BIOCHEMISTRY- BP203T

UNIT: 4 Nucleic acid metabolism and genetic information transfer

CLASS:7

TOPIC: DNA REPLICATION

DNA REPLICATION:

- DNA is the genetic material.
- When the cell divides, the daughter cells receive an identical copy of genetic information from the parent cell.
- Replication is a process in which DNA copies itself to produce identical daughter molecules of DNA.
- Replication is carried out with high fidelity which is essential for the survival of the species.
- Synthesis of a new DNA molecule is a complex process involving a series of steps.
- The salient features of replication in prokaryotes are described first.
- This is followed by some recent information on the eukaryotic replication.

REPLICATION IN PROKARYOTES

- Replication is semiconservative
- The parent DNA has two strands complementary to each other.
- Both the strands undergo simultaneous replication to produce two daughter molecules.
- Each one of the newly synthesized DNA has one-half of the parental DNA (one strand from original) and one-half of new DNA.
- This type of replication is known as semiconservative since half of the original DNA is conserved in the daughter DNA.
- The first experimental evidence for the semiconservative DNA replication was provided by Meselson and Stahl (1958).

Initiation of replication:

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- The initiation of DNA synthesis occurs at a site called origin of replication.
- In case of prokaryotes, there is a single site whereas in eukaryotes, there are multiple sites of origin.
- These sites mostly consist of a short sequence of A-T base pairs.
- A specific protein called dna A (20-50 monomers) binds with the site of origin for replication.
- This causes the double-stranded DNA to separate.

Replication bubbles:

- The two complementary strands of DNA separate at the site of replication to form a bubble.
- Multiple replication bubbles are formed in eukaryotic DNA molecules, which is essential for a rapid replication process.

RNA primer:

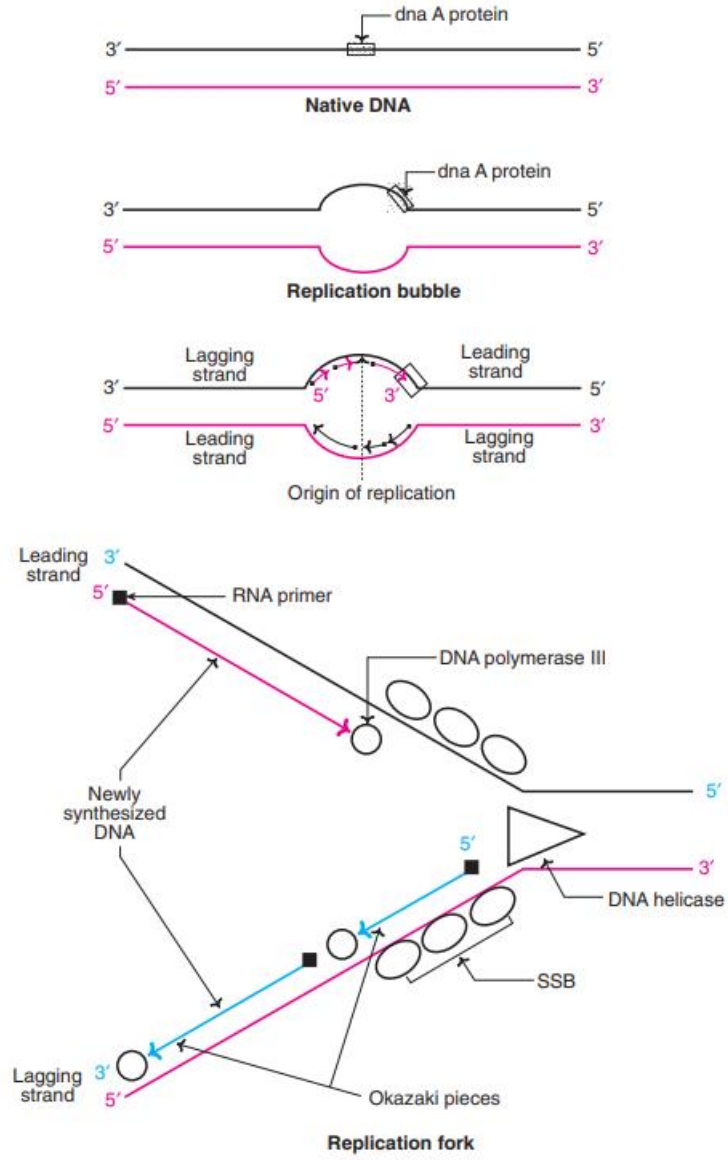
- For the synthesis of new DNA, a short fragment of RNA (about 5-50 nucleotides, variable with species) is required as a primer.
- The enzyme primase (a specific RNA polymerase) in association with single-stranded binding proteins forms a complex called primosome, and produces RNA primers.
- A constant synthesis and supply of RNA primers should occur on the lagging strand of DNA.
- This is in contrast to the leading strand which has almost a single RNA primer.
- DNA synthesis is semi discontinuous and bidirectional
- The replication of DNA occurs in 5’ to 3’ direction, simultaneously, on both the strands of DNA.
- On one strand, the leading (continuous or forward) strand—the DNA synthesis is continuous.
- On the other strand, the lagging (discontinuous or retrograde) strand—the synthesis of DNA is discontinuous.
- Short pieces of DNA (15-250 nucleotides) are produced on the lagging strand.
- In the replication bubble, the DNA synthesis occurs in both the directions (bidirectional) from the point of origin

