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Regulation of Gene Expression

The *lac* Operon

Overview:

Gene expression refers to the multistep process that ultimately results in the production of a functional gene product, <u>either ribonucleic acid (RNA) or protein.</u> The first step in gene expression—the use of DNA for the synthesis of RNA (transcription)—is the primary site of regulation in both prokaryotes and eukaryotes. In eukaryotes, however, gene expression also involves extensive post - transcriptional and posttranslational processes, as well as actions that influence access to particular regions of the DNA. Each of these steps can be regulated to provide additional control over the kinds and amounts of functional products that are produced.

Not all genes are regulated. For example, genes described as constitutive encode products required for basic cellular functions, and so are continually expressed; they are also known as "housekeeping" genes. Regulated genes, however, are expressed only under certain conditions. They may be expressed in all cells or in only a subset of cells, for example, hepatocytes. The ability to regulate gene expression, that is, to determine if, how much, and when particular gene products will be made, gives the cell control over structure and function. <u>It is the basis for cellular differentiation, morphogenesis, and adaptability of any organism.</u> Control of gene expression is best understood in prokaryotes, but many themes are repeated in eukaryotes.

Regulation of Prokaryotic Gene Expression

In prokaryotes such as *Escherichia coli (E. coli),* regulation of gene expression occurs primarily at the level of transcription and, in general, is mediated by the binding of trans-acting proteins to cis-acting regulatory elements on their single DNA molecule (chromosome). [Note: Regulating the first step in the expression of a gene is an efficient approach, as energy is not wasted making unneeded gene products.] Transcriptional control in prokaryotes can involve the initiation or premature termination of transcription.

A. Transcription of mRNA from bacterial operons

In bacteria, the structural genes that code for proteins involved in a particular metabolic pathway are often found sequentially grouped on the chromosome along with the cis-acting regulatory

elements that determine the transcription of these genes. The transcription product is a single polycistronic messenger RNA (mRNA). The genes are thus coordinately controlled, that is, turned on or off as a unit. This entire package is referred to as an **operon**.

B. Role of operators in prokaryotic transcription

Prokaryotic operons contain an operator—a segment of DNA that regulates the activity of the structural genes of the operon. If the operator is not bound by a repressor molecule, RNA polymerase passes over the operator and reaches the protein-coding genes which it transcribes to mRNA. If a repressor molecule is bound to the operator, the polymerase is blocked and does not produce mRNA. As long as the repressor is bound to the operator, no proteins are made. However, when an inducer molecule is present, it binds to the repressor, causing the repressor to change shape so that it no longer binds the operator. When this happens, the RNA polymerase can proceed with transcription. One of the best-understood examples is the lactose operon of *E. coli*, which illustrates both positive and negative regulation.

C. The lactose operon (*Lac* operon) (Inducible Operon)

The lactose (lac) operon contains the genes that code for three proteins involved in the catabolism of the disaccharide, lactose: The lacZ gene codes for β-galactosidase, which hydrolyzes lactose to galactose and glucose; the lacY gene, which codes for a permease that facilitates the movement of lactose into the cell; and the lacA gene that codes for thiogalactoside transacetylase which functions as a possible detoxifier in the cell. Lac A is the only gene which is not required for lactose metabolism.

Structure of *Lac* operon gene | Lactose Metabolism (lactose hydrolysis)
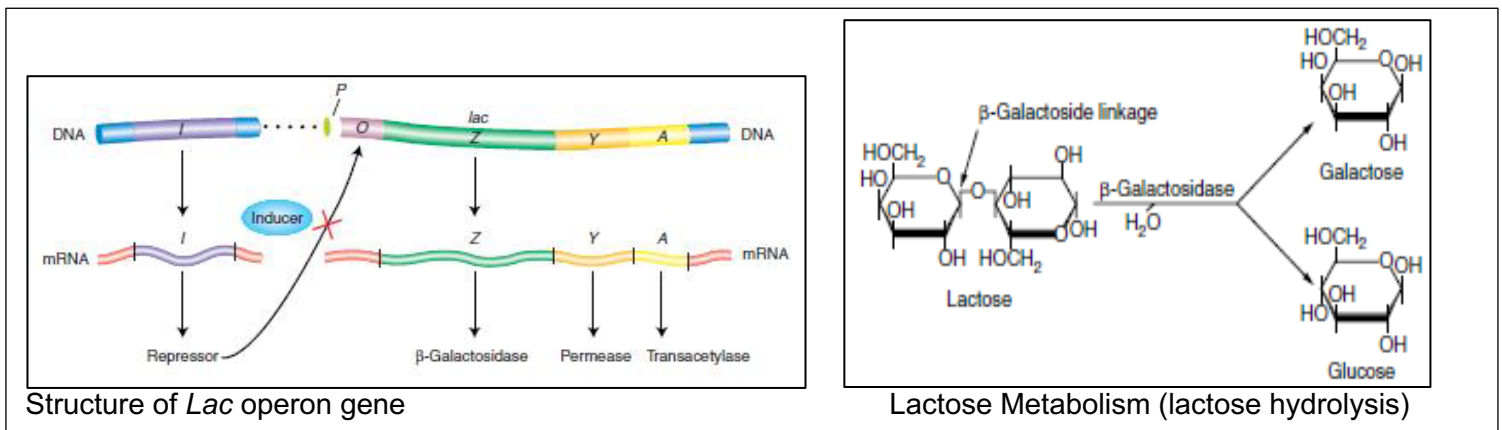
Bacteria use glucose as a fuel in preference to any other sugar.] **why?** The regulatory portion of

