Date:
Page No. 1

L#1 DNA manipulative enzymes :

i) ऐसे enzymes जो DNA में changes लाएँ, उन्हें DNA manipulative enzymes कहते हैं।

ii) They can be grouped into 4 broad classes depending on the type of rxn they catalyze :-

- Nucleases
- Polymerases
- Ligases
- Modifying enzymes

① Nucleases:

i) They degrade the DNA molecule by breaking the phosphodiester bond that link one nucleotide to the next in a DNA strand.

ii) There are 2 diff kinds of nucleases :-

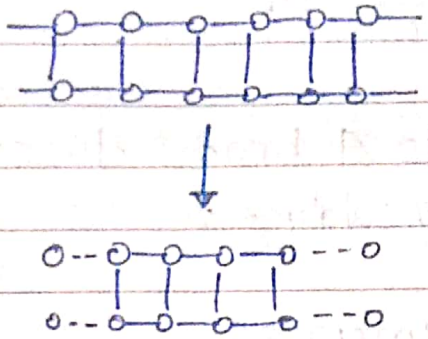
- Exonuclease
- Endonuclease

→ Exonuclease :-

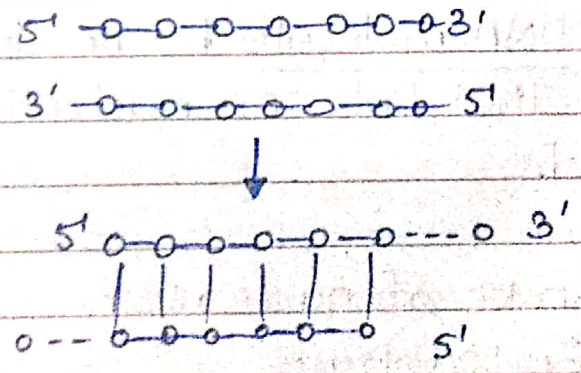
i) They remove nucleotides one at a time from terminal end of DNA molecules.

ii) The main distinction b/w diff exonucleases lies in the no of strands that are degraded when a ds mol is attacked.

a) Bal31 :- It removes nucleotides from both strands of ds DNA mol.



b) E.coli exonuclease III :- It removes nucleotides, only from 5' end of DNA molecule.



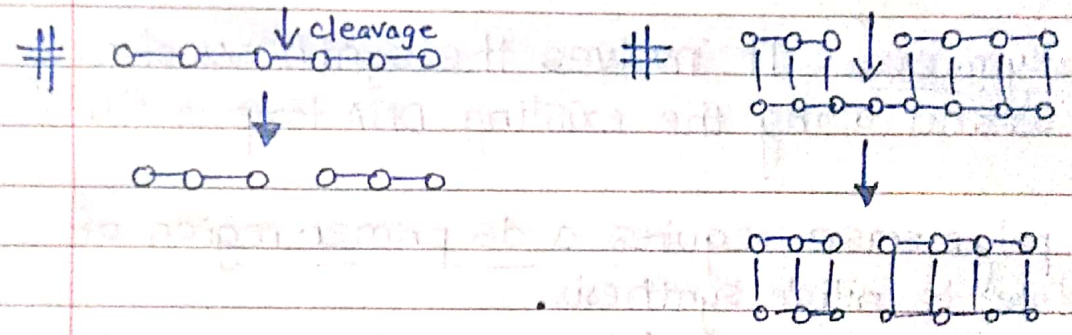
c) Lambda exonuclease III : It removes nucleotides, only from 5' end of DNA molecule.

Note : ① exonuclease : ऐसे enzymes जो DNA के terminal end से nts को cut करें।
② Bal31 : cut nts from both ends of dsDNA mol.
③ Ecoli exonuclease III को ही commonly exonuclease III कहते हैं।

→ Endonuclease : ऐसे enzymes जिससे हम DNA mol. के बीच से nts cut कर पाएँ।

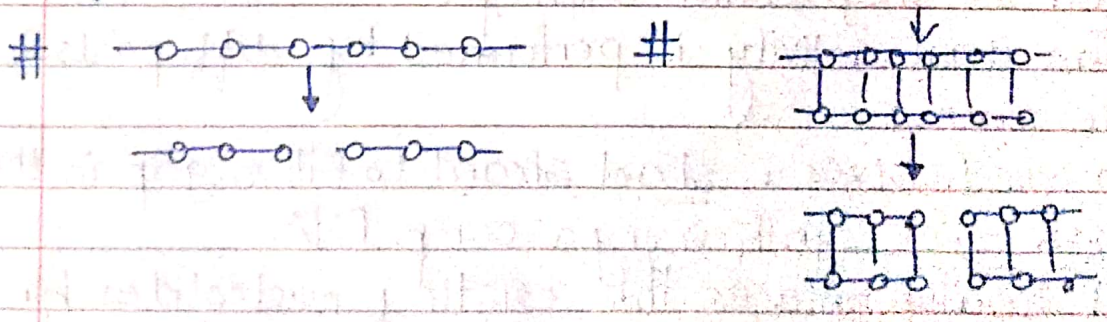
• They are able to break internal phosphodiester bond within a DNA molecule.

Eg: i) S1 endonuclease : It cleaves only ssDNA, including single stranded nicks in dsDNA



* S1 endonuclease केवल ssDNA को cleave करता है।

Eg: ii) DNAse I : It cleaves both ss & dsDNA.



② The special grp of enzymes called Restriction endonucleas cleave dsDNA only at a specific recog. site. It is also called molecular scissor. These enzymes are found in bacteria & Archea & provide a defense mechanism against invading viruses.

Ligase :- It is called "Molecular glue". It is used to seal the nicks that remain in DNA by forming a phosphodiester bond.

Eg: Ty DNA ligase is prep^{d} from E.coli cells infected with Ty phage.

Polymerases: Enzymes that synthesize a new strand of DNA complementary to an existing DNA/RNA template.

Basic polym. rxn : It involves the synthesis of new DNA strand along the existing DNA temp in 5' to 3' dir^{n}.

- Most polymerase requires a ds primer region of template to initiate synthesis.
- In genetics, 3 types of polymerases are commonly used: DNA pol I, Klenow Polymerase, Reverse Transcriptase

i) DNA pol I :- It has dual activity - both synthesis as well as degradation activity.
- The dual activity is performed by diff. parts of the enzyme.
- It synthesizes a short strand to fill in gap in the nick region synthesizing a comp. DNA.
- It finally replaces the existing nucleotides by a process of DNA degrad. immediately followed by DNA pol.

→ Knelow poly:- It is a part of DNA polymerase that retains only the polym. activity but lacks the nuclease activity.

- It is therefore used to fill in the nicks by synthesizing a comp. DNA strand on single stranded template. Since it can't degrade nucleotides, it does not replace existing nucleotides

Note: Polymerase I → Polymerisation + Degradation activity

Knelow → Polymerisation activity only.