Origin of Multicellular Organisms as an Inevitable Consequence of Dynamical Systems

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ABSTRACT

The origin of multicellular organisms is studied by considering a cell system that satisfies minimal conditions, that is, a system of interacting cells with intracellular biochemical dynamics, and potentiality in reproduction. Three basic features in multicellular organisms—cellular diversification, robust developmental process, and emergence of germ-line cells—are found to be general properties of such a system. Irrespective of the details of the model, such features appear when there are complex oscillatory dynamics of intracellular chemical concentrations. Cells differentiate from totipotent stem cells into other cell types due to instability in the intracellular dynamics with cell–cell interactions, as explained by our isologous diversification theory (Furusawa and Kaneko, 1998a; Kaneko and Yomo, 1997). This developmental process is shown to be stable with respect to perturbations, such as molecular fluctuations and removal of some cells. By further imposing an adequate cell-type-dependent adhesion force, some cells are released, from which the next generation cell colony is formed, and a multicellular organism life-cycle emerges without any finely tuned mechanisms. This recursive production of multicellular units is stabilized if released cells are few in number, implying the separation of germ cell lines. Furthermore, such an organism with a variety of cellular states and robust development is found to maintain a larger growth speed as an ensemble by achieving a cooperative use of resources, compared to simple cells without differentiation. Our results suggest that the emergence of multicellular organisms is not a "difficult problem" in evolution, but rather is a natural consequence of a cell colony that can grow continuously. Anat Rec 268:327–342, 2002.

Key words: chaos; cell differentiation; stem cell; positional information; germline; robustness; life-cycle; evolution

A cell has a membrane boundary and an internal metabolic process to sustain it. It reproduces itself, although reproduction may not be necessarily completely faithful. As soon as these basic features of a cell are satisfied, cells continue to replicate, so that they will soon be surrounded by other similar cells. When they are very crowded, the cells will form a colony. This is a step toward a multicellular organism[1]; but some more steps appear to be necessary. What features are required for this colony of cell aggregates to be a multicellular organism? This is the question we address in the present paper.

A cell colony must have the following properties to be regarded as a multicellular organism:

(I) Recursive production of cells at an ensemble level
(I-i) Cell ensemble as a unit: The aggregate of cells has a unity as an ensemble, and a boundary as a multicellular organism. Indeed, this feature is necessary to have

[1]Concerning the origin of multicellular organisms, another hypothesis is proposed by Gordon (1999), in which the first step toward a multicellular organism is the subdivision of a large cell. Since our study is based on universal features of a system of interacting cells with intracellular dynamics and cell divisions, the conclusion to be presented is true even in such case, as long as cell–cell interaction is strong.

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the following recursive production as an ensemble (I-ii); otherwise, cells continue to be aggregated as they grow, without forming a boundary as a multicellular organism.
(I-ii) Recursive generation of the cell colony: The cell colony has to be recursively produced as an ensemble. Recursive production of cells has already been achieved at the origin of a cell, while at the origin of a multicellular organism, recursive production of an ensemble of cells has to be established.

Presently existing multicellular organisms often adopt a well-organized developmental process starting from a specific germ cell, while the mature body thus formed produces germ cells for the next generation (Alberts et al., 1994). An important point here is that, even though multicellular organisms (such as animals, plants, and fungi) have appeared several times independently in evolution (Buss, 1987), they have generally adopted the same basic features to maintain the recursive generation of the cell ensemble, as follows:

II. Cell differentiation: Cells are not identical, and several distinct cell types with different characteristics coexist, derived from the same ancestor cell. With cell differentiation, each different cell type plays a different functional role and helps the others for survival of the cell colony.
III. Robust developmental process: There are a determined developmental path, a determined set of cell types, and a pattern formation process, which are generated from a single or a small number of cells. The final pattern of the cell colony does not change much by each developmental process. The variety of cell types is identical for each developmental process, while the spatial pattern generated by these differentiated cell types, as well as the size of the colony, does not change much in each case. We emphasize that this developmental process is rather robust against molecular fluctuations, as well as external perturbations.
IV. Emergence of germ-line: One of the most important features in multicellular organisms adopted in most cases is that only one or a few types of cells can produce the next generation. As Weissmann (1893) explicitly demonstrated, characters of most cells (called somatic cells) are inherited within their lineage, but not in subsequent generations. The somatic cells are precluded from giving rise to gametes, and only the germ cells are inherited by the subsequent generations. Note that the separation of totipotent cells and somatic determined cells are generally observed even in reproduction processes without the emergence of a germ-line. For example, in asexual reproduction of planarians, only totipotent stem cells produce the next generation. As long as each cell is regarded as a unit for Darwinian evolution, each cell should have competed for reproduction of offspring. In this sense, the "altruistic" behavior of somatic cells, which often occupy a major portion of a multicellular organism, is rather mysterious.

Our questions concerning the origin of multicellular organisms can be summarized as: 1) How does a multicellular recursive system with the above features emerge? 2) Why do most major multicellular organisms satisfy features II, III, and IV? Is there a general restriction on the multicellular organisms to satisfy these features?

The existence of such common features in multicellularity suggests that there is an underlying universal logic for a surviving multicellular organism. The purpose of this study is to ascertain the universal features in a colony of interacting cells, obtained from computer simulations of a simple model of cell society.

Considering that a finely-tuned control mechanism is often adopted in the development of the present organism (Alberts et al., 1994), one might expect that a mechanism that could sustain the above features must be acquired in evolution, to close a recursive life-cycle in a multicellular organism. If this were the case, the origin of multicellular organisms might look rather miraculous. In this work, we present a contrasting viewpoint, by showing that the above features generally appear for an ensemble of very primitive cells with tight cell–cell interactions. For primitive cells, only the following conditions are required:

a. Within a cell, there are biochemical reaction dynamics for the synthesis of chemicals. The reaction process involves mutually catalytic reactions, leading to replication of molecules within, while some nutrient chemicals flow through the cell membrane. Cells grow accordingly.
b. As a result of growth in chemical components in the protocell, a cell divides into two. The number of cells increases accordingly.
c. Some chemicals diffuse through the cell membrane. Hence, the cells exchange some chemicals while they also compete for nutrient chemicals. Accordingly, the cells interact with each other.

In earlier studies (Furusawa and Kaneko, 1998a,b, 2001; Kaneko and Yomo, 1997, 1999) we have shown, in several computer simulations, that cell differentiation and robust developmental processes to form organized patterns generally appear in a system just with the above fundamental properties of a cellular system, if a reaction network is adopted for the internal dynamics of condition a. That is, properties II and III naturally emerge in a system consisting of such primitive cells, without postulating any finely tuned mechanism. Below, we provide some examples of such robust developmental processes, and discuss the nature of this cellular diversification process.

To have recursive production as an ensemble, a clear boundary as a multicellular organism is necessary. For this, some cells have to be mutually adhered. Then, we need some kind of cell adhesion mechanism. Indeed, this adhesion itself is not so surprising, since the cell membrane involves complex molecules, and when the cells are in contact these complex molecules give rise to an adhesion force. This adhesion force depends on the adjacent cell types, and may work only between some combination of cell types. Then, at certain stages of development, some cells no longer adhere to the cell colony. Hence, this selective adhesion can give a boundary of cell ensemble, as a set of adhered cells.

Hence, we impose the following cell adhesion condition to the model with conditions a–c:

d. Adhesion force between cells works selectively between some cell types.

Now, the cell differentiation that generally emerges in a system with conditions a–c, brings about cell-type-dependent adhesion. Due to the difference in adhesion, some differentiated cells may not be attached with other cells. Then, some cells are separated from the colony, from which a colony of the next generation may be generated. Below, we study a model of primitive cells that satisfy just the basic constraints a–d, and show that the recursive generation of cell colony, i.e., the condition I, appears generally.

This recursive generation of a cell colony is found to be stabilized if only one or a very few cells of a specific type are detached from the cell colony. Hence, germ-line cells (IV) generally emerge through the reproduction of the cell colony. The separation of germ cells and somatic cells, generally adopted in multicellular organisms, can be explained as a consequence of having stable repeated recursive production of cell ensembles.

These results show that the emergence of a recursive multicellular life-cycle sustained by (II) cell differentiation, (III) robust developmental process, and (IV) emergence of germ-line is not a difficult problem in a cell society with fundamental properties a–d. The remaining question is why the strategy for multicellularity in existing major multicellular organisms is restricted to this case. To address this question, we studied the relationship between the growth speed of an ensemble of cells and some characteristics of cellular dynamics. The results of simulations provide evidence that a cell colony with a variety of cellular states and robust developmental process has a faster rate of growth than an ensemble of simple cells without differentiation, because of its greater capability for "cooperation" in their growth. This suggests that when competition of resources is tight, the growth of a colony of homogeneous cells is limited, and only a colony with cellular differentiation, which naturally appears under such tight interactions, can overcome such a limit. Hence, it is natural to conclude that emergence of multicellular organisms with differentiated cell types is a necessary course in evolution, once cells are crowded to form aggregates.

MODEL FOR CELL SOCIETY

Before presenting our model, we summarize our standpoint for designing a model to consider the emergence of multicellular organisms.

Although contemporary multicellular organisms have sophisticated mechanisms to keep their life-cycles, at the beginning of multicellularity organisms cannot have such finely tuned mechanisms. To understand the emergence of multicellularity, we must seek an answer that does not rely on detailed cellular processes adopted in existing organisms. Hence, we adopt a simple model containing only the essential features of biological systems, and from this simple, constructive model, we attempt to capture the universal behavior exhibited by all cell societies with such essential features, based on extensive computer simulations of this model.

