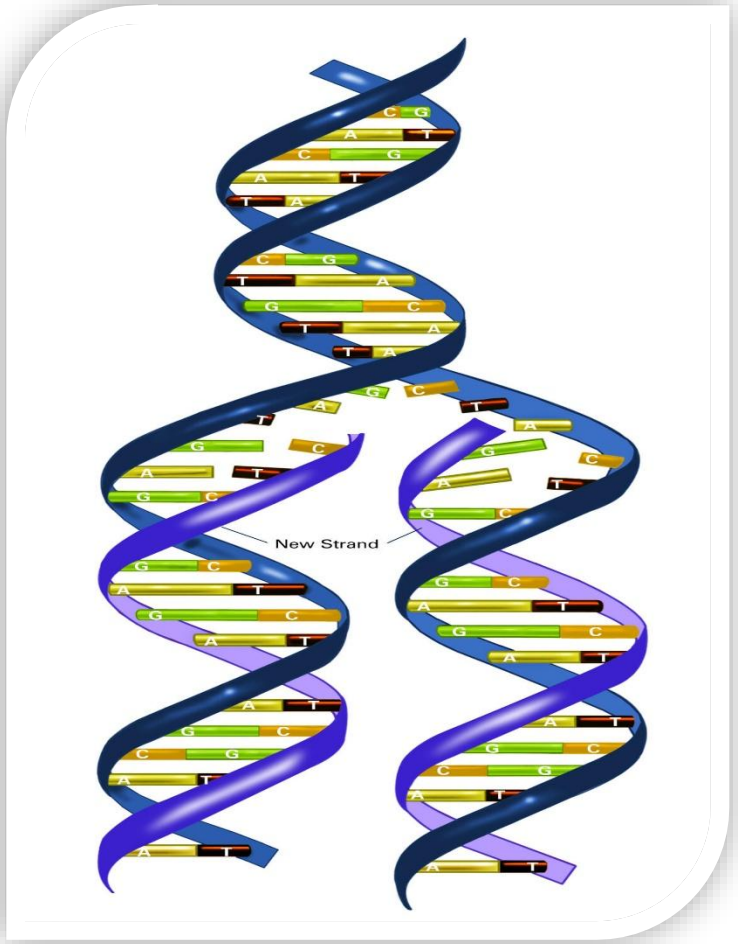
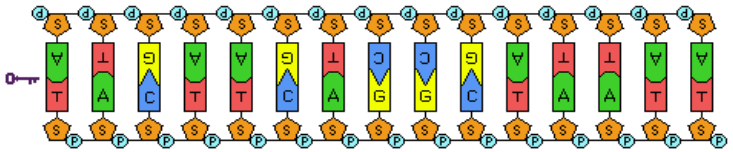
Central Dogma and DNA Replication



By:

Dr/ Aya El-Hanafy

Associate Prof. of biochemistry & Molecular Biology

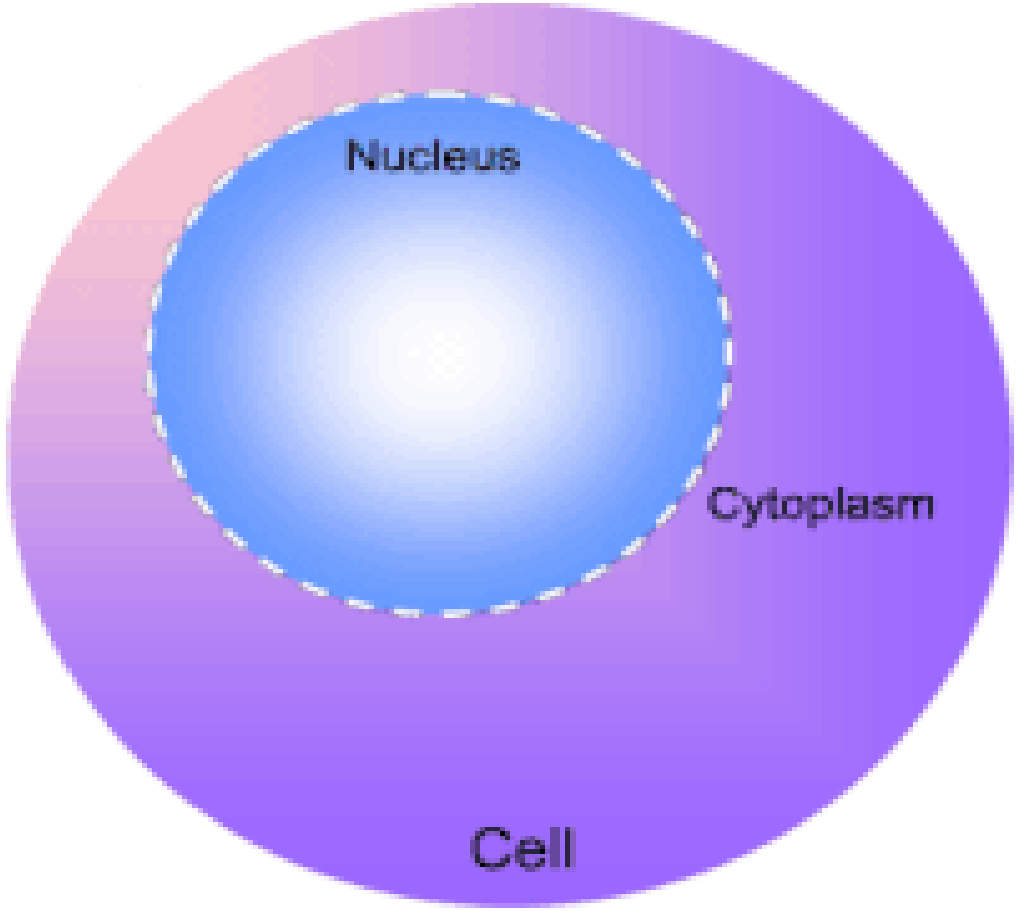


Learning Outcomes

- Define central dogma of molecular biology
- Identify DNA replication & its general criteria
- Recognize the steps of eukaryotic DNA replication
- List enzymes & proteins needed for DNA replication
- Understand the meaning of end replication problem

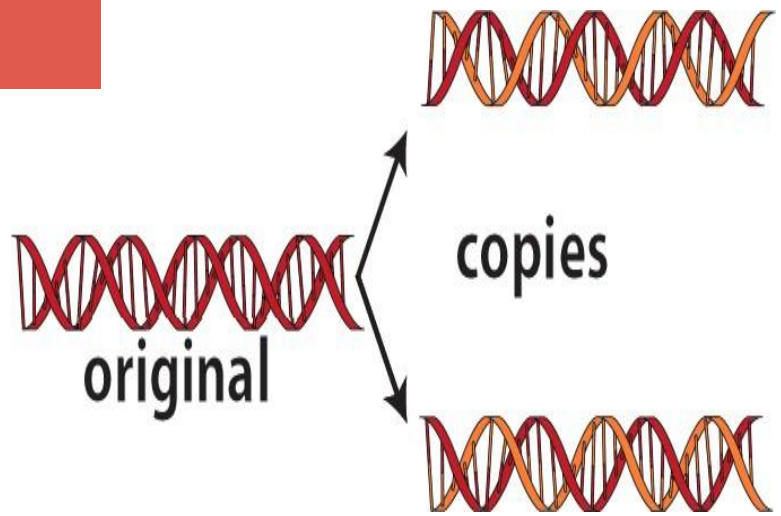
Central Dogma of Molecular Biology

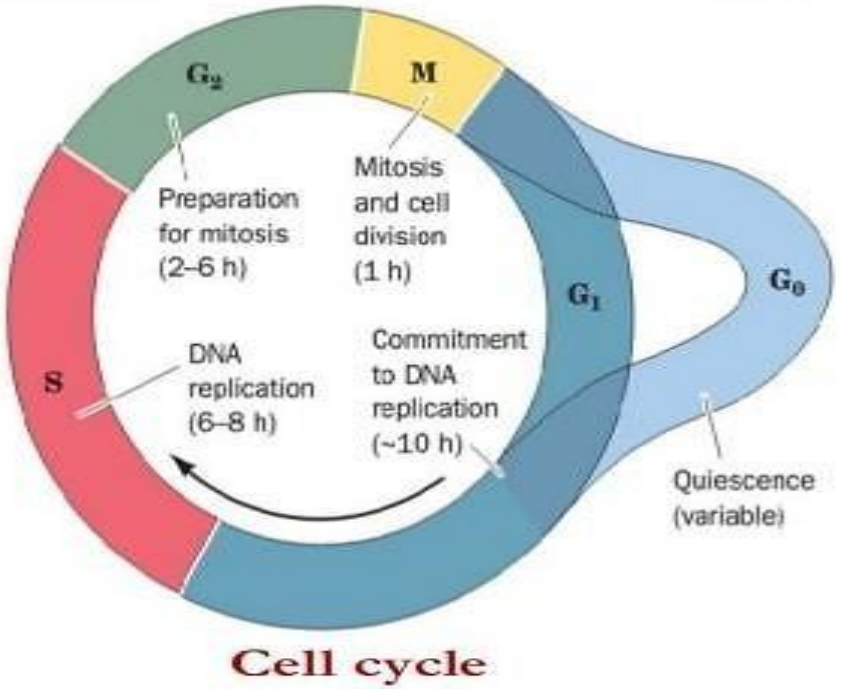
- The biological information flows from DNA to RNA, and from them to proteins.
- DNA in a cell must be duplicated (replicated), and passed down to daughter cells.



