Cell Biology, Genetics and Evolution

DNA Replication

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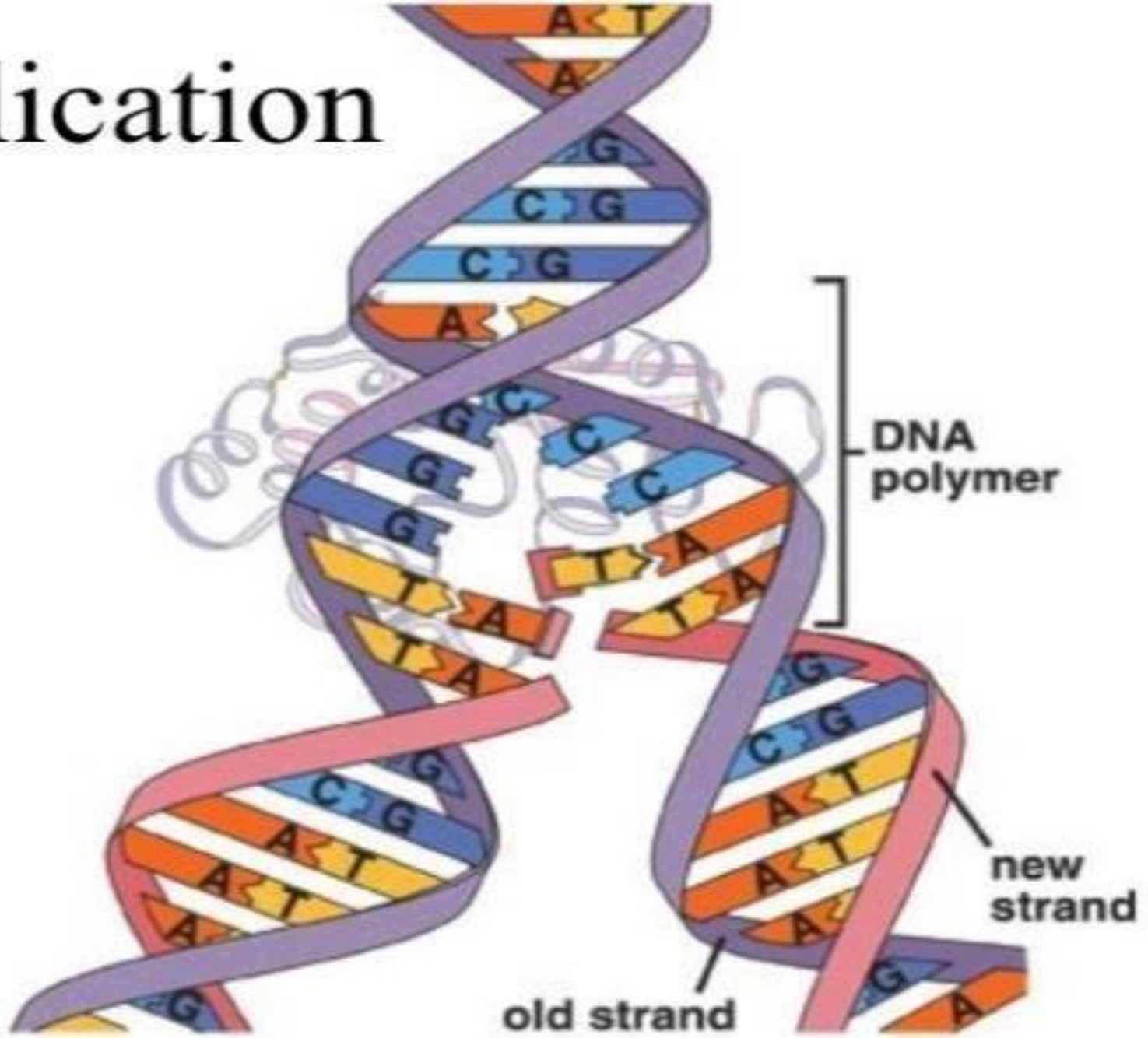
DNA replication

