Operon Modal of Gene Regulation

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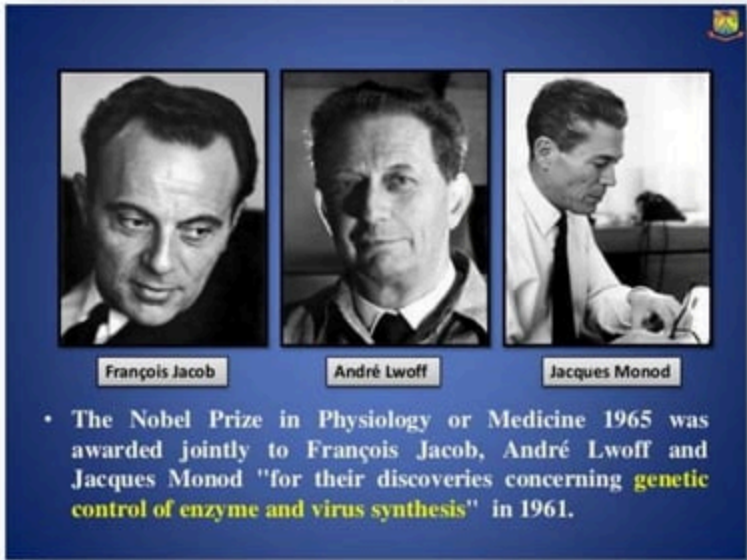
OPERONS

- **An operon is a group of genes that are transcribed at the same time.**
- **They usually control an important biochemical process.**
- **They are only found in prokaryotes.**



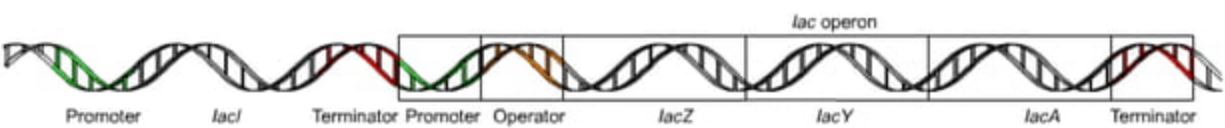
The control of gene expression or Regulation of Gene

- Each cell of multicellular organism contain same genetic material, but the expression of gene is different in different type of cell group. On the basis of expression requirement they are grouped in to
  - **Structural Gene-** Mostly expressed once in a life
  - **Vital Gene-** Involved in of vital biochemical processes such as respiration and need to be expressed all the time
  - **Functional Gene-** Genes are not expressed all the time. They are switched on an off at need

The regulation of Gene required in case of functional gene and its explained by Francois Jacob, Jacques Monod and Andre Lwoff (Nobal Prize in 1961)

An operon is a cluster of coordinately regulated genes. It includes

- **structural genes** (generally encoding enzymes),
- **regulatory genes** (encoding, e.g. activators or repressors) and
- **regulatory sites** (such as promoters and operators).



The *lac* Operon

The *lac* Operon

The lactose operon (lac operon) is an operon required for the transport and metabolism of lactose in humans and many other enteric bacteria. Although glucose is the preferred carbon source for most bacteria, the lac operon allows for the effective digestion of lactose when glucose is not available through the activity of beta-galactosidase

- **The *lac* operon consists of three genes each involved in processing the sugar lactose**
- **One of them is the gene for the enzyme β-galactosidase**
- **One of them is the gene for the enzyme Lipase**
- **One of them is the gene for the enzyme Transacteylase**