DNA replication

- It is the process of making two identical DNA from the original parent DNA molecule.
- DNA has to be copied **before cell division,** so that the new daughter cells have the same genetic information as the parent cell.
- DNA copied during **S phase (DNA synthesis)** of cell cycle
- Site : in the **nucleus**



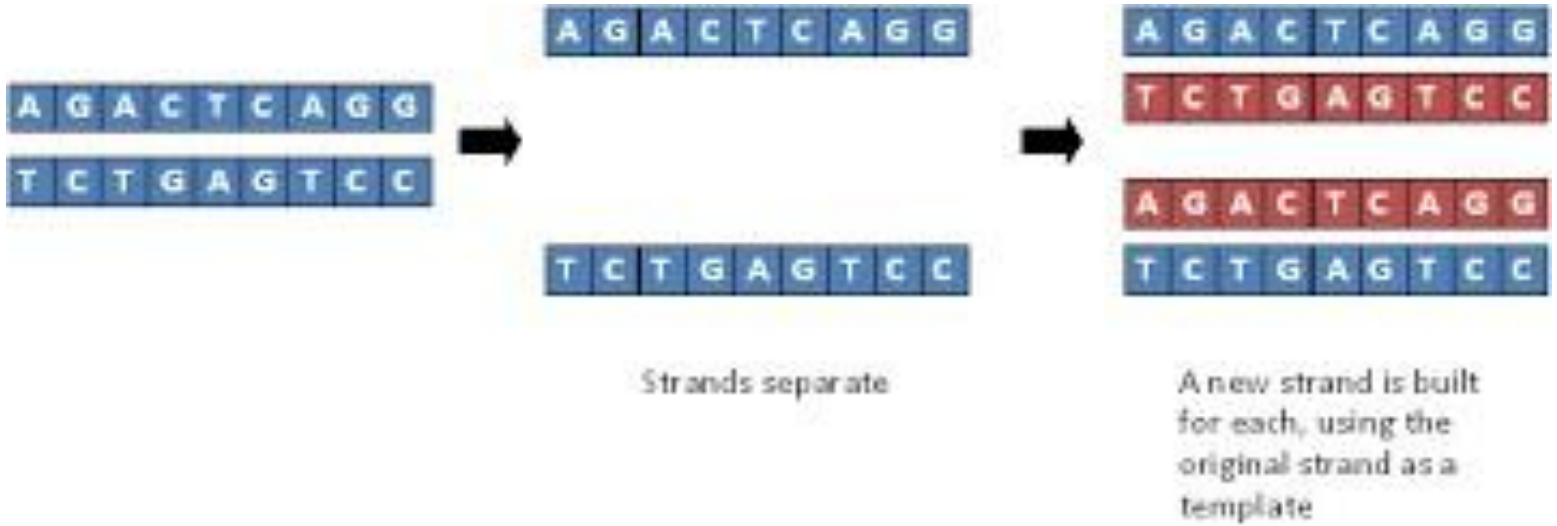


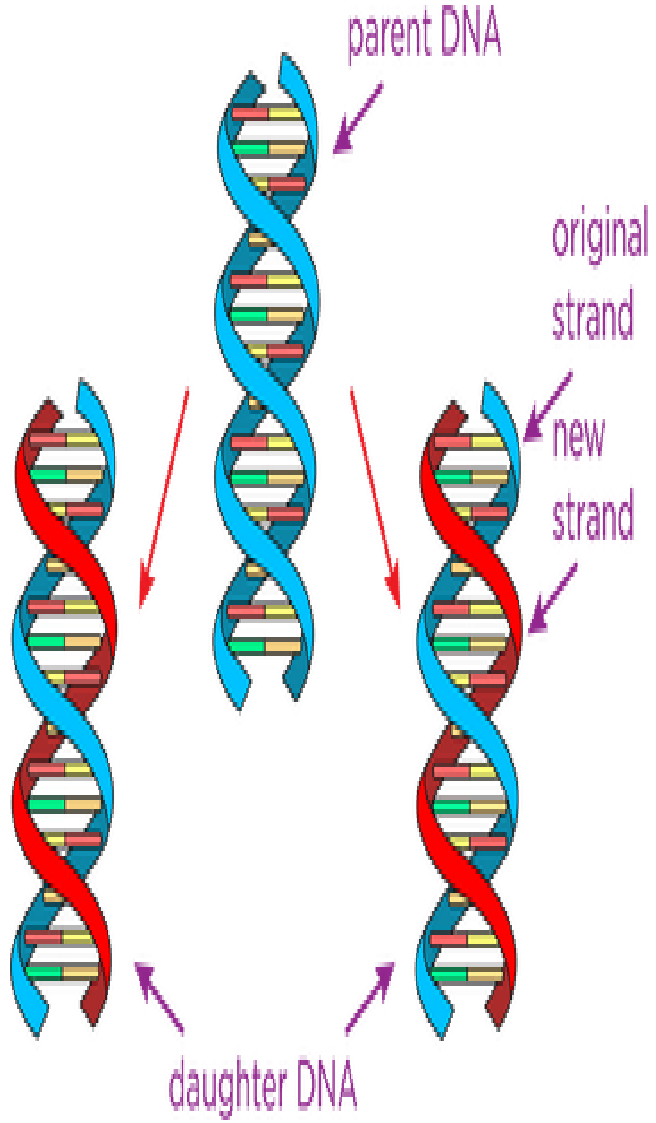
General Criteria of DNA replication

1- Replication is semi-conservative.

As each daughter DNA molecule contain:

- **One old strand** (one parent strand is conserved)
- **One new strand** (from free nucleotides in nucleus)

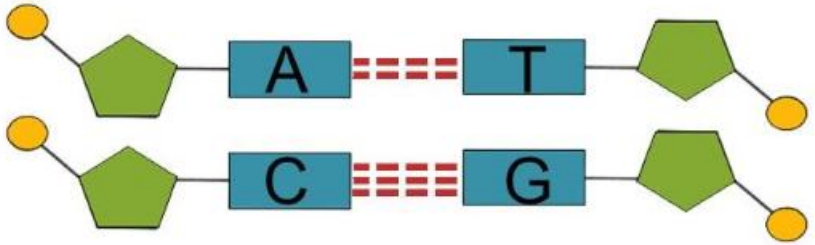


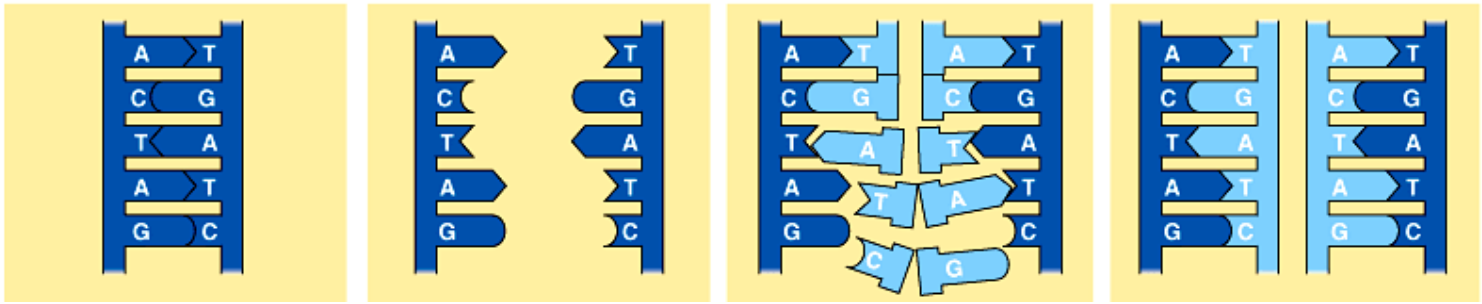


2- Complementary base pairing rule is always maintained

- Adenine pairs only with Thymine [A-T "two H bonds"]
- Cytosine pairs only with Guanine [C-G "three H bonds"]

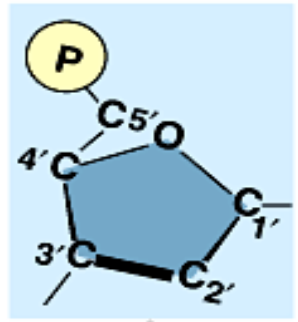
So, this means that in DNA (A+G = T+C)

