Fusion of Dissociated Embryonic Cells in the Teleost, *Oryzias latipes*. IV. Changes in Cell Surface Morphology Related to This Fusion: A Scanning Electron Microscope Study¹

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ABSTRACT. When isolated embryonic cells of the medaka, *Oryzias latipes*, were brought into physical contact within 30 sec of dissociation, cell fusion could be induced, but fusion could no longer be induced between cells that had been isolated for more than 90 sec. Observations with a scanning electron microscope revealed that the cell surface was smooth immediately after dissociation. Cells examined about 30 sec after dissociation, however, had a great wealth of surface folds. The cell surface observed about 90 sec after dissociation was again smooth. Fusing cells were highly plicated. At the contact sites of the two fusing cells many plicae were intertwined in complex patterns. The transitory existence of cell surface folds is believed to be a prerequisite for this cell fusion.

Because fusion is well documented as an important and ubiquitous process in biology, the mechanism of membrane fusion has become a major subject of recent studies in cell biology (8). A method for the induction of frequent fusion has been established in dissociated embryonic fish cells (5, 6). Fusion is induced during the mechanical isolation of blastomeres in normal saline solution by bringing about physical contact between cells within about 30 sec of cell dissociation. Fused cells make up more than half the paired cells (5). In addition to the ease in inducing cell fusion, this high reproducibility of fusion is advantageous for studying spontaneous membrane fusion, which is not mediated by agents such as paramyxovirus.

The scanning electron microscopy (SEM) is an important tool with which to study topographic cell surface morphology. A few reports of SEM studies of cell fusion induced by wheat germ agglutinin (9) and by paramyxovirus (4) have appeared. The latter study pointed out that cell surface microvilli decrease in number during cell fusion and that fusion is always accompanied by cell swelling.

As the first step of a detailed morphological investigation into the mechanism of cell fusion, we have examined changes in cell surface morphology that occur during the fusion process. This communication demonstrates that fusion possibly is induced by bringing about the cell-to-cell contact of isolated cells before or while the cells are highly plicated.

¹ This paper is dedicated to the memory of Dr. J.C. Dan (1910–1978).

MATERIALS AND METHODS

Cell fusion. Developing eggs of the orange-red medaka, *Oryzias latipes*, were used. After dechorionation, blastomeres from the blastula stage were mechanically dissociated into their constituent cells with a #1/3 hypodermic needle. The medium used for dissociation and for a short term cell culture was normal saline solution (0.75% NaCl, 0.02% KCl, 0.02% CaCl₂, and 0.02% NaHCO₃, pH 7.4). Skillful handling produced nearly complete dissociation without disintegrating the cells. The cells used for fusion may be the deep cells. To induce fusion, two dissociated cells must be brought into physical contact. This also was done mechanically with the same needle. The whole procedure of cell dissociation and cell-to-cell contact was performed in a glass well slide with an inverted microscope at room temperature (22–25°C).

Scanning electron microscopy (SEM). For SEM, cells at various stages of the fusion process were immediately fixed with a freshly made cold mixture of 2.5% glutaraldehyde and 1.0% osmium tetroxide in 0.05 M phosphate buffer (pH 7.4) for 30 min at 4°C. This is the improved fixative used to observe the ultrastructure of dissociated embryonic fish cells (1). The cells were dehydrated through a graded series of ethanol solutions, then they were transferred to liquid carbon dioxide and dried to the critical point. The specimens were attached to aluminum plates mounted on aluminum stubs, coated with a thin layer of gold, and examined with a Hitachi S-310 SEM.

RESULTS

As reported previously (5, 6), fusion could only be induced by rapid production of cell-to-cell contact within about 30 sec of cell dissociation. When individual cells remained isolated for more than 90 sec, cell fusion could no longer be induced. Under a light microscope, cells immediately after dissociation were spherical. But about 15–30 sec after dissociation the cell surface began to show fine undulations and/or rapidly to bulge out blebs. Within another 30–60 sec most individual cells retracted their blebs and again became spherical. Within several minutes of the recovery of their spherical shape, cells redeveloped pseudopodia; these were propagated around the cell circumference (2).

Cells examined with the SEM immediately after dissociation were spherical and had very smooth surfaces (Fig. 1). Cells observed about 30 sec after dissociation, however, had a great many surface folds (Fig. 2). These surface folds were plicae, not microvilli (Fig. 2). Individual plicae often had a narrow insertion ridge, but their free edges were somewhat expanded and thickened (Figs. 2 and 5). Examinations of the surface topography of cells that had been isolated more than 90 sec showed that the cell surface was comparatively smooth (Figs. 3 and 4). Some cells had several blebs (Fig. 3).

