Johns Hopkins Engineering

Molecular Biology 585.407

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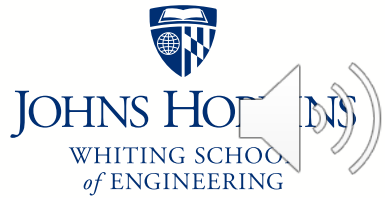
Module 12 / Lecture 2



The Regulation of Gene Expression
Part 2

The lac operon

The lac operon consists of 3 structural genes (lacZ, lacY, and lacA) preceded by a promoter (P**lac**) and a special nucleotide sequence called the operator (O), which actually overlaps the promoter.

Transcription of the lac operon begins at the promoter, which is the site of RNA polymerase attachment, and then proceeds through the operator and all the structural genes until finally ending at a terminator sequence.

The result is a single molecule of mRNA coding for the polypeptide products of all three structural genes. Such mRNA molecules, which code for more than one polypeptide, are called polygenic mRNAs; they occur **only** in prokaryotic cells.

Clustering related genes into an **operon** for transcription into a single polygenic mRNA allows the synthesis of several polypeptides to be controlled in a single step.

The crucial step in this control is the interaction between an operator site in the DNA and a repressor protein.

The interaction between these elements for the specific case of the lac operon is depicted in **Figure 23-4**.

The lac operon

The repressor protein, called the lac repressor, is encoded by the *lac*I regulatory gene, which is located outside the operon (although it happens to be located adjacent to the lac operon it regulates).

The lac repressor is a DNA-binding protein that specifically recognizes and binds to the operator site of the lac operon. When the repressor is bound to the operator (**Figure 23-4a**), RNA polymerase cannot bind to the promoter and thus transcription of the structural genes is not possible.

Binding of the repressor to the operator inactivates the operon and keeps its structural genes turned off.

