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Introduction to Gene Regulation: The Lac Operon BioKit

Overview

This lab introduces gene regulation through exploration of the *lac* operon. You will be given three *E. coli* cultures. One culture was grown in the presence of lactose, one in the presence of glucose, and one in the presence of both these sugars. You will test these cultures for the activity of β-galactosidase, an enzyme that breaks down lactose into two simpler sugars, glucose and galactose. To perform the assay, you will mix some of each culture with ONPG (*o*-nitrophenyl-beta-D-galactopyranoside). ONPG is, like lactose, a substrate for β-galactosidase. β-galactosidase cleaves ONPG into galactose and a yellow compound called *o*-nitrophenol (ONP). The starting material, ONPG, is colorless; a change from clear to yellow indicates β-galactosidase activity. The higher the level of β-galactosidase, the more ONP will be present in solution, and the deeper yellow the solution will be.

Background

Gene regulation is the control of gene expression. A gene is "expressed" when it is transcribed into mRNA and that mRNA is translated into a protein. Gene transcription begins at the gene's promoter. A promoter is a sequence of DNA at the front of the gene where RNA polymerase binds to initiate mRNA synthesis. Many mechanisms of gene regulation, such as the *lac* operon studied in this lab, center around the promoter.

In order to develop correctly and survive, an organism must be able to express genes at specific times and under specific conditions. For example, some people are born with extra digits on their hands. In many people, the presence of the extra digit is associated with the mutation or duplication of a region of DNA that regulates the gene for a protein called "sonic hedgehog." The protein was named after a video game character named "Sonic the Hedgehog." Correct regulation of the sonic hedgehog protein is also important in mice. In mice, if that regulatory region of DNA is mutated, limbs develop abnormally. If the regulatory sequence is deleted completely, the mice develop with no feet. Studies have shown that the timing of expression of the sonic hedgehog gene is critical to normal limb development. In these studies, mice were genetically engineered so that the expression of sonic hedgehog could be shut off at various times in developing mice. Researchers found that the earlier in development the sonic hedgehog gene was shut off, the more severe were the mouse's limb abnormalities. Clearly, the appropriate regulation of this gene is critical for normal development.

Gene regulation accounts for the fact that your cells are different even though they carry the same genome. For example, red blood cells use the hemoglobin protein to carry oxygen throughout your body. Expressing the gene for hemoglobin is critical to their function. In contrast, cells with different functions do not express the gene and therefore do not produce hemoglobin.

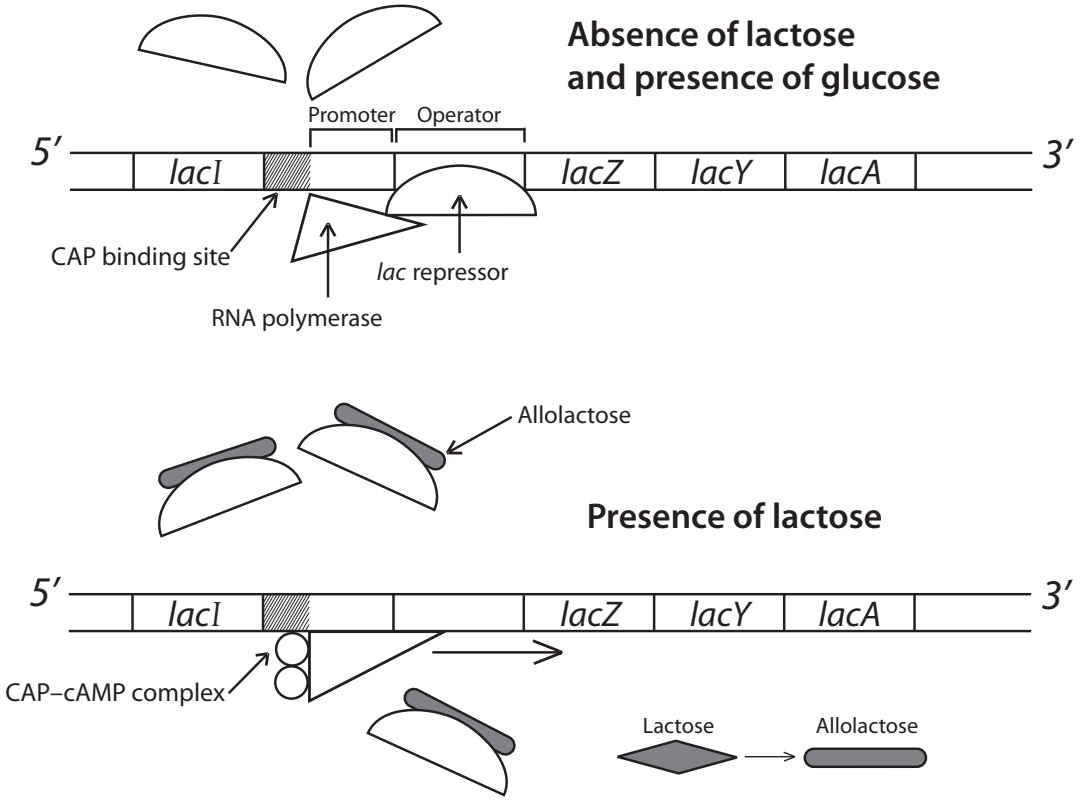
Having mechanisms in place for regulating gene expression saves resources and energy. If a cell produces proteins that it does not need, the cell's resources are depleted with no benefit. The *lac* operon of *E. coli* provides a good example of a regulatory mechanism that allows a cell not to express a protein unless that protein becomes beneficial. The *lac* operon is so-named because it regulates the ability of *E. coli* to use lactose as an energy source. The operon turns on or off the bacterium's production of β-galactosidase, an enzyme that breaks lactose into glucose and galactose. When glucose is available as a food source, the operon is turned off, but when lactose is present, *E. coli* does produce β-galactosidase. How does the bacterium do this?

