Characterizing cilia in sand dollar (*Echinarachnius parma*) embryos after lithium ion treatment

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Cilia are whip-like, cell surface appendages that provide locomotory and sensory capabilities throughout the animal kingdom. During early echinoid development, cilia of different types rapidly form and differentiate on different germ layers. Lithium ion treatment can perturb embryogenesis by shifting germ layer boundaries and thereby aid in characterizing cilia differentiation. Our data suggest the change in cilia phenotype seen on the surfaces of lithium ion-treated "vegetalized" embryos is due to an increase in endodermal expression and endodermal cilia formation compared to untreated embryos.

The many forms and functions of cilia in humans result in a wide array of diseases, or "ciliopathies," when cilia form abnormally. Echinoid embryos provide a valuable model for studying cilia growth and differentiation due to rapid onset of ciliogenesis and multiple cilia types that form. *Echinarachnius parma* globally express cilia on their outer surface 22 hours post-fertilization (hpf). Four hours later, at least three distinct ciliary phenotypes arise on the gastrula stage embryo, while a primitive gut invaginates into the blastocoel lumen. To study cilia of the gut, incubation of *E. parma* embryos in sea water plus 12.5 mM lithium chloride or 6 hours from 4-10 hpf "vegetalized" embryos[1] and produced embryos with exaggerated gut domains with five distinct morphological phenotypes: long endogut (63.3% of embryos), short endogut (11.9% of embryos), unconstructed exogut (1.1% of embryos), constricted exogut (1.5% of embryos), and irregular morphology (22.2% of embryos) (n = 1,449 embryos in one trial; Fig 1). We hypothesized that an exogut region would be composed of endodermal tissue, while mesodermal expression would not be affected[2]. To test this hypothesis, vegetalized embryos were immunofluorescently stained using germ layer specific antibodies (antibody 5c7 for Endo1, gift of D. McClay, Duke University) and imaged by confocal microscopy. Our data show vegetalized embryos overexpressed endoderm in the exogut region, but also throughout the embryo (Fig 1A). Vegetalized embryos that do not exhibit the classic exogut phenotype also showed increased endodermal expression (Fig 1B). Additionally, mesodermal expression was found to be unperturbed between vegetalized and wild type embryos (not shown). These data suggest that for studying cilia types, the current model of interpreting echinoderm vegetalization based on morphology must be revised to account for variance in increased endodermal expression throughout a lithium-perturbed population.

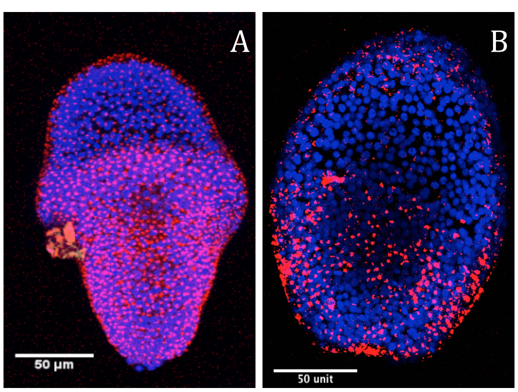

Figure 1. Endodermal expression in vegetalized *E. parma* embryos exceeds the exogut region. Two phenotypically distinct vegetalized *E. parma* embryos illustrate two of the five morphological phenotypes we observed. Blue indicates DNA and red indicates endodermal expression of Endo1. Vegetal pole is oriented downward in images. A) Vegetalized embryo with constricted exogut exhibits a classic "vegetalized phenotype" with exogut narrower than rest of embryo as seen in bottom half of image; surprisingly, Endo1 expression reveals that endoderm domain is not limited to exogut. B) Vegetalized embryo with short endogut exhibits a short invagination at vegetal pole. Endodermal expression is concentrated near the invagination, but is also seen throughout the embryo.

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