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Robust development as a consequence of generated positional information

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Abstract

The origin and robustness of morphogenesis are studied by dynamical system modeling of a cell society, in which cells possessing internal chemical reaction dynamics interact with each other through their mutual interaction with diffusive chemicals in a two-dimensional medium. It is found that stem-type cells differentiate into various cell types (where a cell 'type' is defined by a type of intra-cellular dynamics) due to a dynamic instability caused by cell–cell interactions in a manner described by the isologous diversification theory. The differentiations are spatially regulated by the concentration of chemicals in the medium, while the chemical concentrations are locally influenced by the intra-cell dynamics. Through this reciprocal relationship, chemical concentrations come to exhibit spatial variation as differentiated cell types begin to emerge, and as a result the regulation exercised by the chemical concentrations become spatially inhomogeneous. This reinforces the process of differentiation, through which spatial patterns of differentiated cells appear. Within this reciprocal relationship, the concentration gradients are read and interpreted by the cell as positional information. A spatial order of cells realized in this process represents a stable state of the system governed by this reciprocal relationship, and that the developmental process through which this state is realized is robust with respect to perturbations. The dependence of the morphogenesis on history and the community effect in cell differentiation are also discussed.

Keywords: Morphogenesis; Robustness; Positional information

1. Introduction

In morphogenesis, cells differentiate during the developmental process, and these differentiated cells form an ordered spatial pattern. There are two unsolved fundamental problems of morphogenesis, understanding the origin of positional information and elucidating the reason for the robustness of development. This paper addresses these problems.

With regard to the first of the above stated problems, recent experimental studies have clarified how changes in gene expression take place in the process of differentiation and in the process of spatial pattern formation. However, the description of changes in gene expression alone is not sufficient to fully explain the entire process of morphogenesis, because the manner in which gene expressions are activated or inactivated depends on the chemical state of the cell, and this state is influenced by interactions with surrounding cells.

The second problem referred to above begin from the observation that pattern formation processes in cell societies during normal development are rather stable. Despite the inevitable existence of fluctuations on the molecular level and perturbations on the cellular level, under 'normal' circumstances, pattern formation processes proceed very predictably, with the development of a particular type of tissue in any given organism generally following the same course and resulting in nearly identical patterns in all instances.

Furthermore, some experiments in which the developmental process is perturbed externally through the removal or addition of cells suggest that the cells somehow 'know' their position within a system

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consisting of an ensemble of cells, and with this knowledge they are able to recover the effect of the perturbation. Considering these observations, Wolpert proposed the concept of positional information (Wolpert, 1969): Each cell knows its position by the concentrations of certain diffusing chemicals surrounding the cell, and the gradient of these chemical concentrations is assumed to carry positional information, separated from genetic information of each cell. In fact, the idea that the spatial variation of diffusional chemical concentrations plays a role in morphogenesis was originated by Turing (1952), as discussed later.

Although much has been learned in these pioneering works, the two basic problems mentioned above remain unsolved.

In a cell society, if positional information, embedded in gradients of some chemical concentrations, is externally given, cells can differentiate in appropriate ways to generate a certain order of cell society, as discussed by Wolpert. However, the manner in which such positional information is generated spontaneously is not discussed, within the positional information theory. It is often argued that once the positional information determining the first pattern formation is supplied from outside the system, each subsequently emerging pattern generates increasingly detailed positional information used in the following pattern formations, and the progressive chain of such interconnected formation processes completes the morphogenesis. In fact, there are asymmetric distributions of some components in fertilized eggs, such as bicoid and nanos mRNAs in the Drosophila egg, and it has been asserted that the pattern formation process in the first stage of development is controlled by this initial asymmetry of chemical components (Alberts et al., 2002). However, it cannot be the case that all positional information governing pattern formation is embedded in the initial asymmetry of egg. Indeed, morphogenesis has been found to be robust with respect to changes in such initial asymmetry of components. For example, when the initial concentration gradient of bicoid in the Drosophila egg is altered experimentally, although the subsequent pattern formation in the first stage of development is modified according to this alteration, the final pattern displayed by the fully developed body is unaffected by this perturbation, up to a certain degree (Driever and Nüsselein-Volhard, 1988). This seems to imply that the important positional information is not entirely embedded in the initial conditions of the developmental process, but rather it is to some extent also generated during the developmental process.

In addition to the problem involving positional information, that of elucidating the properties of the system responsible for the robustness of pattern formation with respect to microscopic perturbations also remains unsolved. In the standard picture of developmental biology, cell differentiation is controlled by the "if–then"-type mechanism, given by a threshold condition for concentration of signal molecules to determine whether a differentiation occurs or not. A cell 'reads' signal concentrations and changes its internal state according to the information. However, as discussed in Kaneko and Yomo (1999) and also by Lacalli and Harrison (1991), fluctuations in chemical concentrations introduce a serious problem into the standard picture with "if–then" type mechanism. To see this, first recall that these signal molecules are often present in very low concentrations. Here, the chemical concentration of relevance is the number of molecules per unit volume in the tiny region around the cell. Hence the number of signal molecules associated with cell differentiation is often quite small, while in diffusion processes, each molecule moves randomly. Thus there can be large fluctuations in the concentration of molecules affecting a single cell in general. In fact, Houchmandzadeh, Wieschaus, and Leibler have recently demonstrated such large fluctuations of bicoid concentration in the Drosophila eggs with the same developmental stage (Houchmandzadeh et al., 2002). Such large fluctuations no longer appear at later steps in the developmental process, and robust pattern formation results. It should be stressed here is that, the "if–then" type mechanism has no potential to 'correct' errors arising from such fluctuations to maintain developmental process robustly, as seen in real organisms. One might expect that the errors in determining cell differentiations could be eliminated by proofreading mechanisms existing in a cell, to support the threshold mechanism. However, such proofreading mechanisms consist of a chain of chemical reactions, which also suffer from the fluctuations at a molecular level. Therefore, there will always be fluctuations even if we consider all possible fine tuning of the threshold value.

Taking into account the above considerations, to understand the process of morphogenesis, it is of great importance to construct a model in which both the generation of positional information and the robustness of the developmental process with respect to both macroscopic and microscopic perturbations are incorporated. This paper presents an attempt to realize this.

Note that, in Wolpert's theory, positional information is functionally separated from its interpretation by cells with intra-cellular reaction dynamics. However, to discuss morphogenesis, including the generation of positional information, this separation is not always valid. Here, the inter-dependence between positional information by molecular gradients and intra-cellular dynamics of cells should be considered.

Fifty years ago, Turing proposed a pioneering theory for spontaneous pattern formation, based on chemical reaction and diffusion, without assuming any external mechanism for the imposition of spatial inhomogeneity

(Turing, 1952). This theory partially answers the question concerning the origin of positional information and the inter-dependence between positional information and cellular dynamics, in terms of dynamic instability and diffusive interaction. In the present paper, we adopt a dynamical systems modeling method possessing a certain instability, and in this sense, our theory is along the line of Turing's theory. However, our theory aims to go beyond the standard Turing's theory at some points, considering some insufficiency of the standard theory to solve the questions raised above, as discussed below.

First, in Turing's model, although a spatial pattern of varying chemical concentrations (a periodic striped pattern) is formed, it is not so clear how this variation in concentrations leads to the differentiation into "discrete" cell types, since the variation of chemical concentrations is nearly continuous. Note that a Turing-type dynamics can trigger transitions between discrete cellular states when it couples with other cellular dynamics giving rise to multiple stable states (Miura and Shiota, 2000). However, to regulate the transition between these cellular state elaborately, the Turing-type dynamics generating cellular heterogeneity and cellular dynamics sustaining discrete nature of cellular states must be tightly coupled. Thus, a study of a coupled cell system with rich intra-cellular dynamics having dynamic instability and multistability is required, to discuss irreversibility in cell differentiation straightforwardly.

The second problem is that in the standard Turing model, the developmental process involving the increase of cell number is not considered. In his original model, the system size, i.e. the number of cells, is fixed in time. It should be noted, however, that in development and regeneration processes in multicellular organisms, the regulated growth and differentiation of stem-type cells play an essential role in generating and maintaining spatial and temporal order in the cell society. For example, in the regeneration process of injured tissue, the original spatial pattern is recovered not through simple spread of the remaining tissue, but through elaborately regulated growth and differentiation of undifferentiated cells in the affected region, which appear due to the activation of quiescent stem cells or the de-differentiation of differentiated cells. In these processes, a changing population of each cell type due to growth and differentiations is essential. Although there are some theoretical studies incorporating domain growth into Turing's model (Crampin et al., 1999), the discrete nature of differentiation process is not considered in these studies. A model that fully takes account of cell division process coupled with the intra-cellular reaction dynamics should be studied.

In spite of extensive studies in Turing patterns (Meinhardt and Gierer, 2000), the above two problems were not completely resolved. We have proposed the so-called isologous diversification theory to resolve the problems of the Turing model and to explain the spontaneous differentiation of cells into discrete types in a developmental process in which the number of cells increases (Kaneko and Yomo, 1994, 1997; Furusawa and Kaneko, 1998, 2001). In the theory, each cell state is represented by concentrations of several chemicals that change in time through intra-cellular catalytic reaction processes and cell–cell interactions communicated by the diffusion of penetrating chemicals. Through the reaction processes within a cell, total amount of chemicals in the cell increases, and the cell divides when this amount reaches a certain threshold. As the cell number increases, the state of the cell society consisting of an ensemble of homogeneous cells is destabilized, and due to this dynamic instability, cells become differentiated. The irreversible loss of multipotency through development from stem-type cells also results in the course of development (Furusawa and Kaneko, 2001).

In the theory, the first problem in the standard Turing's theory is resolved by explicitly introducing rich internal dynamics in a cell, with sufficient degrees of freedom. These internal dynamics can have several distinct attracting states, sometimes stabilized by their own, and interpreted as different cell types.

The second problem is resolved by the division process of cells leading to development. On the one hand, this division process leads to successive choice of initial conditions of cellular state of the next generation. With this, differentiation and determination of cell types is discussed. On the other hand, history dependence of developmental process is studied with this introduction of the change of cell numbers, and the transfer of cellular states by cell divisions.

In this theory to this time, we have disregarded spatial dimensions for simplicity. By including a spatially local process in our model, it is possible to study how the dynamic differentiation process and spatial pattern formation develop through mutual dependence, and lead to the formation of stable patterns and stable cell types.[1] In the present paper, we answer the basic questions raised above, by extending the isologous diversification theory to include the effect of spatial extension.

Considering spatially local interactions, we have found that in our model, differentiations are regulated

[1] Another approach to bridge between gene expressions and pattern dynamics is recently proposed by Hogeweg (2000). In this study, Kauffman's (1969) Boolean network model for gene expression patterns is adopted as intra-cellular dynamics, while cell–cell interactions causes transitions into choice of different attractors in the intra-cellular dynamics. Following the increase in the cell number, spatial pattern is formed. One major difference between this study and ours is that her model requires externally given heterogeneity among cells in the first stage of development, while our model exhibits spontaneous diversification caused by instability in dynamics.

by spatial variations of chemical concentrations in the medium, which are sustained by the interactions of cells placed with a given spatial order. In this way, a reciprocal relationship between intra-cellular dynamics and chemical gradients emerges: The formation of gradients of chemical concentrations depends on the intra-cellular states of the cells, and each intra-cellular state is determined under the influence of the concentration gradients that control the growth and differentiation. Only within the process governed by this reciprocal relationship, are the concentration gradients read and interpreted by the cell as positional information. In this process, the resulting spatial pattern of cells can be regarded as a stable state of the system possessing this reciprocal relationship, and we will show that the developmental process leading to this state is generally robust with respect to perturbations. The dependence of the developmental process on history and the community effect in cell differentiation are also discussed.

2. Model

First, we summarize the standpoint from which we designed our model of morphogenesis.

