



S0092-8240(96)00044-4

ISOLOGOUS DIVERSIFICATION: A THEORY OF CELL DIFFERENTIATION

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An isologous diversification theory for cell differentiation is proposed, based on simulations of interacting cells with biochemical networks and the 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 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.

The 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. © 1997 Society for Mathematical Biology

1. Introduction.

1.1. *Biological background.* The development of organisms from their fertilized eggs is one of the most elegant emergence features in biology and has been investigated by many cellular and molecular biologists to elucidate how different types of cells appear and organize the 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 differential physiological states and to produce a variety of cells.

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The gene network picture is expressed by “canalization”. Depending on initial conditions, there are a variety of final states, as expressed schematically by the landscape in Fig. 1a. In terms 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 toward the gene network picture remains: How

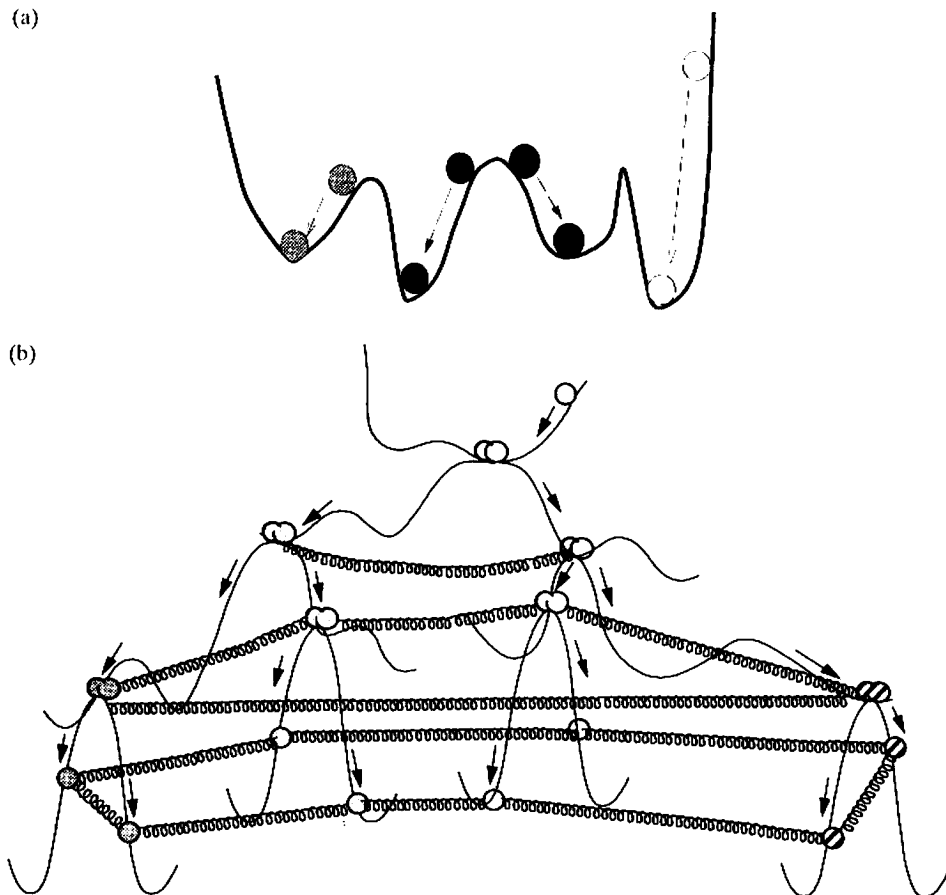


Figure 1. Schematic representation for two pictures of cell differentiation. (a) Fixed landscape; (b) our picture based on interplay between intra- and inter-cellular dynamics.

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, de-differentiate and come to a different cell type. Therefore, the inter-cellular 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 different types of foci in size under the same condition (Yao and Rubin, 1994; Chow *et al.*, 1994, Rubin, 1994a, b). 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 inter-cellular 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 and Comper (1990). However, we believe that no one 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 to give a model with a one-to-one correspondence with biological facts at 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 a 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 modeling 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 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 dynamic clustering in globally coupled chaotic systems (Kaneko, 1989, 1990, 1991, 1994a), 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, 1994a). Even if such 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 interactions¹. 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 a schematic representation).

¹ The use of the term "isologous" is suggested by Susumu Ohno.

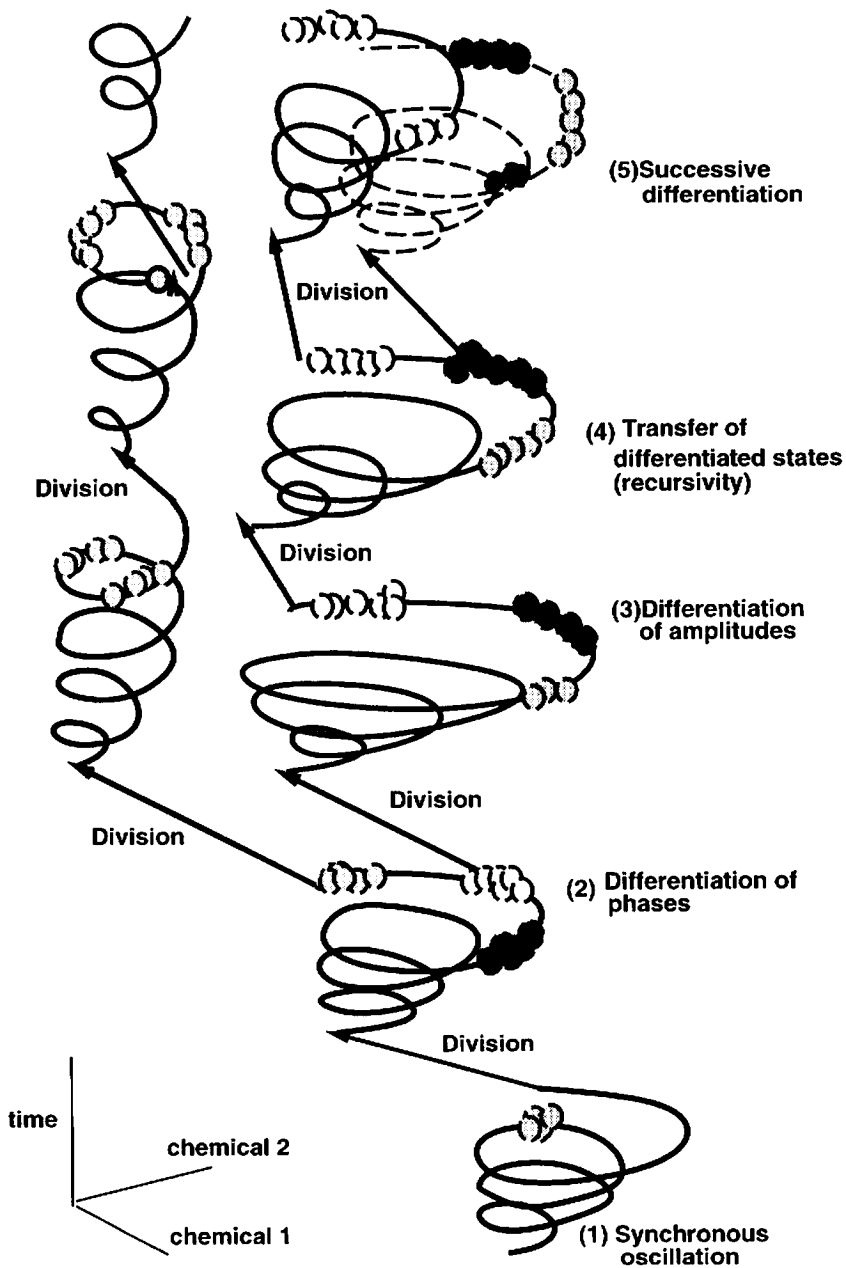


Figure 2. Schematic representation of our isologous diversification. Each spiral represents oscillatory dynamics. In figure, each stage shifts to next by a single reproduction process (e.g. a cell division) for simplicity, but in general, there are several reproductions in each stage. See section 5 for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

Let us take biological units (e.g. cells), interacting with each other, and with the ability to reproduce. A state of each unit has internal dynamics (e.g. biochemical reaction) which allows for non-linear oscillation (through, e.g., autocatalytic reaction). Through this inter- and intra-unit dynamics, the total system consists of coupled non-linear oscillator units. As the number of units increases by the reproduction, they are differentiated spontaneously, through the following stages².

- (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, 1990). 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 the behavior of stages (e.g. composition of chemicals, cycles of oscillations and so on) is differentiated. The clustering of units with regard 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 differentiated cell is preserved to its offsprings. The 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 non-linear oscillations, interaction and the change of degrees of freedom (e.g. the number of cells).

² The later stages are not necessarily chronologically separated. Stage (5) often proceeds with (3) and (4), while stage (4) can occur with (3).

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 remain 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 non-linear dynamical systems, the stability of collective behavior is theoretically confirmed (Kaneko, 1990, 1992).

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 we portrayed. Through this process, a co-operative society of units emerges as a higher level³.

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

³ In this respect, isologous diversification provides a logic for "major transitions" (Szathmary and Maynard-Smith, 1995).

simulations of a specific model. With this demonstration, we provide a coherent answer to the problem raised in section 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, the dynamical systems approach to 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 are activated after some divisions, consistent with expressions of genes by switchings. Since gene expressions are tightly linked with intra-cellular chemical reactions, which are subject to inter-cellular changes, cell differentiation satisfies the postulates of 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 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 the 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 in the differentiation of cells in a well-stirred medium (Ko *et al.*, 1994). It must be noted that the authors do not disregard the spatial effect that is important at a later stage of development for the spatio-temporal organization. Indeed, some preliminary studies including a spatial factor in differ-

entiation suggest the validity of the present scenario and also the amplification of differentiation by spatial inhomogeneity at a later stage.

1.4. *Organization of the paper.* The present paper is organized as follows. In section 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 section 3, in relation with the structure of the chemical network. Explicit examples of dynamic differentiation are given in section 4. Following these results, we propose a general scenario of cellular differentiation in section 5, while some additional analysis of the scenario is given in section 6, based on dynamical systems theory. We discuss the initiation of differentiation, chemical “division of labors”, formation of tumor-like cells and simultaneous multiple deaths. In section 7, some results on the numerical experiments of cell transplantations are given, from which the significance of cellular memory is clarified. Section 8 is devoted to summary and discussions.

