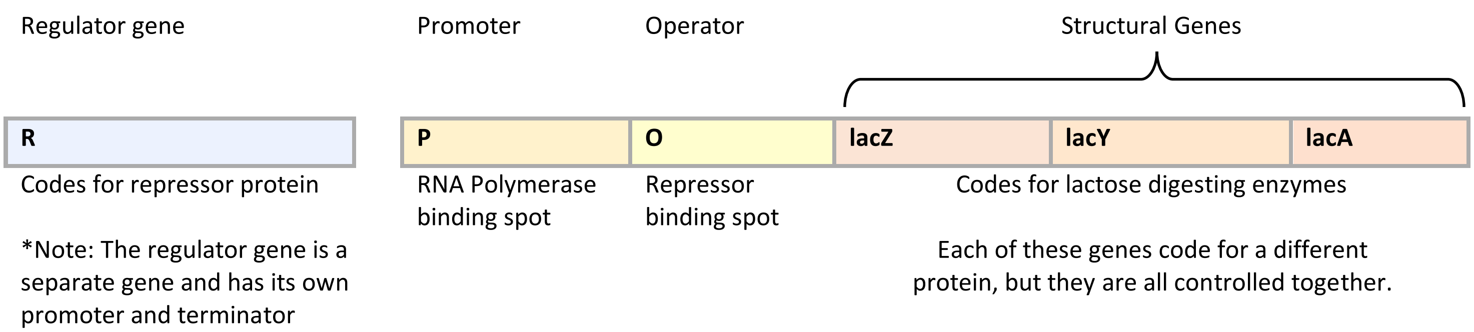
Lactose Operons:

Operons are a set of genes that all have a similar function and are “turned on” and controlled for together via an operator. Many operons exist. Two common operons discussed in genetics courses are the tryptophan (trp) operon and lactose (lac) operon. While there are many different operons, the general structure of all operons is the same. You should review the general structure of an operon before reviewing the details of a lac operon.

A lac operon is an operon that controls genes responsible for the metabolism of lactose. Just like any other operon, lac operons have a promoter, an operator, structural genes, and a repressor gene makes a protein that can halt transcription. To understand when lactose can be metabolized, each part of the lac operon must be understood. Every portion of the lac operon impacts whether lactose metabolism will occur.

Parts of the Lac Operon:



This figure shows what the parts of a lactose operon are. The parts and their functions are important to memorize. Each part is explained in more depth below.

Operator:
The operator can be abbreviated with an O in a genotype and its location is said to be the lactose operator locus, *lacO.* The operator is a specific part of the operon which allows for regulation of gene expression. The operator can turn on and off transcription. The operator is the location where a repressor protein can bind. To regulate transcription and turn it off, the operator acts as a block which hinders transcription from beginning. This will happen in all cases when a repressor is bound. Only when the operator is moved and allows transcription is there a possibility of lactose metabolism. This means transcription can never occur if a repressor is bound to the operator.

A wildtype operator (O^+) will have a repressor protein bound to it when lactose levels are low, but when lactose levels are high the repressor will not be bound. A wildtype operator allows for transcription of the structural genes only when lactose levels are high. This is the method by which the cell is able to prioritize making costly lactase proteins only when they are needed.

A non-functional operator fails to ever turn off transcription because repressor proteins can never bind to it. If a strand of DNA has a non-functional promoter on it, lactose metabolism cannot be regulated by lactose levels. If an operator is non-functional and repressor proteins cannot bind to it, it is constitutively on. Historically, this type of operator is written as O^C, but some professors may also use O^- to represent this type of operator because it is a mutated operator. There are no repressor proteins that can bind to an O^C, so regulation of transcription by lactose levels can never occur with this type of operator.

Promoter:
The promoter in the lac operon is the location where RNA polymerase must bind to start transcription. The promoter is shared for all the structural genes. A wildtype promoter is a functional promoter (P^+) that RNA polymerase has a possibility of binding to while RNA polymerase II will never bind to a non-functional promoter (P^-). Without the binding of RNA polymerase, transcription cannot occur, an RNA transcript will not be made, and proteins needed for lactose metabolism will never be made.

Structural Genes:
While the other parts of the operon dictate when transcription will occur, the structural genes are instructions for specifying what will be made. Three structural genes exist in a lac operon: *lacZ, lacY*, and *lacA*. Each of these three genes codes for a different protein that is required for lactose metabolism. Without all three unique proteins, lactose metabolism does not occur correctly.

All three structural genes can be mutated and become non-functional. If the gene is non-functional and does not code for the correct protein, a superscript minus sign will be written above it in a genotype (e.g. Z^-, Y^-, and A^-). Transcribing a mutated structural gene does not produce a useful, functional, protein for the cell.

Repressor Gene:
The repressor gene is a gene that codes for a repressor protein. This repressor protein helps in the regulation of lactose metabolism. While this gene produces a protein that affects the lac operon, the gene itself has its own promoter and terminator regions separate from the control of the lac operon.

The repressor gene can be wildtype or mutant, just like every other gene discussed. A wildtype repressor gene (I^+) codes for a functional repressor protein. It will bind to an operator when circulating lactose levels are low and be removed from the operator when lactose levels are high. A mutant repressor gene (I^-) codes for a mutated protein that cannot ever bind to the operator. A non-functional repressor gene results in transcription not being repressed.

