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Cell Sorting Model Based on Cell Signaling

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Abstract. We propose a model for cell sorting which accounts for a key phenomenon of histogenesis and morphogenesis. The model reproduces almost all the characteristics of cell rearrangements seen in experimental observations of cell sorting. In particular, global patterns, internal and external positions of an aggregate, seen in cell sorting are reproduced based only on local interactions among the nearest neighbor cells. The model assumes autonomous cell activity, which includes cell communication and active cell movement. The assumptions are fit for current understanding of cell behavior in cell biology, while cell sorting has been discussed based on cell adhesion. Our model gives us not only a consistent explanation for the process of cell sorting, but also a perspective on the development process.

1. Introduction

When dissociated cells from different tissues in embryos are assembled in a randomly mixed aggregate, they adhere to each other and form homogeneous domains of each tissue type by migrating within the aggregate. This phenomenon, called cell sorting, is observed particularly in combinations of two cell types. In a typical case, cells of one tissue type occupy the internal part of the aggregate and are surrounded by cells of the other tissue type, which occupy the periphery (Fig. 1). Moreover, in various combinations of two cell types, one type is always enveloped by the other according to the cell types; it is invariable which cell type envelops the other in each combination of two cell types (STEINBERG, 1963, 1970). In cell sorting, cells derived from different tissues are likely to migrate to reconstruct the original structures of the tissues after they are randomly mixed (TOWNES and HOLTFRETER, 1955). Hence, we can regard cell sorting as a phenomenon which reveals a mechanism of histogenesis and morphogenesis in development.

Cells adhere to each other via adhesion molecules expressed on their cell membranes. Cell adhesion has been thought to be an important factor in the process of cell sorting since early experiments. Currently, there are two major hypotheses which explain cell sorting with cell adhesion. One is the specific adhesion hypothesis (SAH) and the other, the differential adhesion hypothesis (DAH).

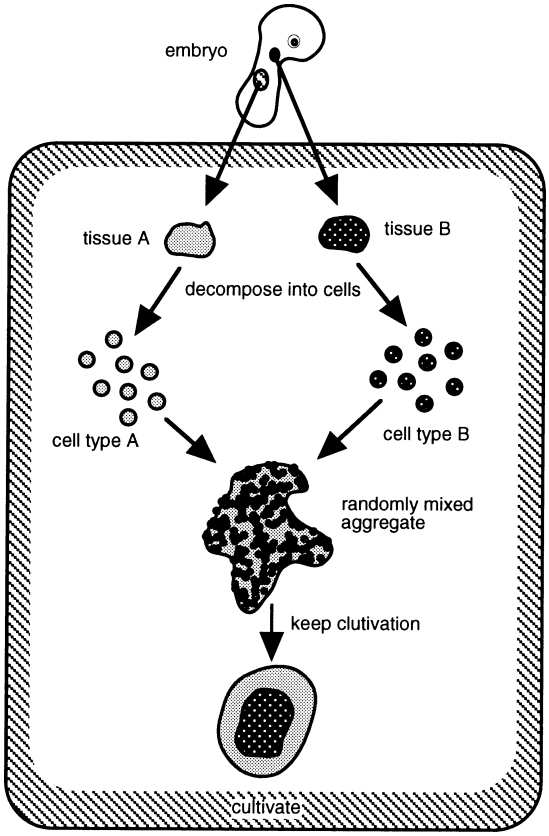

Fig. 1. The outline of experiment of cell sorting.

SAH assumes that selectivity and specificity of cell adhesion cause cell sorting; namely, a domain of particular cells is formed by adhering to themselves specifically in an aggregate, and the domain excludes the other cells by not adhering to them. ROTH and WESTON (1967) showed the specificity of cell adhesion, and several studies have shown evidence of specific adhesion after their work. Moreover, current experimental studies reveal that, at the molecular level, cell adhesions are mostly specific (TAKEICHI, 1990; HYNES, 1992). However, SAH merely assumes that those specific adhesions bring about cell sorting. ARMSTRONG (1989) pointed out the following weaknesses in SAH: Complete cell sorting, transitive relationship, engulfment of tissue fragments and pattern reversal. There have been no theoretical models based on SAH.

On the other hand, DAH attaches importance to the relative strength of cell adhesion, not to the specificity of cell adhesion (STEINBERG, 1963). The strength of cell adhesion is described as cell adhesion energy. The stronger the strength of cell adhesion, the greater the adhesion energy. Cell adhesion differs in strength depending on the combination of cell

types. Thus, adhesion energy varies in the same manner. Cell movements within an aggregate are driven to increase the adhesion energy by thermodynamic processes. Eventually, the total adhesion energy of the aggregate reaches a maximum, and the final configuration is stable. DAH well explains "complete cell sorting", the final configuration where a spherical domain of one cell type is completely enveloped by a domain of the other.