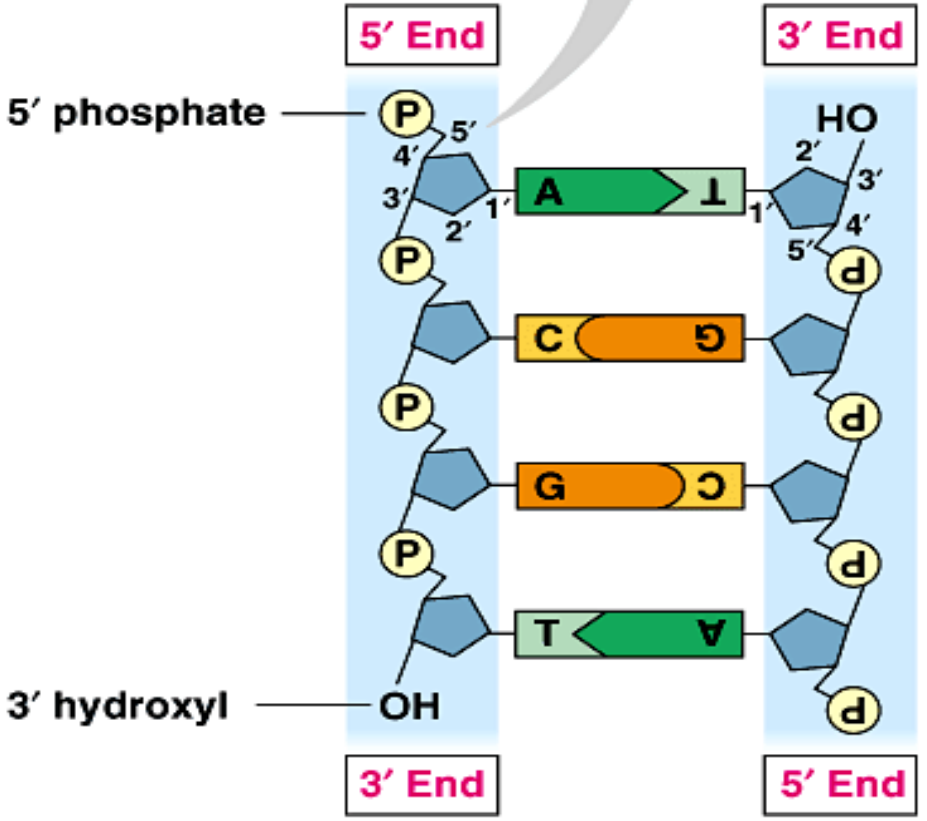




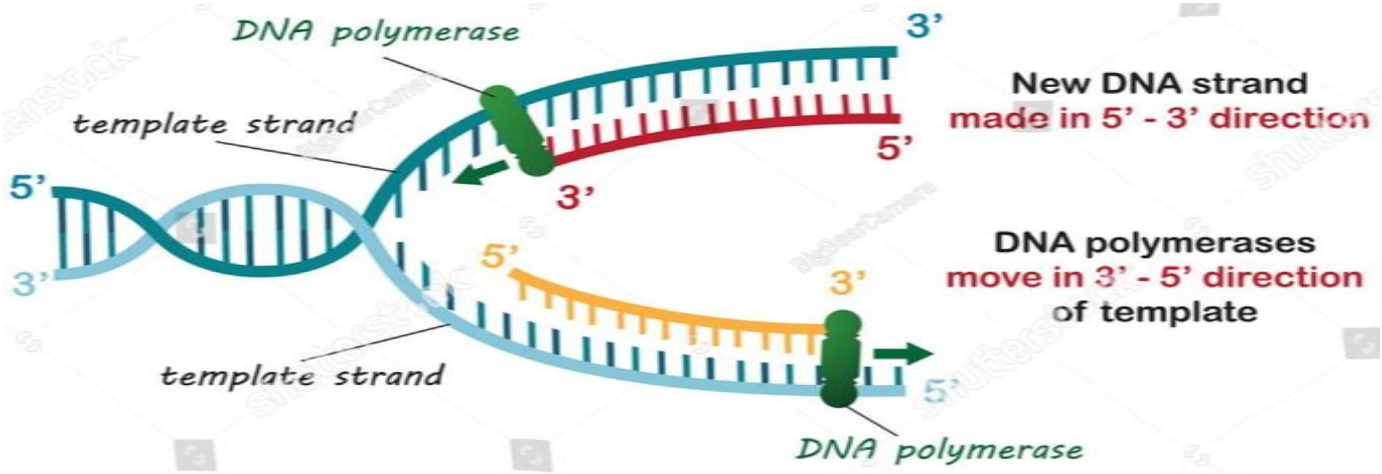
3- Anti-parallel strands

- One strand has a direction “5′-3′”
- complementary strand runs in opposite direction “3′-5′”



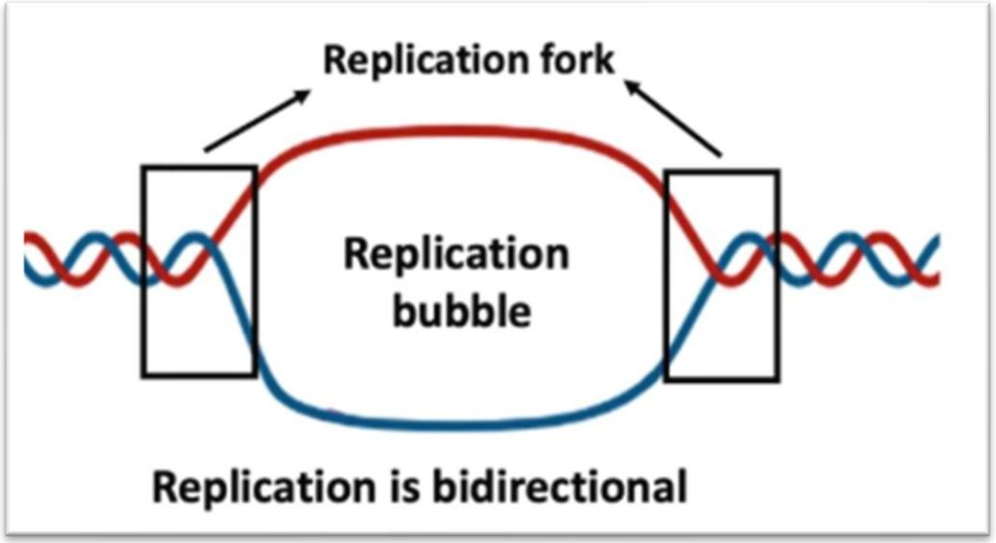


4- Both strands act as template



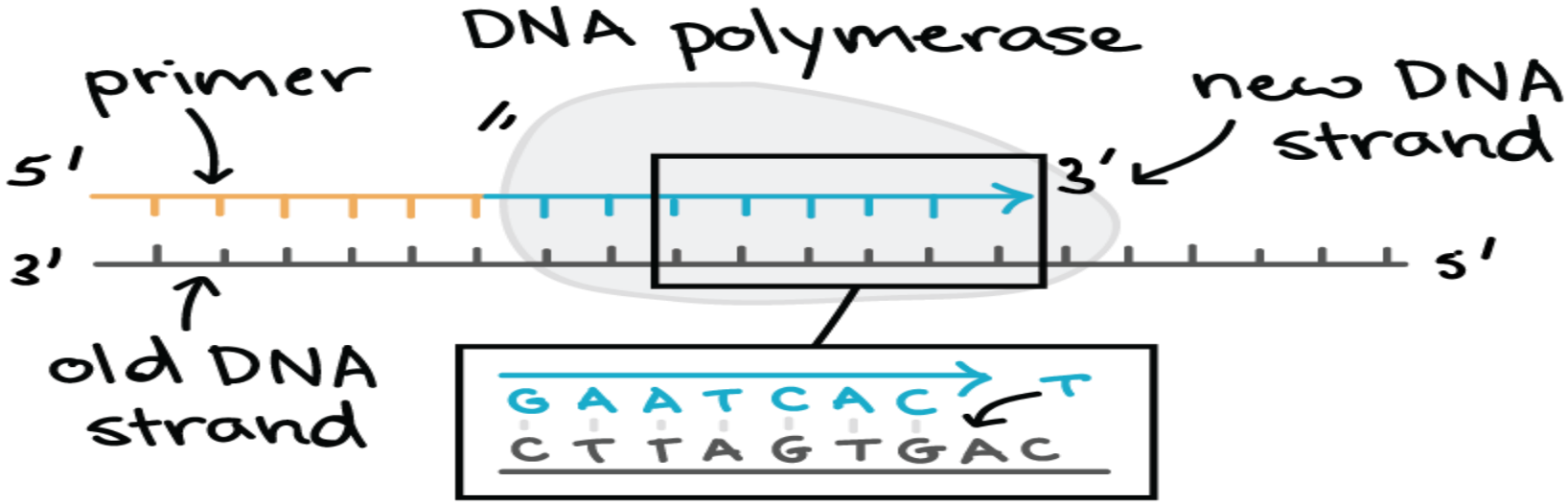
5- Bidirectional process

Replication proceeds in both directions



6- Direction of replication

Synthesis of new strand is done in 5´ to 3´direction, by DNA polymerase



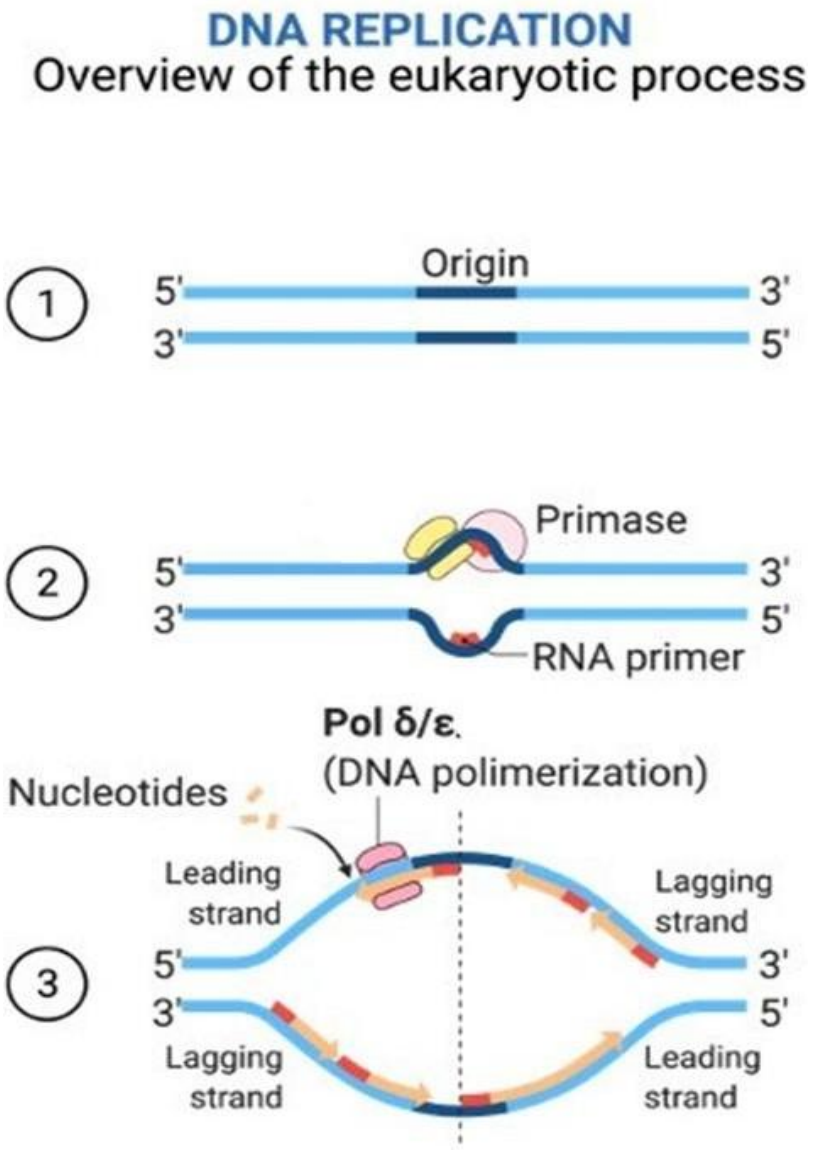
The basic requirements and components of replication