The gene that codes for β-galactosidase is one part of the *lac* operon. An operon is a group of genes regulated as a single transcriptional unit (i.e., transcribed as a single mRNA, which is then translated into multiple proteins). The *lac* operon includes the three genes *lacZ*, *lacY*, and *lacA*, along with a promoter and operator sequence. (The *lacZ* gene codes specifically for β-galactosidase, the enzyme assayed in this lab activity.) Located 5' (upstream) of the three genes are the *lac* promoter, which initiates transcription, and the *lac* operator. Upstream from the promoter is the *lacI* gene, which codes for the *lac* repressor.

In the absence of lactose, the *lac* repressor binds to the operator and blocks almost all transcription of *lacZ* by preventing RNA polymerase from binding to the *lac* promoter correctly. In contrast, when lactose is available, it is converted into a form called allolactose. Allolactose binds to the *lac* repressor, changing the repressor's shape so it can no longer bind to the operator and interfere with transcription of the *lacZ* gene. Then, production of β-galactosidase proceeds freely. This is one level of regulation of the operon.

The expression of the *lacZ* gene is affected also by the amount of glucose in the environment. A protein called CAP (catabolite activator protein), when bound to a molecule called cAMP (cyclic adenosine monophosphate), helps the RNA polymerase bind to the *lac* promoter. In the presence of glucose, the cAMP level is low. Thus, the CAP–cAMP complexes are not available to help the RNA polymerase bind to the promoter, and the *lacZ* gene is not transcribed as efficiently. As a result, less β-galactosidase is produced when glucose is present along with lactose. This regulatory mechanism benefits *E. coli* because the bacterium can exploit energy from glucose more efficiently than from lactose.

Simplified Diagram of the Lac Operon



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Pre-laboratory Questions

1. How does *E. coli* use the β-galactosidase enzyme?

2. Does *E. coli* produce β-galactosidase all the time? Why, or why not?

3. Between 24 and 36 hours before the laboratory period, three bottles of media were inoculated with *E. coli*. One bottle contains glucose, another contains lactose, and the third contains both glucose and lactose. In which culture do you expect to find β-galactosidase activity? Explain.

Qualitative Procedure

Materials

At each lab station:

- 4 dropping pipets
- 3 glass test tubes with rack
- permanent marker
- gloves

Shared:

- ONPG (*o*-nitrophenyl-β-galactosidase)
- *E. coli* grown in the presence of lactose
- *E. coli* grown in the presence of glucose
- *E. coli* grown in the presence of both glucose and lactose
- 37°C incubator

Procedure

Wear gloves during the procedure and avoid contact of the yellow product with your skin.

1. Label three test tubes: one "glucose," one "lactose," and one "glucose+lactose."
2. Transfer 12 drops of *E. coli* from each different culture to the appropriately labeled tube, using a new pipet for each transfer.
3. Add 10 drops of ONPG to each tube (to avoid cross-contamination, do not touch the pipet tip to the tubes).
4. Cover the tubes, gently shake to mix the contents, and place them into a 37°C incubator. Check for color changes at 10-minute intervals. Record the results, in terms of the color of the reaction, in the table below.

Time	Time from start of reaction	Glucose	Glucose + Lactose	Lactose
	10 minutes			
	20 minutes			
	30 minutes			
	40 minutes			
	50 minutes			
	60 minutes			

Laboratory Questions

1. Explain what caused the color change that you observed.

2. How did the level of β-galactosidase activity you observed in the glucose culture compare with that in the other two cultures?

3. How did the level of β-galactosidase activity you observed in the lactose culture compare with that in the other two cultures?

4. How did the level of β-galactosidase activity you observed in the glucose+lactose culture compare with that in the others?

   Given what you know about the *lac* operon, can you explain this result?

5. Assume that you grow a culture of *E. coli* in medium that contains a very small amount of glucose. After 24 hours of growth, the bacteria have completely depleted the supply of glucose. After 30 hours, you add lactose to the medium. You assay the culture for β-galactosidase activity at 20, 26, and 50 hours after you started the culture. What do you expect to find in terms of β-galactosidase activity at each of these times?