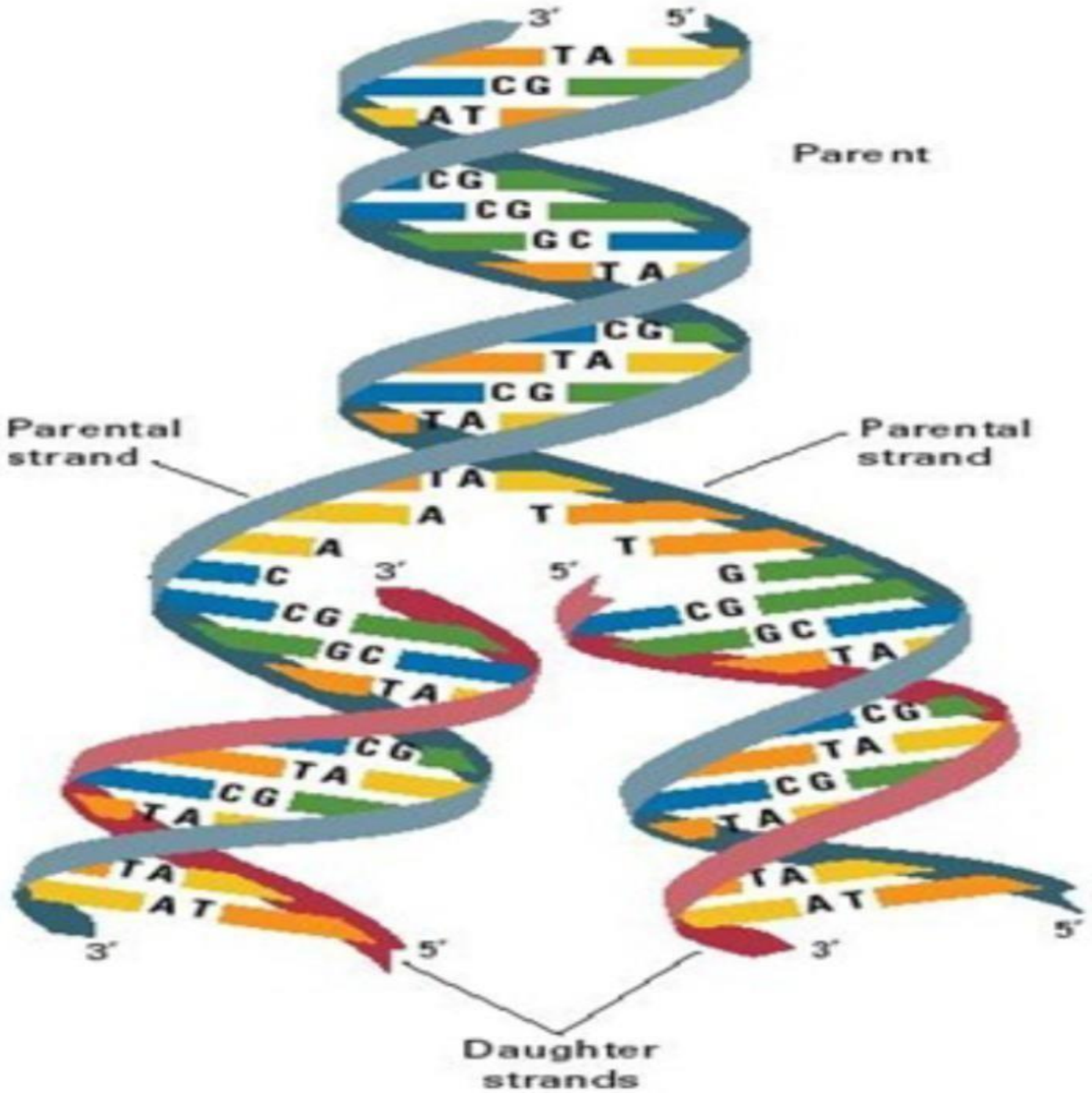


DNA Replication

- DNA replication is a process in which nuclear DNA duplicates itself in different steps so that each daughter cell may get equal amount of DNA after division of parent cell (mitosis).
- DNA, short of Deoxyribonucleic acid, is the self-replicating material which is present in nearly all living organisms as the main constituent of chromosomes. It is the fundamental carrier of genetic information, present in virtually every cell in your body.

