Isologous Diversification: A Theory of Cell Differentiation

Kunihiko Kaneko
Department of Pure and Applied Sciences
University of Tokyo, Komaba, Meguro-ku, Tokyo 153, JAPAN

and

Tetsuya Yomo
Department of Biotechnology
Faculty of Engineering
Osaka University 2-1 Suita, Osaka 565, JAPAN

Abstract

Isologous diversification theory for cell differentiation is proposed, based on simulations of interacting cells with biochemical networks and cell division process following consumption of some chemicals. According to the simulations of the interaction-based dynamical systems model, the following scenario of the cell differentiation is proposed. (1) Up to some threshold number, divisions bring about almost identical cells with synchronized biochemical oscillations. (2) As the number is increased the oscillations lose the synchrony, leading to groups of cells with different phases of oscillations. (3) Amplitudes of oscillation and averaged chemical compositions start to differ by groups of cells. The differentiated behavior of states is transmitted to daughter cells. (4) Recursivity is formed so that the daughter cells keep the identical chemical character. This “memory” is made possible through the transfer of initial conditions. (5) Successive differentiation proceeds.

Mechanism of tumor cell formation, origin of stem cells, anomalous differentiation by transplantations, apoptosis and other features of cell differentiation process are also discussed, with some novel predictions.

(Keywords: differentiation, chemical network, cell division, clustering, open chaos)

1 Introduction

1.1 Biological background

Development of organisms from their fertilized eggs is one of the most elegant emergence in biology and has been investigated by many cellular and molecular biologists to elucidate how different types of cells appear and organize beautiful structure of a matured body. Many of the essential genes for the body plan have been identified. Each gene, responding to the products from the other genes, turns on or off so as to give different physiological states and to produce a variety of cells.

The gene network picture is expressed by “canalization”. Depending on initial conditions, there are a variety of final states as is expressed schematically by the landscape in Fig. 1a). In the term of dynamical system theory, there are many attractors in the
network system. If the initial condition for canalization is given by the initial gene expression and/or by the environments, a cell differentiates into one of the attractors according to their basins. A beautiful and pioneering study is given by Kauffman (1969) to demonstrate that the Boolean networks of genes have a variety of final states. His work clearly shows that with various initial conditions the gene network leads to the existence of a variety of cell types under a single external condition. However, needless to say, a single initial condition embedded in a fertilized egg produces several different cell types. Thus, the following essential question towards the gene network picture remains: How do the different initial and/or external conditions arise, leading to different cell types through the developmental process? How can the differentiation process be robust against perturbations to initial and external conditions?

There are some experiments which point out the relevance of cellular interactions to internal states of a cell and to the robustness against perturbations. One of the authors and his colleagues reported (Ko et al., 1994) that even under a single external condition cells differentiate to some distinct physiological states. In their experiment, E. coli was successively cultivated in a well-stirred liquid culture in order to impose the same external condition on each of the cells. The population of the E. coli was shown to include distinct cell types. The fraction of each cell type exhibited a complex oscillation in the time course. Moreover, it was shown that in a few repeats of the single colony isolation, some colonies inherit the physiological state, while the others present the state other than that their parental colonies exhibit. It is unlikely that each cell of E.coli in the culture from a single cell exhibits different initial conditions in its gene network. Thus, the experiments show that under the same initial and external conditions, the cells can autonomously differentiate.

It has been established that by transplant experiments, some cells, changing their fate, dedifferentiate and come to a different cell type. Therefore, intercellular relationship is essential to the determination of a cell type. The experimental results show that the differentiation process is dynamic in principle, and the fate of a cell is determined dynamically through the interactions with environment or with other cells. Rubin, in a series of papers, has shown that a cell line( NIH 3T3) from a mouse epigenetically transforms to some different types of foci in size under the same condition (Yao and Rubin 1994; Chow et al., 1994, Rubin 1994a,1994b). In addition, the frequency of transformation and the type of transformed cells depend on the cell density and the history of the cell culture before the transformation. This, of course, does not deny possible roles of mutations but it at least suggests the relevance of intercellular interactions to the cell transformation or differentiation. The importance of interaction to differentiation has also been pointed out, for example, by Goodwin (1982), and Newman (1990). However we believe that none has taken fully into account of interaction-induced and dynamic viewpoints successfully. We, hereby propose a novel theory of cell differentiation based on a dynamic and cell-interaction viewpoint.

It may be useful to state the standpoint of our theory and modeling, in advance. We do not aim at giving a model with one-to-one correspondence with biological facts at the present. Rather we present an abstract model and discuss its general features, to show that a prototype of cell differentiation emerges even without implementing a programmed switching process of genes. It should be noted that the differentiation progresses with autonomous choice of initial conditions of internal cellular states through interactions. The process is shown to be a general consequence of dynamical systems of reproducing and interacting units with internal degrees of freedom.

To make one-to-one correspondence with experimental observations, models with detailed physiological information must be required. Our model may look rather premature with regards to such correspondence. However, on the other hand, our theory and mod-
eling are essential to understand how a cell society and differentiated multi-cellular organisms emerge without sophisticated programs implemented in advance. The theory also provides a novel viewpoint to several problems in cell biology, such as the transformation and apoptosis.

1.2 Isologous diversification theory

In the present paper we propose a novel viewpoint of cell differentiation, which satisfies the dynamic, and interaction-based picture and also explains the inheritance of cell types through an initial condition of chemicals in cells.

The background of this theory lies in the dynamic clustering in globally coupled chaotic systems (Kaneko, 1989, 90, 91, 92), where chaos leads to differentiation of identical elements through interaction among them. The relevance of dynamic change of relationships among elements to biological networks has been discussed (Kaneko 1994). Even if each oscillatory element does not show chaotic behavior, the dynamic clustering appears when phase differences of oscillators are amplified through the interaction among them. Cell differentiation provides such an example (Kaneko and Yomo 1994).

One important missing factor in the dynamical systems theory is the change of degrees of freedom. Cells divide to create a new set of dynamical variables. Previously we introduced the term “open chaos” to address the instability in conjunction with the change of the degrees of freedom (Kaneko and Yomo 1994). In open chaos small deviation is amplified, which finally leads to the change in the dimension of the phase space itself unlike those in chaos.

Based on these dynamical systems studies and simulations of the cell differentiation model (to be presented), we propose “isologous diversification theory” as a general mechanism of spontaneous differentiation of replicating biological units. Here we adopt the term “isologous”, in contrast with “homologous”, to stress our general mechanism that any “identical” (rather than similar) units naturally differentiate through interactions.

It is useful to describe the basic framework of the theory here to facilitate the understanding of the later simulation results. The “isologous diversification” is summarized as follows (see also Fig.2 for schematic representation):

Let us take biological units (e.g. cells), interacting with each other, and with ability of reproduction. A state of each unit has internal dynamics (e.g., biochemical reaction) which allows for nonlinear oscillation (through e.g., autocatalytic reaction). Through this inter- and intra-unit dynamics, the total system consists of coupled nonlinear oscillator units. As the number of units increases by the reproduction, they are differentiated spontaneously, through the following stages:

1. **Synchronous oscillations of identical units.** Up to some threshold number of units, all of them oscillate synchronously, and their states are identical.

2. **Differentiation of the phases of oscillations of internal states.** When the number of units exceeds the threshold, they lose identical and coherent dynamics. Although the state of each unit is different at an instance, averaged behaviors over oscillation periods are essentially the same. Only the phase of oscillations differs by units.

The emergence of the stage is a general consequence of the dynamic clustering (Kaneko, 1989,90). It is expected that the oscillators split into clusters with different phases of oscillations, when there is strong interaction among them.

3. **Differentiation of the amplitudes of internal states.** At this stage, the states are different even after taking the temporal average over periods. It follows that

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1 The use of the term “isologous” is suggested by Susumu Ohno.
the behavior of states (e.g., composition of chemicals, cycles of oscillations, and so on) is differentiated. The clustering of units with regards to amplitudes here is again a nature of coupled oscillators when the interaction is suitably chosen.

(4) Transfer of the differentiated state to the offsprings by reproduction.
Each type of a differentiated cell is preserved to its offsprings. Chemical composition of a cell attains recursivity with respect to divisions. Thus a kind of “memory” is formed, through the transfer of initial conditions (e.g., of chemicals) during the reproduction (e.g., cell division). By reproduction, the initial condition of a unit is determined to give a unit of the same type at the next generation.

(5) Hierarchy of organized groups. This stage is the result of successive differentiation with time. Thus, the total system consists of units (cells) of diverse behaviors, leading to a heterogeneous society.

As mentioned, the above stages are based on the general features of coupled dynamical systems. With the reproduction of units, the interaction among them gets stronger and leads to successive diversification of the behavior. Thus the identical elements tend to be diversified through the interplay of nonlinear oscillations, interaction, and the change of degrees of freedom (e.g., the number of cells).

The fourth stage is conceptually new. The observed memory there lies not solely in the internal dynamics but also in the interactions among the units. If one is concerned with the internal dynamics, the memory should be determined by the basin for the attractors of internal dynamics, as in Fig. 1a). The final destination of the balls in the figure corresponds to the memory. However, there remains two factors that need to be considered. One is the division process, which increases the degrees of freedom (Fig. 1b). The other is the interaction among the units, which brings about the differentiation. We believe that this emergence of recursivity or memory is a general feature of coupled dynamical systems with varying degrees of freedom (e.g., the number of cells), and thus is essential to the information and memory in biological systems.

An important consequence of our theory is global stability. The obtained distribution of types of units (cells) is robust against external perturbations. Noise at the division process may change the destiny of some individual cells, but the number distribution of cell types is only weakly influenced by it. Indeed this macroscopic robustness is derived naturally from our interaction-based picture. In the study of coupled nonlinear dynamical systems, the stability of collective behavior is theoretically confirmed (Kaneko 1990,92).

Putting the above processes into biological terms, each unit (cell) takes resources (nutrition) for reproduction (cell division). First, the existence of oscillatory (biochemical) dynamics in each unit (cell) is a natural assumption as will be discussed. The differentiation of phases (at the first stage) is the establishment of the time sharing system for resources, since the ability to get resources generally depends on the internal state of a cell. For example, it may be interesting to note that different regions of the DNA replicate at different characteristic times during the cell cycle (Alberts et al. 1983). It is also known that the cell cycle (of divisions) loses synchronization spontaneously as in our first stage, although it is often attributed to statistical events rather than deterministic instability (Alberts et al. 1983). The third stage is no other than the division of labors in several biochemical reactions in the cell, since the differentiated units utilize different resources (chemicals). The fourth stage where the differentiated feature is epigenetically inherited through reproduction, provides an essential way of maintaining the diversity among the units (cells). Lastly, the fifth stage is simply what gives the complexity of organisms as

2The later stages are not necessarily chronologically separated. The stage (5) often proceeds with (3) and (4), while the stage (4) can occur with (3).
we portrayed. Through this process, a cooperative society of units emerges as a higher level.

