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Constructive and Dynamical Systems Approach to Life

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1. Introduction

Life system is both complex and complicated [12]. These two features have to be distinguished. In a complicated system, many factors are involved and the possible variety of combination of such factors is enormous. It requires hard efforts to disintegrate it into parts, but in principle it is possible. Indeed in the bioinformatics, studies along this direction are carried forward with the aid of computer. On the other hand, in "complex systems", one has to face the circular relationship in which each part is understood only through the relationship with the whole, although the whole, of course, consists of parts. In the complex systems approach, we try to understand universal logic that biological systems have to obey through such dynamic circulation between the whole and parts. Here we are not concerned with determining a specific role of a molecule or a biochemical process; Rather we intend to understand some universal features that a class of biological systems exhibit, irrespectively of details.

In the present paper, we briefly sketch the study of this complex systems approach to biology – both theoretical and experimental – mainly focusing on the studies at a cellular level. For the study of complex systems, we take two standpoints, i.e., constructive biology and coupled dynamical system with internal degrees of freedom.

Constructive approach: Since we are interested in the universal feature of a biological system, we need to study some features that are not influenced by the details of complicated biological processes. The present organisms, however, include detailed elaborated processes that are captured through the history of evolution. Then, for our purpose, it is desirable to set up a minimal biological system, to understand universal logic that organisms necessarily should obey. Hence, the approach that should be taken will be "constructive" in nature. This constructive approach is carried out both experimentally and theoretically. The problems discussed here are summarized in the table.

Some key concepts obtained from coupled dynamical systems

Experimental strategy to understand general questions (to be given later) is construction of prototype system for each problem, while as a theoretical strategy we

adopt dynamical systems theory. We adopt intra-inter dynamics model where each unit (say cell or an individual organism) with internal dynamics (say biochemical reaction dynamics) interact with each other. A characteristic feature common to biological systems is that these units can replicate. Hence the present dynamical systems change their degrees of freedom through temporal evolution. Through extensive studies of this class of models, we have found common features in this class of models;

(i) **isologous diversification** —general tendency of differentiation from identical units [9,18,19],
(ii) **chaotic itinerancy** — emergence of long-term dynamics over several quasi-steady states with some self-organized transition rules [8,9,16,23],
(iii) **minority control**— tendency that replicators with a smaller size in population control the behavior of the total system [20]

These concepts are relevant to understand common nature in biological systems, while the problems in the above table are discussed by these concepts. It should be noted that instead of discussing fitness in replication, we discus recursiveness that emerges from rather plastic replication process.

In the following sections, we discuss each item in the Table 1, both from constructive and dynamical systems viewpoints. At each section we raise a basic question for each item, and propose an answer based on dynamical systems theory. For the first three items in Table 1, we explain briefly a theoretical model, which leads to the proposed answer, while some constructive experiments are briefly described. For the last two items, we describe only the question and outline of the answer, leaving the description of model studies to original papers.

Table 1

Construction of	Experiment	Theory	Question
multicellular system	interaction-induced cell differentiation	isologous diversification in inter-intra dynamics	robustness in development
developmental process (I)	controlled differentiation from stem cell	emergence of differentiation rule by chaos	irreversibility
developmental process (II)	artificial construction of tissues	self-consistency between pattern and dynamics	origin of positional information
replicating system	*in-vitro* replicating system	minority control	origin of information
cell system	dividing liposme with internal reaction	dynamic bottleneck in autocatalytic reaction system	evolvability with recursive production

2. Cell Differentiation

Question: How does an identical cell diversify into a discrete set of cell types through development? Together with this diversification, how are several distinct cell types formed that maintain recursive production? How is a character of each cell type stabilized under molecular fluctuations, and how is the number distribution of cell types stably maintained?

Logic

To answer the question, "**isologous diversification** theory" is proposed. The theory explains how amplification of noise-induced slight difference between cells leads to a noise-tolerant society with differentiated cell types. Through the interaction among cells with internal chemical dynamics, a state with homogeneous cells is destabilized. Cells diversify into discrete types spontaneously [18,19].

