Emergence of Multicellular Organisms with Dynamic Differentiation and Spatial Pattern

Chikara Furusawa
Kunihiko Kaneko
Department of Pure and Applied Sciences
University of Tokyo
Komaba, Meguro-ku
Tokyo 153
Japan
furusawa@complex.c.u-tokyo.ac.jp
kaneko@complex.c.u-tokyo.ac.jp

Abstract The origin of multicellular organisms and the mechanism of development in cell societies are studied by choosing a model with intracellular biochemical dynamics allowing for oscillations, cell–cell interaction through diffusive chemicals on a two-dimensional grid, and state-dependent cell adhesion. Cells differentiate due to a dynamical instability, as described by our "isologous diversification" theory. A fixed spatial pattern of differentiated cells emerges, where spatial information is sustained by cell–cell interactions. This pattern is robust against perturbations. With an adequate cell adhesion force, active cells are released that form the seed of a new generation of multicellular organisms, accompanied by death of the original multicellular unit as a halting state. It is shown that the emergence of multicellular organisms with differentiation, regulation, and life cycle is not an accidental event, but a natural consequence in a system of replicating cells with growth.

Keywords
cell differentiation, multicellular organism, dynamical system

1 Introduction

The development of multicellular organisms is one of the most elegant and interesting processes in biology. Cells that contain the same set of genomes differentiate to several types with exact order and exact location. The determination of cell type is somewhat robust, even though the development process occurs in a thermodynamic environment with molecular fluctuations. Three mechanisms are necessary to sustain a robust developmental process in multicellular organisms. First, an external field, which provides information to control differentiation and proliferation, must be maintained through the interaction among cells. Second, each cell must detect and interpret such external information. Finally, the internal state of each cell must be changed according to this interpreted information, leading to differentiation. Recent advances in molecular biology provide us with a molecular basis for these mechanisms. The gradient of morphogen concentration giving positional information can be identified experimentally, the existence of a signaling pathway from receptor protein on the membrane to the nucleus is verified, and the internal states of cells are reduced to regulations of protein synthesis from DNA molecules.

However, when we focus on the emergence of multicellular organisms in the evolutionary process, it is difficult to argue that such elaborate mechanisms appear independently at the same time. On the other hand, the fossil record shows that the transition to multicellularity has occurred at least three times in fungi, plants, and animals [12]. This suggests that the evolution to multicellularity is not a chance event but a *necessity*

in evolution. The three mechanisms mentioned above must be tightly incorporated, at least at the first stage of multicellularity. Thus, to understand the transition to multicellular organisms, the interplay between interactions among cells and intracellular dynamics must be studied.

The motivation behind this work is not restricted to the origin of multicellularity. Even if it might be possible to describe all detailed molecular processes of the present organism, this does not answer why such a developmental process is robust in spite of the considerable thermodynamic fluctuations occurring at the molecular level, which seems to make machinelike functions such as a "clock" almost impossible. Any rule with a threshold given by a signal molecule's concentration is accompanied by fluctuations and therefore cannot proceed correctly. We need to construct a logic for the development process that, in general, works even under molecular fluctuations. Such a logic is relevant to understanding the level of multicellularity in present organisms, from primitive structures such as *Dictyostellum discoideum* and *Volvox*, to higher organisms.

To understand the emergence of multicellularity as a general consequence of the interplay between inter- and intradynamics of cell societies, we have earlier proposed the "isologous diversification" theory [4,10,11]. This theory is rooted in the "dynamic clustering" observed in globally coupled chaotic systems [8,9]. It provides a general mechanism of spontaneous differentiation of replicating biological units, where the cells (which have oscillatory chemical reactions within) differentiate through interaction with other cells, as their number increases through divisions. This differentiation is due to the separation of orbits in phase space that is not attributed to a specific chemical substance but rather is represented through the dynamic relationships of several chemicals. While the differentiation is triggered by the instability of a nonlinear system, the differentiation process as a whole is shown to be robust against fluctuations.

In this article, we extend previous work to incorporate the formation of *spatial patterns* on a two-dimensional grid. At first glance, our framework may appear similar to previous work [1,3,13], in which cells with internal states are placed on a two-dimensional grid and interact with each other through this environment. In the latter approaches, intracellular dynamics are mainly governed by a set of "if–then"-like (conditional) rules that are specified in advance as genetic control. Although this implementation simplifies the description of the simulation in terms of logical chains, there are three problems that we think will be overcome only by our approach. First, such if–then-like rules are based on the response to signals with some threshold. However, as mentioned above, given the fluctuations in the number of signal molecules, such rules cannot work as anticipated. Second, from the implementation of the rules, one cannot deduce how such a set of rules appears at the first stage of multicellularity. Third, the rules have to be tuned externally to fashion a stable development process.

As mentioned, we propose that the interplay between interactions among cells and intracellular dynamics leads to the emergence of such conditional rules. The rule is found to be tuned spontaneously depending on cell–cell interactions rendering the development process robust against molecular fluctuations and maintaining a degree of order in the cell society. In contrast with previous studies, the rules of our cell society are not given in advance but emerge as a consequence of interactions among cells.

We have studied numerically several models consisting of cells with internal chemical reaction networks and interactions among them through the environment. Three basic problems are discussed: fixation of a spatial pattern of differentiated cells, robustness in the developmental process, and the emergence of a replicating cluster of cells. The first problem is answered by the formation of a ring pattern of differentiated cells, and the second by regeneration of a damaged cell cluster. The solution of the last problem is also given, which is essential to the origin of multicellularity because it treats recursive

generation of an ensemble of cells at a higher level than cell replication. It will be shown that the dynamic order of the cell society is a natural consequence of interacting cells with oscillatory dynamics and cell–cell adhesion forces. Hence, the emergence of multicellularity should occur as a necessity in the course of evolution.

