TEACHER KEY

Gene Regulation Theater—the Lac & Trp Operons

Gene Regulation Notes

Background information video: https://www.youtube.com/watch?v=3S3ZOmleAj0 (Bozeman Science)

I. Terminology and Definitions (answer questions while/after you watch the video)

- **Regulatory gene**: a gene on the DNA that regulates another gene somewhere else on the DNA; when expressed, it creates a regulatory protein (Example: repressor protein)
- **Regulatory protein**: helps either express a gene or not
- **Regulatory sequence**: a section of DNA that regulatory proteins bind to to control expression of genes
- **Positive control**: Example (in bacteria): Lac operon
    - How does the Lac operon work? Draw a diagram and describe.

      The Lac operon has 3 different genes for breaking down lactose (a sugar in milk). Upstream there are promoter and operator sequences. A repressor protein binds to the operator, preventing transcription of the Lac genes. In the presence of lactose, lactose binds to the repressor protein, so it is removed from the operator and the lactose genes can be read by RNA polymerase. Once lactose is gone, the lactose unbinds from the repressor protein, so it fits back on the operator.

    - Why is the Lac operon an example of positive control?

      The *presence* of the substance being regulated - lactose - *allows* for transcription to occur. Lactose binding causes the repressor to *leave* the operator.

- **Negative control**: Example (in bacteria): Trp operon

    - How does the Trp operon work? Draw a diagram and describe.

      The Trp operon has 5 different genes for producing tryptophan (an amino acid for building proteins). When tryptophan is present, then it binds to the repressor protein and allows it to BIND to the operator, blocking the reading of the Trp genes. When Tryptophan IS NOT present, and the bacteria needs to make more, then the repressor cannot bind and RNA polymerase is able to bind and transcribe the Trp genes.

    - Why is the Trp operon an example of negative control?

      The *absence* of the substance being regulated - tryptophan - *allows* for transcription to occur. Tryptophan binding causes the repressor to *stay* on the operator, and only when it is gone does transcription occur.

- **Eukaryotic gene regulation - Transcription Factors**
    - Explain briefly how transcription factors work. Are they more or less complicated than bacterial operons?

      Multiple transcription factors are necessary to initiate transcription of a gene. They help RNA polymerase bind to a promoter region, but they also bind elsewhere in the DNA to enhancer regions. The DNA folds over and these other TFs interact with RNA polymerase, finally allowing transcription to begin. This is much more complex than bacterial operons.

- How is gene regulation by transcription factors similar to operons?

  Both involve regulatory sequences outside of a gene that regulatory proteins bind to and control whether a gene is or is not transcribed.

II. Pre-activity questions:

1. Remember one of the most critical features of biology:
   Central Dogma: DNA→mRNA→Protein
   What step in this process is being regulated by operons and transcription factors? What is it called?

   The first step, when DNA sequences are read by RNA polymerase to create mRNA. This process is called transcription.

2. Why do our genes need to be regulated? What do you think would happen if all our genes were "on" all of the time? (provide a specific example if you want)

   Genes need to be regulated to differentiate specific cells to do certain actions. For example, what makes hair follicle cells produce hair is due to gene regulation. If a hair follicle cell was producing insulin, that wouldn't match with its specific function. Overall there would not be a cohesive system that is efficient.

3. Some genes work together to produce one final product (protein), much like how an assembly line is used to create one product—like a cell phone or a car. What happens when one machine or person in an assembly line is no longer present? (feel free to use the cell phone or car example if that helps!)

   The assembly line can no longer produce the final product. If there was no one to produce glass for a phone, there would be no screen.

4. Another way to describe positive and negative control is **inducible** and **repressible**. An inducible operon is turned on in the presence of some molecule, while a repressible operon is turned off in the presence of a molecule. Consider the two operons you just learned about, the Lac and Trp operons. Which one is inducible, and which one is repressible? Explain why.

   The Lac operon is inducible because the presence of lactose turns on gene transcription. The Trp operon is repressible because the presence of tryptophan turns off gene transcription.

