

Lab 9: Regulation of lactose metabolism

There are clearly different means and levels of regulating gene products, but the first comprehensive description of regulation of genes was that of the enzymes necessary for lactose metabolism in the bacterium, *Escherichia coli*. It has been known since the early 1900s that bacteria make the enzymes needed to metabolize certain energy sources only in the presence of the substrate. If the substrate is absent, gene expression is turned off. Thus, there is no mRNA and no enzyme for metabolizing the substrate. *E. coli* can use a variety of sugars as an energy source; for example: maltose, glucose, lactose. In the 1950s François Jacob and Jacques Monod, two microbiologists at the Pasteur Institute in Paris, determined how *E. coli* regulates the production of β -galactosidase, the enzyme necessary to cleave lactose into glucose and galactose for use as an energy source by the cell.

The basics of the system are as follows. There are three genes that are contiguous on the *E. coli* chromosome. They have a shared promoter region that binds the RNA polymerase and are all transcribed in the same direction into one mRNA molecule. These three genes transcribed together into a single mRNA constitute an **operon**. The genes, in order from the promoter region, are: 1) *lacZ*, 2) *lacY*, and 3) *lacA*. The *lacZ* gene codes for β -galactosidase, the enzyme that is necessary to metabolize the lactose. The *lacY* gene codes for a permease enzyme that enables the transport of the lactose through the cell membrane into the cell. Although the transacetylase, gene, *lacA*, is transcribed with *lacZ* and *lacY*, it is not directly associated with metabolizing lactose and will not be considered further here.

Close to (upstream from) these three genes, but not directly adjacent to them, is another gene, the *lacI* gene, that is crucial in the regulation of the transcription of the *lacZ* and *lacY* genes. The *I* gene codes for a protein, the **repressor** protein, that prevents transcription of *lacZYA*. The *lacI* protein does this by binding to the DNA at a position called the **operator** located directly downstream from and partially overlapping the **promoter** where the RNA polymerase binds to transcribe the three *lac* genes.

The **repressor** protein has two binding sites—one is for binding the DNA of the operator site, the other is specific for binding galactoside molecules (lactose molecules and other galactosides that are analogs of lactose). As long as there is no lactose in the cell media, the repressor protein remains bound to the DNA at the operator. However, when lactose is present, lactose binds the repressor at the lactose site. Binding of the lactose to the repressor protein changes the repressor's shape which reduces the affinity of the repressor for the operator site. This causes the repressor to fall off the operator site and allows the RNA polymerase bound at the promoter site to transcribe the mRNA for the three genes, *lacZ*, *Y*, and *A*. Once all the lactose has been metabolized and the lactose site on the repressor is free, the repressor's conformation allows it to bind again to the operator site and stop the RNA polymerase from initiating transcription of the three genes. Thus, the cell makes β -galactosidase and permease only when lactose is present.

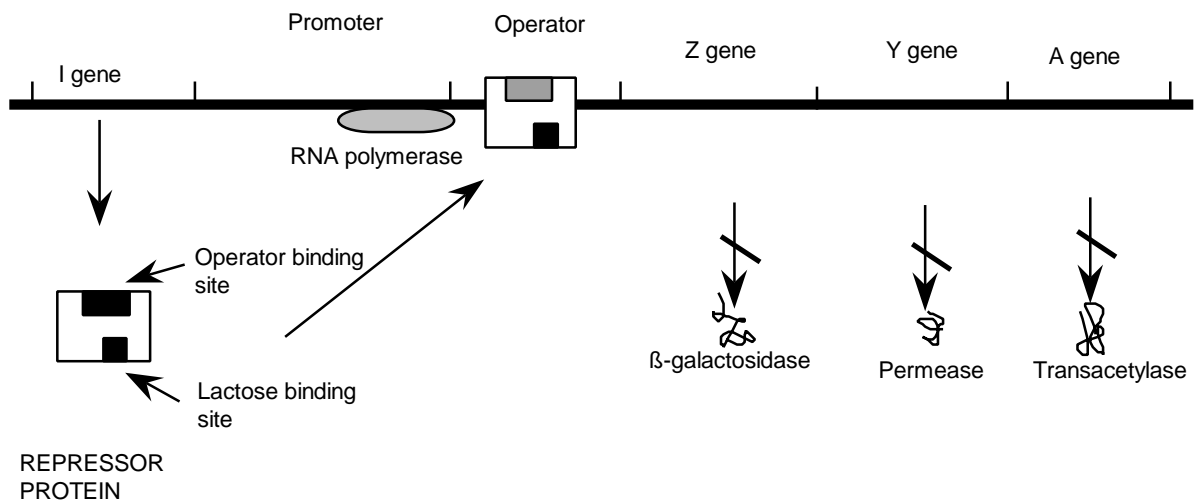


Figure 1. With no lactose present the repressor protein binds to the operator and the RNA polymerase is unable to initiate transcription of the *lac* mRNA. None of the three enzymes can be produced.

