DNA Replication.
An overview.....

For 1st Sem hons.

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The mechanism of DNA replication: overview

- Tightly controlled process,
  – occurs at specific times during the cell cycle.
- Requires:
  – a set of proteins and enzymes,
  – and requires energy in the form of ATP.

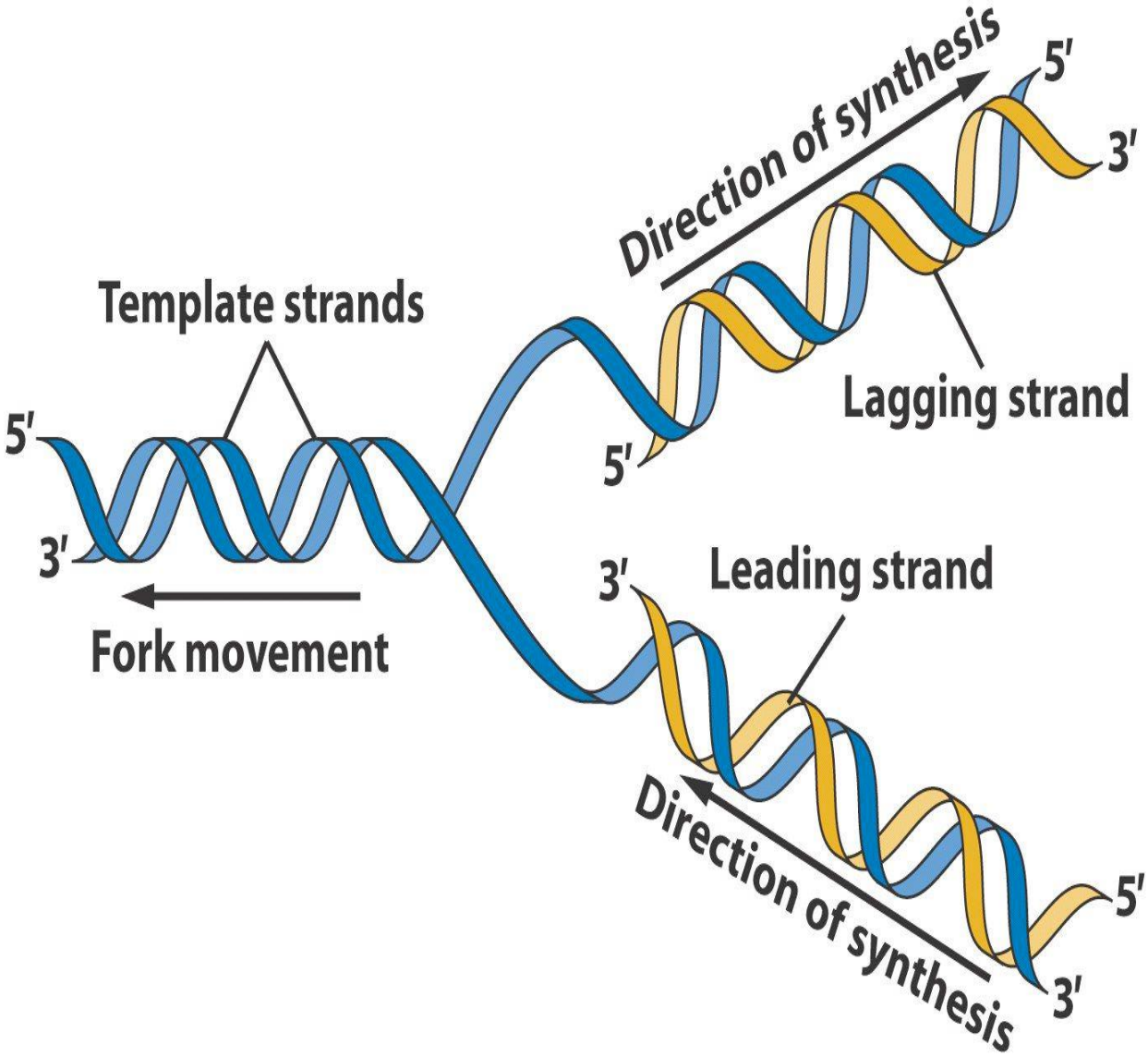
- Three basic steps:
  – Initiation
  – Elongation AND Termination

- Two basic components:
  – template
  – primer.

MECHANISM OF REPLICATION - the process

- The overall process of DNA replication is similar in both prokaryotes and eukaryotes.
- Replication begins at an **origin of replication**, which is the DNA binding site for group of proteins to initiate replication.
- At initiation, DNA unwinds and stabilizes via **topoisomerase, helicase,** and **single-strand binding proteins** and exposes the DNA template .
- **Primase** synthesizes an RNA primer (10-12nt) on each strand.
- **DNA polymerase** binds, triggering elongation in 5'→ 3' direction.
- It reads the DNA **template** strand and synthesizes a **complementary lagging strand** via **Okazaki fragments** discontinuously and **leading strand** continuously.
- During elongation, the replication forks move **bi-directionally** apart toward the end of the chromosome.

