



BHARATHIDASAN UNIVERSITY

**Tiruchirappalli- 620024,
Tamil Nadu, India**

Programme: M.Sc., Biomedical science

Course Title : Molecular Biology

Course Code : BM35C5

Unit-V

**TOPIC: Regulation of gene expression in
Prokaryotes**

Dr. A. S. VIJAYAKUMAR

Guest lecturer

Department of Biomedical Science

Regulation of gene expression in Prokaryotes

Gene is the segment of DNA that controls all traits of organism that may be physical or metabolic. Information encoded in DNA is transcribed into RNA and then translated into proteins. The ability of a cell to switch the genes on and off is of fundamental importance because it enables the cell to respond to the changing environment and it is the basis of cell differentiation. So we can say that:

“Gene Regulation is a process in which a cell determines which genes it will express and when.”

Regulation of gene expression includes a wide range of mechanisms that are used by the cell to increase or decrease the production of specific gene products (protein or RNA). Sophisticated programs of gene expression are widely observed in biology to trigger developmental pathways, respond to environmental stimuli or adapt to new food sources.

Discovery:

The first discovery of a gene regulation system is widely considered to be the identification in 1961 of the *lac operon*, discovered by Jacques Monod, in which some enzymes involved in lactose metabolism are expressed by the genome of *E.Coli* only in the presence of lactose and absence of glucose.

Furthermore, in 1953, **Jacques Monod discovered enzyme repression. He observed the presence of** tryptophan in medium of *E.Coli* which repressed the *synthesis of tryptophan synthetase* and then further studies showed that all the enzymes, present in tryptophan biosynthetic pathway are simultaneously repressed in the presence of end product.

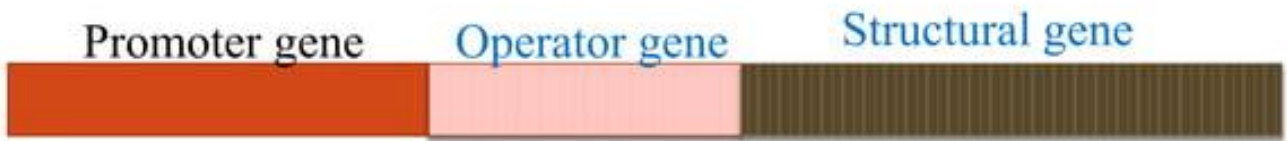
Gene Regulation in Prokaryotes:

The rate of expression of bacterial gene is controlled mainly at level of transcription. Regulation can occur at both the initiation and termination of mRNA synthesis because bacteria obtain their food from the medium that immediately surrounds them. Their regulation mechanisms are designed to adapt quickly to the changing environment. If a gene is not transcribed then the gene product and ultimately the phenotype will not be expressed. In contrast, eukaryotes have much complex and larger genome and cells of higher organisms are surrounded by constant internal milieu. The ability of such cells to respond to hormones and to impulse in nervous system is thus comparatively more important than the ability to respond rapidly in the presence of certain nutrients.

Principles of Transcriptional Regulation:

Gene Expression Is Controlled by Regulatory Proteins:

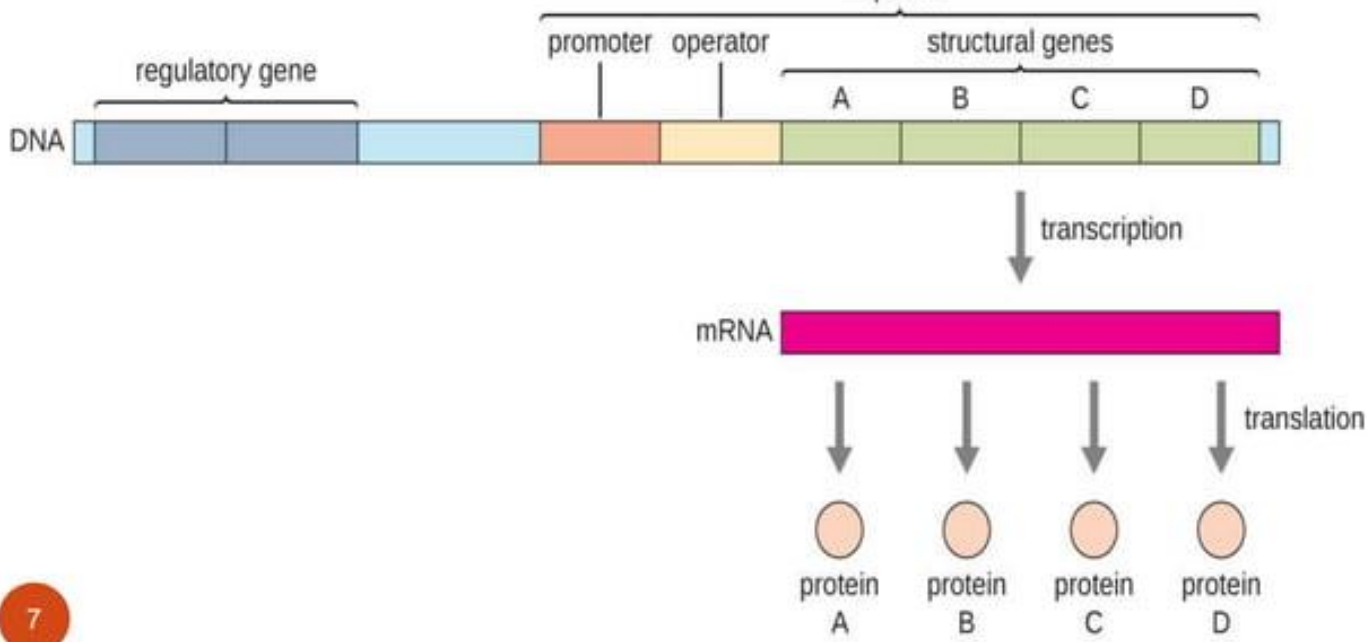
Genes are very often controlled by extracellular signals, in the case of bacteria, this typically means molecules present in the growth medium. These signals are communicated to genes by regulatory proteins, which come in two types: **positive regulators, or activators;** and **negative regulators, or repressors.** Typically these regulators are DNA binding proteins that recognize specific sites at or near the genes they control. An **activator increases transcription of the regulated gene; repressors decrease or eliminate that transcription.** First, RNA polymerase binds to the promoter in a closed complex (in which the DNA strands remain together). The polymerase-promoter complex then undergoes a transition to an open complex in which the DNA at the start site of transcription is unwound and the polymerase is positioned to initiate transcription. This is followed by promoter escape, or clearance, the step in which polymerase leaves the promoter and starts transcribing.



