Short communication

The potency of the first two cleavage cells in echinoderm development: the experiments of Driesch revisited

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Abstract. A hundred years have passed since Driesch performed the classical experiment of separating sea urchin blastomeres from a two-cell-stage embryo, finding that each developed into a complete though smaller larva. The earlier studies of Roux using frogs showed that inactivating one of the two blastomeres by a heated needle resulted, during the early stages of development, in the formation of a half embryo. In this type of experiment, in which the two blastomeres are not separated, the live blastomere continues its development while it is still attached to an inactivated neighbour. In the work reported here, Roux's experimental design was used on two-cell-stage embryos of sea urchins. In contrast to the findings of Roux using amphibians, it was found (as claimed by Driesch) that the living blastomere developed as in the case of separated blastomeres.

Key words: Sea urchin – Blastomere – Totipotency – Ricin A

Introduction

A hundred years have passed since Driesch performed the classical experiment of separating sea urchin blastomeres from a two-cell embryo, finding that each could develop into a complete though smaller larva (Driesch 1892). Further studies demonstrated that blastomeres of many species remain totipotent even after several cleavages (Wilson 1925; Gilbert 1991). These findings clearly indicate that from the two-cell stage, adherence of one blastomere to another conveys information which determines their further development. It is amazing that the contemporary understanding of the possible mechanisms involved in this information transfer is not much greater than that of a century ago. Several mechanisms can be envisaged: (1) transfer of soluble signal molecules from one cell to another, (2) surface to surface interactions between the cells or even (3) changes in surface curvature (adherent cells lose their spherical configuration at the attachment site) may constitute the signal.

The earlier studies of Roux in amphibians showed that inactivating one of the two blastomeres with a heated needle resulted during the early stages of development in the formation of a half embryo (Roux 1888; Sander 1992). In this type of experiment, in which the two blastomeres are not separated, the live blastomere continues its development while it is still attached to an inactivated neighbour. If under these circumstances the molecular basis of cell surface – cell surface organization and cell surface geometry are not damaged, this result indicates the possible importance of these factors in transmitting to one cell the existence of another.

In the present study, Roux's experimental design was used on two-cell-stage embryos of sea urchins. However, since it was impossible to inactivate one blastomere by Roux's method, the blastomere was injected with ricin A instead. It is postulated that if the live blastomere attached to the inactivated one develops into a half embryo this would indicate the importance of cell surface organization. On the other hand, if normal development ensues in the live blastomere other factors should be sought. In contrast to the findings of Roux in amphibians, it was found that normal development took place just as in the case of separated blastomeres.

Materials and methods

Preparation and injection of the sea urchin embryos. Gametes of Lytechinus pictus were obtained from sexually mature sea urchins by intracoelomic injection of a 0.5 M KCl solution. Shed sperm were removed from male sea urchins with a Pasteur pipette and stored in a glass tube on ice until used. Eggs shed from females were collected in seawater (SW) and allowed to settle under gravity. The supernatant was removed by aspiration and eggs were filtered through 100 μm Nitex into acidified (pH 5.0) SW for 5 min to remove the surrounding jelly coat. The supernatant was aspirated and the eggs were washed three times in fresh SW. The eggs were
fertilized in SW by addition of a few drops of a dilute (1/100) sperm solution. The fertilized eggs were cultured till the first cell division. At the two-cell stage, one of the two blastomeres was injected and the embryos were then further cultured at 15°C for 72 h in SW. During this period, the injected embryos were examined for the appearance of morphological characteristics, such as gut structures, spicules and pigment cells. The embryos were scored as positive for gut structures if an organized hollow tube was present in the blastocoel cavity. Spicules could be identified in the embryos as fine birefringent pins, and pigment cells were observed by their orange-red colour.