Replication at FORK site



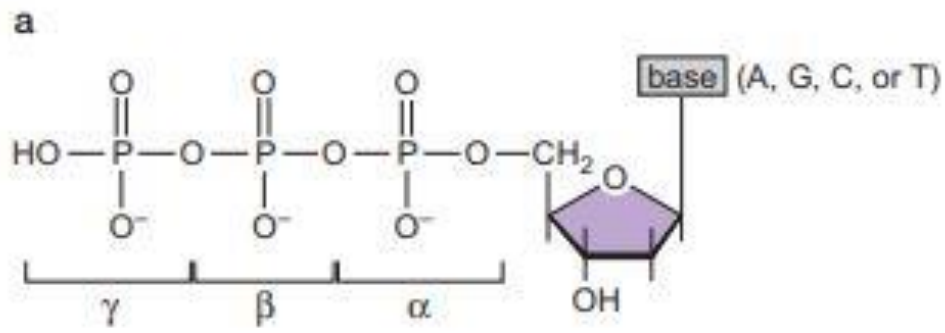
Occurs continuously as **leading strand** And discontinuously as **lagging strand** via **OKAZAKI fragments.**

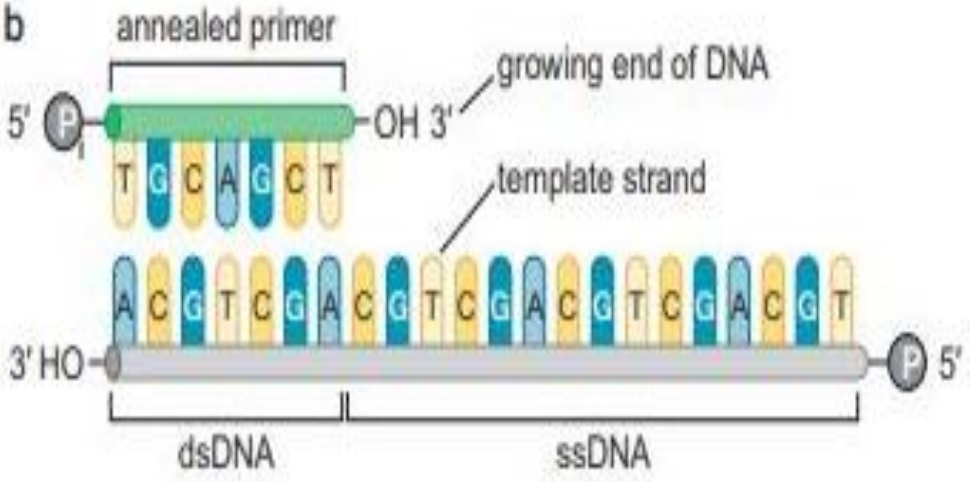
Substrates required for DNA synthesis

Two features are important before moving towards DNA synthesis -

- The general structure of the 2’ -deoxy nucleoside triphosphates. The positions of the α-phosphate, β-phosphate, and γ-phosphate are labeled.

- The structure of a generalized **primer: template junction**. The shorter primer strand is completely annealed to the longer DNA strand and must have a free 3’ -OH adjacent to an ssDNA region of the template from which new DNA synthesis occurs extending the primer.





DNA replication enzymes and Proteins - the other substrates for DNA synthesis

DNA polymerase

- DNA polymerases are enzymes used for the synthesis of DNA by adding nucleotide one by one to the growing DNA chain in both prokaryotic and eukaryotic cells. Besides, polymerization it also responsible for DNA repair mechanisms.

Ssb proteins

- stabilizes the single strand of DNA.

DNA Helicase enzyme

- This is the enzyme that is involved in unwinding the double-helical structure of DNA allowing DNA replication to commence.
- It uses energy that is released during ATP hydrolysis, to break the hydrogen bond between the DNA bases and separate the strands.
- This forms two replication forks on each separated strand opening up in opposite directions.
- At each replication fork, the parental DNA strand must unwind exposing new sections of single-stranded templates.
- The helicase enzyme accurately unwinds the strands while maintaining the topography on the DNA molecule.

DNA primase enzyme

- This is a type of RNA polymerase enzyme that is used to synthesize or generate RNA primers, which are short RNA molecules that act as templates for the initiation of DNA replication.

DNA ligase enzyme

- This is the enzyme that joins DNA fragments together by forming phosphodiester bonds between two adjacent nucleotides.

Exonuclease

- These are a group of enzymes that remove nucleotide bases from the end of a DNA chain.