- **DNA template**: Double stranded DNA
- **Substrates**: 4 Deoxyribonucleosides triphosphate

  (dATP, dGTP, dCTP, dTTP)
- **Enzymes and proteins**: 

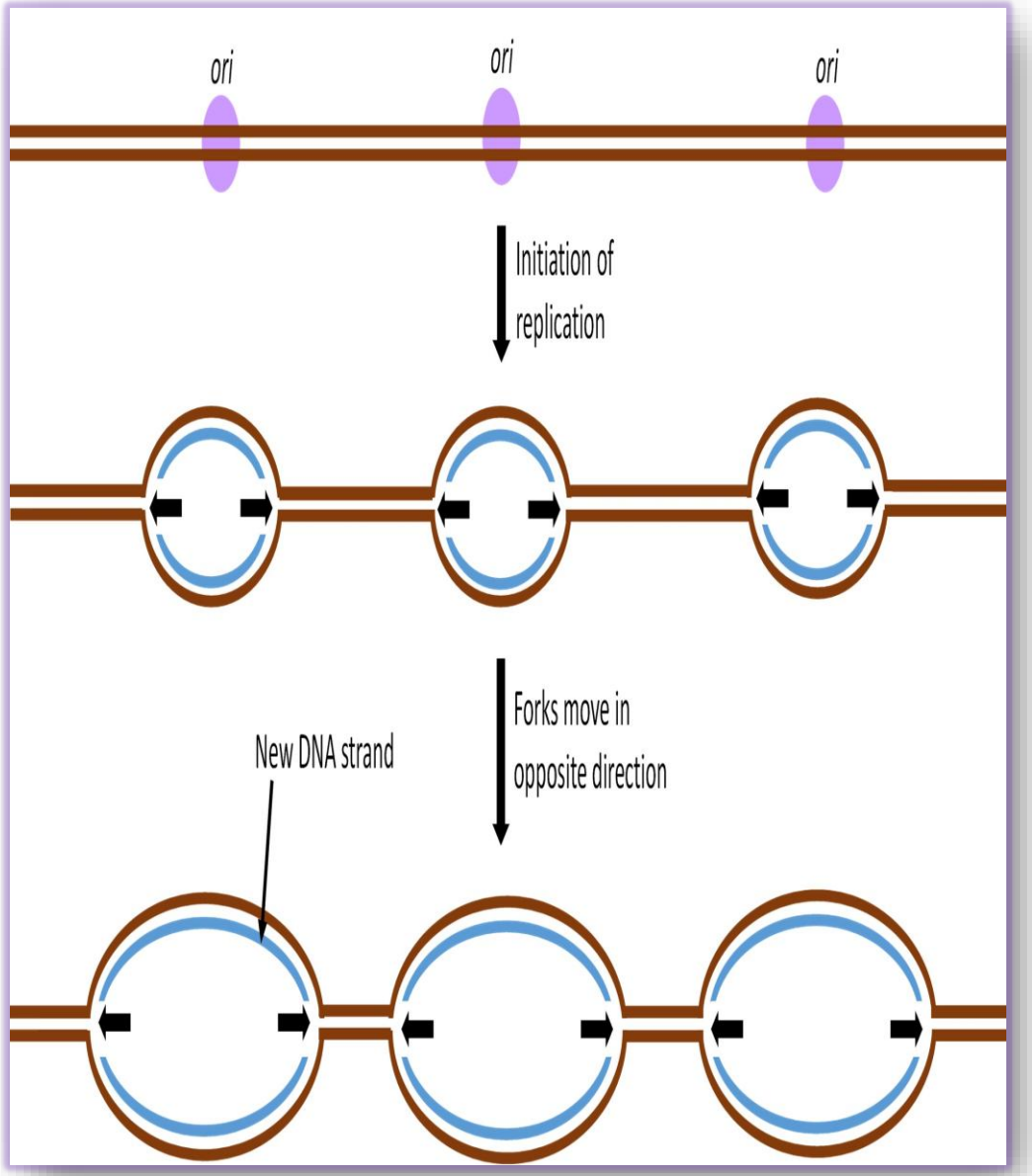
Steps of DNA replication

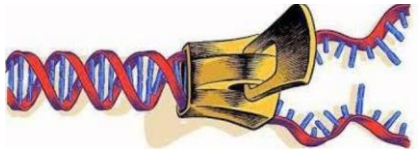
1. Identification of the origin of replication
2. Unwinding of double stranded DNA and formation of the replication fork (replication bubbles)
3. Initiation of DNA synthesis & elongation in 5´-3´ direction
4. Primer removal & ligation of newly synthesized DNA segments
5. Proofreading of newly synthesized DNA strands
6. Reconstitution of chromatin structure



1- Identification of the origin of replication

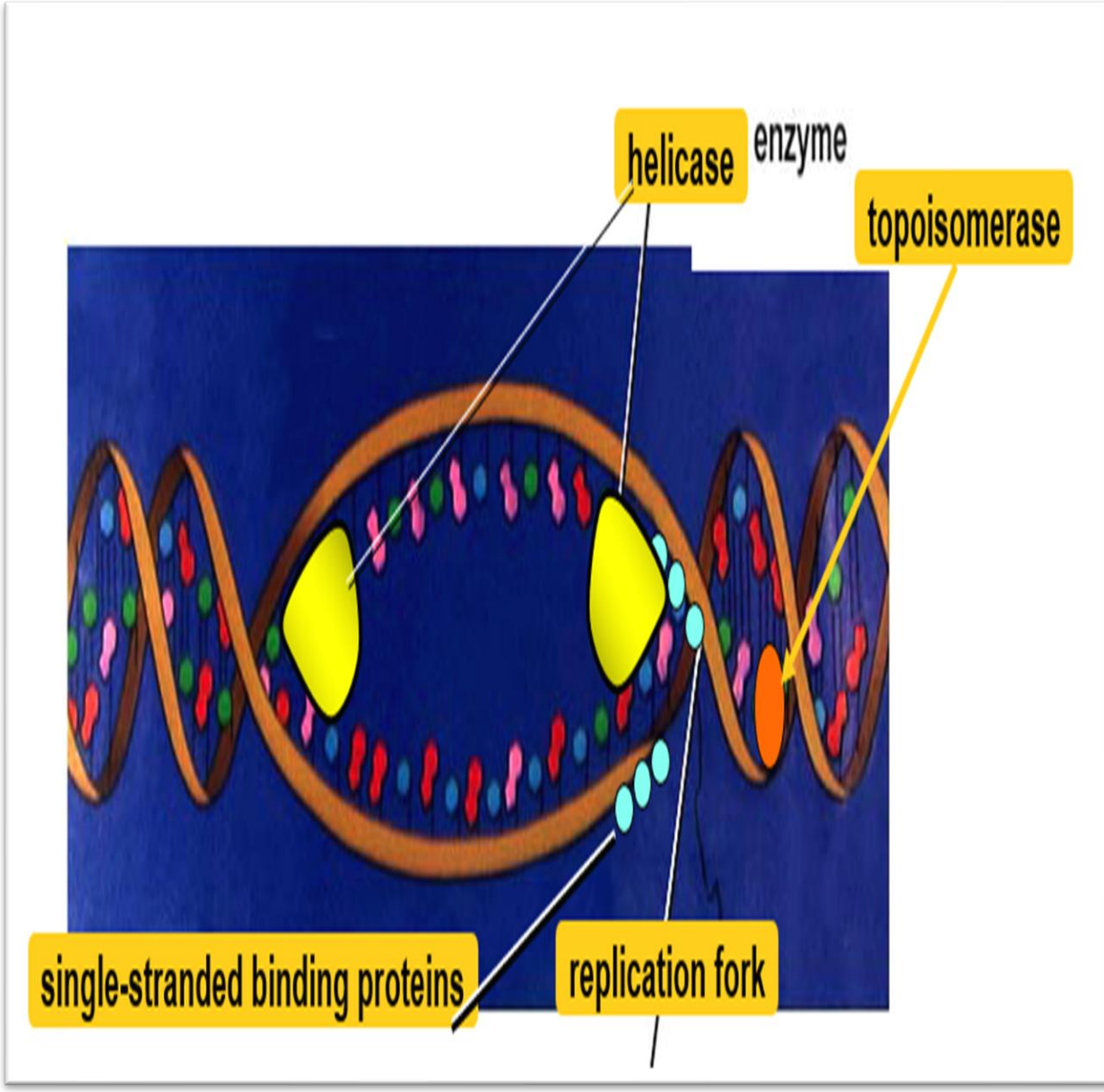
- Replication **starts** at points called ***origins of replication, (ori)***
- These sites contain short sequence composed only of **(AT) base pairs (consensus sequence)**.
- **Specialized proteins** recognize the origin, bind to it, and **open the DNA** making its components accessible for replication