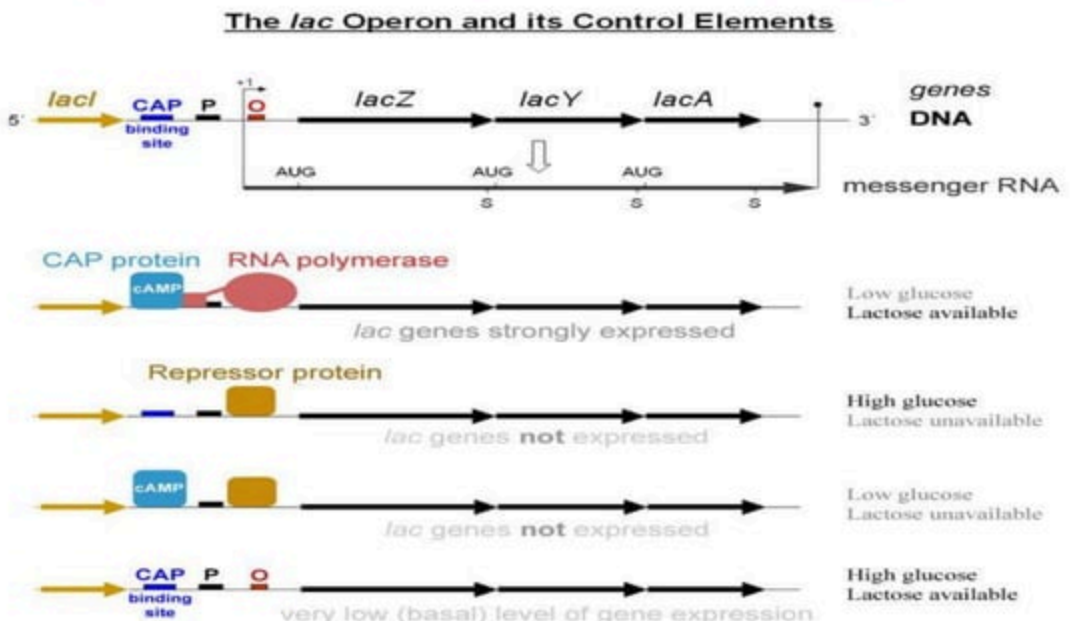
- **β-galactosidase** hydrolyses lactose into glucose and galactose
- **Permease increase the permeability of Cell membrane for Lactose**

ADAPTING TO THE ENVIRONMENT

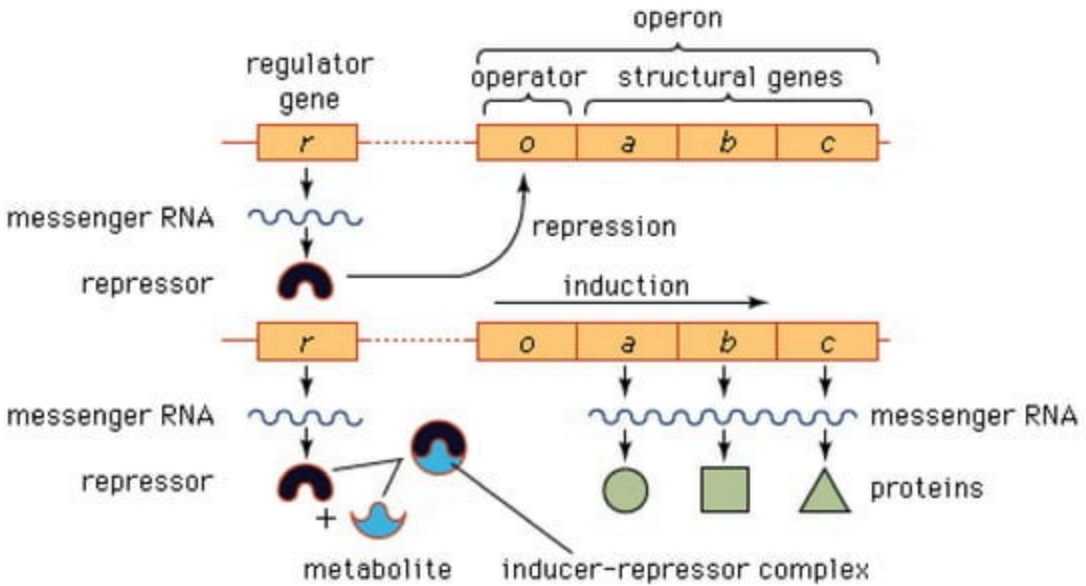
- ***E. coli*** **can use either glucose, which is a monosaccharide, or lactose, which is a disaccharide**
- **However, lactose needs to be hydrolysed (digested) first**
- **So the bacterium prefers to use glucose when it can**

FOUR SITUATIONS ARE POSSIBLE

1. **When glucose is present and lactose is absent the E. coli does not produce β-galactosidase.**
2. **When glucose is present and lactose is present the E. coli does not produce β-galactosidase.**
3. **When glucose is absent and lactose is absent the E. coli does not produce β-galactosidase.**
4. **When glucose is absent and lactose is present the E. coli does produce β-galactosidase**

