LAC OPERON

Genes are DNA sequences that are transcribed into RNA. Regulatory elements are DNA sequences that are not transcribed but affect the expression of genes. Positive control includes mechanisms that stimulate gene expression, whereas negative control inhibits gene expression.

A significant difference between bacterial and eukaryotic gene control lies in the organization of functionally related genes. Many bacterial genes that have related functions are clustered and under the control of a single promoter. These genes are often transcribed together into a single mRNA. A group of bacterial structural genes that are transcribed together (along with their promoter and additional sequences that control transcription) is called an operon.

Negative and Positive Control:

There are two types of transcriptional control: negative control, in which a regulatory protein is a repressor, binding to DNA and inhibiting transcription; and positive control, in which a regulatory protein is an activator, stimulating transcription.

Operons can also be either inducible or repressible. Inducible operons are those in which transcription is normally off (not taking place); something must happen to induce transcription, or turn it on. Repressible operons are those in which transcription is normally on (taking place); something must happen to repress transcription, or turn it off.

The lac Operon of E. coli

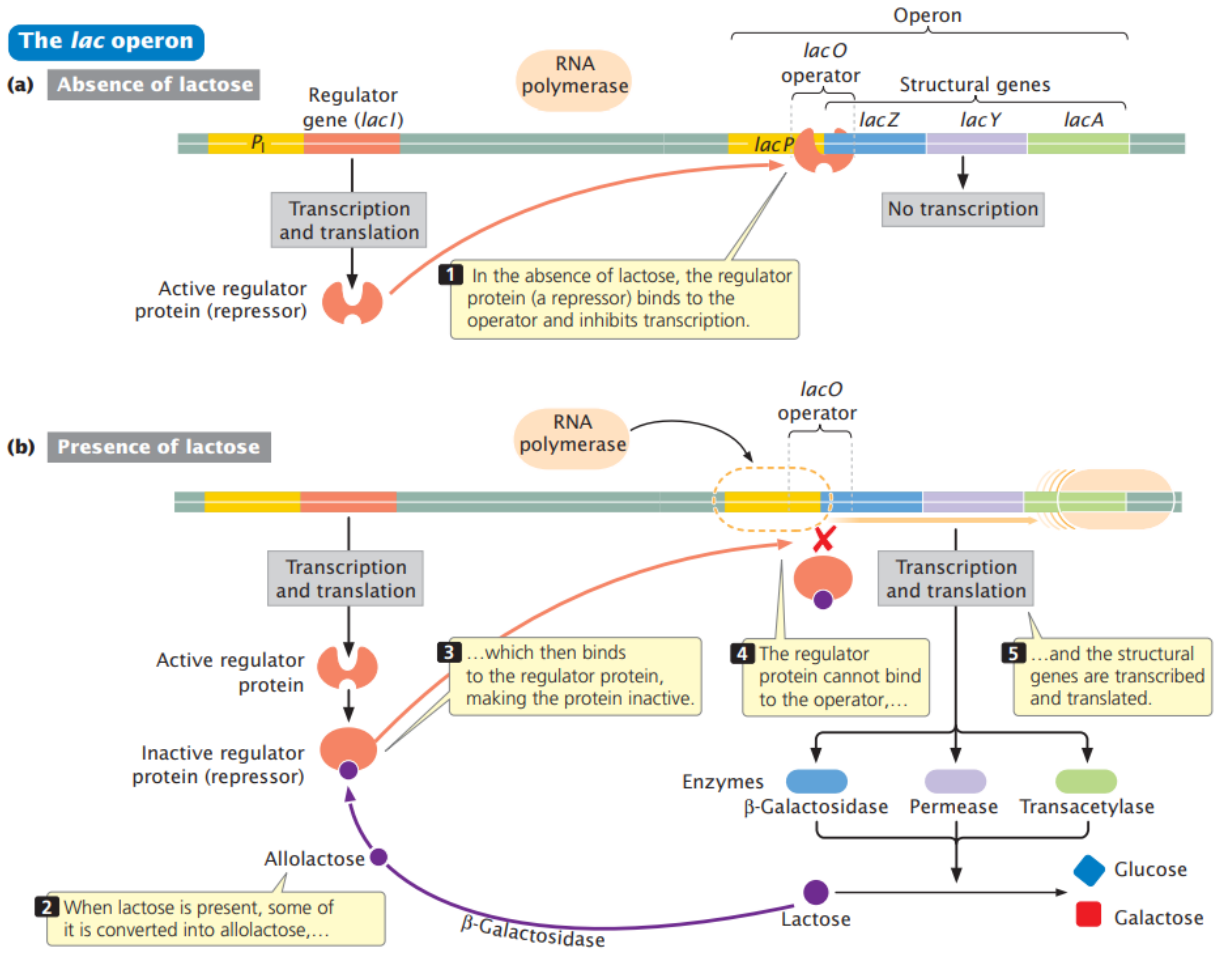
In 1961, François Jacob and Jacques Monod described the "operon model" for the genetic control of lactose metabolism in E. coli. This work and subsequent research on the genetics of lactose metabolism established the operon as the basic unit of transcriptional control in bacteria. Lactose is a major carbohydrate found in milk; it can be metabolized by E. coli bacteria that reside in the mammalian gut.

