Dynamical Systems Basis of Metamorphosis: Diversity and Plasticity of Cellular States in Reaction Diffusion Network

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Abstract

Dynamics maintaining diversity of cell types in a multi-cellular system are studied in relationship with the plasticity of cellular states. By adopting a simple theoretical framework for intra-cellular chemical reaction dynamics with considering the division and death of cells, developmental process from a single cell is studied. Cell differentiation process is found to occur through instability in transient dynamics and cell-cell interaction. In a long time behavior, extinction of multiple cells is repeated, which leads to itinerancy over successive quasi-stable multi-cellular states consisting of different types of cells. By defining the plasticity of a cellular state, it is shown that the plasticity of cells decreases before the large extinction, from which diversity and plasticity are recovered. After this switching, decrease of plasticity again occurs, leading to the next extinction of multiple cells. This cycle of diversification and extinction is repeated. Relevance of our results to the development and evolution is briefly discussed.

keywords: metamorphosis, diversity, plasticity, stability

1 Introduction

In multi-cellular organisms, developmental process from a single cell in embryo or a few homogeneous cells with multi-potency leads to an organism that consists of various cell types. Furthermore, in some multi-cellular organisms such as insects, the developmental process is generally accompanied by metamorphosis. There, after a cell society consisting of specific distribution of some cell types
is achieved and sustained over some time, a transition to a novel society of distribution of different cell types starts through specific process. In other words, there exists several multi-cellular states, each of which is regarded to be quasi-stable distribution of cell types. In the event of metamorphosis, both the number of cells and the number of cell types decrease drastically, and then a different multi-cellular state is realized. After this “extinction”, adult organism makes embryos again to form the next generation. In other words, developmental process here passes through a rather restricted state that is different from the original embryo. Through the process, adult body of each species is formed, from which the next generation is reproduced. With this recursive production, the life cycle is repeated.

In usual developmental process, cells successively lose the multipotency, that is ability to produce a set of different cell types. This ability is regarded as a degree of changeability of a cell, i.e., plasticity. As the development proceeds, the number of cells and cell types increases, while the plasticity of cells generally decreases. Through the metamorphosis, however, some cells recover the ability to produce other type of cells, by regaining the plasticity.

In general, to consider the diversity of tissues and recursive production of a multi-cellular organism, it is important to study how several stable distributions of different cell types are formed, sustained, or collapsed. Then the following two general questions are addressed:

1. What mechanism causes the transition between different quasi-stable states with several cell types?
2. How is the switching process related with the plasticity of cellular states?

Our purpose in the present paper is to answer these questions in terms of dynamics of diversity and plasticity of cell types.

As for the first question, “isologous diversification theory” was proposed as a theoretical framework for robust developmental process of multi-cellular organisms. In these studies, dynamical systems approach is adopted, to show that robust developmental process with various cell types emerges as a result of interplay between intra-cellular dynamics and cell-to-cell interactions. On the other hand, as is mentioned above, there are diversity at a tissue level, i.e., different stable distributions consisting of different cell types, in a usual multi-cellular organism. So far, however, spontaneous formation of several quasi-stable states is not studied so much. Here we study the dynamics to maintain the diversity of cell types and the emergence of switches among several quasi-stable multi-cellular states. We show that all the cells change their states drastically at each switch, accompanied by deaths of multiple cells.

As for the second question, we show that the plasticity of each cell decreases with the increase of diversity of cell types, through the developmental process. We discuss relationship between the two, and elucidate the switching mechanism in terms of the diversity and plasticity.

For the present study, we first need to define plasticity of a cellular state.

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1In ref[5], a preliminary study for a prototype of the life cycle of multi-cellular organisms is reported.
Here we define it as changeability of a cellular state against external environmental change. By choosing a specific model, this plasticity is computed explicitly. By studying a developmental process of cells in the model, we will also find switches of multi-cellular states accompanied by multiple cell deaths. This switching process will be shown to be tightly related with the loss of plasticity. From extensive simulations, the long-term dynamics of cell society and the plasticity we found are summarized as follows:

As the development progresses, several cell types with low plasticity increase their number, which leads to extinction of several types of cells. This extinction brings about a drastic change in environment surrounding cells. Accordingly, internal states of all the surviving cells are changed. Then, different cell types with high plasticity are generated. From this 'undifferentiated state', which is similar as the initial state, a new cell society with a different set of cell types is formed. In some case this cycle is repeated.

We show that the cycle of increasing and decreasing diversity and plasticity generally appears in our dynamical systems model. We reveal a general mechanism underlying commonly in such process, and formulate it in terms of dynamical relationship between diversity and plasticity of cell types.