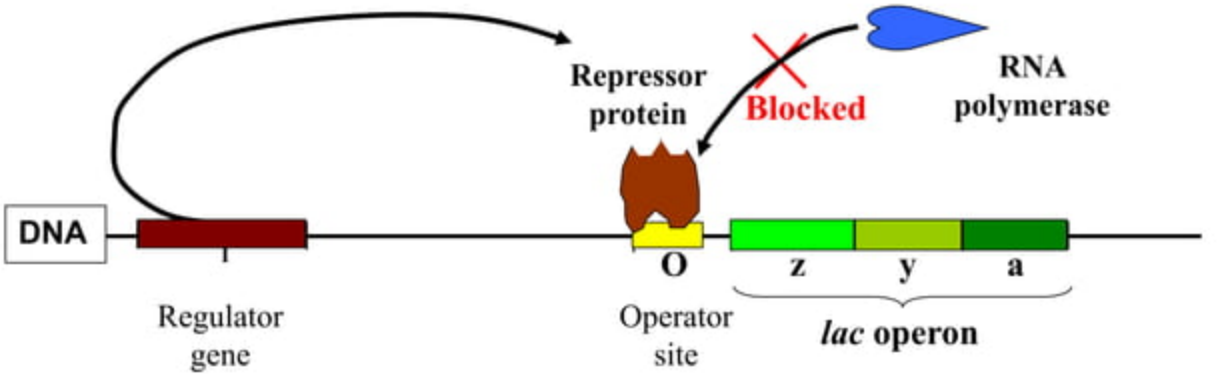


THE CONTROL OF THE *LAC* OPERON



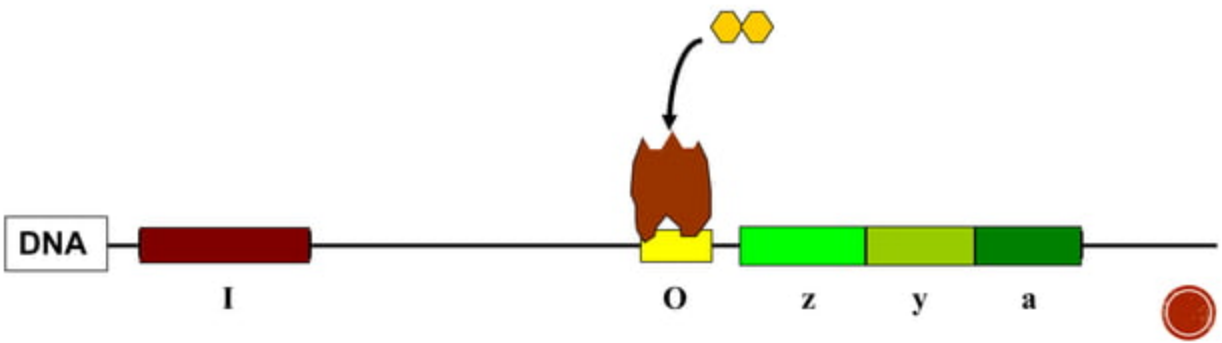
1. WHEN LACTOSE IS ABSENT

- A repressor protein is continuously synthesised. It sits on a sequence of DNA just in front of the *lac* operon, the **Operator site**
- The **repressor protein** blocks the **Promoter site** where the RNA polymerase settles before it starts transcribing



2. WHEN LACTOSE IS PRESENT

- A small amount of a sugar allolactose is formed within the bacterial cell. This fits onto the repressor protein at another active site (allosteric site)
- This causes the repressor protein to change its shape (a conformational change). It can no longer sit on the operator site. RNA polymerase can now reach its promoter site