Lactose does not easily diffuse across the E. coli cell membrane and must be actively transported into the cell by the protein permease. To utilize lactose as an energy source, E. coli must first break it into glucose and galactose, a reaction catalyzed by the enzyme β-galactosidase. This enzyme can also convert lactose into allolactose, a compound that plays an important role in regulating lactose metabolism. A third enzyme, thiogalactoside transacetylase, also is produced by the lac operon, but its function in lactose metabolism is not yet known.

Regulation of the lac operon

The lac operon is an example of a negative inducible operon. The enzymes β-galactosidase, permease, and transacetylase are encoded by adjacent structural genes in the lac operon of E. coli and have a common promoter. β-Galactosidase is encoded by the lacZ gene, permease by the lacY gene, and transacetylase by the lacA gene. Although lactose appears to be the inducer here, allolactose is actually responsible for induction. Upstream of lacP is a regulator gene, lacI, which has its own promoter (PI). The lacI gene is transcribed into a short mRNA that is translated into a repressor. In the absence of lactose (and, therefore, allolactose), the repressor binds to the lac operator site lacO. RNA polymerase binds to the promoter and moves down the DNA molecule, transcribing the structural genes. When the repressor is bound to the operator, the binding of RNA polymerase is blocked, and transcription is prevented. When lactose is present, some of it is converted into allolactose, which binds to the repressor and causes the repressor to be released from the DNA. In the presence of lactose, then, the repressor is inactivated, the binding of RNA polymerase is no longer blocked, the transcription of lacZ, lacY, and lacA takes place, and the lac proteins are

produced.



