13 - The lac Operon

In Part 13, we will begin learning about the mechanisms that regulate **gene expression** in bacteria. Gene expression refers to processes that activate structural genes, producing a mRNA molecule by transcription and a functional protein product by translation. Specifically, we will study the expression of the ***lac* operon** system in the bacterium *E. coli*. The *lac* operon contains the structural genes that metabolize lactose to produce energy.

Some bacterial genes are always transcribed. These genes that are always expressed are called **constitutive** or **housekeeping genes**. Note that constitutive genes produce constitutive or housekeeping proteins. Housekeeping proteins are required for the normal functioning of the bacterial cell, the so-called housekeeping functions.

Regulated genes change expression under different environmental conditions. In one environment the regulated gene is transcribed, while in another environment the regulated gene is silenced. The mRNAs produced from regulated genes are translated to make **inducible proteins**. Inducible proteins are tightly controlled so that thousands of copies of the protein may be present in certain environments, while only a few or no copies of the protein are produced in other environments. Regulated genes and their protein products are advantageous because they allow bacteria to adapt to changing environments and compete for available resources, such as carbon or nitrogen. Other regulated genes activate cell division.

Key Questions

- What is the difference between a constitutive and a regulated gene?
- What are some metabolic processes that are always occurring in a bacterial cell (or, would be governed by housekeeping genes)?

Inducible Genes

Gene regulation in bacteria often involves controlling the initiation of transcription. Transcriptional regulation requires the binding of **regulatory transcription factor proteins** to **regulatory DNA sequences** near the promoter region of a gene. These regulatory transcription factor proteins function to either enhance or inhibit sigma (σ) factor protein and RNA polymerase core enzyme binding to the promoter.

Regulatory transcription factor proteins include:

- **Repressor proteins**. Repressor proteins decrease how often transcription is initiated (**negative control**).
- **Activator proteins.** Activator proteins increase how often transcription is initiated (**positive control**).

Repressor and activator proteins contain DNA binding domains and have additional domains that bind to small organic molecules (sugars, amino acids, or nucleotides) called **effectors**. When an effector molecule binds, the three-dimensional structure of the repressor or activator protein changes. This change in protein shape influences the ability of the activator protein or repressor protein to bind to the DNA.

How do bacteria turn a regulated gene from an off state to an on

state? For example, how does a bacterium produce the enzymes necessary to metabolize the sugar lactose when lactose becomes available in the environment? An effector molecule, called an **inducer**, causes transcription to increase (**figure 13.1**). Inducers can function in two different ways:

- **The inducer can bind to a repressor**. When the inducer binds to the repressor protein, the repressor is released from a binding site on the DNA, and transcription of the structural gene increases.
- **The inducer can bind to an activator**. In this case, the activator protein cannot bind to the DNA unless the inducer is present. When the inducer binds to the activator protein, the activator can bind to the DNA and transcription increases.

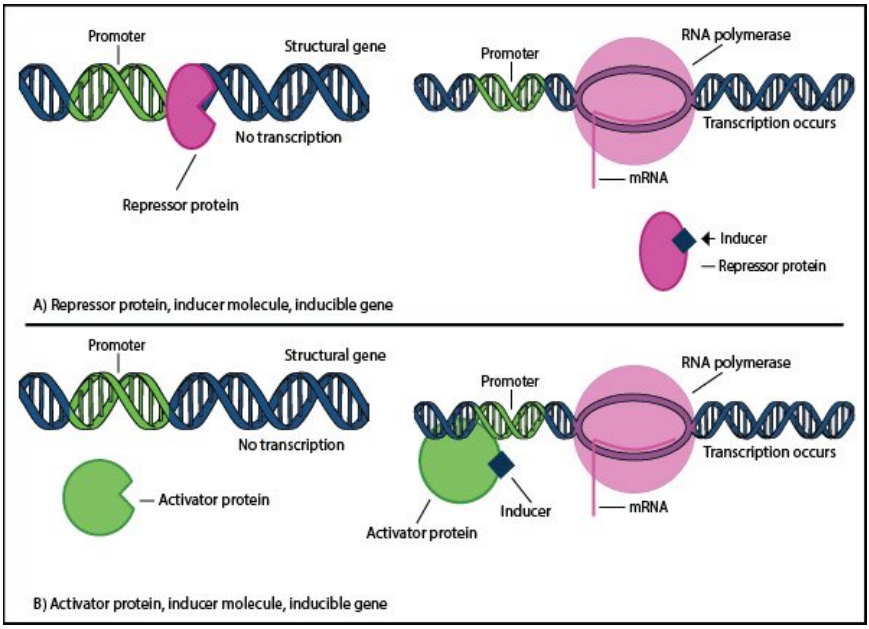


Figure 13.1 ***Inducing a Gene*** *--- Image created by SL*

Key Questions

- How do repressor and activator proteins affect transcription?
- What is an effector molecule?
- Describe two ways that an inducer can turn a gene on.