1.3 Cell differentiation model

Although the isologous diversification is proposed as a rather general scenario for biological systems, it is most important to verify the scenario for the cell differentiation problem through simulations of a specific model. With this demonstration, we provide a coherent answer to the problem raised in §1.1.

First we note that it is rather natural to assume some oscillatory behaviors in cellular chemical reactions. Indeed, oscillations are observed in some metabolic cycles (Hess 1971) as in the concentration of Ca$^{2+}$, cyclic AMP, NADH and so on, while there are cyclic behaviors in cell divisions themselves, as in the oscillations of cyclin and MPF (M-phase-promoting factor) (Alberts et al. 1983). Thus dynamical systems approach to the cell differentiation is a rather natural postulate from physiology. The importance of oscillatory dynamics in cellular systems has been pointed out by Goodwin (1963, 1982).

Here we adopt autocatalytic reaction networks in each cell, while interactions among cells are considered through the medium contacting with cells. It should be noted that the chemicals here include those associated with the genetic expressions, and even the components of DNA. Thus our model is compatible with the picture by a genetic switch network. Indeed, in our simulations, some chemicals turn to be activated after some divisions in consistency with expressions of genes by switchings. Since gene expressions are tightly linked with intracellular chemical reactions, which are subject to intercellular changes, the cell differentiation satisfies the postulates of the isologous diversification.

Previously we proposed a simple model of cell differentiation, by including the cell division process, besides the cellular interactions. Through simulations of this simple model, we have found the clustering of chemical oscillations by cells at the initial stage, and then the differentiation to rich and poor cells at the later stage (Kaneko and Yomo 1994). In the present paper, we extend the model to study how cells are differentiated and determined successively into different types. (see also (Kaneko and Yomo 1995) for a brief report).

The proposed isologous diversification theory and our simulation results capture the essence of differentiation in the view of cellular biology. Our result covers from the loss of totipotency, origin of stem cells, hierarchical organization, differences in growth rates, to the importance of the tiny amount of chemicals that trigger differentiation. Some predictions are also made on the formation of tumors and their trans-differentiation.

In our model, the units are made to interact in a homogeneous environment (well stirred medium in biological sense), and hence there is no spatial variation. The differentiation is proposed to be brought about by a dynamic mechanism, in contrast with the (spatial) Turing instability. Indeed, our dynamic scenario is consistent with the experimental reports on the differentiation of cells in a well stirred medium (E.Ko et al, 1994). It is to be noted that the authors do not disregard the spatial effect that is important at a later stage of development for the spatiotemporal organization. Indeed, some preliminary studies including a spatial factor in differentiation suggest the validity of the present scenario and also the amplification of differentiation by spatial inhomogeneity at a later stage.

\[3\]In this respect, isologous diversification provides a logic for “major transitions” (Maynard-Smith and Szathmary, 1995).
1.4 Organization of the paper

The present paper is organized as follows. In §2, we present our model within a rather general framework. Before showing the cell differentiation process, we give a few remarks on the chemical dynamics within each cell in §3, in relation with the structure of chemical network. Explicit examples of dynamic differentiation are given in §4. Following these results, we propose a general scenario of cellular differentiation in §5, while some additional analysis of the scenario is given in §6, based on dynamical systems theory. We discuss the initiation of differentiation, chemical “division of labors”, formation of tumor-like cells, and simultaneous multipled deaths. In §7, some results on the numerical experiments of cell transplantations are given, from which the significance of cellular memory is clarified. §8 is devoted to summary and discussions.

2 Model

The biochemical mechanisms of cell growth and division are very much complicated, including a variety of catalytic reactions. The reaction occurs both at the inter- and intra-cellular levels. Here we study a class of models which captures such biochemical reaction and inter-cellular interactions.

Our model for cell society consists of

- (1) Biochemical Reaction Network within each Cell: Intra-cellular Dynamics
- (2) Interaction with Other Cells through Media: Inter-cellular Dynamics
- (3) Cell Division
- (4) Cell Death

Our scenario to be proposed is independent of the details of modeling as long as the items (1)-(3) are included. For simulations, however, we need a specific model. Here one example of such models is given, to propose a dynamic scenario of cell differentiations. The basic structure of our model is same as the previous one (Kaneko and Yomo 1994), although the present model includes a biochemical network rather than a simple set of reactions, to cope with the complexity in cell systems. See Fig.3a) for schematic illustration of our modeling.

(A) Internal Reaction

First we adopt a set of \( k \) chemicals’ concentrations as dynamical variables in each cell, and also those in the medium surrounding the cells. Here chemicals are not specified. They may include chemicals associated with genetic expressions, as well as the “metabolic” process in a very broad sense.

Based on the argument in (Kaneko and Yomo 1994), we use the following variables; a set of concentrations of chemical substrates \( x_i^{(m)}(t) \), the concentration of the \( m \)-th chemical species at the \( i \)-th cell, at time \( t \). The corresponding concentration of the species in the medium is denoted as \( X^{(m)}(t) \). We assume that the medium is well stirred, and neglect the spatial variation of the concentration. Furthermore we regard the chemical species \( x^{(0)} \) (or \( X^{(0)} \) in the media) as playing the role of the source for other substrates.

The reactions \( m \rightarrow \ell \) are usually catalyzed by enzymes, which are inductive and are again synthesized with the aids of other chemicals \( x^{(j)} \). If this synthetic reaction is linear in \( x^{(j)} \), the concentration of the corresponding enzyme \( E_j^{m \rightarrow \ell} \) obeys the dynamics

\[
\frac{dE_j^{m \rightarrow \ell}}{dt} = \text{const.} \times x^{(j)} - \delta E_j^{m \rightarrow \ell}.
\]

Assuming, for simplicity, fast dynamics for enzymes, we adiabatically solve the above reaction equation of enzyme concentrations, to get \( E_j^{m \rightarrow \ell} \propto x^{(j)} \).
Let us apply the Michaels-Mentens form for the reaction from \( x^{(m)} \rightarrow x^{(\ell)} \) aided by the enzyme \( E^{m-\ell}_j \). Thus the reaction from the chemical \( m \) to \( \ell \) aided by the chemical \( j \) leads to the term \( e_1 x^{(j)}_i(t)x^{(m)}_i(t)/(1 + x^{(m)}_i(t)/x_M) \), where \( x_M \) is a parameter for the Michaels-Mentens’ form, and \( e_1 \) is the coefficient for the reaction.

Summing up, \( x^{(\ell)} \) is produced with the path from the chemical \( m \), with the aid of chemical \( j \). Here \( j \) and \( m \) depend on \( \ell \), and generally there can be several paths for the production of \( m \). Here we use the notation \( Con(m, \ell, j) \) which takes the value 1 when there is a path from the chemical \( m \) to \( \ell \) catalyzed by the chemical \( j \), and takes 0 otherwise. In the present paper the coefficients \( e_1 \) and \( x_M \) are identical for all paths.

In addition, we assume that there are paths from the source chemical and to a “division factor”. The source is a nutrition-type chemical for others, while the division factor includes chemicals synthesized and to be utilized by the division, such as the lipids for membranes, ATP, or DNA. Here we do not allocate it with a specific chemical, but it is assumed that there is a threshold for the synthesis of the division factor (e.g., DNA) to the cell division as will be given in the process (C).

The paths from the source chemical \( x^{(0)} \) lead to the term \( S(\ell)e_0x^{(0)}_t(t)x^{(\ell)}_t(t) \) where \( S(\ell) = 1 \) when there is a path from 0 to \( \ell \), and 0 otherwise. The path to the final product from some chemicals \( x^{(\ell)} \), leading to a linear decay of \( x^{(\ell)} \), with a coefficient \( \gamma \). This term is expressed by \( \gamma P(\ell)x^{(\ell)}_t(t) \), where \( P(\ell) = 1 \) if there is a path from the chemical \( \ell \) to the final product, and otherwise \( P(\ell) = 0 \). Summing up all these processes, we obtain the following contribution of the chemical network to the growth of \( x^{(\ell)}_t \) (i.e., \( dx^{(\ell)}_t(t)/dt \)):

\[
\text{Met}_{i}^{(\ell)}(t) = S(\ell)e_0x^{(0)}_t(t)x^{(\ell)}_t(t) + \sum_{m,j} Con(m, \ell, j)e_1x^{(j)}_i(t)x^{(m)}_i(t)/(1 + x^{(m)}_i(t)/x_M) - \sum_{m', j'} Con(\ell, m', j')e_1x^{(j')}_i(t)x^{(m)}_i(t)/(1 + x^{(m)}_i(t)/x_M) - \gamma P(\ell)x^{(\ell)}_t(t),
\]

where we note that the terms with \( \sum Con(\cdots) \) represent paths coming into \( \ell \) and out of \( \ell \) respectively. Here the chemical network can include metabolic reactions and/or those related with genetic expressions.

The biochemical reaction here is schematically shown in Fig.3 b). When \( m = \ell \), the reaction is regarded as autocatalytic, in the sense that there is a positive feedback to generate the chemical \( k \). (In general, it is natural to assume that a set of chemicals works as an autocatalytic set.) Later we will study the case with autocatalytic reactions only, in a more detail.

(B) Active Transport and Diffusion through Membrane

A cell takes chemicals from the surrounding medium. Interactions between cells, thus, occur through the medium. It is natural to assume that the rates of chemicals transported into a cell are proportional to their concentrations outside. Further we assume that this transport rate also depends on the internal state of a cell. Since the transport here requires energy (see e.g., Alberts et al (1983)), the transport rate depends on the activities of a cell. To be specific, we choose the following form:

\[
\text{Transp}_{i}^{(m)}(t) = p(\sum_{\ell=1} x^{(\ell)}_i(t))X^{(m)}(t)
\]

The summation \( (\sum_{\ell=1} x^{(\ell)}_i(t)) \) is introduced here to mean that a cell with more chemicals is more active. Here we choose this bi-linear form for simplicity, although nonlinear dependence on \( \sum_{k=1} x^{(k)}_i(t) \) (i.e., with a positive feedback) leads to qualitatively similar results. Besides the above active transport, the chemicals spread out through the membrane with normal diffusion by

\[\text{With regards to the interplay between metabolic reaction and the cell division factor, the present model may have a common feature with the “chemton model” by Gant(1975).}\]
Combining the processes (B) and (C), the dynamics for \( x_i^{(m)}(t) \) is given by

\[
\frac{dx_i^{(0)}(t)}{dt} = -e_0x_i^{(0)}(t)\sum_\ell x_\ell(t) + Transp_i^{(0)}(t) + Diff_i^{(0)}(t),
\]

\[
\frac{dx_i^{(f)}(t)}{dt} = Met_i^{(f)}(t) + Transp_i^{(f)}(t) + Diff_i^{(f)}(t),
\]

Since the present processes are just the transportation of chemicals through the membrane of a cell, the sum of the chemicals must be conserved. If the volume of the medium is \( V \) in the unit of a cell, the chemical in the medium is diluted by this factor, and we get the following equation for the concentration of the medium:

\[
\frac{dX^{(m)}(t)}{dt} = -(1/V)\sum_{i=1}^N\{Transp_i^{(m)}(t) + Diff_i^{(m)}(t)\} - D_{out}X^{(m)}(t),
\]

where the last term corresponds to the outflow (washout) of chemicals to the outside of the medium.