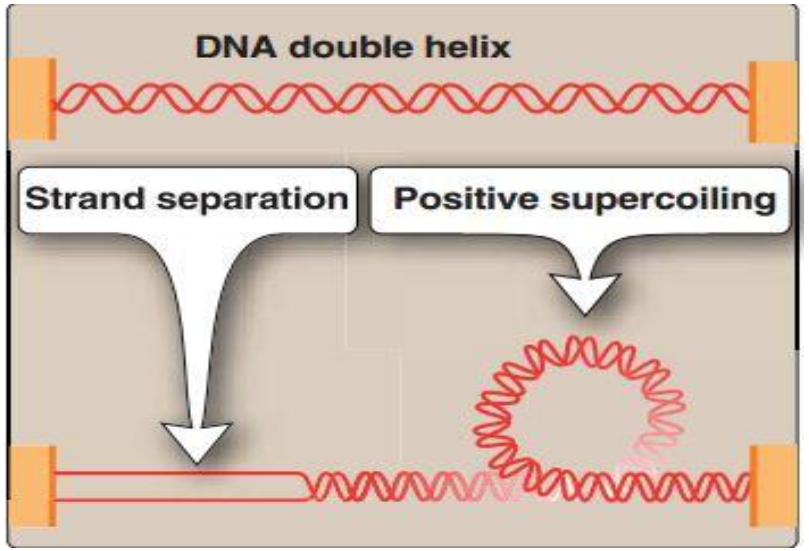


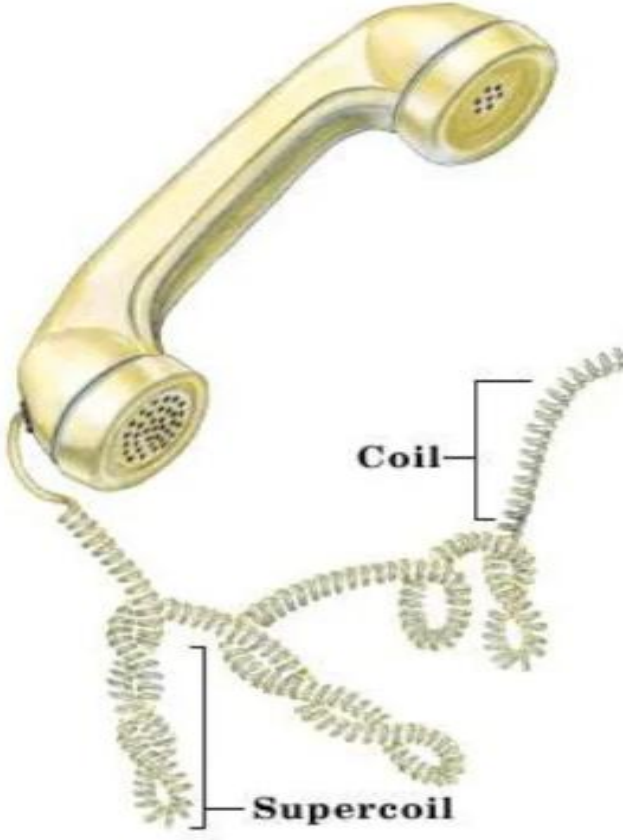
2- formation of replication fork

- The **DNA helicase** break the hydrogen bonds between DNA strands (It uses **ATP** molecules)
- Unwinds (separates) the 2 complementary strands.
- They form "V" shaped structure called **replication fork.**
- The two strands of DNA are kept away and separated by the **single-strand binding proteins.**



- As the 2 strands of DNA are separated, **a problem** happens called **Supercoiling** in the region of DNA **ahead of the replication** fork and interfere with further unwinding of the double helix.

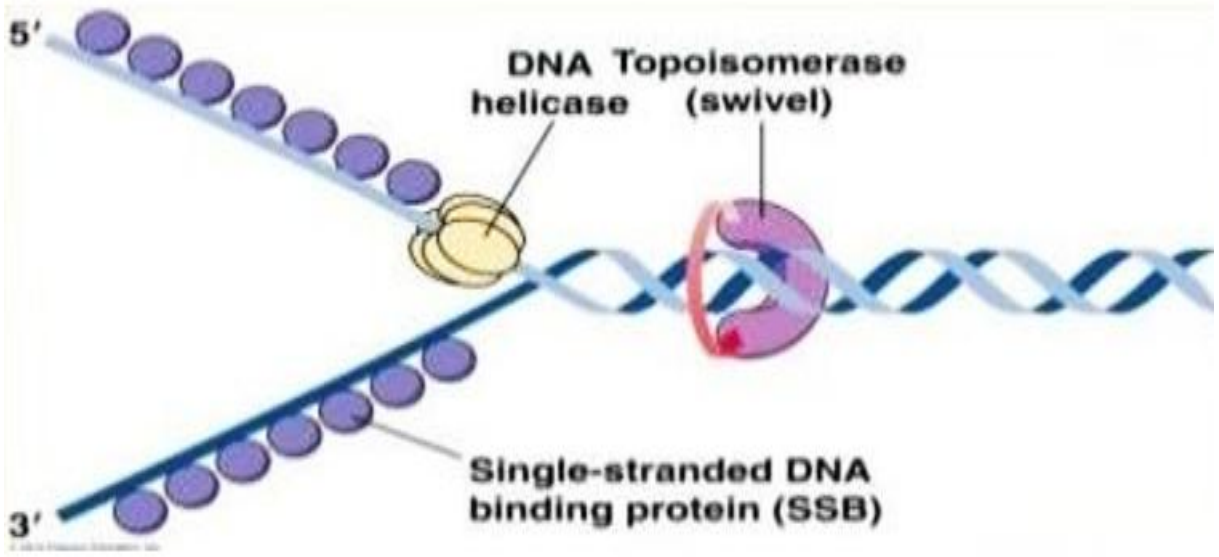


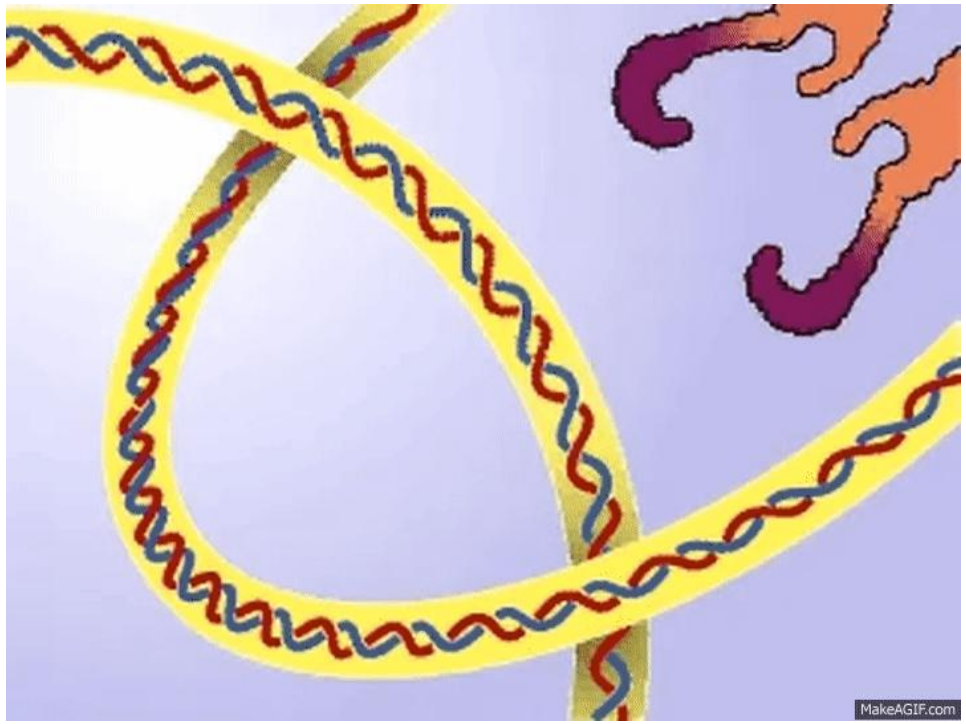


To solve this problem:

there are a group of enzymes called **Topoisomerases**

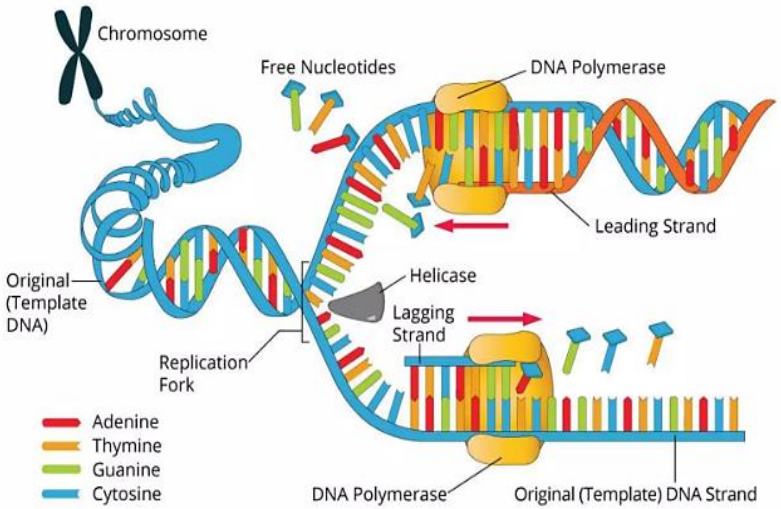
- They have both **nuclease** (strand cutting) and **ligase** (strand resealing) activity