Based on DAH, several mathematical models were formulated (GOEL *et al*., 1970; LEITH and GOEL, 1971; GORDON *et al*., 1972; ANTONELLI *et al*., 1973, 1975; GOEL and ROGERS, 1978; MOCHIZUKI *et al*., 1996). However, some models could not reproduce complete cell sorting (GOEL *et al*., 1970; ANTONELLI *et al*., 1973, 1975; MOCHIZUKI *et al*., 1996). Other models required assumptions such as remote interactions among cells to reproduce complete cell sorting (LEITH and GOEL, 1971; GORDON *et al*., 1972; GOEL and ROGERS, 1978).

The difficulty in reproducing complete cell sorting is due to the existence of configurations which have local maxima in the total adhesion energy. The cell rearrangement is halted at a local maximum in the energy landscape.

GRANER and GLAZIER (1992, 1993) proposed an extended Q-pot model (ANDERSON *et al*., 1984) to overcome the difficulty. GRANER (1993) argued that if cell movement was continuous, the cell configuration could reach the global maximum and bypass irrelevant local maxima. GRANER and SAWADA (1993) proposed a flexible geometrical model as the model in which cell movement is continuous.

DAH deals with cell sorting by using a physical quantity, adhesion energy. Because of this bold simplification, it explains cell sorting briefly in terms of "energetics". However, such simplification seems too rough because cell sorting takes place only when cells are alive in an aggregate. Thus, it is important to take the autonomous activity of each cells into account when constructing a model, whereas under DAH, each cell is regarded as an analogue of a liquid molecule following the gradient of the energy landscape.

Our model is the first model which explains cell sorting consistently with the cell activity. Moreover, it is able to overcome the above weaknesses in SAH, under specific adhesion.

2. Model

2.1. Assumption: The cell as an individual in cell sorting

Current experimental studies are identifying many cell adhesion molecules, proteins which mediate cell adhesion, and are clarifying their functions gradually. Cell adhesion molecules (CAMs) are transmembrane proteins. They cohere selectively according to their conformations. Moreover, they are likely to act as receptors, and affect the inner state of a cell by transmitting external information; that is, they can act as signal transducers (TAKEICHI, 1991).

A cell may be led to a spontaneous movement state by variation of the inner state. We should distinguish spontaneous movement from thermodynamic movement. Spontaneous movement means directional movement observed in cell locomotion (WOLPERT *et al*., 1969; HEATH and HOLIFIELD, 1991; STOSSEL, 1993) and thermodynamic movement refers to that seen in microscopic Brownian motion assumed under DAH. If the cell moves randomly, then the cell alters the direction of the movement randomly for itself. Additionally,

we should distinguish directional movement from movement directed by a chemical gradient that may be present in an aggregate (TOWNES and HOLTFRETER, 1955).

Hence, a cell is an individual which receives signals from its surroundings (adjacent cells), varies its inner state in response to the signals, exerts an affect on its surroundings, and moves spontaneously. Our model is constructed based on these assumptions.

2.2. Assumption: Active cell movement

We assume that each cell has an inner state relevant to spontaneous cell movement. The inner state varies depending on signals from adjacent cells, and each cell becomes activated when the inner state exceeds a certain critical level. The activated cell breaks the adhesions between adjacent cells to move, and thrusts its way among the neighboring cells. The cell is likely to invade a gap between adjacent cells (Fig. 2). The moving cell is most likely to penetrate two adjacent cells where the adherence is weakest.

Whether or not the invasion of the moving cell succeeds depends on the state of the invaded cells. There is one invaded cell on either side of the gap. If neither of the invaded cells are in the activated state, the invading cell fails to migrate. If either cell is in the activated state, that cell changes position with the invading cell. If both are in the activated state, the more active cell changes position. Finally, the adhesions torn by those cells are reestablished (Fig. 3).

In reality, all cells in an aggregate move continuously and simultaneously, and the relative positions of the cells vary gradually. However, for simplification, we assume that the cell movements are discrete, and that the position changes occur exclusively in our model; namely, the cells change their positions by leaping to the adjacent cell position, and more than two position changes do not occur simultaneously.

Here, we do not assume remote interactions, only local interactions via CAMs, and local behavior of cell movement.

