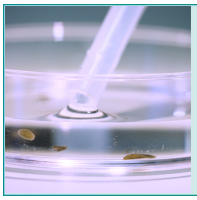

OVERVIEW

In this activity, students use planarians (flatworms in the phylum Platyhelminthes) as a model organism to study the remarkable process of regeneration. Students first observe the planarian's physical and behavioral attributes. Student teams then formulate a hypothesis regarding the regenerative ability of planarians. Over two to three weeks, students care for their planarians and collect data to test their hypothesis.

After completing their experiments, students view the short film *Identifying the Key Genes for Regeneration* and use information from the film to answer questions about the role of stem cells in regeneration.

KEY CONCEPTS

- Regeneration replaces damaged or lost structures in adult organisms.
- Planarians can serve as model systems for the study of regeneration.
- Some organisms possess greater regenerative potential than others as a result of having different types and numbers of stem cells.
- Stem cells are undifferentiated cells. They can proliferate to give rise to other stem cells (self-renew) or to cells that will differentiate into specialized cell types.

STUDENT LEARNING TARGETS

- Design and carry out an investigation.
- Use a model organism to gather evidence to support or refute claims/hypotheses.

CURRICULUM CONNECTIONS

Standards	Curriculum Connection
NGSS (2013)	HS-LS1-2, HS-LS1-4
AP Bio (2015)	2.C.2, 2.E.1, 3.B.1, 3.D.2, 4.A.3, SP3, SP4, SP5
IB Biology (2016)	1.6, 2.1
Common Core (2010)	ELA.RST.9-12.3
Vision and Change (2009)	CC2, DP1

KEY TERMS

anterior, flatworm, multipotency, neoblast, posterior, regeneration, stem cell, totipotency

TIME REQUIREMENTS

- **Part 1** of the activity will take 45 to 60 minutes. This time may vary depending on the amount of introduction provided.
- **Part 2** will extend over two to three weeks. Different amounts of time may be required during each class period depending on your strategy. For example, developing the research plan may require one class period or can be assigned as homework. Each student team's plan should be approved before they conduct their experiment.
- **Part 3** will require about 20 to 30 minutes of class time or can be assigned as homework.

SUGGESTED AUDIENCE

- High School: Biology (General, AP/IB)
- College: Introductory Biology

PRIOR KNOWLEDGE

Students should be familiar with mitotic cell division and cellular differentiation. They should also have a general knowledge of organism anatomy, specifically the anterior-posterior body axis. Some knowledge of regeneration and how it differs from wound healing and scar formation may be helpful but is not necessary.

MATERIALS

For each team (two or three students):

- up to 3 planarians (can be ordered online from biological supply houses such as Carolina Biological Supply and Ward's Science, or collected from a freshwater stream or pond)
- Petri dishes
- small pieces of hard-boiled egg yolk or liver (cut to the size of a grain of rice or small pea)
- bottled water (you may wish to refrigerate some of the water beforehand, as cold water can be used to make planarians move more slowly)
- plastic pipette
- clear ruler (metric, about 10 cm)
- piece of dark paper
- concave microscope slide
- dissecting microscope or magnifying glass
- small soft brush (camel hair works well)
- paper towels
- permanent marker

For Part 2 of the activity, add the following materials:

- plain glass microscope slide
- lens paper
- coverslip or scalpel
- ice (optional)

BACKGROUND INFORMATION

Planarians are flatworms common to many parts of the world. They can live in both saltwater and freshwater ponds and rivers. Some species even live on land.

Many planarians have structures called eyespots in their anterior (head) region, which detect light. Planarians can sense and respond to chemical stimuli in their environment by using chemoreceptors mainly localized on the sides of their bodies (auricles). Their bodies also contain structures called protonephridia, which function like kidneys by removing fluid waste and regulating salts and water intake.

Planarians ingest food through a tube called the pharynx, which is usually kept inside their body. During feeding, this tube extends out of the planarian's ventral midsection. Food that goes into the pharynx enters a branched gastrovascular cavity, where it is digested and distributed to all the cells of the planarian's body. Solid waste is also eliminated through the pharynx.

Some planarians move by beating cilia (protuberances of epithelial cells), which allows them to glide. Others move by contracting muscles and undulating their bodies.

When cut, planarians do not feel pain, only pressure. Each planarian has a bilobate (two-lobed) brain in its anterior region, and two lateral and ventral nerve cords that run through the length of its body.

