Regulation of Gene Expression

The expression of some genes cannot be regulated: these genes are transcribed and translated throughout the life of the cell. Such non-regulated genetic function is referred to as **constitutive protein synthesis**. Even among these genes, however, some are more active than others. Genes that encode enzymes for glucose metabolism, for example, bind RNA polymerase much more readily than do genes for most other proteins. Initiation of transcription occurs at a higher frequency for those genes with promoters that have the highest affinity for the polymerization enzyme.

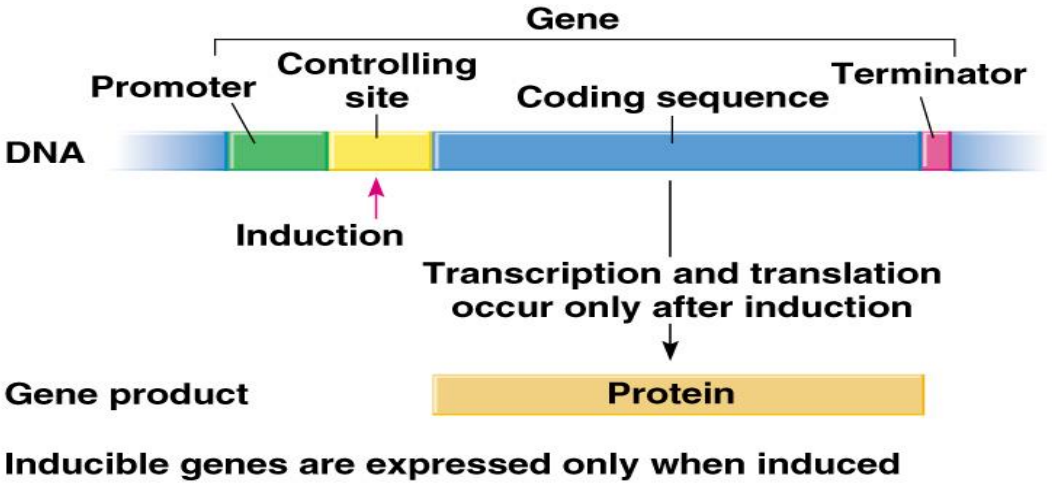


Figure: General organization of an inducible gene

At any given time, many genes in a cell will be shut off and the production of the corresponding gene product temporarily halted. In other words, a cell rarely expresses its entire genetic potential. When *E.coli* is growing on glucose, for example, only 10 to 20 percent of its genes are active. *Resources are conserved by turning off genes for unneeded functions*. Many catabolic enzymes, for example, are not synthesized unless the substrate is available. In the absence of the substrate, transcription is turned off. Residual mRNA deteriorates within 2 minutes; after that the unneeded enzymes are no longer synthesized.

The presence of the substrate turns on transcription of the appropriate gene, the enzyme is produced, and the substrate is utilized. This is control of gene expression by the process of gene **induction**. Inducible genes are turned on by the inducer, which is often the substrate of the corresponding catabolic pathway. Enzymes of biosynthetic pathways, on the other hand, are produced only when the end product of the pathway is needed. When this product is abundant, production of the enzymes is turned off by the process of gene **repression**. The product of the biosynthetic pathway must be present for enzyme synthesis to be repressed.

Induction and repression are methods of regulating enzyme synthesis by controlling transcription of mRNA from the regulated genes in response to varying concentrations of substrates and products. The group of genes that provides a bacterial cell with this ability is called the ***operon***.

The Operon

The operon is a segment on the bacterial chromosome that consists of the following elements:

1- *Structural genes*:
These are genes that direct the synthesis of protein with related functions, such as the enzymes of a single metabolic pathway. The genes are physically adjacent to one another and are regulated as a unit.

2- A *regulator region consisting of a promoter and an operator*. Recall that the promoter is the site to which mRNA polymerase binds to the DNA and initiates transcription-in this case, of a polygenic message. The operator region, which lies between the promoter and the structural genes, is the binding site for a specific repressor protein. When the operator site is occupied by repressor, RNA polymerase cannot bind to the promoter on DNA, transcription is blocked, and protein synthesis is turned off.

