

**A cell-sorting model can explain cone mosaic pattern of  
medaka**

by

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## Abstract

In fish retina, four types of cone photoreceptor cells with different sensitive wave-length of the light (blue, UV, red and green) are arranged in regular pattern, called "cone mosaic". A pair of small cones, one sensitive to red and the other sensitive to green is close in contact and forms a "double cone". The development of cone mosaic has been studied by various experimental techniques, however a mechanism of formation of cone mosaic has not been identified yet.

We study the mechanism of formation by analyzing models in which cells are arranged on the 2-dimensional surface, experience interaction between neighbors, and automatically form the regular pattern. We demonstrated that medaka mosaic pattern can be generated by the cell sorting model in which cells exchanges their location with neighbors at a rate affected by cell-cell adhesions.

## Introduction

Cone photoreceptor cells (or cones) in fish retina are classified into several subclasses differing in shape and the sensitive wave-length and can be identified by their shapes under a microscope (Raymond *et al.*, 1993). They are arranged in a regular mosaic pattern, but the specific pattern of mosaic varies with species (Lyll, 1957; Wagner, 1972). In medaka, there are four kinds of differentiated cells (long single cone, short single cone, and two components of double cones), and are maximally sensitive to blue, ultraviolet, red, and green-light, respectively. A pair of red and green cones is in close contact, constituting a double cone.

In an embryo, the cone mosaic pattern is formed (Raymond *et al.*, 1995), and the mosaic expands as the eye size increases (Stenkamp *et al.*, 1997; Nishiwaki *et al.*, 1997). The pattern is generally regular (as in Fig. 1A), but the number of columns is larger in peripheral than in the central areas of retina because the retina is spherical rather than cylindrical. In medaka, some irregularity of mosaic pattern inevitably caused by the insertion of a new column of cone cells (Nishiwaki *et al.* 1997).

The development of rod and cone photoreceptors has been studied by various experimental techniques. However the mechanism of formation of cone mosaic has not been identified. At this moment there is no clear experimental evidence of the cell-cell interruption in pattern formation procedures or involvement of diffusive molecules.

In the zebrafish retina, as well as medaka, there are four types of cone cells. The arrangement of cone mosaic of zebrafish is, however, different from that of medaka. In the retina, double cones are arranged in rows and two kinds of single cones (sensitive to ultraviolet and blue, respectively) are also arranged in rows as illustrated in Fig. 1B. A model of differential adhesion and cell rearrangement was able to produce regular mosaic patterns in zebrafish retina (Mochizuki, unpublished manuscript). He developed a spatially structured model to examine whether the mechanism of cell sorting was able to explain the formation of zebrafish cone mosaic. He assumed that the rearrangement of differentiated cells was responsible for generating the pattern, and that the stochastic movement of the cell was modified by cell-cell adhesion. He showed the mosaic pattern was formed in a robust manner if the magnitude of cell-cell adhesion between different cell types were chosen appropriately. Using statistical physics techniques, he estimated probabilities of certain patterns by calculating total adhesion and the number of configurations, and confirmed the theoretical predictions by direct computer simulations.

We can show that a model of state transition is able to produce regular mosaic patterns in zebrafish retina (Tohya *et al.* 1999)

In the present paper, we study the system that forms retinal mosaic pattern of medaka (Fig. 1A) by a cell sorting model based on Mochizuki *et al.* (1996, 1997, 1998) and Mochizuki (unpublished manuscript). Differently from the interaction with nearest neighbors adopted by them, we here consider that cells exchange their location with neighbors at a rate affected by cell-cell adhesions not only between nearest neighbors but also between oblique cells.

## Model

We consider a model of differential adhesion and cell rearrangement based on Mochizuki *et al.* (1996, 1997, 1998) and Mochizuki (unpublished manuscript), in which cells move by exchanging their positions with the nearest neighbors. Differently from them, here, we consider that a rate of mobility of cells is affected by not only cell-cell adhesion between nearest neighbors but also cell-cell adhesion between oblique cells, because blue sensitive cones are surrounded by double cones, and directions of which make the round (Fig. 1A).

We regard double cone as single cell unit, because a double cone consists of a pair of red- and green-sensitive cones which are very closely connected. We, however, distinguish double cones by their orientations and assign  $D_{\uparrow}$ ,  $D_{\rightarrow}$ ,  $D_{\downarrow}$  and  $D_{\leftarrow}$ , in which arrows indicate the direction from red- to green-light sensitive cones. Also blue-light and UV sensitive cones are denoted by  $B$  and  $U$ , respectively. Therefore each sites takes one of six states ( $B$ ,  $U$ ,  $D_{\uparrow}$ ,  $D_{\rightarrow}$ ,  $D_{\downarrow}$  and  $D_{\leftarrow}$ ).

We assume that adhesion of cells occur only between cells in contact and cells randomly exchange their locations with the nearest neighbors. Let  $m$  be cell motility. In the absence of differential adhesion, cells exchange their positions at a rate  $m$ . A pair of nearest neighboring cells exchanges their location in a time interval  $\Delta t$  with probability  $m\Delta t$ .

