

## Regulation and Control of Metabolism in Bacteria      Dr.Neihaya Heikmat

Most bacteria are exposed to a constantly changing physical and chemical environment. Within limits, bacteria can react to changes in their environment through changes in patterns of structural proteins, transport proteins, toxins, enzymes, etc., which adapt them to a particular ecological situation.

Bacteria have developed sophisticated mechanisms for the regulation of both catabolic and anabolic pathways. Generally, bacteria do not synthesize degradative (catabolic) enzymes unless the substrates for these enzymes are present in their environment.

For example, synthesis of enzymes that degrade lactose would be wasteful unless the substrate for these enzymes (lactose) is available in the environment. Similarly, bacteria have developed diverse mechanisms for the control of biosynthetic (anabolic) pathways.

Bacterial cells shut down biosynthetic pathways when the end product of the pathway is not needed or is readily obtained by uptake from the environment. For example, if a bacterium could find a preformed amino acid like tryptophan in its environment, it would make sense to shut down its own pathway of tryptophan biosynthesis, and thereby conserve energy. However, in real bacterial life, the control mechanisms for all these metabolic pathways must be reversible, since the environment can change quickly and drastically.

### Conditions Affecting Enzyme Formation in Bacteria

As stated above, bacterial cells can change patterns of enzymes, in order to adapt them to their specific environment. Often the concentration of an enzyme in a bacterial cell depends on the presence of the substrate for the enzyme. **Constitutive enzymes** are always produced by cells independently of the composition of the medium in which the cells are grown. The enzymes that operate during glycolysis and the TCA cycle are generally constitutive: they are present at more or less the same concentration in cells at all times.

**Inducible enzymes** are produced ("turned on") in cells in response to a particular substrate; they are produced only when needed. In the process of induction, the

substrate, or a compound structurally similar to the substrate, evokes formation of the enzyme and is sometimes called an **inducer**.

A repressible enzyme is one whose synthesis is down regulated or "turned off" by the presence of (for example) the end product of a pathway that the enzyme normally participates in. In this case, the end product is called a **corepressor** of the enzyme.

## **Regulation of Enzyme Reactions**

Not all enzymatic reactions occur in a cell to the same extent. Some substances are needed in large amounts and the reactions involved in their synthesis must therefore occur in large amounts. Other substances are needed in small amounts and the corresponding reactions involved in their synthesis need only occur in small amounts.

In bacterial cells, enzymatic reactions may be regulated by two unrelated modes: (1) control or regulation of enzyme activity (feedback inhibition or end product inhibition), which mainly operates to regulate biosynthetic pathways; and

(2) control or regulation of enzyme synthesis, including end-product repression, which functions in the regulation of biosynthetic pathways, and enzyme induction and catabolite repression, which regulate mainly degradative pathways. The process of feedback inhibition regulates the activity of preexisting enzymes in the cells. The processes of end-product repression, enzyme induction and catabolite repression are involved in the control of synthesis of enzymes.

The processes which regulate the synthesis of enzymes may be either a form of positive control or negative control. End-product repression and enzyme induction are mechanisms of negative control, because they lead to a decrease in the rate of transcription of proteins. Catabolite repression is considered a form of positive control because it affects an increase in rates of transcription of proteins.

Bacteria exert control over their metabolism at every possible stage starting at the level of the gene that encodes for a protein and ending with alteration or modifications in the protein after it is produced.

For example, variation in gene structure can vary the activity or production of a protein, just as modifications of a protein after it is produced can alter or change its activity. One of the most important sites for control of metabolism at the genetic level is regulation of transcription.