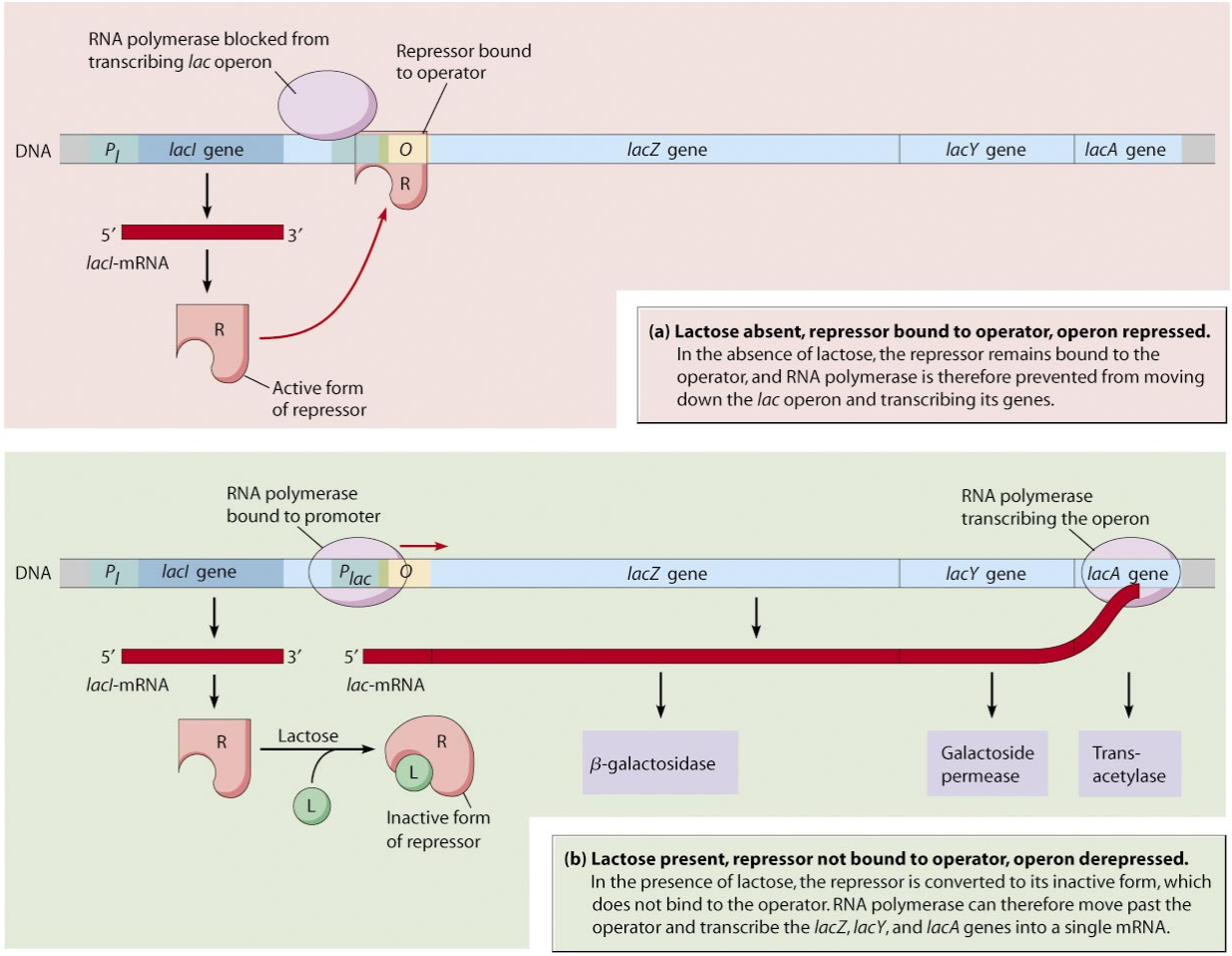


Figure 23-4

Repressor protein

If binding of the repressor to the operator blocks transcription, how do cells turn on transcription of the lac operon, as occurs in the presence of inducers such as lactose?

Answer - inducer molecules bind to the lac repressor, thereby altering its conformation so that the repressor can no longer bind to the lac operator site in the DNA.

Without repressor bound to it, the operator site is unoccupied and RNA polymerase can bind to the promoter and proceed down the operon, transcribing the lacZ, lacY, and lacA genes into a single polygenic mRNA molecule (**Figure 23-4b**).

A crucial feature of a repressor protein, therefore, is its ability to exist in two forms, only one of which binds to the operator.

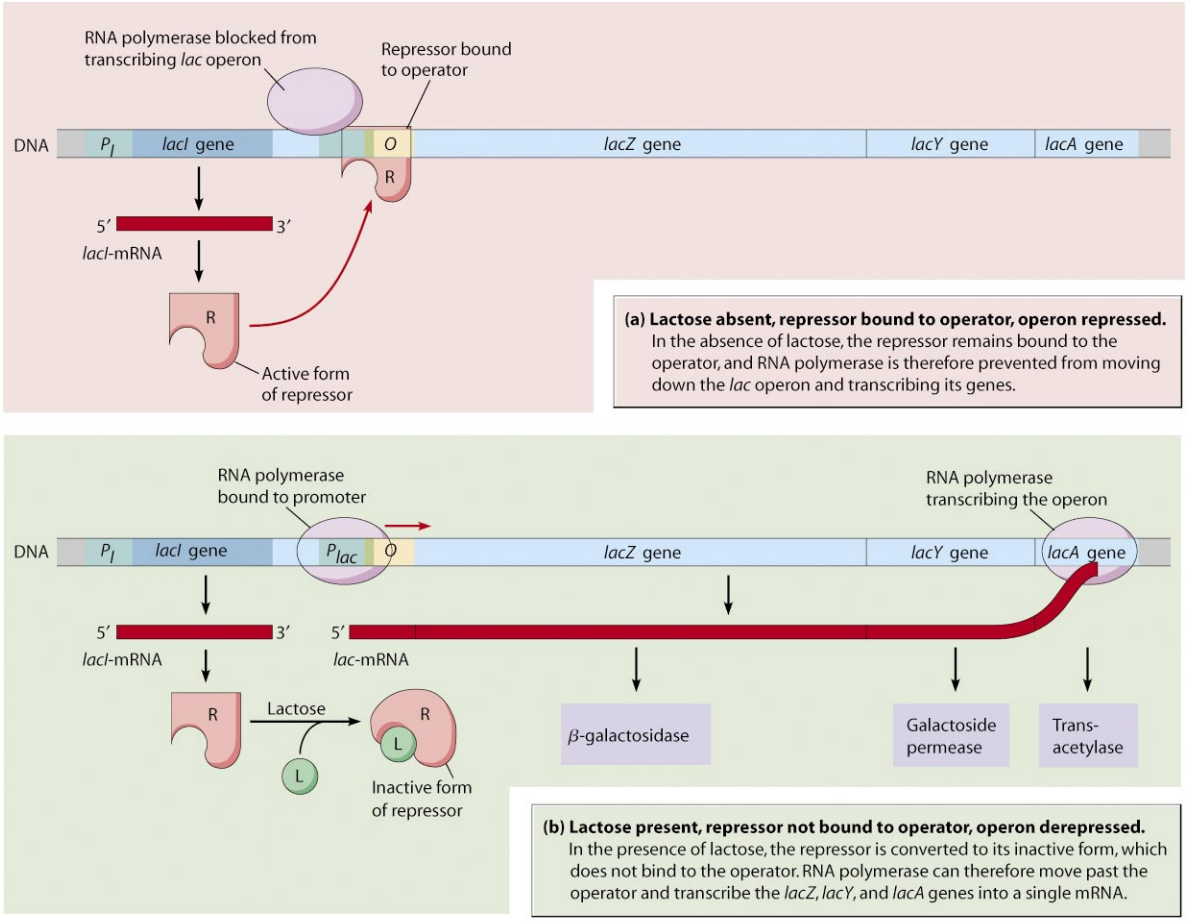


Figure 23-4

Repressor protein

A repressor is an allosteric protein. It can exist in either of **two conformational states**, depending on whether or not an effector molecule is present.

In one state the protein is active; in the other state it is nearly inactive.

When the effector molecule binds to the protein, it induces a change in the conformational state of the protein and therefore in its activity.

The **binding is readily reversible**, however, and departure of the effector results in the protein's rapid return to the alternative form.

Reversible interaction of the lac repressor (notes)

Figure 23-5 shows the reversible interaction of the lac repressor with its effector, called **allolactose**, an isomer of lactose produced after lactose enters the cell.

The conformational form assumed by the repressor protein in the absence of allolactose recognizes and binds to the operator (thereby inhibiting transcription), whereas the form with allolactose attached to it does not.

The result is that the repressor protein inhibits transcription of the lac operon in the absence of allolactose, when there is no need to produce the catabolic enzymes encoded by the lac operon.

In the presence of allolactose, the repressor converts to its inactive form, which does not recognize the operator and hence does not prevent transcription of the lac structural genes by RNA polymerase.

Lactose triggers the induction of the enzymes encoded by the lac operon. Because the lac operon is turned off unless induced, it is said to be an inducible operon.

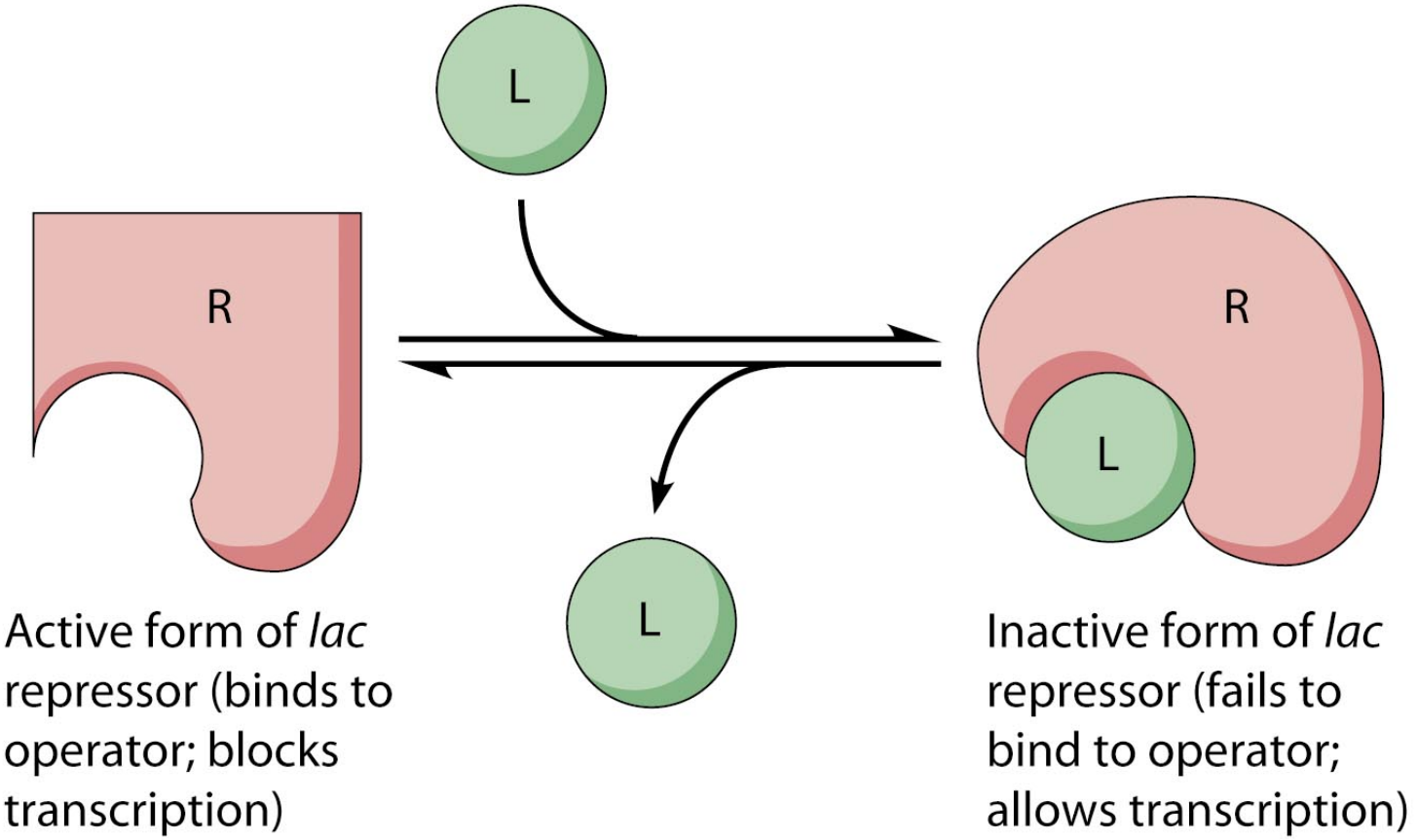


Figure 23-5

The genes involved in tryptophan synthesis are organized into a repressible operon (notes)

Although much of the work leading to the initial formulation of the operon concept involved the lac operon of E. coli, a number of other bacterial regulatory systems are now know to follow the same general pattern, that is, genes coding for enzymes of a given metabolic pathway are clustered together in a group that serves as a unit of both transcription and regulation.

One or more operators, promoters, and regulatory genes are usually involved, although there is sufficient variation from one operon to another to preclude many generalizations.

Operons coding for enzymes involved in catabolic pathways generally resemble the lac operon in being inducible; that is, they are turned on by a specific allosteric effector, usually the substrate for the pathway involved.

In contrast, operons that regulate enzymes involved in anabolic (biosynthetic) pathways are repressible operons; they are turned off allosterically, usually by an effector that is the end-product of the pathway. The tryptophan (trp) operon is a good example of a repressible operon (**Figure 23-6**).

