Metal ion effects on ciliary differentiation in *Lytechinus pictus* and *Echinarachnius parma* embryos

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Cilia are ubiquitous, whip-like cell appendages that drive cell motility and possess receptors for environmental signals. Cilia assemble on human cells to allow for proper organ function, and cilia retract from the cell surface prior to cell division. Echinoid embryos display multiple cilia subtypes that were selectively enriched by different metal ion treatments. Metal ion treatments followed by quantitative light microscopy revealed a novel ciliary retraction pattern in long-length cilia.

Ciliated epithelial cells undergo elaborate cytoskeletal rearrangements as they alternate between a ciliated interphase and non-ciliated mitosis. Such cells recover their basal body and microtubules from the cilium for use in the mitotic spindle through a process that is not well understood. Published reports refer to a resorption or shortening of the cilium in which the axonome is disassembled at its tip and subunits are returned to the cytoplasm by retrograde transport. We observed a different process, ciliary retraction, in which the axoneme is entirely withdrawn into the cytoplasm before microtubule disassembly occurs. To facilitate observation of retracted cilia, *L. pictus* embryos were treated with 125 μM zinc sulfate at 1 hr post-fertilization to produce animalized[1] embryos which display long, apical tuft-like cilia over the entire embryo surface (Fig 1A). Cilia on zinc-treated embryos grew to a length of 52.0 ± SD 2.6 μm (n = 176 cilia on 4 embryos) reflecting normal apical tuft cilia length of 49.9 ± SD 14.2 μm (n = 29 cilia on 3 embryos). Animalized embryos were fixed at the 24-48 hr stage and labeled with anti-acetylated tubulin antibody to identify axonemal microtubules. Confocal microscopy revealed ciliary axonemes were entirely withdrawn into the cytoplasm before disassembly (Fig 1B-D). While the retraction mechanism is unknown, retracted cilia were bent and coiled in the cytoplasm consistent with a cortically-localized motor pushing the cilium into the cell. Retracted cilia formed coiled or hooked structures wrapping under the nucleus as if they deflected off of the basal plasma membrane during retraction.

Treatment with lithium ions facilitates observation of gut cilia of the endoderm by producing vegetalized[1] embryos that exhibit a large, evaginated exogut instead of a smaller invaginated wild-type gut. Embryos at the 8-cell stage were cultured in 12.5 mM or 25 mM lithium chloride for *E. parma* and *L. pictus,* respectively. Lithium-treated *E. parma* showed vegetal cilia averaging 10.5 ± SD 2.5 μm (n = 44 cilia on 3 embryos), significantly longer ($P = 4.85E^{-16}$) than vegetal cilia of *L. pictus* averaging 4.7 ± SD 2.8 μm (n = 44 cilia on 4 embryos). Confocal images of vegetalized *E. parma* revealed distinct cilia boundaries where longer cilia populate the presumptive ectoderm and exogut tip while short cilia are present lining the exterior of the exogut.

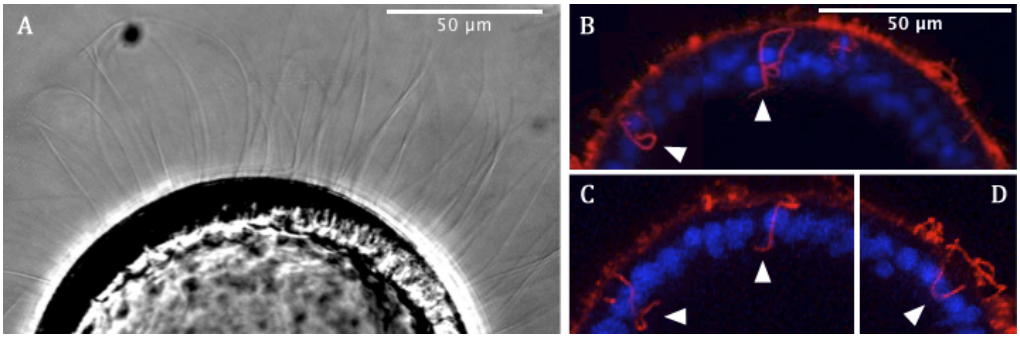

Figure 1. Zinc treatment produces long, apical tuft-like cilia across the entire embryo (A). Confocal microscopy (B-D) showed retracted axonemes in the cytoplasm (arrowheads). Acetylated tubulin of the axoneme is labeled red; DNA is stained blue. B-D are the same scale.

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