2. Model. The biochemical mechanisms of cell growth and division are very 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 proposed scenario is independent of the details of modeling as long as items (1)–(3) are included. For simulations, however, we need a specific model. Here, one example of such a model is given, to propose a dynamic scenario of cell differentiations. The basic structure of our model is the same as the previous one (Kancko 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 a schematic illustration of our modeling.

2.1. *Internal reaction.* First, we adopt a set of k chemical 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.

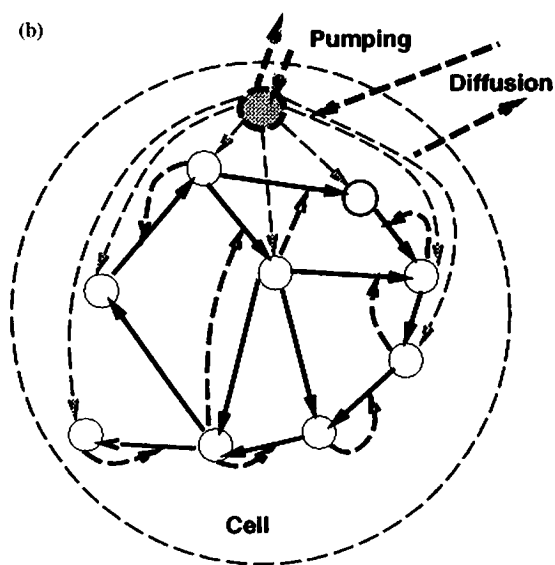


Figure 3. (Continued).

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 $m \rightarrow l$ 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 $E_j^{m \rightarrow l}$ obeys the dynamics $dE_j^{m \rightarrow l}/dt = \text{const.} \times x^{(j)} - \delta E_j^{m \rightarrow l}$. Assuming, for simplicity, fast dynamics for enzymes, we adiabatically solve the above reaction equation of enzyme concentrations, to get $E_j^{m \rightarrow l} \propto x^{(j)}$.

Let us apply the Michaelis–Mentens form for the reaction from $x^{(m)} \rightarrow x^{(l)}$ aided by the enzyme $E_j^{m \rightarrow l}$. Thus, the reaction from chemical m to l aided by chemical j leads to the term $e_1 x_i^{(j)}(t) x_i^{(m)}(t) / (1 + x_i^{(m)}(t) / x_M)$, where x_M is a parameter for the Michaelis–Mentens form, and e_1 is the coefficient for the reaction.

Summing up, $x^{(l)}$ is produced with the path from chemical m , with the aid of chemical j . Here, j and m depend on l , and generally there can be several paths for the production of m . Here, we use the notation $\text{Con}(m, l, j)$, which takes the value 1 when there is a path from chemical m to l catalyzed by 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 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 division⁴, as will be given in process 2.3.

The paths from the source chemical $x^{(0)}$ lead to the term $S(l)e_0x_i^{(0)}(t)x_i^{(l)}(t)$, where $S(l) = 1$ when there is a path from 0 to l , and 0 otherwise. The path to the final product from some chemicals $x^{(l)}$ leads to a linear decay of $x^{(l)}$, with a coefficient γ . This term is expressed by $\gamma P(l)x_i^{(l)}(t)$, where $P(l) = 1$ if there is a path from chemical l to the final product, and otherwise $P(l) = 0$. Summing up all these processes, we obtain the following contribution of the chemical network to the growth of $x_i^{(l)}$ (i.e. $dx_i^{(l)}(t)/dt$):

$$\begin{aligned} \text{Net}_i^{(l)}(t) = & S(l)e_0x_i^{(0)}(t)x_i^{(l)}(t) \\ & + \sum_{m,j} \text{Con}(m,l,j)e_ix_i^{(j)}(t)x_i^{(m)}(t)/(1+x_i^{(m)}(t)/x_M) \\ & - \sum_{m',j'} \text{Con}(l,m',j')e_ix_i^{(l)}(t)x_i^{(j')}(t)/(1+x_i^{(l)}(t)/x_M) \\ & - \gamma P(l)x_i^{(l)}(t), \end{aligned} \quad (1)$$

where we note that the terms with $\sum \text{Con}(\dots)$ represent paths coming into l and out of l , 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. 3b. When $m = l$, the reaction is regarded as autocatalytic, in the sense that there is a positive feedback to generate 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 more detail.

2.2. Active transport and diffusion through membrane. A cell takes chemicals from the surrounding medium. Interactions among 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:

$$\text{Transp}_i^{(m)}(t) = p \left(\sum_{l=1} X_i^{(l)}(t) \right) X^{(m)}(t). \quad (2)$$

⁴ With regard to the interplay between metabolic reaction and the cell division factor, the present model may have a common feature with the "chemton model" by Ganti (1975).

The summation ($\sum_{l=1} x_i^{(l)}(t)$) is introduced here to mean that a cell with more chemicals is more active. Here, we choose this bi-linear form for simplicity, although non-linear dependence on $\sum_{k=1} x_i^{(k)}(t)$ (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

$$\text{Diff}_i^{(m)}(t) = D(X^{(m)}(t) - x_i^{(m)}). \quad (3)$$

Combining processes 2.2 and 2.3, the dynamics for $x_i^{(m)}(t)$ is given by

$$dx_i^{(0)}(t)/dt = -e_o x_i^{(0)}(t) \sum_l x_i^l(t) + \text{Transp}_i^{(0)}(t) + \text{Diff}_i^{(0)}(t), \quad (4)$$

$$dx_i^{(l)}(t)/dt = \text{Met}_i^{(l)}(t) + \text{Transp}_i^{(l)}(t) + \text{Diff}_i^{(l)}(t). \quad (5)$$

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:

$$dX^{(m)}(t)/dt = -(1/V) \sum_{i=1}^N \{ \text{Transp}_i^{(m)}(t) + \text{Diff}_i^{(m)}(t) \} - D_{\text{out}} X^{(m)}(t), \quad (6)$$

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 \bar{X}^0 and its flow rate per volume of the medium by f , the dynamics of source chemicals in the media is written as

$$dX^{(0)}(t)/dt = f(\bar{X}^0 - X^0) - (1/V) \sum_{i=1}^N \{ \text{Transp}_i^{(0)}(t) + \text{Diff}_i^{(0)}(t) \}. \quad (7)$$

2.3. 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

$$\int_{t_0(i)}^T dt \sum_l \gamma P(l) x_i^{(l)}(t) > R \quad (8)$$

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 $(\frac{1}{2} + \epsilon)x_i^{(m)}$ and $(\frac{1}{2} - \epsilon)x_i^{(m)}$, respectively, with a small "noise" ϵ , 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 later⁵. The essence of our cell differentiation lies in the amplification process by open chaos.

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 as big 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 equations (4) and (5), given by $-x_i^{(m)}(t)(dV_{\text{cell}}/dt)/V_{\text{cell}}$, 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

⁵ 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 differentiation process is accelerated initially. Still, essentially the same differentiation process occurs with the unequal partition.

model does not make any essential difference with regard to the qualitative behaviors.

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

$$\sum_{j=1}^k x_i^{(j)}(t) < S_i \quad (9)$$

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 equation (1). Roughly speaking, the dynamics strongly depend 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 a few chemicals are activated, and all the other chemical concentrations vanish. Here, no other chemical paths are active, since the ongoing reaction is just Source $\rightarrow x^{(j)} \rightarrow$ Division Factor, without any reactions $x^{(j)} \rightarrow x^{(l)}$ (see Appendix A). When the number of autocatalytic paths per chemical is small, on the other hand, many chemicals are generated, but the dynamics fall 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 show 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 $x^{(l)}$ by taking only one cell and medium, without imposing the division condition (i.e. the dynamics are given by two sets of chemicals for one cell and the medium), where periodic alternations of dominating chemical 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

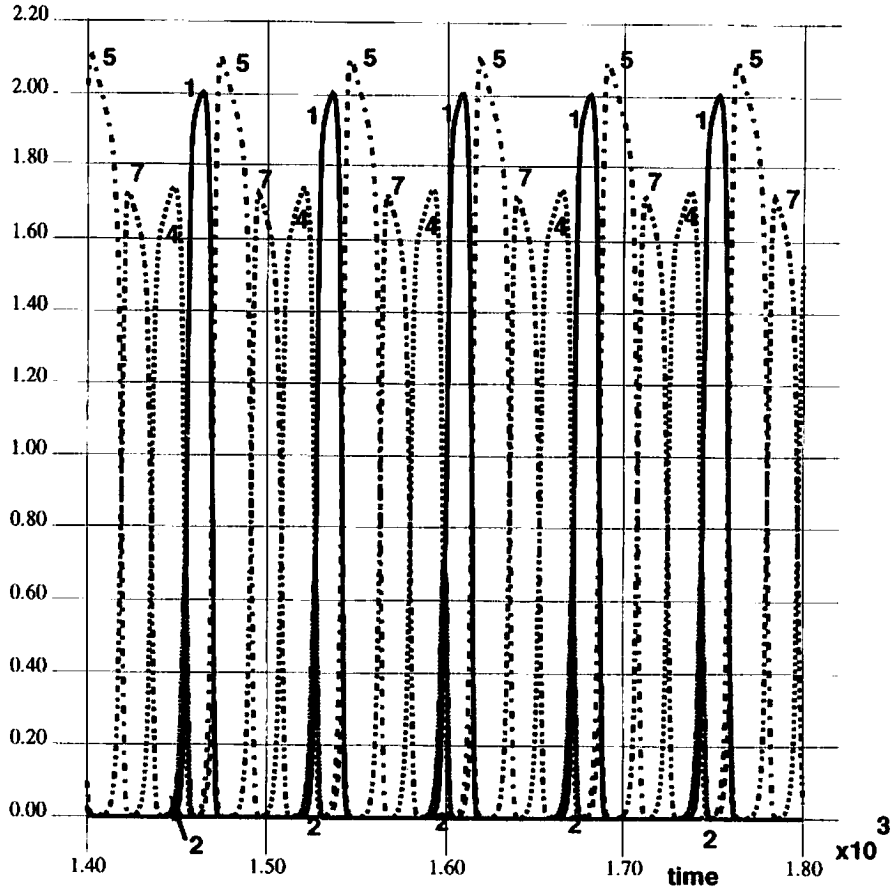


Figure 4. Overlaid time series of $x^{(m)}(t)$ of a single cell in medium, obtained from a network with three connections of eight chemicals whose connection is given in Fig 5a. Each line with number $m = 1, 2, 4, 5, 7, 8$ gives time series of corresponding chemical $x^{(m)}(t)$. Note that chemical 2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