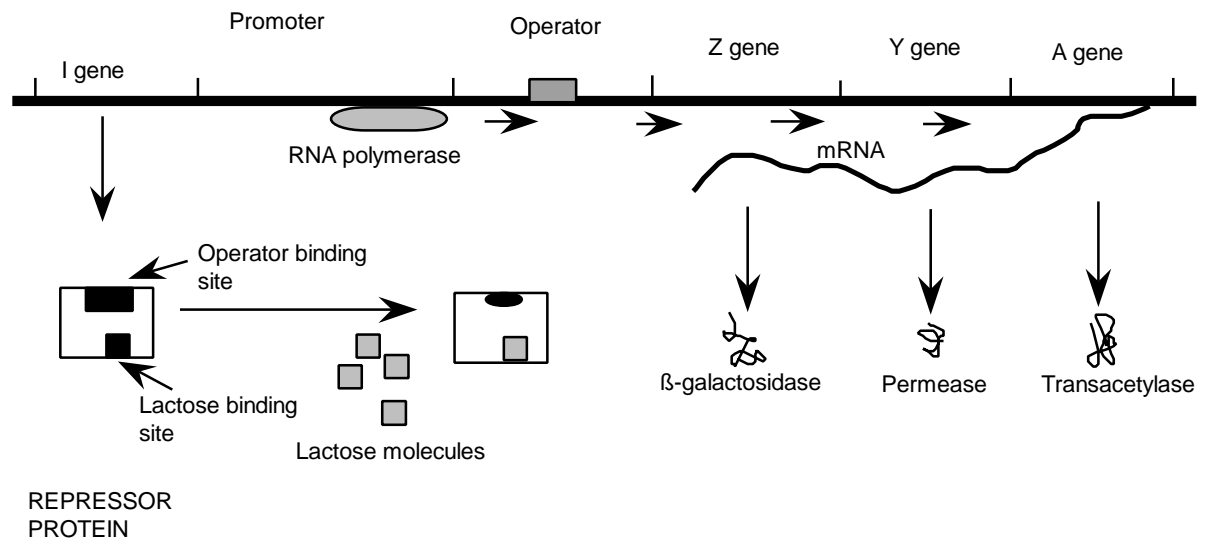


Figure 2. Lactose binds to the binding site on the repressor protein and changes the affinity of the operator binding site for the operator DNA. Now the RNA polymerase can initiate transcription of the *lac* mRNA.

Jacob and Monod deduced the mechanism of the lac operon by looking at the phenotypes of various mutants in the system. A list of various mutants is given below. There are two types of mutants. The first set includes mutations in the DNA that codes for the various structural proteins of the system. They are:

Z^- : encodes a non functional β -galactosidase.

Y^- : encodes a non functional permease.

I^- : encodes a repressor protein that will not bind to the operator site. This mutation is **constitutive**, i.e., β -galactosidase and permease are always transcribed whether lactose is present or not.

I^S : encodes a repressor protein that has a modified lactose binding site that does not allow the lactose molecule to bind. Once the repressor is bound to the operator site, it cannot be removed even if lactose is available in the media. The *lac* genes are not transcribed with or without lactose available.

The next set consists of mutations for binding sites on the DNA for the repressor protein (the operator) and for the RNA polymerase (the promoter). These are called **polar mutations** because their presence affects the production of genes downstream from them in the operon. They are:

O^C : operator site with DNA that has been modified so that the repressor will not bind to it. This mutation is **constitutive**, i.e., β -galactosidase and permease are always transcribed whether lactose is present or not.

P^- : promoter DNA region with a mutation that inhibits the binding of the RNA polymerase.

Table 1. Synthesis of β -galactosidase and Permease in Haploid Strains of *E. coli*.

GENOTYPE	β -galactosidase made		Permease made	
	Lactose present	Lactose absent	Lactose present	Lactose absent
1. $I^+ P^+ O^+ Z^+ Y^+$	+	-	+	-
2. $I P^+ O^+ Z^+ Y^+$	+	+	+	+
3. $I^- P^+ O^+ Z^+ Y^+$	-	-	-	-
4. $I^+ P^- O^+ Z^+ Y^+$	-	-	-	-
5. $I^+ P^+ O^c Z^+ Y^+$	+	+	+	+
6. $I^+ P^+ O^+ Z^- Y^+$	-	-	+	-
7. $I^+ P^+ O^+ Z^+ Y^-$	+	-	-	-

Although bacteria are haploid organisms, it is possible to make a partial diploid, called a merozygote, that help in determining the nature of the mutations. In Table 2 below, Numbers 8 and 9 indicate that the I gene is responsible for making a substance that is diffusible—that can act on its own chromosome as well as the other chromosome. Numbers 10 and 11 illustrate that the O mutant is a polar mutant and affects only the genes distal to it on the same chromosome, but not on the genes on the other chromosome.

