DNA Replication

SUBJECT: MOLECULAR BIOLOGY
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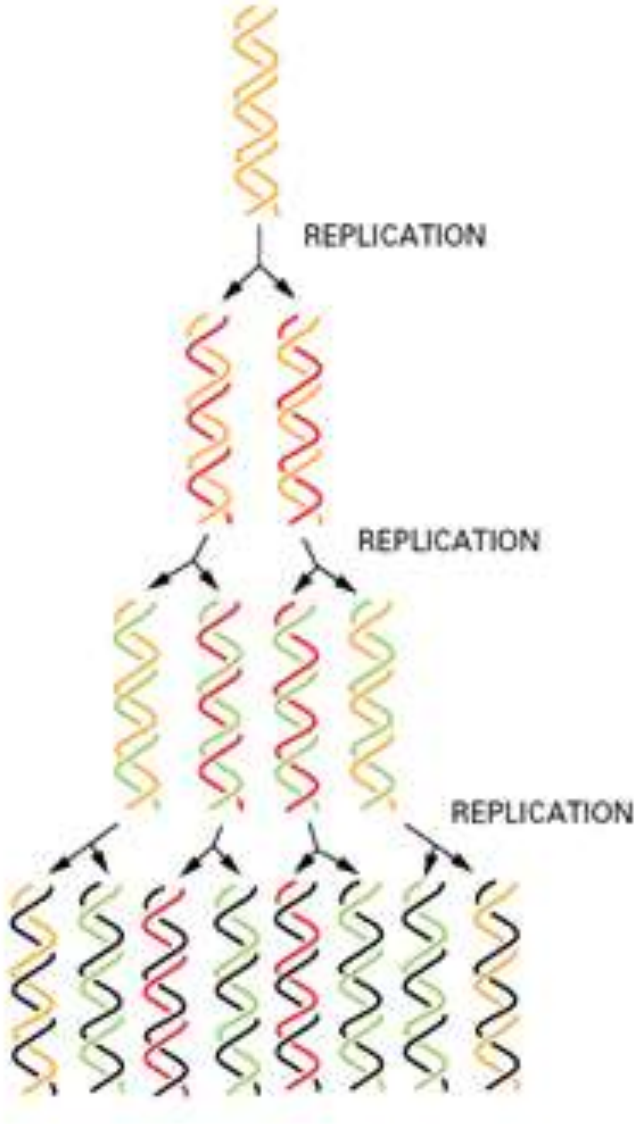
DNA Replication

- **A** process by which a cell makes an identical copy of its DNA.
- Essential for cell division, growth, and reproduction in both prokaryotes and eukaryotes.
- Ensures genetic material is passed on accurately to daughter cells.