Repressible Genes

How do bacteria turn a regulated gene from an on state to an off state? For example, how does a bacterium stop producing the enzymes required to make the amino acid tryptophan, when there is plenty of tryptophan in the environment? The presence of an effector molecule may inhibit transcription in two ways (**figure 13.2**):

- **An effector molecule called a corepressor binds to a repressor protein.** Without the corepressor, the repressor protein does not bind to the DNA. When the corepressor binds the repressor, a conformational change occurs in the repressor. The repressor protein can then bind to the DNA and inhibit transcription.
- **An effector molecule, called an inhibitor, binds to an activator protein.** In this case, the activator protein is normally bound to the DNA and activates transcription. When the inhibitor binds to the activator, a conformational change causes the activator protein to be released from the DNA, and transcription ceases.

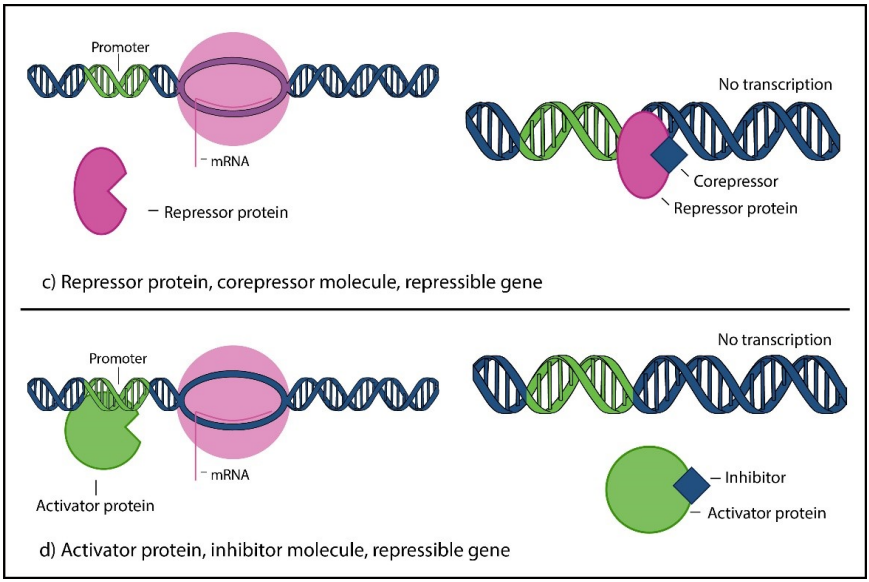


Figure 13.2 ***Repressing a Gene*** *--- Image created by SL*

Key Questions

- Describe how a corepressor and an inhibitor can turn a transcribed gene to an off state.

Enzymes Involved in Lactose Metabolism in *E. coli*

Now we will turn our attention to a specific example of gene regulation in the bacterium *E. coli*. The specific example of gene regulation discussed in this chapter involves lactose metabolism. Lactose is a sugar that can be used as a carbon and energy source for the bacterium *E. coli*. Lactose breakdown by an *E. coli* cell involves three enzymes (**figure 13.3**):

- **Lactose permease.** Lactose permease is a cytoplasmic

membrane protein involved in the transport of lactose from the environment into the cytoplasm of the *E. coli* cell.

- **Beta (β)-galactosidase.** β-galactosidase cleaves the lactose imported by lactose permease, producing the monosaccharides galactose and glucose. Galactose and glucose can then be metabolized by the *E. coli* cell. β-galactosidase can also catalyze a side reaction that converts lactose into the effector molecule **allolactose**. Importantly, allolactose is the inducer that binds to the *lac* repressor protein (see below).
- **Galactoside transacetylase.** Galactoside transacetylase converts atypical isomers of lactose into forms that can be metabolized readily by β-galactosidase.

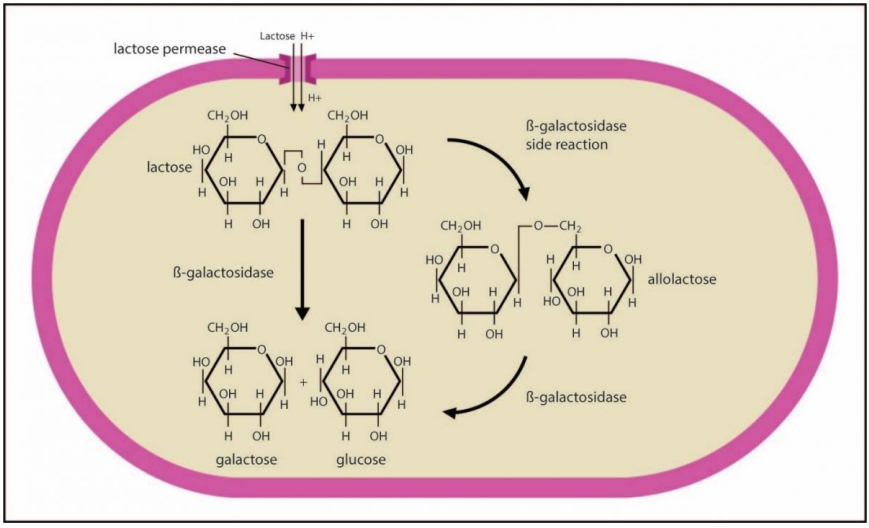


Figure 13.3 ***Two of the enzymes involved in lactose metabolism*** *--- Image created by SL*

Key Questions

- What are the functions of the three bacterial enzymes involved in lactose breakdown?