For the purpose of the present study, we adopt a simple modeling framework for internal chemical reaction dynamics, that is reaction-diffusion system on "chemical species space". By adopting this modeling, one can correspond chemical concentrations with the fixed expression of genes, which is a common representation for each differentiated cell type. We then discuss both the stability of realized cell states and their diversity.

The present paper is organized as follows. In the next section, we describe the details of our model. We present the behavior of developmental process in section III. The condition for the present model to show cell differentiation and switching over several quasi-stable cell societies is presented there. In section IV, we reveal a feedback process leading to this switching, and confirm the relationship between the switching and the plasticity of total cells. After presenting some results on a control of multi-cellular state by external operations in section V, we briefly mention the generality of the present result, and discuss its relevance to development and evolution in section VI.

2 model

In this paper, we adopt a constructive modeling, by taking only some basic features of a problem in concern, to answer general questions. Here, with regards to diversity, stability and plasticity of cell states.

The basic strategy of the modeling follows the previous works [8, 9, 11]. Cells with internal biochemical states compete for resources in the environment for their growth. Following the growth or decrease of cell contents, a cell can divide or die so that the cell number changes in time. Our dynamical systems model consists of the following three parts:

1. intra-cellular chemical reaction network
• cell-cell interaction
• cell division and cell death

Now we describe each process. In Fig.1 we show the schematic representation of our model.

• intra-cellular reaction network
In general, intra-cellular chemical reactions consist of the reactions both of genes and metabolites. Genes are set to be on or off through biochemical reactions. This intra-cellular biochemical reaction constitutes a network both of genes and metabolites, while the time scale for the reactions among genes are relatively much slower than those of metabolites. Here, simple intra-cellular chemical reaction dynamics is chosen as an abstract model, so that it satisfies basic features described above.

First, we assume a reaction network consisting of many product and substrate chemicals. Existence of these two types of chemicals, each constituting reaction networks, are inspired by the genes and metabolites in a cell. The concentration of the \( j \)-th products in \( i \)-th cell is denoted by \( v_j^i(t) \), while that of substrates is denoted by \( u_j^i(t) \). Here, product chemicals are synthesized autocatalytically by consuming corresponding substrate chemicals. We adopt a variant of Gray-Scott model\(^2\) as this autocatalytic reaction scheme\(^2\). In this paper, we mainly present the results of the case where the number of chemical species is commonly set to \( K = 30 \).

In the model \( v_j^i(t) \) is assumed to correspond to the degree of expressions of a gene (or RNA), and \( u_j^i(t) \) to concentration of a metabolite. Besides the reaction dynamics between them, chemical concentrations may change through the reaction dynamics within a gene network or metabolic network. To take into account of such dynamics, we assume reversible reactions in each of product and substrate chemicals, which form two reaction networks. Each chemical is converted to other chemicals by a reversible reaction given by the network. The reaction network is represented by a reaction matrix \( W(i,j) \), which is 1 if there is a reaction from chemical \( i \) to chemical \( j \), and 0 otherwise. Since the reaction network is assumed to be reversible, the matrix is symmetric, i.e., if \( W(i,j) = 1 \), then \( W(j,i) = 1 \). Here, we adopt the same network for products and substrates, and also assume that the network is composed of two reaction paths per chemical to form a single closed-loop structure for simplicity.

The rate constant of reversible reaction \( C_u \) and \( C_v \) are assumed to be common to all resources and all products, respectively. Moreover, we also assume that \( C_u \) is larger than \( C_v \), by considering the difference in the time scales between metabolites and genes. These values are fixed throughout the simulation. Then, these reversible reactions are regarded as ‘diffusion’

\(^2\)Gray-Scott model is a reaction-diffusion system composed of two chemicals, substrate and product. It is a simplified version of autocatalytic Selkov model that explains self-sustained oscillation of glycolysis.
on ‘chemical species space’, so that intra-cellular reaction between products and substrates is regarded as a one-dimensional reaction-diffusion system [13]. In that representation, each attractor of intra-cellular reaction dynamics corresponds to a cell state with a different genetic expression pattern. Since developmental process in real cell system is elucidated as spontaneous cascading processes with different gene expression patterns, we study how different cellular states are selected by including the developmental process to the model.

