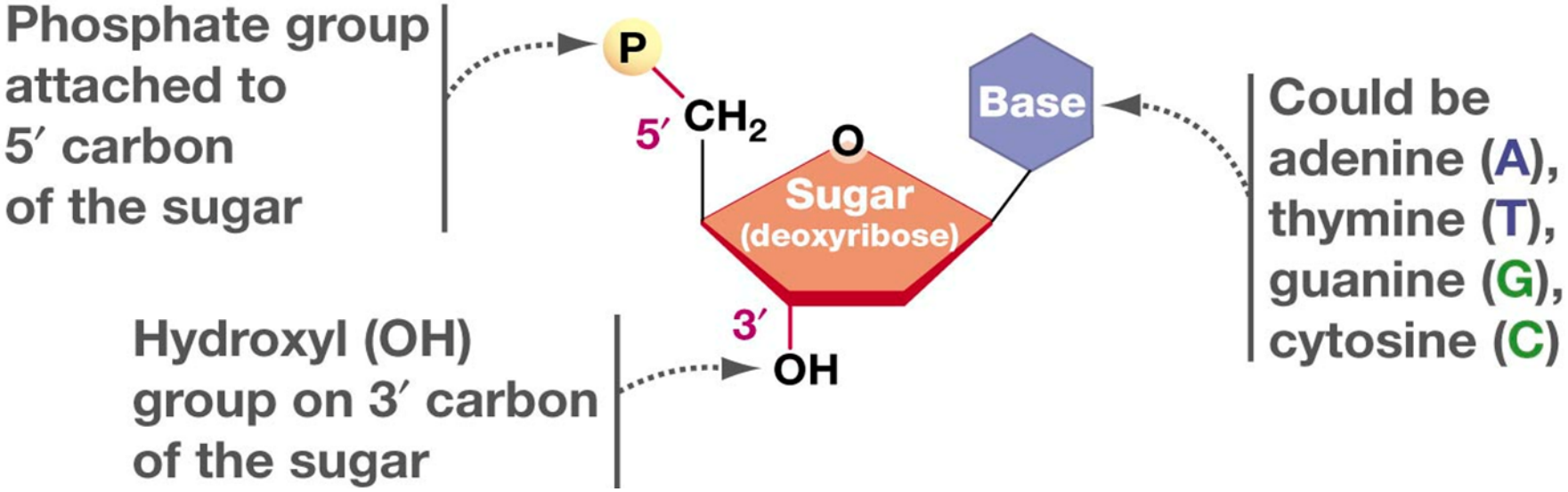
DNA SYNTHESIS & REPLICATION

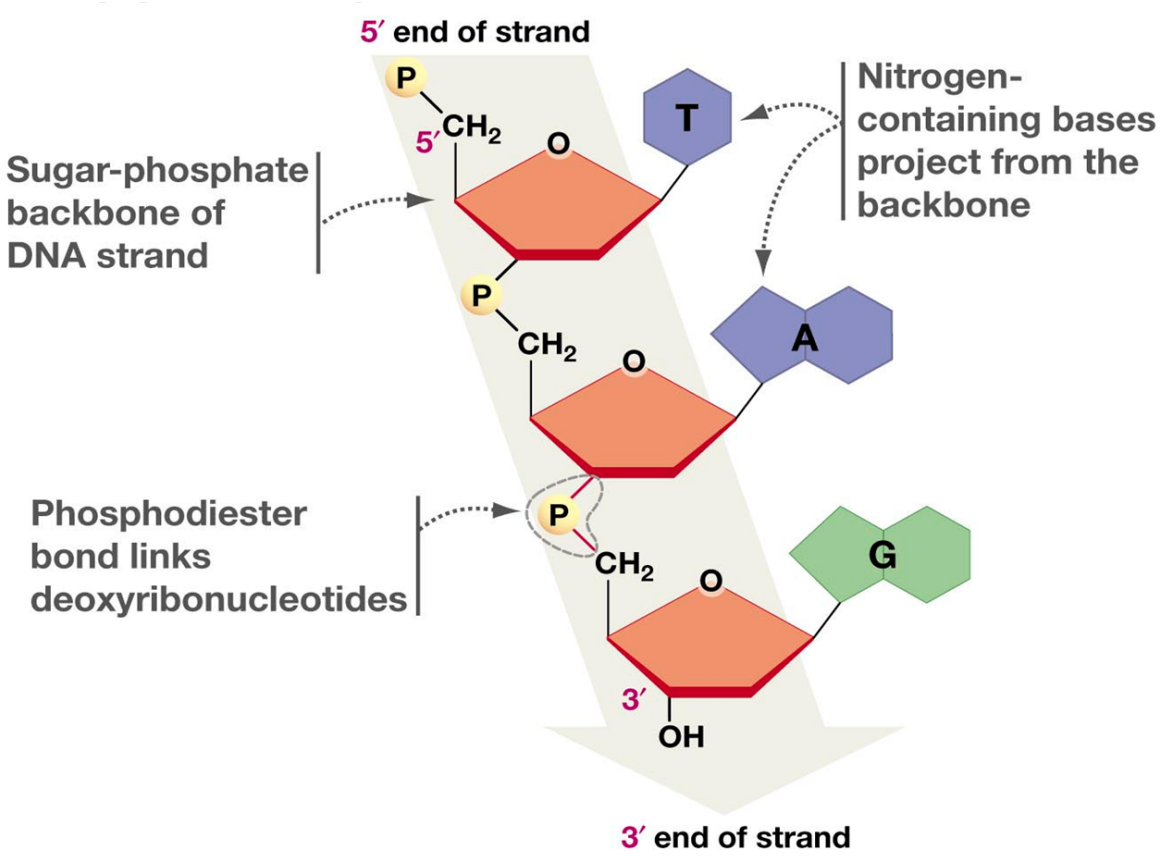
Structure of deoxyribonucleotide

- Primary structure of DNA
- Two components
  - Backbone: sugar + phosphate
  - Nitrogen containing bases: ATGC



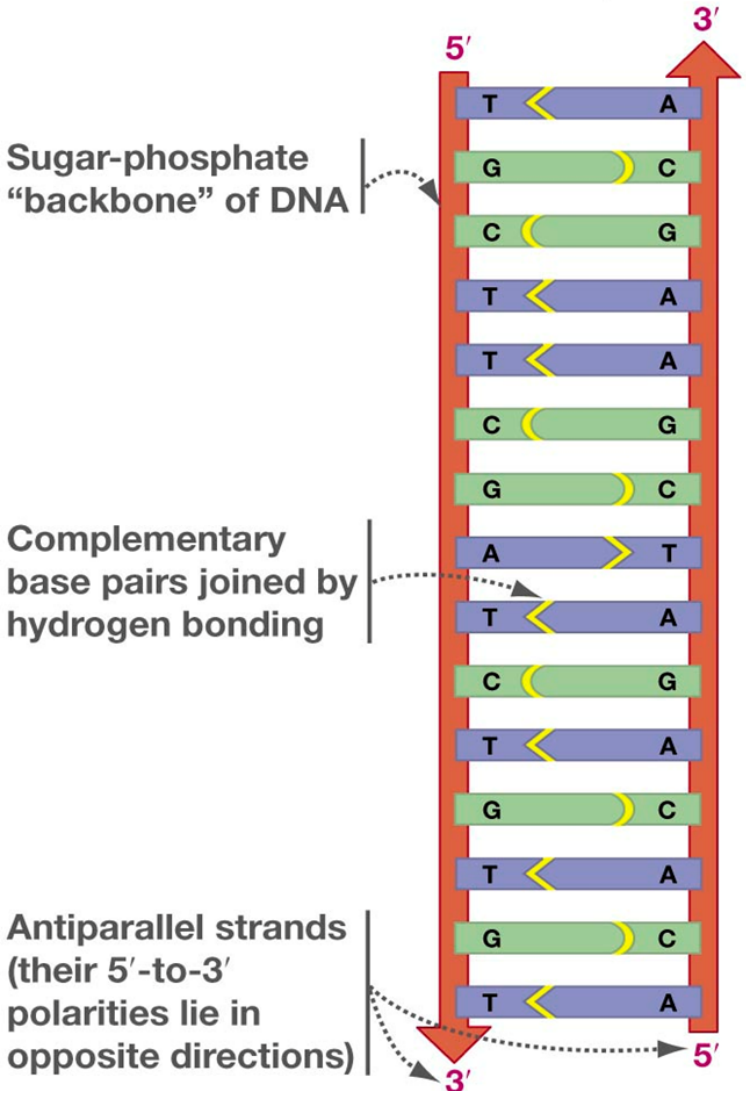
DNA backbone

- Phosphodiester linkages bond nucleotides
- DNA has a direction
  - 5' end: start
    - Phosphate
  - 3' end: finish
    - OH



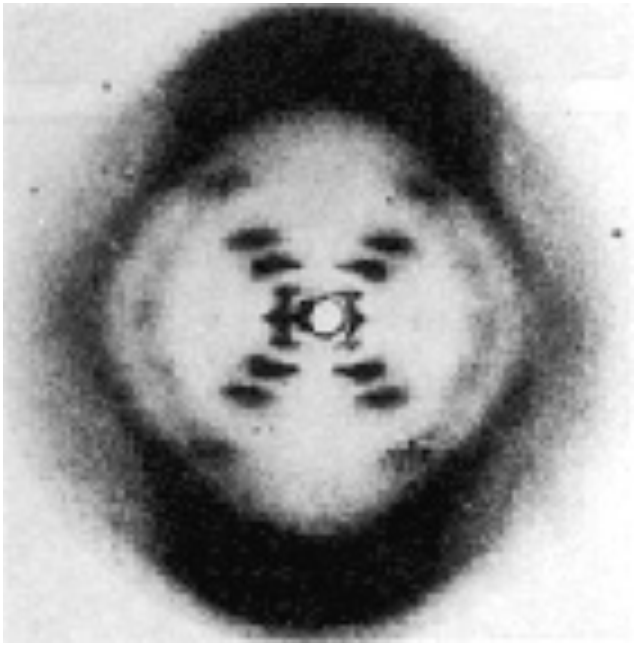
DNA's secondary structure

- Watson and Crick proposed:
  - 2 DNA strands are *antiparallel*
    - line up in opposite direction
    - 5' links with 3'
  - Base pairing is complementary
    - Adenine bonds with Thymine
    - Guanine bonds with Cytosine



