Prokaryotic DNA and Telomere Replication

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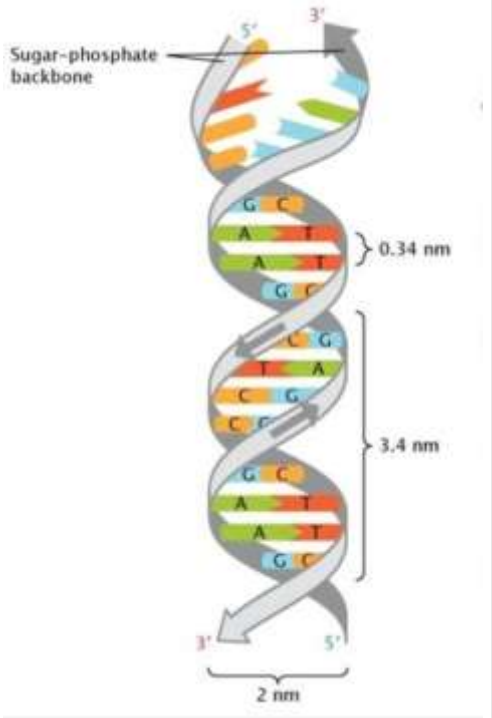


Introduction

- DNA replication is essential before dividing of a cell so each daughter cell has a complete copy of genetic information.
- The enzyme that copy DNA, polymerize nucleotides in the 5'→3' directions although the two polynucleotide strands of DNA run in opposite directions, yet both strands appear to grow in the same directions.
- Replication mechanisms requires lots of enzymes and proteins.

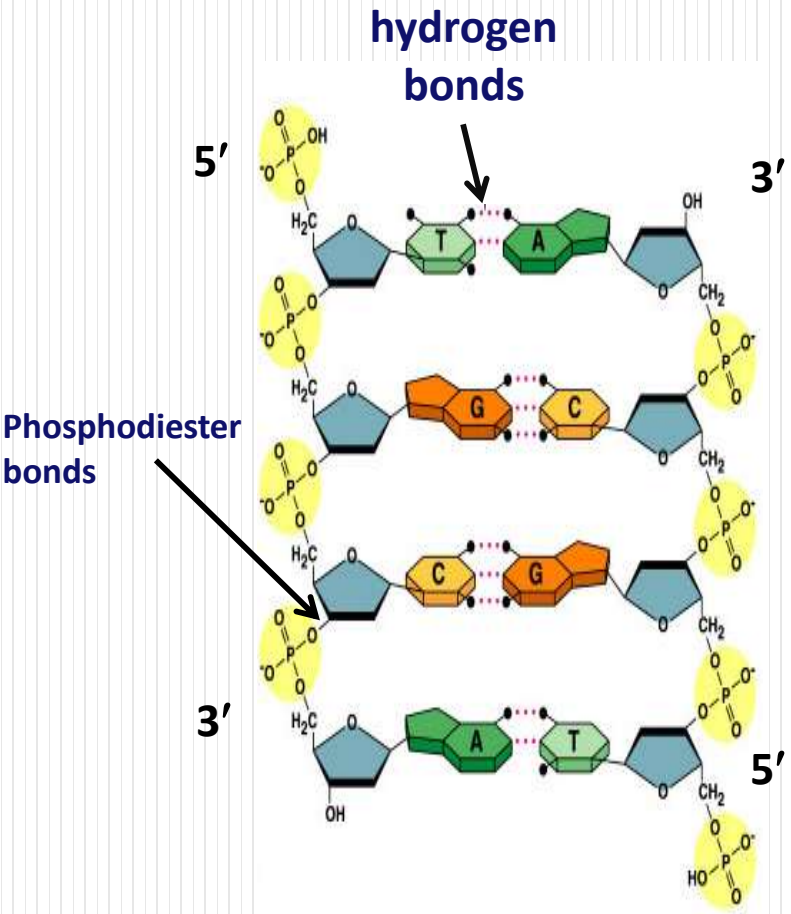
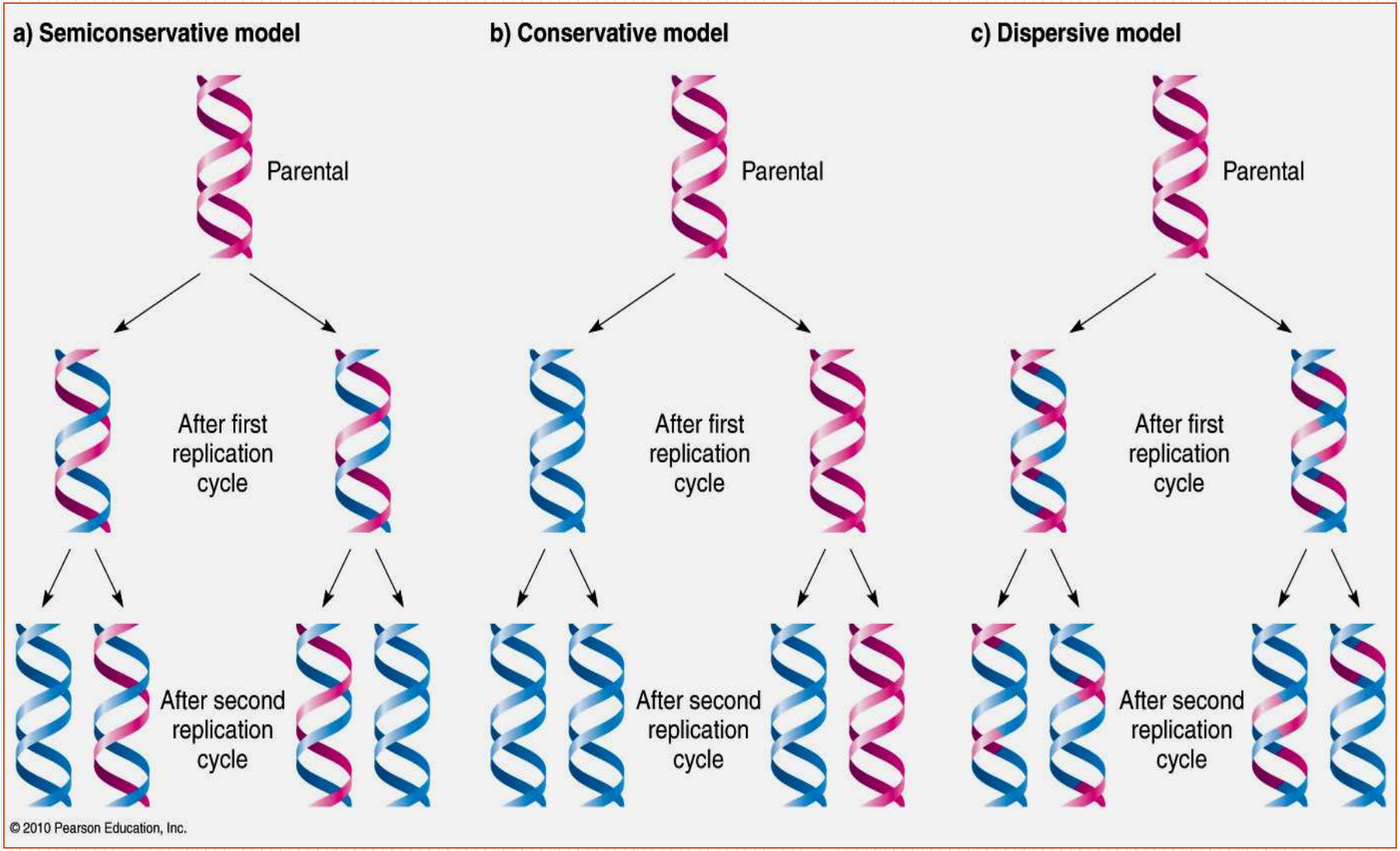