2 Model for Differentiation

Our model for differentiation consists of

- Internal biochemical reaction dynamics in each cell
- Cell–cell interactions through media
- Cell division
- Cell adhesion

In essence we assume a network of catalytic reactions for internal dynamics that also allows chaotic oscillations of chemical concentrations, while the interaction process is just a diffusion of chemicals through media.

We represent the internal state of a cell by k chemicals' concentrations as dynamical variables. Cells are assumed to be surrounded by the medium, where the same set of chemicals is given. Hence, the dynamics of the internal state is represented by a set of variables $c_i^{(m)}(t)$, the concentration of the mth chemical species at the ith cell, at time t. The corresponding concentration of the species in the medium is represented by a set of variables $C^{(m)}(x, y, t)$, where x and y denote the position on the two-dimensional grid.

2.1 Internal Reaction Dynamics

As internal chemical reaction dynamics we choose a catalytic network among the k chemicals. Each reaction from chemical i to j is assumed to be catalyzed by chemical ℓ, determined by a matrix (i, j, ℓ). To represent this reaction-matrix we adopt the notation $\mathrm{Con}(i, j, \ell)$ for the connection matrix, which takes on unity when the reaction from chemical i to j is catalyzed by ℓ, and 0 otherwise. Each chemical has several paths to other chemicals, which act as a substrate to create several enzymes for other reactions. In addition, we assume that all chemicals have the potential to catalyze a reaction to generate itself from another chemical, besides the ordinary reaction paths determined randomly. Due to this auto-catalytic reaction, positive feedback to amplify external signals is made possible, which often leads to oscillatory reaction dynamics.[1] This reaction matrix $\mathrm{Con}(i, j, \ell)$, generated randomly, is fixed throughout the simulation.

Still there can be a variety of choices in the enzymatic chemical kinetics. In this article, we assume quadratic effects of enzymes. Thus, the reaction from chemical m to ℓ aided by chemical j leads to the term $e_1 c_i^{(m)}(t)(c_i^{(j)}(t))^2$, where e_1 is a coefficient for chemical reactions, taken to be identical for all paths.

Of course, the real biochemical mechanisms within cells are very much more complicated. We do not take such details into account here, as our purpose is to show how the differentiation process appears as a general consequence of interacting cells with internal nonlinear dynamics. What is essential here is a biochemical reaction that allows for nonlinear oscillation, which is generally expected as long as there is a positive feedback process. It should be noted that in real biological systems, oscillations are observed in chemical substrates such as Ca, cyclic AMP, and so on [5–7,14].

1 For a more detailed discussion of the role of auto-catalytic reactions, see [4].

Besides allowing for the change in chemical concentrations, we take into account the change in the volume of a cell. The volume is now treated as a dynamical variable, which increases as a result of transportation of chemicals into the cell from the environment. As a first approximation, it is reasonable to assume that the cell volume is proportional to the sum of chemicals in the cell. We note that the concentrations of chemicals are diluted as a result of an increase in the volume of the cell. With the above assumption, this dilution effect is tantamount to imposing the restriction $\sum_{\ell} c_i^{(\ell)} = 1$, that is, the normalization of chemical concentrations at each step of the calculation, while the volume change is calculated from the transport as described later.

2.2 Cell–Cell Interaction Through Diffusion to Media

Each cell communicates with its environment through the transport of chemicals. Thus, cells interact also with each other via the environment. 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.

The transportation or diffusion coefficient should depend on the chemical. Here, we assume that there are two types of chemicals, one that can penetrate the membrane and one that cannot. We use the notation σ_m, which takes the value 1 if the chemical $c_i^{(m)}$ is penetrable, and 0 otherwise.

To sum up all these processes, the dynamics of chemical concentrations in each cell is represented as follows:

$$dc_i^{(\ell)}(t)/dt = \Delta c_i^{(\ell)}(t) - (1/k)\sum_{l=1}^{k} \Delta c_i^{(\ell)}(t)\,, \tag{1}$$

with

$$\begin{aligned}\Delta c_i^{\ell}(t) = &\sum_{m,j} \mathrm{Con}(m, \ell, j)\; e_1\; c_i^{(m)}(t)\; (c_i^{(j)}(t))^2 \\ &- \sum_{m',j'} \mathrm{Con}(\ell, m', j')\; e_1\; c_i^{(\ell)}(t)\; (c_i^{(j')}(t))^2 \\ &+ \sigma_\ell D_m (C^{(\ell)}(p_i^x, p_i^y, t) - c_i^{(\ell)}(t)).\end{aligned} \tag{2}$$

where the terms with $\sum \mathrm{Con}(\cdots)$ represent paths coming into and out of ℓ, respectively. The variables p_i^x and p_i^y denote the location of the ith cell on the x–y grid. The term $\Delta c_i^{(\ell)}$ gives the increment of chemical ℓ, while the second term in Equation 1 summarizes the constraint $\sum_{\ell} c_i^{(\ell)}(t) = 1$ due to growth of volume [2]. The third term in Equation 2 describes the transport in the medium, where D_m denotes the diffusion constant of the membrane, which we assume to be identical for all chemicals.