DNA's secondary structure

- Watson and Crick proposed:
  - Twists to form double helix
- Rosalind Franklin
  - Laboratory technician
  - Provided photographic evidence
  - Research used without her permission

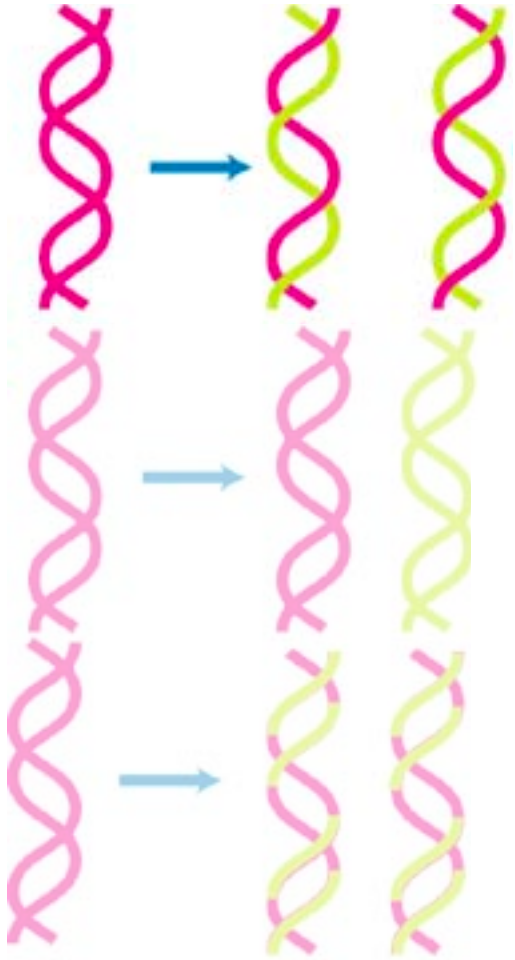


DNA as templates

- Watson and Crick proposed:
  - Strands of DNA served as templates (patterns) for production of new strands
  - According to complementary base pairing

Alt. hypotheses for DNA replication

- Semiconservative replication
  - Parental DNA separate and each strand is template
  - Daughter molecules: 1 old & 1 new strand
- Conservative replication
  - Parental DNA is template for synthesis of new molecule
- Dispersive replication
  - Daughter molecules old DNA interspersed with newly synthesized DNA



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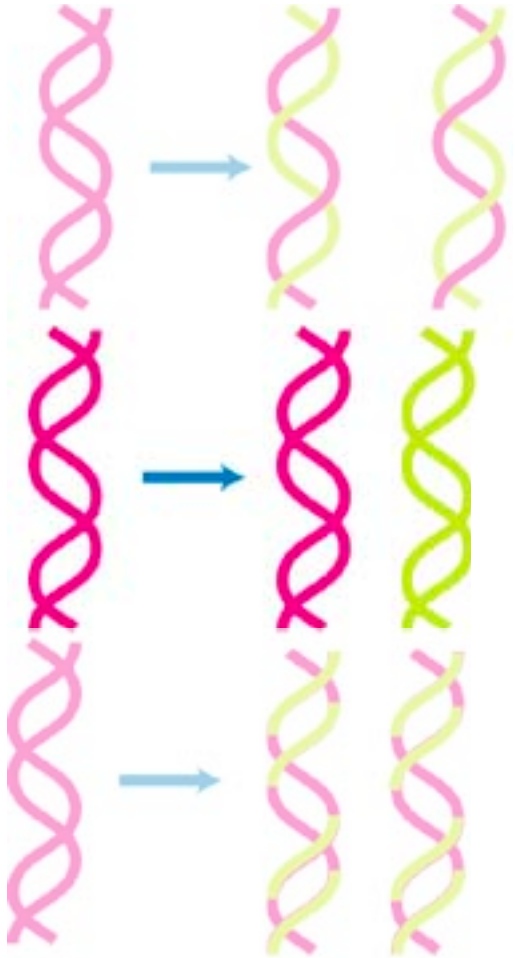

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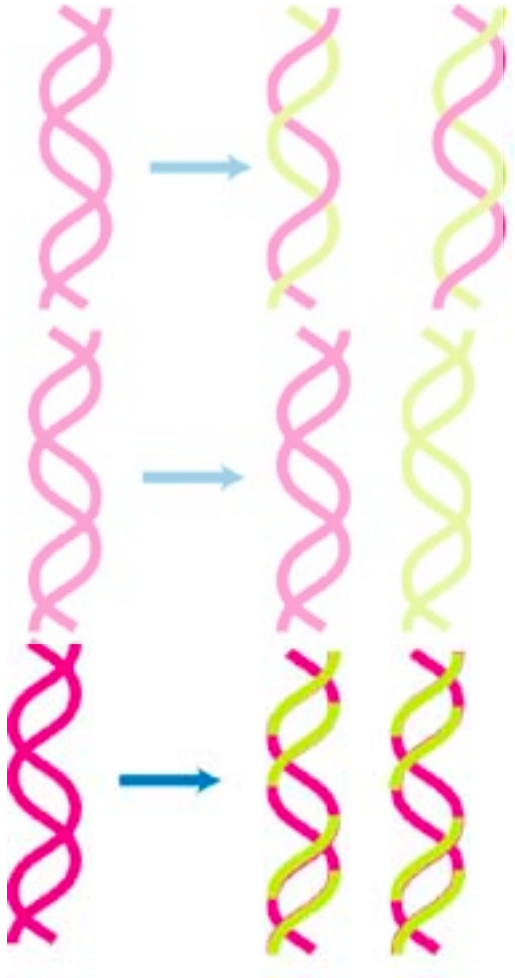
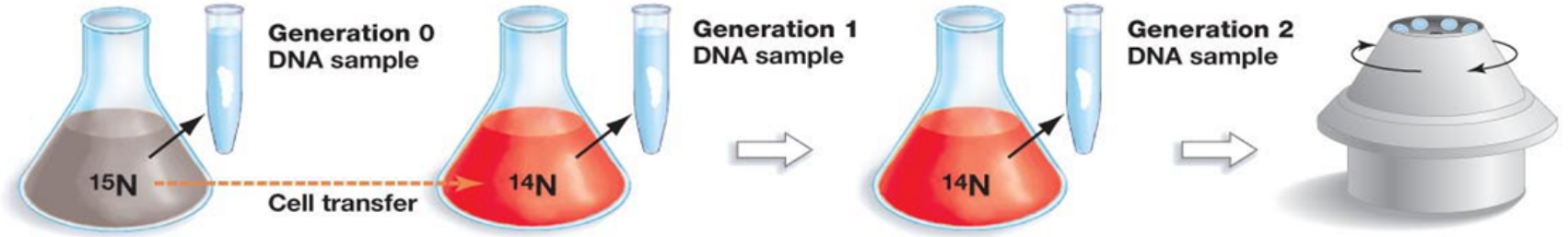


Meselson-Stahl Experiment

- To determine how replication occurs
- Grew *E. coli* in 'heavy' nitrogen (^{15}N)
- After many generations
  - Moved back to 'normal' nitrogen medium (^{14}N)
  - Separated DNA by density

EXPERIMENT

EXPERIMENTAL SETUP:



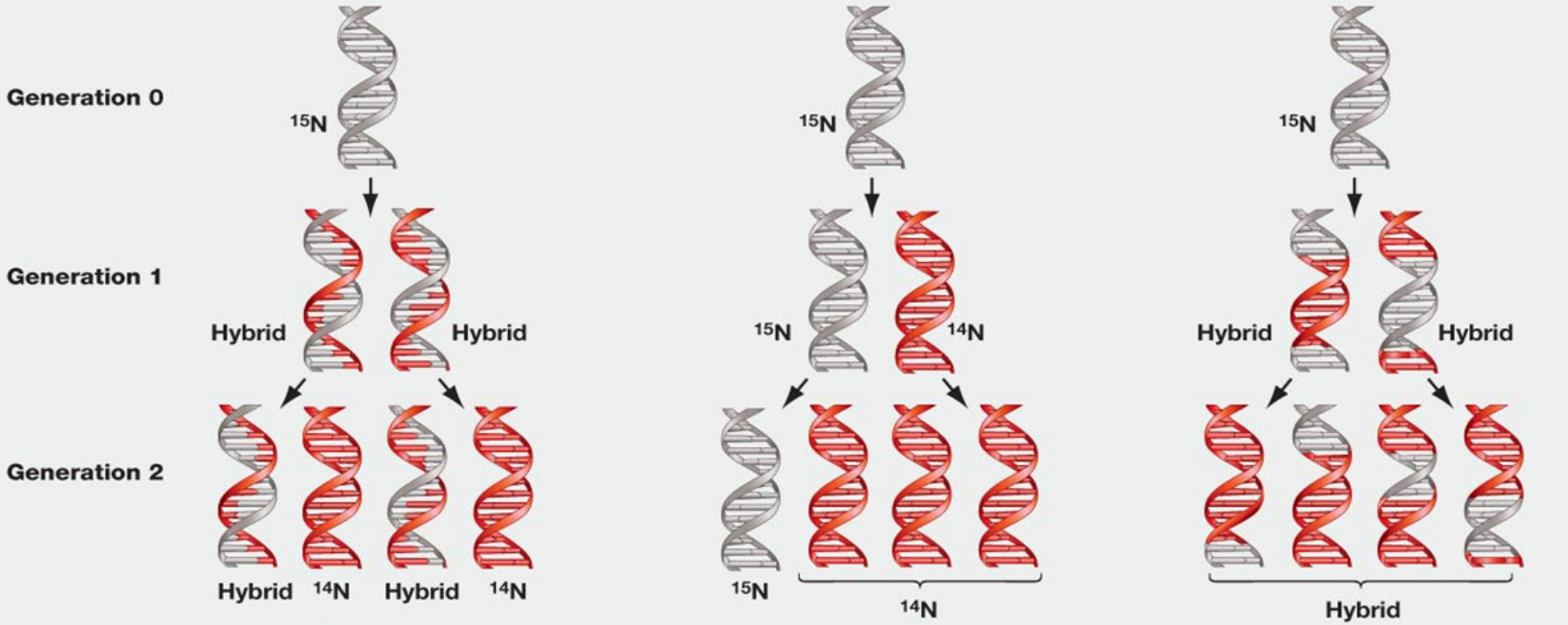
1. Grow *E. coli* cells in ^{15}N medium.

