Morphogenesis, Plasticity, and Irreversibility

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Abstract

Dynamical systems theory of morphogenesis is surveyed, where positional information is generated through intra-cellular reaction dynamics and cell-cell interaction. Cells differentiate as a result of the dynamics, and these differentiated cells form an ordered spatial pattern, which further stabilizes the cellular states, leading to robust morphogenesis and irreversibility in the differentiation. Induction, community effect, gastrulation, and activin-controlled artificial tissue-genesis are discussed from this theory, where relevance of dynamics of cellular plasticity is stressed.

1 Question to be addressed: Origin of positional information

Biological pattern formation (morphogenesis) is understood as a change of genetic expression depending spatially on the gradient of chemicals (Alberts et al. 2002). In the morphogenesis, cells differentiate with developmental process, and these differentiated cells form an ordered spatial pattern. Recent experimental studies have clarified how the change of gene expressions take place in the process of differentiation, and in the spatial ordering process. However, the description of genetic changes only is not sufficient to fully explain the whole morphogenesis, since how some genes are on or off depends on chemical states of a cell, which are also influenced by interaction with surrounding cells.

Note that the pattern formation process during normal development is rather stable. Considering possible fluctuations of molecules and perturbations on cell behaviors, they are quite robust in the normal developmental process (Kaneko and Yomo 1999; Lacalli and Harrison, 1991). Almost identical final patterns are formed and the temporal course for the pattern formation is also repeated. Furthermore, some experiments to perturb externally the process by removal or addition of cells suggest that the cells somehow 'know' their position within the whole system of an ensemble of cells, so that the damage is repaired. Following these observations, Wolpert proposed the concept of positional information, which states that the cell knows its position by concentrations of diffusible chemicals (Wolpert, 1969).

There are some problems in this positional information idea. The first problem is with regards to the origin of positional information. If some chemical gradient is externally given, one can specify the position. However, how the chemical gradient itself is generated is not answered, within the positional information theory. To form a gradient in chemicals, cells at one end and the other end have to

behave differently. However, to ignite such differentiation, the existence of gradient is required in the positional information theory. In other words, how polarity is generated spontaneously is not solved. In this sense, it is desirable to set up a theory in which the formation of positional information and the cell differentiation reinforce each other.

Of course, in some cases, the gradient is given as a condition from its mother, and exists in a fertilized egg, as the concentration of some proteins such as bicoid (Driever and Nüsselein-Volhard 1988) by which the pattern formation processes at the first stage of development is believed to be controlled.

However, not all the axis for the pattern is not given as a maternal effect. Also, in mammals, the homogeneity among cells seems to exist up to 4th divisions. Even in other organisms that are more tightly controlled by maternal gradient, such problem may exist. For example, even though the initial concentration gradient of bicoid in Drosophila egg are changed experimentally, final pattern of the entire body is robust with respect to this perturbation up to a certain range (Driever and Nüsselein-Volhard 1988).

This means that, the positional information is not always embedded in the initial condition of developmental process, but rather it is generated during developmental process.

The second problem is on the robustness. As a standard picture of developmental biology, cell differentiation progresses depending on the concentration of signal molecules. Since this concentration depends on the space, the cell state changes according to its position. In this way, a cell is believed to 'read' the positional information. However, there should be large fluctuations in the chemical concentrations, which may cause a serious problem.

Recall that these signal molecules work often with very low concentration. The quantity of chemical "concentration" is given by the number of molecules per tiny volume around the cell. Here, the chemical concentration of relevance is the number of molecules per unit volume in the tiny region around the cell. Hence the number of signal molecules associated with cell differentiation is often quite small, while in diffusion processes, each molecule moves randomly. Thus there can be large fluctuations in the concentration of molecules affecting a single cell in general. In fact, Houchmandzadeh, Wieschaus, and Leibler (Houchmandzadeh, Wieschaus, and Leibler 2002) have demonstrated such large fluctuations of bicoid concentration in the Drosophila eggs with the same developmental stage. Such large fluctuations no longer appear at later steps in the developmental process, and robust pattern formation results. Accordingly the threshold mechanism, only by itself, is vulnerable under the fluctuation and cannot resolve the error arising from the fluctuation.

In prior to positional information theory, Turing proposed a theory in which spatial inhomogeneity of diffusible chemical concentrations emerge spontaneously (Turing 1952). In fact, the term morphogen was introduced by Turing. In this pioneering study Turing introduced a system of coupled elements interacting diffusively each other. He has shown that spatial pattern is formed with regards to the chemical concentration, starting from spatially homogeneous initial condition.

To be specific, consider two reacting chemicals that activate the reaction ('activator') and inhibits the reaction (inhibitor). When the diffusion constant of the activator is faster than that of the inhibitor, under a certain condition, a homogeneous state with a constant concentration of the chemical is destabilized. Due to the instability of the homogeneous state, a pattern with some wavelength is formed that stabilizes the system. Indeed Turing discussed generally the pattern formation in a diffusion-reaction system. There is a case that the instability leads to temporal rhythm. With more than two chemicals, there is also a case with spatiotemporal pattern dynamics, including spatiotemporal chaos. Turing proposed to call such diffusive chemical relevant to the morphogenesis as "morphogen". Although his theory has not yet fully been appreciated in developmental biology, the theory, on the other hand, is fully appreciated in the physics or chemistry of nonequlibrium systems.

This theory partially had answered the first question for the positional information, by using dynamic instability and diffusive interaction. However, in spite of his pioneering work on morphogenesis, some basic problems remain unsolved, i.e., on the origin and the robustness of the positional information. Furthermore, Turing's theory was not seriously taken into account in biological community,

since the genetic control mechanism discovered since then was thought to be essential.

First, although a periodic stripe pattern with alternation of chemical concentrations is generated by Turing pattern, how the difference in concentrations leads to cell differentiation to "discrete" cell types is not solved yet. Indeed, the change of chemical concentrations is rather continuous, and it is not clear how discrete types represented also by on or off of genetic expressions emerge from it. Since differentiation is not well-defined, the irreversible loss of potentiality is not studied either, in Turing's theory.

Second, in the standard Turing model, the developmental process with the increase of cell numbers is not considered. In his original model, the system size, i.e. the number of cells, is fixed in time. Hence, neither the robustness nor irreversibility of the developmental process is discussed.

In the development, the initial few cells have potentiality to differentiate to all other cell types. As the development progresses, some cells lose the potentiality in time, and in the normal development, this loss is irreversible. This irreversible loss of potentiality is influenced by interaction with surrounding cells, and is related with the pattern formed by cells. Indeed the potentiality generally depends in what type of tissue a cell exists. For some tissues, when some cells are externally removed from the pattern, regeneration can occur. On the other hand, for some other tissues, cells are determined in their type, and such regeneration is not possible. How such irreversible loss of potentiality for differentiation is related with the morphogenesis is not answered yet.

The third problem concerns with the discovery of gene regulatory mechanisms made after the publication of the Turing's paper. For example, in Drosophila, each segmentation corresponds to an expression of some gene. From the gene regulatory dynamics, gene expressions are on or off according to the concentration of some signal molecules. Hence the Turing's mechanism has not been considered seriously. Still, these observations do not necessarily reject the Turing's idea itself. If Turing's idea is generalized, one can say that the interplay between intra-cellular reaction dynamics and the diffusion of chemicals lead to morphogenesis. This generalized viewpoint is consistent with the present molecular biology view based on the interplay between intra-cellular gene regulatory dynamics and diffusive signal molecules. What we need to add to the original Turing's mechanism is sufficient complexity in the intra-cellular reaction dynamics. This is another missing link between Turing's and gene-regulatory network dynamics.

In spite of extensive studies in Turing patterns (Meinhardt and Gierer 2000), the above three problems were not completely resolved yet. Previously, we proposed a theory to overcome such drawbacks and to explain the spontaneous differentiation of cells to discrete types with the increase of cells. In the theory so far, we have disregarded spatial factor for simplicity. By including spatially local process, it is possible to study how dynamic differentiation process and spatial pattern formation are mutually reinforced, and form stable pattern and stable cell types. In the present paper we discuss these problems by also reviewing some of our earlier studies.

