BACTERIOLOGY - CHAPTER NINE

GENETIC REGULATORY MECHANISMS

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REGULATION OF GENE EXPRESSION

Bacteria do not make all the proteins that they are capable of making all of the time. Rather, they can adapt to their environment and make only those gene products that are essential for them to survive in a particular environment. For example, bacteria do not synthesize the enzymes needed to make tryptophan when there is an abundant supply of tryptophan in the environment. However, when tryptophan is absent from the environment the enzymes are made. Similarly, just because a bacterium has a gene for resistance to an antibiotic does not mean that that gene will be expressed. The resistance gene may only be expressed when the antibiotic is present in the environment.

Bacteria usually control gene expression by regulating the level of mRNA transcription. In bacteria, genes with related function are generally located adjacent to each other and they are regulated coordinately (*i.e.* when one is expressed, they all are expressed). Coordinate regulation of clustered genes is accomplished by regulating the production of a polycistronic mRNA (*i.e.* a large mRNA containing the information for several genes). Thus, bacteria are able to "sense" their environment and express the appropriate set of genes needed for that environment by regulating transcription of those genes.

Inducible genes - The operon model

Definition

An inducible gene is a gene that is expressed in the presence of a substance (an inducer) in the environment. This substance can control the expression of one or more genes (structural genes) involved in the metabolism of that substance. For example, lactose induces the expression of the *lac* genes that are involved in lactose metabolism. An certain antibiotic may induce the expression of a gene that leads to resistance to that antibiotic.

Induction is common in metabolic pathways that result in the catabolism of a substance and the inducer is normally the substrate for the pathway.

Lactose Operon

Structural genes
The lactose operon (figure 1) contains three structural genes that code for enzymes involved in lactose metabolism.

- The *lac z* gene codes for β-galactosidase, an enzyme that breaks down lactose into glucose and galactose
- The *lac y* gene codes for a permease, which is involved in uptake of lactose
- The *lac a* gene codes for a galactose transacetylase.

These genes are transcribed from a common promoter into a polycistronic mRNA, which is translated to yield the three enzymes.

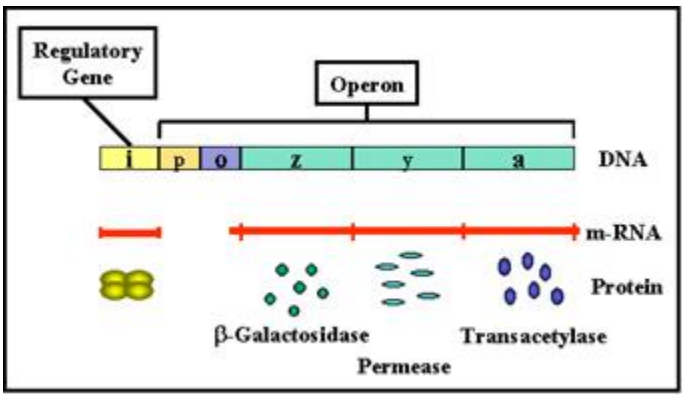

Figure 1

Regulatory gene
The expression of the structural genes is not only influenced by the presence or absence of the inducer, it is also controlled by a specific regulatory gene. The regulatory gene may be next to or far from the genes that are being regulated. The regulatory gene codes for a specific protein product called a REPRESSOR.

Operator
The repressor acts by binding to a specific region of the DNA called the operator which is adjacent to the structural genes being regulated. The structural genes together with the operator region and the promoter is called an OPERON. However, the binding of the repressor to the operator is prevented by the inducer and the inducer can also remove repressor that has already bound to the operator. Thus, in the presence of the inducer the repressor is inactive and does not bind to the operator, resulting in transcription of the structural genes. In contrast, in the absence of inducer the repressor is active and binds to the operator, resulting in inhibition of transcription of the structural genes. This kind of control is referred to a NEGATIVE CONTROL since the function of the regulatory gene product (repressor) is to turn off transcription of the structural genes.