Operons

In bacteria, a group of structural genes can be under the control of a single group of regulatory DNA sequences, a single promoter, and a single terminator. This grouping of structural genes is an **operon** (**figure 13.4**). Operons allow proteins that are involved in certain biochemical pathways (for example, lactose metabolism) to be controlled in a coordinated way. When an operon is transcribed, a **polycistronic mRNA** is produced that contains the coding regions for multiple types of proteins.

Typical operons contain a **promoter**. Recall that the promoter serves as the binding site for σ factor and contains the transcription start site for the operon. Operons also contain an **operator** DNA sequence where a repressor protein binds, an **activator binding site** DNA sequence where an activator protein binds, **structural genes** that encode proteins, and a **terminator** that signals the end of transcription. Recall that the terminators in bacteria work either using the rho (ρ)-dependent or ρ-independent mechanism.

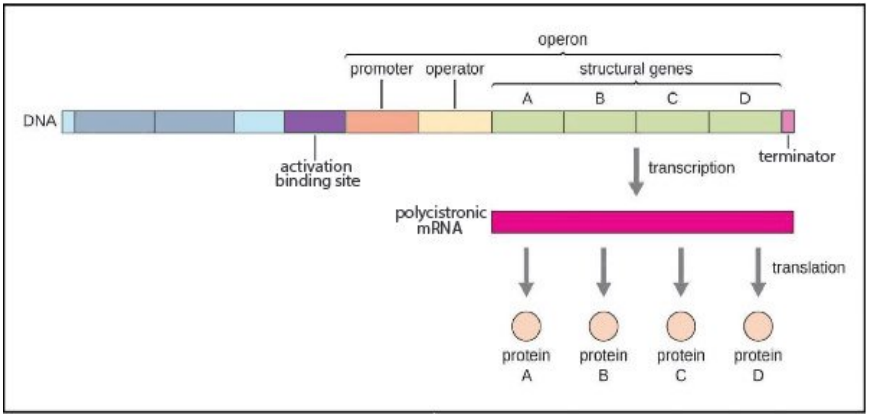


Figure 13.4 ***Operon Structure*** *--- This image is used from OpenStax (access for free at https://books.byui.edu/-vuzA*

Key Questions

- What is an operon?
- Why is it advantageous to organize structural genes into operons?
- What is a polycistronic mRNA?
- What is an operator and an activator binding site?

The Lactose (*lac*) Operon

François Jacob and Jacques Monod first described transcriptional regulation in bacteria by studying lactose metabolism in *E. coli*. Jacob and Monod won the Nobel Prize in 1965 for their work.

Lactose metabolism requires regulating genes within the **lactose (*lac*) operon**. The *lac* operon contains the following DNA sequences and structural genes (**figure 13.5**):

- **CAP site.** The CAP site is a DNA sequence that serves as the binding site for an activator protein called the **catabolite activator protein (CAP)**.
- ***lac* promoter (*lacP*).** *lacP* contains the -35 sequence, the -10 sequence, and the +1 site. *lacP* determines where transcription of the *lac* operon will begin, serving as the binding site for the σ factor protein. Recall that σ factor targets the RNA polymerase core enzyme to the +1 site.
- **Operator site (*lacO*).** *lacO* is the binding site for the *lac* repressor protein.
- ***lacZ*.** *lacZ* is the structural gene that encodes the enzyme **β-galactosidase**.
- ***lacY*.** *lacY* is the structural gene that encodes the enzyme **lactose permease**.
- ***lacA*.** *lacA* is the structural gene that encodes the enzyme **galactoside transacetylase**.

- ***lac* terminator.** The *lac* terminator is a DNA sequence involved in transcriptional termination. The *lac* operon is terminated by the rho (ρ)-dependent mechanism.

Near the *lac* operon is another gene, called ***lacI***, that contains its own promoter and terminator. The *lacI* gene encodes the ***lac* repressor** protein. The *lac* repressor protein binds to the *lacO* sequence and turns off the expression of the *lac* operon (in other words, the *lac* operon displays **negative control** via the *lac* repressor). The *lacI* gene is a constitutive or housekeeping gene and is therefore always transcribed.

