

## MODELING THE REGULATORY SWITCHES OF THE *PITX1* GENE IN STICKLEBACK FISH

### INTRODUCTION

The types and amounts of proteins produced by a given cell in the body are very important and carefully regulated. Transcribing DNA to messenger RNA and translating that RNA to protein is often referred to as gene expression. Regulating that expression simply means turning on or off or increasing or decreasing the production of a given protein. Interestingly, protein production can be regulated at the translational and transcriptional steps, as well as after a protein is produced. In this activity, we will talk about how gene expression is regulated through transcription.

The basic elements of transcription regulation in eukaryotes are similar to the very well-studied lac and trp operon systems found in bacterial cells. In both eukaryotic and bacterial systems a protein, either an activator or repressor, binds to a region of the DNA called an “operator” in prokaryotes and a “regulatory switch” or “enhancer” in eukaryotes. The activator or repressor protein acts like the hand that flips the switch, but it can only turn the switch on or off if it can bind to the specific DNA sequence. Thus, the presence or absence of the activator or repressor and the sequence specificity of that binding are the driving force that regulates gene transcription.

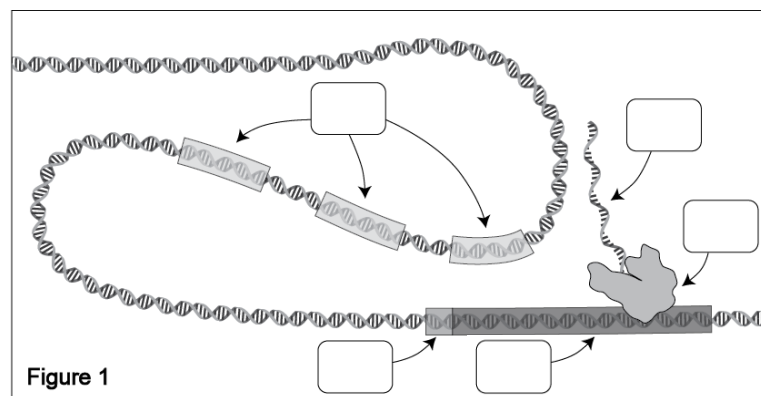
Both prokaryotic and eukaryotic cells regulate gene expression in response to internal and external stimuli. Gene regulation is essential for the cell to perform the functions needed to live. In multicellular eukaryotes, gene regulation is also important in building bodies. During development, different sets of genes need to be turned on and off in the right places, at the right times, and in the right sequence for bodies to be built correctly. How is cell type–specific gene regulation achieved? It depends on two conditions: Does the gene have the appropriate regulatory switch, and does that particular cell have the appropriate activator or repressor? In this activity, you will learn about one particular gene, *Pitx1*, and how its expression is regulated in different tissues. You will then learn how changes in regulatory switches in the *Pitx1* gene lead to changes in expression which ultimately affect the structure of the body.

### PART 1: REVIEWING THE REGULATION OF EUKARYOTIC GENE TRANSCRIPTION

Watch the short film, *The Making of the Fittest: Evolving Switches, Evolving Bodies*. Pay close attention to how the switches regulate the expression of the *Pitx1* gene in stickleback embryos.

Use the information from the film and your knowledge of eukaryotic gene transcription to answer questions 1-6.

1. Figure 1 is a diagram, similar to the one shown in the film (8:00-8:34), showing key components of gene transcription. Label the boxes in Figure 1 with the letters a-e, which correspond to the terms listed below. For example, write letter “a” in the box pointing at the protein-coding region.
  - a. Protein-coding region
  - b. Regulatory switches (or enhancers)
  - c. Promoter
  - d. mRNA
  - e. RNA polymerase



*The Making of the Fittest:  
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**STUDENT MATERIALS**

2. Describe the function of regulatory switches. (**Note:** Some textbooks refer to regulatory switches as enhancers.)

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3. Gene transcription is a complex process that involves specific interactions of proteins and regulatory regions of DNA. The animation in the film (8:00-8:34) and Figure 1 show some of the factors involved but not all. In the cell, a number of proteins bind to different regions on the DNA to regulate gene transcription. Use your textbook to learn about the proteins involved in eukaryotic gene transcription. Circle the proteins that are involved in eukaryotic gene transcription and regulation.

