CONTROL OF GENE EXPRESSION IN PROKARIOT

2c. || Regulation of (Protein/Enzyme) Synthesis

Operon Concept & Lac Operon ||

DNA control enzyme synthesis. Every somatic cell of a species bear some chromosome, but always every cell never produces all enzymes. There must have a controlling system. The controll can be excercised by induction or by repression (Suppression) and either at the transcription level or at translation level.

Jecob & Monod (1960) proposed a hypothesis to explain the control the enzyme synthesis. It is known as Operon concept or model.

* Gene cluster, Promoter, additional sequence that function together in regulation is called operon.

An operon is a group of closely linked structural genes (cistrons) and the associated control genes (operator genes and regulator genes promoter genes) which regulate genetically controlled metabolic activity.

* LAC OPERON →

Lac operon is an operon which associated with lactose metabolism and control the synthesis of β galatooxidase enzyme.

1. Negative Control:

Back with (1967), epstein, Backwith (1968) and Martin (1960) have described the operation of lac operon in E. coli in following ways—

(a) When Inducer (lactose) Absent :—

i) In the absence of Inducer lactose the regulator gene produces a protein repressor which bind strongly to the operator site and prevents its transcription.

The **Control Mechanism** of (lac) Operon divided into-

- ~~Positive~~ Negative Control / Induction & repressor Method
  - Transcription Regulation by repressor Protein
  - When Inducer Present.
  - When Inducer Absent.
- Positive control / (Regulation by activator Protein)
  - Catabolic repression / Glucose effect

As a result the structural genes (z, y, a) do not synthesize mRNA and enzyme β galacto oxidase is not formed & hence there is no question of break down of lactose. *

(b) When Inducer Present :—

ii) When an inducer lactose is introduced in the medium, it enters the cell and gets modified in such a way that it binds to the repressor. The repressor is therefore free and induces the RNA transcription & β galactooxidase is produced which break the lactose.