Where RNA polymerase binds

Where repressor molecule bind

Group of different genes encoding for specific proteins

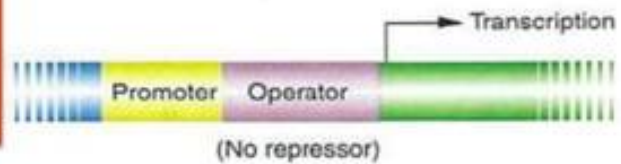
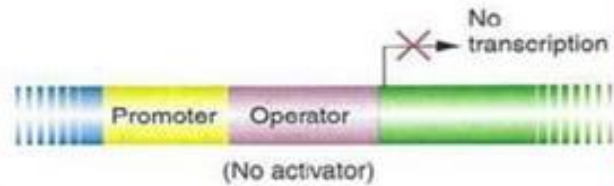


Positive and negative regulation

Positive regulation



Negative regulation



Polymerase Bind DNA and by Repressors That Block That Binding:

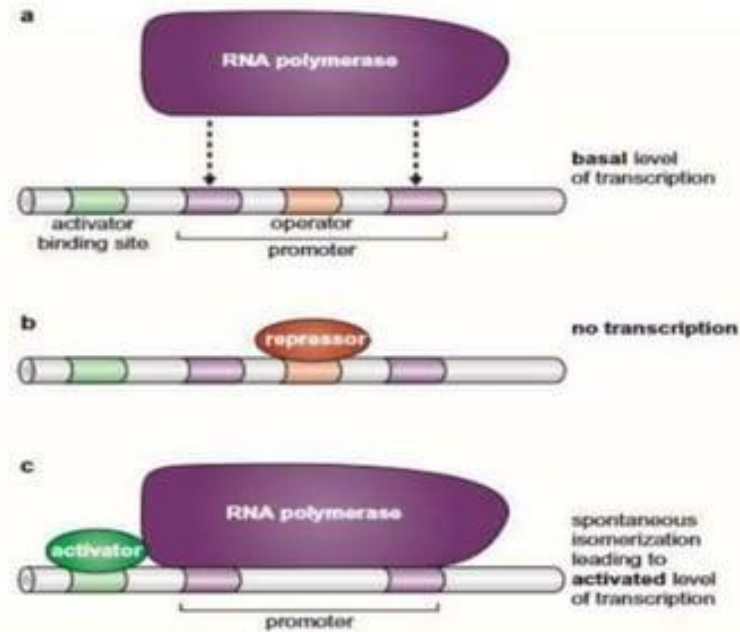


Fig.2. Activation by Recruitment of RNA Polymerase. (a) In the absence of both activator and repressor, RNA polymerase occasionally binds the promoter spontaneously and initiates a low level (basal level) of transcription. (b) Binding of the repressor to the operator sequence blocks binding of RNA polymerase and so inhibits transcription. (c) Recruitment of RNA polymerase by the activator gives high levels of transcription. RNA polymerase is shown recruited in the closed complex. It then spontaneously isomerizes to the open complex and initiates transcription.

The Lac Operon

An Inducible Operon

Operon is the group of the genes that are next to each other in DNA and that can be controlled in a unified manner. The genes of the structural enzymes are always transcribed together into a single polycistronic lac mRNA which explains why they are always expressed together.