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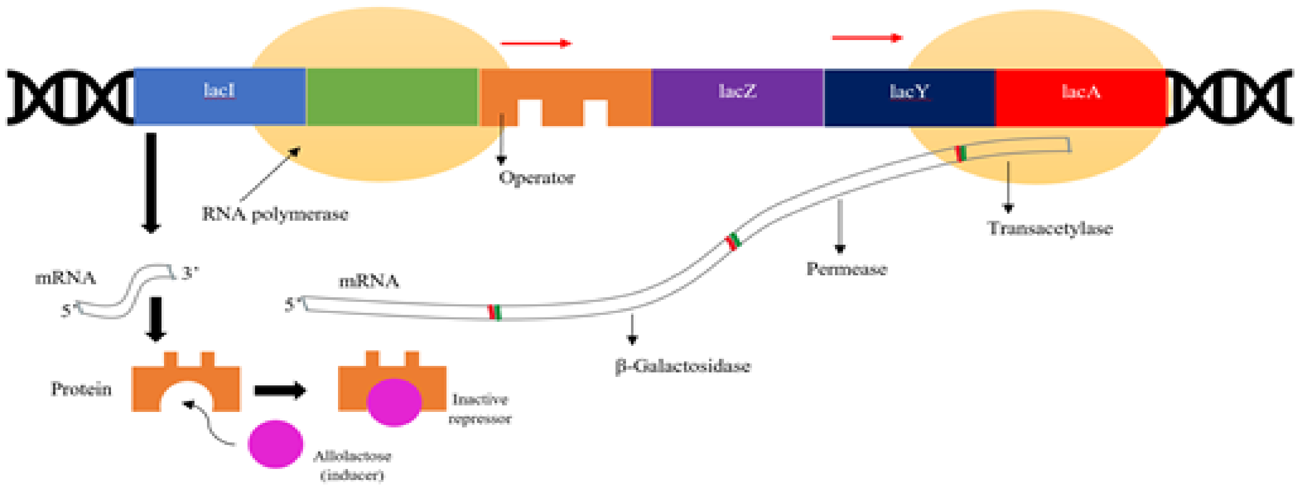
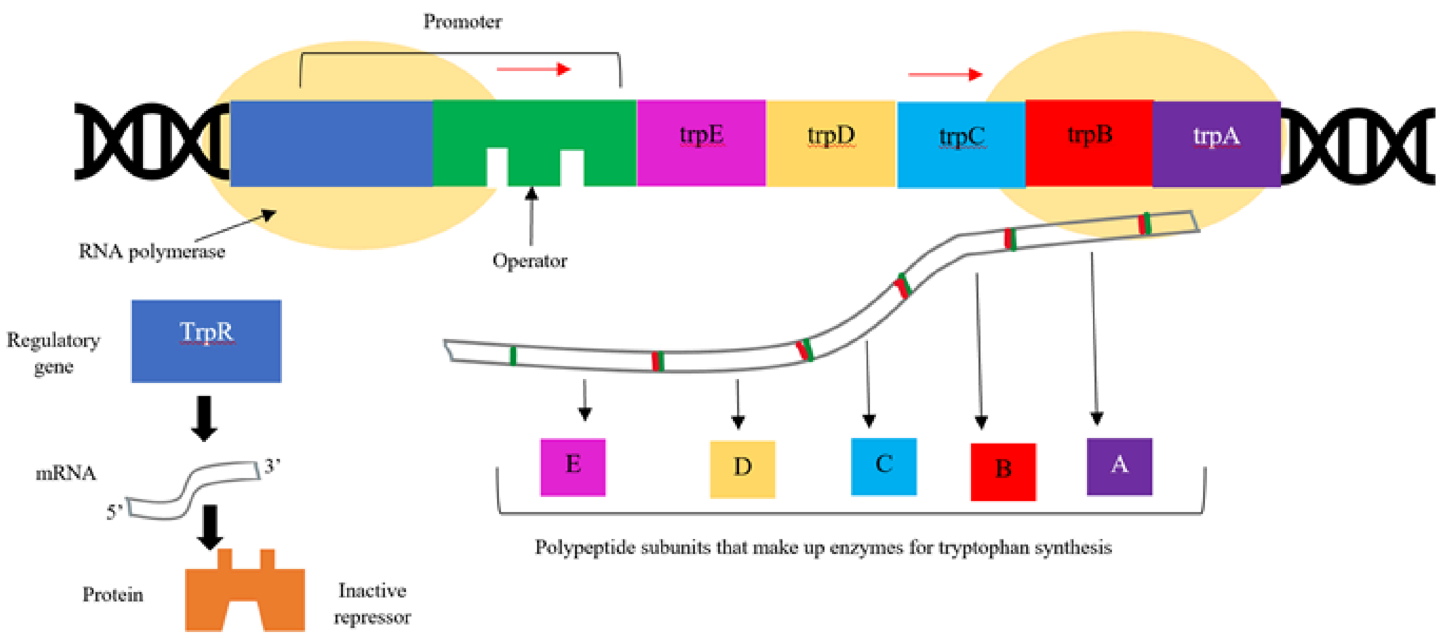


Directions for a Lac Operon skit: (positive control)

1. In groups of 6, give each person a role & write the roles down on pieces of paper or notecards for people to hold. (roles= operator, promoter, gene(s)/ transcription unit, repressor protein, lactose, RNA polymerase)
2. You want to act out the Lac operon, so make sure each person knows their role:
   - **Operator**: this is a segment of the DNA that occurs before the genes that code for the formation of the proteins (enzymes). The operator will be blocked from functioning by the repressor protein when lactose is NOT present.
   - **Promoter:** this is the segment of the DNA that occurs before the 3 lactase genes and before the operator. This is where RNA polymerase will bind to in order to start "reading" the genes.
   - **Genes/ transcription unit:** the 3 Lac genes are what code for the actual creation of the enzymes, which allow lactose to enter the cell (permease) break down lactose (B-Galactosidase or lactase).
   - **Repressor protein**: bind to and block the operator from being accessible when lactose is NOT present.
   - **Lactose:** bind to the repressor protein, preventing it from blocking the operator and therefore freeing the "path" for RNA polymerase to bind and transcribe the gene(s).
   - **RNA polymerase**: bind to the promoter region and proceed past the operator when it becomes unblocked. "read" the DNA template strand and create mRNA that codes for how to make enzymes!
3. Decide how you would like to sequence your skit. Note that you will want to have clear signs for people to hold so we know who represents what. You are welcome to use objects you find around the room if it helps in the understanding and explanation of the operon.
4. You will have 15 minutes to develop your mini skit and no more than 5 minutes to present your material and act out your gene regulation example.



