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Programme: M.Sc., Biomedical Science

Course Code: BM35C5
Course Title: Molecular Biology

Unit-II

Replication

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Unit II:

Replication: Principle and mechanism of replication- Conservative, Semi conservative, Uni- directional and bi-directional mode of replication- Enzymes and accessory proteins involved in replication- Prokaryotic and Eukaryotic DNA replication. DNA damage& repair (Direct repair systems, mismatch repair system, base excision repair and recombination repair)

PRESENTATION: 2

PROKARYOTIC DNA REPLICATION

- DNA replication is the biological process of **producing two identical replicas of DNA from one original DNA molecule.**
- DNA replication occurs in **all living organisms** acting as the most essential part of biological inheritance.
- **Prokaryotes** have been **extensively investigated** in terms of DNA replication, partly due to the tiny size of their genomes and mutations.
- 4.6 million base pairs make up a circular chromosome in the bacteria E. coli, and the entire chromosome is duplicated in around 42 minutes, starting from a single origin of replication and proceeding around the circle in both directions. This means that approximately 1000 nucleotides are added per second.

PROTEINS AND ENZYMES INVOLVED IN REPLICATION

1. **Helicases**: unwinds the ds DNA
2. **DNA topoisomerases**: – relieves the topological stress produced in the helical stuctures of DNA during unwinding
3. **DNA polymerase I**
4. **DNA polymerase III**
5. **DNA primase**: is an enzyme involved in the replication of DNA and is a type of RNA polymerase. segment called a primer complementary to a ssDNA template
6. **DNA ligase**– catalyses the formation of a phosphodiester linkage.
7. **Single stranded DNA binding proteins** – stabilizes the single strands of DNA after unwinding

Initiation

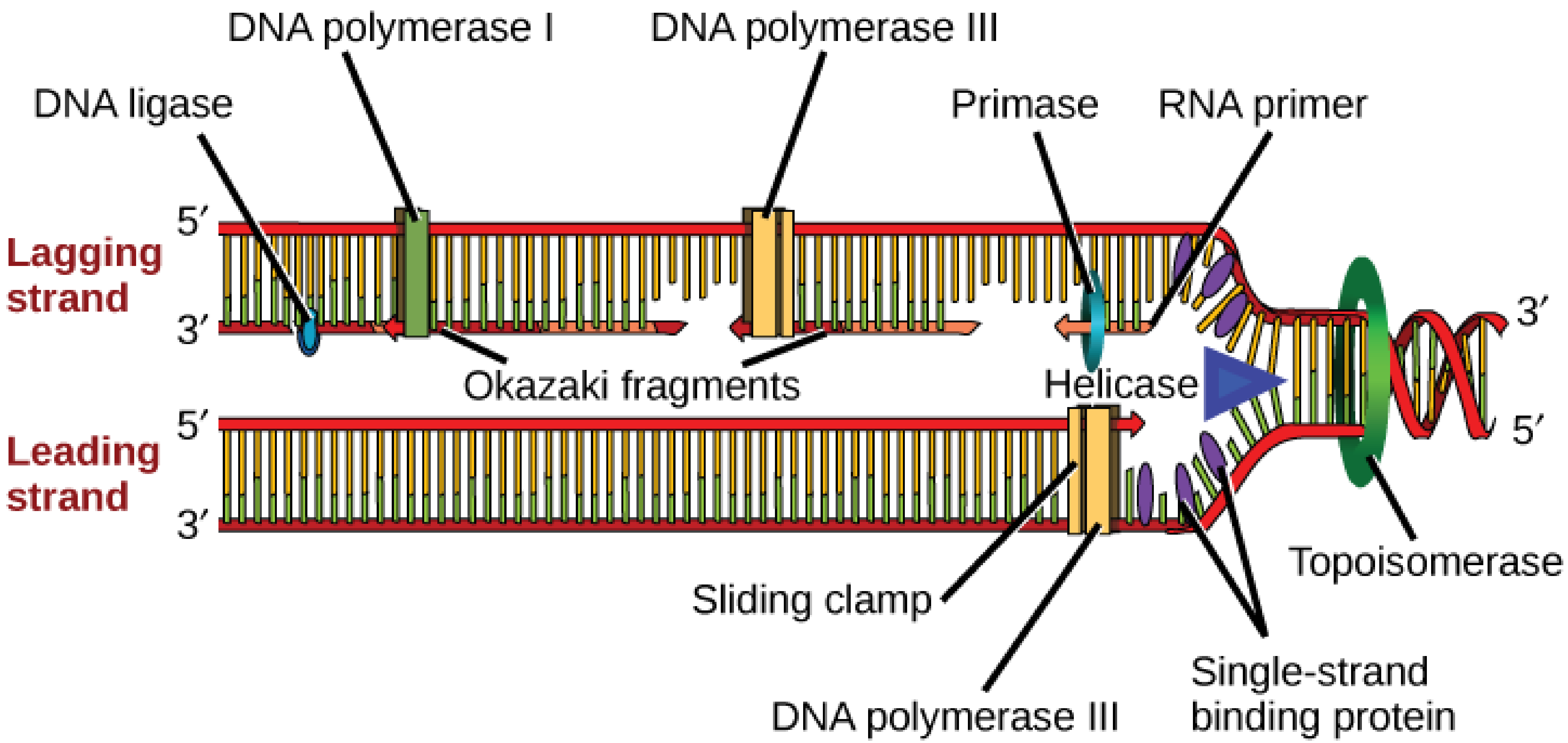
- Replication begins at the origin of replication, a specific sequence of nucleotides in the DNA.
- Initiator proteins recognize and bind to the origin, leading to the unwinding and separation of the DNA strands.
- Formation of a replication bubble where the replication process initiates bidirectionally.
- The replication fork is formed initially, and the **DNA strand is unzipped** by the helicase enzyme, which catalyzes the replication fork formation.
- For a pair of replication forks to develop, the helix must be unwound by the helicase enzyme, and the unwound helix must be stabilized by SSB proteins and DNA isomerases.
- Primase synthesizes RNA primers with a base sequence of 10 bases, which are used to trigger the synthesis of both the leading and lagging strands.

