Prokaryotic Gene Regulation

Despite the simplicity of their form, bacteria have a fundamental need to regulate the expression of their genes.

One of the main reasons is that they are nutritional opportunists.

Consider how bacteria obtain the many important compounds, such as sugars, amino acids, and nucleotides, needed for metabolism.

Bacteria swim in a sea of potential nutrients. They can either acquire these compounds from the environment or synthesize them by enzymatic pathways.

Synthesizing the necessary enzymes for these pathways expends energy and cellular resources; so, given the choice, bacteria will take compounds from the environment instead.

To be economical, they will synthesize the enzymes necessary to produce these compounds only when there is no other option—in other words, when these compounds are unavailable in their local environment.

Bacteria have evolved regulatory systems that couple the expression of gene products to sensor systems that detect the relevant compound in a bacterium's local environment.

The regulation of enzymes taking part in sugar metabolism provides an example.

Sugar molecules can be oxidized to provide energy or they can be used as building blocks for a great range of organic compounds.

However, there are many different types of sugar that bacteria could use, including lactose, glucose, galactose, and xylose.

First, a different import protein is required to allow each of these sugars to enter the cell.

Further, a different set of enzymes is required to process each of the sugars.

If a cell were to simultaneously synthesize all the enzymes that it might possibly need, the cell would expend much more energy and materials to produce the enzymes than it could ever derive from breaking down prospective carbon sources.

Cells need mechanisms that fulfill two criteria:

1. **They must be able to recognize environmental** conditions in which they should activate or repress transcription of the relevant genes.

2. They must be able to toggle on or off the transcription of each specific gene or group of genes.

Bacteria have a simple general mechanism for coordinating the regulation of genes encoding products that participate in a set of related processes: these genes are clustered on the chromosome and are transcribed together.

Many prokaryotic mRNAs are **polycistronic**— multiple genes on a single transcript—and the single promoter that initiates transcription of the cluster is the site of regulation for expression of all the genes in the cluster.

The gene cluster and promoter, plus additional sequences that function together in regulation, are called an **operon**

Lac Operon:

Many of the principles of prokaryotic gene expression were first defined by studies of lactose metabolism in *E. coli, which can use lactose as its sole carbon source*.

In 1960, **François Jacob and Jacques Monod** published a short paper in the *Proceedings of the French Academy* of Sciences that described how two adjacent genes involved in lactose metabolism were coordinately regulatedby a genetic element located at one end of the gene cluster.

The genes were those for b-galactosidase, which cleaves lactose to galactose and glucose, and galactoside permease, which transports lactose into the cell .

The terms “operon” and “operator” were first introduced in this paper.

François Jacob

Jacques Monod, 1910–1976

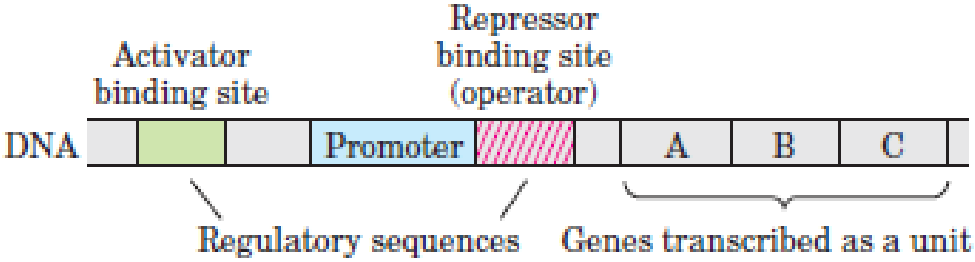

FIGURE 28–5 Representative prokaryotic operon. Genes A, B, and C are transcribed on one polycistronic mRNA. Typical regulatory sequences include binding sites for proteins that either activate or repress transcription from the promoter.

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The lactose (lac) operon includes the genes for b-galactosidase (Z), galactoside permease (Y), and thiogalactoside transacetylase (A).

