RE

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Restriction Endonucleases

① Def^n: Restriction Endonucleases are endonucleases that cleaves the DNA at particular sites within their restriction sequences

② Discovery:

i) The existence of R·E. was first postulated by Werner Arber in early 1960s while studying bacteriophages.

ii) Most bacteria produce Restriction enzymes as a defense against bacteriophages.

iii) They prevent the replication of bacteriophage DNA by cleaving it's DNA at specific sites.

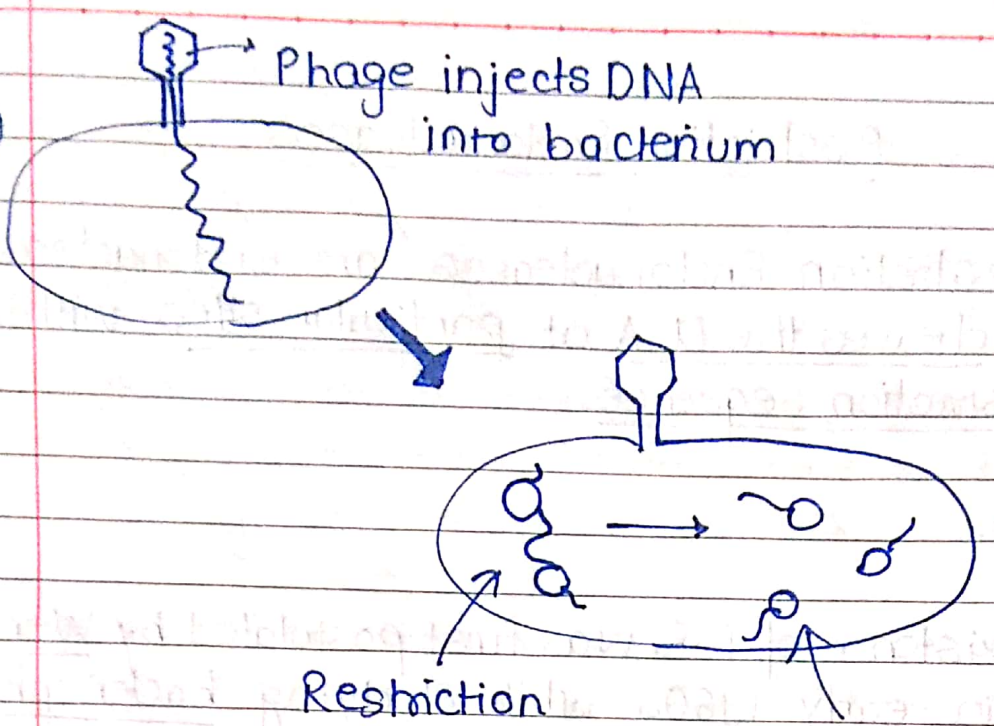
So, Restriction enzymes protected their host from bacteriophages by breaking down the phage DNA at their specific sites.

Q How come these RE don't cleave their host DNA?

Ans. The host DNA is protected by Methylases, which add methyl groups to Adenine or Cytosine bases within the recognition site, thereby modifying the site & protecting the DNA.

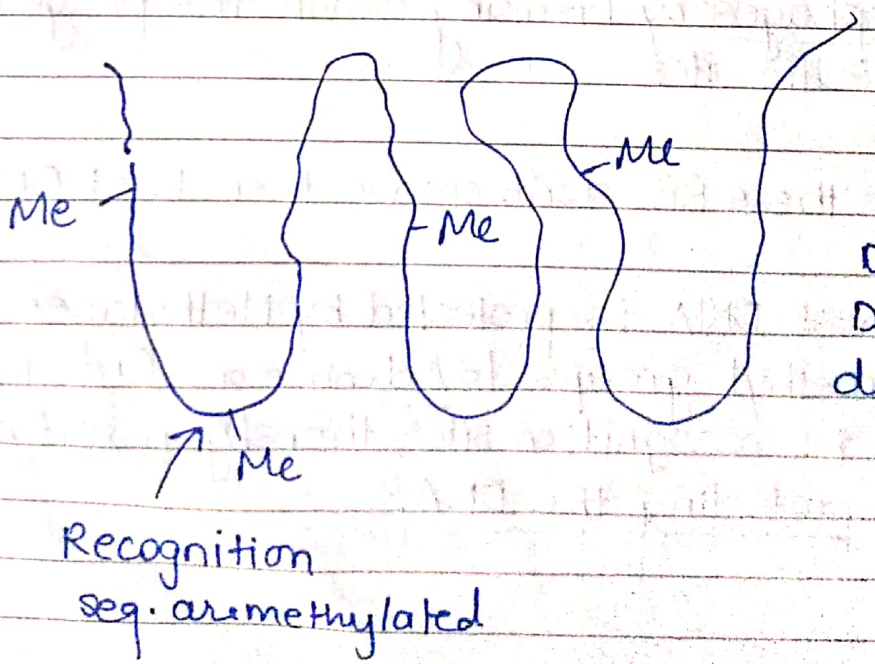
RE.

a)



- This phenomenon, in which some strains of bacteria produce restriction enzyme in response to attack of bacteriophage is known as "Host-Controlled restriction"

b)



RE.

So, restriction enzymes or restriction endonucleases are a class of nucleases which can cleave dsDNA in a precise manner at limited no of sites which have a unique base sequence (recognition sequence).

→ Classification of Restriction Enzymes

① Type I RE ② Type II RE ③ Type III RE

- Type I & Type III:

i) They have both restriction & modification activity.

ii) They cut DNA at sites, some distance away from their recognition sequences.

iii) They need ATP for energy & lack predictability.

Type I & Type III R.E. are not much used in genetic engineering.

- Type II RE are ideal for biotechnology because of their desirable features like:

i) They have only restriction activity, but no modification

ii) They cut the DNA in a predictable & site specific manner, at a site within or adjacent to the restriction sequence

iii) They only req. Mg^{++} as cofactor, not ATP.

→ Nomenclature of RE :-

i) The RE are designated by 3 letter abbreviation for host organism, followed by 4th letter designating the strain.

ii) If required, Roman numerals are used after the 4th letter, used to indicate different restriction-modification system, when more than one enzyme is obtained/isolated from the same organism. More often, the Roman numerals indicate the order of discovery.

iii) Of the 3 letters used :-

- 1st letter denotes the 'Species name' of the organism from which it is isolated.
- 2nd & 3rd letter of RE should be from the 1st & 2nd letter of the Genus name. The letter should be written in lower case & should be in Italics

Eg : RE from E coli will have *Eco* as starting words.

- If the RE is isolated from a particular strain of the organism, then that should be written as 4th letter. It should be in capitals & not in Italics.

RE.

Eg: RE from E coli strain will be written as EcoR

→ Properties of Restriction Enzymes:

i) Restriction enzymes recognize the specific base sequence (restriction sequence) in dsDNA only.

ii) Restriction enzymes recognize the Palindromic sequence.

Eg: EcoRI recognizes 5'-GAATTC-3'
3'-CTTAAG-5'

iii) The size of recognition seq could be 4bp or 6bp or 8bp.

→ Restriction Pattern: