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.

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.

Assaying lactose presence or absence through the Lac repressor

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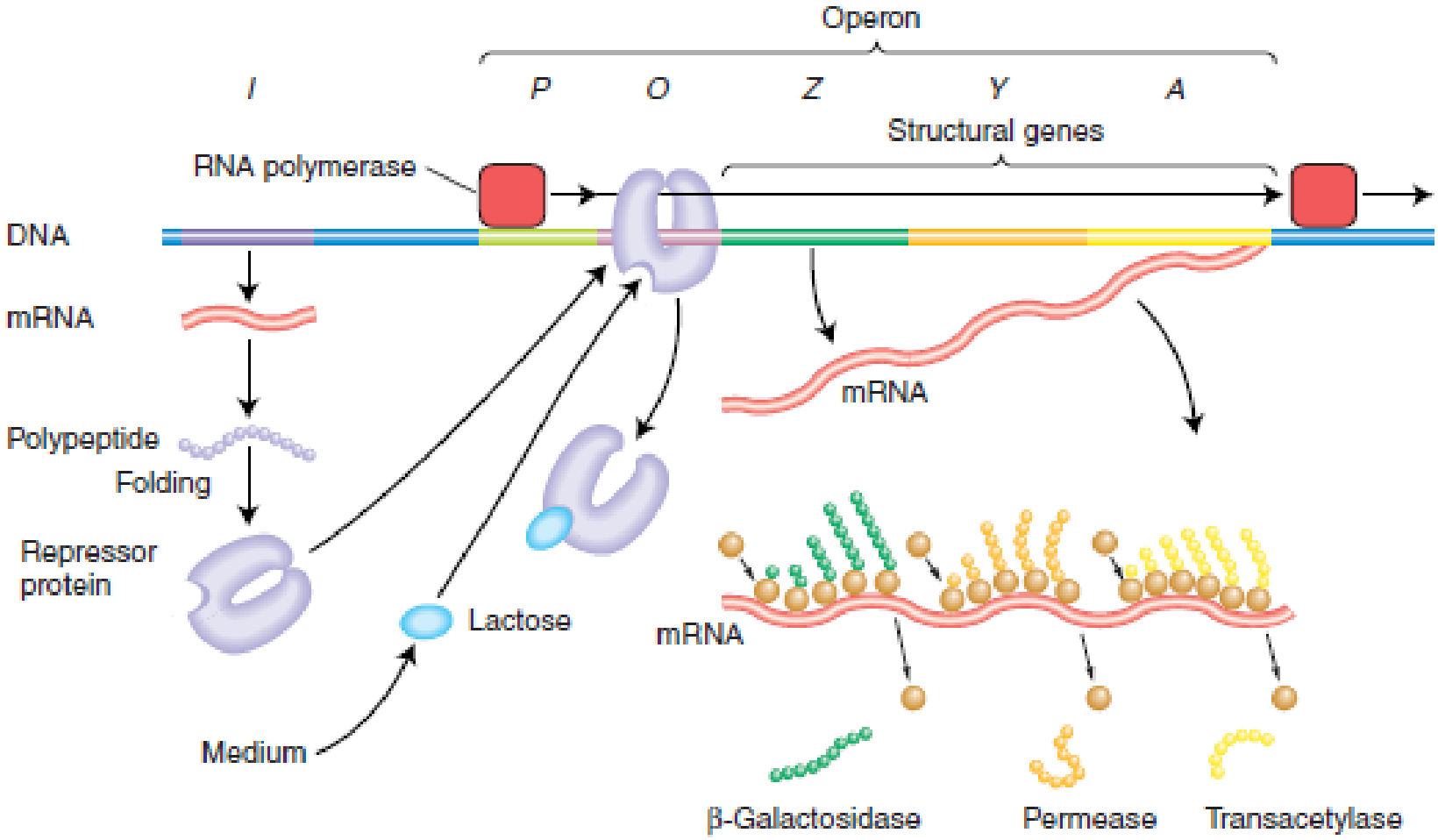


Figure 10-6 Regulation of the *lac* operon. The *I* gene continually makes repressor. In the absence of lactose, the repressor binds to the *O* (operator) region and blocks transcription. Lactose binding changes the shape of the repressor so that the repressor no longer binds to *O*. The RNA polymerase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

Molecular characterization of the Lac repressor and the *lac operator*

- Walter Gilbert and Benno Müller-Hill provided a decisive demonstration of the *lac system in 1966 by monitoring* the binding of the radioactively labeled inducer IPTG to purified repressor protein.
- They first showed that the repressor consists of four identical subunits, and hence contains four IPTG-binding sites.

