

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

OVERVIEW

This hands-on activity supports the short film, *The Making of the Fittest: Evolving Switches, Evolving Bodies*, and aims to help students understand eukaryotic gene regulation and its role in body development using the example of a well-studied gene called *Pitx1*. Students will interpret molecular diagrams of eukaryotic gene transcription to familiarize themselves with the molecular components and mechanisms responsible for regulating gene transcription. They will then create models showing how *Pitx1* gene transcription is regulated in two morphologically different populations of stickleback fish.

KEY CONCEPTS AND LEARNING OBJECTIVES

- Virtually all cells in the body have the same genetic makeup,* but not all genes are expressed in all tissues at all times.
- Whether a gene is expressed is dependent on the regulatory switches that the gene possesses and the activators or repressor proteins present in that cell.
- Activators bind to regulatory switches in a sequence-specific manner. The binding of the activators to the switches activates transcription.
- Some genes, especially genes involved in body development, have multiple switches. Each switch independently regulates the expression of the gene in different parts of the body at different times in the animal's life cycle.
- The presence of multiple switches enables the same gene to be used many times in different contexts and thus greatly expand the functional versatility of individual genes.
- Mutations in regulatory switches affect expression of a particular gene in a particular tissue without affecting the gene's protein product (shape and function).
- Changing the expression of a gene involved in body development can have profound effects in shaping the body.

(* There are some notable exceptions:

- (1) Mature red blood cells have no DNA.
- (2) Germ line cells (sperm and egg) have half the genetic material.
- (3) B cells, which are part of the immune system, have DNA that has been rearranged to make antibodies.
- (4) Cells acquire somatic mutations. In some cases, these mutations confer a selective advantage for that cell and that mutation will be fixed in a set population of cells, like in the case of cancer cells.)

At the end of this activity, students should be able to:

- Correctly interpret visual representations of eukaryotic gene transcription.
- Contrast how mutations in regulatory switches differ from mutations in coding regions of genes.
- Construct models that accurately represent the regulation of *Pitx1* transcription in marine and freshwater stickleback fish.

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CURRICULUM CONNECTIONS

Text/Curriculum	Curriculum Topics
Next Generation Science Standards	HS-LS1.A, HS-LS3.A, HS-LS3.B, HS-LS4.B, HS-LS4.C
AP [®] (2012–2013 Standards)	1.A.1, 1.A.2, 1.A.4, 1.C.2, 2.D.1, 2.E.1, 3.A.1, 3.A.4, 3.B.1, 3.C.1, 4.A.3
IB (2016 Standards)	2.7, 3.1, 5.2, 7.2
Common Core*	CCSS.ELA-Literacy.WHST9-12.1, CCSS.ELA-Literacy.RST.9-12.5, CCSS.ELA-Literacy.RST.9-12.7,

KEY TERMS

transcription, eukaryotic gene regulation, regulatory switches, enhancers, or cis-regulatory regions, activators, repressors, general transcription factors, mediators, promoters, RNA polymerase, phenotype, evolution, natural selection, mutation, adaptation, genes, proteins, protein-coding region, mutations.

TIME REQUIREMENT

This lesson is designed for two 50-minute class periods (see Teaching Tips for more information). Depending on the level of students' background knowledge, certain parts of the activity may be eliminated or may be done as a whole-class activity.

SUGGESTED AUDIENCE

This lesson is designed for high school biology (general bio and especially AP/IB) and introductory college biology.

PRIOR KNOWLEDGE

Students should be familiar with basic genetics and the central dogma. Some knowledge about how lac and trp operons work would be helpful. In addition, students should understand the role of genetic variation in a species in natural selection and evolution.

MATERIALS

Students 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
- Any items that can represent proteins. Any types of materials that come in different colors can be used. See Teaching Tips for ideas.
- Ruler
- Scissors (optional)

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TEACHING TIPS

Key Points to Emphasize:

- Students may wonder why *Pitx1* is expressed in such diverse tissues. This is because the *Pitx1* gene contains multiple regulatory switches that allow for transcription of that gene in multiple tissues. The expression of *Pitx1* is important in various tissues because the Pitx1 protein is itself a regulatory protein that serves many roles in the development of the fish. Pitx1 controls the expression of multiple genes, not just those responsible for forming the hind limb.
- In addition, students should recognize that some activators and repressors are present in multiple tissues. Approximately 5-10% of the human genome encodes regulatory proteins which act in different combinations to produce a great diversity of gene expression patterns.
- When students think of cell-type-specific proteins, they may automatically think of structural proteins like insulin in pancreatic cells, or myosin proteins in muscle cells. Remind students that cells also contain regulatory proteins, like Pitx1. The combination of both structural and regulatory proteins defines the cell type.
- Lead students to understand that the expression of activators that activate *Pitx1* transcription (for example, the activator that binds the pelvic switch) is also under regulatory control. Thinking about development raises chicken-or-egg questions. Although the development of a complex animal from a single cell is not fully understood, great progress has been made in recent decades to understand how different sets of genes move the development of an embryo through different stages to maturity. These processes involve messenger RNA and proteins packed into the egg, and dividing cells having positional information about where they are in the developing embryo. Students are encouraged to read more about embryology, development, and differential gene expression.
- The film uses the term “regulatory switches.” Some textbooks will refer to them as enhancers.

Procedural Tips:

- Have students work in groups of two or three.
- A nice animation of eukaryotic gene transcription can be found at http://www.hhmi.org/biointeractive/evolution/dna_transcription_regulation.html to use as a supplemental resource. Note that the diagram in the animation looks different from the ones used in the film. Make sure that students are comfortable with looking at a variety of schematic illustrations used to represent similar molecular processes.
- You may want to show students the film the day before or assign it as homework to provide enough time to complete Parts 1 and 2.
- In Part 3, have your students tape the models onto a poster board or on four sheets of 8.5 x 11 inch paper. Alternatively, your students can take digital photographs of their models and submit them for review. Be sure to instruct your students on how they should submit the work they do in Part 3.
- Students can use different types of materials (i.e., colored stickers, Play-Doh, Legos, beads, etc.) to model the proteins in Part 3. The proteins needed for this activity are listed below. Suggested colors are in parentheses. Provide students with four sets of each of the proteins, although not every protein will be used:
 - RNA polymerase (purple)
 - Pelvic switch binding activator (green)
 - Jaw switch binding activator (red)

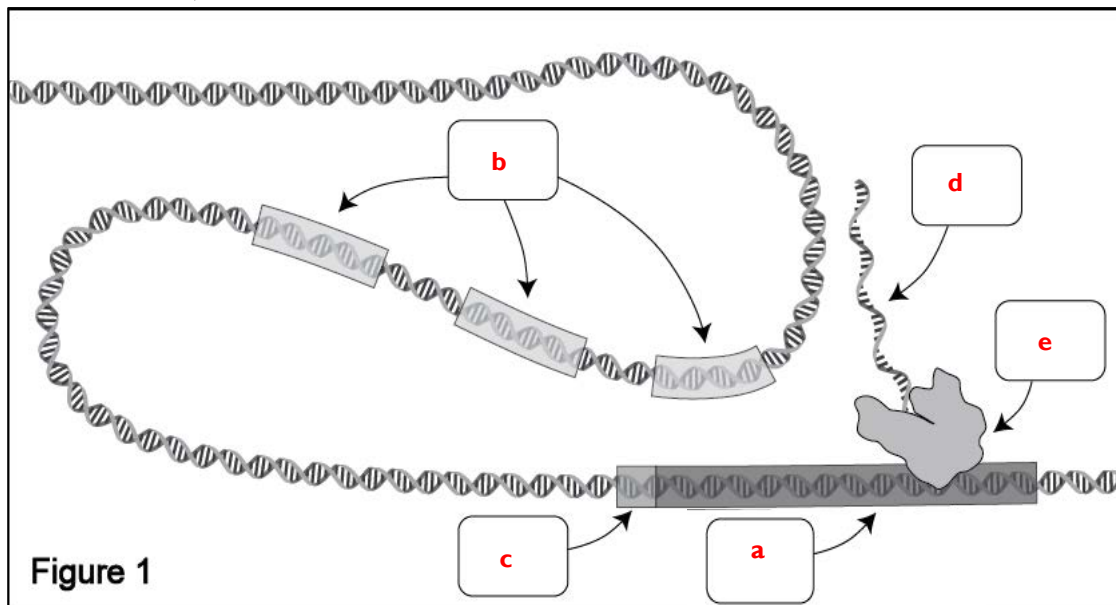
- Pituitary switch binding activator (yellow). This piece will not be used in the activity, but you may want to provide it for students as a “red herring.” You may discuss with your students where this activator would bind and in which tissues you might find it.
- General transcription factors (optional, blue)
- Mediators (optional, orange)

ANSWER KEY

PART 1: REVIEWING THE REGULATION OF EUKARYOTIC GENE TRANSCRIPTION

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 coding region of a gene.