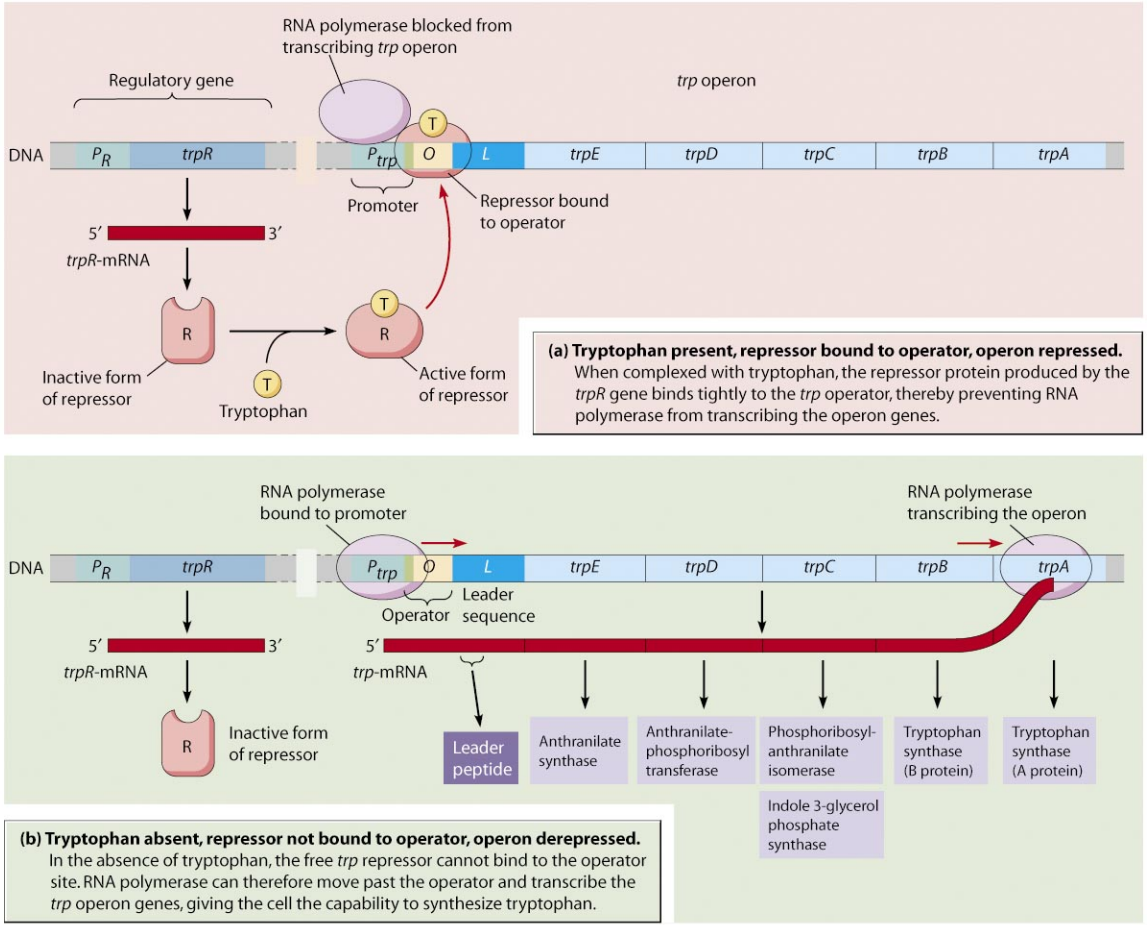


Figure 23-6

The genes involved in tryptophan synthesis are organized into a repressible operon

The trp operon contains the structural genes coding for the enzymes that catalyze the reactions involved in tryptophan biosynthesis, as well as the DNA sequences necessary to regulate the production of these enzymes.

The effector molecule in this case is the end-product of the biosynthetic pathway, the amino acid tryptophan.

Expression of the enzymes produced by the trp operon is repressed in the presence of tryptophan (**Figure 23-6a**) and de-repressed in its absence (**Figure 23-6b**).

Thus, unlike the lac system, the regulatory gene for this operon, called trpR, codes for an allosteric repressor protein that is active (binds to operator DNA) when the effector is attached to it and that is inactive in its free form.

The effector in such systems (in this case, tryptophan) is sometimes referred to as a co-repressor because it is required, along with the repressor protein, to shut off transcription of the operon.