Lac operon is induced 1000 folds by lactose. The cell is an energy efficient unit that makes protein it needs. E. coli has about three thousand protein encoding genes but only a set of them is expressed at any one time. The best illustration of this efficiency is provided by the enzymes involved in the utilization of **disaccharide lactose**. The synthesis of these enzymes may be induced up to 1000 folds in response to the addition of lactose to the culture media. Regulation by enzyme induction has been found in many other bacterial systems that degrade sugars, amino acids and lipids. In these systems, the availability of the substrates stimulates the production of enzymes involved in its degradation. **Induction is the production of a specific enzyme (or set of enzymes) in response to the presence of a substrate. The lac operon is a inducible system.**

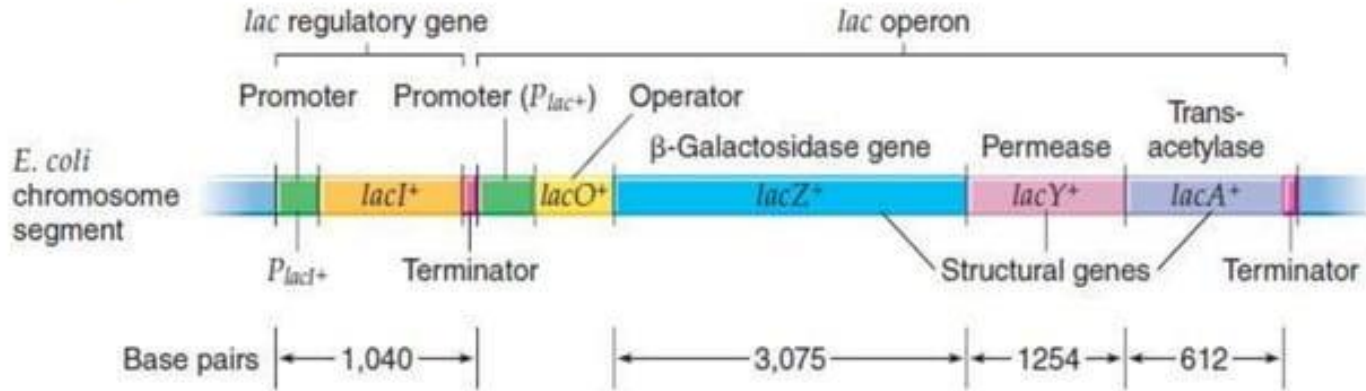
Lactose as a Carbon Source for *E. coli*

E. coli can grow in a simple medium containing salts (including a nitrogen source) and a carbon source such as glucose. The energy for biochemical reactions in the cell comes from glucose metabolism. The enzymes required for glucose metabolism are coded for by constitutive genes. If lactose is provided to *E. coli* as a carbon source instead of glucose, a number of enzymes that are required to metabolize lactose are rapidly synthesized. (A similar series of events, each involving a sugar-specific set of enzymes, is triggered by other sugars as well.) The enzymes are synthesized because the genes that code for them become actively transcribed in the presence of the sugar; the same genes are inactive if the sugar is absent. In other words, the genes are regulated genes whose products are needed only under certain conditions.

Structure of the lac operon:

1. The lac operon consists of the three structural genes, a promoter, regulator, terminator and an operator.
- 2 These three structural genes are; lac Z, lac Y and lac A.
- 3 Lac Z encode β -galactosidase, an intracellular enzyme that cleaves the disaccharide lactose into **glucose and galactose** and catalysis **isomerization of lactose to allolactose**,
- 4 Lac Y encode β -galactosidase permease, an inner membrane bound symporter that pumps lactose into the cell using a proton gradient.
- 5 Lac A encodes β -galactosidase transacetylase, an enzyme that transfer an acetyl group from acetyl-CoA to β -galactosides.
- 6 Only lac Z and lac Y are necessary for lactose catabolism.

Organization of the *lac* genes of *E. coli* and the associated regulatory elements: the operator, promoter, and regulatory gene. The promoter, operator, and three adjacent *lac* genes together constitute the *lac* operon.



lac Operon Gene

I

P

O

lac Z

lac Y

lac A

Gene Function

Gene for repressor protein

Promoter

Operator

Gene for β-galactosidase

Gene for β-galactoside permease

Gene for β-galactoside transacetylase

The three enzymes required for the utilization of the lactose are β -galactosidase, β -galactosidase permease and β -galactosidase transacetylase.

The expression of lac operon is regulated by lac repressor.

The repressor binds closely and specifically to short DNA segment called “**operator**” which is in location very close to the β -galactosidase gene.

