Origin of Complexity in Multicellular Organisms

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Through extensive studies of dynamical system modeling cellular growth and reproduction, we find evidence that complexity arises in multicellular organisms naturally through evolution. Without any elaborate control mechanism, these systems can exhibit complex pattern formation with spontaneous cell differentiation. Such systems employ a "cooperative" use of resources and maintain a larger growth speed than simple cell systems, which exist in a homogeneous state and behave "selfishly." The relevance of the diversity of chemicals and reaction dynamics to the growth of a multicellular organism is demonstrated. Chaotic biochemical dynamics are found to provide the multipotency of stem cells.

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Multicellular organisms consist of differentiated cell types, with rather complex biochemical dynamics exhibited by complex metabolic and genetic networks. Through the course of development, cells differentiate into several types and often form complex patterns. The molecular mechanisms existing of each stage of development have been elucidated experimentally [1]. However, it is still not clear why multicellular organisms should have such complexity, nor why such inhomogeneities in cell types and patterns are common. These are not trivial problems [2], since a simple biochemical network would be sufficient (and perhaps more fit) to produce identical cells rapidly and faithfully. Here we give an answer to the problem of why multicellular organisms in general have diverse cell types with complex patterns and dynamics.

Indeed, by considering dynamical systems of appropriate biochemical networks, it is possible to show that inhomogeneous patterns with diverse cell types can emerge. Such work was pioneered by Turing [3,4], and extended in Refs. [5–7]. Differentiation into several cell types has also been studied by using random genetic networks [8]. Still, it is not known how biochemical and genetic networks allowing for diversity are evolved.

Any theory accounting for such cellular diversity requires a specific choice of the genetic and biochemical reaction networks and parameters. Then, to explain the ubiquitous nature of cellular diversity in multicellular organisms, we must understand how such reaction dynamics allowing for cell differentiation can evolve, without postulating a finely tuned mechanism. Here we study the relationship between the growth of multicellular organisms and some characteristics of intracellular dynamics, to understand the origin of cellular diversity. Since the question we address is rather general, we seek an answer that does not rely on detailed knowledge of specific cellular processes in existing organisms. Instead, we adopt dynamical systems models that display only essential features of the developmental process. In a minimal model of cellular dynamics, we include only intracellular biochemical reactions with enzymes, simple cell-cell interactions through diffusion, and cell division as a result of biochemical reactions within each cell. By studying a class of models with this general and minimal content, we show that cell differentiation from a stem cell and development producing a complex pattern are natural consequences of evolution.

Model.—First, cells are assumed to be completely surrounded by a one-dimensional medium. The state of a cell i is assumed to be characterized by the cell volume and $c_i^{(m)}(t)$, the concentrations of k chemicals ($m = 1, \ldots, k$). The concentrations of chemicals change as a result of internal biochemical reaction dynamics within each cell and cell-cell interactions communicated through the surrounding medium. The corresponding chemical concentration in the medium is denoted by $C^{(m)}(x, t)$, where x denotes the position along the one-dimensional axis. We use a one-dimensional model only for its tractability; conclusions we draw in this case are consistent with the result of preliminary simulations of a two-dimensional model.

Internal reaction dynamics.—For the internal chemical reaction dynamics, we choose a catalytic network among the k chemicals, represented by a matrix $\mathrm{con}(\ell, j, m)$ which takes on unity when the reaction from the chemical ℓ to m is catalyzed by j, and 0 otherwise. The rate of increase of $c_i^{(m)}(t)$ [and decrease of $c_i^{(\ell)}(t)$] through this reaction is given by $c_i^{(\ell)}(t)[c_i^{(j)}(t)]^{\alpha}$, where α is the degree of catalyzation ($\alpha = 2$ in most simulations here). Each chemical has several paths to other chemicals, and thus a complex reaction network is formed.

Besides, we take into account the change in the volume of a cell, that varies as a result of transportation of chemicals into the cell from the environment. For simplicity, we assume that the volume is proportional to the sum of chemicals in the cell. The concentrations of chemicals are diluted as a result of an increase in the volume of the cell. With the above assumption, this dilution effect is tantamount to imposing the restriction $\sum_\ell c_i^{(\ell)} = \text{const}$.

