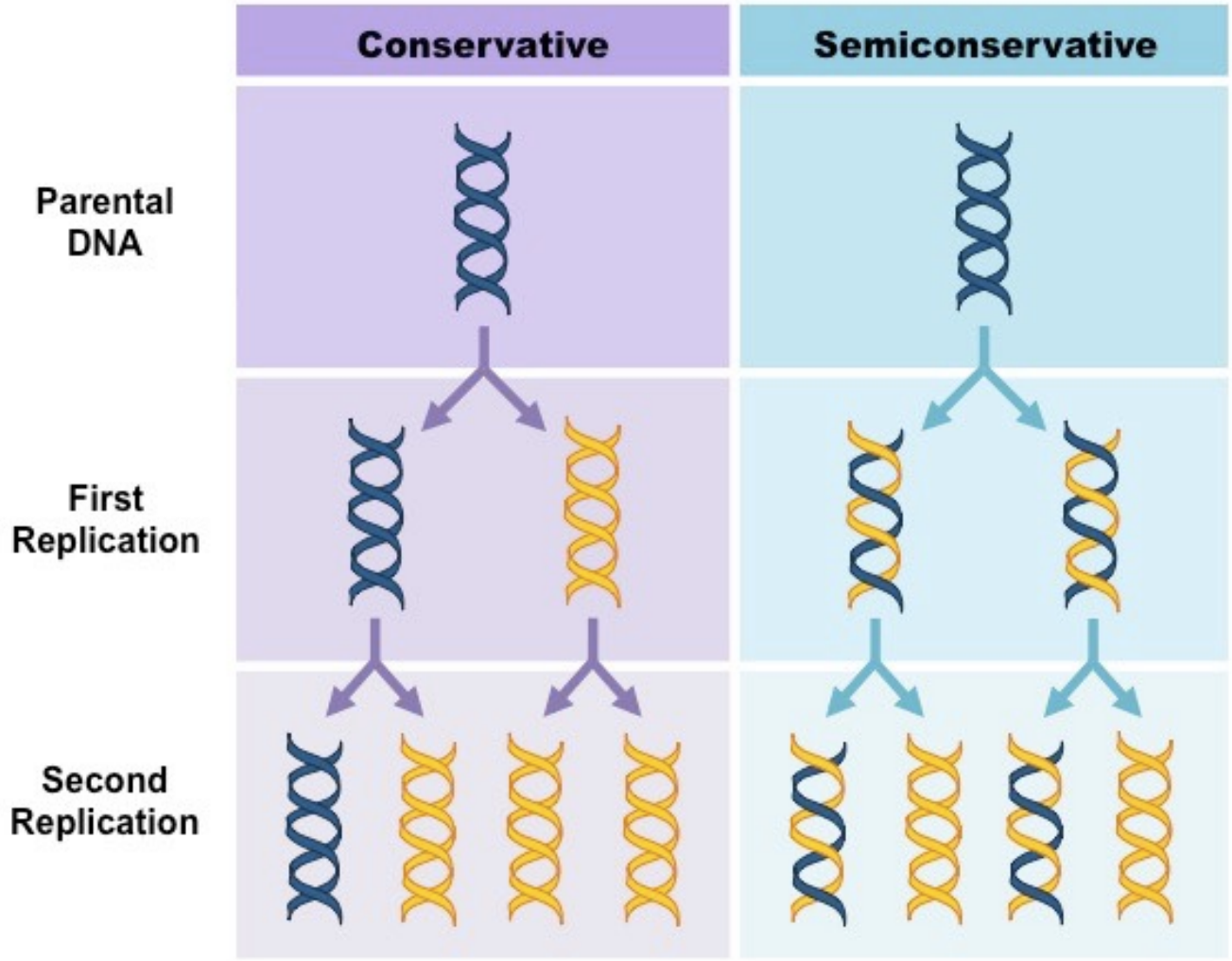
DNA Replication and Repair

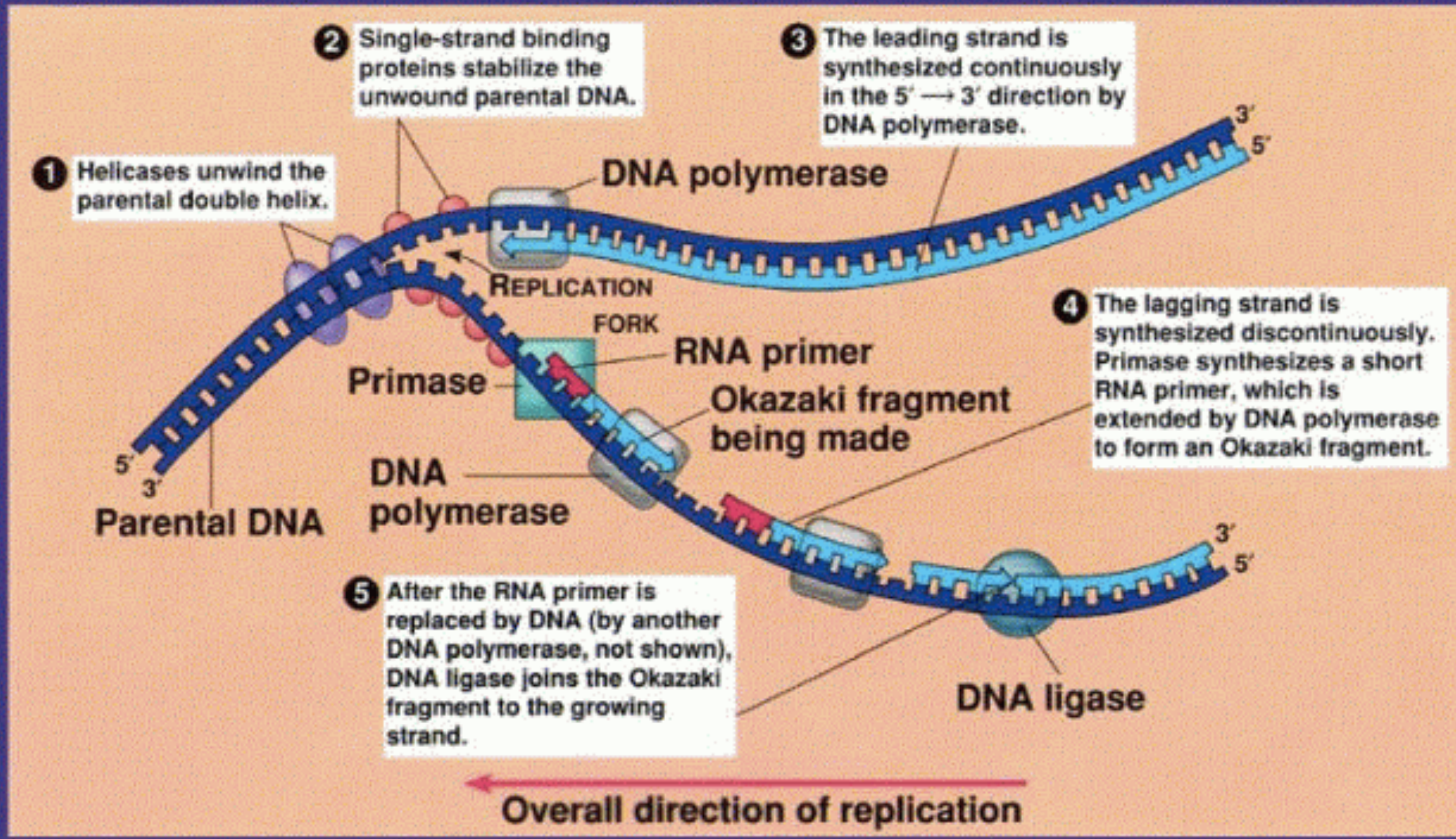
Lesson 2

Semiconservative replication

- A mechanism of DNA replication in which each of the two strands of parent DNA is incorporated into a new strand of DNA