2 Logic

In a cell, there are several chemical components. Then a cellular state depends on chemical compositions within a cell. These chemicals include proteins, RNA, membrane, ans so forth. Through chemical reaction processes, the chemical compositions of a cell can change in time. With the intracellular reactions, some chemicals are converted to products, and at some stage, the cell becomes large enough to be divided into two. Without assuming any sophisticated programs, the two divided cells take almost identical chemical compositions, although there are some differences due to molecular fluctuations.

Note that positive feedback process exists in the intracellular reactions. With nonlinear dynamics in the reaction, the tiny differences between the two cells may be amplified. This amplification depends also on cell-cell interaction, including competition for chemical resources for cell growth. As the cell number is increased, this cell-cell interaction is stronger, and the amplification of tiny difference will be stronger. At some stage of cell numbers, this amplification becomes large enough to make a

macroscopic difference between the two cells. Now homogeneous states of a cell ensemble will be unstable.

On the other hand, these cells interact with each other through diffusion of chemicals across the membrane. Now through unstable dynamics, the cell state changes in time, also depending on the states of other cells. Here, it is expected that cells with similar states compete strongly for resources for growth, whereas by taking a rather different state these cells do not compete each other so much. It may then be expected that differentiation of cells comes to a stage that different types of cells mutually stabilize others' states. This mutual stabilization is possible under suitable cell-cell interactions. (see Fig.1 schematically).

This diversification of cells into a discrete set of types from a single cell type is a general consequence of interacting cells with biochemical networks and cell divisions, as was confirmed by several model simulations (Kaneko and Yomo, 1994;1997;1999; Furusawa and Kaneko, 1998a;1998b), and also based on dynamical systmes theory (Kaneko,1990). According to the theory, differentiation proceeds first by loss of synchrony of intracellular oscillations as the number of cells increases. Then the chemical composition of the cells is differentiated. The differentiated compositions become inherited by the next generation, and such cells are determined. As a result of successive occurrence of the cell differentiation, the cell society will be composed of different cell types. The present mechanism leading to differentiation is called isologous diversification, since it gives a mechanism how identical state (cell) can be diversified through the interplay between internal (reaction) dynamics and interaction. With this mechanism, cell differentiation is shown to be stable against molecular and other external fluctuations, where amplification of noise-induced slight difference between cells leads to a noise-tolerant society with differentiated cell types.

Now, to discuss morphogenesis, we need to consider also the spatial structure of these differentiated cell types. For it we take into account of spatial locations of cells as well as diffusion of chemicals through the space. Then, it is rather natural that the differentiated cell types by the mechanism discussed above are organized spatially to form a pattern, that is robust against perturbation. With this pattern formation, gradients of chemicals are formed that consolidate the differentiation of cell types. Here, cell differentiation by intracellular dynamics lead to a gradient of chemicals, and form positional information, while position information strengthens differentiation of internal states. With this reinforcement, differentiation by inter-intra-cellular dynamics is transferred to a spatial pattern, while by it the differentiation is further stabilized. Cell differentiation is consolidated with the spatial pattern formation. Interplay among internal dynamics, inter-cellular diffusion of chemicals, cell division process, and spatial pattern formation leads to such consolidation.

3 Model

Cells consisting of several chemicals are assumed to locate in space, and chemicals diffuse through the space, so that nearby cells interact more strongly as a result. Chemicals that are permeable from the membrane diffuse through the space with a given diffusion constant, while nutrients are supplied with such diffusion rate from the outside.

(i) Internal chemical reaction dynamics:

Cells are assumed to be completely surrounded by a two-dimensional medium including diffusive chemical substances. To represent cellular reaction dynamics, we assume that each cellular state is represented by concentrations of k chemicals. Due to chemical reactions, the concentrations change over time. For the reaction dynamics, we choose a catalytic network among the k chemicals. Each reaction from some chemical i to other chemical j is assumed to be catalyzed by a third chemical ℓ, which are determined randomly. These reactions form a complicated reaction network. Here we simply choose randomly connected reaction networks, and study common features in such system.

(ii) Cell-cell interaction:

Cells interact with each other through the transport of some chemicals into and out of the surrounding medium. Here we consider only indirect cell–cell interactions via diffusive chemical substances,

as a minimal form of interaction. We assume that the rates of chemicals transported into a cell are proportional to differences of chemical concentrations between the inside and the outside of the cell.

(iii) Cell division:

Each cell receives penetrating chemicals from the medium as nutrients, while the reaction in the cell transforms them into non-penetrating chemicals which comprise the body of the cell. As a result of these reactions, the amount of chemicals in each cell changes. The chemical composition of two divided cells are almost identical (except some molecular fluctuations). Cells have a given size within this space, and when a cell is divided, its offspring cell is located in the neighborhood of the original cell. (The direction of putting cells is chosen randomly).

Cells have a given size within this space, and when a cell is divided, its offspring cell is located in the neighborhood of the original cell. (The direction of putting cells is chosen randomly). As for the details of the model, see (Furusawa and Kaneko, 1998a;1998b;2002;2003) [1]

4 Results

In this case also, as the number of cells increases, first the cells start to differentiate as already seen in the previous chapters. The different cell types take different chemical compositions. Together with this process of differentiation, these different types of cells start to form some spatial pattern. Here, first intra-cellular reaction dynamics and cell-cell interaction lead to cell differentiation, which later are fixed into a spatial pattern.

We discuss two examples for the development in a two-dimensional space. In the example of Fig.2, from the initial stem type-S (represented by red cell in Fig.2), type-X (yellow) and type-Y (green) cells through the differentiations, at the inside of the circle. The concentric circle pattern with type-X, Y cells inside is formed first. As these cells increase, there appears a new type of cell Z (blue) inside the cluster of type-X cells by the differentiations from type-X cells. These cell types again take distinct chemical compositions. The type-S cells have larger chemical diversity have larger variations. Here successive differentiation $(S \to S, X, Y)$, $(X \to X, Z)$, $(Y \to Y)$, $(Z \to Z)$ are observed.

The second example, which we mostly discuss here is a stripe formation. In this case, a single cell placed at the center of the medium exhibits the oscillatory reaction dynamics, and have higher chemical diversity (Fig4(a)). There, type-A (green) cells are first differentiated from the original type-S (red) cells, at one side of the cells as shown in Fig3(b). Along a specific axis S and A are differentiated. At this stage the differentiation rule is given by $(S \to S, A)$ and $(A \to A)$. As the number further increase, the state of type-S cells at the other side of A is destabilized, to differentiate to a new type-B (blue). Hence the stripe pattern with A,S,B is formed, Here the differentiation rules are $(S \to S, A, B)$, $(A \to A)$, and $(B \to B)$. As a result of these differentiations, a spatial pattern of cells consisting of three stripes, each containing cells of just one kind, is formed, as shown in Fig.3(d). Later another type-C is differentiated from type-S near the border of S and A. The intra-cellular dynamics of each cell type are shown in Fig.4.

In a system exhibiting the stripe pattern, the point symmetry is broken, in contrast to the ring pattern (Fig.2(c)). This is due to the development that takes place at the beginning of the differentiation process from type-S to type-A cells when there is still a small number of cells (Fig.3(b)). It is at this stage that the point symmetry is lost, and this asymmetric small cluster of cells expands through further cell divisions. Because of the difference regarding both rates and types of nutrients absorbed and released by type-S and type-A cells, the asymmetric distribution of cells brings about asymmetric concentration gradients of nutrients in the medium, as shown later.

[1] As the initial state, a single cell, whose chemical concentrations are determined randomly, is placed in the medium. According to the process described above, cells divide to form a cluster. With the increase of volume of cells, chemicals in the medium are consumed. To maintain the growth of the organism, the system is contacted with a bath of chemicals from which nutritive chemicals are supplied.