Since the chemicals in the medium can be consumed with the flow to the cells, we need some flow of chemicals (nutrition) into the medium from the outside. Here only the source chemical \( X^0 \) is supplied by a flow into the medium. By denoting the external concentration of the chemicals by \( \overline{X}^0 \) and its flow rate per volume of the medium by \( f \), the dynamics of source chemicals in the medium is written as

\[
\frac{dX^{(0)}(t)}{dt} = f(\overline{X}^0 - X^0) - (1/V)\sum_{i=1}^N\{Transp_i^{(0)}(t) + Diff_i^{(0)}(t)\}.\]

(C) Cell Division

Through chemical processes, cells can replicate, which requires consumption of ATP, formation of membrane, and replication of DNA and so on. In our model the division factor, generated from some chemical species, is assumed to act as the chemical for the cell division. Thus it is rather natural to introduce the following condition for cell division: The cell \( i \) divides when

\[
\int_{t_0(i)}^T dt \sum_\ell \gamma P(\ell)x_\ell(t) > R
\]

is satisfied, where \( R \) is the threshold for cell replication, and \( t_0(i) \) is the time of the birth of the cell (i.e., the previous division). Here again, choices of other similar division conditions can give qualitatively same results as those to be discussed. The essential part for the division condition is that it satisfies an integral form representing the consumption.

When a cell divides, two almost identical cells are formed. The chemicals \( x_i^{(m)} \) are almost equally distributed. “Almost” here means that each cell after a division has \((1/2 + \epsilon)x_i^{(m)}\) and \((1/2 - \epsilon)x_i^{(m)}\) respectively with a small “noise” \( \epsilon \), a random number with a small amplitude, say over \([-10^{-3}, 10^{-3}]\). Although the existence of imbalance is essential to the differentiation in our model and in nature, the mechanism or the degree of imbalance is not important for the differentiation itself. Indeed any tiny difference is amplified to yield a macroscopic differentiation, resulting in the same population distribution of differentiated cells later\[5\]. The essence of our cell differentiation lies in the amplification process by open chaos.

\[5\text{Of course, the (almost) equal partition is not necessary for the differentiation of our simulation. We use this partition, to stress the intrinsic mechanism of differentiation. By adopting unequal division, our} \]
Since \( x_i^{(m)} \) stands for the concentration, rather than the amount, it might look strange to make the concentration half by division. Here we assume that the volume of a cell is approximated to be constant except for a short span for the division. During the short span for the division, the volume gets twice and thus the concentration is made half in the above process. In other words, we approximately separate the stages of the volume expansion and chemical process. Another possible interpretation is that the biochemical reaction process occurs within a limited region of a cell, which is not affected by the growth of a cell size itself.

It is also possible to model the reaction process, including the growth of the cell volume explicitly. In this case, an additional term for dilution is included in eqs.(4) and (5), given by \(-x_i^{(m)}(t) \frac{dV_{cell}}{dt} / V_{cell}\), with \( V_{cell} \) as the cell volume, which increases in proportion to the consumption of the division factor. When this term is included, the division to half should be replaced by the preservation of \( x_i^{(m)}(t) \) by the division. Indeed we have confirmed, in several simulations, that this modification of the model does not make any essential differences with regards to the qualitative behaviors.

(D) Cell Death

In some simulations, we impose a deterministic condition for cell death. Here we adopt the following condition for the death:

\[
\sum_{j=1}^{k} x_i^{(j)}(t) < S, \tag{9}
\]

where \( S \) is a threshold for “starvation”. The choice of the death process is again rather arbitrary. We have assumed that a cell dies when the chemicals included therein are too little, although a choice of similar forms is expected to give the same results. Here, the chemicals inside the dead cells are released into the medium. Thus the concentration \( X^{(j)}(t) \) is added by \( x_i^{(j)} / V \) at every cell death.

3 Internal Chemical Dynamics

Before presenting the dynamics of our cell society, let us briefly describe the nature of chemical (metabolic) reaction given by eq.(1). Roughly speaking, the dynamics strongly depends on the number of autocatalytic paths. Here we choose a random network so that each chemical has a given number of outgoing autocatalytic chemical paths. If the number is large, only few chemicals are activated, and all the other chemicals’ concentrations vanish. Here no other chemical paths are active, since the ongoing reaction is just Source \( \rightarrow x^{(j)} \rightarrow \text{Division Factor} \), without any reactions \( x^{(j)} \rightarrow x^{(\ell)} \) (see Appendix 1).

When the number of autocatalytic paths per chemicals is small, on the other hand, many chemicals are generated, but the dynamics falls onto a fixed point without any oscillatory behavior. In the medium number of autocatalytic paths, non-trivial (metabolic) reactions appear. Some (not necessarily all) chemicals are activated. The concentrations of chemicals oscillate in time, which often shows a switching-like behavior. That is, chemicals switch between low and high values successively. Similar behavior is also seen in randomly connected Lotka-Volterra equations as saddle-connection-type dynamics (Sasa and Chawanya, 1995).

In Fig.4, we have plotted the time series of \( x^{(\ell)} \) by taking only one cell and medium, without imposing the division condition (i.e., the dynamics is given by two sets of chemicals for one cell and the medium), where periodic alternations of dominating chemical differentiation process is accelerated initially. Still, essentially the same differentiation process occurs with this unequal partition.
species are observed.

In the present paper we discuss cases with a medium number of autocatalytic paths, since they lead to non-trivial (metabolic) oscillations. Here the term “autocatalytic path” is not necessarily taken strictly. Chemicals autocatalytic “as a set” can be adopted in the chemical network. See Appendix 2 for an evolutionary account for the choice of autocatalytic networks.

4 Example of Differentiation Process

We have carried out several simulations of our model with the chemical number $k = 8$, $k = 16$, $k = 32$, and $k = 64$, taking a variety of randomly chosen chemical networks with connections from 2 to 6 per chemicals. Since typical behaviors are rather common, we present an example of simulation results by taking the network with $k = 8$ given in Fig. 5a) (with three randomly chosen autocatalytic paths per chemicals).

Up to some cell numbers, all cells have identical chemical concentrations at each instance, and oscillate synchronously. All the cell divisions occur simultaneously, and the cell number increases as $1, 2, 4, 8, \ldots$. When the number exceeds some threshold value, the oscillation is desynchronized as in Fig.6, where the timeseries of chemicals is plotted. In the figure, phases of oscillations of 8 cells split roughly into 2 groups. On the other hand, the snapshot values of chemicals at this stage are plotted in Fig.7 with respect to the cell index defined in the order of birth. (In the example in the figure, the differentiation starts when the cell number is 8). At this stage, the difference by cells, however, is seen only for snapshot values. The average chemical concentrations over several periods are almost identical.

When cells further divide, differences in chemicals start to be fixed by cells. Average chemical concentrations measured over periods of oscillations, as well as their compositions differ by cells. The chemicals averaged from the latest division time are plotted in Fig.8a)-d), for different temporal regimes. In Fig. 8a) (at $t = 280$), the 3rd, 6th, 12th, and 13th cells have different chemical compositions from others in the average. Thus two groups start to be formed around $t = 280$, while the fixation to distinct two groups is seen around $t \approx 400$. In Fig. 8b, three groups are formed that is the group of the 3, 6, 12, 13, 17, 18, 19, and 20th cells, and the group of 4, 8, 15, 16, 29-32nd cells, and that of the other cells. For $t > 600$, the distinction is much clearer as is seen in Fig 8c)-d). One group of cells has a larger ability of taking the source chemical $x^{(0)}$, since they have larger $\sum_j x^{(j)}$, with $\bar{x}$ as the temporal average. We call the term $\sum_j x^{(j)}$ as activity. A cell with larger activity is called strong(er) or active here, which divides faster than other cells.

In Fig. 9a)b) chemical oscillations of two stronger cells are plotted while that of the other group is given in Fig.9c). One can clearly see that the time series of Fig.9a) and 9c) are different in nature, while only the phase of oscillations differs between Fig.9a) and 9b). Time series of chemicals 1, 2, and 3 overlaid for all cells are given in Fig.10 a)-c), respectively, where differentiation to two (or more) groups is again discernible. The orbits of two groups lie in a distinct region of the phase space (see Fig.11), while the phases of oscillations remain different by cells within each group.

Here one notes that the difference by chemicals is prominent for chemicals 2 and 3, while that for chemical 1 is much smaller. (Chemicals 4 and 5 show similar behavior as 1). The clearest difference is seen in chemical 3, whose concentration is the smallest among chemicals. As will be discussed, this suggests the relevance of extremely dilute chemicals to differentiations.

It should be noted that the offspring of one group of cells preserves its feature here. In Fig. 8c), the 29, 30, 31, and 32nd cells are direct offsprings of the 4, 8, 15, and 16th cells.
At these later stages, differentiated features are transmitted to daughter cells. (Both of divided cells are called daughter cells throughout the paper, since there is no principal reason to distinguish the two right after the division).

As the cell number increases, further differentiation proceeds. As shown in Fig.8d), each group of cells further differentiates into two subgroups. There are more types of cells at this stage.

5 Proposed Scenario on Cell Differentiation

Several simulations with a variety of chemical networks show similar behaviors with those given in the previous section. In so far as we have checked, the following differentiation process starts at some cell number when a chosen chemical network allows for oscillations. Summing up these simulation results we arrive at the isologous diversification scenario for the cell differentiation (see Fig.2 for schematic representation). In our model the scenario is summarized as follows, where each item corresponds to each stage of the isologous diversification in §1.2.

(1) Synchronous oscillation of chemicals and synchronized division

Up to some number of cells, the chemical (metabolic) oscillations of all cells are coherent, and they have almost same concentrations of chemicals. Accordingly, the cells divide almost simultaneously, and the number of cells is the power of two. It is interesting to note that mammalian cells are not differentiated up to three times divisions. Our result suggests that the cell differentiation is triggered not by a gene which counts the cell division but through the cellular interaction.

