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Cell Mosaic Patterns under the Direct Lateral Inhibition Rule of Differentiation

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Abstract. A direct lateral inhibition of cell differentiation is one of the mechanisms to generate cell mosaic patterns: there are homogeneous cells initially and once a cell differentiated, it inhibits its immediate neighbors' differentiation. We elucidated the range of variety of mosaic patterns allowed under the direct lateral inhibition rule on a linear and hexagonal cell array. Then we considered processes of these pattern formations. Some of these patterns were being made using the hypotheses of sequential or random determination of cell fates, which are reasonable on the basis of actual observations.

1. Introduction

Formation of cell mosaic patterns on a homogeneous sheet is one of initial stages of the development of an embryo having no noticeable feature to a body full of variety (Honda, 1990; Honda *et al.*, 1986). Among several mechanisms of mosaic pattern formation, lateral inhibition of cell differentiation is worth to notice though it is worn-out and simple, because recent works on early neurogenesis of insects have shown that the lateral inhibition was not merely a theory, but a fact being confirmed by discovery of actual substances which directly mediate

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inhibitory interactions.

That is, initially homogeneous cells are all competent to differentiate into neuroblasts. Once a cell has differentiated as a neuroblast, it inhibits its immediate neighbors from following this pathway. The inibitory interaction among cells is mediated by membrane-bounded proteins at the surface. Thus the interaction is limited to immediate neighbors (Hartenstein and Campos-Ortega, 1985; Doe and Goodman, 1985a, b; Campos-Ortega, 1985, 1988; Wharton *et al.*, 1985; Vassin *et al.*, 1987; Technau and Campos-Ortega, 1987; Kidd *et al.*, 1989).

The mechanism of interaction between immediate neighbors enables us to perform simple computer simulations assuming that the sequence of cell fate determination is at random or along one of body axes. The simulations took place on disordered polygonal patterns which are close to actual cellular polygons (Tanemura *et al.*, 1991), and using a boundary condition of simulation field which is plausible to a neurogenetic region of insect embryos (Honda *et al.*, 1990). These works succeeded in quantitative explanation of the insect neurogenesis, i.e., reasonable estimation of number of neuroblasts formed in the neurogenic region.

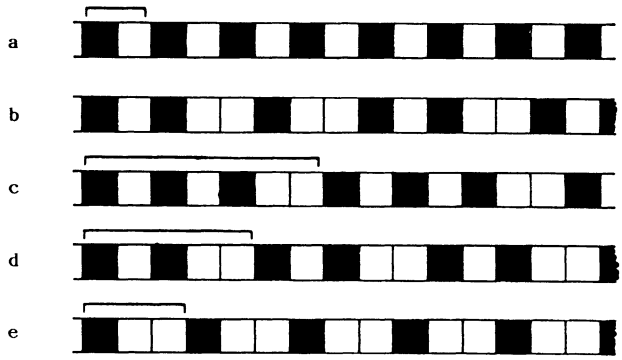
Here we will study the direct lateral inhibition in advanced theoretical aspects. That is, (1) to examine variety range of patterns which were allowed in principle under the direct lateral inhibition rule, and (2) to generate actually some patterns among the above-mentioned theoretical patterns by a hypothesis which was considered to be reasonable based on experimental works.

2. One-Dimensional Cell Array

At first, we will consider a linear array of cells, where cells on a line are initially homogeneous. The direct lateral inhibition rule does not allow an existence of two differentiated cells which are immediately neighboring with each other. In addition, we assume all cells differentiate, which were allowed to differentiate under the rule. Therefore, an array consisting of three neighboring undifferentiated cells does not exist at all, because the middle cell in the array ought to differentiate according to the rule.

Two extreme cases are shown in Figs. 1a and e, where differentiated cells were designated by solid rectangle. Two types of cells were alternated in Fig. 1a, and cell number ratio of undifferentiated to differentiated cells are the minimum (1.0). On the contrary, Fig. 1e shows the maximum cell number ratio (2.0). We can make numerous intermediate patterns between the two. Examples of cell number ratio = 1.33 (4/3) and 1.5 (3/2), which consist of periodic repeats, are shown in Fig. 1c and d.

The alternate pattern (Fig. 1a) can be generated if the cell fate determination whether or not to differentiate takes place sequentially in a cell array, e.g., left to right. That is, the left end cell (the first cell) in Fig. 1a differentiates, the second cell from the left end remains undifferentiated because of the lateral inhibition by the first cell, the third cell differentiates because of no inhibition from the left side,



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and so on. However, other patterns in Fig. 1 except 1a can not be generated by such an above-mentioned method. These patterns could be generated if we postulate that substances mediating the lateral inhibition are diffusible, differentiated cells inhibit cell differentiation, not individually, but in a cell block (a unit consisting of a few cells), cells have the third state in addition to differentiated and undifferentiated states, or any reaction-diffusion model of self-organization system works as a whole (Turing, 1952).