- |                |                               |                |
|----------------|-------------------------------|----------------|
| proteasomes    | general transcription factors | lactase        |
| activators     | operons                       | ribosomes      |
| DNA polymerase | mediators                     | RNA polymerase |

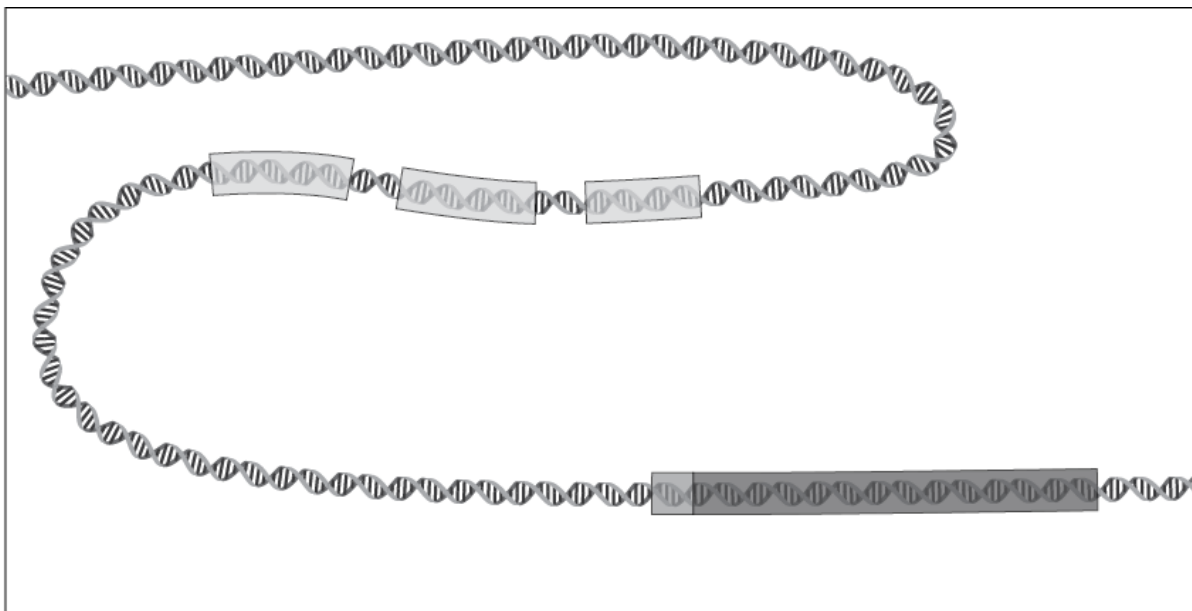
4. Which protein(s) from the list above bind(s) to regulatory switches in a sequence-specific manner?

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5. Which protein(s) from the list above bring(s) bound activators in contact with proteins bound to the promoter?

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6. This drawing is missing all the protein components of eukaryotic gene transcription. Draw in the proteins identified in question #5 to show active eukaryotic gene transcription. Be sure to label the proteins and DNA in your figure. You can use any shape to represent these proteins.



## PART 2: GENE REGULATION IN DIFFERENT TISSUES

As you saw in the film, the presence or absence of pelvic spines in the stickleback fish is controlled by whether the *Pitx1* gene is expressed in the pelvic tissue. However, the *Pitx1* protein is actually important in building other body parts and is therefore expressed in multiple tissues at specific times.