(2) Clustering by phases of oscillations

As cells divide further, the chemical oscillations start to lose their synchrony. Cells separate into several groups with almost same phases of oscillations. As has been discussed (Kaneko and Yomo 1994, Kaneko 1994), this temporal clustering corresponds to time sharing for resources: Since the ability to get them depends on the chemical activities of cells, cells can get resources successively in order with the use of difference of phase of oscillations. Thus the competition is avoided although any control mechanism is not imposed externally.

At this stage, the differentiation is not yet fixed. In other words, only the phases of oscillations are different by cells, but the temporal averages of chemicals, measured over some periods of oscillations, are almost identical by cells. Cells are identical on the average. The difference of phase, however, is a trigger to the fixed differentiation at the next stage.

The cell number starting to show the differentiation depends on parameters of our model. When the nonlinearity in our model (e.g., $e_1$ or $p$ in our model) is weak the differentiation starts only after the cell number is large (e.g., 128), while with stronger nonlinearity, this stage starts around the cell number 8. For higher nonlinearity, this stage is not clearly discerned, and the next stage starts after few divisions.

This clustering corresponds to the second stage of the isologous diversification, and is explained from the studies of globally coupled dynamical systems (Kaneko, 1989, 1990).

(3) Fixed differentiation

After some divisions of cells (for example, at the stage of 32 cells) differences in chemicals start to be fixed by cells. The average chemical concentrations and their ratios differ by cells. Thus cells with different chemical compositions are generated. This leads to differentiation of cells not only with regards to activities (i.e., differentiation between strong and weak cells) (Kaneko and Yomo 1994), but also with regards to the composition of chemicals. As is seen in Fig.8a)-d), two distinct groups of cells are created when the
cell number is 32, and the average chemical compositions are different between the two. (see the item (8) for the further differentiation at the later stage).

Thus we have reached the third stage of the differentiation in the isologous diversification theory. In connection with the theory, the following three points should be noted:

(i) Interaction-induced change of internal dynamics
If cells were independent, one could think that the fixed differentiation would correspond to different attractors in the intracellular dynamics. This is not the case. As will be discussed later, an ensemble consisting of only one type of cells is unstable. There is an admissible range for the ratio between the numbers of two groups of cells, which depends on the parameters of our model, and is determined by the cellular interactions. Thus the differentiation process depends both on intracellular dynamics and interactions.

(ii) Two-level differentiations with phase and amplitude
As is mentioned the phase of oscillations differs by cells even within each group. Hence there are two levels of differences by cells, one for the change of phases of oscillations, and the other for the fixed differentiation. Indeed this two-level differentiation gives a source for the hierarchical organization.

It is interesting to note that the phase difference is given by “analogue” means, while the fixed difference of averages leads to a rather “digitally” distinct separation. Cells’ differences by phases are not rigid, since the phase diffusion can change them: Perturbations brought about by division are enough to shift the phase and destroy the memory of the previous clustering. On the other hand, cells are clearly separated into few groups, distinkted by the average amplitudes of chemicals. This difference by the amplitude of oscillations is more rigid, since it is not shifted continuously as in the case of the phase. Thus digitally distinct groups are formed, which are stable against perturbation such as the division. This emergence of digital information is the basis of the cellular memory. A daughter of a cell of a given type keeps its mother’s character. Indeed, the cells with stronger activities in Fig.8, are successive daughters of a “strong” ancestor cell, as mentioned. (Cells 29-32 are daughters of 4, 8, 15, and 16, while cells 17-20 are daughters of 3, 6, 12, and 13).

The separation by the amplitude is seen in the locus of the orbits in the phase space of chemical values. The orbits of the two groups of cells lie in distinct regimes in the phase space. As is seen in the overlaid orbits of Fig.11, the oscillation phases are different by cells albeit lying on the same locus in each group, while the difference of orbits between the two groups is clearly discernible.

(iii) Separation of inherent time scales
Another important feature here is the differentiation of the frequency. One group of cells oscillates faster than the other group. Typically cells with low activities oscillate more slowly in time with smaller amplitudes, and divide slowly, as is seen in Fig.9 and Fig.10. (In Fig.10a) b), lines taking higher concentrations correspond to stronger cells, while the concentration of chemical 3 is smaller for stronger cells in Fig 10c). Thus faster oscillations correspond to stronger cells for all chemicals in the figures.) Hence inherent time scales differ by cells, which is also seen in the differentiation of speed of division. Indeed, one group of cells divides faster than the other group of cells. It should be noted that the inherent time scales of cells are created spontaneously through cell divisions and differentiations.

(4) Transmission of differentiation to daughter cells
After the above fixed differentiation, chemical compositions of each group are inherited by their daughter cells. In other words, when the system enters into this stage, a cell

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6 Differentiation of time scale is also studied by Volkov et al. (1992) in a coupled oscillator model with growing degrees of elements, corresponding to the clonal growth.
loses totipotency, as will be more clearly shown by the transplantation experiment in §7. By using the term in cell biology (see e.g., Alberts et al. (1983)), we call that the determination of a cell has occurred at this stage, since daughters of one type of cells preserve the type. Hence a cell at this stage is called a determined one.

With the above transmission, the “recursivity” is achieved. In Fig.12, chemical averages of cells between successive divisions are plotted. Initially, chemical compositions change through divisions, but later they come to almost fixed values. In Fig.12a) averages of chemicals are plotted in the order of divisions. Up to around the 90th division, chemical averages differ by divisions, while the averages split into roughly two distinct groups later and keep the averages by divisions. We have also plotted the “return map” in Fig.12b), that is the relation between the chemical averages between the mother and daughter cells. In the return map, the recursivity is seen as points lying around the diagonal \((y = x)\) line. The emergence of recursivity is seen after some divisions.

Note in Fig.12b) that the concentration of chemical 2 is close to zero up to the division to 32 cells, but later is increased to keep the recursivity. From the molecular biology viewpoint, this may be regarded that some genes start to be expressed to produce some proteins. In our result here such expressions start to appear after some divisions without any pre-programming.

This recursivity is not expected from the studies of coupled oscillators. For the division, we have just imposed one condition of an integral type, which itself does not imply any recursive condition. Through the interference between cellular interactions and intracellular dynamics, a cell selects an initial condition after each division, so that it keeps its recursive structure.

It is important to note that the chemical characters are “inherited” just through the initial conditions of chemical concentrations after the division, although we have not imposed any external mechanisms for a genetic transmission. (It should be mentioned, however, that we do not deny roles of genes in the differentiation process, since our chemical can include DNA. Our viewpoint here is neither that genes determine everything nor that they are unimportant, but that they are included as one of the components in networks.)

In our model the inheritance is achieved through the transfer of “initial conditions” at the division. It should be noted that the initial chemical composition after division is not necessarily recursive. Indeed the return map of the snapshot chemical values after divisions does not fall onto the fixed point as in Fig. 12b), but scatters within some range (although it is smaller than that at initial divisions). This is because the phase of oscillation itself is not necessarily relevant to keep the type. The recursivity holds only for the averages but not for the initial condition after each division, although it is attained through the choice of the latter.

Here we have reached the fourth stage of differentiation of the isologous diversification theory, by demonstrating the emergence of epigenetic inheritance through coupled nonlinear dynamics and selection of initial conditions. The almost “digital” distinction of chemical characters, noted previously, is relevant to preservation of them to daughter cells, since analogue differences of phases may easily be disturbed by the division process, and cannot be transmitted to daughters robustly.

(5) Successive differentiation

Due to the determination, it is possible to draw a cell lineage diagram for each differentiation process, by defining cell types by distinct chemical characters. Generation of cell lineage gives rather useful information to be compared with that obtained in the cell biology. From the cell lineage, one can see how the differentiation processes hierarchically, and how cellular memory is sustained.

In Fig.13, we have plotted the cell lineage diagram, where the division process with
time is represented by the connected line between mother and daughter cells. The color in the figure shows the cell type determined according to the chemical averages in Fig. 8. (The “green” cell has $x_i^{(2)} > .125$, while the blue cell has $x_i^{(2)} < .03$ in Fig. 8d), with $\bar{\mu}$ as the temporal average.

Cellular memory is clearly seen in this figure, where green and blue colors are preserved through divisions for $t > 400$. We also note that the same type of determined cells (“green” cells) appears from different branches around $t \approx 500$. Such convergence of cell types from different branches is also known in cell biology. In fact, lineage analysis shows that in C. elegans, as well as in other animals, each class of differentiated cells, such as hypodermis, neuron, muscle, and gonad, is derived from several founder cells originating in separate branches of the lineage tree (Kenyon, 1985).

Successive differentiation and determination of cells are seen in the cell lineage Fig. 13. After two types of cells (i.e., red and green in the figure) are differentiated around $t \approx 550$, the “red” cells are again differentiated into red and blue cells, (see the two levels of “stronger” chemicals in Fig. 8d). Once this differentiation occurs, this character is fixed again, and after some time, such characters are determined by the daughter cells. With the cell divisions, this hierarchical determination of cells successively continues. For example, daughters of “green” cells can later differentiate into “dark green” and “light green” cells. Here the difference between two green-type cells (for example that of chemicals, or the frequency) is smaller than that between red and green cells. (Examples of these successive determination can be seen in Fig 8.)

Initial “red” cells have the potentiality to be either “green” and “blue” cells, while some of the “red” cells remain to be of the same type by the division. Thus one can write down an automaton-like representation as “red” $\rightarrow$ “red”, “green”, or “blue”, while the division allows only for “green” $\rightarrow$ “green”, and “blue” $\rightarrow$ “blue”. Since the “red” cell creates “blue” and “green” besides creating itself by divisions, the red cells may be associated with stem cells. When, for example higher differentiation into dark and light green cells occurs, the green cell will again play the role of a stem cell over green-type cells.

With this hierarchical differentiation, successive diversification proceeds as is postulated in the isologous diversification theory.

6 Further Remarks on Dynamic Differentiation

It is useful to make some remarks about how the above scenario works and make some possible predictions on the stability of differentiation processes.

1) Initial of differentiation

In our simulation, the differentiation starts after some divisions have occurred. Since the division leads to almost equal cells, a minor difference is enhanced to lead to macroscopic differentiation. We have found that small difference of chemicals with very low concentration leads to the amplification of the difference in the concentration of other chemicals. In Fig. 14, snapshot chemical concentrations are plotted at the time step when the phase difference by cells is triggered. We note that the difference of the chemical 7 (with high concentration) is negligible while the difference of the chemical 5 (with very low concentration) is remarkable. It is interesting to note that such chemical with a low concentration is important, rather than that with a high concentration. This observation reminds us of a certain protein that is known to have a signal transmission in...
order to trigger a switch of differentiation with only a small number of molecules.