Fig: Nucleotides in DNA backbone are bonded from phosphate to sugar between 3′ & 5′ carbons.

DNA Replication is Semi-conservative

- Semi-conservative model of Watson and Crick predicts that when a double helix replicates, each daughter molecule will have one old strand and one newly synthesized strand.
- Competing models were the conservative model (the two parent strands rejoin) and the dispersive model (each strand is a mix of old and new).
- Experiments by Matthew Meselson and Franklin Stahl supported the semi-conservative model of DNA replication.
- They labeled the nucleotides of the old strands with a (^{15}N) heavy isotope of nitrogen, while any new nucleotides were labeled with a lighter isotope (^{14}N).

Comparison of three proposed Models



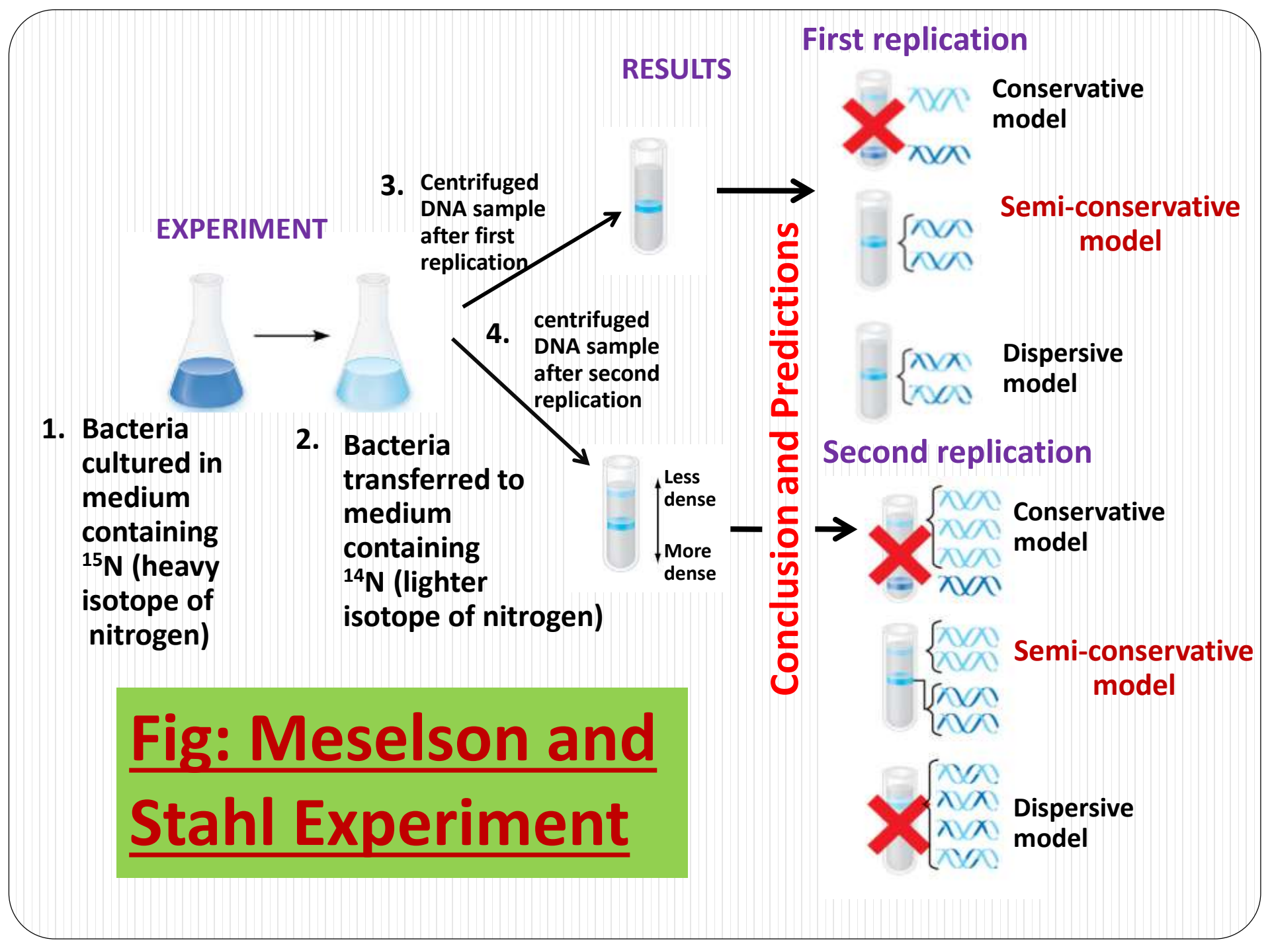


Fig: Meselson and Stahl Experiment

CONCLUSION OF THE EXPERIMENT:

- The first replication produced a band of hybrid DNA, eliminating the conservative model.
- A second replication produced both light and hybrid DNA, eliminating the dispersive model and **supporting the semi-conservative model**.

