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Discovering Prokaryotic Gene Regulation with Simulations of the *trp* Operon

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Learning Objectives

1. *Perturb and interpret a simulation of the trp operon*
2. *Define how simulation results relate to cellular events*
3. *Describe the biological role of the trp operon*
4. *Describe cellular mechanisms regulating the trp operon*
5. *Explain mechanistically how changes in the extracellular environment affect the trp operon*
6. *Define the impact of mutations on trp operon expression and regulation*

The *E. coli* genome contains approximately 4,300 genes that code for metabolic enzymes needed for cellular respiration, transport proteins essential for acquiring nutrients, regulatory proteins needed to control the production of other proteins, and many others. Because protein synthesis requires a tremendous expenditure of energy (ATP), only a subset of the available genes are actively being expressed (turned ON) at any given time (Figure 1). The expression of many of these genes are influenced by external and internal conditions. Natural selection has favored *E. coli* and other prokaryotes that are able to regulate the expression of genes so that they are only expressed when they need to be expressed.

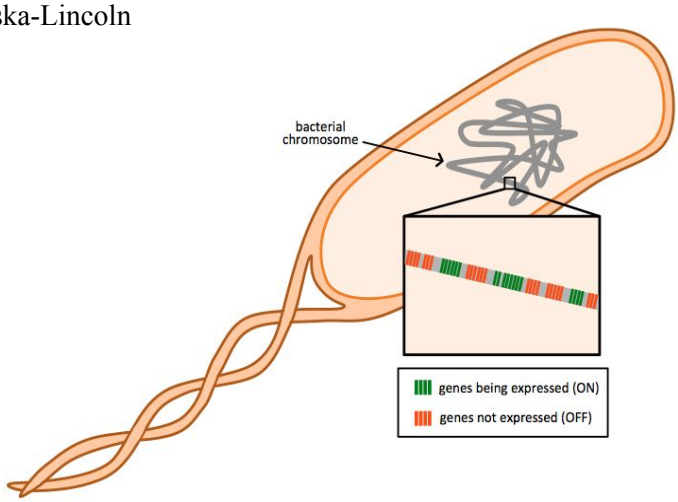

Figure 1 Patterns of Gene Expression in Prokaryotes
Only a subset of the genes in the genome are actively being expressed (turn ON) at any given time. This allows the cells to conserve energy when certain gene products (proteins) are not necessary.

In the activities that follow, you will be exploring the genetic control mechanisms that regulate the production of the amino acid tryptophan, which is needed for protein synthesis, in prokaryotes to exemplify gene expression and regulation present in all organisms.

The Operon Model

In prokaryotes, genes that share a similar function are often clustered together on the chromosome and their expression is *coordinately controlled* (i.e., if one gene is going to be expressed, all of the genes will be expressed) by a single **promoter** and **operator**. This form of gene regulation differs from eukaryotes in that eukaryotic genes are regulated individually. Collectively, the promoter, operator, and protein-coding genes are called an **operon** (Figure 2).

The regulatory or control region of the operon consists of the promoter, operator, and binding sites for regulatory proteins (transcription factors) called **activators** that function to activate transcription. The promoter is a sequence of DNA to which RNA polymerase binds to initiate transcription. The operator is a short sequence of DNA that recognizes transcriptional regulators and is analogous to an ON/OFF switch. Operators typically bind **repressor proteins** that shut off transcription by blocking RNA polymerase from binding to the promoter. Repressor proteins, along with **co-repressors,** will be described in more detail in the following activities.

When the operon is turned ON, the genes within the operon are transcribed by RNA polymerase to produce a single mRNA. The mRNA is then translated into individual polypeptides (proteins).