Due to the complexity of cellular dynamics in real organisms, it is almost impossible to construct a model that gives results in precise agreement with the quantitative behavior of real biological processes. Of course, one could go about constructing a complicated model of a cellular system based on experimental data obtained in cell biology studies and in this way imitate the behavior of living cells. However, such mimicry would get us no closer to understanding the essence of cellular systems, because we could obtain behavior that is as similar to that of biological processes as we desire by simply adding increasingly complicated mechanisms to the model. By contrast, in this study we adopt a simple model containing only the features essential to capture the essence of the developmental process, including the generation of a spatial order of cells, and with this we attempt to understand the universal behavior of pattern formation in the class of multicellular systems.

To investigate pattern formation in multicellular systems, we have considered simple models consisting of only the following basic features of multicellular systems:

- Internal dynamics governed by a biochemical reaction network within each cell.
- Interactions between cells (inter-cellular dynamics).
- Cell division and cell death.
- Cell adhesion.

2.1. Internal chemical reaction dynamics

Within each cell, there is a network of biochemical reactions. This network includes not only a complicated metabolic network but also reactions associated with genetic expressions, signaling pathways, and so forth. In the present model, a cellular state is represented by the concentrations of k chemicals. The dynamics of the internal state of each cell is expressed by a set of variables $\{c_i^{(1)}(t), \ldots, c_i^{(k)}(t)\}$ representing the concentrations of the k chemical species in the i-th cell at time t.

As the mechanism governing the internal chemical reaction dynamics, we consider a catalytic network among the k chemicals. Each reaction producing some chemical j from some chemical i is assumed to be catalysed by a third chemical ℓ, which is determined randomly. 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 catalysed by ℓ, and 0 otherwise. Each chemical acts as a substrate to create several enzymes for other reactions, and from each chemical there are several paths to other chemicals. Thus these reactions form a complicated network. The matrix $Con(i,j,\ell)$ is generated randomly before a simulation and is fixed throughout that simulation.

We denote the rate of increase of $c_i^{(m)}(t)$ (and hence decrease of $c_i^{(j)}(t)$) through a reaction from chemical j to chemical m catalysed by ℓ as $ec_i^{(j)}(t)(c_i^{(\ell)}(t))^{\alpha}$, where e is the coefficient characterizing this chemical reaction and α is the degree of catalysation. For simplicity, we use identical values of e and α for all paths. In this paper, we set $\alpha = 2$, which implies a quadratic effect of enzymes. We note, however, that this specific choice of α is not essential in our model of cell differentiation.

Our model also takes into account the change in 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, i.e. $\sum_m c_i^{(m)} = const$. It follows that the volume of a cell is proportional to the sum of the quantities of all chemicals in the cell. The volume change is calculated from the transport of chemicals, as discussed below.

2.2. Cell–cell interaction

Each cell communicates with its environment through the transport of chemicals into and out of the surrounding medium. Here we consider only indirect cell–cell interactions intermediated by diffusive chemical substances in a two-dimensional medium. The state of the medium is expressed by a set of variables, $\{C^{(1)}(x,y,t), \ldots, C^{(k)}(x,y,t)\}$, whose elements represent the concentrations of the k chemical species at the position denoted (x,y) at time t.

We assume that the rates of transportation of chemicals into a cell are proportional to the differences between the chemical concentrations on the inside and the outside of the cell. Thus, the term in the equation for each $\mathrm{d}c_i^{(m)}(t)/\mathrm{d}t$ describing the transport of the m-th chemical from the medium into the i-th cell is given by $D_m(C^{(m)}(t) - c_i^{(m)}(t))$, where D_m is a transport coefficient of the cell membrane.

In general, the transport (diffusion) coefficients should be different for different chemicals. Here, we consider the simple situation in which there are two types of chemicals, those which can penetrate the membrane and those which cannot. This distinction is made in the equation of motion by the parameter σ_m, which is 1 if the m-th chemical is penetrating and 0 if it is not.

Incorporating all of the above mentioned processes, the dynamics of the chemical concentrations in each cell are represented as

$$\begin{aligned}
&\mathrm{d}c_i^{(\ell)}(t)/\mathrm{d}t \\
&\quad = \sum_{m,j} Con(m,\ell,j) e c_i^{(m)}(t)(c_i^{(j)}(t))^{\alpha} \\
&\qquad - \sum_{m',j'} Con(\ell,m',j') e c_i^{(\ell)}(t)(c_i^{(j')}(t))^{\alpha} \\
&\qquad + \sigma_\ell D_m(C^{(\ell)}(p_i^x, p_i^y, t) - c_i^{(\ell)}(t)) \\
&\qquad - c_i^{(\ell)}(t) \sum_{m=1}^{k} \sigma_m D_m(C^{(m)}(p_i^x, p_i^y, t) - c_i^{(m)}(t)), \qquad (1)
\end{aligned}$$

where the first and second terms with $\sum Con(\cdots)$ 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 p_i^x and p_i^y denote the location of the i-th cell. The last term gives the constraint $\sum_\ell c_i^{(\ell)}(t) = 1$, which is satisfied due to the change of the cell volume as chemicals flow in and out of the cell.

The diffusion of penetrating chemicals in the medium is governed by a partial differential equation for the concentration of each chemical, $C^{(\ell)}(x,y,t)$ as

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

We assume the boundary conditions

$$\begin{aligned}
C(0,y,t) &= C(x_{max},y,t) \\
&= C(x,0,t) = C(x,y_{max},t) = \text{const.} \\
&\quad \times (0<x<x_{max}, 0<y<y_{max}), \qquad (3)
\end{aligned}$$

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 the Dirac delta function. These boundary conditions can be interpreted as representing a chemical reservoir outside the medium that supplies those penetrating chemicals that are consumed by the cells.

2.3. Cell division and cell death

Each cell receives penetrating chemicals from the medium as nutrients, while the reaction in the cell transforms them into non-penetrating chemicals which compose the body of the cell. As a result of these reactions, the volume of the cell increases. In this model, the cell divides into two almost identical cells when the volume of the cell becomes double its original size. During this division process, all chemicals are almost equally divided between the daughter cells, with the differences taking the form of tiny random fluctuations (e.g. $\sim 10^{-6}\ c_i^{(\ell)}$). Although the imbalance represented by these differences in chemical concentration is essential to the differentiation process in our model and in nature, the actual size of this imbalance does not affect the results we present below. In our model, these tiny differences between cells can be amplified, due to the intrinsic instability of the internal dynamics.

Penetrating chemicals can penetrate the cell membrane in both directions, and therefore 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.

2.4. Cell adhesion

As a minimal model of cell–cell adhesion, we assume that two cells positioned within a given distance have a 'connection', so that they adhere to each other. This adhesion force is represented by a 'spring' between two cells whose potential has a minimum as some distance, so that two adjacent cells are separated by this amount. We assume that the magnitude of the adhesion force is a function of only the distance between the interacting cells, and, in particular, is independent of intra-cellular state.

In addition to the adhesion force, a random force, modeling Brownian motion, is applied to all cells. By this random force, the cells making up an ensemble seek a configuration that is stable with respect to perturbations, including these fluctuations. Then, when a cell divides, the two daughter cells are placed at randomly chosen positions close to the mother cell. Each daughter cell creates new connections with the neighboring cells, and the system adjusts into a new stable configuration.

3. Result

In this section, we present numerical results demonstrating the developmental process and the resulting spatial pattern described by our model. As mentioned,

in our model, we adopt a simple type of intra-cellular reaction dynamics governed by a reaction matrix that is determined randomly. The behavior of the cellular system depends on the choice of the random reaction matrix. To extract universal features of the system, which are independent of the detailed structure of the network and parameter values, we have performed simulations using thousands of different reaction networks and parameters. From these simulations, we have found that differentiation resulting from cell–cell interactions and a robust developmental process toward an ordered spatial pattern of differentiated cells is observed for some fraction of randomly generated reaction networks (e.g. 6% of random networks). It is important to note that in these results the diversification process from multipotent stem-type cells to determined cell types is basically the same as that found previously (Furusawa and Kaneko, 1998, 2001) in the simulation of models in which the spatial variation of chemical concentrations in the environment is not accounted for. For this reason, in this paper, we focus on the emerging spatial pattern of differentiated cells and the process by which it develops, while the diversification process itself is described only briefly. A detailed account of the diversification process is given in the above cited papers.

In the present model, cellular diversification processes are observed in the case with the intra-cellular chemical reaction dynamics exhibiting nonlinear oscillatory behavior, as shown in Figs. 1(a) and 3(a).[2] For other cases without oscillatory dynamics, in which the concentrations of chemicals are fixed over time, a homogeneous cell society of nearly identical cells appears. Here, to investigate the emergence of the developmental process in multicellular organisms, we choose the case with oscillatory intra-cellular dynamics in the beginning.[3] One reason for studying reaction networks giving rise to such oscillatory dynamics is discussed in Furusawa and Kaneko (2000) (see also discussion in Section 8). Indeed, cell society starting from a cell with oscillatory intra-cellular dynamics has a higher growth speed as an ensemble, and it is natural to assume that such cell society is selected through evolution.

[2] Although instability in dynamics, which amplifies a microscopic difference among intra-cellular states, is necessary for our diversification process, chaotic intra-cellular dynamics itself is not always necessary for it. In some cases, the cellular dynamics exhibits periodic oscillation without chaos at an early stage of development, but by increasing the cell number, the entire dynamical system including all cells and environment becomes unstable, which results in the differentiations in the same manner as shown later. Furthermore, even without oscillatory dynamics at all, differentiation at the present mechanism is found to work, if there appears some instability in the transient dynamics of cellular state (Takagi and Kaneko, 2003).

[3] Note that even in this case, differentiated cell types that appear later through cell–cell interaction often have fixed chemical concentrations without oscillations.

Note that in real biological systems, such oscillatory dynamics are often observed in some chemical systems that include chemicals such as Ca, NADH, cyclic AMP and cyclins (Tyson et al., 1996; Hess and Boiteux, 1971; Alberts et al., 2002). Such oscillations generally appear in a system that includes positive feedback reactions, which are observed ubiquitously in real biological systems. Indeed, the replication process requires amplification of molecule numbers, for which positive feedback process is required. Thus, it is natural to employ reaction networks that exhibit oscillatory dynamics in our model.[4]

In the remainder of this section, we describe the developmental process by considering two specific reaction networks which exhibit different types of spatial patterns, a concentric ring pattern and a striped pattern of differentiated cells.

3.1. Differentiation process toward a ring pattern

In this section, we present numerical results demonstrating the development toward a concentric ring pattern of differentiated cells. For the simulations that we carried out, employing a variety of reaction networks, this was the most frequently observed non-trivial spatial pattern. Here we consider numerical experiments employing a particular reaction network with the number of chemicals set to $k = 32$ and 9 connections for each chemical. The parameters are set as $e = 1.0$, $D_m = 0.001$, and $D_e = 0.001$. The first, second, and third chemicals are penetrating (i.e. $\sigma_0 = \sigma_1 = \sigma_2 = 1$), and the others are not.

As the initial state, we consider a single cell placed at the center of the medium, whose chemical concentrations $c_i^{(\ell)}$ are determined randomly with the constraint $\sum_\ell c_i^{(\ell)} = 1$. In Fig. 1(a), we show a time series of the concentrations of the chemicals for a single, isolated cell. The attractor of the internal chemical dynamics in this case is chaotic. We call this initial type of cell "type-0" in this section. According to our numerical results, it seems that this state represents the only attractor that can in practice be realized in simulations employing randomly chosen initial conditions.

Now, with diffusion, external chemicals flow into the cell. This happens because there will eventually develop a lower concentration of penetrating chemicals within the cell, since penetrating chemicals are transformed into non-penetrating ones through the intra-cellular 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. As the number of cells increases repeatedly by a factor of two (i.e. $1 \to 2 \to 4 \to 8 \cdots$) with

[4] The importance of oscillatory dynamics in cellular systems has been pointed out by Goodwin (1963).

