Cell surface properties of amphibian embryonic cells

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SUMMARY

Gastrula and neurula embryos of Cynops pyrrhogaster were dissociated into cell suspensions with ethylenediaminetetraacetic acid (EDTA), trypsin, and alkali solution. The cells were cultured in Niu-Twitty's balanced salt solution and their aggregates were examined histologically. The results indicated that the capacity of amphibian embryonic cells for aggregate formation, sorting out, and notochord differentiation was not suppressed by EDTA and alkali treatments. Trypsin treatment, however, virtually suppressed the cell's capacity for aggregate formation. When aggregate formation resulted from trypsin-dissociated cells, the aggregates showed the sorting out and no differentiation of notochord.

INTRODUCTION

The cell surface plays an important role in embryonic development. Changes in cell surface properties, resulting in the formation of stronger intercellular adhesion, are necessary for embryonic morphogenesis (Gustafson & Wolpert, 1967; Ede & Agerbak, 1968; Johnson, 1970; Bellairs, Curtis & Sanders, 1978). Layer specificities and tissue specificities exist upon embryonic cell surfaces (Moscona & Moscona, 1952; Townes & Holtfreter, 1955; Moscona, 1968; Steinberg, 1970; Gottlieb, Rock & Glaser, 1976; Marchase, Vosbeck & Roth, 1976; Tickle, Goodman & Wolpert 1978). Embryonic cells also possess lectin receptors, cell recognizing substances, and specific cell ligands on their surfaces (Goldschneider & Moscona, 1972; Kleinschuster & Moscona, 1972; O'Dell, Tencer, Monroy & Brachet, 1974; Moscona, 1974; Krach, Green, Nicolson & Oppenheimer, 1974; Hausman & Moscona, 1976; McClay, Chambers & Warren, 1977a; McClay, Gooding & Fransen, 1977b; Shur, 1977a, b; Morriss & Solursh, 1978; Nosek, 1978; Ben-Shaul, Hausman & Moscona, 1979; Boucant et al. 1978; Hausman & Moscona, 1979). These data suggest that the embryonic cell surface has a role in cellular adhesiveness during morphogenesis and in embryonic cell differentiation.

Patricolo (1967) indicated that EDTA-dissociated amphibian gastrula cells reaggregated but did not differentiate. The dorsal lip of the amphibian gastrula, however, differentiated into chorda cells (Townes & Holtfreter, 1955; Jones &
M. Matsuda

Elsdale, 1963). These data suggest that changes of embryonic cell surface properties cause abnormal cell aggregation and inhibition of cell differentiation.

To investigate the cell surface properties in relation to embryonic cell differentiation, dissociated amphibian embryonic cells were cultured and examined for aggregate formation, cellular sorting out, and notochord differentiation.

MATERIAL AND METHODS

Cynops pyrrhogaster embryos were obtained according to Matsuda & Oya (1977). Early gastrula (st. 11), middle gastrula (st. 13b), early neurula (st. 15), and neurula (st. 18) embryos were used. Embryo stages were determined from Okada & Ichikawa (1947).

Embryos were sterilized in 70 % alcohol for 50 sec. Chorions and vitellin membranes were removed with iridectomy scissors and watchmaker's forceps. The individual embryo was placed in 2 ml of dissociating medium in a Linbro semimicrotray microwell (FB-16-24-TC), whose bottom was coated with 1 % agar, and incubated for 30 min at 25 °C. After the treatment, the dissociating medium was replaced with Ca$^{2+}$- and Mg$^{2+}$-free Niu-Twitty’s balanced salt solution (CMF) and the embryo was dissociated into a cell suspension by gentle pipetting. Trypsin (0.4 %; Difco 1:250) in CMF, EDTA (10$^{-3}$ M; Katayama Chemical Co.), and alkali solution (CMF of pH 9.8 justified with KOH) were used as the dissociating media. Dispersed cells were given one minute to settle out of the suspension. The medium was removed and replaced five times with Niu-Twitty’s balanced salt solution (BSS; Flickinger, 1949). Dissociated cells of one embryo were cultured in BSS in a well, which was rotated on a gyroratory shaker at 70 rev./min at 20–23 °C. As controls, st. -11, -13b, -15, and -18 embryos, which were dechorioned, were cultured in the same manner as described above. When the control embryos developed at sts. 27–29, the cultures were fixed for 3–5 h in 10 % neutral formalin, rinsed in running tap water, dehydrated, and embedded in paraffin. These paraffin materials were sectioned at 10 μm and stained with picronigrosin (one part of 1 % nigrosin solution and nine parts of saturated picric acid) for 2 h at room temperature.