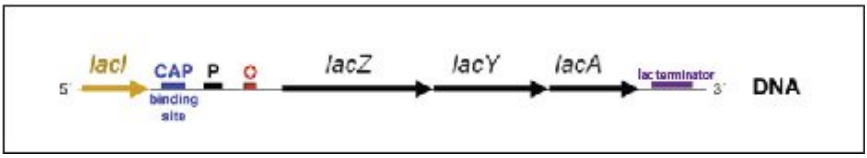


Figure 13.5 ***Lac operon structure*** *--- Image created by G3Pro and modified by SL. Image used under license CC BY 2.0*

Key Questions

- What are names of the three structural genes of the *lac* operon?
- What are the names and functions of the four regulatory DNA sequences within the *lac* operon?
- What is the function of the *lacI* gene?

lac Operon Expression

In the absence of lactose, **repression** of the *lac* operon occurs as follows (**figure 13.6** and **13.7**):

1. *lacI* is a constitutive gene, meaning that it is always transcribed. Transcription of the *lacI* gene produces a *lacI*

mRNA that is then translated to produce the *lac* repressor protein.
2. The *lac* repressor protein binds to the operator (*lacO*) DNA sequence.
3. Sigma (σ) factor and the RNA polymerase core enzyme do not bind efficiently to *lacP* when the *lac* repressor is bound to *lacO*. As a result, the three structural genes (*lacZ*, *lacY*, and *lacA*) of the *lac* operon are transcribed at a low level, producing only a few (5–10) copies each of β-galactosidase, lactose permease, and galactoside transacetylase per cell.

When lactose is present in the environment, the *lac* operon is **induced** as follows:

1. The few copies of lactose permease expressed by *E. coli* move lactose across the cytoplasmic membrane into the cytoplasm of the cell.
2. The few copies of β-galactosidase present convert lactose into **allolactose**.
3. Allolactose binds to the *lac* repressor protein. The binding site for allolactose on the *lac* repressor is called the **allosteric site**.
4. The conformation of the *lac* repressor protein changes.
5. The *lac* repressor protein is released from *lacO*.
6. Sigma (σ) factor and the RNA polymerase core enzyme bind to *lacP* efficiently.
7. The structural genes of the *lac* operon (*lacZ*, *lacY*, and *lacA*) are transcribed to produce a polycistronic mRNA. The polycistronic mRNA is then translated to produce thousands of copies β-galactosidase, lactose permease, and galactoside transacetylase.

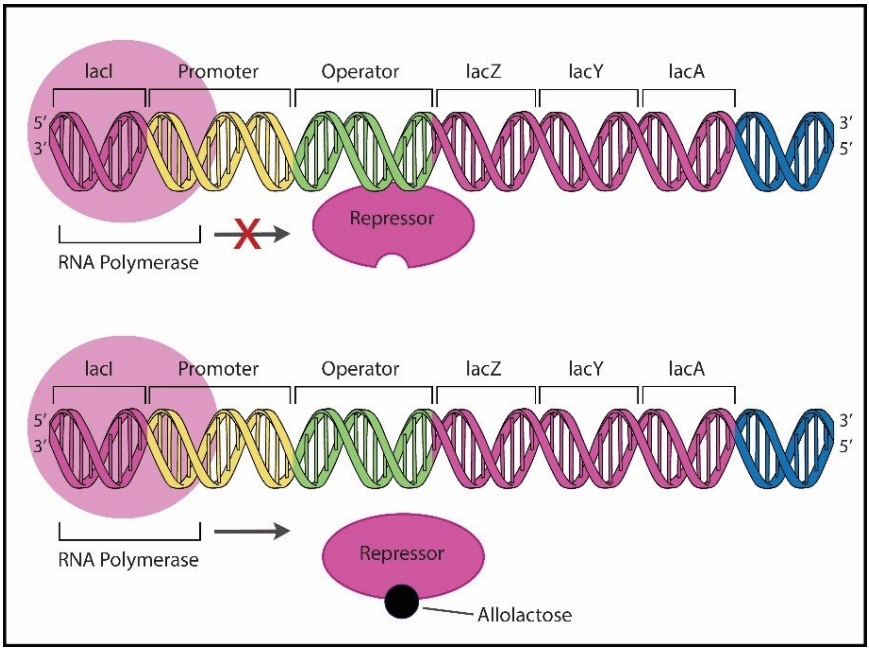


Figure 13.6 ***Lac Operon in the Absence/Presence of Lactose*** *--- This image is used from OpenStax (access for free at https://books.byui.edu/-vuzA modified by SL*