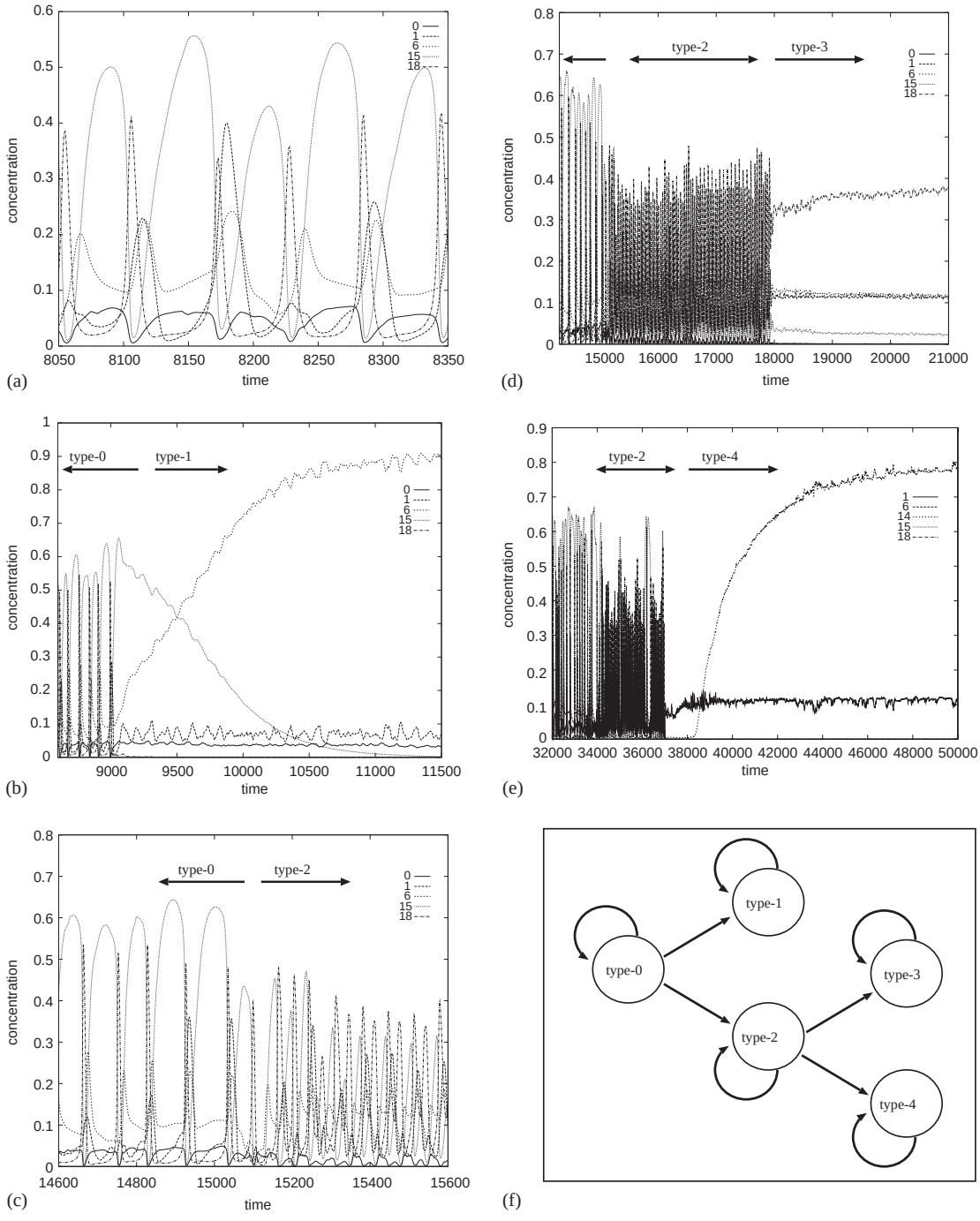

Fig. 1. (a) Overlaid time series of the cellular chemical concentrations $c^{(m)}(t)$ for the type-0 cell in the case of a 'ring pattern'. The vertical axis represents the concentration, and the horizontal axis represents time. In this figure, we have plotted the time series of only 5 of the 32 internal chemicals, for clarity. The lines designated by the numbers $m = 0$, 1, 6, 15 and 18 represent the time series of the concentrations of the corresponding chemicals $c^{(m)}(t)$. (b)–(e) Time series of $c^{(m)}(t)$ in a single cell, representing the process of differentiation to type-1,2,3 and type-4 cells, respectively. (f) Automaton-like representation of the rules of differentiation. The path back to the original node represents reproduction of the same cell type, while the paths to other nodes represent transitions to the corresponding cell types.

further divisions, a cluster of type-0 cells eventually is formed (Fig. 2(a)). Among these cells, although the internal dynamics of each cell correspond to the same attractor, the coherence of the oscillations among individual cells is easily lost due to the chaotic nature of the dynamics in these type-0 cells. The microscopic differences introduced at each cell division are gradually amplified to a macroscopic level, and this destroys the phase coherence of the oscillation among the cells.

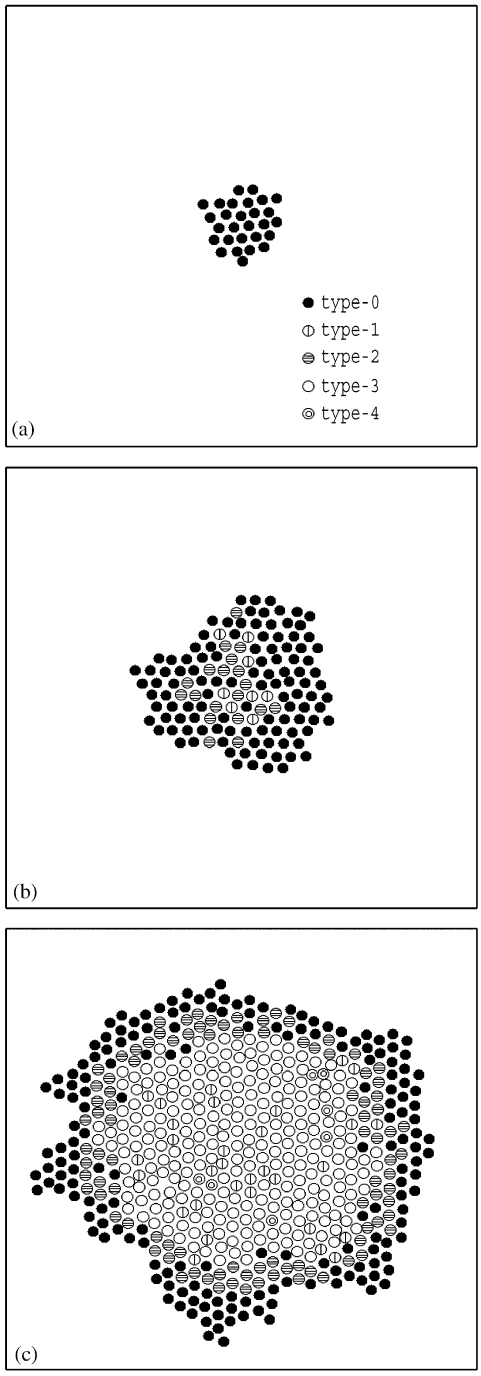

Fig. 2. Development of a cell cluster toward a 'ring pattern' in a two-dimensional medium. Each mark corresponds to a particular cell type, with different cell types distinguished by significantly different internal dynamics.

When the number of cells exceeds some threshold value (here approximately 40), some cells located internal positions within the cluster begin to display different types of dynamics (Fig. 2(b)). In Figs. 1(b) and (c), the time series of the chemical concentrations in these new types are plotted. We call these "type-1" and "type-2" cells, respectively. Figs. 1(b) and (c) show that each of the these attracting states occupies a distinct region in the phase space, and that each can be clearly distinguished as a distinct state. Transitions from the type-0 state to other state are interpreted as the process of differentiation.

Note that this differentiation is not induced directly by the tiny differences introduced at the cell divisions. Also, note the transition from one cell type to another does not occur at the time of cell division, but later, through the interactions among the cells. The phenomenon of differentiation observed here is caused by an instability in the entire dynamical system consisting of all the cells and the medium. It is due to this instability that tiny differences between two daughter cells can be amplified to a macroscopic level through the intracellular dynamics and interactions between cells. Only when the strength of this instability exceeds some threshold does differentiation occur. Then, the emergence of new cell types stabilizes the dynamics of the other cell types. We thus see that the cell differentiation process in our model results from the amplification of tiny phase differences through orbital instability (transient chaos), while the coexistence of different cell types stabilizes the system as a whole.

It should be noted that these differentiated cell types do not necessarily correspond to attractors of the internal dynamics of a single cell. In fact, for a single cell system, there is no attractor corresponding to a differentiated cell type, for the reaction matrix and parameter values adopted in the simulation described above. This implies that when a single differentiated cell is transplanted into a medium containing no other cells, this cell is de-differentiated back into a type-0 cell. Here, a multiple-cell system, the stability of such internal dynamics is sustained only through interactions among cells.

As the cell number increases further, some type-2 cells located internally within a cluster further differentiate into other cell types, which are called type-3 and type-4 here (Figs. 1(d) and (e)). They form the 'inner core' of the cluster as shown in Fig. 2(c). At this stage, a ring pattern consisting of three layers is formed, in which a ring of type-2 cells lies between type-0 cells, located at the periphery, and type-1, 3 and 4 cells, located inside.

The transitions between different cell types through differentiation follow specific rules. These rules originate from a constraint on the transient dynamics exhibited when a cell makes the transition between the attracting states corresponding to two cell types. Fig. 1(f) represents all possible transitions using an automaton-like representation.