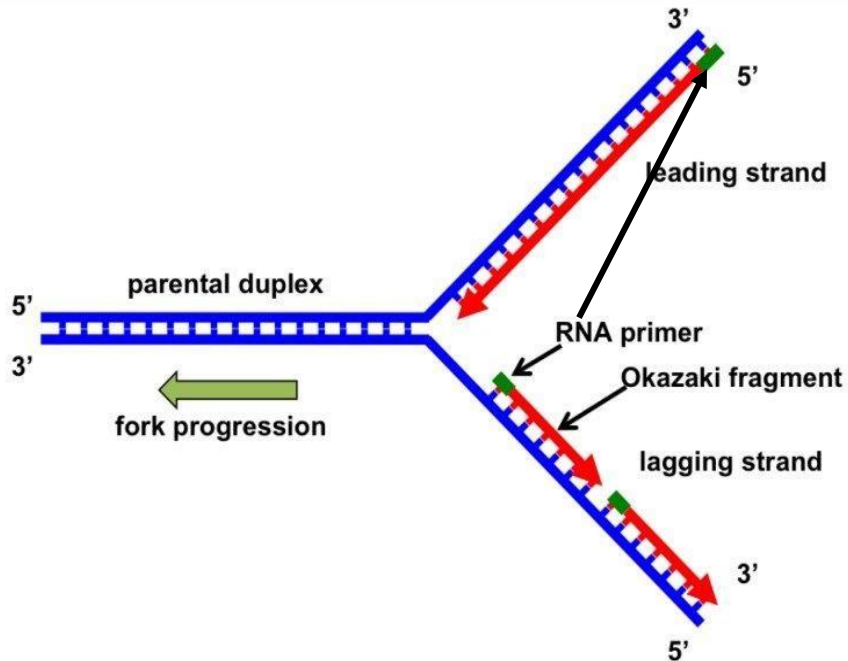




3- Initiation of DNA synthesis & elongation

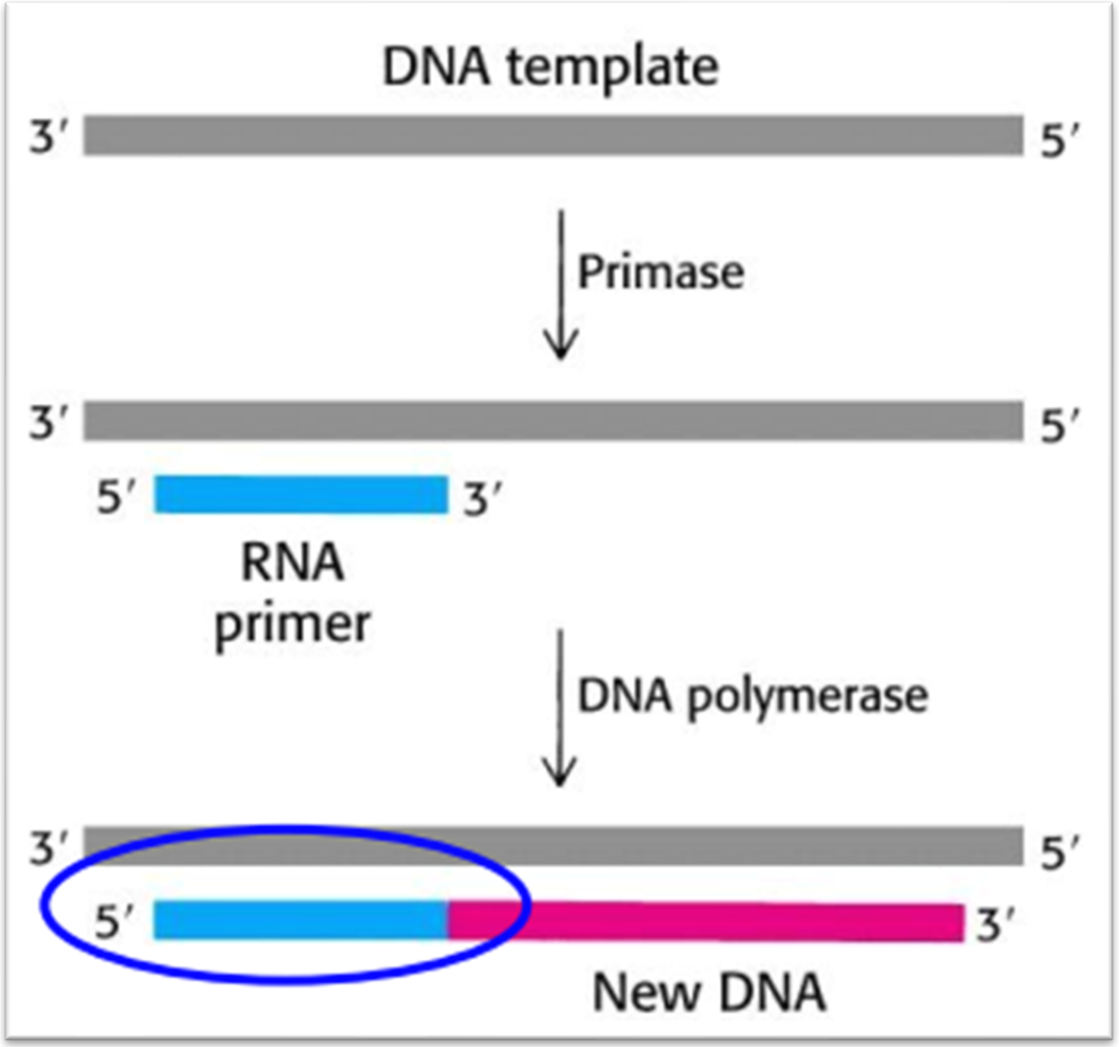
- Each strand will act as a template to direct the synthesis of new daughter DNA strand.
- This occurs by **DNA polymerase enzyme.**
- **The DNA polymerases** responsible for replication are only able to **read** the parental nucleotides sequences in 3´- 5´direction and **synthesize** the new DNA strands only in 5´- 3´ direction.
- DNA polymerases **cannot initiate** DNA synthesis by themselves, rather, it requires a short chain of nucleotides called a **primer** with a free OH group at 3´





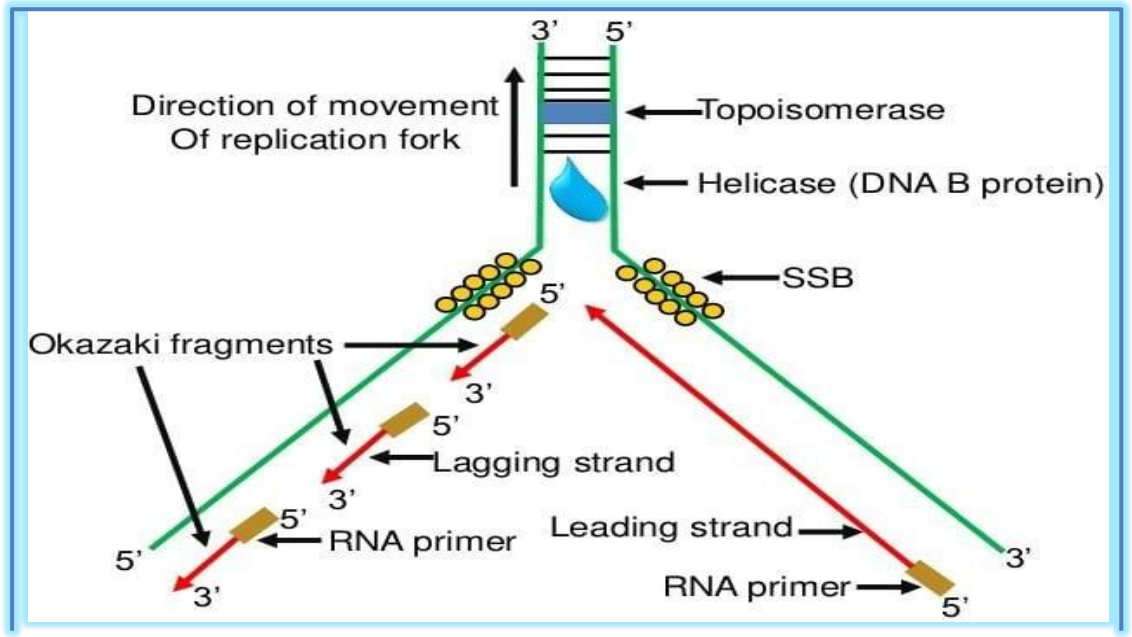
3-Initiation of DNA synthesis & elongation

- **The primase (DNA polymerase α)**: synthesizes a short stretch of RNA that are complementary and antiparallel the template DNA strands



3-Initiation of DNA synthesis & elongation

leading strand	Lagging strand:
This strand copy parental strand is in the direction from Ori **towards** replication fork	This strand copy parental strand is in the direction **away** from the replication fork
Needs One RNA primer	Needs multiple primers
synthesized continuously	synthesized discontinuously in a small fragments known as Okazaki fragment
DNA polymerase ε	**DNA polymerase δ**



- **Lagging strand** is formed in the form of small fragments termed ***Okazaki fragments*** **(each is formed of small DNA segment +RNA primer).**
- Okazaki fragments are connected into one continuous strand by ***ligase enzyme.***

3-Initiation of DNA synthesis & elongation

DNA Polymerases :

In humans :