A SUMMARY OF DNA REPLICATION



DNA Replication Overview

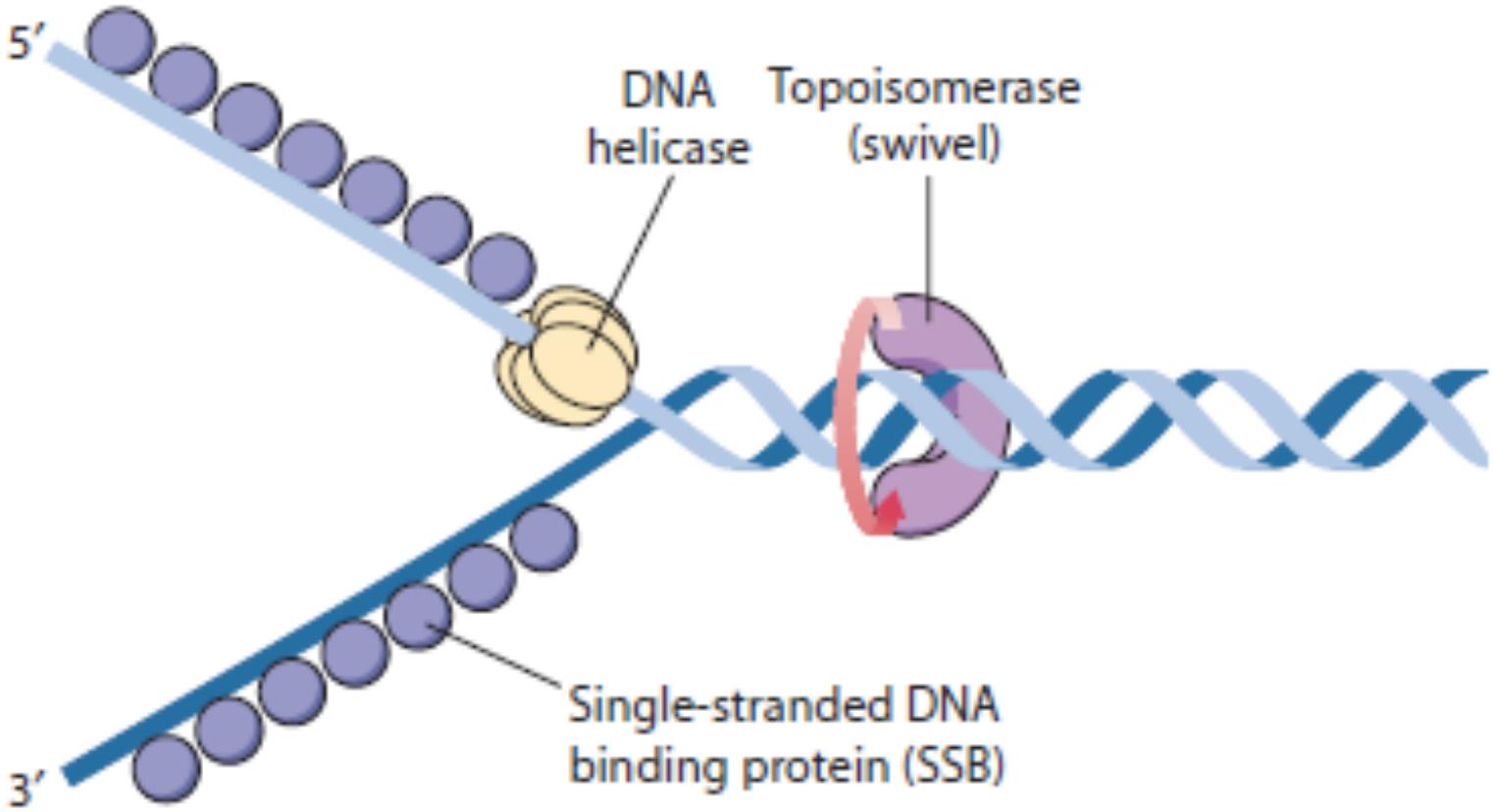
Three steps:

1. **Separation** of the **parent** DNA strands
2. Building of **complementary** (**daughter**) strands
3. **Proofreading** and **repairing** errors in the new strands

Step 1 – Strand Separation

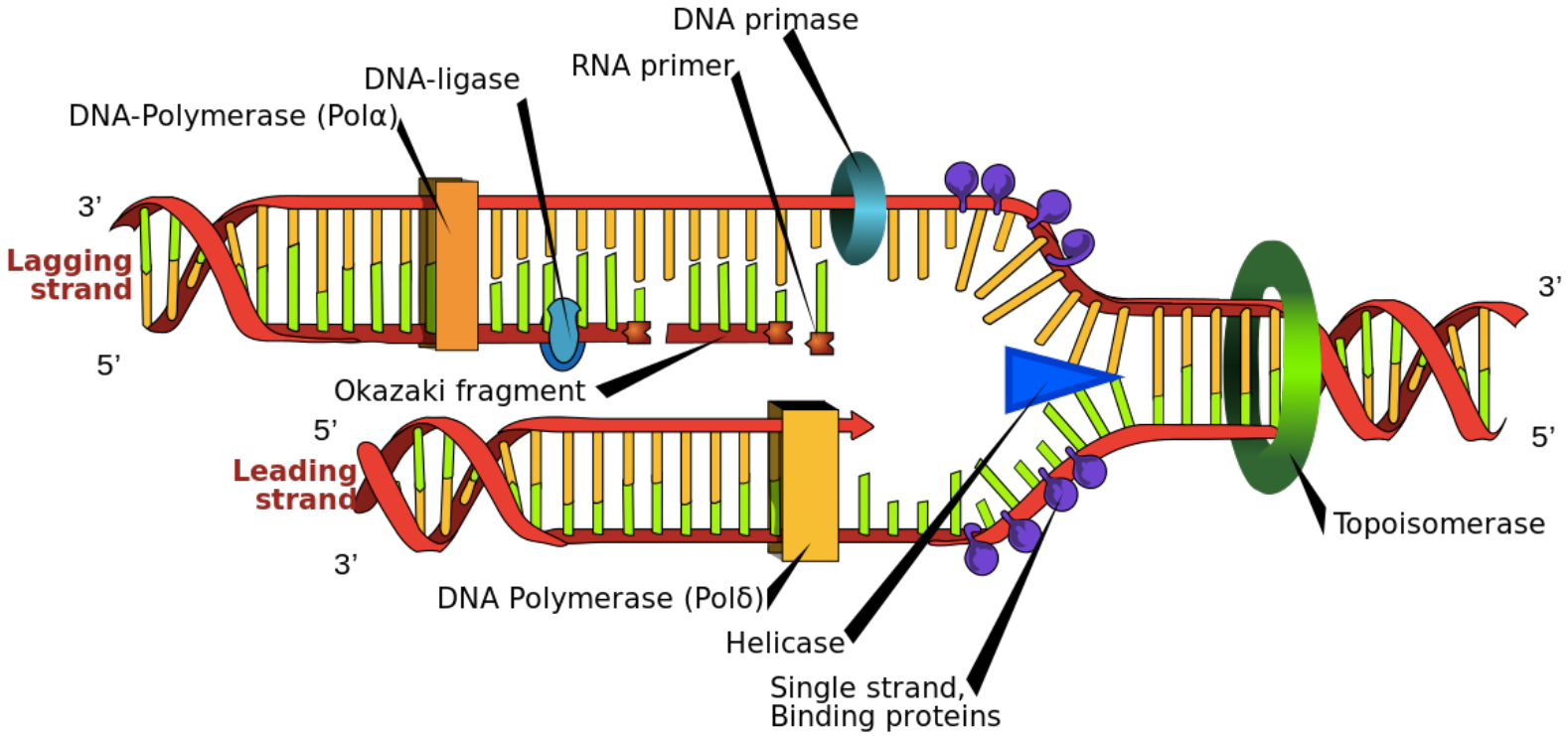
- The **Replication origin** is a specific sequence of DNA = the starting point for replication