- Second, they showed that, in the test tube, repressor protein binds to DNA containing the operator and comes off the DNA in the presence of IPTG.

- Gilbert and his co-workers showed that the repressor can protect specific bases in the operator from chemical reagents.

- They took operon DNA to which repressor was bound and treated it with the enzyme DNase, which breaks up DNA.
- They were able to recover short DNA strands that had been shielded from the enzyme activity by the re-pressor molecule and that presumably constituted the operator sequence

They also showed the incredible specificity of repressor–operator recognition, which can be disrupted by a single base substitution.

Polar mutations

Some of the mutations that mapped to the *Z and Y* genes were found to be polar—that is, affecting genes "downstream" in the operon. For example, polar *Z mu*-tations resulted in null function not only for *Z but also* for *Y and A.*

Polar mutations in Y affected A also but not *Z.*

These polar mutations were the genetic observations that suggested to Jacob and Monod that the three genes were transcribed from one end as a unit.

One condition is that lactose must be present in the environment.
This condition makes sense, because it would be inefficient for the cell to produce the lactose metabolic enzymes if there is no substrate to metabolize.

We have already seen that the cell's recognition that lactose is present is accomplished by a repressor protein.

The other condition is that glucose cannot be present in the cell's environment.

Because the cell can capture more energy from the breakdown of glucose than it can from the breakdown of other sugars, it is more efficient for the cell to metabolize glucose rather than lactose.

Thus, mechanisms have evolved that prevent the cell from synthesizing the enzymes for lactose metabolism when lactose and glucose are present together.

The repression of the transcription of lactose-metabolizing genes in the presence of glucose is an example of **catabolite repression.**

The transcription of proteins necessary for the metabolism of many different sugars is similarly repressed in the presence of glucose.

The results of studies indicate that a breakdown product, or *catabolite, of glucose prevents activation of* the *lac operon by lactose—this is the catabolite repression* just referred to.

However, the glucose catabolite is known to modulate the level of an important cellular constituent—**cyclic adenosine monophosphate (cAMP).**

When glucose is present in high concentrations, the cell's cAMP concentration is low.

As the glucose concentration decreases, the cell's concentration of cAMP increases correspondingly.

A high concentration of cAMP is necessary for activation of the *lac operon.*

Mutants that cannot convert ATP into cAMP cannot be induced to produce b-galactosidase, because the concentration of cAMP is not great enough to activate the *lac operon.*

In addition, there are other mutants that do make cAMP but cannot activate the Lac enzymes, because they lack yet another protein, called **CAP (catabolite activator protein), made by the *crp gene.***

CAP binds to a specific site on the *lac operon.*

The DNA-bound CAP is then able to interact physically with RNA polymerase and increases that enzyme's affinity for the *lac promoter.*

By itself, CAP cannot bind to the CAP site of the *lac operon.*

However, by binding to its allosteric effector, cAMP, CAP is able to bind to the CAP site and activate RNA polymerase.

In this way, the catabolite repression system contributes to the selective activation of the *lac operon*