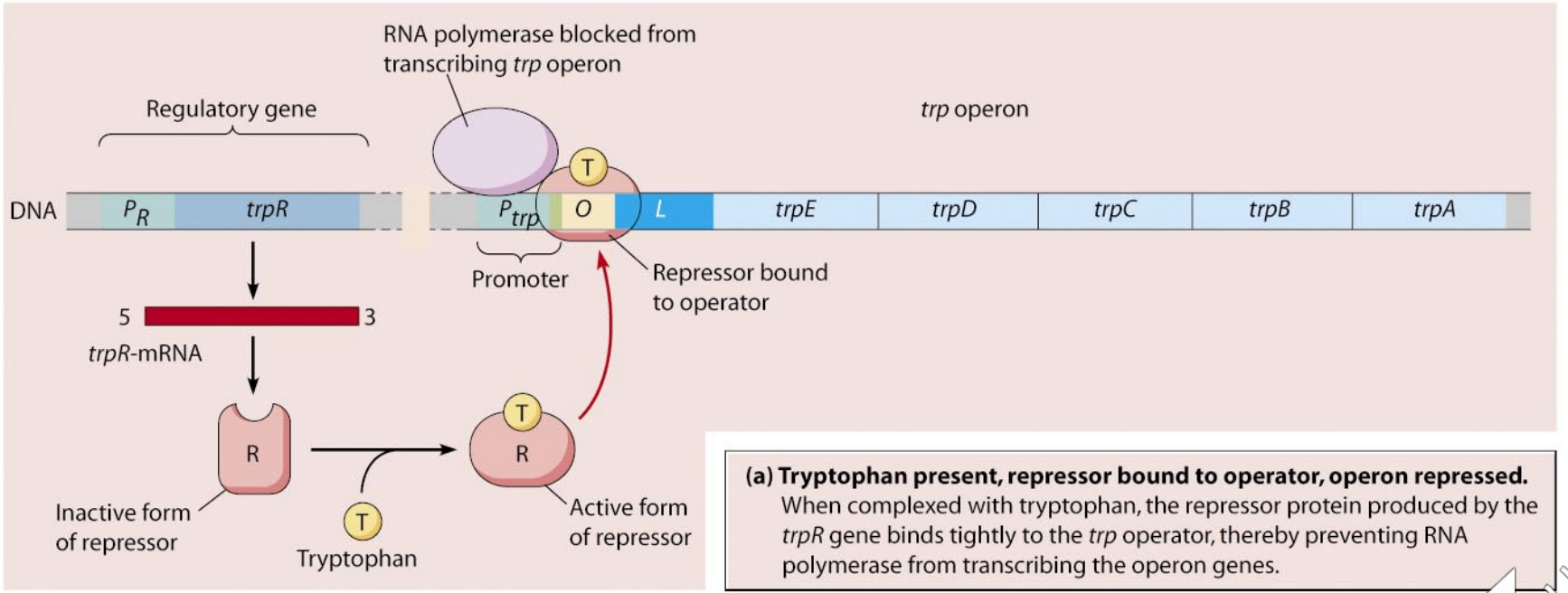
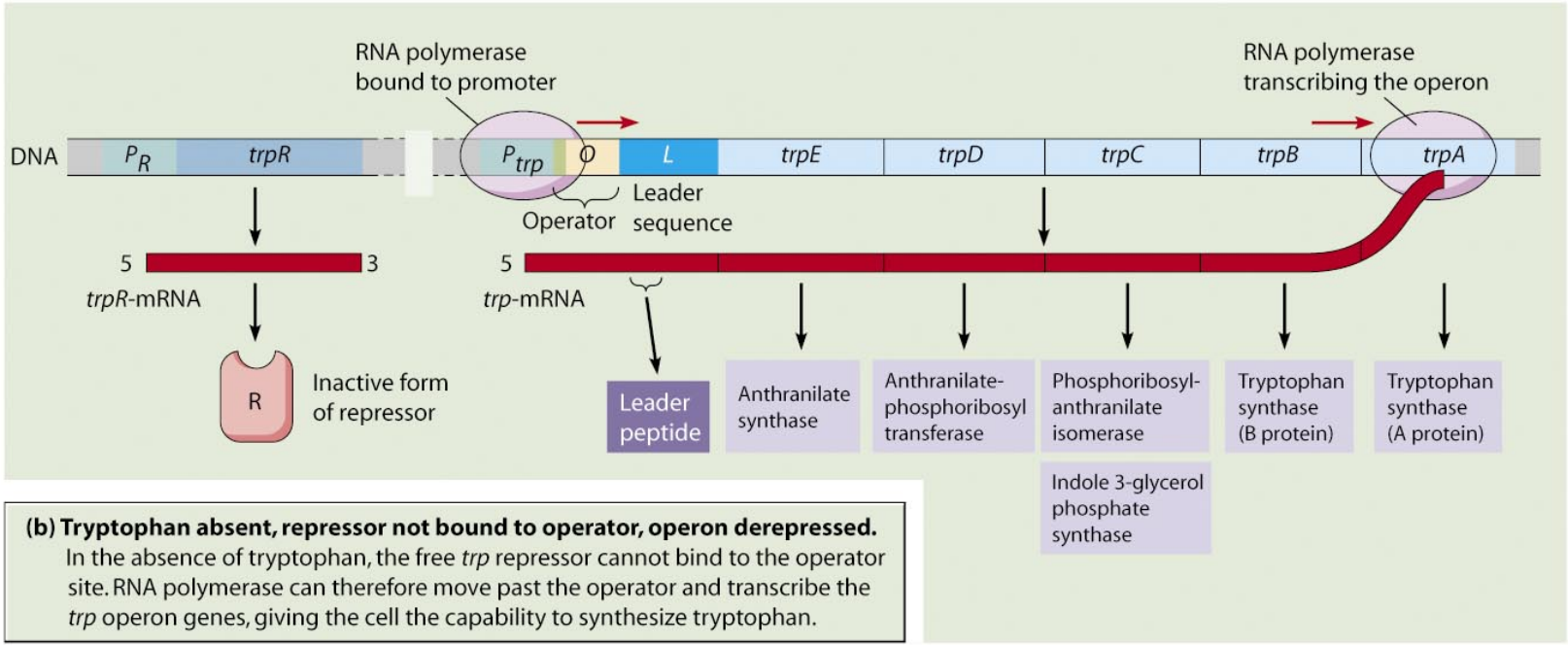