The last of these enzymes appears to modify toxic galactosides to facilitate their removal from the cell.

Two types of DNA–protein interactions are required for regulated transcription. Both take place near the site at which gene transcription begins.

One of these DNA–protein interactions determines where transcription begins. The DNA that participates in this interaction is a DNA segment called the **promoter,** and the protein that binds to this site is RNA polymerase.

When RNA polymerase binds to the promoter DNA, transcription can initiate a few bases away from the promoter site. Every gene must have a promoter or it cannot be transcribed.

The basics of prokaryotic transcriptional regulation

The other type of DNA–protein interaction decides whether promoter-driven transcription takes place. DNA segments near the promoter serve as binding sites for regulatory proteins called **activators and repressors.**

In bacteria, most of these sites are termed **operators.**

For some genes, an activator protein must bind to its target DNA site as a necessary prerequisite for transcription to begin.

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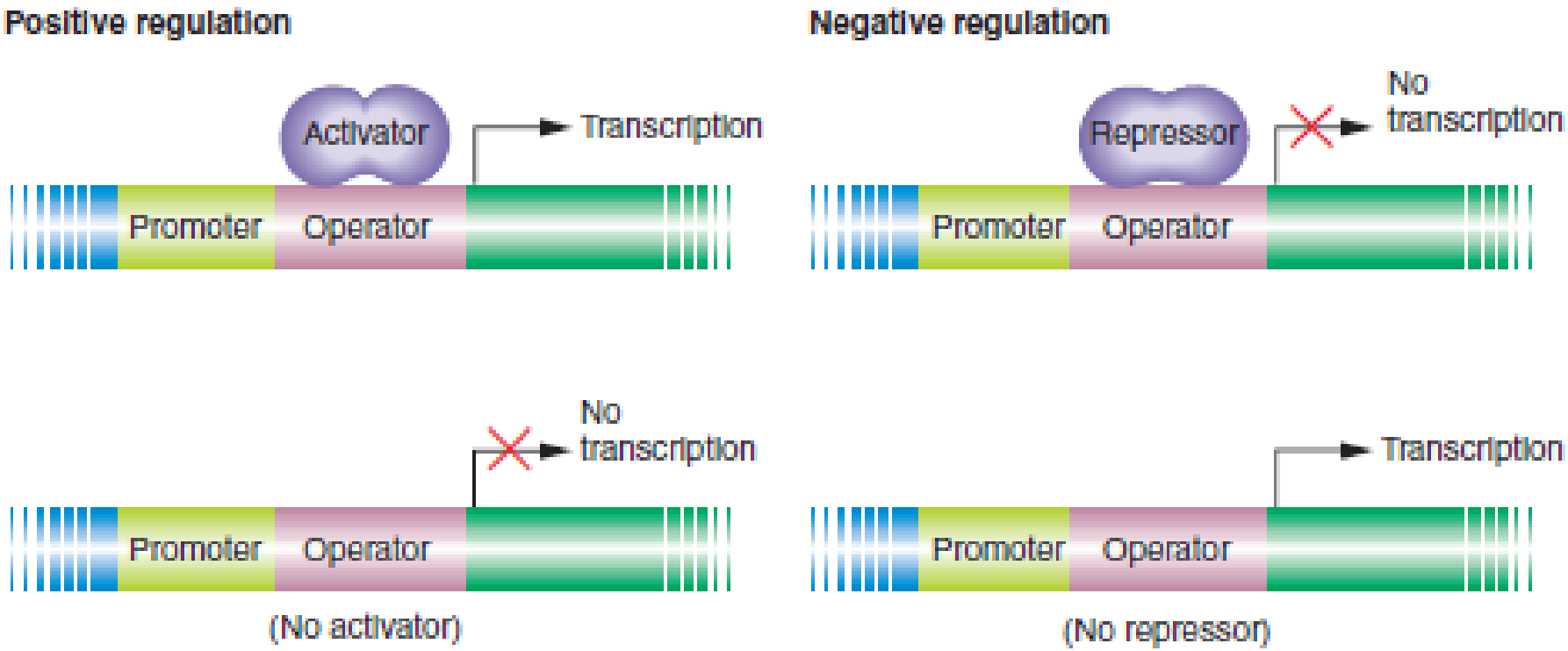
Such instances are sometimes referred to as ***positive regulation*** because the *presence of the bound protein is required* for transcription.