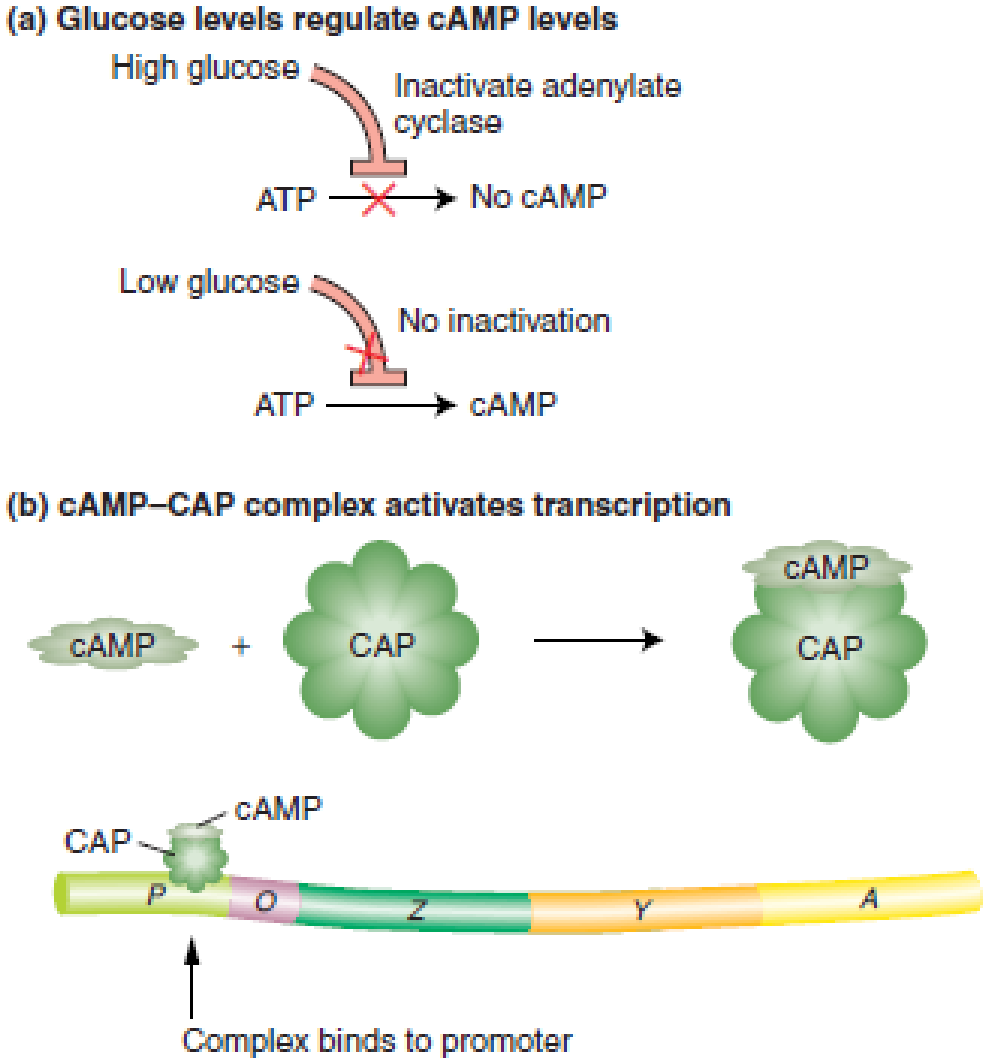


Figure 10-13 Catabolite control of the *lac* operon. (a) Only under conditions of low glucose is adenylate cyclase active and cAMP (cyclic adenosine monophosphate) formed. (b) When cAMP is present, it forms a complex with CAP (catabolite activator protein) that activates transcription by binding to a region within the *lac* promoter.