3.2. *Differentiation process toward striped pattern*

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

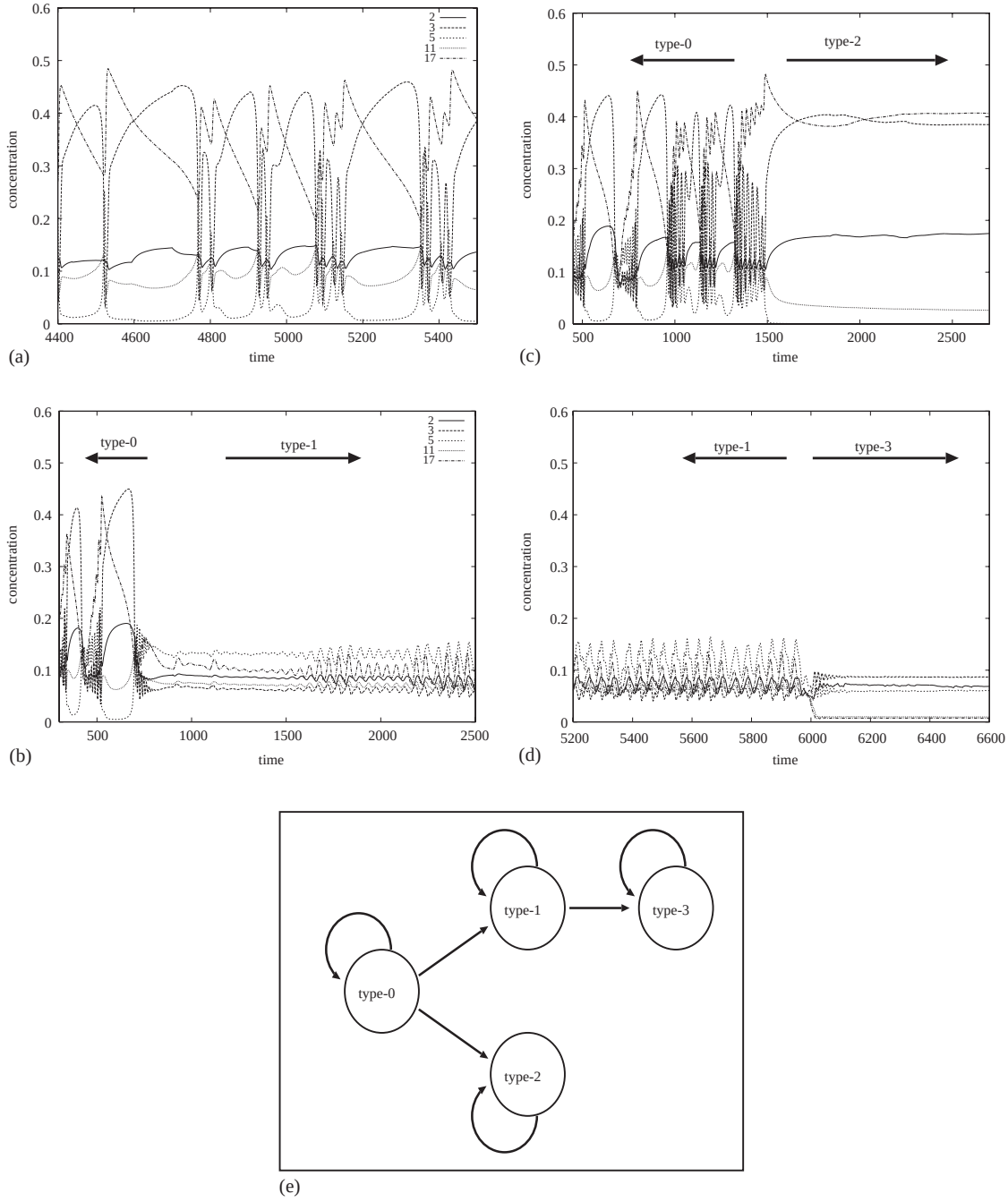

Fig. 3. (a) Overlaid time series of concentrations $c^{(m)}(t)$ for the type-0 cell in the case of a 'striped pattern'. The vertical axis represents the concentration of chemicals and the horizontal axis represents time. In this figure, we have plotted the time series of only five of the 32 internal chemicals, as in Fig. 1. The lines designated by the numbers $m = 2$, 3, 5, 11 and 17 represent the time series of the concentrations of the corresponding chemicals $c^{(m)}(t)$. (b)–(d) Time series of $c^{(m)}(t)$ in a single cell, representing the process of differentiation to type-1,2, and type-3 cells, respectively. (e) Automaton-like representation of the rules of differentiation.

discussed in the previous section. In this section, we describe an example of the development toward a striped pattern, which was obtained in simulations of the model using a reaction matrix, also generated randomly, that is different from that resulting in a ring pattern. Here, we consider numerical experiments employing a particular reaction network for which the number of chemicals is $k = 32$ and there nine connections for each chemical. The parameters are set as $e = 1.0$, $D_m = 0.002$, $D_e = 0.004$. Chemicals 1–3 are penetrating (i.e., $\sigma_\ell = 1$), and the others are not.

In this case, a single cell placed at the center of the medium exhibits the oscillatory reaction dynamics plotted in Fig. 3(a), for all initial sets of chemical

concentrations chosen in our simulations. We call cells in such a state "type-0" here. In this example, when the number of type-0 cells becomes 4 through cell division, the entire system becomes unstable due to cell–cell interactions, and two type-0 cells differentiate into another distinct cell type, called type-1 (Fig. 3(b)). With further divisions of type-0 and type-1 cells, a cluster consisting of cells of these two types is formed (Fig. 4(b)). As the cell number increases further, some type-0 cells located on the side of the type-0 region farthest from the type-1 region differentiate into another distinct cell type, called type-2. At the same stage, some type-1 cells that have migrated into the type-0 region differentiate into another cell type, called type-3.[5] As a result of these differentiations, a spatial pattern of cells consisting of four stripes, each containing cells of just one kind, is formed,[6] as shown in Fig. 4(d).

In a system exhibiting the striped pattern, the disappearance of point symmetry, like that possessed by the ring pattern (Fig. 2(c)), is due to the development taking place at the beginning of the differentiation process from type-0 to type-1 cells when there is still a small number of cells (Fig. 4(b)). It is at this stage that the point symmetry is lost, and this asymmetric small cluster of cells expands through further cell divisions. Because of the difference regarding both rates and types of nutrients absorbed and released for type-0 and type-1 cells, the asymmetric distribution of cells brings about asymmetric concentration gradients of nutrients in the medium, as shown later. These affect subsequent differentiations and thereby cause the system to evolve an asymmetric spatial pattern of cells.

4. Positional information

In the situation considering in the previous sections, the concentration gradients of chemicals in the medium appear to act as "positional information". In the case of the ring pattern (Fig. 2), chemical concentrations vary along the radial direction in a cell cluster, while in the case of the striped pattern (Fig. 4), the variation of chemical concentrations is limited almost entirely to the direction perpendicular to the stripes. Because of the existence of such a spatial dependence of the chemical concentrations, a cell can know its position and the proper manner to evolve (i.e. to make a transition to another cell type or not) in order to maintain or strengthen the existing ordered pattern. An important point here is that such gradients are not imposed on the system from the outside, but, instead, they emerge and are maintained by the cell–cell interactions with appropriate intra-cellular dynamics. It can be clearly shown that, for example, an ordered spatial pattern like that in Fig. 4 and the concentration gradient sustaining this pattern disappear and never return to the original state when the internal states of all cells (i.e. the concentrations of the chemicals within the cells) in the cluster are suddenly changed by assigning them randomly chosen values, even if the concentrations of nutrients in the medium and the locations of the cells are not changed (Fig. 5(b)). These results indicate that there is a reciprocal relationship between the cellular dynamics and the concentration gradients in the medium, in which the dynamics of the cells maintain the gradients and the gradients control the dynamics of the cells.

For chemical concentrations to act as positional information, it must be the case that a cell can determine its position by 'perceiving' this concentration, in the sense that a cell placed at a particular position differentiates in to the cell type that maintains the pattern. In addition to this 'controlling' property of such information, to maintain a robust developmental process, it must be the case that this information itself is preserved even when the system is subject to perturbations. These features of the positional information can be demonstrated by removing some cells and examining the regeneration process. In the next section, we study the reciprocal relationship between positional information and cell differentiation, and clarify how the concentration gradient can act as positional information.

5. Stability of spatial patterns with respect to perturbations

As mentioned above, in our model, an ordered spatial pattern emerges as a result of the interplay between intra- and inter-cellular dynamics. Here we give results indicating that this emergence process is stable with respect to macroscopic perturbations, for example, those consisting of the removal of some cells. This stability is an important implication of this interplay. On

[5] 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. In some cases, by chance, there is no such mixing, and therefore in these cases, no type-3 cells appear.

[6] Starting from different initial conditions (i.e. different initial concentrations of chemicals in original first cell), in some cases, although the intra-cellular dynamics of each cell type and the order of appearance of each cell type are the same as in the case depicted in Fig. 4, a cell cluster evolves into a more disordered spatial pattern. Such a disordered pattern appears when type-0 cells and type-1 cells are intermingled in the first stage of development. This intermingling occurs by chance, due to random determination of the positions of the two daughter cells after each cell division and the random fluctuation force that causes the Brownian motion of cells. This kind of "teratogenesis" can be avoided by introducing the dependence of adhesion force on the intra-cellular dynamics, for example, if the adhesion force between cells of the same type is sufficiently stronger than that between cells of different types, as is often the case in actual organisms.

Fig. 4. Development of a cell cluster toward 'striped pattern' in a two-dimensional medium. Each mark corresponds to a particular cell type, distinguished by its particular type of internal dynamics.

the basis of this observation, we discuss the nature of positional information in the system.

In our model simulations, differentiation occurs when the instability of the system exceeds some threshold through the increase of the cell number, and the emergence of differentiated cells stabilizes the system. As a natural consequence, a large perturbation, such as the removal of cells, causes the system to become unstable. However, we found that after such an alteration of the system, a series of cell differentiations generally occur that lead the system back to the original distribution, so that the system return to its stable state. In our previous model, in which each cell is coupled to each other in an identical manner through a homogeneous environment, stability with respect to the removal of cells results from the regulation of the differentiation probability from stem-type cells into various differentiated types of cells (Furusawa and Kaneko, 1998, 2001). For example, we studied a cell society consisting of multipotent stem-type cells "S" and differentiated cell types "A" and "B", with the possible transitions $S \to S, A, B$. In this system, if the number of type A cells is reduced (for example, by perturbation), the rate of occurrence of the transition $S \to A$ is enhanced, and the original number distribution of cell types is thereby approximately recovered.

Now let us return to the present model with non-trivial spatial dependence. Here, again, the developmental

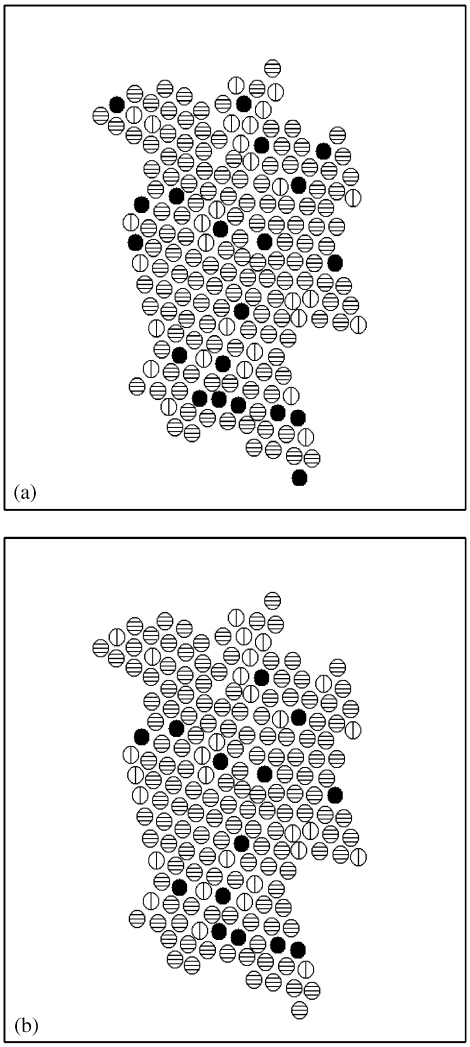

Fig. 5. Examples of spatial patterns of cells that appear in simulations starting from multiple cells, instead of a single cell. In this simulation, the reaction network and parameter values of Fig. 4 were used. Here, 185 cells were placed in the same locations as shown in Fig. 4(d), and the initial concentrations of chemicals were determined randomly and independently for each cell. In (a), the initial chemical concentrations in the medium were set to constant values, independent of the spatial variables. In (b), the initial chemical concentrations in the medium were set to be the same as in Fig. 3(d). For these cases, the definitions of the cell types are the same as those shown in Fig. 3. Note that these spatial patterns of cells never appear as a result of the developmental process from a single cell.

process generally possesses stability with respect to such perturbations. In the present model, however, the regulation of the differentiation process in response to perturbations depends on the existing spatial pattern of cells and the chemicals present in the medium. It is through this regulation that the damaged spatial pattern is regenerated. In this section, we describe regeneration processes involving the striped pattern consisting of three layers of cell types, as shown in Fig. 6(a). This pattern was obtained using the same reaction matrix and parameter values as in the example depicted in Fig. 4, but starting from different initial conditions (i.e. different initial concentrations of chemicals in the first cell). We will consider three typical examples of external perturbations here.

(I) *Recovery from the removal of the entire type-2 region*: In one case, we removed all the type-2 cells (Fig. 6(b)), after the striped pattern in Fig. 6(a) had developed. After this operation, the rate of transitions from type-0 to type-2 cells was enhanced at the side of type-0 region farthest from the type-1 region, and as a result, the striped pattern with three layers gradually reappeared (Fig. 6(c)). Figs. 6(d)–(f) display the concentrations of nutrients in the medium, corresponding to the dotted line in Figs. 6(a)–(c), respectively.

(II) *Recovery the removal of the entire from type-0 region–de-differentiation of type-2 cells into type-0, induced by the interaction with type-1 cells*: In this case, we removed all the type-0 cells that were located in the middle of the striped pattern, and combined the remaining cell clusters consisting of only type-1 and type-2 cells, as shown in Fig. 7(b). After this alteration, type-2 cells located at the boundary between the type-1 and type-2 regions de-differentiated back into type-0 cells, and the striped pattern with three layers was thereby recovered (Fig. 7(c)). It is important to note that de-differentiation from a type-2 cell to a type-0 cell never occurs during the "normal" course of development, i.e. without perturbations. In Figs. 7(d)–(f), the concentrations of nutrients in this case are plotted in the same manner as in Fig. 6.

