

ARUNAI ACADEMY

FOR

PG TRB-BOTANY

DHARMAPURI.9500244679

REPLICATION - ENZYMES

DNA HELICASE

- Separation of two strands- DNA helicase enzyme functions “Unwinds DNA”.
- DNA double helix by breaking the hydrogen bond between two strands of DNA.
- It recruits RNA primase and activate it by forming a complex called **primosome**. (The “Dna.B helicase” and “Dna.G Primase” constitute a functional unit within the replication complex, called the “**primosome**”)
- They have molecular weight 300,000, which contain **six identical subunits**.
- The DNA is around by the Dna.B helicase at the replication fork, DNA primase occasionally associates with Dna.B helicase and synthesizes a short RNA primer.
- ”Helicase” and “Nuclease” activities of the Rec B, C, D enzyme is believed to help initiate homologous genetic recombination in

E.Coli. It is also involved in the repair of double-strand breaks at the collapsed replication fork.

- ➔ **Okazaki fragments**” are short stretches of 1000-2000 bases produced during discontinuous replication, they are later joined into a covalently intact strand

DNA SINGLE- STRANDED BINDING PROTEINS

(SSBP)

- ➔ **Sabilisation of the single strand** created by helicase
- ➔ Single stranded binding protein prevents reforming of the double of the DNA
- ➔ Molecular weight of the SSB protein is 75,600
- ➔ It contains Four identical subunits, which binds single-stranded DNA.
- ➔ The main function of the SSB protein is “**Prevents reannealing**” in the **Replication process**.
- ➔ As Polymerase-III holoenzyme advances, it must displace the SSB protein in order that base pairing of the nucleotide being added can occur.
- ➔ “**RFA**” is a **single-stranded DNA binding protein** equivalent in function to the E.Coli SSB protein

DNA GYRASE(OR)TOPOISOMERASE

- ➔ **Catalyses the formation of negative supercoil**
- ➔ “Topoisomerases” can change the “Linking number”(Lk).
- ➔ Every cell has enzymes that increase (or) decrease the extent of DNA unwinding is called “Topoisomerases” the property of DNA that they change is the linking number.
- ➔ **Topoisomerases”, these enzymes play an especially important role in processes such as “Replication” and “DNA packaging”.**

➤ **DNA gyrase** inserts negative super coils by nicking both strands of DNA, passing the DNA strands through the nick.

- **Topoisomerase I** relieve supercoiling in DNA molecule by transient breaking & releasing of one strand of DNA.
- This act by transiently breaking one of the two DNA strands, rotating one of the ends of the unbroken strand, and rejoining the broken ends; they change Lk in increments of 1.
- **Topoisomerase II** provide a “swivel” in front of each replication fork.
- The enzyme breaks both DNA strands and changes Lk in increments of 2.

Prokaryotic Topoisomerases:

Four different Topoisomerases (I and IV) occur in E.Coli.

1) Type.I (Topoisomerase I and III):

The type I generally relax DNA by removing negative super-coils (increasing Lk)

2) Type.II (Topoisomerase II and IV):

The Topoisomerase II is also called “**DNA gyrase**”, can introduce negative supercoils. (Decrease Lk).

It uses the **energy of ATP** and a surprising mechanism to accomplish this.

The degree of supercoiling of bacterial DNA is maintained by regulation of the net activity of **topoisomerase-I and II**.

Eukaryotic Topoisomerases:

Eukaryotic cells also have type-I and type-II topoisomerases.

Topoisomerases-I & II are both type-I. The two type-II topoisomerases, topoisomerases IIa and IIb, can not unwind DNA (introduce negative supercoils). Although both can relax positive and negative supercoils.

We consider one probable origin of negative supercoils in eukaryotic cells.

The DNA gyrase molecular weight is 400,000, which contain **FOUR** subunits and functions “Supercoiling”.

Supercoiled DNA is a higher-ordered structure occurring in circular DNA molecules wrapped around a core.

What is linking number?

The linking number (Lk) is a topological property. Lk can be defined as “ the number of times the second strand pierces the second strand surface”

PRIMASE

- Synthetises RNA primer.
- Copies a DNA template strand by making an RNA strand complementary to it
- It synthesized a short segment of RNA called RNA primer about 10 nucleotides in 5' to 3' direction.
- DNA primase has molecular weight 60,000 Dalton and contains only single subunit, which functions synthesize RNA primers.
- The RNA primer typically is 15-50 bases long. It synthesizes primers starting with the sequence **pppAG**, opposite the sequence 3'-GTC-5' in the template.

DNA POLYMERASE III

- Catalyzes chain elongation only in 5'-3' direction by adding new nucleotides.
- Have the ability of proof reading.

DNA POLYMERASE I

- DNA polymerase I involved in removing RNA primer In the processing of DNA after replication.
- **5'-3' exonuclease activity**: this enzyme remove ribonucleotide one at a time from 5' end of the primer.
- **3'-5' exonuclease activity / proofreading activity**.

DNA pol I have the ability of 'proof read' means if DNA pol III makes a mistake during DNA synthesis , the resulting unpaired

base at 3' end at the growing strand removed by the DNA pol . I before synthesis continues.

- DNA pol I also **fills the resulting gaps** by synthesizing DNA

DNA POLYMERASE II

- Have **direct polymerase activity** at replication fork
- Capable of synthesis on the damage template.
- Participating in **DNA repairing**.
- Temporary functional when DNA pol I & DNA pol III are not fictional.
- DNA pop II has 3'-5' exonuclease activity.

DNA POLYMERASE α

- 5' to 3' DNA-dependent DNA polymerase
- 5' to 3' DNA-dependent RNA polymerase (primase)

DNA POL. δ

- 3' to 5' exonuclease (proofreading activity)
- 5' to 3' DNA polymerase

DNA POL. ϵ

- 5' to 3' DNA-dependent DNA polymerase
- 3' to 5' exonuclease activity

LIGASE

- Binds the lagging strand
- DNA ligase seals the nicks between Okazaki fragments converting them to a continuous strand of DNA.
- Covalently closes nicks in double stranded DNA
- An enzyme that creates a phosphodiester bond between the 3' end of one DNA segment and the 5' end of another. Once the RNA

primer has been removed and replaced the adjacent Okazaki fragments must be linked together.

- The 3'-OH end of one fragment is adjacent to the 5'-Phosphate end of the previous fragment.
- The responsible for sealing this nick lies with the enzyme DNA ligase.
- **Ligases are present in both prokaryotes and eukaryotes**

**THIS STUDY MATERIAL- ENZYMES INVOLVED IN
DNA REPLICATION-NOT STEPS OF DNA REPLICATION**

➤ **ARUNAI ACADEMY FOR BOTANY PG -TRB.DHARMAPURI.**

(Exclusively for Botany) 9500244679

- **Unit wise study materials for Botany, Education, Psychology and GK.**
- **Topics wise test(daily)-15000 questions and 15 Full test**
- **Classes -Every Sundays 10.am to 5 pm**

ARUNAI ACADEMY FOR PG TRB-BOTANY DHARMAPURI.9500244679/7010753971

**ARUNAI ACADEMY FOR
BOTANY PG -TRB**

DHARMAPURI.

CONTACT:9500244679,

7010753971

ALL POWER IS WITHIN YOU



ARUNAI ACADEMY FOR PG TRB-BOTANY DHARMAPURI.9500244679/7010753971