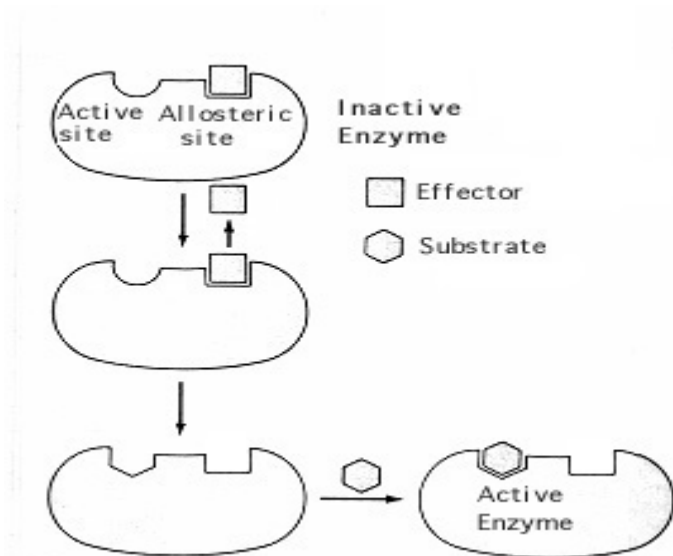
At this level, in positive control mechanisms (e.g. catabolite repression), a regulatory protein has an effect to increase the rate of transcription of a gene, while in negative control mechanisms (e.g. enzyme induction or end product repression), a regulatory protein has the effect to decrease the rate of transcription of a gene.

### **Allosteric Proteins**

The most common points of regulation are at the level of transcription (e.g. enzyme induction and enzyme repression) and changing the activity of preexisting proteins. In turn, these levels of control are usually modulated by proteins with the property of **allostery**.

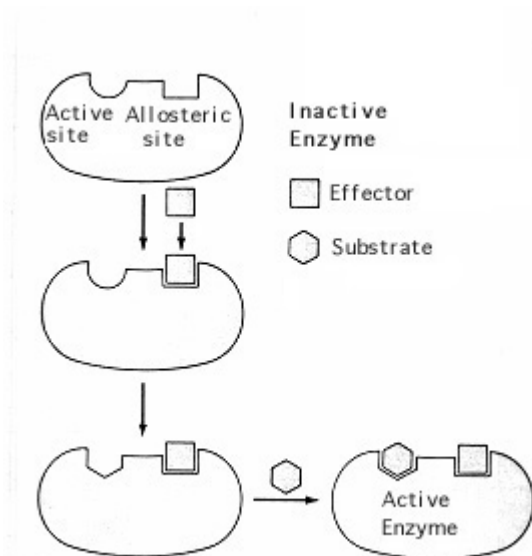
An allosteric protein is one which has an **active (catalytic) site** and an **allosteric (effector) site**. In an allosteric enzyme, the active site binds to the substrate of the enzyme and converts it to a product. The allosteric site is occupied by some small molecule which is not a substrate. However, when the allosteric site is occupied by the effector molecule, the configuration of the active site is changed so that it is now unable to recognize and bind to its substrate (Figure 1). If the protein is an enzyme, when the allosteric site is occupied, the enzyme is inactive, i.e., the effector molecule decreases the activity of the enzyme.

There is an alternative situation, however the effector molecule of certain allosteric enzymes binds to its allosteric site and consequently transforms the enzyme from an inactive to an active state (Figure 2). Some multicomponent allosteric enzymes have several sites occupied by various effectors' molecules that modulate enzyme activity over the arrange of conditions.



**Figure 1. Example of an allosteric enzyme with a negative effector site.**

When the effector molecule binds to the allosteric site, substrate binding and catalytic activity of the enzyme are inactivated. When the effector is detached from the allosteric site the enzyme is active.



**Figure 2. Example of an allosteric enzyme with a positive effector site.**

The effector molecule binds to the allosteric site resulting in alteration of the active site that stimulates substrate binding and catalytic activity. Some allosteric

proteins are not enzymes, but nonetheless have an active site and an allosteric site.

The regulatory proteins that control metabolic pathways involving end product repression, enzyme induction and catabolite repression are allosteric proteins. In their case, the active site is a DNA binding site, which, when active, binds to a specific sequence of DNA, and which, when inactive, does not bind to DNA. The allosteric or effector molecule is a small molecule which can occupy the allosteric site and affect the active site.