Topoisomerase

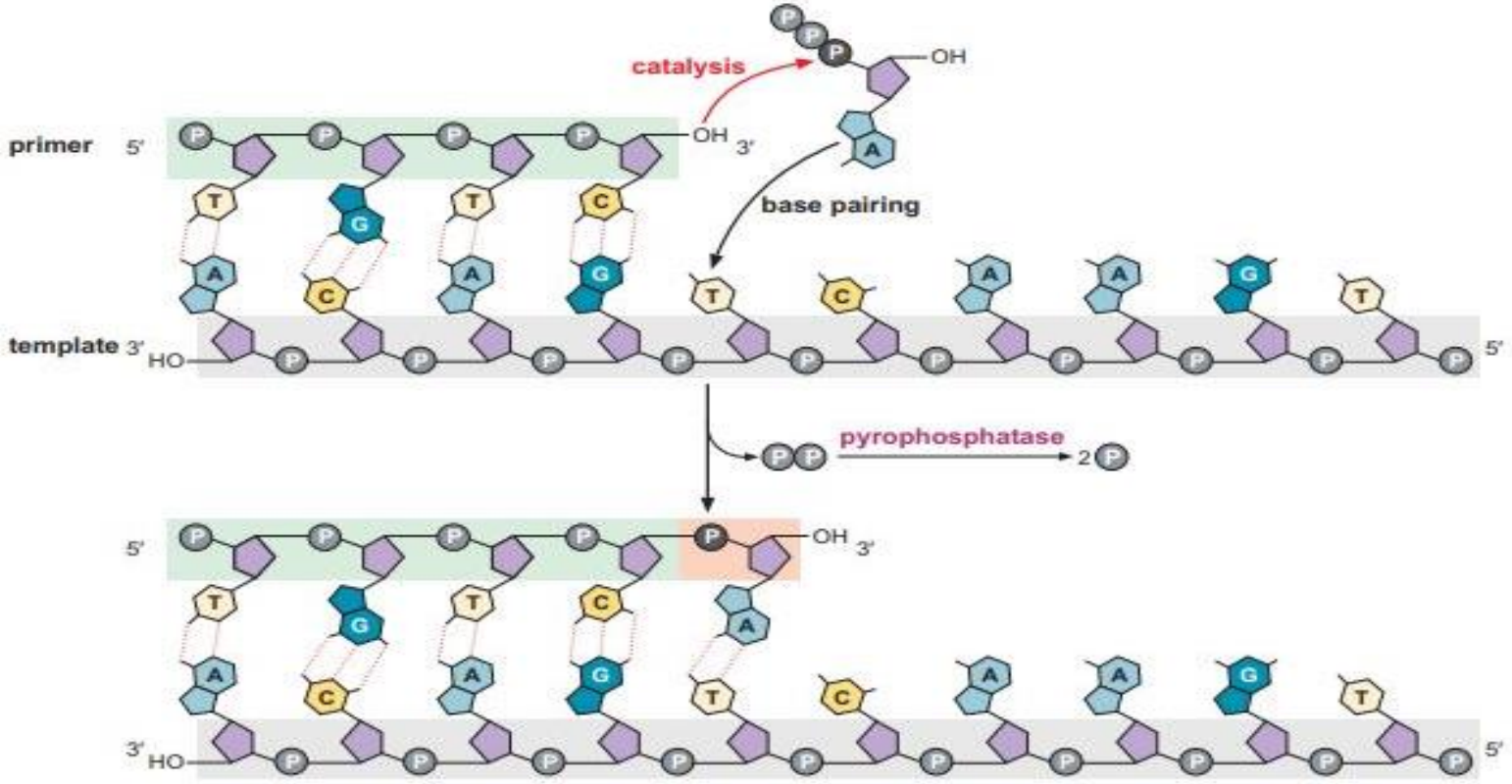
- This is the enzyme that solves the problem of the topological stress caused during unwinding.
- They cut one or both strands of the DNA allowing the strand to move around each other to release tension before it rejoins the ends.
- And therefore, the enzyme catalysts the reversible breakage it causes by joining the broken strands.
- Topoisomerase is also known as DNA gyrase in E. coli.

Telomerase

- This is an enzyme found in eukaryotic cells that adds a specific sequence of DNA to the telomeres of chromosomes after they divide, stabilizing the chromosomes over time.

Mechanism of DNA synthesis – initial step

DNA synthesis is initiated when the 3’ -OH of the primer mediates the nucleophilic attack of the alpha-phosphate of the incoming dNTP. This results in the extension of the 3’ end of the primer by one nucleotide and releases one molecule of pyrophosphate. Pyrophosphatase rapidly hydrolyzes released pyrophosphate into two phosphate molecules



Assemblage and functioning of enzymes and protein at replication fork site during replication process