For other genes, a repressor protein must be prevented from binding to its target site as a necessary prerequisite for transcription to begin. Such cases are sometimes termed ***negative regulation*** because the *absence of the bound repressor allows* transcription to begin.



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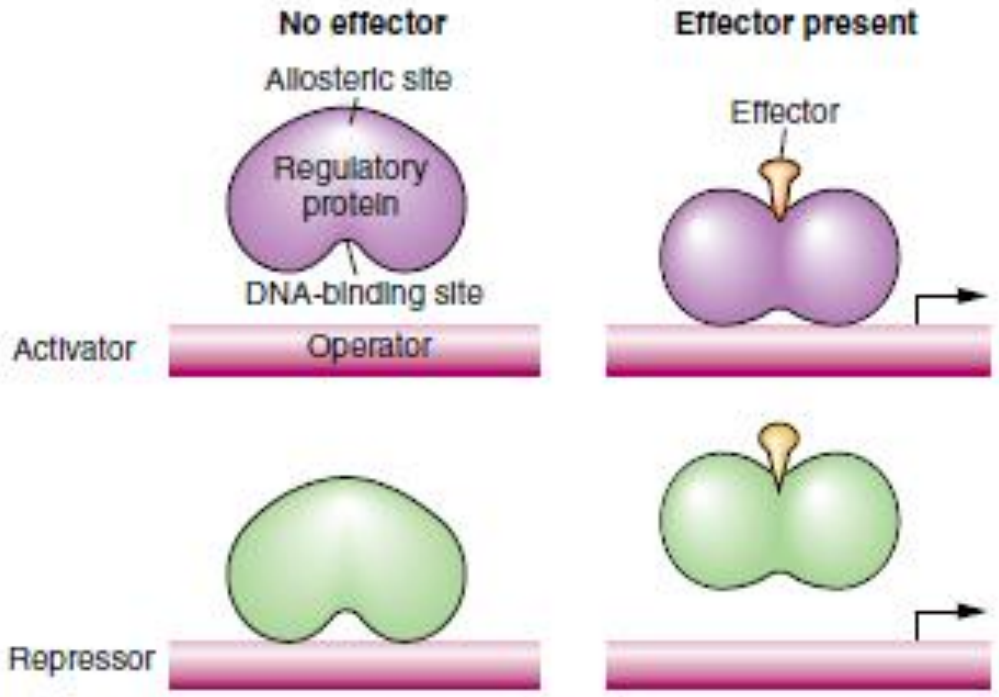


Figure 10-3 The influence of allosteric effectors on the DNA-binding activities of activators and repressors.

How do activators and repressors regulate transcription?

Often, a DNA-bound activator protein physically helps tether RNA polymerase to its nearby promoter so that polymerase may begin transcribing.

A DNA-bound repressor protein typically acts either by physically interfering with the binding of RNA polymerase to its promoter (blocking transcription initiation)

or by impeding the movement of RNA polymerase along the DNA chain (blocking transcription).

The pioneering work of François Jacob and Jacques Monod in the 1950s showed how lactose metabolism is genetically regulated.

THE *lac STRUCTURAL GENES*

The metabolism of lactose requires two enzymes: a permease to transport lactose into the cell and -galactosidase to cleave the lactose molecule to yield glucose and galactose

The gene for the Lac repressor.

A fourth gene, the I gene, encodes the Lac repressor protein, so named because it can block the expression of the *Z, Y, and A genes.*

The I gene happens to map close to the Z, Y, and A genes, but this proximity does not seem to be important to its function.