- cell-cell interaction
  Assuming that environmental medium is completely stirred, we can neglect spatial variation of chemical concentrations in it, so that all the cells share the spatially homogeneous environment. Here we consider only the diffusion of resource chemicals through the medium as a minimal form of interaction. In this model, we assume that only substrate chemicals are transported through the membrane as resources, in proportion to the concentration difference between the inside and the outside of a cell. All the resource chemicals have the same diffusion coefficient $D_u$. Each cell grows by taking resource chemicals from the medium and transforms them to product chemicals. $U^j$ is the concentration of $j$-th resources in the medium. Resource chemicals in the medium are consumed by cells, while we assume that resource chemicals are supplied from external material to the medium, with the rate proportional to the difference between the concentration in the bath and the medium. Again, all the resource chemicals have the same diffusion coefficient $D_U$ in the medium. The concentrations of all resources in the material bath, $U$ are set to be 1. The parameter $Vol_0$ is the volume ratio of a medium to a cell, and $N$ is the number of cells.

- cell division and cell death
  Each cell gets resource chemicals from the medium and grows by transforming them to the product chemicals. Here we assume that cell volume (denoted by $Vol_i$ for cell $i$) is proportional to total amount of product chemicals. For each time step in temporal evolution, we count the change of product chemicals, that is, the change of total cell volume, and each chemical concentration becomes to be normalized by the factor. When the cell volume becomes twice the original, then the cell is assumed to divide, while if it is less than half the original, then the cell is put to death. In real biological system, cell division occurs after replication of DNA (which has smaller diffusion coefficient and cannot penetrate through the membrane). Hence these assumptions are rather natural. After cell division, each cell volume is set to be half, and each cell is divided into two almost equal cells, with some fluctuations. To be concrete, chemical concentration $a$ (a is a representation of $u$, $v$) is divided into $(1 + \eta)a_i^{(j)}$ and $(1 - \eta)a_i^{(j)}$ respectively, where $\eta$ is a uniform random number over $[-10^{-3}, 10^{-3}]$. These fluctuations can give rise to a small variation among cell states, which
eventually leads to cell differentiation by being amplified through cell-cell interaction.

Accordingly, the concentration change of each chemical species is given by

$$\Delta u_i^j(t) = D_u(U^j(t) - u_i^j(t)) - u_i^j(t)v_i^j(t)^2$$

$$\Delta v_i^j(t) = u_i^j(t)v_i^j(t)^2 - Bv_i^j(t)$$

(1)

$$\frac{du_i^j(t)}{dt} = \Delta u_i^j(t) + C_u \sum_{k=1}^{K} W(j, k)(u_k^i(t) - u_i^j(t)) - u_i^j(t)dV ol_i(t)/dt$$

$$\frac{dv_i^j(t)}{dt} = \Delta v_i^j(t) + C_v \sum_{k=1}^{K} W(j, k)(v_k^i(t) - v_i^j(t)) - v_i^j(t)dV ol_i(t)/dt$$

$$\frac{dU^j(t)}{dt} = \frac{D_u}{V ol_0} \sum_{i=1}^{N}(u_i^j(t) - U^j(t)) + D_U(U - U^j(t))$$

$$\frac{dV ol_i(t)}{dt} = \frac{\sum_{j=1}^{K} \Delta v_i^j(t)}{\sum_{j=1}^{K} v_i^j(t)} V ol_i(t)$$

Here, the last term for each equation for $\frac{du_i^j(t)}{dt}$ and $\frac{dv_i^j(t)}{dt}$ represents the dilution of concentration by the increase of the volume.

In the model introduced above, a single cell has many fixed-point attractors, in contrast to the previous studies [8, 9, 4, 5], where a single cell can take only one or a few attractors. Many stable cellular states are realized accordingly, that correspond to different cell types. Furthermore, as will be shown, by cell-cell interaction, cells are differentiated to take different chemical compositions.

### 3 Developmental process with cell division and cell death

The behavior of a single cell state depends on the bifurcation structure of the reaction-diffusion system on ‘chemical species space’ mentioned above. In the region where uniform steady state becomes unstable, a single cell state has multiple attractors. The dependence of the number of attractors on the number of chemical species is expected to be exponential, which is verified in numerical simulations (data not shown). Based on these results, we discuss developmental process with the change of cellular states under the process of cell division and cell death. We study a coupled dynamical system, where the intra-cellular state and the inter-cellular interaction are mutually influenced. By cell-cell interaction, a homogeneous cell society of a single cell attractor may be destabilized, so that novel states may appear. We study such cell differentiation processes here.
3.1 Initial conditions and methods

In all the simulations, we mainly set the parameters $C_u = 2.0$, $C_v = 0.020$, $D_u = 0.50$, $D_U = 1.0$, $Vol_0 = 3.0$, $B = 0.060$. These values of $B \sim C_v$ correspond to one of typical values at which the original Gray-Scott model in one-dimensional space forms a self-replicating spot pattern, so that our model also shows such pattern dynamics. Initial condition of the first cell is chosen as $u_j(0) = 0.50$ and $v_j(0) = 0.250 + 0.01 \times rand(j)$, where $j = 1, 2, ..., K$ and $rand(j)$ is a uniform random variable over [-1,1], although this specific choice is not important.

