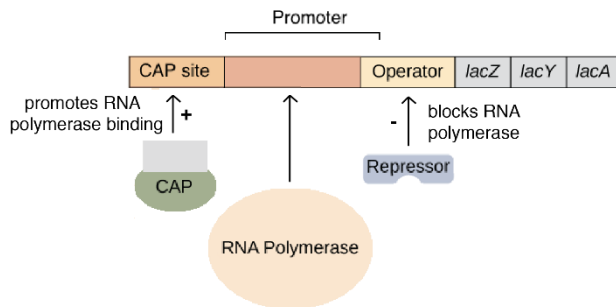# Lac operon

The lac operon is a genetic regulatory system found in bacteria, such as E. coli, that controls the transport and metabolism of lactose. It consists of a cluster of genes that are expressed and produce enzymes when lactose is available, while remaining inactive in the absence of lactose or when a more favorable energy source, like glucose, is present.

Structure of Lac operon:

The components of a lac operon are Structural genes and Regulatory DNA sequences.

Structural genes of lac operon

The lac operon consists of three structural genes: lacZ, lacY, and lacA. These genes are transcribed together as a single polycistronic mRNA from a common promoter.

1. **lacZ**: This gene codes for the enzyme β-galactosidase, which is a tetramer with a molecular weight of approximately 500 kD. β-galactosidase plays a crucial role in lactose metabolism by breaking down β-galactosides, including lactose, into their monosaccharide components. For example, lactose is hydrolyzed into glucose and galactose, which can be further metabolized through glycolysis.
2. **lacY**: This gene encodes the β-galactoside permease, which is a membrane-bound protein with a molecular weight of about 30 kD. β-galactoside permease facilitates the transport of β-galactosides, such as lactose, into the bacterial cell. It is responsible for the uptake of lactose from the environment.
3. **lacA**: This gene codes for β-galactoside transacetylase, although its precise role within the lac operon is not fully understood. β-galactoside transacetylase transfers an acetyl group from acetyl-CoA to β-galactosides. However, its specific function in lactose metabolism remains unclear.

These three structural genes, lacZ, lacY, and lacA, are located adjacent to each other within the lac operon. Together with the promoter, operator, and regulatory elements, they form a functional unit known as an operon. The lac operon provides the necessary genes and enzymes for the uptake and metabolism of lactose and other β-galactosides in bacteria like E. coli.

Regulatory genes of lac operon

The lac operon consists of several regulatory genes that control its activity:

1. **Promoter**: The promoter region is the binding site for RNA polymerase, the enzyme responsible for initiating transcription. It is located upstream of the lac operon and facilitates the binding of RNA polymerase to initiate the transcription of the structural genes.
2. **Operator**: The operator region is a negative regulatory site situated between the promoter and the structural genes. It overlaps with the promoter region. The lac repressor protein binds to the operator, preventing RNA polymerase from transcribing the structural genes. The operator acts as a switch, determining whether transcription should occur or not.
3. **Lac I (Repressor) Gene:** The Lac I gene codes for the lac operon repressor protein. It is located adjacent to the promoter region of the lac operon and has its own promoter and terminator. The repressor protein is a tetramer composed of identical subunits with a molecular weight of 38 kD each. The repressor is continuously synthesized since its gene is always transcribed. The repressor protein can bind to the operator, thereby repressing (turning off) the lac operon by blocking RNA polymerase binding.

4. **Catabolite Activator Protein (CAP) Binding Site:** The CAP binding site is a positive regulatory site found just upstream of the lac operon promoter. The catabolite activator protein (CAP) binds to this site. CAP is a dimeric protein that can bind to cAMP (cyclic adenosine monophosphate) and DNA. When cAMP binds to CAP, its affinity for DNA increases. CAP bound to DNA promotes transcription by enhancing the binding of RNA polymerase to the promoter region, leading to increased gene expression of the lac operon.

These regulatory genes, including the promoter, operator, Lac I repressor gene, and CAP binding site, work together to control the activity of the lac operon, allowing the cell to respond to the presence or absence of lactose and other regulatory signals.

Regulation of lac operon:

In prokaryotes, the Lac-operon system is controlled in two ways:

- Positive control
- Negative control

Positive Control of Lac-Operon

The positive control of the lac operon refers to the regulatory mechanism that activates gene expression when certain conditions are met. In the case of the lac operon, the presence of an inducer, such as lactose, triggers the positive control.