4.1 Generation of positional information

Now we discuss how the positional information is generated with this differentiation process. Before going into specific discussion, we recall again the requisite for 'information'. As discussed in (Kaneko and Yomo 2002), the information carrier has to satisfy (1) controllability and (2) preservation. If chemical concentration gradient works as positional information, we need to discuss (i) how it is generated from dynamics, and (ii) how it controls the differentiation of cells, and (iii) how such gradient is preserved under molecular fluctuations as well as external disturbances.

Hereafter we focus on the example with a stripe pattern formation. In Fig.5, we have plotted the concentration of resource chemicals along the long axis of this cell aggregate. In spite of the constant boundary condition to supply this chemical constant, there appears some chemical gradient. Here the concentration gradient and the region of each type of cell has some relationship. For example, at the region of the type-S and type-B cells the resource chemical 0 has a higher concentration, and at the region of type-A cells higher concentration of chemical 1 is maintained.

Conversely, if we put a type S-cell, in a region with a higher concentration of 1(or 2), differentiation to type-A (or B) follows. In this sense, the gradient of these chemicals plays the role to control a differentiation of a cell into each type. Accordingly, the chemical concentrations are 'read' by each cell, and act as the positional 'information'.

Depending on the concentration of the environment at each cell, the cell state slightly changes even if it belongs to the same type. Although the differentiation into types is discrete, there appears slight modulation of each cell's state depending on its position. In Fig. 6, we plotted $\sum_j (x_j)^2$ of each cell along the axis, where x_j indicates the intra-cellular concentration of j-th chemical. One can first see clear discrete differentiation into types, while the states of the cells of the same type are slightly modulated by their position. This modulation (analogue difference) is discernible well for type-S and B cells. Here it is interesting to note that besides the digital information on type, there is analogue information by the cell position, which brings about a continuous modulation of the cell state. Note that for the type-A cells, this modulation is much smaller. We will come back to this point later.

4.2 Complementary relationship between internal cell state and positional information

Once a pattern is formed together with the generation of positional information by concentration gradient, both the pattern and information are stable against some perturbations, such as removal of some cells, or perturbations on chemical concentrations into some cells. After perturbations, both the pattern and chemical concentration gradient come back to the original. Since the cell state and gradient are mutually stabilized, after small perturbations, they can come back to the original cell state and spatial pattern. Thus, the preservation property required for information is realized.

This, however, does not necessarily mean that only the positional information is sufficient to produce the pattern. For example, if we randomize the cell state, by changing each intra-cellular concentrations, while keeping the environmental chemical gradient, then the generated cell pattern later is quite disordered as in Fig.7. The original regular stripe pattern is totally lost. Hence the positional information only is not sufficient to regenerate the pattern. If the intra-cellular state is too different, the cell cannot read the information. The complementary structure between the internal cell state and positional information is important.

4.3 Regeneration process

Now we discuss the problem of regeneration in relationship with irreversible differentiation. In the present model case of stripe pattern formation, the type-A cell proliferates the same type, and the type-B also. Then what happens if some cells are removed to destroy the field of original positional information. Does the cell ensemble have potentiality for regeneration?

(I) **Recovery from the removal of the entire type-B region**: In one case, we removed all the type-B cells (Fig.8(a)), after the stripe pattern had developed. After this operation, the rate of transitions from type-S to type-B cells was enhanced at the side of type-S region farthest from the type-A region, and as a result, the stripe pattern with three layers gradually re-appeared.

(II) **Recovery the removal of the entire from type-S region – De-differentiation of type-B cells into type-S, induced by the interaction with type-A cells**: In this case, we removed all the type-S cells that ware located in the middle of the stripe pattern, and combined the remaining cell clusters consisting of only type-A and type-B cells, as shown in Fig.8(b). After this alteration, type-2 cells located at the boundary between the type-A and type-B regions de-differentiated back into type-S cells, and the stripe pattern with three layers was thereby recovered. It is important to note that de-differentiation from a type-B cell to a type-S cell never occurs during the "normal" course of development, i.e. without perturbations.

As shown, the recovery is possible after large damage to the system. By this damage, the positional information by concentration gradient is also largely destroyed. However, through the recovery process, the positional information is regenerated. This is possible, because the positional information is not given externally, but rather is generated through intra-cellular chemical reaction dynamics and cell-cell interaction.

(III) **Formation of a new pattern resulting from the removal of the type-A region**: Here, all the type-A cells were removed from a cluster with a striped pattern (Fig.8(c)). In this case, regeneration of the type-A region was not observed. Instead, type-S cells at the periphery of the cluster differentiated into type-B cells. As a result, a sandwich-like BSB structure was formed, and with further development, a ring structure with inner type-S cells and outer type-B cells was formed. In this case, the final cell society consisted only of type-S and type-B cells.

In this example, once a type-B cell is formed the differentiation from S to A is inhibited. In the 'normal' process, the type-A cell is already differentiated before type-B, and the ASB pattern is generated, and the inhibition is not effective. In the present case, without the existence of type-A cells, the type-B cells exist, that inhibit the differentiation into the type A fro the type S. In this way, the ordering (or history) of developmental process is important.

Why were type-B cells, rather than type-A cells, made differentiated when type-A and B cells are attached? Here one should note that the variation of the chemical concentrations of type A cells is much smaller than the other types, as displayed in Fig.6. As discussed in the fluctuation-respons relationship (Sato et al., 2003), the state with smaller fluctuations or variations has a smaller response against external change, and has a lower plasticity. The type-B cells have larger fluctuations and variations against the external change than the type A cells. Hence the type B cells have a higher plasticity, and are easily changed. They have a higher potentiality of de-differentiation when the external condition is varied.

4.4 Importance of the ordering in development

As shown in the above examples, the history how an ensemble of cells has developed with increasing the cell number is essential to the selection of a pattern. The cells that are later generated are put in the field of chemical concentration that is already produced. The selection of cell state is determined accordingly. This point is not well discussed at least in the original Turing pattern. To re-examine this issue, we have put 100 cells initially in the configuration that was generated through the original simulation. Here initial condition is set so that it has a homogeneous chemical concentration. The pattern thus formed is plotted has no regularity, in the same manner as in Fig.7. In the term of dynamical systems, the selection of initial and boundary conditions through the development is essential to form an ordered pattern here.

5 Constructive Experiment

Here we discuss a constructive experiment to form a tissue from undifferentiated cells artificially. To be specific we explain recent controlled tissue generation by Asashima's group (Ariizumi and Asashima, 2001; Uochi and Asashima 1996). They took out some cells from an animal cap of the Xenopus' egg. These cells were isolated first by dstaching each other, and then they are put into the solution of activin for a while. After this operation, the ensemble of these undifferentiated cell, put together, are cultured, to examine how they develop later. Here initial cells are almost homogeneous. Surprisingly, only by changing the concentration of activin, a variety of tissues of the frog, including notochord, heart, and muscle, were generated. By further using the solution of retinoic acid and Con. A (Conacanbailn A), Asashima's group have succeeded in forming over a dozen of tissues of the frog, including the nephron, sensory organs such as eye or ear. These results should be important in the tissue engineering. Indeed they have confirmed that thus generated tissues have original function, by putting them into an adult body. Here, however, we discuss the importance of the result in considering the logic of development.

The following two points should be noted in relationship with our theoretical results. These two features show that naive picture for threshold mechanism for development is not sufficient. The first feature suggests the existence of attractors or attracting states as a dynamical system at a tissue level. The second feature suggests that the differentiation is determined through interplay between intracellular dynamics and cell-cell interaction, as adopted in our study.

(i) Jump-over — generation of tissues, jumping over normal temporal course of development.

Activin concentration is adopted as a control parameter in their experiment. This activin molecule is important in the normal developmental process as Asashima had earlier discovered. Still, the concentration as high as adopted in their experiment is not indeed achieved in the real normal development. In addition, in the normal development several other molecules are involved as controllers. Hence the experimental process they adopted progressed in a rather different situation from the normal development case. The most surprising point in their experiment is that in spite of this difference from the normal course, the final tissue generated is same as that in the normal development. They indeed checked the morphology of tissues, cell types, gene expressions, to show that the tissue generated is normal. The normal function is confirmed, by transplantng these constructed tissues, such as heart or eye.