Another assumption of sequence of the fate determination, which is plausible on the basis of actual observations, is that cells are determined their fate at random. That is, an undifferentiated cells in a linear array is picked up at random and tested whether or not it is allowed to differentiate. If the cell is not inhibited by any immediate neighbor (left or right neighbors), it differentiates. This procedure proceeds until all cells that are allowed to differentiate have done so, providing the final pattern of heterogeneity (Fig. 1b). The number ratio of undifferentiated to differentiated cells in the pattern can be estimated. Our computer simulation, with a one-dimensional cell array of 1000 cells, under a periodic boundary condition at the left and right ends of the cell array, has shown an average ratio of 1.299 ± 0.029 (mean and standard deviation, sample size = 10). The condition which was used in the simulation was identical to that of the mathematical problem that a line was filled sequentially at random with non-overlapping intervals, which has already been solved analytically as the ratio = $[1 + \exp(-2)]/[1 - \exp(-2)] = 1.3130$ by Page (1959) and Mackenzie (1962).

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antero-posterior direction and consists of two types of cells, which are S1 and S2. S1 cells have lost microvilli first on the apical cell surface during the developmental process, while S2 cells keep abundant microvilli for a long time. Most of S1 cells are not neighboring immediately with each other. Thus S1 cells seem to correspond to the differentiated cell in the direct lateral inhibition hypothesis. Cell number ratio of S2 to S1 was reported to be 1.32 (91/69) by Yoshida *et al.* (1989). The value is that expected under the direct lateral inhibition rule in the linear cell array by Page (1959) and us.

3. Two-Dimensional Cell Array

We will consider possible mosaic patterns having periodic repeats under the direct lateral inhibition rule on a regular hexagonal lattice. A block consisting of seven cells was handled as a unit where a differentiated cell was encircled by six undifferentiated cells (Fig. 2a). There are numerous ways to pack these blocks in a plane without gaps (Figs. 2b–d), while overlaps (Figs. 2b and c) of one cell width are allowed. The closest packing is shown in Fig. 3a where the ratio of undifferentiated to differentiated cell numbers is 2. On the other hand, the sparsest pack is in Fig. 3f with cell number ratio = 6. There are many cell arrays between the two. Some of them are shown in Figs. 3b–e.

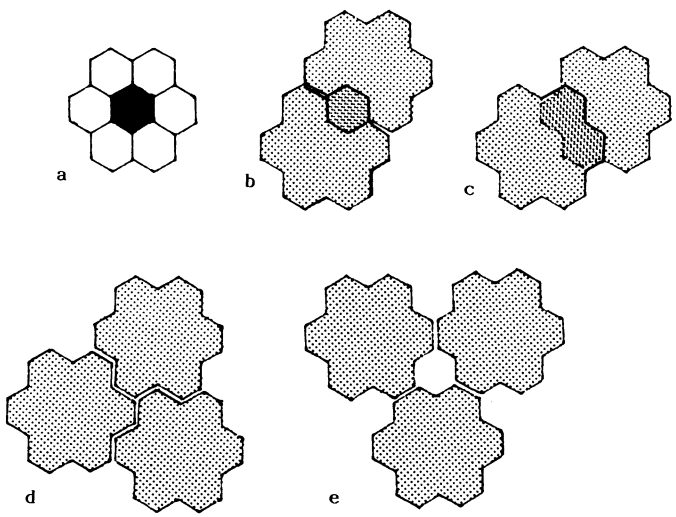

Fig. 2. (a) a block consisting of one differentiated cell and six undifferentiated cells encircling it. (b), (c) overlaps of one cell width between blocks which are allowed under the direct lateral inhibition rule. (d) a neat arrange which corresponds to the pattern of Fig. 3f. (e) a gap which is not allowed under the rule.

