DESMOSOME FREQUENCY: EXPERIMENTAL ALTERATION MAY CORRELATE WITH DIFFERENTIAL CELL ADHESION

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SUMMARY

Differential cell adhesion, a suggested guiding force for tissue rearrangements during embryogenesis, could be affected by desmosome frequency. A model system for studying embryonic tissue-positioning behaviour involves combining different tissues and following their rearrangements. We have previously shown that for one tissue, embryonic chick heart ventricle, direction of tissue positioning can be altered experimentally. Heart tissue pre-cultured for 2.5 days tends to segregate internally, while tissue pre-cultured for just half a day tends to segregate externally. Also, intact fragments of tissue tend to segregate internally, while reaggregates of trypsin-disaggregated tissues tend to segregate externally. We show here that treatments that increase the tendency to internalize also increase the frequency of adherens junctions and treatments that increase the tendency to externalize decrease the frequency of junctions. An identical hierarchical ordering of the 4 experimental tissues occurs with respect to positioning behaviour and desmosome frequency. In the hierarchy, 2.5-day-cultured fragments > 2.5-day-cultured reaggregates > 0.5-day-cultured fragments > 0.5-day-cultured reaggregates, tissues to the left tend to segregate internally and to have more desmosomes. Tissues to the right segregate externally and have fewer desmosomes. This is what is expected if desmosomes are organelles for adhesion and if differential adhesion is a factor in tissue-positioning behaviour.

INTRODUCTION

Desmosomes, or maculae adherentes, are cell junctions believed to be important in cell adhesion (McNutt & Weinstein, 1973; Staehelin, 1974; Weinstein, Merk & Alroy, 1976). They increase in frequency during development as early embryonic tissues become more cohesive (Overton, 1962), but may decrease when tissues appear to become less cohesive as in malignant transformation (McNutt & Weinstein, 1969; Martinez-Palomo, 1970) and in certain skin diseases (see Staehelin, 1974).

Differences in relative intensities of cell adhesion may guide embryonic tissue movements (Steinberg, 1978a, b), and desmosomes, as adhesive junctions between cells, may be involved. When 2 different populations of embryonic cells are intermixed, or when 2 pieces of intact tissue are fused, cell and tissue movements occur, much as in embryonic morphogenesis, until one tissue comes to surround or engulf the other. Steinberg suggests that less cohesive tissue surrounds more cohesive tissue in such experiments.

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Overton (1977) has reported, for one tissue combination, that internally segregating cells have more desmosomes than externally segregating cells. If desmosomes are organelles of cell adhesion, her results are just those expected for a differential adhesion interpretation of tissue positioning. In such a case, however, it is difficult to interpret cause and effect. Do differences in desmosome frequencies indicate differences in relative adhesion or do they merely reflect tissue-specific differences in one of many different cell organelles? A better system might be one in which the same tissue is experimentally treated to alter its positioning behaviour.

We have developed a system using embryonic chick heart ventricle in which the direction of tissue engulfment can be predictably altered (Wiseman, Steinberg & Phillips, 1972; Phillips, Wiseman & Steinberg, 1977). Tissue pre-cultured for several days is engulfed by tissue pre-cultured only overnight. Tissue that is cut intact from an embryo is engulfed by tissue that is trypsin-disaggregated and reaggregated. A hierarchy of direction of engulfment is formed from the 4 possible experimentally produced tissue categories such that, for any binary combination, the tissue to the left is more likely to be engulfed by the tissue to the right than the reverse: 2-5-day-cultured fragment > 2-5-day-cultured reaggregate > 0-5-day-cultured fragment > 0-5-day-cultured reaggregate.

In the present study we counted and measured adherens junctions in tissues treated as described above. An identical hierarchy forms in which tissues more likely to be engulfed or internalized (i.e. more cohesive as interpreted by the differential adhesion hypothesis) have more adherens junctions than tissues more likely to engulf or externalize (less cohesive according to the hypothesis). A preliminary report of some of these results has been published (Strickler & Wiseman, 1978).

MATERIALS AND METHODS

Tissue culture

Heart ventricles from 5-day-old chick embryos were excised in cold Hanks' balanced salt solution. Reaggregates and fragments were formed by standard procedures used in our laboratory (Wiseman et al. 1972). In short, fragments were cut directly from heart tissue and allowed to round up in culture for 0-5 or 2-5 days. Trypsin-dissociated cells were combined in reaggregates and also cultured for 0-5 or 2-5 days. Tissue masses were cultured in 10-ml culture flasks on a gyratory shaker in a 5% CO₂, high humidity atmosphere at 37 °C and 140 rev./min. Eagle's MEM plus 10% horse serum, 100 units/ml penicillin, and 100 μg/ml streptomycin was used throughout.