If the strength of adhesion per contact is dependent upon cell types (differential adhesion cannot be neglected), the transition rate from one pattern to the other is different from the backward transition, because a given exchange of locations of two cells changes the numbers of cell-cell contacts with the surrounding cells. Let  $\lambda_{i,j}$  be cell-cell adhesion between cells of type  $i$  and  $j$ .

Since a double cone consists of red- and green-sensitive cones, the adhesion from the contact with its neighbor should depend on the orientation. For example, in Fig. 1A, a blue cone is in contact with both parts of a double cone. Concerning the adhesion between them, we assume that the adhesion of the contact from this pair is summation of adhesions of a blue cone and each of the two parts of a double cone divided by  $\beta$ , that is  $(\lambda_{BG} + \lambda_{BR}) / \beta$ . Let  $\gamma$  be coefficient of oblique connections. Then adhesion between oblique neighboring cells is given by  $\gamma\lambda$ . We call  $\beta$  and  $\gamma$  shape parameters.

Let  $\pi$  and  $\pi'$  be the spatial patterns before and after a transition event. We assumed the transition rate is an increasing function of the difference in the total adhesion between the two patterns  $\pi$  and  $\pi'$ . Let  $E(\pi)$  be the total adhesion of pattern  $\pi$ , which is the summation of value of  $\lambda$  of all the cell contacts on the lattice. Then the probability of transition from  $\pi$  to  $\pi'$  occurring in a short time interval of length  $\Delta t$  is assumed to be:

$$\Pr(\pi \rightarrow \pi' \text{ in } \Delta t) = \frac{2m}{1 + \exp[-\Delta E/m]} \Delta t, \quad (1)$$

where  $\Delta E$  is the difference in the total adhesion between after and before transition,  $\Delta E = E(\pi') - E(\pi)$ . In a sufficiently short interval  $\Delta t$ , the probability for two or more transition events to occur can be neglected, and the exchanges of cells occur independently between different locations. The factor of 2 in the numerator in Eq. (1) is needed to make the transition rate equal to  $m$  in the absence of differential adhesion ( $\Delta E = 0$ ). The transition rate increases with  $\Delta E$ , but saturates for a very large  $\Delta E$ .

We adopt periodic boundary conditions. The system starts from a lattice with random configuration, though ratio of cells is consistent with regular pattern, that is, B:U:D is equal to 1:1:2.

Different ways to assign the affinities give different cell differentiation rules. If we choose an appropriate set of values of adhesions and shape parameters, the spatial

pattern can converge to regular pattern such as Fig. 1A, whilst the regular pattern is not generated for wrong choices of adhesions. We searched for the combinations of adhesions and shape parameters that can generate the regular cone mosaic pattern as observed in medaka retina (Fig. 1A).

## Result

We performed computer simulations in order to examine that the cell sorting model can generate medaka mosaic.

We introduce a score to indicate the performance of sets of parameters in forming regular patterns illustrated in Fig. 1A. We count the total number of cell-cell contacts that are of "appropriate" types -- meaning the contacts between cell types included in the regular cone mosaic pattern in Fig. 1A. In doing this, we consider both contacts between nearest neighbors and contacts with cells in the oblique positions. The score of the spatial pattern is the number of appropriate contacts in the whole lattice divided by the possible maximum. It is 1 if the pattern is completely regular and it becomes smaller as the pattern becomes irregular.

We stopped calculation when the performance score became greater than 0.95, or when exchanging occur 1,000,000 times. For each set of parameters, we calculated replicate runs with five initial conditions that were fixed.

To decrease parameters, we set the affinity between the same type of cells to zero in the following ( $\lambda_{BB} = \lambda_{UU} = \lambda_{GG} = \lambda_{RR} = 0$ ). Because in the regular pattern in Fig. 1A, there is no contacts of cells of the same types. Thus we have six adhesions between 4 kinds of cells which remain to specify. We varied  $\lambda_{BU}$ ,  $\lambda_{BR}$ ,  $\lambda_{BG}$ ,  $\lambda_{UR}$ ,  $\lambda_{UG}$  and  $\lambda_{GR}$  from 0.0 to 3.0 by step 1.0, varied  $\beta$  from 1.0 to 3.0 by step 0.5 and adopted 0.0, 0.1, 0.25 and 0.5 to  $\gamma$ . Thus we have 81,920 ( $= 4^6 \times 5 \times 4$ ) sets of parameters.

Figure 2 shows dependency of shape parameters,  $\beta$  and  $\gamma$ . Open circles indicate that medaka pattern is generated (the average score is greater than 0.95), if appropriate adhesions are given. On dots, computer simulations were performed but medaka pattern were not represented. We have 4,096 combinations of adhesions per each circles and dots. Generated pattern is clearly changed by  $\beta$ . If  $\beta$  is smaller than 2.0 medaka pattern is produced. It means that adhesion of contact with both parts of a double cone is greater than adhesion of contact with one of part of a double cone. On the other hand, if  $\gamma$  is equal to or close to zero, medaka pattern is not generated. It shows that adhesion between cells in oblique positions is needed.

