**3- The lac Operon of E. coli:

- *Lactose (a disaccharide) is one of the major carbohydrates found in milk; it can be metabolized by E. coli bacteria that reside in the gut of mammals.*

- *The lac operon of E. coli controls the transcription of three genes with a common promoter and are transcribed together in lactose metabolism:*

(A) Lac Z gene: Encodes **β-galactosidase** enzyme that:

1- Break lactose into glucose and galactose.

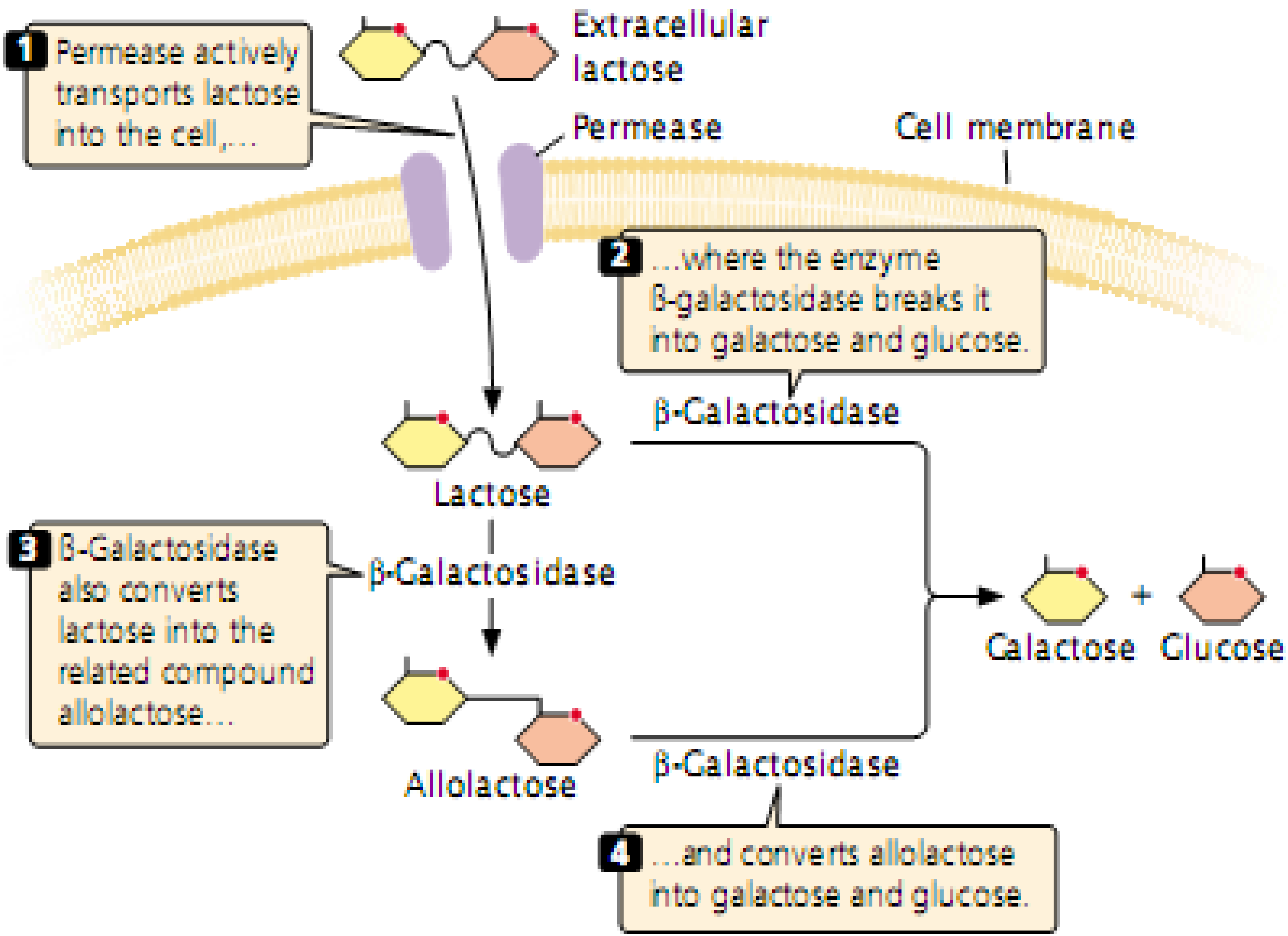
2- Convert lactose into allolactose (a compound that plays an important role in regulating lactose metabolism).

