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A Checkerboard-like Cellular Pattern Due to Difference of Cell Adhesion

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We found a peculiar cellular pattern resembling to a checkerboard in an epithelium of the quail oviduct. It consists of two types of cells, ciliated cells and grand cells. We could understand morphology of the pattern based on an assumption that the adhesion between the different types of cells is stronger than that between the same type of cells. The difference of cell adhesions was obtained quantitatively by using a technique of computer simulations.

INTRODUCTION

Several years ago, we made a geometrical and mechanical model for polygonal cellular patterns in a tissue (Honda & Eguchi, 1980; Honda, 1983). The model has been applied to a few actual tissues, corneal endothelia (Honda, Ogita, Higuchi & Kani, 1982), starfish embryos (Honda, Dan-Sohkawa & Watanabe, 1983) and cultured monolayer cell sheets (Honda, Kodama, Takeuchi, Yamanaka, Watanabe & Eguchi, 1984). Here we will modify our model for adaptation to a peculiar cellular pattern of the oviduct which have been discovered recently (Yamanaka & Honda, in preparation).

Figure 1 is a surface-view of the avian oviduct epithelium. It consists of two types of cells which arrange alternately forming an **ichimatsu** pattern which is one of traditional Japanese patterns. An ichimatsu pattern is just the same as a

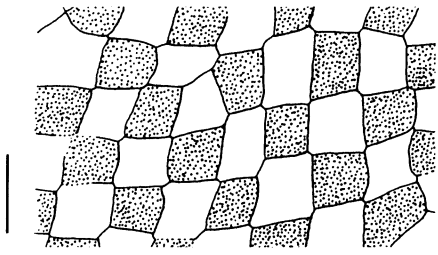

Fig. 1: A surface-view of the oviduct epithelium of Japanese quail. Drawing after a microphotograph. Ciliated cells (designated by stipple) and grand cells are alternately arranged forming a checkerboard-like pattern. Vertical bar, 50 μm.

checkerboard-like pattern.

The purpose of the present report is to indicate that this oviduct pattern is in a balanced state between two factors, (1) the boundary contraction and (2) the differential cell-to-cell adhesion. And, on the basis of this assumption, the difference of cell adhesion is quantitatively estimated.

MATERIALS AND METHODS

Oviduct

Oviducts were isolated from female Japanese quail, and treated for observations as described elsewhere (Yamanaka, in preparation). Luminal surface of the magnum of the oviduct was observed.

Computation

A digital electronic computer Facom OS IV (Fujitsu Co.) was used for computation of s-values by the boundary contraction procedure (Honda & Eguchi, 1980).

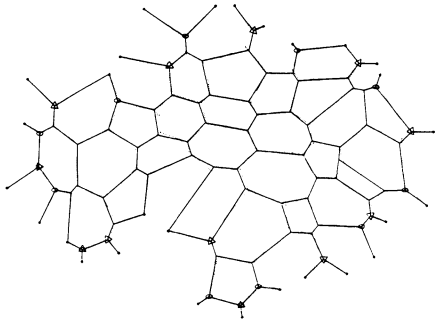

Fig. 2: A polygonal cellular pattern for explanation of the boundary contraction procedure (Honda & Eguchi, 1980).

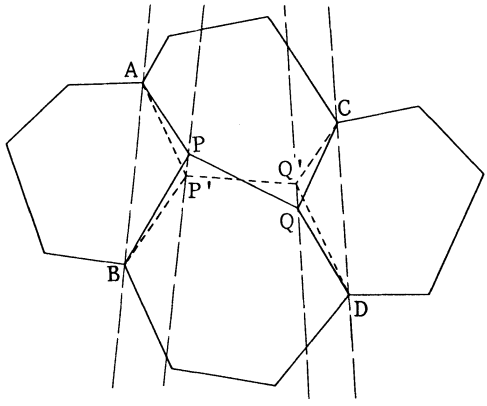

Fig. 3: The elemental step of the boundary contraction procedure. PP' and Q'Q are parallel with AB and CD, recpectively (Honda, Dan-Sohkawa & Watanabe, 1983).