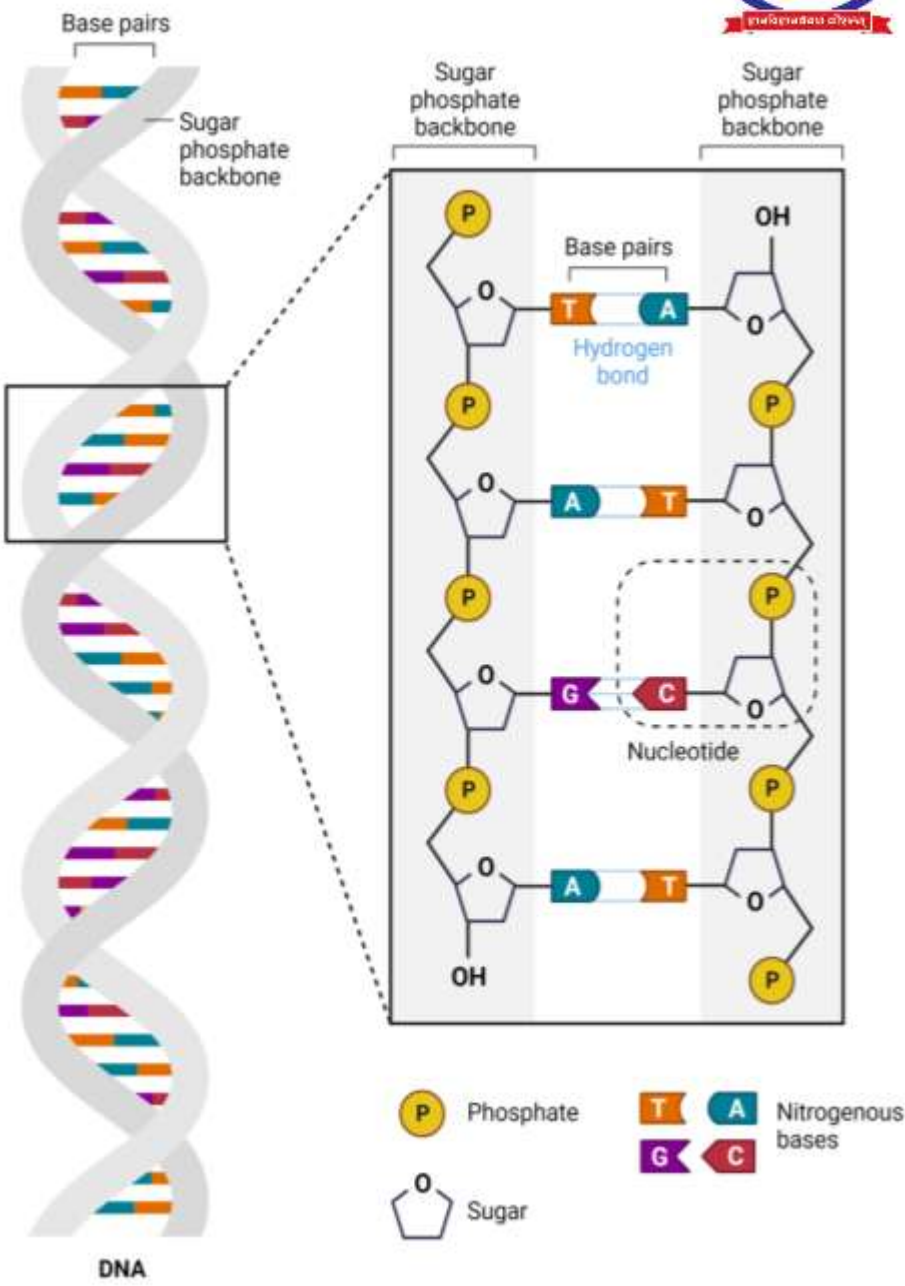


Structure of DNA

- **Double Helix:** DNA is composed of two strands that twist into a double helix.
- **Nucleotides:** Building blocks of DNA, composed of a sugar, phosphate group, and nitrogenous base.
- **Base Pairing:**

➢ Adenine (A) pairs with Thymine (T)

➢ Cytosine (C) pairs with Guanine (G)



Basic rules of replication :

- Semi-conservative
- Starts at the 'origin'
- Synthesis always in the 5'-3'direction
- Can be uni or bidirectional
- Semi-discontinuous
- RNA primers required

