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Morphogenesis of exogut isolated from vegetalised embryo of sea urchin

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It is well known that sea urchin embryos treated with lithium chloride (LiCl) develop to abnormally into vegetalised embryos, in which differentiation of ectodermal cells is inhibited. When embryos of the sea urchins, *Hemicentrotus pulcherrimus* and *Anthocidaris crassispina* were treated with 20 mM LiCl from the 8-cell stage to the corresponding early gastrula stage, they developed to vegetalised embryos with a large exogut 45 h after fertilisation. In these vegetalised embryos, high activity of alkaline phosphatase (AP) was detected histochemically at the end of the exogut where it is attached to the embryo body. High activity of AP is known to be detected specifically in the gut of sea urchin pluteus larvae by the same procedure as used in this study. Hence, we concluded that this part of the exogut is composed of the cells which develop into the cells of the gut in normal development.

When exogut isolated from vegetalised embryos was cultured in the extract obtained from eggs or embryos, the end composed of the cells in which high AP activity was detected, expanded during culture and formed a large spherical structure about 24 h after the initiation of culture. The minimum concentration of extract to cause expansion of isolated exogut was 5×10^3 egg or embryo equivalent/ml ASW (artificial seawater). The extract boiled at 95 °C for 1 h also caused expansion of isolated exogut at the same concentrations as non-boiled extract. On the other hand, the extract obtained from eggs or embryos by chloroform–methanol extraction did not cause any expansion of exogut, but the aqueous phase, heat-dried and dissolved in ASW, induced expansion of isolated exogut. Exogut cultured in medium made from the precipitate obtained by addition of ice-cold ethanol to the extract of eggs or embryos did not expand, but did in the medium from the supernatant. Treatment of the extract by DNase, RNase or peptidase did not exert any effects on the expansion capacity of the extracts obtained from eggs or embryos.

The rate of protein tyrosine phosphorylation in isolated exogut dramatically increased soon after initiation of culture in the extract of eggs or embryos, and reached a peak just before the initiation of its expansion. When isolated exoguts were cultured in egg or embryo extract containing genistein, an inhibitor of protein tyrosine kinase, expansion did not occur. Genistein treatment for 5 h started soon after the initiation of culture blocked the increase in the rate of protein tyrosine phosphorylation and subsequent expansion was not observed, but treatment with genistein initiated after the peak of protein tyrosine phosphorylation did not exert any effects on expansion of exogut. On the other hand, treatment of exogut with cytochalasin D also blocked expansion of exogut when it was carried out after the peak of protein tyrosine phosphorylation.

The observations in the present study indicate that eggs or embryos contain substances which activate protein tyrosine kinase in isolated exogut and the activation of this enzyme causes exogut expansion, which is supported by the formation of cytoskeletal structures. These substances are resistant to heat and are not digested by DNase, RNase or peptidase. It is important to characterise these substances further for an understanding of the signalling system by which isolated exogut expands to make large spherical structures at the end composed of the cells with high AP activity. It is unknown at present whether or not these substances contribute to alteration of cell characteristics in isolated exogut.