MODELLING AND RESULTS

Epithelia

The oviduct epithelium is on an inside-surface of the oviduct tube, and a sheet of one cell thickness. Columnar cells are packed with each other two-dimensionally. An apical or luminal surface of the cell is an inside-surface of the oviduct tube. Just below the apical surface level there are bundles of contractile microfilaments which are consisting of actin filaments and running along cell boundaries. In addition, cells in the epithelium adhere tightly with each other at the level (Honda & Yamakawa, in preparation). This structure is typical of an epithelial cell.

The boundary contraction model

Several years ago, we made a cell model which is called the boundary contraction model (Honda & Eguchi, 1980; Honda, 1983). The model is suitable for describing an epithelium. The model will be explained as follows:

When a polygonal pattern is given (Fig. 2), we can change the pattern so that its total boundary length is shortened. We choose one side in the given pattern, and note a local region around it. A local region is shown in Fig. 3. We will consider the length of five sides, AP, BP, PQ, QC and QD. Points A, B, C and D are fixed. We can move point P to P', and point Q to Q' so that the total length becomes minimum while the four polygonal areas, AP'B, BP'Q'D, DQ'C, and CQ'P'A are kept constant respectively. We call this **an elemental step** of the boundary contraction procedure.

Then we choose a side in the given pattern randomly, and perform the elemental step of the boundary contraction procedure.

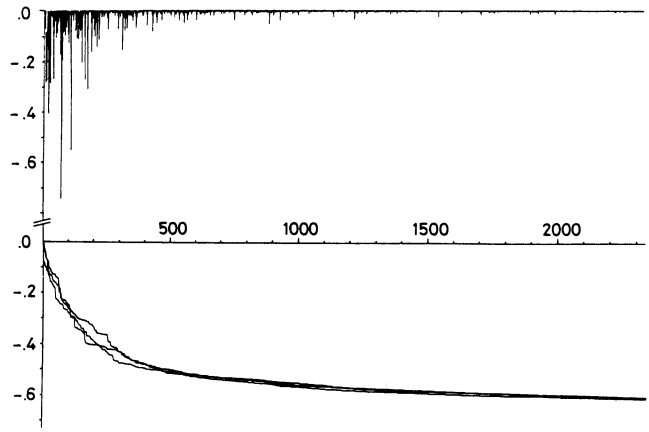

Fig. 4: Process of the boundary contraction procedure of the pattern in Fig. 2. Abscissa, steps of the boundary contraction procedure. Ordinate of the top figure, decrease of the boundary lengths. Ordinate of the bottom figure, -s value. The s value means a percentage of the integrated decrease of the boundary length to the initial total boundary length (Honda & Eguchi, 1980).

After several thousands repetitions of the procedure, the pattern changes into anoher pattern whose total boundary length is almost minimum. The process is shown in Fig. 4. Vertical lines in the

top figure present decreases of boundary lengths at every elemental step. The curve in the bottom figure is an integration of the decrease. In the case of the procedure of Fig. 2, after about two thousand steps, the total boundary length does not decrease anymore. We are interested in an amount of the decrease of boundary lengths by the boundary contraction procedure.

Polygonal patterns whose total boundary length is minimum

In general, a honeycomb pattern, a kind of hexagonal patterns whose inner angles are 120 degree all, is known to have a minimum boundary length. By using the boundary contraction procedure, we have elucidated that epithelial celluar patterns have not been deformed greatly (Honda & Eguchi, 1980; Honda, 1983). That is, epithelial patterns are already contracted due to the bundles of microfilaments at the apical level. The epithelial pattren has contractile properties similar to a honeycomb one.

The differential adhesion hypothesis of cell aggregates

When two types of cells are mixed and cultured in a dish, we often get a cell aggregate in which two types of cells have sorted out into two parts. Steinberg (1963, 1978) gave a hypothesis that differences of cell-to-cell adhesion make several cases.