- The Y-shaped structure that forms as the two strands separate is known as the **replication fork**



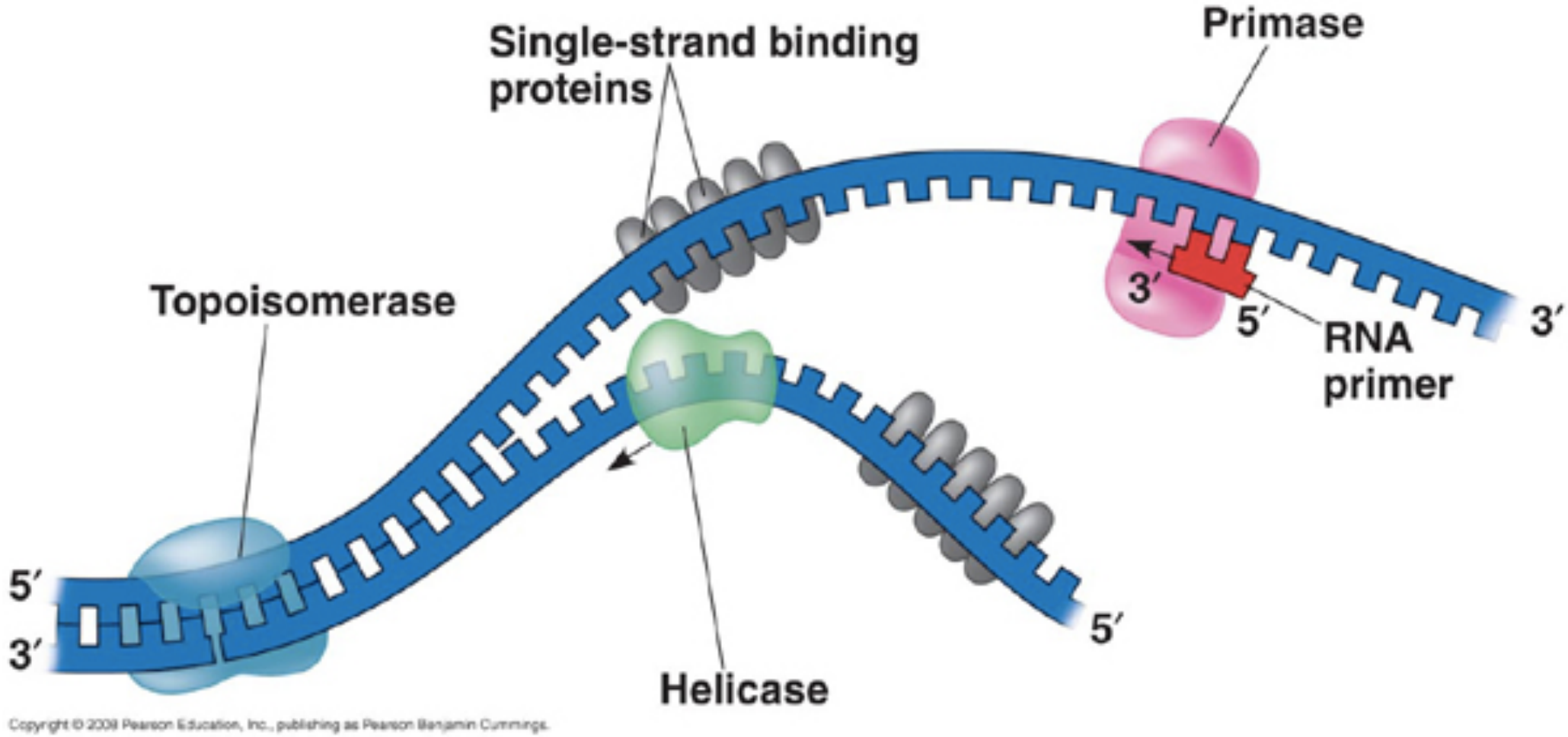
Two challenges:

1. **Tension** is created ahead of the fork
2. Tendency to re-join, or **anneal** (complementary)