In the case of enzyme repression, a positive effector molecule (called a corepressor) binds to the allosteric regulatory protein and activates its ability to bind to DNA. In the case of enzyme induction a negative effector molecule (called an inducer) binds to the allosteric site, causing the active site to change conformation thereby detaching the protein from its DNA binding site.

### **Feedback Inhibition**

Feedback inhibition (or end product inhibition) is a mechanism for the inhibition of preformed enzymes that is seen primarily in the regulation of whole biosynthetic pathways, e.g. pathways involved in the synthesis of the amino acids. Such pathways usually involve many enzymatic steps, and the final (end) product is many steps removed from the starting substrate. By this mechanism, the final product is able to feed back to the first step in the pathway and to regulate its own biosynthesis.

In feedback inhibition, the end product of a biosynthetic pathway inhibits the activity of the first enzyme that is unique to the pathway, thus controlling production of the end product. The first enzyme in the pathway is an allosteric enzyme. Its allosteric site will bind to the end product (e.g. amino acid) of the pathway which alters its active site so that it cannot mediate the enzymatic reaction which initiates the pathway. Other enzymes in the pathway remain active, but they do not see their substrates.

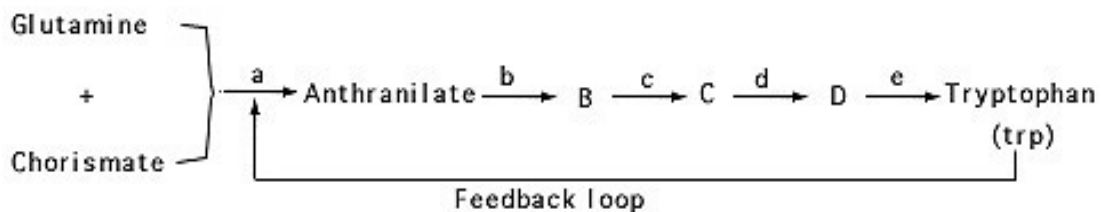
The pathway is shut down as long as adequate amounts of the end product are present. If the end product is used up or disappears, the inhibition is relieved, the

enzyme regains its activity, and the organism can resume synthesis of the end product.

One of the most intensely studied bacterial pathways is the pathway of tryptophan biosynthesis (Figure 3). The pathway of tryptophan biosynthesis is regulated by feed back inhibition. Tryptophan is the effector molecule for allosteric **enzyme a**. When the end product of the pathway (tryptophan) attaches to enzyme a, the enzyme is inactive and can no longer join glutamine and chorismic acid into anthranilate. If tryptophan is disjoined from the enzyme the pathway is resumed, and tryptophan synthesis will continue. Tryptophan biosynthesis is also regulated at a genetic level by the processes of enzyme repression and attenuation.

Note: In the case of feedback inhibition (above), the signal molecule, tryptophan, is a negative effector of Enzyme a in the pathway of tryptophan biosynthesis, because when it binds to Enzyme a, it inactivates the enzyme.

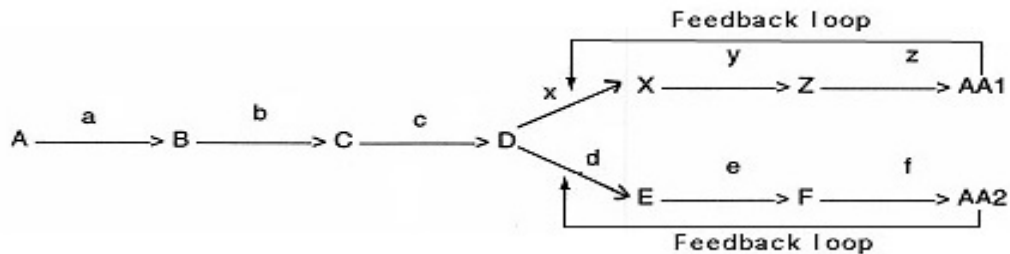
In enzyme repression (below) tryptophan is a signal molecule that acts as a positive effector of the trp repressor protein because when it binds to the repressor it activates the protein, so that it binds to the trp DNA.