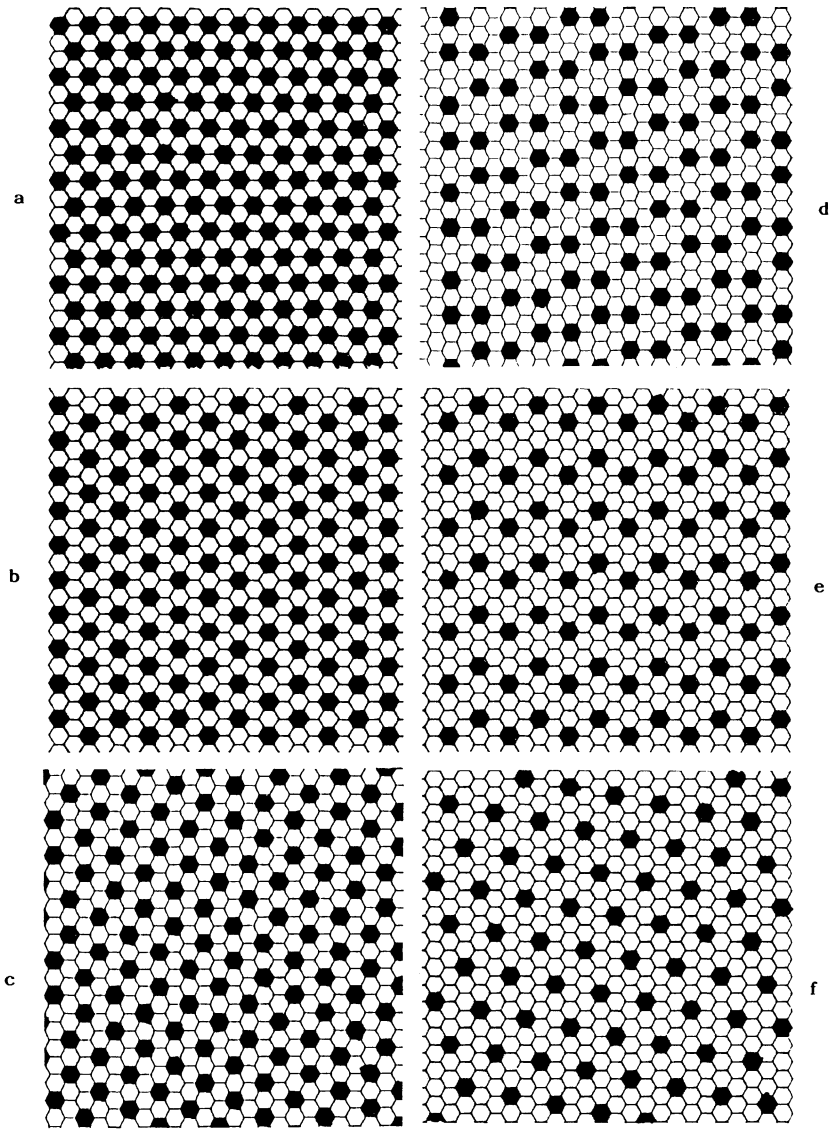

Fig. 3. Mosaic patterns on two-dimensional cell array under the direct lateral inhibition rule. Differentiated cells were designated by a solic hexagon. Number ratio of undifferentiated to differentiated cells is 2.0 (a, the closest), 3.0 (b, c), 4.0 (d), 5.0 (e) and 6.0 (f, the sparsest), respectively.

We know a few actual cell mosaics corresponding to some of these cell number ratios. An example of cell number ratio = 2 is the epidermis of an axolotl embryo which consists of ciliated and non-ciliated epidermal cells. The cell number ratio of non-ciliated cells to ciliated cells is about 2 (Landström, 1977). The second one is neuroblasts being determined among many equivalent ectoderm cells during neurogenesis of insect embyos as mentioned already. Immediate neighbors of the neuroblasts were inhibited from taking on the same fate, then becoming epidermoblasts. The cell number ratio of epidermoblasts to neuroblasts is 3–4 in fruitfly and grasshopper embryos (Campos-Ortega, 1985; Doe and Goodman, 1985b).

We can make the pattern with cell number ratio = 2 under the simple hypothesis that the cell fate determination takes place sequentially in one direction, e.g., from left to right. The hypothesis is reasonable because the cell fate determination is known to take place from the medial and lateral sides of neurogenic region of fruitfly embryos (Hartenstein and Campos-Ortega, 1984; Honda *et al.* 1990). We will consider that a front of differentiation runs along various directions on a polygonal pattern. Cells on the front line is determined whether or not to differentiate under the direct lateral inhibition rule. In Fig. 4a front of differentiation runs from left to right where a hexagonal lattice is rotated. The cells in front in Figs. 4a and b are all not neighboring immediately. Since these cells do not prevent from differentiating with each other, they differentiate all. After the front runs to right, the mosaic pattern with cell number ratio 2.0 as Fig. 3a has been generated (Fig. 4c).

We made various mosaic patterns by rotation of a front line in various degree. Some of results are shown in Figs. 5a–d, where cell number ratios are 2.5 (= 5/2, a), 2.66 (= 8/3, b) and 3.0 (= 3/1 c, d). The pattern of Figs. 5c and d have the maximum cell number ratio among them. We have not succeeded at present in making patterns having more ratio value under the hypothesis of sequential

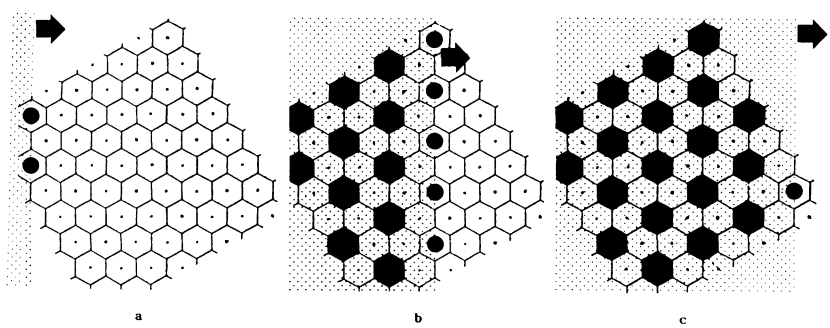

Fig. 4. Generation of a cell mosaic by the hypothesis that the cell fate determination takes place sequentially in one direction under the direct lateral inhibition rule. The front line of determination moves from left to right (a→b→c).