3- A *repressor gene (R gene) located in another portion of the bacterial chromosome*. This gene directs the cell to produce the repressor protein that attaches to the operator region. The repressor protein has the additional ability to bind specifically with a small *effector*, the molecule that determines whether an operon will be turned on or turned off.

Inducible Operon

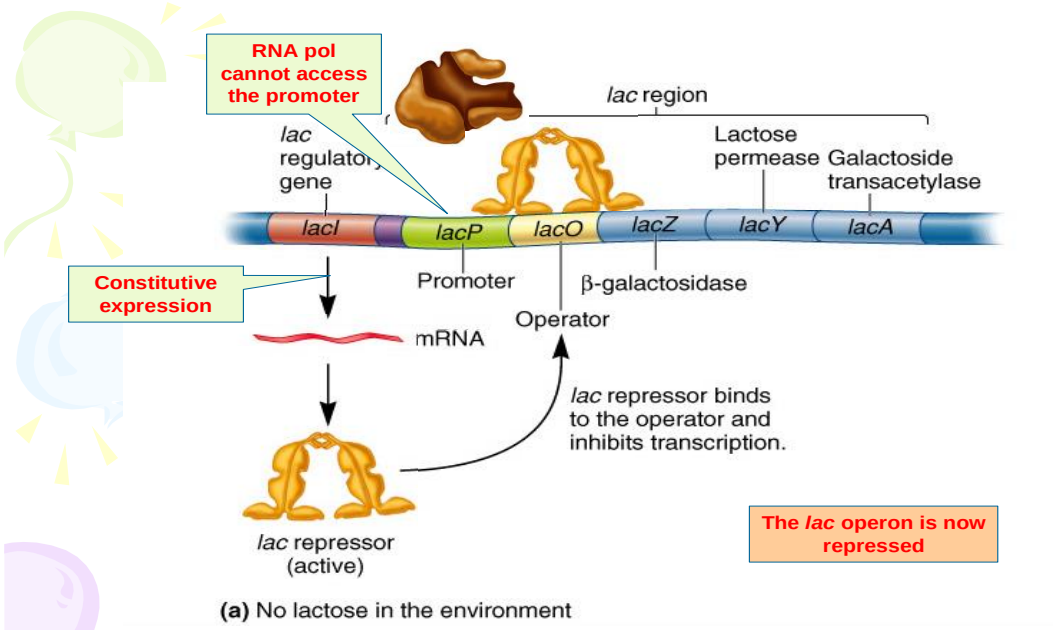
For many catabolic pathways, the substrate must be present before the pathway's enzymes can be produced. The operon is not expressed when the substrate (inducer) is not available to prevent the repressor from binding to the operator, In this state, transcription and enzyme synthesis are blocked. When present, the substrate induces the transcription of the operon by inactivating the repressor and preventing it from binding to the operator. Thus, if a bacterium is inoculated into a medium containing a new catabolite, such as lactose, the cell cannot use the substrate immediately. A lag period occurs until the sugar induces the formation of lactose-digesting enzymes.