This type of modeling is also important for our understanding of present-day organisms. In a pioneering study of pattern formation, Turing (1952) proposed a mechanism whereby a spatially uniform state is destabilized by the cell–cell interactions, leading to the formation of a spatially periodic pattern (Turing, 1952) (see also Newman and Comper (1990)). The existence of multiple stable cellular states in genetic networks was found by Kauffman, which correspond to different cell types (Kauffman, 1969). Although these studies were based on a simple abstract model of a cellular system, they succeeded in extracting the universal logic in all multicellular systems, ranging from a very primitive one to the present sophisticated one.

To investigate the emergence of multicellularity, we considered simple models consisting of the following basic cell features: 1) internal dynamics consisting of a biochemical reaction network within each cell, 2) interactions between cells (intercellular dynamics), 3) cell division and cell death, and 4) cell adhesion.

In the following sections, we briefly explain three basic features of our model. More detailed descriptions of modeling and the model equations are presented in the Appendix and Furusawa and Kaneko (1998a,b).

Internal Chemical Reaction Dynamics

Cells are assumed to be completely surrounded by a two-dimensional medium including diffusive chemical substances. To consider cellular reaction dynamics, we assume that each cellular state is expressed by a set of continuous variables representing concentrations of k chemicals within a cell. Due to chemical reactions, the concentrations change over time. For the reaction dynamics, we choose a catalytic network among the k chemicals. Each reaction path from some chemical i to an other chemical j is assumed to be catalyzed by a third chemical ℓ, which are chosen randomly. These reaction paths form a complicated reaction network. Here we simply choose randomly connected reaction networks, and study common features in such systems. In this study, using thousands of randomly generated reaction networks, we tried to extract universal features of cell societies consisting of interacting cells with intracellular reaction dynamics. Although each network does not correspond to specific examples of real cells, our results are meant to be rather general, and do not require detailed, specific choices of the network structure.

Cell–Cell Interaction

Cells interact with each other through the transport of some chemicals into and out of the surrounding medium. Herein we are not concerned with direct cell–cell interactions (such as gap junctions); rather, we consider only indirect cell–cell interactions via diffusive chemical substances. This indirect interaction is sufficient for all of the differentiations and pattern formations we found. One could also include direct cell–cell interactions, but it would not alter our conclusions. 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 diffusion of a chemical species through a cell membrane depends on the properties of each species. In this model, we simply assume that there are two types of chemicals: one that can penetrate through the membrane, and one that cannot. The medium is regarded as the environment of the cell(s), and could be taken as large as possible. Here the medium chosen is much larger than the cell volume (100 times for most simulations), and inhomogeneity of the medium outside of the region with existing cells can be neglected.

Cell Division and Cell Death

Each cell receives penetrating chemicals from the medium as nutrients, while the reaction in the cell transforms them into nonpenetrating chemicals that comprise the body of the cell. As a result of these reactions, the amount of chemicals in each cell changes. In this model, we assume that the volume of a cell is proportional to the total amount of chemicals in the cell, and a cell divides into two when the cell volume becomes double that of the original.

The chemical compositions of two divided cells are almost identical (except for molecular fluctuations). After cell division, two daughter cells appear around the mother cell's position, and the positions of all cells are adjusted to keep the distance between adjacent cells constant, as discussed below. As a result, the total size of the cell cluster increases. As the initial state, a single cell, whose chemical concentrations are determined randomly, is placed in the medium. According to the process described above, the cells divide to form a cluster.

With the increase of cell volume, chemicals in the medium are consumed. To maintain the growth of the organism, the system is immersed in a bath of chemicals from which nutritive chemicals are supplied. In the bath, the concentrations of nutrient chemicals are kept constant.

Penetrating chemicals can penetrate the cell membrane in both directions, and these chemicals may flow out of a cell. As a result, the volume of the cell can become smaller. In our model, a cell dies when its cell volume becomes less than a given threshold.

Cell Adhesion

As a minimal model for cell–cell adhesion, we assume that cells within a given distance have a "connection," so that they adhere to each other. This adhesion force is given by a "spring" between them, so that the two adjacent cells adhere at the natural length of the spring. The adhesion may depend on chemical states of the adjacent cells. Below we study the case without such dependence, i.e., all adjacent cells adhere with each other, and in a later section we study the case with cell-type-dependent adhesion. When a cell divides, two daughter cells are placed at randomly chosen positions close to the mother cell,[2] and each daughter cell makes new connections with the neighboring cells.

EMERGENCE OF A ROBUST DEVELOPMENTAL PROCESS

In this section, we discuss how the order of a cell society with a variety of cell types emerges, by showing that cellular differentiation and developmental processes form organized patterns. This ordered development of cell society is a common feature in existing multicellular organisms.

As mentioned above, in our model we adopt a simple intracellular reaction dynamic whose rules of reaction are determined randomly and fixed throughout the simulations. The behavior of the cellular system depends on the choice of the random reaction network. To extract the universal features of the system, which are independent of the detailed structure of network and parameters, we performed simulations using thousands of different reaction networks and parameters. As a result, we found that differentiations due to the cell–cell interactions and a robust developmental process toward an ordered spatial pattern of differentiated cells are commonly observed for some of the randomly generated reaction networks.

In the present model, cellular diversification processes are observed when intracellular chemical reaction dynamics show oscillatory behavior, as shown in Figure 1a. For other cases without oscillatory dynamics, in which the concentrations of chemicals are fixed over time, the cells keep an almost identical state, and a cell society of homogeneous cells appears. To investigate the emergence of developmental process in multicellular organisms, we assume that the intracellular dynamics exhibits oscillations. The reasons for studying the networks that give rise to such oscillatory dynamics are as follows.

First, we have found that robust developmental processes with spontaneous differentiation and spatial patterns commonly emerge only if the cells exhibit intracellular reaction dynamics. Second, as discussed below, a cell system characterized by oscillatory intracellular dynamics has a higher growth speed as an ensemble. Since the cells are crowded, only in a cell system with such dynamics can the number of cells continue to increase effectively. For this reason it is expected to be selected through evolution.

In real biological systems, such oscillatory dynamics are often observed in chemicals, such as Ca, NADH, cyclic AMP, and cyclins (Alberts et al., 1994; Hess and Boiteux, 1971; Tyson et al., 1996). Such an oscillation generally appears in a system with positive feedback reactions, which are observed ubiquitously in real biological systems. Indeed, the replication process requires the amplification of molecules, for which a positive feedback process is required. Thus, it is natural to postulate the existence of such oscillatory dynamics in our model system.[3]

Next we present the developmental process by considering two specific reaction networks that exhibit different types of spatial patterns, i.e., the concentric ring pattern and the stripe pattern of differentiated cells.

Developmental Process for the Ring Pattern

In this section, we present numerical results demonstrating the development of the concentric ring pattern of differentiated cells. This spatial pattern is most frequently observed in simulations carried out by taking a variety of randomly generated reaction networks.[4]

As the initial state, we put a single cell, whose internal state (i.e., the chemical concentrations in the initial cell) is determined randomly. In Figure 1a we show a time series of the concentrations of the chemicals for a single, isolated cell. Here, the intracellular dynamics show complex oscillatory dynamics. In this section, we call this initial type of cell "type 0" (represented as a red cell in Fig. 2). This state is the only stable state of intracellular dynamics when the cell is isolated in the medium.

[2]Although the position of the two daughter cells is determined by both intracellular dynamics and cell–cell interactions in real cells, we neglect such factors for simplicity.

[3]The importance of oscillatory dynamics in cellular systems was previously pointed out by Goodwin (1963).

[4]Here we consider numerical experiments employing a particular reaction network with the number of chemicals $k = 32$, and nine catalytic paths for each chemical.