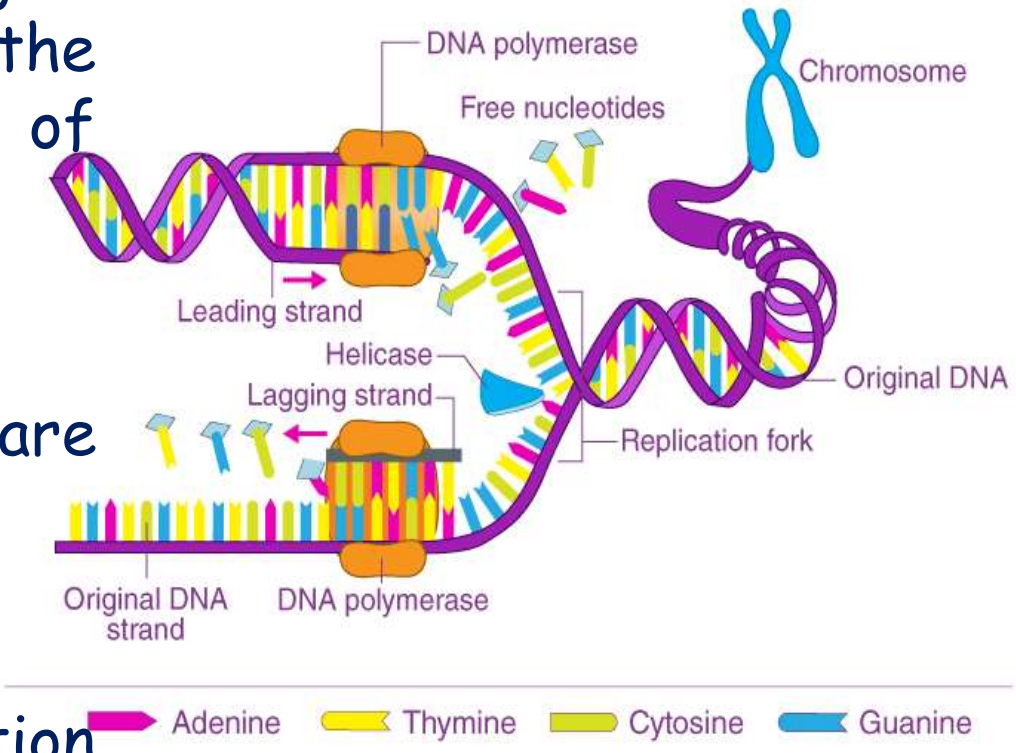
Enzymes	Function
Topoisomerases	Prevents torsion by DNA breaks
Helicase	Unwinding of DNA
Primase	RNA primer synthesis
Single strand binding proteins	Stabilizes DNA in single stranded state
