ENZYMES INVOLVED IN DNA REPLICATION

CELL AND MOLECULAR BIOLOGY
I MSC ZOOLOGY
UNIT IV

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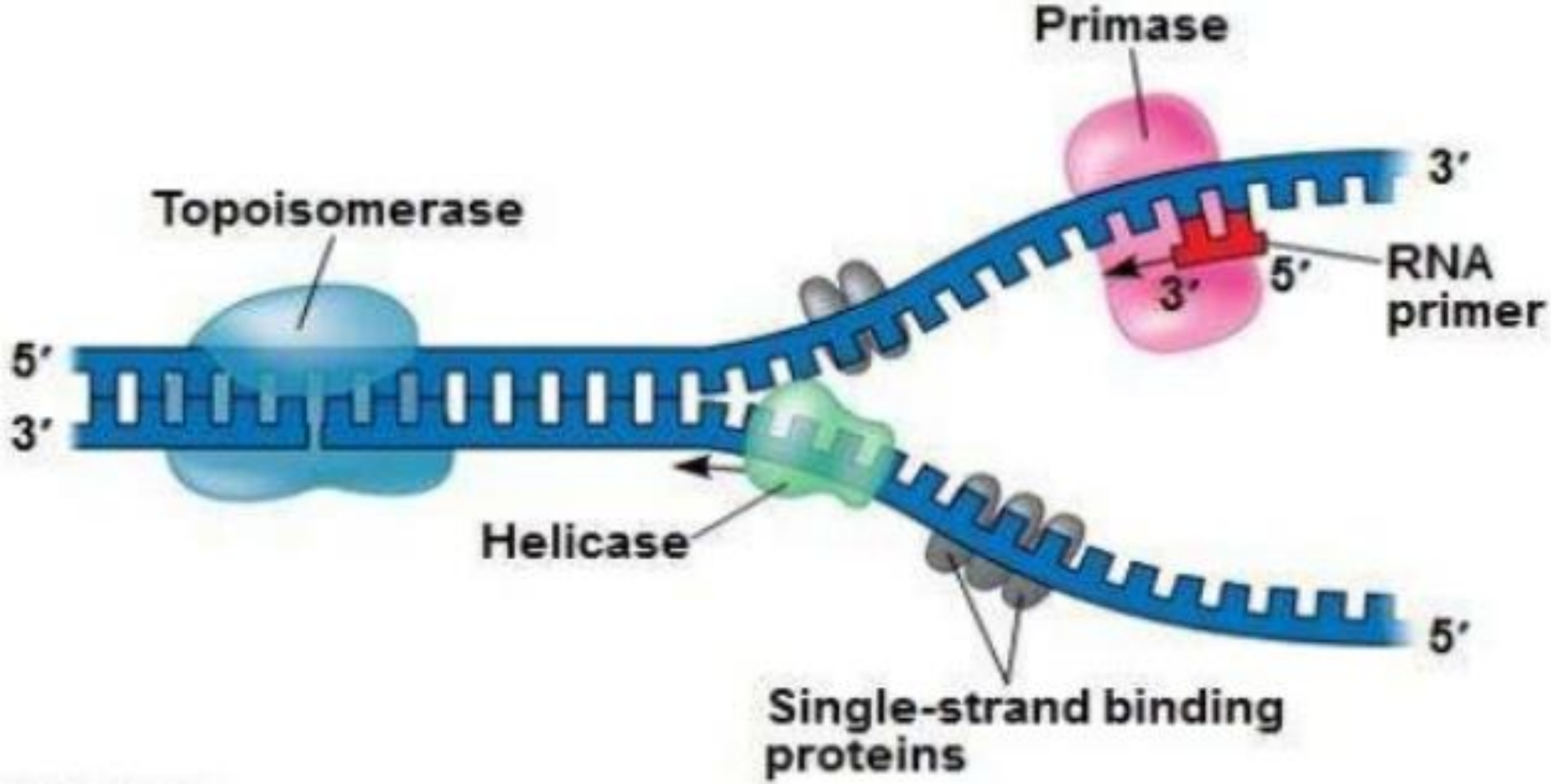
- **Replication** means **"Synthesis of daughter nucleic acid molecules identical to the parental nucleic acids"**.
- Thus accurate and complete replication of the DNA is essential to the ability of a cell organism to reproduce.
- It is the basis for biological inheritance.
- DNA is made up of a double helix of two complementary strands.
- During replication, these strands are separated.
- There are many enzymes they participate in the process of replication.

