Scanning electron microscopy of cells isolated from amphibian early embryos

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SUMMARY

Cells have been dissociated from *Xenopus* and *Ambystoma* late blastulae, allowed to adhere to glass coverslips, and studied by scanning electron microscopy. *Xenopus* ectoderm cells initially show filopodia; later larger single pseudopodia are formed. *Ambystoma* ectoderm cells show fewer filopodia than *Xenopus* ectoderm, but later form pseudopodia. Ectoderm cells of both *Xenopus* and *Ambystoma* show links between adjacent cells. *Xenopus* endoderm cells do not show filopodia initially, but later show large pseudopodia.

INTRODUCTION

When amphibian early embryos are dissociated into single cells, the cells become motile and can reaggregate (Holtfreter, 1946, 1947). Recently, the properties of cells isolated from amphibian embryos have been studied, since they might provide information on the mechanisms involved in normal morphogenesis. These studies have shown that the types of cell movement shown *in vitro* and the proportion of cells exhibiting movement vary with the stage of the embryo from which the cells are taken and are different for different germ layers (Satoh, Kageyama & Sirakami, 1976; Johnson, 1976). This might be expected if such movements are significant to normal morphogenesis. In addition, hybrid embryos which do not gastrulate, or gastrulate abnormally, show altered motility *in vitro* (Johnson, 1969, 1970, 1972), suggesting that gastrulation is effected by the motile properties of individual cells.

The scanning electron microscope has revealed much about the cellular changes which accompany early embryogenesis. Cells of the different germ layers have different surface structures (Smith, Osborn & Stanisstreet, 1976) and during gastrulation the invaginating cells change shape (Tarin, 1971) and form microvilli (Monroy, Baccetti & Denis-Donini, 1976). However, less is known about possible changes in the surface architecture of isolated amphibian cells and so we have used the scanning electron microscope to study cells dissociated from blastulae of *Xenopus laevis* and *Ambystoma mexicanum*. An abstract of this work is being published in *J. Anat*.

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MATERIALS AND METHODS

Xenopus embryos were obtained by injecting pairs of adults with chorionic gonadotrophin (‘Chorulon’, Intervet Ltd.) and were staged according to Nieuwkoop & Faber (1956). The jelly coats were removed chemically by a modification of the method of Dawid (1965): embryos were placed in 2% cysteine hydrochloride in 10% Steinberg saline (Steinberg, 1957) brought to pH 7.8 with 2 M NaOH. The embryos were washed and subsequently cultured in 10% Steinberg saline, pH 7.3. When they had reached the late blastula stage (stage 81/2), the vitelline membranes were removed manually and the embryos were dissected using iris scalpels to obtain ectoderm cells from the animal pole and endoderm cells from the floor of the blastocoel. Ambystoma embryos were obtained from natural matings of laboratory-maintained adults. When the embryos had reached the blastula stage the jelly and vitelline membranes were removed manually, and the embryos were dissected in the same way as the Xenopus embryos.

The tissues were transferred to dissociation medium (Landesman & Gross, 1968) with 4 mM EDTA over an agar substrate for 1 h. At the end of this period the cells were dispersed by gentle micropipetting. They were then transferred to 10 mm glass coverslips in plastic Petri dishes containing Stearns’ medium (Stearns & Kostellow, 1958), allowed to settle, and were cultured for various times (routinely 1/2 h, 1 h and 3 h). The cells were then fixed by replacing the medium with fixative (2.5% glutaraldehyde plus 2% paraformaldehyde with 2.5 mM calcium chloride in 0.1 M cacodylate buffer, pH 7.2, modified from Karnovsky, 1965). Following fixation overnight, the coverslips were washed in changes of cacodylate buffer containing 2.5 mM calcium chloride over 2 h. The cells were dehydrated in an acetone series and the absolute acetone was...
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replaced with liquid CO$_2$. The cells were then dried using the critical point method, and the coverslips affixed to microscope stubs. Finally, the cells were coated with gold-palladium and observed and photographed with a Cambridge ‘Stereoscan’ scanning electron microscope.