2.3. The mathematical formulation

For simplification, our model is two dimensional. We consider finite hexagonal lattice

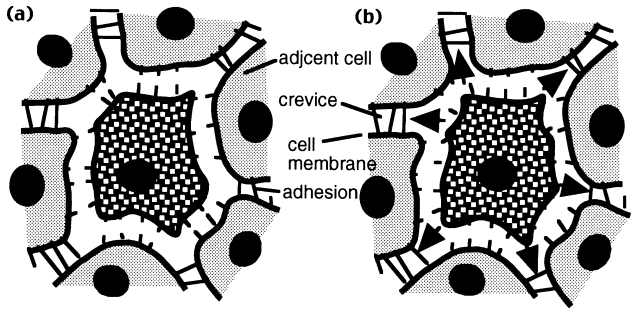

Fig. 2. The direction of cell movement: (a) A centered cell is activated and breaks the adhesions between adjacent cells. (b) The activated cell tries to invade one of the gaps to which arrows point. The chosen gap is likely to be weakest in adhesion.

space. Each lattice point is occupied by one cell of either type, called “dark” and “light” for convenience (Fig. 4).

We denote the inner state of the i-th cell by S_i, which has nonnegative integer values.

$$S_i = 0, 1, 2, \ldots (i = 1, \ldots, n),$$

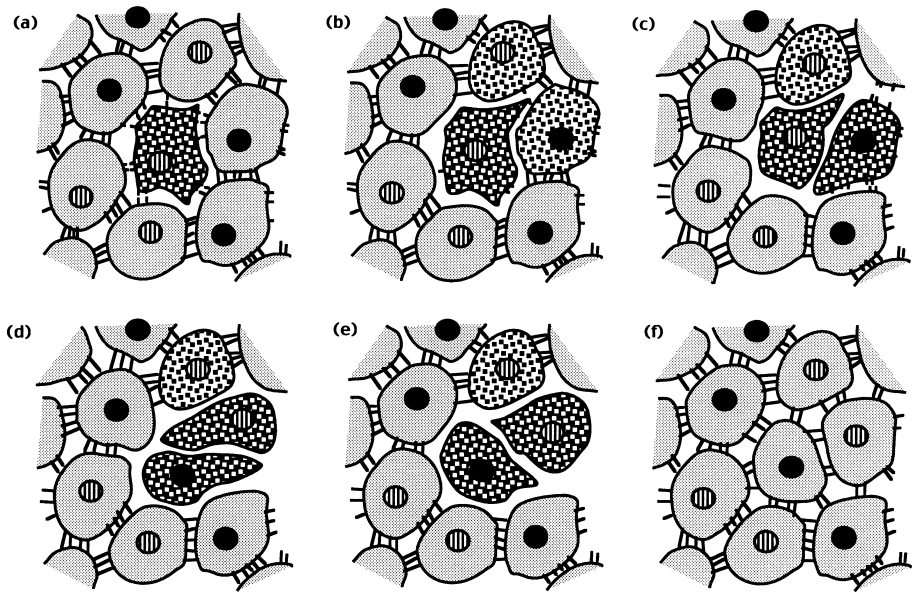

Fig. 3. The process of cell movement of two dimensions: (a) The centered cell was activated. (b) The cell is trying to invade an upper right gap. There are two invaded cells (a little dark). (c)–(e) One of the two became activated and is changing position with the invading cell. (f) Finally, adhesions are restored after the position exchange.

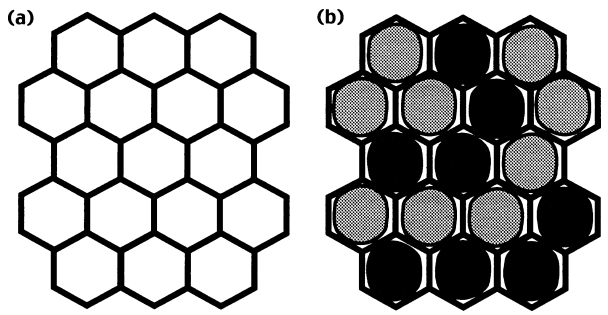

Fig. 4. (a) Finite hexagonal lattice space of two dimensions. (b) Each lattice is occupied by a “dark” or “light” cell.

where n is the number of cells in an aggregate.

The inner state S_i is the sum of inner state increases induced by signals from the adjacent cells:

$$S_i = \sum_{j=K_i} \Delta S_j, \quad K_i = \text{nearest neighbor cells of the } i\text{ - th cell, } \Delta S_j \in \{\Delta S_l, \Delta S_u, \Delta S_a\},$$

where ΔS_l and ΔS_u denote the inner state increases induced by a signal from the like cell and unlike cell respectively, and ΔS_a denotes the increases induced by no signal, which means the absence of an adjacent cell.

In this paper, we assume that $\Delta S_l = 0$, $\Delta S_u = 1$, and $\Delta S_a = 2$. Thus, the signal from a like cell does not increase the inner state, the signal from an unlike cell increases the inner state, and no signal by absence of an adjacent cell increases it further.