ENZYMES INVOLVED IN DNA REPLICATION

- This is the list of Enzymes involved in DNA Replication.
  - **DNA Helicase**
  - **DNA Polymerase**
  - **DNA clamp**
  - **Single–Strand Binding (SSB) Proteins**
  - **Topoisomerase / DNA Gyrase**
  - **DNA Ligase**
  - **Primase**

DNA Helicase

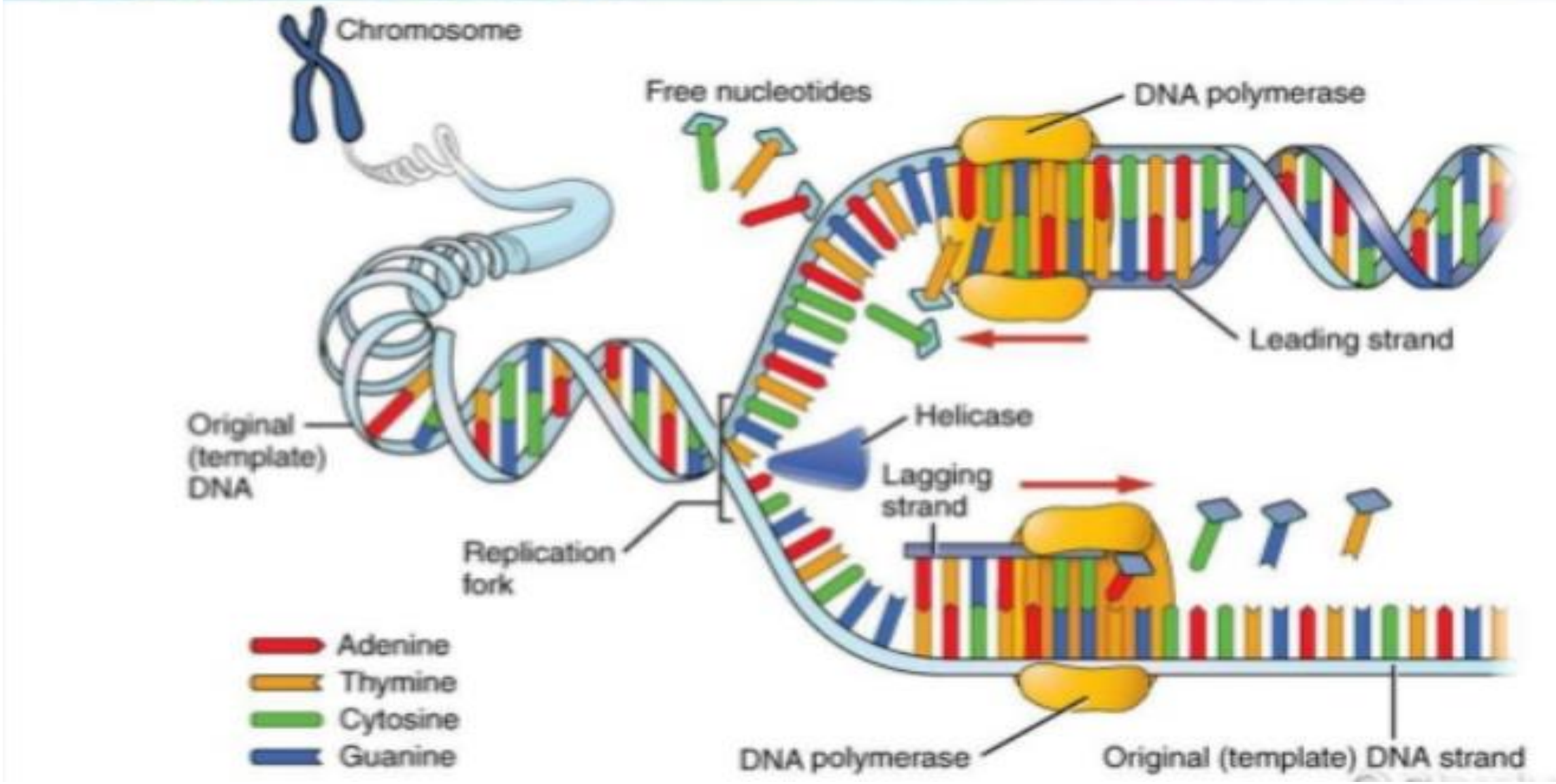
- **Helicases** were discovered in E. coli in 1976 and are a class of enzymes vital to all living organisms.
- Also known as helix destabilizing enzyme, they separates the two strands of DNA at the Replication Fork behind the topoisomerase.
- They are motor proteins that move directionally along a nucleic acid phosphodiester backbone, separating two annealed nucleic acid strands (i.e., DNA,RNA, or RNA–DNA hybrid) using energy derived from ATP hydrolysis.
- They have molecular weight 300,000, which contain SIX identical sub units.