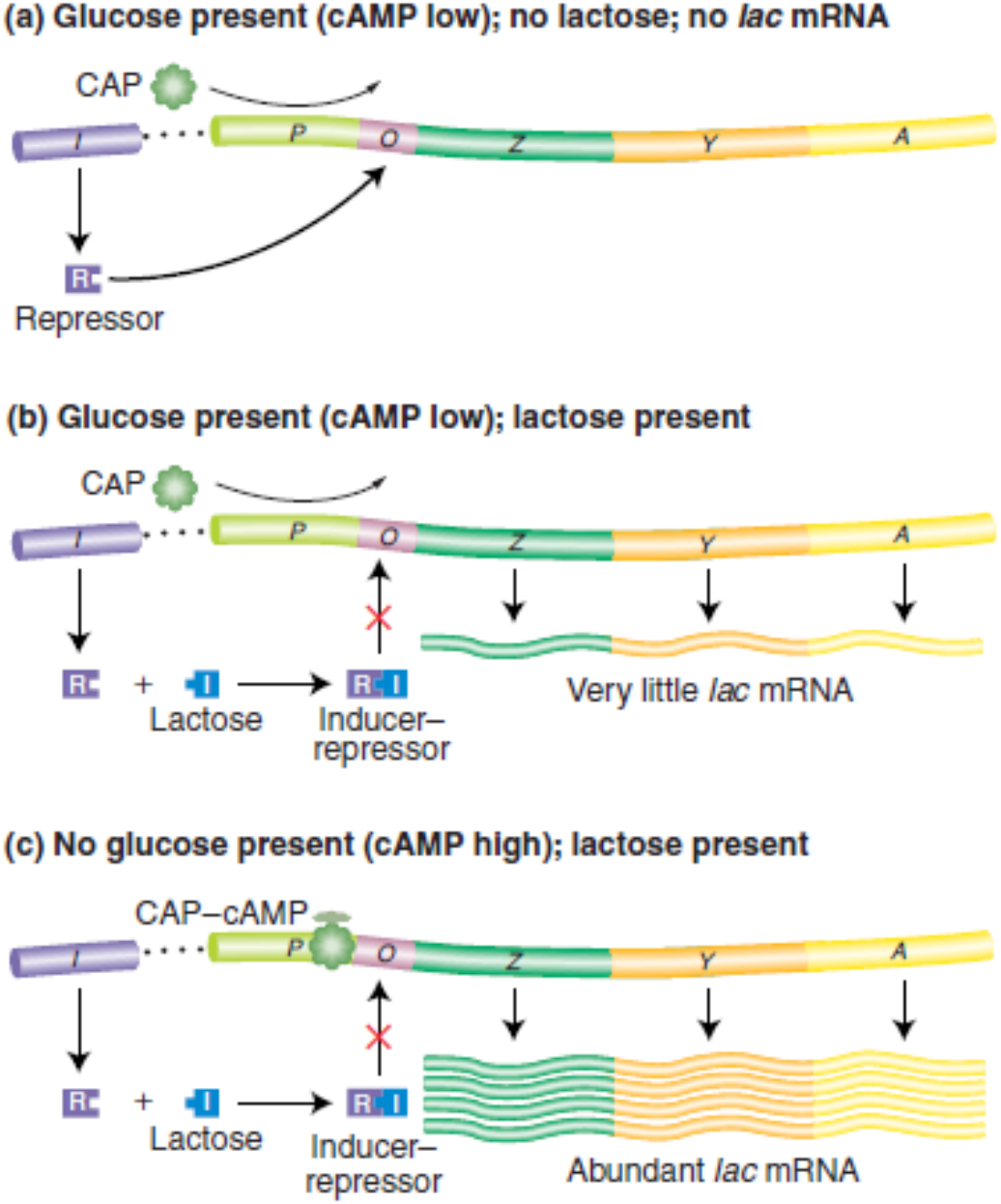
We can now fit the CAP–cAMP- and RNA-polymerase binding sites into the detailed model of the *lac operon*.

The presence of glucose prevents lactose metabolism because a glucose breakdown product inhibits maintenance of the high cAMP levels necessary for formation of the CAP–cAMP complex, which in turn is required for the RNA polymerase to attach at the *lac* promoter site.

Even when there is a shortage of glucose catabolites and CAP–cAMP forms, the mechanism for lactose metabolism will be implemented only if lactose is present.