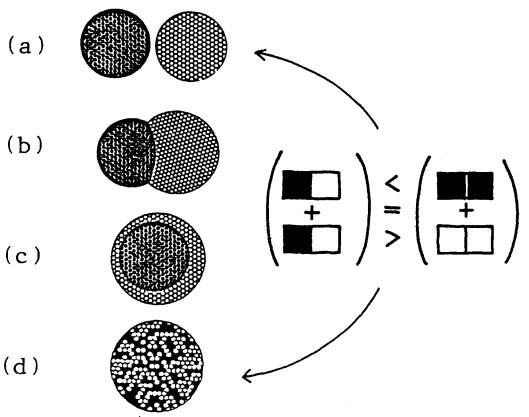

Fig. 5: The differntial ahesion hypothesis of cell aggregates. Relationship of the cell adhesion between the different types of cells to that between the same type of cells makes several cases, (a) two independent cell aggregates to (d) an intermixing cell aggregate (Based on Steinberg, 1978).

When the adhesion between the different types of cells is weak in comparison with the adhesions between the same type of cells, two independent aggregates form (Fig. 5a). When the adhesion becomes strong, one type of cells includes the other (Fig. 5c). And in the extreme case of the strong adhesion, the two type cells intermix with each other (Fig. 5d).

The Steinberg's original hypothesis is three-dimensional. But we will apply the intermixing case of his hypothesis to our two-dimensional cell arrangements.

A balanced state between two factors

We will look at the actual checkerboard-like pattern again (Fig. 6b). When we assume that ciliated cells adhere more

strongly to grand cells, we can understand that the precise checkerboard pattern forms (Fig. 6a). Ciliated cells and grand

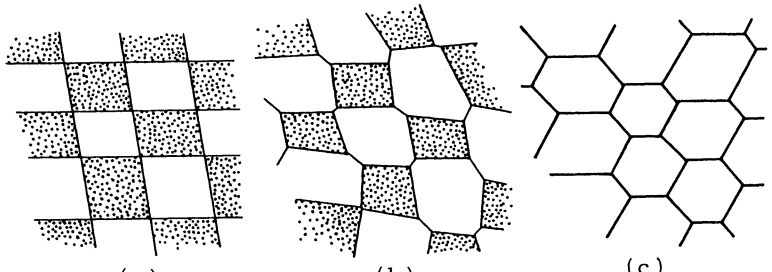

Fig. 6: An actual oviduct epithelial pattern (b) is an intermediate between a checkerboard pattern (a) and a honeycomb one (c).

cells are neighboring alternately. However, this tissue has an epithelial nature so their cell boundary contracts forming a honeycomb-like pattern (Fig. 6c). Therefore, the checkerboard pattern is deformed towards a honeycomb pattern a little. We would like to consider the actual oviduct epithelium is in a balanced state between a checkerboard pattern and a honeycomb one.

Estimation of the difference of cell adhesion

Based on the above-mentioned consideration, we will estimate how much the cell adhesion differs between the different types of cells and the same type of cells.

We will modify the elemental step of the boundary contraction procedure (Fig. 7). We add weight factors a(j) to the summation formula of five boundary lengths,

$$a(j)AP + a(j)BP + a(j)PQ + a(j)QC + a(j)QD.$$

The values of weight factors should be respectively determined according to boundary species. For example, a(CG) is 0.4 for the

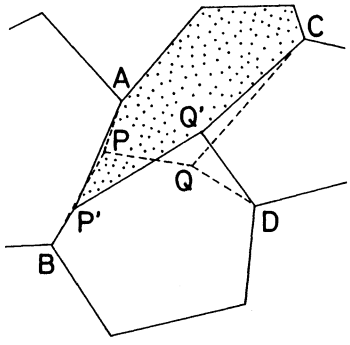

Fig. 7: The modified elemental step of the boundary contraction procedure with weight factors, a(CG) and a(GG). Points P and Q which have been settled by the conventional boundary contraction procedure, that is, with a(CG)=a(GG)=1.0 are moved to P' and Q', respectively, by the modified boundary contraction procedure with a(CG)=0.4 and a(GG)=1.0. Stippled polygon, ciliated cell. Others are grand cells.

boundary between a ciliated cell and a grand cell. And a(GG) is 1.0 for the boundary between two grand cells. When we use these

values of weight factors, the elemental boundary contraction procedure generates a pattern as shown in Fig. 7. We will compare it with the pattern which is generated by the conventional boundary contraction procedure with using a(CG) and a(GG) are 1.0 all. GC boundaries are greatly elongated, and one GG boundary is considerably shortened. In general, when the a(CG) value is smaller than the a(GG) value, we get the pattern in which CG boundaries are elongated and GG boundaries are shorten. The pattern becomes close to a checkerboard pattern.