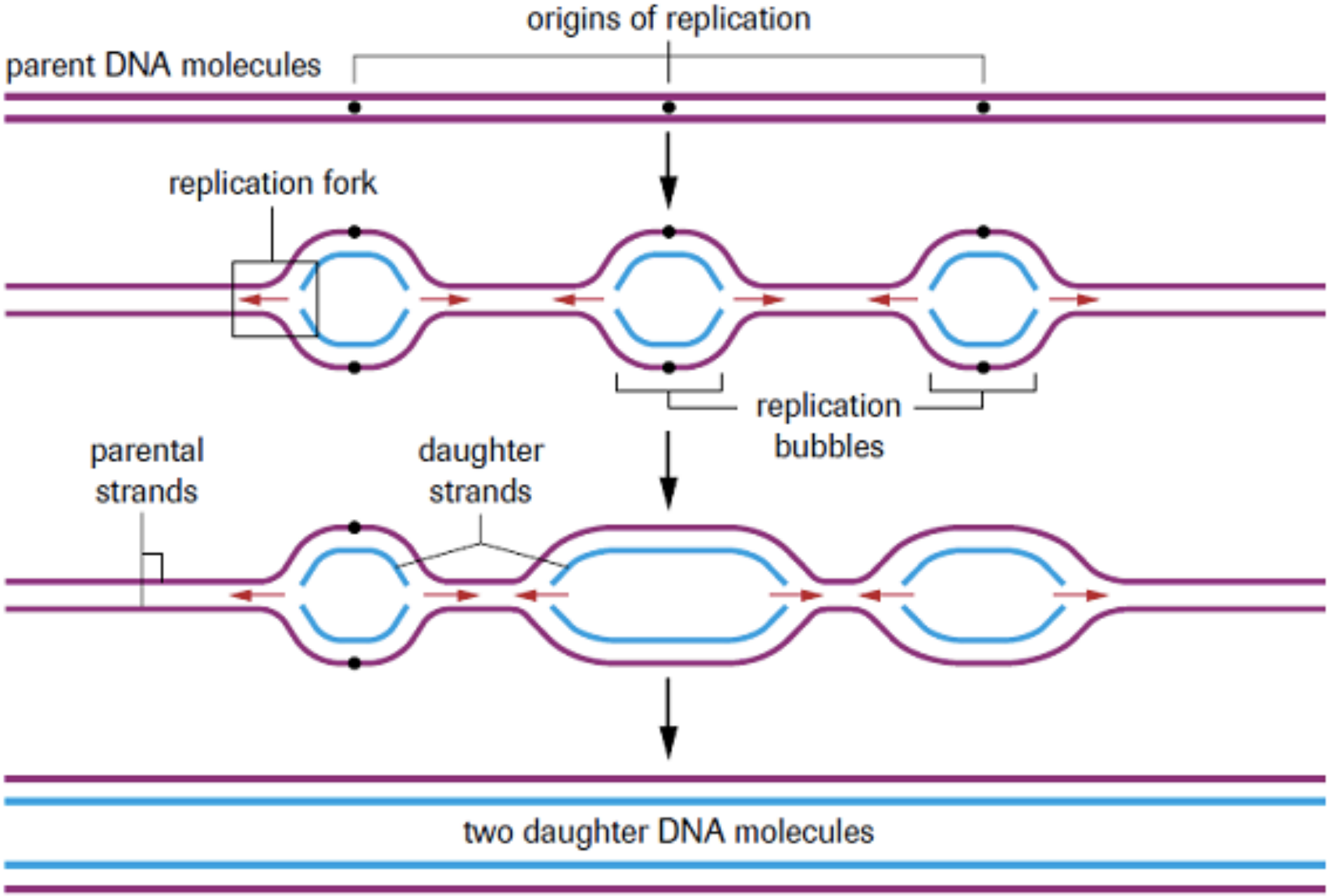


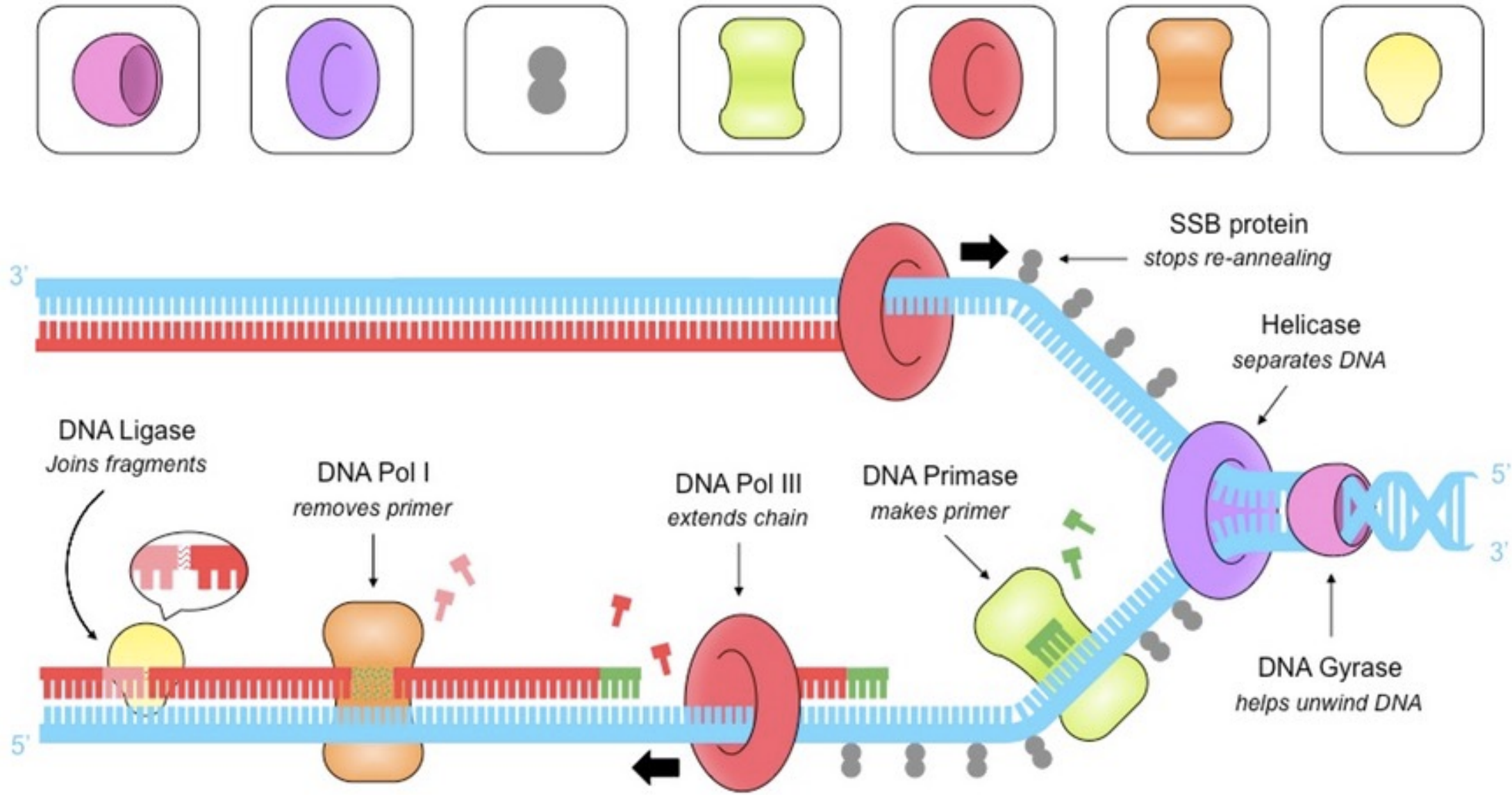
1. **Topoisomerases** relieves tension in DNA strands by cutting one or two strands near the fork, allowing strands to untangle, and then re-join the cut strands
2. **Single-strand binding proteins (SSBs)** prevent annealing by attaching to DNA strands to stabilize them and keep them separated

Single-strand binding proteins (SSBs)



- **Helicase** enzyme binds to these origins are begins to unwind the two strands of DNA from the origin (can separate in both directions) by breaking H-bonds b/w complementary base pairs
- As the forks proceed in opposite directions, the space between them is called a **replication bubble**, soon filled with the newly replicated DNA


origins of replication
parent DNA molecules
replication fork
replication bubbles
parental strands
daughter strands
two daughter DNA molecules