DNA polymerase	synthesis of new strand
DNA ligase	seals nick via phosphodiester linkage

Major steps of DNA Replication

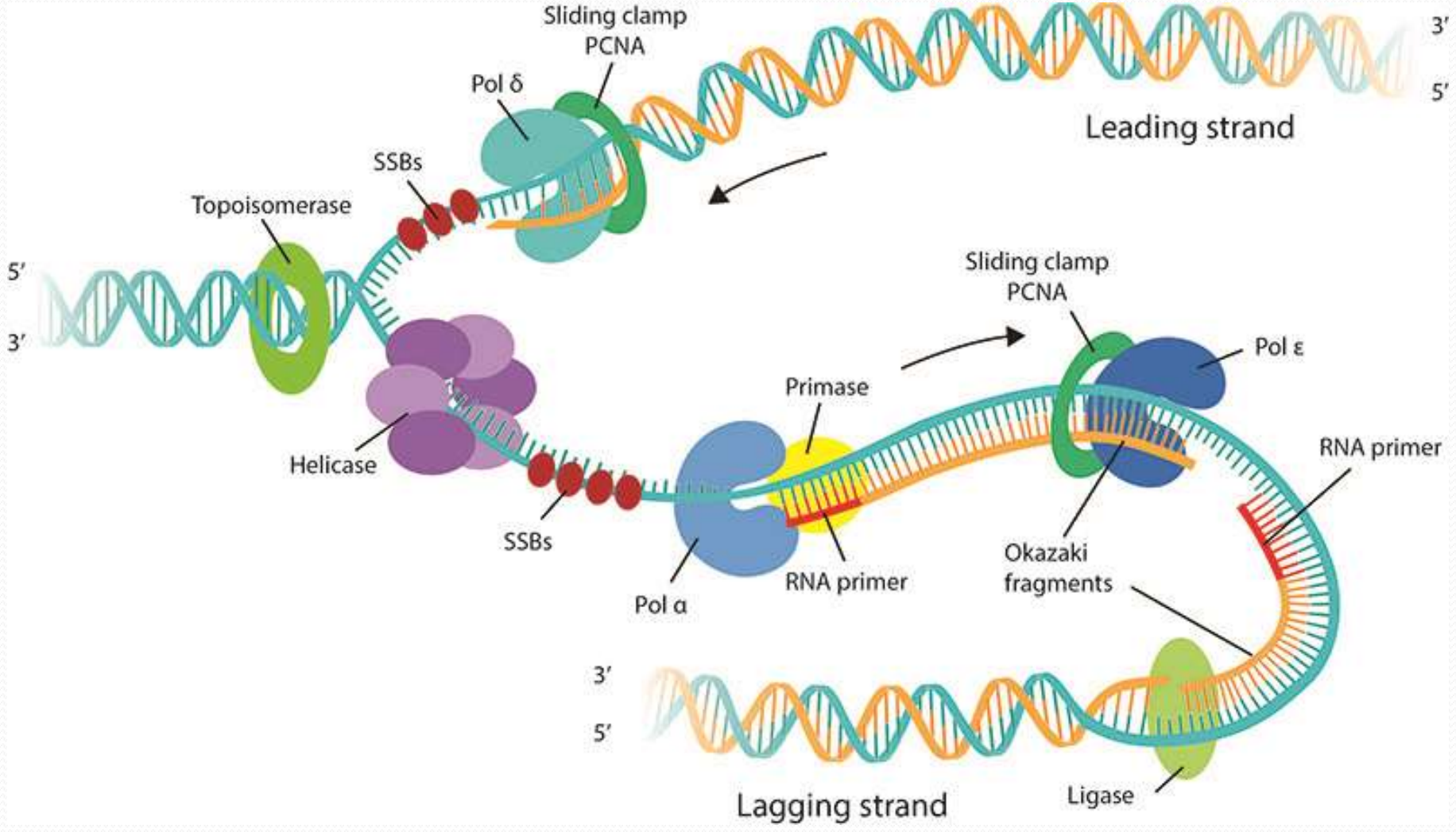
Initiation: The process begins at specific locations on the DNA called "origins of replication"