Prokaryotic Replication- *A closer look*

I. Origin of Replication- The Ori C and Prepriming Complex

- Replication begins at particular sites called **origins of replication**, where the two DNA strands are separated, opening up and form ‘replication bubble’.
- Ori C consists of 245bp and contains 3 repeats of a13bp sequence and 4 repeats of a 9bp sequence called 13mers and 9mers respectively.
- A eukaryotic chromosome may have hundreds or even thousands of origins of replication.
- Replication proceeds in both directions from each origin, until the entire molecule is copied.

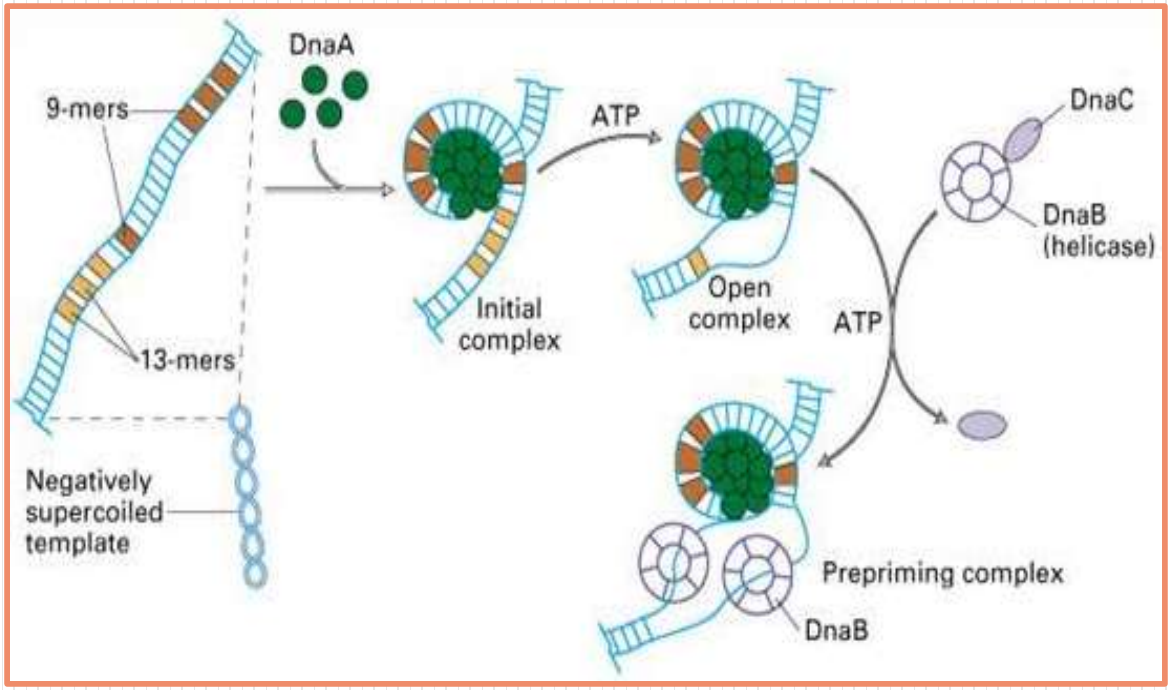


Fig: Structure of Ori C

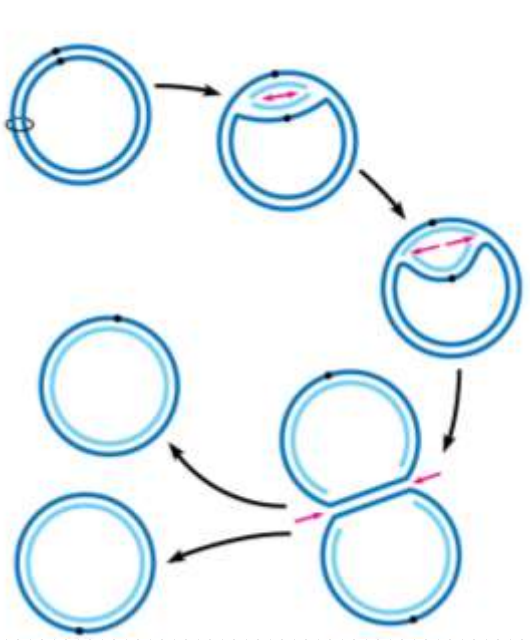
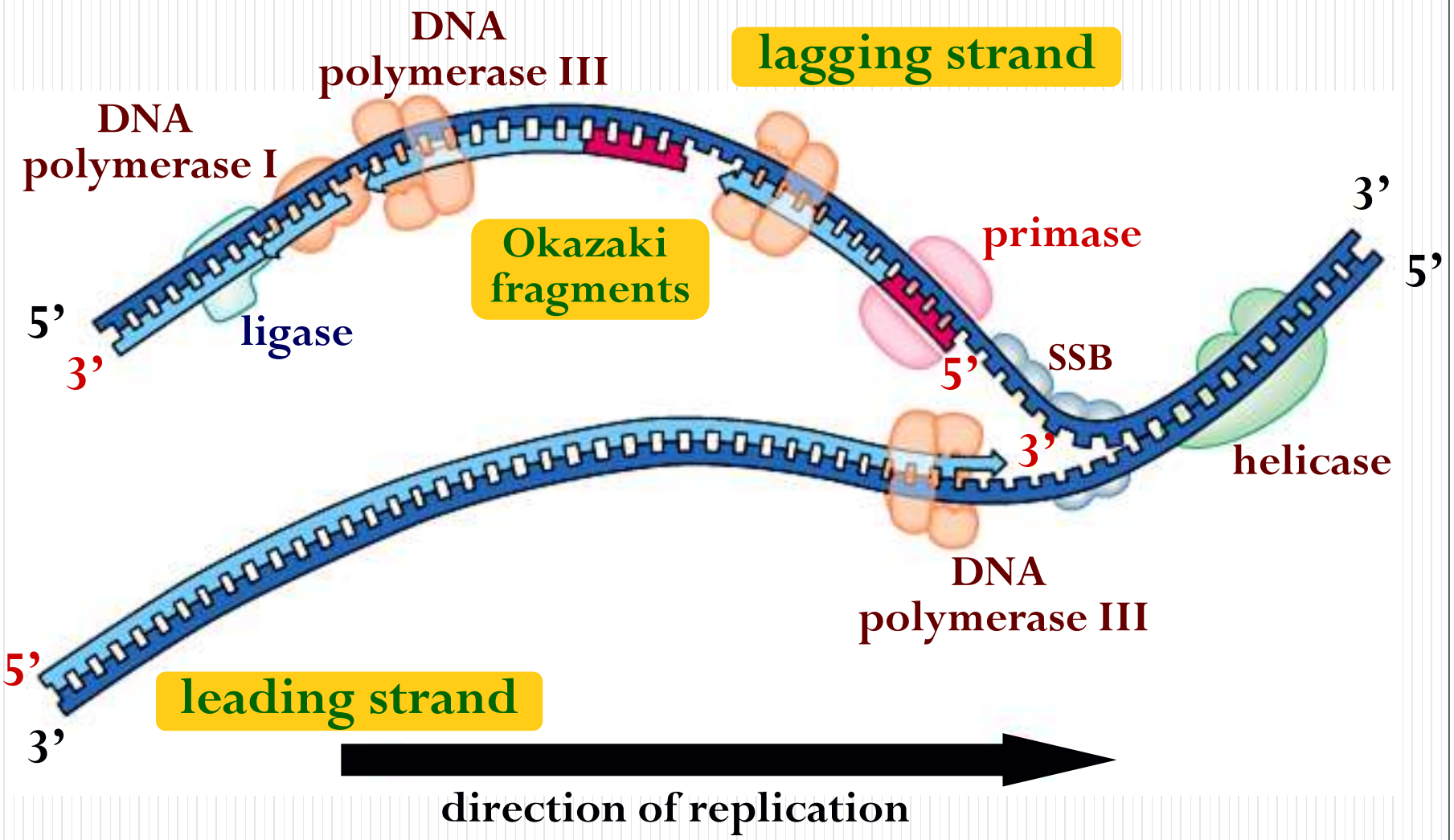


Fig: Bidirectional DNA replication

- At first 20 Dna-A protein molecules bind to the four 9bp repeates,bindind needs ATP, which help to denature 13bp repeats rich in A=T.
- Now Dna –B which serves as a helicase along with Dna –C proteins bound to unbound region in two hexamers that unwinds DNA bidirectionally to create the replication fork. All these processes need ATP.

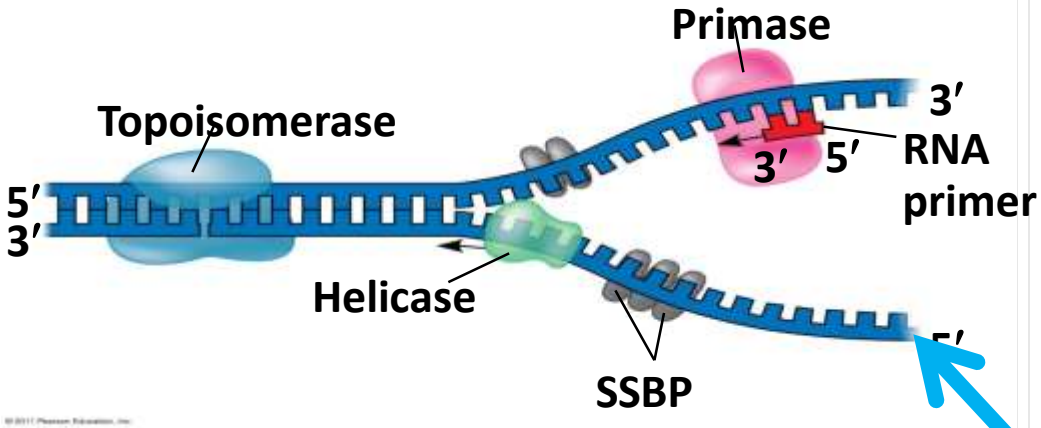
- At the end of each replication bubble there is a aY-shaped region where new DNA strands are elongating, called **replication fork.**
- **Helicase** enzymes that unwind the double helix at the replication forks.
- **Topoisomerase I and II** relax super coiled DNA strands ahead of replication forks by breaking, swiveling, and rejoining DNA strands.
- **Single-strand binding proteins (SSBP)** bind to and stabilize DNA single-strand.

