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Scanning electron microscopy of cells from hydroxyurea-arrested blastulae of *Xenopus laevis*

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Summary. Cells from *Xenopus* embryos blocked at the blastula stage by treatment with hydroxyurea have been isolated and cultured in vitro. The morphology of these cells has been compared with that of cells from normal embryos using scanning electron microscopy. Cells from such hydroxyurea-blocked embryos do not show the features, or changes in features, in culture shown by cells from normal embryos.

Recently the properties of cells isolated from amphibian early embryos have been studied in the hope that they might provide information on the mechanisms involved in normal morphogenesis. The types of cell movement shown in vitro, and the proportions of cells exhibiting movement vary with the stage of the embryo from which the cells are taken, and are different for different germ layers[2,3]. Similarly, work in our laboratory has shown that the scanning electron microscopical appearance of cells isolated and cultured on glass differ between cells from different embryonic tissues[4], and is dependent upon the stage of the embryo providing the cells[5]. These findings are consistent with the idea that the properties shown by cells in vitro are significant to normal morphogenesis. In addition, cells from genetically deficient hybrid embryos which do not gastrulate, or gastrulate abnormally, show altered motility in vitro[6].

Some years ago it was shown that treatment of embryos of echinoderms and amphibians with hydroxyurea blocked development at the 4–8-cell stage and late blastula stage

Percentages of cells from normal and hydroxyurea-blocked blastulae of *Xenopus* showing different features by scanning electron microscopy

		Time in culture	Total number of cells	% of cells in each condition							
				Filopodia	Few filopodia	Flattened	Pseudopodia	Elongated	Webbing	Featureless	'Blebbing'
Ectoderm	Control	3 min	228	48	35	3	–	–	–	14	–
		0.5 h	280	–	7	82	–	–	–	11	–
		3 h	258	–	–	37	37	–	–	26	–
	Cells from hydroxyurea-blocked blastulae	3 min	295	–	–	7	–	–	11	82	–
		0.5 h	228	–	–	3	–	–	4	93	–
		3 h	180	–	–	6	–	–	–	94	–
	Cells cultured in presence of hydroxyurea	3 min	245	–	–	3	–	9	–	23	65
		0.5 h	296	–	3	33	–	5	8	40	11
		3 h	229	–	–	–	–	–	–	94	6
Endoderm	Control	3 min	108	–	–	5	–	–	12	83	–
		0.5 h	145	–	3	39	2	6	–	50	–
		3 h	75	–	–	34	22	12	–	32	–
	Cells from hydroxyurea-blocked blastulae	3 min	213	–	6	5	–	1	3	85	–
		0.5 h	176	–	–	5	–	3	5	87	–
		3 h	217	–	–	10	–	3	–	87	–

respectively[7,8]. Brachet[8] noted that these were the stages at which nuclear RNA-synthesis was first detectable in these embryos, and thus it is possible that hydroxyurea inhibits embryogenesis by inhibiting RNA-synthesis, as it does in prokaryotes[9]. The aim of the present work has been to determine whether the cells from hydroxyurea-treated amphibian embryos, which are prevented from undergoing normal morphogenesis at gastrulation, show the normal changes in appearance under the scanning electron microscope when cultured in vitro.

Materials and methods. *Xenopus* embryos were obtained by artificial fertilization, and were allowed to develop to the 8-cell stage (stage 4[10]). The jelly coats were removed with 1% mercaptoethanol in 100% Steinberg saline[11], brought to pH 9.0 with 2 M NaOH, and the embryos were washed in 10% Steinberg saline, pH 7.3. The embryos were cultured in this saline, with or without 10^{-2} M hydroxyurea, overnight at a slightly lowered temperature (15–18 °C, depending on the individual experiment) until the late blastula stage (stage $8\frac{1}{2}$[10]). The vitelline membranes were removed with watchmakers' forceps, and the embryos dissected to obtain ectoderm cells from the animal pole and endoderm cells from the vegetal pole. The dissected tissues were transferred to dissociation medium[12] with 4 mM EDTA over an agar substrate, and after 1–1.5 h the cells were dispersed by gentle micropipetting. The cells were transferred to glass coverslips in Stearns' medium[13] and were cultured for 3 min, 0.5 h and 3 h. The cells were fixed overnight in paraformaldehyde/glutaraldehyde fixative[14] containing 2.5 mM $CaCl_2$, washed in cacodylate buffer and dehydrated in a graded acetone series. The cells were dried using the critical point method and the coverslips affixed to microscope stubs and coated with gold-palladium. The specimens were observed and photographed with a Cambridge Stereoscan scanning electron microscope. For each condition some low-power micrographs were taken of fields of cells in an attempt to quantify the data. Cells were scored as showing filopodia, few filopodia, webbing or pseudopodia, as being flattened onto the glass (this category includes flattened cells which showed filopodia), or as being featureless (table). In the case of the additional experiment in which cells were cultured in the presence of hydroxyurea, it was necessary to introduce a new descriptive category, since many of the cells showed 'blebs' or 'bulges'.