the operon is upstream of the three structural genes, and consists of the promoter (P) region where RNA polymerase binds, and two additional sites, <u>the operator (O) site and the CAP site</u>, where regulatory proteins bind. **The *lacZ, lacY, and lacA* genes are expressed only when the O site is empty, and the CAP site is bound by a complex of cyclic adenosine monophosphate** (cAMP) and the catabolite gene activator protein or CAP (sometimes called the cAMP regulatory protein or CRP). A regulatory gene, the *lacI* gene, codes for the repressor protein (a trans-acting factor) that binds to the operator site. [Note: The *lacI* gene has its own promoter.]

The *lacI* gene is excluded from the lac operon defining region. However it has an important role in transcriptional regulation in the form of ==interaction between repressor, operator and inducer.==

The lactose operon of *E. coli*.
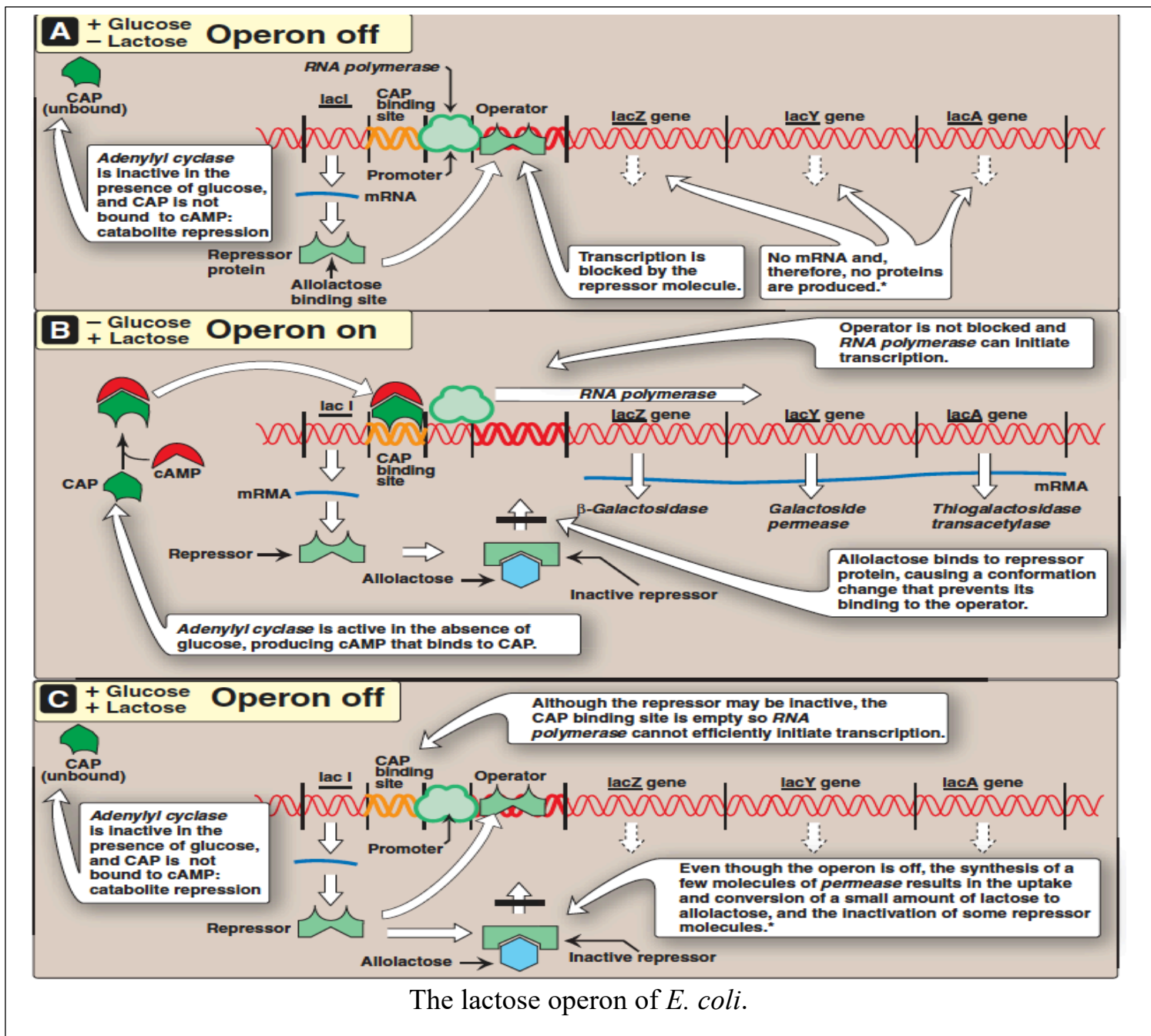
1. When glucose is the only sugar available: In this case, the lac operon is repressed <mark>(turned off).</mark> Repression is mediated by the repressor protein binding to the operator site, which is downstream of the promoter region (Fig-A). Binding of the repressor interferes with the progress of RNA polymerase, and blocks transcription of the structural genes. This is an example of <mark>negative regulation</mark>.
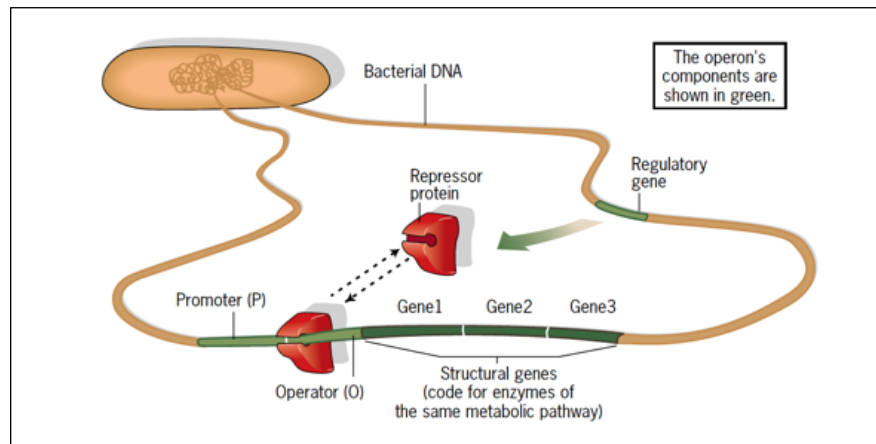

