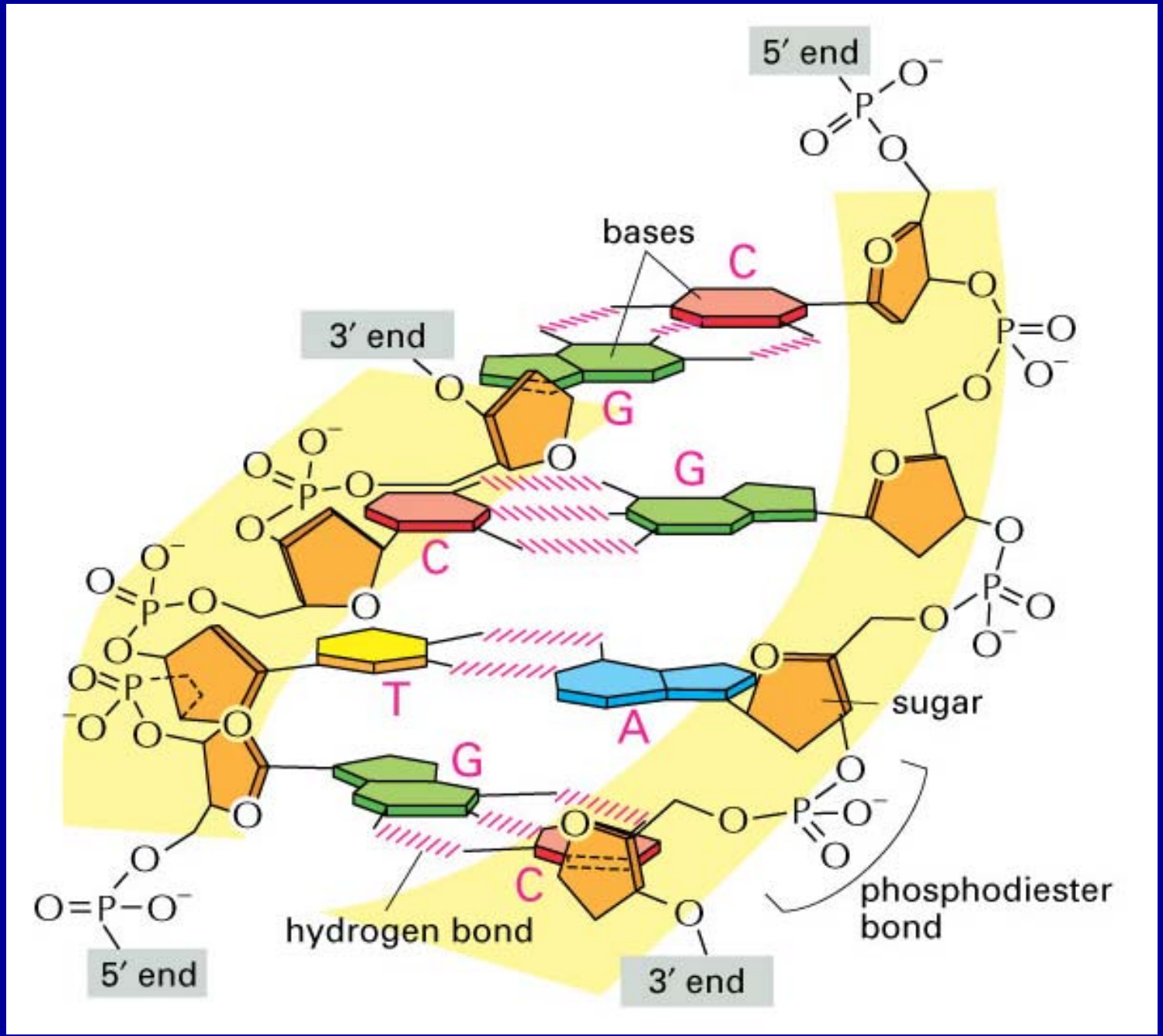


1) DS Helix
2) Uniform Diameter
3) RT handed twist
4) Anti-parallel



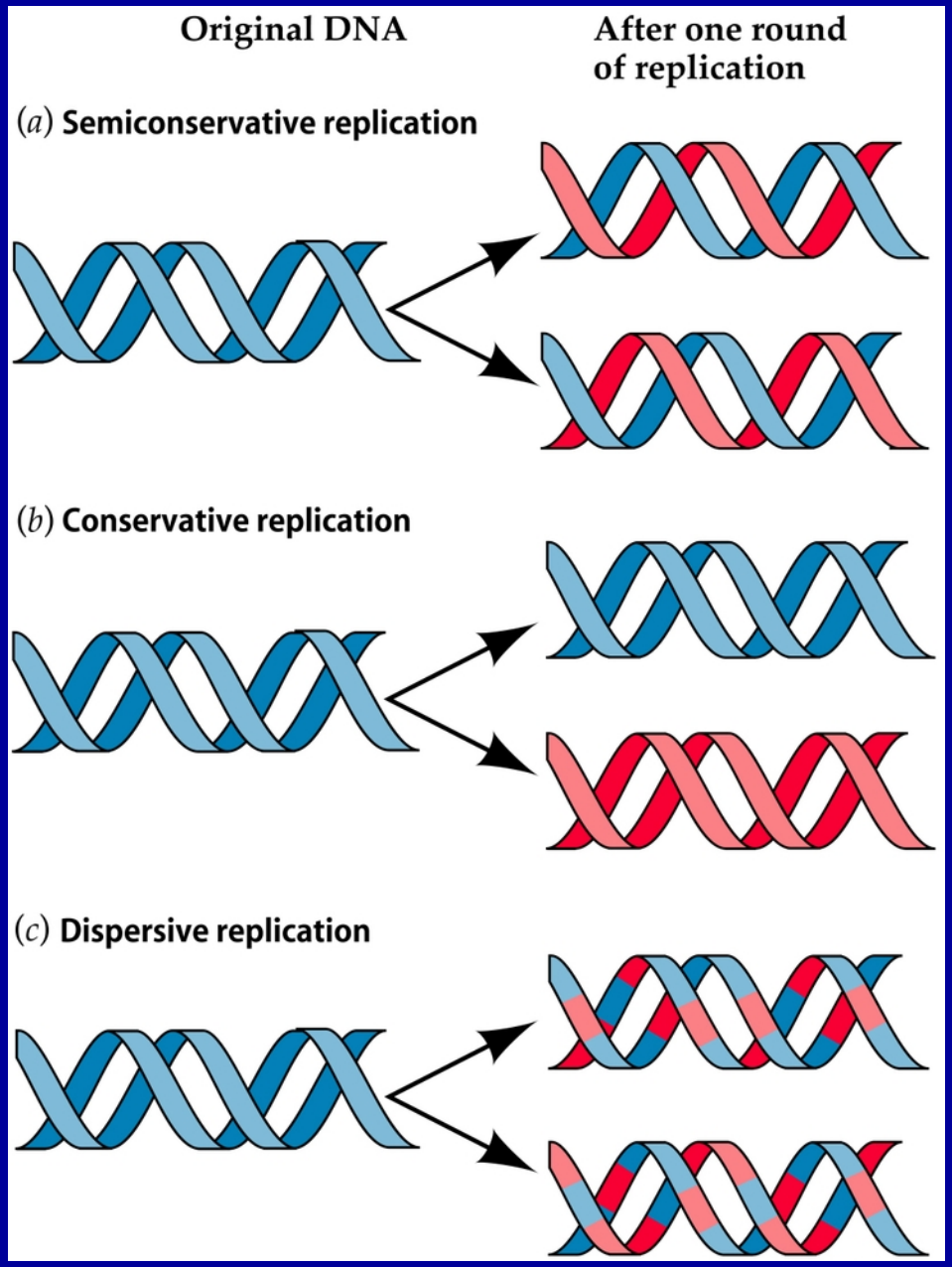
5) Complementary Base pairing

6) Double Helix is Essential to DNA's Function


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

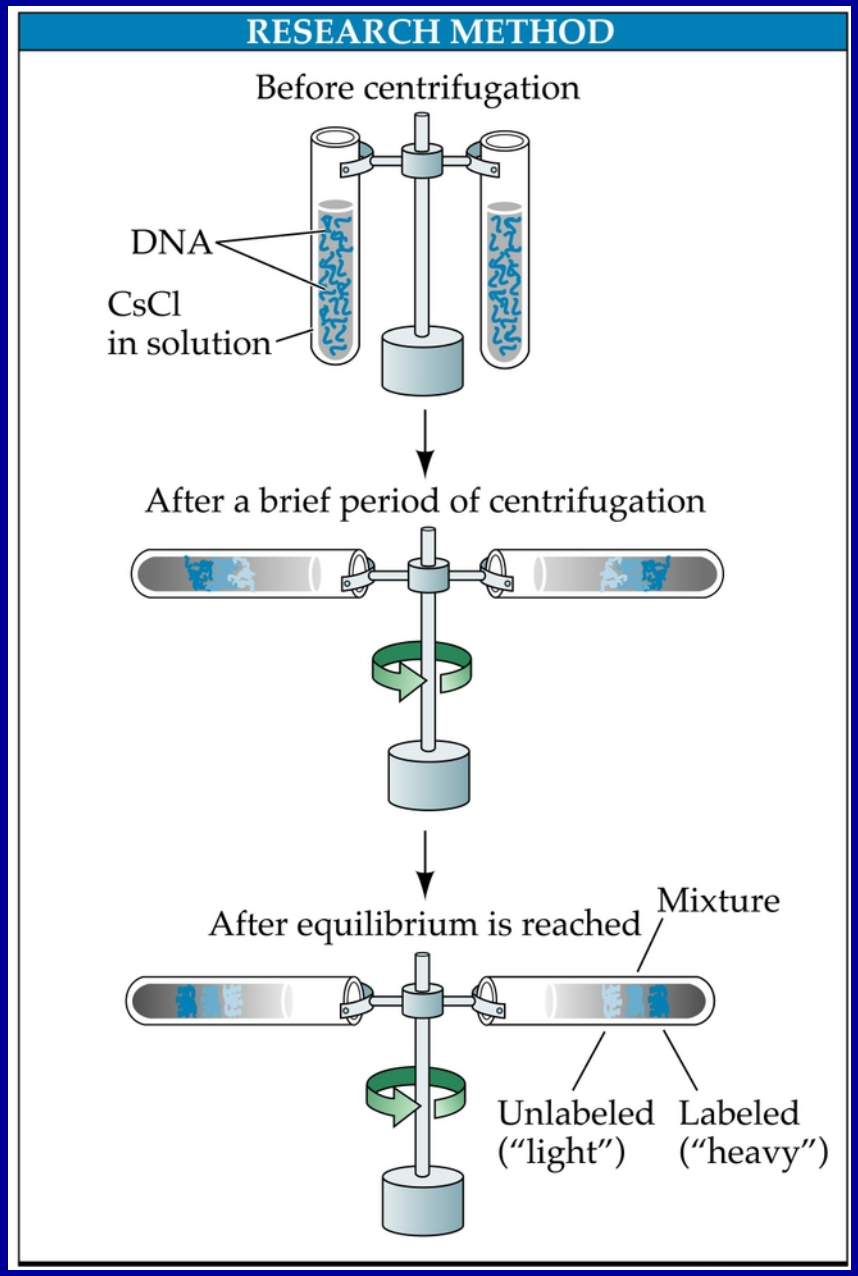
C. DNA Replication

- 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

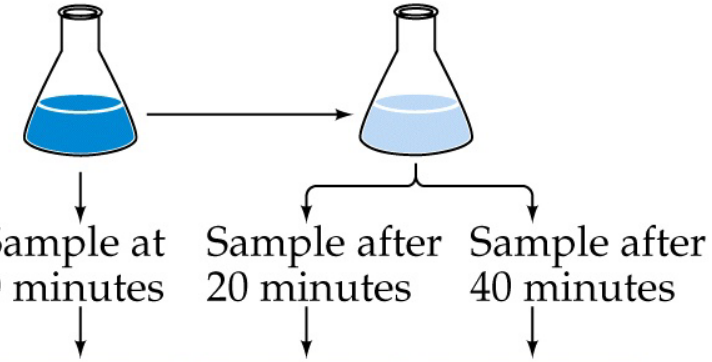
- Kornberg (1956) demonstrated *in vitro* that DNA served as its own template during replication.
- Meselson and Stahl's experiment (1957) proved replication of DNA to be semiconservative. A parent strand is a template for synthesis of a new strand. Two replicated DNA helices contain one parent strand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

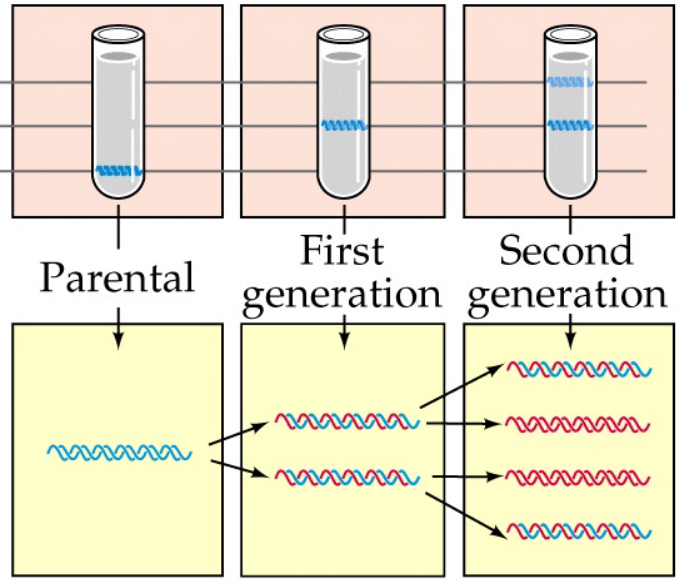
Question: Does DNA replicate semiconservatively, or by some other mechanism?