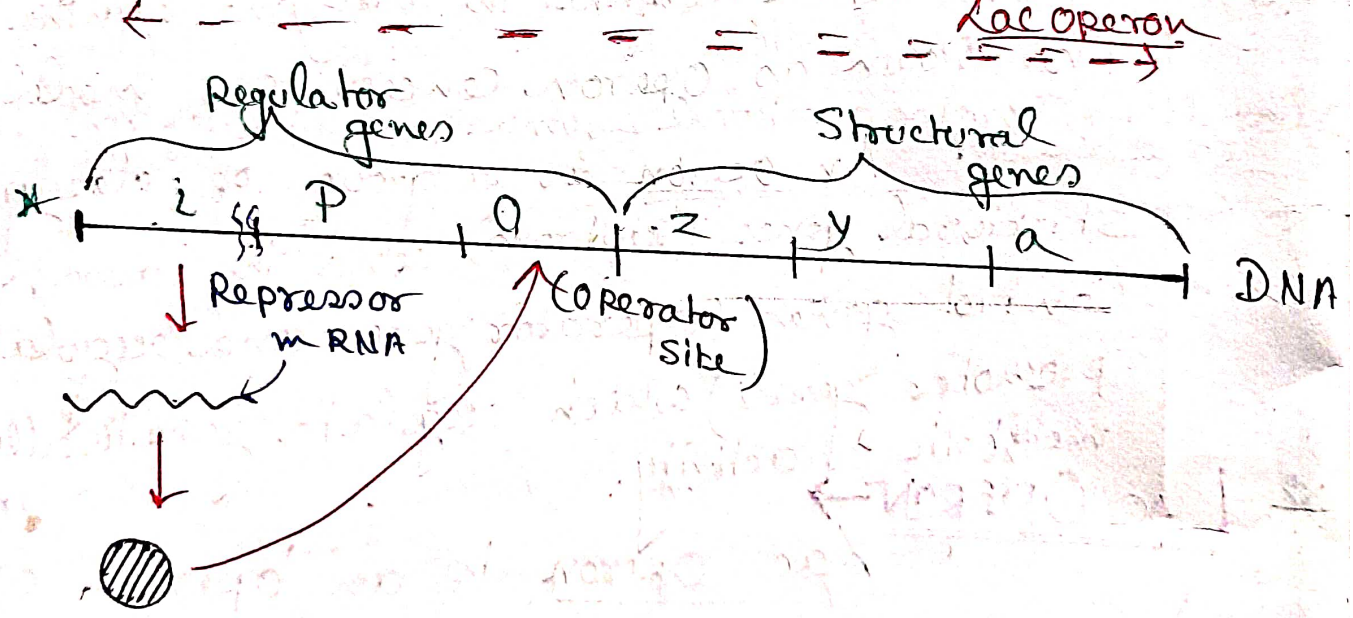
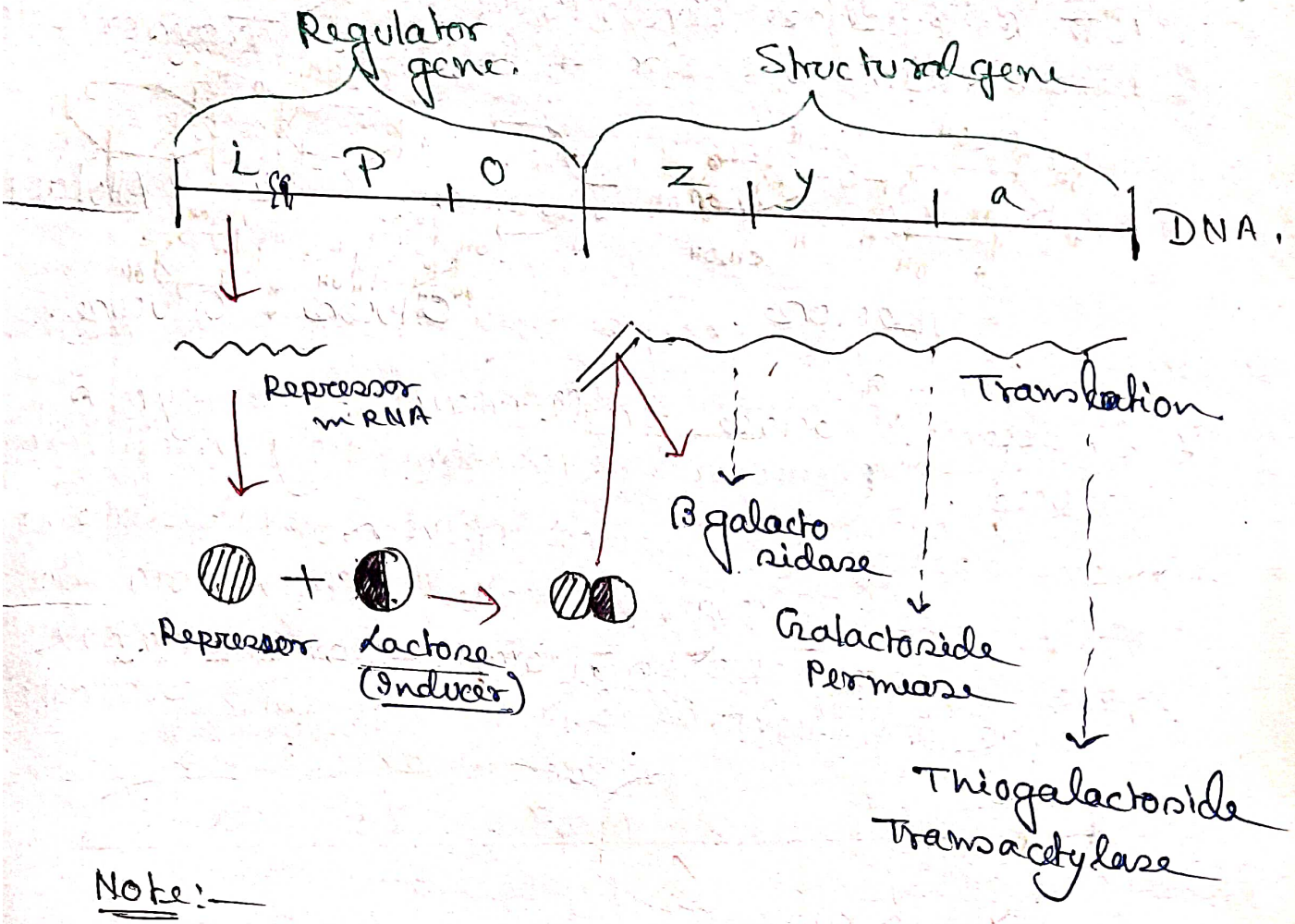