**Figure 3. The pathway of tryptophan biosynthesis in *E. coli*.**

The pathway is regulated by the process of feedback inhibition. Tryptophan (trp), the end product of the pathway, is the effector molecule that binds to the allosteric site of Enzyme a, the first enzyme in the pathway. When trp is bound to the enzyme the catalytic (active) site of Enzyme a is altered so that it is unable to react with its substrates and the synthesis of anthranilate is inhibited.

If a metabolic pathway branches, leading to the synthesis of two amino acids, each end product (amino acid) can control its own synthesis without affecting the other (Figure 4). For example, the amino acids proline and arginine are both synthesized from glutamic acid. Each amino acid can regulate the first enzyme unique to its own synthesis without affecting the other, so that a surplus of arginine will not shut off the synthesis of proline and vice versa.



**Figure 4. Generalized scheme for regulation of a branched metabolic pathway by the process of feedback inhibition.**

### Enzyme Repression

Enzyme repression is a form of negative control (down-regulation) of bacterial transcription. This process, along with that of enzyme induction, is called negative control because a regulatory protein brings about inhibition of mRNA synthesis which leads to decreased synthesis of enzymes.

Although feedback inhibition shuts off synthesis of the end product of a pathway, it still allows some waste of energy and carbon if the cell continued to manufacture enzymes for which it has no use. It is the process of enzyme repression that prevents the synthesis of the enzymes concerned with the synthesis of that particular end product.

In the case of the pathway of tryptophan biosynthesis (Figure 3), the end product of the pathway, tryptophan, serves as an effector molecule that can shut down the synthesis of the Enzymes a, b, c, d, and e that are concerned with tryptophan biosynthesis. This results in saving of many molecules of ATP which must be

expended during protein synthesis, and it conserves amino acid precursors for synthesis of other proteins.

The process is slower to act than is feedback inhibition (which acts immediately) because pre-existing enzymes have to be diluted out as a result of cell division before its effects are seen.

## **Enzyme Induction**

In some cases, metabolites or substrates can turn on inactive genes so that they are transcribed. In the process of enzyme induction, the substrate, or a compound structurally similar to the substrate, evokes the formation of enzyme(s) which are usually involved in the degradation of the substrate.

Enzymes that are synthesized as a result of genes being turned on are called inducible enzymes and the substance that activates gene transcription is called the inducer. Inducible enzymes are produced only in response to the presence of a substrate and, in a sense, are produced only when needed. In this way the cell does not waste energy synthesizing unneeded enzymes.

The best known and best studied case of enzyme induction involves the enzymes of lactose degradation in *E. coli*. Only in the presence of lactose does the bacterium synthesize the enzymes that are necessary to utilize lactose as a carbon and energy source for growth. Two enzymes are required for the initial breakdown of lactose: lactose permease, which actively transports the sugar into the cell, and beta galactosidase, which splits lactose into glucose plus galactose.

The mechanism of enzyme induction is similar to end product repression in that a regulatory gene, a promoter, and an operator are involved, but a major difference is that the lac Repressor is active only in the absence of the inducer molecule (lactose).

In the presence of lactose, the Repressor cannot bind to the operator region, so that the genes for lactose transport and cleavage are transcribed. In the absence of



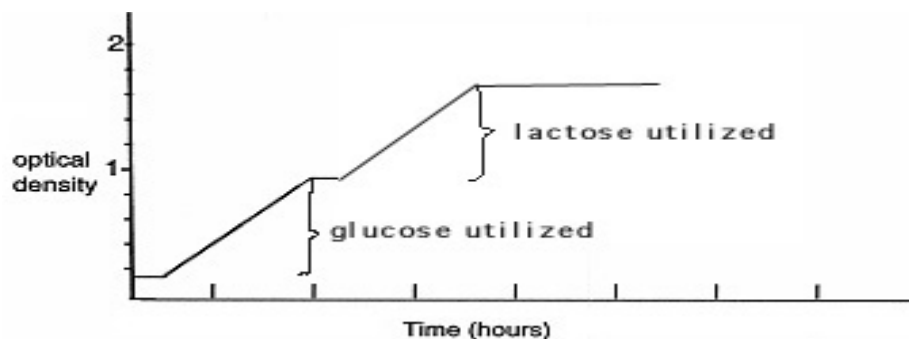
lactose, the Repressor is active and will bind to the operator with the result that the genes for lactose metabolism are not transcribed.