METHOD



RESULTS

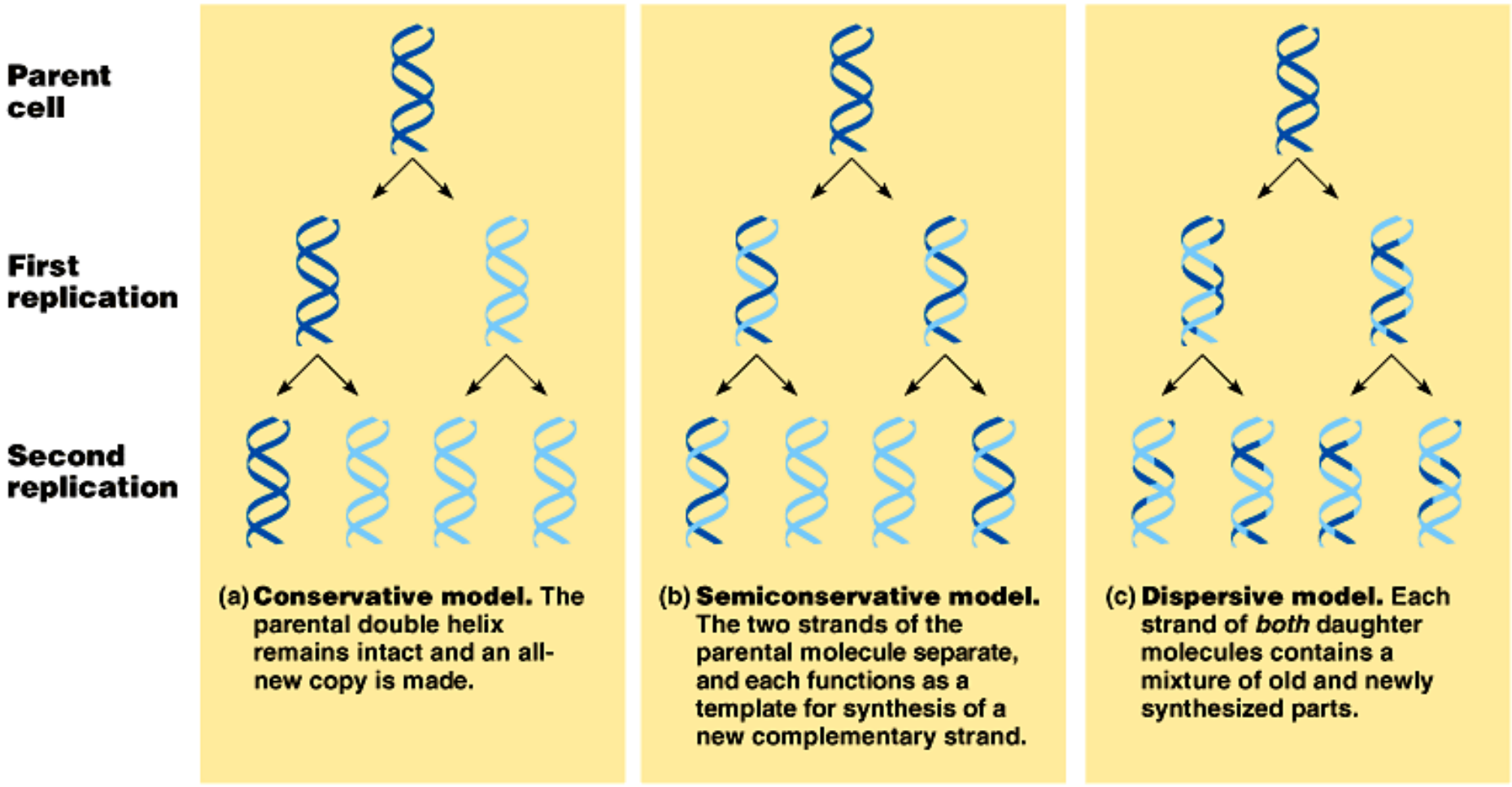
$^{14}N/^{14}N$ (light) DNA
$^{14}N/^{15}N$ (intermediate) DNA
$^{15}N/^{15}N$ (heavy) DNA



INTERPRETATION

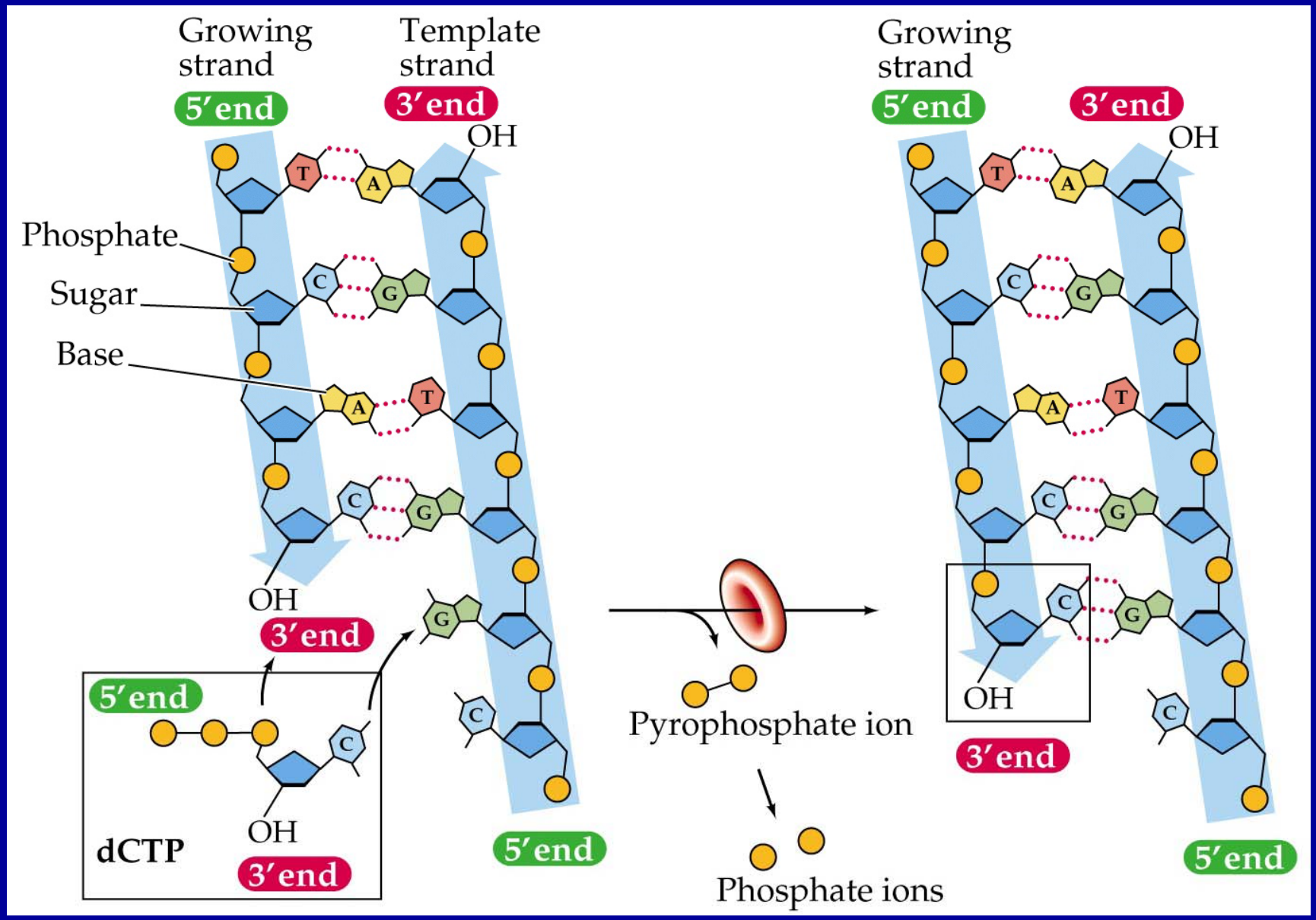
Conclusion: DNA replication is semiconservative.