Fig. Repression of mRNA synthesis

Case I.

(3) ~~Case II → Inducer~~



Note:—

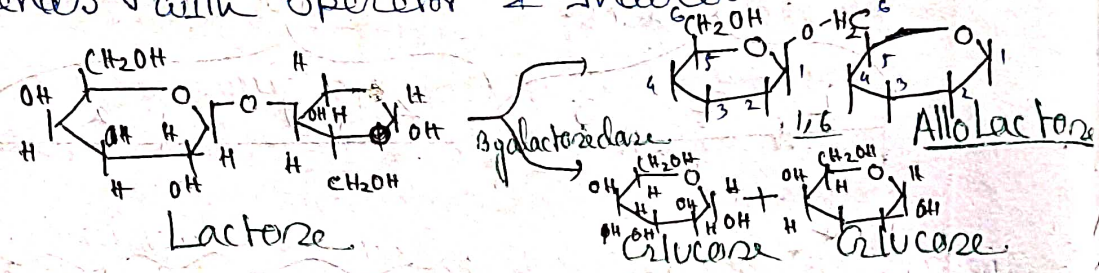
1. The Transcription of structural genes leads to the synthesis of 3 enzyme viz- β galactosidase
galactoside permease
Thiogalactoside Transacetylase

2. These 3 enzymes bring about the metabolism of lactose
β galactosidase splits glucose and galactose.
Galactoside permease facilitates the entry of lactose into cell. The function of Thiogalactoside transacetylase is not known.

3. In the lac operon system, the lactose functions as an inducer for the synthesis of 3 enzymes. Hence the lac operon system is called an inducible system. This system is a negative regulation:-

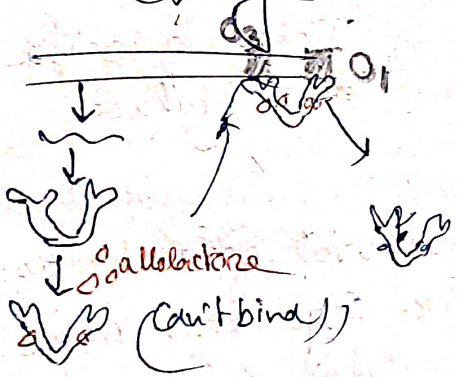
⇒ A few molecules of z, y, a gene products synthesized in uninduced state, Providing low level of enzyme activity, this activity is essential for induction of the operon.

Allolactose, an inducer formed by ~~break~~ reaction with βgalactosidase, binds to repressor & repressor can't binds with operator & induces Transcription.



* In absence of lactose small amount of 3 enzymes Produced. (Repressor does not completely inhibit transcription)

when medium with lactose given small amount transported (Through permease) converts to allolactose & allo binds to repressor, Promotes conformational change & prevent bind repressor with promoter.



3 operons in lac operon.

Repressor tetramer's one dimer bind with O_1
other binds with O_2 or O_3

$O_1 - O_2$ $O_1 - O_3$

Ω hairpin ↓ loop.

Ref - Swasted.

