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## Lithium Chloride Inhibits Development Along the Animal Vegetal Axis and Anterior Midline of the Squid Embryo

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When squid embryos, *Loligo pealeii*, are cultured *in vitro* (1) they may be individually manipulated with classic and molecular techniques, providing insights into the conservation of developmental pathways. Dorsoventral polarity in many embryos is associated with precise gene transduction cascades involving the Wnt signaling pathway (2). Results from experiments with frog (3), fish (4), and mouse (5) embryos suggest that a component of this cascade,  $\beta$ -catenin, plays a major role in axis formation. Additional support for the role of  $\beta$ -catenin in the early development of many embryos comes from studies using lithium chloride (LiCl). LiCl is a known vegetalizing agent for sea urchins (6), and in echinoderms generally, it enhances and expands levels and regions of nuclear  $\beta$ -catenin localization coincident with increases in endoderm and mesoderm (7, 8). In contrast, its effect on amphibian embryos is species- and stage-specific. For example, when gastrulating embryos are treated with LiCl, they develop reduced notochords and enhanced vegetal structures, but when treatments are given at earlier or later cleavage stages, dorsalizing and anteriorizing effects, respectively, are observed (3, 9). In this study, embryos cultured *in vitro* were treated with lithium chloride to determine its effect on development; this is the first step towards understanding the molecular mechanisms of patterning in squid.

Embryos were fertilized *in vitro* (1) and cultured at 17 °C in 60-mm plastic petri dishes (Falcon) that were lined with 0.2% agarose (Type II-A, Sigma), filled with Millipore (0.22  $\mu$ M) filtered seawater (MFSW), and supplemented with bovine serum albumin (BSA) (0.5%). Each LiCl treatment dish also contained 20, 40, or 60 mM LiCl (Sigma). Three trials of 15 embryos per treatment were performed. Dishes and solutions were changed every other day. Embryos were treated with LiCl for the first 6 d of development, by which time epiboly of the outer yolk cell was complete. Development was observed until the control embryos began to hatch from their chorions, at 19–20 d after fertilization. The classical stages of J. M. Arnold (10) were used to describe embryonic development. Embryos cultured in the presence of LiCl exhibited a dosage-dependent inhibition of development that was evident by 6 d in culture (Fig. 1a, b, 6 d post-fertilization (dpf), stage 18), but it was more easily detected later, during organogenesis (Fig. 1d, e, f, 17 dpf, stage 27). Development was inhibited in many structures normally associated with ectodermal tissues, such as tentacles, eyes, mantle, fins, and funnel. Moreover, convergence and inhibition of anterior midline structures was observed in embryos treated with 40 and 60 mM LiCl. In one trial, for example,

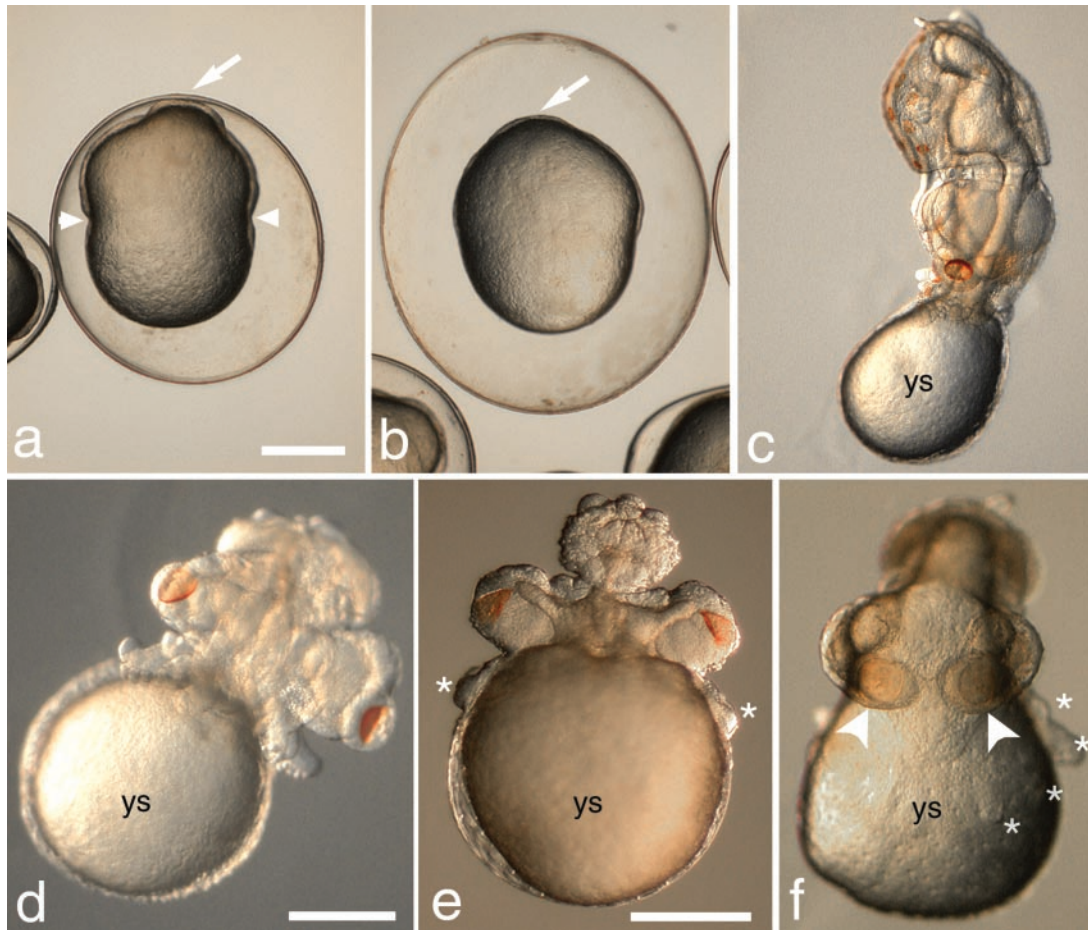
8 of 16 embryos cultured in the presence of 60 mM LiCl had anterior midline structures that were inhibited. As a result, the eyes were abnormally placed: either converged (5/16, Fig. 1f), fused (2/16), or cyclopic (1/16). In contrast, structures that normally form on the posterior body wall such as the funnel or paired statocysts, although reduced, were always present in these embryos. These observations suggest that various regions in the embryo are differentially sensitive to LiCl treatment.