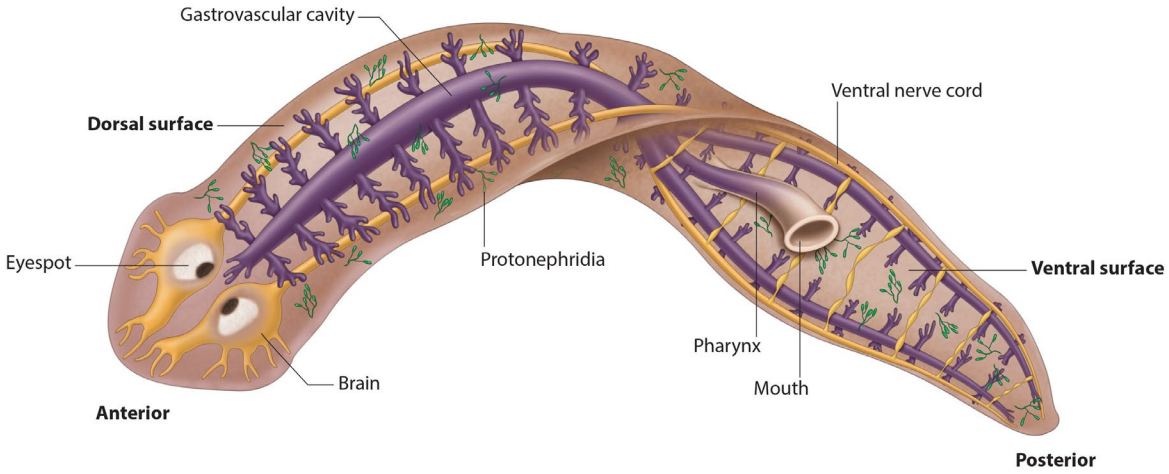

Figure 1. External and internal anatomy of a generic planarian. (Image courtesy of Alejandro Sánchez Alvarado.)

Researchers are greatly interested in the planarian's ability to regenerate missing or damaged body parts. The regenerative process is enabled by adult stem cells called neoblasts, which can collectively differentiate into any of the 40 to 50 different cell types present in a planarian. Neoblasts represent approximately 20% of all cells in a planarian. They are located throughout the body, with the exception of the anterior tip of the head and the pharynx. Individual neoblasts are considered pluripotent, meaning they can make most, but not all, cell types. However, a collection of neoblasts can make any cell type, making them a *collectively* totipotent cell population.

Another remarkable feature of many planarian neoblasts is their high mitotic activity; in other words, they are almost constantly dividing. The frequent division of neoblasts results in a fast turnover of all planarian tissues. As a result, planarians are likely to lack any long-lived cells and can turn over all the cells in their bodies in just a matter of weeks, keeping all their organs "young." Although specific human tissues, such as the small intestine, also have high rates of turnover, most of the cells in adult humans are not replaced as frequently. Planarians can also quickly increase and decrease in body size by changing their overall number of cells, typically in response to food availability.

TEACHING TIPS

General

- You might want to have students complete the "Why Two Heads?" Phenomenal Image activity either before or after doing this activity.
- Have students work in groups of two or three, especially for Part 2 of the activity.
- The film and associated questions (Part 3) can be assigned as homework after completing the hands-on portion of the activity (Parts 1 and 2).

Care and Feeding of Planarians

- Collect or order your planarians to arrive a few days before starting the activity with students. This will allow the animals to acclimate to their new environment beforehand.
- Keep the planarians in a container in a cool area that is dark or dimly lit.
    - A wide, shallow container is better than a narrow, deep one, because the larger surface area of the wide container allows for more aeration.
    - Leave the lid of the container loose or ajar to allow airflow.
- Culture the planarians in bottled water or unpolluted pond/stream water, or as directed in the instructions that accompany the planarians. Do not use tap, salt, or distilled water. (Tap water often contains chlorine or

metal ions that can harm planarians. Distilled water may lack minerals and nutrients that planarians need to survive.)

- Change the water in the container two or three times a week. First, make sure no planarians are floating in the water. If any planarians are floating, carefully make them sink with a plastic pipette. Next, carefully remove most of the water using the pipette, leaving the planarians in place. You can also slowly pour off the water, as the planarians typically stick to the bottom of the dish. Finally, gently add new bottled water.
- Feed the planarians at least once a week. They can be given small pieces of hard-boiled egg yolk or liver, cut to the size of a grain of rice or small pea (one piece of food per dish). Let them eat undisturbed for an hour, or until they stop feeding and swim away from the food.
    - Do not leave the food in the water for any longer than four hours, because the food will begin to decay and pollute the water. After feeding is complete, remove the leftover food and change the water using the method described above.
    - The egg yolk or liver can be frozen and used as needed. Once frozen, it is easy to use a pair of scissors or a small blade to cut off small pieces.
    - Do not let students feed their planarians for three or four days after cutting them.