This diversification of cell types from a single cell type is shown to be a general consequence of interacting cells with biochemical networks and cell divisions, as is confirmed by several model simulations. According to the theory, differentiation proceeds first by loss of synchrony of intracellular oscillations as the number of cells increases. Then the chemical composition of the cells is differentiated. The differentiated compositions become inherited by the next generation, and lead to determined cell types. As a result of successive occurrence of the cell differentiation, the cell society will be composed of different cell types. The whole developmental process is shown to be stable against molecular and other external fluctuations, where amplification of noise-induced slight difference between cells leads to a noise-tolerant society with differentiated cell types. This robustness is a remarkable feature of isologous diversification, in contrast to the conventional threshold-type mechanism for development.

Model

We have studied several models [2,17–19] choosing (a) the internal variables and their dynamics, (b) interaction type, and (c) the rule to change the degrees of freedom (e.g., cell division).

As for the internal dynamics, auto-catalytic reaction among chemicals is chosen, which often leads to nonlinear oscillation in chemical concentrations. As the interaction mechanism, diffusion of chemicals between a cell and its surroundings is chosen.

To be specific, we mainly consider the following model here. First, the state of a cell i is assumed to be characterized by the cell volume and a set of functions $x_i^{(m)}(t)$ representing the concentrations of k chemicals denoted by $m = 1, \cdots, k$. For the internal chemical reaction dynamics, we choose a catalytic network among the k chemicals. The network is defined by a collection of triplets (ℓ,j,m) representing the reaction from chemical m to ℓ catalyzed by j. The rate of increase of $x_i^{\ell}(t)$ (and decrease of $x_i^{m}(t)$) through this reaction is given by $x_i^{(m)}(t)(x_i^{(j)}(t))^{\alpha}$, where α is the degree of catalyzation Each chemical has several paths to other chemicals, and thus a complex reaction network is formed. These reactions can

include genetic processes, where $x_i^\ell(t)$ is regarded as a degree of expression of a given gene.

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 transport rate of chemicals into a cell is proportional to the difference in chemical concentrations between the inside and the outside of the cell, and is given by $D_\ell(X^{(\ell)}(t) - x_i^{(\ell)}(t))$, where D_ℓ denotes the diffusion constant, and $X^{(\ell)}(t)$ is the concentration of the chemical at the medium. With this type of interaction, corresponding chemicals in the medium are consumed. To maintain the growth of the organism, the system is considered to be immersed in a bath of chemicals through which (nutritive) chemicals are supplied to the cells.

As chemicals flow out of and into the environment, the cell volume changes. The volume is assumed to be proportional to the sum of the quantities of chemicals in the cell, and thus is a dynamical variable. Accordingly, chemicals are diluted as a result of the increase of the cell volume.

In general, a cell divides according to its internal state, for example, as some products, such as DNA, are formed, accompanied by an increase in cell volume. Again, considering only a simple situation, we assume that a cell divides into two when the cell volume becomes double the original. At each division, all chemicals are almost equally divided, with random fluctuations.

Of course, there can be a variety of choices on the chemical reaction network. However, the observed differentiation process with the increase of the cell number does not depend on the details of the choice, as long as the network allows for the oscillatory intra-cellular dynamics leading to the growth in the number of cells.

Results from the Model

Up to a certain number of cells (which depends on the model parameters), dividing cells from a single cell have the same characteristics. When the number of cells rises above a certain (threshold) value, the state with identical cells is no longer stable. Small differences introduced by the fluctuation start to be amplified, until the synchrony of the oscillations is broken. Then the cells split into a few groups, each having a different oscillation phase (see Fig. 1a). The cells belonging to each group are identical in phase. This diversification in the phases, however, cannot be called cell differentiation, because the time average of the biochemical concentrations reveals that the cells are almost identical. The change of phases at the second stage is nothing but dynamic clustering studied in coupled nonlinear oscillators [9, 10].

With the further increase of the cell number, the average concentrations of the biochemicals over the cell cycle become different. The composition of biochemicals become different for each group. The orbits of chemical dynamics plotted in the phase space of biochemical concentrations, lie in a distinct region within the phase space (see Fig. 1b).