Appendix B 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 two to six per chemical. Since typical behaviors are rather common, we present an example of simulation results by taking the

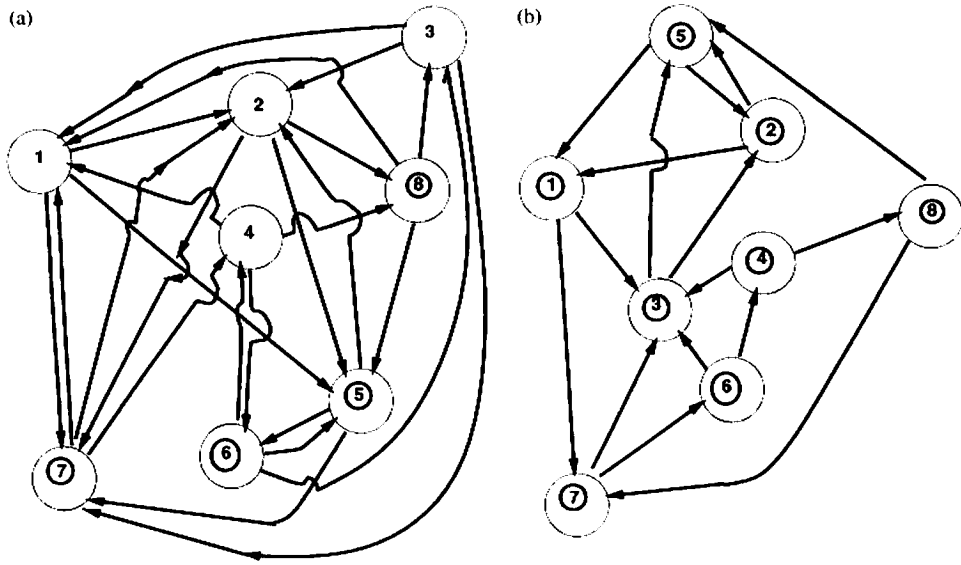


Figure 5. Biochemical network adopted in simulations shown in present paper. (a) Three outgoing connections per chemical; (b) two outgoing connections per chemical. Species with a double circle has arrow to division factor product ($P(t) = 1$), while all chemicals have arrows from source chemical 0 (i.e. $S(m) = 1$ for all m).

network with $k = 8$, given in Fig. 5a (with three randomly chosen autocatalytic paths per chemical).

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 $1, 2, 4, 8, \dots$. When the number exceeds some threshold value, the oscillation is de-synchronized, as in Fig. 6, where the time series of chemicals is plotted. In the figure, phases of oscillations of eight cells split roughly into two 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 two distinct

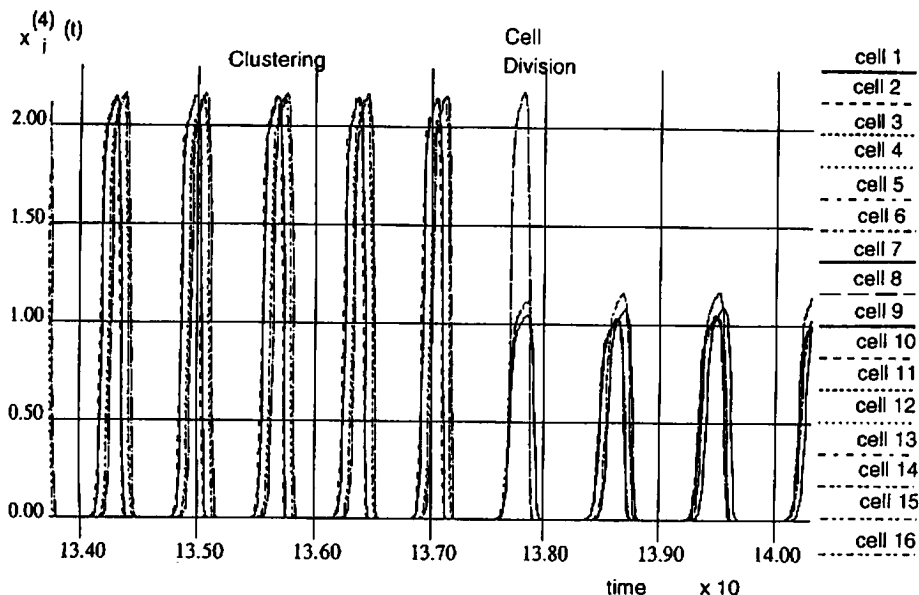


Figure 6. Time series of $x_i^{(4)}(t)$ for $134 < t < 140$, overlaid over all cells. Cell division occurs around $t = 137.8$, when cell number doubles from 8 to 16. Cell index is defined in order that cell is born. In simulations of present section (given in Figs. 6–14 and 22) we adopt chemical network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

groups is seen around $t \approx 400$. In Fig. 8b, three groups are formed, which are the group of cells 3, 6, 12, 13, 17, 18, 19 and 20, the group of cells 4, 8, 15, 16, 29–32 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 $\overline{\sum_j x^{(j)}}$, with $\overline{\cdots}$ as the temporal average. We refer to the term $\overline{\sum_j x^{(j)}}$ as activity. A cell with larger activity is called strong(er) or active here, which divides faster than other cells.

In Fig. 9a and 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 c are different in nature, while only the phase of oscillations differs between Fig. 9a and b. The time series of chemicals 1, 2 and 3 overlaid for all cells are given in Fig. 10a–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 behavior similar to 1). The clearest difference is seen in

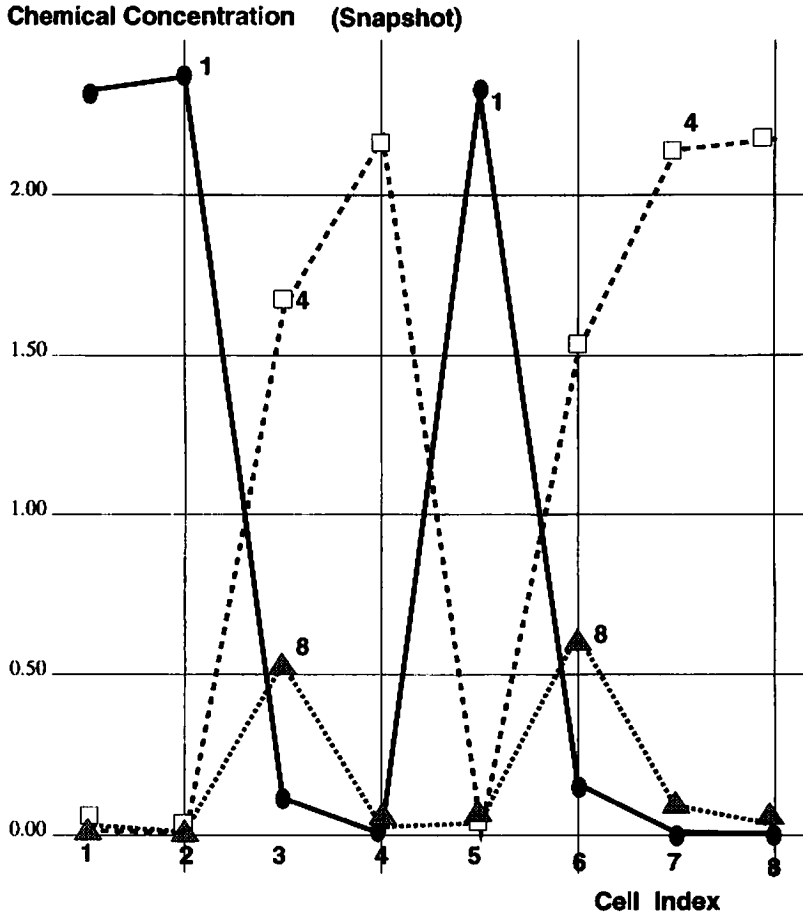


Figure 7. Snapshot chemical concentrations of $x_i^{(m)}(t)$ at $t = 114$, when cell number is 8. Concentration of chemicals $x_i^{(m)}$ for $m = 1, 4$ and 8 are shown, since concentrations of other chemicals are very low at this instant. Lines connecting $x_i^{(m)}$ are just for sake of presentation.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

chemical 3, whose concentration is the smallest among the 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, cells 29, 30, 31, and 32 are direct offsprings of cells 4, 8, 15 and 16. At these later stages, differentiated features are transmitted to daughter cells. (Both of the divided cells are called daughter cells