The microinjection procedure of McMahon et al. (1985) was followed. At the two-cell stage, a few embryos were transferred with a pulled mouth pipette into a plastic culture dish containing SW. The culture dish was previously treated for 1 min with a 1% polylysine solution (Sigma) which caused the embryos to stick to the bottom of the dish by electrostatic attraction. Microinjection needles were made by double pulling capillary tubing on a micropipette puller. The solution to be injected was introduced into the capillary needle, and was drawn to the tip by capillary action. The needle was then partially back-filled with a low-viscosity oil (Fluorinert FC 77) and introduced into an oil-filled microinjection transmission system (Narishige apparatus) attached to a 3 ml Hamilton glass syringe. Micromanipulation was performed using a micromanipulator (Leitz) on an inverted microscope (Zeiss). The flow rate was controlled by turning the syringe screw until a steady flow issued from the needle tip. The flow from the needle was essential for penetration through the fertilization membrane and the cell membrane.

**Inhibition of cell cleavage.** For complete inhibition of cell cleavage of one blastomere of the two-cell-stage sea urchin embryos, the lectin, ricin A chain (Sigma, L-9514), was used. The toxin is known to inhibit protein synthesis by inactivating the 60S ribosomal subunits (Olsnes 1978). A solution of 0.7 mg ricin A in 1 ml of 40% glycerol containing 10 mM phosphate pH 6.0, 0.15 m NaCl, 10 mM galactose and 0.5 mM dithioerythritol was used for injection.

**Results and discussion**

One blastomere of the two-cell-stage sea urchin embryo was injected with approximately 1 pl of ricin A (Fig. 1a). In other cases one cell from the two-cell stage was exploded by injection of a large volume (approximately 5 pl) of the microinjection solution (Fig. 1b). From each culture that was used, a few embryos were taken as controls. In one group of controls, one of the two blastomeres was injected with a physiological solution, whilst in the other group the blastomere was not injected.

The development of the embryos was followed up to 72 h. Out of the 54 embryos in which 1 of their blastomeres was injected with the ricin A solution, 49 survived after 24 h in culture. It was clear that the injected blastomere did not divide whereas the uninjected blastomere divided normally. At the fourth cell division, the injected blastomere produced half the number of cells of a normal embryo (four mesomeres, two macromeres and two micromeres), i.e. all the cell types were presented (Fig. 2a, b). The three dimensional configuration of the developing cells from the uninjected blastomere appeared normal. During the next 20 h of development the injected blastomere was attached very tightly to the dividing uninjected blastomere. Later, when the uninjected blastomere developed into a blastula, the injected blastomere became detached from it (Fig. 2c). The blastula developed into a normal gastrula in which the formation of the archenteron and the appearance of the spicules and some pigment cells could be clearly observed. The embryos that formed from uninjected blastomeres appeared normal but were half the volume of the control embryos (Figs. 2d, e, f). Only a few embryos continued to develop to the pluteus larva stage and formed all organs that characterize a normal pluteus larva, including an almost symmetrical pair of spicules (Fig. 2f).

In 43 embryos, 1 blastomere of the 2-cell stage was exploded with a large volume of the ricin A solution. Thirty-six embryos survived after 24 h in culture and in all of the cases the uninjected blastomeres developed normally. At the fourth cell division, the unexploded blastomere produced half the number but all three cell types of blastomeres (Fig. 3a, b). After about the first 20 h in culture the remains of the exploded blastomere were attached to the uninjected blastomere, but during the blastula stage they became detached. The further development of the blastula was found to be normal (Fig. 3c, d, f). A few embryos continued to develop to the pluteus larva stage and formed a bilateral organization and all organs that characterize a normal pluteus larva (Fig. 3e).

The results indicate that ricin A does not pass from

![Fig. 1. a One blastomere of the two-cell-stage sea urchin embryo (*Lytechinus Pictus*) injected with ricin A solution (arrow). b One blastomere of the two-cell-stage embryo that was injected with a large volume of ricin A solution that causes the cell to explode (arrow). Magnification ×800](image-url)