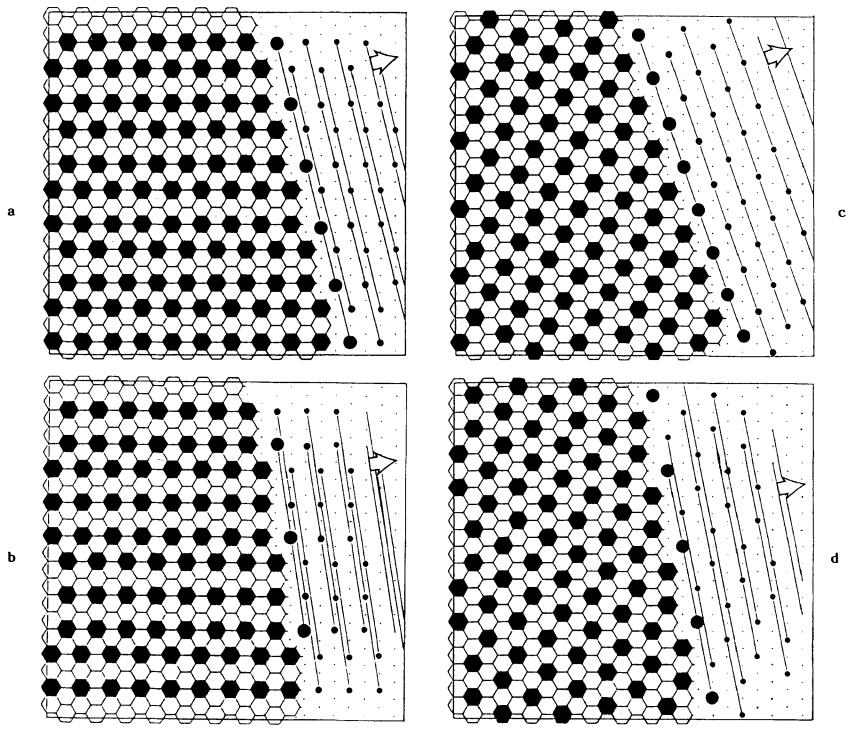

Fig. 5. Cell mosaics made by the hypothesis described in Fig. 4. Cell number ratio is 2.5 (5/2, a), 2.66 (8/3, b), 3.0 (c, d), respectively. d corresponds to Fig. 3c.

determination of cell fates.

Another way to make cell mosaic is to determine cell fates, not sequentially, but at random. This case was already performed (Tanemura *et al.*, 1991). Cells are picking at random on a hexagonal lattice consisting of 50 × 50, 100 × 100 or 150 × 150 hexagons. A picked cell was tested if it differentiates or not under the direct lateral inhibition rule. That is, if the cell has not differentiated and has no differentiated cell among its immediate neighbors, its differentiation is switched on. Otherwise, the cell is left as it is. Once a cell differentiates, the cell and its immediate neighbors are excluded and a new cell is picked at random from the remaining cells. This procedure is repeated until all cells that are allowed to differentiate have done so. The computer simulation was performed using the periodic boundary condition. One of resultant examples is shown in Fig. 6. The ratio of undifferentiated to differentiated cell numbers was 3.32. The value is larger than these by sequential determination of cell fates (Figs. 5a–d), and corresponds to the cell number ratio (3–4) of the insect neurogenesis (Tanemura

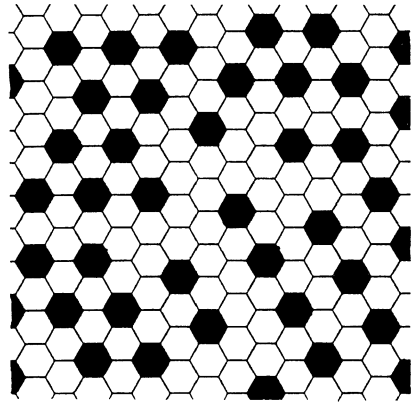

Fig. 6. A cell mosaic by the hypothesis of random determination of cell fates under the direct lateral inhibition rule.

et al., 1991).

We have not made other mosaic patterns where cell number ratio is more than 3.32 as far as using the hypothesis of the simple sequential determination of cell fates. Mosaic patterns having larger cell number ratio could not be generated without assuming further complicated hypotheses of supervising control systems as mentioned in the previous section, "One-dimensional cell array". However, we do not have so solid foundations of actual observations that we perform to build the further complicated hypothesis.