The relevance of chemicals with low concentration is also seen in the determined
differentiation. As noted in Fig.8, the difference is most remarkable for chemicals with low
concentrations. It is interesting to check this proposition from experimental cell biology.
The relevance, on the other hand, is a consequence of our isologous diversification theory.
Since the theory is based on the amplification of tiny difference by a nonlinear mechanism,
difference of “rare” chemicals by cells can be easily amplified to lead to a macroscopic
difference of cells.

2) Specialization of a cell

In our simulation, there are different types of a cell as to the variety of chemicals within.
Often, only few chemicals take high concentrations in a cell with “stronger activity”,
while those in a “weaker” cell are more equally distributed. The latter type of a cell
keeps chemical variety. In Fig.8, the difference by chemical species is smaller in weaker
cells. (recall the comment on Fig.10c), where weaker cells have larger concentrations of
the chemical of tiny amount). Generally, the bias in chemical concentrations is tended to
increase with the cell number. This tendency is seen not only with regards to the number
of such cells, but also to the chemical composition of each specialized cell.

The emergence of different cell types makes possible the division of labor in chemical
reactions, mentioned in the isologous diversification theory. As is expected the number
of cell types is increased with the increase of chemical species $k$, although the increase
seems to be rather slow. The number of cell types could be much larger if one-to-one
correspondence between a chemical and a cell type were adopted. Since chemicals are
connected in a biochemical network and cells originate in a single ancestor, the number
of cell types is radically reduced.

3) Tumor type cell

In simulations with a larger diffusion coupling, a peculiar type of cells appears. They
destroy ordered use of chemical resources, which makes the cell society disorganized. This
type of a cell is an extreme limit of a specialized cell, and has a much higher concentration
of one chemical than other species. In some examples we have observed, one chemical $m$
has more than $10^3$ times concentration of others. The major biochemical (metabolic) path
here is rather trivial, since the reaction is mostly governed by the path Source Substrate
$x^{(0)} \rightarrow x^{(m)} \rightarrow$ Division Factor. Chemical diversity in the cell is largely reduced. On
the other hand, the concentration $x^{(m)}$ is so large that the cell divides faster than other
cells. These cells destroy the mutual relationships among cells, attained through the
successive stages of the isologous diversification. Taking into account this fact that these
cells destruct the chemical order sustained in the cell society, they may be regarded as
“tumor” cells.

The formation of these “tumor” cells may be triggered by the mutation, which, in our
model, is represented by the “noise” term in the division process (the random number
at the division process, when $\epsilon$ is larger). Still the growth of tumor cells depends on the
cellular interactions, for example on the diffusion constant or the density of differentiated
cells. Depending on the interaction term, errors in the division process may or may not
lead to the “tumor”-type cells.

In Fig.15 we have plotted the average chemicals versus the cell index, obtained from
a set of simulations with the same network as in §4, and by choosing a larger diffusion
coupling ($D = 0.2$), and smaller threshold for the division. In Fig.15 b), the “tumor”
cell starts to appear at $t \approx 140$, where the 15th cell (when the total cell number is 32)
has a very high concentration of the chemical 4 . The chemical $x^{(4)}$ of the cell is around
3.6 while concentrations of other chemicals are close to zero. Since the cell divides much
faster than other cells, its offsprings increase the number rapidly, which keep the same
chemical characters. Thus the “tumor” cell starts to dominate the system. As in Fig.
offsprings of the tumor cells keep the property of an extremely high concentration of the chemical $x^{(4)}$, although its concentration can be weaker with the divisions. These cells are again differentiated through divisions, as is seen in Fig.15 d). Here we note that chemicals other than the species 4 are richer in the normal (weaker) cells. Hence, the “tumor” cell here is specialized strongly.

Of course, the extremely high concentration of one chemical here can be due to the simplicity of our biochemical network only with 8 species. For a network with more components, the bias must be weakened. However, it is still expected that there appears a cell type with strongly biased composition of chemicals, even if the bias is not so extreme as in the case here. We propose that the chemical diversity is decreased in a tumor cell in general, which is our prediction, to be tested experimentally.

The cell lineage is given in Fig.16, where the “tumor” cells are plotted at the right side of the lineage, and their division is faster. One can also see the difference of division speeds among the “tumor” cells: Some start to lose their high activity, and start to be differentiated. It should be noted that the recursivity is not satisfied for “tumor” cells. By plotting the return map of the chemical 4 as in Fig.12b), we have found that the fixed point is not achieved for it, while the return maps of other chemicals or cells of a non-tumor type satisfy the recursivity. This loss of recursivity implies the heterogeneity of tumor cells. (see also Rubin (1990)).

In our simulation, since the source chemical is limited, the number of “tumor” cells (with strong activity) cannot grow indefinitely. With the increase of the cell density, some cells lose their strong activity. In Fig. 17, we have plotted the temporal evolution of cell numbers with three levels of activity. Around $t \approx 400$, there is saturation of the number of “tumor” cells, and the loss of activity occurs.

In the experiment of *E. coli* by Ko, Yomo, and Urabe (1994), there appears a long-term oscillation of the numbers of cells with different activities. Such oscillations are observed from the longer time simulation of our model. The complex dynamics of the number ratio of active to weak cells naturally arises through the interaction among cells.

In the example of the network Fig.5a, the “tumor” formation is enhanced when the diffusion $D$ is larger. In this case cellular interactions through medium are stronger, and the competition for resources is more tight. Taking also into account of the “tumor” formation beyond some number of cells, we may conclude that the “tumor” formation is enhanced by the competition for resources by cells, or roughly speaking by the effective density of cells.

Rubin( Yao and Rubin 1994; Chow et al., 1994, Rubin 1994a,1994b), in a series of experiments, has shown that formation of a type of tumors strongly depends on the density of cells, even if the mutation may be relevant to the trigger to it. The enhancement of “tumor” formation in a high density, found in our results, agrees with the experiments. This suggests that some type of tumor formation depends on epigenetical factors, and indeed is a general consequence in an interacting cellular system.

Indeed the formation of “tumor”-type cells is a consequence of isologous diversification theory. In the theory the differentiation process is not programmed explicitly as a rule but occurs through the interaction. Thus, when a suitable condition of the interaction is lost, for example, by the increase of density, “selfish” cells destroying the cooperative use of resources are formed.

In the natural course of the cell differentiation, the interaction among cells through the chemical environment is not global, but is somehow localized in space. Such spatial effect is important when the cell number and the total size of the system are increased. If the range of interaction is limited, the effective density of cells does not increase even if the cell number does. Thus, the cell society can be developed without tumor formations. Another way to avoid the ceaseless growth of the cellular density is the introduction of
cell death. By increasing the cell death threshold ($S$), the tumor formation is avoided.

4) Cell death

When the cell death condition is included, we have observed simultaneous deaths of many cells. Here we give another set of differentiation process, by using a different pathway given in Fig.5b), and by taking a larger threshold $S$ for cell death. The chemical averages are plotted in the order of cells’ indices in Fig.18, which shows the differentiation process clearly. In Fig. 18a), snapshot chemical values are plotted, where slight differentiation has started. In Fig. 18b), the fixed differentiation proceeds. One can see 8 cells with stronger activity: the 31st and 32nd cells are daughters of 7th and 16th, and 33-36th cells are daughters of 7,16,31, and 32nd, respectively. Around the time step 2000, cell deaths start to be observed. Chemical averages after this stage are given in Fig. 18c), where differentiation between strong and weak cells has occurred, as well as slight differentiation among strong cells. Two groups are clearly distinct as in the orbits in the phase space, say in $(x^{(3)}, x^{(6)})$.

After differentiation to three groups, cell death processes start to appear. Through the deaths, the number of cells varies aperiodically around 32 as shown in Fig.19, where spontaneous deaths of multiple cells are often observed.

The above process of cell deaths with differentiation is clearly seen in the cell lineage diagram of Fig.20. In Fig.20a), an early stage of differentiation is shown. Colors correspond to cell types, where the activity of cells is in the order of green, blue and red for $t > 800$. One can see simultaneous deaths of cells of the same type, arising from the same branch. Cell deaths over longer time scales are shown in Fig.20b) and c). At later stage, steady distribution of cell types is formed through the deaths. Weaker cells exist with some ratio.

In the development of organisms from fertilized eggs, some cells deterministically die at certain stages, as termed apoptosis or programmed death. Despite of several studies in molecular genetics, no genes or molecules responsible for the age of an individual cell have been identified, although the presence of apoptosis-related genes had been reported in some works. Thus, whether a certain program or gene network governing cell aging exists or not remains an open question. Our simulation showing simultaneous deaths of multiple cells as in the apoptosis, indicates that cell death can deterministically occur without a special program. In other words, the fate of a cell including its death may be mainly governed by the interaction among the cells, influencing its physiological state. This interaction-based apoptosis will be justified by the transplant experiment. We predict here that a cell, transplanted just before its death can survive longer than as expected, indicating a change in its fate.

In the present model, no spatial structures are included. Cells cannot move in space for richer nutritions. Thus the number of cells is limited, and such multiple deaths are repeated. With the introduction of spatial structure, such repetition may be lost by the loss of spatial coherence. In our model the period for such deaths is not fixed, but fluctuates in time. Such fluctuation is characteristic of an interaction-determined, rather than a genetically determined, system.

5) Dependence on parameters and universality

We have made several simulations changing the parameters in our model. Due to a large number of parameters, it is hard to give roles of parameters clearly, but they are roughly summarized as follows:

When the nonlinearity is weak, the differentiation starts later. It seems that the differentiation will start after the cell number is large enough, as long as there is chemical

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8Simulation with the network of Fig.5a) and with a larger $S$ leads to the same behavior as presented here. We use a different pathway in order also to see the generality of our results.
oscillation, even if the nonlinearity is not large\footnote{Due to limitation of computational resources, it is practically difficult to check if the differentiation starts after the number is larger than 256 where the computation requires much longer time.}.

Increasing the parameter $p$ or $\gamma$ enhances differentiation. These changes increase the amplitude and/or the frequency of oscillation, and may be regarded as the increase of “nonlinearity”. The decrease of the growth threshold $R$, on the other hand, suppresses the differentiation. Cells remain identical, although chemical speciation within is often enhanced, and only few chemicals’ concentrations are larger than zero; in other words an ensemble of “tumor”-type cells tend to be formed. Increase of the diffusion $D$ has a similar effect as the decrease of $R$.

As mentioned, single cell dynamics depends on the network, which reflects on the behavior of cellular society. Generally speaking, our differentiation scenario works as long as a single cellular dynamics allows for the oscillatory dynamics. As for paths to the division factor ($P(\ell)$) and paths from the source chemical ($S(\ell)$), there is a tendency that the differentiation process is enhanced when these connections are not full ($= k$).