Primase
Topoisomerase
3′
5′
RNA primer
3′
5′
5′
3′
Helicase
5′
Single-strand binding proteins

DNA Polymerase

- **DNA polymerases** are enzymes that synthesize DNA molecules from deoxyribonucleotides, the building blocks of DNA.
- These enzymes are essential to DNA replication and usually work in pairs to create two identical DNA strands from a single original DNA molecule.
- During this process, DNA polymerase "reads" the existing DNA strands to create two new strands that match the existing ones
- Also performs proof-reading and error correction.

WHAT IS DNA POLYMERASE?



- These enzymes catalyze the following chemical reaction,

$$\text{deoxynucleoside triphosphate} + DNA_n \rightleftharpoons \text{diphosphate} + DNA_{n+1}$$

- Catalyses DNA–template–directed extension of the 3'– end of a DNA strand by one nucleotide at a time.
- The known DNA polymerases have highly conserved structure.
- The shape can be described as resembling a right hand with thumb, finger, and palm domains.
- DNA polymerases can be further subdivided into two different families.

Family

PROKARYOTES

- DNA polymerase I
- DNA polymerase II
- DNA polymerase III
- DNA polymerases IV
- DNA polymerase V

EUKARYOTES

- More than 15
- DNA polymerases α, δ, and ϵ
- DNA polymerase γ

Prokaryotic DNA-polymerases

Polymerase	Polymerase activity (for all enzymes 5′ → 3′)	Exonuclease activity
DNA polymerase I	Filling if gap after removal RNA primer, DNA repair, removal of RNA primers	5′→3′ and 3′→5′
DNA polymerase II	DNA repair	3′→5′
DNA polymerase III*	Replication, proofreading and editing	3′→5′