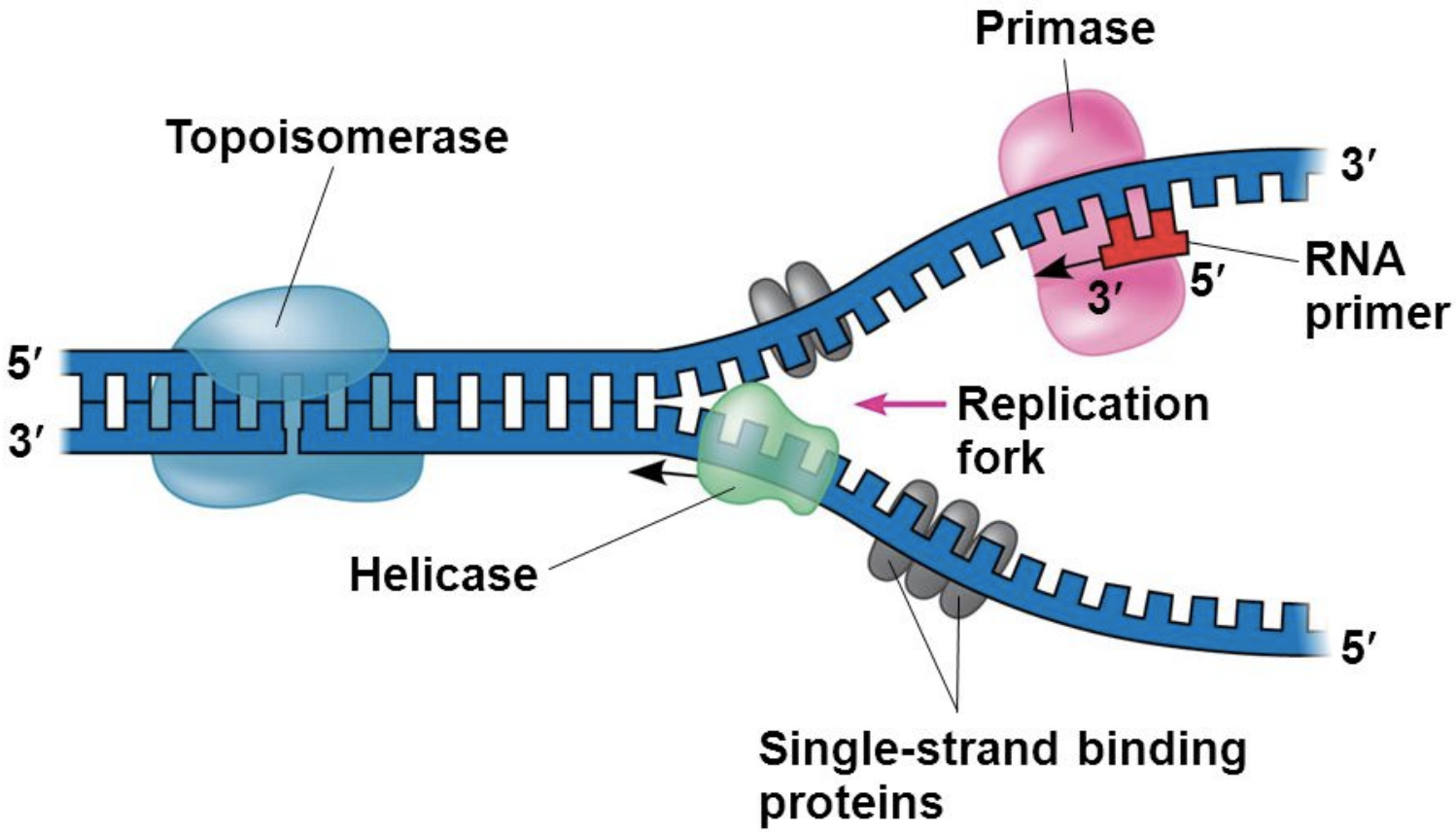

SSB protein
stops re-annealing
Helicase
separates DNA
DNA Ligase
Joins fragments
DNA Pol I
removes primer
DNA Pol III
extends chain
DNA Primase
makes primer
DNA Gyrase
helps unwind DNA
3'
5'
5'
3'

Step 2 – Building Complementary Strands

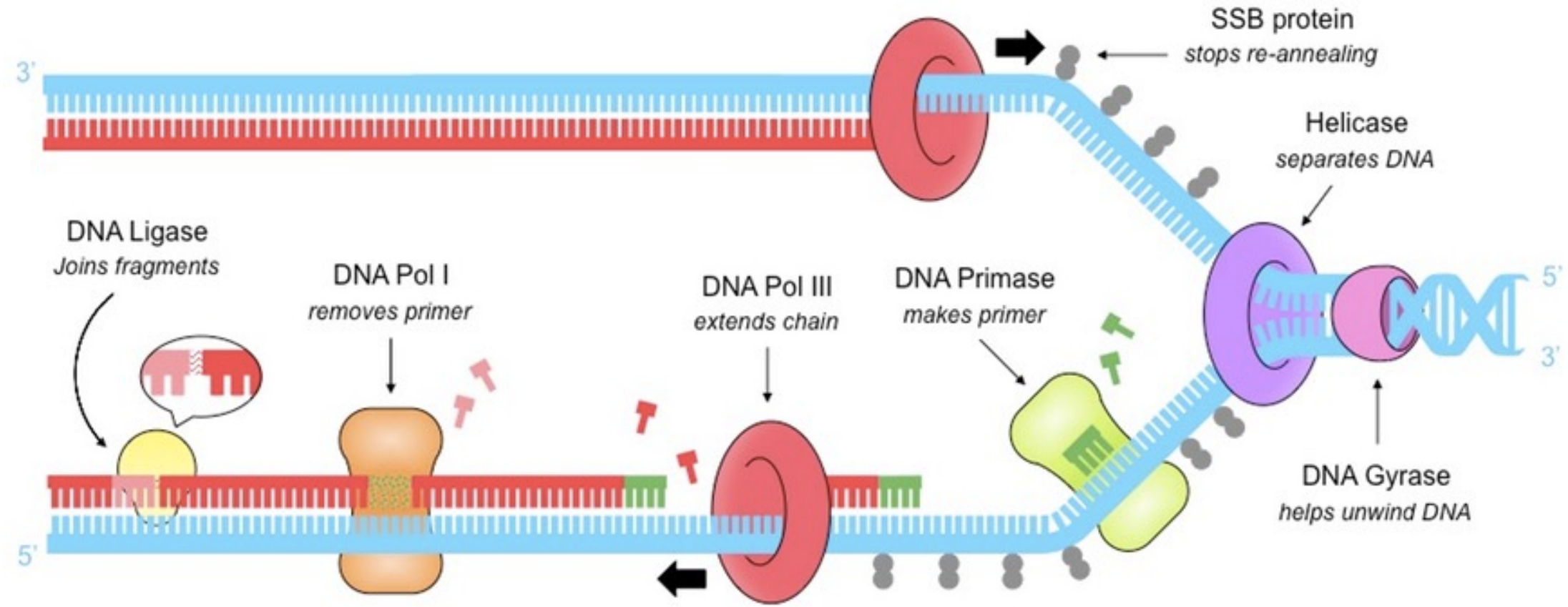
New nucleotides are joined by a group of enzymes called DNA polymerases (prokaryote example will be discussed)

- **DNA polymerases** ”read” the **template** strand in its 3’→5’ direction
- They can only add nucleotides to the 3’ end of an existing DNA strand
- Thus, the **new strand** is always assembled in the 5’ to 3’ direction

