Fusion of dissociated fish embryonic cells

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SUMMARY

The induction of a frequent fusion in dissociated fish embryonic cells is reported. Fusion was induced during mechanical isolation of blastomeres in normal saline solution or Ca2+-free saline solution by quickly bringing about physical contact between cells within about 1-5 min of dissociation. Fused blastomeres were obtained in about 18% yield from early morula cells, in about 68% yield from early blastula cells and in about 5% yield from early gastrula cells.

INTRODUCTION

When dissociated in an appropriate medium, teleostean embryonic cells show unique and remarkable behaviour. In *Fundulus heteroclitus*, Trinkaus (1963) reported that the adhesivity of isolated blastomeres to a glass substrate is higher in cells of the early gastrula than in cells of the blastula. Dissociated embryonic cells of *Oryzias latipes* also show an increase in the degree of re-aggregation with advancing developmental stages (Yokoya, 1966). Fujinami & Kageyama (1975) observed that the pseudopodial activity of isolated *Oryzias* embryonic cells increases during late blastula and gastrula stages. Since the adhesivity and pseudopodial activity of blastoderm cells *in vivo* were described in the morphogenesis of *Fundulus* (Trinkaus, 1973) and of *Oryzias* (Kageyama, 1977), such behaviour changes of isolated embryonic cells are suggested to be due to the increased deformability of the cell surface during development (Trinkaus, 1963, 1973; Fujinami & Kageyama, 1975).

During studies of the formation of binucleate cells in dissociated fish blastomeres (Mizukami, 1971, 1976), Mizukami (1976) unexpectedly observed the fusion of isolated cells. After several trials to reproduce the cell fusion phenomenon, a method for the induction of frequent fusion was successfully obtained. This paper reports the process of fusion and the change in proportion of fused cells yielded with respect to developmental stages.

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MATERIALS AND METHODS

Developing eggs of the orange-red variety of the medaka, *Oryzias latipes*, were used. After dechorionation, whole blastoderms from morula, blastula and early gastrula stages were mechanically dissociated into their constituent cells with sharp watchmaker's forceps. The media used were normal saline solution (0.75% NaCl, 0.02% KCl, 0.02% CaCl₂, and 0.02% NaHCO₃, pH 7.4) and Ca²⁺-free saline solution. In normal saline solution whole blastoderms were dissociated mechanically into several cell masses and then into single cells. In normal saline solution cells showed surface adhesivity to the glass substrate or to each other, while adhesivity of the cells was not observed in Ca²⁺-free saline solution. But the adhesivity of cells in normal saline solution was not so great that cells could not be dissociated or handled. Skilful handling allowed nearly complete cell dissociation without injuring cells. In order to induce cell fusion, two dissociated cells must be brought into physical contact. This was also done mechanically with sharp watchmaker's forceps. The whole procedure of cell dissociation and cell-to-cell contact, followed by observation of the fusion process, was performed in a deep depression slide with the aid of an inverted microscope, without exchanging the medium, at room temperature.

RESULTS AND CONSIDERATIONS

The embryonic cells could be mechanically dissociated in both normal saline solution and Ca²⁺-free saline solution, in which the cells remained healthy and active for about 3 h or more. Cell divisions often occurred in both media, the more frequently in cells of the younger stages. Immediately after cell dissociation cells became spherical, but about 30 sec after dissociation hyaline blebs or pseudopodia bulged out rapidly. Cells of late blastula and early gastrula showed more pseudopodial activity than cells of morula and early blastula. In about 30–60 sec, most individual cells retracted their blebs and became spherical again. Within a few minutes of recovery of their spherical shape, cells of late blastula and early gastrula stages redeveloped blebs and these propagated around the cell circumference. There was little difference in the behaviour of isolated cells or in the fusion processes and fusion ratios between the two media, except for the adhesivity.

Cell fusion could only be induced by rapid production of cell-to-cell contact within about 90 sec of cell dissociation. If individual cells remained isolated for about 2 min cell fusion could no longer be induced, suggesting that isolated embryonic cells quickly lose their ability to fuse readily. This temporal condition is similar to that in a method for the induction of a high fusion frequency in meiotic protoplasts from liliaceous plants (Ito, 1973). In the case of protoplasts, fusion was induced with rapid isolation of protoplasts followed by rapid production of the naked cell-to-cell contact (Ito, 1973).
Fig. 1. Fusion of two dissociated cells of early blastula induced by rapid production of contact between the dissociated cells. The number on each photograph indicates the time in minutes from the mechanical production of cell-to-cell contact. 

*n*, nucleus.
Fusion of isolated embryonic cells

Figure 3. Change in fusion ratios (%) of cells isolated from embryos at different stages of development. The numbers above each pair of experimental points represent the diameters of isolated cells in μm.

Figure 1 shows one type of fusion of two dissociated cells of an early blastula. Fusion at the contact point of the two cells rapidly produced a dumb-bell shape (Fig. 1C, D) followed by formation of an ellipse (Fig. 1F), then a spherical shape (Fig. 1H) within 17 min. In most cases fused cells became completely spherical within 15 min. Isolated cells of blastoderms at every stage of development studied gave this type of fusion. The configurations of fusing cells of this type looked like those of dividing cells. But cell fusion and cell division could be clearly distinguished by the fact that two nuclei were observed in fusing cells during whole processes of fusion (Fig. 1), while in a dividing cell the nucleus disappeared.

Figure 2 shows another type of fusion of two cells of a middle blastula. In this case a hyaline pseudopodium was protruded by either cell in the contact region (Fig. 2A, B). The hyaline pseudopodium then rotated around the other cell circumference (Fig. 2C), the attached cells completed fusion, becoming a cell with a binucleus. This type of fusion was observed to occur with higher frequency in cells of middle and late blastula and early gastrula than in cells of morula and early blastula.

Fusion of two nuclei in a fused cell has been reported (Mizukami, 1976). Fusion ratios of 100 pairings of isolated embryonic cells at each stage of...
development in normal saline solution are plotted in Fig. 3. The result consisted of two series of experiments and similar changing patterns of fusion ratios were obtained. Fused cells were obtained in 16–20% yields from early morula cells. Fusion ratios increased to about 68% at early blastula stage, and then decreased to about 25% at late blastula stage. Only 3–6% pairings of early gastrula cells fused together.

These results imply that the state of cell membrane which allowed fusion when cells were isolated and then brought into contact, gradually altered during late blastula and early gastrula stages. It has been reported that the pseudopodial activity and adhesivity of isolated fish embryonic cells increase during late blastula and early gastrula stages (Trinkaus, 1963; Fujinami & Kageyama, 1975). Therefore it seems that some changes in the physiological properties of the cell surface may occur in embryonic cells during these early stages of development.

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REFERENCES


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