6) Macroscopic stability

To close the section, it should be noted that our scenario, although based on chaotic instability, is rather robust against changes of initial conditions or errors in the division process. Of course, which cell becomes one given type can depend on the initial conditions. On the other hand, the number distribution of each type of cells, as well as the cell lineage diagram, does not depend on initial conditions, as long as very special initial conditions are not adopted, as in the transplantation experiment (see the next section).

The variety of cell types and their number distribution are robust against the noise (error) in the division process (which may be regarded as the mutation when the corresponding chemical is DNA). On the other hand, when the population of one type of cells is decreased (e.g., by external removal), the distribution is recovered through further divisions.

This kind of robustness at an ensemble level, indeed is expected from our isologous diversification theory, since the stability of macroscopic characteristics is attained in coupled dynamical systems (Kaneko 1992,94; Yomo and Kaneko 1994). This robustness gives a key to understand how a stable cell society is formed, without being damaged by somatic mutations.

7 Transplantation Experiments and Cellular Memory

The differentiation in our isologous diversification scenario originates in the interaction among cells, but later, at the third stage, chemical characters of a cell are memorized through the initial condition after division. The differentiation at the former mechanism is reversible, while the latter mechanism leads to determination. It is interesting to note that the determination is not implemented in the model in advance, but emerges spontaneously at some stage of cell numbers.

In the natural course of differentiation and in the simulations in §4, however, it is not possible to separate the memory in the inherited initial condition from the interaction with other cells. To see the tolerance of the memory in the inherited conditions, one effective method is to choose a determined cell and transplant it to a variety of surrounding cells, that are not seen in the “normal” course of differentiation and development. Let us discuss the results of these “transplantation” experiments.

In real biological experiments on differentiation, some “artificial” initial conditions are
adopted by the transplantation of some types of cells. To check the validity of our scenario and to see the tolerance of the memory in the inherited initial condition, we have made several numerical experiments taking such “artificial” initial conditions. Here we have made the following observations, by initially taking cells obtained at the normal diffusion process and putting them into undifferentiated cells at an earlier stage.

i) starting only from few differentiated cells of the same type in addition to undifferentiated cells

Fig. 21a) gives the evolution of average chemical concentrations by cells starting from 4 determined cells (whose cell index is from 1 to 4) and 4 undifferentiated cells. The former cells are sampled from later stages ($t = 691$) in the simulation of §3, while the latter ones come from the former stages ($t = 205$). In Fig.21a), 14,15,16,17th cells are the first daughters of 1,2,3,4th cells and the cells from 25 to 32 are the daughters of the above 8 cells. The former group of cells keeps the type, whose offsprings remain the same type. Thus the determination is preserved, and the memory in the inherited initial conditions is robust against the change of cell interactions. The undifferentiated cells, on the other hand, start to differentiate to form many types of cells, as is seen in Fig.21a).

This robustness of cell memory is kept as long as the ratio of initial determine cells to undifferentiated cells is not too much. (see iii) for the case otherwise).

ii) starting from the mixture of different determined cells

Again the cell memory is preserved, and daughters of a cell keep the same character. In Fig. 21b), two types of determined cells are initially taken, 7 cells for one type (with the index from 1 to 7), and 1 cell (the 8th cell in the figure) of another type, obtained from §3 at $t = 691$. The Fig.21b) shows the average chemical pattern after twice divisions. The 16th, 31st, and 32nd cells, which are offsprings of the 8th cell keep the character, while other cells remain to be the other type. Some other simulations also show that cellular memory is preserved as long as initial distribution of cells is not dominated by only one type of cells (see iii) for the case otherwise).

iii) starting only from few differentiated cells of the same type

In most cases, these cells start to dedifferentiate again to generate different types of cells. Some of them keep their character while others (and their offsprings) become a different type. If initial distribution of cells is dominated by one type of determined cells in cases i) and ii), again some of them start to dedifferentiate and become a different type of cells. In Fig.21c), the average chemical pattern is plotted starting from 20 cells of one type of determined cell of §3 at $t = 691$. Cells with the index from 1 to 16 and 19 preserve their character, while 17th and 18th cells become a different type, and the 20th cell is transdifferentiated to another type. Here their offsprings again keep the character: 22nd and 23rd cells are daughters of 17th and 18th, while the 21st cell is a daughter of 20th, and 41-42nd are those of 20th and 21st. Thus determination again occurs after the process of dedifferentiation.

In an example with a different chemical network (with a larger number (4) of connections), we have found the formation of “tumor” cells with ongoing simple chemical reaction paths. Again, these cells lose the variety of chemicals and destroy the ordered use of resources.

Summing up i)-iii), we can conclude that the cell memory is preserved mainly in each cell, but cellular interactions are also important to sustain it. The achieved recursivity in §4 is understood as the choice of internal dynamics through cellular interactions.

Thus the cellular interactions play the role not only of the trigger to differentiation, but also of the maintenance of diversity of cells. Internal cellular memory is maintained as long as the diversity is sustained. The relevance of interactions to diversification is a
key concept in our isologous diversification.

iv) differentiation of “tumor” cells

Another interesting initial condition is the use of “tumor” cells. Starting only from tumor cells, their offsprings remain to be the same type initially. As the divisions are repeated, some cells’ activities get weaker and start to be differentiated. This is a consequence of our theory that is strongly based on cellular interactions.

Such differentiation of “tumor” cells is promoted by adding undifferentiated cells initially. As an extreme case of “tumor” cell, let us consider a cell with \( x^{(m)} \) is large and \( x^{(\ell)} = 0 \) for \( \ell \neq m \). Such cell can divide faster if there are paths from the source to \( m \) and from \( m \) to the division factor. When there are a large number of autocatalytic paths, \( x^{(\ell)} \) remains to be zero for the cell, whose offsprings keep the same type. In this extreme case, the cellular society remains to consist only of “tumor” cells. Even in this case it is found that “tumor” cells are differentiated by adding undifferentiated cells ( taken at the initial stage of the simulation of our model).

8 Discussions

8.1 Summary and biological relevance

To sum up we have proposed isologous diversification theory on cell differentiation, by introducing a model based on the interacting cells with chemical oscillations and the clustering of coupled oscillator systems. From the simulation of the model, we have observed successive spontaneous differentiations and their transfer to daughter cells, without any external mechanism.

Let us summarize the consequences of our simulations.

- Cell differentiation occurs through the interplay between intracellular chemical reaction dynamics and the interaction among cells through chemical media.
- Cell differentiation is initiated by the clustering of chemical oscillations, appearing at some cell number, which is explained by general features of coupled nonlinear oscillators.
- Chemicals with tiny amounts in cells are relevant to a trigger to differentiations.
- With further divisions cells lose totipotency, and offsprings preserve the same character. This recursive division of cells appears only after some stage of cell divisions.
- Distinct and memorized cell types are formed by the clustering of the amplitude, rather than the phase, of oscillations, which leads to the emergence of digital changes in chemical concentrations.
- Determined cell changes are characterized by the cell activity and chemical compositions.
- Inherent time scales, given by the oscillation period, differentiate by cells. Generally active cells oscillate faster, and divide faster. This separation of time scale brings about the separation of growth speed of cells, and leads to the disparity between rapidly growing and inactive cells.
- Generally speaking, cells whose chemicals are concentrated on few species, are stronger in activities and divide faster. Cells keeping a variety of chemicals divide
slower. There is a negative correlation between the growth speed and the chemical variety within a cell.

- Successive differentiation appears at a later stage, which leads to cells that bring about only a range of cell types successively.

- Determined cell types formed at the later stage are preserved by their transplantation to other cell society as long as there are not too much cells of the same type.

- It is possible to dedifferentiate cells, by putting them in some conditions such as overcrowded cells of an identical type.

- Spontaneous multiplied deaths appear through the interaction of cells, after cells are differentiated.

- A type of tumor-like cells is formed, depending on the cellular interaction. These cells destroy the ordered use of resources attained in the cell society. For example the formation is enhanced by the cell density or the diffusion coupling. Transplantation of cells of the same type may enhance the tumor formation.

- Such type of tumor cells can be differentiated to normal cells, through the interaction with other cells. The differentiation can be enhanced by adding undifferentiated cells.

It is interesting to note that the above picture is consistent with a variety of experimental results such as loss of totipotency, origin of stem cells, hierarchical organization of differentiation, separation of growth speeds by differentiation, tumor formation, importance of tiny amount of chemicals for the trigger of differentiation, and so on. It should be mentioned that these results naturally appear as a general consequence of our isologous diversification theory without pre-programmed implementation, and are independent of detailed modeling. We should also mention that our theory is compatible with the genetic switching mechanism for the differentiation. Here such switching-type expression appears naturally through cellular interactions.

As is described in §6.2, once cell differentiation in our model reaches the fifth stage in which the cells are successively differentiated, the variety of components in each cell is negatively correlated to the growth rate or activity. That is, a cell with a higher division rate tends to have a lower variety of metabolites or simply saying, these cells have a simpler metabolic network. This proposition can possibly be examined by cell biologists. For instance, in the process of development of the organism, one can sample existing various types of cells in several stages of successive differentiation. Then it is possible to determine the variety of the components including macromolecules and the growth rate for each cell type and check the correlation between the two. Thus, the authenticity of the isologous diversification theory can easily be tested in the laboratory scale.

Furthermore, the theory can be extended for medical application. As mentioned in §6.4, the tumor cells in our model are in the extreme case of the loss of the variety, where the cells lose some of the metabolites or become to have a simpler network to achieve the faster growth rate. One way to bring a tumor cell back to normal is to supply the metabolites which they lost through the development. The cell will then recover its normal network and hence, will grow harmoniously with the surrounding cells. Similarly, in order for cancer cells to regain the normal physiological state even with some mutations on their DNA, they are fused with the liposomes encapsulating the cytosol of normal cells or undifferentiated cells, which are expected to include some of metabolites or macromolecules that the cancers lack.
8.2 Isologous diversification

It should be noted that the introduction of tiny difference at the division is not essential to our differentiation scenario. A system composed of identical cell states is unstable when the interaction is strong. Even if parameters or initial conditions of a cell are different, they may not be essential to the differentiation. Indeed in the clustering of coupled dynamical systems, it is known that an element with a different parameter can oscillate almost coherently with others, while elements with identical parameters split to two (or more) groups (Kaneko 1994). Here the parameter variation of elements is not essential to the grouping of them. Thus it can happen that a cell with rather different parameters or initial conditions remains to be of the same type with other cells, while some cells with identical parameters or close initial conditions become a different type. In this perspective, it is expected most somatic mutations are irrelevant to differentiation, unless it brings about a drastic change in the parameter for the interaction and intracellular dynamics.

Our proposal here is that the differentiation and diversification are not due to variations by reproducing errors but by the dynamical instability. This is the reason why we have called our theory “isologous diversification”, to stress the inherent tendency of differentiation of identical elements.