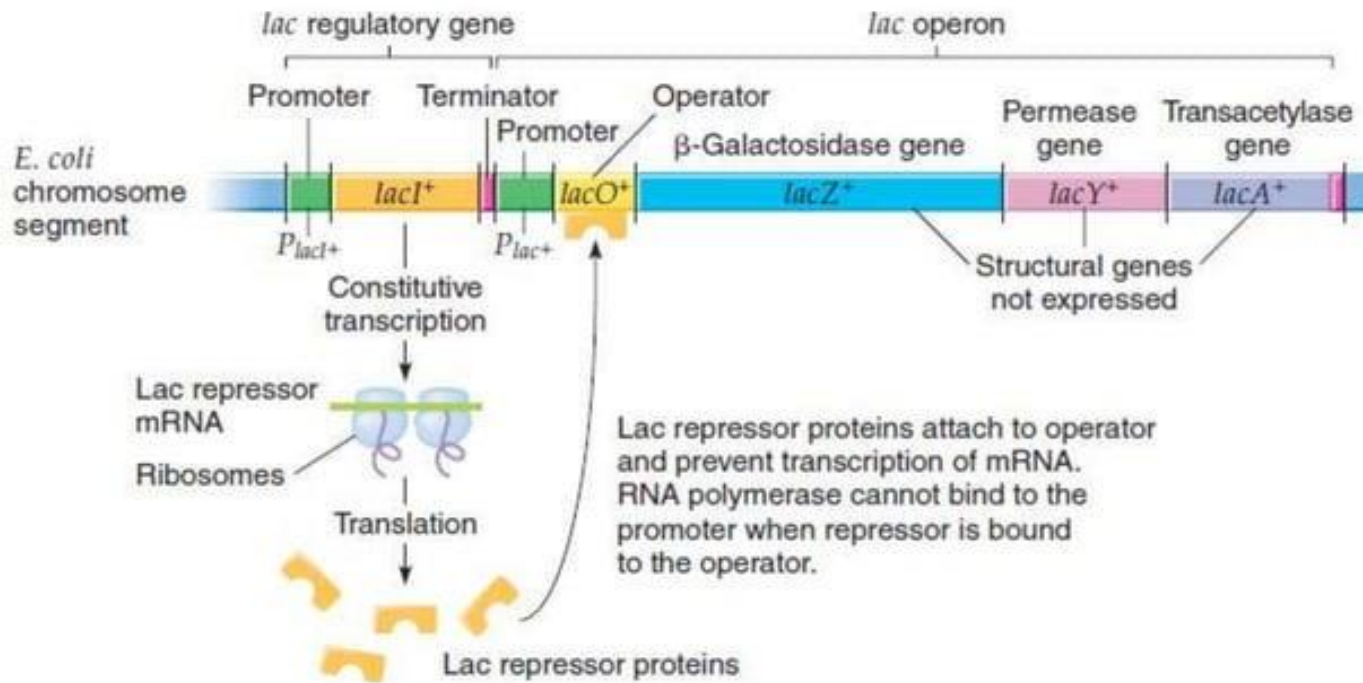
The affinity of the repressor to bind to the operator is regulated by inducer, a small molecule that can bind to the repressor. The natural inducer of lactose is **allolactose**, a metabolite of lactose. However, the analogue IPTG (isopropyl thiogalactoside) is a more powerful inducer that is preferred in the laboratory. Each subunit of the repressor has one binding site for inducer and upon binding it undergoes a conformational change by which it becomes unable to bind to the operator. In this way the presence of the inducer permits transcription of lac operon which is no longer block by the repressor.

Promoter is the DNA segment to which RNA polymerase binds when initiating transcription. **Repressor binds within the promoter and prevents attachment of RNA polymerase**

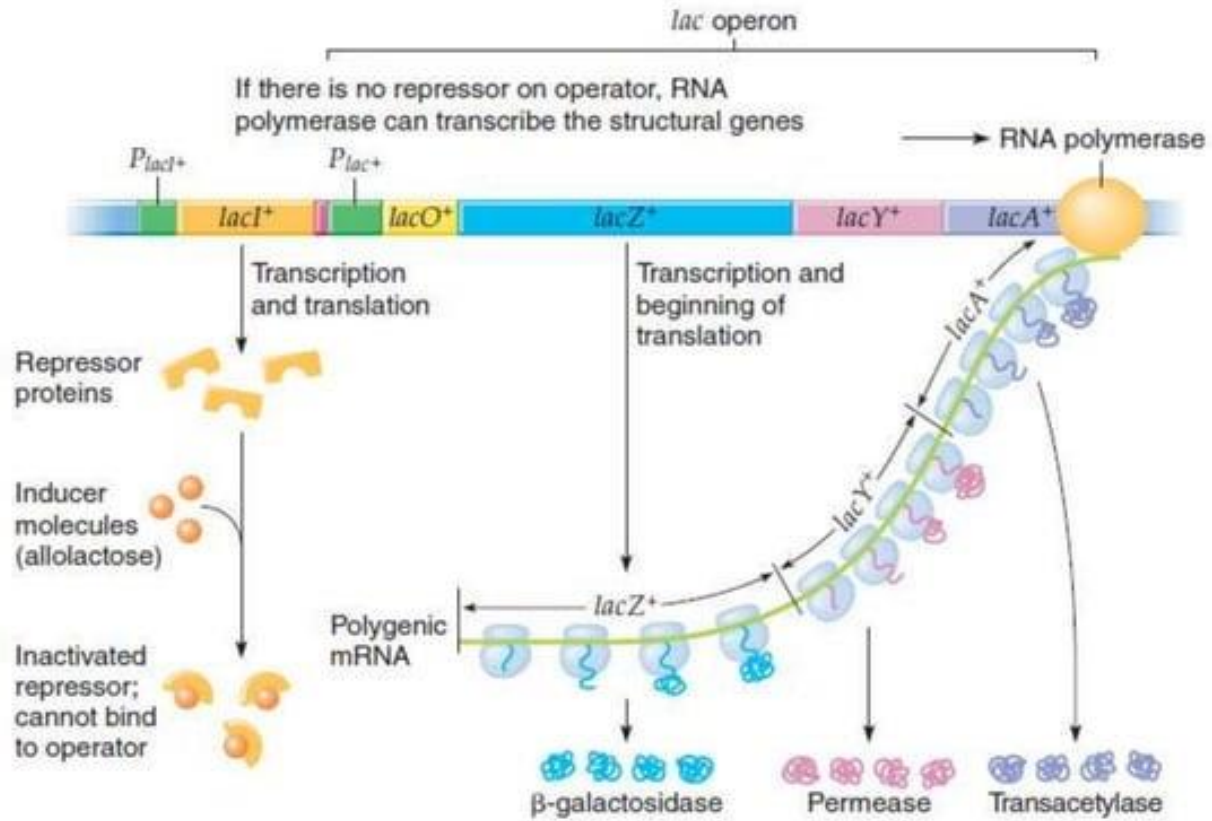
The way in which the repressor work is very simple.