2. *The lac promoter site. The promoter (P) is the site on* the DNA to which RNA polymerase binds to initiate transcription of the *lac structural genes (Z, Y,* and *A).*

3. *The lac operator site. The operator (O) is the site on* the DNA to which the Lac repressor binds. It is located between the promoter and the *Z gene near* the point at which transcription of the multigenic mRNA begins.

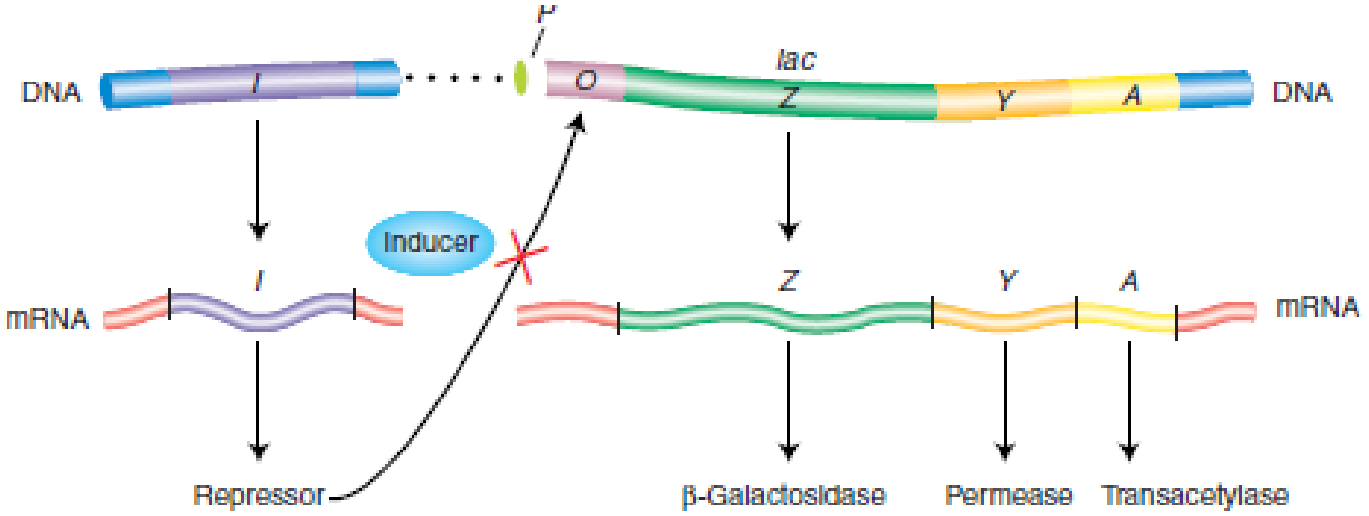
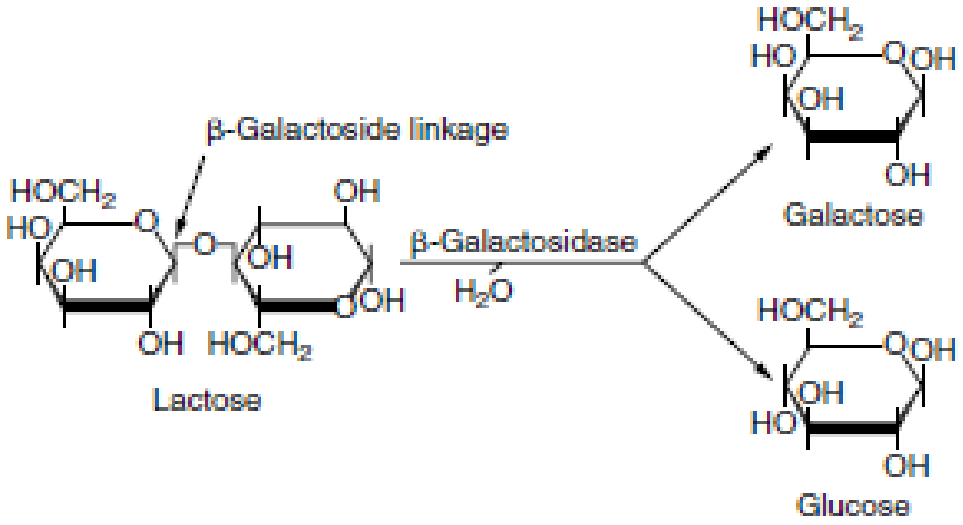