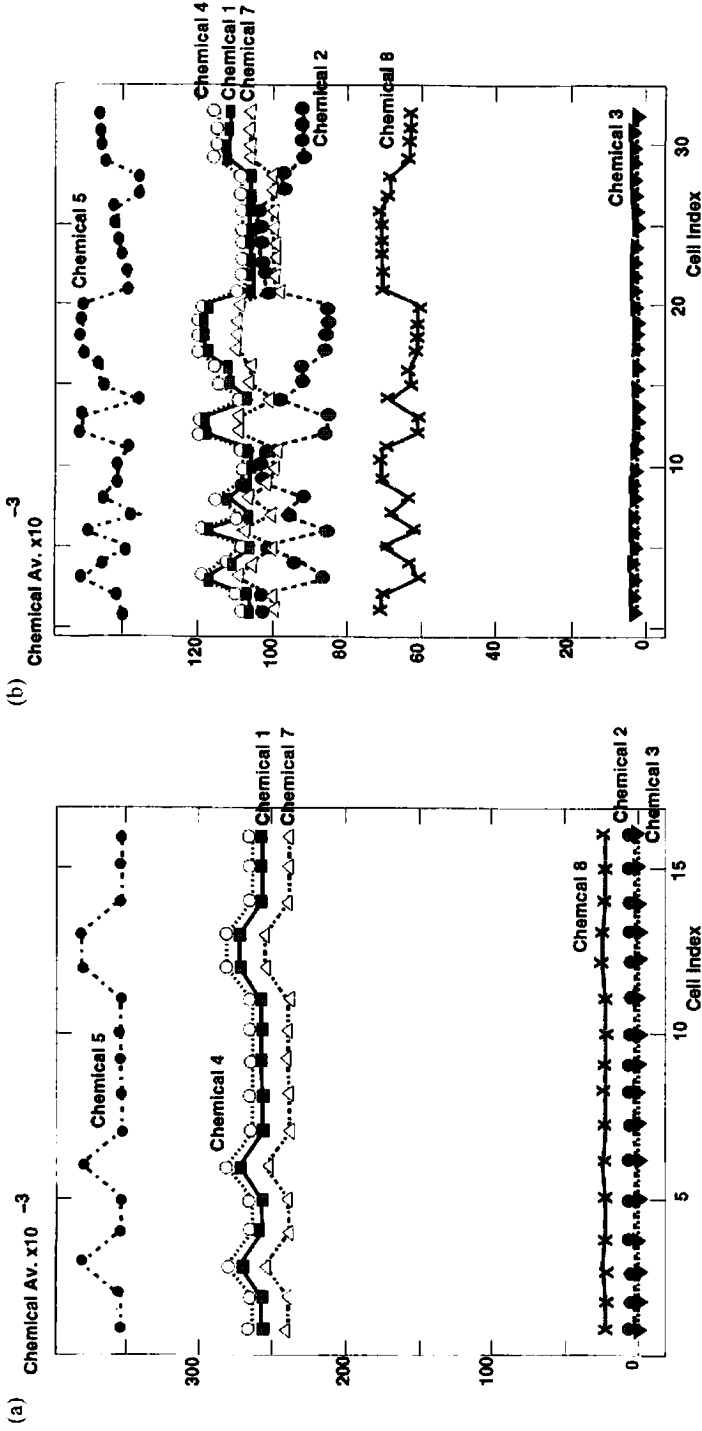


Figure 8. Average chemical concentrations of $x_i^{(m)}$. Average is taken over time steps from latest cell division (or since its birth, when it has not experienced division yet). Each line corresponds to each chemical $x_i^{(m)}$ for $m = 1, 2, \dots, 8$, with \dots as average. Note that line is plotted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

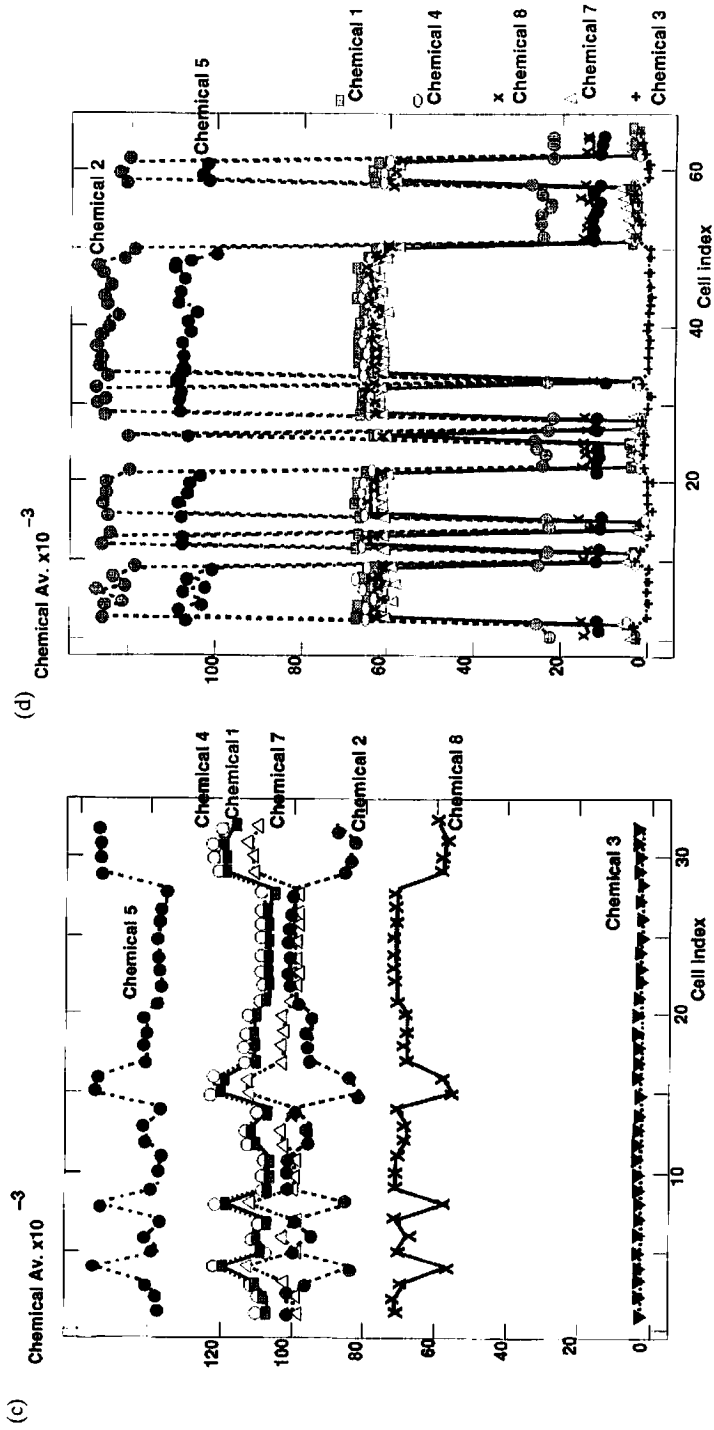


Figure 8. (Continued).

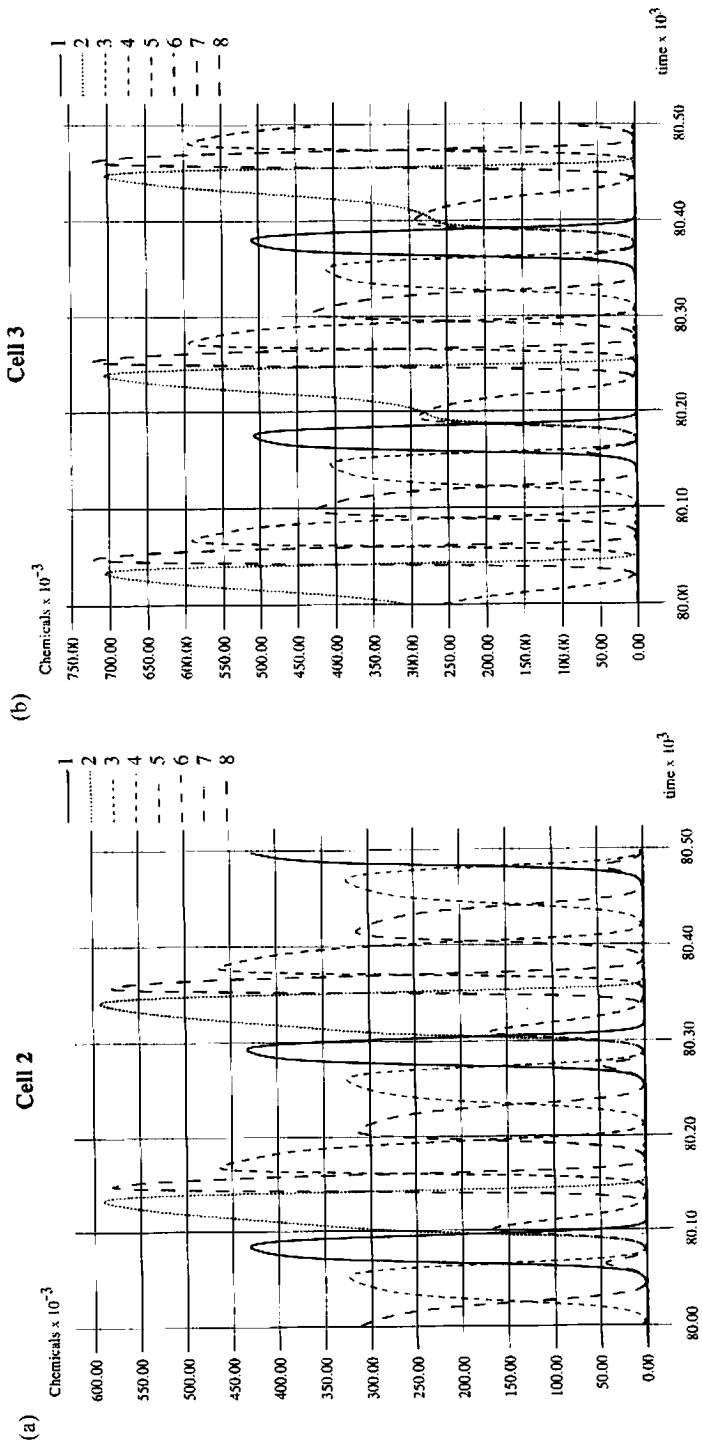
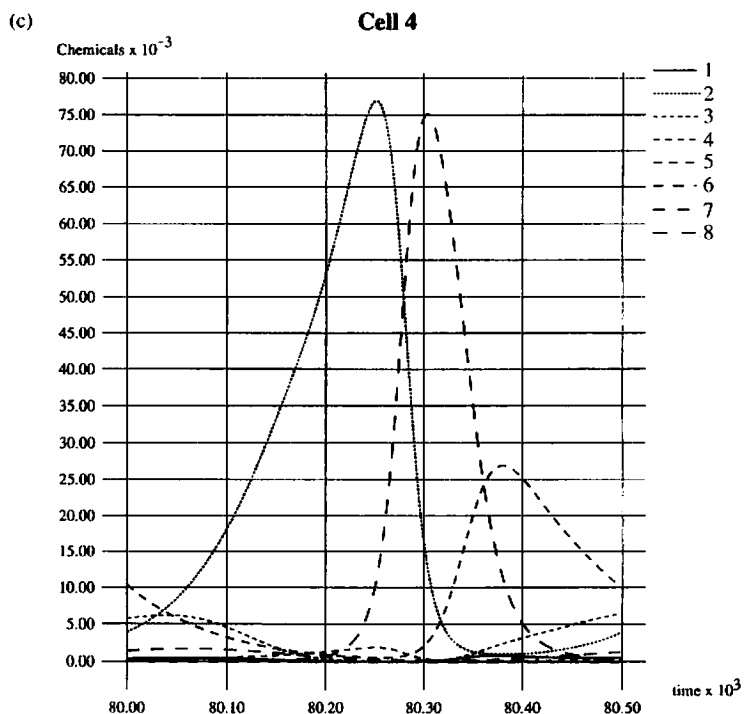


Figure 9. Time series of $x_i^{(m)}(t)$ for $800 < t < 805$, overlaid over all chemical species m . (Each line corresponds to each chemical). (a) For cell $i = 2$; (b) for cell $i = 3$; (c) for cell $i = 4$.



throughout the paper, since there is no principal reason to distinguish the two right after division.)

