

DNA REPLICATION

DNA replication involves the unwinding of the two polynucleotide strands, followed by the duplication of two new complementary strands via a template mechanism. Each DNA strand acts as a template for the newly synthesized molecule. DNA replication takes place only once in the cell cycle, which starts with the separation of two DNA strands which permits the nucleotides to fall into place and to be linked by the action of enzyme DNA polymerase. The two nucleotide strands can be separated because they are joined by relatively weak hydrogen bonds.

DNA is known to be the master molecule that controls activities of the cell. It performs two main functions.

- 1) Synthesis of another molecule of DNA (Replication)
- 2) Synthesis of protein molecules (Protein Synthesis)

Semi conservative method of DNA replication

The Watson and Crick model of DNA immediately suggested that the two strands of DNA should separate. Each separated strand serves as a template for formation of a new but complementary strand. Thus the new or daughter DNA molecules formed would be made of one old and another newly formed complementary strand. This method of formation of ~~two~~ new DNA is called semi conservative method of DNA replication.

Mechanism of DNA Replication - DNA replication involves four major stages.

- 1) Initiation of DNA replication

② Unwinding of helix.

- 3/ Formation of Primer strand and
- 4/ Elongation of new strand.

1) Initiation of Replication - Replication of DNA in *E. coli* always begins at a definite site called the replication origin while in Eukaryotes there are several thousand origins of replication called the replicons.

2) Unwinding of helix - Unwinding is brought about by enzyme helicase known as Rep protein. This unwinding results in formation of supercoils which are then sep. removed by the action of another enzyme DNA gyrase or topoisomerases. Unwinding of DNA molecules separate two strands to form Y shaped replication fork. These exposed single strands are stabilized by single strand binding protein or helix destabilizing protein. The SSB protein holds open the two separated strands to form replication fork.

Formation of Primer Strand - A new strand is now synthesized opposite the parental strand that acts as a template strand. DNA polymerase III is the true replicase in *E. coli*, another enzyme known as primase initiates the synthesis by producing a short primer strand of RNA. This primer RNA is removed enzymatically once the initiation of DNA synthesis is completed.

Elongation of new strand - After the formation of primer strand, DNA

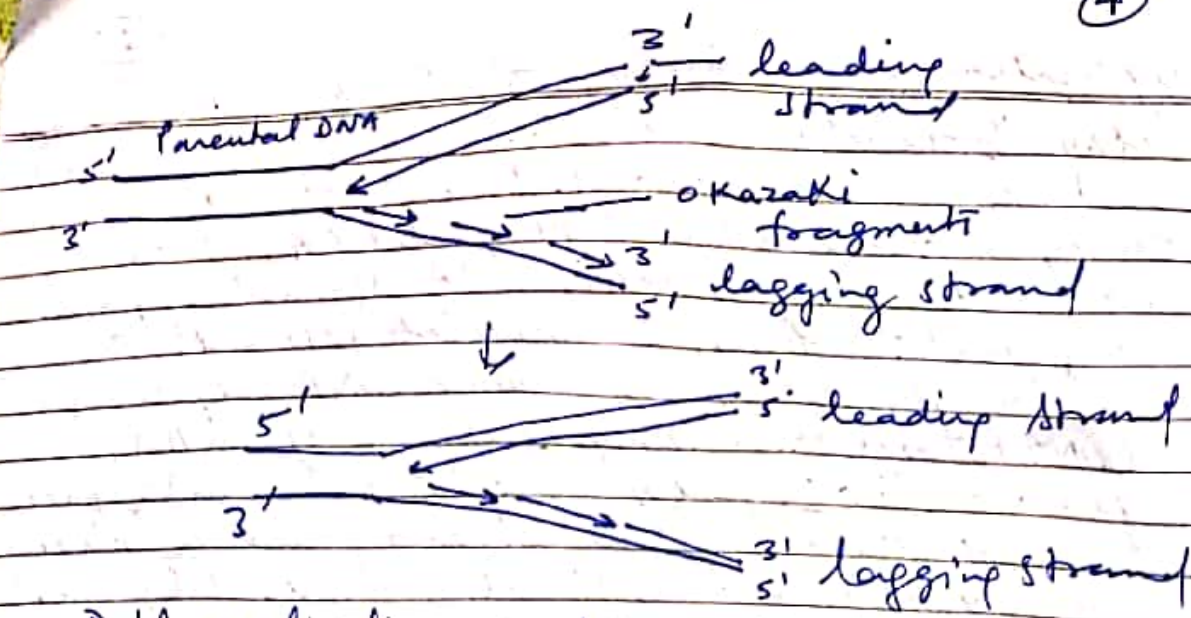
Replication occurs in 5' - 3' direction, i.e. deoxyribonucleotides are added only to the 3' OH end of the new strand. During this process for eg. deoxyadenosine triphosphate (dATP) is added to an elongating DNA strand opposite thymine (T) base in template strand. This is brought about by enzyme DNA replicase (DNA Polymerase). The phosphate of dATP forms a 3' → 5' phosphodiester bond with free 3' OH of the elongating strand, and a molecule of pyrophosphate is simultaneously released. Thus the nucleotide at 3' carbon of sugar is always the most recently added nucleotide to the chain.