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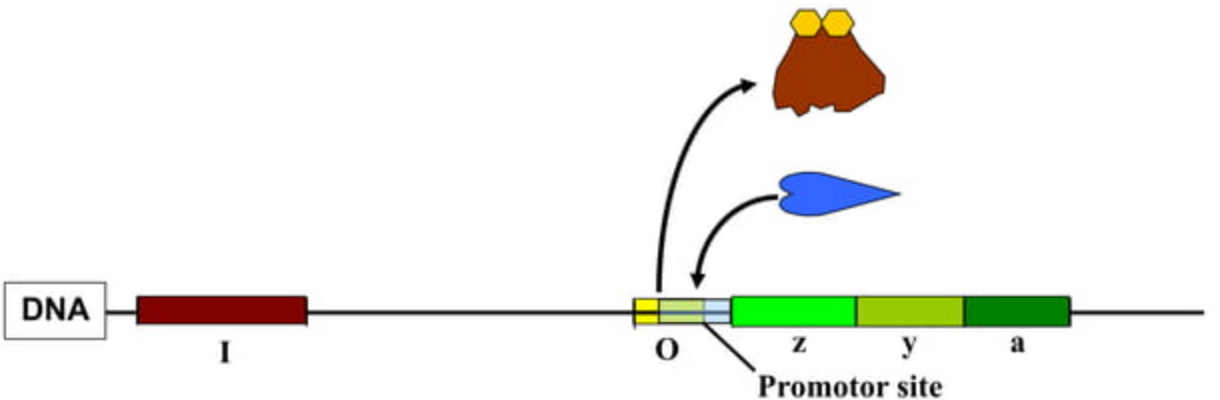
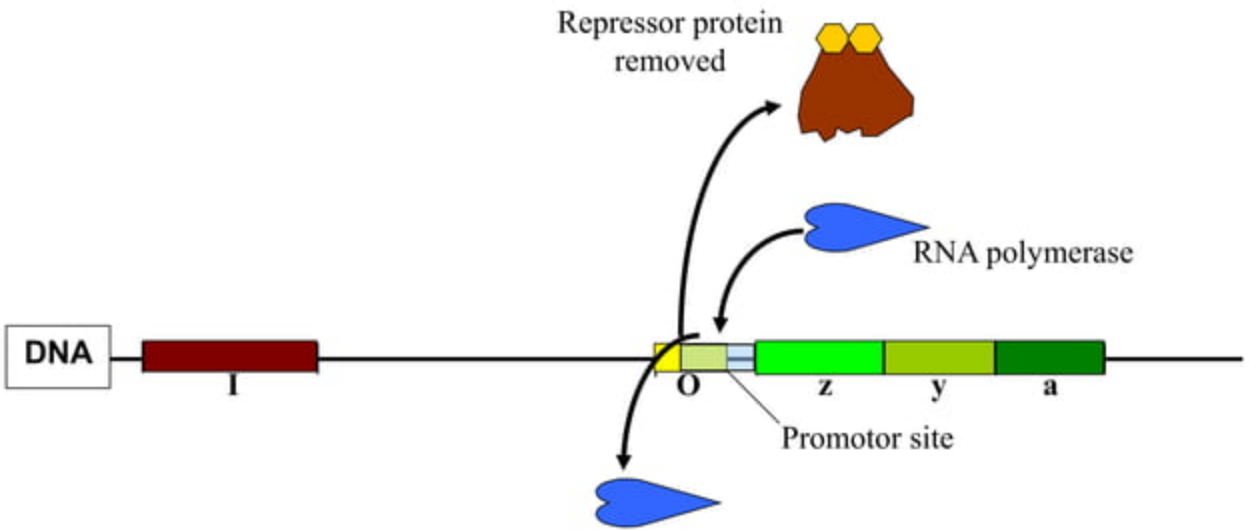


3. WHEN BOTH GLUCOSE AND LACTOSE ARE PRESENT

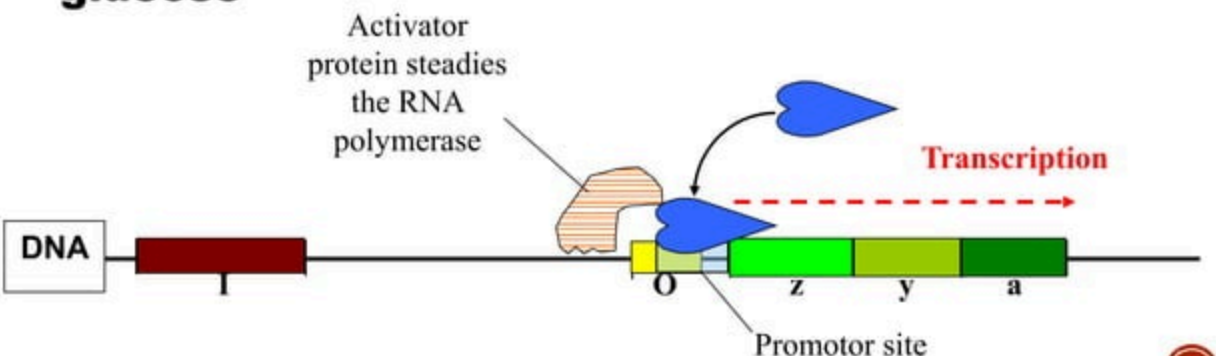
- **This explains how the *lac* operon is transcribed only when lactose is present.**
- **BUT..... this does not explain why the operon is not transcribed when both glucose and lactose are present.**