Results and discussion. 1. Cells from normal blastulae: Observations on the appearance of cells isolated from normal *Xenopus* blastulae and studied under the scanning electron microscope have been published in detail elsewhere[4], and so will be described here only briefly for comparison with cells from the hydroxyurea-arrested blastulae. Ectoderm cells fixed after 3 min showed many fine filopodia (figure 1). After 0.5 h in culture, the cells still showed filopodia, but were more flattened onto the glass substrate. After 3 h many of the cells were polarised and had formed single, large pseudopodia (figure 2). Endoderm cells fixed after 3 min showed a raspberrylike appearance due to the collapse of the cell membrane onto the underlying yolk platelets, and generally did not show filopodia. After 0.5 h in culture the cells were slightly flattened onto the glass. After 3 h some of the endoderm cells had formed pseudopodia.

2. Cells from hydroxyurea-arrested blastulae: It was apparent upon dissection that the hydroxyurea-treated embryos had larger blastocoels than normal embryos, since the endodermal floor of the blastocoel was concave. The ectoderm of the treated embryos dissociated more readily than that of the controls. In addition, the pattern of pigment granules was abnormal in hydroxyurea-blocked blastulae, suggesting that the calcium balance of the cells might be disturbed[15].

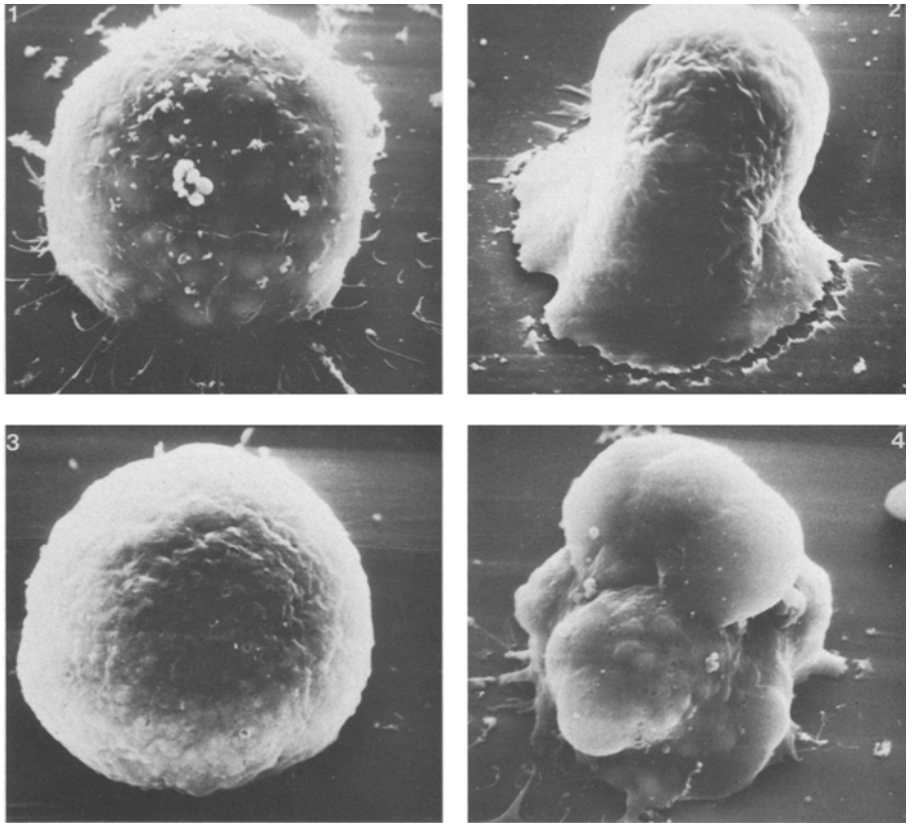

Fig. 1–4. Scanning electron micrographs of ectoderm cells isolated from *Xenopus* blastulae. Fig. 1. Cell cultured for 3 min shows filopodia. × 1330. Fig. 2. Cell cultured for 3 h shows pseudopodium. × 1450. Fig. 3. Cell isolated from hydroxyurea-blocked blastula and cultured for 3 min does not show filopodia. × 1660. Fig. 4. Cell dissociated and cultured for 3 min, both procedures in the presence of hydroxyurea, shows large bulges of surface. × 1160.