How is *Pitx1* expressed in different tissues? The *Pitx1* gene has multiple regulatory switches that control the expression of the gene in different tissues: the pituitary, jaw, and pelvic tissues. Having multiple switches enables *Pitx1* to be used many times in different contexts and expands the versatility of that gene. These switches are part of the DNA upstream of the *Pitx1* coding region. Activators present in a particular tissue bind to a specific sequence on the DNA and turn *Pitx1* on in the appropriate tissues. For example, in the cells that develop into the pelvis there is a specific activator (activator 2) that binds in a sequence-specific manner to the pelvic switch to transcribe *Pitx1* in that tissue. In the jaw, there is a different activator (activator 1) that binds to a different sequence called the jaw switch to turn on *Pitx1* in the jaw tissue. However, *Pitx1* is not transcribed in the eyes because it does not have a sequence that can bind to activators present in the eyes. As you can see, while the DNA is the same in all cells of the body, the activators that are present differ from tissue to tissue. By having multiple regulatory switches, *Pitx1* can be used many times in different tissues to build specific body parts.

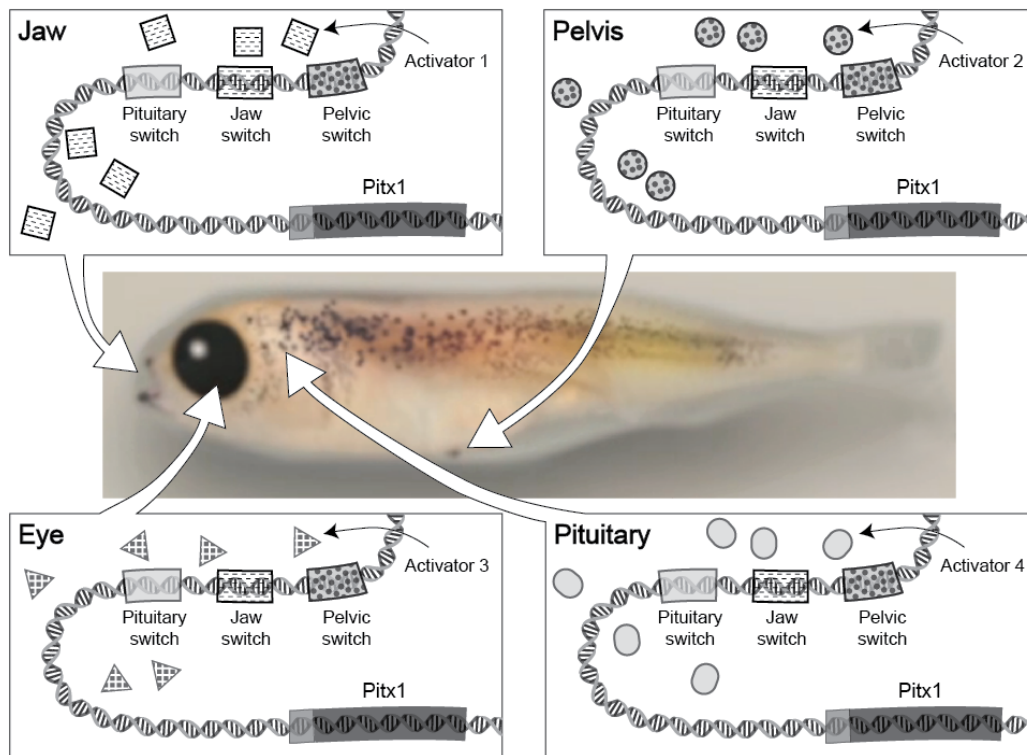


Figure 2

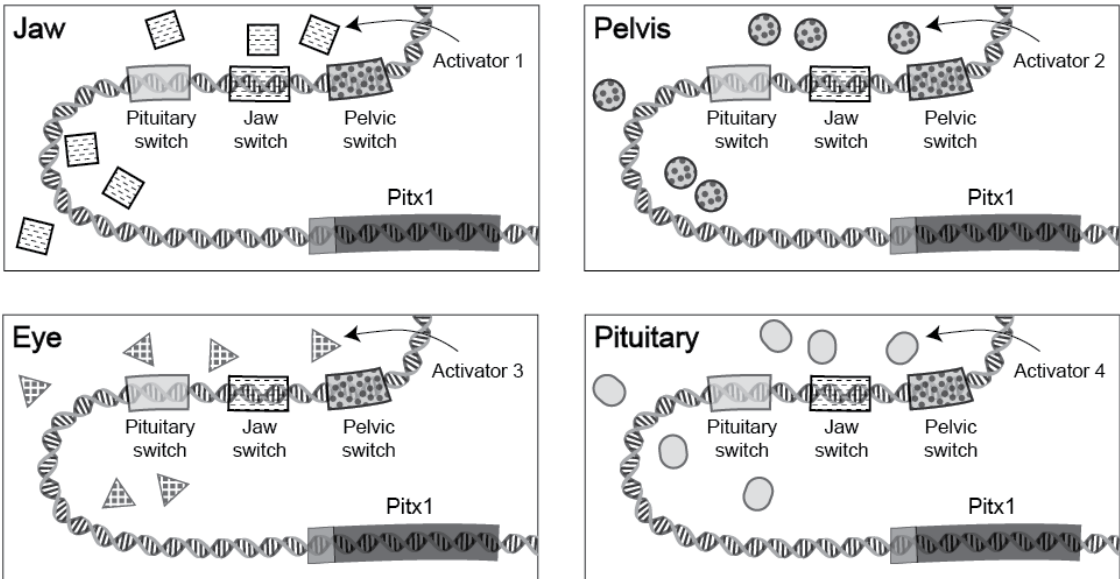
Figure 2 illustrates how *Pitx1* transcription is regulated in different tissues. The center image is that of a stickleback embryo. The drawings in the surrounding boxes show the *Pitx1* gene region and activator proteins present in the jaw, pelvis, eye, or pituitary tissues. Note that for simplicity, we are only showing one activator molecule present in a particular tissue. In reality, many activators are present in a particular tissue at any one time. Activator molecules with specific shading can bind to switches with the same shading.