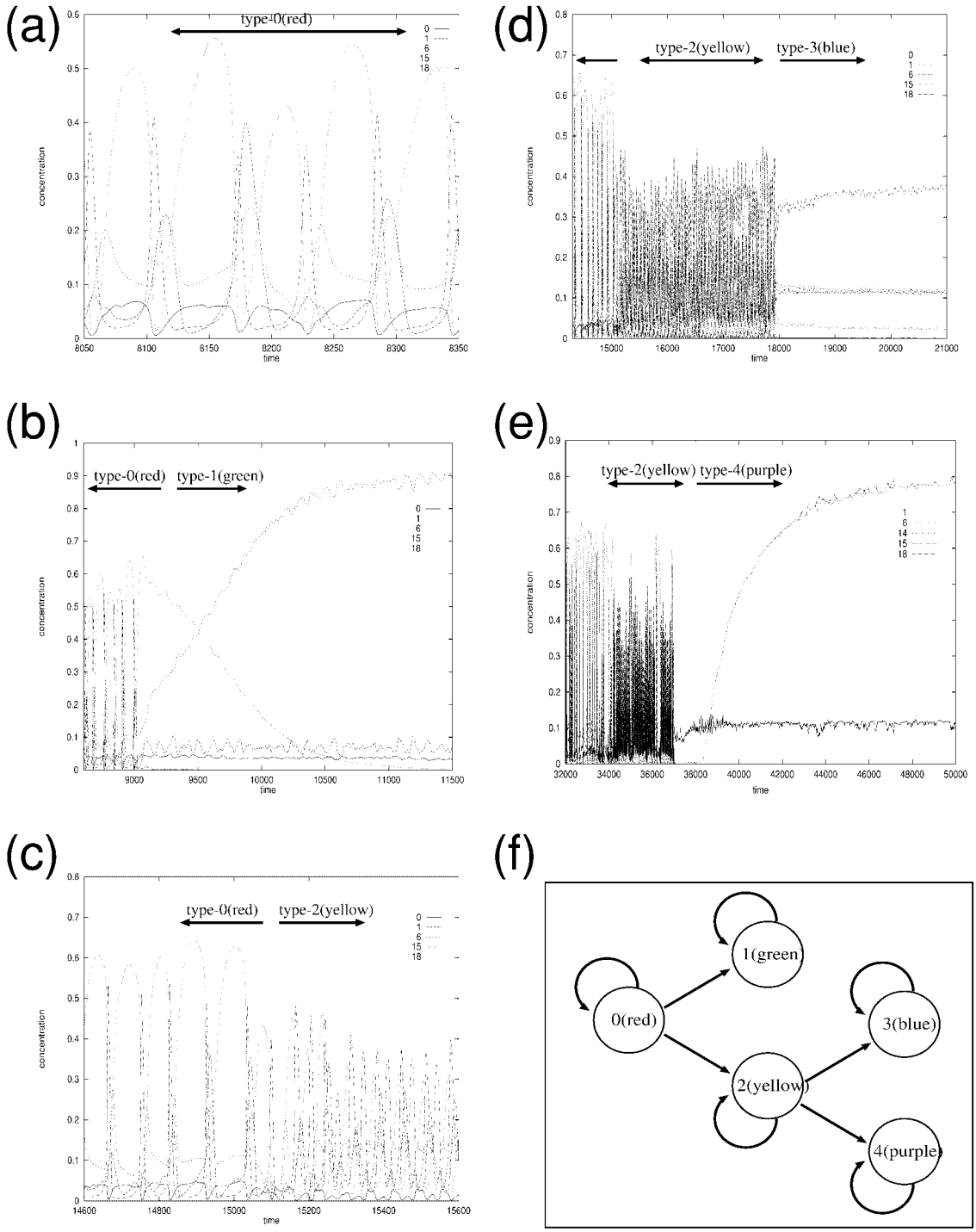

Fig. 1. **a:** Time series of concentrations for the type 0 cell in an example of a ring pattern. The ordinate represents concentrations of chemicals, plotted as a function of time. For clarity, we have plotted the time series of only six of the 32 internal chemicals. **b–e:** Time series of concentrations in a cell, representing the course of differentiation to cell types 1–4, respectively. **f:** The rules of differentiation. The path back to the original cell type represents the reproduction of the same type, while the paths to other types represent the potential for differentiation to the corresponding cell type.

Now, with diffusion, external chemicals flow into the cell. There is a lower concentration of penetrating chemicals in the cell because penetrating chemicals are transformed into nonpenetrating ones within the intracellular reaction. This flow leads to the increase of the cell volume. If this volume exceeds a given threshold, the cell divides into two, with almost identical chemical concentrations, and the daughter cells exhibit the same complex oscilla-

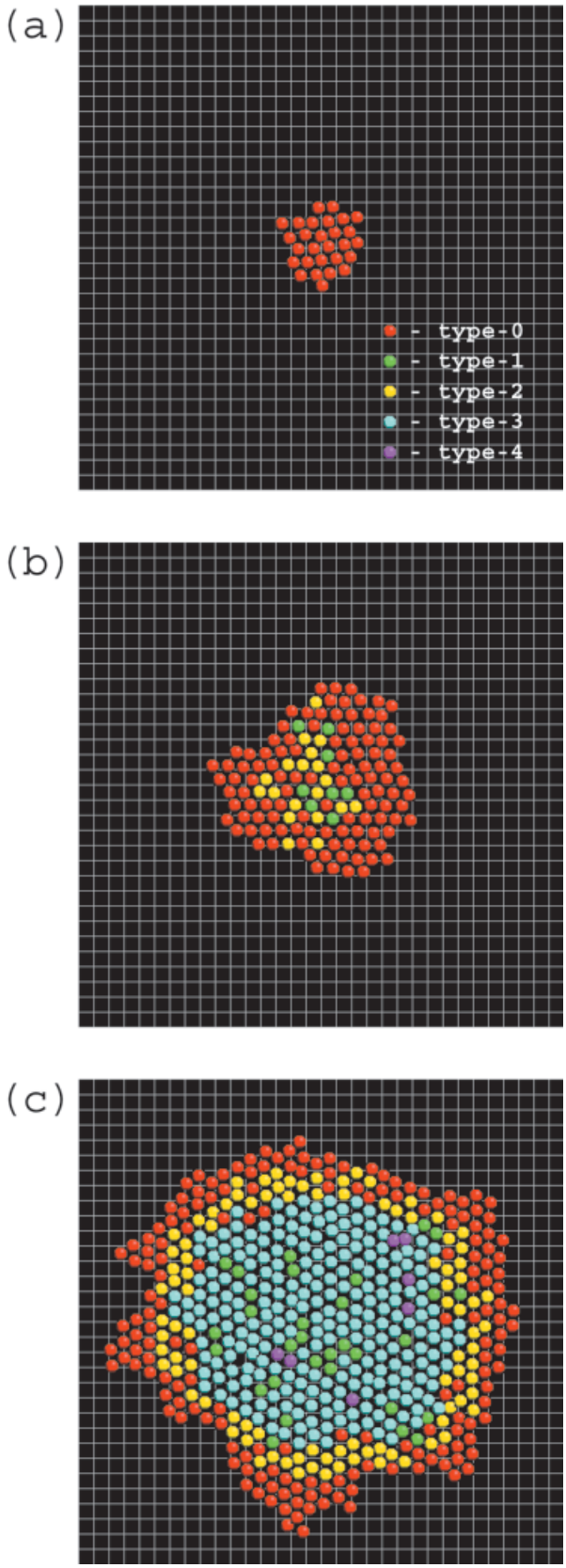

Fig. 2. Development of the cell cluster toward the ring pattern. Each color of a cell represents a particular cell type, determined as distinct states of chemical compositions and dynamics, as shown in Figure 1.

tory dynamics corresponding to type 0. At the first stage of development, a cluster of type 0 cells is formed, as a result of further cell divisions (see Fig. 2a). At this stage, although all cells in the cluster exhibit the same complex oscillatory dynamics corresponding to type 0, the coherence of the oscillations among individual cells is easily lost. The microscopic differences among cells, which arise due to the differences in their positions in the medium and the fluctuations at the cell division, are amplified due to instability in the intracellular dynamics and cell–cell interactions. The amplification of microscopic differences among cells makes the cells to take various different phases of intracellular dynamics. Note that the instability in the dynamical system leading to the heterogeneity of the cellular state is not exceptional in randomly chosen reaction networks in our model. It is generally observed in interacting cells with nonlinear reaction networks, even when the reaction networks are generated randomly.[5]

When the number of cells in the cluster exceeds some threshold value (in the present work, approximately 40), some type 0 cells located at the inside of the cluster begin to display different types of dynamics (Fig. 2b). In Figure 1b and c, the time series of the chemical concentrations in these new types are plotted. We call these "type 1" and "type 2" cells (represented in Fig. 1 as green and yellow cells, respectively). These cell types have different chemical compositions, and there are no stable intermediate states between these types. The chemical compositions and dynamics of the cells converge to form one of these discrete types. By plotting the chemical compositions, each type can be clearly distinguished as a distinct state.

This differentiation is not induced by the imbalance of chemical concentrations at the division process, or any external control mechanism, such as concentration gradients of signal molecules given from the outside of the system. Also, the transition from one cell type to another does not occur at the time of cell division. Rather, it occurs later through the interaction among the cells. This phenomenon is caused by instability in the dynamical system, which consists of all the cells and the medium. When the total cell system becomes unstable, due to the increase of the cell number, differentiations starts. Then, the emergence of another cell type stabilizes the dynamics of each cell again. Indeed, this differentiation is a general feature of a system of interacting units, each of which possesses some nonlinear internal dynamics. This has been clarified by isologous diversification theory (Furusawa and Kaneko, 1998a; Kaneko and Yomo, 1997).[6]

As the cell number increases further, some yellow cells located inside the cluster further differentiate to other cell types (types 3 and 4) (Fig. 1d and e), and are represented as blue and purple cells, respectively. Since cells are dif-

[5]In a pioneering study of cellular inhomogeneity Turing (1952) found that instability of a homogeneous state arises from reaction, and diffusion leads to pattern formation. In terms of molecular biology, the cell–cell interactions that cause such instability, leading to a heterogeneous cellular state, are called *lateral inhibition* (Alberts et al., 1994).

[6]It should be noted that the differentiation process presented here can occur in a system without spatial variation of chemicals, even when cells interact through an identical environment (see Furusawa and Kaneko (1998) for details). The theoretical background of this cellular diversification lies in the study of coupled dynamical systems (Kaneko, 1990, 1994).