We know the cell number ratio = 4.8 (= [91 + 241]/69) of early stage of epithelial cells forming scale rows on the wing of a butterfly *Pieris rapae* (Yoshida and Aoki, 1989). However, in this case, very large differentiated cells having lost microvilli were encircled by small cells having microvilli. The cell size difference results in increase of the cell number ratio, because many small cells are necessary to encircle a large cell. Thus, cell number ratio of the epithelium on early stage of butterfly wing development is an exceptional case. At present in ordinary systems, there is no actual observation of cell mosaics having large cell number ratio (e.g., more than 3.32) under the simple direct lateral inhibition rule.

We have to mention that the direct lateral inhibition is not a unique factor for the mechanism of cell mosaic formation, though it has solid foundations of actual observations and is convincing. There are several possible factors to influence the mosaic formation, e.g., diffusible substances which mediate the lateral inhibition, appearance of new cells through cell division, rearrangement of cells in a tissue, and intrinsic determination of cell fates through cell lineage. Generally speaking, we have to investigate cell mosaic carefully before application of the direct lateral inhibition of cell differentiation.

Part of the present investigation has been presented in Honda (1990).

REFERENCES

Campos-Ortega, J. A. (1985). Genetics of early neurogenesis in *Drosophila melanogaster*. *Trends Neurosci.* **8**: 245–250.

Campos-Ortega, J. A. (1988). Cellular interactions during early neurogenesis of *Drosophila melanogaster*. *Trends Neurosci.* **11**: 400–405.

Doe, C. Q. and Goodman, C. S. (1985a). Early events in insect neurogenesis I. Development and segmental differences in the pattern of neuronal precursor cells. *Devel. Biol.* **111**: 193–205.

Doe, C. Q. and Goodman, C. S. (1985b). Early events in insect neurogenesis II. The role of cell interactions and cell lineage in the determination of neuronal precursor cells. *Devel. Biol.* **111**: 206–219.

Hartenstein, V. and Campos-Ortega, J. A. (1984). Early neurogenesis in wild-type *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **193**: 308–325.

Hartenstein, V. and Campos-Ortega, J. A. (1985). Fate-mapping in wild-type *Drosophila melanogaster* I. The spatio-temporal pattern of embryonic cell divisions. *Roux's Arch. Dev. Biol.* **194**: 181–195.

Honda, H. (1989). Cell sheet transformation during body formation of living organisms. *Forma* **4**: 13–20.

Honda, H. (1990). Spatial images of living organisms and a cell sheet. (In Japanese). *Suurikagaku* No. **327**: 58–64.

Honda, H., Tanemura, M. and Yoshida, A. (1990). Estimation of neuroblast numbers in insect neurogenesis using the lateral inhibition hypothesis of cell differentiation. *Development* **110**: 1349–1352.

Honda, H., Yamanaka, H. and Eguchi, G. (1986). Transformation of a polygonal cellular pattern during sexual maturation of the avian oviduct epithelium: computer simulation. *J. Embryol. Exp. Morph.* **98**: 1–19.

Kidd, S., Baylies, M. K., Gasic, G. P. and Young, M. W. (1989). Structure and distribution of the Notch protein in developing *Drosophila*. *Genes Devel.* **3**: 1113–1129.

Landström, U. (1977). On the differentiation of prospective ectoderm to a ciliated cell pattern in embryos of *Ambystoma mexicanum*. *J. Embryol. Exp. Morph.* **41**: 23–32.

Mackenzie, J. K. (1962). Sequential filling of a line by intervals placed at random and its application to linear adsorption. *J. Chem. Phys.* **37**: 723–728.

Page, E. S. (1959). The distribution of vacancies on a line. *J. Roy. Statist. Soc.* **B21**: 364–374.

Tenemura, M., Honda, H. and Yoshida, A. (1991). Distribution of differentiated cells in a cell sheet under the lateral inhibition rule of differentiation, *J. Theor. Biol.* **153**: 287–300.

Technau, G. M. and Campos-Ortega, J. A. (1987). Cell autonomy of expression of neurogenic genes of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **84**: 4500–4504.

Turing, A. M. (1952). The chemical basis of morphogenesis. *Philos. Trans. R. Soc. London* **B237**: 37–72.

Vassin, H., Bremer, K. A., Knust, E. and Campos-Ortega, J. A. (1987). The neurogenic gene Delta of *Drosophila melanogaster* is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. *EMBO J.* **6**: 3431–3440.

Wharton, K. A., Johansen, K. M., Xu, T. and Artavanis-Tsakonas, S. (1985). Nucleotide sequence from the neurogenic locus Notch implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell* **43**: 567–581.

Yoshida, A. and Aoki, K. (1989). Scale arrangement pattern in a lepidopteran wing. 1. Periodic cellular pattern in the pupal wing of *Pieris rapae*. *Develop. Growth Differ.* **31**: 601–609.