- a. Protein-coding region
- b. Regulatory switches (or enhancers)
- c. Promoter
- d. mRNA
- e. RNA polymerase



2. Describe the function of regulatory switches. (**Note:** Some textbooks refer to regulatory switches as enhancers.) **Regulatory switches are regions of DNA that can be bound by a particular activator or repressor in a sequence-specific manner. It can either be near the coding region or many megabases away. The switch controls the transcription of genes in different tissues and at different times in development.**

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3. Gene transcription is a complex process that involves the interactions of proteins and regulatory regions of DNA. The animation in the film (8:00-8:24) 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. Answers are underlined and bold.

proteasomes

activators

DNA polymerase

general transcription factors

operons

mediators

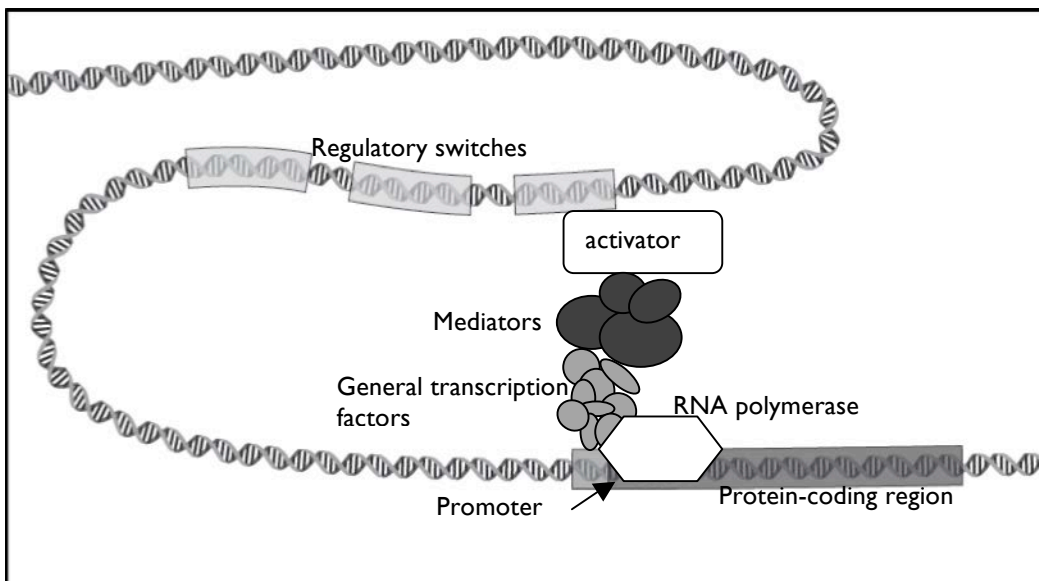
lactase

ribosomes

RNA polymerase

4. Which protein(s) from the list above bind(s) to regulatory switches in a sequence-specific manner?
Activators
5. Which protein(s) from the list above bring(s) bound activators in contact with proteins bound to the promoter?
Mediators
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 regions in the figure. You can use any shape to represent these proteins.

Students should label all the proteins and DNA regions. Students may choose to draw mediators and general transcription factors as a single circle, which is acceptable as long as they are bound to the correct proteins and DNA.