Figure 10-4 A simplified *lac* operon model. Coordinate expression of the *Z*, *Y*, and *A* genes is under the negative control of the product of the *I* gene, the repressor. When the inducer binds the repressor, the operon is fully expressed.



THE INDUCTION OF THE *lac SYSTEM The P, O, Z, Y,* and *A segments together constitute* an **operon,** defined as a segment of DNA that encodes a multigenic mRNA as well as an adjacent common promoter and regulatory region.

The *lac I gene,* encoding the Lac repressor, is *not considered part of the lac operon itself.*

The Lac repressor has a ***DNA binding* site** that can recognize the operator DNA sequence and an ***allosteric site*** *that binds lactose or* analogs of lactose that are useful experimentally.

When lactose or its analogs bind to the repressor protein, the protein undergoes an **allosteric transition, a** change in shape.

This slight alteration in shape in turn alters the DNA-binding site so that the repressor no longer has high affinity for the operator.

Thus, in response to binding lactose, the repressor falls off the DNA.

The relief of repression for systems such as *lac is termed* ***induction; lactose and its*** analogs that allosterically inactivate the repressor and lead to the expression of the *lac genes are termed* **inducers.**

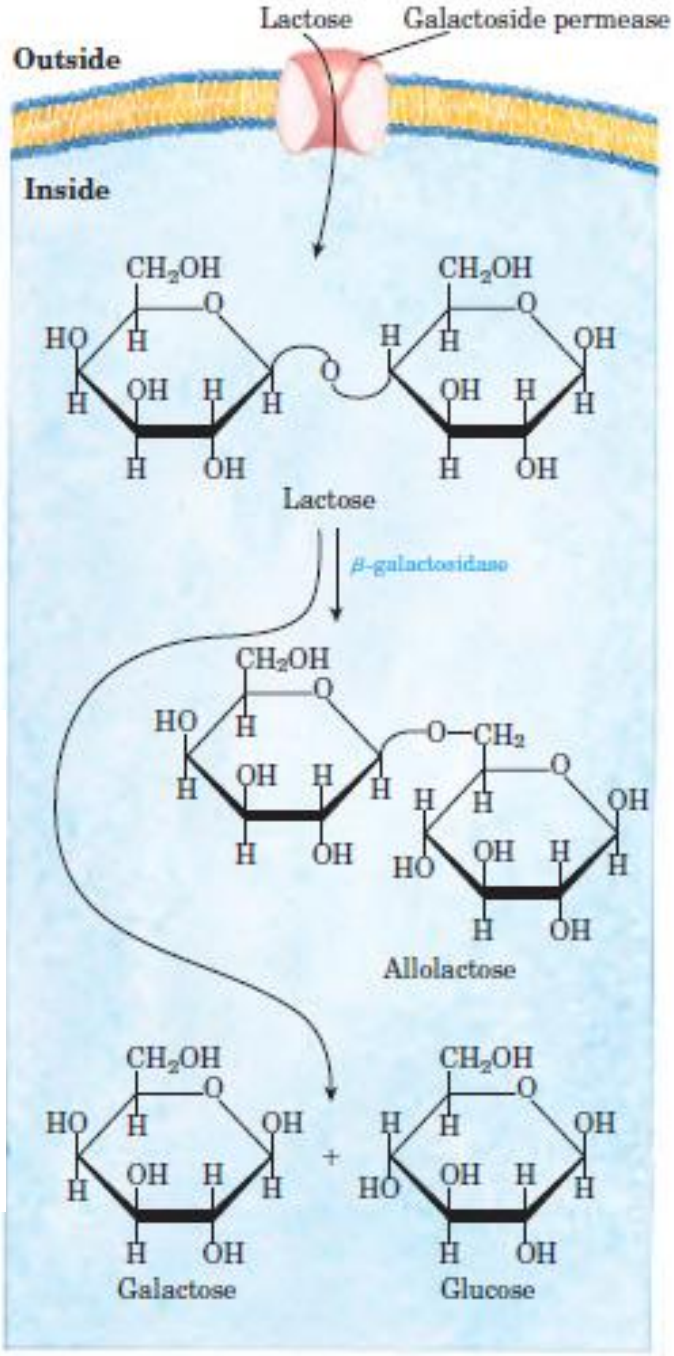


FIGURE 28-6 Lactose metabolism in *E. coli*. Uptake and metabolism of lactose require the activities of galactoside permease and β-galactosidase. Conversion of lactose to allolactose by transglycosylation is a minor reaction also catalyzed by β-galactosidase.

TO SUMMARIZE

In the absence of an inducer (lactose or an analog), the Lac repressor binds to the *lac operator* site and prevents transcription of the *lac operon* by blocking the movement of RNA polymerase.

In this sense, the Lac repressor acts as a roadblock on the DNA.

Consequently, all the structural genes of the *lac operon* (the *Z, Y, and A genes) are repressed, and there is no b*-galactosidase, -galactoside permease, or transacetylase in the cell.

In contrast, when an inducer is present, it binds to the allosteric site of each Lac repressor subunit, thereby inactivating the site that binds to the operator.

