Machinery of DNA Replication

Machinery of DNA Replication and the Enzymes

In living cells, the process of replication requires a set of catalysts (enzymes). The main enzyme is referred to as DNA-dependent **DNA polymerase**, since it uses a DNA template to catalyse the polymerisation of deoxynucleotides.

DNA polymerases are highly efficient enzymes, as they catalyse polymerisation of a large number of nucleotides in a very short time. *E. coli* with 4.6×10^6 bp, completes its replication within 38 minutes; that means the average rate of polymerisation is approximately 2000 bp per second. these polymerases are fast and catalyse the polymerization reaction with very high degree of accuracy. Any mistake during replication would result into mutations.

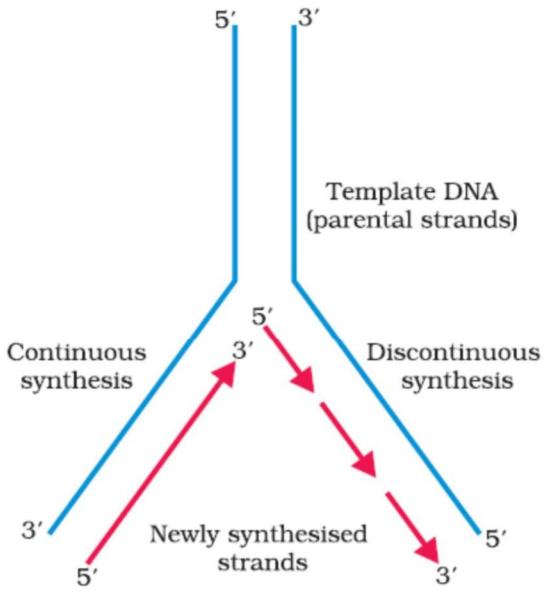

Figure Replication Fork

energetically replication is a very expensive process. Deoxyribonucleoside triphosphates serve dual purposes. Firstly, it acts as substrates, and secondly, they provide energy for polymerisation reaction (the two terminal phosphates in a deoxynucleoside triphosphates are high-energy phosphates, same as in case of ATP).

In addition to DNA-dependent DNA polymerases, there are many additional enzymes involved in completing the process of replication with high degree of accuracy. For long DNA molecules, since the two strands of DNA cannot be separated in its entire length (due to very high energy requirement), the

replication occur within a small opening of the DNA helix, which is called **replication fork**. The DNA-dependent DNA polymerases catalyse polymerisation only in one direction that is 5′→ 3′.

This creates additional complications at the replicating fork.

Consequently, on one strand (the template with polarity 3′ → 5′), the replication is **continuous** and is called leading strand, while on the other (the template with polarity 5′ → 3′), it is **discontinuous and called lagging strand**. The discontinuously synthesised fragments, which are called Okazaki fragment, are later joined by the enzyme **DNA ligase**.

The DNA polymerases on their own cannot initiate the process of replication. They require a primer strand called RNA primer for the initiation of replication. There is a definite region in *E. coli* DNA where the replication originates. Such regions are termed as **origin of replication**. It is because of the requirement of the origin of replication that a piece of DNA if needed to be propagated during recombinant DNA procedures, requires a vector. The vectors provide the origin of replication.

In eukaryotes, the replication of DNA takes place at S-phase of the cell-cycle. The replication of DNA and cell division cycle should be highly coordinated. A failure in cell division after DNA replication results into polyploidy (a chromosomal anomaly).