Of course, real biochemical processes within a cell are much more complicated. Instead of taking such details into account, we employ this simple model, and consider a

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variety of reaction networks created randomly, to elucidate a general relationship between the growth of cells and cellular dynamics.

Cell-cell interactions through a medium.—Cells interact with each other through the transport of chemicals out of and into the surrounding medium. As a minimal case, we consider only indirect cell-cell interactions through diffusion of chemicals via the medium. The diffusion coefficient should depend on the chemical. Here, we assume that there are two types of chemicals, one which can penetrate the membrane and one which cannot. We use the notation σ_m, which takes the value 1 if the chemical $c_i^{(m)}$ is penetrable, and 0 otherwise.

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

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

with

$$\Delta c_i^{\ell}(t) = \sum_{m,j} \mathrm{con}(m, j, \ell) e_1 c_i^{(m)}(t) [c_i^{(j)}(t)]^{\alpha} - \sum_{m',j'} \mathrm{con}(\ell, j', m') e_1 c_i^{(\ell)}(t) [c_i^{(j')}(t)]^{\alpha} + \sigma_{\ell} D [C^{(\ell)}(p_i^x, t) - c_i^{(\ell)}(t)]. \quad (2)$$

The variable p_i^x denotes the location of the ith cell. The second term in Eq. (1) represents the dilution effect by changing the volume of the cell. On the other hand, the diffusion of chemicals in the medium are governed by the following equation for $C^{(\ell)}$:

$$\partial C^{(\ell)}(x,t)/\partial t = -\tilde{D} \nabla^2 C^{(\ell)}(x,t) + \sum_i \delta(x - p_i^x) \sigma_{\ell} D \times [C^{(\ell)}(x,t) - c_i^{(\ell)}(t)], \quad (3)$$

where the boundary condition is chosen to be $C(0,t) = C(x_{\max}, t) = \mathrm{const}$, $\tilde{D}$ is the diffusion constant of the environment, and $x_{\max}$ denotes the extent of the medium. This boundary condition can be interpreted as a chemical bath outside of the medium, which supplies those penetrable chemicals that are consumed to the medium via a constant flow to the cell.

Cell division.—Each cell takes penetrable chemicals from the medium as the nutrient, while the reaction in the cell transforms them to unpenetrable chemicals which construct the body of the cell. As a result of these reactions, the volume of the cell is increased. In this model, the cell is assumed to divide into two almost identical cells when the volume of the cell is doubled.

During this division process, all chemicals are almost equally divided, with tiny random fluctuations (e.g., $\sim 10^{-6} c_i^{(\ell)}$). The case of equal division is assumed, since we do not intend to introduce an elaborate mechanism, while the fluctuations are introduced because we wish to study development processes that are robust with respect to molecular fluctuations. After cell division, two daughter cells appear around their mother cell's position, and the positions of all cells are adjusted so that the distances between adjacent cells are 1. As a result, the total length of the chain of cells increases.

As the initial state, a single cell, whose chemical concentrations are determined randomly, is placed. According to the process described above, cells divide to form a chain. Of course, the behavior of the model depends on each intracellular reaction network. We have carried out simulations of the model by considering 800 different reaction networks, generated randomly. Throughout the paper, the number of chemical species k is 20, and each chemical has 6 reaction paths to other chemicals, chosen randomly. Each chemical reaction path is catalized by other (or the same) chemical, again chosen randomly. Among the 20 chemicals, 3 chemicals can penetrate cell membranes. The parameters are fixed at $e_1 = 1.0$, $D = 1.0$, $\tilde{D} = 2.0$, and $x_{\max} = 500$.

As results, we found that each growth curve using a different reaction network can be classified into two classes: (I) "fast" growth in which the increase of cell number grows exponentially in time t, and (II) "slow" growth in which the cell number grows linearly in time. Under the parameters presented above, approximately 5% of randomly chosen reaction networks show the case (I) behavior. These two classes, indeed, are also distinguished by the nature of the corresponding cellular dynamics.

In case (II), the chemical compositions and dynamics of all cells are identical. These dynamics fall into either a fixed point or a limit-cycle attractor. In this situation, only a few cells around the edges of the chain can divide. Since cells are not differentiated, chemicals required for cell growth are identical for all cells. Thus, once the cells at the edges consume the required resources, the remaining cells can no longer grow. This is the reason for the linear growth.

