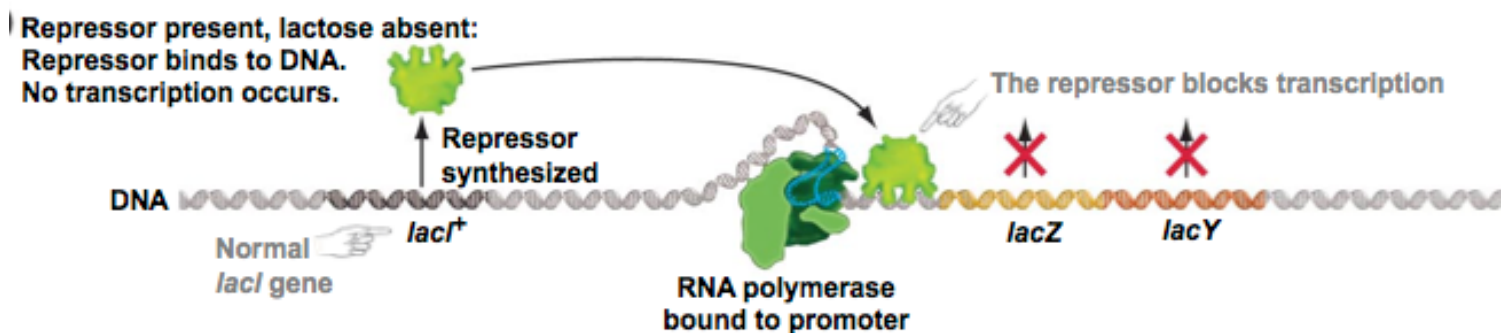


# Bio 230 General Genetics Experiment 4

## Induction of the *lac* operon

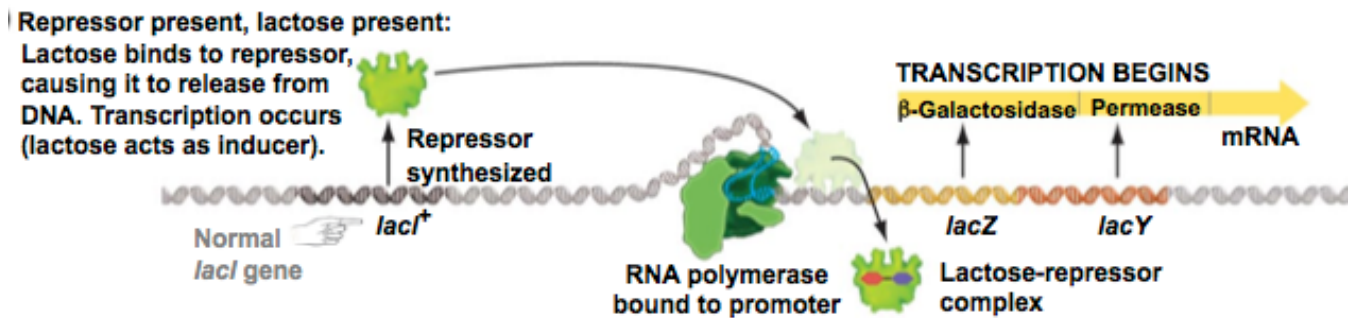
Bacteria have evolved for efficiency, and for adaptation to changing conditions. Environmental factors may alter a cell's physiology, by temporarily turning gene systems on or off. The *lac* operon in *E. coli* is one of the simplest and best examples of such gene regulation. In this operon, lactose induces the synthesis of the enzymes involved in its breakdown, particularly beta galactosidase. This enzyme breaks lactose down to glucose + galactose. In the absence of lactose, these enzymes are not required, and thus are not synthesized, as it would be inefficient to do so. The primary regulator of the *lac* operon is a "negative control element" called the *lac* repressor. This protein is a negative regulator because when it binds to a gene, it turns the expression of that gene off. When lactose is present in the cell, the sugar combines with and inactivates the repressor. Under these conditions, the genes of the lactose operon are expressed, and the enzyme required to utilize the lactose is synthesized. This process, whereby an environmental factor activates a gene, is called gene or transcription *induction*.

The repressor of the lactose operon is coded for by the *lac i* gene. The repressor binds to the operator area, *o*, and blocks the attachment of the RNA polymerase to the promoter site, *p*.



The RNA polymerase must attach to the promoter site and move through the operator site if the lactose operon is to be transcribed. Thus, for our purposes we will consider the repressor an "on/off switch", whereby when the repressor is bound to

the DNA the gene is switched OFF, and when repressor is not bound, it is ON and being transcribed.



The regulation of the lactose operon is not only under the negative control of the repressor, but also under positive control of another protein called the *catabolite activator protein* [CAP]. In the *absence of glucose*, CAP binds to the promoter site, and increases the level of transcription by helping RNA polymerase to bind. In the presence of glucose, CAP fails to bind, and transcription remains low.

This level of regulation is for efficiency. If there is already sufficient glucose in the cell, why invest the energy and time in manufacturing enzymes to create more glucose? Glucose is the preferred energy source, so if it's available, cells don't need to break down lactose.

You will measure the amount of enzyme produced by the gene under various conditions. In order to do so, you need to be able to measure one of the products of the enzyme's reaction as it is made. Unfortunately, there is no easy assay for glucose or galactose. However,  $\beta$  galactosidase will cleave other similar molecules, such as orthonitrophenylgalactoside (ONPG). One of the cleavage products created when the enzyme operates on ONPG is bright yellow, and thus can easily be seen, and measured using a spectrophotometer at its peak absorbance (420nm).

Finally, you will look at the effect of the 'superinducer' isopropylthiogalactoside (IPTG). This molecule also binds to the repressor protein and alters its binding to the promoter. However, unlike lactose, it is not broken down by  $\beta$  galactosidase. Consequently, once in the cell, it remains there for the duration of the experiment.

## Procedure

Add 1 ml of bacterial culture to all tubes

At the times shown on the flow diagram, add 0.1 mls water to the Control tube and 0.1 mls of the appropriate substrate (all 1% solutions) to each of the T0 experimental tubes.

Cover **all** tubes with Parafilm and incubate in the shaking water bath

At the times shown, add 0.1 mls of substrate to the experimental tubes, replace in the water bath

At 80 minutes, remove all tubes, add 0.1 mls 1% SDS to each, followed by 2 mls of 0.5% ONPG in 50mM phosphate buffer, pH 7.2

Cover all tubes, invert three times and return to the water bath for 20 minutes

Remove all tubes, add 1 ml of 1M Na<sub>2</sub>CO<sub>3</sub> to each tube. Cover and invert twice

Read the absorbance of all tubes at 420 nm, using the Control as a blank

Plot the absorbance for each substrate tube as a function of time using your data and also the class average.

Hand in the graphs and your interpretation of the results next lab.

Add 1 ml of bacterial culture to each tube