Hence distinct groups of cells are formed with different chemical characters. Each group is regarded as a different cell type, and the process to form such types is called differentiation. With the nonlinear nature of the reaction network, the difference in chemical composition between the clusters is amplified. By the formation of groups of different chemical compositions, each intra-cellular biochemical dynamics is again stabilized.

After the formation of cell types, the chemical compositions of each group are inherited by their daughter cells. In other words, chemical compositions of cells are recursive over divisions. The biochemical properties of a cell are inherited by its progeny, fixed or determined over the generations. After several divisions, such initial condition of units is chosen to give the next generation of the same type as its mother cell.

Experiment

The above theory can be generally applied to developmental process in the present multi-cellular organisms. However, the present organisms adopt further detailed mechanisms in addition to the above process, to stabilize it. Then, it is important to study experimentally a multicellular system as simple as possible. As this constructive experiment, Yomo's group, [21], by using *E. coli* cells, found that in a highly dense culture (with strong interaction), the *E. coli* cells spontaneously differentiate into a few groups with different enzyme activities, although they have identical genes.

3. Differentiation from Stem Cell

Question: In development of multi-cellular organisms, there exists a cell, called the stem cell, that either proliferates or differentiates to several other cell types. There exists a rule for such differentiation from multipotent stem cells. How are such differentiation rules generated? How can several cell types coexist stably under fluctuations?

Logic

We discuss this problem by extending the isologous diversification to include chaotic dynamics. Two fundamental features of stem cell systems - stochastic differentiation of stem cells and the robustness of a system due to regulation of this differentiation - are found to be general properties of a system of interacting cells exhibiting **chaotic itinerancy** for intra-cellular reaction dynamics.

Results from Model Simulations

The most interesting example in the model discussed in §2 is the formation of stem cells. In an example given in Fig.1c [2], a cell type, denoted as "S", either reproduces the same type or forms different cell types, denoted for example as type A and type B (i.e., $S \to S, A, B$). iType B cell replicates as $B \to B$), while the type A cell either eplicates or differentiates as $A \to A, A1, A2$. The type A1 and A2 cells are determined (i.e., $A1 \to A1$, $A2 \to A2$). This hierarchical organization is often observed when the internal dynamics have some complexity, such as chaos.