Three alternative models of DNA replication



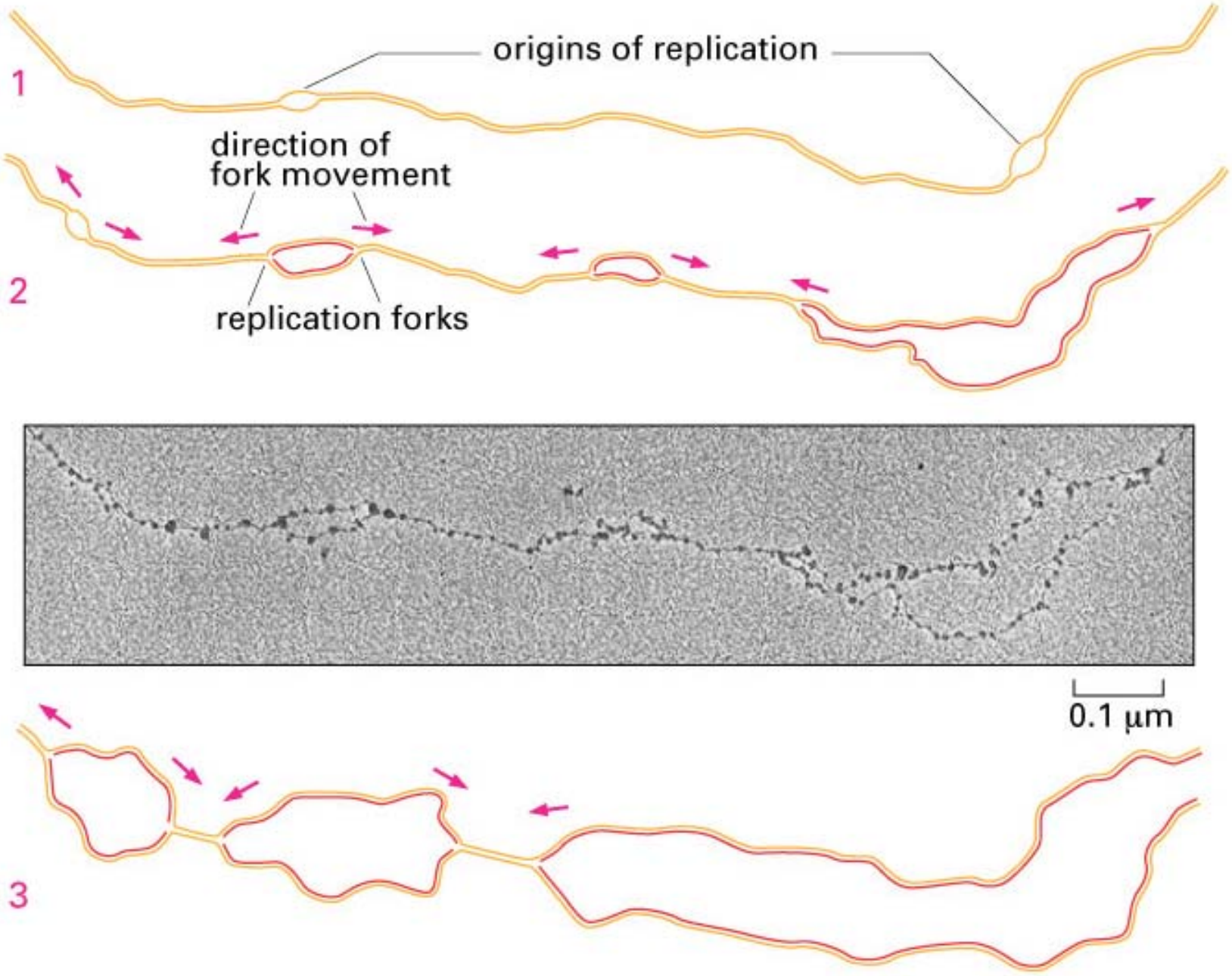
D. The Mechanism of DNA Replication

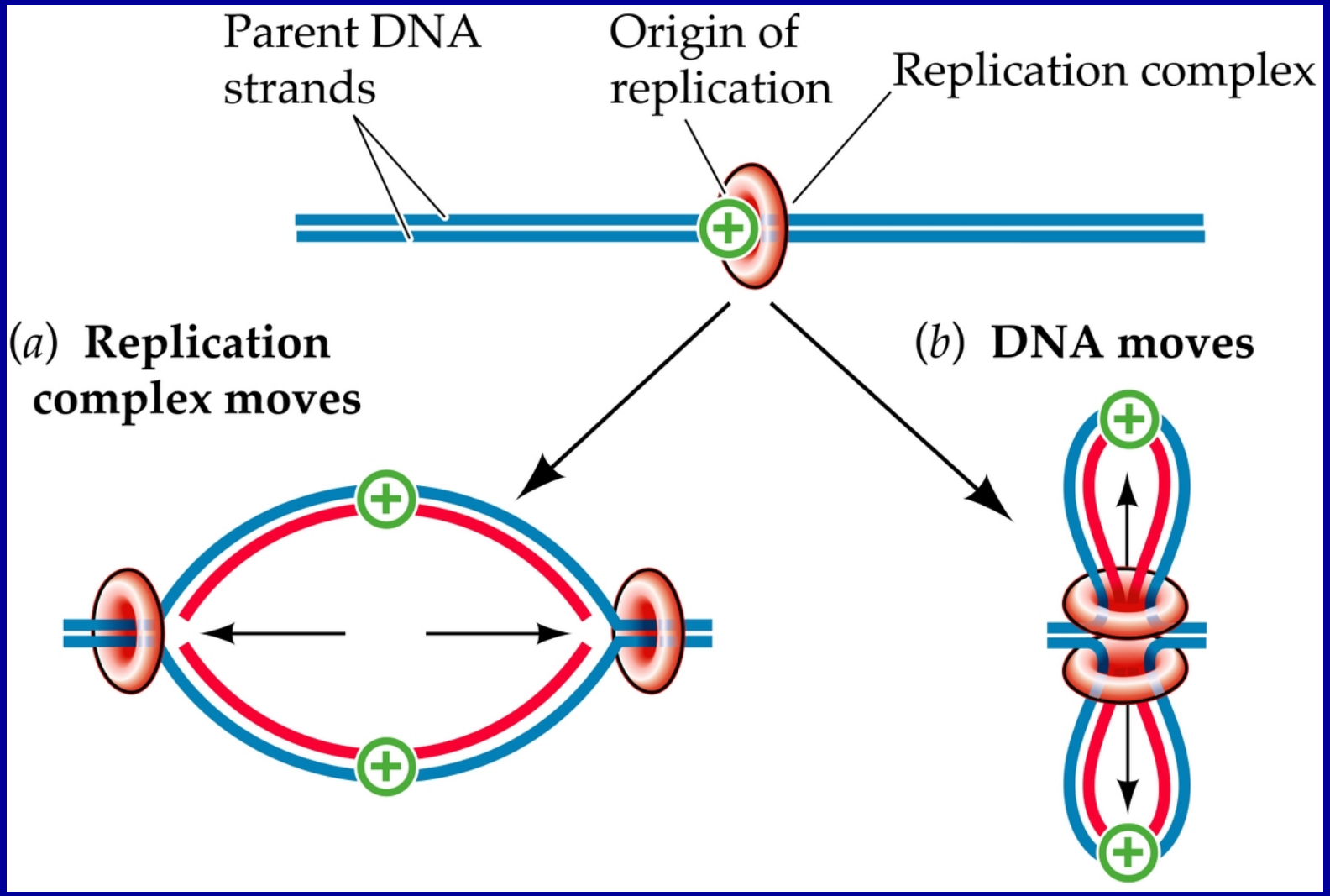
- DNA polymerase catalyzes nucleotides from the 5′ to the 3′ end.
- Nucleotides are added by complementary base pairing with the template strand.
- The substrates, deoxyribonucleoside triphosphates, are hydrolyzed as added, releasing energy for DNA synthesis.


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

D. The Mechanism of DNA Replication

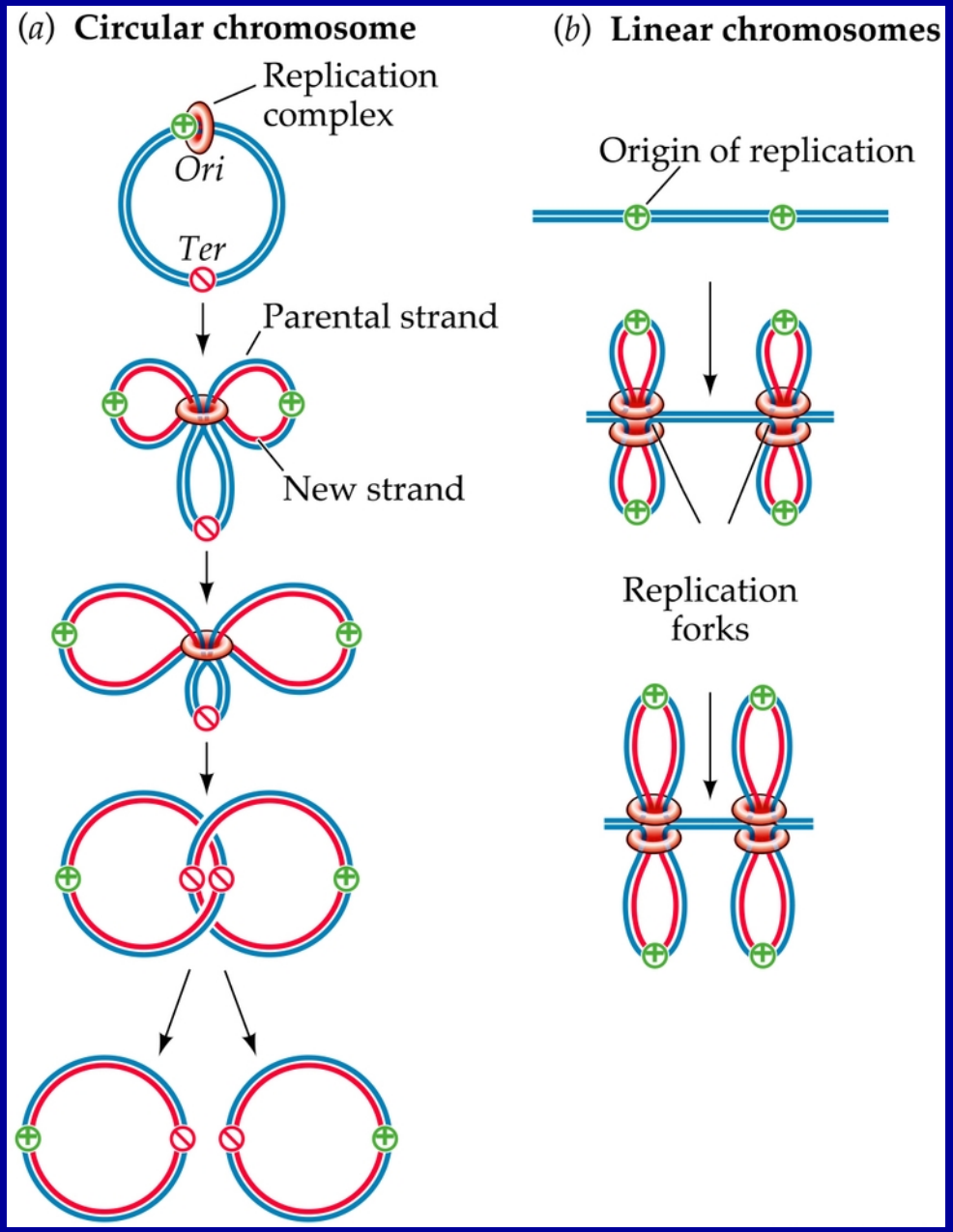
- News Flash: The DNA replication complex is in a fixed location and DNA is threaded through it for replication.
- Old idea was via moving replication forks.

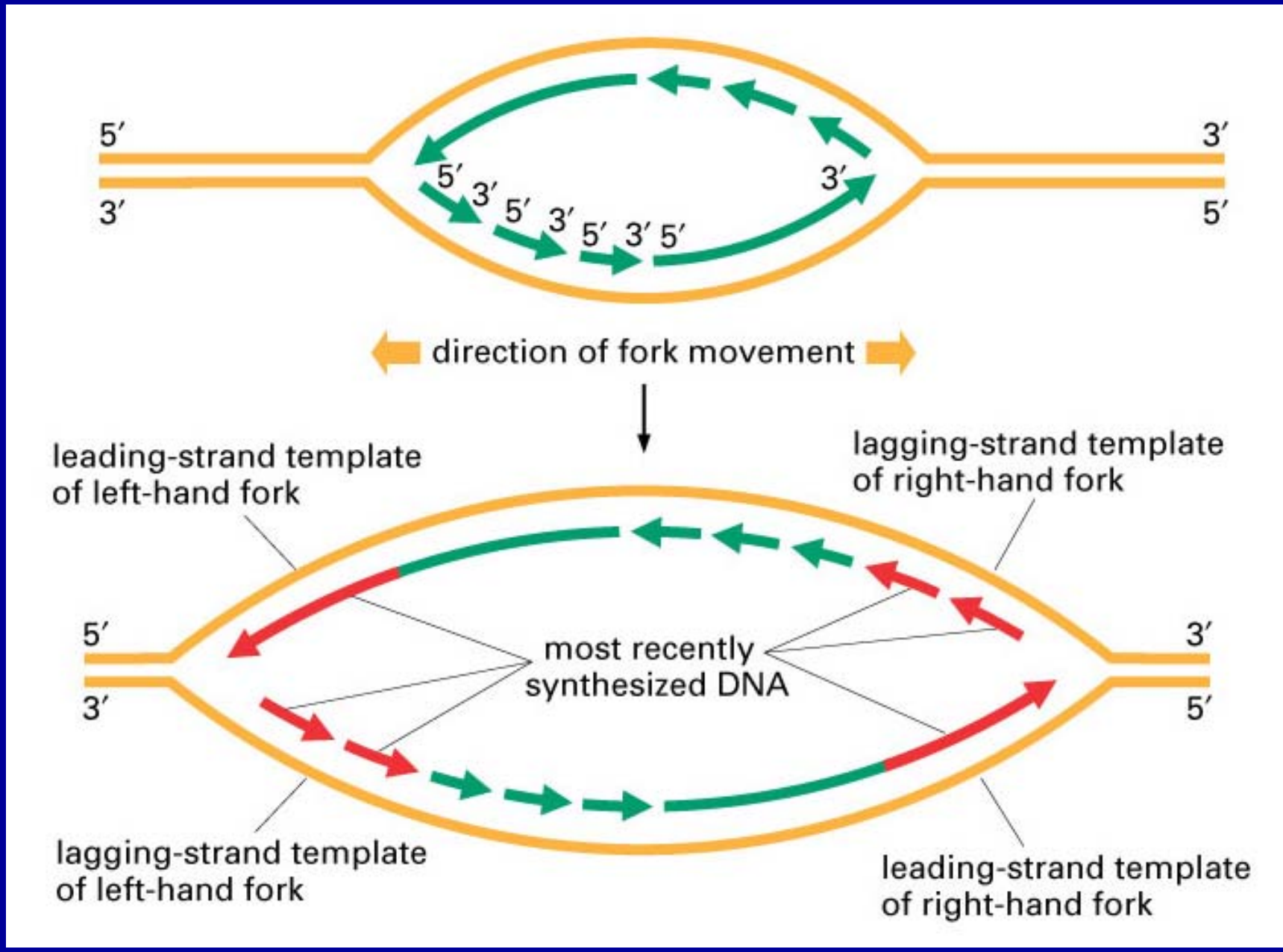

origins of replication
1
direction of
fork movement
2
replication forks
0.1 µm
3


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

D. The Mechanism of DNA Replication

- Prokaryotes have a single origin of replication; eukaryotes 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