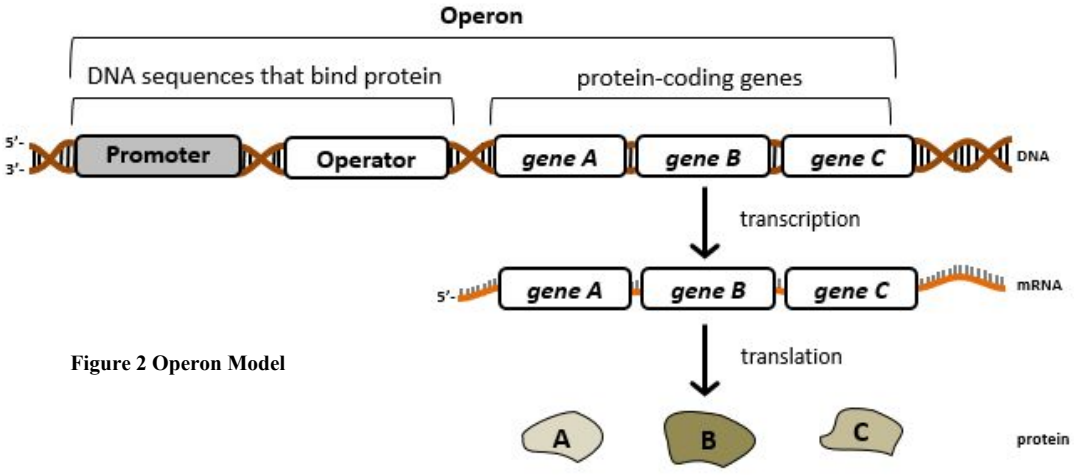

Figure 2 Operon Model

The *trp* Operon

The ***trp* operon** is a cluster of genes that function together to produce the amino acid tryptophan (trp) and is one of the most basic examples of gene regulation in response to changes in both the external and internal environment. Most bacteria obtain tryptophan by either synthesizing it from precursor molecules within the cell or by transporting it into the cell from the environment. For example, *E. coli* cells living in the gut of an omnivore such as a grizzly bear will experience fluctuations in environmental tryptophan depending on the food sources available to the bear. When the bear is feeding on a rich protein source such as salmon, tryptophan would be readily available to the *E. coli* cells. However, when the bear is primarily feeding on berries (low protein), environmental tryptophan would be low and the *E. coli* cells would synthesize their own tryptophan. To do so, the cell must express genes of the *trp* operon.

Regulation of the *trp* Operon

The activity of the *trp* operon is controlled by a regulatory protein called the ***trp* repressor** and by intracellular levels of **tryptophan** (an amino acid), which acts as a **corepressor**. The *trp* repressor is produced from the regulatory gene called *trpR* that is located upstream of the *trp* operon. Cells can respond rapidly to changes in cellular tryptophan levels because the *trpR* gene is continuously expressed. When cellular tryptophan levels are low, RNA polymerase is able to bind the DNA at the *trp* promoter and transcribes the protein-coding genes of the *trp* operon (Figure 3). The five genes are transcribed into a single mRNA that is translated into five individual proteins. *TrpE* and *trpD* (enzyme 1), *trpC* (enzyme 2) and *trpB* and *trpA* (enzyme 3) form three enzymes that produce tryptophan from precursor molecules within the cell.

When tryptophan levels are high, the tryptophan binds to and activates the *trp* repressor. Activation causes the *trp* repressor to change shape and allows it to bind to the DNA in the ***trp* operator**. This binding shuts off the *trp* operon by blocking the RNA polymerase (Figure 4). The *trp* operon is considered a **repressible operon** and is an example of **negative gene regulation** because it is actively being transcribed (*trp* operon is considered ON) in the absence of its regulatory protein, the *trp* repressor. The *trp* repressor is required to stop transcription of the *trp* operon (*trp* operon is considered OFF).