Answer questions 1-8 using your knowledge of *Pitx1* gene regulation, gene switches, and the information in Figure 2.

- List all the tissues shown in Figure 2 that express the *Pitx1* gene.

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- If a fish does not produce activator 1 proteins because of a mutation in the gene that encodes those proteins, *Pitx1* will be expressed in which of the following tissues? (Put a check mark next to the tissue(s) that will express *Pitx1*.)  
 jaw \_\_\_\_\_                      pelvis \_\_\_\_\_  
 eye \_\_\_\_\_                      pituitary \_\_\_\_\_
- If a fish does not produce activator 3 proteins, *Pitx1* will be expressed in which of the following tissues? (Put a check mark next to the tissue(s) that will express *Pitx1*.)  
 jaw \_\_\_\_\_                      pelvis \_\_\_\_\_  
 eye \_\_\_\_\_                      pituitary \_\_\_\_\_
- Assume a fish inherits a deletion mutation in the pituitary switch which inactivates that switch. You isolate DNA from jaw, pelvic, eye, and pituitary tissues. In the DNA of which tissue(s) would you expect to see the pituitary switch mutation? Draw an "X" over the mutated switch in the appropriate tissue(s).



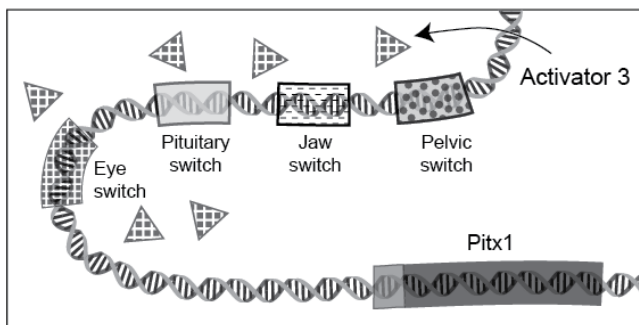
When a mutation in the pituitary switch prevents activator 4 from binding, where would you expect *Pitx1* to be expressed? (Put a check mark next to the tissue(s) that express *Pitx1*.)