In some cases, a special type of repressor protein is made that gets stuck to the operator. While lactose molecules can remove other repressor proteins, this is not the case for a super repressor. High lactose levels cannot remove this repressor protein. This occurs when there is a super repressor (I^S). When a super repressor is bound, lactose metabolism can never occur, regardless of lactose levels.

It is important to note that repressor proteins function through regulating at the operator. Both the type of repressor protein and the operator determine transcription regulation. Repressor proteins can only bind to functional repressors. A repressor protein, even a super repressor, cannot bind to an operator that is constitutively on. When reading genotypes to determine when lactose metabolism occurs, all parts of the operon must be considered.

Cis-acting and Trans-acting Elements:

In cells with two copies of lac operons, it is important to consider what each lac operon can do to affect lactose metabolism. An operator is only able to block transcription on its own strand of DNA. The promoter can be recognized to start transcription, but it only starts transcription on that strand of DNA. Once transcription is started on that strand, it continues by making transcripts of any structural genes on that strand. The repressor gene, however, acts differently. The repressor gene produces a repressor protein that is allowed to float around in the cell and can affect any operon. The repressor protein can repress the strand it is on but also other strands of DNA featuring an operon.

The terms cis-acting and trans-acting can be used to explain how the elements of the operon affect each other. An element is considered to be cis-acting if it can only affect other elements on the same DNA strand it is on. Elements that are trans-acting are able to affect DNA strands they are not a part of. The repressor protein is said to be trans-acting because it could repress any operon by binding to lactose operators even on other strands of DNA.

Using a Genotype to Determine Lactose Metabolism:

It can be helpful to come up with a set of steps to work through when looking at a genotype and determining if lactose metabolism will occur. Some helpful hints for doing this are:

- Check all structural genes in the same step.
- Think about what can happen at each part of the operon.
- Think about how the parts of the lac operon rely on each other.
    - To begin this process, think about how repressor proteins and operators interact. Does one “override” the other/ affect what impact the other has?
    - Your steps should take into consideration this hierarchy.
- Your set of steps can feature crossing things out, putting squares or boxes around parts of a genotype, or any other notes you need.
- Remember to include what happens when there are copies of an operon and when there are one.

The worksheet for this material has a place for you to write out what steps you want to use. Your steps might not be identical to someone else’s steps. Just having a set of steps can make lac operon problems easier.

Lactose Metabolism by cAMP:

Lactose metabolism is a costly process. While the lactose sugar can be broken down to use as energy, the cell favors using smaller easier to metabolize molecules. When glucose and lactose are both present in a cell, the cell will use glucose for energy first. When glucose runs out, cAMP levels will rise. When the cell has high levels of cAMP, cAMP can bind to the catabolite activator protein (CAP) and together they will promote RNA polymerase binding. This is how low levels of glucose is able to regulate lactose metabolism and allow the cell to favor the breakdown of glucose over the costly breakdown of lactose.

Learning Goals:

- Understand how operons work.
- Be able to name and describe the function and relative location of all parts of the lac operon.
- Understand how to use information about the genotype of a haploid bacteria or partial diploid bacteria to identify if lactase can be made.
- Successfully complete problems asking about lactase production in different environmental conditions (with or without lactose or high cAMP levels).

Order of Activities:

1. Watch this video. This is a good overview to discuss how operons work and to allow you to see how lac and trp operons are related: https://youtu.be/10YWgqmAEsQ
2. Review this website. This is a walk through to understand how each part of the operator alters the transcription and translation of lactase genes: https://dnalc.cshl.edu/view/15884-The-lac-operon.html
3. Test yourself by completing the corresponding worksheet for this material. Complete this worksheet in order. First complete the table and develop a set of steps for how to use genotypes to determine when lactose metabolism will occur. Only after you have created a set of steps should you attempt the practice problem section of the worksheet. If you are lost, you can view the key to look at a possible set of steps to approach lac operons. Attempt to first complete the worksheet on your own, then pair up with a partner or group to discuss when possible. There is an answer key provided so you can check your work. Any questions you get wrong or confused about you should attempt to explain why the answer is correct and then complete again after you finish the activities in this guide.
4. To wrap up your understanding of lac operons, view the following practice problems: https://online.ucpress.edu/abt/article/77/1/30/18711/Teaching-the-Big-Ideas-of-Biology-with-Operon
5. Most of the genetics courses cover other operons besides lac operons. Look through your notes and identify which other operons you must understand. Use what you learned about lac operons to explain these other operons to yourself or a friend. It can be useful to review the general parts of an operon.
6. After reviewing any topic, it is a good idea to have a metacognition check. Ask yourself the following questions:
    a. What are my emotional responses to learning this material? Which material am I frustrated with and need aid in understanding?
    b. What difficulties have I had with the learning tasks? What specific tasks will I do to master this content?
    c. Do I understand all of the learning goals? Can I explain each of them out loud to someone clearly and concisely?
    d. How is what I learned related to other things I have learned in this class? How is it related to other classes, my career, and my life?
7. If you would like to have more aid in learning this material, please reach out. There are numerous individuals who want to help you feel confident in your understanding. If your course has learning assistants or teaching assistant(s), you should reach out to them to review concepts you want to learn more about. Your professor is also a great resource to go to when you do not understand a topic. You can study with your peers or receive academic support through the LRC as well. If you would like help identifying how to receive the support you need, do not hesitate to contact the CU Denver Learning Resources Center at LRC@ucdenver.edu or stop by our front desk in the learning commons building.