As the DNA replication proceeds, of the two parental strands, synthesis of new strand occurs continuously along the upper strand (5' P - 3' OH) it is known as leading daughter strand. Synthesis of another daughter strand along the lower parental strand (3' OH - 5' P) takes place in the short pieces called the lagging daughter strand.

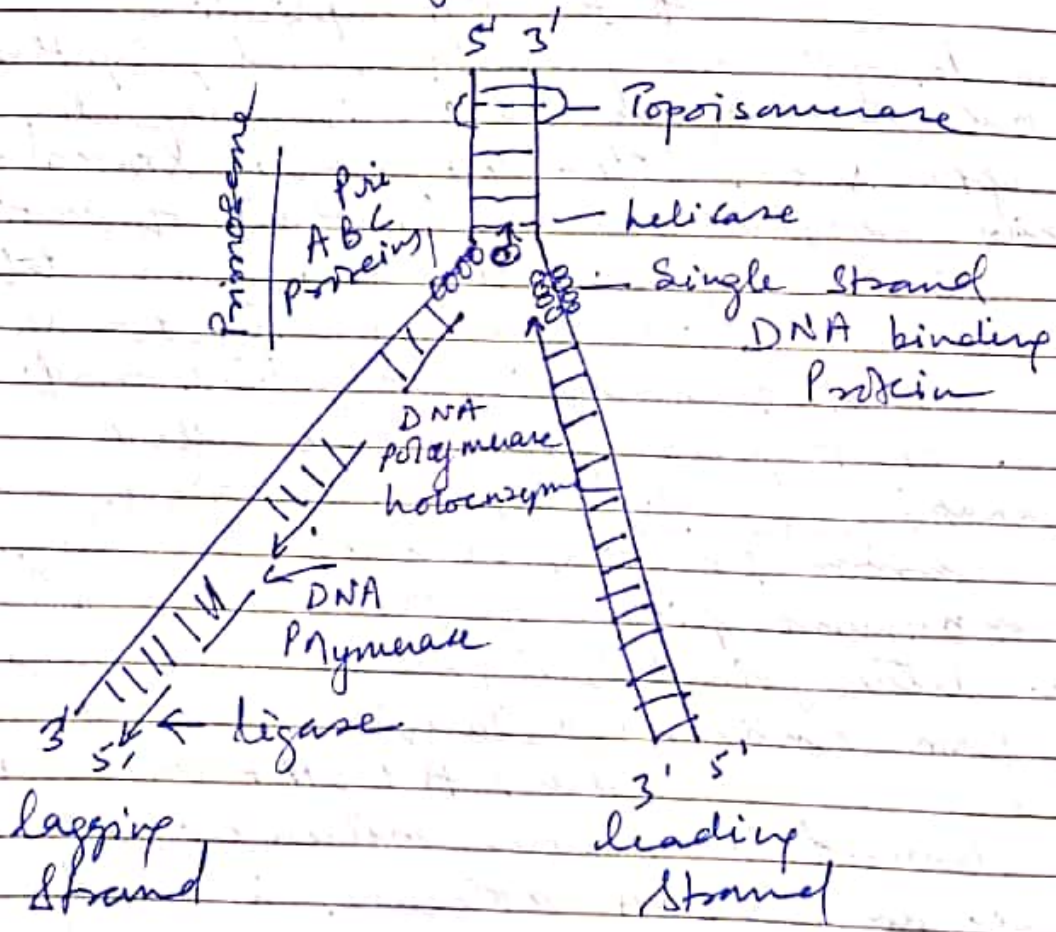
These short pieces of DNA are called Okazaki fragments, segments or pieces and are about 1,000 - 2,000 nucleotides long. Discontinuous pieces of the lagging strand are later joined by the enzyme ligase to form continuous daughter strand.

Thus two DNA molecules are now formed from one molecule. Each of these daughter DNA molecules is made up of two strands out of which one is old and other one is new.

DNA Polymerase copies DNA strand very accurately and it also has a error correcting mechanism called Proof reading.



DNA replication continuous and discontinuous DNA synthesis in leading and lagging strands.



A schematic model of a replication fork in *E. coli* showing the major components of replication apparatus.

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