3.2 Cell differentiation

Now we show an example of the differentiation process. Fig.2 shows a typical temporal evolution of the concentration of $v_j(j = 1, .., K)$ for all the cells starting from a single cell, in which cell differentiation occurs. A series of snapshots are shown by using a gray scale for the concentration of product chemicals. Each group of distinct cellular states with a different set of values $v_j$ corresponds to a quasi-stable cell type, which makes recursive production. (Time for reproduction is much longer than typical transient time to reach each quasi-stable state.) The figure also includes the cell states that appear at a later stage of the temporal evolution(Fig.2(f)). There exist eleven types, all of which are fixed points. Under the instability of transient state of cells, small fluctuations in cell division process are amplified through the competition for resources in environment among all the cells, which leads to cell differentiation. From extensive simulations of the present model, the necessary conditions for cell differentiation are summarized as follows 3:

(1) Inter-cellular interaction is stronger than some threshold.
(2) The ratio of the reaction rates for $u^j$ to that for $v^j$, i.e., $C_u/C_v$, is in the intermediate range.
(3) The number of chemicals is larger than some threshold.
Details on these conditions are shown in appendix.

3.3 Switching between quasi-stable ensembles consisting of several cell types

Next we investigate the long time behavior of cell differentiation process. We show an example of typical temporal evolution by plotting existing cell types and their population at every 1000 time unit in Fig.3. A multi-cellular state consisting of several cell types is formed at a very early stage, and is maintained over a long period, which is much longer than typical transient time of a single cell state. Then a sudden crisis occurs, and after some generations, a new multi-cellular state with different cell types is recovered. This crisis is accompanied

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3If we set our initial condition of the first cell exactly on an attractor of a single cellular state, differentiation does not occur. In this case, cells will go extinct after several divisions.
by the decrease of the cell number and diversity in cell types. This switching between diversification and extinction can occur repeatedly.

4 Further Analysis of the switching process

4.1 quantitative characterizations of the plasticity of cell types

Now we investigate the switching among several quasi-stable multi-cellular states in more detail. We define recursiveness of each cellular state through the reproduction, by comparing the average concentrations of product chemicals between a mother cell and its daughter cell. This similarity of chemical compositions between a mother and its daughter cell is defined as follows. Let \( \overrightarrow{V_i(n)} \) denote the vector representation of all the average concentration of product chemicals \( v_i^j \) of the \( i \)-th cell between (n-1)-th and n-th cell division, that are denoted by \( v_i^j \) for \( j = 1, 2, \ldots, K \). As an index for the recursiveness of cellular state, we introduce an inner product denoted by \( H_i(n) \) between \( \overrightarrow{V_i(n)} \) and \( \overrightarrow{V_i(n-1)} \).

\[
H_i(n) = \frac{\overrightarrow{V_i(n)} \cdot \overrightarrow{V_i(n-1)}}{|\overrightarrow{V_i(n)}||\overrightarrow{V_i(n-1)}|}, \quad \overrightarrow{V_i(n)} = (v_i^1, v_i^2, \ldots, v_i^K)
\]

If \( H_i(n) \approx 1 \), the cell is regarded to keep the same type between successive cell divisions, that means recursive reproduction. In the present model, each differentiated cell type is represented as distinctly separated chemical states of cells where each cell type can produce its own type recursively. The cell differentiation processes in our model are well represented with these two quantities, by which we can distinguish all cell states correctly.

Next we introduce a measure of plasticity of cell type to study the switching process in terms of the temporal change of plasticity of cell types. Here we define the plasticity of each cell type as changeability of the cell state against environmental fluctuations, which are the change of concentrations of resource chemicals in the environment caused by cell divisions and deaths. We characterize the plasticity of cell state in the following manner:

First we take \( v(o)_i^j \), the concentration of \( j \)-th product chemical of the type \( i \) cell, while we define \( v(f)_i^j \) as the concentration of \( j \)-th product chemical of the attractor of the cell type \( i \) by eliminating cell division and death processes, to check the attractor state at the fixed environment. Then we define the **attractor distance** \( \text{Dist}_i \) of the type \( i \) cell, as the Euclid distance between \( v(o)_i^j \) and \( v(f)_i^j \), namely,

\[
\text{Dist}_i = \sqrt{\sum_{j=1}^{K} (v(f)_i^j - v(o)_i^j)^2}
\]

As the distance is smaller, the cell type is closer to a specific attractor reached under a fixed environment by fixing cell-cell interactions.