Here are the steps involved in the positive control of the lac operon:

1. Expression of the Repressor Protein: The regulatory gene of the lac operon expresses the lac repressor protein. The repressor protein is continuously synthesized and present in the cell.
2. Production of Repressor Proteins: The expression of the regulatory gene leads to the production of repressor proteins.
3. Binding of the Inducer: The repressor protein has binding sites for both the operator and the inducer (lactose). When lactose is present in the cellular environment, it acts as an inducer and binds to the repressor protein.
4. Formation of the R+I Complex: The binding of the inducer (lactose) with the repressor protein forms a complex called the R+I complex. This complex alters the conformation of the repressor protein.
5. Prevention of Repressor Binding: The R+I complex no longer binds to the operator region. As a result, it no longer blocks the binding of RNA polymerase to the promoter region of the lac operon.
6. Transcription and mRNA Production: With the repressor protein no longer blocking the operator, RNA polymerase can bind to the promoter region and initiate transcription. This leads to the production of mRNA from the lac operon genes.

By the presence of an inducer, such as lactose, the positive control of the lac operon allows for the switch-on of gene expression. The inducer prevents the repressor protein from binding to the operator, enabling RNA polymerase to transcribe the genes of the lac operon and produce the necessary enzymes for lactose metabolism.

Negative Control of Lac-Operon

The negative control of the lac operon refers to the regulatory mechanism that inhibits gene expression in the absence of an inducer, such as lactose. It involves the action of the lac repressor protein. Here are the steps involved in the negative control of the lac operon:

1. **Expression of the Repressor Protein:** The regulatory gene of the lac operon expresses the lac repressor protein. The repressor protein is continuously synthesized and present in the cell.
2. **Production of Repressor Proteins:** The expression of the regulatory gene leads to the production of repressor proteins.
3. **Binding of Repressor to Operator:** In the absence of an inducer or lactose, the repressor protein binds directly to the operator region of the lac operon. This binding physically obstructs the movement of RNA polymerase and prevents its attachment to the promoter region.
4. **Blockage of Transcription:** The binding of the repressor protein to the operator effectively blocks the transcription of the lac operon genes. RNA polymerase is unable to proceed with transcribing the mRNA.
5. **Switching Off the Lac Operon:** In the absence of an inducer, the lac operon remains switched off. The absence of lactose as an inducer allows the repressor protein to remain bound to the operator, inhibiting gene expression.

Overall, the negative control of the lac operon ensures that the genes involved in lactose metabolism are not transcribed when lactose is absent. The repressor protein plays a key role in blocking the movement of RNA polymerase, effectively switching off the lac operon. The presence of an inducer, such as lactose, will bind to the repressor protein and relieve its inhibitory action, leading to the activation of gene expression.

TRYPTOPHAN OPERON

- **Tryptophan operon** was the first repressible operon discovered.
- Tryptophan operon is the cluster of genes that code for the elements essential for the synthesis of tryptophan which is an essential amino acid.
- Here note the difference between <u>Lac operon</u> and the Tryptophan operon abbreviated as the trp operon; Lac operon works for the catabolism of the lactose while the trp operon works for the synthesis of enzymes needed for the synthesis of tryptophan.
- Therefore, **trp operon** is an anabolic type of operon.

STRUCTURE OF THE TRYPTOPHAN OPERON
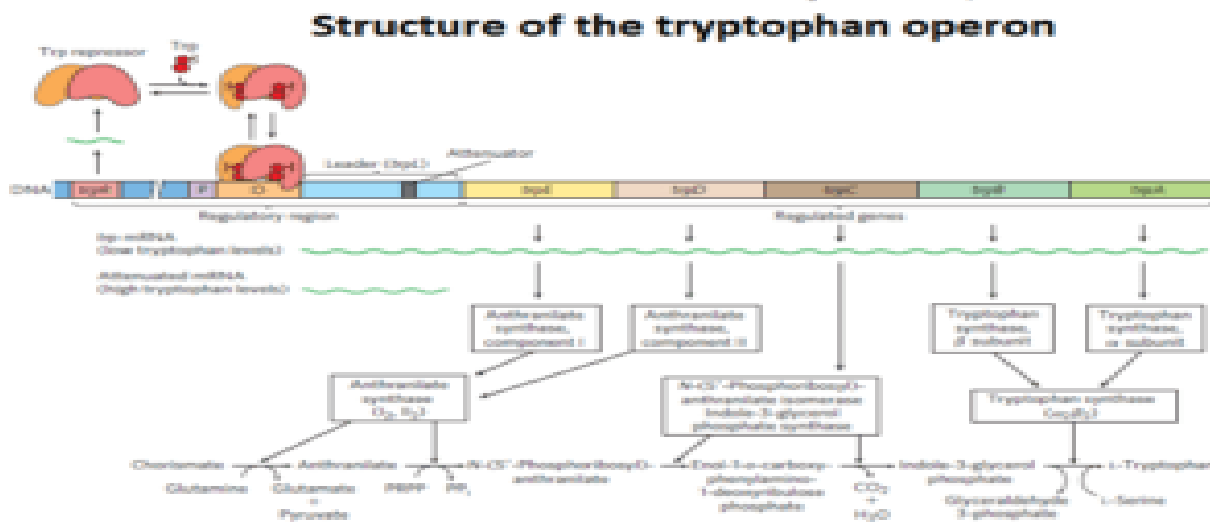
The above figure elaborates the **structure of the trp operon** from *E. coli*. The tryptophan operon consists of **promoter**, **operator** which overlaps with the promoter followed by the leader sequence of about 162 nucleotides and **five structural genes** which code for enzymes that are essential for the synthesis of tryptophan.