We will apply this modified boundary contraction procedure to actual oviduct epithelia. When we use various a(CG) values, the oviduct epithelium is deformed in various degree after the modified boundary contraction procedure. In these processes, we could find the definite a(CG) value at which the actual oviduct epithelium is not deformed.

The result is shown in Fig. 8. The ordinate is an s-value which is the percentage of decrease of the total boundary length after the boundary contraction procedure. The s-value is used as

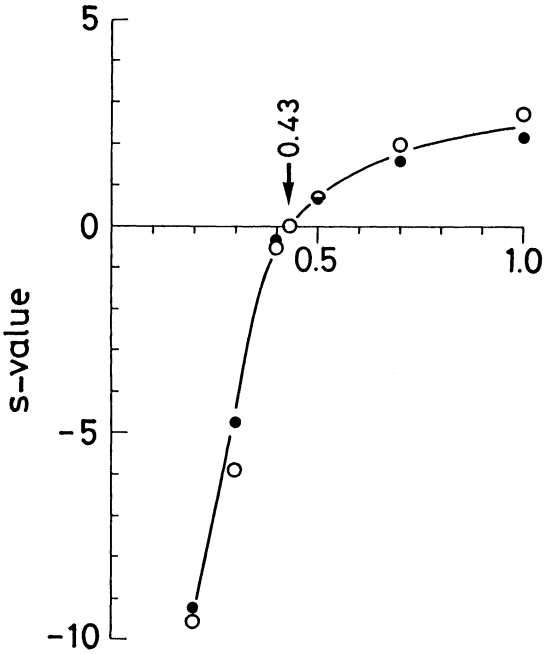

Fig. 8: a(CG)=0.43 at which oviduct epithelial patterns are not deformed by the modified boundary contraction procedure with a(GG)=1.0. Abscissa, the value of a(CG). Ordinate, the s value which is used as an indicater of pattern deformation during the procedure. Open and solid circles, data by using two independent actual oviduct epithelial patterns.

an indicater of the pattern deformation. While a(GG) is kept at 1.0, we decrease a(CG) from 1.0. The deformation of the pattern decrease, and, at a(CG)=0.43, the pattern was scarecely deformed. We defined the a(CG) value is 0.43. This value was confirmed by using two individual actual oviduct epithelia (solid and open circles in Fig. 8).

DISCUSSION

We have to make an interpretation of the a(CG) value here. We will consider the stabilization of the oviduct epithelium. First, the boundary shortening contributes to the stabilization of the epithelium. Second, since the epithelium contains CG boundaries more than GG boundaries, it is reasonable to consider the decrease of a unit length of CG boundaries contributes to the stabilization less than the decrease of a unit length of GG boundaries. Quantitatively speaking, the decrease of 2.33 units (which is an inverse of a(CG)=0.43) of CG boundary length is the same contribution to the stabilization as the decrease of a unit of GG boundary.

This interpretation may not be so clear in respect to physical meanings. But at present, we do not have any method to measure, in situ, the cell-to-cell adhesion in a tissue. So, I believe this estimation of a(CG) is worth to do.

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Q: From the mathematical point, resulting patterns of your shortening procedure can be dependent strongly on the boundary condition of the system. How did you treat the boundary condition? If you make your system large enough and look at cells inside far from the boundary, the resulting pattern is insensitive to the preparation of the boundary. (K. Kitahara)

A: We had performed the boundary contracting procedure a few times on a single pattern with variation of the boundary condition. Differences were small (Figs. 2-3 in J. Theoret. Biol. 84: 575-588, 1980). However, we have usually used for analysis polygonal patterns consisting of more than fifty polygons as you mentioned.