Inducer

Transcription of the lac genes is influenced by the presence or absence of an inducer (lactose or other β-galactosides) (Figure 2).

e.g	+ inducer	expression
	- inducer	no expression

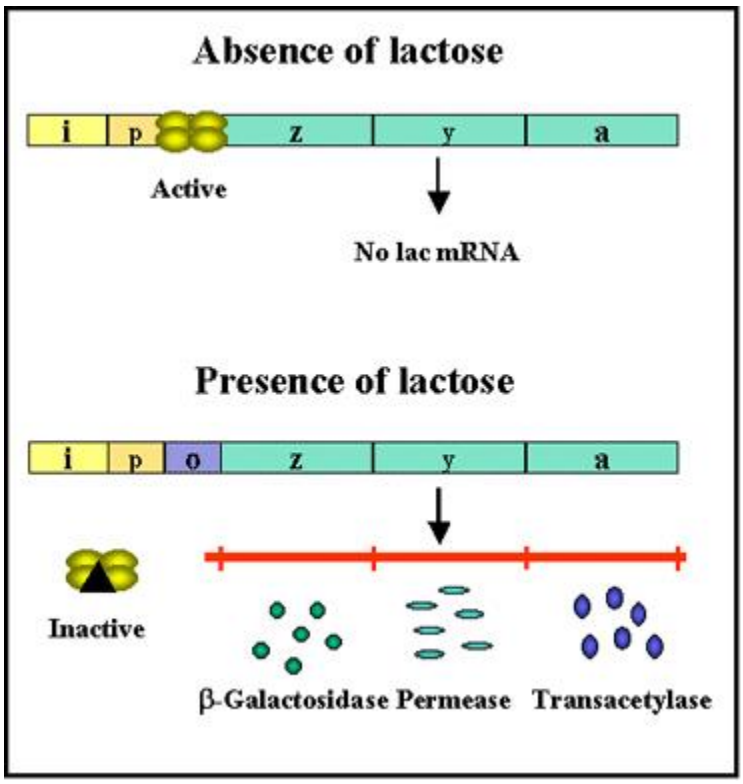

Figure 2

Catabolite repression (Glucose Effect)

Many inducible operons are not only controlled by their respective inducers and regulatory genes, but they are also controlled by the level of glucose in the environment. The ability of glucose to control the expression of a number of different inducible operons is called CATABOLITE REPRESSION. This is illustrated in Figure 3.

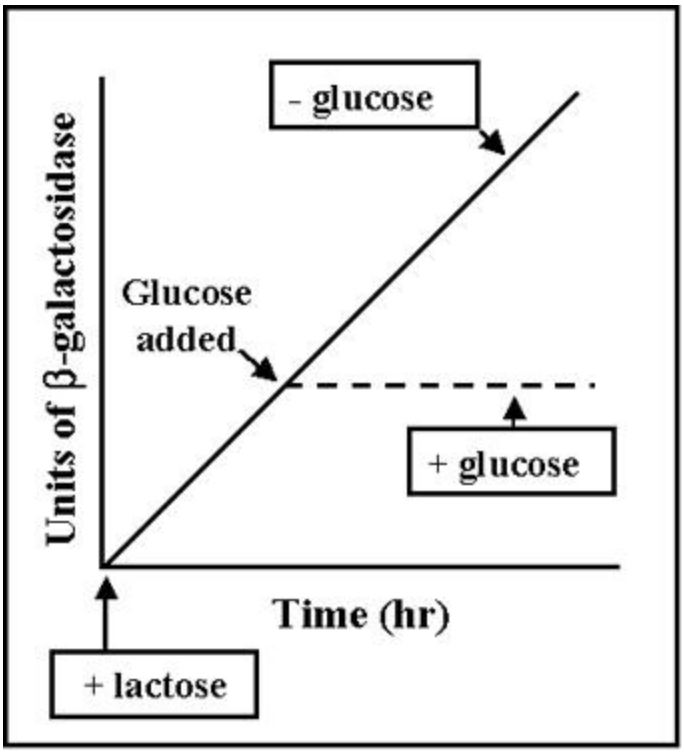

Figure 3

Catabolite repression is generally seen in those operons which are involved in the degradation of compounds used as a source of energy. Since glucose is the preferred energy source in bacteria, the ability of glucose to regulate the expression of other operons ensures that bacteria will utilize glucose before any other carbon source as a source of energy.