PART 2: GENE REGULATION IN DIFFERENT TISSUES

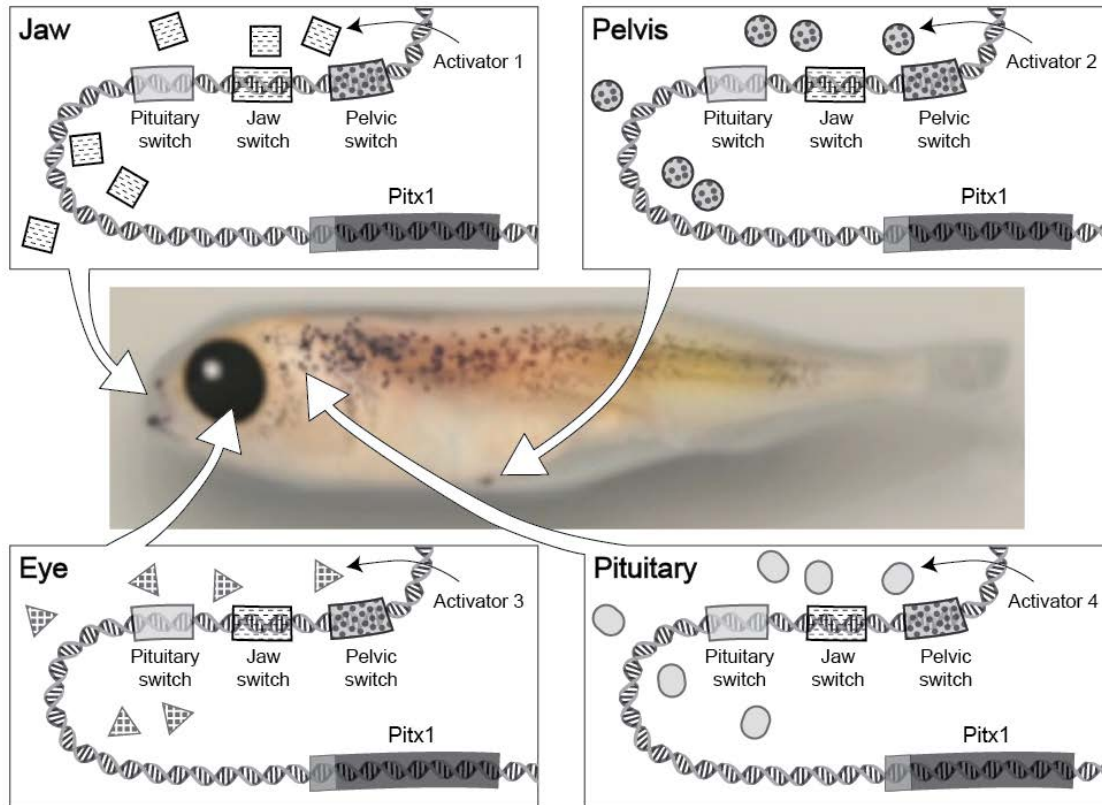


Figure 2

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. While the diagram only shows one activator in one tissue, many activators are present in a particular tissue at any one time. For simplicity, we are only showing one activator molecule present in a particular tissue. Activator molecules with specific shading can bind to switches with the same shading.

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

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

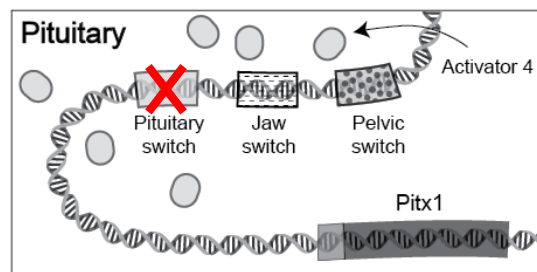
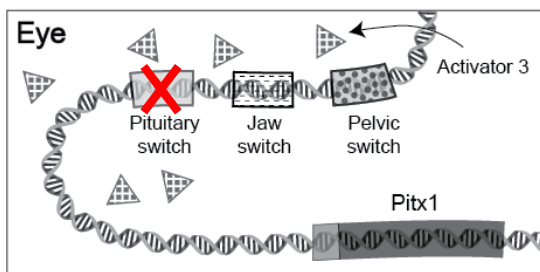
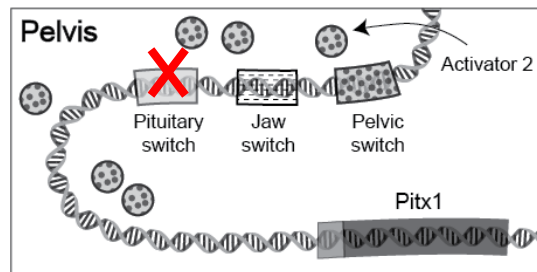
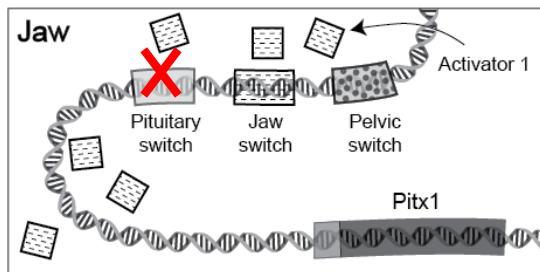
The jaw, pelvis, and pituitary