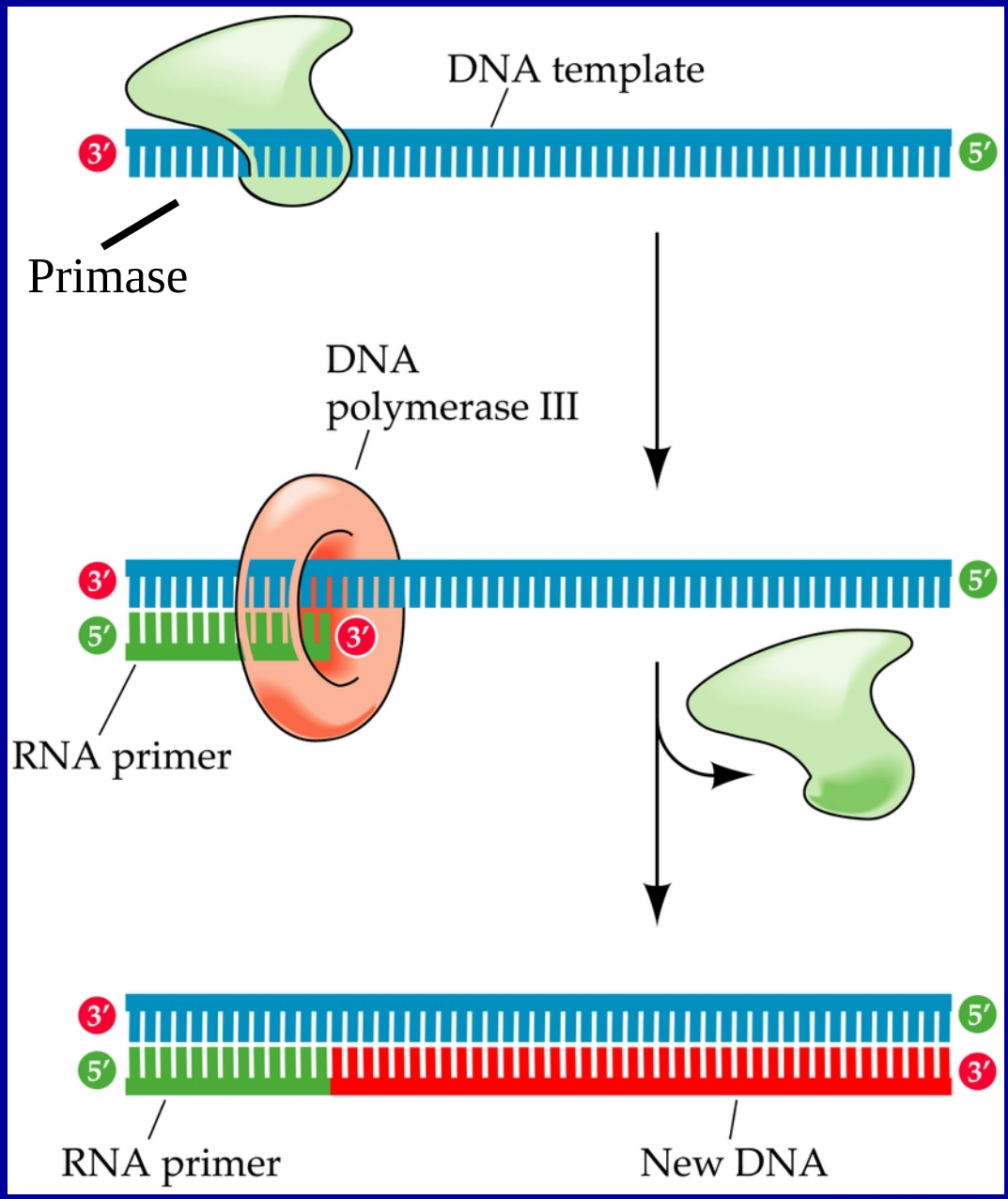

5′
3′
5′ 3′ 5′ 3′ 5′ 3′ 5′
3′
3′
5′
direction of fork movement
leading-strand template
of left-hand fork
lagging-strand template
of right-hand fork
5′
3′
most recently
synthesized DNA
3′
5′
lagging-strand template
of left-hand fork
leading-strand template
of right-hand fork

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.

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.

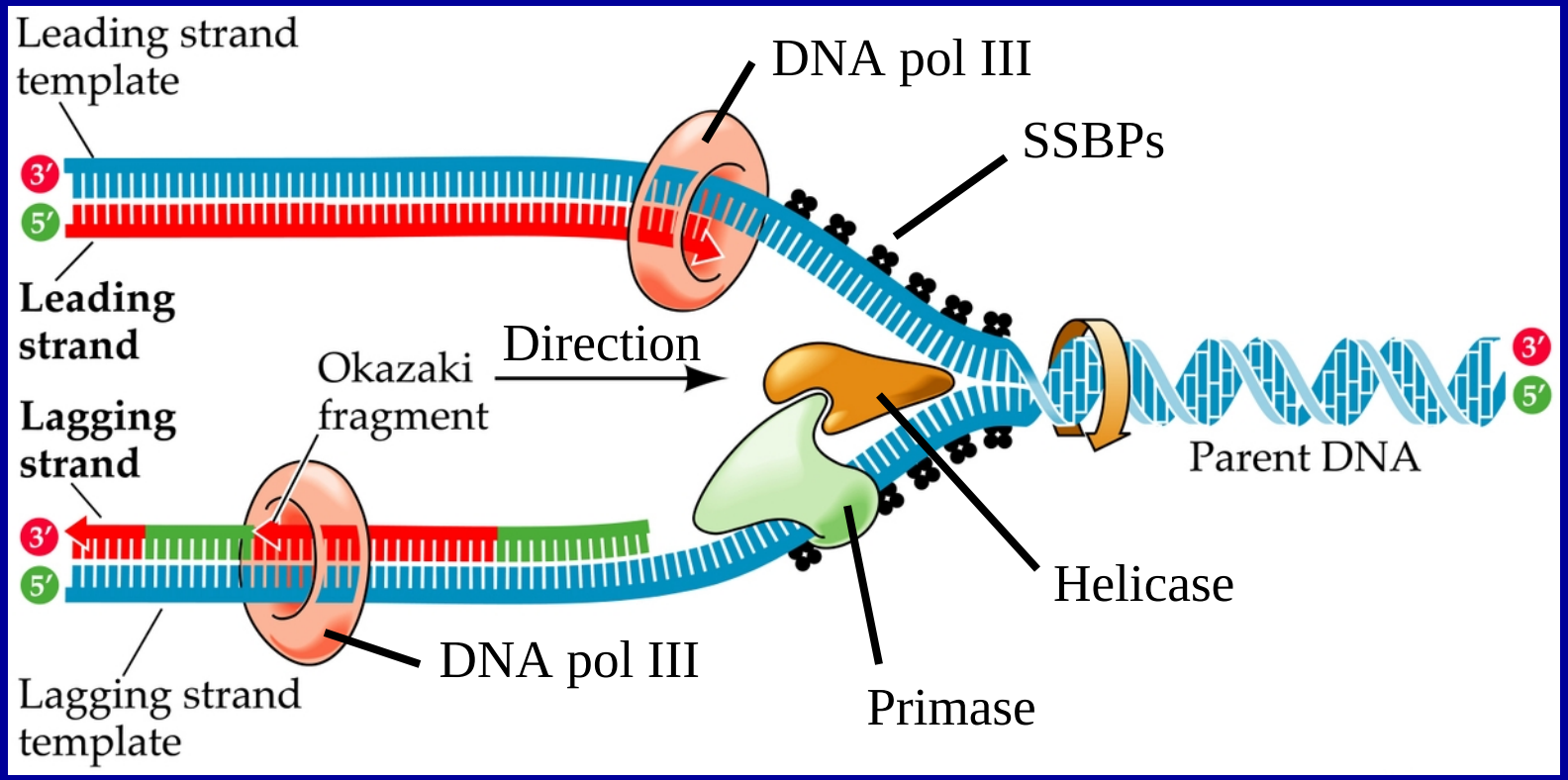
- RNA primer is degraded and DNA is replaced by DNA polymerase I.

D. The Mechanism of DNA Replication

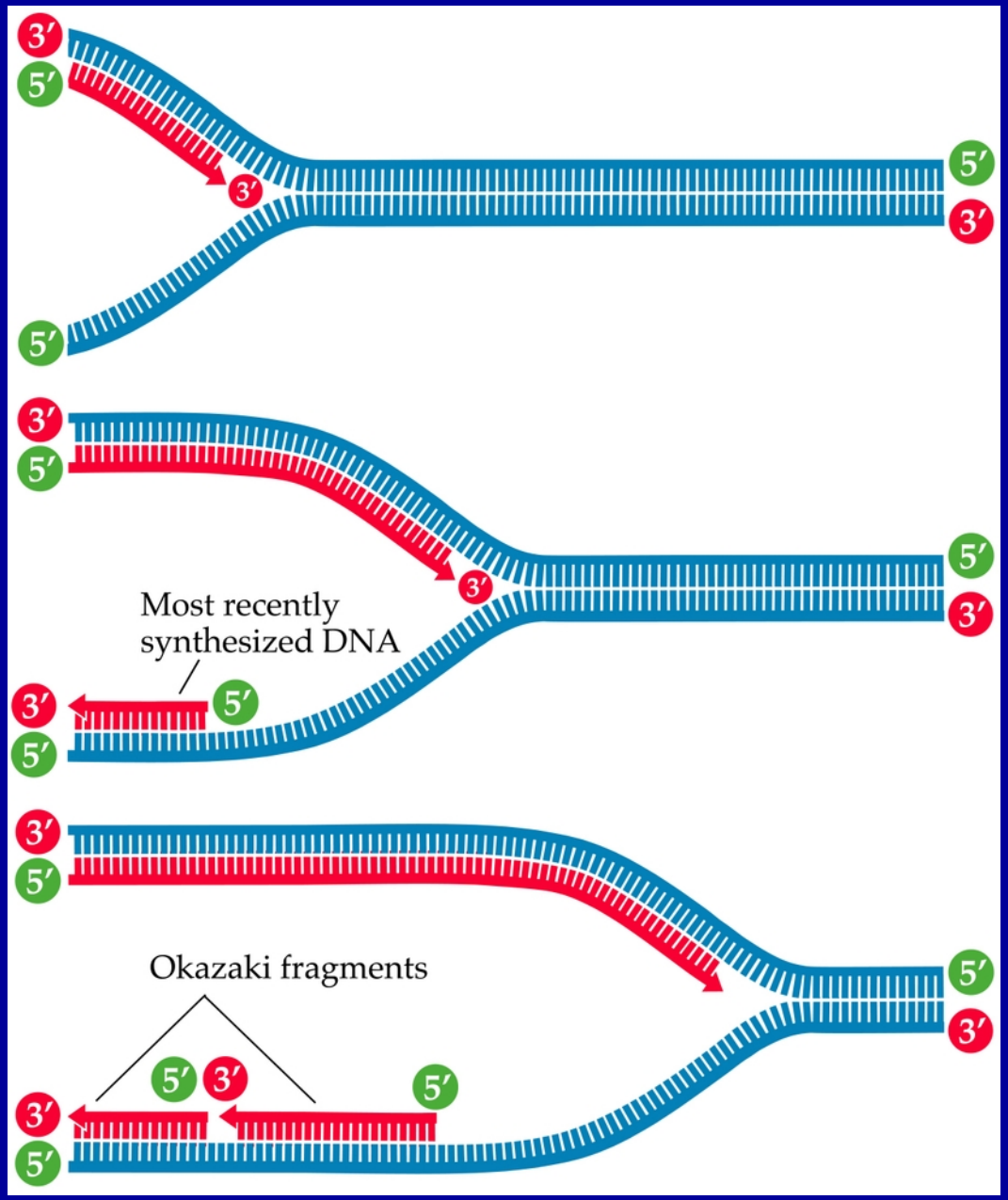
- On the lagging strand, growing in the other direction, DNA is made in the 5′-to-3′ direction but synthesis is discontinuous: DNA is added as short Okazaki fragments to primers, then DNA polymerase III skips past the 5′ end to make the next fragment.