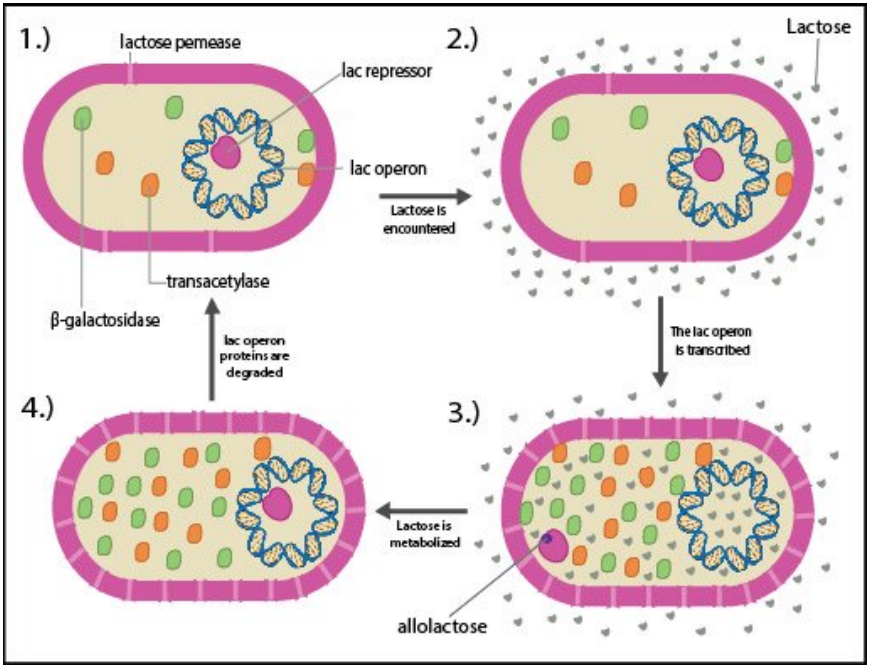


Figure 13.7 ***Summary of lac Operon Induction*** *--- Image created by SL*

Key Questions

- Describe the steps involved in inducing the *lac*
- Describe the steps involved in turning off the *lac*

lacI$^-$ —Constitutive Expression of the *lac* Operon

To gain an appreciation of the genetics involved in *lac* operon regulation, let us focus on the experiment that determined the function of the *lacI* gene product, which we now know makes the *lac* repressor protein.

When François Jacob and Jacques Monod first started studying lactose metabolism, they identified a mutant strain of *E. coli* that they named *lacI*$^-$. In this *lacI*$^-$ mutant strain, the enzymes involved in lactose metabolism were always produced, even in the absence of lactose. Thus, the *lacI*$^-$ mutation is a **constitutive mutation** producing constitutive expression of the *lac* operon. How could this phenotype be explained?

Jacob and Monod suggested that the *lacI*$^-$ constitutive phenotype could be explained in two ways:

- The *lacI*$^-$ mutation produces a defective protein that activates transcription in all environmental conditions (constitutive activator hypothesis).
- The *lacI*$^-$ mutation produces a defective protein that fails to inhibit transcription (defective repressor hypothesis).

Mutant and Merozygote Strains of *E. coli*

To distinguish between the two hypotheses indicated above, Jacob and Monod examined two strains of *E. coli*. Jacob and Monod studied the *lacI*$^-$ strain described above, and they examined an unusual strain of *E. coli* called a **merozygote**, or partial diploid.

Bacteria typically have a single circular chromosome. However, bacteria often contain small circular DNA molecules in addition to the chromosome. These small DNA molecules are the **plasmids** that are used in gene cloning experiments (see Part 12). A common type of plasmid is the **F plasmid** that functions in bacterial fertility (i.e., DNA transfer between bacteria). The merozygote strain that Jacob and Monod examined in their experiments contained a modified F plasmid (**F' plasmid**), which contained a *lacI* gene and the *lac* operon. Thus, *E. coli* cells that contain an F' plasmid are merozygotes (partial diploids), containing a copy of *lacI* and the *lac* operon genes on both the chromosome and on the F' plasmid.

The merozygote strain used by Jacob and Monod contained *lacI*$^-$ on the chromosome and a wild-type copy of the gene (*lacI*$^+$) on the F' plasmid; this *E. coli* strain was in essence a *lacI*$^+$/*lacI*$^-$ heterozygote. The other DNA sequences within the *lac* operon (*lacP*, *O*, *Z*, *Y*, and *A*) were wild-type and were found on both the chromosome and the F' plasmid. Thus, the *E. coli* merozygote strain was homozygous for these other DNA sequences).

Key Questions

- What is a merozygote?
- Why it might be advantageous for an *E. coli* cell to have two copies of each *lac* operon gene?

The Jacob and Monod Experiment

The Jacob and Monod experiment compared a *lacI*$^-$ strain (*lac* operon is always expressed) to the *lacI*$^+$/*lacI*$^-$ merozygote strain. The experiment was done as follows:

1. The mutant (*lacI*$^-$) and the merozygote (*lacI*$^+$/*lacI*$^-$) strains were grown in separate flasks.

2. Each bacterial culture was then split into two smaller flasks, a control flask and an experimental flask. For example:

    - Mutant strain (*lacI*$^-$)
        - Control (flask 1)
        - Experimental (flask 2)
    - Merozygote strain (*lacI*$^+$/*lacI*$^-$)
        - Control (flask 3)
        - Experimental (flask 4)

3. Lactose was added to the experimental flasks (tubes 2 and 4).

4. The bacterial cultures were incubated to allow transcription and translation of the β-galactosidase, lactose permease, and galactoside transacetylase proteins.

