

Protocol adapted from: The effects of sulfate on sea urchin development: Matthews
Banda, F&M 2002
Look Ma, No Archenteron! Sulfate's role in sea urchin early development.
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Objective

To observe the role sulfate plays in sea urchin gastrulation, and to replicate the findings of Karp and Solursh that the archenteron fails to form without sulfate(1974). To test whether endoderm differentiation can occur in the absense of the movements of gastrulation.

Introduction

Sea urchin development progresses in a predictable and easily observable way (Gilbert, 2000). The vegetal plate thickens and primary mesenchyme cells ingress and form spicules, the urchin skeleton (Figure 1). Then, the vegetal plate invaginates, forming the archenteron, and this archenteron migrates up the sea urchin's blastocoel wall with the help of secondary mesenchyme cells (Figure 1).

The migration of the archenteron depends not only on signals and proteins already present in the egg, but also on extracellular materials that have been incorporated into the organism. Karp and Solursh have hypothesized that secondary mesenchyme cells, which form the filopodia of the developing archenteron (primary gut) require sulfate (to form sulfated proteoglycans which act as something like an adhesive) in order to migrate along the extracellular matrix within the blastocoel of a developing sea urchin (1974).

Presumably, sea urchin embryos incorporate sulfate from the environment into their extracellular matrixes. The extracellular matrix contains acid mucopolysaccharide, which when bound to sulfated proteoglycans, is rough in appearance (Karp and Solursh, 1974). This roughness is akin to velcro's roughness, allowing secondary mesenchyme cells to pull the archenteron up along the blastocoel cavity. If sulfate is not present, it has been observed that an archenteron does not form (Karp and Solursh, 1974). Fixed and stained embryos will indicate if these cells are still present within the embryo (and have simply failed to migrate).

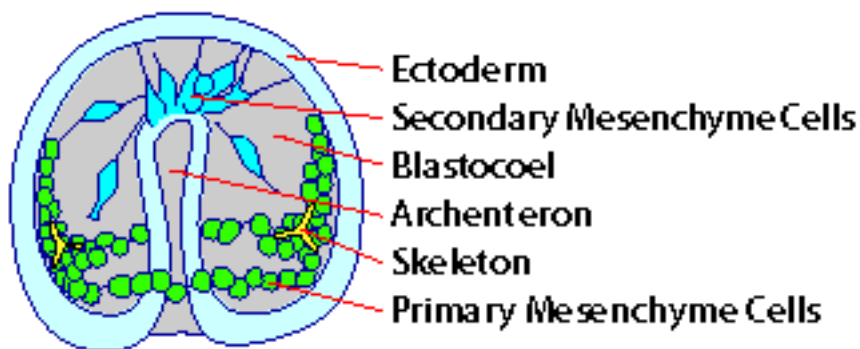


Figure 1: A schematic drawing of a gastrulating sea urchin embryo. Note the secondary mesenchyme cells, in the form of a filopodia, attaching to the blastocoel wall to pull the archenteron up through the blastocoel to form the gut cavity.

Protocol

1. *Lytechinus pitus* or *Strongylocentrotus purpuratus* (from the Pacific coast), *Arbacia punctulata* (from the Atlantic) or *Lytechinus variegates* (from Florida) can be used for this experiment.
2. Induce sea urchin gamete release by injection of KCL and obtain eggs and sperm in separate beakers as described in basic protocol "sea urchin gamete collection"
3. Wash eggs 4xs in sulfate-free water

4. Fertilize eggs as described in the basic protocol "sea urchin fertilization"
5. Check for fertilization under the microscope
6. Fill (and label) a beaker with ASW and a beaker with sulfate-free water and pipette fertilized eggs into each beaker.
7. Cover and incubate overnight.
8. The next day, extract swimming embryos and place them on labeled depression slides.
9. Observe and photograph embryos.
10. Fix samples of each group of embryos as described by "preparation of fixed embryos..." for staining next week.

from DB lab website

Preparation of fixed embryos for immunocytochemistry and AP staining

1. Transfer 50 ml of embryo cultures to centrifuge tubes. Spin at 1500 rpm for 5 minutes. Check that you can see a pellet of embryos at the bottom. Quickly pour off the ASW. Try to remove as much as possible, but don't worry about a little ASW left in the tube.
2. Gently swirl tube to resuspend the embryos. Add 40 ml of ice cold methanol and allow to fix on ice for no more than 20 min. By this time, the embryos should have settled to the bottom of the tube.
3. Decant off the methanol and resuspend the embryos about 25 ml ice cold ASW.
4. ON ICE, let the embryos settle to the bottom of the tube by gravity.
5. Decant off the ASW and resuspend the embryos in fresh ice cold ASW. (At this point embryos can be stored in refrigerator)

Immunofluorescent staining of Sea Urchin embryos

1. Transfer fixed embryos to microfuge tubes. Allow to settle for 10 minutes. Gently remove most of the liquid.
2. Add 100 ul antibody to one tube and 100 ul 10% normal goat serum to the other. Let sit for 45 minutes at room temperature. Embryos will settle.
3. Remove most of liquid. Add 1 ml SW to wash. Allow to settle, remove liquid.
4. Add 100 ul Fluorescein- or Texas red-conjugated anti-mouse IgG (diluted according to manufacturer's recommendations) to both tubes. Let sit for 45 minutes at room temperature. Embryos will settle.
5. Remove most of liquid. Add 1 ml SW to wash. Allow to settle, remove liquid. Add 100 ul PBS.
6. Transfer 10 ul of each sample to microscope slides. Check that there are embryos. Coverslip. Examine using epifluorescence.

Histochemical staining of sea urchin embryos for alkaline phosphatase (AP) enzyme activity

1. Obtain embryo samples, tube of AP substrate buffer and tube of phosphate buffered saline (PBS) for each group. Allow embryos to settle. Carefully remove supernatant.
2. Resuspend in 0.5 ml AP substrate buffer. Allow embryos to settle for 10 min. Remove excess buffer.
3. Add 100 ul AP substrate to tubes. Check for staining after 5 minutes by transferring a small sample to depression slide and observing on 4X. Be careful not to get AP substrate on your hands (wear gloves) or on your microscope. Do not leave light turned on between observations. To stop the reaction, return embryos to the tube and add 0.5 ml PBS.
4. Allow embryos to settle for 10 minutes. Remove buffer to about 100 ml, return to depression slides and observe. Look for evidence of morphogenesis (archenteron invagination) and tissue differentiation (gut alkaline phosphatase activity and spicule

formation). Document your observations by capturing images of your stained embryos.

Works Cited

Experiments in Developmental Biology.

http://www.swarthmore.edu/NatSci/sgilber1/DB_lab/DB_lab.html Cebra-Thomas, 2004.

Gilbert, S.F. 2000. Developmental Biology (6th ed). Sinauer Associates, Inc., Massachusetts, p. 238-241.

Karp, G. C. and M. Solursh. 1974. Acid Mucopolysaccharide Metabolism, the Cell Surface , and Primary Mesenchyme Cell Activity in the Sea Urchin Embryo. Developmental Biology. 41: 110-123

image from: <http://sdb.bio.purdue.edu/dbcinema/images/Miller00.gif>