The 'process' reaching this tissue is quite different from the normal case. In the normal development, by starting from undifferentiated cells, first mesoderm including notochord and muscles are formed from which neural systems are generated, and later some sensory organs are formed. In the present 'constructive experiment', without going into these steps, the goal tissue is generated, by jumping over processes to form earlier tissues.

The present result contests with the viewpoint that developmental process should constitute in succession of finely tuned stepwise processes. Rather, it is more natural to adopt the viewpoint that the final tissue is a kind of attractor in a very large dynamical systems, and there can be several paths to reach the final attractor other than the normal developmental process. Of course, such paths to attractors are rather intermingled and complex. Then the basin for such attractors need some control. The result of Asashima's group suggests that their change of activin concentration is not a finely tuned control but rather is used to perturb initial state by destabilizing the cellular state so that it moves toward a basin of attraction to some other tissues.

(ii) community effect – the formation of tissue is highly dependent on the number of cells.

In the experiments of Asashima's group, a number of undifferentiated cells were taken from the animal cap of Xenopus, and with these the construction of several types of tissue (e.g. heart, notocord, muscle and so forth) was caused by controlling the concentration of activin. In these experiments, it was found that how the construction of tissues proceeds crucially depends on the number of cells used. Indeed, the normal tissue is generated only for some range of initial cell numbers. As for the

nephros formation by the soution of activin (with 10 $ngm\ell$) and retinoic acid, the normal tissue is formed only if the number of cells is around $300 \sim 500$. When the number of the initial cells is less that 100, the cells will die, while by starting from cells larger than 500, inhomogeneous tissue are generated. The construction of the heart tissue by activin is also possible only for a range of cell numbers. This experimental result cannot be understood solely by signalling to one of the cells, and cell-cell interaction is essential as has been discussed in the previous section.

6 Summary and Discussion: Development as Dynamics of Plasticity

6.1 Summary

We now summarize the logic of our theory and results of our simulations, and discuss their relevance to biological morphogenesis.

In our theory, cells possessing internal non-linear reaction dynamics differentiate into several distinct cell types when the cell number exceeds a threshold value. The transition from one cell type to another is regulated by the position of the cell in question. This regulation leads to an ordered spatial pattern consisting of differentiated cells, as shown in Figs.2 and 3. Each cell "reads" information from the external field and reacts to this information by modulating its intra-cellular dynamics in accordance with it. This modulation controls the rates of differentiation into various cell types, while such cell differentiations, in turn, alter the state of the environment. With this circular relationship between intra- and inter-cellular dynamics, the gradients of chemicals in the medium act as positional information controlling the fate of each cell. This circular relationship of intra- and inter-cellular dynamics is responsible for the robustness of the developmental process.

6.2 Community effect

Gurdon showed that the commitment, i.e., determination to a certain cell type, depends on the total cell number, and termed this cell-number effect as community effect (Gurdon, Lemaire, and Kato 1993). When the number of cells is small, the differentiation is not determined, and the cell state changes in time, while as the cell number is larger, the cell state is mutually stabilized, and is maintained. The experiment by Asashima also shows clearly the cell number dependence of the tissue formation.

Since our theory is based on cell-cell interaction, such community effect is naturally expected. Indeed, we have studied this problem by taking the stripe case again. For example, when a single type-B cell (such as that in Fig.3) is placed in a medium containing no other cells, this cell de-differentiates back into a type-S cell. However, as shown in Fig.9, the type-B cell state is stable when such a cell is surrounded by a sufficient number of other type-B cells. To confirm this point, we have carried out simulations with the same model, but in this case by placing a large number (larger than 10) of type-B cells in an otherwise unoccupied medium. In this case, the cell colony consisting of only type-B cells grows.

As discussed previously (Furusawa and Kaneko, 1998), there can be multiple stable cell colony states. A cluster consisting only of type-B cells corresponds to one such stable state, but this state never appears in the ordinary developmental process starting from a single cell. In this case, only when the number of type-B cells initially placed in the medium is more than approximately 15, do these cells remain in their type-B states. Otherwise, all the cells de-differentiate into type-S cells. This result clearly indicates that the states of the cells existing in a cluster can be mutually stabilized by their cell-cell interactions.

6.3 Induction and plasticity

In development, the term 'induction' is often adopted. When some group of cells start to interact with another group, cells of one group change their state to form a different tissue, we use this term.

In fact, both cell groups interact mutually, but it is regarded that only one group of cells is changed, induced by the other group. This means that 'changeability' of the cell state is higher for the former than the latter group. This is nothing but the problem of the degree of plasticity in cells.

One might wonder that the "plasticity" here might look ambiguous. Still, in several models of cell differentiations (Furusawa and Kaneko, 2000;2001), we have empirically observed that plasticity can be quantitatively represented by the following measures;

(i) a cell with larger fluctuations or temporal variation of chemical concentrations (say gene expressions) has higher plasticity, i.e., its susceptibility against external condition's change is larger. The fluctuations and susceptibility (changeability) are correlated according to the fluctuation-response relationship (Sato et al., 2003).

(ii) a cell with diverse chemicals has higher plasticity. In other words those with weak but diverse gene expressions has higher plasticity.

Hence the concept "plasticity" can be measured experimentally by the current tool of experimental biology. Assuming the quantitative measure on plasticity, we propose the following hypothesis:

When two groups of cells with different plasticity meet, the group of cells with higher plasticity is changed more. In other words, the group with lower plasticity brings about the induction of the other group of cells having a higher plasticity.

Now we check the validity of this hypothesis from our simulation. In the model we studied here, when the type-A and B cells are put adjacent, the type-B cells are de-differentiated, and change to type-S cells, while when S and B cells are adjacent, there occur differentiations from S cells. Then the 'changeability' of cells are in the order of $S > B > A$. Now recall the data of variation of chemical concentrations among cell types. The variations, as well as fluctuations of each cell type decrease in the order of $S > B > A$ again. Hence the hypothesis that the variation or fluctuation gives the degree of plasticity is confirmed. When two types of cells, generally those with higher plasticity are changed (induced), and this plasticity is quantitatively measured as variation or fluctuation of cellular states.

6.4 transformation of state differentiation into spatial pattern

It should be noted again that the pattern formation here is not predetermined from the spatial information, but rather through intra-cellular dynamics and interaction. Spatial patterns and intra-cellular states mutually stabilize to from robust pattern formation consisting of several cell types. Here differentiation by intra-cellular dynamics is consolidated to a pattern.

Although we have here adopted oscillatory catalytic reaction dynamics for intra-cellular dynamics, the present mechanism does not necessarily require oscillatory dynamics. As long as there exists some instability in intra-cellular dynamics, this mechanisms works completely. Indeed, Takagi and Kaneko (Takagi and Kaneko, 2005) have shown that the present mechanism works starting from the intra-cellular dynamics with multiple fixed points. Another choice for the intra-cellular dynamics, that straightforwardly corresponds to gene regulatory dynamics, is the use of threshold dynamics so that each gene expression takes either onto high or low values , corresponding to on or off of genes respectively (Mjolness, Sharp and Reinitz, 1991; Salazar-Ciudad, Garcia-Fernandez, and Sole 2000). Even with these dynamics, instability in the intra-cellular state can occur (Ishihara and Kaneko, 2004). By using such gene regulatory dynamics, and applying the present scheme, it is shown that the present scenario for morphogenesis works. In this sense, the third question addressed to the Turing's mechanism — how dynamical systems mechanism and gene regulatory mechanisms are consistent — is answered.

With this regards, we should mention that Newman put forward the idea that Turing pattern will be consolidated to gene expression (Newman and Comper 1990;Newman 1994;2003). The present theory is consistent with his idea.

6.5 destabilization of intra-cellular state and regain of plasticity; an interpretation of gastrulation

In the development of vertebrate, there is an important epoch, that is gastrulation. As the number of cells increase beyond some value, the cells start to move, triggerred by mechanical instability like buckling, so that some cells start to move inside of the other group. Although the origin of the gastrulation itself could be mechanical, an important consequence of this is that cells that were apart so far and did not interact with each other start to contact directly and this novel cell-cell interaction leads to a drastic change of cellular states.