Experimental Design and Observations

- Some students may be uncomfortable cutting the planarians. Reassure them that the planarians will not feel pain or bleed. You may also suggest having a classmate do the cutting.
- Replacing the planarians' water with cold water (e.g., refrigerated bottled water) will make them move more slowly. This can make the planarians easier to measure and observe.
- You can use the following tips to determine when certain body parts have regenerated.
    - To determine when a planarian's **digestive system** has regenerated, place the Petri dishes on a white surface. This will help students see the planarian's dark-colored digestive tract more easily.
    - To determine when a planarian's **pharynx** has regenerated, place the Petri dishes on a dark surface and add a small piece of food to the water. The pharynx, which looks like a white tube, will be extended when the planarian is eating.
    - One way to test the regeneration of **eyespots** is to place the Petri dish on a dark surface, then cover half of the dish with dark paper. Students may notice the planarian moving from the uncovered side of the dish to the darker, covered side. Students can also shine a light on the planarian and observe its response; only planarians with functioning eyespots should react to the light by moving away (negative phototaxis).
    - Students should *not* rely solely on pigmentation to identify newly regenerated tissue. Newly regenerated tissue is often not pigmented at first, as the addition of pigmentation is actually the last step in planarian regeneration.
- Students may find extra planarians or pieces of a planarian in their Petri dishes. This may be due to the planarian reproducing asexually. (Planarians can reproduce both asexually, through fission, and sexually.) To reproduce asexually, a planarian will stick its posterior end (tail) to the bottom of the dish. The planarian will then swim away until a small section of its tail breaks off, giving rise to a new independent animal.
- Students may wish to learn more about planarians after finishing their experiments. You can encourage your students to research planarians using resources like the ones in the "References" section at the end of this document.

Planarian Disposal

- At the end of the experiment, planarians can be kept in the classroom, donated to another classroom, or disposed of in a safe way. **Do not release the planarians into the wild.**

- You can safely dispose of the planarians by putting them in a sealed container, placing the container in the freezer for 24 hours, and then disposing of the container in the trash. Alternatively, you can add 10% bleach to the container, wait one hour, and then dump the contents down the sink.

DISCUSSION POINTS

Before the Activity

- Ask your students to think about what happens when they cut one of their fingers. Is the scar that forms an example of regeneration? Could they grow back a whole new finger?
  - In human adults, physical injuries like cuts trigger a wound-repair process that leads to the formation of a scar. The scar tissue is different from the tissue that was there before.
  - In contrast, regeneration produces new tissues that have the original tissues' architecture and functions. Humans can regenerate lost tissues during prenatal development and, to a lesser extent, in early life. However, we almost completely lose the ability to regenerate as adults, except for in the skin and the liver.
- Ask your students why scientists might want to study planarians. How are humans and planarians the same or different?

After the Activity

- Ask students the following question: "When a planarian is cut in half and two new planarians develop, how do their genomes compare?"
  - The genomes of the two planarians should be identical because they arose from the same planarian.
- Ask students to research whether other animals can regenerate or have any adult stem cells. They should discover that planarians are not unique in possessing adult stem cells, but that there is a spectrum of cell types and regenerative abilities among animals.
  - On one end of this spectrum are planarians, *Hydra*, and starfish. These animals have adult stem cells throughout their bodies, which give them the ability to regenerate lost body parts.
  - The other end of the spectrum includes the roundworm *C. elegans*, which completely lacks adult somatic stem cells.
  - At the middle of the spectrum are vertebrates, including humans. These animals have tissue-specific stem cells and are thus capable of some tissue-specific cell turnover.

ANSWER KEY

Part 1: Getting to Know Your Planarian

4. Measure the length and width of your planarian. This could be tricky! Hint: Cold water may make the planarian move more slowly.

   a. Record your planarian's measurements.

   Planarians vary in size. Their average length is about 0.5 to 2 cm, and their average width is about 0.2 to 0.3 cm.

   b. Describe the technique you used to measure your planarian below, so that others will be able to repeat the process and obtain similar data.