Electron microscopy

Experimentally treated tissues and small pieces of heart ventricle cut directly from 5- and 7-day-old embryos were fixed at room temperature for 1 h in 4% glutaraldehyde in 0.2 M phosphate buffer at pH 7.4. Tissues were postfixed in 1% OsO₄ for 1 h at room temperature, stained overnight at 4 °C with uranyl acetate, dehydrated in a graded series of acetone, and embedded in Epon 812. Thin sections were cut with a diamond knife on an LKB-III ultramicrotome. Sections were stained with lead citrate, placed on 1-hole grids, and examined with a Zeiss EM 9 S transmission electron microscope.
Counting and measuring junctions

Five tissue masses in each of the 4 experimental categories and the 2 control categories were used. At least 3 of the 5 in each category were from separate embryos isolated on different days. Ten equally spaced photographs were taken along the diameter of a section through the centre or widest part of each tissue mass (average diameter, 0.25-0.5 mm). The primary magnification of the micrographs was × 10000 and 8 in. × 10 in. study prints at a magnification of about 41000 were scored.

Desmosomes (maculae adherentes) were characterized by an intercellular space about 20-35 nm and especially by 2 very electron-dense cytoplasmic plaques (McNutt & Weinstein, 1973; Staehelin, 1974; Weinstein et al. 1976). They are discrete parallel specializations of apposed cell membranes (Fig. 1).

Zonulae and fasciae adherentes are difficult to discriminate between in thin section. It is impossible to know if the junctional complex encircles the cell or not. These 2 kinds of junctions were counted together and characterized by an intercellular space around 15-25 nm and considerable amorphous electron-dense material on the cytoplasmic side (Fig. 2). They were nearly always longer in section than maculae adherentes (0.5-0.8 μm as compared to 0.12-0.18 μm).

We did not observe obviously well-developed intercalated discs (Sjöstrand, Andersson-Cederqvist & Dewey, 1958; Fawcett & McNutt, 1969), although we did see a few fasciae adherentes close to muscle filament bundles. Because desmosomes go through various morphological stages in their formation (Lentz & Trinkaus, 1971), and because it may be that embryonic...
The lengths of apposed cell membranes were determined with a map measurer and junctions were classified and counted. The length of each junction was measured because the extent of junctional contact between cells depends upon junction size as well as number. Results were converted to numbers of junctions and lengths of junctions per 100 \( \mu m \) of apposed cell membranes.

Study prints were labelled with code numbers by one of us and scored by the other without knowledge of which tissue was being examined.

RESULTS

Figs. 1, 2 show a macula adherens and a fascia or zonula adherens by our techniques. Table 1 presents numbers and lengths of macula adherentes and of fasciae and zonulae adherentes combined. Table 2 presents the totals for all adherens junctions. As expected, the lengths of maculae adherentes were fairly uniform across tissue categories (0.13–0.17 \( \mu m \)), while the fasciae and zonulae adherentes were more variable in length (0.47–0.76 \( \mu m \)).

The frequencies and total lengths of junctions per 100 \( \mu m \) of apposed cell membranes conform to expectations of the differential adhesion hypothesis. The most internalizing of the tissues in fragment fusion studies (2.5-day-cultured fragment) has the most junctions, while the most externalizing (0.5-day-cultured reaggregate) has the least.

Interestingly, there are more junctions in tissues, both fragments and reaggregates, which have developed for 5 days in the embryo followed by 2 days in culture than in tissues which have developed in the embryo for the entire 7 days. More desmosomes form in culture than in the embryo over a similar length of time.

A 2-way analysis of variance testing fragment versus reaggregate, and 0.5 versus 2.5 days of culture for combined junctions (Table 2) shows the numbers significant for one comparison and highly significant for the other three. For numbers of junctions the level of significance for fragment versus reaggregate is 0.062, and for 0.5 versus 2.5 days of culture is 0.013. There is no significant interaction between the 2 effects. For total lengths of junctions per 100 \( \mu m \), the level of significance for fragment versus reaggregate is 0.022, and for 0.5 versus 2.5 days of culture is 0.002. Again, there is no interaction of effects.

There was no apparent difference in frequency or extent of junctions from the surface to the interior of tissue masses.