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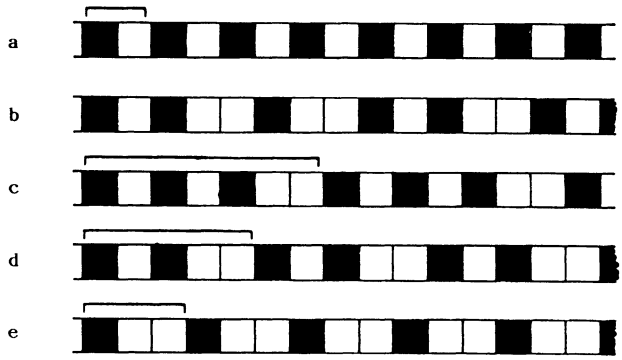

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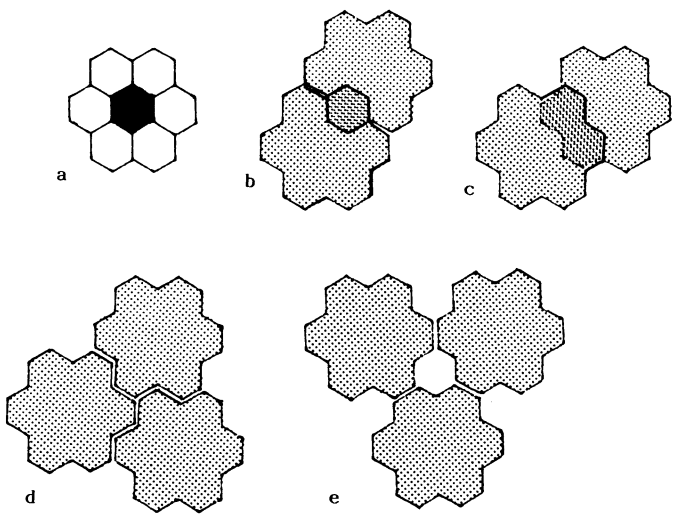

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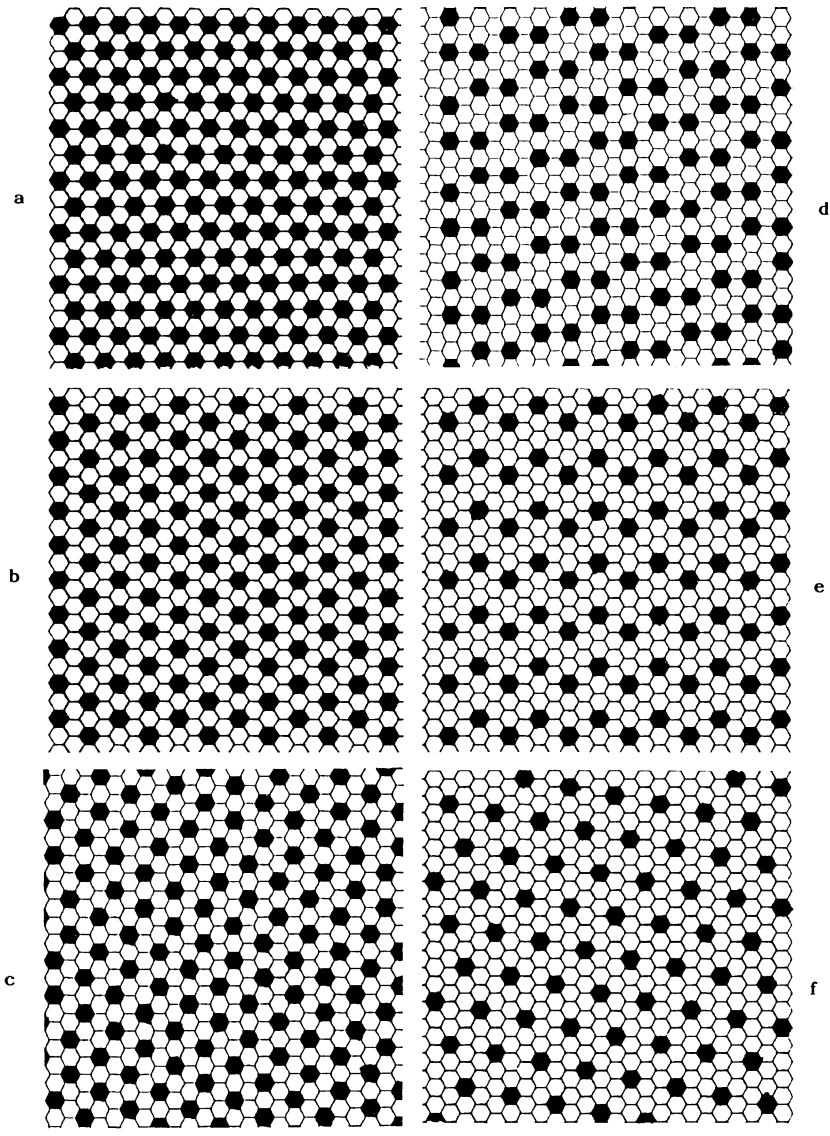