Now, the stability of the cell state is not solely determined by the intra-cellular state, but also through the interaction with neighboring cells. Then when cells start to be contacted with some other cells that were far and not contacted, the cell state can be destabilized which may lead to novel differentiation.

Note that the plasticity in a 'closed' system generally decreases (or does not change) in the temporal course. All of our simulation results of cell differentiation process support this proposition. As long as the change of chemical states of cell follows their intra-cellular chemical reaction dynamics and cell-cell interaction in a given spatial configuration, the plasticity will be decreased in time. In the case of gastrulation, however, it seems that the plasticity is regained, following the destabilization of the cell state, as is triggered by novel contacts of cells, that were not adjacent before. Here mechanical instability of cell configurations leads to cell motions, and novel arrangements of cells. Hence, novel type of dynamics that was not included in the original chemical system sets in here. In this, sense the system is 'open' to a new dimension of interaction, and allows for the recovery of plasticity.

Now we reconsider the experiment of Asashima's group from this viewpoint. We propose here that, after cells are put in the solution of activin, the cell state is destabilized to recover some degree of plasticity by this operation. Through this destabilization, a new path to states that were inaccessible are opened. Then, as the concentration of activin is higher, a path to a tissue that was hard to be reached are opened. Now, in the process of gastrulation, contact with new cell aggregates will lead to a similar destabilization. Then, the longer the contact is, the instability is larger and a developmental path to a tissue that was harder to reach should be opened. If the argument here is correct, the increase of contact time with cells that were apart in the course of gastrulation and the increase of the concentration of activin in the artificial development experiment must have similar effect. In fact, the ordering of notochord, nephros, muscle, in the order of concentration of activin in Asashima et al's experiment corresponds exactly to the order of tissues generated in the gastrulation. (here the cells that first contacted with new cells in the gastrulation have a longer time of contact, which are developed to the muscle). Although the discussion here is still premature, it will be important in future to understand the relationship between plasticity of cellular state and the ordering of induction as well as the tissue formation by external control.

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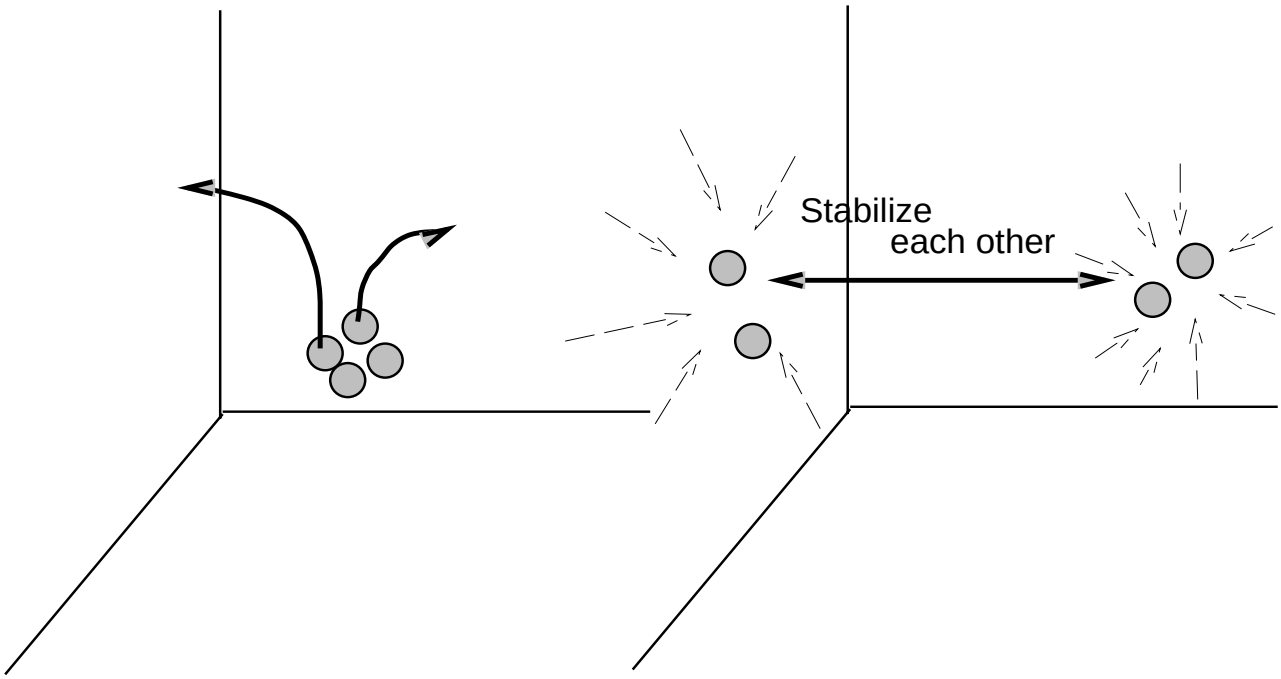

Figure 1: Schematic representation of state space and differentiation. A homogeneous cell state is destabilized, and by taking distinct states, the cells mutually stabilize others' state.

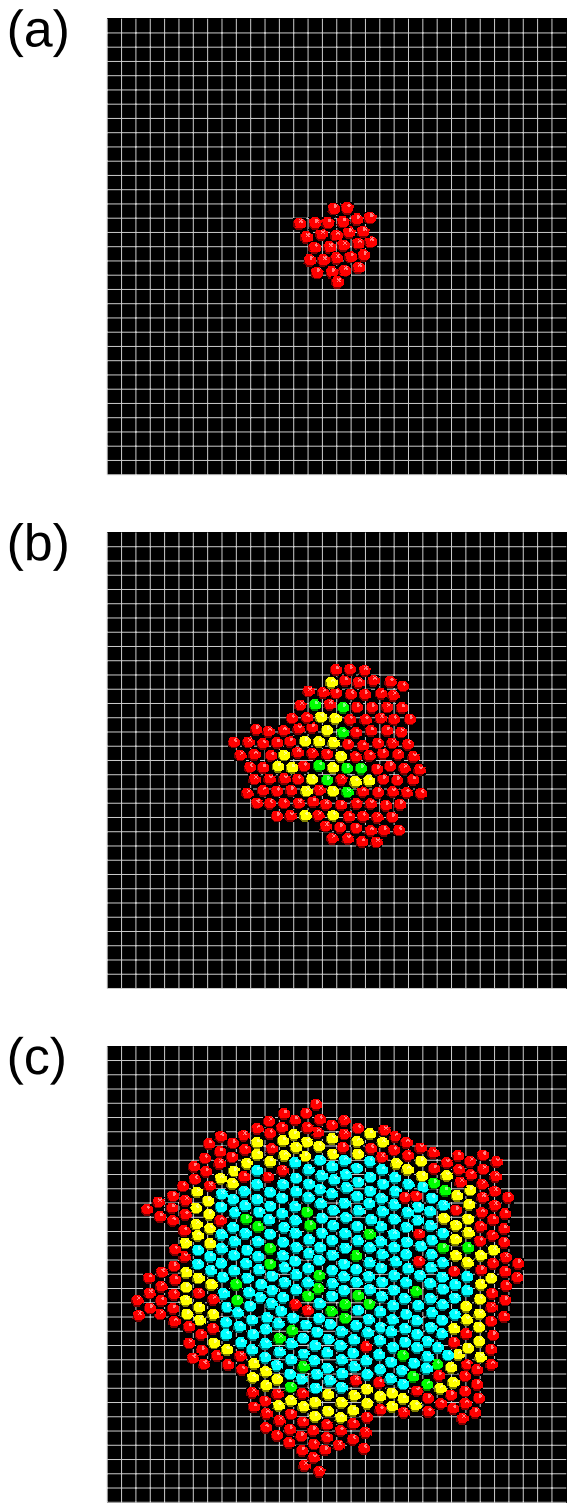

Figure 2: Development of a cell cluster toward a 'ring pattern' in a 2-dimensional medium. Each mark corresponds to a particular cell type, with different cell types distinguished by significantly different internal dynamics. adopted from [Furusawa and Kaneko, 1998b] with permission