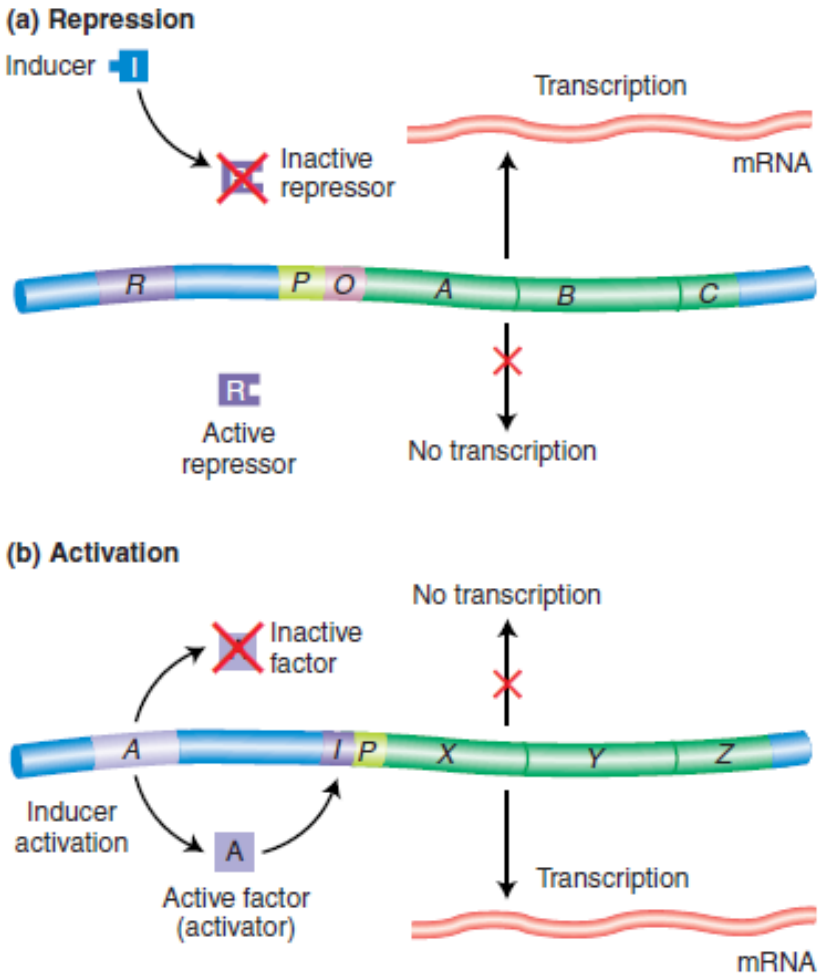
This level of control is accomplished because lactose must bind to the repressor protein to remove it from the operator site and permit transcription of the *lac* operon.

Thus, the cell conserves its energy and resources by producing the lactose-metabolizing enzymes only when they are both needed and useful.


(a) Glucose present (cAMP low); no lactose; no lac mRNA
CAP
I
P
O
Z
Y
A
R
Repressor
(b) Glucose present (cAMP low); lactose present
CAP
I
P
O
Z
Y
A
R
+
I
Lactose
R I
Inducer–
repressor
Very little lac mRNA
(c) No glucose present (cAMP high); lactose present
CAP–cAMP
I
P
O
Z
Y
A
R
+
I
Lactose
R I
Inducer–
repressor
Abundant lac mRNA

Inducer–repressor control of the *lac operon is an* example of repression, or **negative control, in which** expression is normally blocked.

In contrast, the CAP–cAMP system is an example of activation, or **positive control, because it acts as a signal activating expression—** in this case, the activating signal is the interaction of the CAP–cAMP complex with the CAP site.



DUAL POSITIVE AND NEGATIVE CONTROL: THE ARABINOSE OPERON.

- The regulation of the arabinose operon provides an example in which a single DNA-binding protein may act as *either a repressor or an activator.*