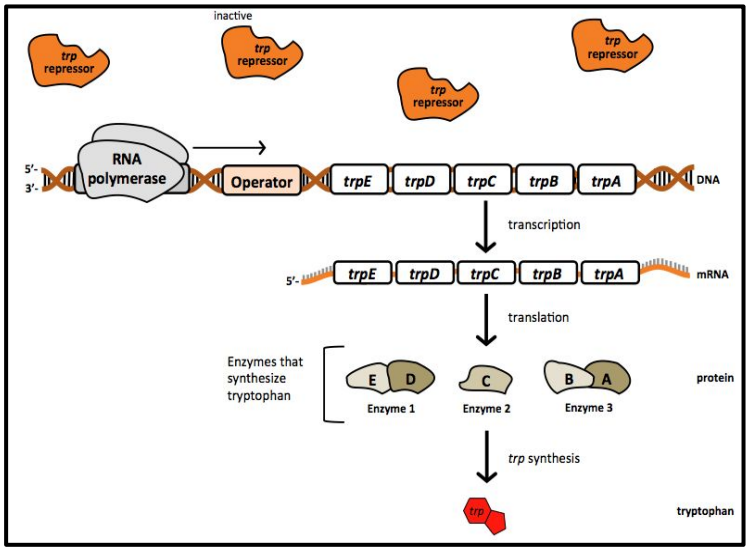

Figure 3 *trp* Operon Expression
When tryptophan levels are low, RNA polymerase transcribes the *trp* operon.

Further, the *trp* operon demonstrates **feedback inhibition** at the level of gene expression. When tryptophan levels are low, the operon is ON which leads to the production of tryptophan. When tryptophan accumulates to a sufficient level, it activates the *trp* repressor which then inhibits further production of tryptophan. As the cell translates other proteins, it will use up its store of tryptophan. When tryptophan levels drop, the *trp* repressor will no longer bind tryptophan and no longer block the RNA polymerase from transcribing the *trp* operon. This cycle will continue indefinitely unless the cell is able to acquire an adequate amount of tryptophan from the environment ,which would then inhibit the *trp* operon.

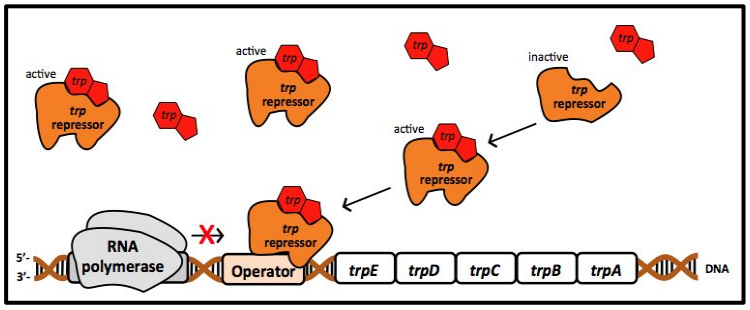

Figure 4 Repression of the *trp* Operon
When tryptophan levels are high, transcription of the *trp* operon is inhibited.

Throughout this lesson on the *trp* operon, you will be asked to provide mechanistic explanations to justify your predictions/conclusions. What is a mechanistic explanation? A mechanistic explanation explains HOW something happens by including the components involved and how those components behave or interact. For example, when tryptophan levels are high, the *trp* operon is OFF; but HOW does that happen? Here is an example of an appropriate mechanistic explanation: tryptophan binds to and activates the *trp* repressor; the *trp* repressor then binds to the promoter region of the *trp* operon and blocks transcription of the *trp* operon by RNA polymerase.

Homework Questions

Complete the following questions:

1. What is tryptophan?

2. What cellular signal does the *trp* operon detect?

3. Collectively, what is the function of the proteins encoded in the *trp* operon?

4. Use your own words to define a repressible operon.

trp Operon Simulation

Dynamic Simulation

In this activity you will be using a computational modeling and simulation software called the Cell Collective to explore the components and dynamics of the *trp* operon. Figure 5 represents a simplified computational model of the *trp* operon that was built using the Cell Collective. Before running the simulation, it is important that you understand the components and properties represented in the model (Figure 5). When building models in the Cell Collective, arrows represent positive regulation (activation) and blunted lines represent negative regulation (inhibition). This convention allows scientists to build models that incorporate different types of regulation. In a biological context, however, the interactions between components of a system are diverse and complex. Therefore, arrows connecting components in models like shown in Figure 5 often represent diverse interactions and relationships. **To begin, complete Table 1 by describing/defining what each arrow and blunted line in the model represents.**