RESULTS

**Xenopus ectoderm**

*Xenopus* ectoderm cells fixed after $\frac{1}{2}$ h in Stearns’ medium appeared slightly flattened and attached to the glass by cytoplasmic ‘skirts’ (Fig. 1). Some cells were covered with filopodia attached to the coverslip (Figs. 1 and 2) and, in some cases, to adjacent cells (Fig. 3). After 1 h in Stearns’ medium, some cells appeared similar to those in the $\frac{1}{2}$ h sample, but some cells had become polarized and were attached to the glass coverslip by single large pseudopodia (Fig. 4). After 3 h, most of the cells were polarized and attached by single pseudopodia (Fig. 5), and in some cases these pseudopodia formed a ‘stalk’ which raised the cell from the substrate. Thus *Xenopus* ectoderm cells appeared to change their method of attachment to glass over the 3 h period studied.

With these cells, an additional experiment was performed in an attempt to determine the time of appearance of the filopodia. Cells were fixed after 3 min in Stearns’ medium. It was found that the cells were attached to the glass after this time, although scanning electron microscopy showed that they were less flattened than the cells of the $\frac{1}{2}$ h samples, and the cells possessed many fine filopodia (Fig. 6). The upper surface of the cells showed collapsed filopodia, and thus it is probable that the cells had developed filopodia while in the dissociation medium.

**FIGURES 9-16**

Fig. 9. Ectoderm cell isolated from *Ambystoma* blastula and cultured on glass for $\frac{1}{2}$ h. Cell surface is covered with projections. × 730.

Fig. 10. Ectoderm cell isolated from *Ambystoma* blastula and cultured on glass for 1 h. Like *Xenopus* cells, cell has become polarized and has formed a single pseudopodium. × 670.

Fig. 11. Ectoderm cell isolated from *Ambystoma* blastula and cultured on glass for 3 h. Cell shows single pseudopodium. × 780.

Fig. 12. Ectoderm cells isolated from *Ambystoma* blastula and cultured on glass for 3 h. Links are seen between adjacent cells. × 670.

Fig. 13. Endoderm cells isolated from *Xenopus* blastula and cultured on glass for $\frac{1}{2}$ h. Cells have a ‘raspberry-like’ appearance and, unlike ectoderm, show no filopodia. × 380.

Fig. 14. Endoderm cell isolated from *Xenopus* blastula and cultured on glass for 3 h. Cell has formed a large pseudopodium. × 775.

Fig. 15. Endoderm cell isolated from *Ambystoma* blastula and cultured on glass for $\frac{1}{2}$ h. Cell is relatively featureless. × 370.

Fig. 16. Endoderm cells isolated from *Ambystoma* blastula and cultured on glass for 1 h. Cells at 3 h are similar in appearance. × 175.
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Ambystoma ectoderm

Ambystoma ectoderm cells fixed after $\frac{1}{2}$ h in Stearns' medium showed fewer filopodia than the corresponding Xenopus cells (Fig. 7). Two types of cells were present; one with a smooth surface (Fig. 8) and the other with a surface covered with short protrusions (Fig. 9). Since the blastula ectoderm is more than one cell thick at the stage used, it is possible that these two cell types correspond to inner and outer ectoderm, and further experiments will be undertaken to attempt to clarify this problem. After 1 and 3 h, some cells had become polarized and showed single pseudopodia (Figs. 10 and 11 respectively). Thus Ambystoma ectoderm cells, like those of Xenopus, change their appearance over the 3 h period studied. As was observed in Xenopus cultures, some adjacent cells were linked by fine processes although these appeared somewhat different from the links between Xenopus cells (Fig. 12).

Xenopus endoderm

In general, isolated Xenopus endoderm cells appeared similar to the endoderm of intact embryos (Smith et al. 1976)—the cell membrane had collapsed onto the underlying yolk platelets to give a 'raspberry-like' appearance. Unlike ectoderm, isolated endoderm cells did not show filopodia or obvious cytoplasmic 'skirts'. Cells fixed after $\frac{1}{2}$ h and 1 h in Stearns' medium appeared similar and were slightly flattened onto the glass (Fig. 13). Endoderm cells fixed after 3 h in Stearns' medium presented a different appearance—like the 3 h ectoderm cells they had formed pseudopodia (Fig. 14).

