CONCEPT MAP

DNA REPLICATION

Replication is the process of formation of carbon copies of DNA. The primary function of DNA replication is to provide same genetic material as possessed by the parent to the progeny. Thus, the replication of DNA must be complete and carried out in such a way as to maintain genetic stability within the organism and the species. For replication, DNA itself functions as template, therefore, DNA replication is an autocatalytic function of DNA. It occurs during S-phase of the cell cycle and is a multistep complex process which requires over a dozen of enzymes and protein factors.

Semi-conservative Replication

DNA replication is **semi-conservative** *i.e.*, a type of replication in which one strand of the daughter duplex is derived from the parent while the other strand is formed anew. The parent strands are separated and act as template for synthesis of new daughter strand. The new strand has complementary base pairs to template strand. (A opposite T and G opposite C).

DNA Polymerase

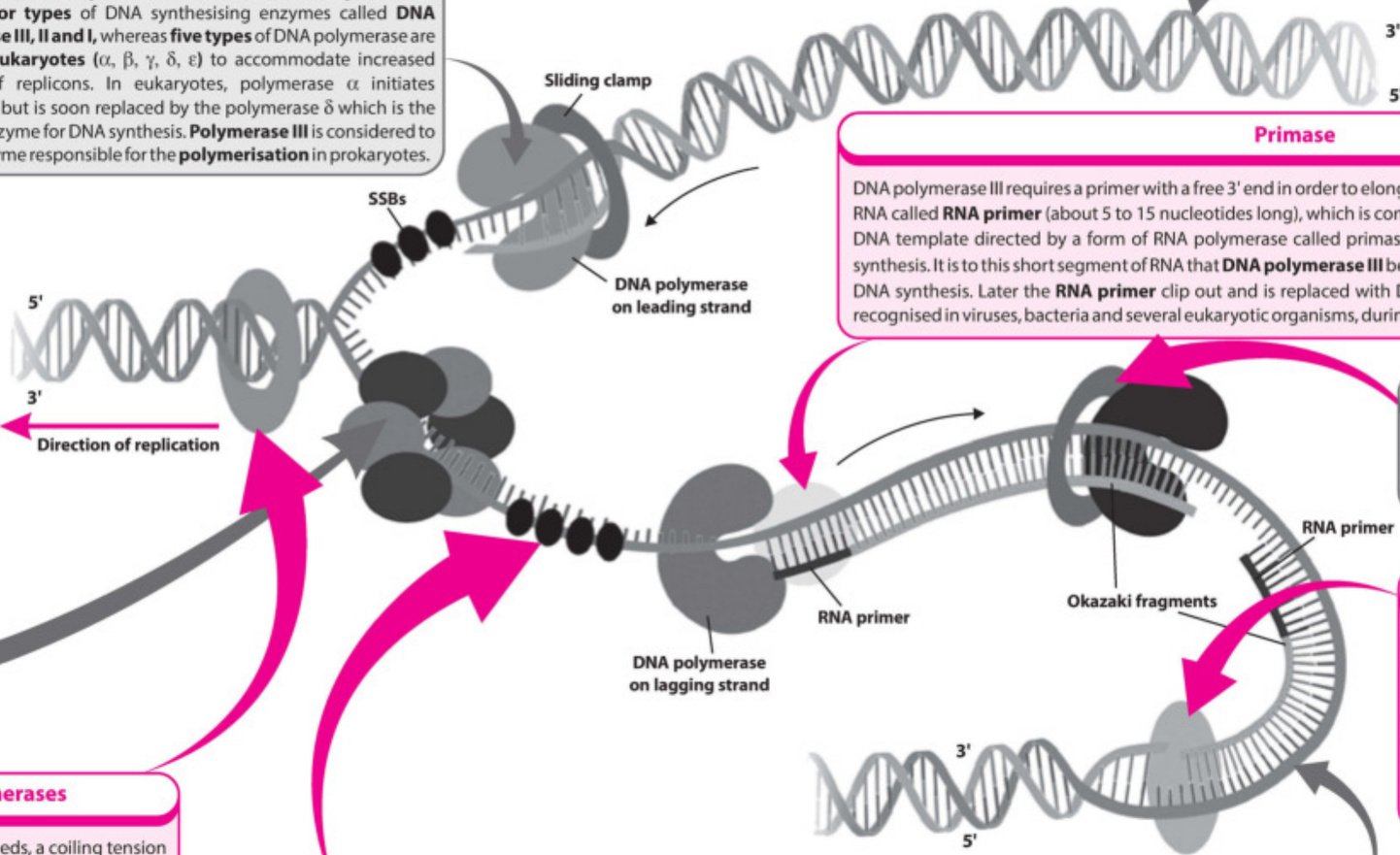
The main enzyme of replication is **DNA dependent DNA polymerase** that catalyses the polymerisation of deoxynucleotides to synthesise a new strand. **Prokaryotes** have **three major types** of DNA synthesising enzymes called **DNA polymerase III, II and I,** whereas **five types** of DNA polymerase are found in **eukaryotes** (α, β, γ, δ, ε) to accommodate increased number of replicons. In eukaryotes, polymerase α initiates replication but is soon replaced by the polymerase δ which is the primary enzyme for DNA synthesis. **Polymerase III** is considered to be the enzyme responsible for the **polymerisation** in prokaryotes.