The *lac* Operon

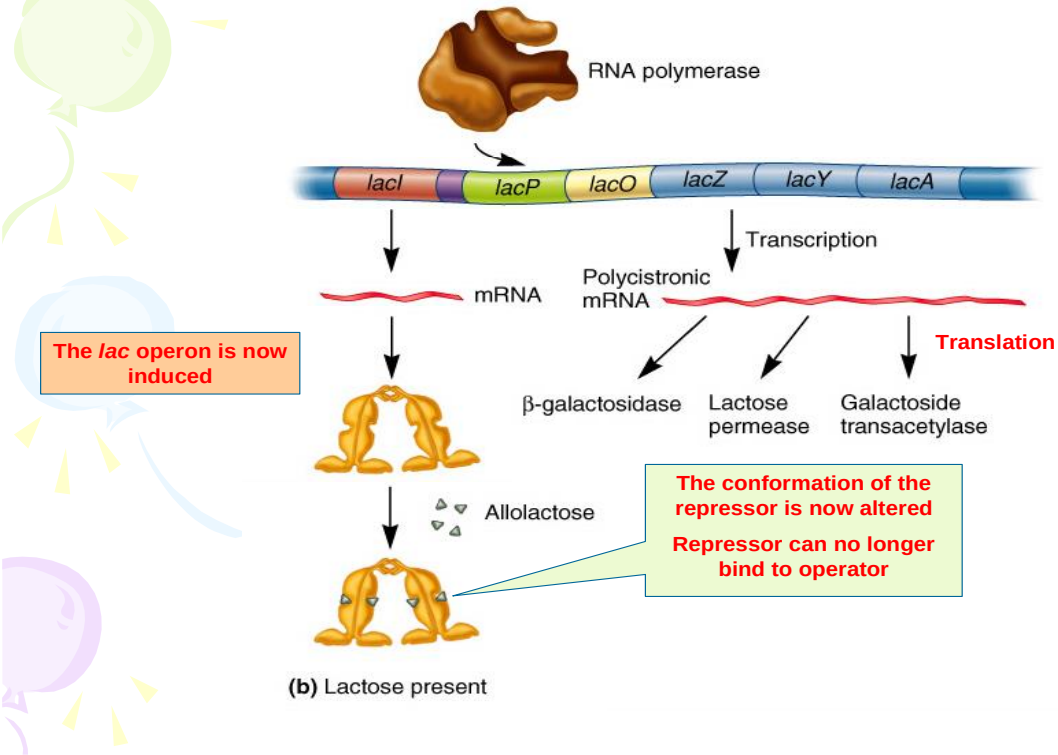
The *lac* operon consists of:

- T**hree structural genes** each involved in processing the sugar lactose
  - One of them is the gene for the enzyme **β-galactosidase** (tetrameric enzyme). This enzyme hydrolyses lactose into glucose and galactose
  - **Permease** (single unit protein)(Carrier protein)
  - **Transacetylase** (Two subunits)
- **Regulatory gene (*i*)** code for regulatory protein (repressor) consist of four subunits (tetramer).
- **Control region** consists of **Promoter** and **Operator**

All these gene are adjacent to each other (regulatory gene, control region and structural gene).