Figure 23-6a



(b) Tryptophan absent, repressor not bound to operator, operon derepressed. In the absence of tryptophan, the free *trp* repressor cannot bind to the operator site. RNA polymerase can therefore move past the operator and transcribe the *trp* operon genes, giving the cell the capability to synthesize tryptophan.

Figure 23-6b

Eukaryotic gene expression is regulated at multiple levels

Gene expression in eukaryotic cells acts at several different levels.

Figures 23-11 (a and b) trace the flow of genetic information from genomic DNA in a eukaryotic nucleus to functional proteins in the cell's cytoplasm.

Five main levels of control: (1) the genome, (2) transcription, (3) RNA processing and export from nucleus to cytoplasm, (4) translation, and (5) post-translational events.

Regulatory mechanisms in the last three categories are all examples of posttranscriptional control which encompasses a wide variety of different processes.

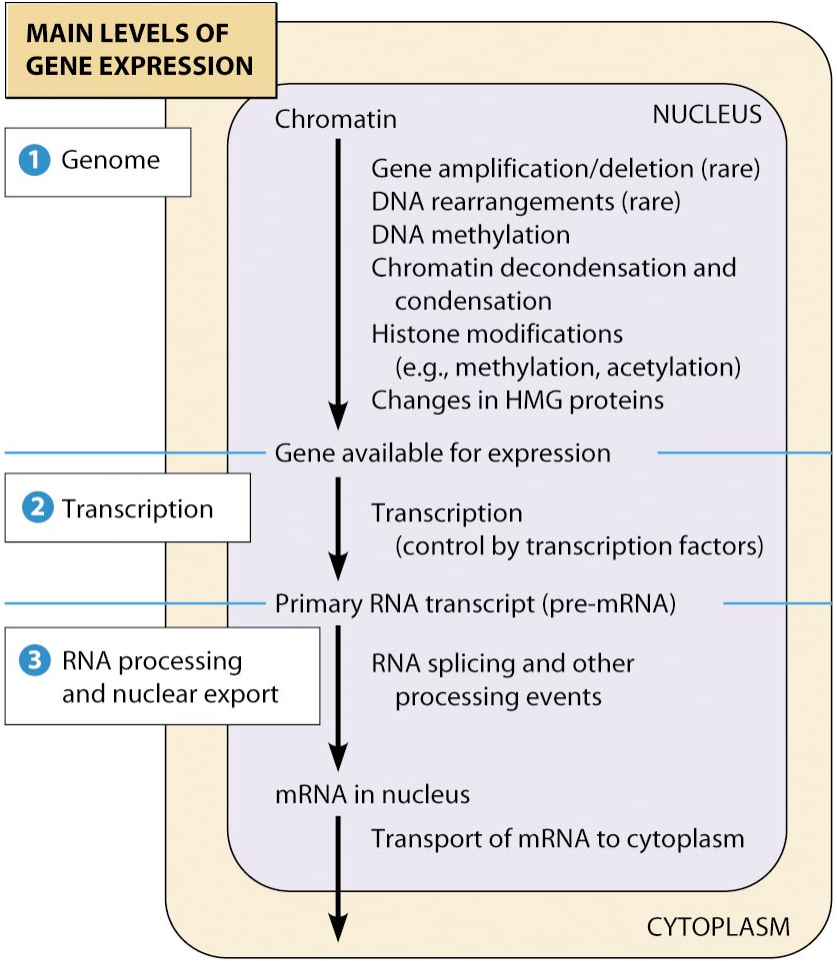


Figure 23-11a

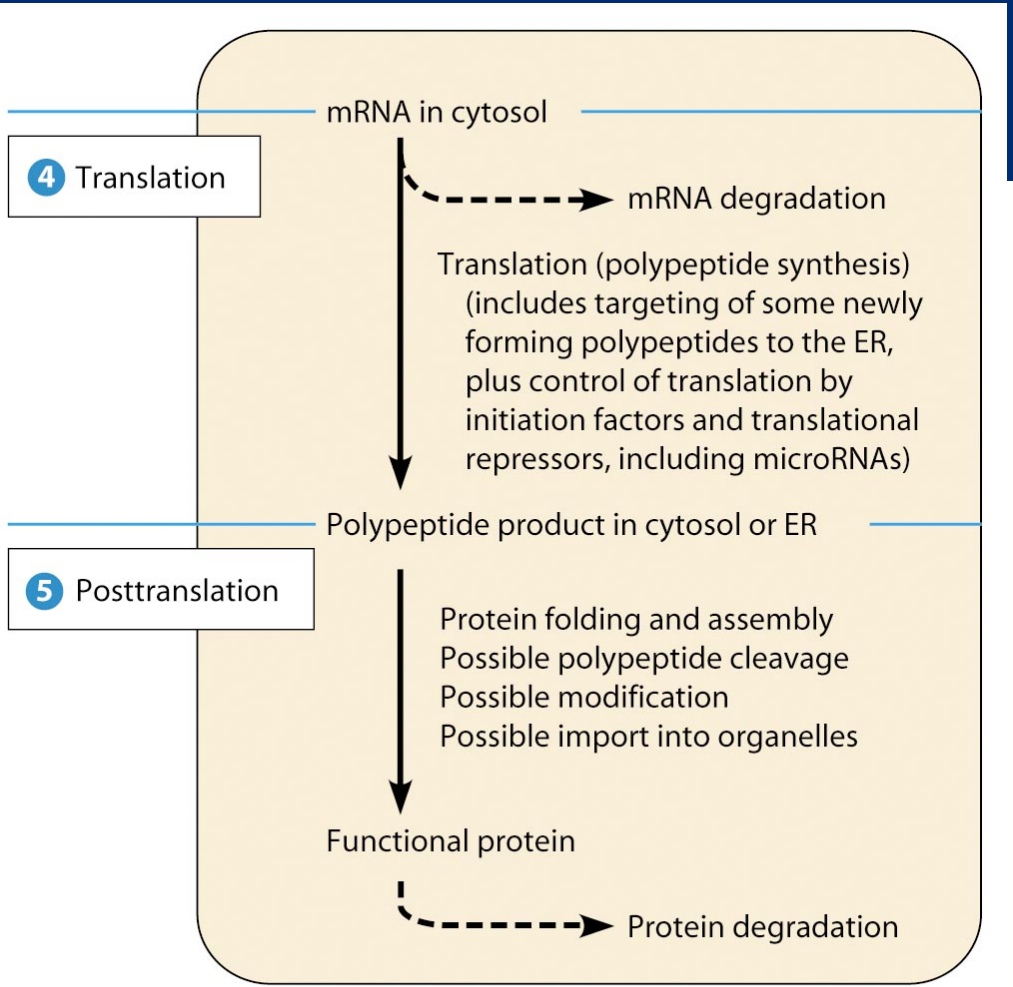


Figure 23-11b