Now, we conjecture that

(1) the cell plasticity (changeability) is characterized by this attractor distance (i.e., distance from an attractor) and that
the potentiality of differentiation decreases as this attractor distance is smaller.

We will demonstrate these conjectures by measuring the temporal fluctuations of cells and frequency of differentiation.

**Conjecture (1):** First, we note that the plasticity of a cell means the changeability of the state against the environmental change. Here, the conjecture means that this changeability is smaller as the state is closer to a given attractor. In the present system, the environment fluctuates according to the birth and death of surrounding cells. Hence, the changeability against environment is computed by the temporal fluctuations of chemical compositions of each cell type. (Such relationship between fluctuation and response against environment is known as fluctuation-dissipation theorem in physics, and is extended to a biological system recently.)

Hence, we demonstrate the conjecture (1) by checking if there is positive correlation between the attractor distance and the degree of temporal fluctuations of each cell state. We compute the temporal fluctuation of each cell type for some periods where several cell types coexist stably in each temporal evolution. This temporal fluctuation of each cell type is computed as the difference of chemical concentrations (i.e., Euclid distance) between two cells at a given time span. Here we take the time span $t = 1000$, and temporal fluctuations are calculated from the average over 100 data samples. An example of this temporal fluctuation plotted as a function of the attractor distance is shown in Fig.4, which shows positive correlation between the two. We investigate other data over ten periods from five temporal evolutions including an example of Fig.3, and all of them show positive correlations. (The correlation coefficient ranges from 0.46 to 0.84.) This result shows positive correlation between the temporal fluctuations of the chemical concentrations of a cell type and its attractor distance.

**Conjecture (2):** The second conjecture will be confirmed by the positive correlation between the attractor distance and the frequency of the occurrence of re-differentiation event when a cell ensemble consisting of cells of this single cell type are put to a new environment. We compute the average of this frequency for each cell type, over cells within a given range of attractor distance. The relationship between the frequency of re-differentiation and the attractor, thus obtained, is plotted in Fig.5 using the same data as above. As shown in Fig.5, there is a sigmoidal function dependence, between the attractor distance and the frequency of re-differentiation. There is a threshold for the attractor distance below which the corresponding cell type loses the ability of re-differentiation drastically.

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4 Here the population of each cell type is set to be 80. If the number is much larger, the homogeneous cell ensemble grown from the group goes extinct by the lack of the resources. On the other hand, if the number is much smaller, then the effect to internal cell states caused by the change of the environment is so large that the cells lose the original character when they are selected. Hence we choose this medium number for cell population.

5 This also supports the initial condition dependence of cell differentiation event mentioned previously.
By summing up these two results, it is confirmed that the attractor distance introduced above is valid as a measure of plasticity of cell type.

4.2 Mechanism for switching through extinction of many cells

Now we discuss the switching with multiple cell deaths in relationship with the loss of plasticity.

First, we summarize our results discussed here as the following scenario: At each stage of given quasi-stable multicellular states, cell types with different degrees of plasticity coexist. Then at each stage, cell types with relatively high plasticity (i.e., with larger attractor distance) differentiate to other cell types with lower plasticity, so that the ratio of cell types with lower plasticity increase gradually. Then the distribution of cell types allowing for effective use of resource chemicals is destroyed, resulting in extinction of many cell types. With these multiple deaths, the concentrations of environmental resource chemicals change drastically, leading to re-differentiation of some of surviving cells with low plasticity into a new state with high plasticity. Then, emergence of novel cell types with high plasticity gives rise to a novel multi-cellular state, and effective use of resources becomes possible again. With this drastic change, switch to a novel multi-cellular state follows.

This scenario is verified by computing the temporal change of the following five quantities using the data for the temporal evolution of Fig.3: the total diversity of cell types (Fig6(a)), the average recursiveness of cell types over all cells (Fig6(b)), the total number of cells (Fig6(c)), the average of attractor distances over all of the existing cell types at each moment(Fig7(a)) and the number of cells at each bin of attractor distances(Fig7(b)). As shown in Fig6 and Fig7 the average attractor distance as well as the number of cell types decreases first. With these decreases, the recursiveness of cells increases on the average. Then, the attractor distance and the number of cell types stays at low values, and almost complete recursive production is sustained, since the most existing cells have low plasticity. After slight decrease of the attractor distance and diversity, then, these two values go up to higher values, accompanied by multiple cell deaths and switching of cell types. Now, high plasticity and diversity of cell types are recovered. After this recovery, the attractor distance and diversity again decrease gradually, until the next multiple cell deaths occur. This cycle consisting of the decrease of diversity, extinction, and recovery is repeated.