2. 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 ☒

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3. 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 ☒
4. Assume that a fish inherits a deletion mutation in the pituitary switch which inactivates the switch. You isolate DNA from jaw, pelvic, eye, and pituitary tissues. In the DNA of which tissue(s) would you expect the pituitary switch mutation? Draw an "X" over the mutated switch in the appropriate tissue(s).



Make sure that students have placed an "X" over the pituitary switch in all four tissues. The mutation will be the same in all tissues because it is an inherited mutation.

5. In which tissues would you expect Pitx1 to be expressed in the above scenario? (Put a check mark next to the tissue(s) that express Pitx1.)

jaw ☒ pelvis ☒
eye _____ pituitary _____

6. 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.

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 ☒

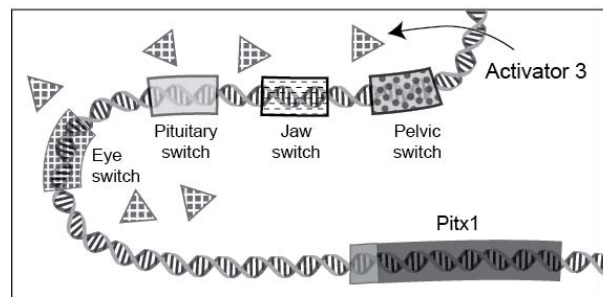
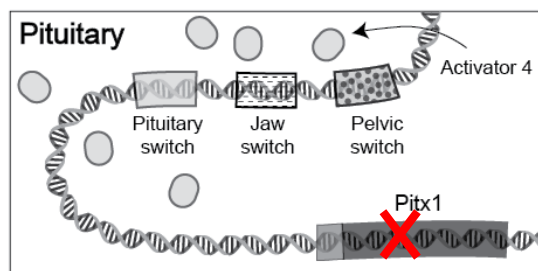
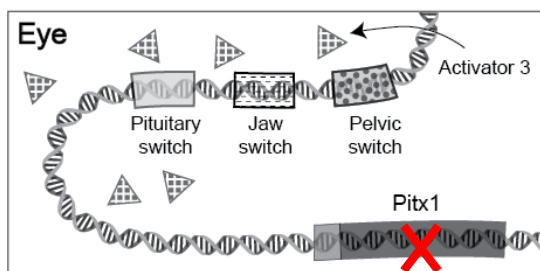
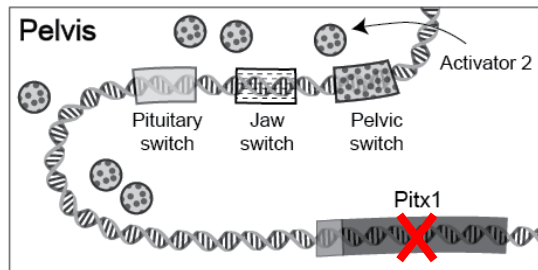
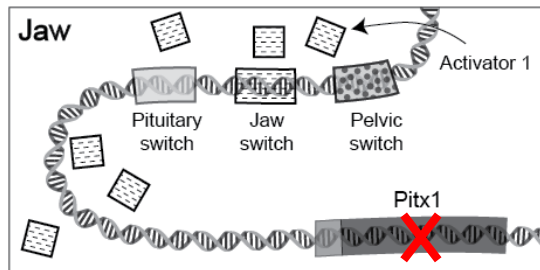


Figure 3

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7. 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.

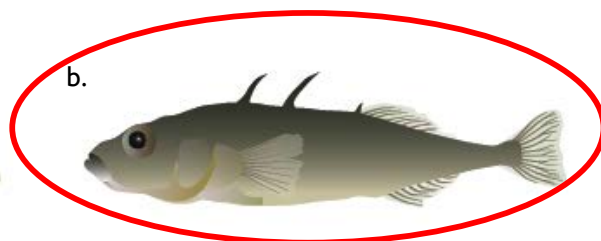
Make sure that students have placed an "X" over the *Pitx1* coding region in all four tissues. The mutation will be the same in all tissues because it is an inherited mutation.