Table 2. Synthesis of β -galactosidase and Permease in Haploid Strains of *E. coli*.

GENOTYPE	β -galactosidase made		Permease made	
	Lactose present	Lactose absent	Lactose present	Lactose absent
8. $I^s P^+ O^+ Z^+ Y^+ / I^+ P^+ O^+ Z^+ Y^+$	-	-	-	-
9. $I P^+ O^+ Z^+ Y^+ / I^+ P^+ O^+ Z Y$	+	-	+	-
10. $I^+ P^+ O^+ Z^+ Y / I^+ P^+ O^c Z Y^+$	+	-	+	+
11. $I^+ P^+ O^+ Z Y^+ / I^+ P^+ O^c Z^+ Y$	+	+	-	+

The lac operon system thus far described shows **negative control** that is **inducible** by the substrate, lactose. There is also a **positive control** system operating in the *lac* operon. Maximal induction of the *lac* operon also requires the **CAP- cAMP** (catabolite activator protein complexed with cyclic adenosine monophosphate) complex that binds to the DNA at the CRP recognition site at the beginning of the promoter site. The role of CAP-cAMP is to help the RNA polymerase activate *lacZYA* transcription. CAP is made by the *crp* gene which is located elsewhere in the genome. The cAMP comes from the conversion of ATP (adenosine triphosphate), but in the presence of breakdown products from glucose, this conversion is inhibited. In high concentrations of glucose, cAMP is not made. When there is no cAMP, the CAP-cAMP complex cannot form and bind the CRP recognition site. Therefore, if both glucose and lactose are available for the cell, β -galactosidase will not be produced until all the glucose has been used up and cAMP formation is no longer inhibited.

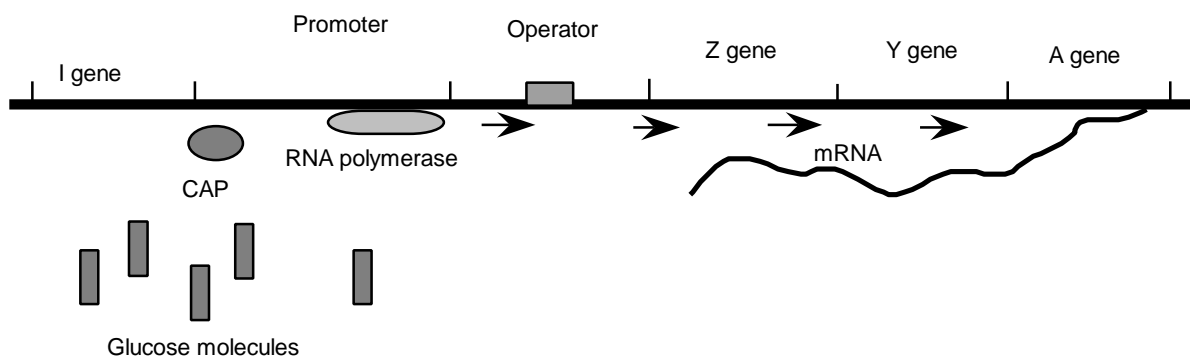


Figure 3. With glucose available, ATP is not converted to cAMP; thus, the CAP-cAMP complex cannot form and bind to the promoter. Very little *lac* mRNA can be made.

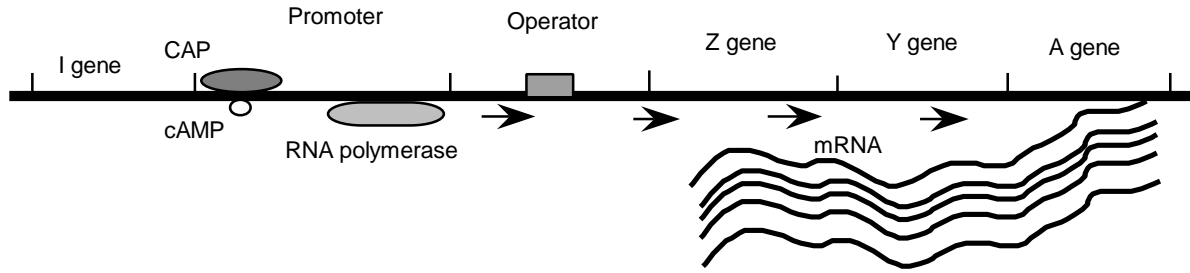


Figure 4. With no glucose available, ATP can be converted to cAMP and the CAP-cAMP complex can be formed and bind to the promoter. Large amounts of *lac* mRNA are produced.