RESULTS

Recovery of the dissociated cells. Dissociated middle gastrula cells are shown in Fig. 1. Yolk platelets derived from broken cells are evident. Recovery of these cells after the dissociation is summarized in Table 1. Our results showed that almost all cells of intact embryos were recovered after EDTA and alkali dissociations, (see Suzuki, Kuwabara & Kuwana, 1976) but only half the cells were recovered after trypsin dissociation.

Viability of the dissociated cells. Results of viability tests of dissociated cells are summarized in Table 2. Viability of dissociated cells with each treatment at
Fig. 1. Dissociated middle gastrula cells. A, EDTA-dissociated cells; B, alkali-dissociated cells; C, trypsin-dissociated cells. (Phase contrast.)
Table 1. Number of dissociated cells recovered from one embryo

<table>
<thead>
<tr>
<th>Stages</th>
<th>EDTA-dissociated cells</th>
<th>Trypsin-dissociated cells</th>
<th>Alkali-dissociated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>26000 ± 800*</td>
<td>14700 ± 1400</td>
<td>20800 ± 2400</td>
</tr>
<tr>
<td>13b</td>
<td>56000 ± 3600</td>
<td>30800 ± 2800</td>
<td>51600 ± 2000</td>
</tr>
<tr>
<td>15</td>
<td>64800 ± 2800</td>
<td>26000 ± 800</td>
<td>60800 ± 1600</td>
</tr>
<tr>
<td>18</td>
<td>73600 ± 1600</td>
<td>35600 ± 4000</td>
<td>74800 ± 2800</td>
</tr>
</tbody>
</table>

* ± Standard deviation.

Table 2. Viability of dissociated amphibian embryonic cells

<table>
<thead>
<tr>
<th>Stages</th>
<th>EDTA-dissociated cells</th>
<th>Trypsin-dissociated cells</th>
<th>Alkali-dissociated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>87.3 ± 4.89%*</td>
<td>95.4 ± 1.90</td>
<td>77.7 ± 7.30</td>
</tr>
<tr>
<td>13b</td>
<td>87.2 ± 4.36</td>
<td>95.8 ± 1.49</td>
<td>76.8 ± 4.45</td>
</tr>
<tr>
<td>15</td>
<td>89.7 ± 4.13</td>
<td>98.7 ± 0.52</td>
<td>90.7 ± 5.43</td>
</tr>
<tr>
<td>18</td>
<td>92.8 ± 6.23</td>
<td>97.8 ± 1.60</td>
<td>89.4 ± 8.67</td>
</tr>
</tbody>
</table>

* ± Standard deviation.

Table 3. Formation of aggregates from dissociated cells

<table>
<thead>
<tr>
<th>Stages</th>
<th>EDTA-dissociated cells</th>
<th>Trypsin-dissociated cells</th>
<th>Alkali-dissociated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>74(32/43)</td>
<td>0(0/44)</td>
<td>77(37/48)</td>
</tr>
<tr>
<td>13b</td>
<td>100(42/42)</td>
<td>20(9/43)</td>
<td>91(20/22)</td>
</tr>
<tr>
<td>15</td>
<td>93(41/44)</td>
<td>8(2/44)</td>
<td>92(22/24)</td>
</tr>
<tr>
<td>18</td>
<td>88(21/24)</td>
<td>0(0/24)</td>
<td>88(21/24)</td>
</tr>
</tbody>
</table>

each developmental stage was over 85 % except for the alkali-dissociated gastrula cells.

Formation of aggregates. The results of aggregate formation from the dissociated cells are summarized in Table 3. Dissociated cells usually formed one aggregate per well. In the cultures of EDTA-dissociated and alkali-dissociated cells, most cells joined the aggregate, while only a few hundred remained on the agar and failed to participate. The majority of these cells did not exclude trypan blue.
Embryonic cell surface properties

Table 4. Histological observation of aggregates from dissociated amphibian embryonic cells

<table>
<thead>
<tr>
<th>Stages</th>
<th>Type of dissociation</th>
<th>No. of aggregates</th>
<th>Aggregates which showed the sorting out</th>
<th>No. of aggregates which differentiated notochord</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>EDTA</td>
<td>13</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Alkali</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
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<td>EDTA</td>
<td>22</td>
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<td>Alkali</td>
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<td>EDTA</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Alkali</td>
<td>26</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>18</td>
<td>EDTA</td>
<td>22</td>
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<tr>
<td></td>
<td>Alkali</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>

In the cultures of trypsin-dissociated cells, hardly any aggregates were formed although the majority of individual cells excluded trypan blue.

Histological observation of the aggregates. Histological observations of the aggregates are summarized in Table 4. All the aggregates exhibited smooth spherical forms.