As the cell number increases, further differentiation proceeds. As shown in Fig. 8d, each group of cells further differentiates into two sub-groups. 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. Insofar 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 cell differentiation (see Fig. 2 for the schematic representation). In our model, the scenario is summarized as follows, where each item corresponds to each stage of the isologous diversification in section 1.2.

5.1. *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 the same concentrations of chemicals. Accordingly, the cells divide almost simultaneously, and the number of cells

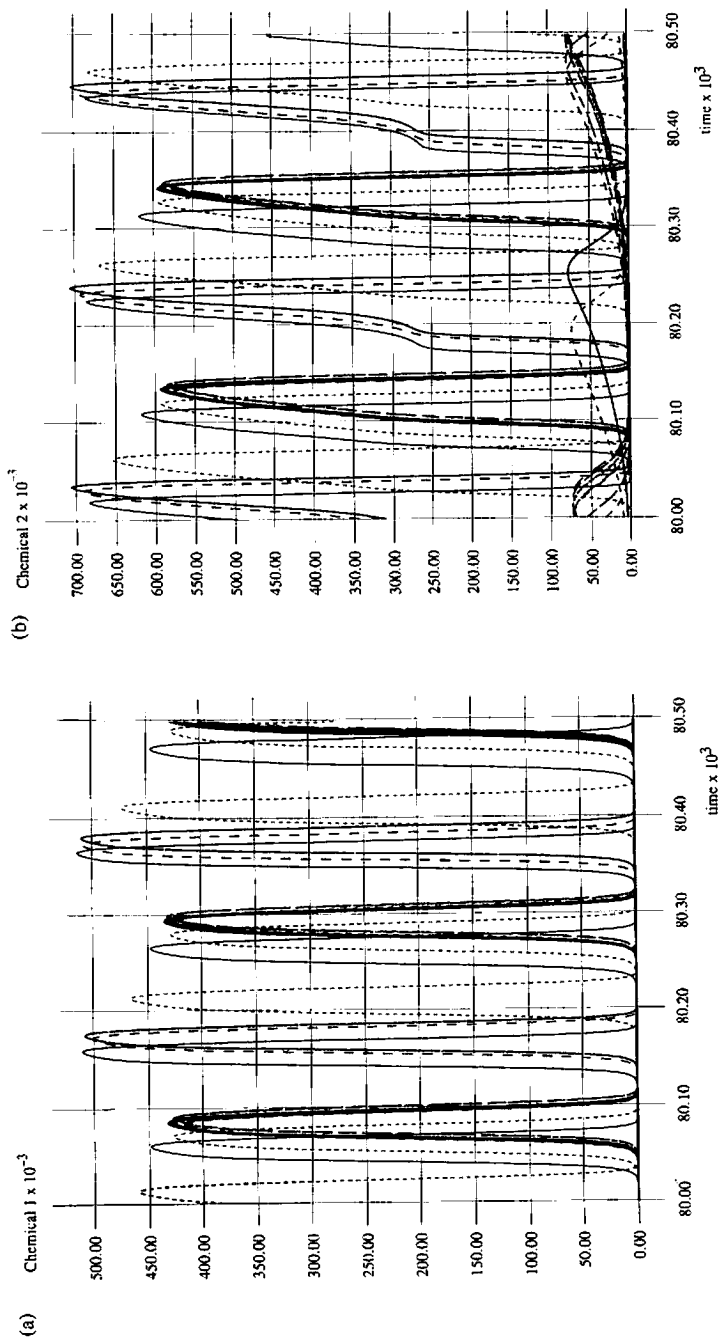


Figure 10. Time series of $x_i^{(m)}(t)$ for $800 < t < 805$, overlaid over all cells i . Each line corresponds to time series of each cell. (a) For chemical species $m = 1$; (b) for chemical species $m = 2$; (c) for chemical species $m = 3$. In (a) and (b), oscillations with larger amplitude correspond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

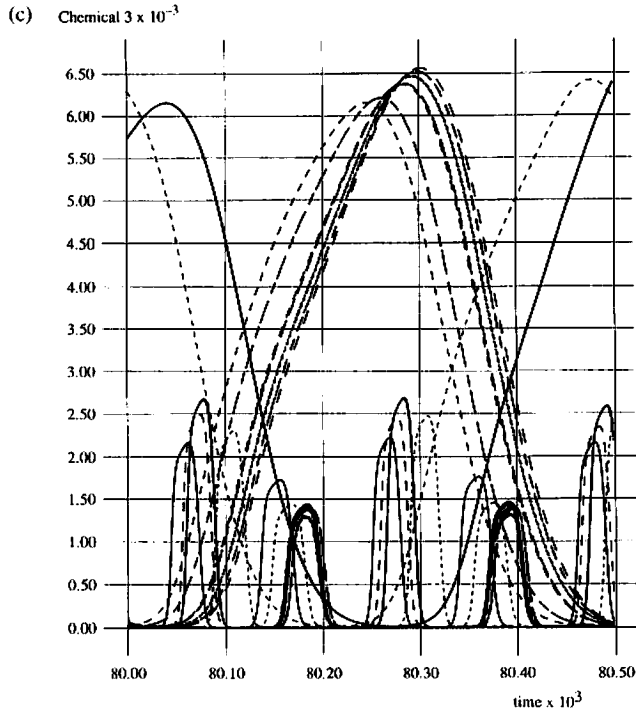


Figure 10. (Continued).

is the power of 2. It is interesting to note that mammalian cells are not differentiated up to the third divisions. Our result suggests that the cell differentiation is triggered not by a gene which counts the cell division but through the cellular interaction.

5.2. Clustering by phases of oscillations. As cells divide further, the chemical oscillations start to lose their synchrony. Cells separate into several groups with almost the same phases of oscillations. As has been discussed (Kaneko and Yomo, 1994; Kaneko, 1994a), 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 the difference of phase of oscillations. Thus, competition is avoided, although any control mechanism is not imposed externally.

At this stage, 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.

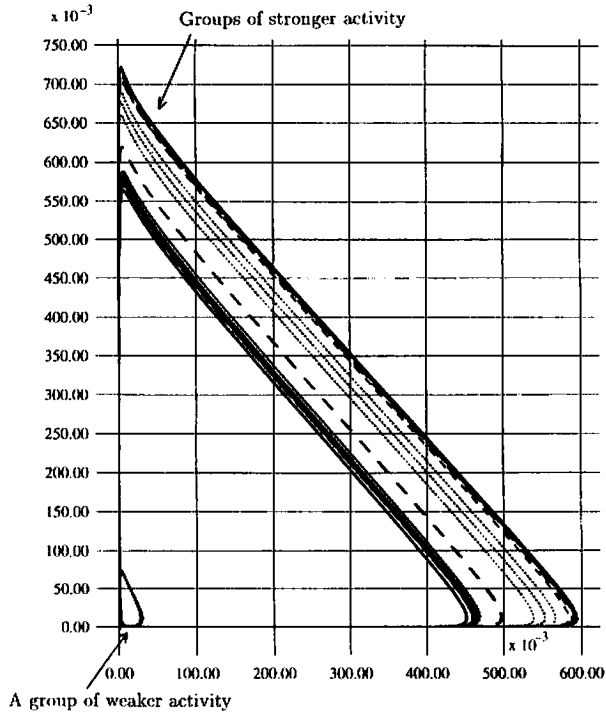


Figure 11. Orbits of chemical oscillations. Plotted are $(x_i^{(5)}(t), x_i^{(8)}(t))$ for $800 < t < 900$ overlaid over all cells (whose number is 64). Two groups are clearly seen.

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

5.3. 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 regard to activities (i.e. differentiation between strong and weak cells) (Kaneko and Yomo, 1994), but also with regard to the composition of chemicals. As 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 section 5.5 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.

5.3.1. *Interaction-induced change of internal dynamics.* If cells are independent, one could think that the fixed differentiation would correspond to different attractors in the intra-cellular 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 intra-cellular dynamics and interactions.

5.3.2. *Two-level differentiations with phase and amplitude.* As mentioned, the phases 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 a few groups, distinguished 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 characteristics. 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 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 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.

5.3.3. *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 seen in Fig. 9 and Fig. 10. (In Fig. 10a), lines taking higher concentrations correspond to stronger cells, while the concentration of chemical 3 is smaller for stronger cells in Fig. 10b. 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 differentiations⁶.

5.4. *Transmission of differentiation to daughter cells.* After the above fixed differentiation, chemical compositions of each group are inherited by their daughter cells. In the other words, when the system enters into this stage, a cell loses totipotency, as will be more clearly shown by the transplantation experiment in section 7. By using a term from cell biology (see e.g. Alberts *et al.*, 1983), we say that the determination of a cell has occurred at this stage, since daughters of one type of cell preserve the type. Hence, a cell at this stage is called a determined one.

With the above transmission, "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 7th division (to create the 90th cell), 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 a molecular biology viewpoint, this may be regarded as some genes starting 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 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

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.