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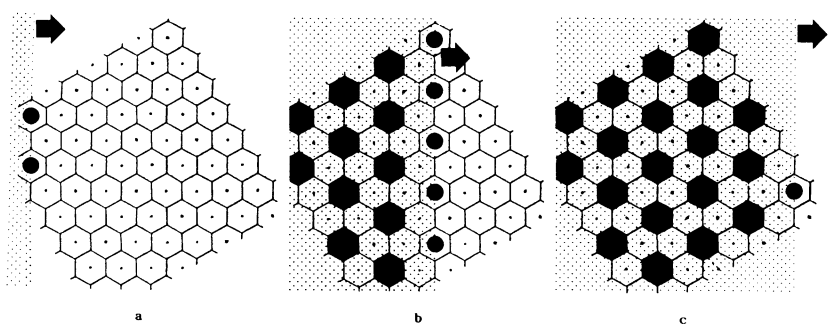

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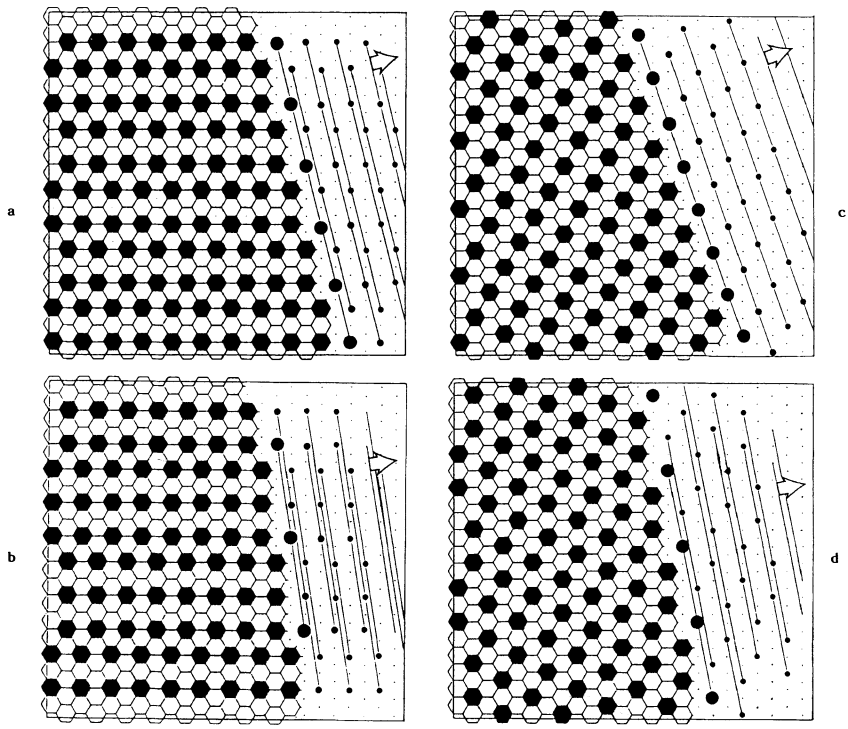

Fig. 5. Cell mosaics made by the hypothesis described in Fig. 4. Cell number ratio is 2.5 (5/2, a), 2.66 (8/3, b), 3.0 (c, d), respectively. d corresponds to Fig. 3c.

determination of cell fates.

Another way to make cell mosaic is to determine cell fates, not sequentially, but at random. This case was already performed (Tanemura *et al.*, 1991). Cells are picking at random on a hexagonal lattice consisting of 50 × 50, 100 × 100 or 150 × 150 hexagons. A picked cell was tested if it differentiates or not under the direct lateral inhibition rule. That is, if the cell has not differentiated and has no differentiated cell among its immediate neighbors, its differentiation is switched on. Otherwise, the cell is left as it is. Once a cell differentiates, the cell and its immediate neighbors are excluded and a new cell is picked at random from the remaining cells. This procedure is repeated until all cells that are allowed to differentiate have done so. The computer simulation was performed using the periodic boundary condition. One of resultant examples is shown in Fig. 6. The ratio of undifferentiated to differentiated cell numbers was 3.32. The value is larger than these by sequential determination of cell fates (Figs. 5a–d), and corresponds to the cell number ratio (3–4) of the insect neurogenesis (Tanemura

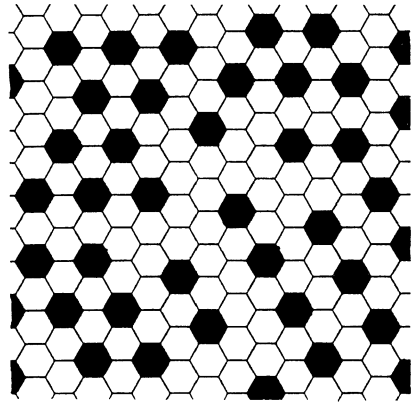

Fig. 6. A cell mosaic by the hypothesis of random determination of cell fates under the direct lateral inhibition rule.

et al., 1991).

We have not made other mosaic patterns where cell number ratio is more than 3.32 as far as using the hypothesis of the simple sequential determination of cell fates. Mosaic patterns having larger cell number ratio could not be generated without assuming further complicated hypotheses of supervising control systems as mentioned in the previous section, "One-dimensional cell array". However, we do not have so solid foundations of actual observations that we perform to build the further complicated hypothesis.