From these results we concluded that the changes in surface morphology occur continuously in most of the individual cells within a few minutes after isolation.

The cell surface at the early stage of fusion was highly plicated (Fig. 5). At the contact sites of the two fusing cells many plicae were intertwined in complex patterns (Fig. 5b). Thin cytoplasmic strands sometimes were observed to extend onto the adjacent cell (Fig. 5b). The two cells, fusion of which progressed to a degree, also had many surface folds (Fig. 6). The surface folds were interlaced and fused at the contact site. The fusion process was usually completed within 10 min. The cell surface at the late stage of fusion still had thin plicae (Fig. 7).

Figs. 1-4. Scanning electron micrographs of dissociated embryonic fish cells showing changes in cell surface morphology after isolation. $\times 1,600$

Fig. 1. Cells immediately after dissociation are spherical and have smooth surfaces.

Fig. 2. Cells examined about 30 sec after dissociation have many surface folds (plicae, not microvilli).

Figs. 3 and 4. Cells observed about 90 sec after cell dissociation. Surfaces of the cells are comparatively smooth. Some cells have developed several blebs (b).

DISCUSSION

In dissociated embryonic fish cells, there is a time restriction for cell fusion when isolated cells are brought into cell-to-cell contact (5). The relation of the time restriction to changes in the morphology of the cell surface after dissociation, as shown in this study, suggests that the surface folds which rapidly appeared after dissociation then promptly disappeared, may play an important role in cell fusion. The fact that all the fusing cells had many surface folds which intertwined at the fusing sites substantiates this suggestion. Poste and Allison (8) have divided the membrane fusion process into four stages: contact (the close approximation of the membranes), induction (the displacement of Ca^{2+} from the membranes), fusion (the establishment of stable intermembrane linkages), and stabilization (the recovery of the newly fused membrane to the normal condition). They pointed out that successful contact between

Figs. 5-7. Scanning electron micrographs of the fusion of dissociated embryonic fish cells.

Fig. 5. The early stage of fusion. Fusing cells are highly plicated. At the contact sites of the two fusing cells, plicae are intertwined in complex patterns. Thin cytoplasmic strands sometimes extend onto the adjacent cell (arrow). (a) $\times 1,130$, (b) $\times 3,000$

Fig. 6. The middle stage of fusion. The surfaces of the fusing cells are covered with many folds which are interlaced and fused at the contact sites. $\times 870$

Fig. 7. The late stage of fusion. The cell surface still has thin plicae. $\times 1,300$

the plasma membranes is achieved because of the low radius of curvature of the microvilli. The results of our SEM study provides experimental evidence for this hypothesis. The occurrence of surface folds and their low radius of curvature are considered to make successful contact between the membranes possible. We also believe that membrane fusion is a highly ordered process restricted to specific situations and conditions and that the specificity of the contact interaction between two membranes may in part determine their ability to fuse (8). We have shown that fusion of dissociated embryonic fish cells is spontaneous (5, 6). For example, fusion is induced in a Ca^{2+} -free saline solution and is not affected by a range of pH (6.0-9.0). Therefore, if membrane contact is to occur successfully, the subsequent steps of the fusion reaction must progress automatically. In discussion of their study of myoblast fusion *in vitro*, Knudsen and Horwitz (3) have suggested that cell-to-cell adhesion is not only the first step but also is an integral part of myoblast fusion.

In paramyxovirus-induced cell fusion, cells with a large number of microvilli on their surface fuse together more rapidly than do those with few microvilli, but the presence of microvilli is not essential for fusion to occur (7, 10). It has been thought

that many haemagglutinin spikes are present in the envelope of the paramyxovirus, which explains why this virus can cause cell agglutination when allowed to interact with cells at a high density. Knutton *et al.* (4) observed with an SEM that agglutination results from the cross-bridging of adjacent cells by virus particles. The cell swelling observed with an SEM during virus-induced fusion (4), however, was not detected in the present SEM study.

As to the possibility that the plicae of the surface of fusing cells is an artifact of the preparative technique that probably occurs during the critical point drying stage, we can say it seems slight. Cells other than those with plicae were similarly treated for SEM observations, and the cells were observed to have smooth surfaces. Although the present study with SEM shows the correlation between changes in the surface morphology of dissociated cells and their fusion, the initial change for membrane fusion could not be detected because the first fusion event had already taken place by the time the sites of fusion were recognized in the SEM photographs. A thin section study of this fusion is contemplated.

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