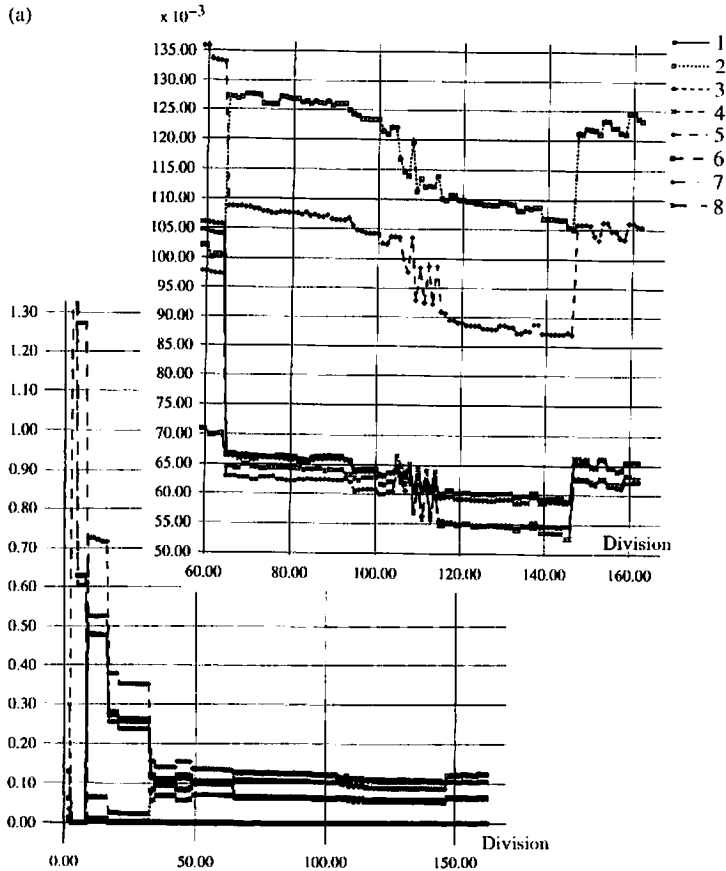


Figure 12. (a) Chemical concentrations $x_i^{(m)}$, averaged over two successive divisions, are plotted in order of divisions. Upper column shows the expansion of lower for divisions later than 60th. Different marks correspond to different chemicals, while lines are plotted only for convenience; (b) return map of chemical concentrations $x_i^{(m)}$ averaged over two successive divisions. Daughter cell's average concentration is plotted versus its mother's cell's average before division to the daughter. Chemicals 2, 5 and 8 are plotted with different marks, while dotted lines are drawn only for convenience. Lower column is the expansion of upper column.

between cellular interactions and intra-cellular 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 characteristics 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 every-

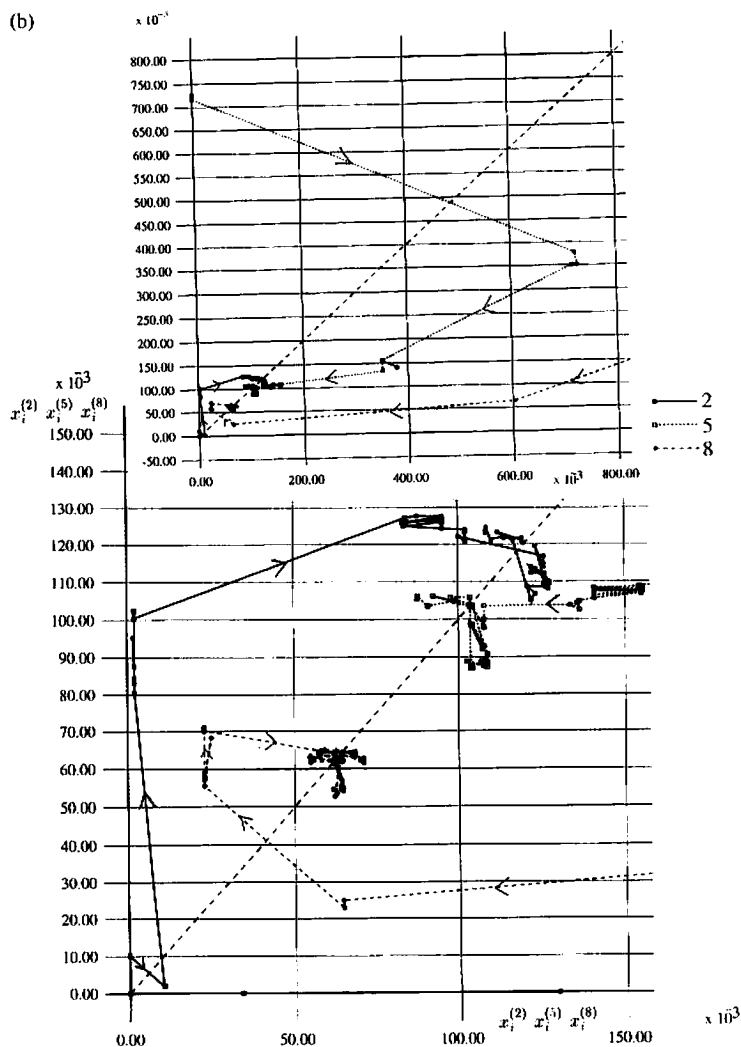


Figure 12. (Continued).

thing 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 non-linear dynamics and selection of initial conditions. The almost "digital" distinction of chemical characteristics, 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.

5.5. *Successive differentiation.* Due to determination, it is possible to draw a cell lineage diagram for each differentiation process, by defining cell types by distinct chemical characteristics. The generation of cell lineage gives rather useful information to be compared with that obtained in cell biology. From the cell lineage, one can see 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 $\overline{x_i^{(2)}} > .125$, while the blue cell has $\overline{x_i^{(2)}} < .03$ in Fig. 8d, with $\overline{\dots}$ 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 diagram (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 characteristic is fixed again, and after some time, such characteristics 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 determinations can be seen in Fig. 8.)

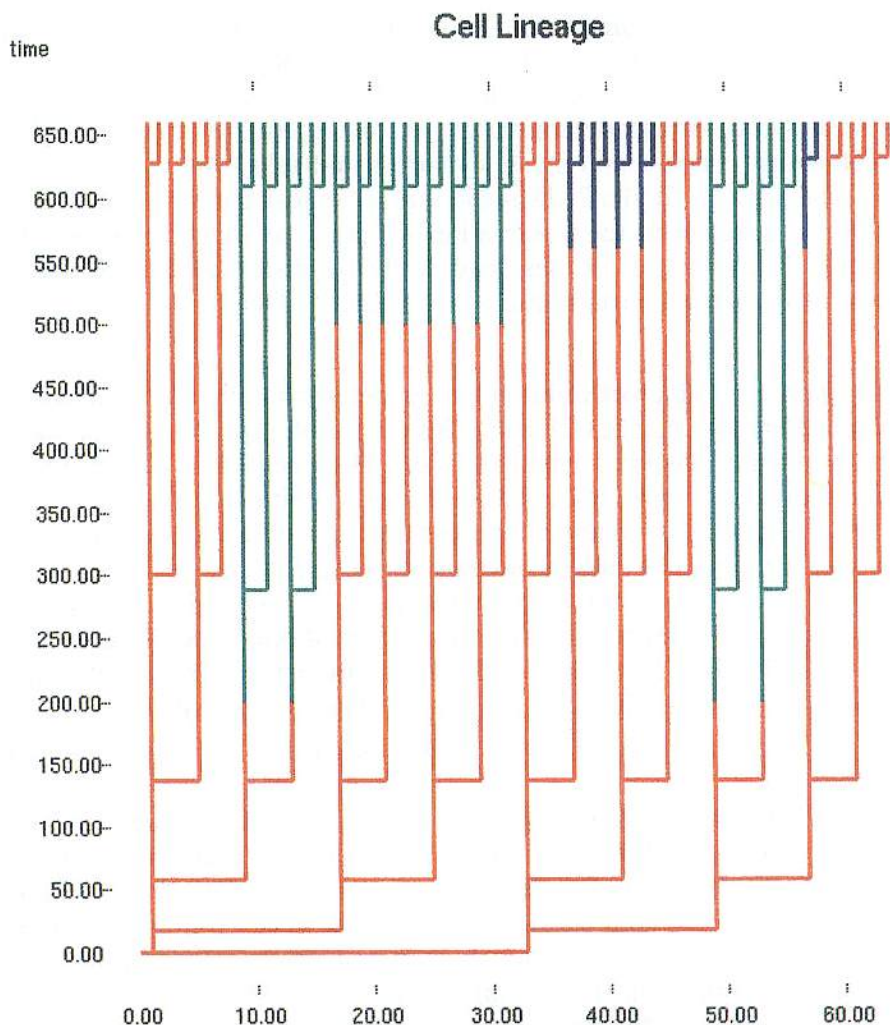


Figure 13. Cell lineage diagram corresponding to simulation in Figs. 6–12. Vertical axis shows time, while Horizontal axis shows a cell index. (Only for practical purpose of keeping track of branching tree, we define index for lineage as follows: when daughter cell j is born from cell i 's k th division,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

Initial “red” cells have the potential to be either “green” and “blue” cells, while some of the “red” cells remain as the same type by the division. Thus, one can write down an automaton-like representation as “red” → “red”, “green” or “blue”, while the division allows only for “green” → “green” and “blue” → “blue”. Since the “red” cell creates “blue” and “green” besides creating itself by divisions, the red cells may be associated with stem cells⁷. 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.

6.1. Initiation 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 a 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 chemical 7 (with high concentration) is negligible, while the difference of chemical 5 (with very low concentration) is remarkable. It is interesting to note that such a chemical with low concentration is important, rather than that with 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 differences by a non-linear mechanism, the difference of “rare” chemicals by cells can be easily amplified to lead to a macroscopic difference of cells.

⁷ In some simulations with a different network, two types of cells (with strong and weak activities) are formed, where the division of a “strong” cell brings about one strong cell and one weak cell each. In this case, the association of the strong cell with the stem cell may be more transparent.

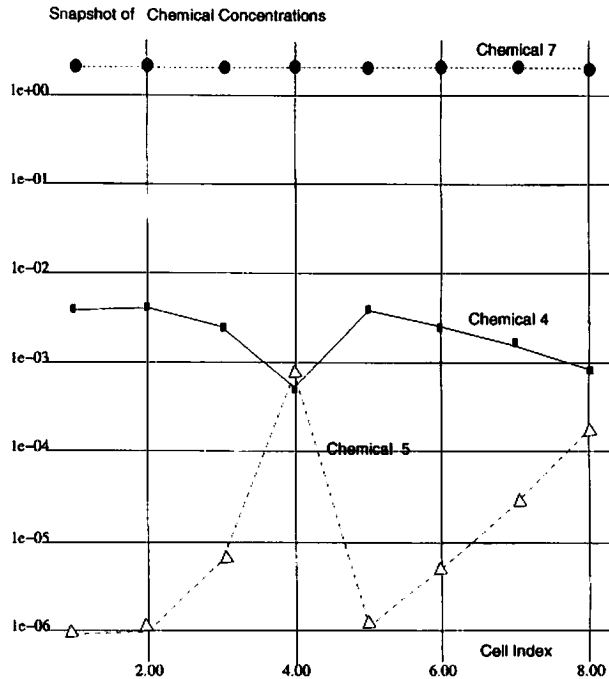


Figure 14. Snapshot chemical concentrations of $x_i^{(m)}$, at $t = 63$, just at onset of chemical difference by cells (clustering). Chemicals 4, 5 and 7 are plotted in a logarithmic scale, in order of cell index (order that cell is born).