2. Transfer cells to ^{14}N medium. Collect samples. Purify DNA.

3. Leave cells in ^{14}N to divide again. Collect sample. Purify DNA.

4. Centrifuge samples.

PREDICTIONS:



Semiconservative replication

After 2 generations:
1/2 low-density DNA (^{14}N)
1/2 intermediate-density DNA (hybrid)

Conservative replication

After 2 generations:
1/4 high-density DNA (^{15}N)
3/4 low-density DNA (^{14}N)

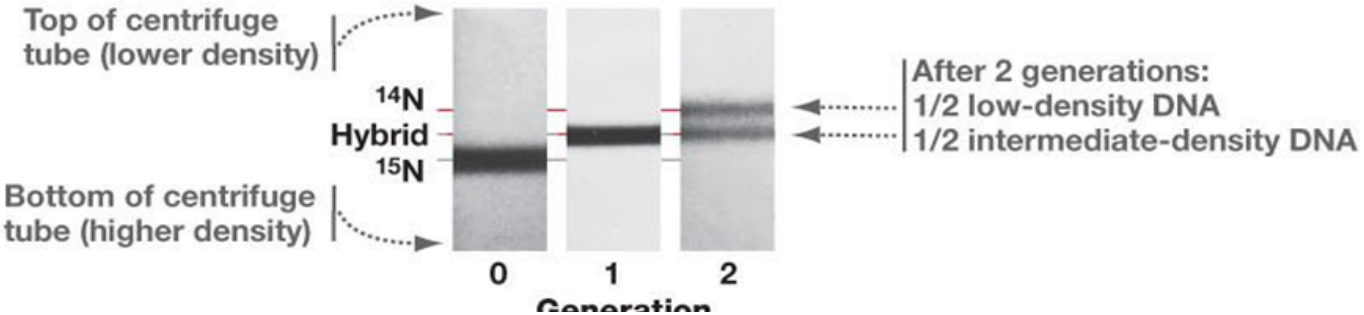
Dispersive replication

After 2 generations:
All intermediate-density DNA (hybrid)

Meselson-Stahl Experiment

EXPERIMENT

RESULTS:

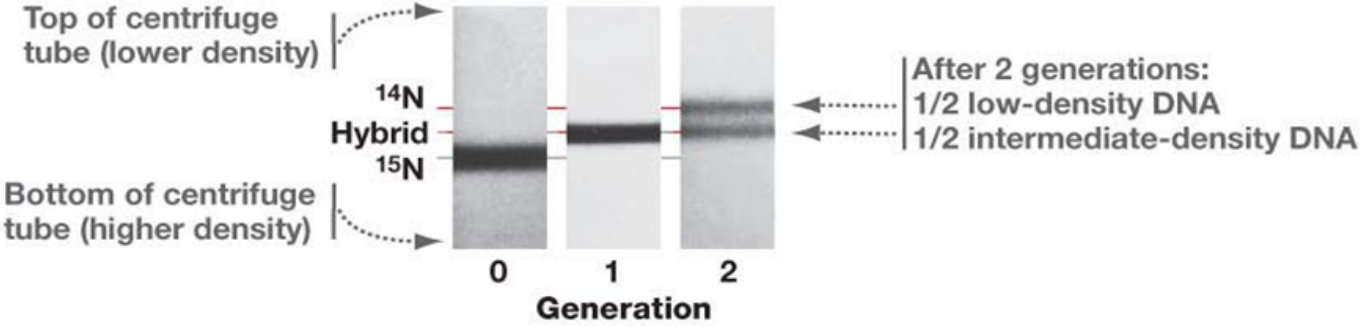


What was their conclusion?

Meselson-Stahl Experiment

EXPERIMENT

RESULTS:



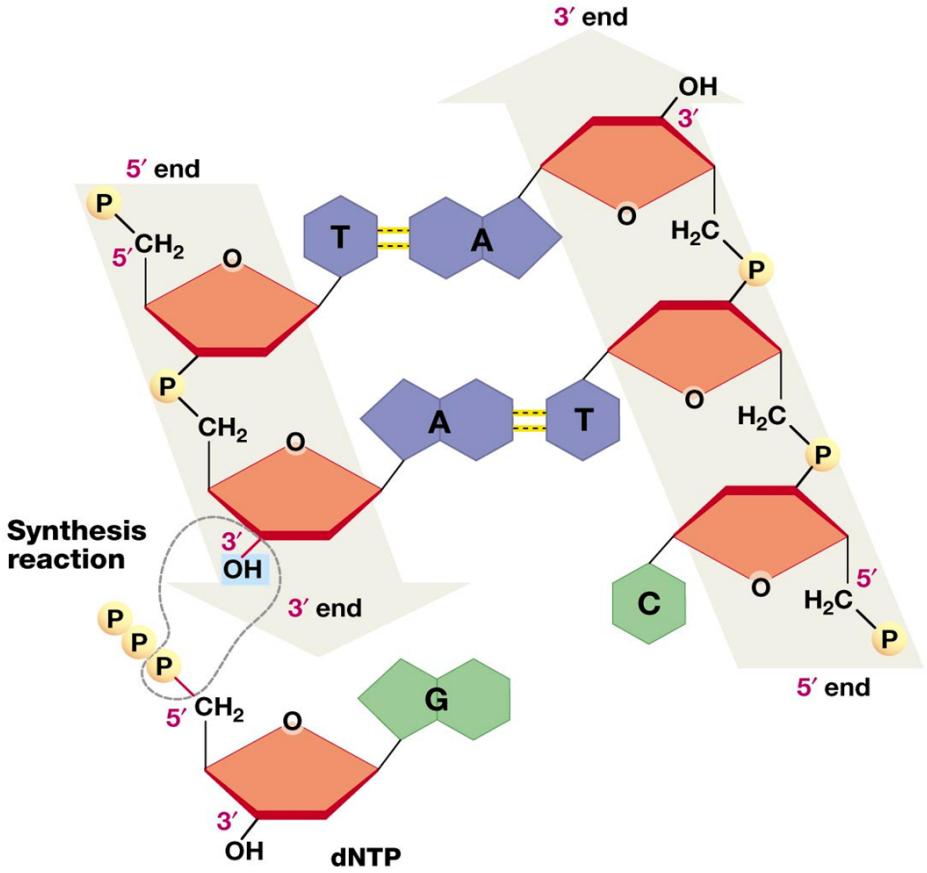
CONCLUSION: Data from generation 1 conflict with conservative-replication hypothesis. Data from generation 2 conflict with dispersive-replication hypothesis. Replication is semiconservative.

DNA polymerase

- Meselson & Stahl showed
  - each parental DNA strand is copied in entirety
  - Did not give a mechanism
- DNA polymerase discovery
  - Cleared way for understanding DNA synthesis

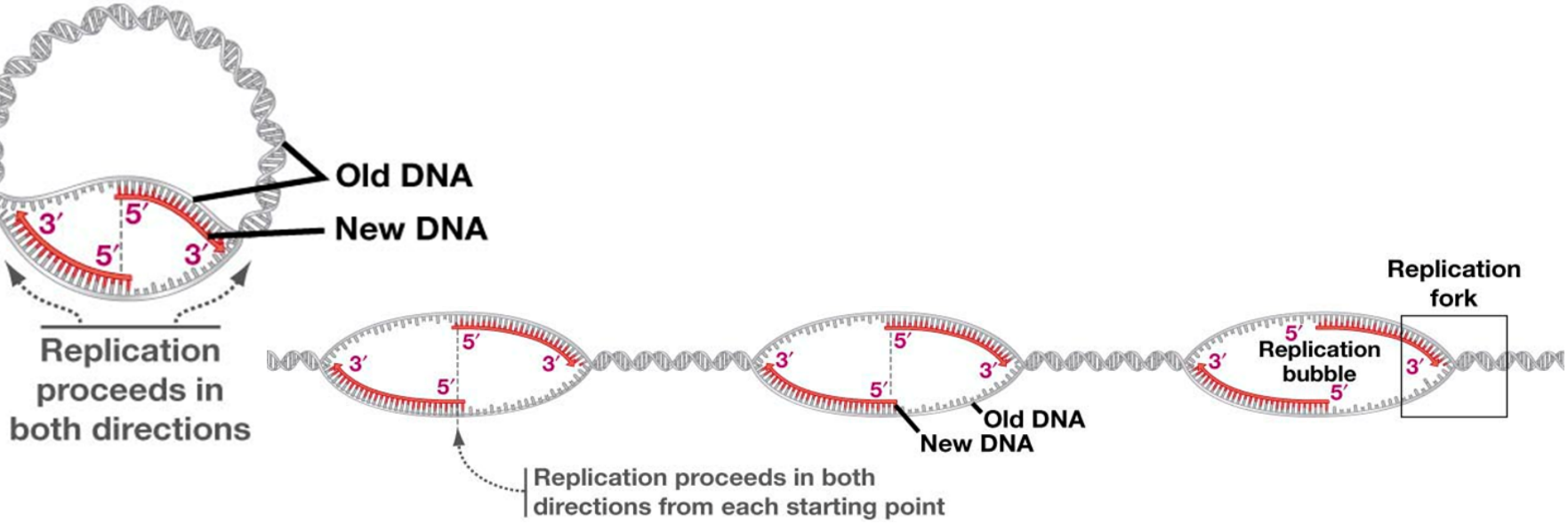
DNA polymerase

- Only work in one direction
- Add nucleotides only to 3' end
- DNA synthesis always proceeds
  - 5′ → 3′ direction