Structure of DNA

- Double-helix DNA is made of two asymmetrical strands. Each strand is made of nucleotides lined up one after the other, and these nucleotides are bound to corresponding once on the other strand to create a ladder-like structure.
- DNA is made up of nucleotides—the building blocks of nucleic acid – which are composed of a nitrogenous base, a five carbon sugar (ribose or deoxyribose), and at least one phosphate group.
- Adenine (A) , Thymine (T) , Guanine (G) and Cytosine (C) are called “nitrogenous bases”.


3'
5'
Parent
Parental strand
Parental strand
3'
5'
5'
3'
3'
5'
Daughter strands

Role of Enzymes in DNA Replication

- Protein (Enzymes) Assisting the DNA replication
- Following proteins assist in the synthesis of DNA:
- DNA polymerase
- Ligase
- Primase
- Helicase
- Single strand binding protein

Phases of DNA Replication

- There are three main phase for the completion of DNA replication:

- I. Initiation Phase
- II. Extension/Elongation/Polymerization Phase
- III. Termination Phase

I. Initiation Phase

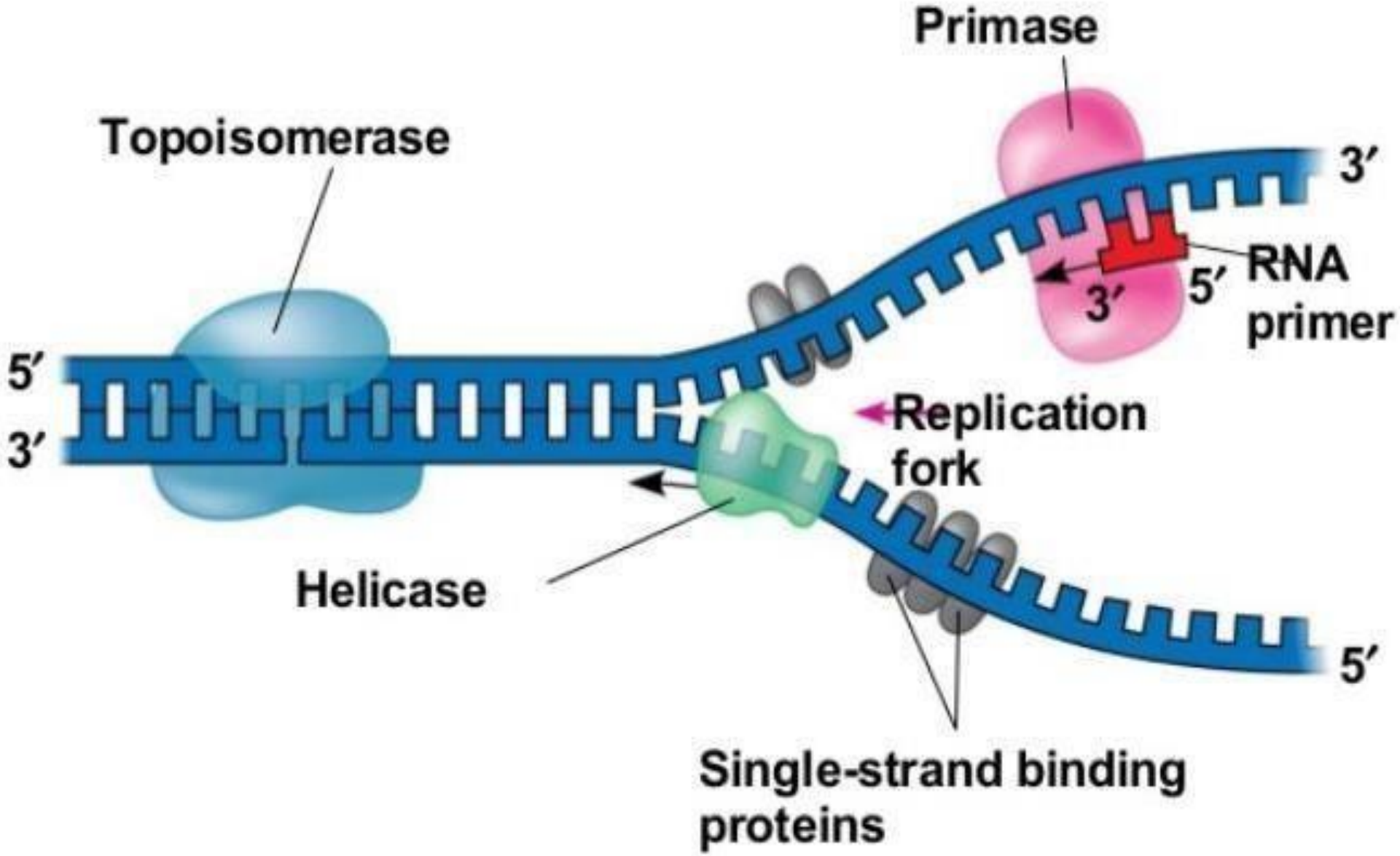
- Initiation phase is the starting phase of DNA replication. It completes in the presence of different enzymes and protein.
- The process of DNA replication initiates at a specific site on DNA strand which is known as origin of replication.
- **Origin of Replication**
- The origin of replication of DNA molecule begins at special sites, these special sites are specific sequence of nucleotides and are called origin of replication.