Although we show only one example here, qualitatively the same behavior is generally observed at each switching event in the present model. The scenario mentioned above is rather universal.
5 Transition of states by external operations

So far, we have studied the switching process with regards to relationship between diversity and plasticity of cell types. In this section, we study the behavior of the present cell system after some kind of operation is applied to the cell system. First we add noise into intra-cellular chemical reactions. Here the noise is regarded as the fluctuation of the number of molecules, and is assumed to be Gaussian white noise. The stochastic differential equations for resource and product chemicals in a cell are expressed by adding a noise term to the ordinary difference equations (1):

\[ \Delta v^j_i(t) = u^j_i(t)v^j_i(t)^2 - Bv^j_i(t) + \eta \sqrt{v^j_i(t)} \]
\[ \Delta u^j_i(t) = D_u(U^j_i(t) - u^j_i(t)) - u^j_i(t)v^j_i(t)^2 + \eta \sqrt{u^j_i(t)}. \]

Here \( \eta \) is a Gaussian white noise satisfying \( \langle \eta(t)\eta(t') \rangle = \sigma^2 \delta(t-t') \).

We study how developmental process changes with the change of the noise amplitude. When the noise amplitude is too large, multi-cellular states change almost randomly in time. If the amplitude is small, stable multi-cellular states of several cell types are formed eventually, and the switching process does not appear any more. An example of such long time behaviors is plotted in Fig.8 and the corresponding change of the average attractor distance is also plotted in Fig.9. Starting from different initial conditions, different multi-cellular states consisting of different cell types are realized, which have different plasticity. It is now shown that multi-cellular states (with relatively low plasticity) are stabilized by the noise.

Next we study the behavior against external change of the environment. As an example, we decreased the supply of some resource chemicals. The change of multi-cellular states as a result of the restriction of some resources is shown in Fig.10. When concentration of five chemical resources are reduced, given by the arrow in the figure, the original cell types that have low plasticity become unstable, and some of the cell types regain the plasticity. Then the cell differentiation process is restarted, leading to a novel multi-cellular state. The corresponding change of the average attractor distance is also plotted in Fig.11, which clearly shows that the plasticity is regained by the external change of environment. Thus, a multi-cellular states that was stable and fixed is destabilized by external operation, which leads to the change of environment.

6 Summary and discussion

In this paper, we have studied a dynamical systems model of developmental process, by introducing a new framework, namely, reaction-diffusion system on ‘chemical species space’ for intra-cellular chemical reaction dynamics. By taking the developmental process into account further, it is shown that cells are differentiated into several types. The condition for the cell differentiation by cell-cell interaction is obtained.

As a long-term behavior, we have found the switching over several multi-
cellular states that maintain diverse cell types. In each multi-cellular state, diverse cell types coexist to reduce the competition for chemical resources, while the switching is characterized by multiple cell deaths arising from the loss of diversity of cell types and higher competition for the resources. This switching behavior is first discovered in the present model.

Then, we propose that this switching behavior is characterized by the loss of plasticity of total cells, that is a general consequence of our dynamical systems theory. The irreversible loss of plasticity is a general course in the developmental process, i.e., differentiation from a cell type with relatively high plasticity to that with lower plasticity, so that the ratio of cell types with lower plasticity increases gradually. Then, effective use of resource chemicals by a suitable distribution of different cell types is destroyed, which leads to multiple cell deaths. Drastic change of the composition of environmental resource chemicals is resulted. Cells with low plasticity are replaced by those with high plasticity. As a result, a new multi-cellular state with novel cell types is generated. This process is repeated. Schematic representation of the above scenario is summarized in Fig.12.

The existence of several quasi-stable multi-cellular states is important to consider the origin of several tissues in multi-cellular organisms. In multi-cellular organisms, several tissues coexist that are represented as a cell ensemble with a different composition of consisting cell types, with a common gene set. Then, the switching over several multi-cellular quasi-stable states will be important to study how the life cycle of a multi-cellular organism is formed. As a first step toward such study, we impose some external change to the system to make a transition between different multi-cellular states. In future, the search for a rule of transitions between successive multi-cellular states will be important. The dynamics of metamorphosis can be discussed along the line.

In our switching process, all cells with low plasticity are changed to those with high plasticity, through multiple, simultaneous cell deaths. In real metamorphosis, such kind of “extinction” of many cells is also observed generally, as seen for example in insects. According to our results, fluctuations of cell states have positive correlation with the plasticity of cell types.