5. The bacterial cells in each flask were then lysed using ultrasonic sound waves.

6. β-galactosidase levels in each of the four lysates were measured. β-galactosidase can convert the chemical β-O-nitrophenylgalactoside (β-ONPG), which is colorless, into galactose and O-nitrophenol, which is yellow. Note that if a yellow product is formed, β-galactosidase is present.

7. The O-nitrophenol (yellow product) levels in each lysate were measured using a spectrophotometer.

Key Questions

- Explain the purpose of flasks 1–4 in the Jacob and Monod experiment.
- Explain what is occurring when a reaction turns yellow.

lacI$^+$ Produces a Diffusible Repressor Protein

Yellow color was observed in flasks 1 and 2 for the mutant strain (*lacI*-). Thus, in the *lacI*$^-$ strain, β-galactosidase is produced in the absence and in the presence of lactose (i.e., expressed constitutively).

In the merozygote strain, no yellow color was produced in the absence of lactose; however, two times the yellow color was produced in the

presence of lactose. This means that β-galactosidase is not produced when lactose is absent (flask 3) because the *lacI*$^+$ gene on the F’ plasmid produces a protein factor (i.e., the *lac* repressor protein) that binds to both copies of *lacO,* and thus inhibits expression of both the chromosomal and F’ plasmid genes that make β-galactosidase. Remember that bacteria do not have a nuclear membrane, so both the host chromosome and the F' plasmid are found in the cytoplasm. This *lac* repressor protein diffuses throughout the cytoplasm of the cell and can bind to **any** *lac* operator. Because the *lac* repressor can bind to any operator in the cell, the *lac* repressor is said to be an example of a ***trans*-acting factor**.

When lactose is present, lactose is converted to allolactose, and allolactose releases the *lac* repressor proteins from both copies of the operator. The *lac* operons on both the chromosome and on the F’ plasmid are now expressed (flask 4). The expression of two copies of the *lac* operon produces two times the yellow color in flask 4.

This experimental result provided supporting evidence for the defective repressor hypothesis. It is worth noting that in the case of the *lacI*$^-$ constitutive activator hypothesis, the *lac* operon should have been expressed in the merozygote strain in both the absence (flask 3) and in the presence of lactose (flask 4).

Key Questions

- Explain how the flask 3 result showed that the *lac* repressor protein is an example of a *trans*-acting factor.

Glucose and the *lac* Operon

The *lac* operon can also be regulated by glucose. Glucose is the

preferred carbon and energy source used by *E. coli*. The genes involved in glucose breakdown (catabolism) are expressed constitutively (always transcribed). In the presence of glucose, the *lac* operon is not needed, so transcription of the *lac* operon is turned off (**catabolite repression**). When glucose becomes limited and lactose is present, this catabolite repression is alleviated, and the *lac* operon is turned on. Lactose is then used by the *E. coli* cell as the carbon and energy source.

The sequential use of sugars—first glucose, followed by lactose—is called **diauxic growth** (**figure 13.8**).

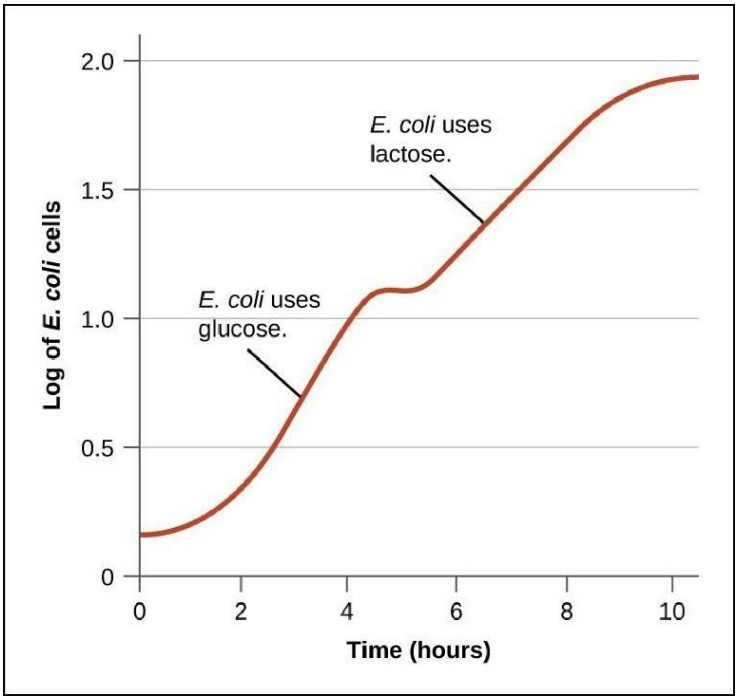


Figure 13.8 ***Diauxic growth*** *--- Image created by SL*

How is the *lac* operon repressed (turned off) by glucose? Recall that

there are two factors involved in gene regulation in bacteria:

- **Regulatory transcription factor protein**. The regulatory transcription factor protein that is responsive to glucose levels is the **catabolite activator protein (CAP).** The CAP protein binds to the CAP binding site in the *lac* operon, which is located immediately upstream of the *lac* operon promoter (*lacP*).
- **Effector molecule**. It would be reasonable to assume that glucose is the effector molecule involved in catabolite repression; instead, the effector involved in glucose regulation is **cyclic AMP (cAMP)** (**figure 13.9**).

cAMP is produced from ATP by the enzyme **adenylyl cyclase**. When glucose is present is present in the environment, adenylyl cyclase activity is inhibited, and cellular cAMP levels are low. When glucose levels in the environment are low, adenylyl cyclase activity increases, resulting in higher levels of cAMP in the cell.