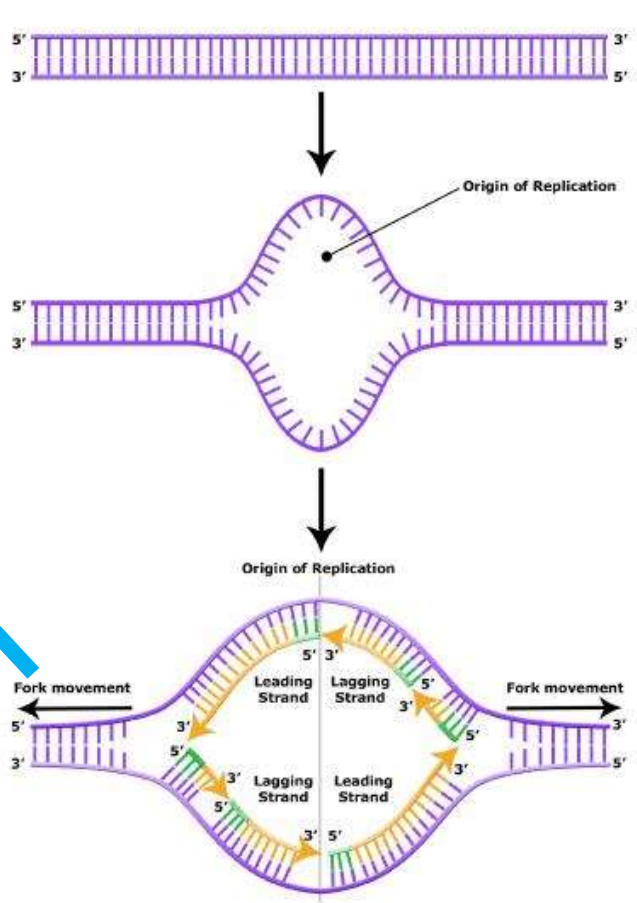
Replication fork



SSB = single-stranded binding proteins

II. Chain Elongation





- RNA Primase :Adds small section of RNA (RNA primer) to the 3′ end of template DNA.
- DNA polymerase III add nucleotides to the 3′ end and help to builds new DNA strand.
- The initial nucleotide strand is a short RNA **primer** (5–10 nucleotides long), and the 3′ end serves as the starting point for the new DNA strand.
- An enzyme called **primase** adds RNA nucleotides one at a time using the parental DNA as a template.

Structure of DNA Polymerase III

- DNA polymerase III is a holoenzyme, which has two core enzymes (Pol III), each consisting of three subunits (**α, ε and θ**), **a sliding clamp that has two beta subunits**, and a clamp-loading complex which has multiple subunits (**δ, τ, γ, ψ, and χ**).
- Core enzyme catalyzes DNA polymerization activity.
- **ß subunit** binds DNA at the junction between template and primer as a dimeric ring. The ring holds the catalytic core polymerase and functions like a clamp.

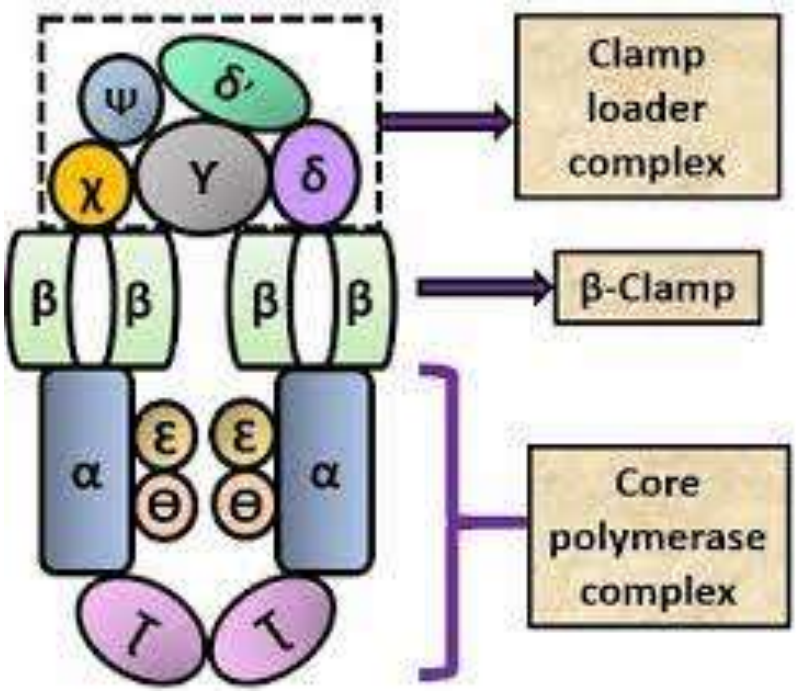


Fig: Structure of DNA Polymerase III

Leading & Lagging strands

- DNA polymerases add nucleotides only to the free 3′ end of a growing strand; therefore, a new DNA strand can elongate only in **the 5′ to 3′ direction.**
- Along one template strand of DNA, the DNA polymerase III synthesizes a **leading strand** continuously, moving toward the replication fork.
- The lagging strand is synthesized as a series of segments called **Okazaki fragments**, which are joined together by **DNA ligase.**