The Lac repressor falls off the DNA, allowing the transcription of the structural genes of the *lac operon to* begin.

The enzymes b-galactosidase, -galactoside permease, and transacetylase now appear in the cell in a coordinated fashion.

The study of *lac operon mutants has revealed some* details of the workings of the operon's regulatory system.

In the absence of lactose, the *lac operon genes are* repressed.

Mutations in the operator or in another gene, the *I gene, result in constitutive synthesis of the gene* products.

When the *I gene is defective, repression can* be restored by introducing a functional *I gene into the* cell on another DNA molecule, demonstrating that the *I gene encodes a diffusible molecule that causes gene* repression.

This molecule proved to be a protein, now called the Lac repressor, a tetramer of identical monomers.

The *I gene is transcribed from its own promoter (PI) independent* of the *lac operon genes.*

Despite this elaborate binding complex, repression is not absolute. Binding of the Lac repressor reduces the rate of transcription initiation by a factor of 10^3

Even in the repressed state, each cell has a few molecules of b-galactosidase and galactoside permease, presumably synthesized on the rare occasions when the repressor transiently dissociates from the operators.

This basal level of transcription is essential to operon regulation.

Operator mutations reveal that such a site is **cis-acting**; that is, it regulates the expression of an adjacent transcription unit on the same DNA molecule.

In contrast, mutations in the gene encoding a repressor protein reveal that this protein is **trans-acting**; that is, it can act on any copy of the target DNA site in the cell.

Jacob and Monod isolated another class of repressor mutation, called **superrepressor** (*IS) mutations.*
IS mutations cause repression even in the presence of an inducer.

Unlike the case for *I, IS mutations* are dominant to *I.*

This key observation led Jacob and Monod to speculate that *IS* mutations alter the allosteric site so that it can no longer bind to an inducer.

As a consequence, *IS-encoded repressor* protein continually binds to the operator—preventing transcription of the *lac operon even when the* inducer is present in the cell.