In case (I) with exponential growth, it is found that the chemical reaction dynamics of the cells are more complex than in case (II). The microscopic differences between the chemicals in two daughter cells are amplified through the internal biochemical dynamics and the cell-cell interaction, leading to chaotic chemical dynamics. For most such cases, the cells differentiate into various cell types that are defined by distinct chemical dynamics and compositions (see Fig. 1). Even though no external mechanism of differentiation is implemented, the instability that results from the intracellular dynamics and the cell-cell interactions brings about transitions to various cell types [9]. Indeed, this differentiation is a general feature of a system of interacting units that each possess some nonlinear internal dynamics [10,11], as has been clarified by isologous diversification theory [5–7].

In case (I), the division of cells is not restricted to the edge of the chain. There is a flow of the nutritive chemicals into the inside of the chain, that can maintain the growth of internal cells. This flow is sustained by the diffusion

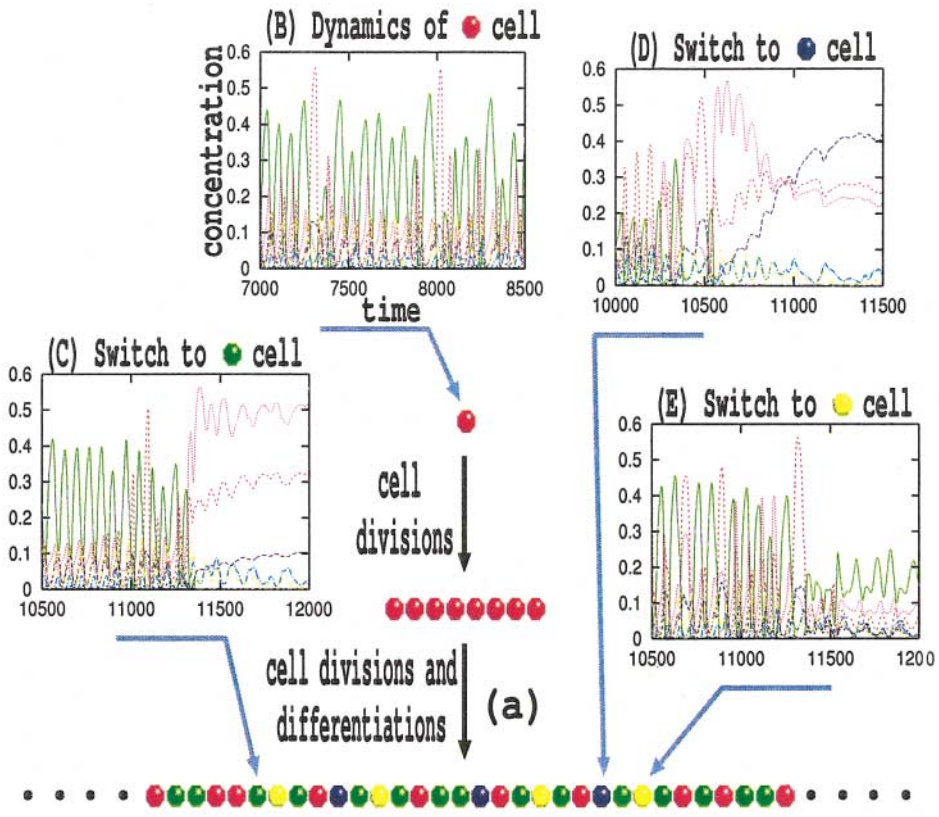

FIG. 1 (color). (a) The developmental process of the spatial pattern with differentiated cells, starting from a single cell. Up to several divisions, a single type of cell, represented as a "red" cell, reproduces, maintaining its characteristic type of dynamics. The time series of the chemical concentrations of this type of cell are plotted in (b), where the time series of only six chemical concentrations among the 20 are overlaid, to avoid an indistinct figure. With a further increase of cell number, some of the "red" cells start to exhibit different types of chemical dynamics, due to the cell-cell interactions. The transitions from the "red" cells to three distinct cell types represented by "green," "blue," and "yellow" are plotted in (c), (d), and (e). In this example, only these four types of cells appear in the developmental process, while no intermediate types exist. Here, the "red" cells are regarded as the stem cells that have the potential both to reproduce themselves and to differentiate into other cell types, while the differentiated cells reproduce only the same type.

between cells possessing different chemical compositions and exhibiting different phases of chemical oscillations. Here, the capability for cell division is also differentiated as the development progresses. The total increase in cell number is due to the division of certain type(s) of cells, and most other cell types stop dividing.