- jaw \_\_\_\_\_                      pelvis \_\_\_\_\_  
 eye \_\_\_\_\_                      pituitary \_\_\_\_\_

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**STUDENT MATERIALS**

5. A fish inherits a mutation that results in a new regulatory switch ("eye switch") that regulates *Pitx1* expression in the eye. This new switch binds a particular activator found in the tissues of the eye ("activator 3"). See figure 3.

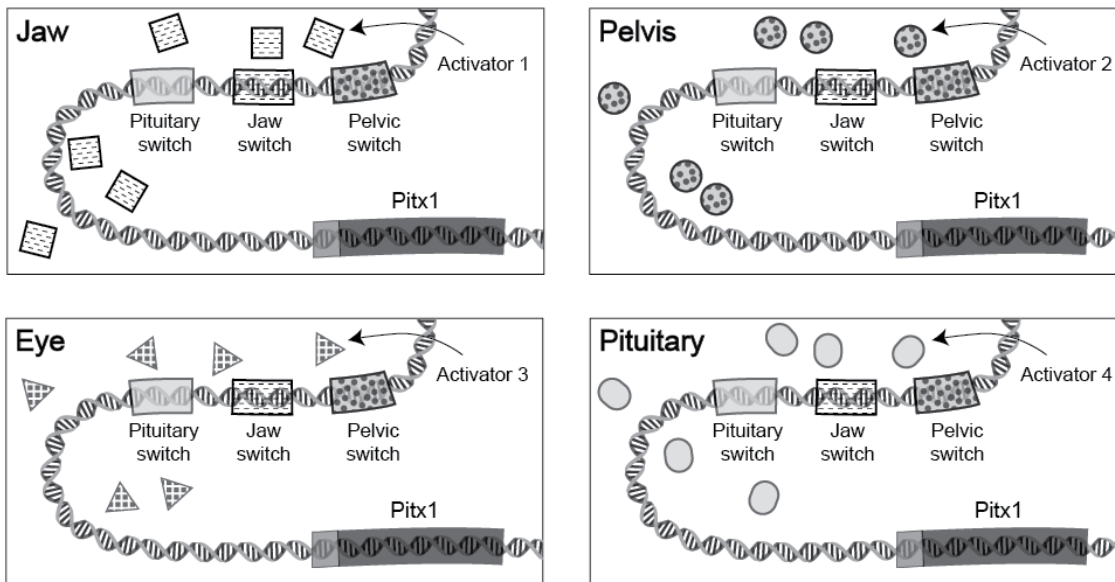


**Figure 3**

Where would you expect *Pitx1* to be expressed? (Put a check mark next to the tissue(s) that will express *Pitx1*.)

jaw \_\_\_\_\_                      pelvis \_\_\_\_\_  
eye \_\_\_\_\_                      pituitary \_\_\_\_\_

6. A fish inherits a mutation in the *Pitx1* coding region. This is a nonsense mutation that introduces a premature stop codon, resulting in a nonfunctional truncated protein. You isolate DNA from jaw, pelvic, eye, and pituitary tissues. In which tissue(s) would you expect to see this *Pitx1* coding region mutation? Draw an "X" over the *Pitx1* coding region in the tissues where you would expect to see the mutation.



Where would you expect *Pitx1* will be expressed? (Put a check mark next to the tissue(s) that express *Pitx1*.)