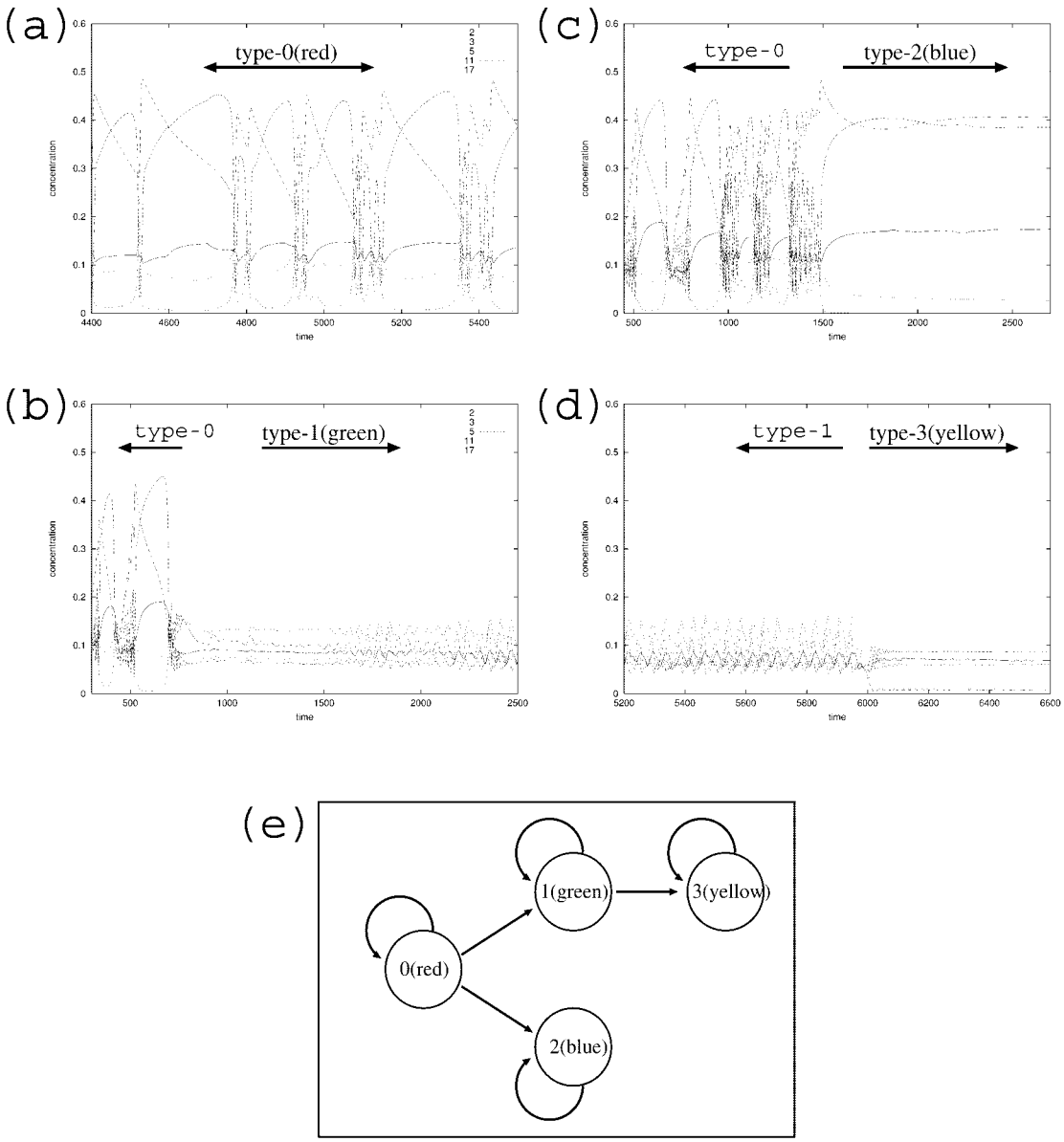

Fig. 3. **a:** Time series of concentrations of the type 0 cell in the example of a stripe pattern. As in Figure 1, the ordinate represents the concentrations of chemicals, and the abscissa represents time. **b–d:** Time series of concentrations in a cell, representing the course of differentiation to cell types 1–3, respectively. **e:** The rules of differentiation in this example.

ferentiated clearly into a few discrete types, they can be represented by a few colors. They form the "inner core" of the cluster, as shown in Figure 2c. At this stage, a ring pattern consisting of three layers is formed, in which the ring of type 2 cells lies between type 0 cells at peripheral region, and an inner core consisting of type 1, 3, and 4 cells.

The transitions between different cell types through differentiation follow specific rules that depend on the random network chosen. These rules are generated from a constraint for the transition dynamics between the states corresponding to each cell type. Figure 1f represents such potentiality using a tree-like representation. In this example, type 0 cells are regarded as totipotent stem cells that have the potential both to reproduce themselves and to differentiate into all other cell types, while the differentiated cells have lost totipotency and only reproduce the same type or differentiate into cell types of lower hierarchy in the tree of the rule. The emergence of totipotent stem cells is a common feature of this cellular diversification process, and is also commonly seen in real multicellular organisms. As discussed below, the regulation of growth and differentiation of the stem cell population is essential to maintain robust development.

Differentiation Process for the Stripe Pattern

In our model simulation, possible spatial patterns of differentiated cells are not restricted to the ring pattern as

presented in the previous section. In this section, we present an example of the development for the stripe pattern, which is obtained by simulations of the model using a different reaction network that is also randomly generated.[7]

In this example, a single cell placed at the center of the medium shows oscillatory reaction dynamics (plotted in Fig. 3a). We call this a type 0 cell (represented as a red cell in Fig. 4).

In this example, when the number of type 0 cells becomes four by cell divisions, the homogeneous state becomes unstable by cell–cell interactions, and two type 0 cells differentiate into another distinct cell type (type 1; represented as a green cell in Fig. 3b). With further divisions of type 0 and type 1 cells, a cluster consisting of these two cell types is formed (Fig. 4b). Because of the differences in absorption of nutrients between type 0 and type 1 cells, the asymmetric distribution of cells brings about an asymmetric concentration gradients of nutrients in the medium. These gradients of chemical concentrations control the further differentiations. As the cell number increases further, some type 0 cells located at the opposite side to the type 1 region differentiate into another distinct cell type, called type 2 cell (blue cell). At the same stage, some type 1 cells that migrated into the type 0 region[8] differentiate into another cell type, called the type 3 cell (yellow cell). As a result of these differentiations, a stripe pattern with four different cell types is formed, as shown in Fig. 4c.

Positional Information

In the morphogenesis of organisms, concentration gradients of chemicals in space are thought to generate and control the pattern (Alberts et al., 1994). This is one model for positional information (Wolpert, 1969). In our model, such gradients exist, and control the growth and differentiation of cells toward a spatial order of differentiated cells. An important point is that such gradients are not imposed on the system from the outside, but, instead, are generated spontaneously and maintained by the cell–cell interactions, as shown in Figure 5. Indeed, the concentration gradients sustaining these patterns disappear when the internal states of all cells (i.e., concentration of chemicals within cells) in the cluster are chosen randomly, even if the concentration of nutrients in the medium and the location of all cells are not changed.

Our results indicate that there is a circular relationship to sustain the development toward a spatially ordered organism, as follows: 1) Depending on the existence and state of the cells, the concentration gradients in the medium are formed by the absorption and release of chemicals by the cells. 2) According to the concentration of chemicals at the corresponding position, the growth and differentiation of each cell is determined.

The positional information emerges through the interplay between intracellular dynamics, and cell–cell interaction (unpublished results). This generation of positional information leads to robustness of the cell society and the developmental process, as discussed in the next section.

Robustness of a Cell Society

An important feature of this developmental process is that a cell society with various cell types is stable with respect to certain types of perturbations.

Indeed, the robustness of the developmental process seems to be a necessary condition for multicellular systems to close their life-cycle successfully under perturbations from external environment or fluctuations at a molecular level. For example, when a cell differentiation is triggered by a small number of biochemical molecules, as is often seen in real organisms, stochastic errors due to molecular fluctuations are inevitable. Thus, the "if-then"-type mechanism for the differentiation, given by a threshold condition for concentration of a signal molecule, cannot proceed the whole development process robustly in the presence of errors that arises inevitably by fluctuations of molecules in the number.[9] To repeat developmental processes over generations and to close the life-cycle of multicellularity successfully, a robust development process against such perturbations is a necessary condition.

In the developmental process presented above, we examined the nature of stability with respect to the molecular (microscopic) level and the cell-population (macroscopic) level.

To investigate the stability with respect to the molecular fluctuations, we added microscopic "noise" to the concentrations of chemicals during the developmental process. Although we used continuous variables for concentrations, the term "noise" here represents the fluctuations in the number of molecules. As a result, we found that the developmental process toward a cell society with the same set of cell types and a similar distribution of cell types is observed as long as the amplitude of noise is less than a certain threshold. This stability comes from the stabilization of cellular states by the cell–cell interactions. As a rough estimate from our theory, the minimal number of molecules necessary for robust development is around 100–1,000 per cell. This number is consistent with observations for present-day organisms, and is not so difficult to realize in the beginning of multicellular organisms, since a reasonable-sized cell can contain at least as many molecules (for details, see Furusawa and Kaneko, 2001 and Kaneko and Yomo, 1997).

In addition to the stability at a microscopic level, our cell society is also stable with respect to macroscopic perturbations caused, for example, by death or damage of some cells.

[7]Here we employ a particular reaction network, again with the number of chemicals $k = 32$ and nine catalytic paths for each chemical. The choice of these numbers is not important. The differentiations are observed as long as the initial cell states have diverse chemicals and allow for complex dynamics (see text). Such dynamics are observed when the number of catalytic paths is neither too large nor too small (say $4 \sim 12$).

[8]This mixing of type 0 and type 1 cells at the boundary between these two regions is caused only by the random determination of the positions of the two daughter cells after cell division and the random fluctuation force applied to each cell. Thus, in some cases, this mixing of cells at the boundary does not occur, and the type 3 cells do not emerge.

[9]One might argue that the error could be eliminated by proofreading mechanisms existing in a cell. However, such proofreading mechanisms consist of chemical reactions, which also suffer from the fluctuations at a molecular level. Hence, there will always be fluctuations even if we consider all possible fine-tuning of the threshold value through evolution. It is also hard to imagine that such proofreading mechanisms, even if possible, could exist in the beginning of multicellular organisms.