(B) Lac Y gene:

encodes **permease** for active transport of lactose into the E. coli cell membrane.

(C) Lac A gene:

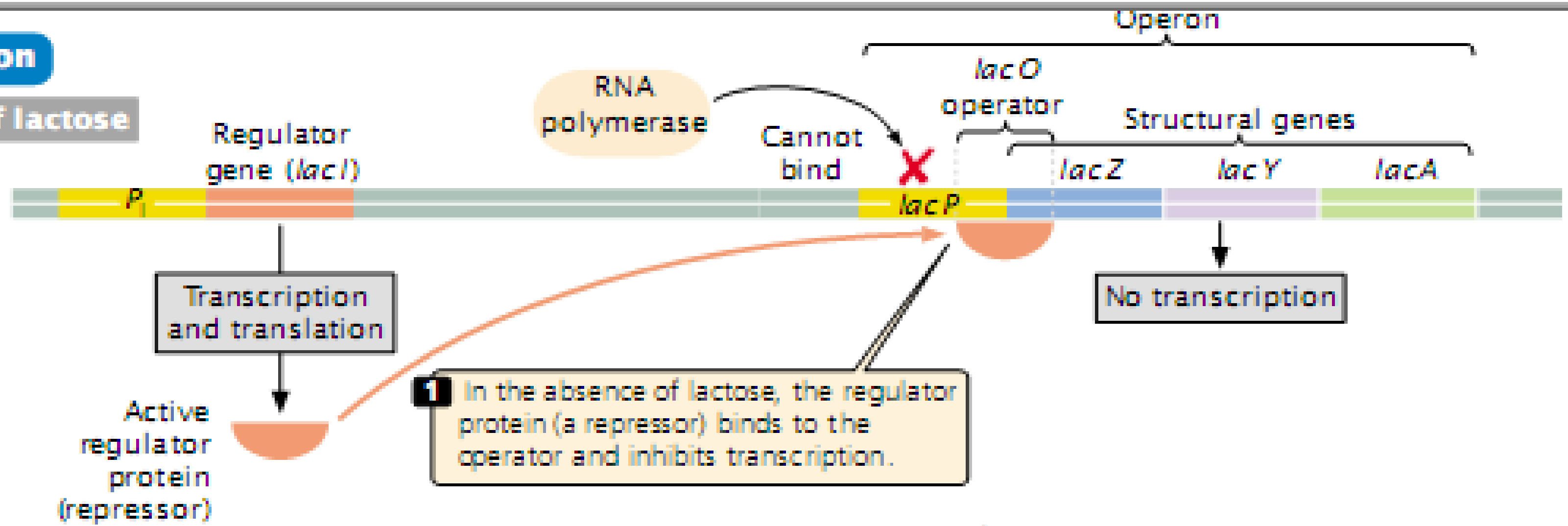
encodes **thiogalactoside transacetylase** but its function in lactose metabolism is not



1 Permease actively transports lactose into the cell,...
Extracellular lactose
Permease
Cell membrane
2 ...where the enzyme β-galactosidase breaks it into galactose and glucose.
β-Galactosidase
Lactose
3 β-Galactosidase also converts lactose into the related compound allolactose...
β-Galactosidase
Galactose
Glucose
Allolactose
β-Galactosidase
4 ...and converts allolactose into galactose and glucose.

- The lac operon is inducible: a regulator gene (lacI) which has its own promoter (P) produces a repressor that binds to the operator site, so the binding of RNA polymerase is blocked, and transcription of the structural genes is prevented.