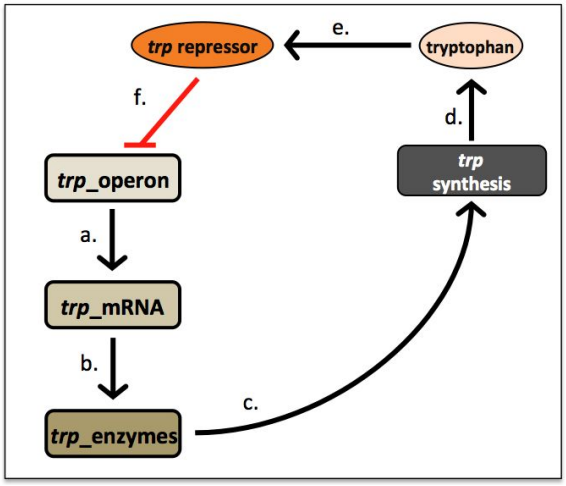

Figure 5 Diagram of *trp* Operon Computational Model

Table 1 Describe/define what each interaction represents in Figure 5.	
a.	The *trp* operon is transcribed by RNA polymerase to produce *trp*_mRNA.
b.	The *trp*_mRNA is _____ into proteins, which assemble to form the _____.
c.	The *trp*_enzymes are _____ for tryptophan synthesis from precursor molecules within the cell.
d.	
e.	
f.	

trp Operon Simulation

In this activity you will be simulating how the external environment and intracellular (inside the cell) conditions influence the expression of the *trp* operon.

Part 1: Access to the Dynamic Model of Cellular Respiration

a. Go to learn.cellcollective.org
b. Register and login to Cell Collective.
c. From the Home page, select "**Prokaryotic Gene Regulation: the trp operon**" (Figure 6).
d. Click "Model" on the top middle menu bar.
e. Within the "Model" tab, click "**Simulation**" to enter the simulation workspace (Figure 7). In this workspace you will be able to observe dynamic behavior of the *trp* operon.
f. You are now ready to simulate the model.

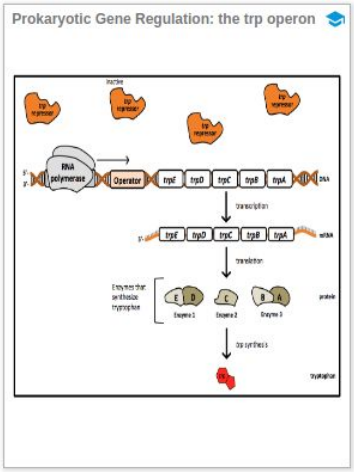

Figure 6 Model Selection

Part 2: Simulation Setup

a. To begin, adjust the "**Sliding Window Size**" to 1 in the **Simulation Control** panel (Red box, Figure 7) and in the **Internal Components** panel (Blue box, Figure 7), check the box under the " 👁 " icon next to "trp_operon". Do NOT adjust External Components activity levels at this time.
b. Click the play (▸) button under **Simulation Control** to start the simulation.
c. You can now evaluate the activity (shown on the y-axis) of the *trp* operon when no tryptophan is present in the external environment.
d. To see the activity of other components in the model just check the " 👁 " icon box next to its name. By checking the components in the order in which they are activated you will be able to observe the dynamics of the *trp* operon.
e. Reset your simulation by clicking the stop (◉) button under **Simulation Control**.
f. Continue this activity by completing the questions on the next page.