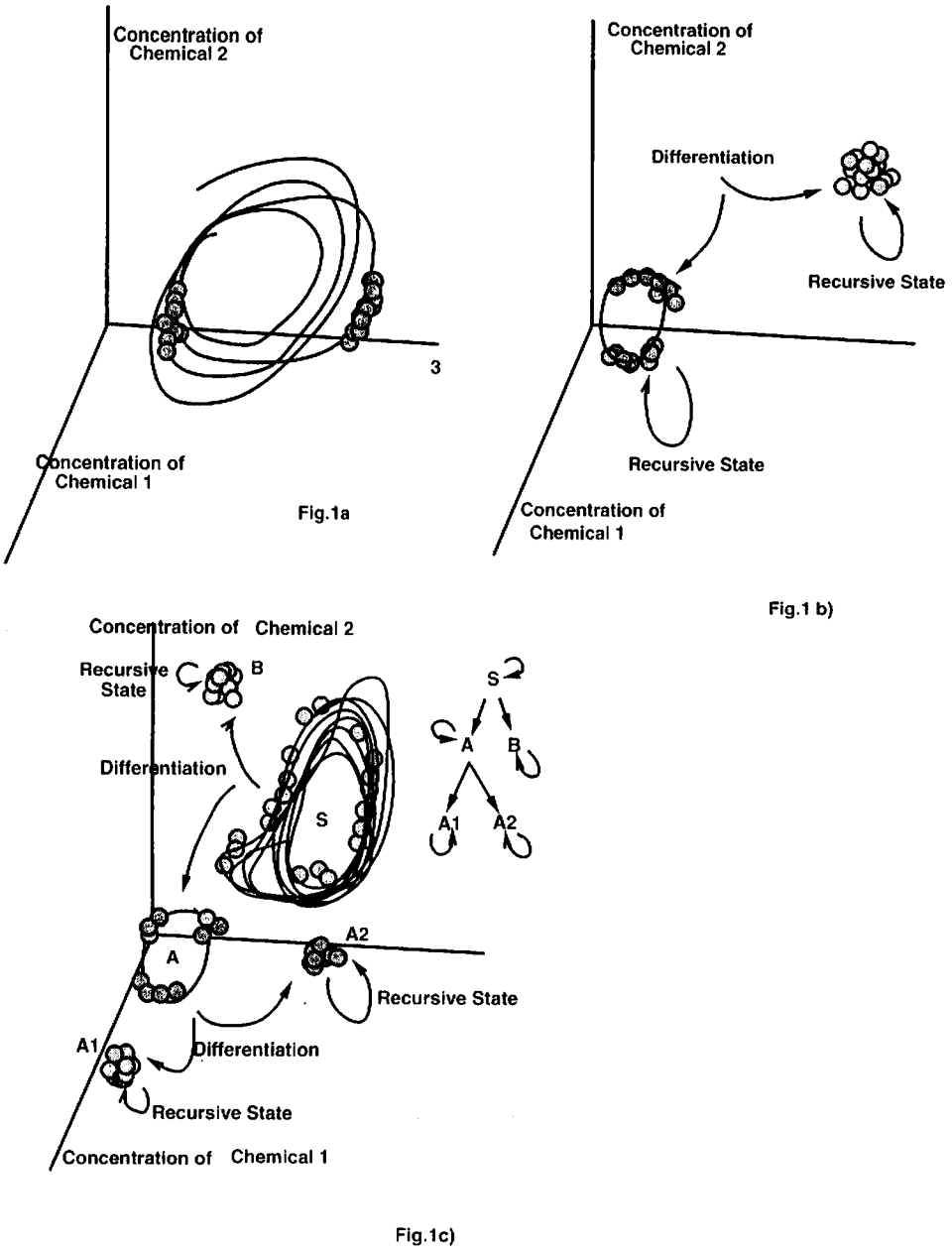

FIGURE 1. Schematic representation of cell differentiation process, plotted in the phase space of chemical concentrations.

It is found that stem cells differentiate into other cell types stochastically due to instability caused by cell-cell interactions.

The differentiation here is "stochastic", arising from chaotic intra-cellular chemical dynamics. The choice for a stem cell either to replicate or to differentiate looks like stochastic as far as the cell type is concerned. Since such stochasticity is not due to external fluctuation but is a result of the internal state, the probability of differentiation can be regulated by the intra-cellular state. This stochastic branching is accompanied by a regulative mechanism, as will be discussed in the next section.

4. Stability and Irreversibility in Development of a Cell Society

Question: In development of multi-cellular organisms, there is stability both at microscopic and macroscopic levels. The developmental process is stable against molecular fluctuations, and also against macroscopic damage such as elimination of some cells. How does stability emerge in a multi-cellular system? Also, there is a successive determination from totipotent ES cell, to multipotent stem cells, and finally to several determined cell types. There exists a clear timeline in the direction of the loss of potency to form a variety of cell types, in the normal development course. How is such irreversibility generated and how is it characterized quantitatively? Is such irreversibility related with developmental stability?

Logic

Using the model studies in §3 and 4, and also by examining several other models, it is shown that the cell types and their number distribution are robust against perturbations [3,6,15] Furthermore, it is shown that the irreversible loss of multipotency accompanying the change from a stem cell to a differentiated cell is a general course of interacting cell systems, and is characterized by a decrease in the intracellular chemical diversity and in the complexity of the cellular dynamics.

Results from Model Simulations

First, intra-cellular dynamics of each cell type in our models are stable against such perturbations. Then, one might think that this selection of each cell type is nothing more than a choice among basins of attraction for a multiple attractor system. If the interaction were neglected, a different type of dynamics would be interpreted as a different attractor. In our case, this is not true, and cell-cell interactions are necessary to stabilize cell types. Given cell-to-cell interactions, the cell state is stable against perturbations on the level of each intra-cellular dynamics. The concept of "partial attractor" is proposed in [13], to explain this situation.