The diffusion of penetrable chemicals in the medium is governed by a partial differential equation for the concentration of chemical $C^{(\ell)}(x, y, t)$. For each chemical $C^{(\ell)}$, at a particular location:

$$\begin{aligned}\partial C^{(\ell)}(x, y, t)/\partial t = &-D_e \nabla^2 C^{(\ell)}(x, y, t) \\ &+ \sum_i \delta(x - p_i^x, y - p_i^y)\sigma_\ell D_m (C^{(\ell)} - c_i^{(\ell)}(t)).\end{aligned} \tag{3}$$

We assume the following boundary condition:

$$C(0, y, t) = C(x_{\max}, y, t) = C(x, 0, t) = C(x, y_{\max}, t) = \text{const.} \qquad (0 < x < x_{\max}, 0 < y < y_{\max}), \quad (4)$$

where D_e is the diffusion constant of the environment, $x_{\max}$ and $y_{\max}$ denote the extent of the lattice, and $\delta(x, y)$ is Dirac's delta function. This boundary condition can be interpreted as a chemical bath outside of the medium, which supplies those penetrable chemicals that are consumed to the medium via a constant flow to the cell. In practice, the variable $C^{(\ell)}(x, y, t)$ is discretized on an $n \times n$ grid, to reduce the diffusion equation to n^2 differential equations.

2.3 Cell Division

Each cell takes penetrable chemicals from the medium as the nutrient, while the reaction in the cell transforms them to unpenetrable chemicals that construct the body of the cell such as the membrane and DNA. As a result of these reactions, the volume of the cell is increased by a factor $(1 + \sum_\ell \Delta c_i^\ell(t))$ per dt. In this article, the cell is assumed to divide into two almost identical cells when the volume of the cell is doubled.

The chemical composition of two divided cells is almost identical with their mother's, with slight differences between them due to random fluctuations. In other words, each cell has $(1+\epsilon)c^{(\ell)}$ and $(1-\epsilon)c^{(\ell)}$, respectively, with a small "noise" ϵ given by a random number with a small amplitude, say from $[-10^{-6}, 10^{-6}]$. Although the existence of this imbalance is essential to differentiation in our model and in nature, the mechanism or the degree of imbalance is not important for the differentiation itself. The important feature of our model is the amplification of microscopic differences between the cells through the instability of the internal dynamics.

2.4 Cell Adhesion

In cell biology, each cell adheres to its neighbor cells through binding to proteins on its membrane surface. The nature of membrane proteins depends on the internal state of the cell, and it is natural to assume that whether adhesion occurs or not is determined by a combination of the cell types of the two neighbors. As a minimal model for adhesion, we assume that cells within a given threshold distance have a "connection," where a "spring" is put between them so that they adhere within the natural length of the spring if the combination of the two cell types satisfies a given condition. For example, cells with the same cell type will be connected by the same spring (with the same strength and natural length) if a distance condition is satisfied, while pairs with any combination of two different cell types do not adhere.

In addition to the adhesion force, a random fluctuation force is applied to all cells as expected from molecular Brownian motion. We seek a configuration that is stable against perturbations including these fluctuations. When a cell divides, two daughter cells are placed at randomly chosen positions close to the mother cell, and each daughter cell makes new connections with the neighbor cells.

3 Results Without Spatial Information

We have performed several simulations of our model with different chemical networks and different parameters. Because typical behaviors are rather common as long as nonlinear oscillatory dynamics are included, we present our results by taking a specific chemical network with $k = 20$ chemicals. First we show some results of simulation

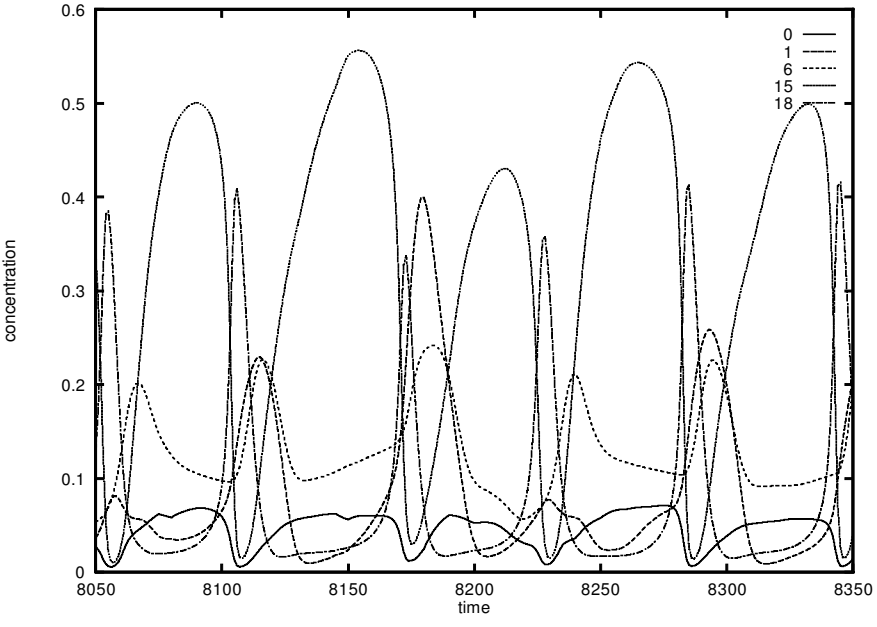

Figure 1. Overlaid time series of $c^{(m)}(t)$ in the type-0 cell, obtained from a network with 20 chemicals and seven connections in each chemical. We have plotted only the time series of 5 of the 20 internal chemicals to avoid a crowded figure. Each line with number $m = 0, 1, 6, 15, 18$ represents the time series of concentration of the corresponding chemical $c^{(m)}(t)$. The parameters are set as $e_1 = 0.7$, $D_m = 0.1$, $D_e = 0.2$; chemicals $c^{(\ell)}(t)$ for $\ell \leq 3$ are penetrable (i.e., $\sigma_\ell = 1$), while the others are not. The reaction network Con(i, j, ℓ) is randomly chosen and is fixed throughout the simulation results of the present article.

without spatial information, to demonstrate the essence of our differentiation process based on dynamical instabilities.