- The presence of allolactose (Although lactose appears to be the inducer here, allolactose is actually responsible for induction) inactivates the repressor and the binding of RNA polymerase is no longer blocked, the transcription of lacZ, lacY, and lacA takes



(b) Presence of lactose

lacO operator

RNA polymerase

Transcription and translation

Active regulator protein

Inactive regulator protein (repressor)

3 ...which then binds to the regulatory protein, making the protein inactive.

4 The regulatory protein cannot bind to the operator,...

Transcription and translation

5 ...and the structural genes are transcribed and translated.

Enzymes

β-Galactosidase

Permease

Transacetylase

Glucose

Galactose

Allolactose

Lactose

β-Galactosidase

2 When lactose is present, some of it is converted into allolactose,...

6 The enzymes convert lactose into glucose and galactose.

**4- Positive Control and Catabolite Repression

- *E. coli and many other bacteria will metabolize glucose preferentially in the presence of lactose and other sugars. They do so because glucose requires less energy to metabolize than do other sugars.*

- *When glucose is available, genes that participate in the metabolism of other sugars are repressed, in a phenomenon known as* ***catabolite repression.***

- For example, the efficient transcription of the lac operon takes place only if lactose is present and glucose is absent.

- Catabolite repression results from positive control in response to glucose:

*** Positive control is accomplished through the binding of a protein called the catabolite activator protein (CAP) to the promoter of the lac genes and stimulates transcription.*

*** RNA polymerase does not bind efficiently to many promoters unless CAP is first bound to the DNA.*

*** Before CAP can bind to DNA, it must form a complex with a modified nucleotide called cyclic AMP. The binding of cAMP–CAP to the promoter activates transcription by facilitating the binding of RNA polymerase.*

*** Low concentrations of glucose stimulate high levels of cAMP, resulting in increased cAMP–CAP binding to DNA. This increase enhances the binding of RNA polymerase to the promoter and increases transcription of the lac genes.*

*** High concentration of glucose within the cell lowers the amount of cAMP, and so little cAMP–CAP complex is available to bind to the DNA. Subsequently, RNA polymerase has poor affinity for the lac promoter, and*

When glucose is low

1 When glucose level is low, cAMP levels are high.

2 CAP readily binds cAMP, and the CAP–cAMP complex binds DNA,…

3 …increasing the efficiency of polymerase binding.

cAMP cAMP cAMP cAMP cAMP cAMP

CAP

RNA polymerase

lacO operator

cAMP CAP

cAMP CAP

P_i *lacI*

lacP *lacZ* *lacY* *lacA*

Transcription and translation

4 The result is high rates of transcription and translation of the structural genes…

Enzymes

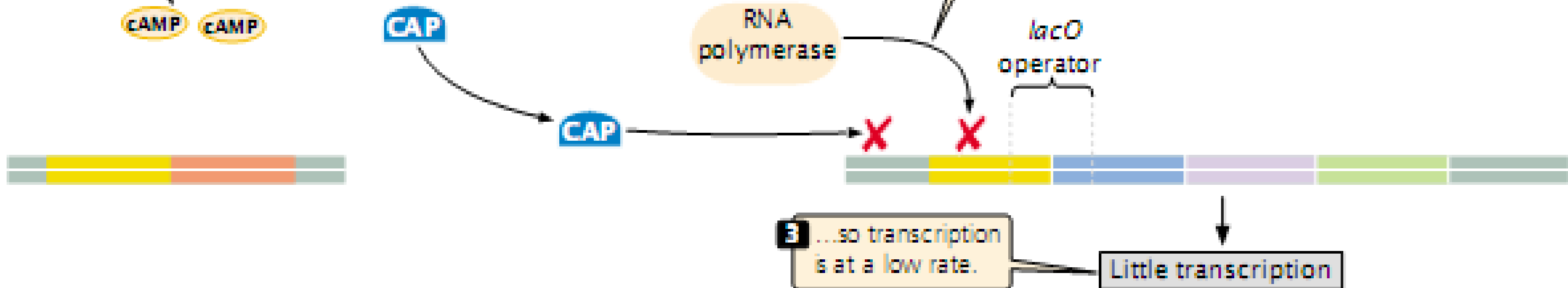
β-Galactosidase Permease Transacetylase

Lactose

Glucose

Galactose

5 …and the production of glucose from lactose.