The present scenario of loss of plasticity and recovery by multiple cell deaths can be experimentally verified, by measuring the gene expression as indicators of chemical concentrations. One can measure the variance of gene expressions, for example by using fluorescent protein and cell sorter, during the course of the developmental process. Consider generally growth of a colony of cells. By measuring the change of variance of gene expressions over cells through the developmental process of cells, one can examine whether there is a decrease in fluctuations, and moreover, whether there is a recovery when multiple cell deaths lead to a novel ensemble of cells, as in the event of metamorphosis.

In the present paper, we discussed spontaneous cell differentiation and switching processes in a well-stirred medium, i.e., in a spatially homogeneous medium. To discuss morphogenesis with spatial pattern formation, it will also be interesting to include spatial inhomogeneity in the medium.

The present results also have some implications to the evolution. Indeed, one can extend our model to regard each unit as an organism, instead of a
cell, and include genetic change (mutation) as that of parameters in the model. With this extension, different types in our model can be regarded as different species. Indeed, a theory of sympatric speciation with using phenotypic plasticity is recently proposed along this line [10, 13]. In a preliminary study of the present model including genetic mutation process, we have observed sympatric speciation process to form several species, while with this process, the plasticity of each species defined in this paper decreases. With this extension, successive extinction events of some cell types in the present model correspond to mass extinction of species through evolution. Note that in the theory of punctuated equilibrium [2], evolution process consists of long quasi-stationary regime and rapid temporal change accompanied by extinctions, as discussed above.

Here, the recovery of plasticity of species after extinction of many cells is relevant to open-ended evolution, as will be discussed elsewhere.

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A The condition for cell differentiation

Here we study conditions for cell differentiation. First, we study the initial condition dependence of differentiation event. As is mentioned above, it is necessary for an initial cellular state not to be exactly at an attractor of a single cell. If we start precisely from an attractor of a single cell state, then the cells that are derived from its successive divisions cannot differentiate. Once the state is on an attractor, the cell division gives two identical cells, as long as the fluctuation in cell division is not large to make different attractor. Then, with the increase of cell numbers, all the cells compete for the same resources, and eventually they come to the stage that all the cells cannot take enough resources leading to the decrease in the chemical contents in a cell. Hence all the cells die, almost simultaneously at some stage. On the other hand, cell differentiation, however, generally emerges as long as the initial condition is not chosen precisely on an attractor of a single cell state.

Second, we study dependence of the differentiation frequency on $C_v/C_u$, the ratio of the time scale of species-changing reaction among resource chemicals to the one among product chemicals. We changed the parameter $C_v$ from 0.002 to 20 while fixing $C_u = 2.0$.

If $C_v$ is much smaller than $C_u$, all the cells increase their number with keeping almost the same chemical composition, and competition for the resources is too strong for cells to survive. Whereas, if $C_v$ is a comparable order of $C_u$, all the cells take the same uniform state with $w^j \neq 1$ and $v^j \neq 0$, so that extinction event occurs. Cell differentiation events occur most probably in the intermediate case between above two cases.

Third, we study the dependence of differentiation event on the strength of
interaction between cell and environment. As an index of it, we change the parameter $D_u$ from 0.1 to 1.9. For $D_u \leq 0.4$, cell-cell interaction is too weak to amplify small differences among cell states, so that differentiation event cannot occur. Hence it is necessary that the strength of cell-cell interaction is stronger than some threshold value.

Fourth, we study the dependence of differentiation event on the number of chemicals $K$. The frequency of cell differentiation rises with the increase of $K$. Hence it is necessary that the number of chemicals is larger than some value. The necessary conditions for cell differentiation obtained in this appendix are summarized in Sec.III.B:

(0) Initial condition is not set to be exactly on an attractor of a single cell.
(1) Inter-cellular interaction is stronger than some threshold.
(2) The ratio of the reaction time scale among $u^j$ to that among $v^j$ is in the intermediate range.
(3) The number of chemicals is beyond some level.
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synthesized autocatalytically
role in cells
source of supply
environment
interaction in each chemical species
chemical species
Existing
not exist
time scale
faster
slower
cell division
cell death
when total V becomes twice
when total V become half

| role in cells | resource                  | product                                      |
|--------------|---------------------------|----------------------------------------------|
| source of supply | flow from the environment | synthesized autocatalytically                |
| environment | exist | not exist                                |
| chemical species | species-exchanging reaction (symmetrical = 'diffusion') | species-exchanging reaction (symmetrical = 'diffusion') |
| time scale | faster | slower                                    |
| cell division | | when total V becomes twice |
| cell death | | when total V become half |