They bind within the promoter and prevents the attachment of RNA polymerase

Functional state of the *lac* operon in wild-type *E. coli* growing in the absence of lactose.



Functional state of the *lac* operon in wild-type *E. coli* growing in the presence of lactose as the sole carbon source.



Tryptophan Operon

(A repressible operon)

E. coli has certain operons and other gene systems that enable it to manufacture any amino acid that is lacking in the medium in which it is placed, so that it can grow and reproduce. When an amino acid is present in the growth medium, though, the genes encoding the enzymes for biosynthetic pathway for that amino acid are turned off. Unlike the *lac operon*, wherein gene activity is induced when a chemical (lactose) is added to the medium, **in this case gene activity is repressed when a chemical (an amino acid) is added.** We call amino acid biosynthesis operons controlled in this way repressible operons. In general, operons for anabolic (biosynthetic) pathways are repressed (turned off) when the end product is readily available

In tryptophan operon, regulation of transcription occur at initiation and termination. In *E. coli* the *five contiguous trp genes encode enzymes that* synthesize the amino acid tryptophan. These genes are expressed efficiently only when tryptophan is limiting.

- **Five structural genes (A–E)**
- The **promoter and operator** regions are upstream from the *trpE* gene.
- Between the promoter–operator region and *trpE* is a short region called ***trpL*, the leader region.**
- Within *trpL*, close to *trpE*, is ***an attenuator site (att)*** that plays an important role in the regulation of the *trp* operon.
- The regulatory gene for the *trp* operon is ***trpR***, located some distance from the operon. **The product of *trpR* is an aporepressor protein**

The entire *trp* operon is approximately 7,000 base pairs long. Transcription of the operon results in the production of a polycistronic mRNA for the five structural genes.

STRUCTURE

Trp operon gene	Gene function
trp R	Codes for repressor
P	promoter
O	Operator, site of attachment of RNA polymerase
TrpE	Codes for Anthranilate
TrpD	Codes for Phosphoribosyl anthranilate transferase
TrpC	Codes for Phosphoribosyl anthranilate isomerase
TrpB	Codes for Trp β synthetase
trpA	Codes for Trp α synthetase