Two out of 13 aggregates, which were formed by EDTA-dissociated early gastrula cells, showed mixtures of endodermal, ectodermal, and mesodermal cells. Differentiated notochord was not observed in these two aggregates. Ten out of the remaining 11 aggregates showed central cell masses which were formed by ectodermal and mesodermal cells, easily distinguished from the surrounding endodermal cells by differences in cellular shape and size. Differentiated notochords were observed in these cell masses (Fig. 2). The pattern of this cell arrangement was termed pattern A. One out of the 11 aggregates did not form the central cell mass constituted by ectodermal and mesodermal cells. These cells remained close to the surface of the aggregate, differentiating into notochords, surrounding mesenchymes, and epidermis at the periphery, endodermal cells centrally. This cell arrangement was termed pattern C (Fig. 3). The cell arrangement of the control embryos (Fig. 4) resembled pattern C. Twenty-two aggregates from EDTA-dissociated middle gastrula cells were examined. The sorting out of endodermal cells and ectodermal and mesodermal cells occurred in all aggregates. Eleven out of 22 aggregates showed pattern A. In the remaining aggregates, ectodermal and mesodermal cells were distributed in the central portion, embedded in endodermal cells, but these cells accumulated and reached the surface (Fig. 5). This arrangement was termed pattern B. As the developmental stage of donor embryos proceeded, the proportion of pattern B and C aggregates to total aggregates became larger.

The sorting out of endodermal cells and ectodermal and mesodermal cells
Fig. 2. Section of aggregate formed by EDTA-dissociated early gastrula cells (pattern A). Notochord indicated by arrow.

Fig. 3. Section of aggregate formed by alkali-dissociated middle gastrula cells (pattern C). Notochords indicated by arrows.

Fig. 4. Section of control embryo. Early neurula embryo was cultured and fixed at st. 28.

Fig. 5. Section of aggregate formed by EDTA-dissociated middle gastrula cells (pattern B). Notochords indicated by arrows.

Fig. 6. Section of aggregate formed by trypsin-dissociated middle gastrula cells. The section shows sorting out of endodermal and other cells. No significant differentiation is observed.
occurred in all aggregates formed by alkali-dissociated cells. The majority of aggregates showed pattern C. Differentiated notochords were observed in all aggregates.

Eleven aggregates of trypsin-dissociated middle gastrula cells were observed. All aggregates showed segregation of endodermal cells and other cells. These segregation patterns resembled pattern C. Histological examination, however, did not demonstrate any significant differentiation (Fig. 6).

**DISCUSSION**

In this experiment, the differentiation of notochords was shown within the aggregates of EDTA-dissociated early gastrula cells, although Patricolo (1967) reported that the aggregates of dissociated early gastrula cells did not demonstrate any significant differentiation. Jones & Elsdale (1963) showed that EDTA-dissociated dorsal-lip cells from a gastrula of *Triton alpestris* differentiated into chorda cells in *in vitro* culture. The difference between our results and those of Patricolo (1967) probably derive from differences in cell-dissociating procedures and culture methods.

Formation of aggregates from trypsin-dissociated cells resulted in fewer and smaller aggregates. Differentiated notochord was not observed in these aggregates. The small volume of aggregates was caused by the decrease in the number of reaggregated cells. Moscona & Moscona (1967) commented that trypsin dissociation of chick embryonic cells was superior to EDTA dissociation with regard to cell dissociation, cell recovery, and cell differentiation. The results of this experiment, however, showed that trypsin dissociation of amphibian embryonic cells reduced recovery of cells, formation of aggregates, and differentiation of cells, compared to EDTA and alkali dissociations.

The results of the present experiment also indicated that the capacity for aggregate formation, sorting out, and notochord differentiation was not suppressed in amphibian embryonic cells by EDTA and alkali treatments. Trypsin treatment, however, virtually suppressed the capacity of cells for aggregate formation. These data suggested that trypsin-sensitive component(s) for cell reaggregation existed upon embryonic cell surfaces. Trypsin-sensitive cell-surface component was required for the aggregation of embryonic neural retina cells and for the adhesion of those cells and optic tectum (McClay *et al*. 1977b; Hausman & Moscona, 1979; Ben-Shaul *et al*. 1979). When aggregate formation resulted from trypsin-dissociated cells, sorting out of endodermal cells and other cells occurred but notochord differentiation was not observed. These results indicated that the capacity for cellular sorting out was not suppressed by trypsin treatment, whereas that of notochord differentiation was suppressed. Chorda cells differentiated autonomously from dorsal lip cells (Jones & Elsdale, 1963). The cell surface of presumptive notochordal cells is possibly changed by trypsin treatment, and these cells may not aggregate
(including the cells dissociated on agar or those dying) and/or may not differentiate.

REFERENCES


Embryonic cell surface properties


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