Eukaryotic DNA-polymerases

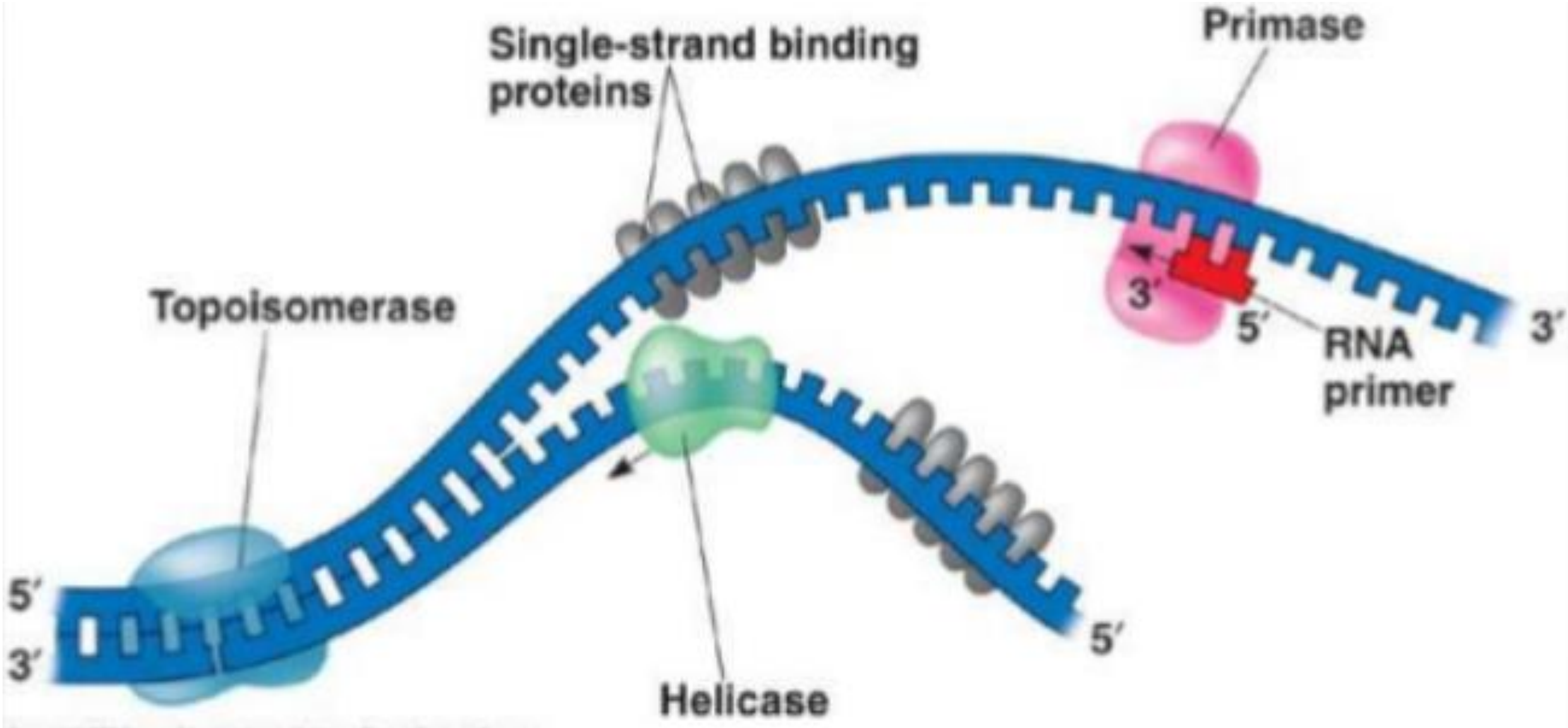
Polymerase*	Polymerase activity (for all enzymes 5′ → 3′)	Exonuclease activity
DNA polymerase α	replication, DNA repair	no
DNA polymerase β	DNA repair	no
DNA polymerase γ	replication in mitochondria	3′→5′
DNA polymerase δ**	replication, DNA repair	3′→5′
DNA polymerase ε	replication	3′→5′

DNA clamp

- A **DNA clamp**, also known as a **sliding clamp**, is a protein fold that serves as a processivity–promoting factor in DNA replication.
- As a critical component of the DNA polymerase III holoenzyme, the clamp protein binds DNA polymerase and prevents this enzyme from dissociating from the template DNA strand.
- The clamp–polymerase protein–protein interactions are stronger and more specific than the direct interactions between the polymerase and the template DNA strand.

Single–Strand Binding (SSB) Proteins

- Single stranded binding proteins prevent reannealing (binding of complementary DNA sequences), protect the single–stranded DNA from being digested by nucleases, and prevent secondary structure formation, thereby allowing other enzymes to function effectively on the single strand.
- Molecular weight of the SSB protein is 75,600.
- It contains FOUR identical subunits, which binds single stranded DNA.


Single-strand binding proteins
Primase
Topoisomerase
3′
5′
RNA primer
3′
5′
3′
5′
Helicase

Topoisomerase

- Every cell has enzymes that increase (or) decrease the extent of DNA unwinding are called "Topoisomerases.
- Topoisomerase is also known as "DNA Gyrase" and that act on the topology of DNA.
- "Topoisomerases bind to double-stranded DNA and cut the phosphate backbone of either one or both the DNA strands, this intermediate break allows the DNA to be untangled or unwound, and, at the end of these processes, the DNA backbone is resealed again.
- Topoisomerases" is an enzyme that can change the "Linking number"(Lk).