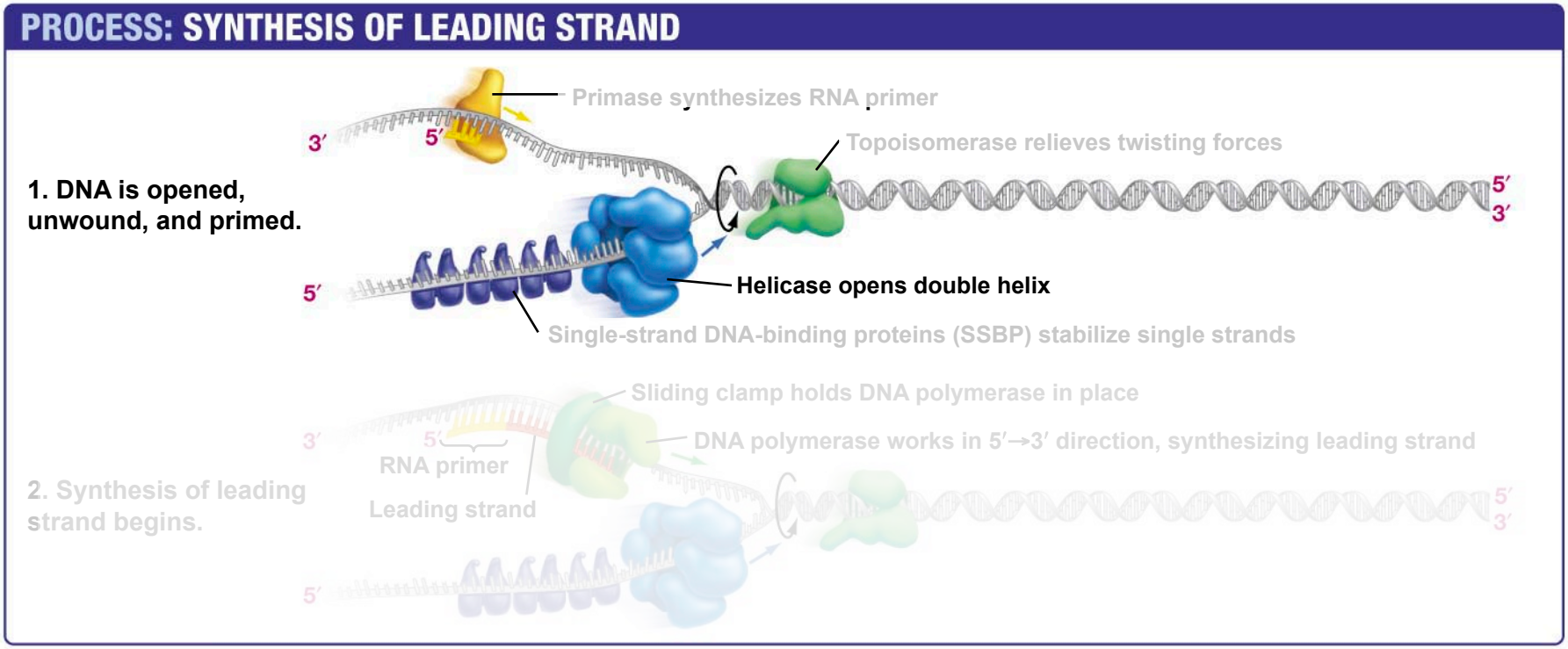
Initiation of replication

- Replication bubble forms in chromosome
- Synthesis proceeds bidirectionally
  - Bacteria have a single origin of replication
  - Eukaryotes have multiple origins of replication



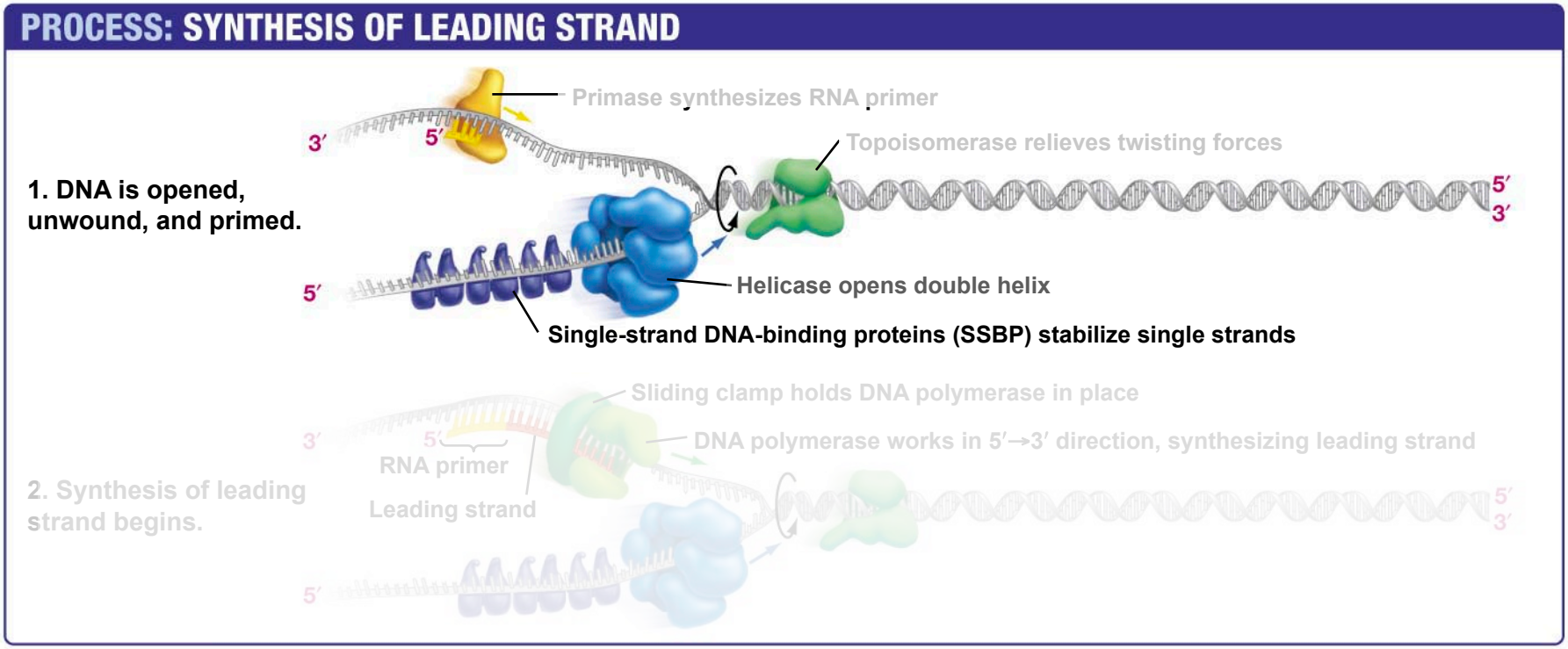
Initiation of replication

- Helix is opened by:
  - Helicase
    - Enzyme that breaks bonds b/n DNA strands



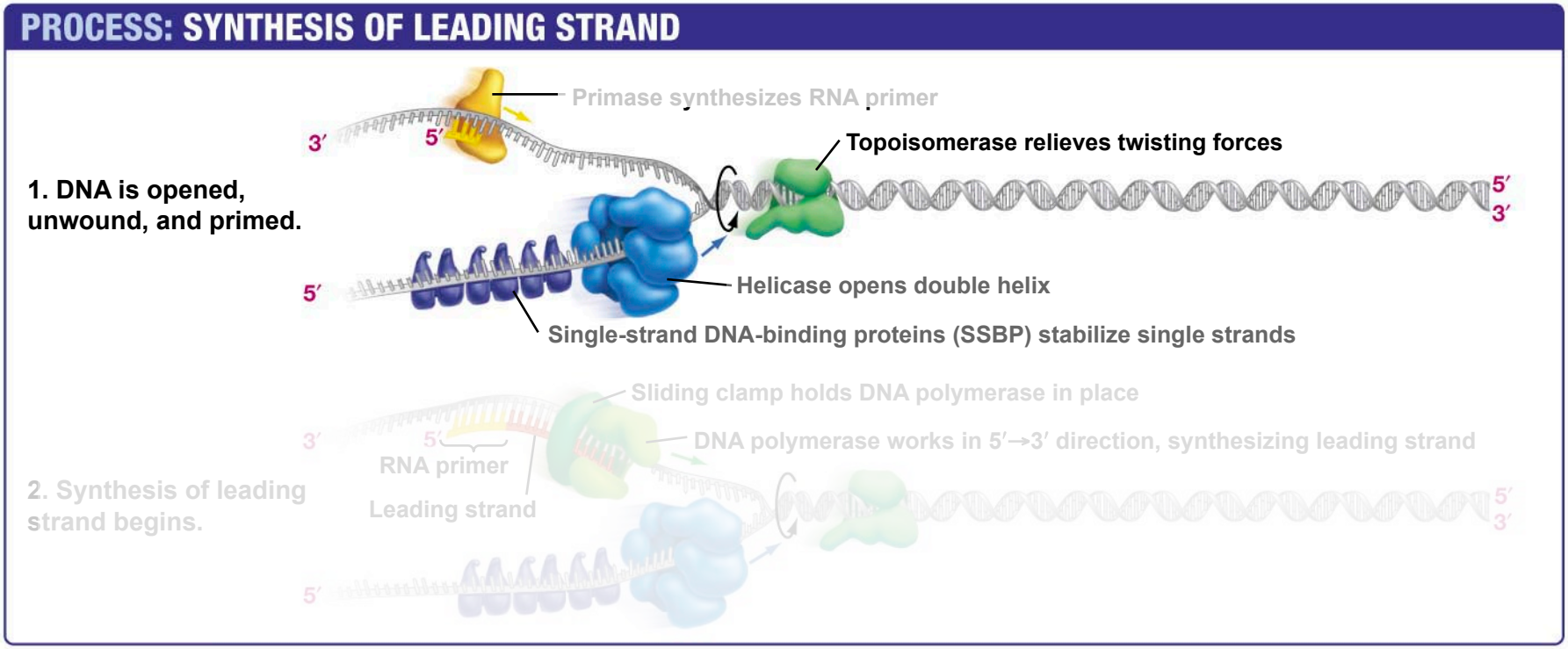
Initiation of replication

- Helix is stabilized by:
  - Single-strand DNA-binding proteins (SSBPs)
    - Attach to separate strands to prevent closing