- **When glucose and lactose are present RNA polymerase can sit on the promoter site but it is unstable and it keeps falling off**



4. WHEN GLUCOSE IS ABSENT AND LACTOSE IS PRESENT

- **Another protein is needed, an activator protein. This stabilises RNA polymerase.**
- **The activator protein only works when glucose is absent**
- **In this way *E. coli* only makes enzymes to metabolise other sugars in the absence of glucose**

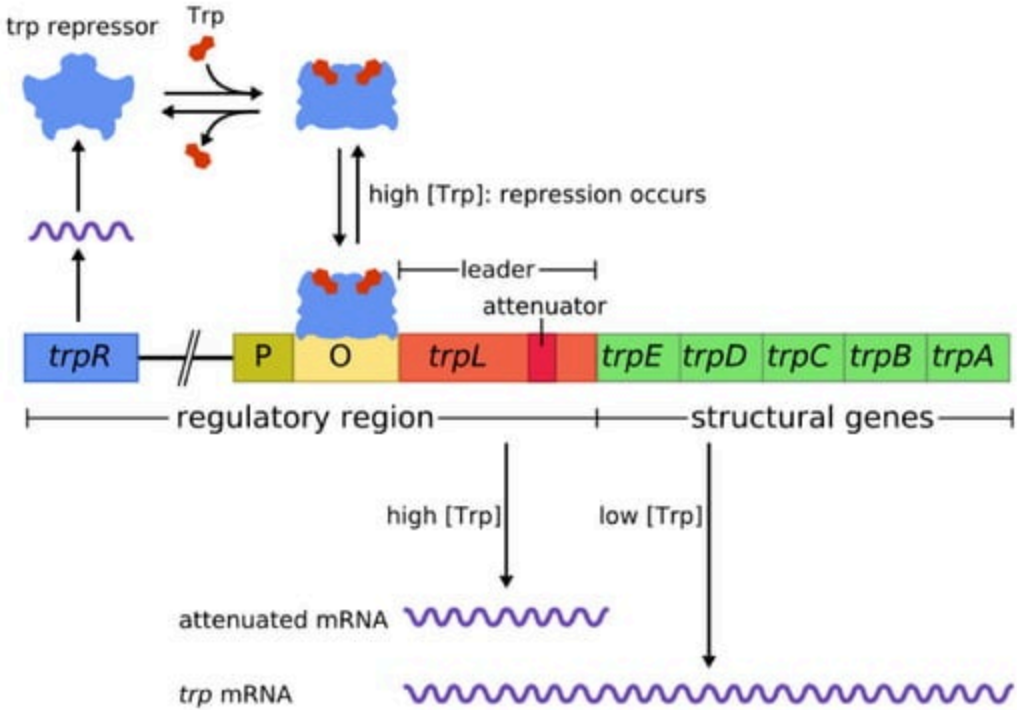


The *trp* Operon

The trp operon is an operon—a group of genes that is used, or transcribed, together—that codes for the components for production of tryptophan. The trp operon is present in many bacteria, but was first characterized in Escherichia coli. The operon is regulated so that when tryptophan is present in the environment, the genes for tryptophan synthesis are not expressed. It was an important experimental system for learning about gene regulation, and is commonly used to teach gene regulation.

Trp operon contains five structural genes: trpE, trpD, trpC, trpB, and trpA, which encode enzymatic parts of the pathway. It also contains a repressive regulator gene called trpR. trpR has a promoter where RNA polymerase binds and synthesizes mRNA for a regulatory protein. The protein that is synthesized by trpR then binds to the operator which then causes the transcription to be blocked. In the trp operon, tryptophan binds to the repressor protein effectively blocking gene transcription. In this situation, repression is that of RNA polymerase transcribing the genes in the operon. Also unlike the lac operon, the trp operon contains a leader peptide and an attenuator sequence which allows for graded regulation.