In the developmental processes in case (I), initial cell types exhibit transitions to other types. Cells of the initial cell types either proliferate or switch to other types stochastically. They can be regarded as a stem cell [12]. The dynamics of a stem-type cell, that can produce different cell types after divisions, exhibit chaotic oscillation (see Fig. 1, and also [7]). The type of differentiated cells maintain their type after divisions.

Next, we compare the growth of a single cell with that of an ensemble of cells. In Fig. 2 the growth speeds of a single cell and an ensemble of cells are plotted. Here each point corresponds to a different reaction network.

The points around the peak of the growth for an ensemble correspond to case (I) of exponential growth. Here, the growth speed of a single cell is not large. In each cell, a variety of chemicals coexist, supporting complex reaction dynamics and cell differentiation. In case (I), organisms with cellular diversity, plotted by green points, have a larger growth speed of an ensemble than those without diversity. Indeed, in the former case, cells differentiate in the use of nutrients to take them efficiently.

In case (II), there are much simpler intracellular reaction processes, with only a few autocatalytic reactions used dominantly, that can produce the rapid replication of a single cell. In this case, the growth speed of a single cell is often large (represented by some of the blue points in Fig. 2), while the growth speed of an ensemble always remains small. In some sense, simple cells with rapid growth are "selfish": Although such simple cells with low diversity of chemical species can exhibit large growth speeds as single cells, they cannot grow cooperatively, and their growth speeds as ensembles are suppressed because of strong competition for resources.

The developmental process presented in case (I) is irreversible. Initially, cells have complex chaotic internal dynamics and a variety of chemicals. They have the potential to differentiate into cell types with simpler cellular dynamics, for example, with a fixed-point and regular oscillation (see Fig. 1 as an example). This type of cell with simpler dynamics can produce cells of the same type or stop dividing. Hence, the loss of multipotency, known to exist in the developmental process of real organisms [1], is explained in terms of the decrease in time of diversity in the intracellular dynamics and chemicals. Indeed, the decrease in complexity of dynamics with the loss of multipotency has been confirmed quantitatively, for example by computing the Kolmogorov-Sinai (KS) entropy of the intracellular dynamics. Using the data in Fig. 2, we have found that case (I) has a positive KS entropy while for the case (II) it is zero. Furthermore, there is positive correlation between the KS entropy and the growth speed as an ensemble. The decrease of KS entropy in going from a stem cell to other differentiated cells has also been confirmed. For example, the KS entropy of the stem-type

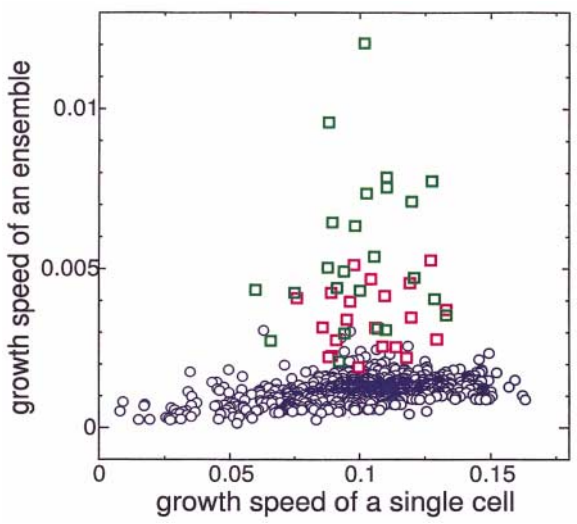

FIG. 2 (color). Relationship between the growth speed of a single cell and that of an ensemble. The ordinate shows the growth speed of an ensemble, measured as the inverse of the time for the cell number doubles from 100 to 200, while the abscissa represents the inverse of the time for a single cell to divide. Each point is obtained by using a different chemical reaction network. The blue circles correspond to case (II), where the state of cells are identical and the growth of an ensemble is linear in time. The dynamics of the chemical concentrations of these cases are mostly of the fixed point type, with a few cases of limit cycles. The green and red rectangles correspond to the case with chaotic cellular dynamics, where the growth of an ensemble is exponential in time. The green points correspond to the case with cell differentiation into several types. For the red points, cell differentiation into distinct types is not observed (up to the time when the cell number reaches 200).