In this section, we assume that the medium is well stirred and all cells interact through an identical environment.[2] Later, simulations with spatial information and diffusive chemicals are shown to allow discussion of pattern formation and the emergence of multicellularity. As initial condition, we take a single cell with randomly chosen chemical concentrations $c_i^{(\ell)}$ satisfying $\sum_\ell c_i^{(\ell)} = 1$. In Figure 1, we have shown the time series of concentration of the chemicals in a cell when only a single cell is in the medium. We call this state "type-0" in this article. This is the only attractor of internal cellular dynamics, detected from randomly chosen initial conditions.

With diffusion, external chemicals flow into the cell, which leads to an increase in the volume of the cell. Thus, the cell is divided into two, with almost identical chemical concentrations. The chemicals within the two daughter cells oscillate coherently, with the same dynamical behavior as their mother cell (i.e., attractor-0). As the number of cells increases by a factor of two (i.e., 1, 2, 4, 8, $\cdots$) with further divisions, the coherence of oscillations is easily lost. Such loss of synchrony is expected from the studies of coupled nonlinear oscillations. The microscopic differences introduced at each cell division are amplified to a macroscopic level through interaction, which destroys the phase coherence.

1 Here, the results are described only briefly. More detailed accounts are given in a prior publication [4], where we adopted a different biochemical network.

3.1 Differentiation

When the number of cells exceeds a threshold value, some cells start to display differing types of dynamics. In Figures 2a and b, the time series of chemicals in these cells are plotted. We call these states "type-1" and "type-2" cells, respectively. Note that these states are not an attractor of the internal dynamics of a single cell. Rather, these states are stabilized by the coexistence of cells with a *different* type. In Figure 3, orbits of chemical concentrations at the transition from type-0 to type-1 are plotted in phase space. It shows that each attractor occupies a distinct regime in phase space. These two types of cells are clearly distinguishable as "digitally" separated states, so that they can be identified computationally.[3] Hence, this phenomenon is regarded as differentiation. Here, the type-0 cells can potentially differentiate to either "1" or "2," while some of the type-0 cells remain the same type after division.

As the cell number further increases, some type-2 cells further differentiate to another distinct cell type, which is called type-3 here (Figure 2c). At this stage, hierarchical differentiation occurs. The type-2 cells also can potentially differentiate back to type-0 cell. Thus, type-2 cells have three choices at division: to replicate, and to differentiate to a type-0 or type-3 cell. All in all, four distinct cell types coexist in this system. In addition, there is a limitation on the number of cells to allow for the diversity in cell types. When the number of cells exceeds this limit, all cells turn into type-1 or type-3 cells, where the chemical dynamics is described by fixed points.

Note that this differentiation is not induced directly by the tiny differences introduced at the division. The switch from one cell type to another does not occur precisely at cell division but occurs later through the interaction among the cells. This phenomenon is caused by an instability in the full dynamical system consisting of all the cells and the medium. Thus, tiny differences between two daughter cells are amplified to a macroscopic level through the interaction. Only when the instability exceeds a threshold does differentiation occur. Then, the emergence of another cell type stabilizes the dynamics of each cell again. The cell differentiation process in our model is due to the amplification of tiny differences by orbital instability (transient chaos), while the coexistence of different cell types stabilizes the system.

3.2 Emergence of Rules for Differentiation and Global Stability

The switch from type to type by differentiation follows specific rules. These rules originate in a constraint on the transient dynamics between attractor states corresponding to each cell type. In Figure 4, we show an automatonlike representation of these rules. As mentioned earlier, cell-type "0" can undergo three transitions: to reproduce itself, and to differentiate to types "1" or "2." A cell of type-2 also has three possibilities. Cell-types "1" and "3" replicate without any further differentiation.

When there are multiple choices of differentiation processes (as in "0" → "0", "1", "2", "3"), the probability of choosing a particular path is neither fixed nor random but is governed by the distribution of coexisting cell types in the system.

It should be noted that the information on the distribution of cell types in the cell society is embedded in each internal dynamics. In other words, each attracting state of internal dynamics is gradually modified with the change of distribution of other cells. This modification of internal dynamics is much smaller than the differences between different cell types. Thus, there are two types of information in internal dynamics, analogue, which embeds the global distribution of cell types, and digital, which gives each cell its type.

This analogue information controls the occurrence of the differentiation, because the

3 In practice, each cell type is distinguished by computing the average of concentrations $c^{(m)}(t)$ over a certain period to obtain the average position of each orbit. With this average, temporal fluctuations from oscillatory dynamics are smeared, and the average positions form clusters clearly separated in phase space, which correspond to the cell types. See [4] for details.