Indeed we believe this “isologous diversification” can be generally applied to a variety of biological systems, because it is based on our study of coupled dynamical systems, which is expected to be universal in a class of interacting, reproducing, and oscillatory units. In particular we have succeeded in showing a mechanism of division of labors through differentiation and segregation into active and inactive groups. Since the picture is based on coupled dynamical systems theory, it is expected to be applied to economics and sociology, which enables us to discuss the origin of differentiation, diversity, and complexity there.

In biology, the origin of multicellular organism is directly related with the above picture and our result here. For its origin, some mechanism of differentiation of identical cells is necessary which leads to divisions of labor, while the differentiation reaches at the stage that only one group of cells brings about its offsprings. According to our results, this feature of a multicellular organism spontaneously emerges as a consequence of strongly coupled reproducing units. It is not a product of chance, but of the necessity in the course of the evolution, since reproducing units should reach a strong coupling regime by their growth. It should be noted that our study explains not only the origin, but also the maintenance of diversity (see also Kaneko and Ikegami (1992) for the relevance of weak chaos to the maintenance).

8.3 Some future problems

Our theory of differentiation raises some basic problems. To close the paper we discuss five of them, the first two of which are related with the extension of our modeling, while the latter three are of more fundamental issue.

**Universality and simpler models**

Our results are rather universally observed as long as individual dynamics allows for some oscillations. Since globally coupled dynamical systems are known to show spontaneous differentiation as the clustering (Kaneko 1989,90), we may expect that our differentiation is universally observed in a large class of a coupled system of nonlinear reproducing units.

To search for a simpler model, we have also checked a model only with a phase variable (Kaneko 1994). So far this model shows the stage of phase clustering and can explain its
relevance to time sharing for resources, but cannot show the stage of the disparity and fixed differentiation. For the stage, a model with amplitude clustering is required, as in ours.

Note that the clusterings in our model occur in a dual space, that is, in chemical species and in the cell index. Indeed one can construct a coupled map model with dual space, which shows the clustering in cell index and/or chemical species. Here the clusterings between cell indices and chemical species compete with each other. Construction of minimal models with the differentiation process will be an interesting problem as dynamical systems theory.

**Introduction of spatially local interaction and development**

In the paper we have assumed that chemical medium is well stirred, and all cells interact with all others uniformly through the medium. In the developmental process of a multicellular organism, spatially local interactions among cells are, of course, important, as the development proceeds.

We have made some preliminary simulations including spatial inhomogenization of the medium. So far the result shows that the differentiation process starts in the same manner as that presented here. First, the phase of oscillation is differentiated according to its division. At a later stage, cells close to each other start to be differentiated following the scenario in the present paper. Then a cell’s character is fixed, depending also on the locality in space. At later stage, due to the local interaction, spatial organization of differentiated cells occurs, leading to the pattern formation, as in the pioneering study by Turing.

Our proposal along the present paper is that the temporal organization of cells occurs first, leading to cell differentiation, and later the pattern formation follows. Hence we have focused on the global interaction case here, although, of course the spatial organization is the next important issue, as will be studied in future.

Since distant cells do not interact directly with each other, differentiation as well as its determination is often enhanced. Another consequence of the spatial separation is the suppression of competition for chemical resources, which makes the simultaneous cell deaths smaller in number, and localized in space.

It will be of interest to include the cell motility following an intercellular force, to study cellular rearrangements leading to the pattern formation. This, for example, may result in a simple model for the differentiation process of Dictyostellum discoideum.

Another extension of our model is the use of a “batch-type” simulation where chemical resources do not flow into the media but are kept constant. Indeed, there is no flow of chemical resources from the outside, during the early developmental process of an egg such as the sea urchin. Our model can directly be extended to this “batch” type simulation, by cutting the flow from the outside, i.e., by setting $D_{out} = f = 0$ and taking a higher density of nutrition initially. Since the nonlinear dynamics and the interaction are still included, it is expected that the differentiation process follows the stages of the present theory, as long as the initial nutrition is sufficient. Here, the final number of cells depends on the initial amount of nutrition, while, the distribution of the cell types should be almost independent of it as long as it is not too small. This robustness may give a prototype of the developmental stability found in Driesch’s experiment on the sea urchin.

**Cellular memory**

The emergence of the cell memory, found in our system, raises an important issue in coupled dynamical systems. Is the memory stored in each cell or in an ensemble of interacting cells? Our proposal here is that it is preserved through intra-inter-dynamics, that is partly within each cell, and partly distributed in the cell society. The existence of multiple cellular types can be related with coexisting attractors corresponding to different basins for initial conditions, while the stability of differentiation is sustained by the inter-
action. Indeed, with the interaction, the distribution of cell types is almost independent of initial conditions, and is also robust against perturbations such as removal of some cells, or other possible environmental changes.

This is a novel form of memory in dynamical systems. Due to the interplay between intracellular dynamics and interactions, the fixation of memory and diversification are compatible. It is important to clarify the condition of the emergence of cell memory, as well as to search for applications of this type of memory to other biological systems such as immune or neural networks.

**Recursivity through choice of initial conditions**

The next problem is the initial condition selection with recursivity. As several divisions proceed, each cell enters into the stage whose daughter keeps the same character. It is recursive in the sense that the initial condition of a cellular state after a given division leads to the next initial condition after the division so that it has the same cellular character. With this sequence of initial conditions some condition must be satisfied to keep the same character. For this, some chemicals should remain at some range, although not necessarily are completely identical. We note that the initial condition itself after each division does not fall on to a fixed point. The phase of oscillations at each division is rather arbitrary. The recursivity is achieved as a fixed point of the average motion as given in Fig. 12.

A novel framework is required to discuss the stability at the average level, and the selection mechanism of initial conditions so that the system is recursive. To be recursive, a set of initial chemicals should be determined rather precisely while others are loosely determined. In our problem, this choice is also dependent on the environment (medium), which depends on other cells' states. The formation of tumor cells is understood as the loss of recursivity, in this context. Detailed discussions on this initial condition problem will be discussed elsewhere, where the problem of separation of egg (DNA) and chicken (protein) will be reconsidered along this line.

**Open chaos**

Besides this viewpoint of coupled dynamical systems, it should be noted that our system is “open-ended” in the sense that the degrees of freedom increase with the cell division, where the notion of “open chaos” (Kaneko 1994b) will be useful to analyze the mechanism of cell differentiation problems.

The last, but important question is the evolution of metabolic network. In the present paper, we have chosen randomly connected autocatalytic networks. Even among the random networks, the dynamic behavior depends on the topology of the network, as well as the number of autocatalytic paths, which is most relevant. The metabolic network in a cell is constructed through the evolution, and differs from that constructed as a random graph. The network is history dependent, and is constrained by the survivability within a cell society. Evolution of metabolic pathways within the cellular interactions and intracellular dynamics should be studied in future.

**acknowledgements**

The authors are grateful to T.Ikegami, S. Sasa, N. Nakagawa, T. Yamamoto, and I. Urabe for stimulating discussions. The work is partially supported by Grant-in-Aids for Scientific Research from the Ministry of Education, Science, and Culture of Japan. The authors would like to thank Chris Langton for their hospitality during their stay at Santa Fe Institute.

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9 Appendix 1: Winner-takes-all mechanism in chemical reaction dynamics

In this appendix, we briefly discuss the chemical reaction dynamics of our model, for a single cell.

When there is a two-way connection between chemical species, the winner-takes-all mechanism can be expected. This can be understood by taking a simple example with two chemical species:

\[
\begin{align*}
\frac{dx(1)}{dt} &= x(2)x(1)/(1 + x(2)) - x(1)x(2)/(1 + x(1)) \\
\frac{dx(2)}{dt} &= x(1)x(2)/(1 + x(1)) - x(2)x(1)/(1 + x(2))
\end{align*}
\]

As is easily seen from this equation, the difference \(x(1) - x(2)\) is amplified with time, and goes to a state with either \(x(1) = 0\) or \(x(2) = 0\). Thus, the bidirectional connection tends to lead to competition of chemical species in the present model. Indeed selection of few chemicals is seen when there are many bidirectional pathways.

As the extreme limit, let us consider a case with full connections of paths. In this case, we have observed that as an attractor only one chemical species has a finite value, and others vanish in a strong nonlinearity regime (i.e., with large \(e_1\)). Here the dynamical process is just the selection of one chemical species through the competition for resources.

10 Appendix 2: Choice of Internal chemical dynamics

When the number of autocatalytic paths is large, the mechanism mentioned in the Appendix 1 works, and only one or few chemicals are activated. When the number of autocatalytic paths is small, on the other hand, many chemicals are generated, but the dynamics is stabilized and goes to a fixed point state. In the medium number of autocatalytic paths, non-trivial metabolic reactions appear as mentioned in §2\(^{10}\). Periodic alternations of dominating chemical species are observed. Depending on the nature of connections, we have seen several types of oscillations, although chaotic ones are not found so often. When the number of chemicals is larger, the alternations are more complicated as in Fig 22 a)b) where the dynamics is possibly aperiodic.

If there are a few non-autocatalytic (i.e., Con\((m, j, \ell)\) with \(j \neq \ell\) paths, fixed-point states are stabilized, and oscillations are hardly observed. We adopt the intracellular dynamics consisting only of the autocatalytic paths whose number is medium per chemicals (from 2 to 4), since they provide examples with ongoing non-trivial metabolic reactions.

There is indeed a reason for this choice from an evolutionary point of view. In the evolutionary process of metabolic reactions, novel chemicals are successively included in the network. Let us consider the inclusion process of a new chemical \(J\). Its chemical concentration must be amplified through the chemical network process, otherwise, it is diluted and disappears through divisions. Since the new chemical \(J\) did not exist before, \(dx^J/dt = 0\) if \(x^J = 0\). On the other hand, for the growth of the concentration of chemical \(J\) in its presence, \(dx^J/dt > 0\) must fold for \(x^J > 0\). Hence the condition \(\frac{\partial}{\partial x^J} \frac{dx^J}{dt} > 0\) must be satisfied. Thus it is expected that \(\frac{dx^J}{dt} \propto (x^{(J)})^\alpha\) with \(\alpha > 0\). Thus, some kind of autocatalytic processes

\(^{10}\) Besides the number of autocatalytic connections, there is further dependence on each pathway of the network. We have examined several random networks of 2 autocatalytic paths per chemicals for \(k = 8\). Some of the networks lead to oscillatory dynamics, while others show fixed point dynamics with few chemicals of high concentrations, although the number of autocatalytic paths is identical. So far we have not found a simple criterion for the oscillatory behavior.
for the chemical \( J \) must exist. In this way, it is expected that chemicals with autocatalytic processes are adopted successively, through the evolution of metabolic process.