I. Initiation Phase

- The eukaryotic DNA contains thousand of such replication origins. A protein initiates DNA replication.
- An enzyme known as DNA Gyrase or Topoisomerase it attaches to the origin of replication, recognizing the sequence of nucleotides. It opens the turns of DNA from spiral ladder to straight ladder.

I. Initiation Phase

- DNA Helicase is an enzyme that breaks down the H-bonds of nitrogenous bases present at replication bubble. When the bonds have broken the nitrogenous bases are exposed for the attachment of new nitrogenous base and to form bonds.
- A protein called single strand binding protein, SSB is used to prevent reunion of unzipped strands.


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

II. Elongation Phase

- In extension phase of DNA replication, nucleotides align with complementary basis on “old” template strands of DNA.
- An enzyme DNA polymerase catalyzes elongation of new DNA at a replication fork. The new nucleotides are added by DNA polymerase one by one. The rate of elongation is about 500 nucleotides per second in human cells.

Types of DNA Polymerase

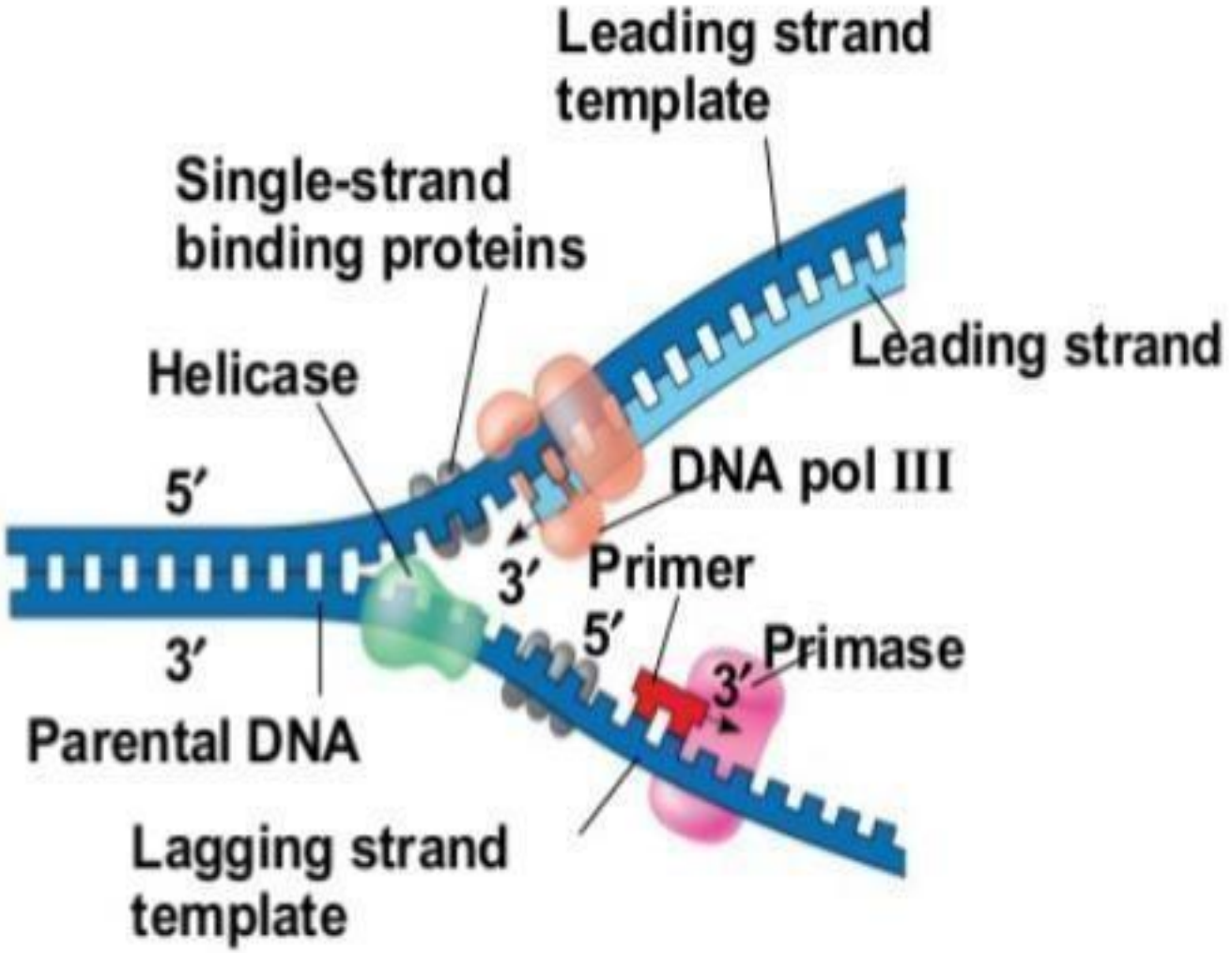
- There are three types of DNA polymerase, which are useful in DNA polymerization process:
- DNA Polymerase I (helps in termination of DNA during replication, also support DNA Polymerase III).
- DNA Polymerase II (helps in repairing of DNA strand, if any damage occurs during lifetime).
- DNA Polymerase III (main enzyme, that helps in synthesis of new strands)