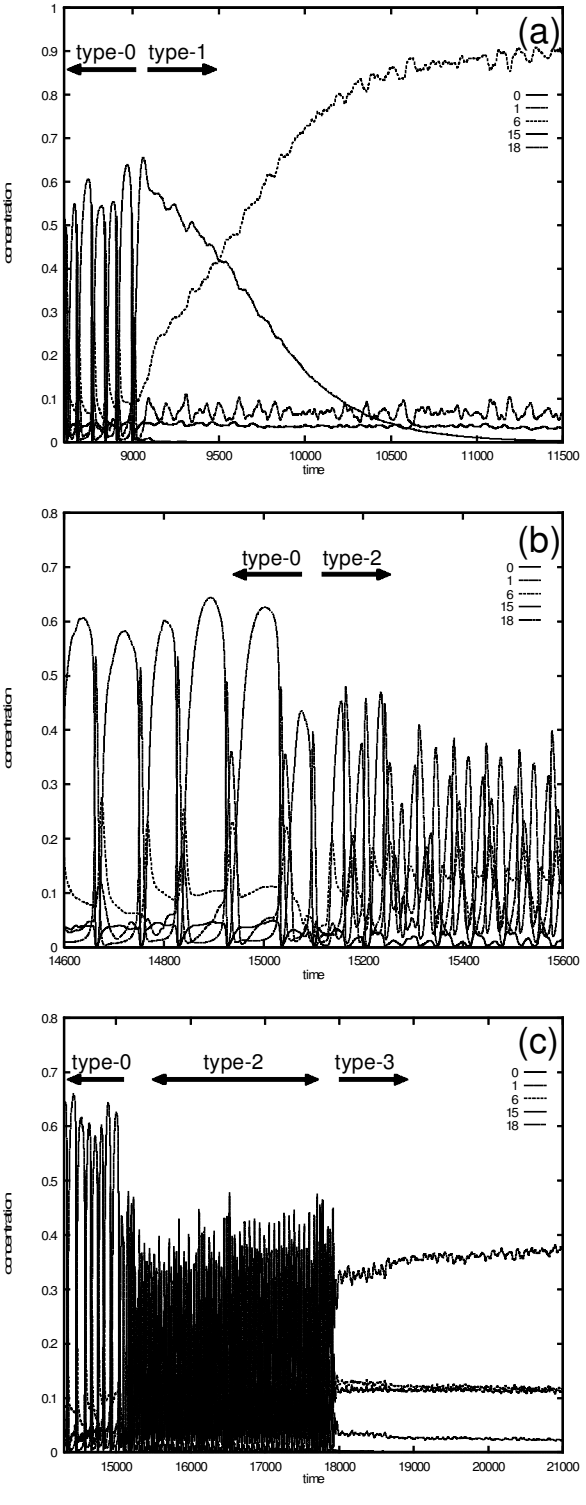

Figure 2. Time series of $c^{(m)}(t)$, overlaid for the 5 chemicals (as given in Figure 1) in a cell. (a)–(c) represent the course of differentiation to type-1, type-2, or type-3 cells, respectively. The differentiation to type-3 always occurs starting from type-2 cells. Note the difference in scale of the *x* axes.

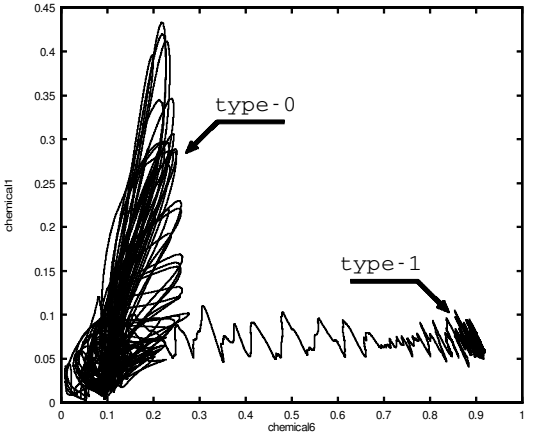

Figure 3. Orbit of internal chemical dynamics in phase space. The orbit of chemical concentrations at a transient process from type-0 to type-1 cells is plotted in the projected space $(c^{(6)}(t), c^{(0)}(t))$. Each cell type is clearly distinguishable in phase space.

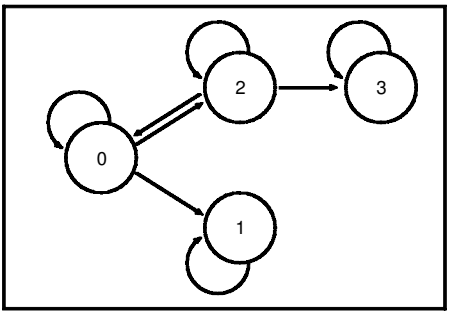

Figure 4. Automatonlike representation of the rules of differentiation. The path back to the own node represents the reproduction of type, while the paths to other nodes represent the potential of differentiation to the corresponding cell type.

transition between the types depends on their modification. On the other hand, change of the distribution of distinct cell types is embedded again as analogue information. For example, the orbit of type-0 cells in Figure 3 is shifted toward the direction of that of type-1, as the number of type-1 cells is reduced. With this shift, the differentiation from type-0 to type-1 is increased.

As a result of this interplay between two types of information, the higher level dynamics emerges, which controls the rate of the division and differentiation depending on the number of each cell type. This dynamics can be represented by the population dynamics of the number of the type-k cells n_k $(k = 0, \cdots, 4)$. The behavior of this dynamics should be stochastic, because only the information on the number of cell types is extracted, by neglecting the lower level information on the internal state (of chemical concentrations).