** 5-The trp Operon of E. coli:

- The trp operon is a repressible operon that controls the biosynthesis of tryptophan.

- Repression is accomplished through the binding of tryptophan to the repressor, which renders the repressor active. The active repressor binds to the operator and prevents RNA polymerase from transcribing the structural genes.

** Eukaryotic Gene Regulation:

1. Chromatin Structure:

Condensed chromatin represses gene expression; chromatin structure must be altered before transcription.

The physical structure of the DNA, as it exists compacted into chromatin, can affect the ability of transcriptional regulatory proteins (termed transcription factors) and RNA polymerases to find access to specific genes and to activate transcription from them.

2. Transcriptional Initiation:

This is the most important mode for control of eukaryotic gene expression.

Specific factors that exert control include:

1- The strength of promoter elements within the DNA sequences of a given gene

2- The presence or absence of enhancer sequences (which enhance the activity of RNA polymerase at a given promoter by binding specific transcription factors)

3- Some regulatory proteins repressors and inhibit transcription.

3. Messenger RNA Processing:

Alternative splicing, which generate biologically different proteins from the same gene.

4. RNA Stability:

The stability of mRNA influences gene expression by affecting the amount of mRNA available to be translated.

Unlike prokaryotic mRNAs, whose half-lives are all in the range of One to five minutes, eukaryotic mRNAs can vary greatly in their stability.

5. Translational Initiation:

The ability of ribosomes to recognize and initiate synthesis from the correct AUG codon can affect the expression of a gene product.

Some eukaryotic proteins initiate at non-AUG codons.

6. Translational and Posttranslational Control:

Several factors affect the rate of translation:

1- The availability of ribosomes.

2- tRNAs,

3- Initiation and elongation factors.

3- Other components of the translational

7. Protein Transport:

In order for proteins to be biologically active following translation and processing, they must be transported to their site of action.

8. Protein Stability:

Many proteins are rapidly degraded due to specific amino acid sequences , whereas others are highly stable.

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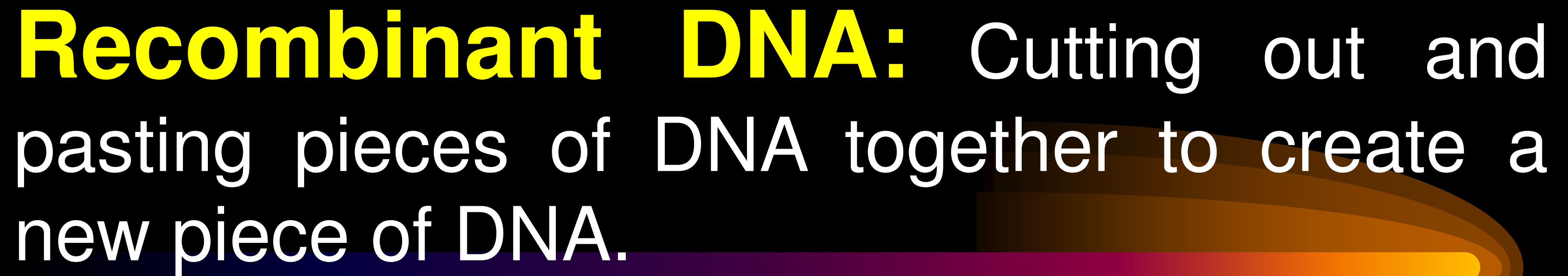
Recombinant DNA Techniques

Recombinant DNA: Cutting out and pasting pieces of DNA together to create a new piece of DNA.



Recombinant DNA technology is a set of molecular techniques used to locate, analyze, alter, study, and recombine DNA sequences.

-It is used to:

* Probe the structure and function of genes.
* Create commercial products.
* Diagnose and treat diseases.

I. Restriction Endonucleases

*The key development that made recombinant DNA technology possible was the discovery in the late 1960s of restriction enzymes (also called restriction endonucleases) that recognize and make double-stranded cuts in the sugar–phosphate backbone of DNA molecules at specific nucleotide sequences.