- The linking number (Lk) is a topological property, it can be defined as " the number of times the second strand pierces the second strand surface".
- There are two classes of topoisomerases.
  - —a) Type–I Topoisomerases
  - —b) Type–II Topoisomerases

a) Type–I Topoisomerases.

- This act by transiently breaking one of the two DNA strands, rotating one of the ends about the unbroken strand, and rejoining the broken ends; they change Lk in increments of 1.

b) Type–II Topoisomerases.

- The enzyme breaks both DNA strands and change Lk in increments of 2.

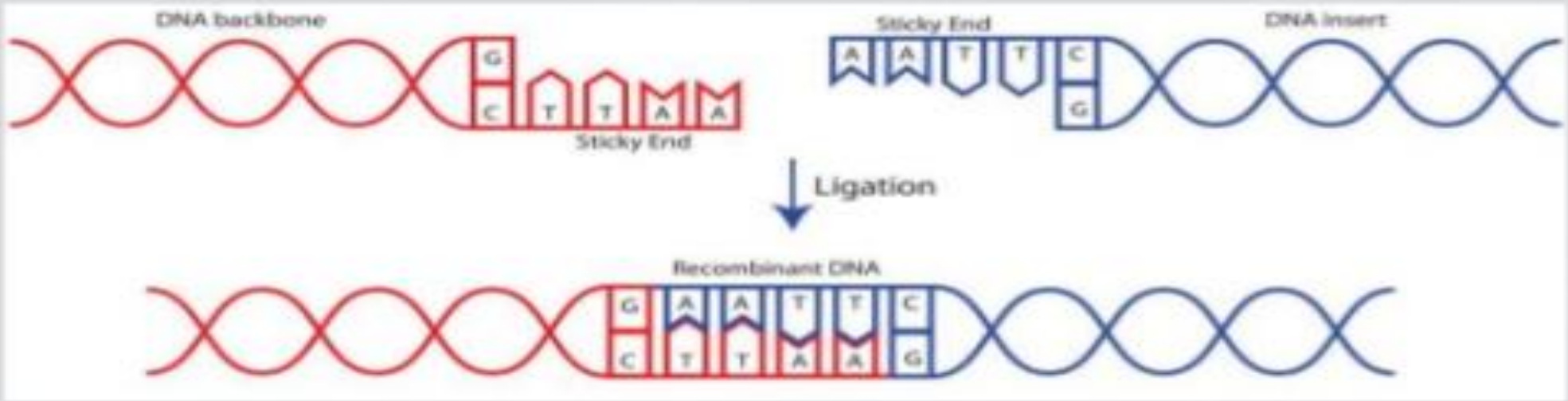
DNA Ligase

- **DNA ligase** is a specific type of enzyme, a ligase, that facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond.
- DNA ligase is used in both DNA repair and DNA replication.
- The mechanism of DNA ligase is to form two covalent phosphodiester bonds between 3' hydroxyl ends of one nucleotide, ("acceptor") with the 5' phosphate end of another ("donor").
- ATP is required for the ligase reaction, which proceeds in three steps:

i. Adenylation (addition of AMP) of a lysine residue in the active center of the enzyme, pyrophosphate is released;