We know the cell number ratio = 4.8 (= [91 + 241]/69) of early stage of epithelial cells forming scale rows on the wing of a butterfly *Pieris rapae* (Yoshida and Aoki, 1989). However, in this case, very large differentiated cells having lost microvilli were encircled by small cells having microvilli. The cell size difference results in increase of the cell number ratio, because many small cells are necessary to encircle a large cell. Thus, cell number ratio of the epithelium on early stage of butterfly wing development is an exceptional case. At present in ordinary systems, there is no actual observation of cell mosaics having large cell number ratio (e.g., more than 3.32) under the simple direct lateral inhibition rule.

We have to mention that the direct lateral inhibition is not a unique factor for the mechanism of cell mosaic formation, though it has solid foundations of actual observations and is convincing. There are several possible factors to influence the mosaic formation, e.g., diffusible substances which mediate the lateral inhibition, appearance of new cells through cell division, rearrangement of cells in a tissue, and intrinsic determination of cell fates through cell lineage. Generally speaking, we have to investigate cell mosaic carefully before application of the direct lateral inhibition of cell differentiation.

Part of the present investigation has been presented in Honda (1990).

REFERENCES

Campos-Ortega, J. A. (1985). Genetics of early neurogenesis in *Drosophila melanogaster*. *Trends Neurosci.* **8**: 245–250.

Campos-Ortega, J. A. (1988). Cellular interactions during early neurogenesis of *Drosophila melanogaster*. *Trends Neurosci.* **11**: 400–405.

Doe, C. Q. and Goodman, C. S. (1985a). Early events in insect neurogenesis I. Development and segmental differences in the pattern of neuronal precursor cells. *Devel. Biol.* **111**: 193–205.

Doe, C. Q. and Goodman, C. S. (1985b). Early events in insect neurogenesis II. The role of cell interactions and cell lineage in the determination of neuronal precursor cells. *Devel. Biol.* **111**: 206–219.

Hartenstein, V. and Campos-Ortega, J. A. (1984). Early neurogenesis in wild-type *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **193**: 308–325.

Hartenstein, V. and Campos-Ortega, J. A. (1985). Fate-mapping in wild-type *Drosophila melanogaster* I. The spatio-temporal pattern of embryonic cell divisions. *Roux's Arch. Dev. Biol.* **194**: 181–195.

Honda, H. (1989). Cell sheet transformation during body formation of living organisms. *Forma* **4**: 13–20.

Honda, H. (1990). Spatial images of living organisms and a cell sheet. (In Japanese). *Suurikagaku* No. **327**: 58–64.

Honda, H., Tanemura, M. and Yoshida, A. (1990). Estimation of neuroblast numbers in insect neurogenesis using the lateral inhibition hypothesis of cell differentiation. *Development* **110**: 1349–1352.

Honda, H., Yamanaka, H. and Eguchi, G. (1986). Transformation of a polygonal cellular pattern during sexual maturation of the avian oviduct epithelium: computer simulation. *J. Embryol. Exp. Morph.* **98**: 1–19.

Kidd, S., Baylies, M. K., Gasic, G. P. and Young, M. W. (1989). Structure and distribution of the Notch protein in developing *Drosophila*. *Genes Devel.* **3**: 1113–1129.

Landström, U. (1977). On the differentiation of prospective ectoderm to a ciliated cell pattern in embryos of *Ambystoma mexicanum*. *J. Embryol. Exp. Morph.* **41**: 23–32.

Mackenzie, J. K. (1962). Sequential filling of a line by intervals placed at random and its application to linear adsorption. *J. Chem. Phys.* **37**: 723–728.

Page, E. S. (1959). The distribution of vacancies on a line. *J. Roy. Statist. Soc.* **B21**: 364–374.

Tenemura, M., Honda, H. and Yoshida, A. (1991). Distribution of differentiated cells in a cell sheet under the lateral inhibition rule of differentiation, *J. Theor. Biol.* **153**: 287–300.

Technau, G. M. and Campos-Ortega, J. A. (1987). Cell autonomy of expression of neurogenic genes of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **84**: 4500–4504.

Turing, A. M. (1952). The chemical basis of morphogenesis. *Philos. Trans. R. Soc. London* **B237**: 37–72.

Vassin, H., Bremer, K. A., Knust, E. and Campos-Ortega, J. A. (1987). The neurogenic gene Delta of *Drosophila melanogaster* is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. *EMBO J.* **6**: 3431–3440.

Wharton, K. A., Johansen, K. M., Xu, T. and Artavanis-Tsakonas, S. (1985). Nucleotide sequence from the neurogenic locus Notch implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell* **43**: 567–581.

Yoshida, A. and Aoki, K. (1989). Scale arrangement pattern in a lepidopteran wing. 1. Periodic cellular pattern in the pupal wing of *Pieris rapae*. *Develop. Growth Differ.* **31**: 601–609.