Directions for a Trp Operon skit: (negative control)

1. In groups of 6, give each person a role & write the roles down on pieces of paper or notecards for people to hold. (roles= operator, promoter, gene(s)/ transcription unit, repressor protein, tryptophan, RNA polymerase)
2. You want to act out the Lac operon, so make sure each person knows their role:
   - **Operator**: this is a segment of the DNA that occurs before the genes that code for the formation of the proteins (enzymes). The operator will be blocked from functioning by the repressor protein when tryptophan IS present (so the cell doesn't make more Tryptophan than it needs).
   - **Promoter:** this is the segment of the DNA that occurs before the 5 tryptophan genes and before the operator. This is where RNA polymerase will bind to in order to start "reading" the genes.
   - **Genes/ transcription unit:** the 5 Trp genes are what code for the actual creation of the Trp enzymes, which synthesize Tryptophan.
   - **Repressor protein**: fits to the operator when Tryptophan is present (blocks RNA polymerase) so that unnecessary Trp isn't made.
   - **Tryptophan:** the molecule of interest! This is an amino acid and it will only be synthesized when needed. It binds to the repressor protein when it is present.
   - **RNA polymerase**: bind to the promoter region and proceed past the operator when it becomes unblocked. "read" the DNA template strand and create mRNA that codes for how to make enzymes for Trp synthesis!
3. Decide how you would like to sequence your skit. Note that you will want to have clear signs for people to hold so we know who represents what. You are welcome to use objects you find around the room if it helps in the understanding and explanation of the operon.
4. You will have 15 minutes to develop your mini skit and no more than 5 minutes to present your material and act out your gene regulation example.

IV. Post-Activity Questions:

1. Consider the two different operons you just learned about. The lac operon is inducible (positive control) while the trp operon is repressible (negative control). What would happen if these operons lost the ability to be controlled? That is, what would happen if the lac operon no longer responded to lactose and the trp operon no longer responded to tryptophan? Compare and contrast the two outcomes.

   The loss of positive control by lactose would mean the lactose genes are never expressed (cannot be induced). Therefore the cell could never make the enzymes needed to break down lactose, and lactose would accumulate, interfering with cell function.

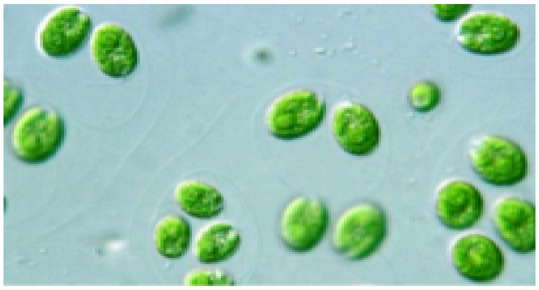
   If the trp operon was no longer controlled by tryptophan, it would express all of its genes all of the time (no longer repressible). The cell would therefore produce too much tryptophan and no switch would ever be triggered to turn it off. This would be energetically costly for the cell.

   Both of these situations result in a loss of homeostasis - the loss of control, or feedback, means that amounts of substances are not kept in an appropriate balance and a certain factor accumulates too much (lactose or tryptophan). The mechanisms are different because one results in no gene expression, while the other results in constant gene expression, but both represent a loss of balance and an ability to respond to changes.

2. Operons are the mechanism of gene regulation in prokaryotes (like bacteria). Recall that eukaryotes use a different system for gene regulation, transcription factors, that work in the same stage of the central dogma (transcription of DNA to mRNA). The green algae, *C. reinhardtii*, that we are interested in for biofuel production is a eukaryote. What is one group of genes that performs some essential function that a green algae might want to "control", and why? Consider what cellular systems algae need to live and how conditions might change.

   Many answers are possible. One example - chlorophyll content may be regulated depending on light intensity. This is because algae is trying to control/maximize its photosynthetic efficiency and needs to control light absorbancy depending on the environment.

3. Green algae like *C. reinhardtii* can produce lipid that can be used as biofuel (in place of gasoline), which presents an opportunity to decrease the world's dependence on fossil fuels. If you were to design your own green algae to create biofuel, what would be a gene you might want to be able to turn on whenever you wanted? Why is understanding gene regulation in green algae so important when designing a species to be used as biofuel?



Pictured: Green algae, *Chlamydomonas reinhardtii*

I would want to control the gene that regulates the growth rate during nutrient starvation conditions. I would want to be able to turn on algae growth even during starvation, so that while it is producing a lot of lipids, it is still growing and dividing, thus producing more useful biofuel more quickly.

Understanding gene regulation is important because it requires specialized knowledge to effectively rewrite genetics and alter the control systems.