Elongation: New strands are synthesized by enzymes.

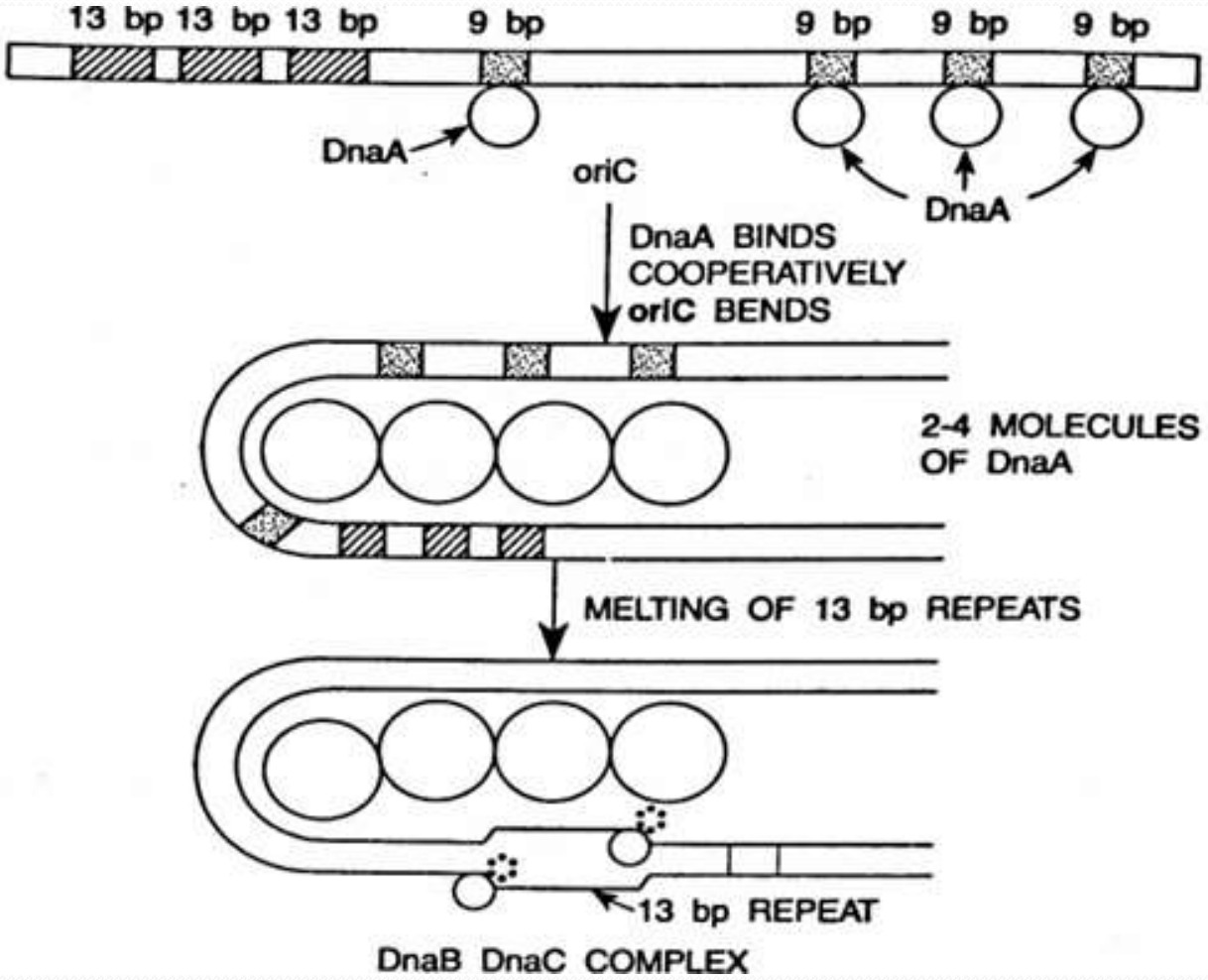
Termination: The replication process ends once the entire molecule is copied.