Second, each cellular state is also stable against perturbations on the cell distribution. The macroscopic stability is clearly shown in the spontaneous regulation of differentiation ratio in the last section. Depending on the distribution of the other cell types, the orbit of internal cell state is slightly deformed. For a stem cell case, the rate of the differentiation or the replication (e.g., the rate to select an arrow among $S \to S, A, B$) depends on the cell-type distribution. For example, when the number of "A" type cells is reduced, the orbit of an "S-"type cell is shifted towards the orbits of "A", with which the rate of switch to "A" is enhanced [2].

If each cellular state were an attractor of internal chemical dynamics, the microscopic stability of a cellular state would be achieved. In this case, however, neither the macroscopic stability of a cellular state nor the stability of developmental process (how each cell type appears in the time course) is explained. Thus the cell-cell interaction is important. On the other hand, if the cell type number distribution (accordingly the interaction) is changed up to some degree, each intracellular dynamics keep its type. Hence, discrete, stable types are formed through

the interplay between intra-cellular dynamics and interaction. The recursive production is attained through the selection of initial conditions of the intra-cellular dynamics of each cell, so that it is rather robust against the change of interaction terms as well.

In a real organism and in our model, there is a clear temporal flow, as for the loss of multipotency. Initial ES cells have totipotency to create all other types of cells, then stem cells have limited multipotency. The cells at later stage lose their multipotency. In our model simulation, this process is characterized by the change of the following quantities [6,15]:

(I) Increase of the stability of intra-cellular state
(II) Decrease in the diversity of chemicals in a cell
(III) Loss of chaotic instability in the intra-cellular dynamics

The degree of (I) could be determined by a minimum change in the interaction to switch a cell state, by properly extending the "attractor strength" introduced in [11]. The degree of determination is roughly measured as the minimum perturbation strength required for a switch to a different state. The diversity (II) is computed, for example, by the Shanonn entropy of the chemical concentrations over all chemical species, i.e. $-\sum_j p(j) log p(j)$ with $p(i) = x(i)/\sum_j x(j)$. Initial totipotent cells have high diversity, which decreases in the course of differentiation. For details and for (III), see [6].

As a related constructive experiment, note controlled tissue generation by Asashima's group [1]. After taking undifferentiated cells from the animal cap of *Xenopus*, they put them into the solution of a protein "activin" for a while, and then cultured the cells, to examine how they develop later. By changing only the concentration of activin or few other chemicals, they succeeded in forming almost all tissues of the frog. The following two points should be noted in relationship with theoretical results. (i) jump-over — generation of tissues, jumping over normal temporal course of development. (ii) community effect – the formation of tissue is highly dependent on the number of cells. These two features show that naive picture for threshold mechanism for development is not sufficient. The feature (i) suggests the existence of attractors or attracting states as a dynamical system at a tissue level. The feature (ii) suggests that the differentiation is determined through interplay between intracellular dynamics and a cell ensemble state, as adopted in our study.

5. Pattern Formation and Origin of Positional Information

Question: Biological pattern formation (morphogenesis) is understood as a change of genetic expression depending spatially on the gradient of chemicals. To encompass the macroscopic pattern formation, it is often discussed that the positional information is formed according to the gradient of some chemical concentration which influences the concentration of such signal molecules that changes genetic

expressions. However, how is such positional information generated? To form gradients, is spatial differentiation of cells necessary. Therein lies a question, which is the first, positional information or differentiation of cells in space?

Logic

Including the spatial structure with diffusion of chemicals, we show that the differentiated types by the mechanism discussed so far are organized partially to form a pattern that is robust against perturbation. With this pattern formation, gradients of chemicals are formed that consolidate the differentiation of cell types. Accordingly positional information and dynamic differentiation mutually reinforce and stabilize each other. Dynamic differentiation is transferred to a spatial pattern.

Indeed, by extending the models in §2-4, to include spatially local interaction, the above logic for the emergence of positional information is obtained. See [3–5, 7] for details.