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

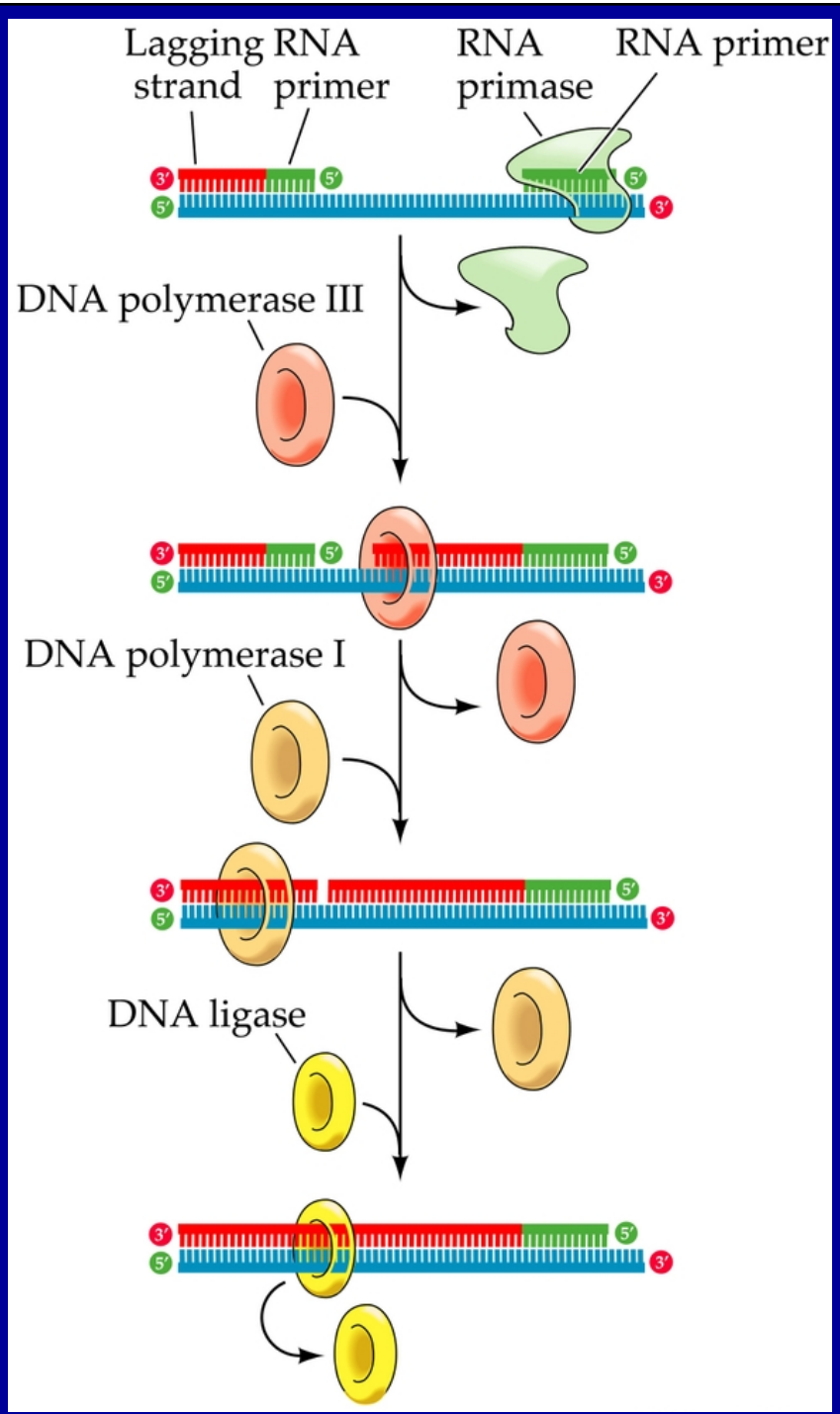
Many Proteins Collaborate at the Replication Fork



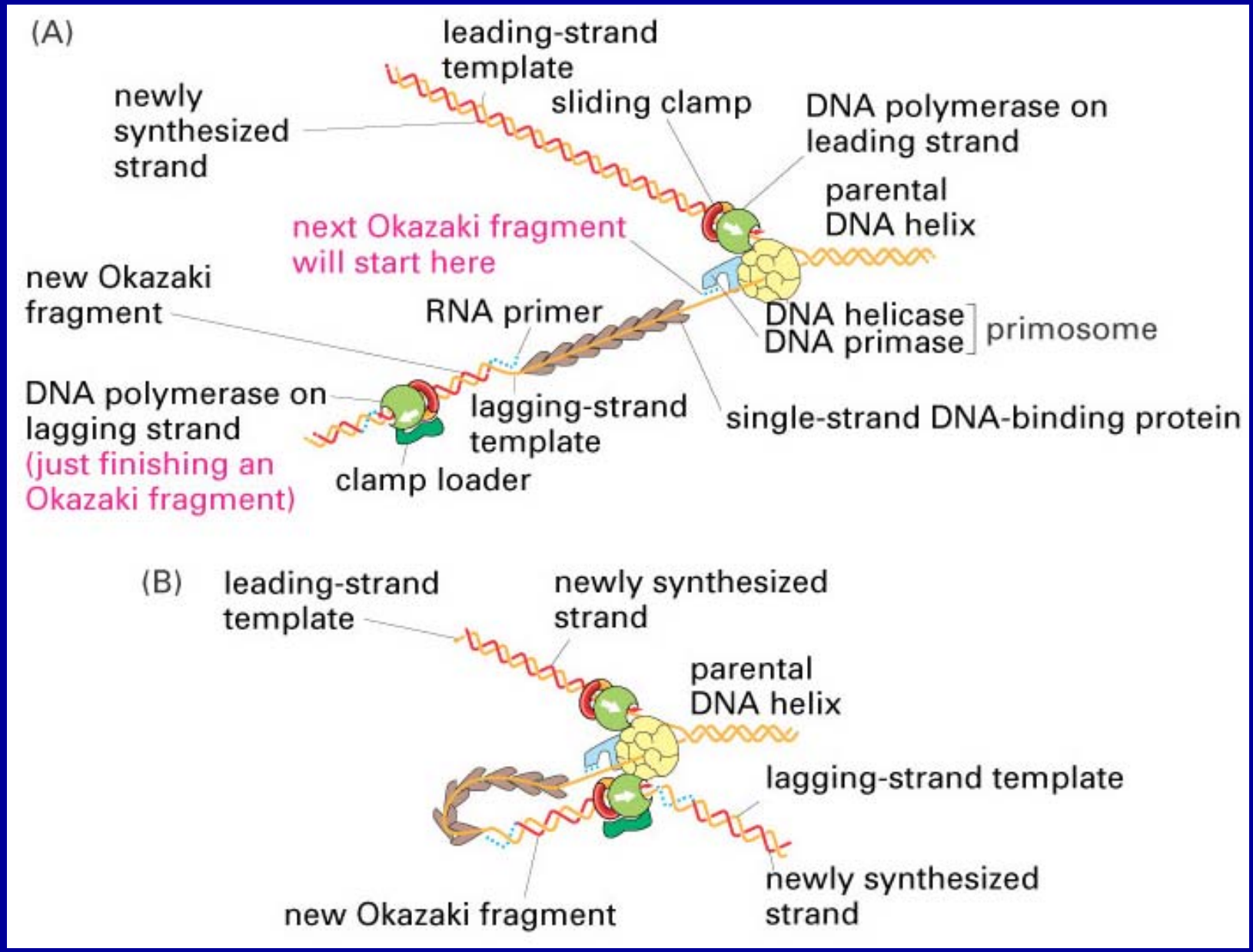
Two Daughter Stands form Different Ways



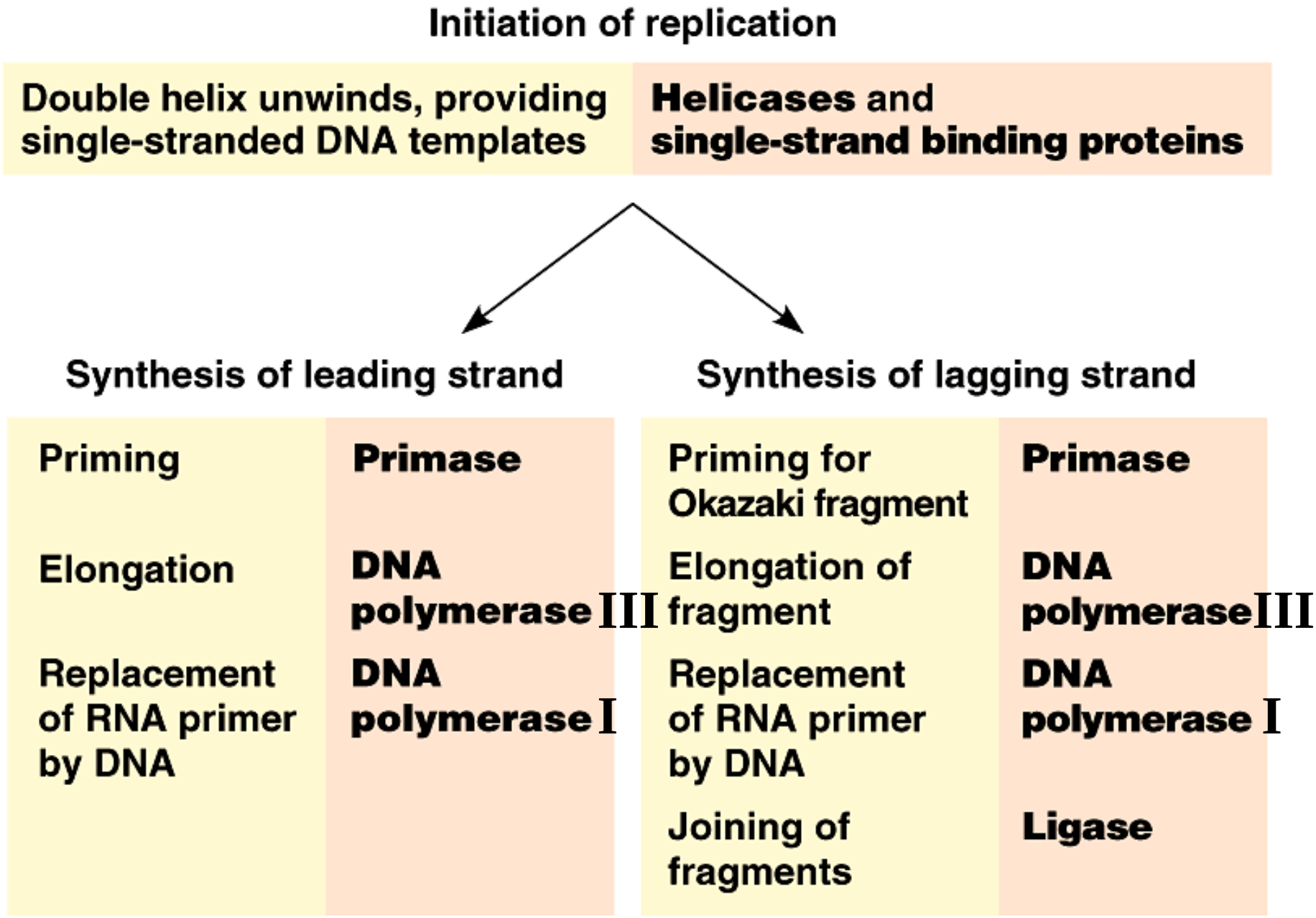
Continuous vs. Discontinuous!



Finishing touches on the discontinuous or lagging strand


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

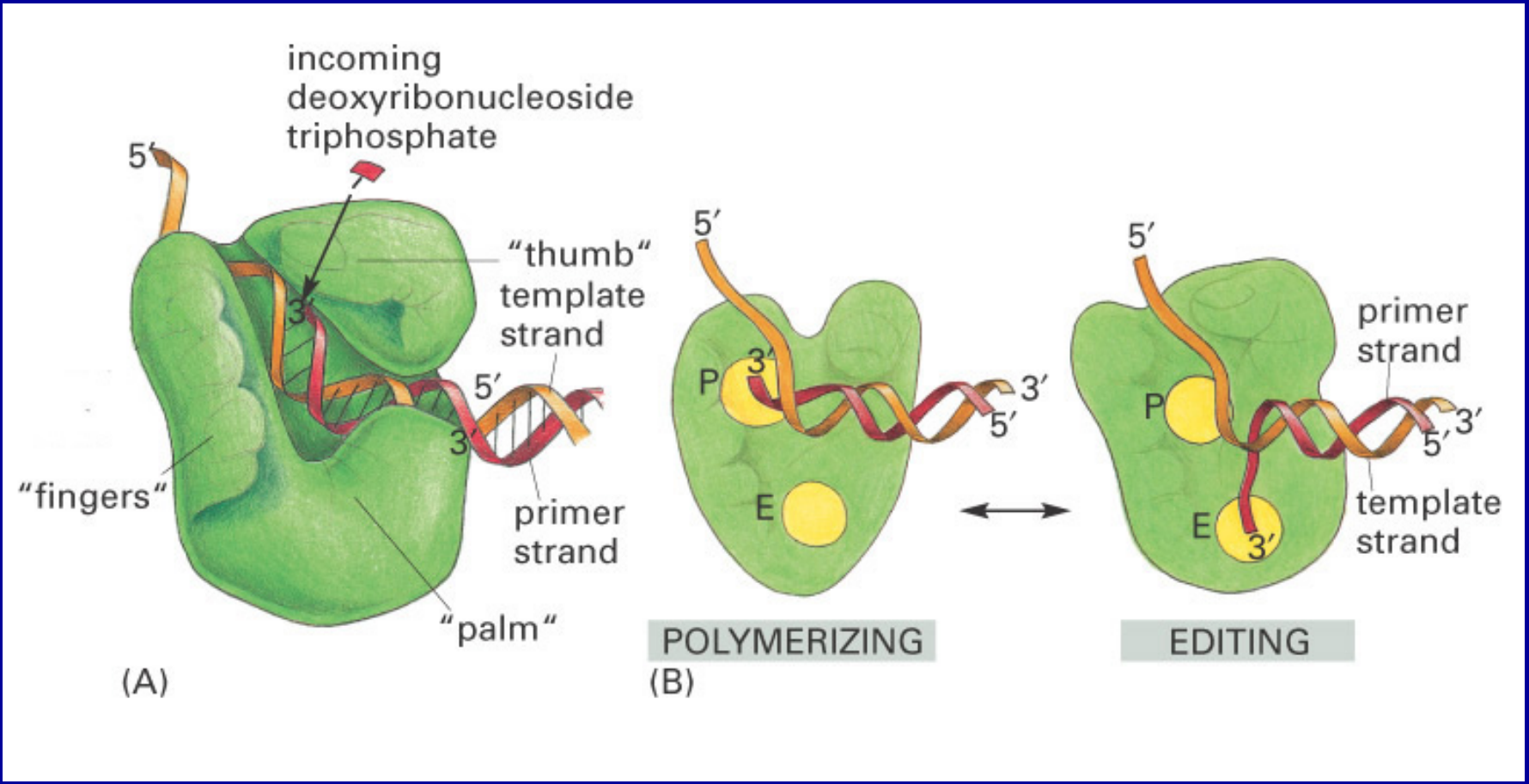
The main proteins of DNA replication and their functions