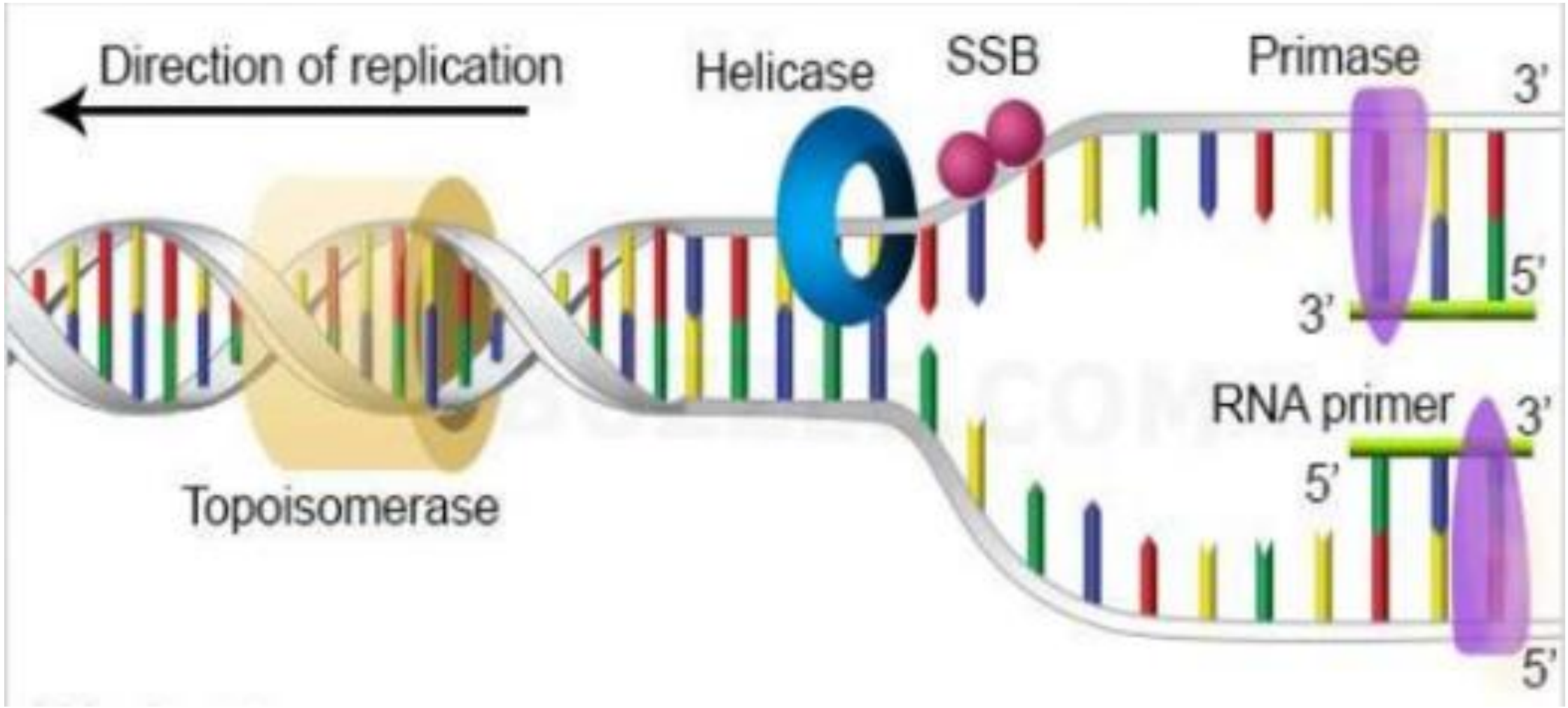
ii. Transfer of the AMP to the 5' phosphate of the so-called donor, formation of a pyrophosphate bond;