   It can be difficult to measure the planarians because they extend and contract quickly. Students often wait for the planarians to extend and then take a quick measurement. They should try to determine the length and width several times and use the average. Putting the planarians in cold water will also make them move more slowly, so that it is easier to measure them.

5. Observe your planarian as it moves around in the Petri dish. If your planarian is not moving, try touching it gently with your pipette, stirring the water gently with the pipette, or removing most of the water.

    a. Describe how the planarian moves. For example, does it lead with only one end or both ends? Does it wiggle from side to side, squeeze and stretch its body, or both?

Planarians have two modes of locomotion. They can use cilia to glide along the substrate. They can also extend and contract their body muscles as they move.

    b. Do you think the planarian uses muscles for these movements? Explain your reasoning using evidence from your observations.

If the "gliding" motion is observed, students may respond that there does not seem to be any evidence of muscles being used for movement. If the "extension and contraction" motion is observed, it would be reasonable to infer that muscles are involved in movement.

6. Cover half of the Petri dish's top with a piece of dark paper, then observe your planarian's behavior for several minutes. Do you think that planarians can detect light? Do planarians prefer light or dark? Explain your reasoning using evidence from your observations.

Students should find that planarians are able to detect light and that they prefer the dark. Evidence may include the observation that planarians move away from the uncovered area of the dish (light) to the area of the dish covered by the paper (dark). If the paper is moved over a different part of the dish, the planarians should again move from the light area to the dark area.

7. Add a small piece of hard-boiled egg yolk or liver to the Petri dish. Again, observe your planarian for several minutes. Do you think that planarians can detect food in their environment? How might they do this? Explain your reasoning using evidence from your observations.

Students should find that their planarians move toward the food, which is evidence that planarians are able to detect food in their environment. Student explanations for how planarians are able to do this may vary. For example, students could suggest that planarians detect food by sensing chemical signals in their environment (which is often actually the case), or that the planarians see the food with their eyespots.

9. Observe the planarian under a dissecting microscope or with a magnifying glass. Sketch your planarian in the box below.

Students' drawings will vary. The drawing should include the anterior auricles (earlike projections on the sides of the head) and eyespots. The posterior end should taper. Shading should indicate a slightly darker middle region.

Part 2: Designing an Experiment to Investigate Planarian Regeneration

2. Describe a **hypothesis** that your group will test to investigate your question. Remember that a hypothesis has to be testable. In this case, pick a hypothesis that you will be able to test in two to three weeks with up to three planarians, using materials approved by your teacher.

Student hypotheses will vary depending on their question. Some possible hypotheses might be:

- *If a planarian is cut in half, each half will regenerate into a planarian of the same size and appearance as the original animal within three weeks.*
- *To regenerate into a complete planarian within three weeks, a piece cut from a planarian has to be bigger than a third of the original planarian.*
- *A regenerated planarian will respond to light and move in the same way as the original animal.*
- *If a planarian is cut into three pieces, all three pieces will regenerate at the same rate.*

3. Develop a research plan for testing your hypothesis. Your plan should include a description of the independent variable(s), dependent variable(s), control(s), materials needed, and a detailed procedure.

Research plans will vary depending on the students' hypotheses. For example, for the first hypothesis shown above, students may choose to cut two planarians in half and leave one whole as a control. They may predict that at the end of the experiment, they will have five planarians of the same size and appearance.

5. Record the data you decided to collect in a notebook, spreadsheet, or other data table.

Student data will vary depending on their research plan. They may include dated length and width measurements, pigmentation intensity evaluations, behavioral observations, and other notes for each planarian in their experiment. A table of sample data is shown below.

Date	General Notes	Planarian 1	Planarian 2	Planarian 3
10/14/18	All planarians responded to a small piece of liver added to their dishes. After 1 hour, the worms were placed in new dishes containing clean water. The old dishes were washed to be used next time.	1.5 cm long	2.23 cm long	2.0 cm long
10/16/18	Two of the planarians were cut with a coverslip.	cut into two sections #1 (anterior) is 1.2 cm, has eyespots #2 (posterior) is 0.3 cm, no eyespots	cut into two sections #1 (anterior) is 1.3 cm, has eyespots #2 (posterior) is 0.6 cm, no eyespots	control, not cut 2.0 cm long
10/18/18	Fed all planarians, then put them in new dishes with clean water.	#1 ate and moved around the dish #2 did not eat and did not move	#1 did not eat but moved around the dish #2 spent time on the liver, moved around the dish	ate and moved around the dish
10/19/18	Covered half of each dish with a dark piece of paper and observed how the planarians responded.	#1 moved from the light side to the dark side #2 stayed on the light side, did not move to the dark side	#1 moved from the light side to the dark side #2 stayed on the light side, did not move to the dark side; #2 also split into two sections, #2a (larger) and #2b (smaller)	moved from the light side to the dark side
10/20/18	Fed all planarians, then put them in new dishes with clean water.	#1 ate #2 ate	#1 ate #2a and #2b did not eat	split into two sections #1a = 1.0 cm, ate #1b = 0.3 cm, did not eat