- The tryptophan operon is the regulation of transcription of the gene responsible for biosynthesis of tryptophan.
- The tryptophan (trp) operon contains five structural genes encoding enzymes for tryptophan biosynthesis with an upstream trp promoter (Ptrp) and trp operator sequence (Otrp).

The Five Structural Genes And Their Products Are As Follows:

Structural genes are TrpE, TrpD, TrpC, TrpB and TrpA
1. trpE: It enodes the enzyme Anthranilate synthase I
2. trpD: It encodes the enzyme Anthranilate synthase II
3. trpC: It encodes the enzyme N-5'-Phosphoribosyl anthranilate isomerase and Indole-3-glycerolphosphate synthase
4. trpB: It encodes the enzyme tryptophan synthase-B sub unit
5. trpA: It encode the enzyme tryptophan synthase-A sub unit
- The trp operator region partly overlaps the trp promoter.
- The operon is regulated such that transcription occurs when tryptophan in the cell is in short supply.

Mechanism of regulation of gene expression in Eukaryotes

1) Chromatin Remodeling

Chromatin structure provides an important level of control of gene transcription. Large regions of chromatin are transcriptionally inactive while others are either active or potentially active. With few exceptions, each cell contains the same complement of genes (antibody-producing cells are a notable exception). The development of specialized organs, tissues, and cells and their function in the intact organism depend upon the differential expression of genes. Some of this differential expression is achieved by having different regions of chromatin available for transcription in cells from various tissues. For example, the DNA containing the B-globin gene cluster is in "active" chromatin in the reticulocyte but in "inactive" chromatin in muscle cells.

Formation and disruption of nucleosome structure

The presence of nucleosomes and of complexes of histones and DNA certainly provides a barrier against the ready association of transcription factors with specific DNA regions. The dynamics of the formation and disruption of nucleosome structure are therefore an important part of eukaryotic gene regulation and the processes involved are as follows:
i) Histone acetylation and deacetylation is an important determinant of gene activity. Acetylation is known to occur on lysine residues in the amino-terminal tails of histone molecules . This modification reduces the positive charge of these tails and decreases the binding affinity of histone for the negatively charged DNA. Accordingly, the acetylation of histones could result in disruption of nucleosomal structure and allow readier access of transcription factors to cognate regulatory DNA elements. Different proteins with specific acetylase and deacetylase activities are associated with various components of the transcription apparatus.
Thus, histone acetylation can activate transcription through a combination of three mechanisms: by reducing the affinity of the histones for DNA, by recruiting other components of the transcriptional machinery, and by initiating the active remodeling of the chromatin structure.
ii) Modification of DNA-The modification of DNA provides another mechanism, in addition to packaging with histones, for inhibiting inappropriate gene expression in specific cell types. Methylation of deoxycytidine residues methylate in DNA may affect gross changes in chromatin so as to preclude its active transcription. Acute demethylation of deoxycytidine residues in a specific region of the tyrosine aminotransferase gene-in response to glucocorticoid hormones-has been associated with an increased rate of transcription of the gene. However, it is not possible to generalize that methylated DNA is transcriptionally inactive, that all inactive chromatin is methylated, or that active DNA is not methylated.

iii) DNA binding proteins- The interactions between DNA-binding proteins such as CAP and RNA polymerase can activate transcription in prokaryotic cells. Such protein-protein interactions play a dominant role in eukaryotic gene regulation. In contrast with those of prokaryotic transcription, few eukaryotic transcription on their own. Instead, each factor recruits other proteins to build up large complexes that interact with the transcriptional machinery to activate or repress transcription. transcription factors have any effect on

A major advantage of this mode of regulation is that a given regulatory protein can have different effects, depending on what other proteins are present in the same cell. This phenomenon, called combinatorial control, is crucial to multicellular organisms that have many different cell types.