Leading Strand

- The enzyme DNA Polymerase III can only add nucleotides to the free 3’ end of DNA strand. It can never add it to the 5’ end. Thus, a new DNA strand is formed in 5’—3’ directions.
- The DNA polymerase can synthesize a continuous complementary strand along 5’—3’ direction. This DNA strand is called “leading strand”. It is also called continuous strand.

Lagging Strand

- The DNA polymerase III that move away from the replication fork to elongate in 3-5 strand of DNA. The DNA synthesized as a series of segments.
- These pieces are called “Okazaki fragments”. These fragments were discovered by Japanese scientist Okazaki.
- These fragments are about 100 to 200 nucleotides long in eukaryotes. It is also called discontinuous strand.

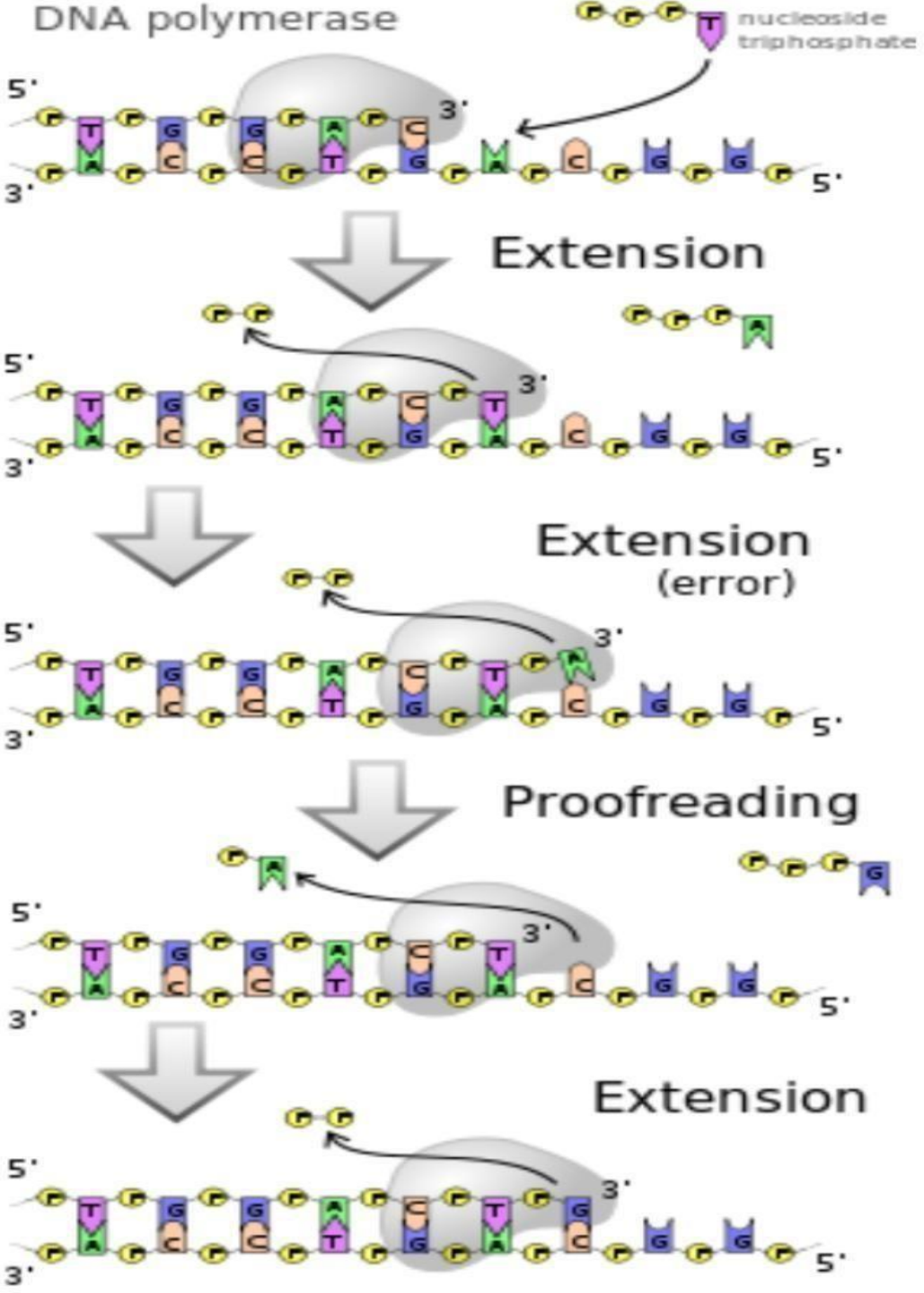

Leading strand
template
Single-strand
binding proteins
Leading strand
Helicase
DNA pol III
5′
3′
Primer
5′
3′
Primase
3′
Parental DNA
Lagging strand
template

RNA Primer

- There is an other problem for DNA polymerase. It can only add a nucleotide to a polynucleotide that is already correctly paired with the complementary strand. This means that DNA polymerase cannot actually initiates synthesis of DNA strand by its own. Nucleotides must me added to the end of an already existing chain. This chain of nucleotides is called a primer.
- The primer is the short stretch of RNA. It is synthesized by an other enzyme primase. It is about 10 nucleotides long in eukaryotes.
- Only one primer is required for the leading strand of new DNA. Each Okazaki fragment must have separate primer in lagging strand.

Proof Reading

- If any error occurs in DNA molecule, that has to be corrected, it is done by an enzyme called DNA polymerase II.
- These errors are only one in billion nucleotides. This process of removing errors by enzyme is called “proofreading”.