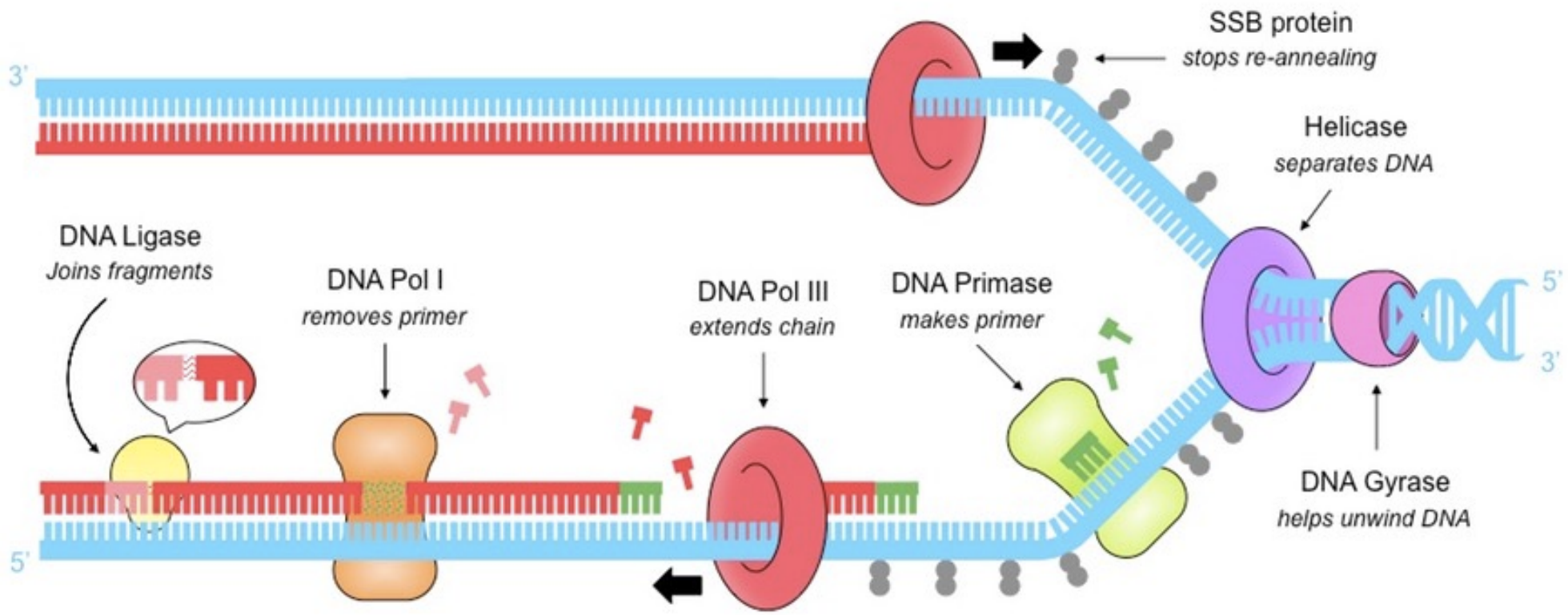
- **RNA primase** enzymes begin the replication process by building a small complementary RNA segment called **RNA primers** (10-60 ribonucleotides long)



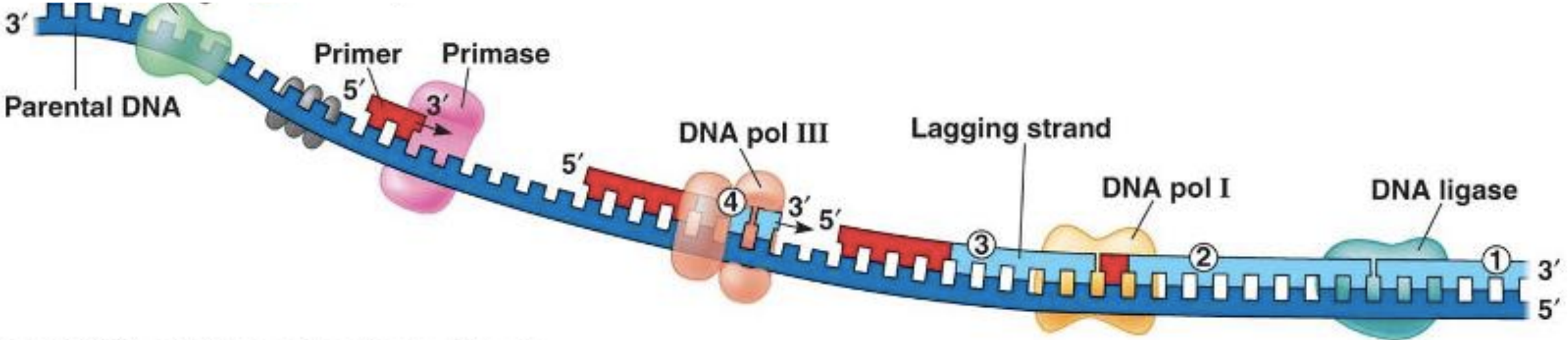
- **DNA polymerase III** begins to add DNA nucleotides to the primer
- Since DNA polymerase III only builds 5'→3', the two new strands begin to be assembled in opposite directions



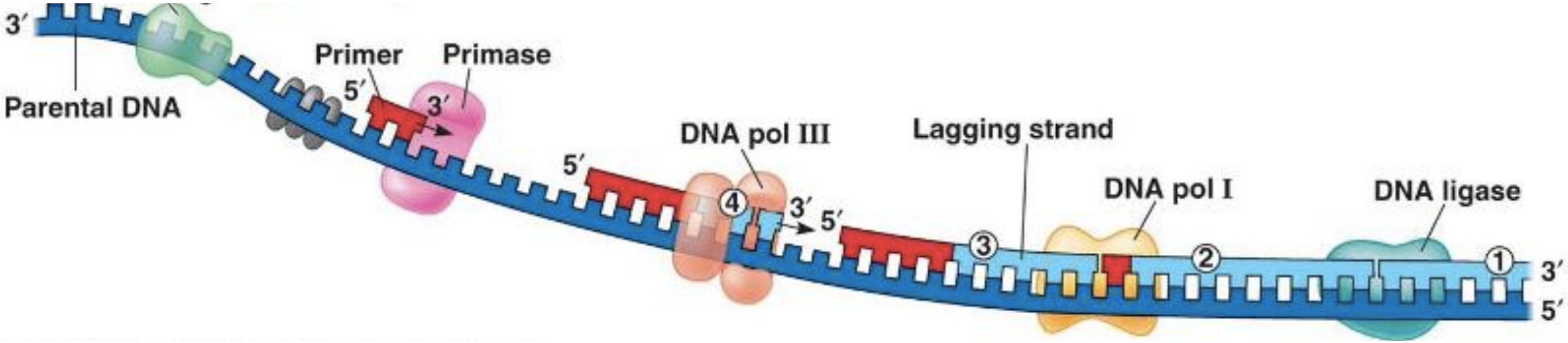
- DNA polymerase III is able to continue continuously
- No need for the RNA primase to add additional primers
- This is called the **leading strand**