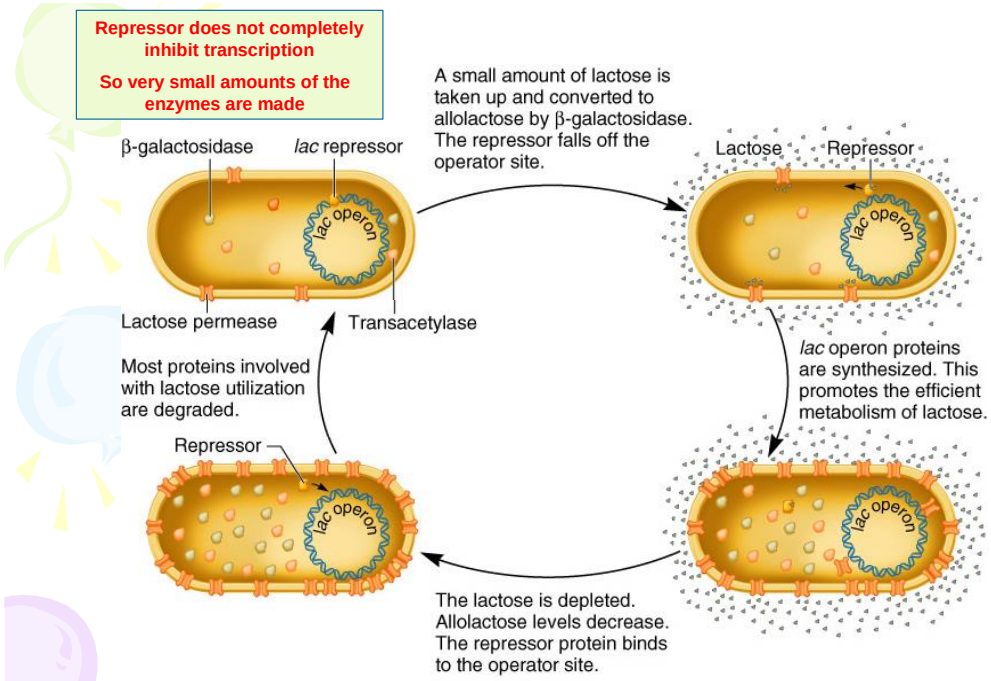


In the absence of inducer allolactose, the repressor protein is tightly bound to the operator site, thereby inhibiting the ability of RNA polymerase to transcribe the operon. This is called **negative control** of lactose operon.



When there is sufficient allolactose, it binds to the repressor. This alters the conformation of the repressor protein in such a way as to prevent it from binding to the operator site. Therefore, RNA polymerase can transcribe the operon.

This is called **positive control** of lactose operon.



The cycle of *lac* operon induction and repression

The promoter of lac operon is weak (RNA polymerase can not easily find promoter region), in this case need helping protein (CAP " catabolite repressor). In *E.coli* cell the cAMP concentration is inversely proportion with glucose concentration. The helping protein has high affinity to bind with cAMP, fprming a complex called cAMP-CAP, this complex binds to the promoter region and advice the RNA polymerase to bind with it (with promoter).

Under condition of low extracellular glucose, cAMP is made. This small effector molecule binds to CAP, causing it to change its conformation and bind to the region adjacent to the *lac* promoter. The binding of the CAP-cAMP complex to this region facilitates the binding of RNA polymerase to the promoter. This leads to an enhanced rate of transcription of the *lac* operon. By comparison, the *lac* repressor protein binds to the operator and inhibits the operon when lactose levels are low. The next figure illustrates how the lac operon will be regulated depending on the levels of cAMP and lactose within the cell.