Quantitative Procedure

In this assay, you will quantitate the amount of β-galactosidase enzyme produced by each culture. The amount of enzyme present in a sample is often described by the level of its "activity." In this case, "activity" corresponds to the cleavage of ONPG to ONP (*o*-nitrophenol) and galactose. Enzyme activity is often reported in units. β-galactosidase activity is typically reported in Miller units, named after the man who developed the assay.

$$1 \text{ Miller unit} = 1000 \times \frac{(A_{420} - (1.75 \times A_{550})}{t \times v \times A_{600}}$$

Where A_{420} = the absorbance of the yellow ONP

A_{550} = a correction factor to take into account that there are bacterial cells in the assay that will scatter light.

t = the reaction time (from the start of the assay until the measurements are made)

v = volume (in mL) of the culture used in the assay. (In this case, v equals 2.4 mL.)

A_{600} = the optical density (OD) of the original culture.

This equation takes into account many of the variables that would invalidate a direct comparison of the β-galactosidase activity levels in two different cultures, so that a direct and accurate comparison can be made between them.

Materials

At each lab station:

- pipets for measuring 4.5, 2.4, and 2.0 mL
- pipetting device
- 6 glass test tubes with rack
- permanent marker
- gloves

Shared:

- ONPG (*o*-nitrophenyl-β-galactosidase)
- *E. coli* grown in the presence of lactose
- *E. coli* grown in the presence of glucose
- *E. coli* grown in the presence of both glucose and lactose
- spectrophotometer
- 37°C incubator
- cuvettes (if the assay is not done in test tube cuvettes)

Procedure

Wear gloves during the procedure and avoid contact of the yellow product with your skin.

1. Label three of the test tubes: one "glucose," one "lactose," and one "glucose+lactose." These tubes will hold your *E. coli* samples. Label the other three tubes (for the assay) as follows: "ONPG glucose," "ONPG lactose," and "ONPG glucose+lactose."
2. Add 4.5 mL of the appropriate culture to each of your *E. coli* tubes—"glucose," "lactose," or "glucose+lactose." Before pipetting your sample from the shared culture, be sure to resuspend the culture by gently swirling the bottle or by pipetting up and down a few times. Do not mix or cross-contaminate the cultures—use a different pipet for each.
3. Following the instructions provided by your instructor, measure the A_{600} of each of the cultures and record the readings in the appropriate box in the top row of the table on the next page. Save these three culture tubes after measuring the absorbance. You will transfer a portion of each of these cultures to a fresh tube to perform your assays.

4. Using the pipetting device and pipets provided to you, transfer 2.4 mL of the *E. coli* from each tube to its appropriate fresh tube that you labeled "ONPG glucose," "ONPG lactose," or "ONPG glucose+lactose." Remember to resuspend each culture before transfer and to use a new pipet for each.
5. Add 2 mL of ONPG to each tube (to avoid cross-contamination, do not touch the pipet tip to the assay tubes).
6. Cover the tubes, gently tip to mix the contents, and place the tubes into a 37°C incubator. Read the A_{420} and the A_{550} of each tube at 10-minute intervals. Be sure that each sample has incubated for the same amount of time when you take your readings. Record the results in the following table.

Time	**Time from start of reaction**	**Glucose** $\mathbf{A_{600}}$ **=**	**Glucose + lactose** $\mathbf{A_{600}}$ **=**	**Lactose** $\mathbf{A_{600}}$ **=**
	10 minutes	A_{420} = A_{550} =	A_{420} = A_{550} =	A_{420} = A_{550} =
	20 minutes	A_{420} = A_{550} =	A_{420} = A_{550} =	A_{420} = A_{550} =
	30 minutes	A_{420} = A_{550} =	A_{420} = A_{550} =	A_{420} = A_{550} =
	40 minutes	A_{420} = A_{550} =	A_{420} = A_{550} =	A_{420} = A_{550} =
	50 minutes	A_{420} = A_{550} =	A_{420} = A_{550} =	A_{420} = A_{550} =
	60 minutes	A_{420} = A_{550} =	A_{420} = A_{550} =	A_{420} = A_{550} =

Laboratory Questions

1. Explain what caused the color change that you observed.

2. Calculate the quantity of β-galactosidase enzyme present in each of the three cultures in Miller units. Use the equation described in the introduction to the Quantitative Procedure.

3. According to your measurement of Miller units of enzyme for each culture, which culture produced more β-galactosidase—the glucose culture, or the lactose culture?

4. According to your Miller unit measures, how did the level of β-galactosidase activity in the glucose+lactose culture compare with that in the other two cultures?

   Given what you know about the *lac* operon, can you explain this result?

5. Assume that you grow a culture of *E. coli* in medium that contains a very small amount of glucose. After 24 hours of growth, the bacteria have completely depleted the supply of glucose. After 30 hours, you add lactose to the medium. You assay the culture for β-galactosidase activity 20, 26, and 50 hours after you started the culture. What do you expect to find in terms of β-galactosidase activity at each of these times?

6. Assume that you added the same amount of lactose to two different cultures of *E. coli* (the cultures contain different strains). There is no glucose in the media. Eighteen hours after lactose was added to the cultures, you assay the cultures for β-galactosidase activity. The only data you collect are the absorbances of the assay at 420 nm. From these data, you conclude that each bacterium in one culture is producing five times more β-galactosidase than each bacterium in the other culture. Is your conclusion valid?