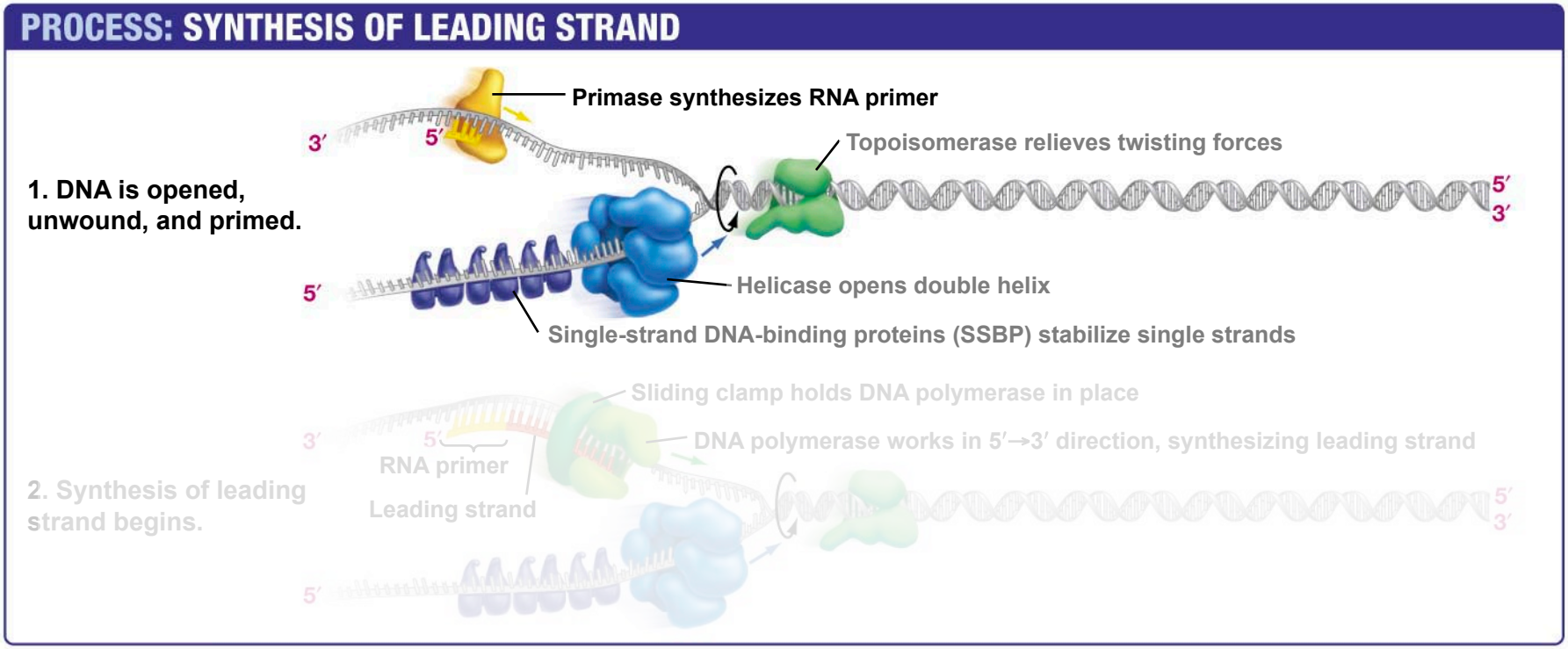
Initiation of replication

- Unwinding of helix creates tension
  - Topoisomerase
    - Cuts and rejoins DNA downstream of replication fork
    - Relieving tension



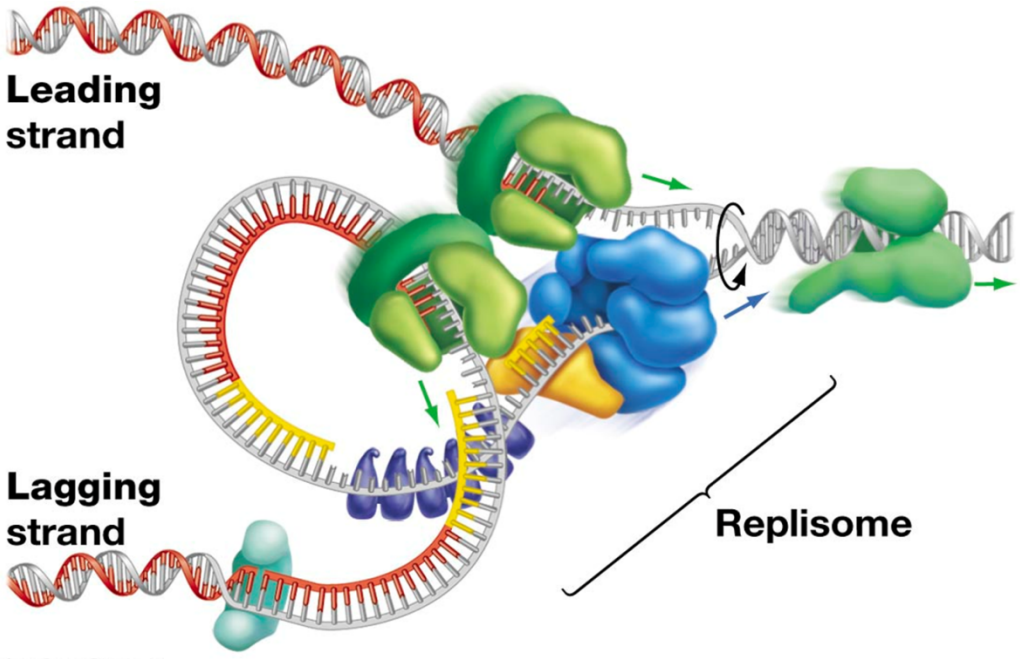
Initiation of replication

- DNA polymerase requires a primer (Primase)
  - Provides a 3' hydroxyl (OH) group that can combine with a nucleotide to form first phosphodiester bond



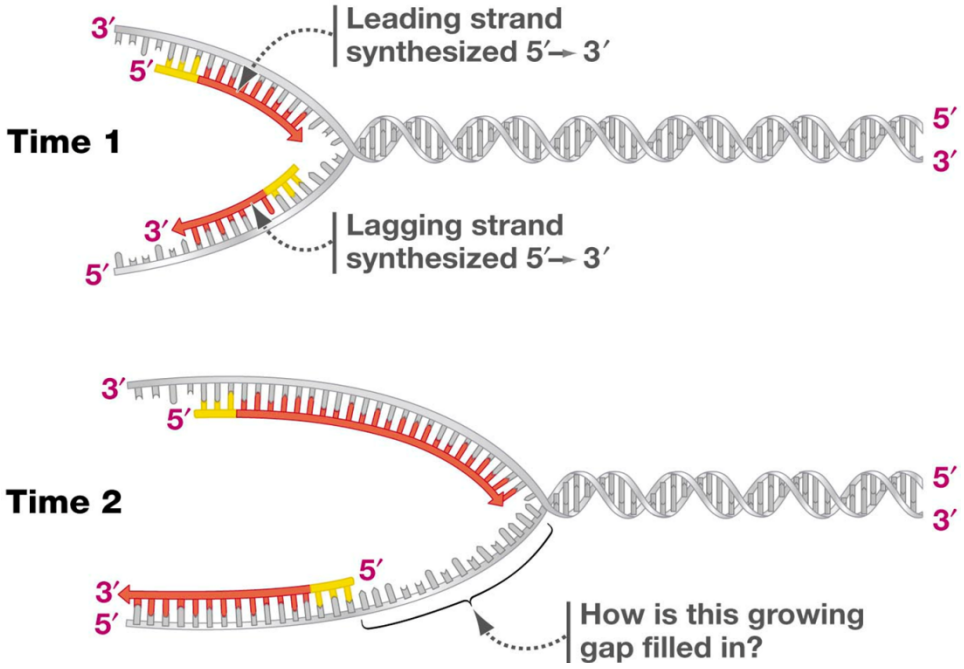
Replisome

- One multi-enzyme machine
  - Allows for the synthesis of a new DNA strand
  - 1 for the leading strand; 1 for the lagging strand

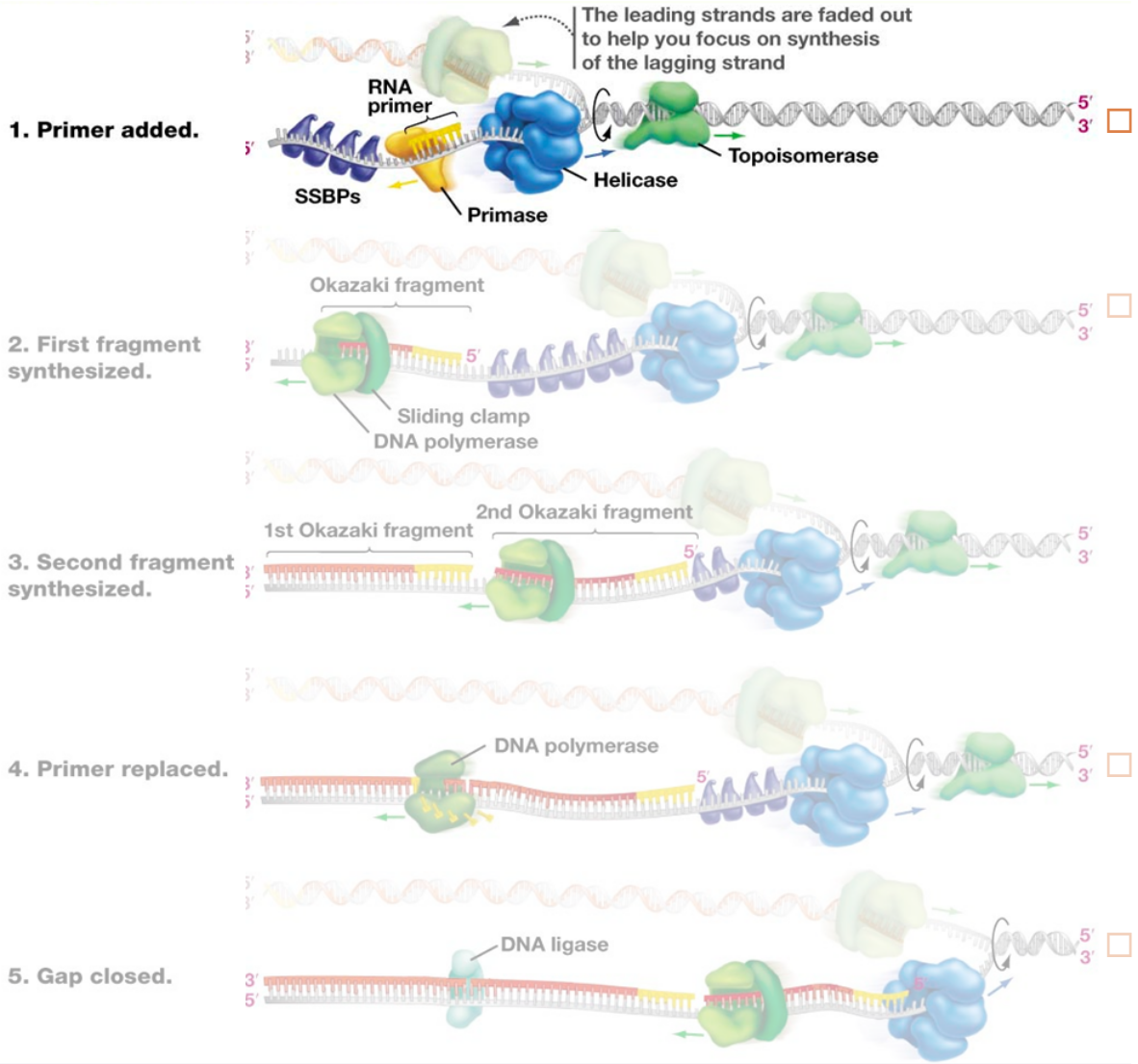


Lagging strand

- Other strand
- Synthesized discontinously
  - In direction away from fork
  - Lags behind fork
    - b/c 5' → 3'