Figure 3 shows values of adhesions for forming medaka pattern. An x-axis is  $(\lambda_{BG} + \lambda_{BR})/2$  and a y-axis is  $(\lambda_{UG} + \lambda_{UR})/2$ . Open circles indicate that medaka pattern is generated if shape parameters and remaining adhesions are appropriately given. Dots indicate medaka pattern is not generated. From this figure, we can conclude that the average of  $\lambda_{BG}$  and  $\lambda_{BR}$ , should be greater than the average of  $\lambda_{UG}$  and  $\lambda_{UR}$ , to form cone mosaic of medaka.

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### Figure Captions

Figure 1 In fish retina, there are four kinds of differentiated cells. A pair of red-sensitive and green-sensitive cones are in close contact, constituting a double cone. Here four kinds of cones are indicated by capitals (R, G, B, U). (A) Regular cone mosaic pattern of medaka. (B) Regular mosaic pattern of zebrafish. The rows of double cones and those of single cones (blue- and UV-sensitive cones) are in parallel and alternately.

Figure 2 Dependency of  $\beta$  and  $\gamma$  (shape parameters). Open circles indicate that the average scores of five runs are greater than 0.95. On dots, computer simulations were performed but medaka pattern was not represented. We varied  $\lambda_{BU}$ ,  $\lambda_{BR}$ ,  $\lambda_{BG}$ ,  $\lambda_{UR}$ ,  $\lambda_{UG}$  and  $\lambda_{GR}$  from 0.0 to 3.0 by step 1.0, thus we have 4,096 combinations of adhesions per each circles and dots. Generated pattern is clearly changed by  $\beta$ , that is,  $\beta$  is smaller than 2.0 medaka pattern is produced.

Figure 3 The combination of adhesions for generating medaka mosaic pattern. An x-axis is  $(\lambda_{BG} + \lambda_{BR})/2$  and a y-axis is  $(\lambda_{UG} + \lambda_{UR})/2$ . Open circles indicate that medaka pattern is generated if shape parameters and remaining adhesions are appropriately given. Dots indicate medaka pattern is not generated. The average of  $\lambda_{BG}$  and  $\lambda_{BR}$ , is higher than the average of  $\lambda_{UG}$  and  $\lambda_{UR}$ , when medaka pattern is generated.

Fig.1

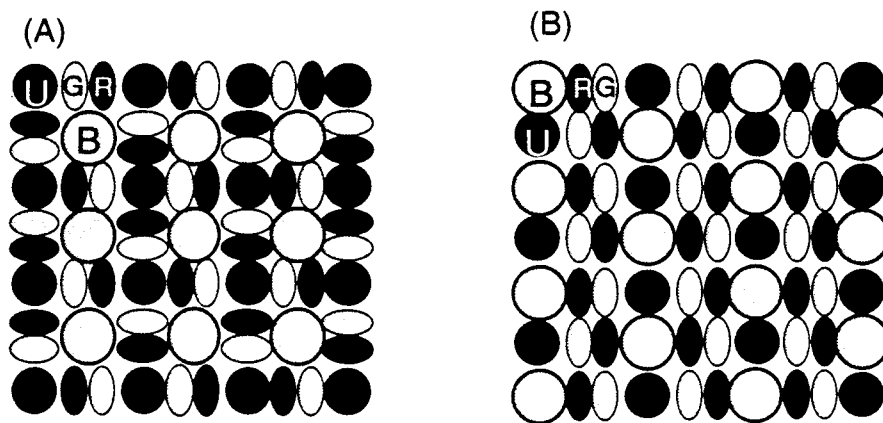


Fig.2

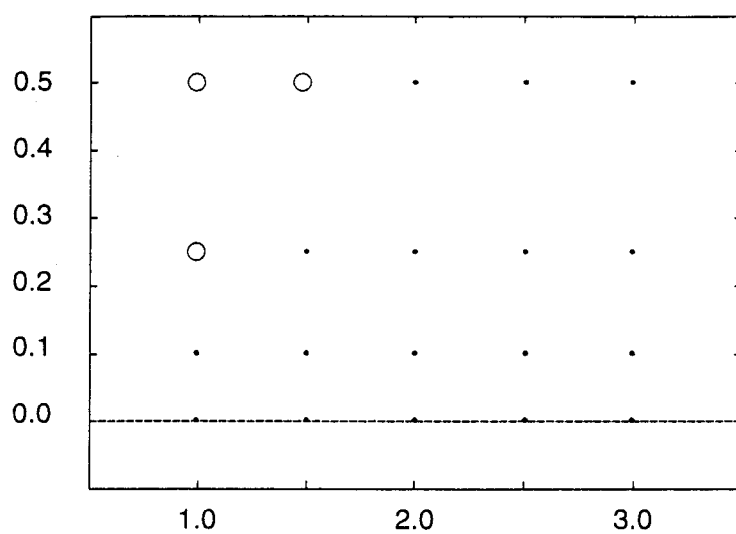


Fig.3