It is an example of repressible negative regulation of gene expression. Within the operon's regulatory sequence, the operator is bound to the repressor protein in the presence of tryptophan (thereby preventing transcription) and is liberated in tryptophan's absence (thereby allowing transcription).


trp repressor
Trp
high [Trp]: repression occurs
leader
attenuator
trpR
P
O
trpL
trpE
trpD
trpC
trpB
trpA
regulatory region
structural genes
high [Trp]
low [Trp]
attenuated mRNA
trp mRNA

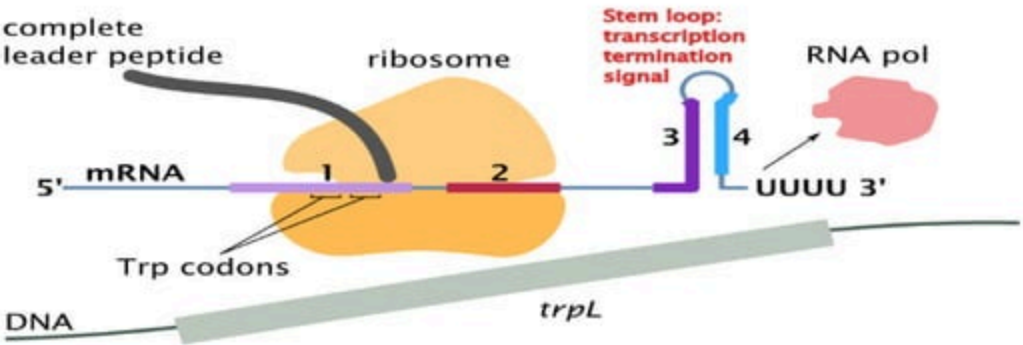
Genes

Trp operon contains five structural genes. Their roles are:

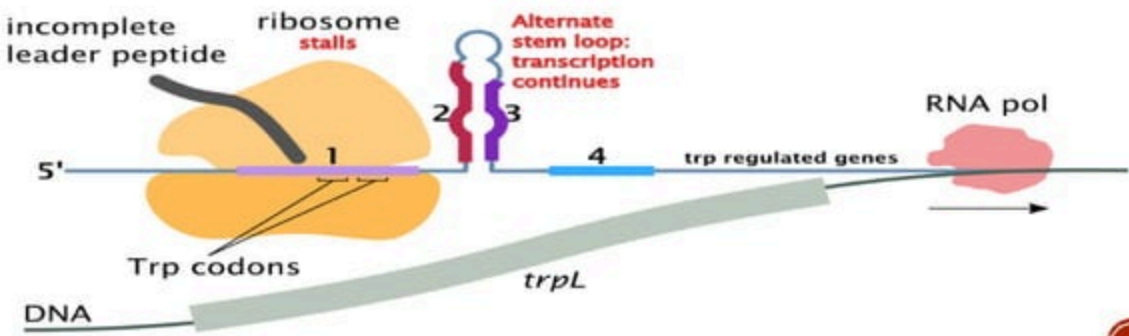
- **TrpE (P00895): Anthranilate synthase produces anthranilate.**
- **TrpD (P00904): Cooperates with TrpE.**
- **TrpC (P00909): Phosphoribosylanthranilate isomerase domain first turns N-(5-phospho-β-D-ribosyl)anthranilate into 1-(2-carboxyphenylamino)-1-deoxy-D-ribulose 5-phosphate. The Indole-3-glycerol-phosphate synthase on the same protein then turns the product into (1S,2R)-1-C-(indol-3-yl)glycerol 3-phosphate.**
- **TrpA (P0A877), TrpB (P0A879): two subunits of tryptophan synthetase. Combines TrpC's product with serine to produce tryptophan.**

- **TrpE & TrpD- Anthranilate synthase**
- **TrpC- Phosphoribosylanthranilate isomerase**
- **TrpB & TrpA- tryptophan synthetase**