DISCUSSION

A mature macula adherens is not difficult to identify, but distinguishing a fascia from a zonula adherens, or different stages in the development of a particular junction is not always easy (Lentz & Trinkaus, 1971; Dembitzer et al. 1980; McNutt & Weinstein, 1973). All adherens-type junctions probably contribute to cell adhesion. In this study there is a positive correlation between direction of engulfment in frag-
Table 1. Numbers and lengths of maculae adherentes, and fasciae and zonulae adherentes per 100 μm of apposed cell membranes in sections of experimentally treated embryonic chick heart tissue

<table>
<thead>
<tr>
<th>Type of tissue</th>
<th>Maculae adherentes</th>
<th></th>
<th>Fasciae and zonulae adherentes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number per 100 μm</td>
<td>Length in μm</td>
<td>Length per junction, μm</td>
<td>Number per 100 μm</td>
</tr>
<tr>
<td>2.5-day fragment</td>
<td>13.0 ± 4.30*</td>
<td>2.1 ± 0.65</td>
<td>0.16</td>
<td>18.9 ± 0.78</td>
</tr>
<tr>
<td>2.5-day reaggregate</td>
<td>9.1 ± 1.04</td>
<td>1.5 ± 0.15</td>
<td>0.16</td>
<td>11.3 ± 1.62</td>
</tr>
<tr>
<td>0.5-day fragment</td>
<td>8.5 ± 1.78</td>
<td>1.1 ± 0.26</td>
<td>0.13</td>
<td>8.8 ± 2.21</td>
</tr>
<tr>
<td>0.5-day reaggregate</td>
<td>4.7 ± 1.06</td>
<td>0.7 ± 0.19</td>
<td>0.15</td>
<td>8.4 ± 2.45</td>
</tr>
<tr>
<td>5-day intact embryo</td>
<td>4.2 ± 1.72</td>
<td>0.7 ± 0.26</td>
<td>0.17</td>
<td>8.3 ± 2.10</td>
</tr>
<tr>
<td>7-day intact embryo</td>
<td>4.1 ± 2.43</td>
<td>0.7 ± 0.41</td>
<td>0.17</td>
<td>12.9 ± 1.83</td>
</tr>
</tbody>
</table>

* Standard error of the mean. Counts and measurements were made on 5 separate tissue masses for each type of tissue.

Table 2. Combined numbers and lengths of all adherens-type junctions per 100 μm of apposed cell membranes in sections of experimentally treated embryonic chick heart tissue

<table>
<thead>
<tr>
<th>Type of tissue</th>
<th>Total adherens-type junctions</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number per 100 μm</td>
<td>Length in μm</td>
<td>Length per junction, μm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5-day fragment</td>
<td>31.9 ± 5.03*</td>
<td>14.8 ± 1.19</td>
<td>0.46</td>
<td></td>
<td></td>
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<tr>
<td>2.5-day reaggregate</td>
<td>20.4 ± 1.86</td>
<td>9.7 ± 1.07</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5-day fragment</td>
<td>17.3 ± 2.89</td>
<td>7.7 ± 1.95</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5-day reaggregate</td>
<td>13.1 ± 3.43</td>
<td>4.7 ± 1.37</td>
<td>0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-day intact embryo</td>
<td>12.4 ± 2.42</td>
<td>5.5 ± 1.03</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-day intact embryo</td>
<td>17.0 ± 2.82</td>
<td>7.8 ± 1.05</td>
<td>0.46</td>
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</tbody>
</table>

* Standard error of the mean. Counts and measurements were made on 5 separate tissue masses for each type of tissue.
ment fusion experiments and the numbers and/or lengths of maculae adherentes or total adherens junctions. These results are exactly what one would expect from a differential adhesion interpretation of tissue positioning; tissues judged more cohesive by the differential adhesion hypothesis have more junctions (Wiseman et al. 1972; Phillips et al. 1977; Steinberg, 1978 a, b).

Our experimental treatments produce predictable shifts in tissue-positioning behaviour and predictable changes in frequency and extent of adherens junctions. If desmosomes function in adhesion, their relative frequencies in differently treated tissues may reflect changes in relative tissue cohesiveness.

Overton (1977) showed earlier that desmosome frequency is greater for internalizing tissues in certain cell-sorting experiments. Later, however, she suggested that desmosomes are not required for such behaviour (Overton, 1979). In our experiments, the identical ordering of 4 experimental tissues in terms of desmosome frequency and tissue-positioning behaviour might suggest that junctions are involved in guiding tissue positioning. However, of course, we have not demonstrated that our treatments directly alter desmosome frequency and thereby cause a change in tissue segregation behaviour. We show only a strong correlation between the two.

One of the most intriguing observations shown here is that desmosomes increase in number more in culture than in the embryo. Certain factors in culture media might stimulate junction proliferation (Weinstein et al. 1976). Liebrich & Paweletz (1976) report that HeLa cell aggregates have few, if any, desmosomes when grown in complete medium, but have more desmosomes when grown in nutritionally poor medium. Overton & DeSalle (1980) demonstrated that desmosome formation requires de novo protein synthesis, a process which can be affected by culture conditions. At any rate, in vitro conditions do affect desmosome formation and we are investigating this problem now.

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REFERENCES


Desmosomes and cell adhesion


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