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

***Pitx1* is not expressed in any tissue**

jaw _____ pelvis _____
eye _____ pituitary _____

8. The *Pitx1* protein has many important functions in various tissues during stickleback development. The complete absence of *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.



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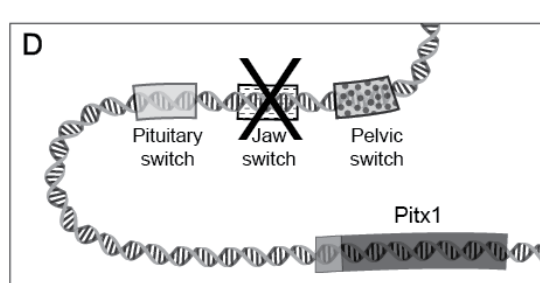
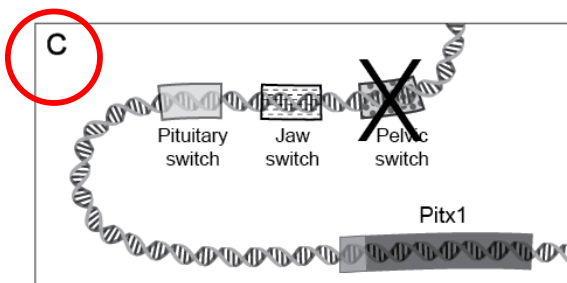
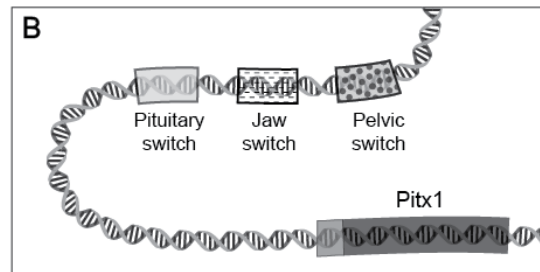
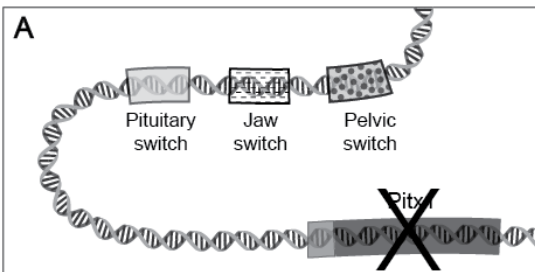
9. A quarry in Nevada contains fossil stickleback fish that once lived in an ancient freshwater lake at this site about 10 million years ago. By examining many stickleback fossils in each rock layer, Dr. Michael Bell has determined that over many generations the skeletons of stickleback fish living in 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.



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PART 3: MODELING GENE SWITCHES IN STICKLEBACK FISH

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?

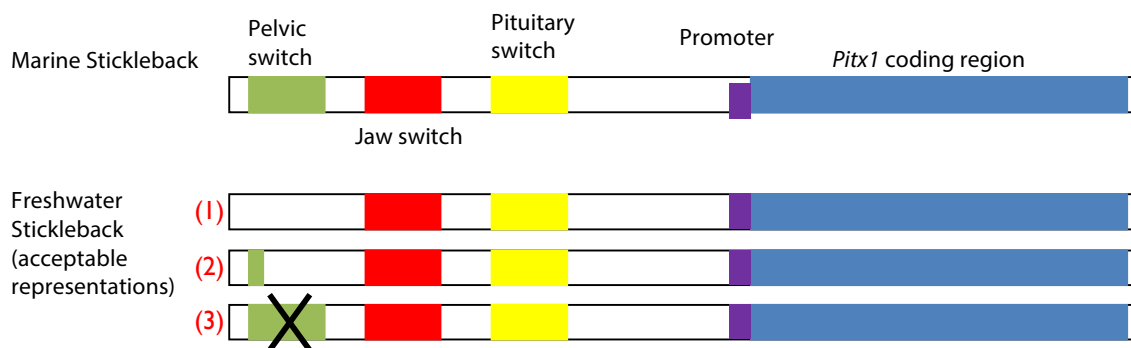
The *Pitx1* DNA of marine stickleback fish contains a pelvic switch, while the *Pitx1* DNA of freshwater stickleback fish does not.