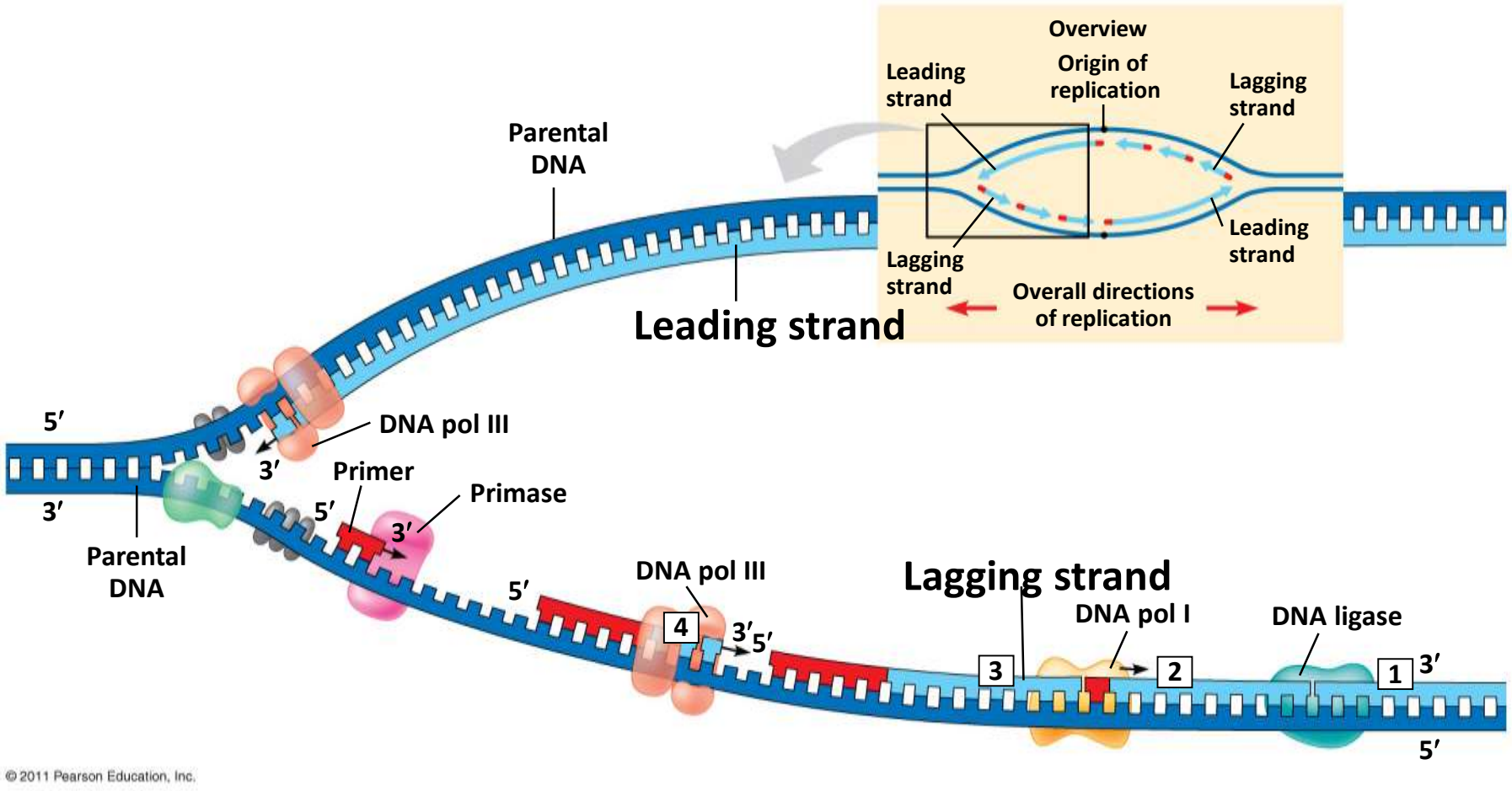


Fig : Leading and Lagging strands synthesis

