The differentiation of cilia subtypes during early stages of sea urchin (*Lytechinus pictus*) embryogenesis

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Cilia are ubiquitous and versatile appendages of cells that move fluid over surfaces and sense signals in the environment. Improper ciliary assembly leads to a variety of human diseases including polycystic kidney disease. Because sea urchin embryos elaborate several forms of cilia from one initial type as development progresses, the sea urchin provides an ideal model for studying the process of ciliary differentiation. Our data suggest that cilia on sea urchin embryos change form by rapid retraction and disassembly at mitosis followed by regrowth on daughter cells in a new molecular form.

In sea urchin (*Lytechinus pictus*) embryos, the onset of ciliogenesis occurs on all blastomeres simultaneously just before hatching and is regulated thereafter in a tissue-specific manner to generate cilia with different lengths, forms, and behaviors. To understand the process of ciliary growth and differentiation during development, we employed heavy metal ion treatments known to trigger developmental fate changes in echinoid embryos[1] in order to enrich for cells with specific cilia types. In control *L. pictus* embryos at the hatching blastula stage, all blastomeres grow a single motile cilium averaging 18 μm in length to propel the embryo through the water. Confocal microscopy of embryos stained with anti-acetylated tubulin to identify ciliary axonemes revealed that at the mesenchyme blastula stage, as the primary mesenchyme cells ingress, they lose their cilia and remain unciliated as they migrate within the blastocoel and initiate skeletogenesis. To study the long apical tuft cilia of the animal pole ectoderm easily, embryos were grown in sea water containing 125 μM zinc sulfate from 1 hr post-fertilization onward to produce "animalized" embryos[1] in which cells produced long immotile cilia (preliminary average 59.9 ± 9.3 SD μm, n=1) yet still retracted fully into the cytoplasm prior to mitosis. To study short gut cilia of the endoderm easily, embryos were grown in sea water containing 25 mM lithium chloride from 16-cell stage to mid blastula to produce "vegetalized" embryos[1] in which most cells differentiated into endoderm producing a large evaginated exogut instead of a smaller invaginated wild-type gut (Fig 1). On these embryos, the diminished ectodermal region continued to elaborate cilia of two lengths as in untreated controls, (preliminary average 47.2 ± SD 9.1 μm distal from vegetal pole, and 12.9 ± SD 4.1 μm in region close to the transition to presumptive endodermal cells, n=1). The extensive vegetal endoderm cells elaborated the short cilia typical of the wild type archenteron (preliminary average 1.9 ± SD 0.8 μm, n=1). Applying methods to alter developmental fates of echinoderm embryo cells has allowed us to establish models for ciliogenesis in which ciliary differentiation can be more easily observed, manipulated, and understood.

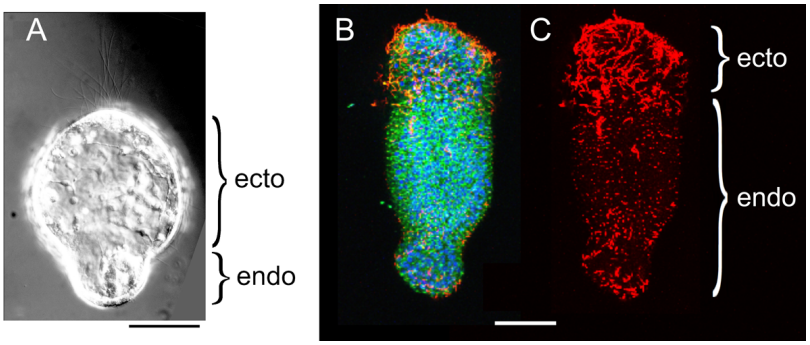

Figure 1. Lithium treatment produces vegetalized embryos with reduced ectoderm ("ecto") and extensive endoderm ("endo") evaginated to form an exogut with short cilia that are turned out to the seawater rather than into the archenteron. The short vegetal cilia then can be easily studied by DIC (A) or immunofluorescence (B, C). B and C show one embryo in which DNA is labeled blue, microtubules green, and acetylated tubulin of the cilia red. Scale bars = 50 μm.

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