Usually, the absence of a cell occurs on the periphery of the aggregate. However, we assume another situation where adjacent cells are absent. While trying to move, the cell breaks the adhesion between adjacent cells. Therefore, CAMs torn by the cell movement no longer receive signals from adjacent cells. Consequently, when a cell tries to move, it always increases the inner state of the adjacent cells.

When S_i exceeds a critical level, the i-th cell becomes activated. The critical level is a parameter that we varied by setting different values for, on which we based our simulations.

We assume that the inner state value of an activated cell directly indicates the movement activity of the cell. Therefore, in the process of position change, if both of the invaded cells are activated, and $S_j > S_k$, then the j-th cell changes position with the invading cell.

The moving cell invades a gap between the cells whose adherence to each other is weakest. If there are two or more such gaps, one of them is chosen at random.

As for the strength of cell adhesion, both homotypic adhesions are 2 and heterotypic adhesion is 1 for all simulations, indicating that the heterotypic adhesion is weaker than both homotypic adhesions. This condition satisfies the specific adhesion.

Within one step, whether or not the position change of each cell occurs is examined in turn, but the order of the examination is decided randomly at each step.

Simulations in this study were carried out from 3000 to 40000 steps.

3. Results

The number of hexagonal lattice points on an aggregate is 1600 (40 by 40). Each cell type has a different critical level, so we denote the critical level of dark cell and light cell by C_d and C_l, respectively.

Initially, two configurations of aggregates were prepared: Randomly mixed aggregates, and aggregates composed of two domains of different cell types.

In the first, when $C_d = C_l = 4$, cell sorting was incomplete (Fig. 5). This condition means that cells of neither type become activated, even if the majority of adjacent cells are unlike cells. At a certain point, the aggregate configuration seemed to mostly freeze, although many cells changed their positions (Fig. 6).

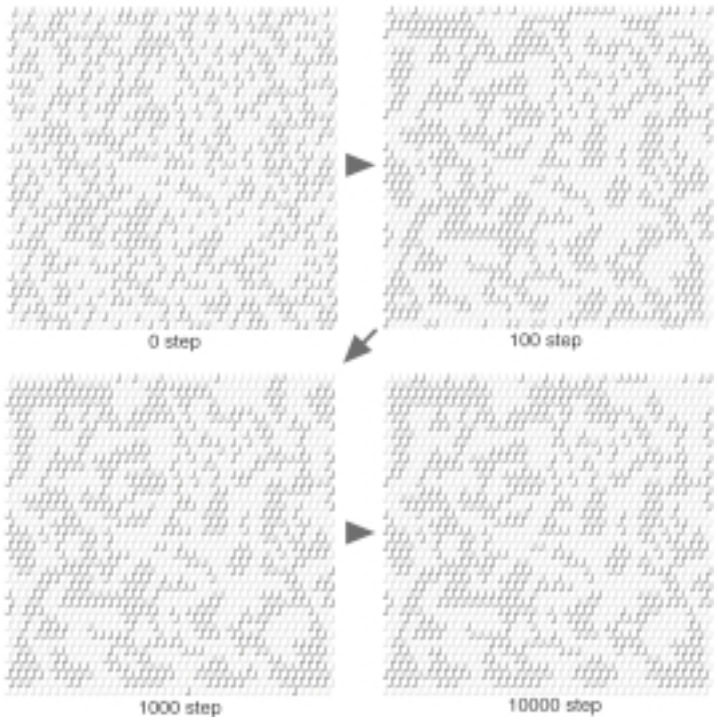

Fig. 5. A simulation result: Initial configuration is randomly mixed. When $C_d = C_l = 4$, cell sorting was incomplete.

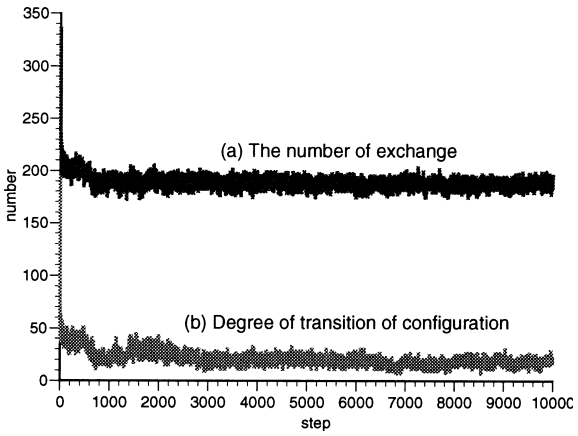

Fig. 6. (a) is the number of position exchange occurring within one step. (b) is the Hamming distance between the present configuration and the preceding one at each step.