2. Positive Control:- (By Cyclic AMP)

Expression of 'lac' Operon is inhibited when more efficient Source of energy (i.e- Glucose) present in the Medium. Presence of Glucose lowers the concentration of intercellular cyclic AMP. (ATP $\xrightarrow{\text{Adenyl cataluse}}$ CAMP

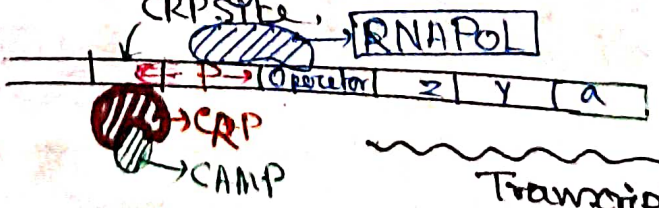
*Diauxic growth: Glucose metabolise 1st than 2nd sugar (sequential use of 2 sugar)

(Effector molecule): AMP binds with an activator protein Catabolite Activator Protein (CAP) or CAMP receptor Protein (CRP).

(a) When Glucose Absent:-

CAMP ↑ / Lac ↓ / CAMP.

CAMP-CRP complex recognize & bind with Lac control region, bound CRP creats Conformational change in DNA makes Possible RNA polymerase to Transcript for Lac Operon.



Transcription occur.

(b) When Glucose Present:-

When bacterium expose to glucose, trans Port of glucose stimulate signaling pathway cause CAMP decrease, because it inhibits Adenyl Cotase, so CAMP no longer available to bind to CRP cacses Transcription rate decrease

Lac operon transcription never exceeds 2%.

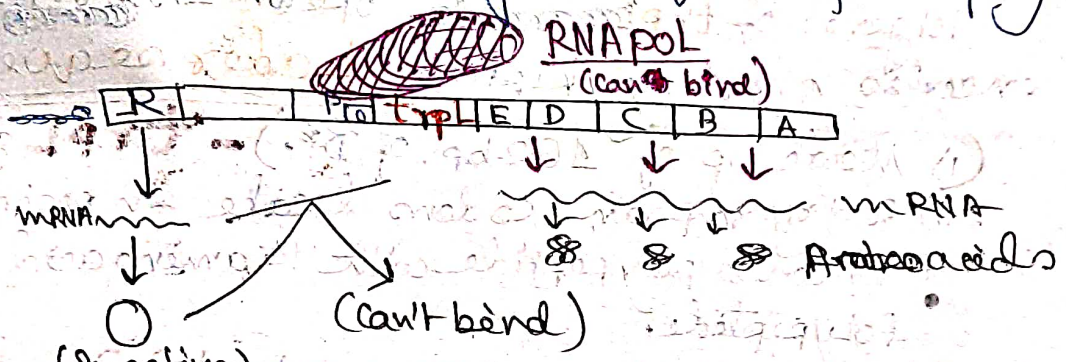
No Transcription occurs.

TRYPTOPHAN OPERON

The trp operon encodes enzyme needed for biosynthesis of amino acid tryptophan, trp-E,D,C,B,A gene encodes enzymes involved tryptophan biosynthesis. here trpL & trpR genes regulate operon different ways.

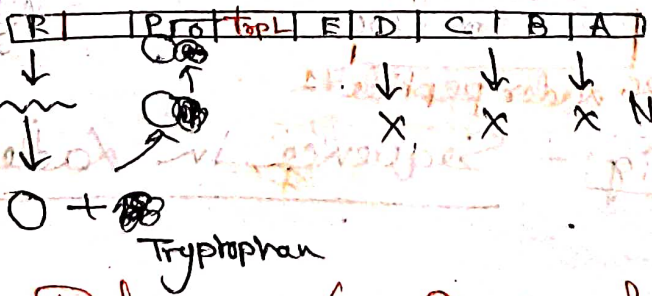
1) When tryptophan Absent:—

trpR gene encode repressor protein, In low tryptophan concentration can't became active & can't bind operator site. Then RNA polymerase Transcribe operon & cell express gene required for Trp synthesis.



2) When Tryptophan Present:—

In presence of Tryptophan in high concentration act as 'co repressor' bind with trp repressor protein, allow to bind in promoter region & inhibit ability to RNA POL to transcribe operon & trp operon turned off.