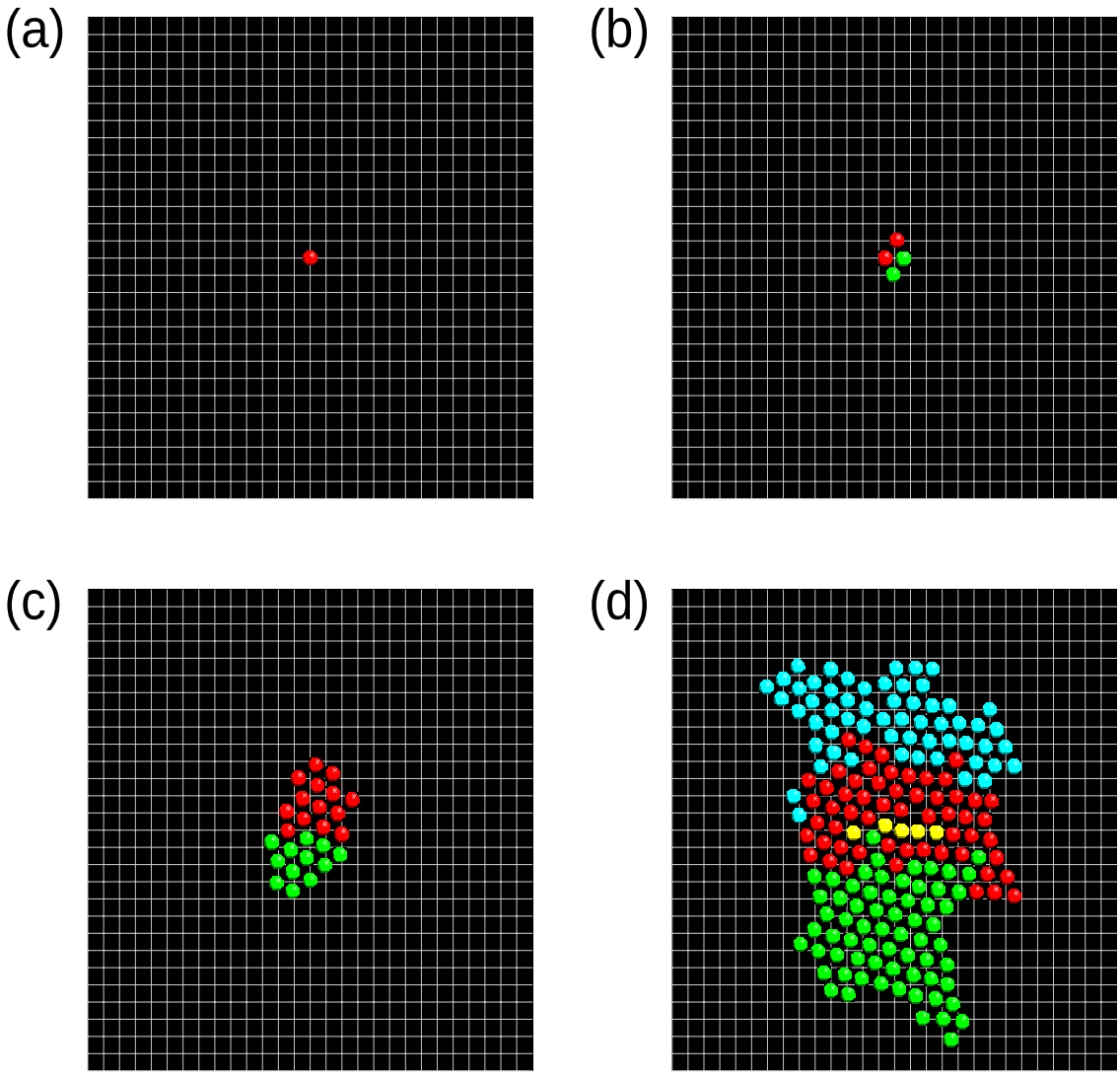

Figure 3: Development of a cell cluster toward 'striped pattern' in a 2-dimensional medium. Each mark corresponds to a particular cell type distinguished by its particular type of internal dynamics, as shown in figure.4. ¿From the stem-type cell S (red cell), type-A (green cell) is differentiated, and forms a cluster as shown in (c). With further increase of the number of cells, another type of cell B is differentiated which forms a cluster at the upper side. With these processes, a stripe pattern is formed (d).

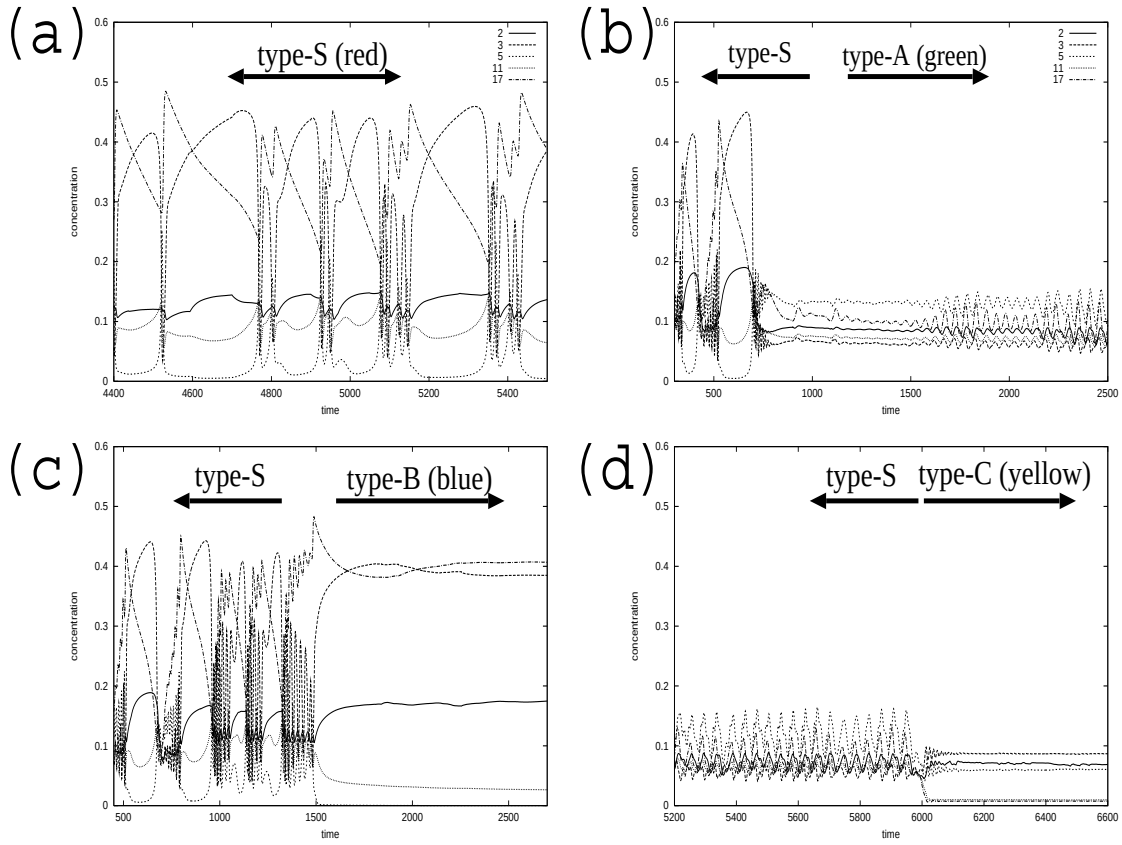

Figure 4: (a): Time series of concentrations for the type-S cell in the example of **striped pattern**. The ordinate represents concentrations of chemicals, plotted as a function of time. In this figure, we have plotted the time series of only 6 of the 32 internal chemicals, for clarity. (b)-(d): Time series of concentrations in a cell, representing the course of differentiation to type-A, B, C, respectively.