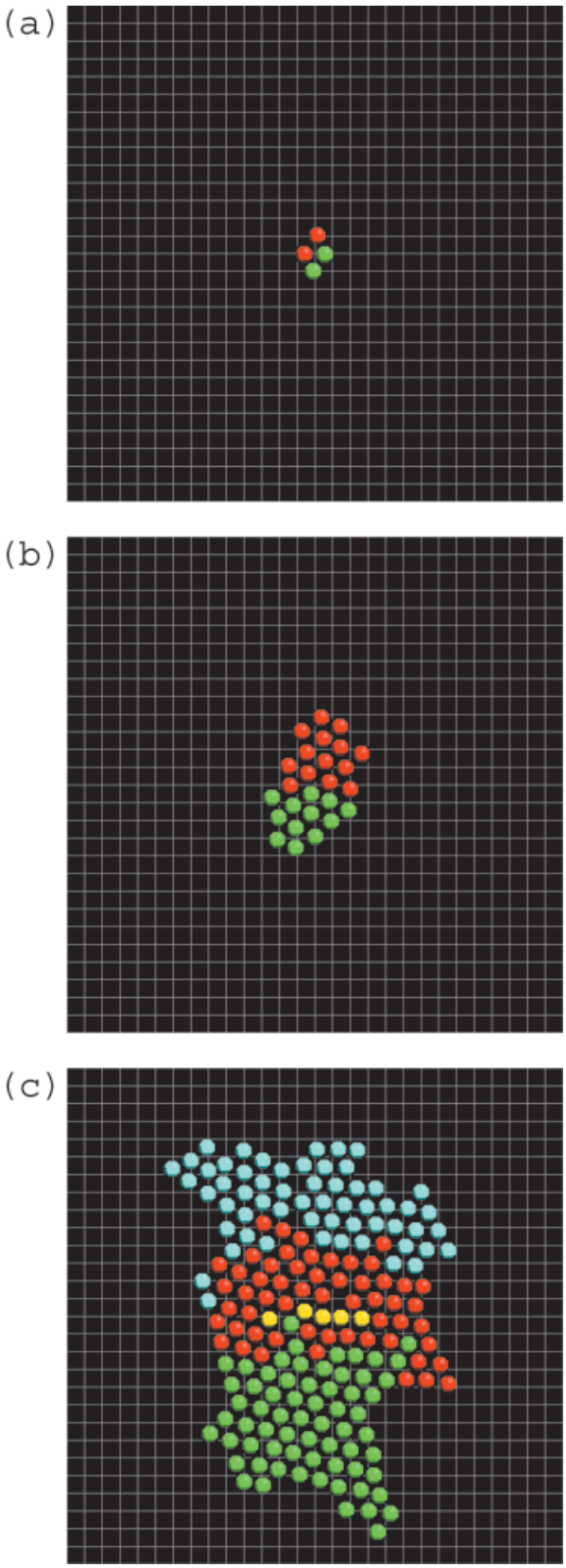

Fig. 4. Development of a cell cluster toward a stripe pattern. Each color represents a particular cell type as determined by distinct states of internal chemical dynamics, as shown in Figure 3.

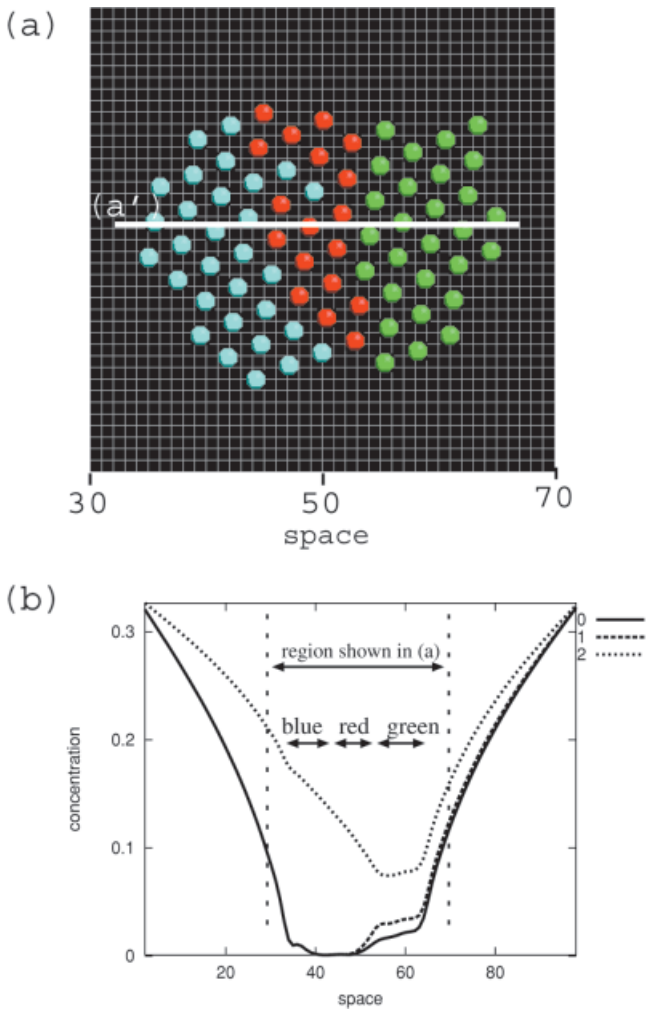

Fig. 5. Concentration gradients along the stripe pattern. **b:** Concentrations of three chemicals in the medium are plotted as functions of position along the segment **a′** in part **a.** In **b**, the regions of different cell types (i.e., blue, red, and green cells) are also shown by the arrows. 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.

As shown in Figure 1f, the differentiations of stem cells (e.g., red cells in Figs. 2 and 4) obey a specific rule. Here, the frequency of differentiation is neither fixed nor random; instead, it is regulated depending on the interactions among the surrounding cells. This regulation of differentiation sustains the stability at a macroscopic level. For example, when all type 2 (blue) cells are removed from a cell society with the stripe pattern (Fig. 4), the rate of differentiation from type 0 to type 2 (red $\rightarrow$ blue) cell is enhanced at the opposite side of the region of green cells, and the original pattern is recovered.

It should be stressed that development with this dynamic differentiation process is always accompanied by this kind of stability with respect to perturbations, without any sophisticated program implemented in advance. In this process, the differentiations occur when the instability of the system exceeds some threshold through the

increase of the cell number, and the emergence of differentiated cells stabilizes the whole system. Therefore, a large perturbation, such as removal of cells, makes the system unstable again, and then the differentiations toward the stable state occur.[10] In other words, only the cell types that have this regulation mechanism to stabilize the coexistence with other cell types can appear in this developmental process.

EMERGENCE OF THE LIFE-CYCLE AS A MULTICELLULAR ORGANISM

In the previous section, we showed that the robust developmental process toward a complex cell society with various cell types is a general feature of a system containing the interacting cells with reaction dynamics. The result is obtained from a class of simple models, but it is expected to be rather general, from theoretical arguments based on dynamic systems (Furusawa and Kaneko, 1998a; Kaneko, 1990; Kaneko, 1994; Kaneko and Yomo, 1999). This means that the emergence of such a developmental process, which is commonly observed in existing multicellular organisms, is not a difficult problem in evolution. In this section, we show that for a system with such a robust developmental process, the recursive generation of cell societies is also not a difficult problem in evolution, by showing the emergence of a replicating unit as an ensemble in our simple model.

To consider this problem, we study the case of differential cell adhesion. Otherwise, all cells remain to form a single colony, as described above, and one cannot discuss separation of cells from a colony. As an example of differential cell adhesion, consider the case that cells of the same type adhere, so that the two cells are located at a distance of the natural length of the spring, while pairs of some different cell types may not adhere with each other.

A cell is under random fluctuations, which lead to its Brownian motion. Thus, in addition to the adhesion force, a random force is applied to all cells. With this random force, we seek a configuration that is stable against perturbations or fluctuations.

As shown in the previous section, the developed cell colony with a variety of cell types generally has a population of totipotent stem cells, which remain until a certain stage of development (e.g., type 0 (red) cells in Figs. 2 and 4). The stem cells keep the potential to rebuild the entire body pattern. Therefore, when such a population of stem cells are released from an organism, they develop toward the same pattern of differentiated cells again. Thus, a life-cycle of replicating multicellular units can emerge by adding a mechanism for cell-type dependent adhesion that joins a class of cells together in unity, even without any more sophisticated mechanism. In this section, we show an example of such emergence of multicellularity by changing the adhesion properties of cells in the previous example of a concentric ring pattern (Fig. 2).

In the examples of spatial patterns shown in Fig. 2, the cells adhere to all adjacent cells with the same strength. Since the force of adhesion depends on the membrane proteins on the cell surface, it is natural to include dependence of adhesion force on the internal states of two adjacent cells. For example, in real organisms, each cell type expresses a different set of adhesion molecules (e.g., CAMs and cadherins) on their cell membranes, which control its adhesion with adjacent cells. As a simple example, we assume that no connection is allowed between a type 2 (yellow) cell and a type 3 (blue) cell in Fig. 2, while the connections for all other combinations are preserved. By this restriction on the connection, the second layer of yellow cells and the inner core consisting mainly of blue cells no longer adhere to each other.

We performed several simulations with these adhesion rules, and 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 (red) cells grows through cell divisions, and type 1 (green) and type 2 (blue) cells appear at the inside of this cluster by differentiation, until the inner core is formed as a result of further differentiation. When the growth of the inner core that consists mainly of type 3 (blue) cells reaches the edge of the cell cluster, however, a small cluster of type 0 (red) and type 2 (yellow) cells, or a solitary cell, are released from the periphery of the mother cluster (Fig. 6a), since there is no adhesion between type 2 (yellow) and type 3 (blue) cells. The released small clusters move away as a result of the random force added to all cells as Brownian motion. (Note that a colony consisting of many cells hardly moves, since the random forces for all cells cancel out on the average.) Now the released cells can come to a region with richer chemical substances in the medium, and start to divide actively. In the new clusters, development proceeds as in their mother cluster. The cells at the inside of a cluster of red cells differentiate to green and blue cells, while the inner core is formed through further differentiations (Fig. 6c), until their peripheral cells are released again. Hence a life-cycle of multicellular replicating units is observed.

The emergence of such a life-cycle is not restricted to the simulations with the adhesion rules mentioned above. In general, this kind of life-cycle appears as long as there are mechanisms to release the stem cells. Here, only some types of cells can be the origin of the next generation of multicellular organism. Cells are separated between those that can produce a new generation and those that cannot. A primitive form of such separation is seen in *Dictyostelium,* in which cell differentiations start to form spore cells when nutrition condition is poor. Only these cells can produce offspring.