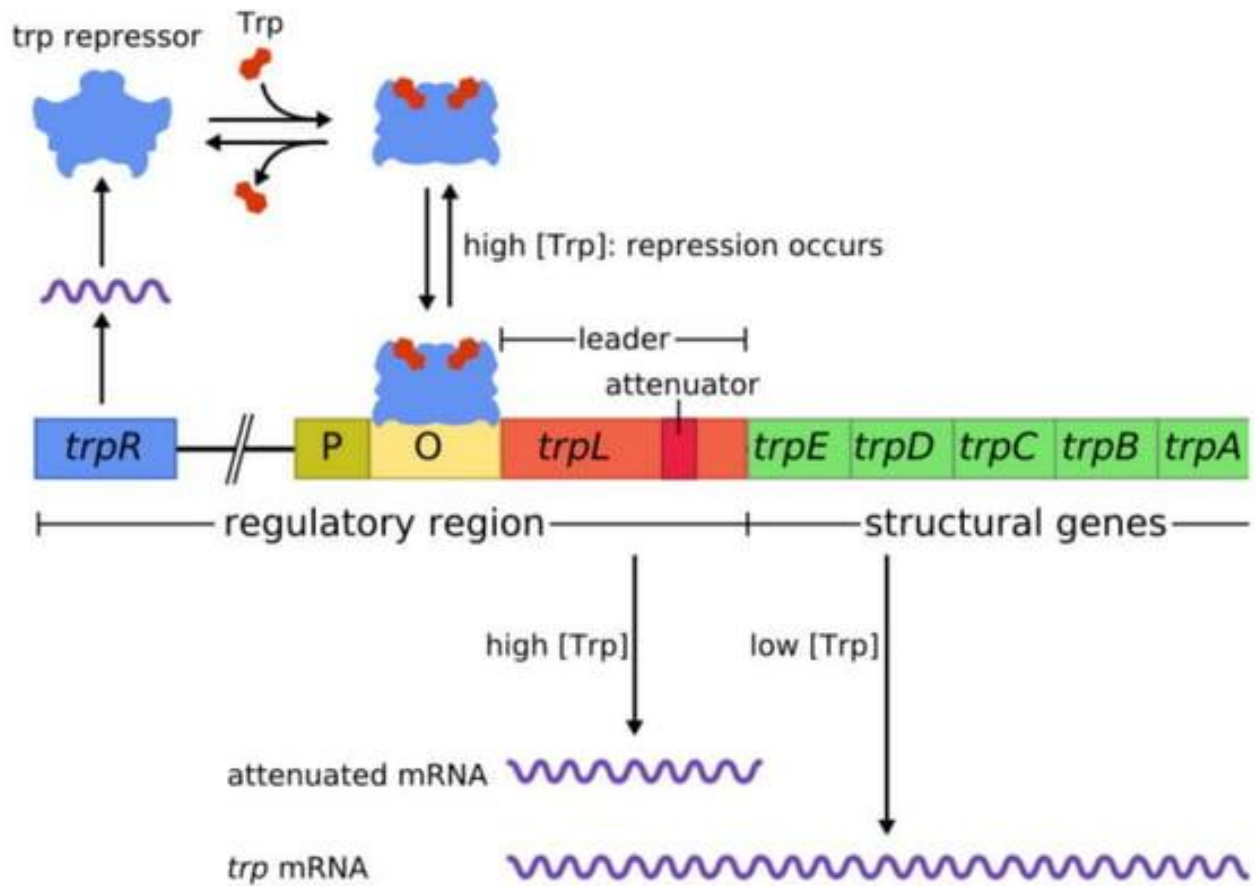
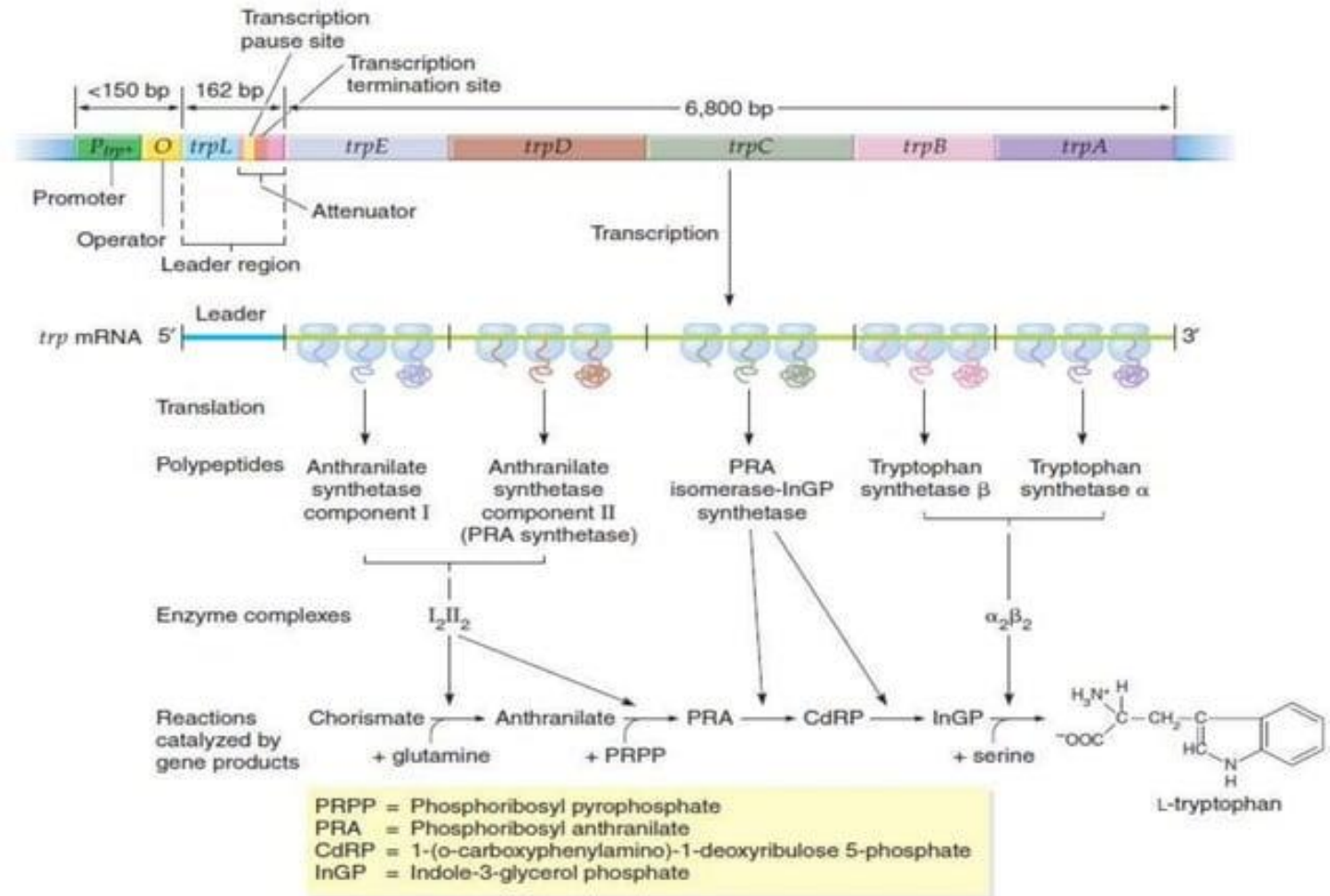