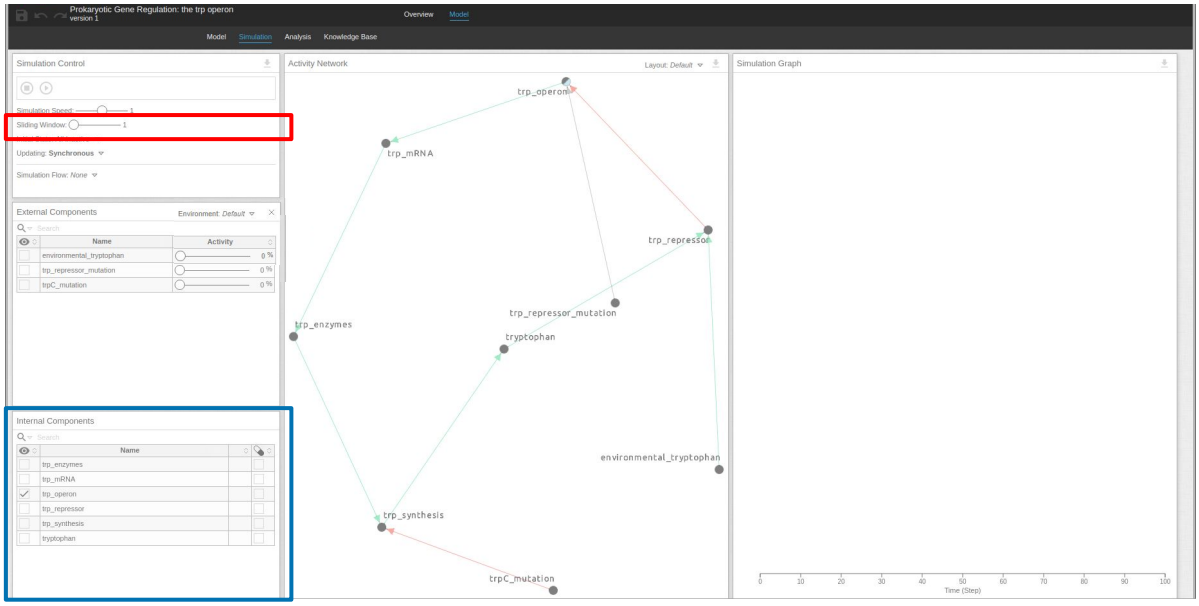

Figure 7 Simulation Setup

Part 3: Experiments using the *trp* Operon Model

The purpose of this section is to learn how the components of the *trp* operon system are interconnected. You will be conducting simulations to verify your own predictions about the dynamic interactions between the components of *trp* operon system. Consider the following investigations and complete each statement thoroughly.

Investigation 1:

A. **Prediction.** How does the presence of tryptophan in the environment influence the expression of the genes in the *trp* operon? (For each prediction select one of the options below)

1.	The *trp* operon will be	active (ON)	inactive (OFF)	alternating between active and inactive
2.	The *trp* repressor will be	active	inactive	alternating between active and inactive

B. **Defend your prediction.** To support your prediction, use the information provided in Figure 3-4 (Pg2) and Figure 5 (Pg3) to complete a mechanistic description in the space below (i.e., circle either active or inactive, and fill in the blanks). This mechanism describes **HOW** the components involved would interact when environmental tryptophan is available to a cell.

The tryptophan (trp) would enter the cell, bind to and **activate/inactivate** the _____. The *trp* repressor would then bind to the _____. As a result, the *trp* operon would become **active/inactive** because the *trp* repressor would block the _____ from transcribing the genes in the operon.

C. **Test your prediction**

1. In the **External Components** panel, adjust the "**environmental_tryptophan**" slider to **100**.
2. Monitor the activity levels of the *trp* repressor and *trp* operon by checking the box under the "👁" icon next to "trp_repressor" and "trp_operon".
3. Start the simulation by clicking the play (▸) button.

D. **Record the results.** Record your simulation results by selecting one of the options for each component you observed.

1.	The *trp* operon is	active (ON)	inactive (OFF)	alternating between active and inactive
2.	The *trp* repressor is	active	inactive	alternating between active and inactive

E. **Evaluate your prediction.** Do your simulation results match your prediction? (circle one) Yes No

If your prediction was not correct, continue to play with the simulation to understand the following:

1) how simulation results translate to events inside the cell

2) how environmental tryptophan affects the *trp* operon

F. **Describe the mechanism correctly.** Describe what your results indicate is occurring in the cell (use your results to support a mechanistic explanation). Specifically, describe **HOW** the components of the *trp* operon system are affected by adding environmental tryptophan.