"red" cells in Fig. 1 is approximately 2.4×10^{-4}, while it is 3.1×10^{-5} for "green," 4.5×10^{-5} for "blue," and less than 1.0×10^{-5} for "yellow" cells.

Our results are robust against the change in model parameters, as long as the internal reaction and intracellular diffusion terms have a comparable order of magnitude. The results are also independent of the details of the model, in particular, of the choice of catalyzation degree $\alpha = 1$ or 3. The same two classes and the same relation between the growth and cellular diversity are obtained, as long as the number of chemicals is sufficient (say for $k > 10$) and the number of the reaction paths is in the medium range (e.g., $3 \sim 9$ for each chemical at $k = 20$; otherwise, the intracellular dynamics fall onto a fixed point for most cases without differentiation).

To sum up, our study has provided evidence that an ensemble of cells with a variety of dynamics and stable states (cell types) has a larger growth speed than an ensemble of simple cells with a homogeneous pattern, because of the greater capability of the former to transport and differentiation in the use of nutritive chemicals. Note that no elaborate mechanism is required for the appearance of such heterogeneous cell ensembles. Some fraction of the randomly chosen biochemical networks we considered exhibit dynamics sufficiently complex to allow for spontaneous cell differentiation.

Our result suggests that complexity of multicellular organisms with differentiated cell types is a necessary course in evolution, once a multicellular unit emerges from cell aggregates. In fact, by carrying out the evolution experiment numerically, with mutation to reaction networks and selection of the cell ensembles with higher growth speed, we have confirmed that cells of the case (I) emerge and survive through evolution.

Our result concerning the relationship between the diversity of chemicals and dynamics and the growth speed of a single cell and ensemble provides experimentally testable predictions. Since even primitive organisms such as *Anabena* [13] and *Volvox* [14] exhibit differentiation in cell types and some spatial pattern, the relationship can be verified. In fact, for a mutant of *Volvox* that possesses only homogeneous cells, the growth becomes slower in comparison to the wild type [15].

Chaotic intracellular dynamics and the diversity of chemicals present in a stem cell are also experimentally verifiable. A decrease of diversity in chemical composition and of complexity in their temporal variation is expected with the decrease of multipotency of a cell.

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[1] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, *The Molecular Biology of the Cell* (Garland, New York, 1994), 3rd ed.
[2] J. Maynard-Smith and E. Szathmary, *The Major Transitions in Evolution* (W. H. Freeman, San Francisco, 1995).
[3] A. M. Turing, Philos. Trans. R. Soc. (London) B **237**, 5 (1952).
[4] S. A. Newman and W. D. Comper, Development **110**, 1 (1990).
[5] K. Kaneko and T. Yomo, Bull. Math. Biol. **59**, 139 (1997).
[6] K. Kaneko and T. Yomo, J. Theor. Biol. **199**, 243 (1999).
[7] C. Furusawa and K. Kaneko, Bull. Math. Biol. **60**, 659 (1998).
[8] S. A. Kauffman, J. Theor. Biol. **22**, 437 (1969).
[9] Under the adopted parameters, we have observed up to six types of cells. As the number of chemicals is increased, the number of cell types slowly increases, although it is hard to define the cell types in such high-dimensional cellular dynamics and measure the maximal number of distinct cell types.
[10] K. Kaneko, Physica (Amsterdam) **41D**, 137 (1990).
[11] K. Kaneko, Physica (Amsterdam) **75D**, 55 (1994).
[12] M. Ogawa, Blood **81**, 2353 (1993).
[13] H-S Yoon and J. W. Golden, Science **282**, 935 (1998).
[14] D. L. Kirk and J. F. Harper, Int. Rev. Cytol. **99**, 217 (1986).
[15] L. Tam and D. L. Kirk, Development **112**, 571 (1991).