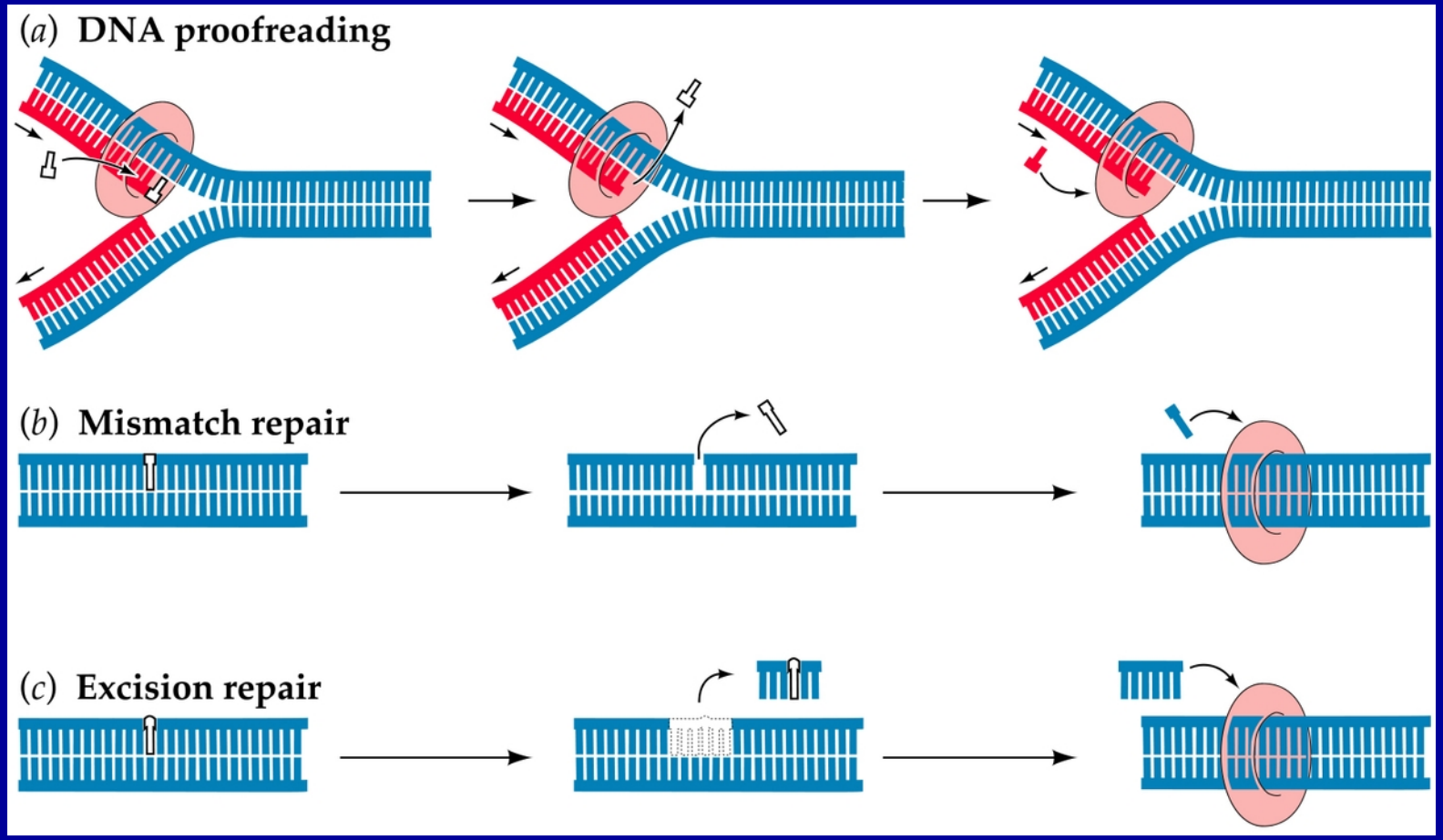


E. DNA Proofreading and Repair

- There is about about one error in 10^6 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.