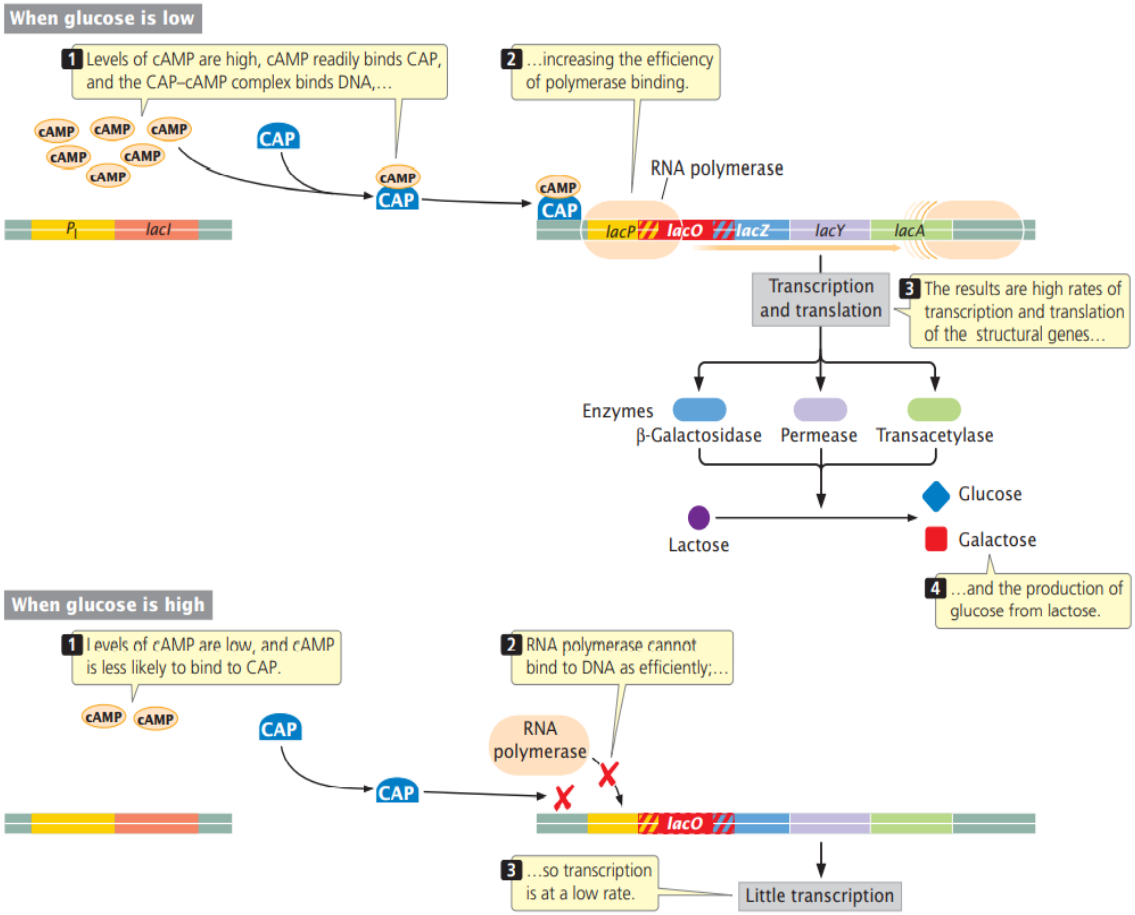
16.8 The *lac* operon regulates lactose metabolism.

Positive Control and Catabolite Repression

E. coli and many other bacteria metabolize glucose preferentially in the presence of lactose and other sugars. They do so because glucose enters glycolysis without further modification and therefore requires less energy to metabolize than do other sugars. When glucose is available, genes that participate in the metabolism of other sugars are repressed, in a phenomenon known as catabolite repression. For example, the efficient transcription of the lac operon takes place only if lactose is present and glucose is absent. Catabolite repression results from positive control in response to glucose. (This regulation is in addition to the negative control brought about by the repressor binding at the operator site of the lac operon when lactose is absent.) Positive control is accomplished

through the binding of a dimeric protein called the catabolite activator protein (CAP) to a site that is about 22 nucleotides long and is located within or slightly upstream of the promoter of the lac genes. RNA polymerase does not bind efficiently to many promoters unless CAP is first bound to the DNA. Before CAP can bind to DNA, it must form a complex with a modified nucleotide called adenosine-3′, 5′-cyclic monophosphate (cyclic AMP, or cAMP), which is important in cellular signaling processes in both bacterial and eukaryotic cells. In E. coli, the concentration of cAMP is regulated so that its concentration is inversely proportional to the level of available glucose. A high concentration of glucose within the cell lowers the amount of cAMP, and so little cAMP–CAP complex is available to bind to the DNA. Subsequently, RNA polymerase has poor affinity for the lac promoter, and little transcription of the lac operon takes place. Low concentrations of glucose stimulate high levels of cAMP, resulting in increased cAMP–CAP binding to DNA. This increase enhances the binding of RNA polymerase to the

promoter and increases transcription of the lac genes by some 50-fold.



16.13 The catabolite activator protein (CAP) binds to the promoter of the *lac* operon and stimulates transcription. CAP must complex with adenosine-3′,5′-cyclic monophosphate (cAMP) before binding to the promoter of the *lac* operon. The binding of cAMP–CAP to the promoter activates transcription by facilitating the binding of RNA polymerase. Levels of cAMP are inversely related to glucose: low glucose stimulates high cAMP; high glucose stimulates low cAMP.