* 2 molecule of Tryptophan binds allow helix-turn-helix of repressor carrying recognition site to fit into adjacent major groove of DNA.

* Difference Between Lac Operon & Trp Operon:—

(I) In trp Operon it (Tryptophan) acts as co repressor rather than inducer in 'Lac' Operon.

(II) trp operon encodes bio synthetic enzyme whearas Lac Operon encodes degradative enzyme.

(III) CRP – cAMP complex not required for Trp Operon.

* Attenuation:-

The Other mechanism of regulatory mechanism of Trp discovered by Yanofsky. This mechanism mediated by trp L gene. This mechanism called Attenuation →

It is a process only occurs in bacteria because of coupling of Transcription & translation. A 28 base pair of DNA that terminate transcription, called Attenuator.

1st gene of trp operon is trpL gene

Made up of with Leader sequence →

(i) Made up of 162 bp. of Bp.) - trpL gene.

ii) 2 tryptophan codons locate in position 10, 11.

iii) encodes polypeptide with 14 amino acid, Leader Polypeptide.

iv) Leader RNA have 4 segment 1, 2, 3, 4. forming either b.p 1,2 or 3,4 or 2,3 of stem loop structure

(* presence of Uric rich region in 4 act as intrinsic terminator)

The 3,4 stem loop is unique it can act as a transcriptional termenator.

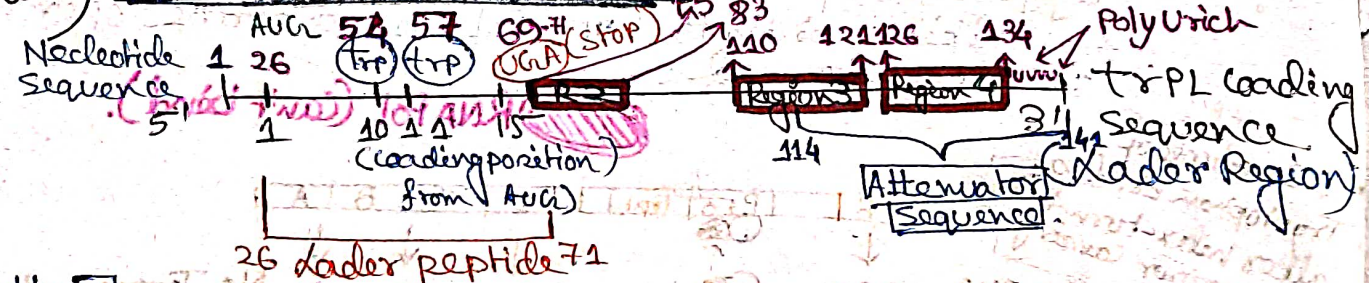


Fig:- Sequence in Leader Region

10, 11 UGA UGA Tandem repeat of Tryptophan codones.

*

* Condition in favour of formation 3-4 stem loop ultimately depends on translation of trpL gene. There are three possible scenarios —

1. When No Translation occurs:— When mRNA free

When mRNA free or in other words transcription not coupled with translation then region 1 hidrogen bonds with region 2 and 3 left hydrogen bond with 3. Terminator stem loop forms.