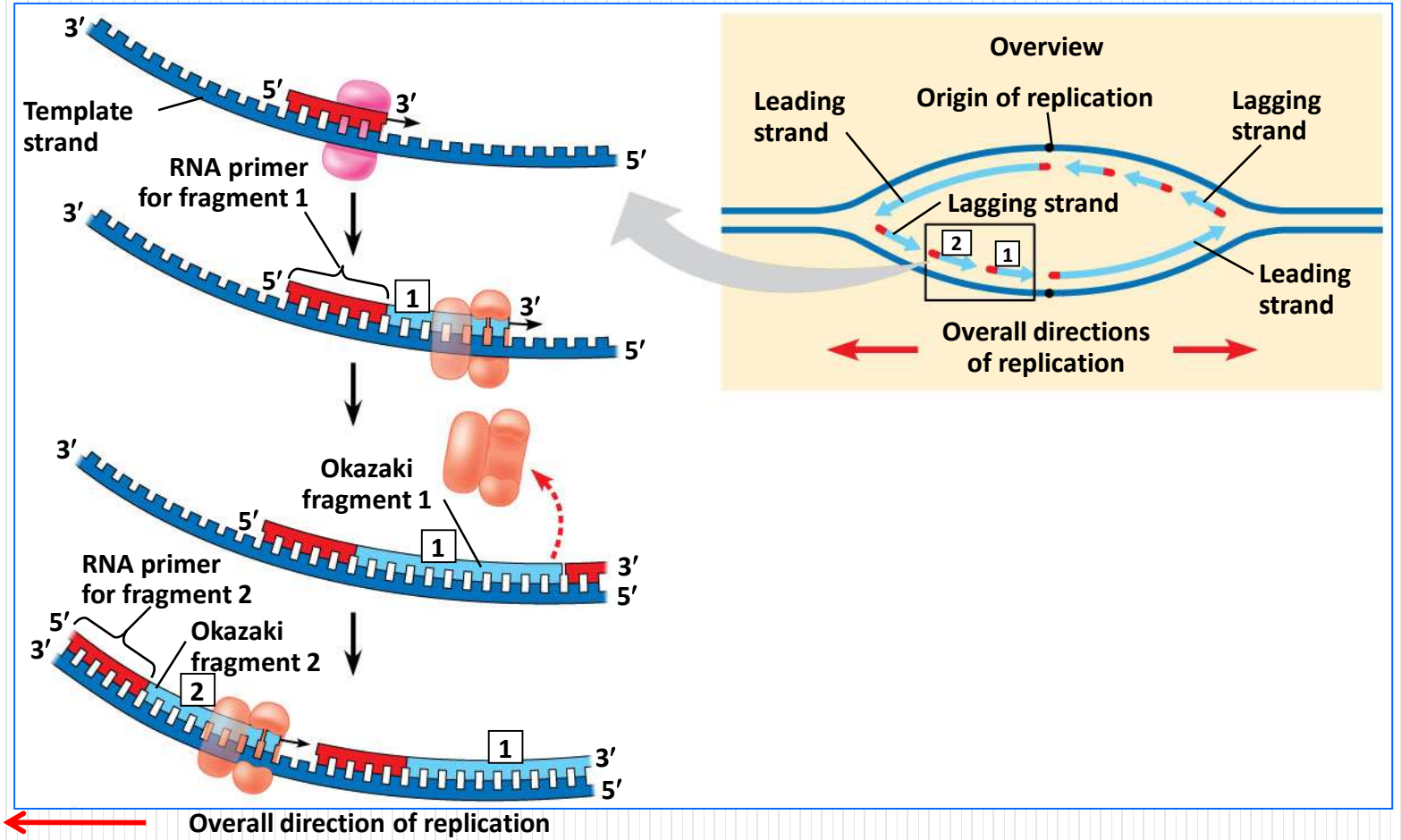
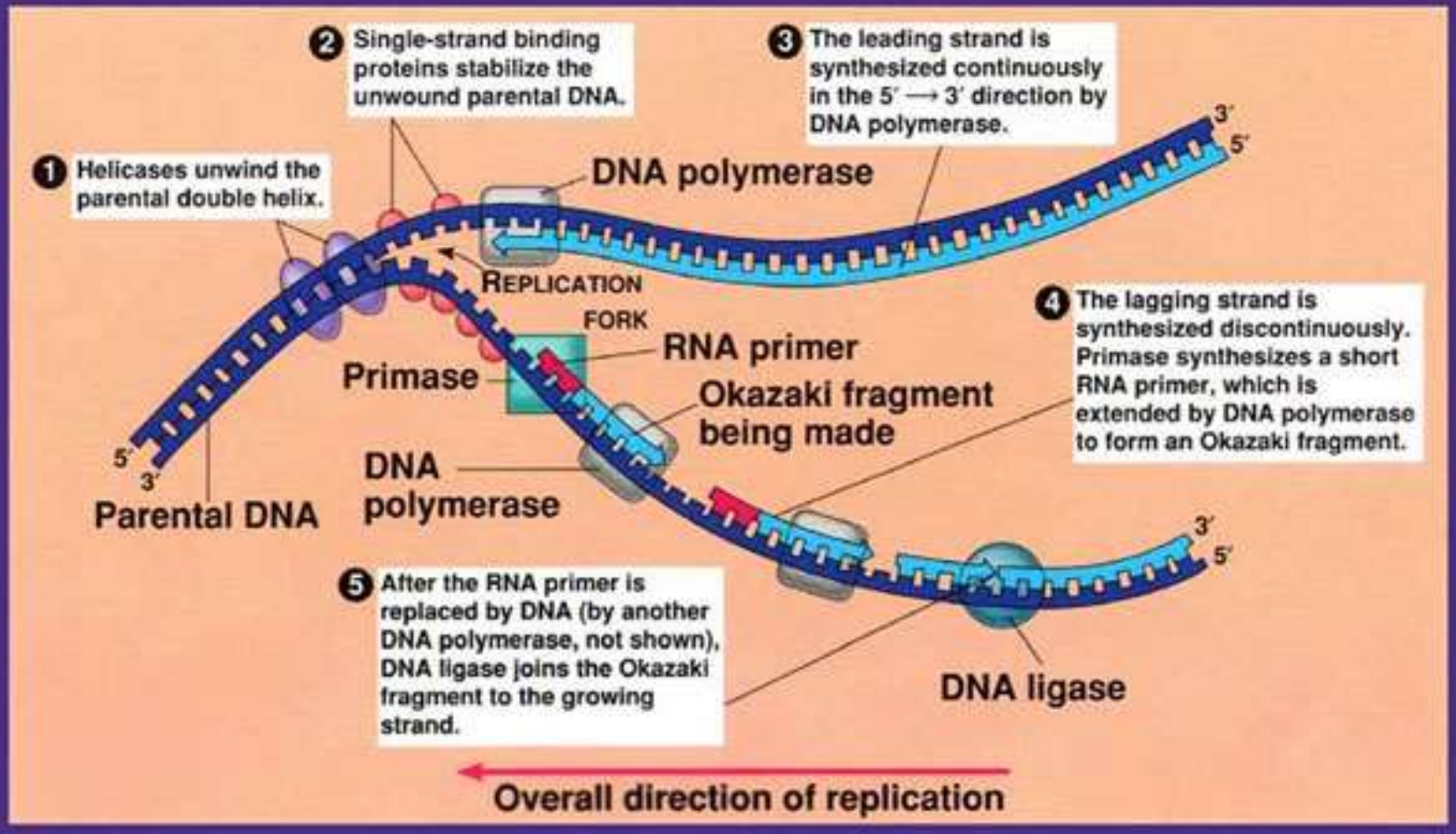