## Catabolite Repression

Enzyme Induction is still considered a form of negative control because the effect of the regulatory molecule (the active repressor) is to decrease or downregulate the rate of transcription. Catabolite repression is a type of positive control of transcription, since a regulatory protein affects an increase (upregulation) in the rate of transcription of an operon.

The process was discovered in *E. coli* and was originally referred to as the glucose effect because it was found that glucose repressed the synthesis of certain inducible enzymes, even though the inducer of the pathway was present in the environment.

The discovery was made during study of the regulation of lac operon in *E. coli*. Since glucose is degraded by constitutive enzymes and lactose is initially degraded by inducible enzymes, what would happen if the bacterium was grown in limiting amounts of glucose and lactose? A plot of the bacterial growth rate resulted in a **diauxic growth** curve which showed two distinct phases of active growth. During the first phase of exponential growth, the bacteria utilize glucose as a source of energy until all the glucose is exhausted. Then, after a secondary lag phase, the lactose is utilized during a second stage of exponential growth.



**The Diauxic Growth Curve of *E. coli* grown in limiting concentrations of a mixture of glucose and lactose**

During the period of glucose utilization, lactose is not utilized because the cells are unable to transport and cleave the disaccharide lactose. Glucose is always metabolized first in preference to other sugars. Only after glucose is completely utilized is lactose degraded. The lactose operon is repressed even though lactose (the inducer) is present. The ecological rationale is that glucose is a better source of energy than lactose since its utilization requires two less enzymes.

Only after glucose is exhausted are the enzymes for lactose utilization synthesized. The secondary lag during diauxic growth represents the time required for the complete induction of the lac operon and synthesis of the enzymes necessary for lactose utilization (lactose permease and beta-galactosidase).

Only then does bacterial growth occur at the expense of lactose. Since the availability of glucose represses the enzymes for lactose utilization, this type of repression became known as **catabolite repression** or the glucose effect.

Glucose is known to repress a large number of inducible enzymes in many different bacteria. Glucose represses the induction of inducible operons by inhibiting the synthesis of cyclic AMP (cAMP), a nucleotide that is required for the initiation of transcription of a large number of inducible enzyme systems including the lac operon.

As a form of catabolite repression, the glucose effect serves a useful function in bacteria: it requires the cells to use the best available source of energy. For many bacteria, glucose is the most common and readily utilizable substrate for growth. Thus, it inhibits indirectly the synthesis of enzymes that metabolize poorer sources of energy.

### **Regulation of glycogen metabolism in yeast and bacteria.**

Microorganisms have the capacity to utilize a variety of nutrients and adapt to continuously changing environmental conditions. Many microorganisms, including yeast and bacteria, accumulate carbon and energy reserves to cope with the starvation conditions temporarily present in the environment. Glycogen biosynthesis is a main strategy for such metabolic storage, and a variety of

sensing and signaling mechanisms have evolved in evolutionarily distant species to ensure the production of this homopolysaccharide. At the most fundamental level, the processes of glycogen synthesis and degradation in yeast and bacteria share certain broad similarities.

However, the regulation of these processes is sometimes quite distinct, indicating that they have evolved separately to respond optimally to the habitat conditions of each species. The mechanisms, both at the transcriptional and at the post-transcriptional level, that regulate glycogen metabolism in yeast and bacteria, focusing on selected areas where the greatest increase in knowledge has occurred during the last few years.

In the yeast system, we focus particularly on the various signaling pathways that control the activity of the enzymes of glycogen storage. In the case of bacterial glycogen, special emphasis is placed on aspects related to the genetic regulation of glycogen metabolism and its connection with other biological processes.