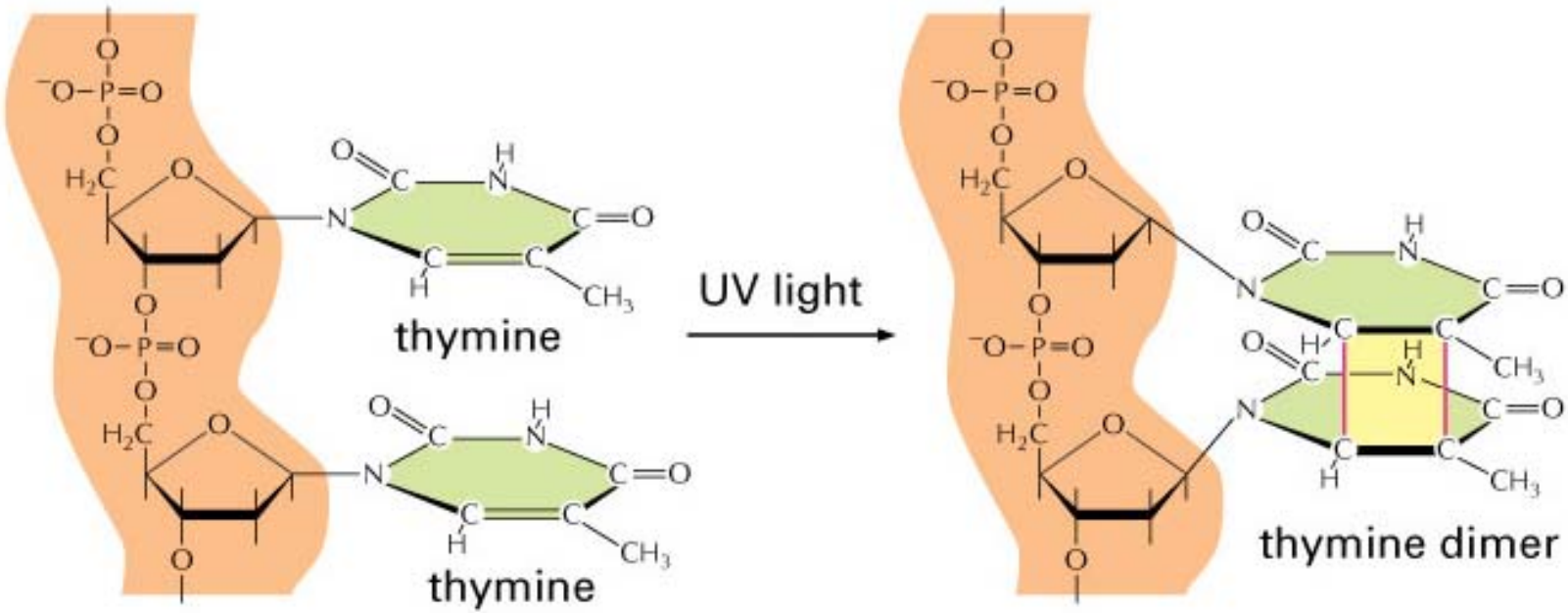
Proofreading: 3′ to 5′



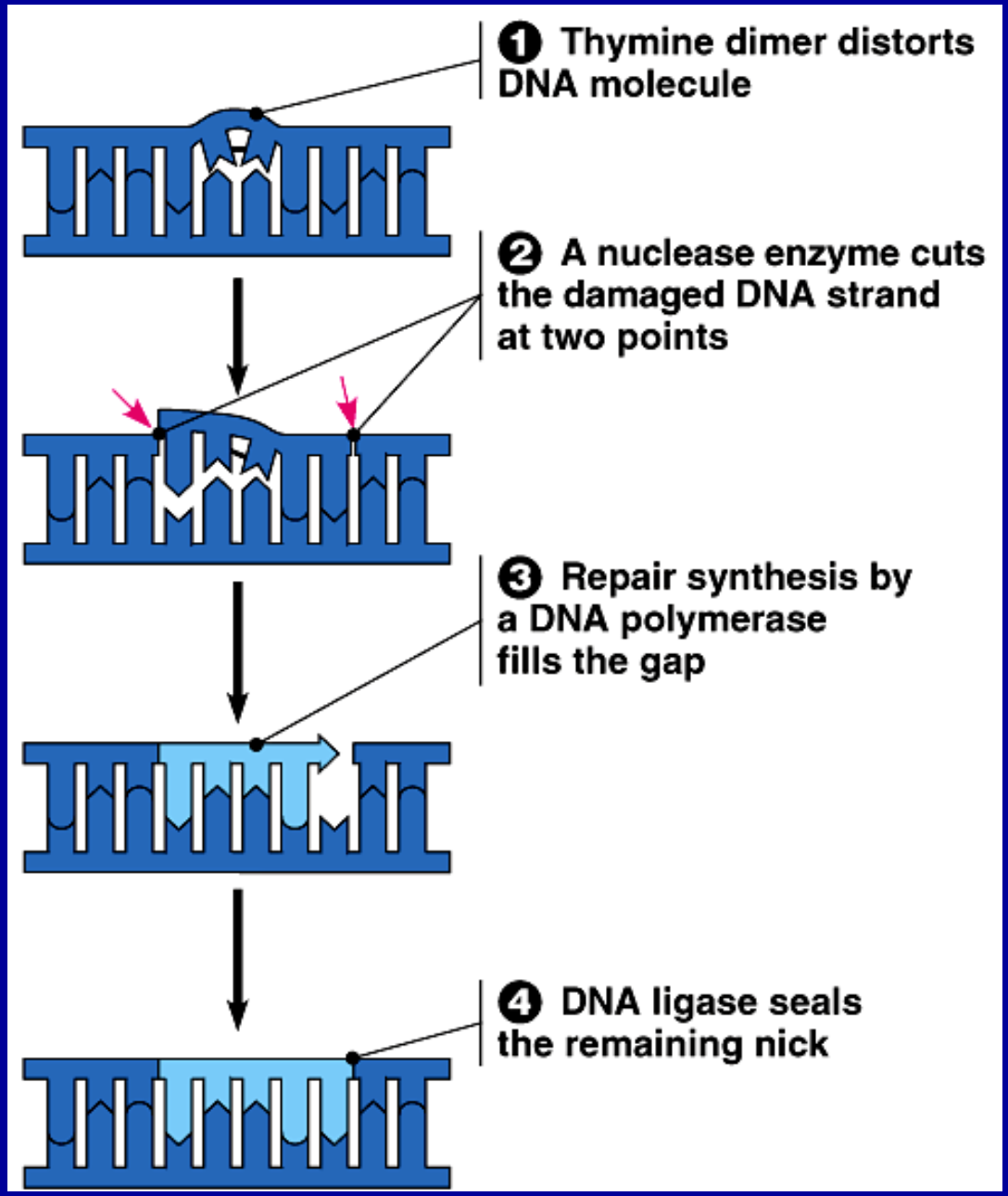

(a) DNA proofreading
(b) Mismatch repair
(c) Excision repair

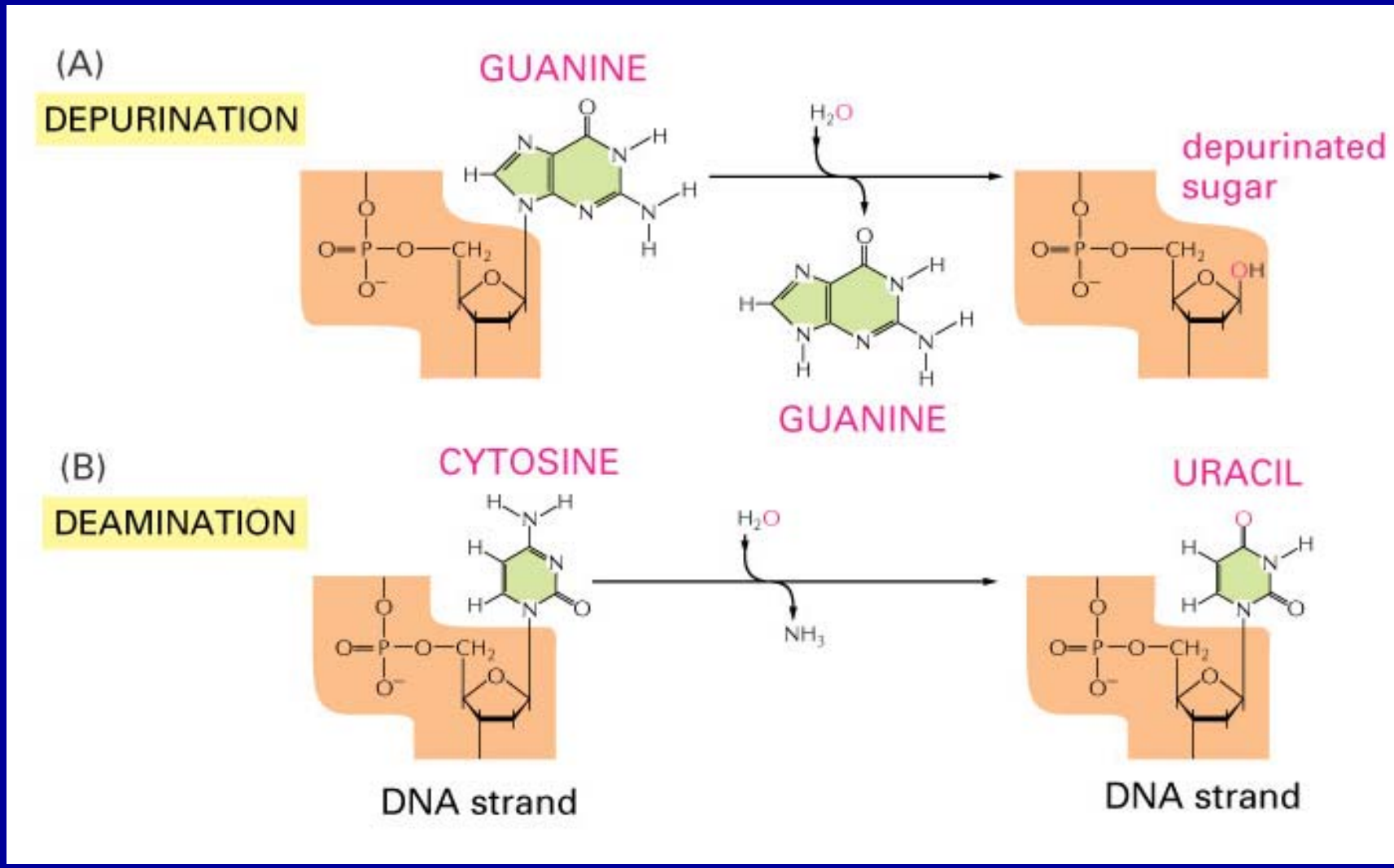
E. DNA Proofreading and Repair

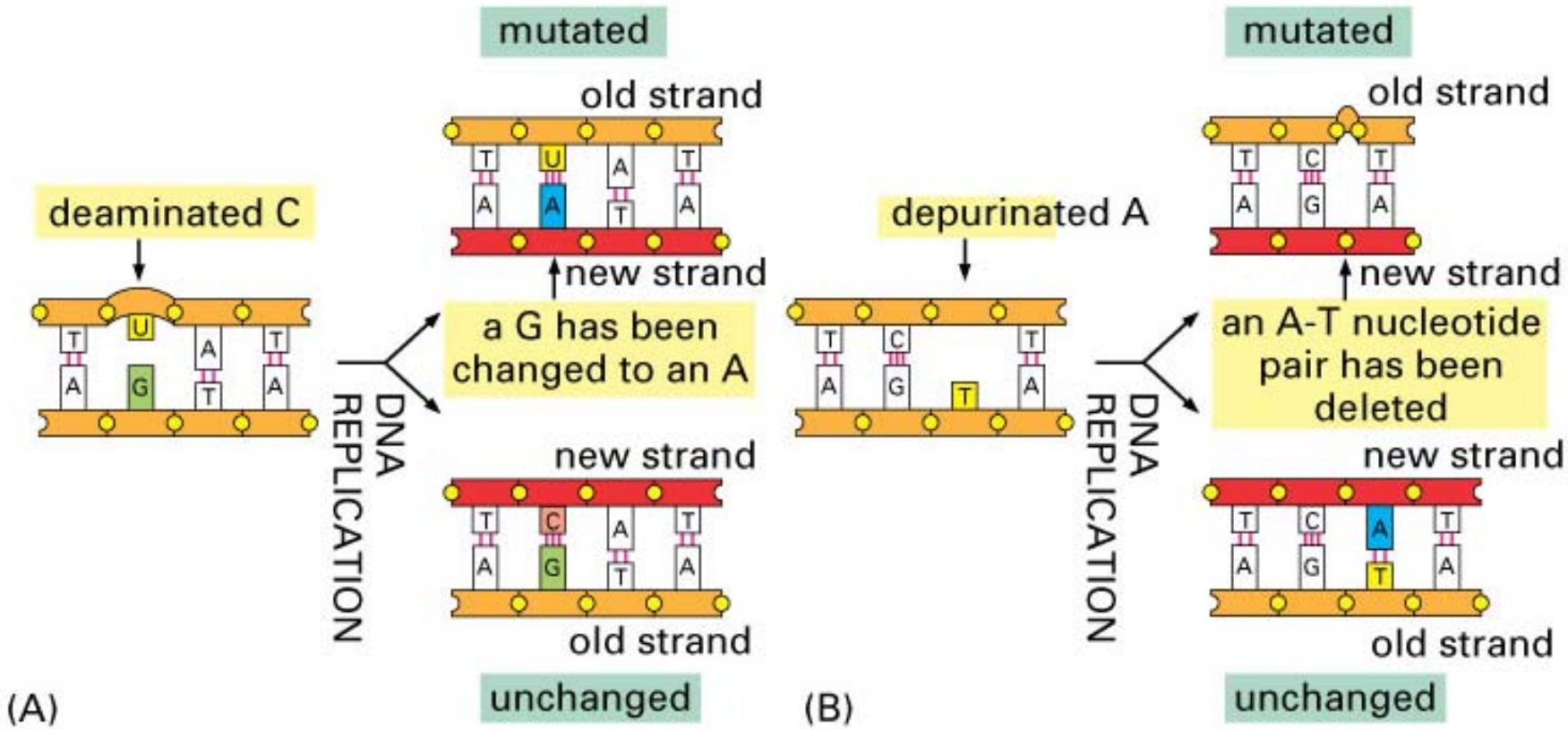
- Although energetically costly and somewhat redundant, DNA repair is crucial to the survival of the cell.


thymine
thymine
UV light
thymine dimer

Nucleotide excision repair of DNA damage



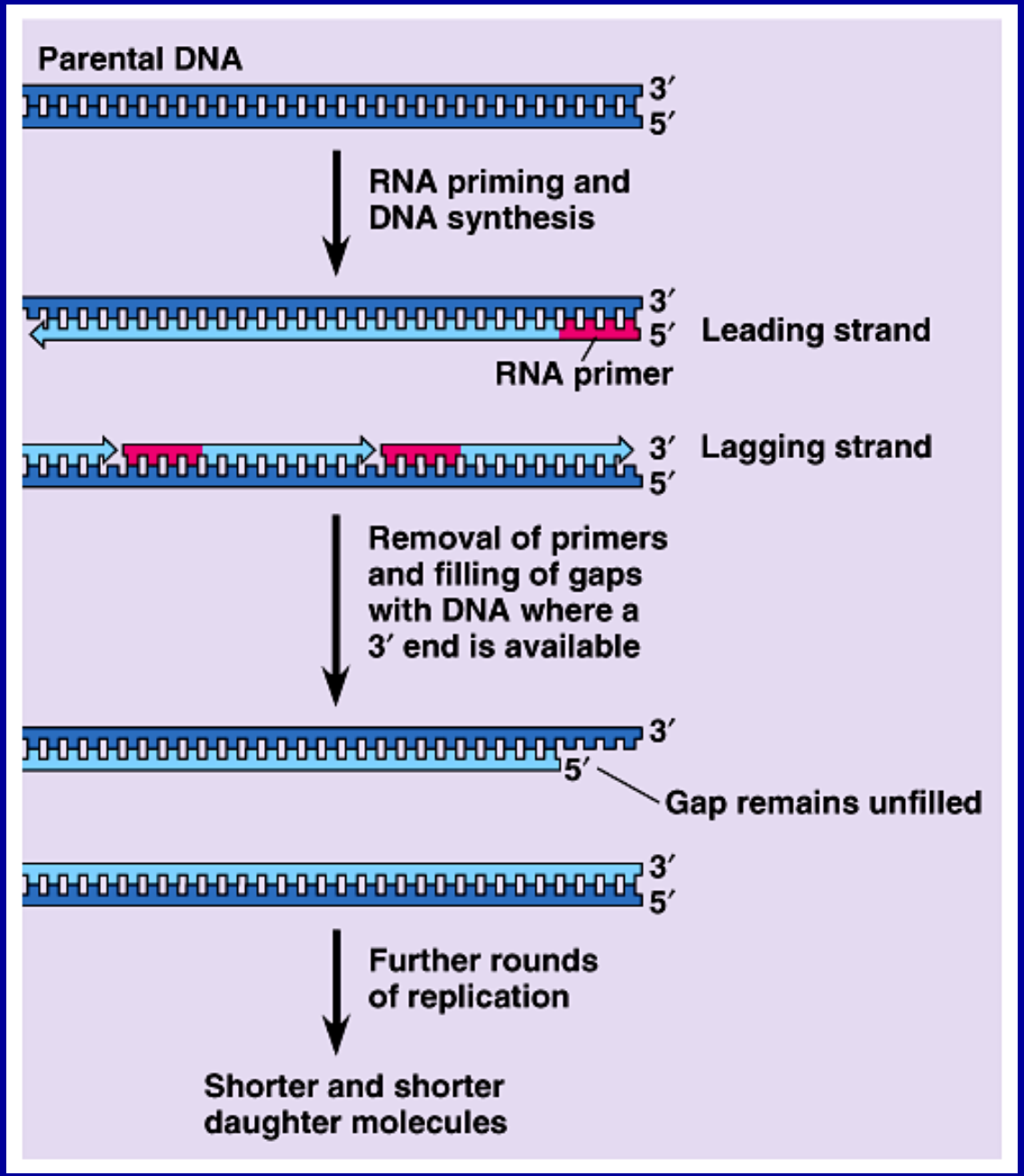

(A)
DEPURINATION
GUANINE
H_2O
depurinated sugar
GUANINE
(B)
DEAMINATION
CYTOSINE
URACIL
H_2O
NH_3
DNA strand
DNA strand


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)

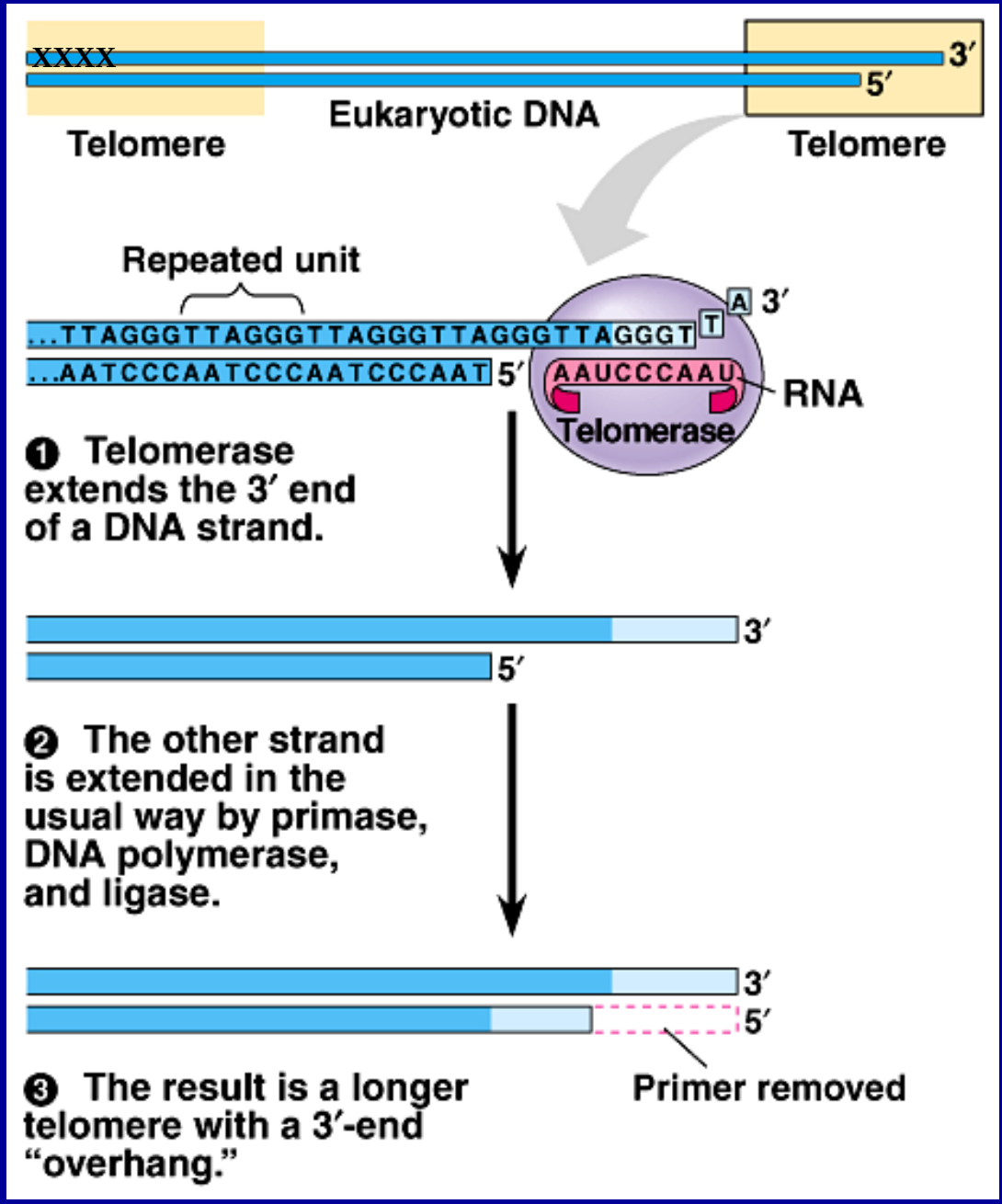
E. DNA Proofreading and Repair

- 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.