Elongation

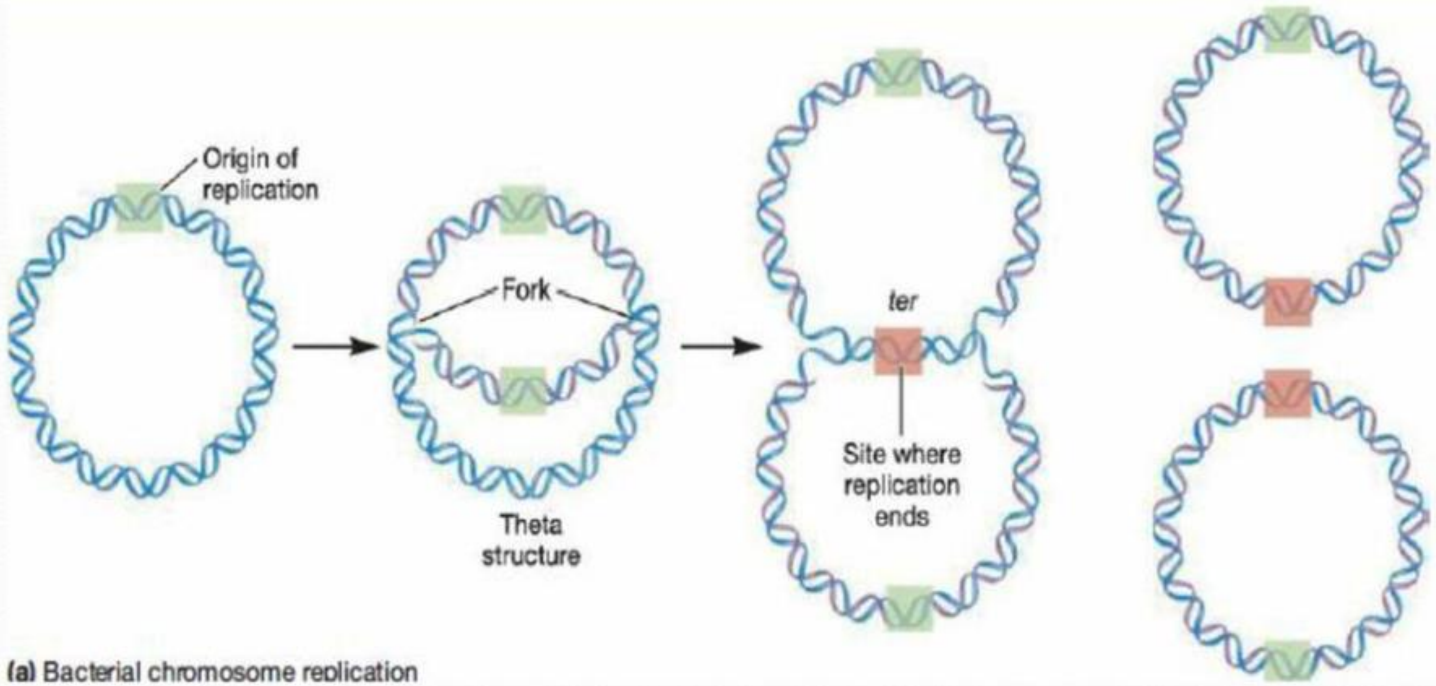
- In parallel with the separation of the strands of DNA, the **polymerase enzymes begin synthesizing the corresponding sequence in each strand of DNA that has been split**.
- The **parental strands will serve as a template for the synthesis of daughter strands** that are produced in the future.
- In addition, it should be emphasized that **elongation is unidirectional**, meaning that DNA is always polymerized in just one direction, from 5′ to 3′.
- As a result, on one strand (the template 3’5′), replication is continuous, therefore the term “continuous replication,”
- whereas on the other strand (the template 5’3′), replication is discontinuous, so the term “discontinuous replication.”
- They are found in the form of pieces known as **Okazaki fragments**. Later on, an enzyme known as **DNA ligase** links them together.