(III) *Formation of a new pattern resulting from the removal of the type-1 region*: Here, all the type-1 cells were removed from a cluster with a striped pattern (Fig. 8(b)). In this case, regeneration of the type-1 region was not observed. Instead, type-0 cells at the periphery of the cluster differentiated into type-2 cells. As a result, a sandwich-like 2-0-2 structure was formed, and with further development, a ring structure with inner type-0 cells and outer type-2 cells was formed. In this case, the final cell society consisted only of type-0 and type-2 cells (Fig. 8(c)). The concentrations of nutrients for this process are plotted in Figs. 8(d)–(f).

From these results, we identify the following characteristics of the development and regeneration process for the present model. First, the differentiation of a type-0 cell into a type-2 cell occurs when the concentrations of chemicals around the type-0 cell approximately satisfy the conditions $C^{(0)}(x,y,t)<0.02$, $C^{(1)}(x,y,t)<0.02$, and $C^{(2)}(x,y,t)>0.13$, where $C^{(i)}(x,y,t)$ denotes the concentration of the i-th chemical in the medium. Next, because type-0 cells absorb chemicals 0 and 1 strongly and chemical 2 only weakly, when a cluster consisting entirely of type-0 cells exceeds a certain

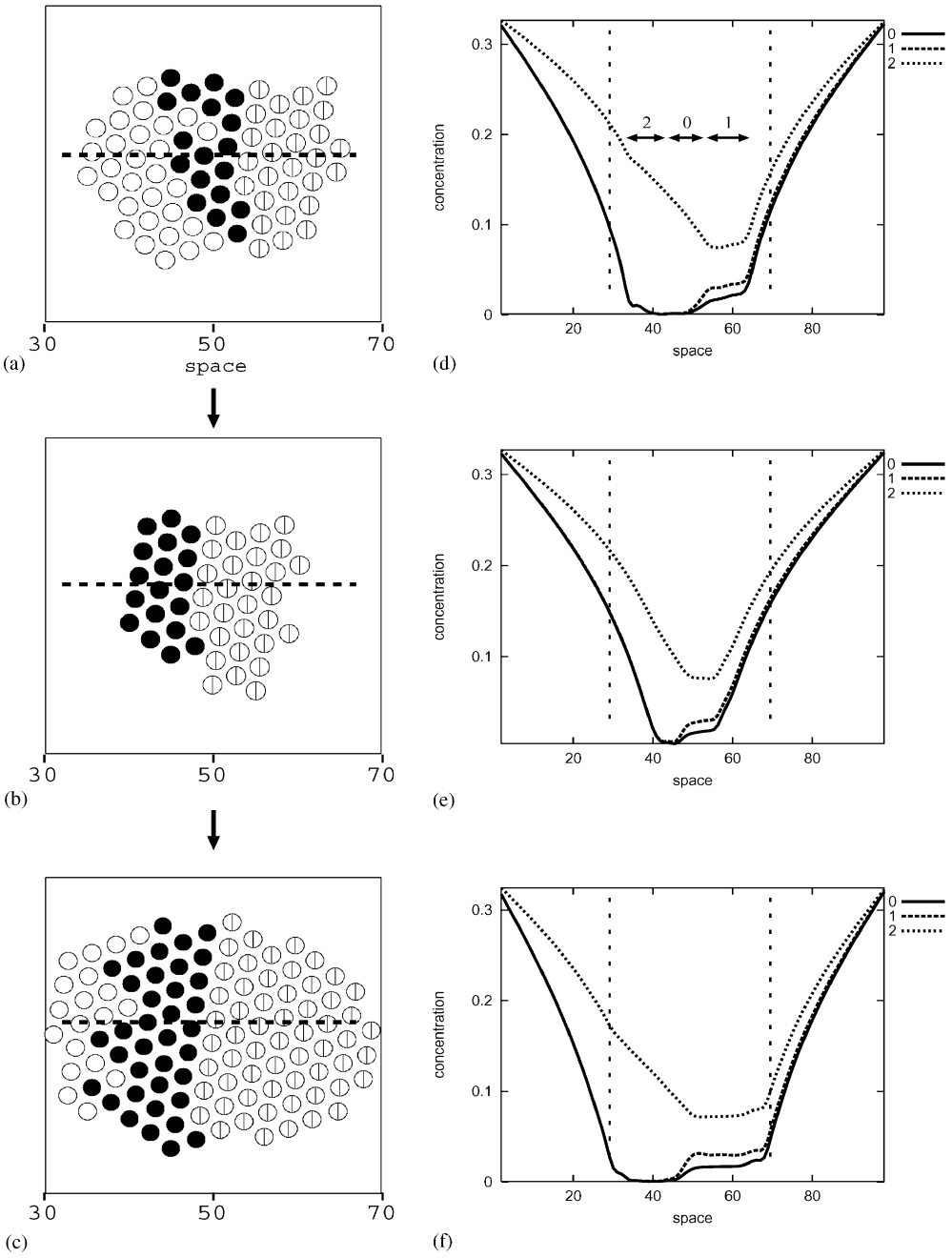

Fig. 6. Regeneration of striped pattern following the removal of the entire type-2 region. In the case depicted in (b), all type-2 cells were removed from the striped pattern of cells depicted in (a). In (c), the effect on the development caused by this operation is shown. It is seen that rate of transitions from type-0 to type-2 cell is enhanced on the side of the type-0 region that is opposite to the type-2 region, and the striped pattern with three layers thereby reappears. In (d)–(f), the concentrations of the nutrients in the medium along the dotted line in (a)–(c), respectively, are plotted.

number of cells, the peripheral region of this cluster come to satisfy the condition for the emergence of type-2 cells, and a ring pattern consisting of type-0 cells on the inside and type-2 cells on the outside appears. The cluster in Fig. 8(c) corresponds to this situation. Then, because type-1 cells absorb chemical 2 more strongly than do type-0 cells, $C^{(2)}$ decreases in a region of type-1 cells (see Fig. 6(a)). As a result, differentiation from type-0 into type-2 cells is suppressed near such a region of type-1 cells. This is the reason why only type-0 cells that are on the side of the type-0 region opposite to the type-1 region differentiate into type-2 cells. Additionally, the decrease of $C^{(2)}$ in the vicinity of type-1 cells brings about the de-differentiation of type-2 cells into

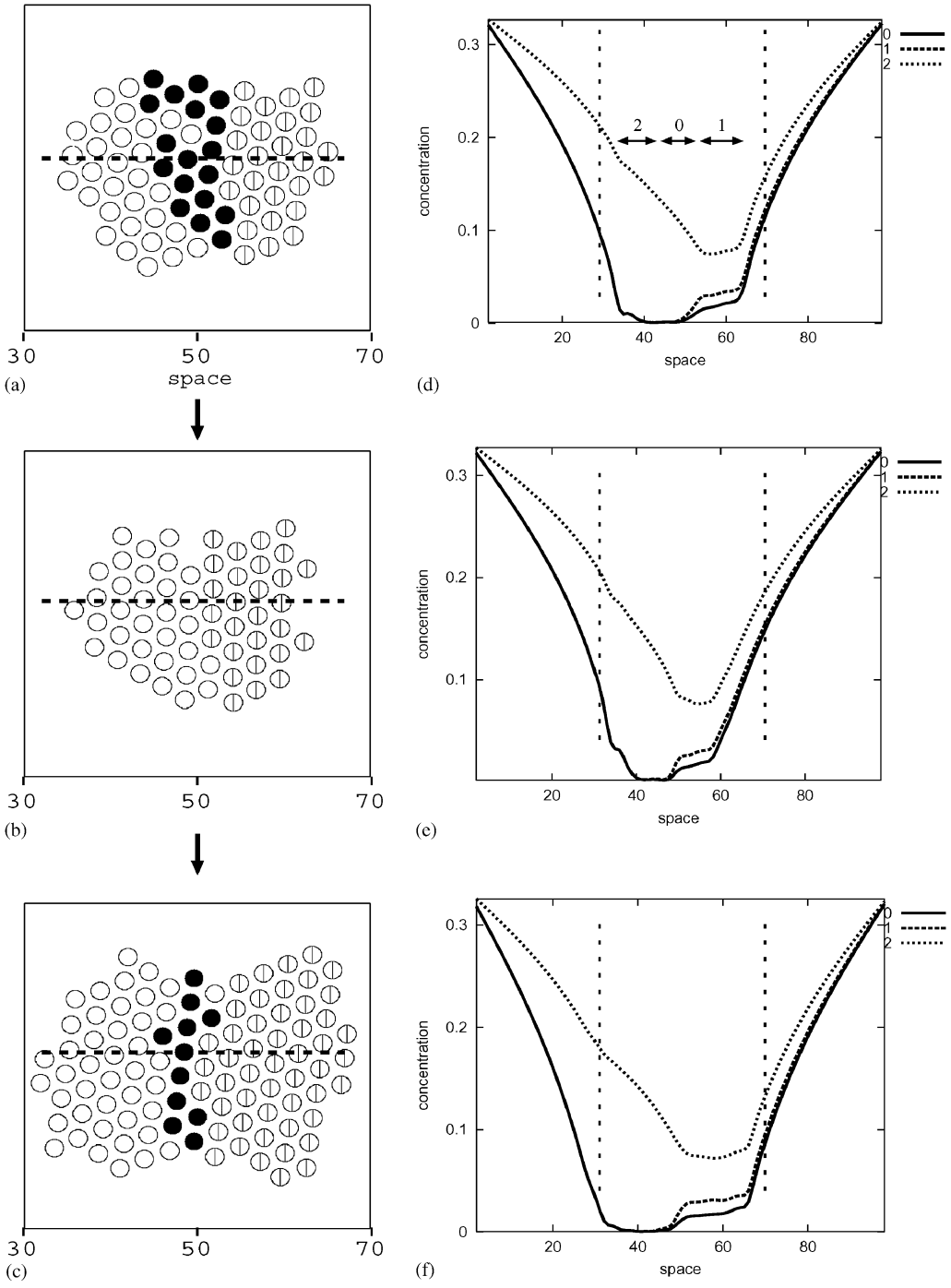

Fig. 7. Regeneration of a striped pattern following removal of the entire type-0 region. In the case depicted in (b), all type-0 cells were removed from the striped pattern of cells depicted in (a). In (c), the effect on the development caused by this operation is shown. We see that the transitions from type-2 to type-0 cells, which never occur in normal development, occur at the boundary of the type-1 and type-2 regions, and the striped pattern with three layers reappears as a result. In (d)–(f), the concentrations of the nutrients in the medium along the dotted line in (a)–(c), respectively, are plotted.

type-0 cells when a type-2 region is placed beside a type-1 region (Fig. 7(c)).

These results indicate that there is a reciprocal relationship sustaining the development and regeneration processes that can be described as follows:

(i) In a manner that depends on the types of cells present, the concentration gradients in the medium are formed.

(ii) The growth and differentiation of each cell is determined by the concentrations of chemicals at the corresponding position.