Synthesis of lagging strand



- Primase
  - Synthesizes RNA primer
- DNA polymerase
  - Adds bases to the 3' end of primer
  - Moves away from replication fork
  - Replicating single bit of DNA (Okazaki fragment)
- Process repeats at fork with further unwinding
- DNA segments are linked by DNA ligase

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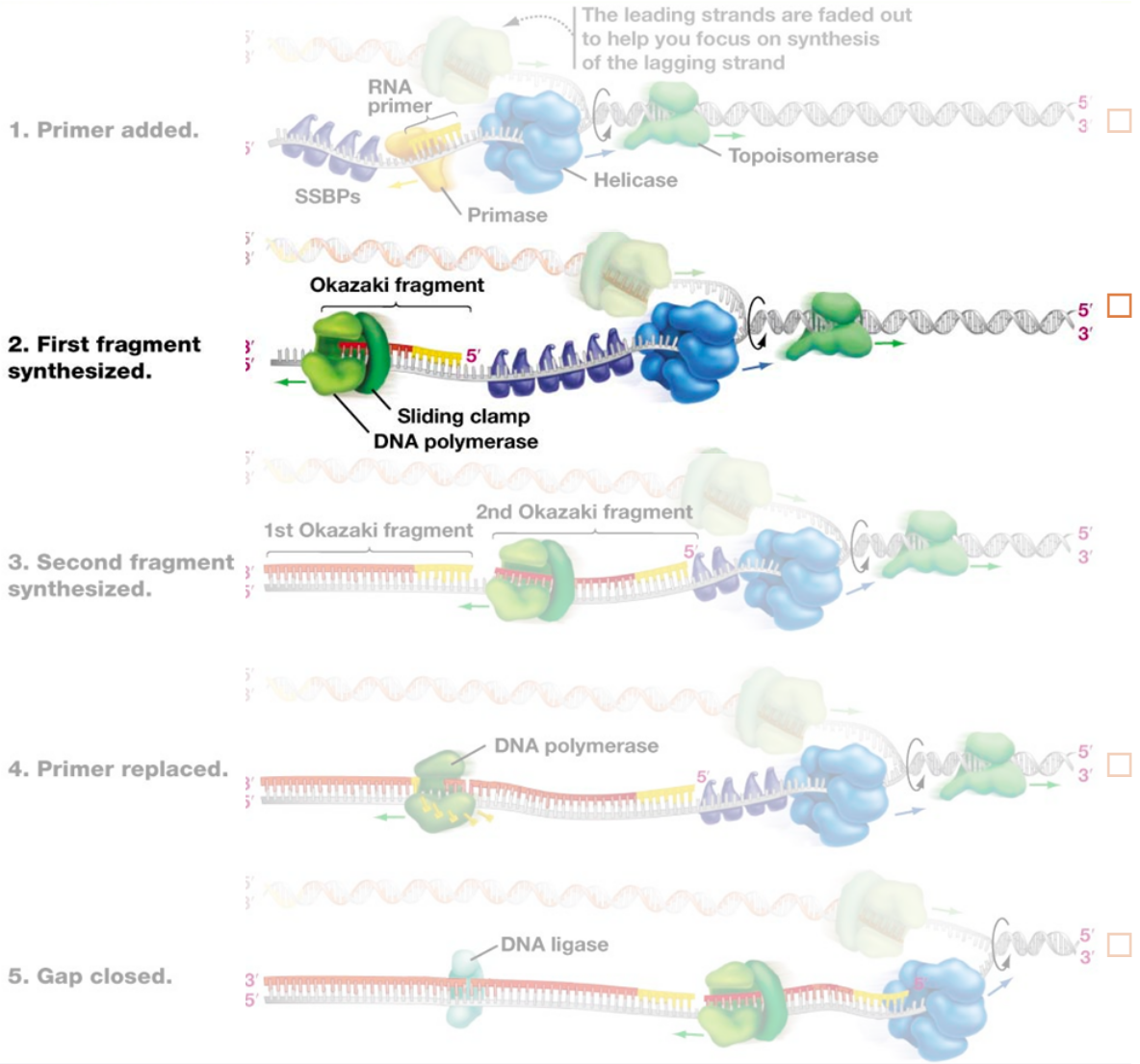

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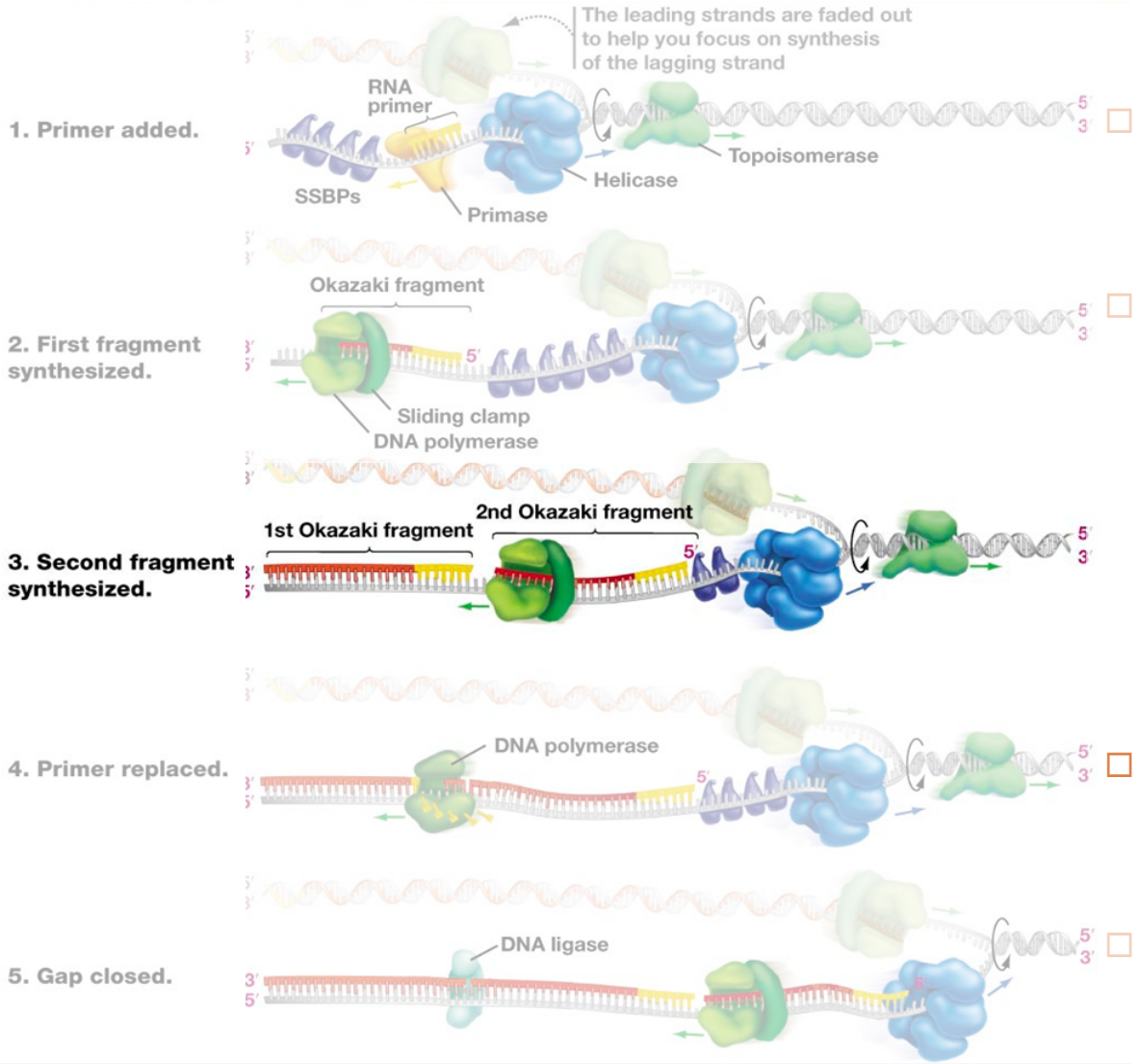

