The Effects of $NiCl_2$on Spicule Formation

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Objective: To observe the developmental effects on the formation of spicules on the larval skeleton in sea urchin embryos. To observe the spicules in the gastrula stage and the larval skeleton using polarized light. The hypothesis is the nickel chloride will affect skeleton formation by the primary mesenchyme cells during gastrulation.

Background

Sea urchins exhibit radial holoblastic cleavage, eventually forming a blastula. Shortly after the blastula hatches from the fertilization membrance, the embryo begins gastrulation. Gastrulation begins when the vegetal side of the blastula begins to thicken and flatten. This flat sheet of cells is called the vegetal plate. In the center of the vegetal plate a small group of cells begins to change. These cells extend and contract long, thin filopodia. These cells then break off from the epithelium and migrate into the blastocoel. These migrating cells are known as the primary mesenchyme cells (PMCS). Before the PMCs migrate, there are equatorial ring patterns that are formed in certain regions of the embryo for the PMCs to navigate to where skeletogenesis can occur. The ring formed by the PMCs during gastrulation is composed of two aggregates of cells: the ventrolateral clusters and the ventral and dorsal chains (Guss, 1997). Eventually the migrating cells localize within the ventrolateral region of the blastocoel. It is in this area that the PMCs fuse together to form syncytial cables. Syncytial cables will eventually form the axis of the calcium carbonate spicules of the larval skeleton.

Spicules are the rods of the sea urchin larval skeleton that are formed from skeletogenic mesenchyme cells in the early gastrula stage and are composed of calcium carbonate (Gilbert, 2010). In normal sea urchin development during gastrulation, the spicules and skeleton morphogenesis is influenced by the interaction between the underlying ectoderm, the PMCs, and the rest of the embryo. There are several PMC-species gene products that participate in the synthesis of the skeleton, but the four proteins that are part of the spicule matrix are msp130, SM50, SM30, and PM27 (Guss, 1997).

The purpose of this lab is to explore the development of spicules in a sea urchin's larval skeleton. NiCl2 is one of the few chemicals that can interfere with the dorsoventral axis and the development of the spicule and skeletal formation. Nickel chloride interferes with spicules development by altering the interaction between the PMCs and the rest of the embryo. Nickel chloride also disrupts the location and amount of dorsal and ventral tissues (Hardin et. al., 1992). Nickel chloride influences the ectoderm of the sea urchin blastula wall (Armstrong et. al., 1993). By

introducing half of the embryos into a solution of NiCl2, we hope to observe the effects of blocking skeleton formation.

We will stain all of the embryos with an Ig8 immunofluorescent antibody. Ig8 will stain or 'tag' the primary mesenchyme cells of the developing embryo. Another way is to observe spicule foramtion by exposing the sea urchin embryos to polarizing light under a compound microscope because the calcium carbonate from the spicules bends polarized light and the spicules become visible.

Procedure:

Fertilization of Urchins:

1. Gamates are collected and then washed in artificial salt water (ASW) several times to removal the jelly-coat layer and gently poor the excess water off.
2. Transfer the eggs in a 50 mL beaker with 10 mL of ASW and collect the eggs in a petri dish.
3. Dilute 50 uL of the sperm in 5 mL of ASW and use 0.1 mL of the diluted sperm to fertilize the eggs in 5 or 10 mL of ASW.
4. Check of the presence of fertilization membranes after 5 to 10 minutes.
5. Discard the ASW containing the sperm and replace with 40 mL of fresh ASW.

Nickel Chloride Treatment:

1. Create the 2, 5, 7, and 10mM $NiCl_2$ solutions from dilutions from a stock solution of 100mM $NiCl_2$.
    a. The 2mM $NiCl_2$ solution is made by a 1/5 dilution.
    b. A ½ dilution is used to make the 5mM $NiCl_2$ solution.
    c. For the 7mM $NiCl_2$ solution, use a 7/10 dilution.
    d. The 10mM $NiCl_2$ solution is made by a 1/10 dilution.

2. After fertilization has occurred (takes 5 minutes and use a compound microscope), transfer half of the embryos into solutions of 2, 5, 7, 10, and 100mM $NiCl_2$ and the other half into ASW (control).
3. Leave the two groups to develop for 1 day and be sure to track the development through the mesenchyme blastula stage. Then transfer the embryos to two separate petri dishes.
4. Fixed the two groups for staining when the control (ASW) reaches gastrulation and has formed the pluteus larva.
5. Remove the liquid from both tubes and add 1 mL of salt water and remove (do this multiple times).
6. Transfer 10 uL of each sample to a microscope slide and put a coverslip over the embryos.
7. While observing the embryos under the compound microscope, use polarized light to examine spicule development in the gastrula and the larva stages.
8. Observe the differences between the embryos in the control and the $NiCl_2$ solutions.
9. The spicule formation was revealed by exposing the embryos to polarized light on the compound microscope.
    a. Light Polarizing Film (Bausch & Lomb, Catalog No. 31-52-62-26)

<u>Immunofluorescent staining of Sea Urchin embryos</u>

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 1% normal goat serum to the other as a control. 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-congugated anti-mouse IgG (diltuted 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.

Antibodies from David McClay

Ig8, 1D5 -PMCs and spicules

Ecto V, 295 - ventral ectoderm

5C7 - vegetal plate and posterior archenteron

Secondary antibody

Texas Red-congugated Goat anti-mouse IgG from Jackson ImmunoResearch (1/100)