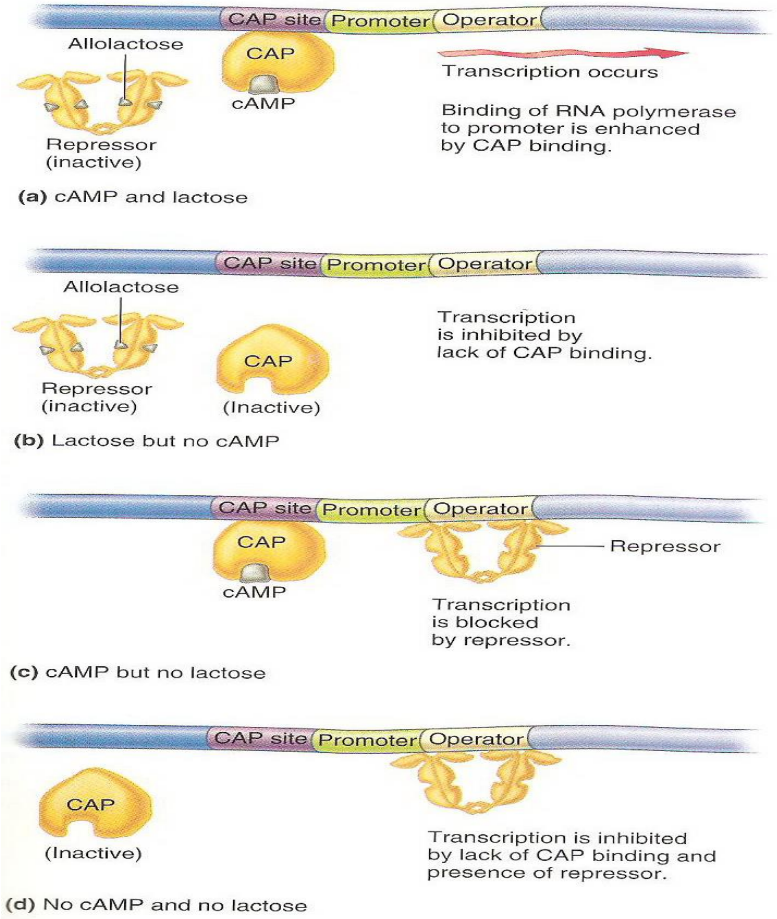


Figure: The roles of *lac* repressor and catabolite activator protein (CAP) in the regulation of the *lac* operon.

Catabolite Repression

Although enzymes for lactose metabolism are inducible, the glucose-digesting glycolytic enzymes are constitutive. What enzymes, then, would you expect to find in *E.coli* growing on a mixture of glucose and lactose? Next figure shows the growth curve observed under such circumstances. The bacteria initially use glucose, and for the first several hours few lactose-catabolizing enzymes are present.

Only when the glucose is depleted are the enzymes that allow lactose metabolism induced. Despite the presence of the inducer (lactose), the lactose operon is not activated during growth on glucose. This phenomenon, which is referred to as **catabolite repression**, provides the bacterium with considerable energy saving. As long as glucose is available, the bacterium avoids the expense of making the lactose-utilizing enzymes (lactose is not needed because glucose supplies all the cells' energy needs).

How can a cell "choose" to preferentially use glucose over lactose? This occurs because transcription of the lactose genes requires more than the presence of the inducer. For RNA polymerase to be attached to the open promoter, another molecule, called *cyclic AMP* (cAMP), must be present. In the presence of glucose, cAMP is converted to ATP, which depletes cAMP levels and prevents the induction of the lactose operon. Once glucose is consumed, the levels of cAMP in the cell increase, and the lactose operon is induced.

