**The LacOp Simulation**

***An Online ‘Lab’ on Prokaryotic Transcriptional Regulation:***

The Lac Operon is most famous for its role in helping geneticists understand transcriptional gene regulation in prokaryotes. In this exercise, you will use an interactive online simulation called ‘LacOp’ to model the metabolism of a single *E.coli* cell. Our goal is to help you understand the dynamics of transcriptional gene regulation in a more fun and effective way than you could from a textbook.

**First, a bit of background:**

Bacteria often have genes whose proteins work together in biochemical pathway(s). Often those genes are clustered and transcribed together on a single RNA. This sort of structure is called on **operon**. Operons are thought to simplify transcriptional regulation because proteins that will be needed together in a pathway are transcribed together in a single RNA.

The lac operon consists of a set of ‘genes’ *E.coli* uses to metabolize lactose into chemical energy.



Since we are here to study gene regulation, the molecular ‘stars’ of this exercise are the *regulatory* molecules; repressor(lacI), operator (lacO) and inducer (allolactose). Specifically, Repressor protein (lacI) binds to the operon’s operator element (lacO), thereby repressing transcription of the operon. However, if lactose is available in the environment, repressor binds lactose’s derivative Allolactose (aka. inducer). When repressor binds this allolactose ‘inducer’, it releases from the operator element, thus inducing transcription of the *structural* genes. These structural genes encode three enzymes (lacZ , lacY and lacA). These structural genes (unlike the regulators) are the ones that directly metabolize lactose into useful energy. As a byproduct, LacZ also metabolizes lactose into Allolactose, which feeds back into the regulation of transcription.

Much of this information is concisely (re) summarized in a figure displayed in the simulation that follows.

Using that simulation is a fun way to get more comfortable with the role(s) of each molecules and explore gene regulation. Recording your findings in this document as you work will help you go in the right direction and get the most out the simulation. Ok, Let’s get to it!.

**Part A:  Understanding the Pathway and testing a promoter mutant.**

The Lac Op Simulation can be found on the web here:

<http://flask-env.rnwhymamqf.us-west-2.elasticbeanstalk.com/lacop>

Read the header and take a look at the graphic of the pathway to familiarize yourself with the key molecules in this pathway, and what each molecule does in the cell. In this simulation, we have omitted the enzyme lacA. *Although LacA plays a role in lactose metabolism of lactose* (like lac Z and Y), it’s role in regulation of the operon is minor. For that reason, in the interest of keeping things less complex, this simulation does not display lacA.

Now that you have done that, you are ready to run the simulation, which has been preset to conditions where wild type cell encounters a lot of valuable lactose (200 units) all of which is outside of a cell.

**A1**. Run the simulation by clicking on the Blue button labelled “Run Pathway and make graph”. Doing so should produce a new graph displaying some colored lines just below that button.

Examine the graph, noting that each line represents the amount of an important molecule in the pathway over time.

Play around a bit with the graph: it should be interactive. Note that you can hide a curve(s) from view by clicking on the corresponding colored boxed(s) in the legend, thereby reducing the number of curves you need to look at. Similarly, you can get a more detailed view of the underlying data by hovering the cursor over a specific point(s) on each a line.

OK, let’s restore the graph to the initial view (or just rerun it by pushing the “Run Pathway..” button again, which will do the same thing…). Now save an image of your results by pasting it below this paragraph. You can use up to a page of space. You will refer back to this graph as you work through this exercise.

Briefly summarize your understanding of what this graph shows in the box below(1-4 sentences max).

**A2**. Do you think the cell would respond differently if the cell has a loss of function mutation in its promoter? Provide a one sentence prediction below of what you might see:

Ok, now test your prediction by (re)running the simulation with those conditions. Specifically: click on the “Promoter” tab, then check the box next to the ‘LacP-‘ label. Then press the “Run Pathway and make graph” button. Examine the results. Save an image of your results, pasting it below:

Summarize these results in the box below, commenting whether they matched your hypothesis: (1-3 sentences)

**Part B: Regulatory Mutants**

Now that you are getting familiar with the LacOp simulation, let’s put it to work to understand gene regulation.

**B1**. Examine the behavior a cell in the absence of lactose. (To do this, click on the ‘Sugar’ tab and adjust ‘Lactose outside’ from 200 to down to zero.), then run the pathway and generate a new graph.

Note how the cell’s metabolism differs from the results you obtained in A1. A quick tip: when the graphs note that the Y axis might be different (LacOp’s software automatically shifts the axis based on the data). Optional: Feel free to paste in an image of that graph below if that is helpful:

Summarize these results of these results in the box below: (1-3 sentences)

**B2**. Now examine the behavior of a cell with a Repressor mutation in the absence of lactose (set Lactose outside to ‘0’ and add a loss of function mutation to Repressor, which is known as LacI-). Run the simulation, and generate a new graph. (Reminder: when comparing graphs, note the units on the Y axis in case they differ). If you are doing this right you should see a very high level of B-galactosidase and Permease being made. This is because, without repressor present, transcription occurs whether or not lactose/allolactose is present. This sort of ‘runaway’ unregulated transcription is known as ‘**constitutive**’ expression. (Optional) Feel free to paste in an image of that graph below if that is helpful to your understanding:

Summarize these results of these results in the box below: (1-3 sentences)

If you haven’t already done so, compare the results for B-galactosidase and Permease in this experiment to your results from ‘A1’ where repressor (lacI) was functional. Can you see why repressor is valuable to *E.coli* in the wild? Explain your thoughts on this: (1 sentence)

**B3**. The other main regulatory region to understand is the ‘**Operator**’(lacO). To do that, repeat the analysis in you did in B2, but mutate LacO instead. Specifically, repeat the work above but instead select the lacO loss of function mutant, which is known as lacOc. (The ‘C’ stands for ‘Constitutive’.) How does the phenotype(s) of lacOc c compare to lacI---?