In this case, in order for the life-cycle to be repeated successfully, the cluster to be released must consist of a small number of cells. When a cluster with a large number of cells is released, the developmental process from the cluster depends on the cell-type distribution of the cluster. For example, when a released cluster consists of a quarter of the cells in the mother cluster with the ring pattern, it is difficult for the cluster to develop into the same pattern as its mother cluster. As an extreme case, when a cluster consisting only of type 3 (blue) cells (i.e., the inner core) is released, they only proliferate the same type of cells, and the cellular diversification process never occurs. Thus, to close a life-cycle successfully by releasing such large clusters, the cell-type distribution in the clusters must be elaborately controlled. Otherwise, the error in the cell-type distribution is accumulated over the generations, by which the recursive life-cycle is eventually destroyed. The existence of such finely-tuned mechanisms, which are re-

[10]Since this cellular system often has multiple stable states, a large perturbation may cause differentiations toward a different stable state. For details of the transition between multiple stable states of cell society, see Furusawa and Kaneko (1998).

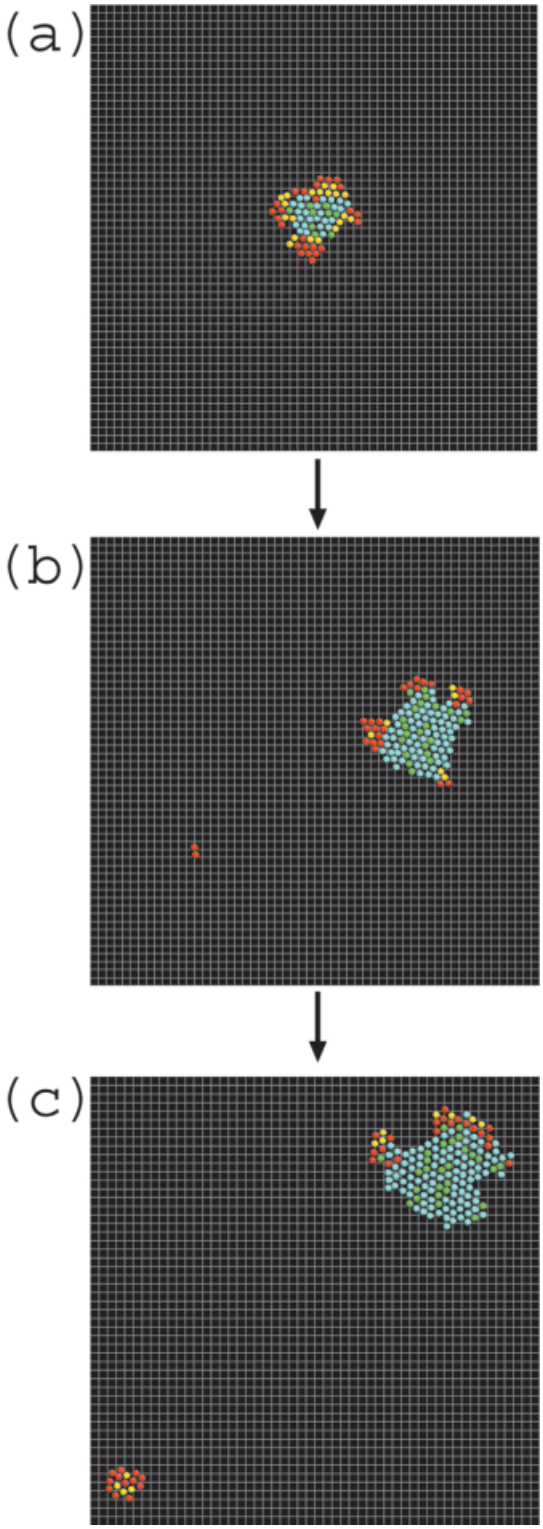

Fig. 6. Releasing peripheral cells from a cluster with a ring pattern. By imposing the inhibition of adhesion between type 2 (yellow) and type 3 (blue) cells, (**a**) when the ring pattern is formed, (**b**) some peripheral cells of the cluster are released. The released small cluster moves away due to random fluctuation force, and when it reaches a new environment with richer nutrients, it starts to grow and the ring pattern is formed again.

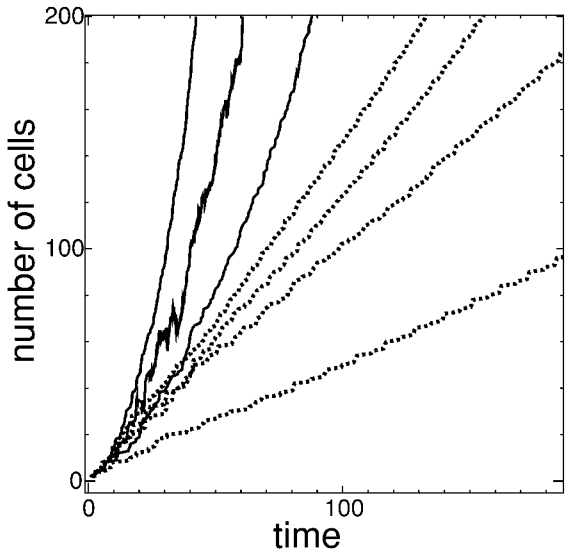

Fig. 7. Growth curves of cell numbers. Temporal evolutions of the cell number are plotted. Each growth curve was obtained by using a different chemical reaction network, chosen randomly. The solid curves correspond to "fast" growth, where several differentiated cell types coexist, while the dotted curves correspond to "slow" growth without cellular diversity.

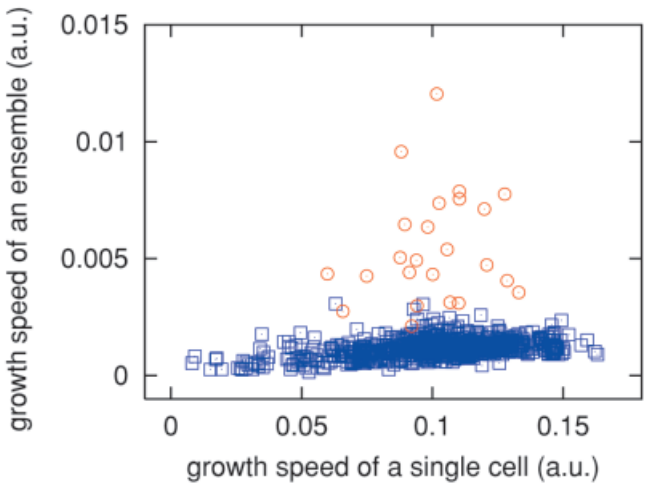

Fig. 8. Relationship between the growth rate of a single cell and that of an ensemble. The ordinate shows the growth rate of an ensemble, measured as the inverse of the time required for the cell number to double from 100 to 200. The abscissa represents the inverse of the time required for a single cell to divide. Each point is obtained by using a different chemical reaction network. The blue points correspond to case 2, with "slower" growth, where the state of cells are identical. The red points correspond to case 1, with "faster" growth as an ensemble, where cells have a variety of internal states. In this case, the cells generally differentiate into several distinct cell types, as shown in Figures 2 and 4.

quired to control the release of distribution of cell types in a large cluster, is not plausible—at least at the first stage of multicellularity. On the other hand, the developmental process forms a cluster with a small number of totipotent cells does not suffer from such error, because the cluster is free from the history of the cell society. We conjecture that this is why the next generation of a multicellular organism is generally generated from one or a few germ cells.

As discussed above, existing multicellular organisms generally adopt the same basic strategies to maintain their life-cycle, i.e., complex organization with various cell types, robust development, and separation of germ cells. Our results from computer simulations suggest that the emergence of a multicellular life-cycle with such strategies is not a difficult problem in evolution, but is a necessity in a system containing intracellular reaction dynamics, cell–cell interactions, and cell divisions. The robust developmental process with totipotent stem cells is a general feature of this system, which emerges even in randomly chosen reaction networks. By imposing a mechanism for differential cell adhesion, totipotent cells are released so that the recursive life-cycle as a multicellular organism is repeated.[11] In the next section, we study how the reaction network that gives rise to the above features is selected, and show that these requirements must be satisfied for multicellular organisms to develop through evolution.

COMPLEX ORGANIZATION IN MULTICELLULARITY AS A NECESSITY FOR EVOLUTION

As previously mentioned, a robust developmental process with various cell types can emerge even when the chemical reaction networks within the cells are generated randomly. In our model system (with suitable parameter values), approximately ~5% of randomly chosen reaction networks result in the developmental process with a variety of cell types, as shown in Figures 2 and 4. One may ask why we should select such cases of the complex organisms with various cell types to describe the general mechanism for the emergence of multicellularity in evolution, while only a small fraction of randomly chosen reaction networks leads to such developmental dynamics. It may be reasonable to assume that multicellular systems emerge from cell colonies without cellular heterogeneity, since such cell colonies are obtained from most reaction networks chosen randomly. If so, can the reaction networks evolve so that differentiation occurs, starting from a cellular system that allows only for a homogeneous cell colony? To clarify what kind of cellular organisms can possibly appear through evolution, we studied the relationship between the growth rate of an ensemble of cells, and some characteristics of intracellular dynamics.

Here, we adopt a model of interacting cells in a one-dimensional medium, instead of the two-dimensional case in the model presented above (we use a one-dimensional model only for its tractability, i.e., just to save CPU time for simulation, since we study thousands of models with different reaction networks. The preliminary results for the two-dimensional model support the conclusions drawn here). The other rules of the model, i.e., internal chemical reactions, transportation of chemicals through the cell membrane, and cell division, are not changed. The one-dimensional medium is considered to be in contact with a bath of chemicals at each end of the medium, through which nutritive chemicals are supplied to the medium. As the initial state, a single cell, whose chemical concentrations are determined randomly, is placed in the medium. After cell division, two daughter cells appear around their mother cell's position, and the positions of all cells are adjusted so that the distances between adjacent cells are constant. As a result, the total length of the chain of cells increases. We measure the growth rate as an ensemble by the increase of cell number in the chain of cells.[12] Since this simple model consists only of the essential features of cellular system, and the results presented below are robust against changing the details of the model and parameters, we believe the results indicate universal characteristics of a cell society.[13]

Classification of Growth Behavior: Fast (Exponential) Growth and Slow (Linear) Growth

To examine how the natures of cell growth and dynamics are correlated, we carried out simulations of the model by considering 1,000 different reaction networks, generated randomly.[14] In Figure 7 some examples of the growth curve of cell number are plotted for different reaction networks. It is demonstrated that the growth can be classified into two classes: 1) fast growth, in which the increase of cell number grows exponentially in time t; and 2) slow growth, in which the cell number grows linearly in time. These two classes are also distinguished by the nature of the corresponding intracellular dynamics.