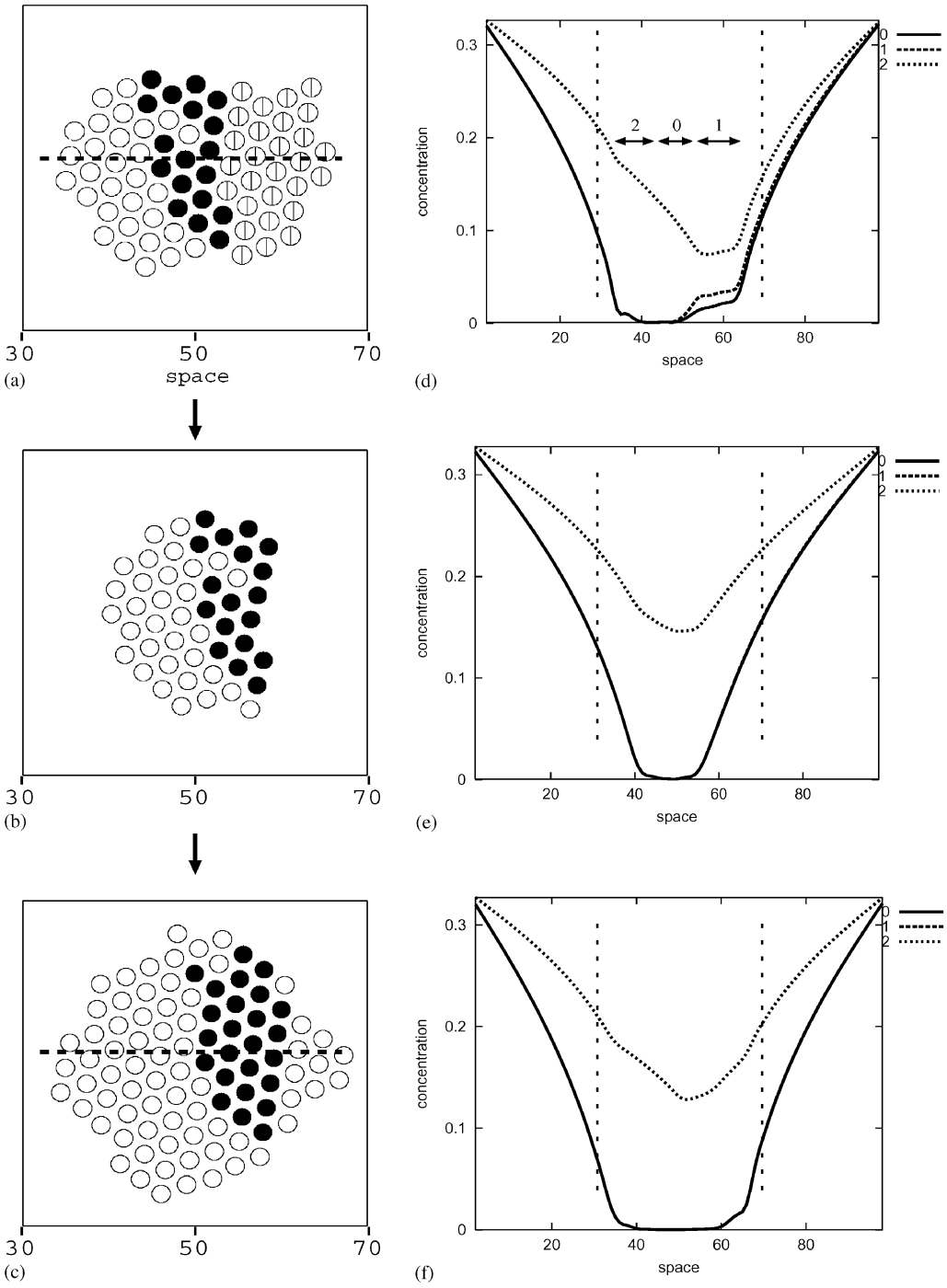

Fig. 8. Regeneration of a striped pattern following the removal of the entire type-1 region. In the case of (b), all type-1 cells were removed from the striped pattern of cells displayed in (a). In (c), the effect on the development caused by this operation is shown. In (d)–(f), the concentrations of the nutrients in the medium along the dotted line in (a)–(c), respectively, are plotted.

To complete this reciprocal relationship, the cells "read" the chemical concentrations in the medium in their neighborhood and change their internal state accordingly. In this system, the cells read the concentration of chemicals as modification of their intra-cellular dynamics. That is, each attracting state of the internal dynamics, corresponding to a distinct cell type, is modified by the existence and the states of surrounding cells. This modification consists of the change of orbits (or average position) in phase space. In Fig. 9 we plotted the dependences of the cellular dynamics on their position, using the cell society with a striped pattern considered in Fig. 4(d). To quantify the change of the intra-cellular dynamics, we determine the average

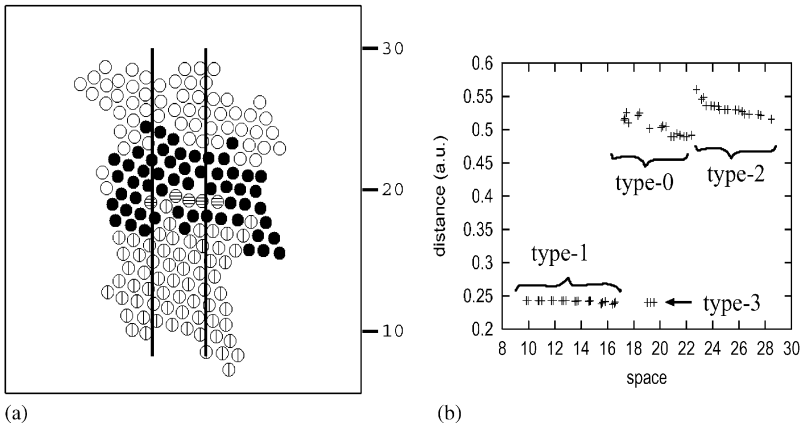

Fig. 9. Variation of intra-cellular dynamics with respect to position. To measure the change undergone by the intra-cellular dynamics when the position of a cell is changed, we determined the average position of the orbit of each cell in the k-dimensional phase space, which was calculated as the averages of the k concentrations $c^{(m)}(t)$ over a certain period for that cell. Each point corresponds to a cell placed between the two solid lines in (a), and the Euclidian distance in the phase space between the average position for a cell and the origin is plotted as a function of the position of the cell in the medium. Note that, the distance from the origin in the phase space does not necessarily indicate the distance between two different cellular states. For example, the variation in the position of a type-0 cell's orbit is much smaller than the distance between average positions of type-0 and type-2 cells.

position of the orbit of each cell in the k-dimensional phase space, which is calculated as the average of the concentrations $c^{(m)}(t)$ in this cell over a certain period. We use this average to smear out temporal fluctuations of chemical concentrations resulting from the oscillatory nature of the intra-cellular dynamics. Additionally, to obtain the long time average without the complication introduced by the change in the number of cells, we disallowed cell division here.

In Fig. 9, the Euclidian distance between the average position of the orbit of the intra-cellular dynamics and the origin in the k-dimensional phase space is plotted as a function of the position in the medium for each cell. As shown in Fig. 9, the intra-cellular dynamics of each cell are gradually modified as its position changes. However, it should be noted that these modifications of a given type of internal dynamics are much smaller than the differences among the dynamics of different cell types. Thus, there are effectively two types of information contained in the internal dynamics of a cell: "analogue" information, reflecting the states of the surrounding cells, and "digital" information corresponding to the cell type. This analogue information controls differentiation through the modification of intra-cellular dynamics. On the other hand, a change in the distribution of cell types, resulting from cell division and differentiation, brings about a change in the concentrations of chemicals in the medium. This change then affects a change in the intra-cellular dynamics, thereby altering the analogue information.

Within this reciprocal relationship, the concentration gradients of chemicals are read and interpreted by the cells, and they react by adjusting their internal states to match the existing spatial pattern. The chemical gradient in the medium is maintained by the configuration of cell types, while, inversely, it controls the appearance of cell types at each position. Thus, in this context, the gradient of chemicals can be regarded as constituting positional information for the cells. An important point in this regard is that this positional information is not imposed from outside the system. Instead, this positional information in the form of concentration gradients of chemicals naturally emerges in the developmental process through instabilities in the dynamics that result from cell–cell interactions.

The spatial pattern that develops through the process described above can be regarded as a stable state of the reciprocal relationship between intra- and inter-cellular dynamics. It should be stressed that this state is generally stable with respect to perturbations up to a certain degree, and thus, the system recovers its original pattern, when some perturbations (such as the removal of cells) are applied to it. The regeneration depicted in Figs. 6 and 7 is manifestation of this stability.

Now we discuss the reason that type-1 cells are not regenerated in case III (in Fig. 8(c)), in contrast to the situation for type-0 and type-2 cells. The fact that there is no regeneration in this case does not imply a loss of stability. Indeed, the potential for regeneration of the type-1 region depends on the stage of development: type-1 cells are regenerated from type-0 cells when the number of type-0 cells is not large. Interestingly, although differentiated cell types generally possess the potential for regeneration when they are removed at the same stage of their appearance (e.g. cell number ~ 4 for type-1 cell), the potential for regeneration is lost for some types at a later stage of development as is the case for type-1 cells.

We have found that thus the potential for regeneration depends on the number of cells present, while the possibility of generating a different types of cell colony (i.e. ring pattern consisting of type-0 and 2 cells) depends on the initial conditions. These two issues—the community effect and the dependence on history—are discussed in the following two sections.

6. Dependence on history of the developmental process

As discussed in the previous section, the spatial order of a cell cluster is realized as a stable state of a cell society governed by the reciprocal relationship between intra- and inter-cellular dynamics. In this developmental process, different stable states of the cell colony are realized in succession, as the number of cells changes. It should be noted, however, that the ordered spatial pattern realized at any given stage, as in Fig. 4, is not always the unique stable state of the system. Instead, there are generally multiple stable states, although one (or only a few) of them can be selected through the developmental process from a single, isolated cell.

The existence of multiple stable states of a cell colony is suggested by the result we found in case III of the last section. To check the dependence of the selected cell colony systematically, we have carried out several simulations, starting with multiple cells, instead of from a single, isolated cell, while using the same reaction matrix and parameters values as used in the case of Fig. 4.

In one simulation, as the initial conditions, we introduced 185 cells and positioned them in the same positions as the cells shown in Fig. 4(d). Then, the chemical concentrations of these cells were determined randomly, and the total cell number was fixed at 185 by disallowing the cell division process. The initial chemical concentrations in the medium were set to constant values, independent of the spatial variables. Fig. 5(a) displays an example of the spatial pattern exhibited after the system settles into a stable state, starting from randomly chosen intra-cellular chemical concentrations that were determined independently for each cell. As shown in the figure, the cell clusters realized in this case from multiple cells fall into homogeneous pattern consisting mainly of type-3 cells, without any spatial structure.

We also performed simulations using different initial conditions for the intra-cellular dynamics with the same number of cells, and observed qualitatively the same results as shown in Fig. 5(a). In these simulations, when the initial cell number was more than approximately 20, the disordered pattern consisted mainly of type-3 cells as Fig. 5(a) represents.

We have also carried out simulations starting from different types of initial conditions in the simulation that cell division is allowed. The results are summarized in Table 1. As seen there, when the initial cell number is between 4 and 20 and the initial states of cells are determined randomly and independently for each cell, a cell colony consisting entirely of stem-type cells (i.e. type-0) emerges. This cell colony eventually develops into a ring pattern of cells, with an outer region of type-2 cells and an inner region of type-0 cells, while for smaller number of initial cells, 'normal development' in which the system consists of a striped pattern colony progresses.

One might expect that by imposing the appropriate positional information, the ordered pattern as depicted in Fig. 4(d) could be regenerated. To check this possibility, we carried out simulations by initially imposing the chemical gradient in the medium that existed in the case of Fig. 4(d) (in which case it was realized through the normal developmental process), while the initial chemical concentrations in the cells were chosen randomly and independently for each cell, as in the case of Fig. 5(a). Again, here the pattern realized asymptotically was disordered as shown in Fig. 5(b). We found that in this situation, the positional information initially imposed is soon disturbed. As mentioned above,

Table 1
Results of simulations starting from different initial conditions

Initial cell state	Initial cell number	Developed cell colony
Random	Small ($\lesssim 4$)	Normal development (striped pattern with type-0, 1, and 2 cells)
	Medium ($\gtrsim 4$, $\lesssim 20$)	Ring pattern with type-0 and 2 cells
	Large ($\gtrsim 20$)	Disordered consisting mainly of type-3 cells
Type-0	Small ($\lesssim 4$)	Normal development (striped pattern with type-0, 1, and 2 cells)
	Medium ($\gtrsim 4$, $\lesssim 20$)	Ring pattern with type-0 and 2 cells
	Large ($\gtrsim 20$)	Disordered consisting mainly of type-3 cells
Type-1	Small ($\lesssim 4$)	Normal development (striped pattern with type-0, 1, and 2 cells)
	Large ($\gtrsim 4$)	Only type-1 cells
Type-2	small ($\lesssim 4$)	Normal development (striped pattern with type-0, 1, and 2 cells)
	Medium ($\gtrsim 4$, $\lesssim 15$)	Ring pattern with type-0 and 2 cells
	Large ($\gtrsim 15$)	Only type-2 cells

positional information is generated through the reciprocal relationship between the cellular dynamics and the pattern, and as these simulation show, positional information alone cannot force the regeneration of the pattern to which it corresponds.