- **DNA Polymerase α (Primase):**

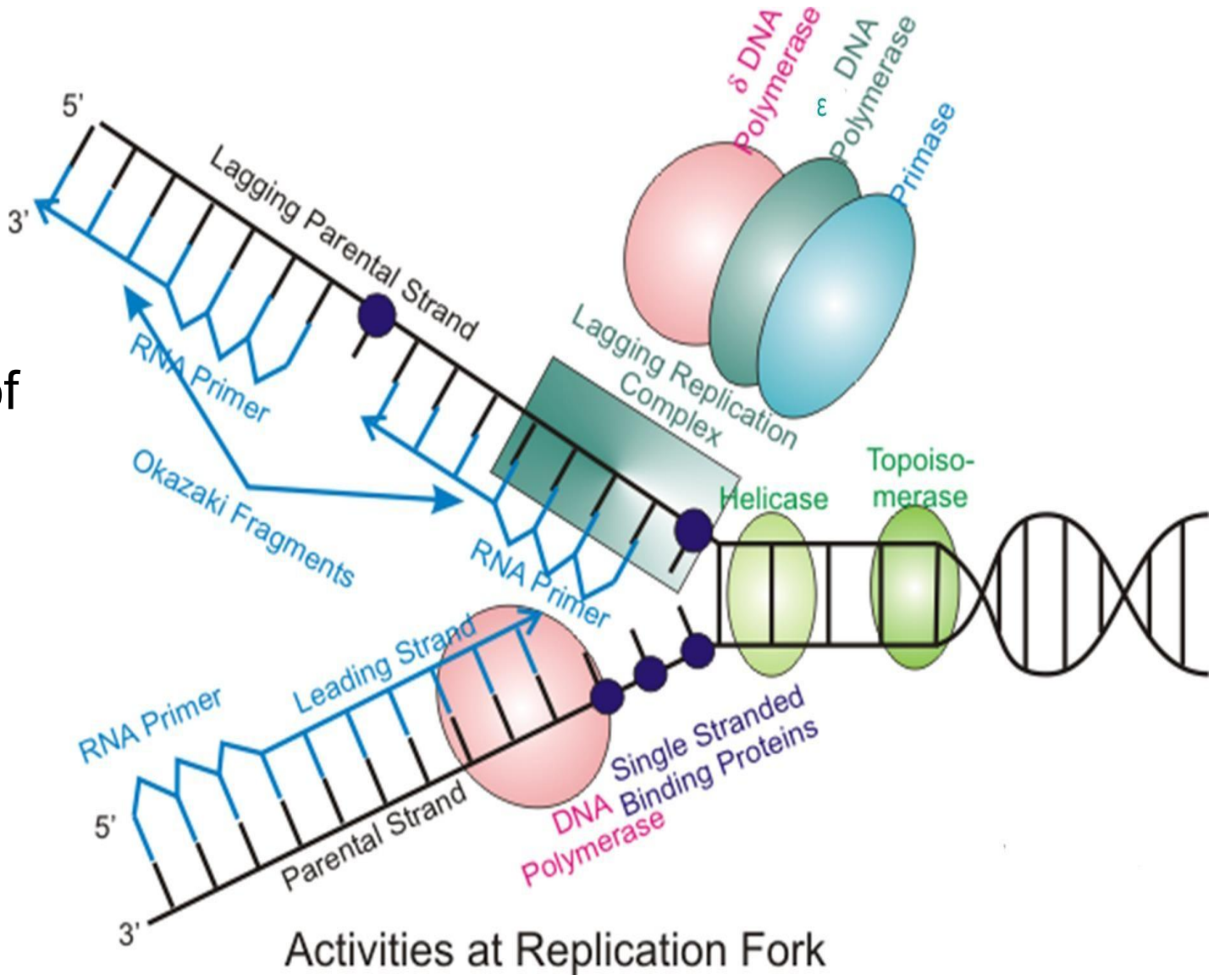
  Synthesize short RNA primers of Okazaki fragments

- **DNA Polymerase δ:**

  Replicate DNA lagging strand in discontinuous manner

- **DNA Polymerase ε:**

  Replicate DNA leading strand in continuous manner



Activities at Replication Fork

Replication Bubble : Bidirectional replication

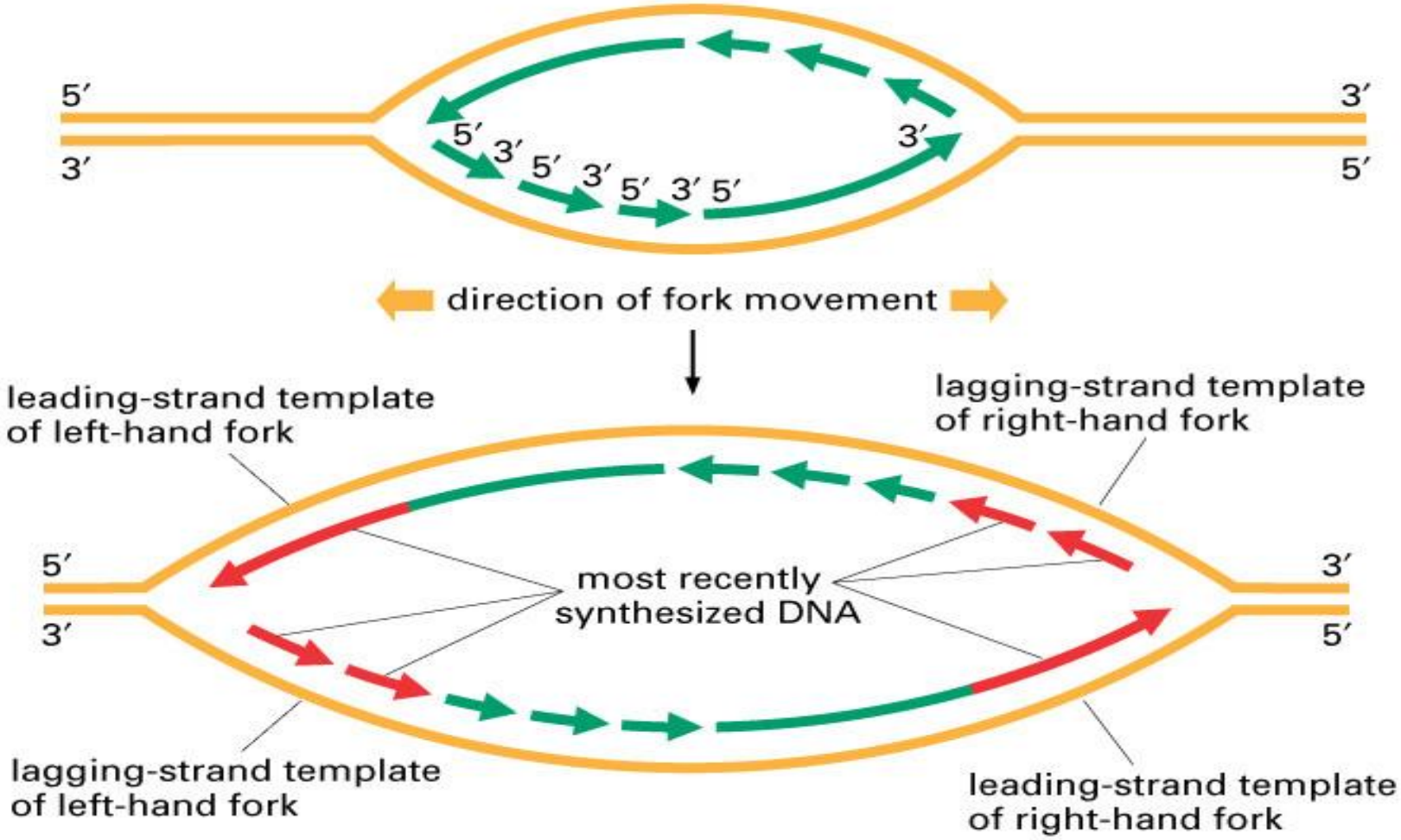


Figure 6-12 Essential Cell Biology, 2/e. (© 2004 Garland Science)

4- Primers removal and ligation of newly synthesized DNA segments

-Excision of RNA primers:

- This occurs by **Exonucleases.**
- Once the primers are removed, ***DNA polymerase δ*** binds to the 3' end of the preceding DNA fragment and extends the DNA over the gap.

-Ligation of different DNA fragments:

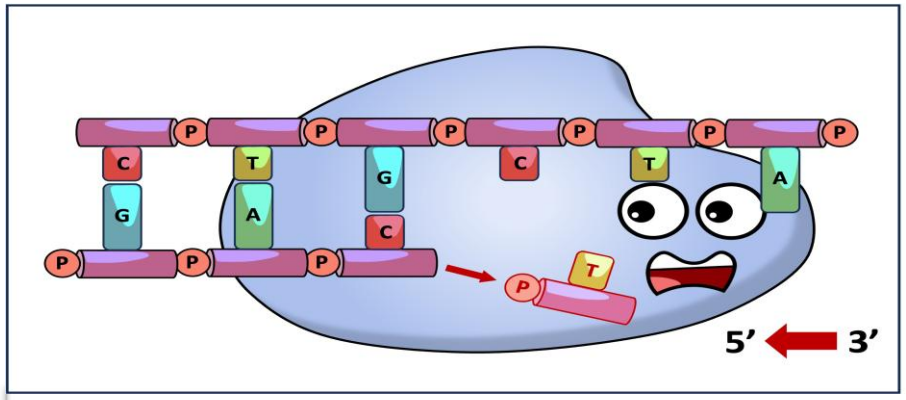
- By **DNA ligase.**

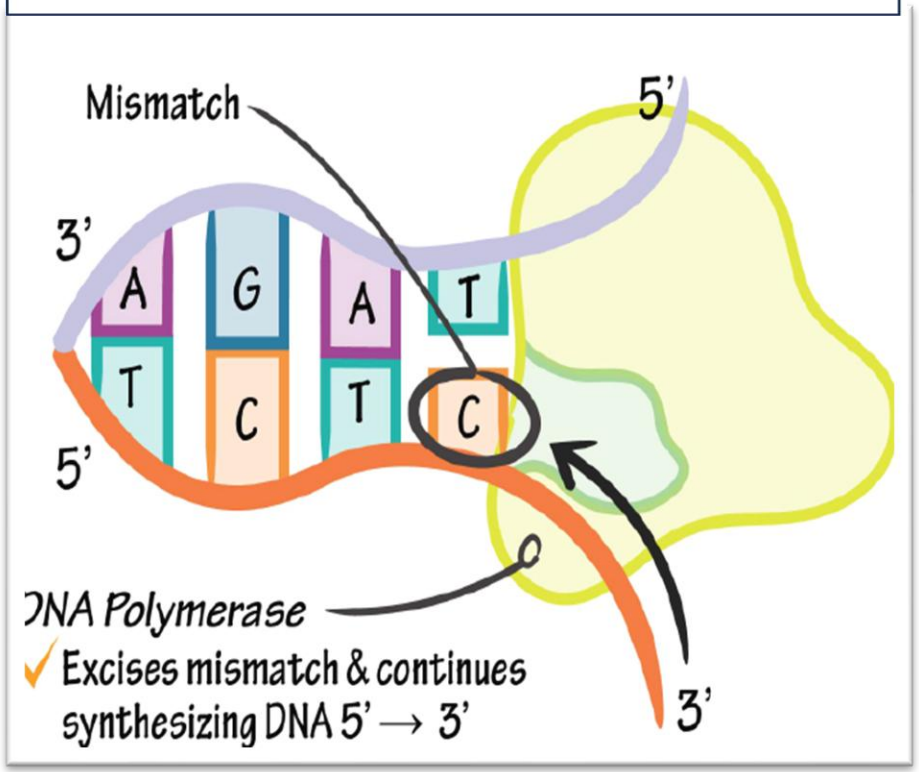
5- Proofreading of Newly Synthesized DNA Strands