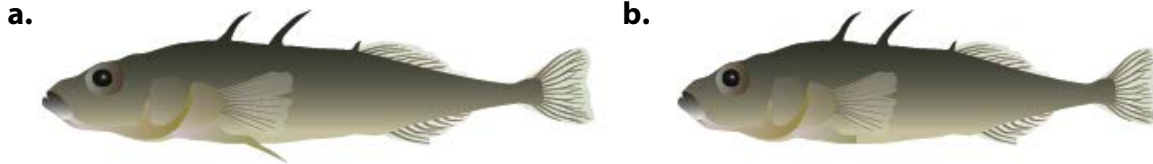
jaw \_\_\_\_\_                      pelvis \_\_\_\_\_  
eye \_\_\_\_\_                      pituitary \_\_\_\_\_



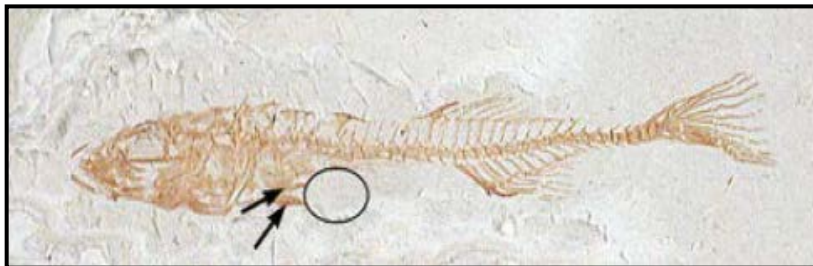
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**STUDENT MATERIALS**

7. The Pitx1 protein has important functions in various tissues during stickleback development. The complete absence of the Pitx1 protein from all tissues is lethal to the organism. However, as shown in the film, Pitx1 protein can be absent in the pelvis alone, and the fish survives. The absence of Pitx1 in the pelvis confers a unique phenotype. Circle the fish below that lacks Pitx1 expression in the pelvis.



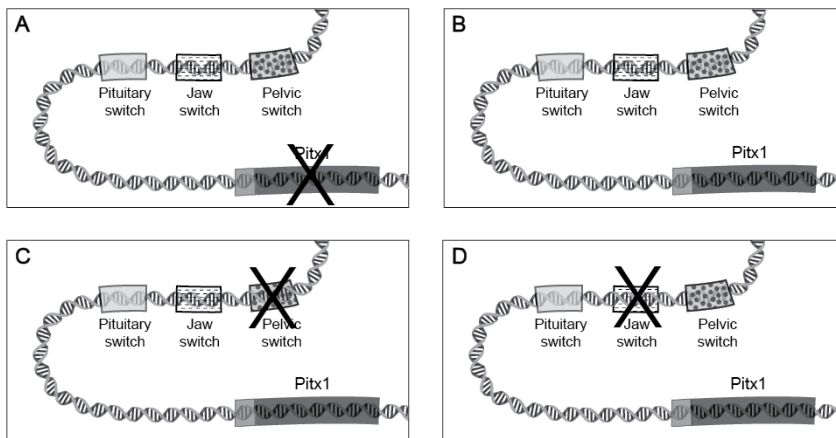
8. A quarry in Nevada contains fossil stickleback fish that once lived in an ancient freshwater lake at this site about 10 million years ago. By examining many stickleback fossils in each rock layer, Dr. Michael Bell has determined that over many generations the skeletons of stickleback living in the lake changed. In some rock layers, most of the stickleback fossils lack pelvic spines, as pictured below.



**Fossil stickleback fish lacking pelvic spines.**

The circle shows the region where you would expect to find the pelvis and pelvic spines; the arrows point to a set of bones that are not part of the pelvis.

Based on what you know about the molecular mechanisms that control the development of stickleback pelvic spines, circle the figure below that likely represents what the *Pitx1* gene region looked like in these stickleback fish that lacked pelvic spines. The X represents a mutation that inactivates that particular gene region.