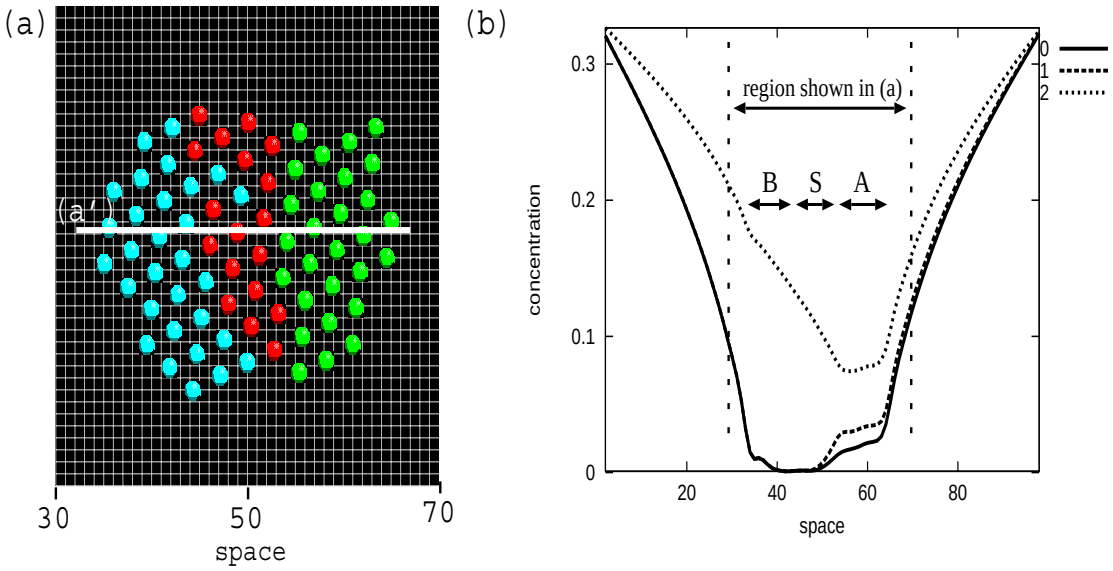

Figure 5: Concentration gradients along the stripe pattern. In (b), concentrations of three chemicals in the medium are plotted as functions of position along the segment (a)' in figure (a). In (b), the regions of different cell types (i.e., region of blue, red, green cells) are also shown by the arrows. At each end of the medium, the concentrations of all chemicals are fixed, because these chemicals are continuously supplied into the medium from a chemical bath at each end, which has fixed concentrations of chemicals.

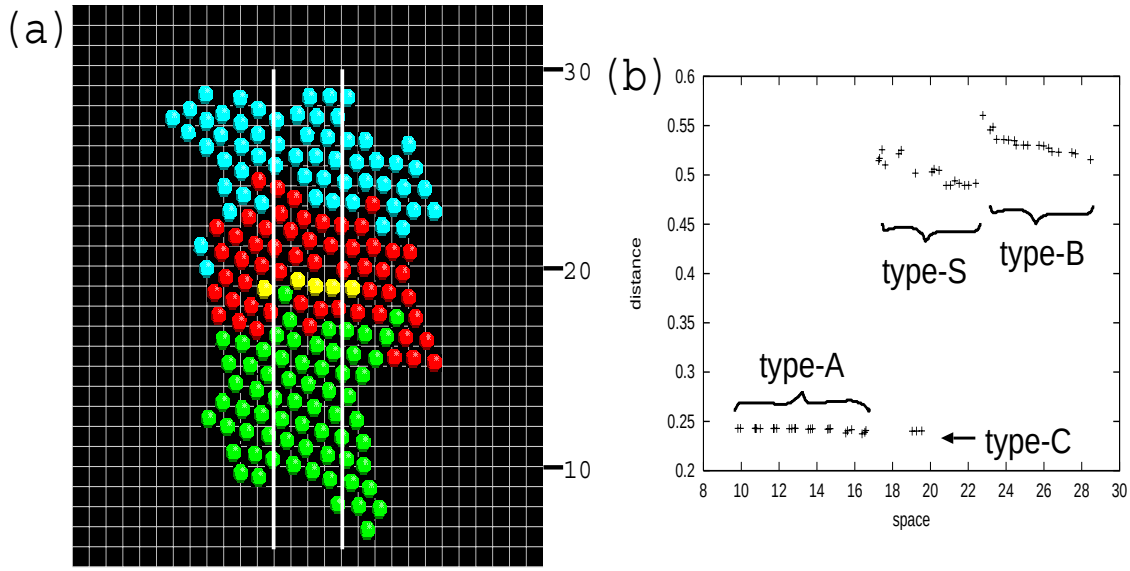

Figure 6: Variation of intra-cellular states with respect to position. The vertical axis shows the Euclidian distance between the average position of the orbit of the intra-cellular dynamics and the origin in the k-dimensional phase space is plotted as a function of the position in the medium for each cell. To measure the distance, we determined the average position of the orbit of each cell in the k-dimensional phase space, which was calculated as the averages of the k concentrations over a certain period for that cell.

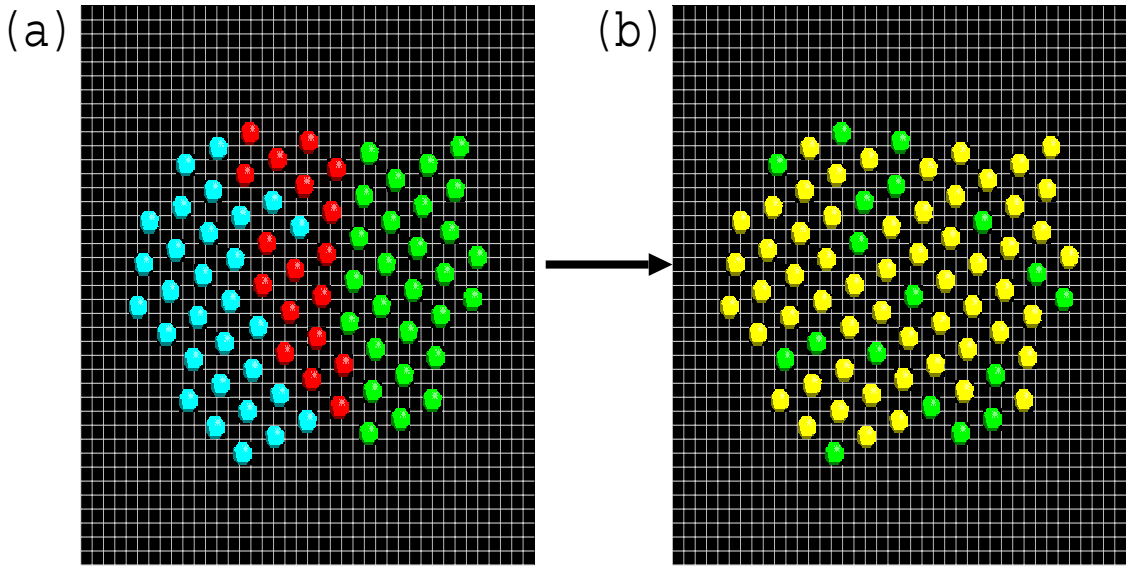

Figure 7: Spatial patterns of cells when multiple cells, instead of a single cell, are placed in the generated positional information (i.e., chemical gradient). The initial chemical concentrations in the medium were set to be the same as in Fig.5. Note that positional information only is not sufficient to reproduce the original pattern.