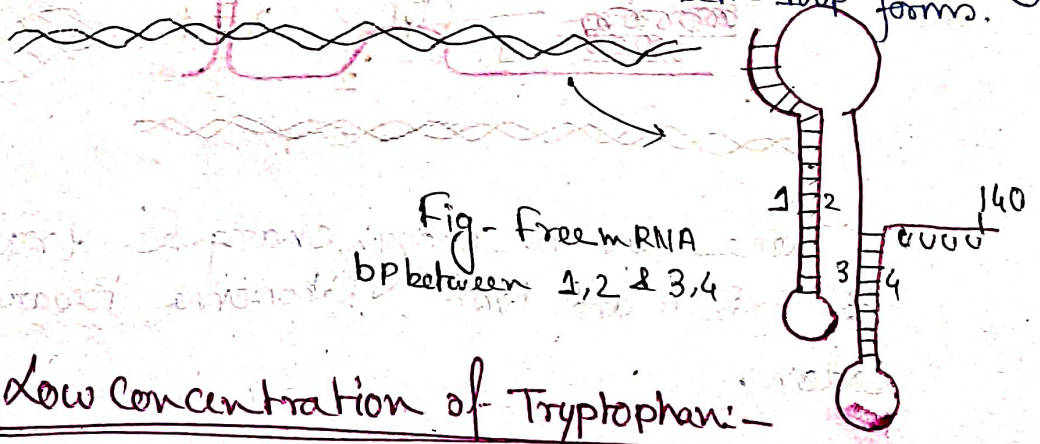
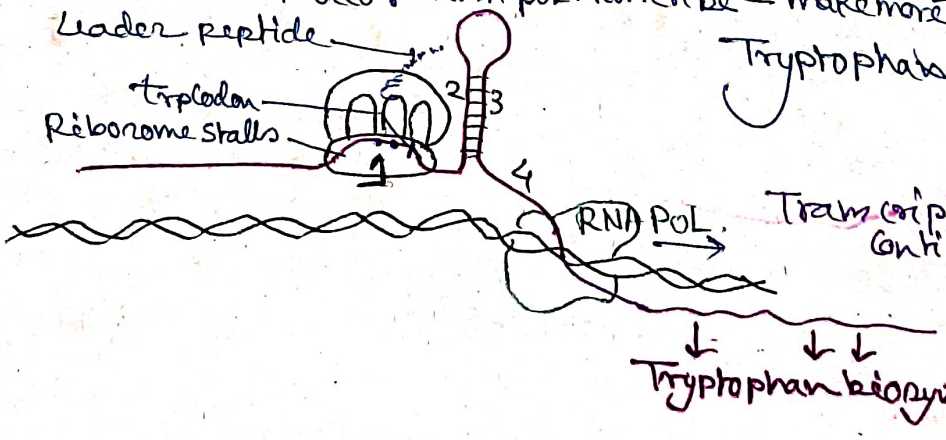


Fig- Free mRNA
bp between 1,2 & 3,4

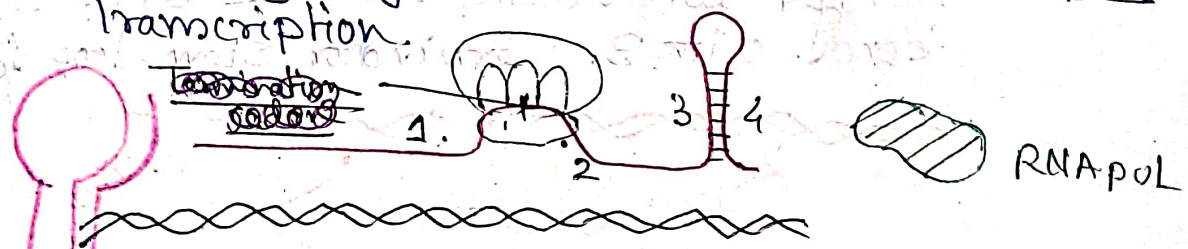
2. Low Concentration of Tryptophan:—

When Tryptophan Conc. low cell can't make sufficient amount of charged $tRNA^{trp}$. Ribosome pauses at trp codons in trpL gene (waiting for charged $tRNA^{trp}$) In the position (10, 11) ribosome shields region 1 & as for it 2 & 3 region formed hair pin loop. (3-4 structure can't form) termination does not occur RNA pol transcribe & make more Tryptophan.



3. High Tryptophan Concentration:—

presence of high level of Tryptophan Transcription of Ribosome progress to ~~stop codon~~ ~~where ribosome pause~~. preventing region 2 form hidrogen bond with either 1 and 3. region region Then 3 hydrogen bond with 4 and terminate Transcription.



There must be enough charged tRNA to in RNA and Push Ribosome towards Termination Codon.