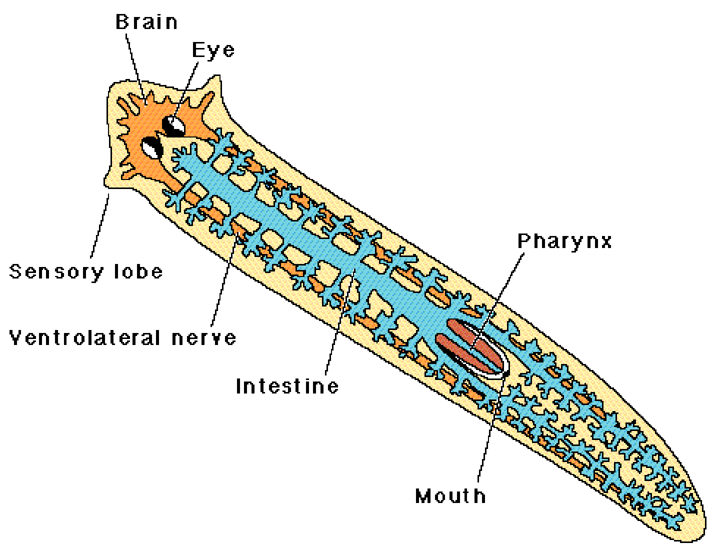
Modeling the Organism: The Cell in Development
Laboratory 5: Regeneration in *Planaria*

<u>Introduction</u>

At the present time there is a huge interest in *regenerative medicine* – the potential of triggering/enhancing the body's capacity to rebuild and repair damaged tissues. While this seems like science fiction, ability of starfish to regenerate a lost arm, of some lizards to shed and re-grow a tail, and of salamanders to replace a damaged foot are striking examples of the ability of some animal tissues to proliferate, reorganize and re-differentiate to replace lost structures.

A classic example of this process is regeneration in the flatworm *Planaria*. *Planaria* is a very simple metazoan (a multi-celled animal) that exhibits bilateral symmetry. It lives in ponds and streams, feeding on decaying plant and animal material in the streambed. While the species of Planaria we will be using in the lab is harmless, some of its relatives are parasites (tapeworms and flukes) that cause a variety of diseases in humans and other animals around the globe.

Flatworms are organized around a simple, tube-like morphology of three primary tissues: ectoderm, mesoderm and endoderm. They possess a simple nervous system with two anterior photoreceptors (that appear uncannily like eyes) on the head, a mid-body trunk with a pharyngeal (mouth) opening, and a posterior tail-like extension.



Source: http://sps.k12.ar.us/massengale/planarian_characteristics.htm , cached 060407

<u>Planaria</u> normally reproduce asexually by simply pulling apart; the two masses of tissue then regenerate the "missing" structures over a period of several days, resulting in two free-living clones.

In this week's lab, each group will select several *Planaria* from the lab culture and transfer them to 3-4 small labeled Petri dishes half-filled with spring water.

First, examine these organisms, identifying the head/trunk/tail. How are they moving around the surface of your (their) Petri dish? Are they light sensitive? Design an experiment to test whether they move toward the light or away from it. Why might this behavior be adaptive in the natural environment? Do you think these organisms are active during the day or night? Why?

Next, plan your surgeries. Draw an outline of a planarian in your notebook, and then sketch the plane along which you will bisect the organism. Is there a difference between severing the head vs. the tail from the rest of the body? Should you cut right/left across the middle of the organism (a lateral incision), or just a small part at one end? What of a longitudinal incision that runs from head to tail, separating right from left? You might also try a partial incision across the animal to see if you can create a two-headed or two-tailed organism. Once you have diagrammed your plans and briefly described your expectations for the outcome, carefully perform your surgeries using a fragment of a single edged razor blade. If the blade becomes sticky with mucilage from the animals, carefully clean it against a folded Kimwipe wetted with 70% Ethanol (being very careful not to cut yourself!).

Once you have completed your surgeries and marked your Petri dishes appropriately, carefully seal them with Parafilm and transfer them to the plastic tray on your bench. You should plan to drop by the lab every few days to observe their recovery, making notes in your lab notebook as you follow the course of regeneration. It will take about 7-10 days to see the results of this remarkable process. During this time the *Planaria* will not eat (so we won't add any food because it will just decompose); if the water seems to turn cloudy (or loses volume), carefully replace it with fresh spring water.

Recent work examining the cell biology of regeneration in *Planaria* has revealed that the bodies of these simple animals contain specialized stem cells called neoblasts. The neoblasts are responsible for contributing to cell division at the site of injury, and then directing the cell differentiation within that mass to replace missing structures. A recent paper exploring the molecular biology of this system is available so that you can read more about this amazing process:

> Ledford, H. (2007) Flatworms' starring role in stem-cell research. Nature 448, 522
>
> Gurley, K. A., Rink, J. C. & Sánchez Alvarado, A. Beta-catenin defines head versus tail identity during planarian regeneration and homeostasis. Science 319, 323–327 (2008)

As an interesting aside, over a hundred years ago Thomas Hunt Morgan was intrigued by regeneration in *Planaria*, but grew discouraged by the complexities of this process and turned to *Drosophila* instead. Some of the questions that intrigued Morgan still remain a mystery . . . for now.

Lab report:

Each student will be responsible for submitting a one page lab "brief" describing the results from one of the following lab modules: (1) this *Arabidopsis* experimental module (started last week); (2) the *Planaria* regeneration experiment (starting this week) or (3) the *Drosophila* patterning investigation (starting next week). Your brief should include some hand drawings of your results and will be due at the start of lab in the last week of the semester. Note that everyone is responsible for completing all three units and entering data and describing results in their lab notebook; however, you are only responsible for writing up one of these three experiments. Lab partners are free to write up the same or different modules, but each student should complete their own brief.