6. Origin of Bioinformation

Question: Among many chemicals in a cell only some chemicals (e.g., DNA) are regarded to carry genetic information. Why do only some specific molecules play the role to carry the genetic information? How have roles in molecules been separated into information carrier and metabolism? Is the separation a necessary course of a replicating cell with internal biochemical reaction dynamics?

Logic

As a step in an investigation of the origin of genetic information, we study how some species of molecules are preserved over cell generations and play an important role in controlling the growth of a cell. We consider a model consisting of protocells. Each protocell contains mutually catalytic molecule species. Through divisions of the protocells, the system reaches and remains in a state in which there are only a few highly catalytic molecules, preserved through selection of very rare fluctuations. In this state, the molecules with high catalytic activities, minor in population, are shown to control the behavior of the protocell. This minority molecule species acts as the carrier of heredity, due to the relatively discrete nature of its population, in comparison with the majority species which behaves statistically in accordance with the law of large numbers. The relevance of this minority controlled state to evolvability is discussed [20].

Experiment

As an experiment corresponding to this problem, note the experiment by Matsuura et al. [22], who succeeded in constructing an *in-vitro* replication system. The system consists of enzymes for the synthesis of DNA, DNA, and RNA. Through mutual catalytic process the whole set of chemicals is replicated by itself. It is also shown that the system has evolvability only if the number of DNA in the system is only few. Otherwise, the system gradually loses its activity to replicate itself. These experimental results are consistent with the minority control theory presented.

7. Origin of a Cell with Recursive Growth

Question: A cell consists of several replicating molecules that mutually help the synthesis and keep some synchronization for replication. At least a membrane that partly separates a cell from the outside has to be synthesized, keeping some degree of synchronization with the replication of other internal chemicals. How is such recursive production maintained, while keeping diversity of chemicals? Furthermore this recursive production is not complete, and there appears a slow "mutational" change over generations, which leads to evolution. How are evolvability and recursive production compatible?

Logic

To answer the above question, we study a system with many chemicals which catalyze mutually. These mutual catalyzations form a network. The dynamics of chemical concentrations are often chaotic in nature, and for some conditions, they show chaotic itinerancy in which a quasi-recursive chemical state is maintained over generations, and then switches from it through unstable chemical states. Here, concentrations of some chemicals are so low, and the number discreteness is important. Then the above chaotic itinerancy is highly influenced by whether the number of such minority chemicals is zero or not. Through the on-off switch of such minority molecule numbers, the system shows successive switches over quasi-recursive states. This leads to the evolution of recursive states [14].

8. Discussion

To sum up the studies on cell differentiation and development so far, supports are given to the following conjecture.

Assume a cell with internal chemical reaction network whose degrees of freedom is large enough. Cells interact with each other through the environment. Some chemicals are transported from the environment. Through this process the cell volume increases and the cell is divided. If the initial cell state is quite stable, no differentiation occurs, when the competition for chemical resources is so high that the cell division stops at some stage. On the other hand, for some reaction networks, cells differentiate and the cell number continues to increase. Hierarchical rule for cell differentiation is generated. The initial cell types have large chemical diversity with temporal change of chemical concentrations. As the number of cells increases and the differentiation progresses, irreversible loss of multipotency follows. The realized states of cell types are temporally fixed while the number distribution of such cell types are stable against perturbations, following the spontaneous regulation of differentiation ratio.

When spatial factor is involved, the above differentiation induced by dynamical instability is consolidated to spatial pattern. With this process gradients of chemical concentrations are organized that provide positional information. The pattern formation with differentiation and positional information stabilize each other.

For the above development, the role of genes is not explicitly implemented. Still, the gene expression is relevant to stabilize the dynamics-originated differentiation. In a system with complex reaction network, it is shown that some molecules in minority start to carry the information, with regards to control of other molecules, and preserved transfer to the next generation. Whether the number of minority molecule is zero or non-zero is crucial to the behavior of a cell, which provides a basis for "programmable behavior" in a biological system.

To sum up, our viewpoint can be summarized as "*plastic dynamics first, consolidation to information later*". Throughout the present research, the importance of plastic dynamics in a biological system is emphasized, in contrast to a viewpoint of life as a tightly determined, highly programmed system.

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