In case 2, with slower growth, the chemical compositions and dynamics of all cells are almost identical. For most such cases, the concentrations of all chemicals in each cell are fixed over time.[15] In this case, only a few cells around the edges of the chain can divide. Since cells are not differentiated, the chemicals required for cell growth are identical for all cells. Thus, once the cells at the edges consume the required chemicals, which are supplied from each end of the one-dimensional medium, the remaining cells can no longer grow. This is why the growth is so slow in case 2.

In case 1, with a faster growth, it is found that the chemical reaction dynamics of the cells are more complex than in case 2. The concentrations of chemicals in the cells are not fixed, but show complex oscillatory dynamics with a variety of chemicals. In this case, cells in the organism assume various different states. The microscopic differences between the chemicals in the two daughter cells are amplified, as previously mentioned. This amplification makes the cells take on different phases of intracellular dynamics.

For most such cases, the cells differentiate into various distinct cell types, as shown in Figures 2 and 4. Here, the increase of cell number makes the state of homogeneous cells unstable by cell–cell interactions, and cells differen-

[11]Again, the result here is obtained from a class of specific models. Still, it is expected that the result is rather general, considering the simplicity of the model and the universality of the theoretical mechanisms.

[12]The same growth curve is obtained by measuring the sum of cell volume in the chain of the cells.

[13]For details of the modeling and results in this section, see Furusawa and Kaneko (2000).

[14]In the simulations presented here, the number of chemical species k is 20, and each chemical has six reaction paths to other chemicals, chosen randomly. Among the 20 chemicals, there are five chemicals capable of penetrating cell membranes, while three chemicals are supplied as nutrition.

[15]In rare cases, the concentrations of all chemicals show a simple periodic oscillation.

tiate. In this case, the division of cells is not restricted to the edge of the chain. With differentiation, cells begin to play different roles and come to require different chemicals as nutrition. Now, chemicals flow into the inside of the chain, and internal cells are also supplied with the nutritive chemicals they require. Hence, even the internal cells are able to grow. This flow is sustained by the diffusion process between cells possessing different chemical compositions and exhibiting different phases of chemical oscillations.

Growth Rates of a Single Cell and an Ensemble

It should be noted that the faster growth in the complex organisms with various cell types is based on the interplay between complex cellular dynamics and cell–cell interaction. Therefore, this faster growth cannot be elucidated by the dynamics of a single cell, but is a property of the whole system. To clarify the relationship between the dynamics of a single cell and those of an ensemble, we compare the growth rate of an isolated cell with that of an ensemble of cells.

In Figure 8, the growth rate of a single cell and of an ensemble of cells are plotted. Here each point corresponds to a result from a different reaction network, generated randomly. The growth rate of a single cell is computed from the time required for an isolated single cell to divide, and that of an ensemble is from the time for a single cell to reach a given number of cells (here 200). As shown in the figure, the growth rate of an ensemble is not monotonically related to that of a single cell.

The points around the peak of the growth speed for an ensemble correspond to case 1, with a variety of cell types, plotted by red points. Here, the growth speed of a single cell is not large. In each cell, a variety of chemicals coexist, supporting complex reaction dynamics and cell differentiation, and the growth rate of an ensemble is higher than that without diversity (case 2).

In case 2, the growth rate of a single cell is often high (represented by some of the blue points in Fig. 8), while the growth rate of an ensemble always remains low. In such cells with rapid growth as single cells, chemicals concentrations are biased to a few species, and only a small number of autocatalytic reaction paths are used. Such simple cells with rapid growth are regarded as "selfish." Although cells with such a low diversity of chemical species can exhibit high rates of growth as single cells, they cannot grow cooperatively, and their growth rates as ensembles are suppressed because of strong competition for resources.

This relationship between growth as an ensemble and the characteristics of intracellular dynamics is robust against changes in the parameters, and is also independent of the details of the model. Thus, we believe that the relationship between growth and cellular dynamics is a universal characteristic in a system containing replicating units with internal dynamics and a competition for resources.

The results of the simulations provide evidence that an ensemble of cells with a variety of dynamics and cell types has a higher growth rate than an ensemble of simple cells in a homogeneous state, because the former cells have greater capability to transport and share nutritive chemicals. We conjecture that this is why existing multicellular organisms share the basic strategies for multicellularity, i.e., complex organization with a variety of cell types, robust developmental process, and emergence of a germline for the next generation, even though the multicellular organisms may have appeared several times independently.

To sum up, we show that complex organisms with various cell types emerge as a result of strong cell–cell interactions, and such organisms have two clear advantages with regard to their continuous growth as an aggregate of cells, over a simple organism without cellular diversification. First, such complex organisms have a higher rate of growth due to their ability to transport and share nutritive chemicals. Second, by releasing totipotent stem cells, such organisms can replicate themselves recursively, which enables the released cells to explore new environments with rich resources. Note that no elaborate mechanism is required for the appearance of the complex cell system with these advantages. On the other hand, simple organisms without cellular heterogeneity may not survive as an ensemble, due to the lack of "cooperation." The competition for the same resources limits their growth, when no special organ for transportation of nutrition has been developed. Hence, it is natural to conclude that complexity of multicellular organisms with differentiated cell types is a necessary course in evolution, once a cell aggregate emerges.

SUMMARY AND DISCUSSION

In the present work, we have shown that salient features of multicellular organisms naturally emerge as the number of very primitive cells increases. Here the primitive cells have internal reaction dynamics (for example, metabolic reaction or genetic expression) and simple cell–cell interaction, and potential for division. Let us recall the four conditions required for multicellular organisms:

I. Recursive production of cells at an ensemble level: The other three features generally emerge in a system of interacting primitive cells.
II. Cell differentiation: Cells differentiate triggered by instability of the homogeneous cell ensemble, when the cell–cell interactions become strong enough as a result of the increase of cell number. A cellular state is destabilized for some cells, and changes into another state. The appearance of new cell types stabilizes the overall cellular system again. The differentiated pattern leads to the generation of "positional information," which is maintained by the interplay between intra- and intercellular dynamics.
III. Robust developmental process: Since the cellular state is determined to make the whole system stable, the developmental process is generally robust with respect to certain types of perturbations. We have confirmed that the developmental process is stable with respect to microscopic perturbations corresponding to molecular fluctuations and macroscopic perturbations, such as the removal of some cells. In this developmental process, the emergence of new cell types is always accompanied by this kind of robustness.
IV. Emergence of a germ-line: In this developmental process, totipotent stem-type cells generally appear, while other cell types have lost the potential to differentiate into all other cell types. By imposing an adequate mechanism for differential cell adhesion, some cells are released to reconstitute an

entire body as the next generation, so that the recursive life-cycle as a multicellular organism is repeated. This robust recursive life-cycle is maintained only when the number of released cells is small.

An important point is that such features naturally emerge in a system of primitive cells without postulating any fine-tuned mechanism. The results clearly show that the emergence of a recursive life-cycle of multicellularity is not a difficult problem.

The question of the origin of multicellularity is often discussed from the viewpoint of fitness for survival, say as a condition for a multicellular organism to have better fitness. In contrast, we suggest that in regards to the origin of multicellular organisms, it is more important to consider how recursive production has arisen through evolution from a loosely reproduction system. The fitness is defined only after the condition for recursive production and evolvability is satisfied (Fontana and Buss, 1994).

We recall that recursive production and differentiation take place at some stage between intracellular processes and cell–cell interaction. A cell state committed to a type is not so much influenced from the cell–cell interaction, while differentiation from a stem cell is influenced more strongly by cell–cell interaction. We should now discuss the degree of relevance of interaction to a cell state.

If the cell-cell interaction is very weak, each cell maintains its state almost independently of other cells. On the other hand, under strong cell-cell interaction, cells are highly interdependent and cannot live on their own. In the former case, the same kind of cell is reproduced, and the number increases. This increase may be rather fast, as in the case 1 above. Although these cells may look successful in the beginning, they may soon encounter a difficult condition for growth, since the increase of the cell number drastically changes the environment, and the resources would be reduced. Since these cells are identical as to their behavior, they compete for the same resources. Therefore, their growth would soon be suppressed.

In the latter case with strong cell-cell interaction, interdependency among cells is essential to survival. Here, the recursiveness as a single cell is partially lost, but recursive production as an ensemble is achieved. Then cell differentiation, a robust development process, and recursive production of the next generation from a single (or few) cells naturally appear as a consequence of a system with cell–cell interaction and intracellular dynamics. The recursive production of a multicellular colony is further strengthened by the emergence of germ-line cells, as discussed above. Now, the basic properties (II–III) appear not by chance but as a necessity in the history of life, as a general result of interacting cells with internal biochemical reaction dynamics, which allows for continual growth in crowded conditions. With differential cell adhesion, a cell colony of a finite size is formed. From this cell colony, one or a few cells of a given type are released, from which the next generation of multicellular cell colonies is formed recursively. This results in the recursive production of a multicellular unit (I) and the emergence of a germ-line (IV). These two features are necessary for a system to continue reproduction even if cells become crowded.

Now let us come back to the origin of a cell in the history of life. Here both the recursive production of cellular contents and the compartmentalization that forms individual cells must begin, starting from a set of molecules reacting with each other. Recently, we have discussed a system with chemical reaction and diffusion which can produce a spot structure, characterized by higher concentration of chemicals than that of background. Each spot reproduces similar (or the same) spots (Takagi and Kaneko, 2001). These spots, when produced, are connected with each other, as in a form of algae. Here, the individuality of each "cell" is not established yet, and the boundary between two spots is shared by the adjacent spots. The spots exist only as a connected chain (or surface),[16] and are not separated. Still, this primitive state of multiple spots may be regarded as analogous to a connected set of primitive cells.