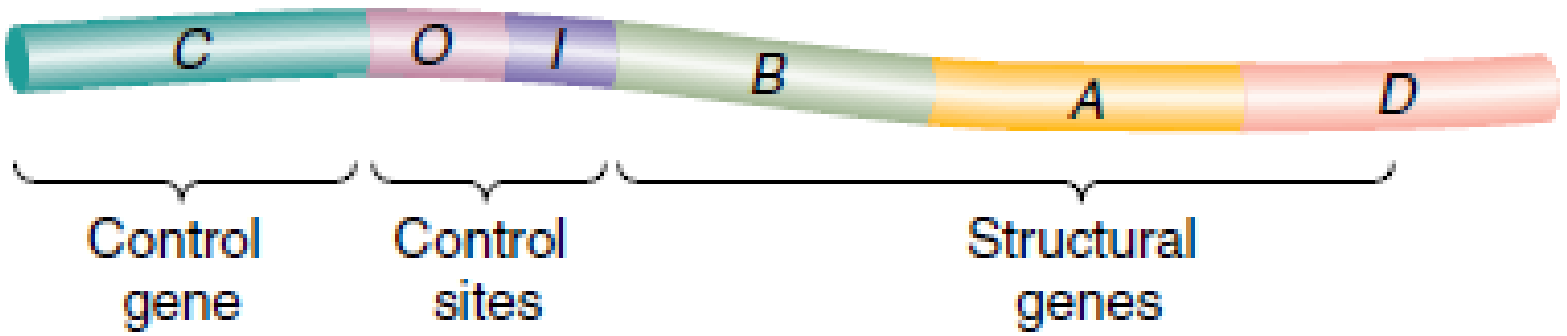


Figure 10-19 Map of the *ara* region. The *B*, *A*, and *D* genes together with the *I* and *O* sites constitute the *ara* operon. *I* is *araI*.

The structural genes (*araB, araA, and araD) encode* the metabolic enzymes that break down the sugar arabinose.

The three genes are transcribed in a unit as a single mRNA.

Transcription is activated at *aral, the* ***initiator*** region, which contains both an operator site and a promoter.

The ***araC*** *gene, which maps nearby, encodes an* activator protein. When bound to arabinose, this protein activates transcription of the *ara operon, perhaps by* helping RNA polymerase bind to the promoter.

In addition, the same CAP–cAMP catabolite repression system that regulates *lac operon expression also regulates expression* of the *ara operon*.

In the presence of arabinose, both the CAP–cAMP complex and the AraC–arabinose complex must bind to the operator region of *araI in order for RNA polymerase* to bind to the promoter and transcribe the *ara operon*

In the absence of arabinose, the AraC protein assumes a different conformation and represses the *ara* operon by binding both to *araI and to a second operator* region, *araO, thereby forming a loop*

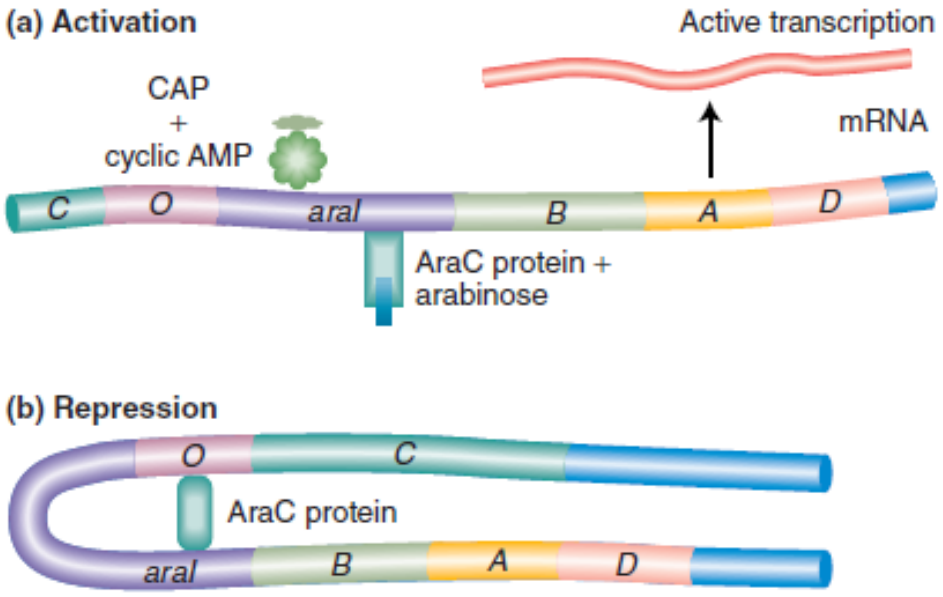


Figure 10-20 Dual control of the *ara* operon. (a) In the presence of arabinose, the AraC protein binds to the *araI* region. The CAP–cAMP complex binds to a site adjacent to *araI*. This binding stimulates the transcription of the *araB*, *araA*, and *araD* genes. (b) In the absence of arabinose, the AraC protein binds to both the *araI* and the *araO* regions, forming a DNA loop. This binding prevents transcription of the *ara* operon.