6.2. *Specialization of a cell.* In our simulation, there are different types of cells with regard to the variety of chemicals within. Often, only a 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 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 a tiny amount of a chemical). Generally, the bias in chemical concentrations tends to increase with the cell number. This tendency is seen not only with regard 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 expected, the number of cell types increases 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 a 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.

6.3. *Tumor-type cell.* In simulations with a larger diffusion coupling, a peculiar type of cell appears. These cells destroy the ordered use of chemical resources, which makes the cell society disorganized. This type of 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 the 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 destroy 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 mutation, which, in our model, is represented by the “noise” term in the division process (the random number at the division process, when ϵ 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 cell.

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 section 4, and by choosing a larger diffusion coupling ($D = 0.2$), and smaller threshold for the division. In Fig. 15b, 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 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 in number rapidly, and keep the same chemical characteristics. Thus, the “tumor” cells start to dominate the system. As in Fig. 15c, 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 seen in Fig. 15d. Here, we note that chemicals other than the species 4 are richer in the normal (weaker) cells. Hence, the “tumor” cell here is strongly specialized.

Of course, the extremely high concentration of *one* chemical here can be due to the simplicity of our biochemical network with only eight species. For a network with more components, the bias must be weakened. However, it is still expected that there will appear a cell type with a 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.

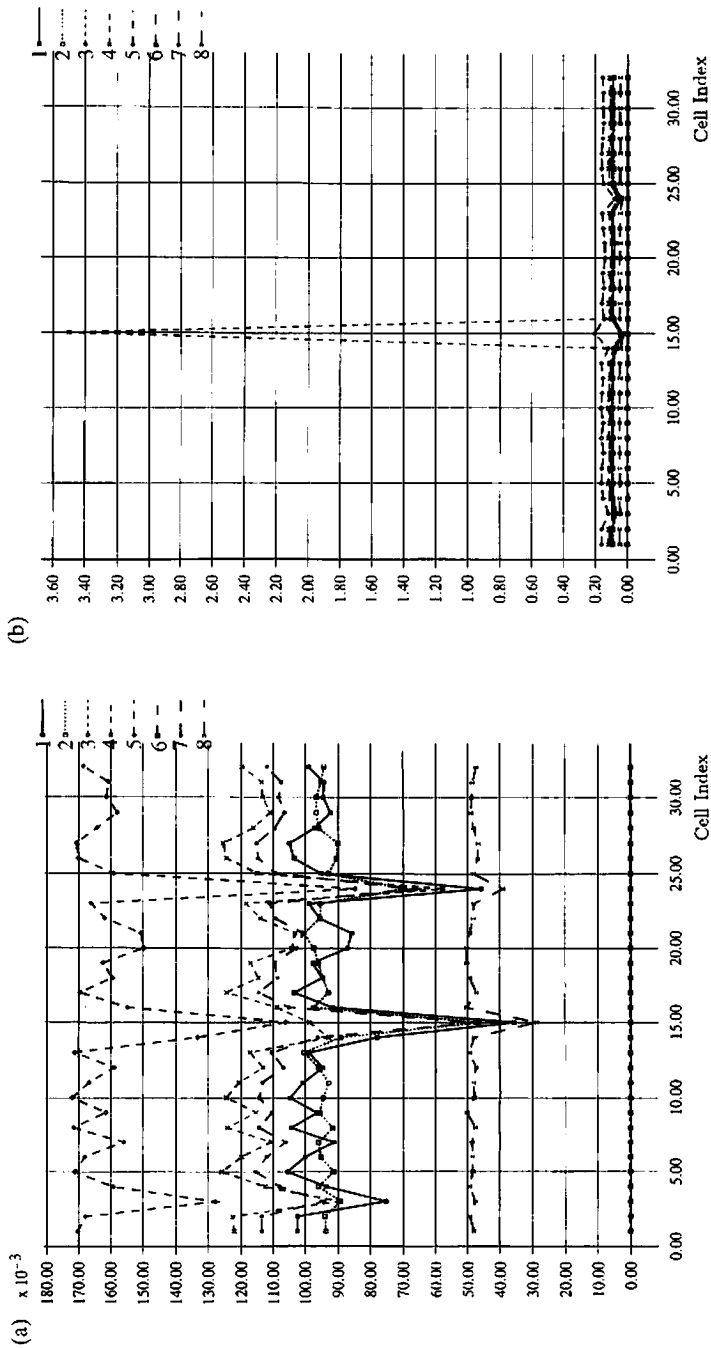


Figure 15. Average chemical concentrations of $x_i^{(m)}$. Average is taken over time steps from latest cell division (or since its birth, when it has not experienced division yet). We adopt chemical network of Fig. 5a and use parameters as for Fig. 6, except D and R , which are taken to be $D = 0.2$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

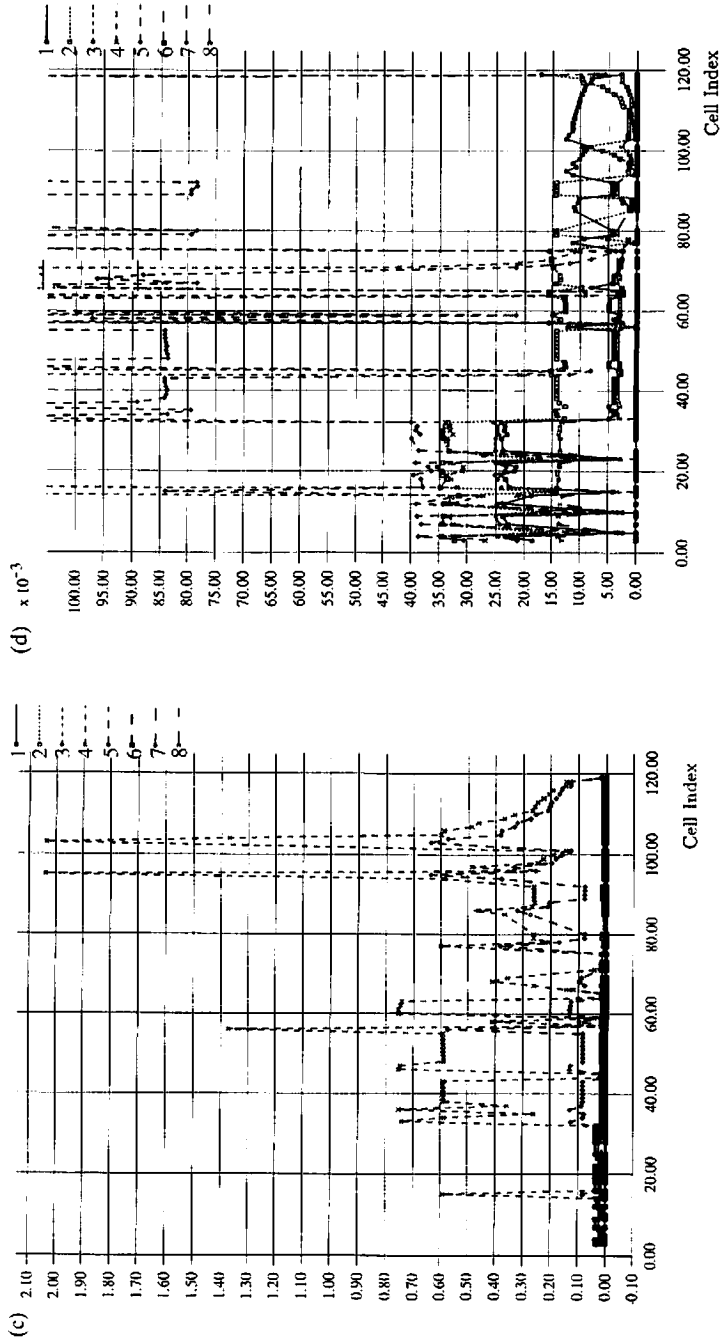


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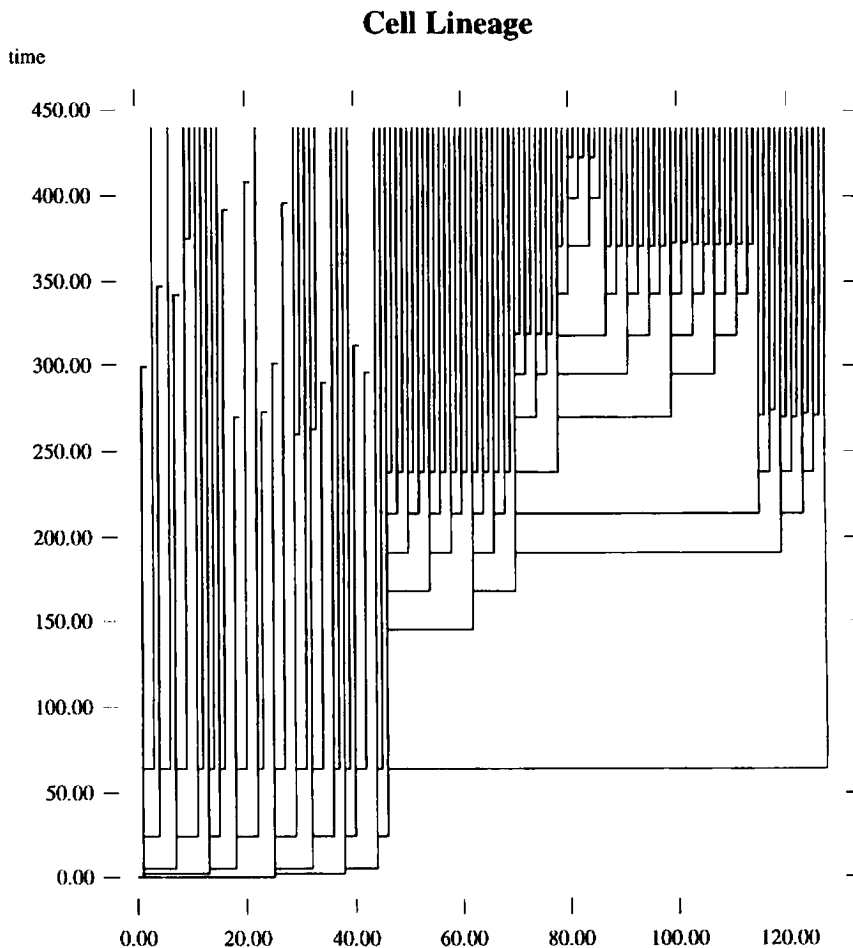


Figure 16. Cell lineage diagram corresponding to simulation of Fig. 15. Vertical axis shows time, while horizontal axis shows cell index, defined as in Fig. 13. The cell death condition is included here, which leads deaths of some non-tumor cells in the left side.