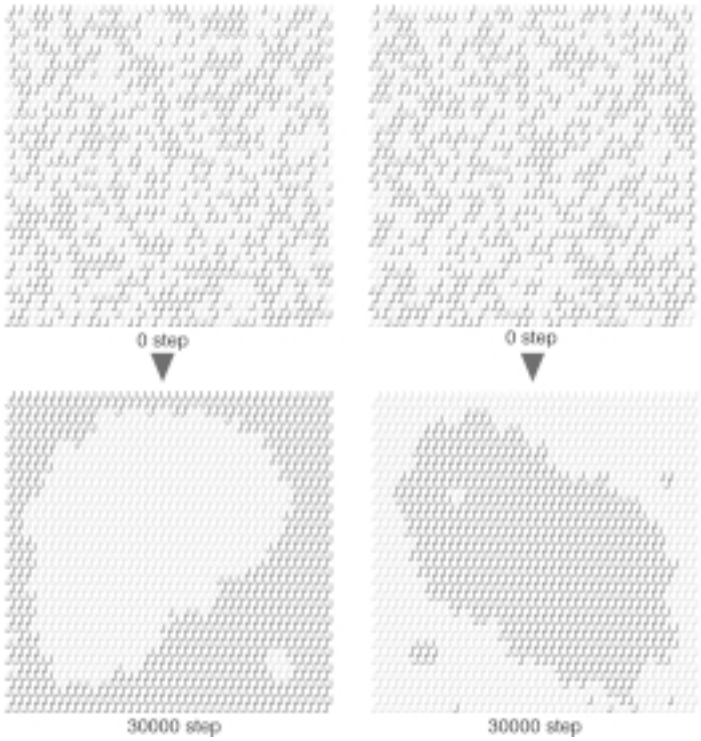

Fig. 7. Two examples of the result when $C_d = C_l = 2$: Complete cell sorting was attained but it was variable which cell type was enveloped by the other.

When $C_d = C_l = 2$, complete cell sorting was attained (Fig. 7). This condition means that cells of both types become activated, even when few of the adjacent cells are unlike cells. However, it was not fixed which cell type enveloped the other in each simulation because these two cell types had an equal critical level and cell adhesion.

When $C_d = 2$ and $C_l = 1$, complete cell sorting occurred. The dark cell type always occupied the inner part of the aggregate, and was surrounded by the light type (Fig. 8). This condition means that cells of both types are easily activated by few unlike cells, but the dark ones are relatively difficult to activate. Under these conditions, randomly mixed cells formed some domains of like cells in the early stage (Fig. 8b). The light cell domain seemed to be connected, and the dark cell domains were disconnected and seemed to be islands in a sea of light cells. The relative positions of these domains did not change much, but the domains altered their shape. Once contours of the domains contacted each other, they coalesced and formed a larger domain (Figs. 8c–f). The above process of formation of the larger domains was similar to that described by TRINKAUS and LENTS (1964). Finally, repeated coalescence and transformation of the domains resulted in one large, central, dark domain, enveloped by the domain of light cells.