- **Proofreading:**

Removal of the misplaced nucleotide and replacing it with the correct nucleotide

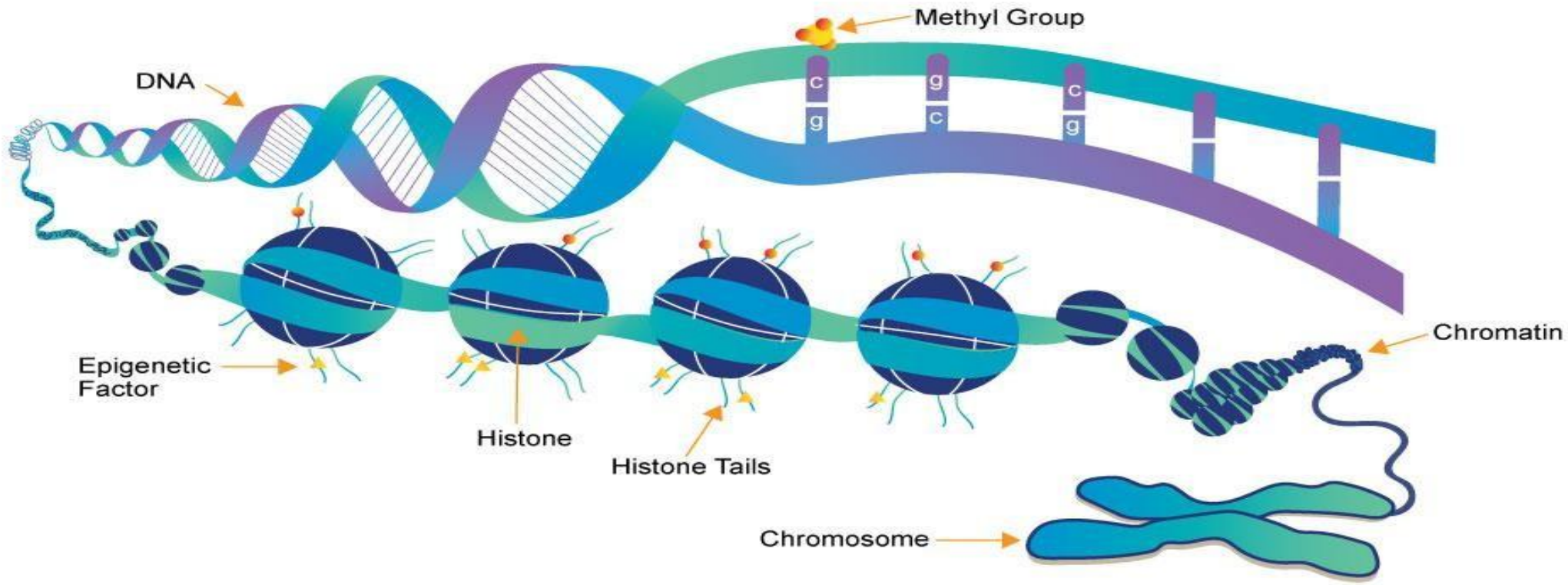
- As each nucleotide is added to the chain, the *DNA polymerases (δ and ε)* check the complementary base on the template and hydrolytically remove the misplaced nucleotides





6- Reconstitution of chromatin structure

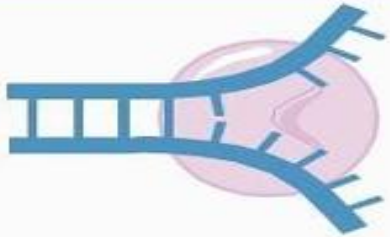
Recoiling of the parent and newly synthesized strand will result in completion of the replication process .



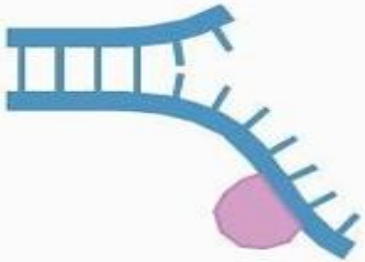
Enzymes and proteins

1- DNA helicases	• Unwinding of dsDNA
2- Topoisomerase	• Relieve tortional strain results from helicase induced unwinding • cutting and joining single strand or both strands
3- single stranded DNA binding protein (ssDB)	• Bind single stranded DNA and stabilize it
4- Primase	• RNA primer
5- DNA polymerase	Chief enzymes required for: • DNA chain elongation • DNA repair • Proofreading require a template and a primer (starter) and synthesize DNA in the 5' to 3' direction.
6- Exonuclease	• Excision of RNA primers
7- DNA ligase	• Join okazaki fragments

Enzymes in DNA replication

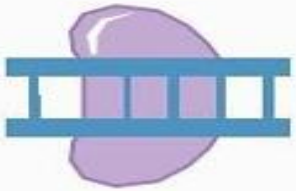


Helicase unwinds parental double helix



Binding proteins stabilize separate strands

Primase adds short primer to template strand



DNA polymerase binds nucleotides to form new strands

(Exonuclease) removes RNA primer and inserts the correct bases

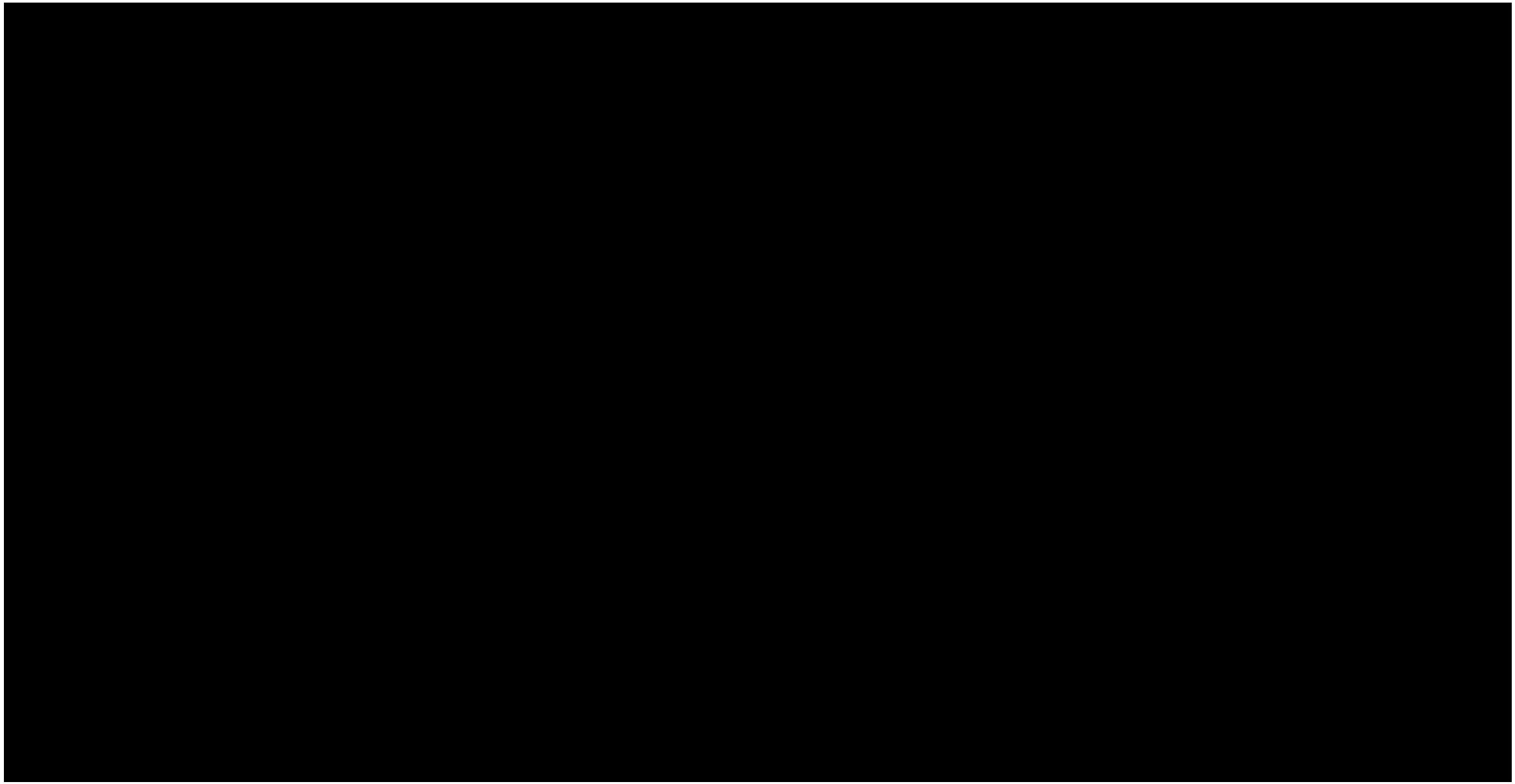
Ligase joins Okazaki fragments and seals other nicks in sugar-phosphate backbone

+ topoisomerase

What is the end replication problem?

Solution to
end replication problem:

Telomerase enzyme




Thank You