High level of tryptophan



Low level of tryptophan



The *arb* Operon

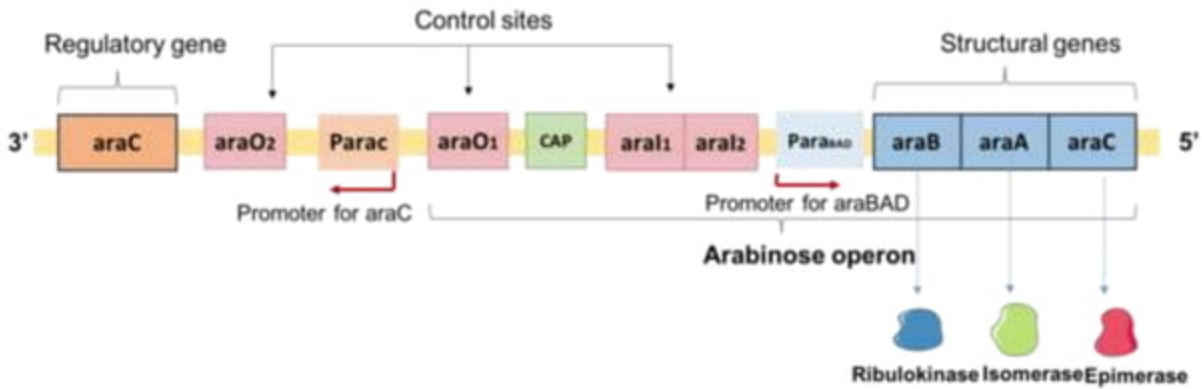
L-arabinose operon

The L-arabinose operon, also called the ara or araBAD operon, is an operon required for the breakdown of the five-carbon sugar L-arabinose in Escherichia coli. The L-arabinose operon contains three structural genes: araB, araA, araD (collectively known as araBAD), which encode for three metabolic enzymes that are required for the metabolism of L-arabinose. AraB (ribulokinase), AraA (an isomerase), AraD (an epimerase) produced by these genes catalyse conversion of L-arabinose to an intermediate of the pentose phosphate pathway, D-xylulose-5-phosphate.

The structural genes of the L-arabinose operon are transcribed from a common promoter into a single transcript, a mRNA. The expression of the L-arabinose operon is controlled as a single unit by the product of regulatory gene araC and the catabolite activator protein (CAP)-cAMP complex. The regulator protein AraC is sensitive to the level of arabinose and plays a dual role as both an activator in the presence of arabinose and a repressor in the absence of arabinose to regulate the expression of araBAD. AraC protein not only controls the expression of araBAD but also auto-regulates its own expression at high AraC levels.

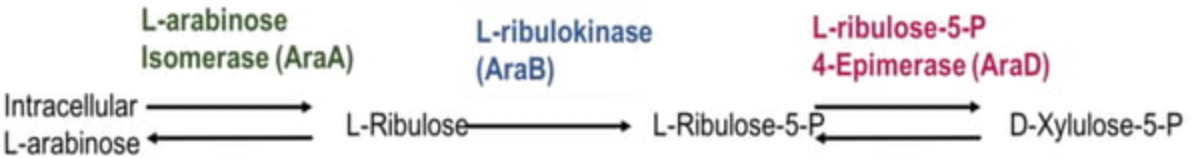
Structure

L-arabinose operon is composed of structural genes and regulatory regions including the operator region (araO1, araO2) and the initiator region (araI1, araI2). The structural genes, araB, araA and araD, encode enzymes for L-arabinose catabolism. There is also a CAP binding site where CAP-cAMP complex binds to and facilitates catabolite repression, and results in positive regulation of araBAD when the cell is starved of glucose



Function

- **araA encodes L-arabinose isomerase, which catalyses isomerization between L-arabinose and L-ribulose.**
- **araB encodes ribulokinase, which catalyses phosphorylation of L-ribulose to form L-ribulose-5-phosphate.**
- **araD encodes L-ribulose-5-phosphate 4-epimerase, which catalyses epimerization between L-ribulose 5-phosphate and D-xylulose-5-phosphate.**



Catabolism of arabinose in E. coli				
Substrate	Enzyme(s)	Function	Reversible	Product
L-arabinose	AraA	Isomerase	Yes	L-ribulose
L-ribulose	AraB	Ribulokinase	No	L-ribulose-5-phosphate
L-ribulose-5-phosphate	AraD	Epimerase	Yes	D-xylulose-5-phosphate