This dynamics of differentiation allows for stability at the level of ensemble of cells. The variety and the population distribution of cell types are robust against the perturbations. As an example, let us consider the stage with three cell types ("0," "1," "2" in Figure 4). When the type-2 cells are removed to decrease the population, events of differentiations from "0" to "2" are enhanced, and the original distribution is recovered,

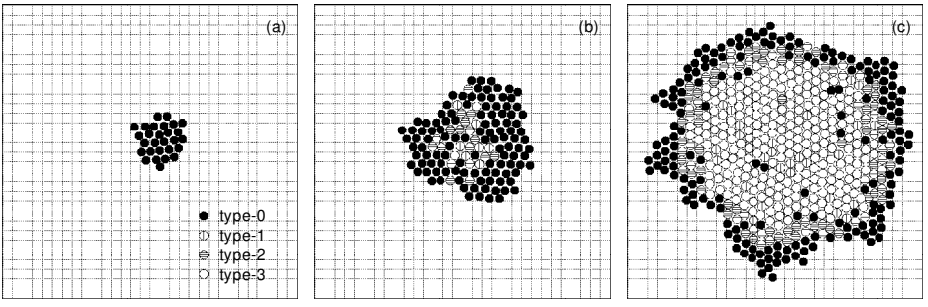

Figure 5. Development of a cell cluster on a two-dimensional grid. Each mark corresponds to a particular cell type determined by differing internal dynamics. The grid indicates the unit of discretization on the diffusive chemicals $C^{(\ell)}(x, y, t)$.

with the mechanism of the shift of the orbits mentioned already. In this case, the stability is sustained by controlling the rate of differentiation from "0" to other types, and this behavior of type-0 cells can be regarded as a stem cell.

4 Simulation Results with Spatial Information

Here, we present results of simulations including the motion of cells and diffusive chemicals on a two-dimensional grid. The reaction matrix and the parameters of internal dynamics are the same as those in the previous section, while the parameters related to the dynamics of the surrounding medium are tuned so that the same set of cell types is obtained with almost identical reaction dynamics and rules for differentiation. For cell types, the same nomenclature is adopted as in the previous section.

4.1 Spatial Pattern of Differentiated Cells

In this subsection, we assume that all cells adhere to each other with the same strength, irrespective of their type, when their distance is within a given threshold.

The first cell, initially placed in the medium, shows type-0 dynamics and divides into two almost identical daughter cells, in the manner described in the previous section. These two daughter cells then make a new connection and adhere. With further divisions, a cluster of type-0 cells is formed (Figure 5a).

When the size of the cell cluster exceeds a threshold value, some cells located at the inside of the cluster start to differentiate to type-1 and type-2 cells (Figure 5b). As the cell number further increases, type-2 cells at the inside differentiate to type-3 cells, to form the inner core of the cluster shown in Figure 5c. At this stage, a ring pattern consisting of three layers is formed. The ring of type-2 cells lies between peripheral cells with type-0 dynamics and an inner core consisting of type-1 and type-3 cells. Positional information giving rise to such a spatial pattern naturally appears through competition for nutrients, without any sophisticated programs implemented in advance. Note that the pattern formation originates from temporal differentiation. It is not a diffusion-induced pattern like Turing's mechanism.

At this stage, the growth of cell clusters is only due to divisions of peripheral type-0 cells. The cell division of type-1 or type-3 cells located at the inner core has stopped, due to their slower growth speed and the limited nutrients therein. As the size of the cell cluster increases, the size of the inner core also increases through differentiations from type-0 to type-1 or type-3 cells. Finally, the growth of the inner core by differentiation overcomes the growth by divisions. At this stage, the ring structure is broken and all

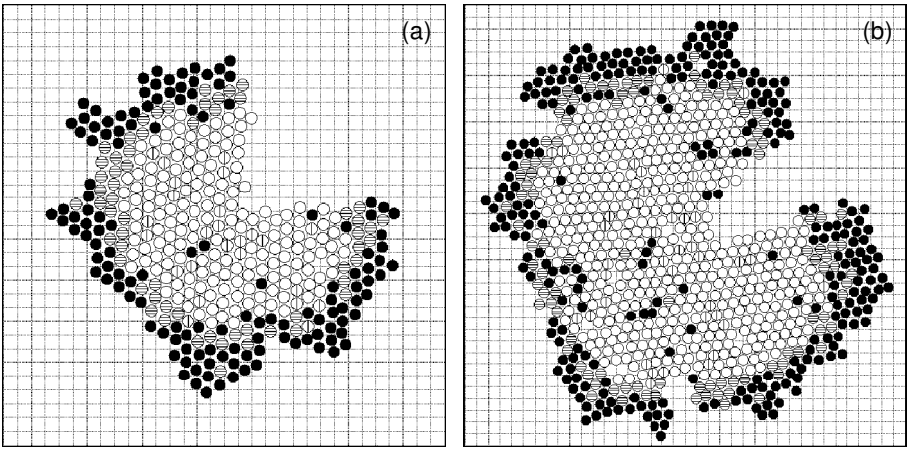

Figure 6. Regeneration of a cell cluster. (a) A fourth of the cell cluster is purposefully removed during the developmental process. (b) The growth in the damaged part is enhanced, and this part is gradually recovered. Note that the layer of peripheral cells at the damaged part becomes thicker.

cells differentiate to type-1 or type-3 cells. The growth of cell clusters almost stops and the internal dynamics of all cells falls into fixed points.

4.2 Regeneration of Damaged Structure

A biological system often has robustness against some perturbations, such as, for example, the processes in regulative development. Such robustness is difficult to realize only by successive execution of preprogrammed "commands" on DNA. The mechanism for robustness must include the interplay between intracellular dynamics and interactions among cells. As an example of such robustness, we discuss the regeneration of a damaged cell cluster, which is a natural consequence of our dynamic differentiation process.

When a cluster develops into the stage with a ring pattern as is shown in Figure 5c, we remove a quarter of the cells from this cluster externally (see Figure 6a) and see what happens later with our model dynamics. After this operation, the division of peripheral cells at the damaged part is enhanced because they receive more nutrition than other cells. Besides this increase, cells also differentiate toward the original pattern, and the damaged part is gradually recovered (Figure 6b).