* These enzymes are produced naturally by bacteria, where they are used in defense against viruses. In bacteria, restriction enzymes recognize particular sequences in viral DNA and then cut it up. A bacterium protects its own DNA from a restriction enzyme by modifying the recognition sequence.

* Restriction enzymes are the work horses of recombinant DNA technology and are used whenever DNA fragments must be cut or joined.

* Types of restriction enzymes :

1. Type I restriction enzymes:

Recognize specific sequences in the DNA but cut the DNA at random sites that may be some distance (1000 bp or more) from the recognition sequence.

2. Type II restriction enzymes:

Recognize specific sequences and cut the DNA within the recognition sequence.

Virtually all work on recombinant DNA is done with type II restriction enzymes.

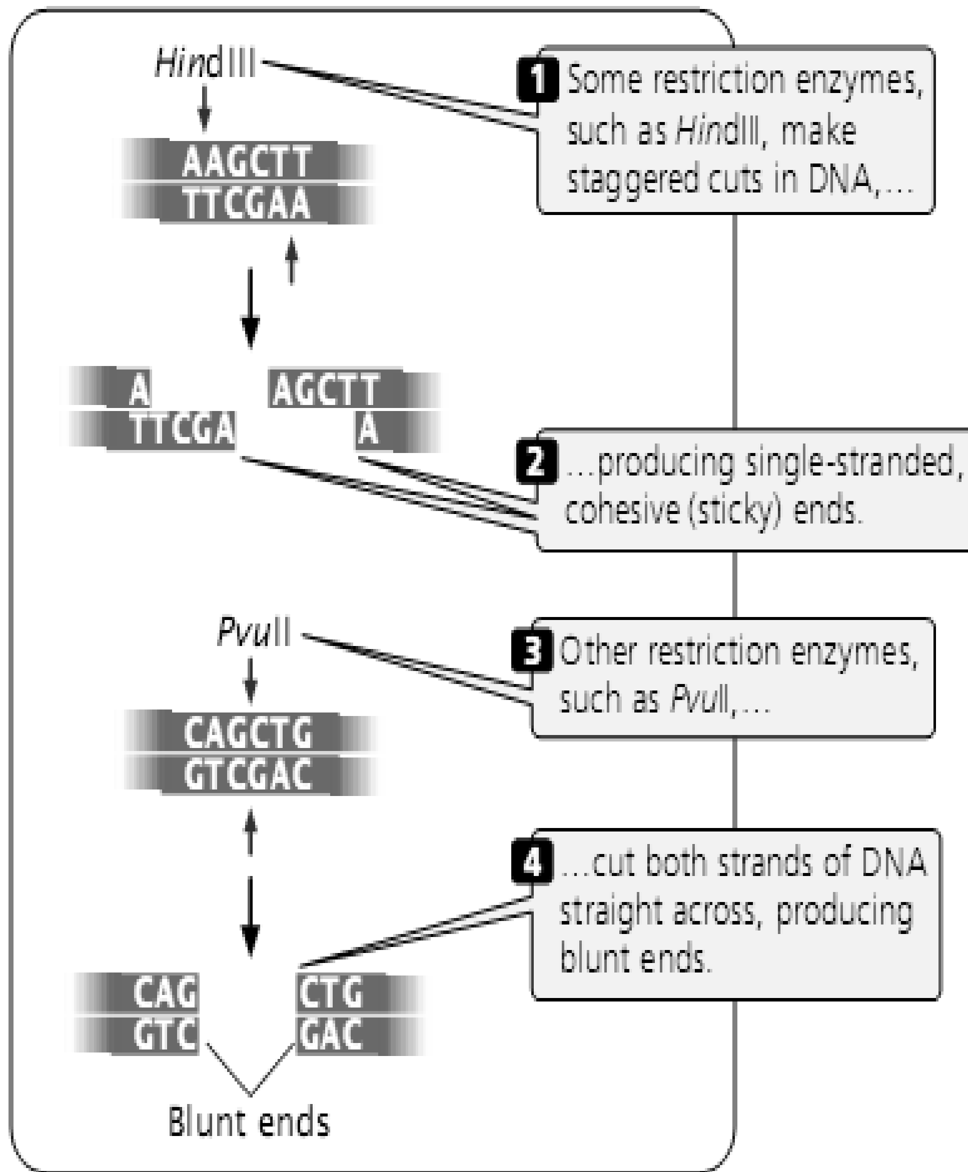
3. Type III restriction enzymes:

Recognize specific sequences and cut the DNA at nearby sites, usually about 25 bp away.

enzymes are usually from 4 to 8 bp long; most enzymes recognize a sequence of 4 or 6 bp.

*Most recognition sequences are palindromic sequences (the sequence on the bottom strand is the same as the sequence on the top strand, only reversed). All type II restriction enzymes recognize palindromic sequences.

Some restriction enzymes make staggered cuts, (Example, HindIII) producing DNA fragments with cohesive ends or sticky ends; others producing blunt-ended fragments



(b)

AAGCTT
TTCGAA

AAGCTT
TTCGAA

Digestion with HindIII

Digestion with HindIII

AGCTT
A
A
TTCGA

AGCTT
A
A
TTCGA

Combine fragments

Gap in sugar–phosphate backbone

AAGCTT
TTCGAA

Gap in sugar–phosphate backbone

5 DNA molecules cut with the same restriction enzyme have complementary sticky ends that pair if fragments are mixed together.

Ligase

AAGCTT
TTCGAA

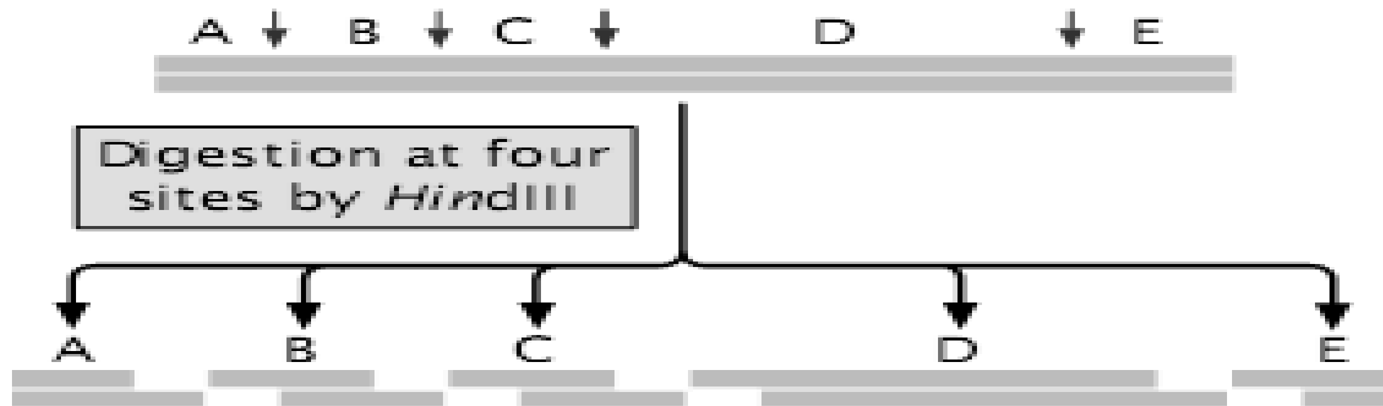
Ligase

6 The nicks in the sugar–phosphate backbone of the two fragments can be sealed by DNA ligase.

Characteristics of some common type II restriction enzymes used in recombinant DNA technology