As mentioned in §2 the term “autocatalytic path” is not necessarily taken strictly, but may assumed to represent chemicals autocatalytic “as a set” (see Fig.23 schematically). In such case, one may approximately represent the set of chemicals by one variable \( x^{(t)} \), and adopt an autocatalytic reaction for \( x^{(t)} \). Thus our chemical reaction may be interpreted to represent the network composed of a set of autocatalytic networks, expected from the evolutionary process.
Figure Caption

Fig.1 Schematic representation for two pictures of cell differentiation. a): fixed landscape. b): Our picture based on the interplay between intra- and inter- cellular dynamics.

Fig.2 Schematic representation of our isologous diversification. Each spiral represents oscillatory dynamics. In the figure each stage shifts to the next by a single reproduction process (e.g., a cell division) for simplicity, but in general there are several reproductions in each stage. See §5 for details.

Fig.3 Schematic representation of our model: a) the whole dynamics of our system; b) chemical reaction within each cell.

Fig.4 Overlaid time series of $x^{(m)}(t)$ of a single cell in medium, obtained from a network with three connections of 8 chemicals whose connection is given in Fig. 5a). Each line with the number $m = 1, 2, 4, 5, 7, 8$ gives the timeseries of the corresponding chemical $x^{(m)}(t)$. Note that the chemical 2 has a lower concentration and appears only around the bottom of the figure, while the concentrations of chemicals 3 and 6 are too low to be discernible in the figure. The parameters are set as $p = 10.0$, $e_0 = e_1 = 1$, $\bar{X}_0 = 40$, $\gamma = 0.2$, $x_M = 10.0$, $D_{out} = f = 0.005$, and $V = 1000$, while the division and death processes are not included.

Fig.5 Biochemical network adopted in the simulations shown in the present paper. (a) 3 outgoing connections per chemical (b) 2 outgoing connections per chemical. The species with a double circle has an arrow to the division factor product ($P(\ell) = 1$), while all chemicals have arrow from the source chemical 0 (i.e., $S(m) = 1$ for all $m$).

Fig.6 Time series of $x_i^{(4)}(t)$ for $134 < t < 140$, overlaid over all cells. Cell division occurs around $t = 137.8$, when the cell number doubles from 8 to 16. The cell index is defined in the order that the cell is born. In the simulations of the present section (given in Fig.6–14, and 22) we adopt the chemical network of Fig.5a) and use the parameters $p = 10.0$, $e_0 = e_1 = 1$, $\bar{X}_0 = 40$, $D = 0.02$, $\gamma = 0.2$, $x_M = 10.0$ $R = 2000$, $S = 0.05$, $D_{out} = f = 0.005$, and $V = 1000$.

Fig.7 Snapshot chemical concentrations of $x_i^{(m)}(t)$ at $t = 114$, when the cell number is 8. The concentration of chemicals $x_i^{(m)}$ for $m = 1, 4, 8$ are shown, since the concentration of other chemicals are very low at this time instance. Lines connecting $x_i^{(m)}$ are just for the sake of presentation. As described in the text, cell’s index is labeled in the order of the birth: when the first division occurs, one of the cells remains to be denoted as 1, while the other is labeled as 2. When the next division occurs for the cell 2, for example, one of the divided cells has the cell index 3, while the other remains to be 2, and so forth. Data for Fig.6–14 are obtained from a simulation with parameters given in Fig.6.

Fig.8 Average chemical concentrations of $x_i^{(m)}$. The average is taken over the time steps from the latest cell division (or since its birth, when it has not experienced a division yet). Each color corresponds to each chemical $\bar{x}_i^{(m)}$ for $m = 1, 2, \cdots, 8$, with $\bar{m}$ as the average. Note that the line is plotted only for visualization, and the values at integer cell indices give corresponding $x^{(m)}(i)$. The concentration of the chemical 6 vanishes after some time, and is not plotted. (a) $t = 280$, when the cell number $N = 16$, (b) $t = 400$ and $N = 32$ (c) $t = 600$ and $N = 32$ (d) $t = 940$ and $N = 64$.

Fig.9: Time series of $x_i^{(m)}(t)$ for $800 < t < 805$, overlaid over all chemical species $m$. (Each line corresponds to each chemical). (a) for the cell $i = 2$ (b) for the cell $i = 3$ (c) for the cell $i = 4$. 
Fig. 10
Time series of \( x_i^{(m)}(t) \) for \( 800 < t < 805 \), overlaid over all cells \( i \). Each line corresponds to the time series of each cell. (a) for the chemical species \( m = 1 \) (b) for the chemical species \( m = 2 \) (c) for the chemical species \( m = 3 \). In (a) and (b), oscillations with a larger amplitude correspond to cells with larger activity (\( \sum_m x_i^{(m)} \)), while they take smaller value for the chemical 3 given in (c).

Fig. 11
Orbits of chemical oscillations. Plotted are \( (x_i^{(5)}(t), x_i^{(8)}(t)) \) for \( 800 < t < 900 \) overlaid over all cells (whose number is 64). Two groups are clearly seen.

Fig. 12
(a) Chemical concentrations \( \bar{x}_i^{(m)} \), averaged over two successive divisions, are plotted in the order of divisions. The upper column shows the expansion of the lower for divisions later than 60th. Different marks correspond to different chemicals, while lines are plotted only for convenience.

(b) Return map of chemical concentrations \( \bar{x}_i^{(m)} \) averaged over two successive divisions. A daughter cell’s average concentration is plotted versus its mother’s cell’s average before the division to the daughter. Chemicals 2, 5, and 8 are plotted with different marks, while the dotted lines are drawn only for convenience. The lower column is the expansion of the upper column.

Fig. 13
Cell lineage diagram corresponding to the simulation in Fig. 6–12. The vertical axis shows the time, while the horizontal axis shows a cell index. ( Only for practical purpose of keeping track of the branching tree, we define the index for the lineage as follows: when a daughter cell \( j \) is born from a cell \( i \)’s \( k \)-th division, the value \( s_j = s_i + 2^{-k} \) is attached to the cell \( j \) from the mother cell’s \( s_i \). The cell index for the cell \( j \) is the order of \( s_j \), sorted with the increasing order. Note that the index for the lineage diagram is different from the cell index adopted in other figures, where the index is given just as the order of birth). In the diagram, the horizontal line shows the division from the cell with index \( n_i \) to \( n_j \), while the vertical line is drawn as long as the cell exists (until it dies out). Color corresponds to the cell’s character defined from the average chemical pattern. After differentiation the activity of a cell is in the order of green, red, and blue, while initial red cells correspond to undifferentiated ones. The “green” cell has \( x_i^{(2)} > .125 \), while the blue cell has \( x_i^{(2)} < .03 \) in Fig. 8d).

Fig. 14
Snapshot chemical concentrations of \( x_i^{(m)} \), at \( t = 63 \), just the onset of chemical difference by cells (clustering). Chemicals 4, 5, and 7 are plotted in a logarithmic scale, in the order of cell index, the order that the cell is born.

Fig. 15
Average chemical concentrations of \( x_i^{(m)} \). The average is taken over the time steps from the latest cell division (or since its birth, when it has not experienced the division yet). We adopt the chemical network of Fig. 5a) and use the parameters as for Fig. 6, except \( D \) and \( R \), which are taken to be \( D = 0.2 \) and \( R = 500 \). (a) \( t = 130 \), when the cell number \( N = 32 \), (b) \( t = 140 \) and \( N = 32 \) (c) \( t = 380 \) and \( N = 119 \) (d) blowup of (c), with the blowup of the vertical axis.

Fig. 16
Cell lineage diagram corresponding to the simulation of Fig. 15. The vertical axis shows the time, while the horizontal axis shows a cell index, defined as in Fig. 13.
Fig. 17
Temporal change of the number of cells $N$, and very strong, strong, and weak cells for the simulations of Fig. 15 and 16. They are defined by $\sum_m x_i^{(m)}>10$, $1<\sum_m x_i^{(m)}<10$, and $\sum_m x_i^{(m)}<1$, respectively.

Fig. 18
Snapshot (a) and average (b,c) of chemical concentrations of $x_i^{(m)}$. In the simulations for Figs. 18–21, we adopt the chemical network of Fig. 5b) and use the parameters $p=10.0$, $e_0=e_1=1$, $\bar{X}_0=10$, $D=0.02$, $\gamma=0.2$, $x_M=10.0$, $R=100$, $S=0.01$, $D_{out}=f=0.005$, and $V=1000$. For $t>700$, concentrations of chemicals 4 and 8 almost vanish, which are not plotted in the figure. The values at (integer) cell indices give corresponding chemical concentrations while lines are drawn only for the clarity of the figure. The circle or square is plotted for $x_1^{(1)}(i)$ to show the existing cell clearly. (a) Snapshot: $t=717$, when the cell number $N=32$, (b) Average over $718 < t < 1822$ and $N=64$ (c) Average over $2355 < t < 2962$; Those cells are dead whose indices do not have corresponding circles for $x_1^{(i)}$ (such as the cells between 7 and 15 or between 37 and 64).

Fig. 19
Time series of the number of cells $N$, for the simulation of Fig. 18.

Fig. 20
Cell lineage diagram corresponding to the simulation in Fig. 18–19. The diagram is plotted in the same manner as Fig. 13. (a) for $t < 2600$ (b) for $t < 4400$ (c) for $t < 20000$. In (a), color corresponds to the cell’s character defined from the average chemical pattern. After differentiation, the activity of a cell is in the order of green, blue, and red while initial red cells correspond to undifferentiated ones. The “green” cell has $x_1^{(1)}>0.12$, while the blue cell has $x_1^{(2)}<0.11$ in Fig. 18b).

Fig. 21
Average chemical concentrations of $x_i^{(m)}$. The average is taken over the time steps from the latest cell division (or since its birth, when it has not experienced the division yet). The network and parameters are same as for Fig. 6. (a) $t=48$, when the cell number $N=48$, starting from 4 determined cells and 4 undifferentiated cells (b) $t=30$ and $N=32$, starting from 7 determined cells of one type, and one determined cell of another type. (c) $t=40$ and $N=42$, starting from 20 determined cells of one type.

Fig. 22
Overlaid time series of $x^{(m)}(t)$ of a single cell in medium. The network is given by (randomly chosen) 4 autocatalytic connections from 64 chemicals. The dynamics of 4 chemicals from (a) is given in (b). The parameters are set as $p=10.0$, $e_0=e_1=1$, $\bar{X}_0=5$, $\gamma=0.2$, $x_M=10.0$ $D_{out}=f=0.005$, and $V=1000$, while division and death processes are not included.

Fig. 23 Schematic representation of the evolutionary process of metabolic networks. The network (b) is added to (a). Note that the part (b) is autocatalytic as a set.