4.3 Emergence of Multicellularity

In this section, we change the condition of adhesion between the cells, to see continuous growth in our cell society. As is mentioned, the ring pattern with three layers is formed when all cell types can connect to each other. The growth, however, stops at a certain stage, and new cell clusters are not formed. Thus, such a cellular system cannot be sustained for long. If a change in the adhesion properties allows for the continuous growth and formation of a new generation of cell clusters, such cellular systems will come to dominate.

To study this problem we introduce a dependence of the adhesion force on cell types. Because the force of adhesion should depend on the membrane proteins on the cell surface, it is natural to include dependence of adhesion on the relative internal states of two adjacent cells. As a simple example, we assume that no connection is allowed between a type-2 cell and a type-3 cell, while the connections for all other combinations are preserved. This restriction on the connection implies that the second

layer of type-2 cells and the inner core in Figure 5c lose their capability to adhere to each other.

We have made several simulations with these adhesion rules and have found that cell clusters divide into multiple parts during development. The first stage of the developmental process is unchanged from the previous example. A cluster of type-0 cells grows through cell divisions, and type-1 and type-2 cells appear at the inside of this cluster by differentiations until the inner core is formed as a result of further differentiations. When the growth of the inner core that consists mainly of type-3 cells reaches the edge of the cell cluster, however, a small cluster of cells, or a solitary cell, is released from the periphery of the mother cluster, as shown in Figure 7c. This figure depicts the process that gives rise to the fourth generation from the third generation of our multicellular organism. As will be shown, the formation of the second generation proceeds in the same way. The peripheral layer of type-0 and type-2 cells is cut off by the growth of the inner core, and the type-2 and type-3 cells at the contact surface of these layers do not adhere any more according to our model assumption.

The released small clusters move away by the force of random fluctuations. They encounter a new environment with rich chemical substances and start to divide actively. The increase in cell number in these clusters makes their random motion slower, because the fluctuation force is added to each cell independently, and thus tends to cancel out when the cell number is larger. In the new clusters, development proceeds as in their mother-cluster: The cells at the inside of a type-0 cluster differentiate to type-1 and type-2 cells, while the type-3 core is formed through further differentiations, until their peripheral cells are released again (Figure 7c). Hence a life cycle of multicellular replicating units is observed, which emerges without explicit implementation. Thus, we observe the emergence of a replicating unit on a higher hierarchical level than individual cell replication. Note that this emergence of replicating cell societies is a natural consequence of a system with internal cellular dynamics with nonlinear oscillation, cell–cell interaction through media, and cell-type dependent adhesion.

4.4 Death of Multicellular Organisms

After the release of peripheral cells, the remnant core with type-1 and type-3 cells stops cell divisions after intracellular chemical oscillations cease (Figure 7b). This determines the lifetime of the replicating multicellular unit, given by its cell configurations and the deficiency of nutrition. This fact provides an interesting point of view with respect to the death of multicellular organisms. As is well known, the death of a multicellular organism is not identical with the death of cells in the organism but rather coincides with the death of the organism as a "system." For example, cells in a dead body often survive for a while. Thus, the emergence of multicellularity must be accompanied with such a "halting" state of the system. This halting state limits the size and the lifetime of an organism. Such emergence of limitation is required to complete a life cycle and to give rise to a new generation. Indeed it is expected that when the size reaches a critical value, such a halting state is brought about by the lack of nutrition, at the first stage of multicellularity, where no special organ for transportation of nutrition is yet developed. In fact, our results show that there is a halting state in a cell cluster when it reaches a size where even cells at the boundary of the cluster lose their activity and stop reproducing.

At the first stage of multicellularity in evolution, two daughter cells fail to separate after division, and a cluster of identical cell types is formed first. To survive as a unit, differentiation of cells has to occur, and subsequently the multicellular cluster needs to release its active cells before the system falls into the halting state. Hence, germ-cell segregation and a closed life cycle are expected to emerge simultaneously with a multicellular organism, as our simulations have demonstrated.