Lithium chloride treatment inhibits development along the animal-vegetal axis in the squid embryo and causes convergence and fusion of anterior cephalic structures. This result is consistent with the notion that LiCl treatment induces vegetalization of the squid embryo and thereby enhances mesodermal and endodermal structures at the expense of ectodermal derivatives, as it does in other invertebrate and vertebrate embryos (3, 6, 7, 8, 9). That LiCl treatment may induce convergence, fusion, and cyclopia in squid embryos strengthens this interpretation (see 11 for a survey of the early literature on cyclopia produced by experimental means) and provides insights into the possible conservation of developmental mechanisms in cephalopods.

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### Literature Cited

1. Crawford, K. 2002. *Biol. Bull.* **203**: 216–217.
2. Sokol, S., J. L. Christian, R. T. Moon, and D. A. Melton. 1991. *Cell* **67**: 741–752.
3. Schneider, S., H. Steinbeisser, R. M. Warga, and P. Hausen. 1996. *Mech. Dev.* **57**: 191–198.
4. Haegel, H., L. Larue, M. Ohsugi, L. Fedorov, K. Herrenknecht, and R. Kemler. 1995. *Development* **121**: 3529–3537.
5. Kelly, G. M., D. F. Erezylimaz, and R. T. Moon. 1995. *Mech. Dev.* **53**: 261–273.
6. Lallier, R. 1975. Pp. 473–507 in *The Sea Urchin Embryo: Biochemistry and Morphogenesis*, G. Czihak, ed. Springer, New York.
7. Logan, C. Y., J. R. Miller, M. J. Ferdowicz, and D. R. McClay. 1999. *Development* **126**: 345–357.
8. Kitazawa, C., and S. Amemiya. 2001. *Dev. Growth Differ.* **43**: 73–82.
9. Kao, K. R., and R. P. Elinson. 1989. *Dev. Biol.* **132**: 81–90.
10. Arnold, J. M. 1965. *Biol. Bull.* **128**: 24–32.
11. Rogers, K. T. 1963. *Dev. Biol.* **8**: 129–150.



**Figure 1.** Lithium chloride inhibits the animal-vegetal body axis and normal organogenesis in squid embryos. (a) Control embryo, 6 days post-fertilization (dpf) stage 18; an arrow marks the developing tail bud. Note the well-defined indentation that indicates the boundary between the embryo and yolk sac (arrowheads). (b) Embryo treated with 20 mM LiCl, 6 dpf. The arrow indicates the developing tail bud. When compared to the control, this embryo is rounder and less well developed. (c and d) Embryos from control (side view), 17 dpf, stage 27, and 20 mM LiCl (anterior view) cultures, respectively. The mantle and body have failed to develop properly in the LiCl-treated embryo, although differentiation has occurred in the eyes and tentacles. (e) Anterior view of an embryo treated with 40 mM LiCl, 17 dpf. The mantle and organs within it are inhibited; eyes and tentacle primordia (\*) are present but poorly differentiated. (f) Embryo treated with 60 mM LiCl, 17 dpf. Large arrowheads indicate lightly pigmented eyes with lenses visible in their centers that have converged toward the anterior mid-line. An \* indicates each of the four tentacle primordia on the left side of the embryo. ys = yolk sac. a, b and c, and e and f are the same magnification. Scale bars = 500  $\mu$ m in each view.

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### HNK-1/N-CAM Immunoreactivity Correlates with Ciliary Patterns During Development of the Polychaete *Capitella* sp. I

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*Capitella* sp. I is an opportunistic polychaete that is widespread and is among the early colonizers of disturbed areas that are high

in organic content. Larvae are lecithotrophic and are competent to settle within a few moments of emerging from the brood tube, although they can remain viable in the water column without feeding for two or three days if environmental cues for settling are not present. Under experimental conditions larvae demonstrate a

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