Fig : Lagging strand synthesis

III.The Termination

- *E. coli* chromosome and several other plasmids carry specific sequence, called ‘Ter sites’, diametrically opposite from OriC;where TBP (ter binding protein) binds.
- There are three Ter sites (**ter A, ter D and ter E**) for counter clockwise fork and three Ter sites (**ter B, ter C and ter F**) for clockwise fork .
- These six Ter sites stall the fork, by inhibiting DNA helicase.

A SUMMARY OF DNA REPLICATION



Proofreading and Repairing DNA

- DNA polymerase I having 5’→3’ and 3’→5’ exonuclease activity and 5’→3’ polymerization activity.
- DNA polymerase I proofread newly synthesized DNA, replacing any incorrect nucleotides and increasing accuracy by a hundred to a thousand fold.
- **mismatch repair** enzymes help to correct errors in base pairing in DNA.
- DNA can be damaged by exposure to harmful chemical or physical agents, which causes spontaneous changes.
- **nucleotide excision repair**, a **nuclease help to** cuts out and replaces damaged part of DNA.

Replication of Telomeres

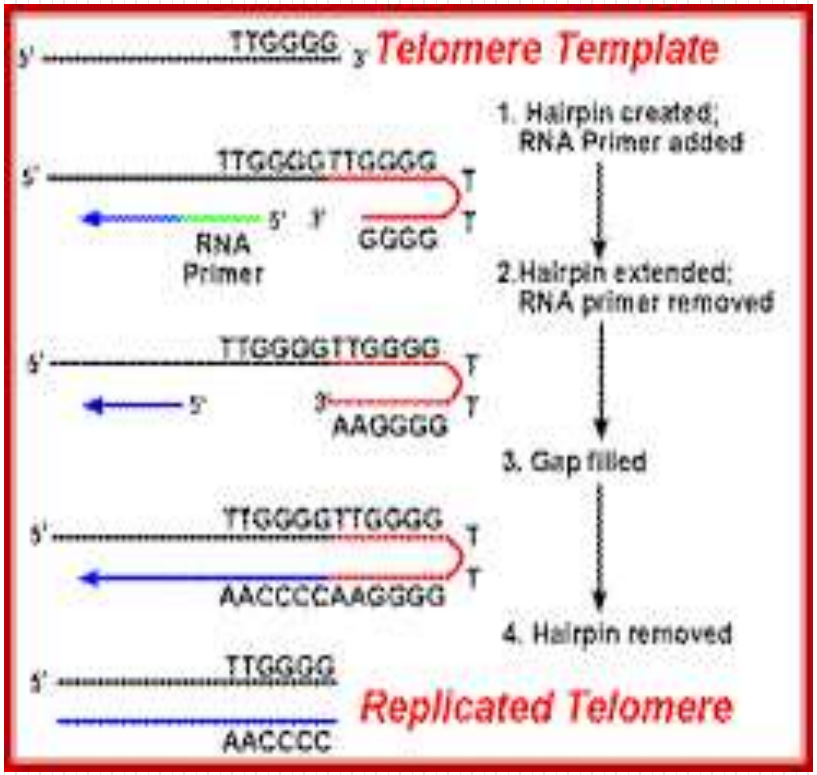
- Eukaryotic chromosomal DNA molecules have special nucleotide sequences (5’TTGGGG3’) at their ends called **telomeres.**
- Limitations of DNA polymerase create problems for the linear DNA of eukaryotic chromosomes. This is not a problem for prokaryotes, as most of which have circular chromosomes.
- The usual replication machinery unable to complete the 5′ ends, so repeated rounds of replication produce shorter DNA molecules with uneven ends in the chain.