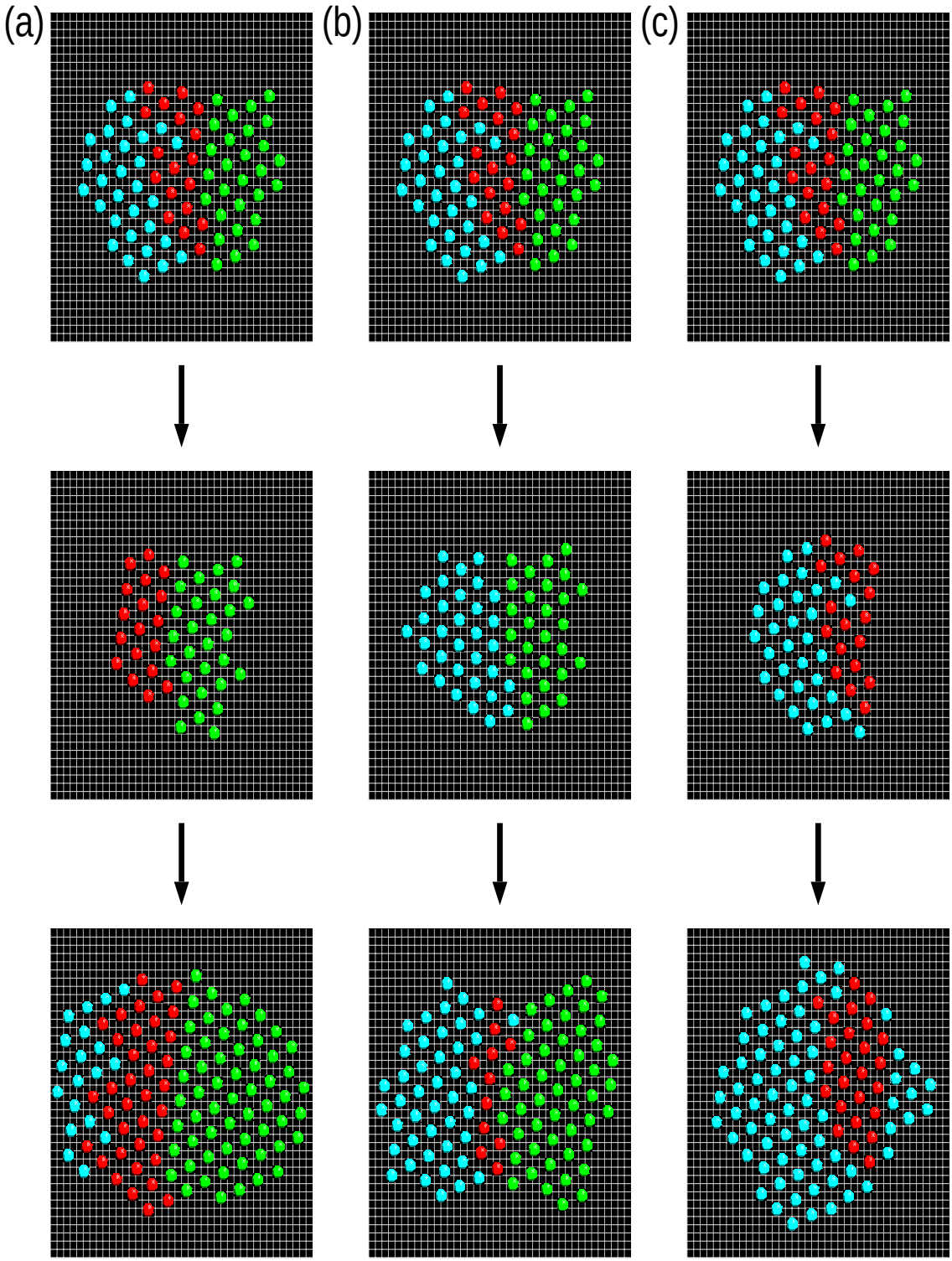

Figure 8: (a) When the type B cells are removed, the type B cells are differentiated from the type S cells to reproduce the original stripe pattern. (b) When the type S cells are removed, the type S cells are de-differentiated from the type B cells to reproduce the original stripe pattern. (c) When the type A cells are removed, the type B cells are differentiated from the type S cells to produce a novel BSB pattern. Based on [Furusawa and Kaneko, 2003b]

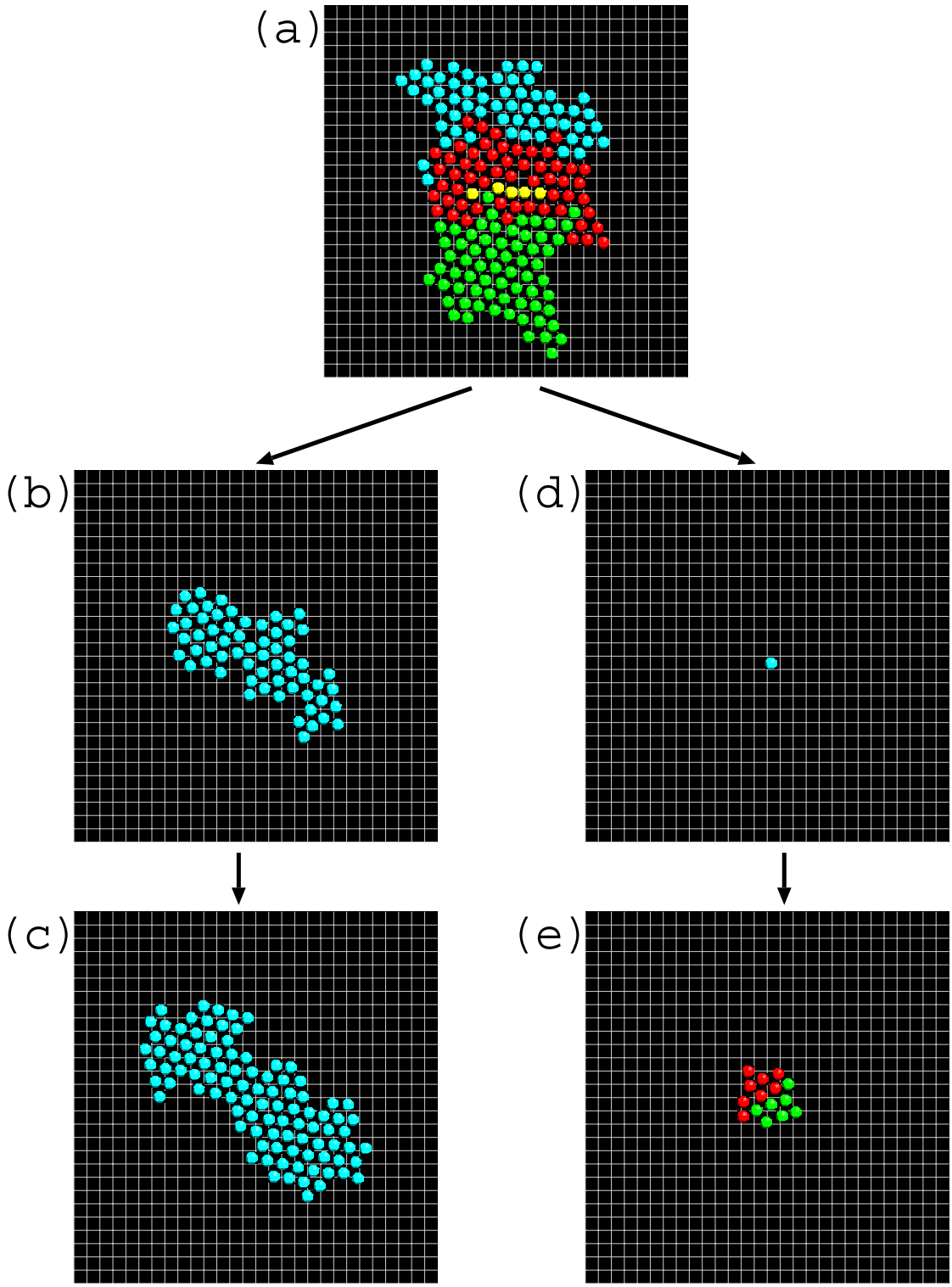

Figure 9: The community effect in the case of a striped pattern. ¿From an ensemble of cells exhibiting a striped pattern, a group of type-B cells or a single type-B cell were transplanted into a new, otherwise unoccupied medium, and they developed under the same rules and parameter values with the previous case. The type-B cells transplanted as a group remain type-B cells, while the type-B cell transplanted as a single cell transforms into a type-S cell and then, eventually develops into a striped pattern.