Replication fork and DNA synthesis:

- The separation of the two strands of parent DNA results in the formation of a replication fork.
- The active synthesis of DNA occurs in this region.
- The replication fork moves along the parent DNA as the daughter DNA molecules are synthesized.

DNA helicases:

- These enzymes bind to both the DNA strands at the replication fork. Helicases move along the DNA helix and separate the strands.
- Their function is comparable with a zip opener. Helicases are dependent on ATP for energy supply.
- Single-stranded DNA binding (SSB) proteins: These are also known as DNA helix-destabilizing proteins. They possess no enzyme activity.



∴STRUCTURE OF DNA REPLICATION

proteins. They possess no enzyme activity.

- SSB proteins bind only to single-stranded DNA (separated by helicases), keep the two strands separate and provide the template for new DNA synthesis.
- It is believed that SSB proteins also protect the single-stranded DNA degradation by nucleases.

DNA synthesis catalysed by DNA polymerase III

The synthesis of a new DNA strand, catalysed by DNA polymerase III, occurs in 5'→3' direction.

This is antiparallel to the parent template DNA strand.

The presence of all the four deoxyribonucleoside triphosphates (dATP, dGTP, dCTP and dTTP) is an essential prerequisite for replication to take place.

The synthesis of two new DNA strands, simultaneously, takes place in the opposite direction—

one is in a direction (5'→3') towards the replication fork which is continuous,

the other in a direction (5'→3') away from the replication fork which is discontinuous.

The incoming deoxyribonucleotides are added one after another, to 3' end of the growing DNA chain. A molecule of pyrophosphate (PPi) is removed with the addition of each nucleotide.

The template DNA strand (the parent) determines the base sequence of the newly synthesized complementary DNA.

Polarity problem:

- The DNA strand (leading strand) with its 3'-end (3'-OH) oriented towards the fork can be elongated by sequential addition of new nucleotides.
- The other DNA strand (lagging strand) with 5'-end presents some problem, as there is no DNA polymerase enzyme (in any organism) that can catalyse the addition of nucleotides to the 5' end (i.e. 3'→5' direction) of the growing chain.
- This problem however is solved by synthesizing this strand as a series of small fragments.
- These pieces are made in the normal 5co3c direction, and later joined together.

Okazaki pieces:

- The small fragments of the discontinuously synthesized DNA are called Okazaki pieces.
- These are produced on the lagging strand of the parent DNA.
- Okazaki pieces are later joined to form a continuous strand of DNA.

DNA polymerase I and DNA ligase are responsible for this process (details given later).

Proof-reading function of DNA polymerase III

Fidelity of replication is the most important for the very existence of an organism.

Besides its 5'→3' directed catalytic function, DNA polymerase III also has a proof-reading activity.

It checks the incoming nucleotides and allows only the correctly matched bases (i.e. complementary bases) to be added to the growing DNA strand.

Further, DNA polymerase edits its mistakes (if any) and removes the wrongly placed nucleotide bases.

Replacement of RNA primer by DNA:

- The synthesis of new DNA strand continues till it is in close proximity to RNA primer.
- Now the DNA polymerase I comes into picture.
- It removes the RNA primer and takes its position.
- DNA polymerase I catalyses the synthesis (5'o3' direction) of a fragment of DNA that replaces RNA primer.
- The enzyme DNA ligase catalyses the formation of a phosphodiester linkage between
- the DNA synthesized by DNA polymerase III and the small fragments of DNA produced by DNA polymerase I.
- This process—nick sealing-requires energy, provided by the breakdown of ATP to AMP and PPi.
- Another enzyme—DNA polymerase II—has been isolated. It participates in the DNA repair process.