

SODIUM IONS AFFECT AXIS FORMATION AND EPIBOLY IN FISH EMBRYOS

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Early development of fish embryo involves of the dorsoventral axis formation and the movement of epiboly. Some mutations were found in large-scale mutant screen affected these processes in zebrafish embryogenesis. There are evidences that dorsalizing signal may function during a prolonged period of cleavage stages (Long W.L., 1983, Stachel S.E., et al., 1993). The nature of this signal remain obscure. Our current work is an attempt to find a possible way for study of this problem.

We had found that embryos of *Misgurnus fossilis* (Cobitidae) which was placed in 100mM NaCl solution at early blastula stage can not begin normal epiboly and axis formation, 50mM concentration has not any effect on embryos (Figure 1, a, b). NaCl in concentration 150mM disrupts embryos but they save viability for hours (Figure 1, c). So we examined the synthesis of proteins in the embryos after 24h in 150mM NaCl and in the control embryos by immunoprecipitation method. The embryos were radiolabeled by ^{14}C -aminoacid mixture, homogenized and immunoprecipitate with anti-annexin antibody and ProteinA-Sepharose. Figure 2 shows the fluorogram of blot of these probes after SDS-PAGE. The same proteins are synthesised in both types of embryos.

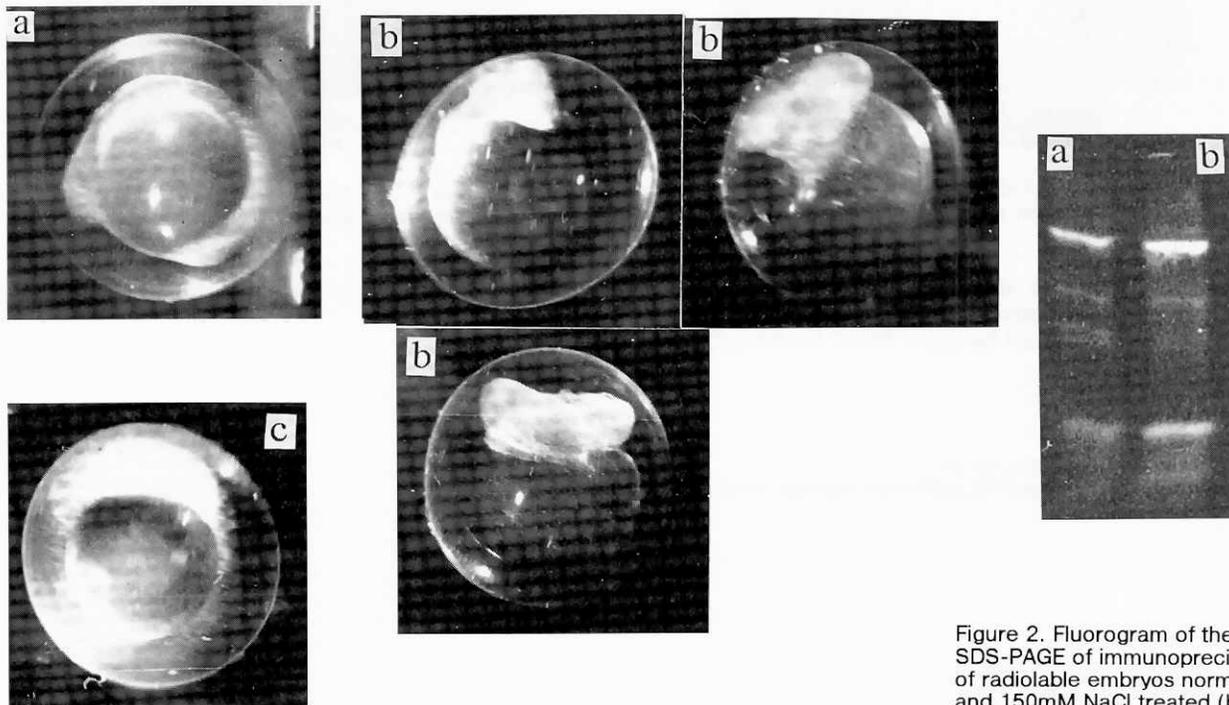


Figure 2. Fluorogram of the blot of SDS-PAGE of immunoprecipitates of radiolabeled embryos normal (a) and 150mM NaCl treated (b).