2. When only lactose is available: In this case, the lac operon is induced (maximally expressed or <mark>turned on</mark>). A small amount of lactose is converted to an isomer, allolactose. This compound is an inducer that binds to the repressor protein, changing the conformation of the repressor protein so that it can no longer bind to the operator. In the absence of glucose, adenylyl cyclase is active, and sufficient quantities of cAMP are made and bind to the CAP protein. The cAMP–CAP trans-acting complex binds to the CAP-binding site, causing RNA polymerase to more efficiently initiate transcription at the promoter site (Figure-B). This is an example of <mark>positive regulation</mark>. The transcript is a single polycistronic mRNA molecule that contains three sets of start and stop codons. Translation of the mRNA produces the three proteins that allow lactose to be used for energy production by the cell. [Note: In contrast to the inducible *lacZ, lacY, and lacA* genes, whose expression is regulated, the *lacI* gene is constitutive. Its gene product, the repressor protein, is active unless the inducer is present.]

3. When both glucose and lactose are available: In this case, transcription of the lac operon is negligible, even if lactose is present at a high concentration. Adenylyl cyclase is deactivated in the presence of glucose—a process known as catabolite repression— so no cAMP–CAP complex forms and the CAP-binding site remains empty. RNA polymerase is, therefore, unable to

effectively initiate transcription, even though the repressor may not be bound to the operator region. Consequently, the three structural genes are not expressed (Figure-C).