- An enzyme called **telomerase , a ribonuclease protein** catalyzes the lengthening of telomeres and bind to G rich sequence and add '**TTGGGG**' to end of telomere sequence of template lagging strand by built in RNA strand template.

- This '**TTGGGG**' repeats form hairpin loop .

- This loop provide3'OH end to DNA polymerase-I to fill the gap after the removal of RNA primer.

- After this hairpin is removed and thus shortening of DNA is prevented.



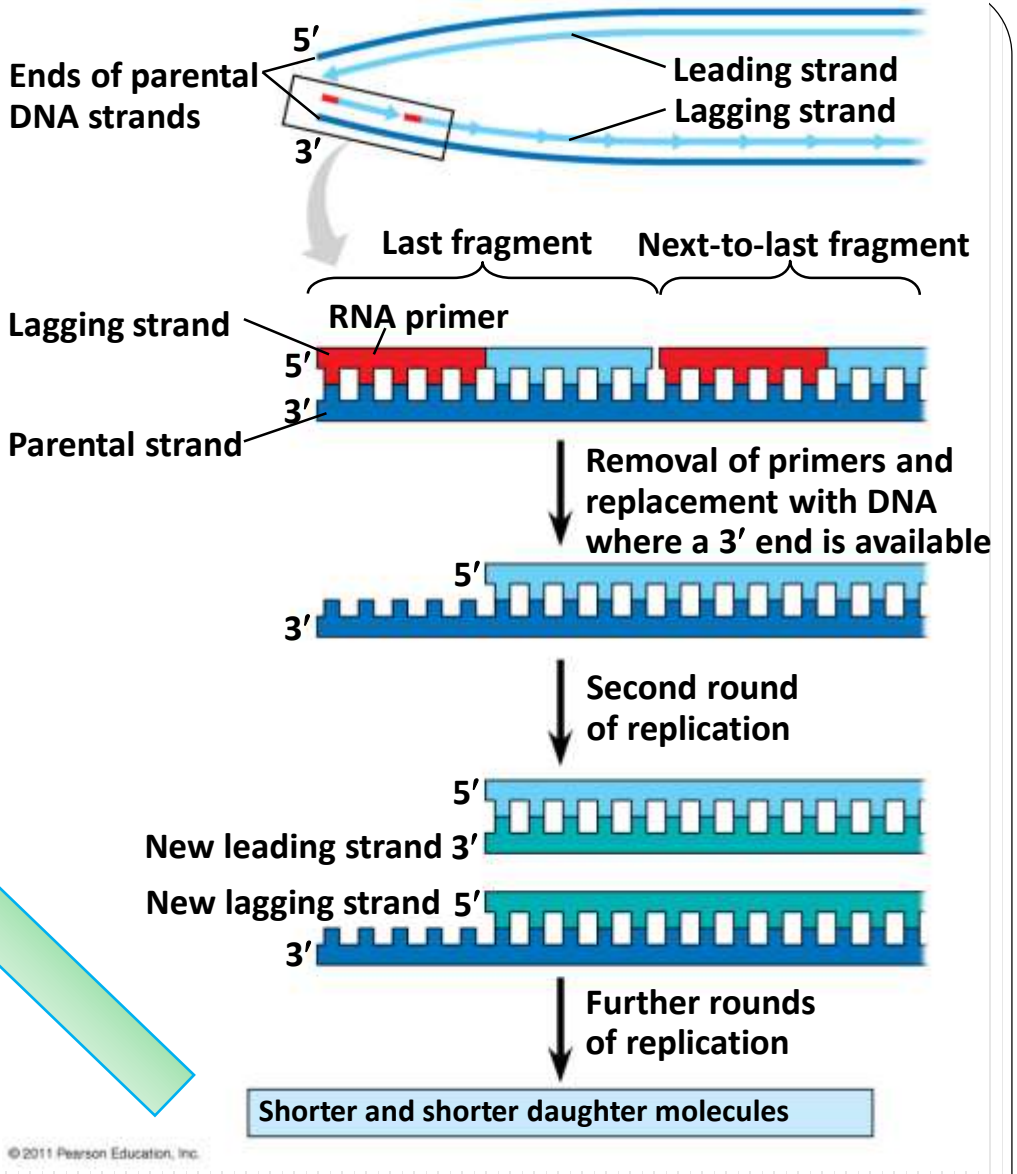


Fig: Telomere Replication

Significance

- Telomeres do not prevent the shortening of DNA molecules, but they do postpone the erosion of genes near the ends of DNA molecules in germ cells.
- Telomere prevent DNAse from degrading the end of linear DNA molecules.
- Facilitates replication of the end of linear DNA molecule without loss.
- It gives stability and integrity to chromosome.
- It has been proposed that the shortening of telomeres is connected to aging
- There is evidence of enhanced telomerase activity in cancer cells, which may allow cancer cells to persist.


Thank You