10/23/18	Fed all planarians, then put them in new dishes with clean water.	#1 ate #2 ate	#1 ate #2a and #2b did not eat	#1a ate #2b did not eat
10/25/18	Fed all planarians, then put them in new dishes with clean water.	#1 developing a tail, ate #2 developing eyespots, ate	#1 developing a tail, did not eat #2a developing eyespots, did not eat #2b did not eat	#1a developing a tail, ate #1b developing eyespots, did not eat
10/28/18	Fed all planarians, then put them in new dishes with clean water.	#1 and #2 ate	#1 and #2a ate #2b did not eat	#1a and #1b ate
10/30/18	Measured and observed all planarians.	#1 is a complete worm, 1.3 cm long #2 split into two sections, #2a and #2b. #2a is 0.7 cm long, has eyespots but no "tail." #2b is 0.3 cm long and has a "tail."	#1 is a complete worm, 1.2 cm long #2a is 0.6 cm long and has eyespots #2b is 0.3 cm long	#1a is a complete worm, 1.1 cm long #1b lost during transfer

7. At the end of your experiment, answer the following questions.
   a. Did your experiment support or refute your hypothesis, or were your results inconclusive? In the space below, justify your reasoning using data from your experiment.

Answers will vary depending on the students' hypothesis and results. For example, students with the first hypothesis shown under Answer 2 above might claim that their hypothesis was supported based on the following evidence. They may have observed that each half regenerated missing parts; for example, the anterior half developed a new "tail," and the posterior half developed auricles and eyespots. They may have also observed that both halves eventually responded to stimuli, such as light or food, and that the pigmentation was fully restored to both halves.

   b. What are two things you have learned about planarian regeneration? Use the results of your experiment to provide evidence for your claims.

Answers will vary. Students should summarize the findings of their experiment to provide evidence for their claims.

   c. What is one question you still have about planarian regeneration?

Answers will vary.

Part 3: Watching a Film about Planarian Regeneration

2. Planarians served as model organisms in both your experiment and in the film. Answer the following questions based on what you learned from the film.
   a. Which cells are responsible for regeneration in planarians?

Adult stem cells are responsible for regeneration.

b. Why are planarians' regeneration abilities greater than those of humans?

Human adults have very few stem cells, whereas up to a fifth (20%) of planarian cells are stem cells.

c. How can scientists identify genes important in regeneration?

Scientists can identify genes important in regeneration by inactivating different genes in planarians and then observing how that affects regeneration. In the film, the scientists used a technique called RNA interference (RNAi) to inactivate two different genes.

d. How does a planarian "know" whether to regenerate a head or a tail? What might cause the planarian to regenerate the wrong body part?

When a planarian loses its head or its tail, stem cells multiply and differentiate to replace the missing part. The stem cells "know" which part to replace because they receive molecular signals from nearby differentiated cells. These signals regulate the stem cells' differentiation into the specific cell types required to regenerate the correct body part. The planarian may regenerate the wrong body part if the normal signaling process is disrupted. In the film, the scientists showed that having either too little or too much beta-catenin, a protein involved in the signaling process, can cause planarians to regenerate wrong parts.

3. Explain how understanding regeneration in planarians might help improve human health.

Planarians provide important information about stem cells and regeneration. Scientists are identifying genes, proteins, and pathways involved in the regeneration of planarians. Through this research, similar genes, proteins, and pathways might also be found in humans.

REFERENCES

This activity was adapted from:
Accorsi, Alice, Monique M. Williams, Eric J. Ross, Sofia M. C. Robb, Sarah A. Elliott, Kimberly C. Tu, and Alejandro Sánchez Alvarado. "Hands-On Classroom Activities for Exploring Regeneration and Stem Cell Biology with Planarians." *The American Biology Teacher* 79, 3 (2017): 208–223. https://doi.org/10.1525/abt.2017.79.3.208.

More information about planarians is available on the Cutting Class website.

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