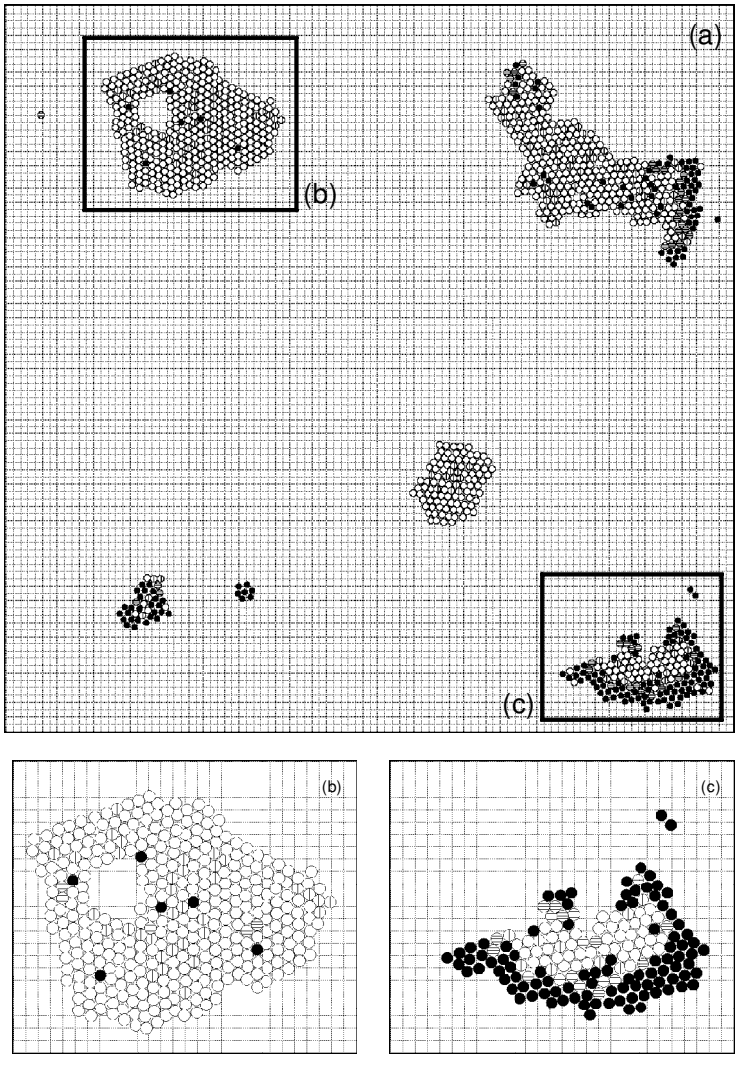

Figure 7. Emergence of a life cycle in a multicellular organism. (a) Part of the snapshot pattern of our model, while (b) and (c) are each an expansion of the corresponding area in (a). The cluster in (b) has already fallen into a halting state, where the oscillation and the growth of almost all cells have stopped. The cluster in (c) is the second-next generation from the cluster in (b). It is just releasing its peripheral cells (the two type-0 cells at the upper part of (c)), which will lead to the next generation.

5 Summary and Discussion

In the present article, we have studied a dynamical model to show that a prototype of cell differentiation occurs as a result of internal dynamics, interaction, and division. We have made several simulations choosing several chemical networks, with a different number of chemical species, and were able to observe the same scenario for cell differentiation. With the same parameters as used in the previous example [4], approximately 40% of randomly chosen chemical networks show oscillatory behavior in our system, while others fall into fixed points. Furthermore, approximately 20% of these oscillatory dynamics are destabilized through cell divisions, where some of the cells differentiate following specific rules, such as those shown in Figure 4.

Let us summarize the consequences of our simulations. First, cells differentiate, caused by a dynamical instability due to cell–cell interactions when the cell number exceeds a threshold value. The initial state is destabilized for some cells and changes into another state. Several discrete cell states appear, whose coexistence restabilizes the overall cellular dynamics. The differentiated states are transmitted to the daughter cells or switch to a different state, obeying a set of hierarchical rules (depicted in Figure 4) that are not preprogrammed but rather emerge from the cell–cell interactions. In addition, the rate of differentiation is modulated by the distribution of different cell types. Information about this distribution is embedded into the dynamics of each cell as a slight (analogue) modulation of the intracellular dynamics for each type.

During simulations on a two-dimensional grid with diffusive chemicals, we have found ring patterns of differentiated cells such as is shown in Figure 5c. This positional information is not imposed on the system from the outside but is sustained by cell–cell interactions through competition for nutrients. Each cell can detect information about this external "field" by modulating its own internal dynamics. This modulation controls the rate of transitions among cellular states, and the transitions in turn change the external field. This feedback maintains the spatiotemporal order of the cell society and also provides the robustness of the multicellular system. As can be seen in Figure 6b, a damaged structure is recovered both by an increase in cell divisions at the damaged part and by cell differentiations to recover the original pattern. It should be noted that this overall stability is an intrinsic feature of our dynamic differentiation process. No external regulation mechanisms are required; rather, this robustness is a feature of the differentiation mechanism itself.

Our results also suggest a novel view of the emergence of multicellularity. With an adequate cell–cell adhesion force, active peripheral cells are released when the process of development reaches a particular stage through divisions and differentiations. These released cells start to develop in the same manner as their mother cluster and release their peripheral cells again. On the other hand, a cluster that has lost the peripheral cells stops its growth, and all the intracellular dynamics fall into fixed points. At the level of the cluster, this inactive state can be regarded as death of the multicellular system. Thus, a life cycle emerges for these multicellular organisms, alternating replication and death of cell clusters. We would like to stress again that this ordered cell society with a closed life cycle appears not from a particular implementation of internal reaction dynamics, but from the interplay between inter- and intracell dynamics.

Still, one might ask in which way multicellularity carries an advantage in natural selection. At this stage, this question is unimportant for our scenario of the emergence of multicellularity. Our results show that when the number of cells increases and the interactions among cells become tight, the diversity of cell types naturally emerges. The tight coupling between cells can easily appear, for example, when the cell separation after division fails due to an adhesive force. Then, it is found that only cell clusters that have a diversity of cell types and adequate cell-type dependent adhesion forces can avoid death as a cluster and keep on growing to give rise to new generations. Thus, the emergence of multicellularity appears to be a natural consequence of an evolutionary process with never-ending reproduction.

It is often believed that the rules that determine when, where, and what type of cells appear in a multicellular organism should be precisely specified beforehand as a successive switching of genes in the DNA, depending on external signals. Our scenario is not necessarily inconsistent with such a switching mechanism because our biochemical dynamics and the emergent regulation mechanisms can include those associated with DNA. However, the essential point of this theory is not the formation of rules consistent with such an explanation. Note that a rule-based explanation cannot answer important questions in the development process, such as: Why did a particular development pro-

cess evolve? Why is such a process robust with respect to thermodynamic fluctuations at a molecular level? Why does a multicellular organism have cell differentiation and death? The mechanism proposed here giving rise to ordered cell societies is able to answer such questions simultaneously.

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