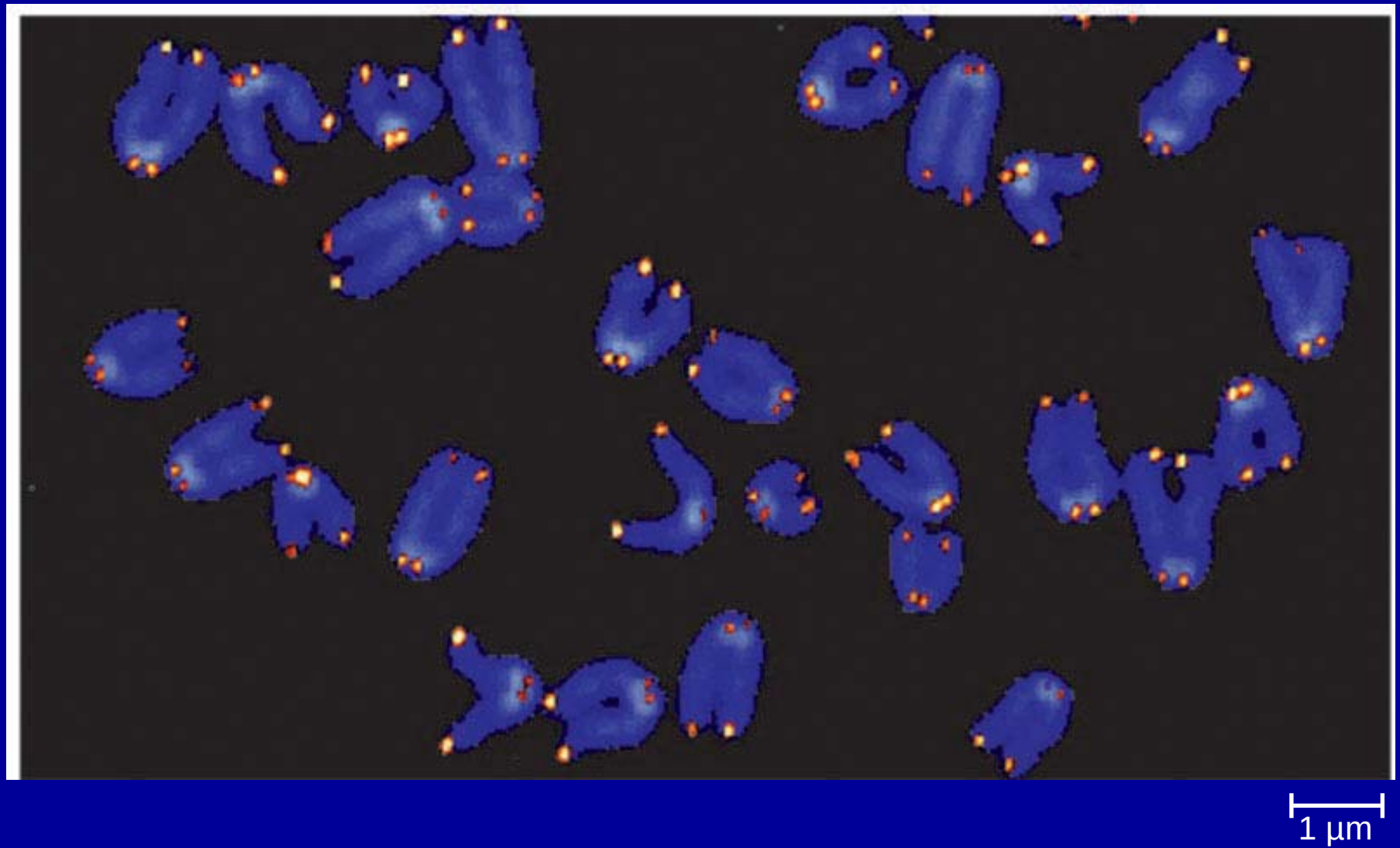
The linear end-replication problem



Telomeres and telomerase



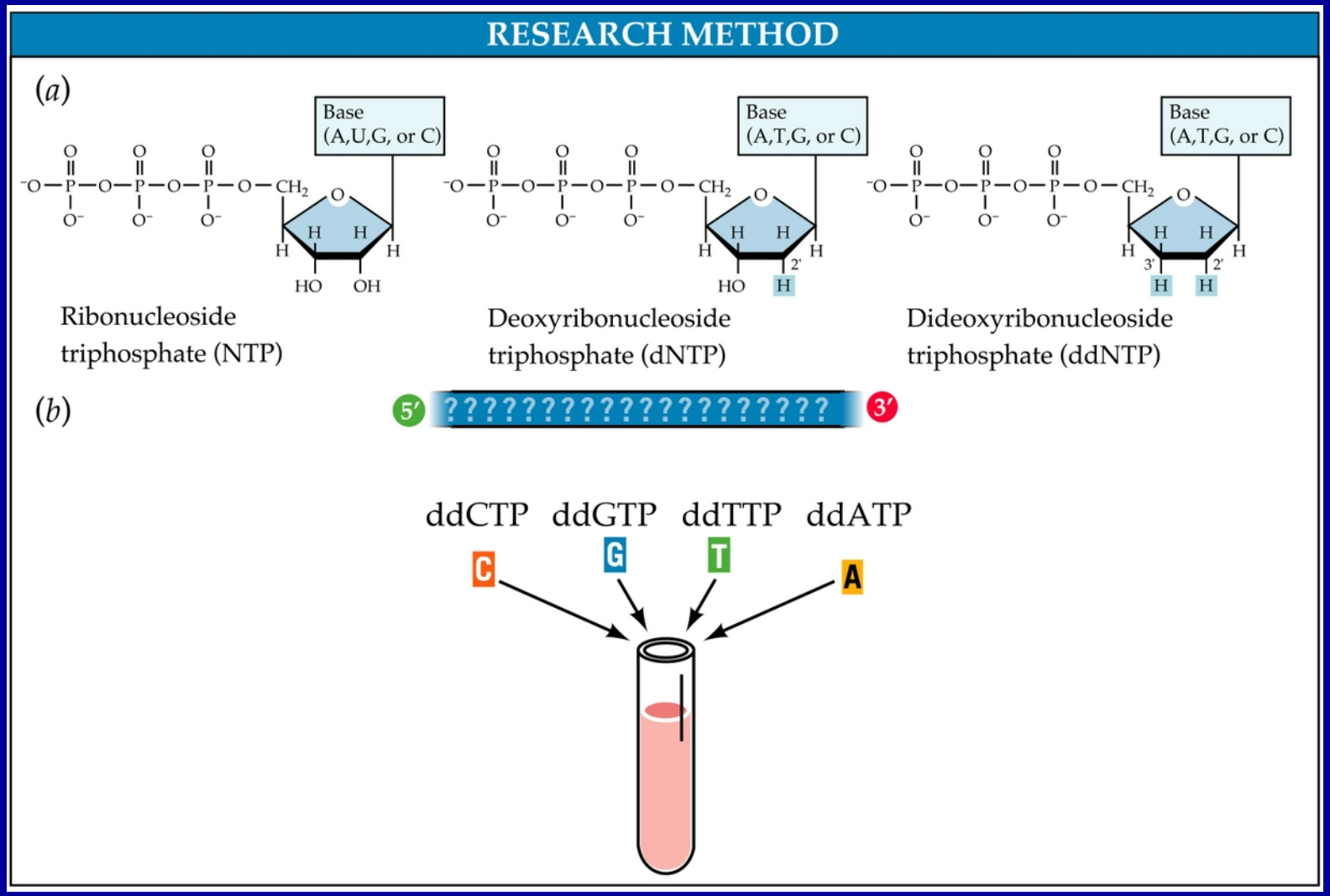
Telomeres (aka telomeric DNA)

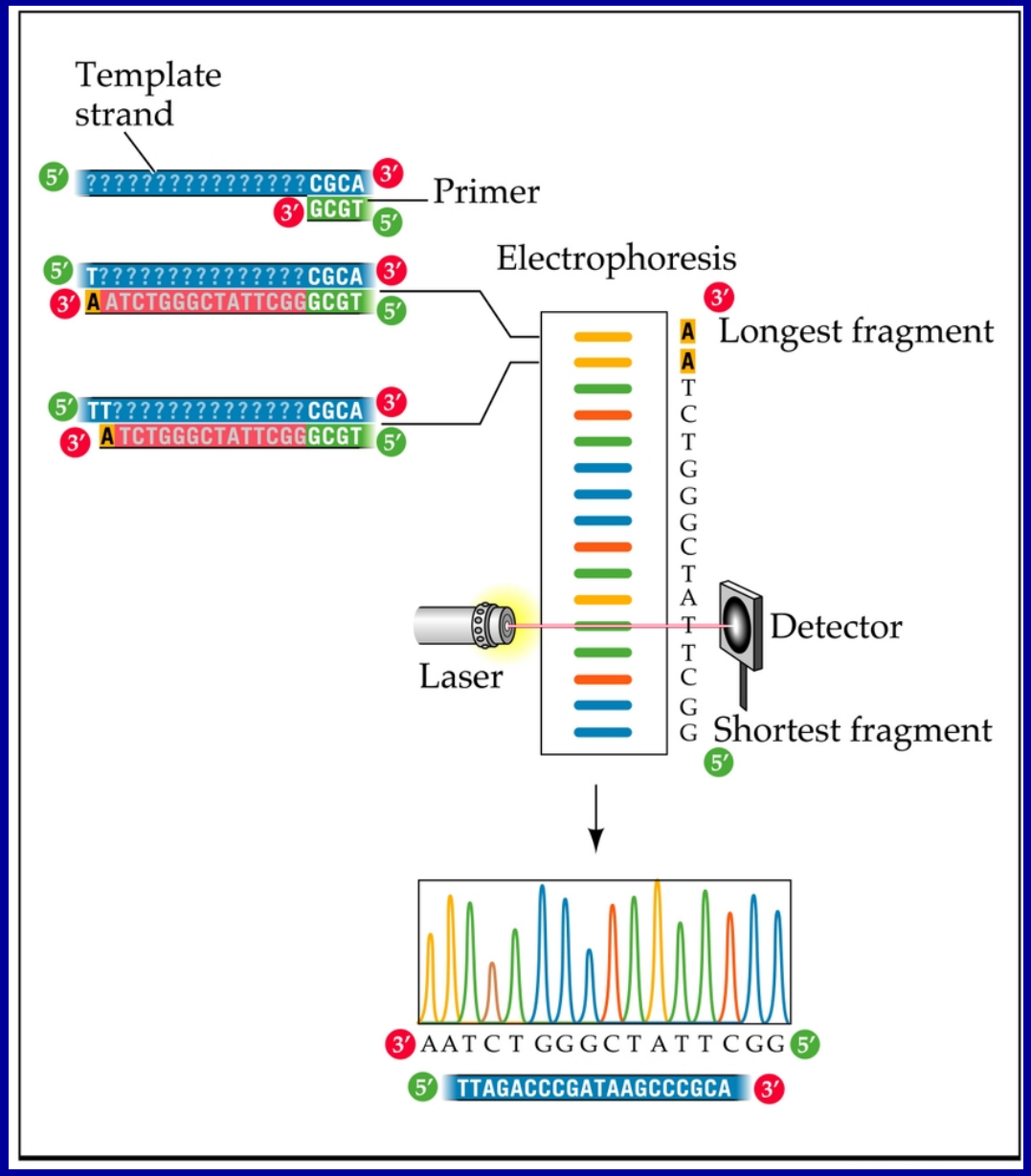


F. Practical Applications of DNA Replication

- 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′
Laser
Detector
Shortest fragment
3′ AATCTGGGCTATTCGG 5′
5′ TTAGACCCGATAAGCCCGCA 3′

Polymerase Chain Reaction: PCR