Mechanism

There is an inverse relationship between glucose levels and cyclic AMP (cAMP) levels in bacteria. When glucose levels are high cAMP levels are low and when glucose levels are low cAMP levels are high. This relationship exists because the transport of glucose into the cell inhibits the enzyme adenyl cyclase which produces cAMP. In the bacterial cell cAMP binds to a cAMP binding protein called CAP or CRP. The cAMP-CAP complex, but not free CAP protein, binds to a site in the promoters of catabolite repression-sensitive operons. The binding of the complex results in a more efficient promoter and thus more initiations of transcriptions from that promoter as illustrated in Figures 4 and 5. Since the role of the CAP-cAMP complex is to turn on transcription this type of control is said to be POSITIVE CONTROL. The consequences of this type of control is that to achieve maximal expression of a catabolite repression sensitive operon glucose must be absent from the environment and the inducer of the operon must be present. If both are present, the operon will not be maximally expressed until glucose is metabolized. Obviously, no expression of the operon will occur unless the inducer is present.

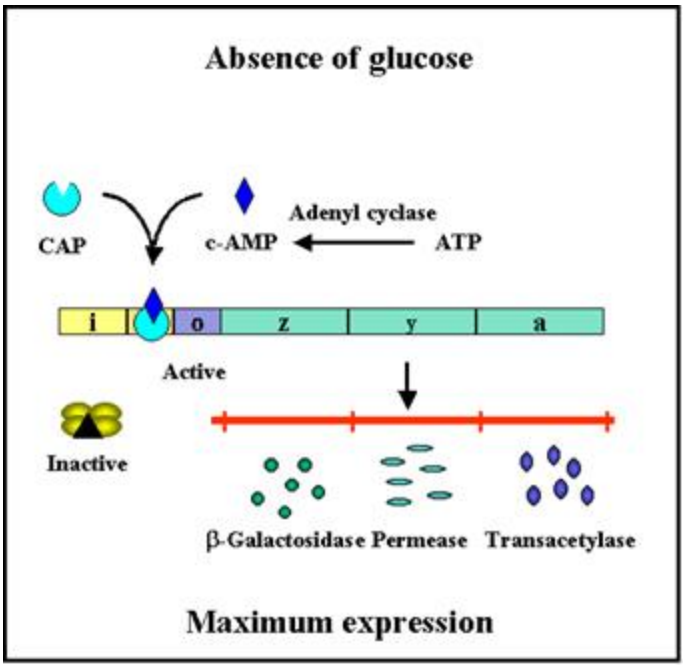

Figure 4

Repressible genes - The operon model

Definition

Repressible genes are those in which the presence of a substance (a co-repressor) in the environment turns off the expression of those genes (structural genes) involved in the metabolism of that substance.

e.g., Tryptophan represses the expression of the trp genes.

Repression is common in metabolic pathways that result in the biosynthesis of a substance and the co-repressor is normally the end product of the pathway being regulated.