Termination

- Replication of bacterial genome proceeds bi- directionally which terminates at a position diametrically opposite to the origin of replication.
- Replication terminates at the **terminus region containing multiple copies of a 20bp sequence called Ter (Terminus) sequence**.
- The completed chromosomes then partitioned into two daughter cells during cell division.



DNA polymerase I
DNA polymerase III
DNA ligase
Primase
RNA primer
5′
Lagging strand
3′
3′
Okazaki fragments
Helicase
5′
Leading strand
3′
5′
Topoisomerase
Sliding clamp
Single-strand binding protein
DNA polymerase III



(a) Bacterial chromosome replication

REPLICATION IN EUKARYOTES

- DNA replication in eukaryotes is the process by which a **cell duplicates its DNA**, ensuring that **each daughter cell receives a complete set of genetic information during cell division**.
- This process is highly regulated and involves multiple enzymes and proteins.

Replication in Eukaryotic Cell

- Replication in E. coli begins at only one site along the single, circular chromosome.
- Eukaryotes have thousand times bigger DNA yet their polymerases incorporate nucleotides into DNA at much slower rates.
- Eukaryotic cells replicate their genome in small portions, termed replicons.
- Each replicon has its own origin from which replication forks proceed outward in both directions.
- In a human cell, replication begins at about 10,000 to 100,000 different replication origins.
- Approximately 10 to 15 percent of replicons are actively engaged in replication at any given time during the S phase of the cell cycle

Features of Eukaryotic DNA Replication

- **Replication is bi-directional** and originates at **multiple origins of replication (Ori C)** in eukaryotes.
- DNA replication uses a **semi-conservative method** that results in a double-stranded DNA with one parental strand and a new daughter strand.
- It occurs only in the **S phase** and at many chromosomal origins.
- Takes place in the **cell nucleus**.
- Synthesis occurs **only in the 5′to 3′direction**.
- Individual strands of DNA are manufactured in different directions, producing a **leading and a lagging strand**.
- Lagging strands are created by the production of small DNA fragments called **Okazaki fragments** that are eventually joined together.
- Eukaryotic cells possess **five types of polymerases** involved in the replication process.