Ambystoma endoderm

The isolated Ambystoma endoderm cells were relatively featureless and showed neither filopodia, 'skirts' nor pseudopodia (Fig. 15). They were fixed after $\frac{1}{2}$ h, 1 h and 3 h in Stearns' medium, and did not alter their appearance over this time (Fig. 16).

Mixed Xenopus ectoderm and Ambystoma ectoderm

In an attempt to determine whether the links between adjacent ectoderm cells were the mechanism by which cells were rejoining or the result of incomplete separation of cells during dissociation, mixed cultures of Xenopus and Ambystoma were examined, in the hope that size differences between Xenopus and Ambystoma would act as a marker (see Discussion). In practice, size was not an absolutely critical marker, and so other markers are being sought.
DISCUSSION

The present results show that the ultrastructural appearance of cells isolated from different regions of amphibian early embryos is different, and confirms early light microscopical observations that when such cells are cultured in vitro their appearance changes over the first few hours (Holtfreter, 1946, 1947). Holtfreter (1947) described isolated neurula ectoderm cells as forming 'tapering pseudopodia' which become 'web-like membranes', and these could correspond to the filopodia and cytoplasmic 'skirts' reported here on ectoderm cells. More recently, filopodia have been demonstrated by both transmission and scanning electron microscopy on mesoderm cells undergoing migration in amphibian gastrulae (Nakatsuji, 1975, 1976; Monroy et al. 1976) and are probably one of the mechanisms by which these cells invaginate (Nakatsuji, 1976). However, in a recent comprehensive S.E.M. study of Xenopus embryos, Keller & Schoenwolf (1977) showed that blastula ectoderm cells in situ do not have filopodia, whereas migrating mesoderm cells of gastrulae do. Our finding that filopodia are already present after only 3 min in Stearns' medium suggests therefore that they are formed during dissociation in EDTA. Two possibilities are that cells in EDTA become motile and therefore form filopodia, or that during the time required for dissociation, cells age to become gastrula cells, and that those cells with filopodia are those that would have become mesoderm cells. The fact that animal pole ectoderm was used in our experiments favours the former of these possibilities, and the observations that embryonic neural retina cells produce filopodia after dissociation, and that cell surface structure is partly dependent upon the method of dissociation, show that the morphology of cells can alter upon isolation (Ben-Shaul & Moscona, 1975).

In the present experiments Xenopus endoderm and ectoderm and Ambystoma ectoderm formed pseudopodia by which they adhered to the glass. Similar pseudopodia have been observed with light microscopy on isolated cells (Johnson, 1976). They are also found on migrating cells in situ, and have been ascribed a functional role in cell migration (Nakatsuji, 1974).

Spiegel & Spiegel (1977) have recently reported the results of S.E.M. observations of cells dissociated from sea-urchin early embryos, and they described structures very similar to those which we show here. Sea-urchin cells are initially covered with microvilli, but after isolation become polarized and form pseudopodia and cytoplasmic 'skirts'. The pseudopodia may form stalks which raise the cell above the substratum, and we have observed similar forms in isolated amphibian cells.

The conditions under which the cells were cultured in our experiments are the same as those which allow reaggregation (Stanisstreet & Smith, 1978). In some cases, filopodia were observed linking adjacent cells in both Xenopus and Ambystoma ectoderm. Similar features have been seen in reaggregating sea-urchin cells (Spiegel & Spiegel, 1977), and in reaggregating embryonic chick
heart cells (Shimada, Moscona & Fischman, 1974), and it is tempting to suggest that cells make initial contact in this way. However, with amphibian ectoderm, particularly *Xenopus* ectoderm, it is difficult to ensure complete separation of cells, since the outer layer of pigmented ectoderm is quite resistant to EDTA dissociation; and it could be that such links are the result of incomplete separation of blastomeres following dissociation or cell division. For this reason, mixed cultures of *Ambystoma* and *Xenopus* ectoderm were made, in the hope that the size difference of the cells could be used as a marker. In long term cultures mixed aggregates did form, but size difference was not an absolutely critical marker for S.E.M. Under the conditions used, amphibian cells would not only reaggregate, but would also sort out (Townes & Holtfreter, 1955; Stanisstreet & Smith, 1978), and it is of interest that endoderm cells did not form filopodia. It is not known what controls the sorting of amphibian cells, but it might be significant that cells of different tissues show different surface features when isolated.

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