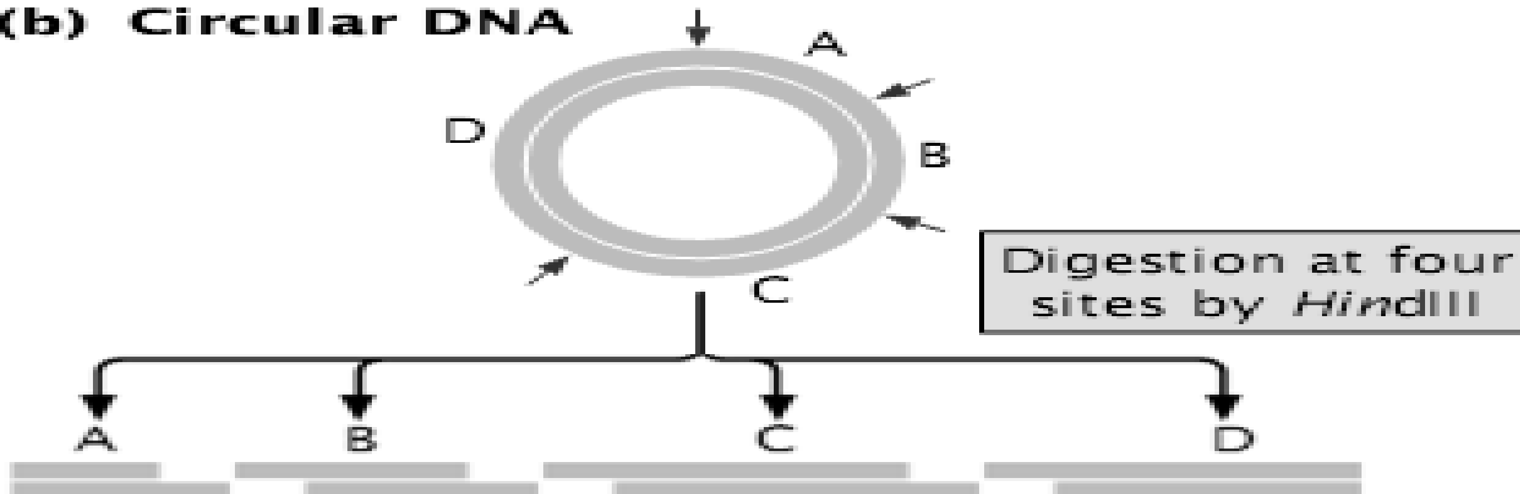
Enzyme	Microorganism from Which Enzyme Is Isolated	Recognition Sequence	Type of Fragment End Produced
*Bam*HI	*Bacillus amyloliquefaciens*	5′–GGATCC–3′ 3′–CCTAGG–3′	Cohesive
*Cof*I	*Clostridium formicoaceticum*	5′–GCGC–3′ 3′–CGCG–5′	Cohesive
*Dra*I	*Deinococcus radiophilus*	5′–TTTAAA–3′ 3′–AAATTT–5′	Blunt
*Eco*RI	*Escherichia coli*	5′–GAATTC–3′ 3′–CTTAAG–5′	Cohesive
*Eco*RII	*Escherichia coli*	5′–CCAGG–3′ 3′–GGTCC–5′	Cohesive
*Hae*III	*Haemophilus aegyptius*	5′–GGCC–3′ 3′–CCGG–5′	Blunt
*Hind*III	*Haemophilus influenczae*	5′–AAGCTT–3′ 3′–TTCGAA–5′	Cohesive
*Hpa*II	*Haemophilus parainfluenzae*	5′–CCGG–3′ 3′–GGCC–5′	Cohesive
*Not*I	*Nocardia otitidis-caviarum*	5′–GCGGCCGC–3′ 3′–CGCCGGCG–5′	Cohesive
*Pst*I	*Providencia stuartii*	5′–CTGCAG–3′ 3′–GACGTC–5′	Cohesive
*Pvu*II	*Proteus vulgaris*	5′–CAGCTG–3′ 3′–GTCGAC–5′	Blunt
*Sma*I	*Serratia marcescens*	5′–CCCGGG–3′ 3′–GGGCCC–5′	Blunt

(a) Linear DNA



With a linear piece of DNA, the number of fragments produced is one more than the number of restriction sites.

(b) Circular DNA



With a circular piece of DNA, the number of fragments produced is equal to the number of restriction sites.

والسلام عليكم و رحمة الله و بركاته

THANK

YOU