DNA Polymerase in Prokaryotes and Eukaryotes

DNA polymerase I demonstrates 5′→3′ exonuclease activity apart from 3′→5′ exonuclease activity. Polymerase I is believed to be responsible for removing the primer, as well as for filling the gaps which naturally occur as primers are removed. Its exonuclease activity also allows for proofreading during this process, a form of DNA repair. **Polymerase II** is also involved in DNA repair. The **3′→5′ exonuclease** activity of polymerase III provides its **proofreading function**. **Polymerase** ε in eukaryotes may help in synthesis of lagging strand along with other roles and **polymerase** β helps in DNA repair.

Leading Strand

DNA polymerase can polymerise nucleotides only in **5′→3′ direction** because it adds them at the 3′ end. Since the two strands of DNA run in **antiparallel direction,** the two templates provide different ends for replication. Replication over the two templates thus, proceeds in **opposite direction.** The strand with polarity 3′→5′ forms its **complementary** strand continuously because 3′ is always open for elongation. It is called **leading strand** with polarity 5'→ 3'.



Primase

DNA polymerase III requires a primer with a free 3' end in order to elongate a polynucleotide chain. A short segment of RNA called **RNA primer** (about 5 to 15 nucleotides long), which is complementary to DNA, is first synthesised on the DNA template directed by a form of RNA polymerase called primase. It does not require a **free 3′ end** to initiate synthesis. It is to this short segment of RNA that **DNA polymerase III** begins to add 5′-deoxyribonucleotides, initiating DNA synthesis. Later the **RNA primer** clip out and is replaced with DNA. RNA priming is a universal phenomenon recognised in viruses, bacteria and several eukaryotic organisms, during the initiation of DNA synthesis.

Sliding clamp or DNA clamp

It is an important protein of DNA polymerase III holoenzyme that prevents the dissociation of polymerase from template strand of DNA.

DNA Ligase

Discontinuous synthesis of DNA requires enzyme DNA ligase that both removes the RNA primer and unites the Okazaki fragments into the lagging strand. DNA ligase, catalyses the formation of the **phosphodiester bond** that seals the nick between the 3′-hydroxyl of the growing strand and 5′-phosphate of an Okazaki fragment.

Origin of Replication

Replication begins at a particular region called **origin of replication** or *Ori* on the chromosome. Most of the bacterial DNA has single *Ori* hence functions as a **replicon** while eukaryotes have multiple *Ori* (multirepliconic). The interaction of specific proteins with *Ori* defines the start site of replication and provides a short region of ssDNA to initiate synthesis of the nascent DNA at each point along the chromosome where replication is occurring.

Helicases

These are the proteins/enzymes which act over the *Ori* site in order to unwind the two strands of DNA by breaking hydrogen bonds. One particular protein **DnaA** initiates first step in unwinding of DNA helix**.** This facilitates the subsequent binding of **DnaB** and **DnaC** proteins that further open and destabilise the helix. The energy required by the proteins to break hydrogen bonds or denaturing the double helix is supplied by the hydrolysis of ATP. The separation of strands create a **replication fork**. Such a fork will initially appear at the point of origin of synthesis and then move along the DNA duplex as replication proceeds. Replication is **bidirectional** and **two forks**, migrate in opposite directions away from the origin.

Topoisomerases

As unwinding proceeds, a coiling tension is generated ahead of the replication fork, often producing supercoiling. Such supercoiling can be relaxed by **DNA gyrase**, a member of a larger group of enzymes referred to as DNA topoisomerases. The gyrase makes either single or double stranded "cuts" and catalyses localised movements that have the effect of "undoing" the twists and knots created during supercoiling. The strands are then resealed. These various reactions are driven by the energy released during ATP hydrolysis.

Single Strand Binding Proteins

The separated strands are stabilised and maintained by **single strand binding proteins (SSBs)** which prevent premature reannealing of ssDNA to dsDNA. This allows enzymes including helicase, primase and DNA polymerase, to bind and initiate DNA synthesis.

Proofreading and Error Correction

Although the action of DNA polymerase is very accurate, synthesis is not perfect and a noncomplementary nucleotide is occasionally inserted erroneously. To compensate for such inaccuracies, all DNA polymerases possess **3′→5′ exonuclease activity.** This property enables them to detect and excise a mismatched nucleotide (in the 3′→5′ direction). Once the mismatched nucleotide is removed, 5′→3′synthesis can again proceed. This process **increases the fidelity** of synthesis.

Lagging Strand

Lagging strand is the strand synthesised In direction opposite to the growing replication fork, *i.e.*, (3' → 5'). Here, the DNA is synthesised discontinuously in short (1-5 kb) fragments, known as **Okazaki fragments**. Several Okazaki fragments (upto a thousand) must be sequentially synthesised for each replication fork. To ensure that this happens, the helicase associates with the primase. This allows the RNA primer to be made and, in turn, the polymerase begin replication of DNA. An RNA primer is required every time to form a new Okazaki fragment.