When the CAP protein binds to cAMP, the CAP protein changes conformation (shape) and can then bind to the CAP site in the DNA. *Lac* operon transcription is activated. In fact, for sigma (σ) factor and the RNA polymerase core enzyme to bind efficiently to the *lac* promoter and transcribe the *lac* operon, CAP must be bound to the CAP site.

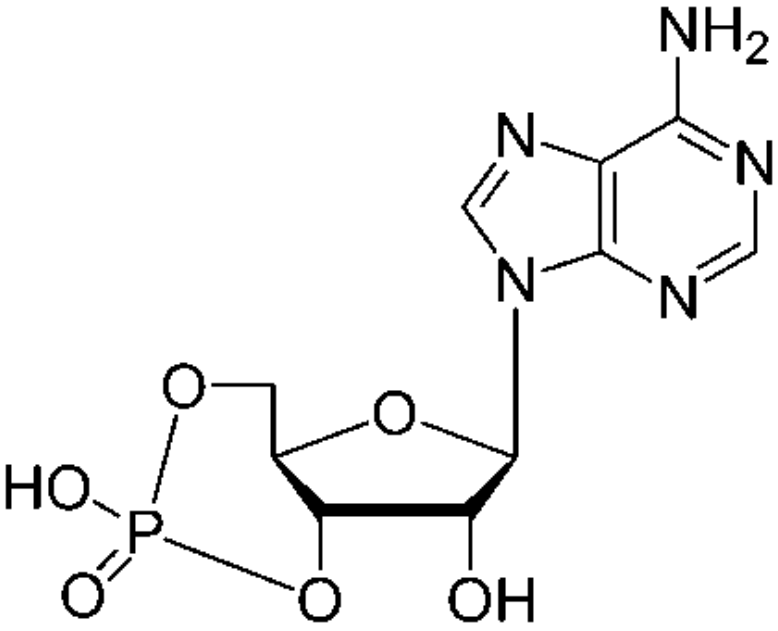


Figure 13.9 ***Cyclic AMP (cAMP) structure***

Key Questions

- Describe how the *lac* operon responds when glucose levels are high or low (assuming that lactose is present).
- What is the function of adenylyl cyclase?
- Explain the relationship between cAMP levels and CAP function.
- Describe the relationship between CAP binding to the CAP site and the transcription of the *lac operon.*

Regulation of the *lac* Operon by the *lac* Repressor and CAP

The interaction between a positive regulatory signal (CAP) and a negative regulatory signal (*lac* repressor) makes transcriptional regulation of the *lac* operon more complicated. What happens to *lac* operon expression when an *E. coli* cell encounters the following conditions? (**figure 13.10**)

- **No glucose or lactose in the environment.** In this environment, cAMP levels are high, and the cAMP is bound to the CAP protein. The cAMP:CAP complex binds to the CAP site on the DNA and tries to promote transcription. However, in the absence of lactose, there is no allolactose, so the *lac* repressor is bound to the operator DNA sequence, preventing transcription. **In this environment, the *lac* operon is not transcribed efficiently**.
- **Glucose is present in the environment; however, there is no lactose.** In this case, cAMP levels are low. At low cAMP levels, the CAP protein does not bind to the CAP site on the DNA. The absence of bound CAP protein is a negative signal that inhibits transcription. Since there is no lactose, there is no allolactose, so the *lac* repressor is bound to the operator DNA sequence. **In this environment, the *lac* operon is not transcribed efficiently.**
- **Glucose and lactose are present in the environment.** In the presence of glucose, cAMP levels are low. Thus, cAMP is not bound to the CAP protein, and CAP does not bind to the CAP site on the DNA. The absence of bound CAP protein is a negative signal that inhibits transcription. In the presence of lactose, allolactose binds to the *lac* repressor, releasing it from the operator. The release of the lac repressor tends to promote transcription. **In this environment, the inability of the CAP protein to bind to the CAP site results in inefficient *lac***

operon transcription.

- **Glucose is absent but lactose is present in the environment.** In this case, cAMP levels are high. cAMP binding to CAP protein changes the conformation of CAP, allowing the CAP protein to bind to the CAP site on the DNA. This serves as a positive signal that tends to promote transcription. When lactose is present, allolactose is present. The *lac* repressor protein binds to allolactose and therefore is released from the operator DNA site. **In this environment, the *lac* operon is transcribed.**

In summary, there is only one way the *lac* operon is transcribed: glucose must be absent from the environment, and lactose must be present.