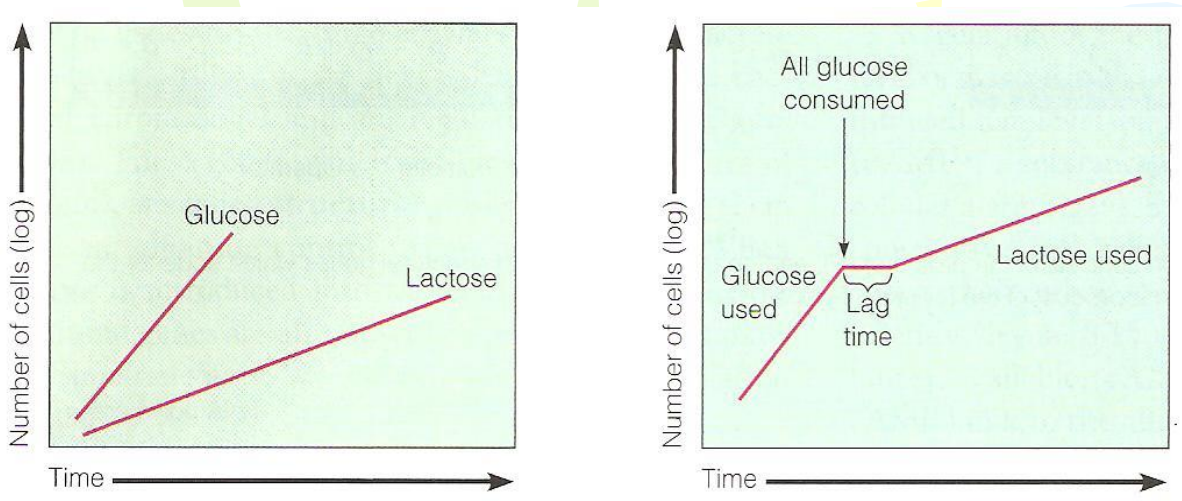
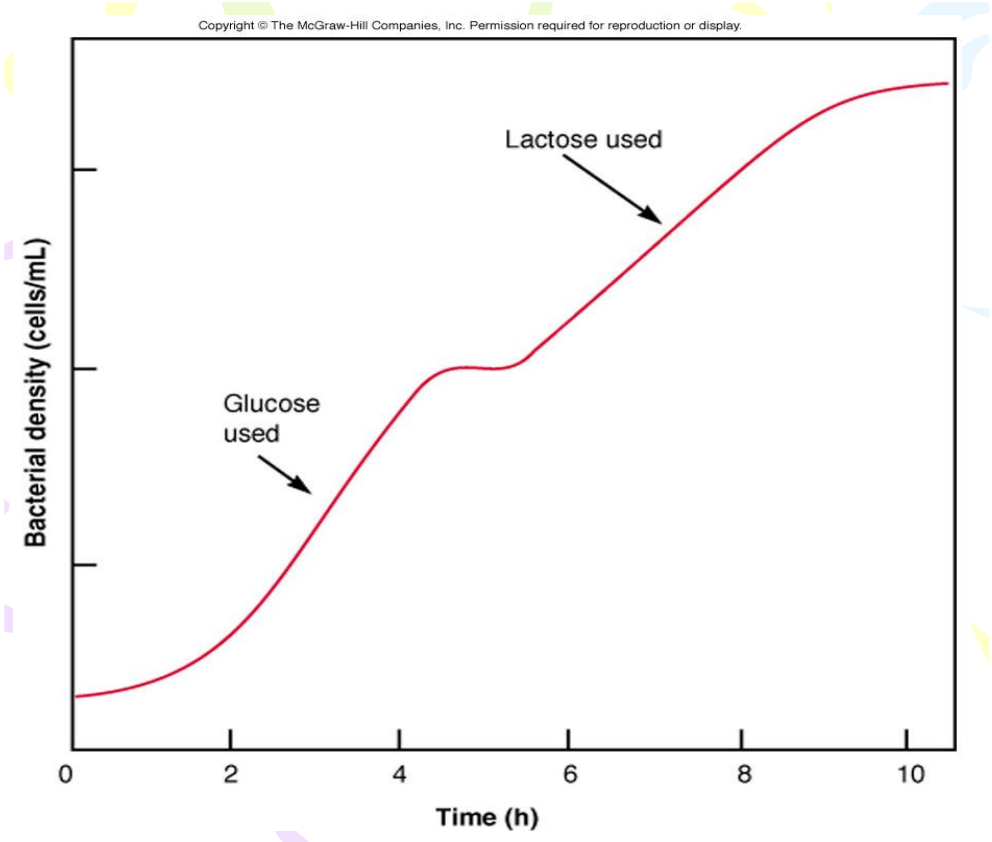


FIGURE **The growth rate of *E. coli* on glucose and lactose.** The steeper the straight line, the faster the growth. **(a)** Bacteria growing on glucose as the sole carbon source grow faster than on lactose. **(b)** Bacteria growing in a medium containing glucose and lactose first consume the glucose, and then, after a short lag time, the lactose. During the lag time, intracellular cyclic AMP increases, the *lac* operon is transcribed, more lactose is transported into the cell, and β-galactosidase is synthesized to break down lactose.



Repressible Operon

Repressible operons differ from inducible operons in several ways:

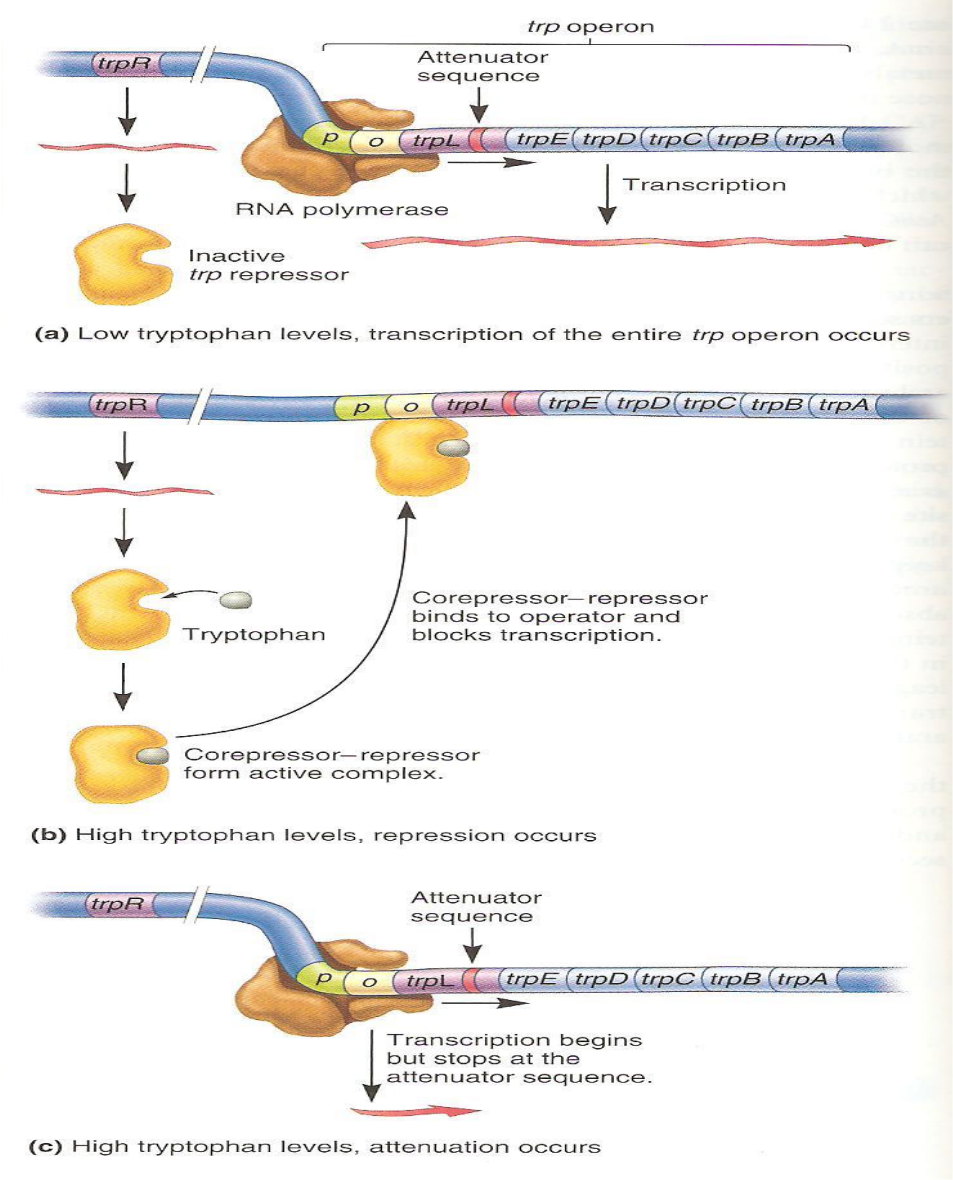
1- They produce and regulate the enzymes of biosynthetic pathways instead of catabolic ones.
2- The effector is the end product of the pathway, not the substrate.
3- The repressor protein is inactive in the absence of the effector. The effector therefore functions as a corepressor that must be present before the operon can be turned off.

The enzymes of repressible pathways are thus produced whenever the pathway's final product (P), which is also the corepressor, is in low concentration and therefore needed by the cell. Under such conditions, the repressor protein cannot bind to the operator, and the operon is expressed. When the product is in ample sypply, it activated the repressor, which binds to the operator and blocks transcription of the genes. This is a state of enzyme (or gene) repressor.

Tryptophan Operon

E. coli normally synthesizes the amino acids it needs for polypeptide synthesis; however, it can save energy by using amino acids available in its environment. In such cases *E. coli* represses the genes for a given amino acids synthesis pathway.