Fig.7. Structure of *trp* operon

The regulatory sequences and structural genes of the *E. coli trp* operon, and the functions of the structural gene products.



Regulation of the *trp* Operon

Two regulatory mechanisms are involved in controlling the expression of the *trp operon*. **One mechanism uses a repressor–operator interaction**, and the **other determines whether initiated transcripts include the structural genes or are terminated before those genes are reached**.

Expression of the *trp* Operon in the Presence of Tryptophan.

The product of *trpR* is an **aporepressor protein**, which is basically an inactive repressor that alone cannot bind to the operator. When tryptophan is abundant within the cell, it interacts with the aporepressor and converts it to an active Trp repressor.

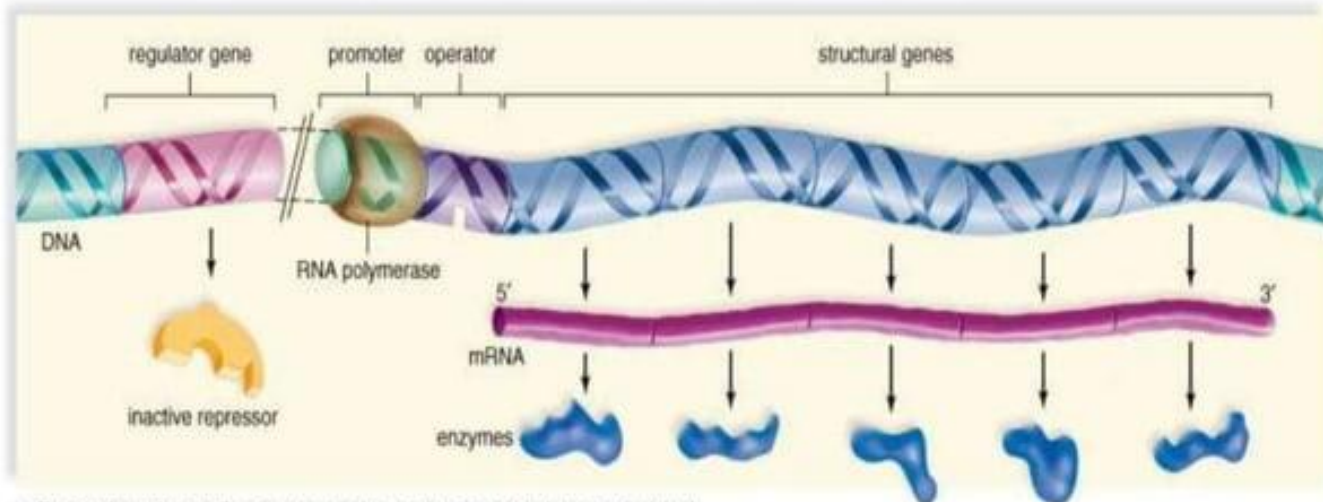
The active Trp repressor binds to the operator and prevents the initiation of transcription of the *trp* operon protein-coding genes by RNA polymerase. As a result, the tryptophan biosynthesis enzymes are not produced. By repression, transcription of the *trp operon* can

Expression of the trp Operon in the Presence of Low Concentrations of Tryptophan.