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

Students should have two sets of pipe cleaners.

- The marine stickleback DNA should have the pelvic, jaw, and pituitary switch, as well as promoter and *Pitx1* coding region designated in the appropriate colors.
- The freshwater stickleback DNA should have all the components listed above but designate an absent or mutated pelvic switch. All of the following representations are acceptable.
 - (1) No pelvic switch at all (no green area).
 - (2) A smaller pelvic switch (green area will be smaller than 1 inch) to represent a deletion.
 - (3) Pelvic switch is present (1-2 inch green area) but indicates that it is inactive, possibly with a large X over the switch.

The exact sizes of and distance between the regions are unimportant.



The student models should contain the following:

1. The *Pitx1* coding region and promoter are represented.
2. The regulatory switches are included and placed correctly.
3. Freshwater stickleback fish have freshwater *Pitx1* DNA containing no pelvic switch, and marine stickleback fish have marine *Pitx1* DNA with all three switches.
4. DNA and proteins are clearly and accurately labeled.
5. The appropriate activator proteins are in the appropriate tissue. (For example, there should be no jaw-specific activators in the pelvic region.)
6. The activators are associated with the appropriate switches.
7. RNA polymerase is placed correctly on the DNA.
8. Students correctly indicate whether *Pitx1* is transcribed.

Optional

9. General transcription factors are labeled and clearly represented.
10. Mediators are labeled and clearly represented.

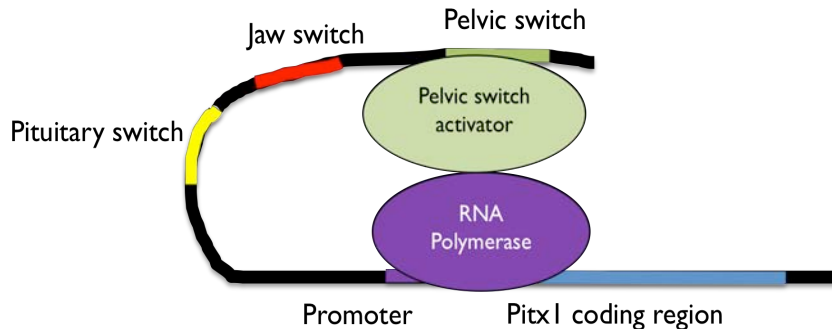
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Sample Models:

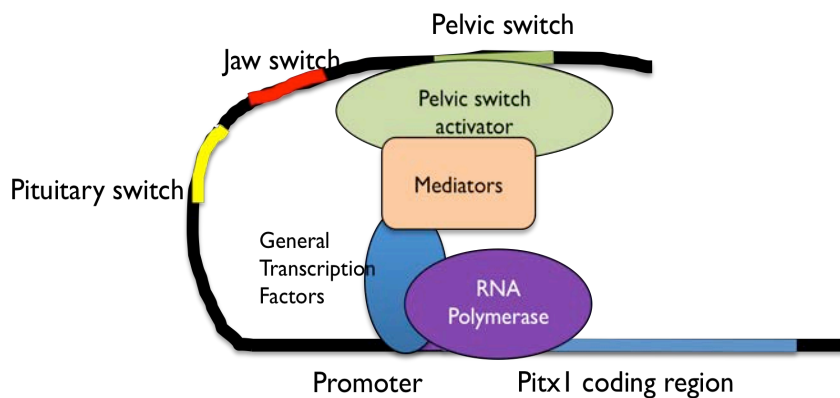
4. Marine Stickleback – Pelvis

Transcription is ON

Note to Teachers: Students may also have general transcription factors and mediators in their model. Both models are acceptable.