During their research Jacob and Monod discovered that there were analogs of lactose (other β -galactosides) that were even stronger inducers of the system than lactose (but were not actually substrates for the β -galactosidase enzyme). One of these we will use in our experiment today is called IPTG (isopropyl- β -D-thiogalactoside).

Another analogue of lactose that is readily cleaved by β -galactosidase is ONPG (o-nitrophenyl β -galactopyranoside). One of the products of the cleavage of ONPG produces a bright yellow color. This will be used our assay for determining the activity of the β -galactosidase.

Lac Operon Experiment

In this lab, you will be provided with three different bacterial strains. The strains are wild type E. coli strain K12, a mutant of E. coli K12 that has a defective *lacZ* gene (Z^-), and a mutant of E. coli K12 that has a defective *lacI* gene (I^-). The strains have been grown in glycerol media. Your job will be to determine which strain is wild type, which is Z^- , and which is I^- , based on your knowledge of the *lac* operon. To do this, you will determine the relative levels of β -galactosidase in each strain after the addition of potential inducers which are either lactose, IPTG (a non-metabolizable lactose analogue), or glucose as follows.

1. Potential induction of the lac operon.

1.1. You will be provided with 12 tubes containing 2 ml of glycerol medium.

1.2. Label all 12 tubes with your group name.

1.3. Label 4 of the tubes with the letter A for strain A, 4 with the letter B for strain B, and 4 with the letter C for strain C.

1.4. Within each strain set, mark 1 tube as Control, 1 tube as + lactose, 1 tube as + IPTG, and 1 tube as + glucose. These will be the potential inducers that you use to induce the lac operon.

1.5. Aliquot 0.5 ml of the bacterial culture of strain A to the 4 tubes labeled A. Repeat for cultures of strain B and strain C.

1.6. Add 100 μ l of either lactose, IPTG, or glucose to the appropriately labeled tubes. For the control tubes, add 100 μ l water.

1.7. Incubate the tubes at 37°C in a water bath for 90 minutes. This incubation allows the cells to take in the sugar and/or inducer molecules and allows time for the induction of the lac operon.

2. Assay for β -galactosidase activity.

2.1. Permeablizing the cell membranes

It is necessary to permeabilize the bacterial membranes so that the substrate ONPG (see below) can rapidly diffuse into the cell. This is done by treatment with SDS/chloroform.

2.1.1. Remove tubes from the water bath.

2.1.2. Add 10 μ l of 0.1% SDS and 20 μ l of chloroform to first tube.

2.1.3. Vortex for 5-10 sec.

2.1.4. Repeat steps 2.1.2 - 2.1.3 for each tube. This should take about 5 minutes. If it takes less than 5 minutes, wait until 5 minutes after treating the first tube has elapsed before continuing.

2.2. Adding ONPG, a substrate for β -galactosidase.

Typically, the substrate for β -galactosidase is lactose in the bacterial cell. However, we will be using an analog of lactose called ONPG (o-nitrophenyl β -D-galactopyranoside). One of the products produced when β -galactosidase cleaves ONPG is yellow in color. Thus, β -galactosidase activity can be assayed by following yellow color development in a reaction containing β -galactosidase and ONPG.

2.2.1. Add 1 ml of 1% ONPG to first tube.

2.2.2. Vortex briefly.

2.2.3. Repeat steps 2.2.1- 2.2.2 for each tube.

2.3. Monitoring β -galactosidase activity.

One of the products produced when β -galactosidase cleaves ONPG is yellow in color. Thus, β -galactosidase activity can be assayed by following yellow color development in a reaction containing β -galactosidase and ONPG.

2.3.1. Monitor the tubes for yellow color development. Some of the tubes will immediately turn yellow. For others it may take as long as 30 min. Some of your samples will not turn yellow.

2.3.2. Place the tubes in front of a white background and determine the relative "yellowness" of each tube.

RESULTS OF LAC OPERON EXPERIMENT

	STRAIN A	STRAIN B	STRAIN C
TUBE 1 - CONTROL			
TUBE 2 - LACTOSE			
TUBE 3 - IPTG			
TUBE 4 - GLUCOSE			
IDENTITY OF STRAIN			

- = no yellow color
 -/+ = very light yellow
 + = light yellow
 ++ = medium yellow
 +++ = dark yellow

References:

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