It is important to note here that the homogeneous states depicted above never appear when a cell cluster is developed from a single cell. In that case, only a striped pattern is obtained, while different types of cell colonies are obtained from initial conditions consisting of multiple cells, as shown in Table 1. These results indicate that there are several stable states realized with various initial conditions for multiple cells, and that the stable state corresponding to the spatial pattern depicted in Fig. 4(d) has a relatively small basin of attraction. Nevertheless, when the system starts from a single stem-type cell (or a sufficiently small number), the "narrow path" leading this state is always selected through the developmental process. Indeed, we have performed several simulations with different reaction networks and parameter values, and found that this requirement of developmental process to form the ordered spatial pattern as Figs. 2 and 4 is a general feature of the developmental process in our model.

One of the most remarkable features of development and regeneration processes in real organisms is that such "narrow paths" to an ordered body pattern are always selected, despite the fact that such systems are subject to considerable perturbations, both at the microscopic level (e.g. molecular fluctuations) and at the macroscopic level (e.g. removal of cells). Our study suggests that the existence of such a narrow path to an ordered pattern and the stability of this path with respect to the perturbations are consequences of the interplay between intra- and inter-cellular dynamics.

7. Community effect

In our model, the differentiated cell types do not necessarily correspond to attractors of the internal dynamics of a single cell, and the interaction with other cells is important. For example, when a single type-2 cell (such as that in Fig. 4) is placed in a medium containing no other cells, this cell de-differentiates back into a type-0 cell. However, as shown in Fig. 10, the type-2 cell state is stable when such a cell is surrounded by a sufficient number of other type-2 cells. To confirm this point, we have carried out simulations with the same model, but in this case placing a large number (larger than 10) of type-2 cells in an otherwise unoccupied medium. In this case, the cell colony consisting of only type-2 cells grows.

As discussed in the previous section, there are multiple stable cell colony states. A cluster consisting only of type-2 cells corresponds to one such stable state, but this state never appears in the ordinary developmental process starting from a single cell. In this case, only when the number of type-2 cells initially placed in the medium is more than approximately 15, do these cells remain in their type-2 states. Otherwise, all the cells de-differentiate into type-0 cells. This result clearly indicates that the states of the cells existing in a cluster can be mutually stabilized by their cell–cell interactions.

The state of a developed cell colony depends on the number of initial cells, also for the case of type-0 and type-1 cells, as given in Table 1.

In real organisms, the stability of the state of a single, isolated cell is quite different from that of a cell existing as part of an ensemble of cells. Gurdon et al. demonstrated this difference for several cell types, using transplantation experiments, and named this change in stability the "community effect" (Gurdon et al., 1993). For example, they transplanted muscle progenitor cells of the Xenopus into other tissue and found that cells transplanted as a group differentiate into muscle cells, as in their normal development, while a single cell transplanted alone changes in response to cell–cell interactions. Although this effect can be explained simply by introducing an autocrine factor to keep the stability of their own cell type, the fact that several cell types have this community effect suggests that this characteristic is a general and intrinsic property of cell societies, independent of the specific choice of such factors.

The dependence of the developed tissue on the initial cell number has also been observed in experiments involving the artificial construction of tissue through the control of the activin concentration by Asashima's group (Ariizumi and Asashima, 2001; Uochi and Asashima, 1996). In these experiments, a number of undifferentiated cells were taken from the animal cap of Xenopus, and with these the construction of several types of tissue (e.g. heart, notocord, muscle and so forth) was caused by controlling the concentration of activin. In these experiments, it was found that the type of tissues constructed depends on the number of cells used. The tissue is generated only for some range of initial cell numbers. These experiments also suggest that the community effect is a generic property of an ensemble of cells.

According to our results, 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 each intracellular state in the ensemble. In the process of development, the dynamics of each cell type are determined in such a way that the entire cell society become stable. The community effect is thus understood as a natural consequence of the developmental process of interacting cells with intra-cellular chemical dynamics.

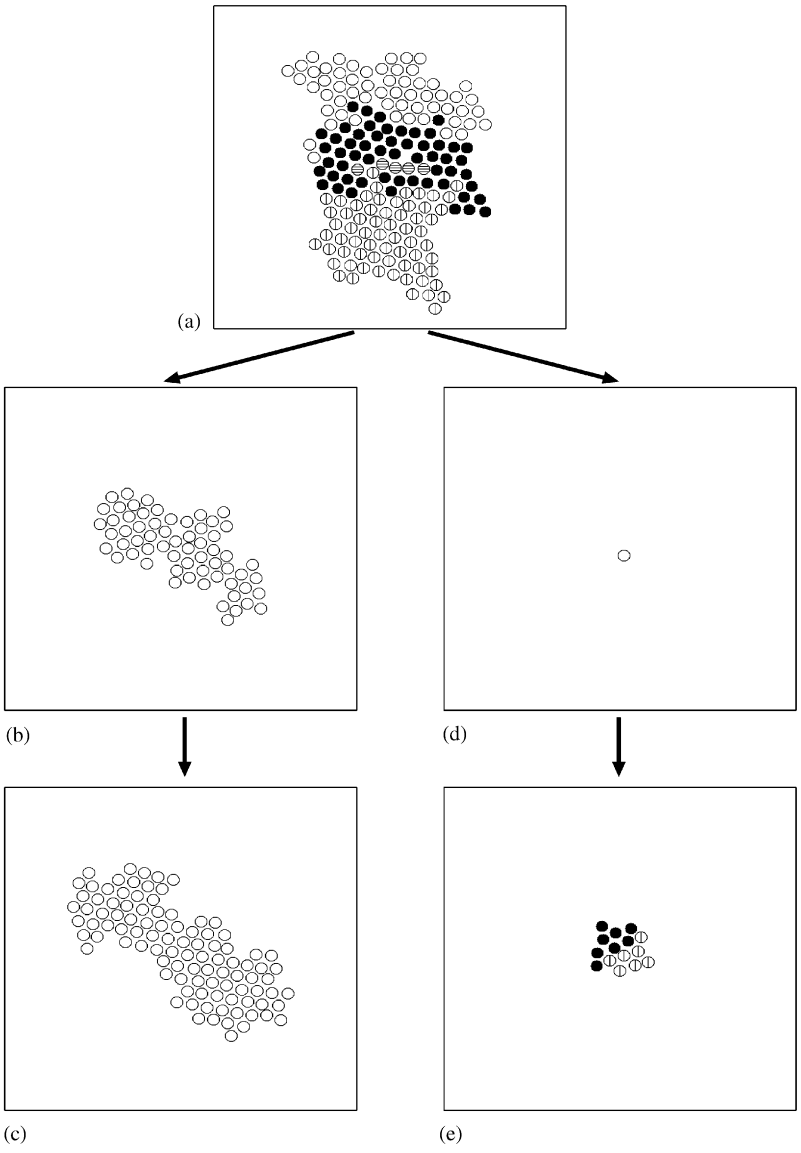

Fig. 10. The community effect in the case of a striped pattern. From an ensemble of cells exhibiting a striped pattern, (b) a group of type-2 cells and (d) a single type-2 cell were transplanted into a new, otherwise unoccupied medium, and they developed under the same rules and parameter values with the case depicted in Fig. 4. As shown in (c), the type-2 cells transplanted as a group remain type-2 cells, while the type-2 cell transplanted as a single cell transforms into a type-0 cell and then, eventually develops into a striped pattern, as shown in (e).

8. Discussion

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

First, in our model, cells possessing internal nonlinear reaction dynamics differentiate into several distinct cell types when the cell number exceeds a threshold value. The transition from one cell type to another is regulated by the position of the cell in question. This regulation leads to an ordered spatial pattern consisting of differentiated cells, as shown in Figs. 2 and 4. An important point here is that the positional information that controls the fate of a cell is not imposed from the outside of the system but, rather, emerges and is maintained through cell–cell interactions communicated through the exchange of diffusive chemicals. Each cell "reads" information from the external field and reacts to this information by modulating its intra-cellular dynamics in accordance with it. This modulation controls the rates of differentiation into various cell types, while such cell differentiations, in turn, alter the state of the environment. With this reciprocal relationship between intra- and inter-cellular dynamics, the gradients of chemicals in the medium act as positional information controlling the fate of each cell. Here, three interdependent processes maintaining the ordered spatial pattern of cells, the generation of positional information, its interpretation, and differentiation in accordance with

this interpretation, are tightly incorporated. Note that in the pattern formation process of Turing's model, such a reciprocal relationship of intra- and inter-cellular dynamics is not clearly discernible, because the internal state of the cells and the environmental state are not well separated.

The reciprocal relationship of intra- and inter-cellular dynamics is responsible for the robustness of the developmental process. In this process, differentiations occur when the homogeneous state of the cell ensemble becomes unstable through the increase of the cell number, while the emergence of differentiated cell types stabilizes the system. Then, when a large perturbation, such as the removal of cells, is applied, the entire system becomes unstable, and as a result, differentiations lead the system back toward the original stable state. In other words, ordered cell patterns generated as a result of this developmental process correspond to a fixed, self-consistent relationship between the intra- and inter-cellular dynamics, which is stable with respect to perturbations up to a certain degree. Indeed, the potential for "regeneration" of a spatial pattern, as described by Fig. 6, is a general feature of this system, which appears without any finely-tuned mechanisms. As discussed in the Introduction, even when the chemical gradient emerging through normal development is severely disturbed experimentally, the original gradient is regenerated, to recover normal development. We think that this regeneration is a manifestation of the dynamic reorganization of positional information demonstrated in this paper.

The developmental process of our model is robust with respect to microscopic perturbations, such as molecular fluctuations, which are inevitable when the number of chemical species is small. In the study reported in Furusawa and Kaneko (2001), to investigate the robustness with respect to such microscopic perturbations, we introduced noise in simulations of this developmental process[7] to represent molecular fluctuations, described by a Langevin equation. In that study, it was found that the developmental process is stable as long as the amplitude of this noise is not too large. This is also true in the system studied presently, displaying spatial patterns. Now even if the number of signal molecules is not very large, the robust pattern formation is possible, and the problem raised in the introduction is resolved. As a rough estimate, the minimal number of signal molecules per cell, necessary for robust development, is around 100–1000. This explains why the developmental process is robust with a quite small number of signal molecules.

An illuminating demonstration of developmental stability has recently been given in a sequence of experimental investigations of tissue construction by Asashima's group (Ariizumi and Asashima, 2001; Uochi and Asashima, 1996). In those experiments, a cell ensemble consisting of undifferentiated cells of Xenopus were put into a solution of activin with a given concentration for some time. These ensembles were allowed to develop and it was found that several different types of tissue were generated, depending on the concentration of activin. The types of tissues that developed include heart, muscle, notocord, and so forth. One of the remarkable findings of their experiments is that, in each case, normal tissue is developed, even though the developmental path leading to the construction of each type of tissue is far from the path of the normal developmental process. This result strongly suggests that normal tissue types correspond to 'attractors' of intra- and inter-cellular dynamics, as also found in our model.