Investigation 2:

A. **Prediction.** Predict the activity of the *trp* operon if tryptophan was no longer supplied to a cell by the environment. (For each prediction select one of the options below)

1.	The *trp* operon will be	active (ON)	inactive (OFF)	alternating between active and inactive
2.	The *trp* repressor will be	active	inactive	alternating between active and inactive

B. **Defend your prediction.** To support your prediction, use the information provided in Figure 3-4 (Pg2) and Figure 5 (Pg3) to complete a mechanistic description in the space below. This mechanism should describe **HOW** the components involved would interact when environmental tryptophan is no longer available to a cell.

C. **Test your prediction.**

1. In the **External Components** panel, adjust the "**environment_tryptophan**" slider to **0.**
2. Monitor the activity levels of the "trp_repressor" and "trp_operon".
3. Start the simulation.

D. **Record the results.** Record your simulation results by selecting one of the options for each component you observed.

1.	The *trp* operon is	active (ON)	inactive (OFF)	alternating between active and inactive
2.	The *trp* repressor is	active	inactive	alternating between active and inactive

E. **Evaluate your prediction.** Do your simulation results match your prediction? (circle one) Yes No

If your prediction was not correct, continue to play with the simulation to understand the following:

1) how simulation results translate to events inside the cell

2) how environmental tryptophan affects the *trp* operon

F. **Describe the mechanism correctly.** Describe what your results indicate is occurring in the cell (use your results to support a mechanistic explanation). Specifically, describe **HOW** the components of the *trp* operon system are affected by removing environmental tryptophan.

Investigation 3: The *trpR* gene, which is upstream of the *trp* operon, codes for the *trp* repressor and is continuously expressed to produce the *trp* repressor protein.

A. **Prediction.** Predict the activity of the *trp* operon and *trp* repressor if a cell acquires a mutation in its *trpR* gene that no longer allows its gene product, the *trp* repressor protein, to bind to the *trp* operator.

1.	The *trp* operon will be	active (ON)	inactive (OFF)	alternating between active and inactive
2.	The *trp* repressor will be	active	inactive	alternating between active and inactive

B. **Defend your prediction.** To support your prediction, use the information provided in Figure 3-4 (Pg2) and Figure 5 (Pg3) to complete a mechanistic description in the space below. This mechanism should describe HOW the components involved interact.

C. **Test your prediction.**
1. In the **External Components** panel, adjust the "**trp_repressor_mutation**" slider to **100**
2. Monitor the activity levels of the "trp_operon" and "trp_repressor"
3. Start the simulation.

D. **Record the results.** Record your simulation results by selecting one of the options for each component you observed.

1.	The *trp* operon is	active (ON)	inactive (OFF)	alternating between active and inactive
2.	The *trp* repressor is	active	inactive	alternating between active and inactive

E. **Evaluate your prediction.** Do your simulation results match your prediction? (circle one) Yes No

If your prediction was not correct, continue to play with the simulation to understand the following:

1) how simulation results translate to events inside the cell

2) how a mutation in the *trpR* gene affects the *trp* operon

F. **Describe the mechanism correctly.** Describe what your results indicate is occurring in the cell (use your results to support a mechanistic explanation). Specifically, describe **HOW** the components of the *trp* operon system are affected by the mutation.

Investigation 4: The *trpC* gene is part of the *trp* operon and codes for an enzyme important for the synthesis of tryptophan.

A. **Prediction.** In a situation where tryptophan is NOT supplied to a cell by its environment, **predict** the activity of the *trp* operon if that cell had acquired a mutation that made the *trpC* gene product (the enzyme) nonfunctional.

1.	The *trp* operon will be	active (ON)	inactive (OFF)	alternating between active and inactive
2.	The *trp* repressor will be	active	inactive	alternating between active and inactive
3.	Tryptophan synthesis will be	active	inactive	alternating between active and inactive
4.	The *trp* mRNA will be	present	absent	alternating between present and absent
5.	Tryptophan will be	present	absent	alternating between present and absent

B. **Defend your prediction.** To support your prediction, use the information provided in Figure 3-4 (Pg2) and Figure 5 (Pg3) to complete a mechanistic description in the space below. This mechanism should describe HOW the components involved interact.