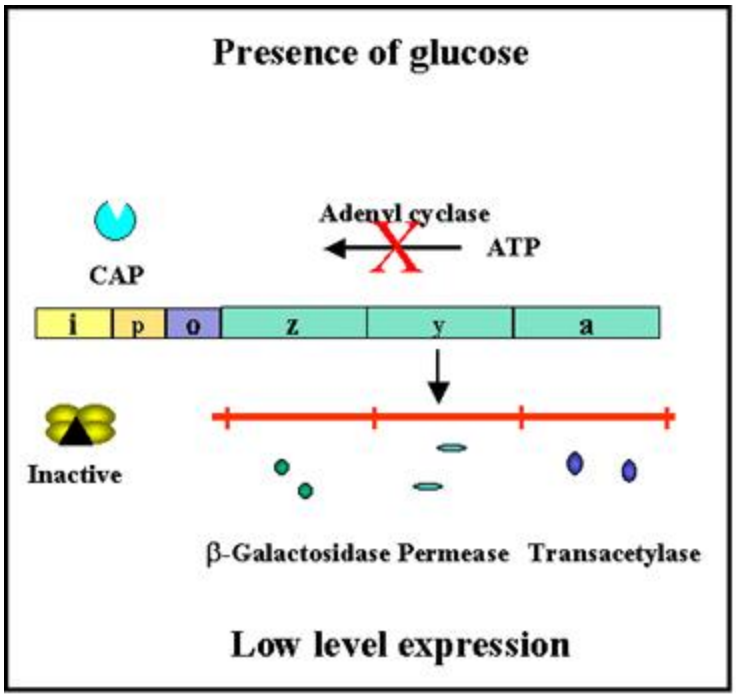

Figure 5

Tryptophan operon

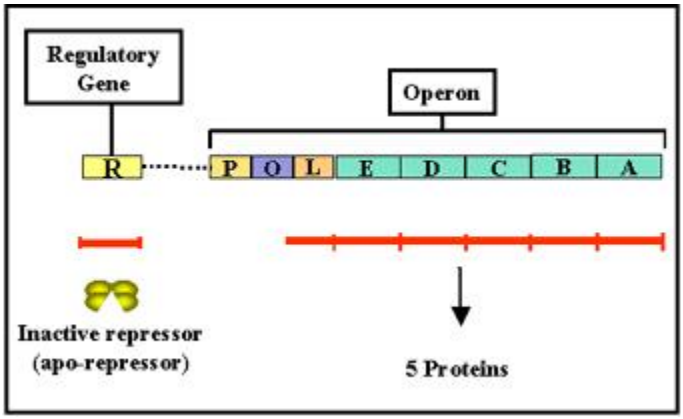

Figure 6

Structural genes

The tryptophan operon (figure 6) contains five structural genes that code for enzymes involved

in the synthesis of tryptophan. These genes are transcribed from a common promoter into a polycistronic mRNA, which is translated to yield the five enzymes.

Regulatory gene

The expression of the structural genes is not only influenced by the presence or absence of the co-repressor, it is also controlled by a specific regulatory gene. The regulatory gene may be next to or far from the genes that are being regulated. The regulatory gene codes for a specific protein product called an REPRESSOR (sometimes called an apo-repressor). When the repressor is synthesized it is inactive. However, it can be activated by complexing with the co-repressor (*i.e.* tryptophan).

Operator

The active repressor/co-repressor complex acts by binding to a specific region of the DNA called the operator which is adjacent to the structural genes being regulated. The structural genes together with the operator region and the promoter is called an OPERON. Thus, in the presence of the co-repressor the repressor is active and binds to the operator, resulting in repression of transcription of the structural genes. In contrast, in the absence of co-repressor the repressor is inactive and does not bind to the operator, resulting in transcription of the structural genes. This kind of control is referred to a NEGATIVE CONTROL since the function of the regulatory gene product (repressor) is to turn off transcription of the structural genes.

Co-repressor

Transcription of the tryptophan genes is influenced by the presence or absence of a co-repressor (tryptophan) (Figure 7).