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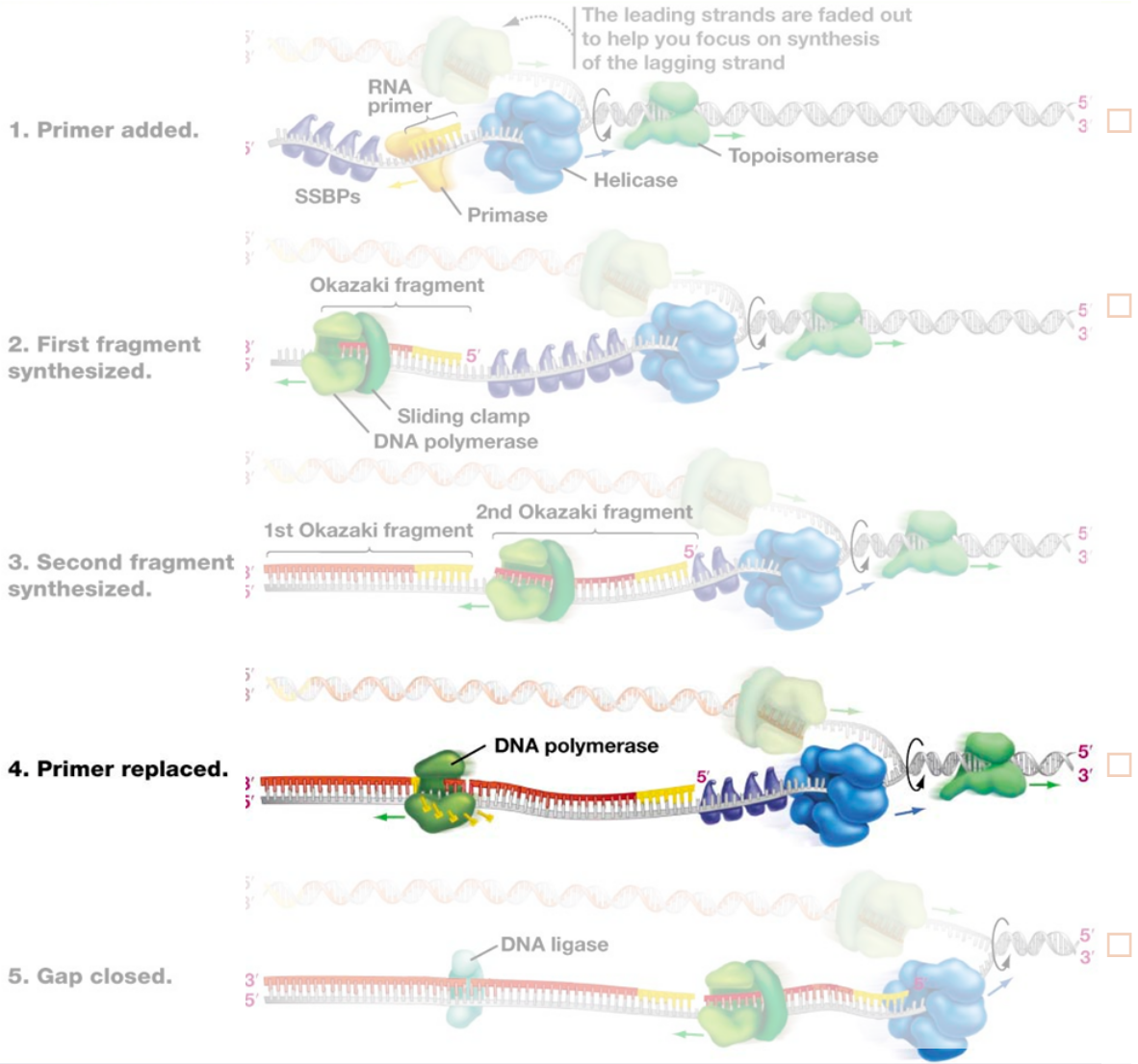

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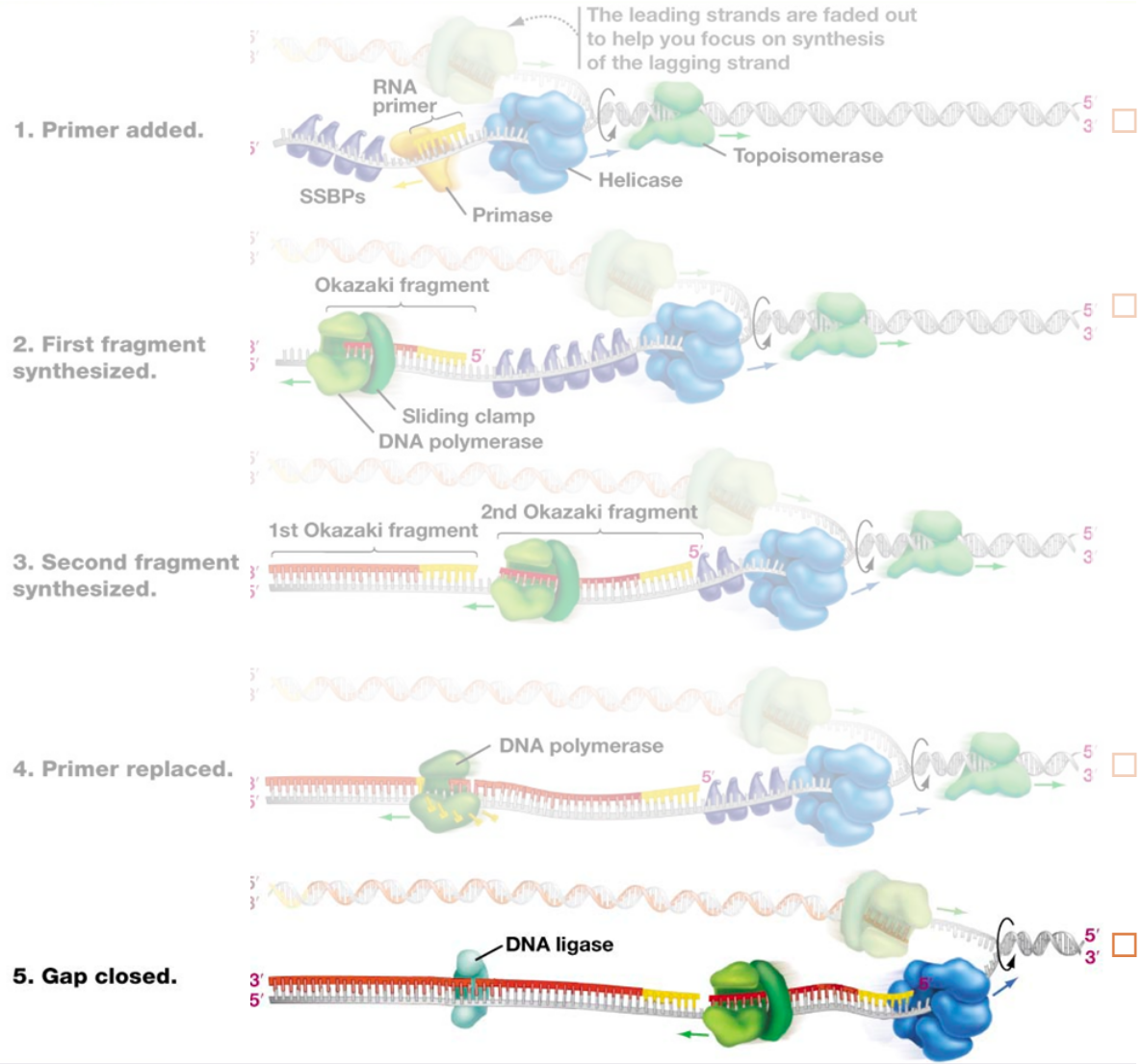

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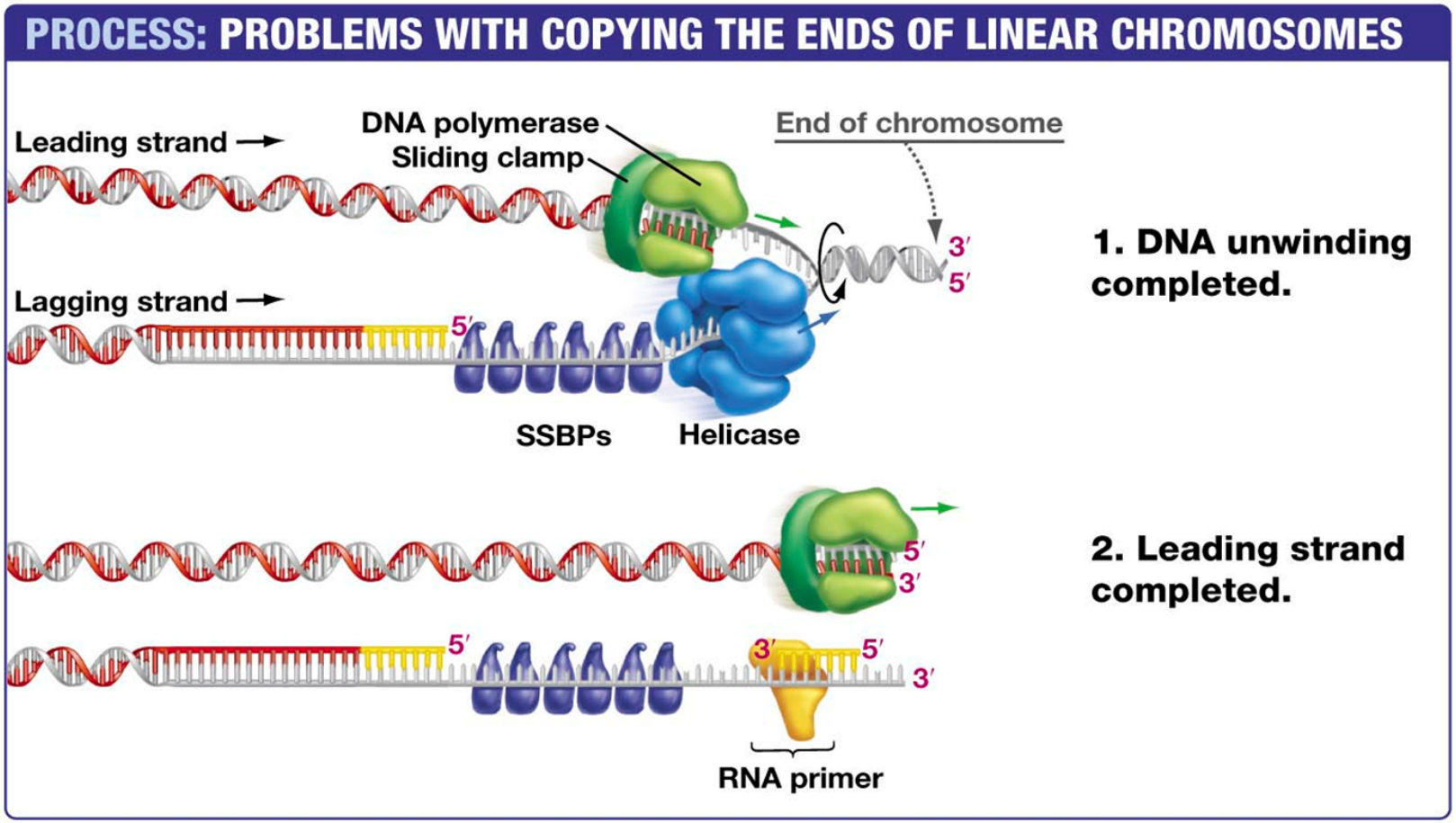
Replicating telomeres

- *Telomeres*
  - End regions of linear chromosomes
- At end
  - No way to replace RNA primer from lagging strand with DNA, b/c no available primer
- Primer is removed
  - Leaves single-stranded section of DNA
  - Eventually clipped off, shortening chromosome