C. **Test your prediction.**

1. In the **External Components** panel, adjust the "**trpC_mutation**" slider to **100**
2. Monitor the activity levels of the "*trp*_operon", "trp_mRNA", "trp_enzymes", "trp_synthesis", "tryptophan" and "trp_repressor"
3. Start the simulation.

D. **Record the results.** Record your simulation results by selecting one of the options for each component you observed.

1.	The *trp* operon is	active (ON)	inactive (OFF)	alternating between active and inactive
2.	The *trp* repressor is	active	inactive	alternating between active and inactive
3.	Tryptophan synthesis is	active	inactive	alternating between active and inactive
4.	The *trp* mRNA is	present	absent	alternating between present and absent
5.	Tryptophan is	present	absent	alternating between present and absent

E. **Evaluate your prediction.** Do your simulation results match your prediction? (circle one) Yes No

If your prediction was not correct, continue to play with the simulation to understand the following:

1) how simulation results translate to events inside the cell

2) how a mutation in the *trpC* gene affects the *trp* operon

F. **Describe the mechanism correctly.** Describe what your results indicate is occurring in the cell (use your results to support a mechanistic explanation). Specifically, describe **HOW** the components of the *trp* operon system are affected by the mutation.

Insight: Attenuation

At first, scientists believed that regulation of the *trp* operon was only controlled by the repressor-operator interaction. However, later experimental findings indicated a secondary mechanism of control. This secondary mechanism is now known as **transcription attenuation**, or attenuation for short.

During the process of attenuation, transcription is halted before RNA polymerase reaches the protein-coding genes of the *trp* operon. This is possible because transcription and translation happen simultaneously in prokaryotes. Before transcription of the *trp* operon genes, RNA polymerase must first transcribe a segment of DNA called the **leader region**, located in between the operator and the *trpE* gene. The leader mRNA contains self-complementary regions (colored green, purple, yellow, and blue segments in Figure 8) that fold into different hairpin structures depending on the availability of tryptophan in the cell. Depending on which hairpin forms, transcription will either continue or terminate before reaching the *trpE* gene. When tryptophan levels are high, the ribosome translates the leader mRNA quickly, leading to the formation of the **terminator** hairpin (Figure 8A). This terminator hairpin causes RNA polymerase and the mRNA chain to be released from the *trp* operon, terminating transcription. When tryptophan levels are low, the ribosome stalls while translating the leader mRNA. This encourages the formation of the **antiterminator** structure, allowing for transcription of the *trp* operon to continue (Figure 8B).