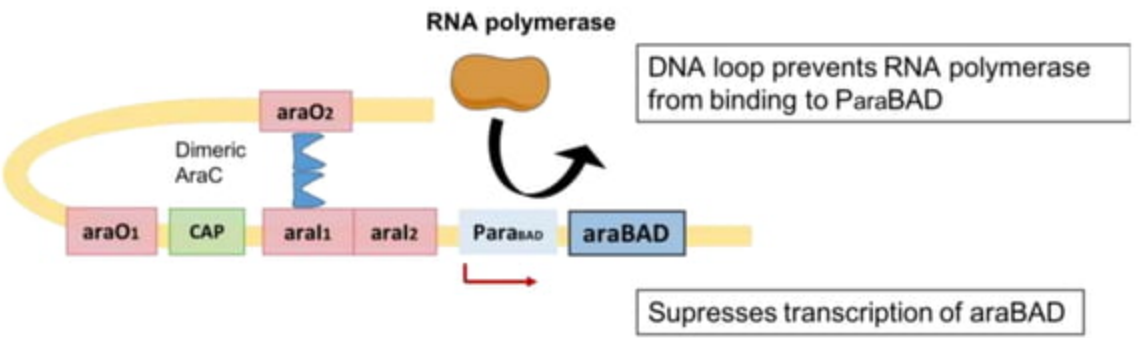
Regulation

The L-arabinose system is not only under the control of CAP-cAMP activator, but also positively or negatively regulated through binding of AraC protein. AraC functions as a homodimer, which can control transcription of araBAD through interaction with the operator and the initiator region on L-arabinose operon. Each AraC monomer is composed of two domains including a DNA binding domain and a dimerisation domain. The dimerisation domain is responsible for arabinose-binding. AraC undergoes conformational change upon arabinose-binding, in which, it has two distinct conformations.[6] The conformation is purely determined by the binding of allosteric inducer arabinose.

AraC can also negatively autoregulate its own expression when the concentration of AraC becomes too high. AraC synthesis is repressed through binding of dimeric AraC to the operator region (araO1).

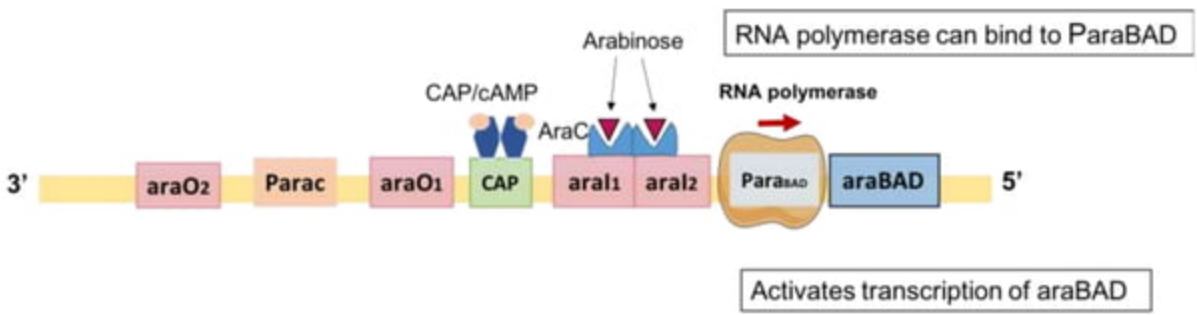
Negative regulation of *araBAD*

When arabinose is absent, cells do not need the araBAD products for breaking down arabinose. Therefore, dimeric AraC acts as a repressor: one monomer binds to the operator of the araBAD gene (araO2), another monomer binds to a distant DNA half site known as araI1. This leads to the formation of a DNA loop. This orientation blocks RNA polymerase from binding to the araBAD promoter. Therefore, transcription of structural gene araBAD is inhibited



Positive regulation of *araBAD*

Expression of the *araBAD* operon is activated in the absence of glucose and in the presence of arabinose. When arabinose is present, both AraC and CAP work together and function as activators



Via CAP/cAMP (catabolite repression)

CAP act as a transcriptional activator only in the absence of E. coli's preferred sugar, glucose. When glucose is absent, high level of CAP protein/cAMP complex bind to CAP binding site, a site between araI1 and araO1. Binding of CAP/cAMP is responsible for opening up the DNA loop between araI1 and araO2, increasing the binding affinity of AraC protein for araI2 and thereby promoting RNA polymerase to bind to araBAD promoter to switch on the expression of the araBAD required for metabolising L-arabinose.

Autoregulation of AraC

The expression of *araC* is negatively regulated by its own protein product, AraC. The excess AraC binds to the operator of the *araC* gene, $araO_1$, at high AraC levels, which physically blocks the RNA polymerase from accessing the *araC* promoter.Therefore, the AraC protein inhibits its own expression at high concentrations