**B4**. Your work in B2 and B3 should show that LacI-- and lacOc c show an identical phenotype: where high levels of transcription occur even when lactose is not present (known as ‘constitutive’ expression). (If that is not what you saw, redo B2 and B3 more carefully…)

The similarity of these two mutations was a puzzle. However, their discoverers won the Nobel prize by devising a clever way to see that LacI and LacO were fundamentally different types of genes, thereby leading the discovery of the first transcription factor (lacI) and the first binding element (lacO). They did this by introducing a second copy of the lac operon on a plasmid, creating an artificial ‘partial diploid’ state that would let them see the interactions between the two copies, and therefore how regulation was working. In these experiments, they often also used a different genotype on the plasmid, so they could tell whether transcription was coming off the main locus or the copy on the plasmid.  For example, if the main chromosome was (lacZ+, lacY-) and the plasmid was (lacZ- and lacY+), if they saw lacZ activity, they could know that the chromosome’s operon was being transcribed. Alternatively, if they saw lacY activity, the could know the plasmid was being transcribed.

Fortunately for us, with LacOp simulation makes it simple to simulate these experiments. Use the LacOp simulation to look for differences between lacI- and lacOc using this approach. (Hint you can do this add another lac operon by selecting the ‘plasmid’ tab).

Summarize your results in the box below in a few sentences:

Lastly, to consolidate your knowledge, complete the following summary table:

|  |  |  |  |
| --- | --- | --- | --- |
| Locus | Molecule Name | Can this molecule regulate a different DNA or just its own? | Is this molecule a Transcription Factor (TF) protein or a TF binding site element on DNA? |
| lacI | Repressor |  |  |
| lacO | Operator |  |  |

**B5. The Unstoppable Force vs. the Immovable Object.**

To better understand these two loci, let’s construct a ‘double mutant’ with two alleles that give opposite phenotype.

**B5.1** Generate a graph of a LacOc mutant’ (or just refer back to B3, where you did it before). Note the phenotype.

B5.2 Now generate a graph of a LacIS ‘super repressor’ mutant’ . Super repressor is mutant allele of the repressor protein that scientists discovered. Note the phenotype.

B5.3 Now for the fun part (or at least ‘fun’ part. Predict the phenotype of a double mutant, that is a cell possessing both a LacIS and a LacOc mutations.    State that hypothesis below. (It is ok to be wrong)

Now, use LacOp the LacOp simulation construct the double mutant and “Run the Pathway” and examine the resulting graph.

Based on the results, explain how these two alleles interact and list a hypothesis of how the underlying mutations might work.

**Optional Bonus Exercises**

**C : Positive Transcriptional Regulation:**

Now examine default conditions that you used in part “A”, above, but this time, click on the ‘cAMP CAP complex’ to activate this important regulator.  Note that CAP (like repressor/lacI) is a transcription factor but that it activates transcription and does so in response to high cAMP levels (indicating a need for more ATP, often due to glucose depletion). Note that wildtype cells have active CAP, but we initially set CAP to inactive to focus our learning on one transcription factor lacI and its DNA binding domain, lacO, rather than the combined actions of both lacI and CAP. Typically, a wild type *E.coli* lac operon’s transcription is often very low but can be dramatically increased when the cell is in dire need for immediate energy (with high cAMP activating CAP) and when the cell detects that lactose is readily available (eliminating repressor lacI binding to lacO).

 In light of this, make the predictions below for CAP and Promoter. Feel free to use the simulation to test partial diploid (sometimes also called ‘merodiploid’) if desired.

|  |  |  |  |
| --- | --- | --- | --- |
| Molecule | Name | Can regulate a different DNA(trans) or just it’s own (cis) | Transcription Factor protein or TF binding site on DNA |
| LacP | Promoter |  |  |
| CAP | Catabolite Activator |  |  |

**D. Compare a Promoter Mutant to a Permease Mutant**

**D1**. Default parameters (as in A1) but with a **LacY- permease** mutation instead. Before you run this simulation, write what you predict will happen. After running the simulation, save an image of your results below and write a brief discussion.

Each line represents the relative concentration of an important molecule through time; taken together the lines give you a systems level molecular picture of the phenotype. Take some time to think about the phenotype. Which two mutants show similar generally phenotypes under these conditions? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Are there any minor differences? If so explain.

**D2**. Imagine you have the two mutants you identified above, but can only detect the production of Glucose as a way to discriminate them. Find a set of conditions (by modifying the parameters and/or introducing mutations) that allows you to tell the difference between the two mutants; those conditions will also reveal their different functions of each mutant.

Show the resulting graphs and explain what you did. (If you are stumped by this, you might find inspiration from examining how ‘Beadle and Tatum’ ordered the compounds in their pathway in *Neurospora*). You can find this is most genetics textbooks .