Assaying lactose presence or absence through the Lac repressor

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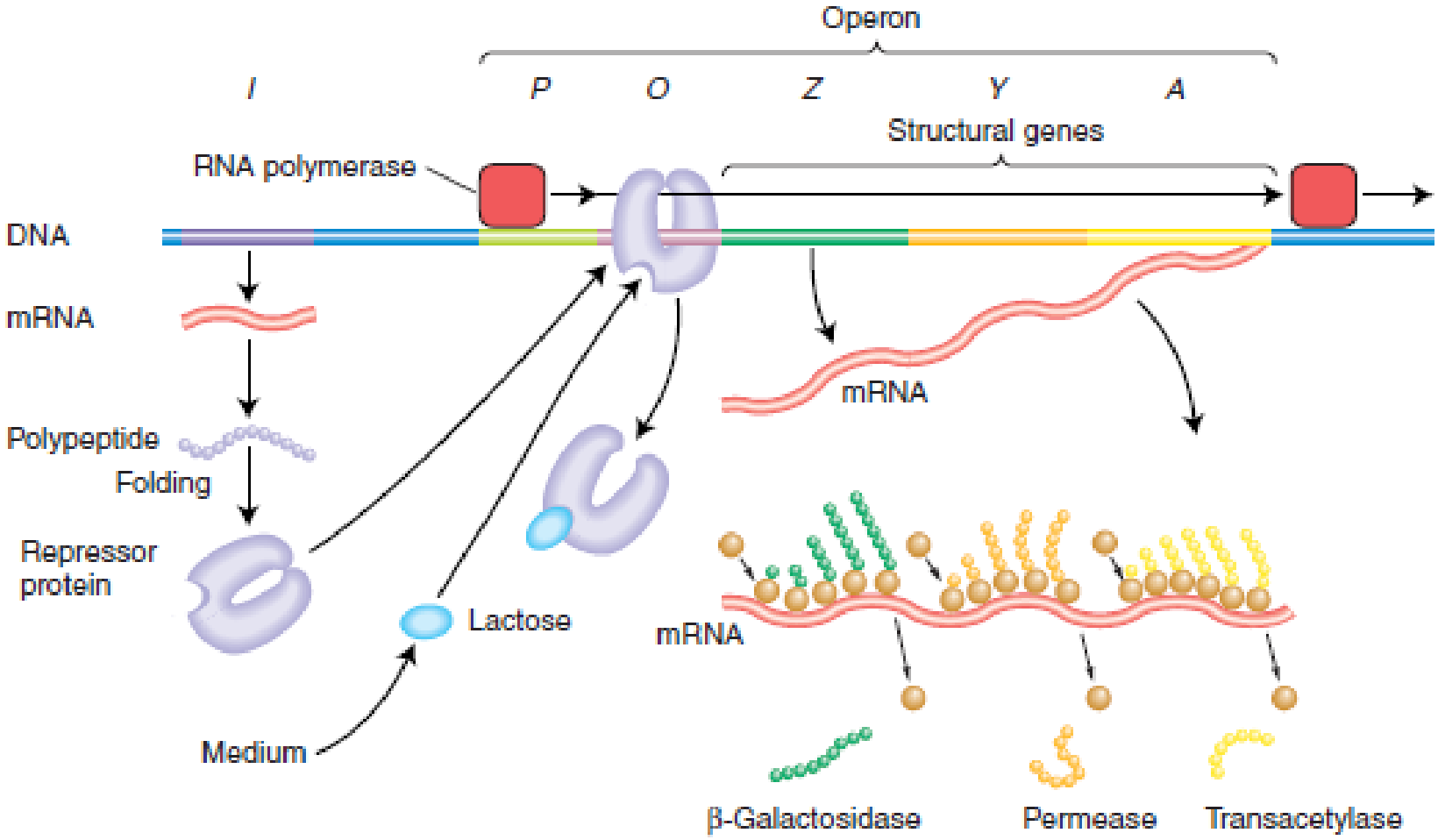

Figure 10-6 Regulation of the *lac* operon. The *I* gene continually makes repressor. In the absence of lactose, the repressor binds to the *O* (operator) region and blocks transcription. Lactose binding changes the shape of the repressor so that the repressor no longer binds to *O*. The RNA polymerase is then able to transcribe the *Z*, *Y*, and *A* structural genes, and so the three enzymes are produced.