DNA Replication in Prokaryotes

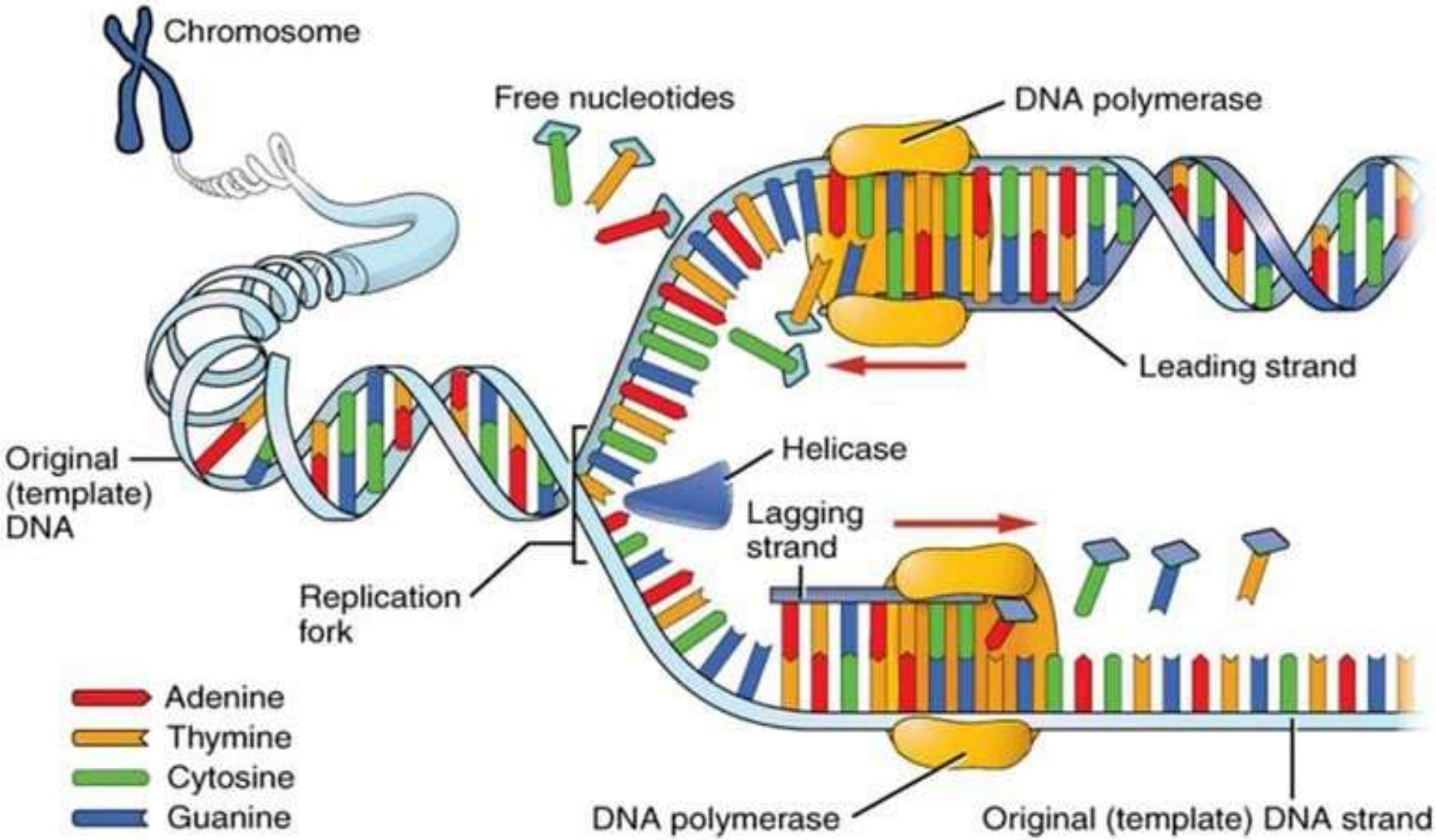


1. Initiation



- DNA replication starts with the formation of replication origin which is highly conserved among bacteria.
- The oriC replication origin of E. coli 245 base pairs.
- The key sequences oriC are two series of short repeats; **three repeats of a 13 base pair sequence** and **four repeats of a 9 base pair sequence.**
- DnaA protein binds to 9-mer DnaA box consensus sequence, 5′-TTATnCACA-3′ and results in unwinding of DNA at oriC.
- Unwinding results in the recognition of DNA by other replication proteins that act subsequently in the initiation process.