Components of Eukaryotic DNA Replication

- 1. Origin of Replication: Specific regions on the DNA where replication begins. Eukaryotic chromosomes have multiple origins of replication to ensure the entire genome is replicated efficiently within the limited time during the S-phase of the cell cycle.
- 2. Replication Fork: The Y-shaped structure formed when DNA is unwound at the origin, allowing replication to occur on both strands.
- 3. Helicase: The enzyme that unwinds the DNA helix by breaking hydrogen bonds between the two strands.
- 4. Single-Stranded Binding Proteins (SSBs): Proteins that bind to the separated DNA strands, keeping them apart and protecting them from degradation.
- 5. Primase: An RNA polymerase that synthesizes a short RNA primer, which serves as a starting point for DNA synthesis.

- 6. DNA Polymerase: The enzyme responsible for synthesizing new DNA strands by adding nucleotides complementary to the template strand. In eukaryotes, there are several types of DNA polymerases involved in replication:
  - DNA Polymerase α: Initiates DNA synthesis by extending the RNA primer.
  - DNA Polymerase δ: Takes over from DNA polymerase α and elongates the lagging strand.
  - DNA Polymerase ε: Primarily responsible for synthesizing the leading strand.
- 7. DNA Ligase: Enzyme that joins Okazaki fragments (short DNA fragments synthesized on the lagging strand) by forming phosphodiester bonds.
- 8. Topoisomerase: Enzymes that prevent the overwinding (supercoiling) of DNA ahead of the replication fork by cutting and rejoining the DNA strands.