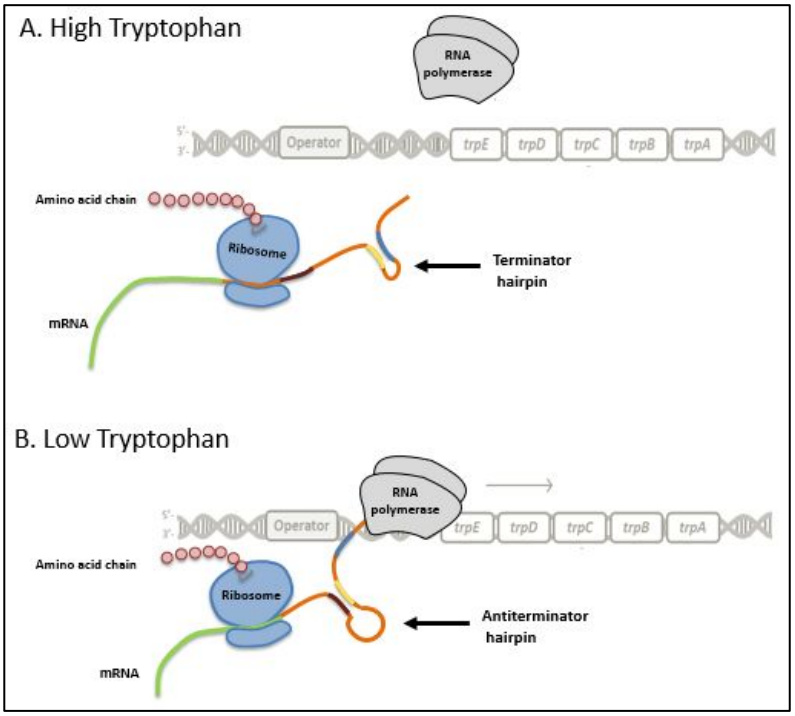

Figure 8 Attenuation and *trp* operon regulation
A) When tryptophan is abundant in the cell, the ribosome translates the leader mRNA quicker. This results in the formation of the terminator hairpin structure, causing dissociation of the mRNA chain and RNA polymerase from the *trp* operon.
B) When there is low tryptophan in the cell, the ribosome stalls when translating the leader mRNA, allowing for the formation of the antiterminator hairpin structure.

Data Analysis

You are a researcher in the late 1960's examining the *trp* operon. Your colleagues have found that the *trp* operon is controlled by a repressor-operator interaction; however, these findings do not fully explain the phenomena of regulation in the *trp* operon. Your hypothesis is that *trp* expression is controlled by an additional regulatory mechanism (regulation of the *trp* operon by attenuation has not been discovered yet).

You have created two mutant strains that contain mutations in the leader region of the *trp* operon (Mutant 1 and Mutant 2) with the goal to demonstrate that this region is important for regulation of the operon. You decide to compare the production of *trp* enzyme anthranilate synthase, which is dictated by the levels of *trpE* expression, in these mutant strains to wild-type ("normal") cells. You produce the following table (Table 2) summarizing your results. Use Table 2 to answer the questions on the next page.

% activity of *trp* operon enzyme	Anthranilate Synthase (*trpE*)
Low Tryptophan	
Wild type	100%
Mutant 1	21-44%
Mutant 2	31-46%
High Tryptophan	
Wild type	4-10%
Mutant 1	5-10%
Mutant 2	5-10%

Table 2. Enzyme activity from wild-type cells in a low-tryptophan environment were set as 100% activity. 2ug/ml of tryptophan was added in the low tryptophan environment; 50ug/ml of tryptophan was added in the high tryptophan environment. Modified from Hiraga et al., 1967.

1. You know that the *trp* operon is regulated by the repressor-operator interaction. Compare the activity of wild type under low tryptophan and high tryptophan. Do your data reflect this type of regulation on the *trp* operon? Explain why below.

2. When looking at your data, under which conditions do you notice the biggest difference in enzyme expression between the wild-type and mutant strains? (Circle one)

   Low tryptophan High tryptophan

3. Should the *trp* repressor be active or inactive under the environmental conditions you selected above? Does this indicate that the *trp* repressor is involved in decreasing Mutant 1 and Mutant 2's enzyme activity in comparison to wild type? Why or why not?

4. Do your data support the hypothesis that there is a secondary mechanism regulating *trp* operon expression? How do the data either support or discredit this hypothesis?

5. With your current knowledge of attenuation, how do you think the mutations in Mutant 1 and Mutant 2 impact the leader region of the *trp* operon to generate the data in Table 2?

6. Another lab has also published findings involving mutations of the *trp* operon leader region. This lab, however, has created a mutant strain that expressed an increase in *trp* operon activity under low tryptophan conditions compared to wild type. Explain how a mutation in the leader region can lead to this increase in *trp* expression.

Discussion question

We now know that the leader region encodes for a peptide (a chain of 2-10 more amino acids) that contains two tryptophan amino acids. You also know that the formation of the antiterminator vs. terminator hairpin is dependent on the ribosome stalling during translation of the leader region, but what causes the ribosome to stall? With these two pieces of information, describe the mechanism of how tryptophan levels regulates transcription of the *trp* operon by means of transcription attenuation.

References

Hiraga S, **Ito K**, **Hamada K**, **Yura T**. 1967. A new regulatory gene for the tryptophan operon of Escherichia coli., Biochem Biophys Res Commun. 26:522-7.