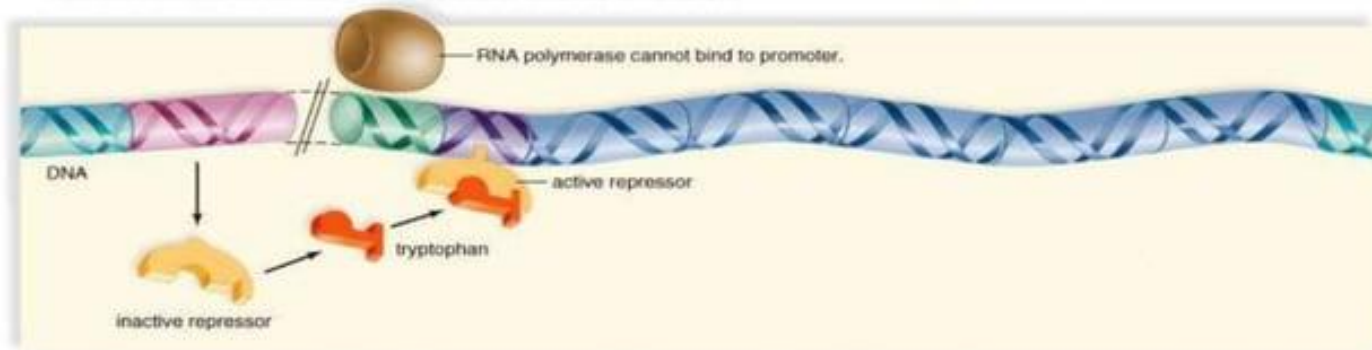
The second regulatory mechanism is involved in the expression of the *trp operon* under conditions of **tryptophan starvation or tryptophan limitation**. Under severe tryptophan starvation, the *trp genes* are expressed maximally; under less severe starvation conditions, the *trp genes* are expressed at less than maximal levels. This is accomplished by a **mechanism that controls the ratio of full-length transcripts that include the five *trp* structural genes to short, 140-bp transcripts that have terminated at the attenuator site within the *trpL* region**. The short transcripts are terminated by a process called attenuation. The proportion of the transcripts that include the structural genes is inversely related to the amount of tryptophan in the cell; the more tryptophan there is, the greater is the proportion of short transcripts. **Attenuation can reduce transcription of the *trp operon* by a factor of 8 to 10. Thus, repression and attenuation together can regulate the transcription of the *trp operon* by a factor of about 560 to 700.**

Mechanism:

The genes are controlled by a repressor, just as the lac genes are, but in this case the ligand that controls the activity of that **repressor (tryptophan)** acts not as an inducer but as a **co-repressor**. That is, when tryptophan is present, it binds the trp repressor and induces a conformational change in that protein, enabling it to bind the trp operator and prevent transcription. When the tryptophan concentration is low, the trp repressor is free of its co-repressor and vacates its operator, allowing the synthesis of trp mRNA to commence from the adjacent promoter. Surprisingly, however, once polymerase has initiated a trp mRNA molecule it does not always complete the full transcript. Indeed, most messages are terminated prematurely before they include even the first trp gene (*trpE*), unless a second and novel device confirms that little tryptophan is available to the cell.



a. **Tryptophan absent.** Enzymes needed to synthesize tryptophan are produced.

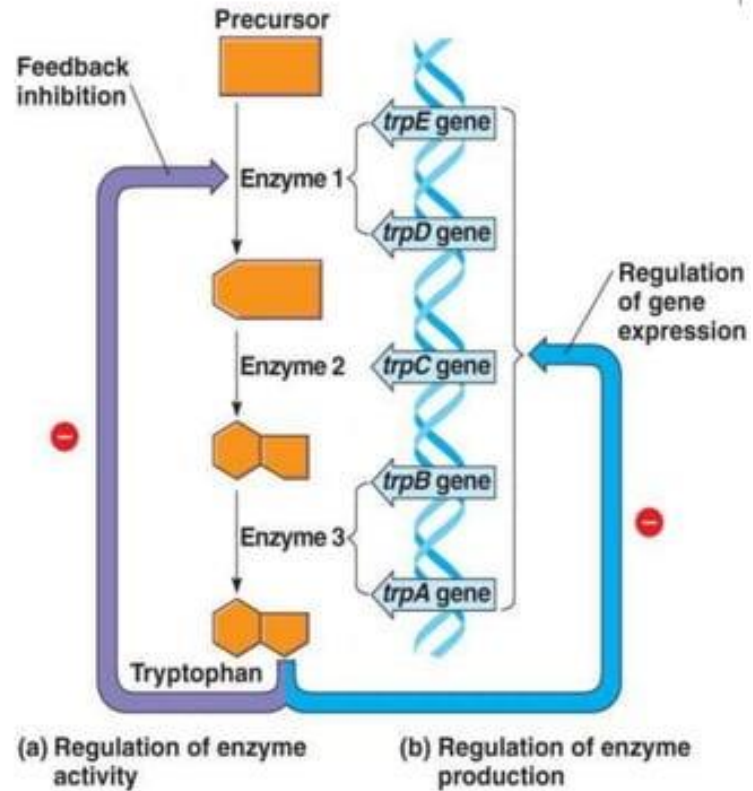


b. **Tryptophan present.** Presence of tryptophan prevents production of enzymes used to synthesize tryptophan.

Fig.8. trp operon; system turned on when tryptophan is absent, system turned off when tryptophan is present.

Regulating Biochemical Pathway for Tryptophan Synthesis.

1. Produce something that will interfere with the function of the enzyme in the pathway.
2. Produce a gene regulator that can inhibit the transcription of one biochemical pathway enzymes.



REFERENCE

- **Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter. (2002). Molecular biology of the Cell. Garland publishing Inc**
- **Ajoy Paul. (2011). Text book of Cell and molecular biology.**
- **Rastogi, S.C. (2004). Cell Biology, 2nd Edition, New Age International Publishers, New Delhi.**
- **Randall H. Morse. (2023). Chapter 8 - Chromatin and transcription, Editor(s): Randall H. Morse.**

Thank you