Figure 1. Normal embryos on body somite stage(a), embryos after incubation during the same time and temperature in 100mM NaCl (b) and in 150mM NaCl (c).

100mM NaCl treated embryos have irregular structures (Figure 1, b) and different cell types (Figure 3), so they have some similarity with the normal embryos.

If we took choline chloride instead of NaCl we had no any influence on embryogenesis so effect of NaCl appear to be specific for Na^+ and is not osmotic.

The action of NaCl to embryogenesis take place on early stages of development only.

If the embryos was in fresh water till the beginning of epiboly, following development in NaCl solution till the hatching was normal. The effect of NaCl was reversed if we placed the embryos from saline to fresh water before gastrulation.

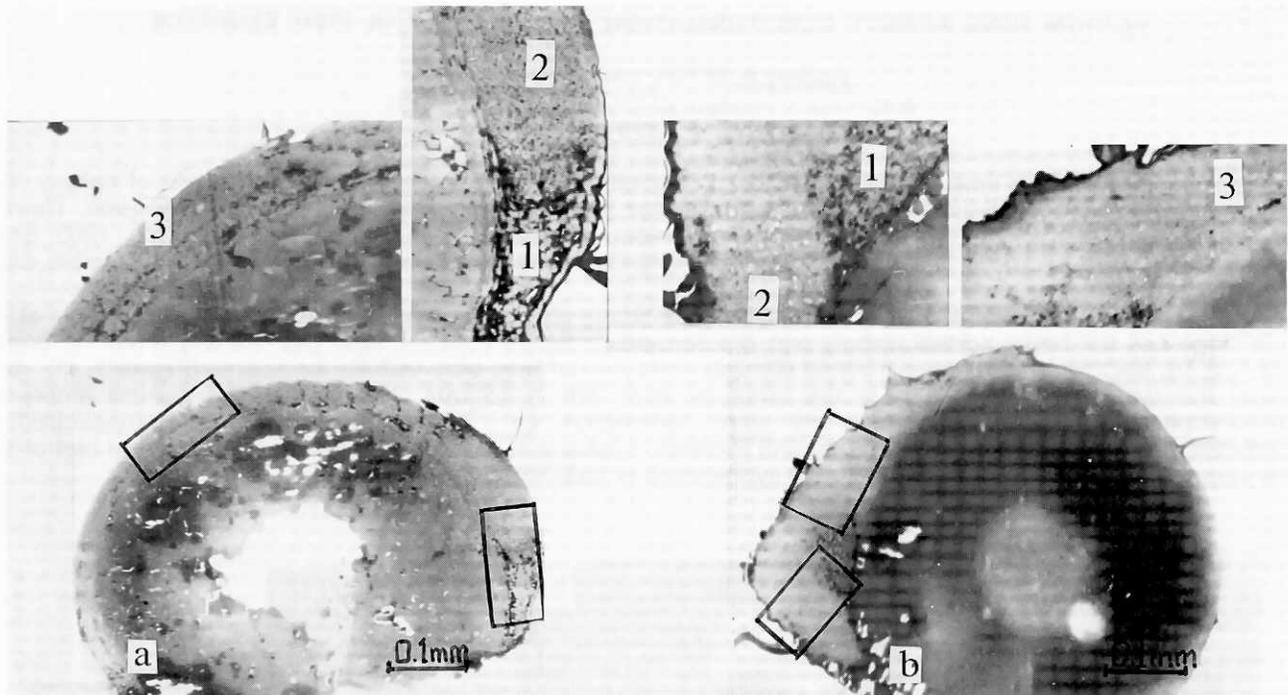


Figure 3. Normal (a) and 100mM NaCl-treated (b) embryos. Embryos were fixed in 2.5% glutaraldehyde in PBS, treated as usual. Semithin sections (2 μ m) were stained with methylene blue, 1, 2 and 3 - similar cell types in normal and NaCl-treated embryos.

All these observations allow us to suppose that ions of sodium have a specific role in the pattern formation in early fish embryos, perhaps in the polarization of blastula cells.

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References

- Long W.L. (1983) *J. Exp. Zool.* 228, 91-97.
 Stachel S.E., Grunwald D.J., Meyers P.Z. (1993) *Development* 117, 1261-1274