Another important feature of the developmental process in our model is its dependence on history. The reciprocal relationship described above generally has multiple stable states, although a specific type of cell society always appears in the developmental process from a single stem cell. Such a dependence on history also exists in real developmental processes, in which the emergence of an ordered cell society of an adult body requires a developmental process that begins from a single or a small number of puripotent cells, while a simple aggregate of differentiated cells never develops into a well-organized adult body. How is the narrow path from a small number of undifferentiated cells to an ordered society of differentiated cells with a certain order is chosen even under microscopic and macroscopic fluctuations? Elucidating the selection mechanism is one of the most important problems in developmental biology. Our results suggest that a spatially ordered cell society is a stable state of a cellular system governed by a reciprocal relationship between intra- and inter-cellular dynamics, and the successive change of this stable state as the number of cells increases is responsible for selecting the narrow path of 'correct' development. Note that without incorporating a mechanism allowing for the change of the number of cells, as is the case in the standard Turing model, such robustness in selecting a developmental path cannot be discussed.

The dependence on history that we found shows that there are several stable states of a cell colony in our model. For example, using the same reaction matrix and parameter values as in the case depicted in Fig. 4, we observed at least five stable cell colonies: (i) a striped pattern in most cases consisting of three types of cells; (ii) a ring pattern consisting of two type of cells; (iii), (iv)

[7] In the simulations of Furusawa and Kaneko (2001), there was no spatial dependence of the environmental chemical concentrations. However, except for the spatial dependence of the regulation of differentiations, the cellular diversification process observed in those simulations is essentially the same as that observed in the system studied in this paper.

colonies consisting of homogeneous cell types (two cases with different types); (v) disordered patterns with two types of cells. In other words, different types of 'tissue' can be generated from the same reaction dynamics. In the 'normal' developmental process starting from a single cell, the striped pattern (i) is generated, while from initial conditions with multiple cells, the other patterns (ii)–(v) can be generated. Thus differentiation of tissues from a different initial cell ensemble is also a general consequence of our theory.

In the present context, it is interesting to reconsider the experiments by Asashima's group. In those experiments, the formation of different types of tissue was brought about by only changing the concentration of activin in the medium. In other words, in the biological system studied there, different stable states of cell aggregates exist, as in our model. Also, in those experiments, the type of tissue generated was found to depend on the number of cells prepared initially. This number dependence corresponds to what we have shown in our model as the community effect. In the experiments, the path leading to the generation of each type of tissue was selected in accordance with the concentration of activin in the medium. In the future, it is important to study the selection of the developmental path and its dependence on external conditions, both theoretically and experimentally.

To confirm the universality of the mechanism identified here as that governing pattern formation with differentiated cells, we have carried out numerical experiments using several different sets of parameter values and choosing thousands of different reaction networks. As a result, we have found that the cellular diversification process leading toward a spatial pattern that we have discussed in this paper is observed for some fraction of randomly chosen reaction networks. Essentially the same results are obtained as long as the magnitudes of the internal reaction term and the cell–cell interaction term are of comparable size, and the intra-cellular reaction dynamics exhibit oscillations that in some case can be chaotic. The results are found to be independent of other details of the model. For example, the essence of developmental processes is unchanged if we change the catalysation degree α to 1 or 3. With the parameter values used in the example considered in Figs. 2, approximately 30% of randomly chosen reaction networks result in oscillatory behavior, while others converge to fixed points. Then, approximately 20% of the system with these oscillatory dynamics are destabilized through cell division, resulting differentiations into distinct cell types. Among the systems with the spontaneous diversification, more than half of them exhibit non-trivial spatial patterns of differentiated cells, as in the examples depicted in Figs. 2 and 4. The majority of these non-trivial spatial pattern is a ring pattern as Fig. 2, since gradients of chemical concentrations along the radial direction in a cell cluster generally appear as a result of absorption/release by cells and diffusion of nutrients. The results show that the emergence of non-trivial spatial pattern is not a special case of our results, but is a general property as long as the spontaneous differentiation by cell–cell interactions occurs.

One might ask why we chose to consider reaction networks of the kind we used, even though only a small fraction of randomly chosen reaction networks lead to complex oscillatory dynamics, which are necessary to realize pattern formation. To address this question, we have studied the growth speed of an ensemble of cells to determine what kind of reaction networks can possibly appear through evolution using a model with cells possessing internal dynamics described by Eq. (1) in a one-dimensional medium. As is shown in Furusawa and Kaneko (2000), if in such a system the internal dynamics can realize chaotic state and if the cell ensemble contains a variety of cell types differentiated from stem-type cells, the cell society can maintain a larger growth speed as an ensemble by realizing a cooperative use of resources than if the colony consists of only a single type of cells, because in the latter case cells strongly compete with each other for the same resources. This difference in growth speed of a colony is also observed in the present simulations using two-dimensional medium. These results suggest that the emergence of a cell society with complex cellular dynamics and a diversity of cell types generated through differentiation of stem-type cells is a necessary course of evolution, because separate aggregates of cells must compete for finite resources necessary for growth and reproduction, and a colony with higher growth speed must be selected. Hence, the use of reaction networks leading to complex oscillatory dynamics for a general model for morphogenesis is supported by considerations from the evolutionary viewpoint. In this sense, our results provide a novel standpoint to understand the emergence of multicellularity in evolution, as discussed in Furusawa and Kaneko (2000, 2002).

It is commonly believed that pattern formation in the development of biological systems is maintained by two functions of cells, generation of positional information and interpretation of this information to change the cell state, and that in this process there is a succession of switches of gene expression that are precisely controlled and finely tuned. Our dynamical system model of cells is not necessarily inconsistent with such a genetic switching process, since the reaction network we adopted in the model can include those associated with genetic expressions. However, the essential point of our results is that the above stated two functions acting in pattern formation cannot be separated in the cellular dynamics of our model, and they emerge as a natural consequence of interacting cells with intra-cellular reaction dynamics,

without the institution of any finely tuned mechanisms. The mechanism at work in our model, consisting of the interplay between intra- and inter-cellular dynamics provides a novel standpoint to understand the robustness of spatial patterns of cells and tissue differentiation. As emphasized throughout the paper, we believe that the robust and spontaneous pattern formation of interacting cells with intra-cellular dynamics provides a fundamental mechanism for morphogenesis of multicellular organisms.

One may still wonder if the differentiation and pattern formation in our theory are relevant to biological function, since any specific functional role of each chemical process is not prescribed in our model. Still, each cell type has a specific chemical composition different from the other type, and this specialized chemical composition, diffusing out from the cell, works as a boundary condition for the other cells, and stabilizes the chemical state of the other cell-type. Organized pattern and specialized chemical composition of each cell-type is used by transport of chemicals necessary for other cells, so that a cell colony can keep growing. This mutual cooperation of cells with different cell types provide one of the most primitive form of functional integration. From this point of view, isologous diversification may contribute to the development of biological function.

To fully answer the questions of how and to what extent contemporary organisms utilize isologous diversification, however, evolutionary processes together with present-day mechanisms of cell differentiation have to be further studied (see Kaneko and Yomo, 1999, for this direction). The following comments about the evolution of development are relevant to this question. In comparison with developmental process presented in the paper, modern developmental processes are much more sophisticated and hierarchically organized, which is under the control of "program-like" routine of gene expression. Still, for evolution to sophisticated processes, at least primitive processes are necessary. Otherwise, evolutionary potential does not exist. Here, we have shown the basis for the evolution, by showing generality of cell differentiation and morphogenesis.

In order to bridge the gap between modern developmental processes and our diversification process, it is important to study how the morphogenesis by the present dynamical processes is embedded into "program-like" behavior as a routine of gene expressions, through evolution (Newman, 2002). With such "program", a complicated and hierarchical organization of developmental process is made possible.

Furthermore, it is also important to study how an initial state of fertilized egg is chosen so that the life cycle of an organism continues recursively. Such study may provide a novel insight about the difference between development starting from finely tuned initial condition (e.g. Drosophila egg with pre-existing asymmetry) and that starting from relatively loosely determined initial condition as in mammalian development. Since the choice of initial conditions among a large number of chemicals is discussed in connection with growth of organisms in our model, it will provide an appropriate framework to study the evolution of morphogenesis, in future.

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References

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2002. The Molecular Biology of the Cell. Garland, New York.

Ariizumi, T., Asashima, M., 2001. In vitro induction systems for analyses of amphibian organogenesis and body patterning. Int. J. Dev. Biol. 45, 273–279.

Crampin, E.J., Gaffney, E.A., Maini, P.K., 1999. Reaction and diffution on growing domains: scenarios for robust pattern formation. Bull. Math. Biol. 61, 1093–1120.

Driever, W., Nüsselein-Volhard, C., 1988. A gradient of bicoid protein in Drosophila embryos. Cell 54, 83–93.

Furusawa, C., Kaneko, K., 1998. Emergence of rules in cell society: differentiation, hierarchy, and stability. Bull. Math. Biol. 60, 659–687.

Furusawa, C., Kaneko, K., 2000. Origin of complexity in multicellular organisms. Phys. Rev. Lett. 84, 6130–6133.

Furusawa, C., Kaneko, K., 2001. Theory of robustness of irreversible differentiation in a stem cell system: chaos hypothesis. J. Theor. Biol. 209, 395–416.

Furusawa, C., Kaneko, K., 2002. Origin of multicellular organisms as an inevitable consequence of dynamical systems. Anatomical Rec. 268, 327–342.

Goodwin, B., 1963. Temporal Organization in Cells. Academic Press, London.

Gurdon, J.B., Lemaire, P., Kato, K., 1993. Community effects and related phenomena in development. Cell 75, 831–834.

Hess, B., Boiteux, A., 1971. Oscillatory phenomena in biochemistry. Ann. Rev. Biochem. 40, 237–258.

Hogeweg, P., 2000. Evolving mechanisms of morphogenesis: on the interplay between differential adhesion and cell differentiation. J. Theor. Biol. 203, 317–333.

Houchmandzadeh, B., Wieschaus, E., Leibler, S., 2002. Establishment of developmental precision and proportions in the early Drosophila embryo. Nature 415, 798–802.

Kaneko, K., Yomo, T., 1994. Cell division, differentiation, and dynamic clustering. Physica D 75, 89–102.

Kaneko, K., Yomo, T., 1997. Isologous diversification: a theory of cell differentiation. Bull. Math. Biol. 59, 139–196.

Kaneko, K., Yomo, T., 1999. Isologous diversification for robust development of cell society. J. Theor. Biol. 199, 243–256.

Kauffman, S.A., 1969. Metabolic stability and epigenesis in randomly constructed genetic nets. J. Theor. Biol. 22, 437–467.

Lacalli, T.C., Harrison, L.G., 1991. From gradient to segments: models for pattern formation in early Drosophila embryogenesis. Semin. Dev. Biol. 2, 107–117.

Meinhardt, H., Gierer, A., 2000. Pattern formation by local self-activation and lateral inhibition. Bioessays 22, 753–760.

Miura, T., Shiota, K., 2000. TGFbeta2 acts as an "activator" molecule in reaction-diffusion model and is involved in cell sorting phenomenon in mouse limb micromass culture. Dev. Dyn. 217 (3), 241–249.

Newman, S., 2002. Developmental mechanisms: putting genes in their place. J. Biosci. 27 (2), 97–104.

Takagi, H., Kaneko, K., 2003. Dynamic relationship between diversity and plasticity of cell types in multi-cellular state. Phys. Rev. E, submitted for publication.

Turing, A.M., 1952. The chemical basis of morphogenesis. Philos. Trans. R. Soc. B 237, 37–72.

Tyson, J.J., et al., 1996. Chemical kinetic theory: understanding cell-cycle regulation. TIBS 21, 89–96.

Uochi, T., Asashima, M., 1996. Sequential gene expression during pronephric tubule formation in vitro in Xenopus ectoderm. Dev. Growth Differentiation 38, 625–634 private communications.

Wolpert, L., 1969. Positional information and the spatial pattern of cellular differentiation. J. Theor. Biol. 25, 1–47.