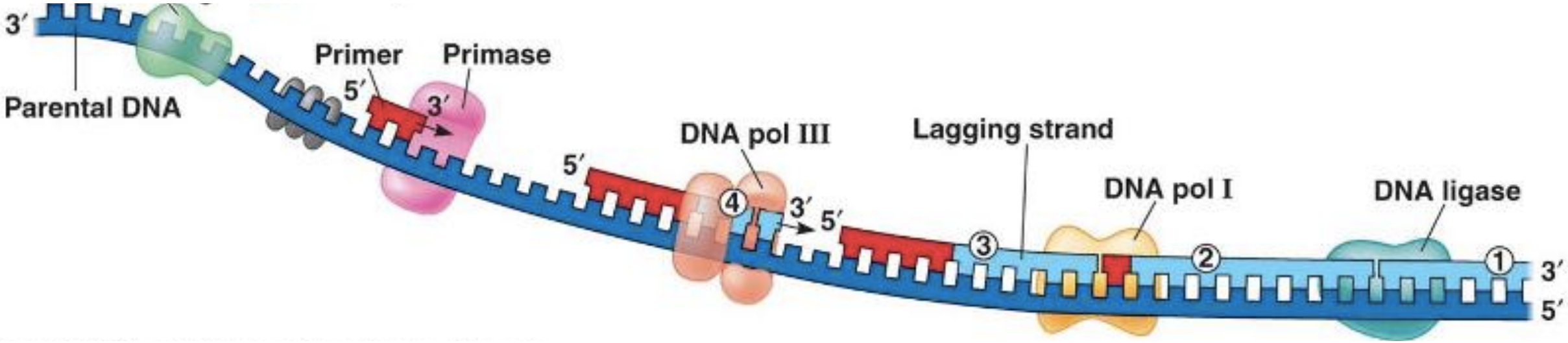
- On the opposite strand, DNA polymerase III is moving away from the replication fork → **lagging strand**
- RNA primase attaches another primer allowing DNA polymerase III to begin from a new point



- The pattern created on the second strand is a series of RNA primers and short DNA fragments called **Okazaki fragments**
- As each fragment extends in the 5’→3’ direction, it eventually runs into the RNA primer attached to the Okazaki fragment ahead



- **DNA polymerase I** removes the RNA nucleotides and replaces them with DNA nucleotides
- **DNA ligase** catalyzes the formations of **phosphodiester bonds** to seal the strand

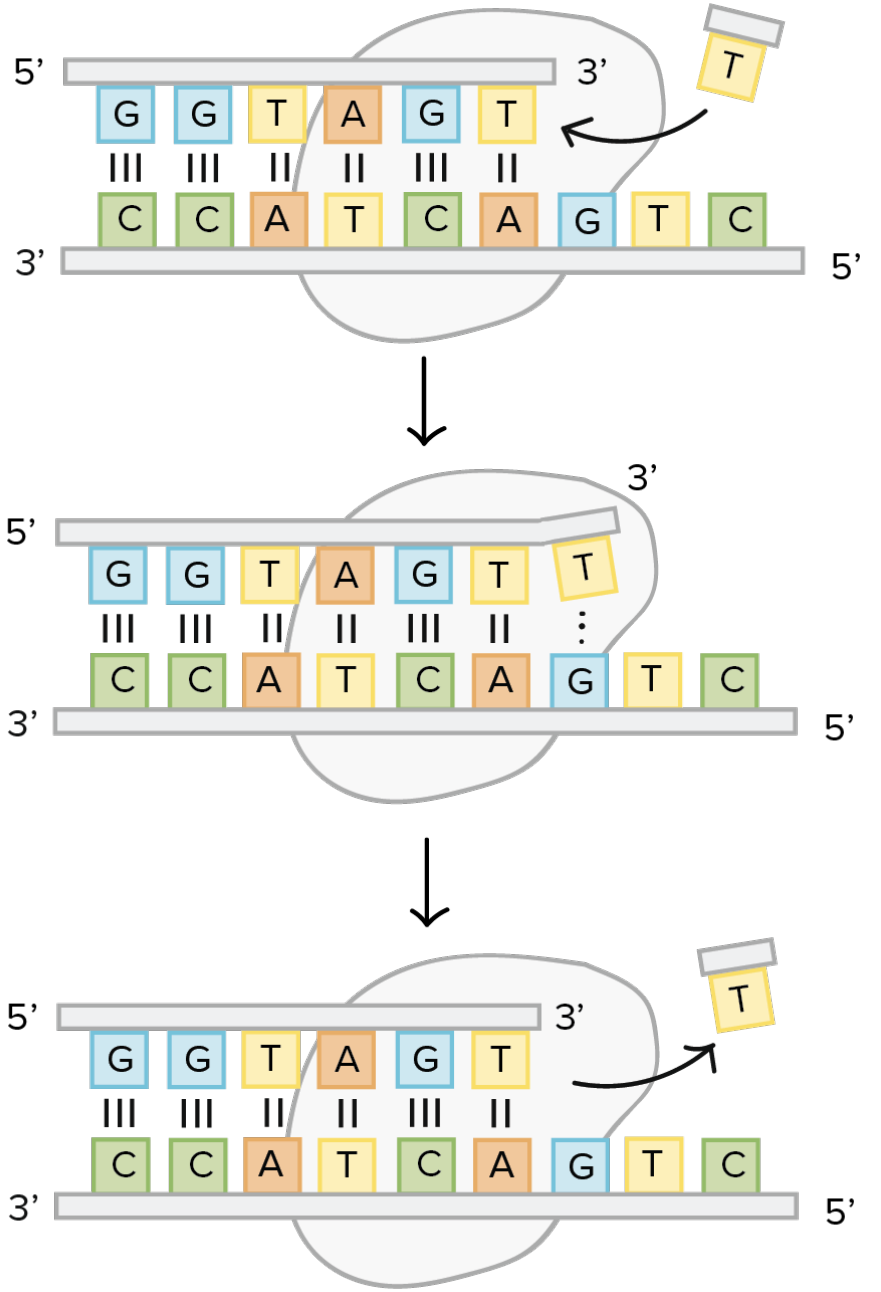


Step 3 – Dealing with Errors (repairing)

- DNA polymerases that carry out replication also play another important role
- As they assemble new DNA strands, they **proof-read** and **correct** errors (base-pair mismatches)
- **Proof-reading:** While creating the complementary strand, if a mismatch occurs, DNA polymerase III may back up, repair, and continue
- **Repairing**: DNA repair mechanisms of proteins and enzymes (such as DNA Polymerase I and II) may locate distortions in the strands between replication events and remove a piece of the strand, DNA polymerases will fill the gap, and DNA ligase will seal the strand