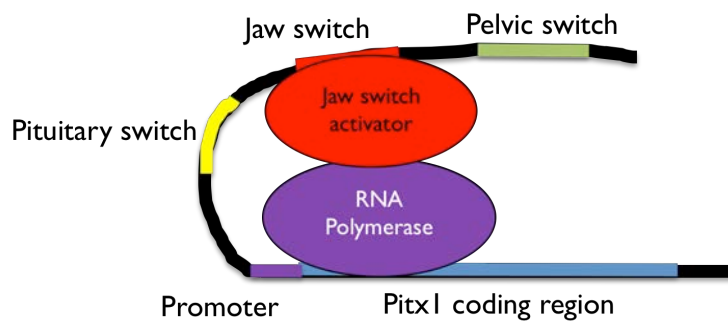


OR



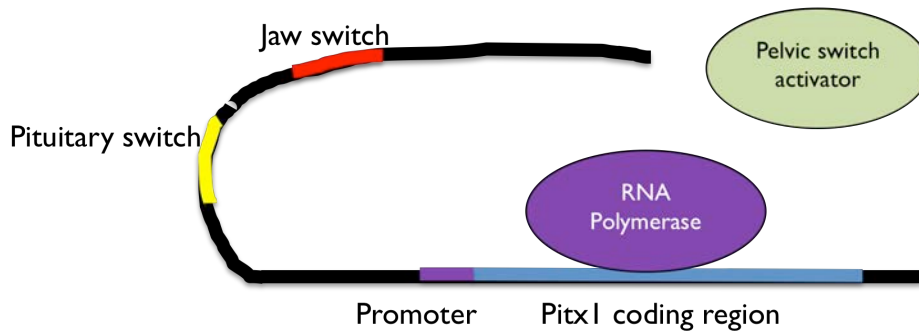
6. Marine Stickleback – Jaw

Transcription is ON



7. Freshwater Stickleback – Pelvis

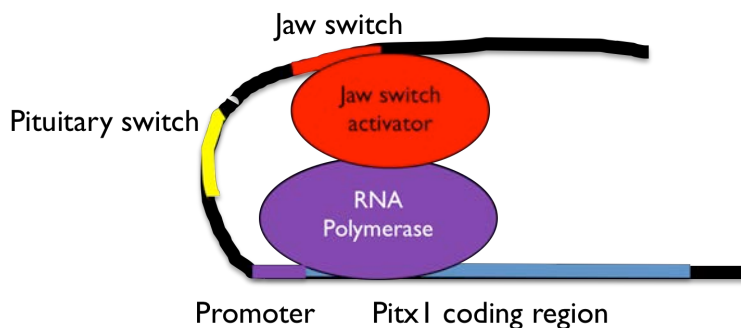
Transcription is OFF



Note: Make sure students use the appropriate freshwater stickleback DNA for the next two models.

8. Freshwater Stickleback – Jaw

Transcription is ON



PART 3 ANALYSIS QUESTIONS

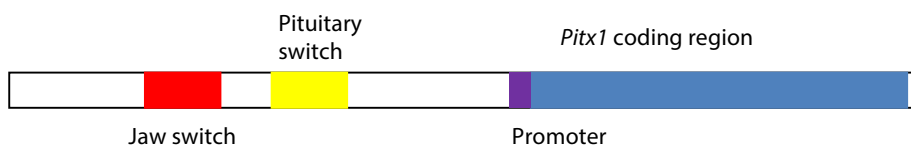
1. Explain the role that regulatory switches play in determining whether stickleback embryos will develop pelvic spines.
A mutation in the pelvic switch shuts off the *Pitx1* gene in the pelvis. This prevents the development of pelvic spines.
2. According to the film, what is the selective pressure that led to freshwater stickleback fish losing their pelvic spines?
Freshwater stickleback fish without pelvic spines had a selective advantage over stickleback fish with pelvic spines because dragonfly larvae would capture young stickleback fish by grabbing onto their protruding 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.

Sample student drawing:

Make sure that students show or designate an absence of the pelvic switch and that there are no switches other than the jaw or pituitary switch. Some students may be tempted to draw a “heart switch.” Take this opportunity to reiterate that the genetic code is the same in all tissues other than the exceptions listed in the Key Concepts and Learning Objectives.



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.

Student answers will vary. Have students discuss in groups and make a list. Possible answers could include:

- There are no histones represented in the model.
- There are other proteins involved in transcription that are not shown in the model (including proteins involved in moving or modifying histones and unwinding DNA).
- The entire stickleback genome is not represented in the model.
- In the cell, there are many different transcription factors interacting in different ways.
- The general transcription factor is made up of a number of different proteins, not all of which are represented in the model.

REFERENCES

Carroll S.B., Prud'homme B., and Gompel N. (2008). "Regulating Evolution." *Scientific American*, May 2008: 60-67.

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