From this "proto-multicellular structure," there can be two routes in evolution. One is to enhance the individual autonomy of a cell. As the individuality of a cell is established, it will divide into two separated cells, each of which can survive on its own. The other possible route is to keep some degree of looseness at the boundary of each cell, so that interdependence between cells is important. In the latter case, the cell boundary should not be as rigid as in the former case. Then the cells may join, or one cell may absorb (eat) another. The membrane of this type of cell is not so rigid, which makes phagocytosis possible. This gives a route to endosymbiosis, leading to a eukaryotic cell. Since this type of cell is influenced more strongly by cell–cell interaction, a route to multicellular organisms will open, as outlined in the present paper.[17]

At this point, it is interesting to note that all multicellular organisms satisfying the condition of recursiveness as a colony (i.e., property I) consist of eukaryotic cells. Indeed, a colony of bacteria cannot establish multicellular organisms to satisfy the requirement of recursiveness as a colony (I). On the other hand, the differentiation itself (stated as the property II) has a broader generality (Ko et al., 1994; Shapiro and Dworkin, 1997). When bacteria are put into a condition with very strong cell–cell interaction, they can be differentiated into distinct types of enzyme activities (i.e., they satisfy property II) (Ko et al., 1994). Still, for prokaryotes, individuality by each cell is so strong that they cannot reach stage I (recursiveness as a colony).

To sum up, two possible routes are discussed for the evolution of cells. In the first case, the influence of cell–cell interaction is not as strong, which corresponds to the prokaryotes. In the other case, cellular states are strongly influenced by cell–cell interactions; this direction leads to phagocytosis, cell symbiosis, and multicellular organisms. The exchange of internal contents between cells will also be easier for these cells, and hence the evolution of sex will also be easier. In fact, multicellularity, eukaryotes, and sex are highly correlated among organisms. As soon as the cell interaction is strong, properties I–III naturally appear for cells to continue to increase their number.

Note that within the developmental process, plasticity (given by variability of cellular states) is decreased

[16]In this respect, it is interesting to doubt that the first organisms were unicellular in the present sense, and to consider the possibility that the first cells formed cell aggregates, and might be regarded as a kind of primitive proto-multicellular organisms.

[17]Establishment of multicellularity requires strong interaction, brought about by crowded population conditions. Possibly $\sim 10^9$ years passed before the population density reached such a high level, in the history of life on Earth.

through cell–cell interaction. Irreversible differentiation progresses from totipotent cells to lineage-restricted stem cells, and to committed cells. This irreversible differentiation is also a general course in a system of internal dynamics and interaction, if the interaction influence is strong enough. Thus the degree of irreversible differentiation is expected to increase with the interaction. The irreversibility of differentiation from stem cell to determined cell progresses more in animal cells than in plant cells, while the cell wall of animal cells is less rigid than in plant cells.[18]

Finally, we discuss the emergence of the germ-line. Most multicellular organisms have established mechanisms to generate germ-line cells, but some of them do not. In our theory and model, through release of one (or a few) cells the next generation begins, which has the same set of cell types as its mother, and similar patterns. On the other hand, if many cells were released together, the recursiveness of the next generation would not be guaranteed. For example, if released cells are determined cells of the same type, they can no longer differentiate. Hence the organism of the next generation, even if it is formed, consists of only homogeneous cells, and is not recursive from the mother. When mixed cells are released, the behavior of the next generation may crucially depend on the number distribution of mixed types, and again recursiveness would not be guaranteed.

Hence, recursiveness as a multicellular organism is facilitated when a specific type of cell is released to give rise to the next generation. According to our theory, this accounts for the origin of a germ-line. Although the separation of a germ-line is not necessary for all multicellular organisms, most complex multicellular organisms have this process. In fact, it is more and more difficult to maintain the recursive generation of organisms, as they become complex, i.e., have more cell types and tissues. Specific control of the initial conditions required to generate such a complex structure is possible only through the segregation of the germ-line.

In some multicellular organisms, reproduction without segregation of germ cells is often observed. For example, in asexual reproduction of planarians, entire bodies of the next generation are reconstituted from half pieces of the mother's body after spontaneous body fission. Even in reproduction without segregating germ cells, the recursive production of the next generation is generally maintained only by totipotent stem cells; differentiated cells do not participate in this reproduction. In the example of the planarians, the reproduction of the body is maintained only by the regulated growth and differentiation of totipotent stem cells existing ubiquitously in the body. Recall that in our results, the separation of totipotent stem cells and differentiated cells is generally observed in the developmental process. Our results show that these totipotent stem cells maintain the potential for "regeneration." For example, when some cells are removed, the regulated differentiation of the stem cells generally recovers the injured part.

The emergence of a germ-line favors evolvability. If mutation to a germ cell occurs, it has a large influence because it is transferred to the next generation. Consider, on the other hand, the case of regeneration from some part of an organism that consists of many cells (for example, when some part is cut from a planarian, it forms a new, complete organism). In this case, all the mutations to the many cells are transferred to the next generation. Since the mutation is random, their influence will cancel each other, and on the average it will be decreased. (The average deviation of N elements decreases as $1/\sqrt{N}$ with N.) Then evolvability, i.e., the potential to create different phenotypes, is decreased. The emergence of germ-line cells facilitates evolvability, which may be another reason why complex multicellular organisms often adopt it. The importance of elements with a minority in the population (such as the germ cell) has previously been discussed as "minority control" (Kaneko and Yomo, 2002), in relationship to the control and preservation of the information molecule, DNA, which is a minority chemical in a cell.

[18]For the details of irreversibility in this differentiation process, see Furusawa and Kaneko (2001).

ACKNOWLEDGMENTS

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APPENDIX

Model Equations

Here we give the explicit model equations used in the simulations in the previous sections. In the present model, a cellular state is expressed by a set of variables $\{c_i^{(1)}(t), \cdots, c_i^{(k)}(t)\}$ representing the concentrations of the k chemical species in the i-th cell at time t. The corresponding chemical concentration in the medium is represented by a set of variables $\{C^{(1)}(x,y,t), \cdots, C^{(k)}(x,y,t)\}$, where x and y denote the position in the two-dimensional medium.

As the internal chemical reaction dynamics, we chose a catalytic network among the k chemicals. To represent the reaction matrix we use the notation $Con(i,j,\ell)$, which takes the value 1 when the reaction from chemical i to chemical j is catalyzed by ℓ, and 0 otherwise. Using this reaction matrix, we denote that the rate of increase of $c_i^{(m)}(t)$ (and decrease of $c_i^{(j)}(t)$) through a reaction from chemical j to chemical m catalyzed by ℓ as $ec_i^{(j)}(t)(c_i^{(\ell)}(t))^{\alpha}$, where e is the coefficient for the chemical reaction and α is the degree of catalyzation.

We take into account the change in the volume of a cell, which varies as a result of the transportation of chemicals between the cell and the environment. For simplicity, we assume that the total concentration of chemicals in a cell is constant, $\Sigma_m c_i^{(m)} = const$. It follows that the volume of a cell is proportional to the sum of the quantities of all chemicals in the cell.

In this model, we consider only indirect cell–cell interactions in the form of the diffusion of the chemical substances in the system, as a minimal form of interaction. Thus, the term describing the transport from the medium into the i-th cell for m-th chemical is given by $D(C^{(m)}(t) - c_i^{(m)}(t))$, where D is a transport coefficient. Furthermore, we assume the simple rule of cell transport, in which 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 of these processes, the dynamics of chemical concentrations in each cell is represented as follows:

$$dc_i^{(\ell)}(t)/dt = \sum_{m,j} Con(m,\ell,j) ec_i^{(m)}(t)(c_i^{(j)}(t))^{\alpha} - \sum_{m',j'} Con(\ell,m',j') ec_i^{(\ell)}(t)(c_i^{(j')}(t))^{\alpha} + \sigma_\ell D(C^{(\ell)}(p_i^x,p_i^y,t) - c_i^{(\ell)}(t)) - c_i^{(\ell)}(t) \sum_{m=1}^{k} \sigma_m D(C^{(m)}(p_i^x,p_i^y,t) - c_i^{(m)}(t))$$

where the terms with $\Sigma\, Con(\cdots)$ represent paths coming into and out of ℓ, respectively. The third term describes the transport of chemicals out of and into the surrounding medium, where D denotes the diffusion constant of the membrane, and the coordinated (p_i^x,p_i^y) denotes the location of the i-th cell. The last term gives the constraint of $\Sigma_\ell\, c_i^{(\ell)}(t) = 1$ due to the growth of the volume.

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:

$$\partial C^{(\ell)}(x,y,t)/\partial t = -D_e \nabla^2 C^{(\ell)}(x,y,t) + \sum_i \delta(x - p_i^x, y - p_i^y)\sigma_\ell D_m(C^{(\ell)} - c_i^{(l)}(t)). \quad [1]$$

We assume the following boundary condition:

$$C(0,y,t) = C(x_{max},y,t) = C(x,0,t) = C(x,y_{max},t) = \text{const.} \quad (0 < x < x_{max},\ 0 < y < y_{max}) \quad [2]$$

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 this cellular system, when the number of paths in the reaction matrix is small, the cellular dynamics generally fall into a steady state without oscillation, in which a small number of chemicals are dominant, while other chemicals' concentrations vanish. On the other hand, when the number of reaction paths is large, all chemicals are generated by other chemicals, and the chemical concentrations come to realize constant values (which are almost equal for many chemicals). Only when there is an intermediate number of reaction paths (e.g., nine connection paths for $k = 32$) do nontrivial oscillations of chemicals appear, as seen in Fig. 1a.

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