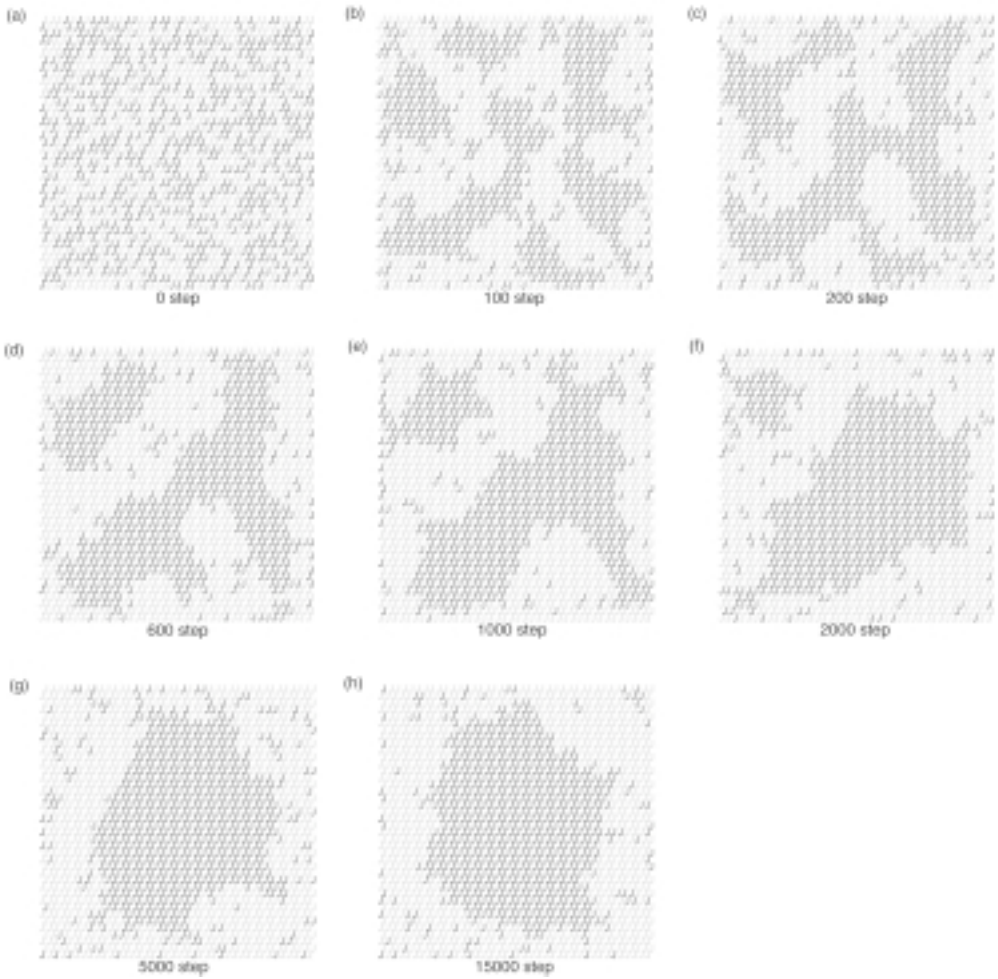

Fig. 8. When $C_d = 2$, $C_l = 1$, complete cell sorting was attained and the dark cell type was always enveloped by the light one.

GLAZER and GRANER (1993) predicted that in the case of active cell movement, the progress of cell rearrangement was ergodic. In our simulations, however, the progress was not ergodic.

According to experimental observation, cells belonging to domains do not detach themselves from the domains (TRINKAUS and LENTZ, 1964). However, detached cells were seen in our simulations. This may arise from a disadvantage of our model, in which cells leap to adjacent positions, changing places with adjacent cells locally. Figure 9 shows how some cells break away from a domain.

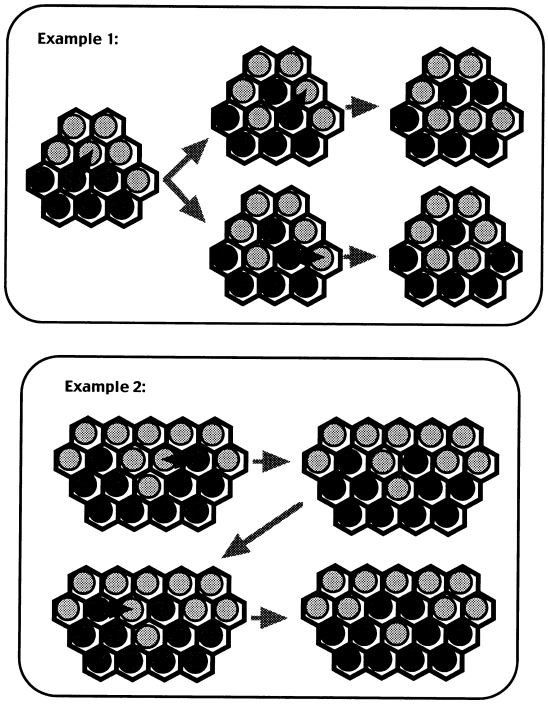

Fig. 9. This figure shows how cells break away from a domain.

In the second initial configuration, an aggregate was composed of two domains of different cell types. Under the conditions that $C_d = 2$ and $C_l = 1$, the domain of the dark cell type was always engulfed by the domain of the light cell type (Fig. 10). The engulfed cell type was identical to that enveloped in the simulations of cell sorting.

4. Discussion

There are four observations to consider relating to cell sorting as follows: 1) rounding of an aggregate, 2) transitive relationship, 3) engulfment of tissue fragments, and 4) pattern reversal. These observations are discussed in detail with our model.

4.1. Rounding of an aggregate

When an aggregate is cultured, even if its shape is initially jagged, it becomes rounded in the course of time.