The binding of specific transcription factors to certain DNA elements may result in the disruption of nucleosomal W structure. Many eukaryotic genes have multiple protein-binding DNA elements. The serial binding of transcription factors to these elements may either directly disrupt the structure of the nucleosome or prevent its re-formation. These reactions result in chromatin-level structural changes that in the end increase DNA accessibility to other factors and the transcription machinery.

2) Enhancers and Repressors- Enhancer elements are DNA sequences, although they have no promoter activity of their own they greatly increase the activities of many promoters in eukaryotes. Enhancers function by serving as binding sites for specific regulatory proteins. An enhancer is effective only in the specific cell types in which appropriate regulatory proteins are expressed. In many cases, these DNA-binding proteins influence transcription initiation by perturbing the local chromatin structure to expose a gene or its regulatory sites rather than by direct interactions with RNA polymerase.

Enhancer elements can exert their positive influence on transcription even when separated by thousands of base pairs from a promoter, they work when oriented in either direction, and they can work upstream (5') or downstream (3') from the promoter. Enhancers are promiscuous; they can stimulate any promoter in the vicinity and may act on more than one promoter.

The elements that decrease or repress the expression of specific genes have also been identified. Silencers are control regions of DNA that, like enhancers, may be located thousands of base pairs away from the gene they control. However, when transcription factors bind to them, expression of the gene they control is repressed.

Tissue-specific gene expression is mediated by enhancers or enhancer-like elements. Many genes are now recognized to harbor enhancer or activator elements in various locations relative to their coding regions. In addition to being able to enhance gene transcription, some of these enhancer elements clearly possess the ability to do so in a tissue-specific manner. Thus, the enhancer element associated with the immunoglobulin genes between the J and C regions enhances the expression of those genes preferentially in lymphoid cells.

Translation control of gene expression refers to the mechanisms that regulate the process of converting genetic information from mRNA (messenger RNA) into proteins. In the context of ferritin mRNA regulation, ferritin is a protein responsible for iron storage in cells.

Translation control of gene expression - ferritin m RNA regulations

Various factors can influence the translation of ferritin mRNA, including regulatory proteins, RNA-binding proteins, and non-coding RNAs. These factors can either enhance or inhibit the translation process, ultimately determining the amount of ferritin protein produced in the cell.

Mechanisms involved in ferritin mRNA regulation

The regulation of ferritin mRNA involves several mechanisms that control its translation. Some key mechanisms include:

1. Iron response elements (IREs): These are specific RNA sequences found in the untranslated regions (UTRs) of ferritin mRNA. They interact with iron regulatory proteins (IRPs) in response to cellular iron levels. When iron is abundant, IRPs bind to IREs and block translation, reducing ferritin synthesis. Conversely, under low iron conditions, IRPs dissociate from IREs, allowing translation and increasing ferritin production to store iron.
2. Transcriptional regulation: The expression of ferritin mRNA can be controlled at the transcriptional level. Various transcription factors and regulatory elements can influence the rate of ferritin gene transcription, affecting the abundance of ferritin mRNA available for translation.
3. Micro RNAs (miRNAs): miRNAs are small non-coding RNAs that can bind to specific regions on ferritin mRNA, leading to translational repression or mRNA degradation. The presence of certain miRNAs can modulate ferritin protein levels in response to cellular conditions.
4. RNA-binding proteins (RBPs): Certain RBPs can interact with ferritin mRNA and influence its stability and translation efficiency. These RBPs may either promote or inhibit translation, depending on cellular signals and conditions.
5. Post-translational modifications: Some regulatory mechanisms involve modifications of translation factors or ribosomal proteins, which can impact the translation of ferritin mRNA.

These mechanisms work together to tightly control ferritin mRNA translation, ensuring appropriate cellular iron levels and preventing iron overload or deficiency. The complex interplay of these regulatory pathways allows cells to adapt to changing iron levels and maintain iron homeostasis.