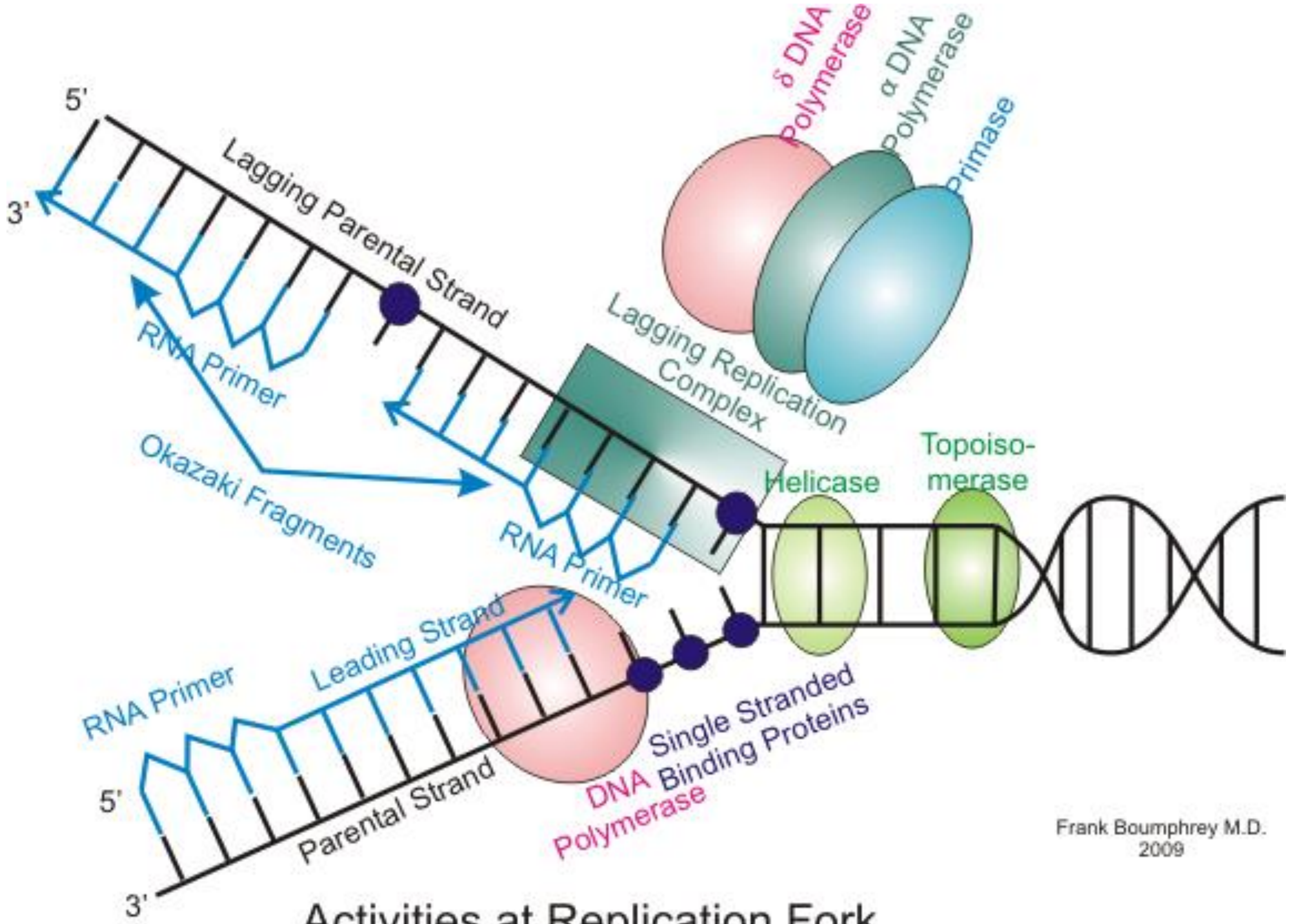
THE ENZYMES OF DNA REPLICATION

- **1. Helicases:** Unwind the DNA helix at the start of replication.
- **2. SSB proteins:** Bind to the single strands of unwound DNA to prevent reformation of the DNA helix during replication.
- **3. DNA Polymerases:**

Eukaryotic cells contain five different DNA polymerases; α, β, γ, δ and ε.

- **4. Telomerase,** a DNA polymerase that contains an integral RNA that acts as its own primer, is used to replicate DNA at the ends of chromosomes (telomeres).
- **5. DNA topoisomerase I:** Relaxes the DNA helix during replication through creation of a nick in one of the DNA strands.
- **6. DNA topoisomerase II:** Relieves the strain on the DNA helix during replication by forming supercoils in the helix through the creation of nicks in both strands of DNA.
- **7. DNA ligase:** Forms a 3′-5′phosphodiester bond between adjacent fragments of DNA.

DNA REPLICATION



Activities at Replication Fork

Stages of Eukaryotic DNA Replication

a. **Initiation**

- **Origin Recognition:** Replication begins at multiple origins of replication across the eukaryotic genome. These origins are recognized by the origin recognition complex (ORC), a protein complex that binds to the DNA.
- **Formation of Pre-Replication Complex (Pre-RC):** Once the ORC binds to the origin, additional proteins, including cell division cycle 6 protein (Cdc6), chromatin licensing and DNA replication factor 1 protein (Cdt1), and the (Minichromosome maintenance) MCM helicase complex, are recruited, forming the pre-replication complex. The helicase is loaded onto the DNA and ready to begin unwinding it.

- **Activation of Helicase:** The helicase is activated by kinases (e.g., cyclin-dependent kinase 2 (Cdk), Dbf4-dependent kinase (DDK)), leading to the unwinding of the DNA at the origin of replication. This creates single-stranded DNA templates for replication.
- **Replication protein A (RPA)-** Binds to the exposed single-stranded DNA molecule and prevents rewinding.
- **Priming:** Once the DNA is unwound, primase synthesizes short RNA primers on the template strands. These primers provide a starting point for DNA polymerase.