The simulation results showed that the dark domain rounded, although its shape was jagged in the middle state (Figs. 8c–f). The contour of the light domain, however, remained square. This may also arise from a disadvantage of the model. We did not vary the shape of the aggregate, because the model operates only on the changes in cell positions. This

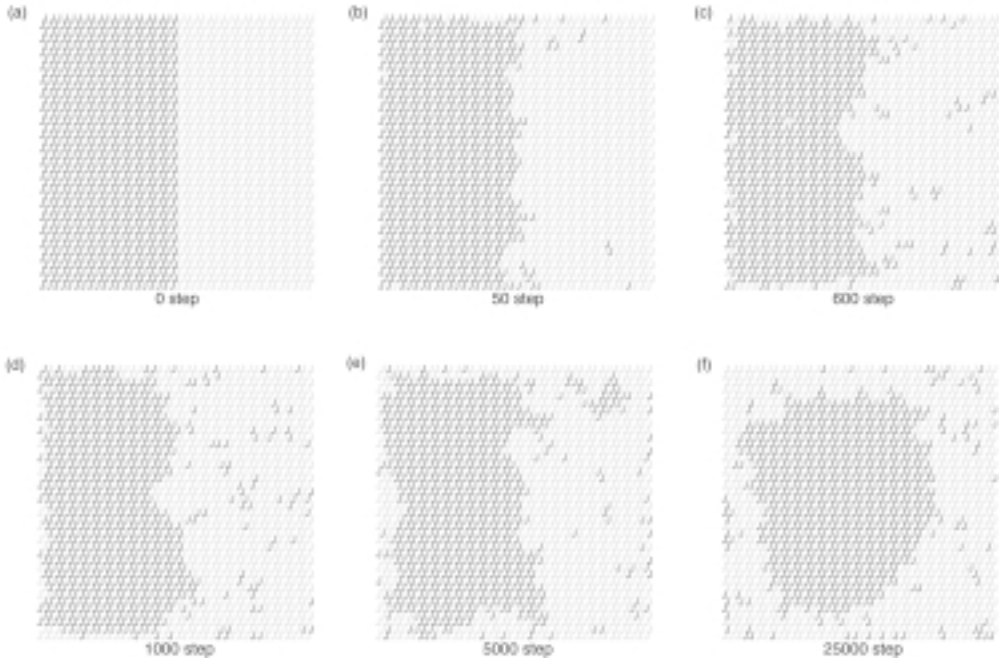

Fig. 10. Initial configuration is composed of two domains of different cell types. When $C_d = 2$, $C_l = 1$, the light cell type always engulfed the dark one.

disadvantage may be eliminated by making cell movement continuous and allowing cells to change shape.

4.2. Transitive relationship

Transitive relationship means that if cell type A envelops cell type B and type B in turn envelops type C, then type A envelops type C. Transitive relationship was reported in several cell types (STEINBERG, 1970).

Our model can account for this relationship using critical levels. The simulation results showed that in combinations of two cell types, the cell type whose critical level was higher was enveloped by the other. Conversely, the cell type whose critical level was lower enveloped the other. If A envelops B, and B envelops C, then the critical level of cell type A, C_A is lower than the critical level of cell type B, C_B, and C_B is lower than the critical level of cell type C, C_C. Thus, C_A is lower than C_C, and so A envelops C. Thus, transitive relationship can be explained by the magnitude of the critical levels.

4.3. Engulfment of tissue fragments

When two different tissue fragments are apposed in culture, the fragment of one tissue type engulfs the other. In various combinations of two tissue types, one is always engulfed

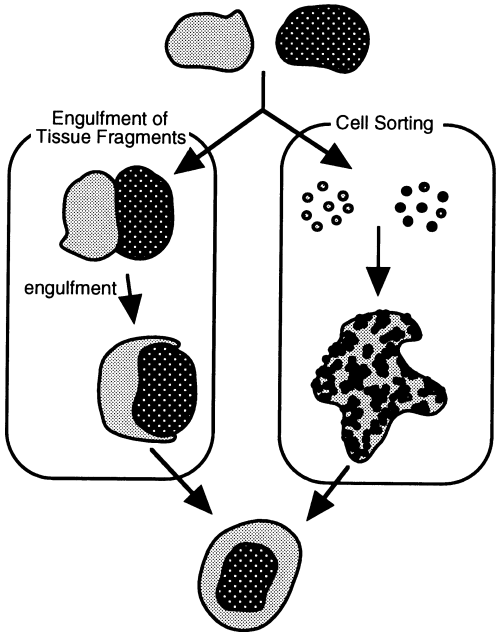

Fig. 11. The rough sketch of engulfment of tissue fragments and cell sorting.

by the other according to each combination. The engulfed tissue type is the same as the cell type enveloped in cell sorting if the two cell types are derived from the same two tissues (Fig. 11).

Our model can reproduce the engulfment under the specific adhesion. The engulfment took place in our model in the same way as in experimental observations (Fig. 10).