e.g.	+ co-repressor	no expression
	- co-repressor	expression

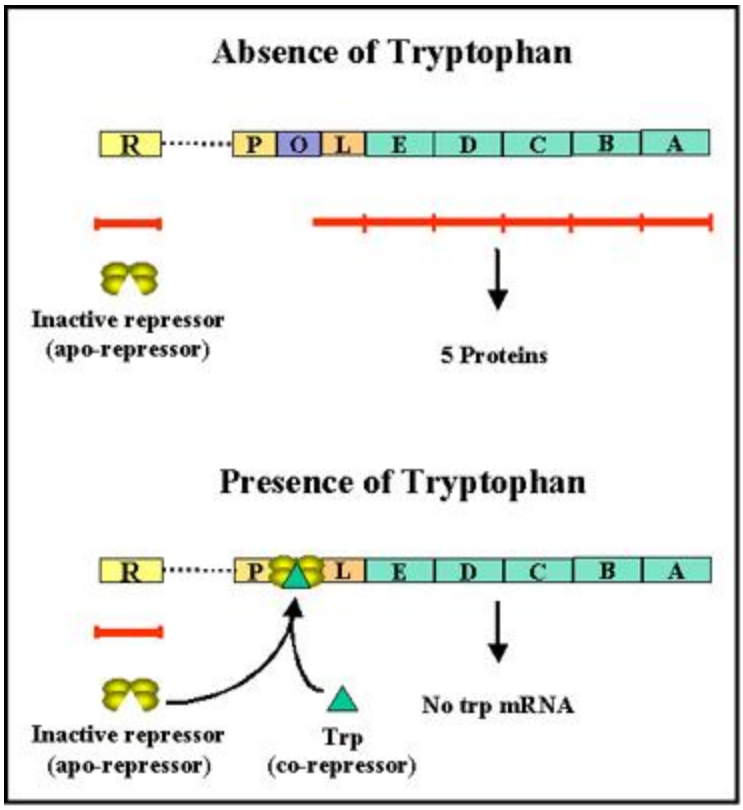

Figure 7

Attenuation

In many repressible operons, transcription that initiates at the promoter can terminate prematurely in a leader region that precedes the first structural gene. (*i.e.* the polymerase terminates transcription before it gets to the first gene in the operon). This phenomenon is called ATTENUATION; the premature termination of transcription. Although attenuation is seen in a number of operons, the mechanism is best understood in those repressible operons involved in amino acid biosynthesis. In these instances attenuation is regulated by the availability of the cognate aminoacylated t-RNA.

Mechanism (See Figure 8)
When transcription is initiated at the promoter, it actually starts before the first structural gene and a leader transcript is made. This leader region contains a start and a stop signal for protein synthesis. Since bacteria do not have a nuclear membrane, transcription and translation can occur simultaneously. Thus, a short peptide can be made while the RNA polymerase is transcribing the leader region. The test peptide contains several tryptophan residues in the middle of the peptide. Thus, if there is a sufficient amount of tryptophanyl-t-RNA to translate that test peptide, the entire peptide will be made and the ribosome will reach the stop signal. If,

on the other hand, there is not enough tryptophanyl-t-RNA to translate the peptide, the ribosome will be arrested at the two tryptophan codons before it gets to the stop signal.

The sequence in the leader m-RNA contains four regions, which have complementary sequences (Figure 9). Thus, several different secondary stem and loop structures can be formed. Region 1 can only form base pairs with region 2; region 2 can form base pairs with either region 1 or 3; region 3 can form base pairs with region 2 or 4; and region 4 can only form base pairs with region 3. Thus three possible stem/loop structures can be formed in the RNA.

region 1:region 2

region 2:region 3

region 3:region 4

One of the possible structures (region 3 base pairing with region 4) generates a signal for RNA polymerase to terminate transcription (*i.e.* to attenuate transcription). However, the formation of one stem and loop structure can preclude the formation of others. If region 2 forms base pairs with region 1 it is not available to base pair with region 3. Similarly if region 3 forms base pairs with region 2 it is not available to base pair with region 4.

The ability of the ribosomes to translate the test peptide will affect the formation of the various stem and loop structures Figure 10. If the ribosome reaches the stop signal for translation it will be covering up region 2 and thus region 2 will not available for forming base pairs with other regions. This allows the generation of the transcription termination signal because region 3 will be available to pair with region 4. Thus, when there is enough tryptophanyl-t-RNA to translate the test peptide attenuation will occur and the structural genes will not be transcribed. In contrast, when there is an insufficient amount of tryptophanyl-t-RNA to translate the test peptide no attenuation will occur. This is because the ribosome will stop at the two tryptophan codons in region 1, thereby allowing region 2 to base pair with region 3 and preventing the formation of the attenuation signal (*i.e.* region 3 base paired with region 4). Thus, the structural genes will be transcribed.

REGULATION OF ENZYME ACTIVITY

Bacteria also have ways of regulating the activities of their enzymes.

Feedback Inhibition

The activity of bacterial enzymes is often subject to feedback inhibition. Usually it is the end

product of a pathway that is the inhibitor and the first enzyme in the pathway is the step that is regulated.

Epigenetic Modification

The activities of bacterial enzymes can also be regulated by covalent modifications of enzymes. Such modifications are called EPIGENETIC MODIFICATIONS.

e.g. Adenylation of glutamine synthetase

Phosphorylation of glycogen synthetase

Usually these modifications are reversible so that the activities of the enzymes can be turned on and off.