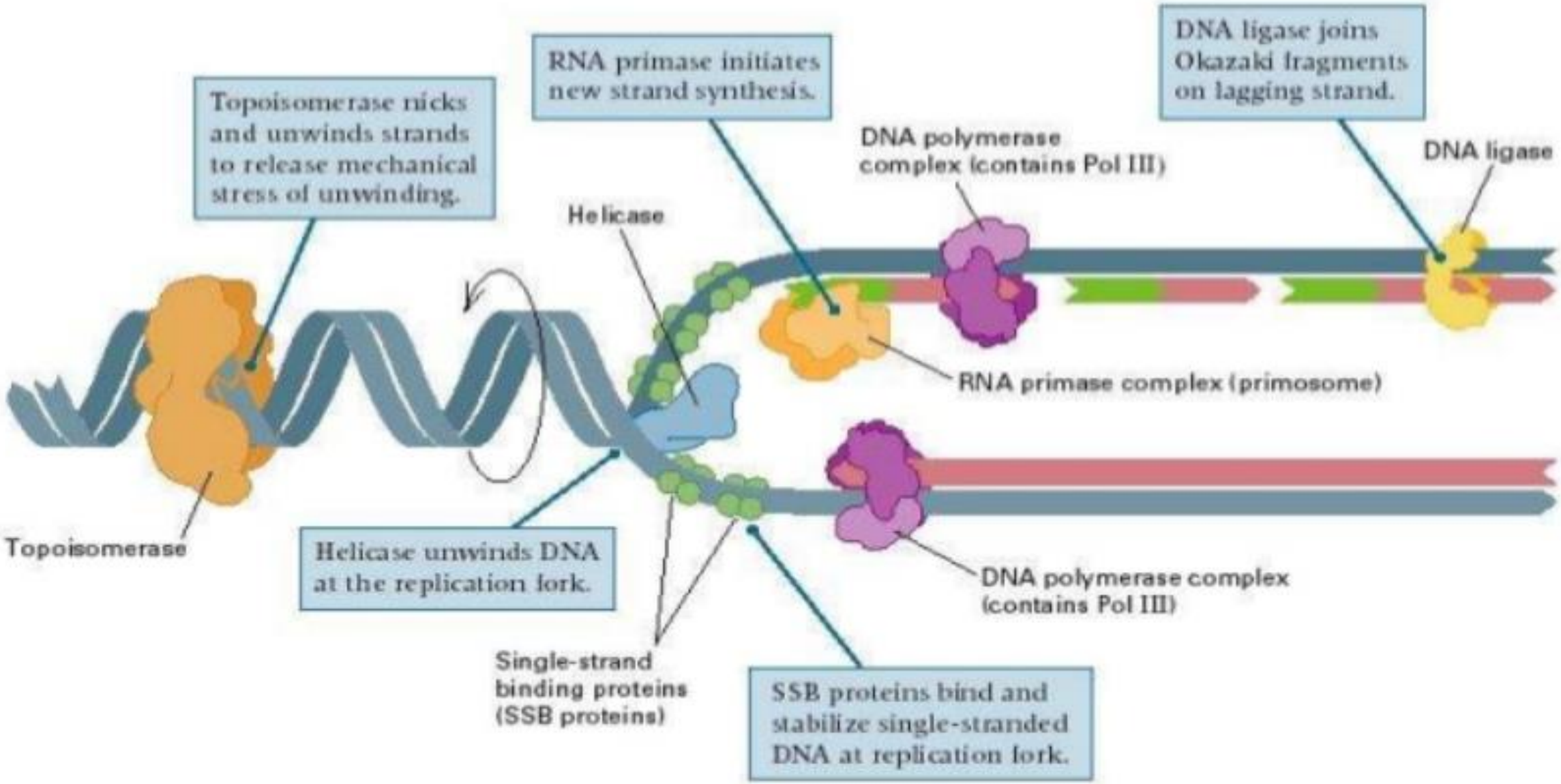
iii. Formation of a phosphodiester bond between the 5' phosphate of the donor and the 3' hydroxyl of the acceptor.



Primase

- **DNA primase** is an enzyme involved in the replication of DNA and is a type of RNA polymerase.
- Primase catalyzes the synthesis of a short RNA (or DNA in some organisms) segment called a primer complementary to a ssDNA template.
- Primase is of key importance in DNA replication because no known replicative DNA polymerases can initiate the synthesis of a DNA strand without an initial RNA or DNA primer (for temporary DNA elongation).
- After this elongation the RNA piece is removed by a 5' to 3' exonuclease and refilled with DNA.


Direction of replication
Helicase
SSB
Primase
3'
5'
3'
RNA primer
3'
5'
5'
Topoisomerase


Topoisomerase nicks and unwinds strands to release mechanical stress of unwinding.
RNA primase initiates new strand synthesis.
DNA ligase joins Okazaki fragments on lagging strand.
DNA polymerase complex (contains Pol III)
DNA ligase
Helicase
RNA primase complex (primosome)
Topoisomerase
Helicase unwinds DNA at the replication fork.
DNA polymerase complex (contains Pol III)
Single-strand binding proteins (SSB proteins)
SSB proteins bind and stabilize single-stranded DNA at replication fork.