4.4. Pattern reversal

In certain combinations of two cell types, varying certain experimental conditions brings about reversal of the envelopment relationship (ARMSTRONG and NIEDERMAN, 1972). This phenomenon, called pattern reversal, is explained by DAH as follows: Ingredients of culture medium affect cell metabolisms, or proteins expressed by cells of one type affect the metabolism of the other cell type. In response to the ingredients or proteins, the cells express other proteins which change the adhesion strength. These changes in adhesion strength are due to pattern reversal (WISEMAN *et al.*, 1972; ARMSTRONG and NIEDERMAN, 1972; ARMSTRONG, 1980; ARMSTRONG and ARMSTRONG, 1984).

In our model, pattern reversal is explained as follows: When the cell metabolisms are affected by the ingredients or proteins, the critical levels of the cells vary. Consequently, pattern reversal occurs when the critical levels are reversed.

4.5. Advantages of our model

Our model is an exchange model, in which cell movements are discrete, and changes in cell position occur locally, instantaneously and exclusively. The change in position has often been thought to be an obstacle in reproducing complete cell sorting. In fact, the existing exchange models based on DAH could not reproduce both complete cell sorting and tissue engulfment without remote interactions among cells. Thus, ours is the sole model that can reproduce both complete cell sorting and tissue engulfment with only local interactions among cells in all exchange models.

There are two obvious differences between our model and existing models. The first difference is the perception of a cell. Movement of a cell in the existing models is restricted by adhesive strengths between itself and neighboring cells, and the cell is also so driven that the adhesive strengths increase. This means that adhesion forces pull cells, and the cells are merely driven by the adhesions. Cell movement is passive, and is completely dependent on the surroundings even if the cell motion is active. The movement can be described by the total adhesion energy, which is a function of the relative positions of the cells. The cell position and strength of adhesion are enough to describe cell behavior in cell sorting.

On the other hand, movement of a cell in our model is unrelated to the adhesions between itself and neighboring cells. The cell receives external signals, changes its inner state in response to the signals, and moves actively according to the inner state. The cell motion is so active as the cell can detach itself from the neighboring cells. In describing cell behavior in our model, we considered the information system, which includes the inner state, of each cell. Our model assumes that cell behavior is not so simple as to be described only by its position and adhesion.

The second difference is the existence of a predetermined final destination in the configuration of the whole aggregate. The existing models assume complete cell sorting to be a configuration which has the global maximum in total adhesion energy, and that sorting is aimed at the global maximum as the final destination. Therefore, in these models, reproducing complete cell sorting is predictable although many models were elaborated on to reproduce complete cell sorting.

On the other hand, in our model, a predetermined final destination does not exist. Each cell movement is decided independently by the inner state of each cell and local information from the nearest neighbor cells. Needless to say, there was no global information on the aggregate such as positional information. Nevertheless, the global pattern of complete cell sorting, central and peripheral domains, emerged. Therefore, autonomous individual behaviors of cells as a whole generated the regulated configuration of the aggregate without global information. Our model is advantageous in that it allows for self-organization.

Another advantage of our model lies in its expandability. Since we regard cell behavior in response to signal transduction as important in the construction of the model, it can be expanded to model the process of “development”. Our model can account for “induction”; the transformation of cell type through signal transduction, although we assumed that cell behavior is relevant to cell movement in this paper. Induction is one of the main factors of “differentiation” in the development process. Our model can also accommodate alterations of cell adhesion and motility (HYNES, 1992; HYNES and LANDER, 1992). That gives a new perspective on the development process.

5. Conclusions

We assumed that the individual cell activity of each cell is intrinsic in cell sorting when constructing the model. Our model can reproduce or explain, (i) rounding of an aggregate, (ii) cell rearrangements seen typically in cell sorting, (iii) engulfment of tissue fragments, (iv) similar configurations in cell sorting and in tissue engulfment, (v) transitive relationship, and (vi) pattern reversal based only on local interactions among cells, and local behavior of each cell. The conditions of the simulations in the model can satisfy specific adhesion which has been supported by experimental studies.

A cell is assumed to, (a) receive information from its surroundings (adjacent cells), (b) change its inner state in response to the information, (c) affect the adjacent cells actively, and (d) move, or rather thrust its way through the weakest adhesion point spontaneously. These assumptions are fit for the current perception of a cell in cell biology.

Assumption (c) includes a situation that whenever a cell attempts moving, it activates the adjacent cells, facilitating the position change. Whether such activation is required for complete cell sorting needs to be investigated in future study.

The discrete cell movements caused cells to detach from domains. To eliminate this disadvantage, an adjustment which makes cell movement continuous or quasi-continuous may be needed.

Finally, our model is expandable for application to the development process.

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