### PART 3: MODELING *PITX1* TRANSCRIPTION REGULATION IN STICKLEBACK FISH

#### MATERIALS

You will need:

- 4 white pipe cleaners, 12-18 inches long (or 18-inch white twine)
- Magic markers (blue, green, red, yellow, purple)
- Scotch tape
- Poster board or 4 sheets of 8.5 x 11 paper
- Items that represent proteins (ask teachers for instructions)
- Ruler
- Scissors (optional)

#### PROCEDURE AND QUESTIONS

You are now familiar with the components involved in regulatory switches, how *Pitx1* transcription is regulated, and how its expression affects the phenotype. From the film, you learned that the marine stickleback fish have pelvic spines while many freshwater stickleback fish do not. This is due to the differential expression of the *Pitx1* gene as a result of a mutation in the regulatory switch. In this activity, you will build four models that represent *Pitx1* gene transcription in two different tissues in both marine and freshwater stickleback populations.

1. Based on the information from the film, what is the difference in the DNA around the *Pitx1* gene region between marine and freshwater stickleback fish?

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2. Make four models of the *Pitx1* gene region. Two models will represent the *Pitx1* gene region of the marine stickleback. Two models will represent the *Pitx1* gene region of the freshwater stickleback.

Use magic markers and white pipe cleaner to make the models. Be sure to include the appropriate regulatory switches for the marine and freshwater stickleback as mentioned in the film. (You may use the lengths indicated in the parentheses as a guide for your DNA model.) Color in the following DNA regions on each of the pipe cleaners:

*Pitx1* coding region = blue (~4 inches)

Promoter = purple (0.25 inches)

Pelvic switch = green (1-2 inches)

Jaw switch = red (1-2 inches)

Pituitary switch = yellow (1-2 inches)

Note: Make sure you keep track of which two models represent the marine stickleback DNA and which two represent the freshwater stickleback DNA.

3. Use Play-Doh, colored stickers, or other objects as instructed by your teacher to represent the following proteins:

RNA polymerase, jaw switch activator, pelvic switch activator, and pituitary switch activator.

General transcription factors and mediators are optional. Make sure that you keep the colors and shapes of the proteins consistent in each model. For example, if your RNA polymerase is a purple circle, make sure all RNA polymerases in your models are purple circles.

4. Use one of the marine stickleback DNA, one set of protein models, and one piece of paper (or part of the poster board) to model *Pitx1* gene transcription in the pelvic tissues of a developing stickleback that HAS pelvic spines. (Note: You might not use all the protein models in the set.)
5. Title the model with the stickleback type (marine or freshwater) and body region. For example, as a header, you might write "marine stickleback – pelvis". If *Pitx1* is transcribed, write "ON" next to your title. If *Pitx1* is not transcribed, write "OFF." Label the DNA and proteins on your model.
6. Repeat steps 4-5 to model *Pitx1* gene transcription in marine stickleback in the jaw tissues.
7. Now, use one of the freshwater stickleback DNA, one set of protein models, and one piece of paper (or part of the poster board) to model *Pitx1* gene transcription in the pelvic tissues of a developing stickleback that LACKS pelvic spines.
8. Title the model with the stickleback type (marine or freshwater) and body region. If *Pitx1* is transcribed, write "ON" next to your titles. If *Pitx1* is not transcribed, write "OFF." Label the DNA and proteins on your model.
9. Repeat steps 7-8 to model *Pitx1* gene transcription in freshwater stickleback fish in the jaw tissues.

**ANALYSIS QUESTIONS**

1. Explain the role that regulatory switches play in determining whether stickleback embryos will develop pelvic spines.

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2. According to the film, what is the selective pressure that led to freshwater stickleback fish losing their pelvic spines?

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3. You isolate the DNA from the heart of the freshwater stickleback that lack pelvic spines. In the space provided below, draw what the *Pitx1* gene region looks like in the heart tissue of that freshwater stickleback. Be sure to include the appropriate switches and *Pitx1* coding region and label your drawing.



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**STUDENT MATERIALS**

4. Models serve many purposes. In this activity, you used a model to visualize a process that is too small to see. Most models have some limitations and don't include all the details of a complex process. List three limitations that your models have in representing the molecular process of *Pitx1* gene transcription.

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