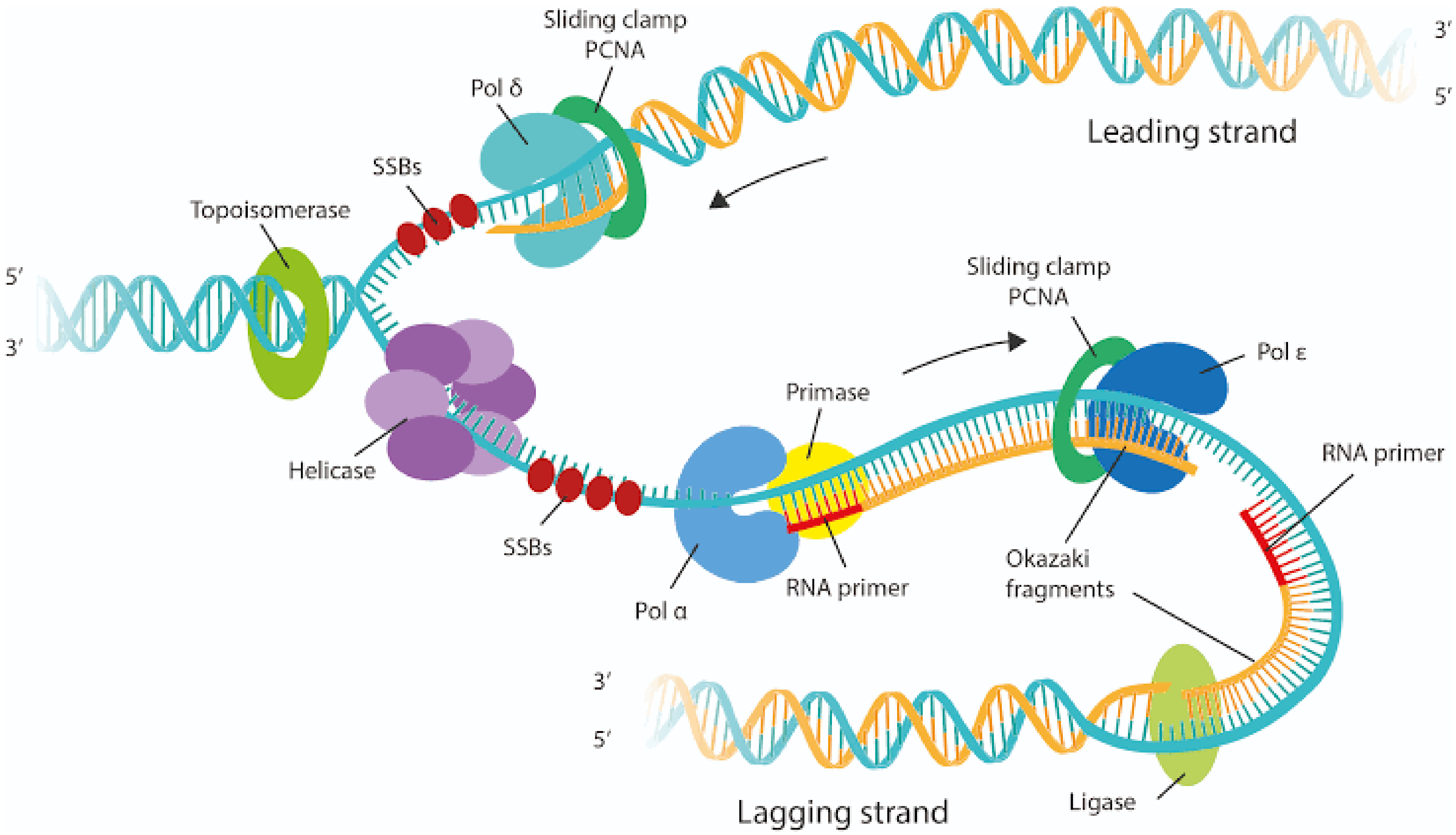
b. Elongation

- **PCNA**- Ring shaped subunit of DNA polymerase holoenzyme that clamps polymerase to DNA
- **Leading Strand Synthesis:** DNA polymerase ε synthesizes the leading strand continuously in the 5’ to 3’ direction, using the original strand as a template.
- **Lagging Strand Synthesis:** DNA polymerase δ synthesizes the lagging strand discontinuously in short segments called Okazaki fragments. This is because the lagging strand is oriented in the 3’ to 5’ direction, opposite to the direction of the replication fork movement. Each Okazaki fragment is initiated with a new RNA primer.
- **RNA Primer Removal:** After the Okazaki fragments are synthesized, the RNA primers are removed by RNase H and FEN1 (flap endonuclease). DNA polymerase δ then fills in the gaps with DNA.
- **Joining of Okazaki Fragments:** DNA ligase seals the nicks between adjacent Okazaki fragments, creating a continuous strand.

c. Termination

- DNA replication continues **until two replication forks meet**, and the replication machinery disassembles.
- The group of cellular **enzymes that remove RNA primers** include the proteins **FEN1 (flap endonuclease 1) and RNase H**.
- In the final stage of DNA replication, the enyzme **ligase joins the sugar-phosphate backbones** at each nick site. After ligase has connected all nicks, the new strand is **one long continuous DNA strand, and the daughter DNA molecule is complete.**

DNA REPLICATION



Acknowledgement

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