The *tryptophan (trp) operon*, which consists of promoter, an operator, leader sequence, and five genes that code for enzymes involved in the synthesis of tryptophan, is an example of such a repressible operon.



Organization of the trp operon and regulation via the trp repressor protein.

a- When tryptophan levels are low, tryptophan does not bind to the repressor protein; the latter thus cannot bind to the operator site. Under these conditions, RNA polymerase can transcribe the operon, which leads to the expression of the *trpE*, *trpD*, *trpC*, *trpB*, *trpA* genes. These genes encod3e enzymes involved in tryptophan biosynthesis.

b- When tryptophan levels are high, tryptophan acts as a corepressor that binds to the *trp* repressor protein. The tryptophan-*trp* repressor complex then binds to the operator site to inhibit transcription.

c- Another mechanism of regulation is attenuation. When attenuation occurs, the RNA is transcribed only to the attenuator sequence, and then transcription is terminated.

Attenuation

Some gene regulation occurs by attenuation, premature termination of transcription within the leader region of the mRNA. Several amino acids, for example, turn off their own production by attenuating genes that direct their synthesis. Messenger RNAs that are controlled by attenuation contain complementary nucleotide sequences in the region between the promoter and structural genes. These sequences can complex with each other, forming loops in the mRNA as it is transcribed.

One of these loops causes the RNA polymerase to detach from its template, and transcription ceases. The formation of this termination loop occurs only when there is a high concentration of the amino acids end product of the biosynthesis pathway. Remember that in prokaryotes, ribosome attach to the newly formed mRNA as soon as the leader sequence has been transcribed. When amino acid is present in high concentrations, there is also a pool of charged tRNA for that amino acids so the ribosomes move along the message without interruption.

As a consequence, the complementary sequences are transcribed and form a termination loop near the end of the leader sequence, the RNA polymerase is ejected from the DNA, and further synthesis of mRNA stops. This prevents wasteful overproduction of enzymes designed to synthesize a product already in ample supply in the cell. But when the amino acid end product is scarce, the concentrations of tRNA charged with that amino acid are low. So when the ribosome comes to the corresponding codon on the emerging mRNA, it pauses until the scarce tRNA is inserted. The complementary sequences exposed at that point fold in a different manner, and a termination loop is not formed. RNA polymerase continues transcribing the DNA template.

Control of enzyme synthesis by induction, repression, catabolite repression, and attenuation supplement feedback inhibition of enzyme activity regulation of cellular metabolism. Unlike feedback inhibition, the operon prevents the synthesis of unneeded enzymes, thereby conserving valuable resources.

The significance of operons to bacterial success goes far beyond their ability to respond to the presence of a nutrient or the need for an end product. Consider *Vibrio cholerae*, for example. This pathogen has one repressor protein that represses virulence genes that encode functions not needed until the pathogen enters a human host.

The repressor releases its grip on these virulence genes in response to some signal that "informs" the pathogen that it has entered a human host (the signal may be a temperature elevated to 37˚C, normal human body temperature). Once the repressor detaches from the operon, the bacterium begins producing its virulence factors. Several genes for pilli production are induced, allowing the pathogen to attach to intestinal epithelial cells.

The cholera toxin genes are expressed, creating in the host a profuse diarrhea that promotes the spread of the organism. None of these virulence properties are needed by the organism growing in water, its major reservoir, where the operon's repressor protein prevents their expression.

In other bacteria, endospore formation, diphtheria toxin production, siderophore (iron-binding protein) induction in response to changing iron concentrations, and countless other properties are regulated by operons.

(((Comparative properties of inducible and repressible operons)))

	Inducible	**Repressible**
Type of pathway regulated	Catabolic	Anabolic (biosynthesis)
Type of effector	Inducer	Corepressor
Relationship of effector to pathway	Substrate	End product
In absence of effector operon is	Repressed	Derepressed
In presence of effector operon is	Induced	Repressed
Native repressor is	Active	Inactive
Repressor-effector complex is	Inactive	Active