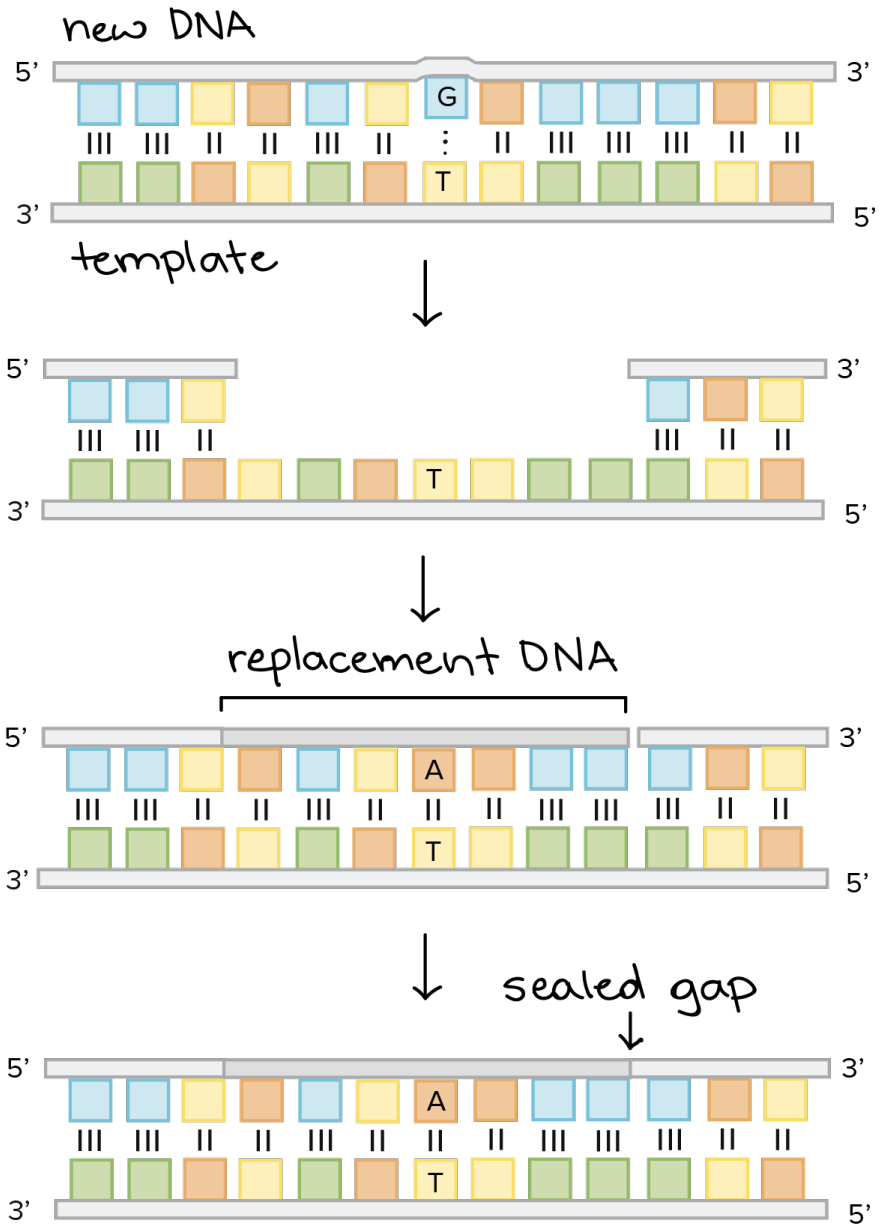
Proof-reading

- **DNA polymerase III** continues adding nucleotides in the forward direction
- If the enzyme adds a mismatched nucleotide, the enzyme acts as a **exonuclease** (cleave nucleotides) to remove the mismatched nucleotide
- The enzyme resumes activity as DNA polymerase



Repairing

- **DNA polymerase II** repairs damage to DNA that occurs between replication events
- Repair complexes remove several to many bases, leaving a gap in the DNA
- Gap is filled in by a DNA polymerase, using the template as a guide
- Nick is sealed by **DNA ligase** to complete repair

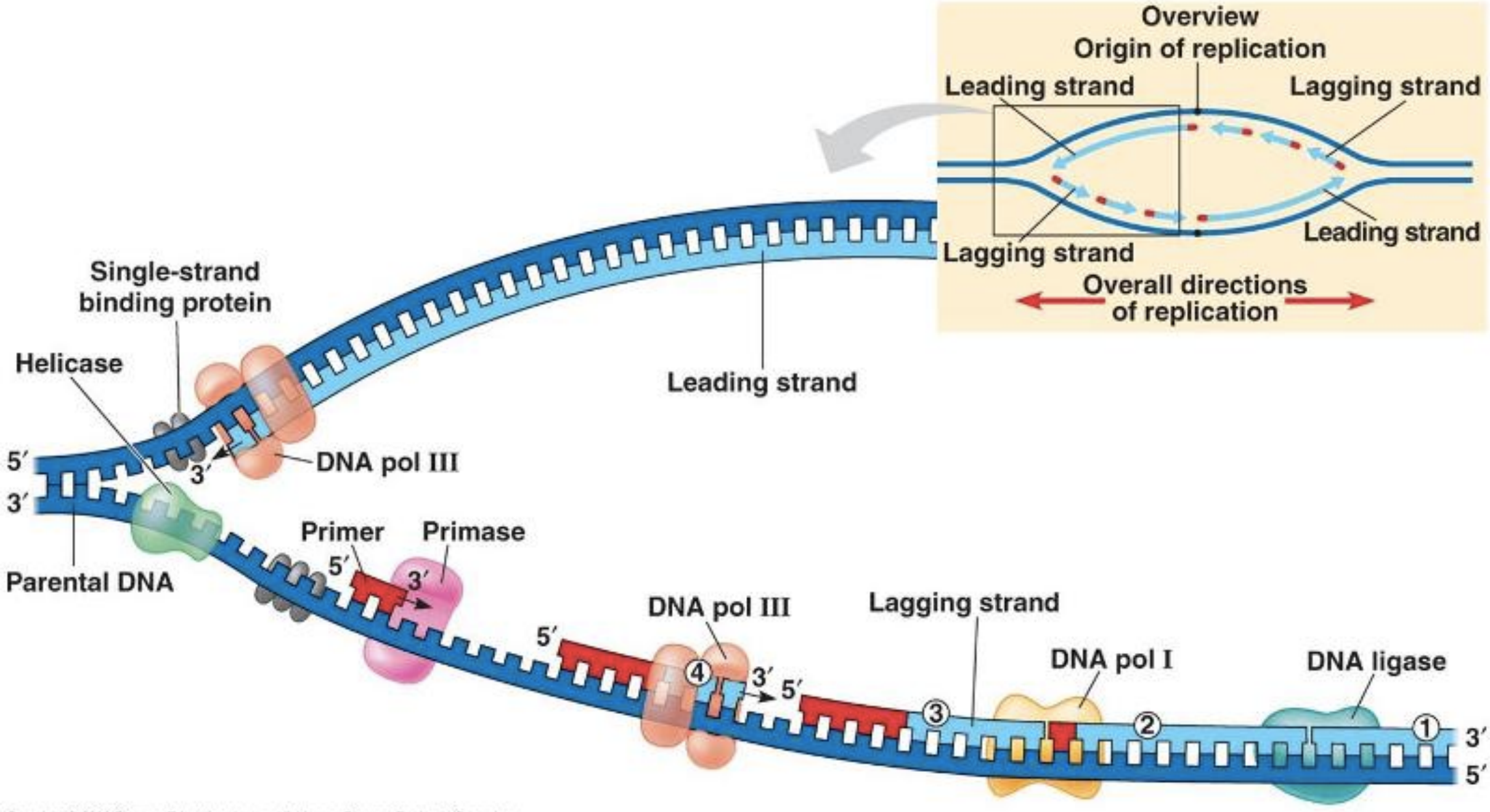


A mismatch is detected in newly synthesized DNA.

The new DNA strand is cut, and the mispaired nucleotide and its neighbors are removed.

The missing patch is replaced with correct nucleotides by a DNA polymerase.

A DNA ligase seals the gap in the DNA backbone.


Overview
Origin of replication
Leading strand
Lagging strand
Leading strand
Lagging strand
Overall directions of replication
Single-strand binding protein
Helicase
Leading strand
5′
3′
3′
DNA pol III
Parental DNA
Primer
Primase
5′
3′
5′
DNA pol III
Lagging strand
DNA pol I
DNA ligase
4
3′
5′
3
2
1
3′
5′

Homework

Investigation 6.4.1

- Work in groups to create a role play for DNA replication

  (role-play and questions due **Friday Nov 17**)