Figure 1: Schematic representation of our model.
Figure 2: An example of the time series of all the cells. Snapshot patterns of the concentrations of all the product chemicals are overlaid with a gray scale at every $t = 50(a) \sim (e)$ except for $f$ at $t = 10000$. The horizontal axis represents the number of product chemicals, whereas the vertical axis represents the indices of cells, which are sorted so that the cells of the same type are aligned. Unless otherwise mentioned, we adopt the parameter values $A = 0.020, B = 0.060, C_u = 2.0, C_v = 0.020, D_u = 0.50, D_U = 1.0, Vol_0 = 3.0$ and $K = 30$ for later figures.
Figure 3: An example of the long time behavior of cell differentiation process. Simulation is carried out up to $t = 1000000$ starting from a single cell, while the data for the total cells are sampled by every 1000 time, to classify all cell types and to get the temporal change of population of each cell type. All cell types are shown in the order of their appearance; a cell type that appears earlier in the simulation has a smaller index for the cell type. The population of each cell type is represented with a gray scale. Here the unit time of the figures is 1000.
Figure 4: An example showing the relation between the attractor distance and the temporal fluctuation of each cell type. Over a period when several types coexist stably in a temporal evolution, we measured the temporal fluctuation of each cell type as the Euclid distances of chemical concentrations between two cells of the same type, chosen at different time, separated by a time span $t = 1000$. The temporal fluctuations are computed over 100 data samples, and the average over the samples are plotted.

Figure 5: The relation between the attractor distance and the frequency of re-differentiation. We compute attractor distance of all the cell types appeared in each temporal evolution, and take an ensemble of cells whose members have the same initial condition. This cell ensemble is put into a new environment to check whether cell differentiation occurs or not. By sampling the data for the attractor distance by 0.1 bin size, we compute the average ratio of the frequency of re-differentiation event for each bin, to get the relationship between the differentiation ratio and the attractor distance.
Figure 6: (a): Temporal change of the number of cell types. (b): Temporal change of the average recursiveness over all cells. (c): Temporal change of the total cell number. We computed all the quantities for the data given in the temporal evolution of Fig. 3. Here the unit time of the figures is 1000.
Figure 7: (a): Temporal change of the average attractor distance. The average attractor distance is obtained by averaging over all existing cells. (b): Temporal change of attractor distances of all the cell types existing simultaneously are plotted with their population. We computed all the quantities for the data given in the temporal evolution of Fig. 3. Each time when extinctions of many cells occur is shown by the arrow. Here the unit time is 1000.
Figure 8: An example of the long time behavior of cell differentiation process by adding noise with the amplitude 0.00010. Simulation is carried out up to $t = 1000000$ starting from a single cell, while the data for the total cells are sampled by every 1000 time, to classify all cell types. The initial condition and method for the classification of cell types are mentioned in the text. All cell types are shown in the order of their appearance; a cell type that appears later in the simulation has a larger index for the cell type. The population of each cell type is represented with a gray scale. Here the unit time is 1000.

Figure 9: The time series of the average attractor distance, corresponding to the temporal evolution of Fig.8. Here the unit time of the figures is 1000.
Figure 10: An example of the temporal behavior of cell differentiation process after external change of environmental resources shown by the arrow. Initially, cell distribution was given by the quasi-stable multi-cellular state at $t = 10^6$ in the simulation of Fig.8. Then, the simulation is carried out up to $t = 50000$, by reducing the supply of five resource chemicals at $t = 5000$, as shown by the arrow. The states of all cells are sampled by every 1000 time unit, to classify all cell types. The method for the classification of cell types is mentioned in the text. Here, indices of cell types are numbered in the order of their appearance. The population of each cell type at each time is represented with a gray scale.

Figure 11: The time series of the average attractor distance, corresponding to the temporal evolution of Fig.10. Here the unit time of the figures is 1000.
Figure 12: Schematic representation of our scenario for the mechanism to keep the diversity of cell types.
Figure 13: The frequency of cell differentiation plotted as a function of the parameter $C_v$ with fixing $C_u = 2.0$. For each point of the figure, we took 20 different initial conditions, and carried out simulations up to $t = 20000$, to check the differentiation. Plotted are the number of events with cell differentiation.
Figure 14: The frequency of cell differentiation plotted as a function of the parameter $D_u$. The parameter $D_u$ is changed from 0.1 to 1.9 with the bin size 0.2. For each point of the figure, we took 20 different initial conditions, and carried out simulations up to $t = 20000$, to check the differentiation. Plotted are the number of events with cell differentiation.

Figure 15: Dependence of cell differentiation on the number of chemicals $K$. We study the cases with $K = 8, 10, 12, 14, 16$ and 18. For each point of the figure, we take 20 different initial conditions and carry out a simulation up to $t = 20000$ with the same parameters mentioned in the text. Accordingly we plot the number of the event where cell differentiation occurs.