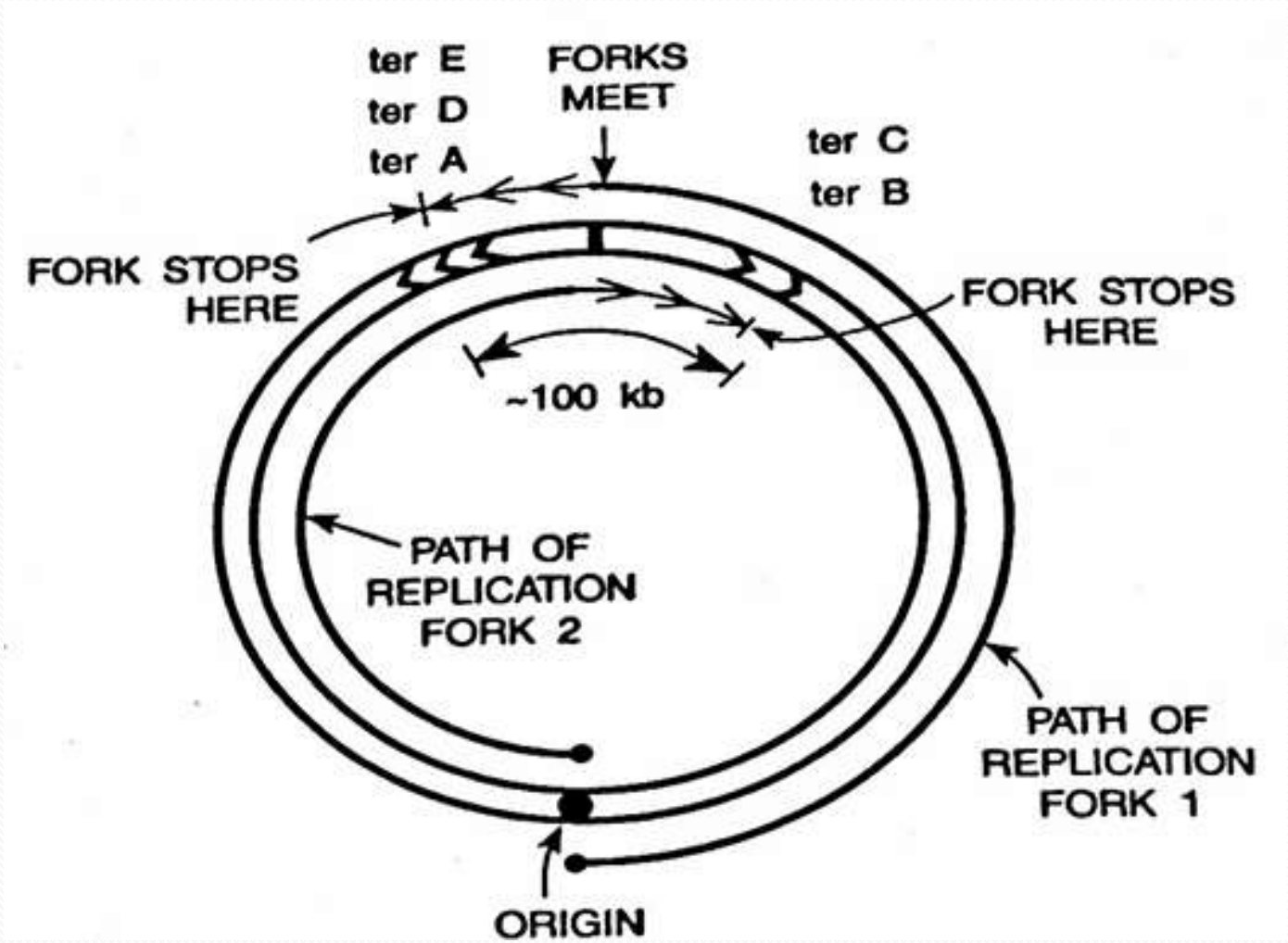
2. Elongation



- DNA polymerase III starts adding nucleotides at the end of the primers.
- Leading strand synthesis is continuous whereas lagging strand is synthesized in fragments.
- DNA polymerase can only synthesize new strands in the 5' to 3' direction.
- The "leading strand" is synthesized continuously toward the replication fork as helicase unwinds the template double-stranded DNA.
- The "lagging strand" is synthesized in the direction away from the replication fork and away from the DNA helicase unwinds.

- This lagging strand is synthesized in pieces because the DNA polymerase can only synthesize in the 5′ to 3′ direction.
- The pieces are called as Okazaki fragments.
- Length of Okazaki fragments in prokaryotes are 1000-2000 nt.
- DNA ligase seals the nicks between Okazaki fragments.
- It requires close and free 3′-OH and 5′-P and proper base-pairing.
- NAD+ required in prokaryote

3. Termination



- Termination of replication takes place at the termination site in the prokaryotic DNA.

- DNA replication terminates when replication forks reach specific 'termination sites', i.e. replication forks meet each other on the opposite end of the parental circular DNA.

- The two replication forks are synchronized by 10-23 bpTer sequences that bind Tus proteins.

- Tus protein binds to terminator sequences (Ter sequence) and acts as a counter-helicase when it comes in contact with an advancing helicase.

- The bound Tus protein effectively halts DNA polymerase movement.

Steps of the DNA replication in prokaryotes

1. Unwinding:

- ✓ DNA unwinds at the origin of replication.
- ✓ Helicase opens up the DNA-forming replication forks; these are extended bidirectionally.
- ✓ Single-strand binding proteins coat the DNA around the replication fork to prevent rewinding of the DNA.
- ✓ Topoisomerase binds at the region ahead of the replication fork to prevent supercoiling.

2. Primer synthesis:

- ✓ Primase synthesizes RNA primers complementary to the DNA strand.

3. DNA synthesis:

- ✓ DNA polymerase starts adding nucleotides to the 3'-OH end of the primer.
- ✓ Elongation of both the lagging and the leading strand continues.
- ✓ RNA primers are removed by exonuclease activity.
- ✓ Gaps are filled by DNA pol by adding dNTPs.

4. Fragment Joining:

- ✓ On the lagging strand, DNA polymerase synthesizes Okazaki fragments, which are later joined by ligase.
- ✓ The gap between the two DNA fragments is sealed by DNA ligase, which helps in the formation of phosphodiester bonds.

5. Proofreading:

- ✓ DNA polymerase checks for errors and corrects them.


Thank you