# Perturbation of gastrulation in sea urchin embryos

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## Introduction

Gastrulation is extensive cell rearrangement where cells undergo dramatic movements and change relative positions. From this ordered movement, layers of cell are created. The cells that will form the endodermal and mesodermal layers, and then organs, are brought inside the embryo, while the cells that will form the skin and nervous system spread over the outside surface. The three germ layers - outer ectoderm, inner ectoderm, and interstitial mesoderm - are produced during gastrulation (Gilbert, 2000).

After the sixth cleavage in normal sea urchin, two tiers of eight cells each are formed in the vegetal half of the embryo. The top tier is termed veg1 and the bottom tier is termed veg2. Veg1 lineages have been shown to contribute portions of the definite hindgut, midgut, and the ectoderm that surrounds the blastopore at the completion of gastrulation (Cameron, 1997). Veg2 and micromere lineages normally contribute to secondary mesenchyme, the coelomic sacs, as well as most of the archenteron (Cameron, 1997). High levels of β -catenin in micromeres prior to gastrulation suggest that β-catenin plays a signaling role (Miller and McClay, 1997).

Embryos that have been treated with lithium chloride accumulate β -catenin in every cell (Gilbert, 2000). $LiCl_2$ inhibits GSK-3, a β -catenin regulating molecule, leading to higher b-catenin levels (Logan et al., 1999). Developmental abnormalities in gut formation accompany increased nuclear β -catenin levels achieved with $LiCl_2$ treatment.
This study examined the effects of $LiCl_2$ on sea urchin gastrulation by examining embryo development in different concentrations of a LiCl solution. It is expected that allowing sea urchin embryos to develop in LiCl will disturb normal vegetal cell processes due to excess β-catenin (Cameron and Davidson, 1997).

Gastrulation in sea urchin embryos 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).

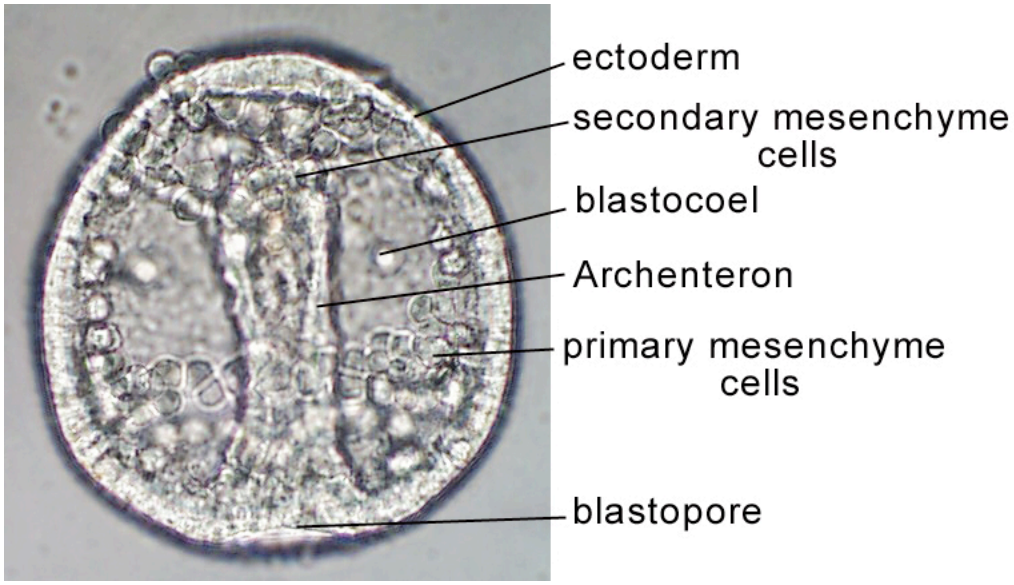


Figure 1: A gastrulating sea urchin embryo. Note the secondary mesenchyme cells, at the top of the archenteron. They send out filopodia, that attach to the extracellular matrix lining the blastocoel wall and pull the archenteron up towards the site of the future mouth to form the gut cavity.

**A. Sulfate-deprivation (Karp & Solursh, 1974)**

1. *Lytechinus pictus 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 3 times 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. Allow embryos to develop until control group has gastrulated.
10. Fix samples of each group of embryos as described below for staining next week.

**B. Lithium treatment (Cameron & Davidson, 1997)**

1. Use a 60 mM solution of LiCl in ASW to prepare 30 mM and 15 mM solutions.
2. At the two-cell stage, transfer embryos from ASW to seawater with each of the LiCl concentrations. A third of the embryos should remain in ASW as a control group.
3. At the mesenchyme blastula stage, transfer 50 ml of embryos in finger bowl to ASW.

4. Observe samples of embryos from a control (ASW with no LiCl added) culture and each of the experimental cultures the next day.
5. Use 2-3 drops eggs/embryos on a depression slide to compare developmental rate and morphology of the groups; focus on vegetal area morphology of embryos.
6. Allow embryos to develop until control group has gastrulated.
7. Fix samples of each group of embryos as described below for staining next week.

**C. Preparation of fixed embryos for 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 embyros can be stored in refrigerator)

**D. Histochemical staining of sea urchin embryos for alkaline phosphatase (AP) enzyme activity (from Drawbridge, 2003)**

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 ul, return to depression slides and observe. Look for evidence of morphogenesis (archenteron invagination) and tissue differentiation (gut alkaline phosphatase activity and spicule formation).

**References**


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