Replicating telomeres



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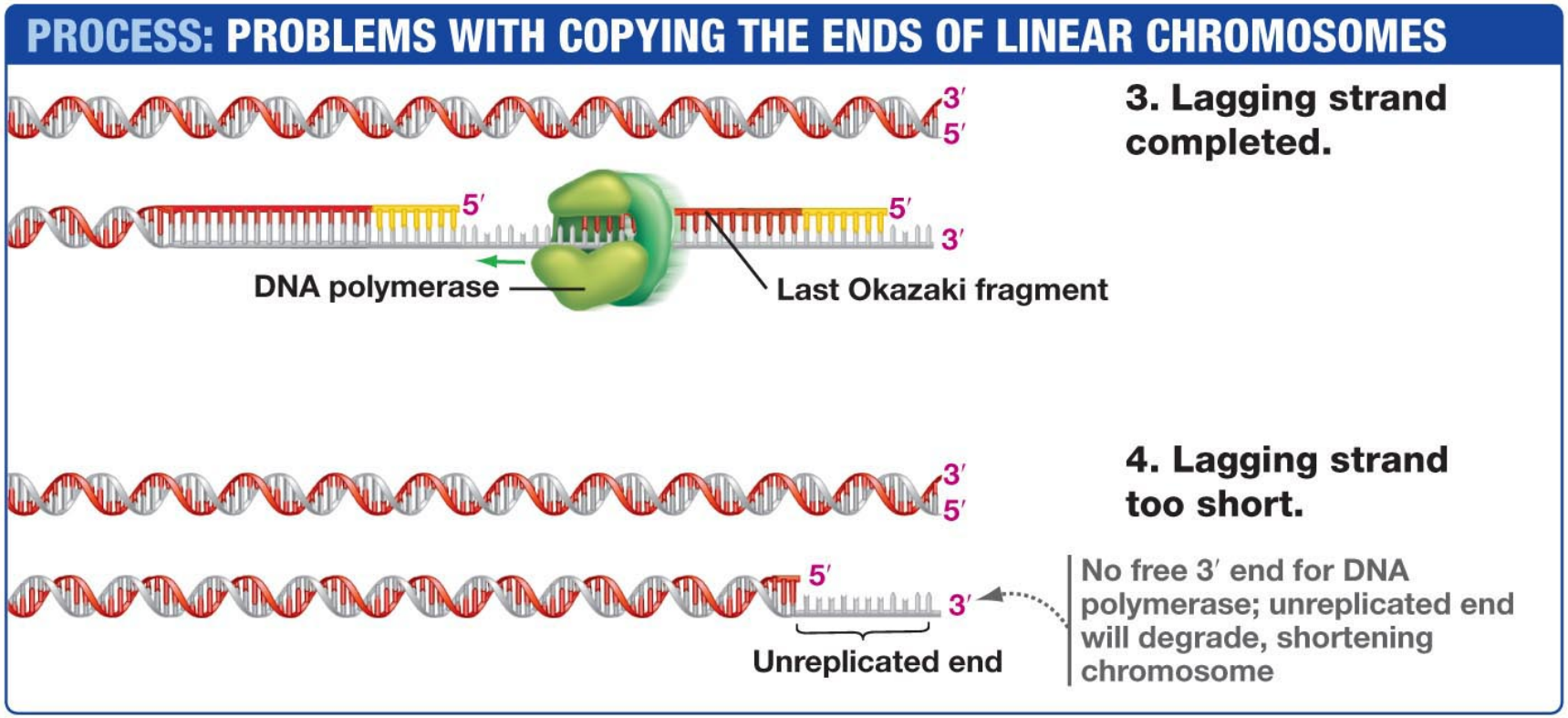
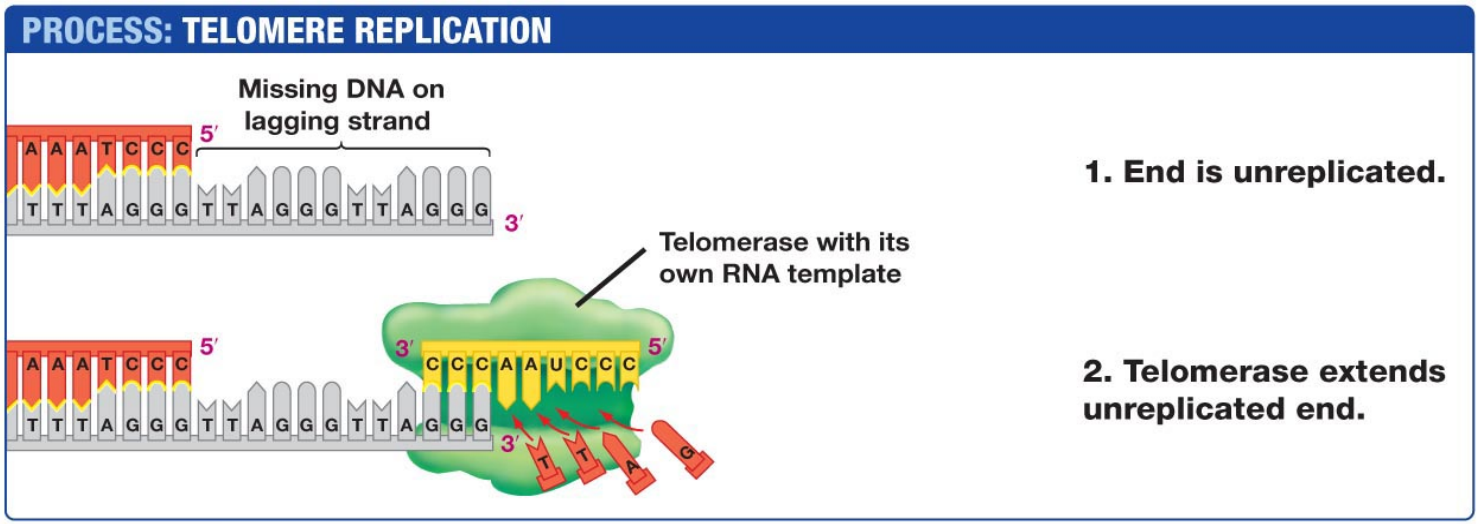


Replicating telomeres

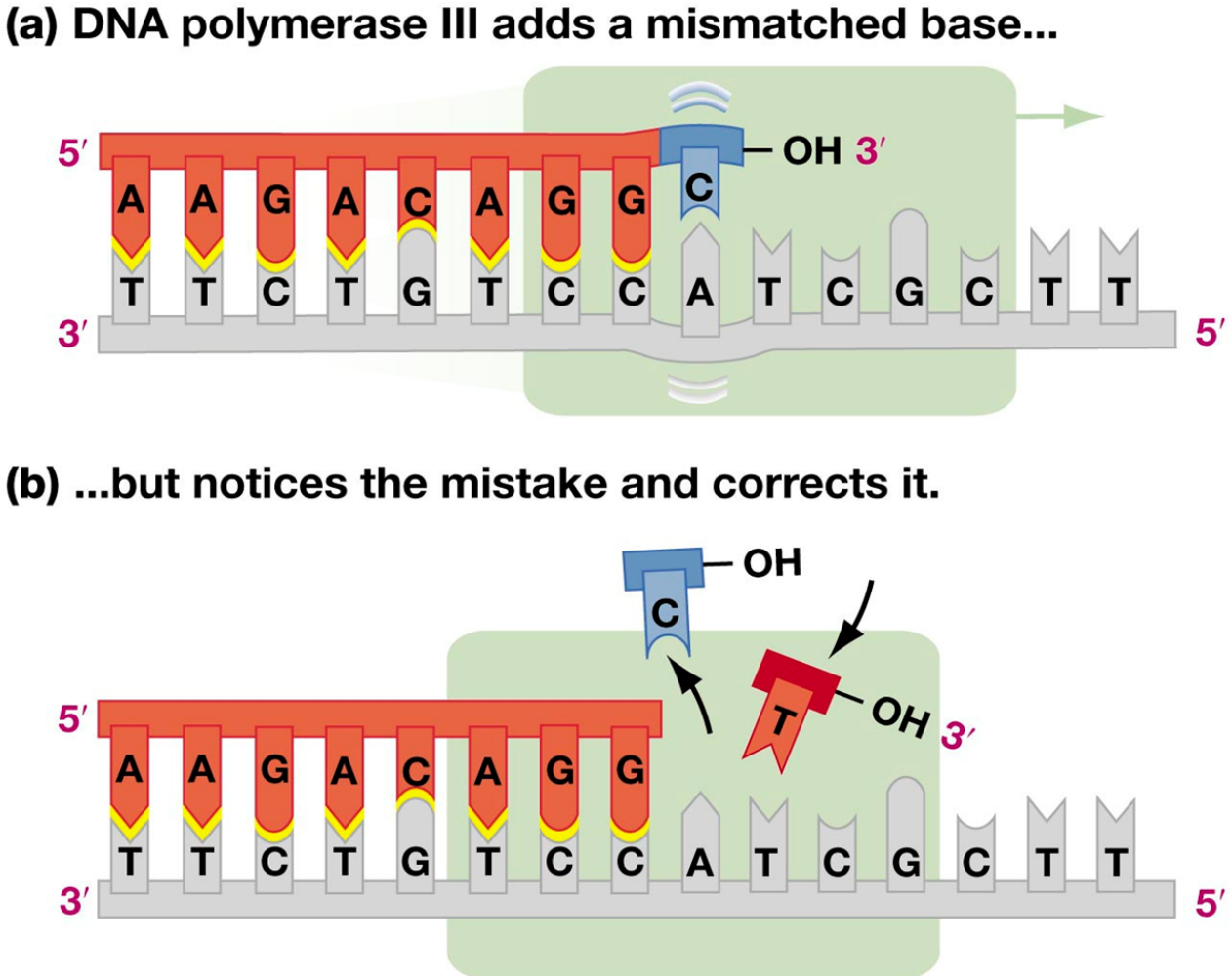
- Telomere don't contain genes
  - But short repeating stretches of bases
- Telomerase
  - Enzyme that adds more repeating bases to end of lagging strand



DNA edits mistakes

- Replication is very accurate
  - 1 mistake for every billion bases
- When mistakes occur after synthesis
  - Repair enzymes remove defective bases and repair them

DNA edits mistakes



DNA edits mistakes

- Even DNA polymerase misses mismatched pairs
- Mismatch repair enzymes
  - Recognize mismatched pairs and fix them
- But nothing is perfect