The *lac* operon: a model of gene regulation in *E. coli*
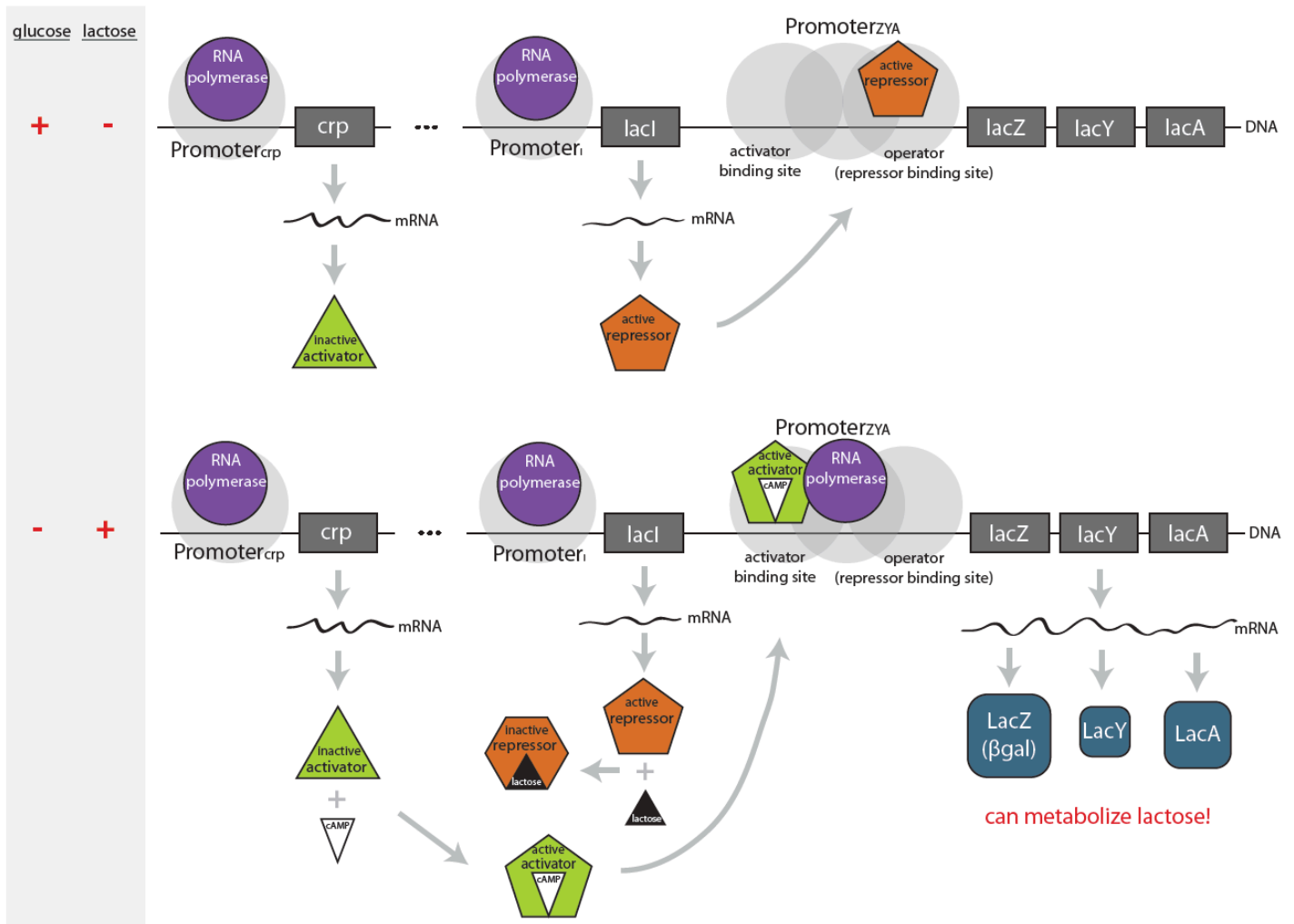
Thus, the activity of the *lac* operon is controlled by two different regulatory proteins.

The first is the **lac repressor** which is produced by the regulatory gene called *lacI*.

The second regulatory protein is an **activator** (activates transcription) called **Catabolite Activator Protein (CAP)**.

The two different regulatory proteins, the lac repressor and CAP, allow the *lac* operon to integrate two different environmental signals, cellular lactose and glucose levels.