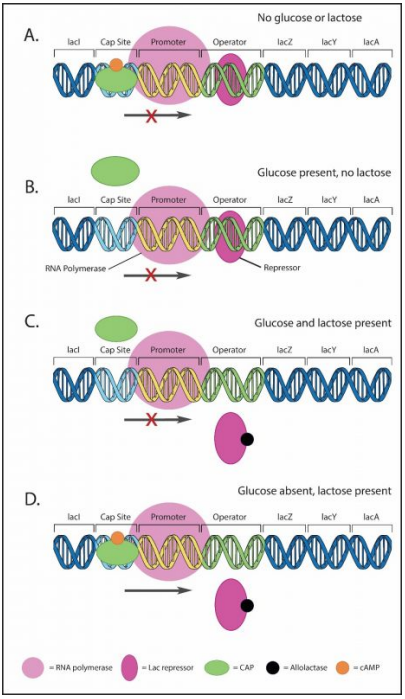


Figure 13.10 ***Lac Operon Expression Under All Cellular Conditions*** *--- Image created by SL*

Key Questions

- Why is the *lac* operon **off** if neither glucose nor lactose are present in the cell?
- Why is the *lac* operon **off** if glucose is present and lactose is absent in the cell?
- Why is the *lac* operon **off** if both glucose and lactose are present in the cell?
- Why is the *lac* operon **on** if glucose is absent and lactose is present in the cell?

Mechanisms of Gene Regulation in Bacteria

The *lac* operon is one example of how bacteria can control gene expression. There are many ways to control the expression of a gene in bacteria:

- **Regulation of transcription**.
    - **Controlling how often transcription starts**. Controlling how often transcription starts involves regulating σ factor (and the RNA polymerase core enzyme) binding to the promoter. Regulatory transcription factors (activator and repressor proteins) enhance or inhibit σ factor binding. The *lac* operon is an example of this type of gene regulation.
    - **Attenuation**. Attenuation involves activating transcription to begin producing a mRNA molecule; however, transcription is terminated prematurely before the entire mRNA is made.
- **Regulation of translation**.

- **Translation repressor proteins.** Translation repressor proteins prevent the initiation of translation. These repressor proteins bind to the Shine-Dalgarno sequence on the mRNA, preventing the 16S rRNA component of the ribosome from binding to the mRNA.
- **Antisense RNA.** Antisense RNA molecules are produced by *E. coli* cells to form hydrogen bonds with the Shine-Dalgarno sequence of a particular mRNA, preventing the 16S rRNA component of the ribosome from binding to the mRNA. Antisense RNA molecules are examples of **noncoding RNAs (ncRNAs)**. ncRNAs are not translated to make protein products.

- **Posttranslational regulation**.

  - **Feedback inhibition**. Feedback inhibition is a situation in which the biochemical products of a metabolic pathway inhibit the first enzyme in the pathway.
  - **Covalent modification**. Covalent modification involves altering the structure and function of a protein by attaching phosphate groups, methyl groups, sugars, or lipids.

Key Questions

- What is an advantage of transcriptional regulation?
- What is an advantage of posttranslational regulation?

Review Questions

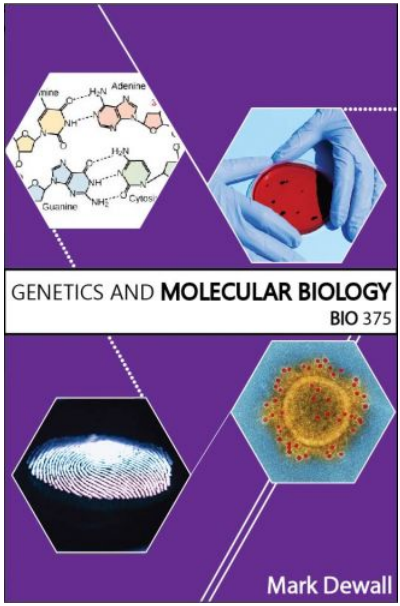
Fill in the blank:

1. _____ is a type of regulatory transcription factor protein that increases transcription.

2. _____ molecules are organic compounds that bind to a repressor or activator protein to change its three-dimensional shape.
3. _____ is the enzyme that catalyzes the cleavage of the disaccharide lactose into two simple sugars.
4. Identify each of the following genes of the *lac* operon:

- The _____ gene encodes the *lac* repressor.
- The _____ gene encodes lactose permease.
- The _____ gene produces an enzyme that converts atypical isomers of lactose into forms that can be used in the bacteria cell.

5. The binding site for the *lac* repressor protein is called _____.
6. The two inducers of the *lac* operon are _____ and _____.
7. A merozygote contains two copies of a gene; one gene is located on the _____ while the other gene is located on the _____.
8. _____ is a yellow compound that is produced when β-galactosidase is active.
9. _____ is an enzyme that is inhibited by glucose.
10. Lactose and glucose regulate expression of the lactose operon. The highest expression of the *lac* operon occurs when _____ is absent and _____ is present.



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