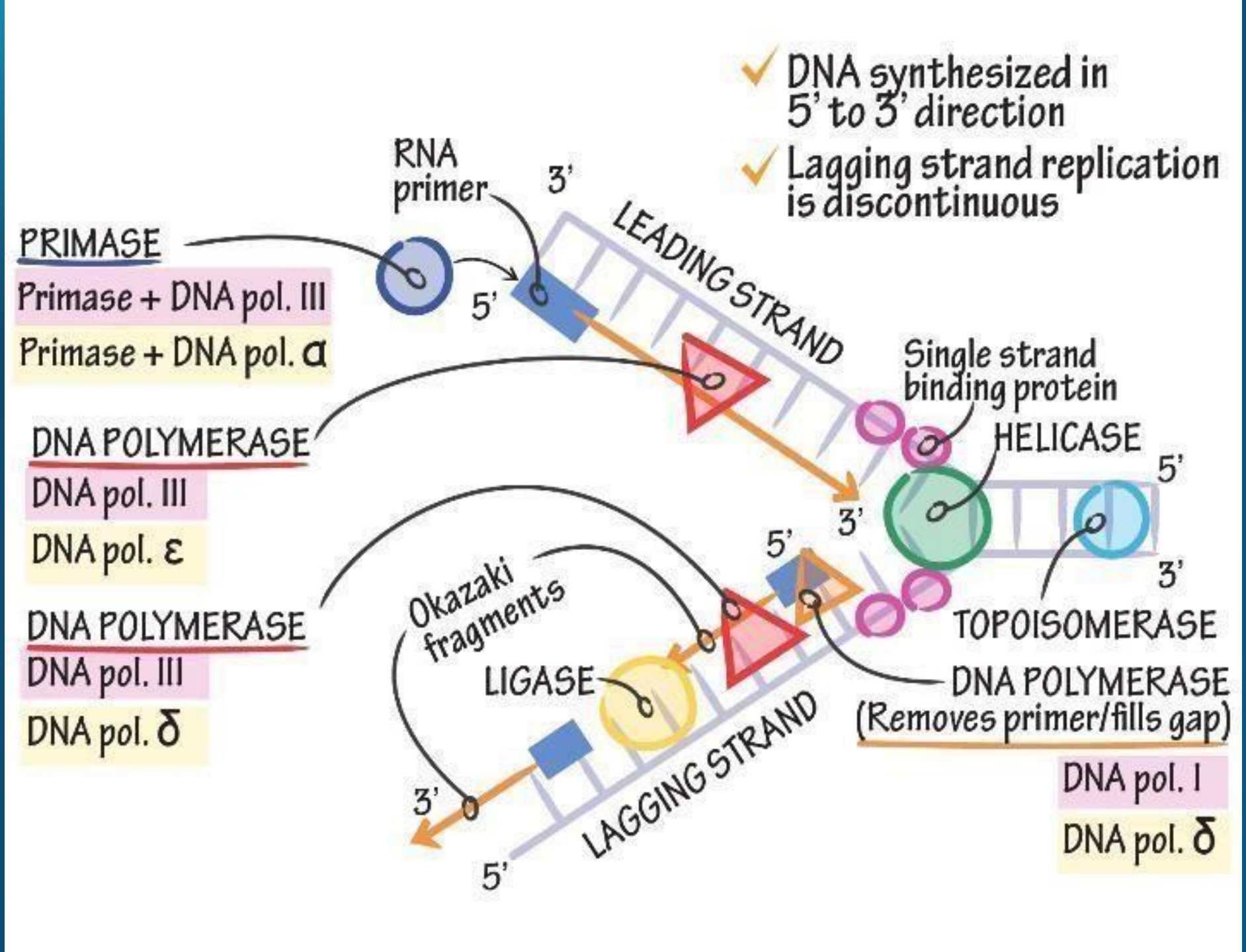

DNA polymerase
nucleoside triphosphate
5'
3'
Extension
Extension (error)
Proofreading
Extension

III. Termination Phase

- Termination phase is the end phase of DNA replication. In this phase new DNA molecules are formed properly.
- The small, newly formed molecules of DNA which were called Okazaki fragments, have to be combined to form a long chain.

III. Termination Phase

- An enzyme called DNA polymerase I or exonuclease removes the primers present between fragments, by breaking the bonds between the small daughter strands and primers.
- It also fills up the spaces between fragments by placing nucleotides in spaces, but it does not join the fragments chemically.
- An other enzyme called Ligase creates the bonds between the extended segments and forms a continuous strand.


DNA synthesized in 5' to 3' direction
Lagging strand replication is discontinuous
RNA primer
3'
5'
LEADING STRAND
PRIMASE
Primase + DNA pol. III
Primase + DNA pol. α
Single strand binding protein
HELICASE
DNA POLYMERASE
DNA pol. III
DNA pol. ε
5'
3'
5'
3'
Okazaki fragments
DNA POLYMERASE
DNA pol. III
DNA pol. δ
LIGASE
TOPOISOMERASE
DNA POLYMERASE
(Removes primer/fills gap)
DNA pol. I
DNA pol. δ
3'
5'
LAGGING STRAND


Thank
You!