The results of the scanning electron microscopy are summarised in the table. Ectoderm cells from the hydroxyurea-treated embryos were different from ectoderm cells from normal embryos, since after 3 min in culture they showed no filopodia (figure 3). After 0.5 h in culture the cells remained featureless and had not flattened onto the glass substrate, and after 3 h the cells had not formed pseudopodia. Endoderm cells from the hydroxyurea-treated embryos were also different from endoderm cells from normal embryos, since they were featureless at all times of culture and did not show pseudopodia at 3 h. Since it was possible that cells were featureless because they were moribund, cell viability was determined by trypan blue exclusion. Cells were prepared as for electron microscopy, and after 3 min and 3 h of culture they were exposed to 1% trypan blue in dissociation medium for 10 min. The results confirmed that at least 87%, and usually 95%, of the cells from both control embryos and hydroxyurea-treated embryos remained viable.

Thus cells from embryos which were prevented by hydroxyurea treatment from undergoing normal morphogenesis did not show the features and changes in vitro shown by cells from normal embryos.

3. Cells cultured in hydroxyurea: An additional experiment was performed to determine whether hydroxyurea had any direct effect on the appearance of *Xenopus* embryonic cells. Ectoderm cells were dissected from normal embryos, and dissociated and cultured in the presence of 10^{-2} M hydroxyurea. Cells after 3 min in culture with hydroxyurea (that had been exposed to hydroxyurea during dissociation) were seen to be different from cells from both normal and hydroxyurea-treated embryos - many ectoderm cells showed smooth 'blebs' or 'bulges' (figure 4). After 0.5 h in culture in the presence of hydroxyurea many of the cells were featureless, and a few still showed 'blebs'; after 3 h more of the cells were featureless (table). This finding might suggest that hydroxyurea has 2 effects on cells from *Xenopus* early embryos; a more immediate effect which produces 'blebs', followed by a longer-term effect which prevents the formation of surface features such as filopodia and pseudopodia.

These results suggest that cells from embryos which are prevented from undergoing normal morphogenesis at gastrulation show a deficiency in their properties in vitro, and are compatible with the idea that the study of cells isolated from early embryos and cultured in vitro may increase our understanding of the mechanisms and controls of morphogenesis.

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Junctions of polytene chromosomes and the inner nuclear membrane[1]

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Summary. Polytene chromosomes in salivary gland nuclei from chironomid larvae of defined stages are connected along their entire length, by some 100 chromatin fibers, to the inner nuclear membrane.

Chromatin forms structural attachments on the inside of the nuclear envelope, which may serve to hold the interphase chromosomes in fixed positions relative to one another. This was stated for the first time by DuPraw, for honey bee embryonic cells[2]. It is a matter for intensive discussion, that chromosomes are in contact with the nuclear envelope during replication[3]. The first report on salivary gland chromosomes attached to the nuclear envelope was based on the observation of unfixed cells of *Chironomus dorsalis* with phase contrast optics[4]; stereomicroscopical investigation of Feulgen-stained glands yielded more information[5]. In *Chironomus thummi,* strings of connecting material could be determined, with the help of the scanning electron microscope (SEM), in freeze-dried cells which had been forced open[6].

In the present investigation it has been ascertained that salivary gland chromosomes of *Acricotopus lucidus* (raised in the laboratory) and *Chironomus* sp. (collected near Stuttgart) are connected to the inner nuclear membrane, as well as to each other. Light microscopical examination of these connections in isolated polytene nuclei is feasible. Using this method, fibers of 0.2–0.5 µm diameter and up to 45 µm in length have been observed. In addition, gland nuclei from larvae of the last 3 instars have been isolated with a modification of Robert's method using nonionic detergents[7]. Nuclei isolated in such a way show no cytoplasmic contamination (figure, a, c–e); this was assured after SEM-examination of deep-frozen carbon-coated nuclei at magnifications up to 10,000 times[8].

Stretched and beaded fibers between euchromatic and heterochromatic chromosomal segments and the inner nuclear membrane were observed in nuclei during transition from the 3rd to the 4th larval instar. Telomeres are always connected to the nuclear membrane or to each other as described by Bauer[9]; strings deriving from them have the largest diameter of all the fibers, about 0.5 µm. In order to make sure that the fibers are not artefacts produced by the method of isolation[7], single nuclei were excised microsurgically[10]. The nuclei thus obtained are bordered by cytoplasm (figure, b, f, g). This is the case in particular with *Chironomus* nuclei, where the cytoplasm impedes the observation of nuclear structures in an unfixed state. Many fibers tear during prolonged fixation with ethanol-acetic acid (3:1); the micrographs in figure, f–g were taken 30 sec