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 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

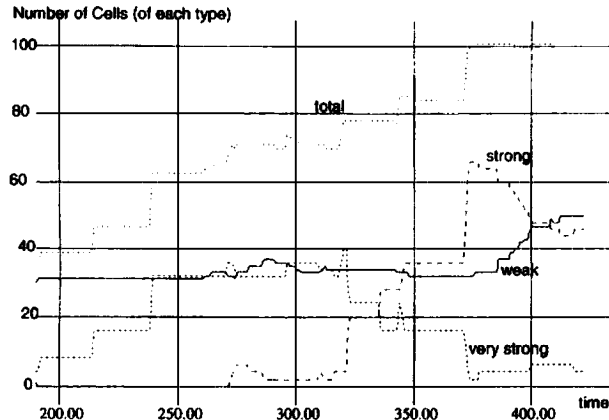


Figure 17. Temporal change of number of cells N , and very strong, strong and weak cells for simulations of Figs. 15 and 16. They are defined by $\sum_m x_i^{(m)} > 10$, $1 < \sum_m x_i^{(m)} < 10$ and $\sum_m x_i^{(m)} < 1$, respectively.

increase of 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 *et al.* (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 the medium are stronger, and the competition for resources is tighter. Taking also into account 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, b), in a series of experiments, has shown that formation of a type of tumor strongly depends on the density of cells, even if the mutation may be relevant to the triggering of 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 co-operative use of resources, are formed.

In the natural course of cell differentiation, the interaction among cells through the chemical environment is not global, but is somehow localized in space. Such a 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), tumor formation is avoided.

6.4. *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 death⁸. 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 eight cells with stronger activity: the 31st and 32nd cells are daughters of the 7th and 16th, and cells 33–36 are daughters of 7, 16, 31 and 32, 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 (thick line), blue (dashed line) and red (thin line) 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 a 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, termed apoptosis, or programmed death. Despite several studies in molecular genetics, no genes or molecules

⁸ Simulation 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 to also see the generality of our results.

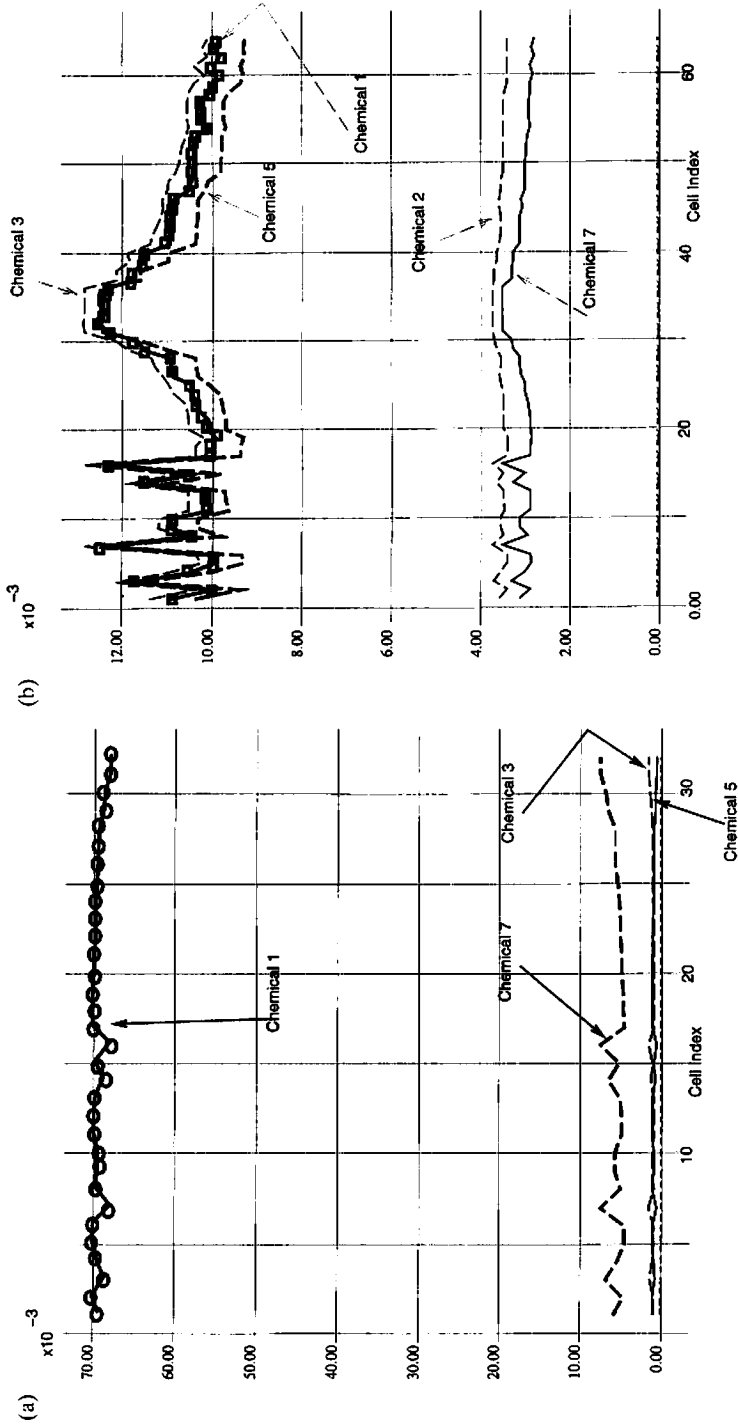


Figure 18. Snapshot (a) and average (b), (c) of chemical concentrations of $x_i^{(m)}$. In simulations for Figs. 18–21, we adopt chemical network of Fig. 5b and use parameters $p = 10.0$, $e_0 = e_1 = 1$, $\bar{X}_0 = 10$, $D = 0.02$, $\gamma = 0.2$, $x_M = 10.0$, $S = 0.01$, $D_{out} = f = 0.005$ and $V = 1000$. For $t > 700$, concentrations of chemicals 4 and 8 almost vanish, which are not plotted in figure. Values at (integer) cell indices give corresponding chemical concentrations, while lines are drawn only for clarity of figure. A circle or square is plotted for $x_i^{(i)}$ to show existing cell clearly. (a) Snapshot: $t = 717$, when 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 $x_i^{(i)}$ (such as cells between 7 and 15 or between 37 and 64).

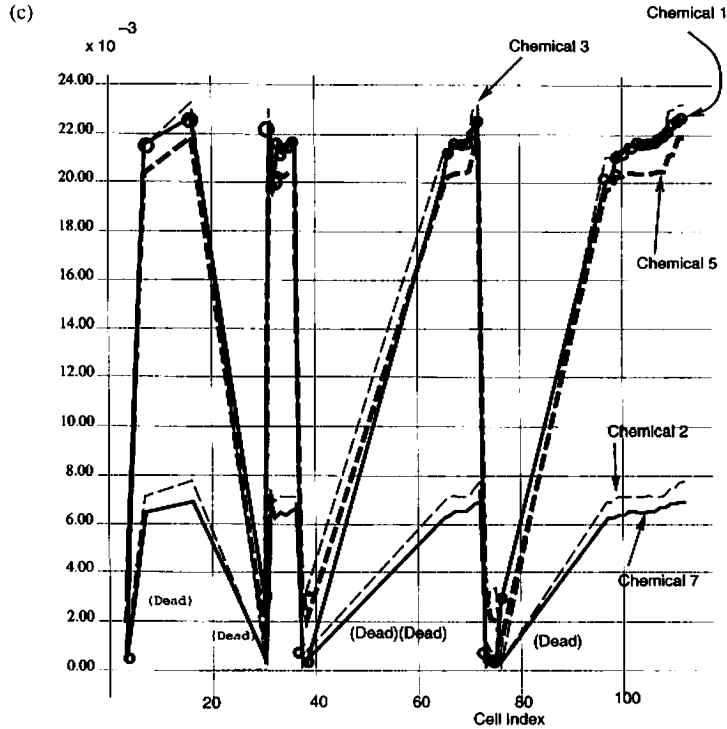


Figure 18. (Continued).

responsible for the age of a 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 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 expected, indicating a change in its fate.

In the present model, no spatial structures are included. Cells cannot move in space for richer nutrition. 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.

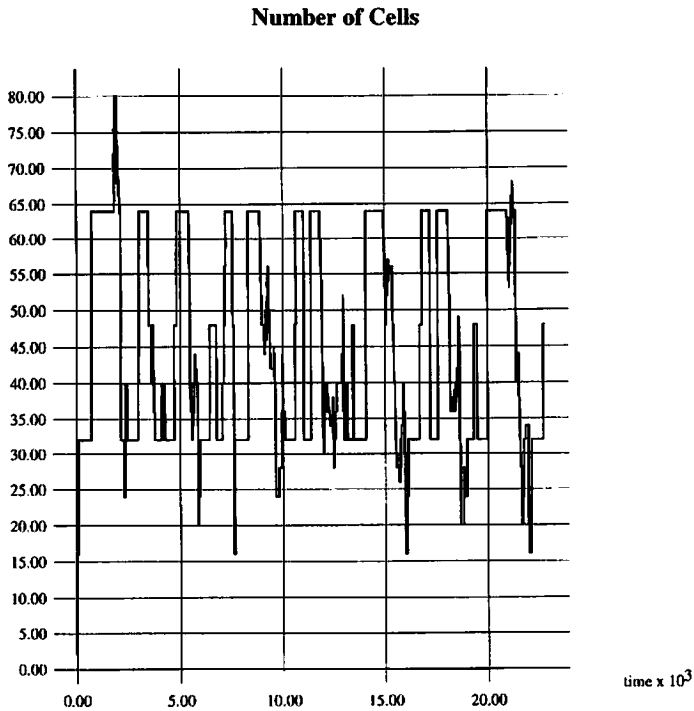


Figure 19. Time series of number of cells N , for simulation in Fig. 18.

6.5. 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 non-linearity 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 oscillation, even if the non-linearity is not large⁹.

Increasing the parameter p or γ enhances differentiation. These changes increase the amplitude and/or the frequency of oscillation, and may be regarded as the increase of “non-linearity”. 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 a few chemical concentrations are larger than zero; in other words, an ensemble of “tumor”-type cells tends 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

⁹ Due to the limitation of computation resources, it is difficult to practically check if the differentiation starts after the number is larger than 256 where the computation requires a much longer time.

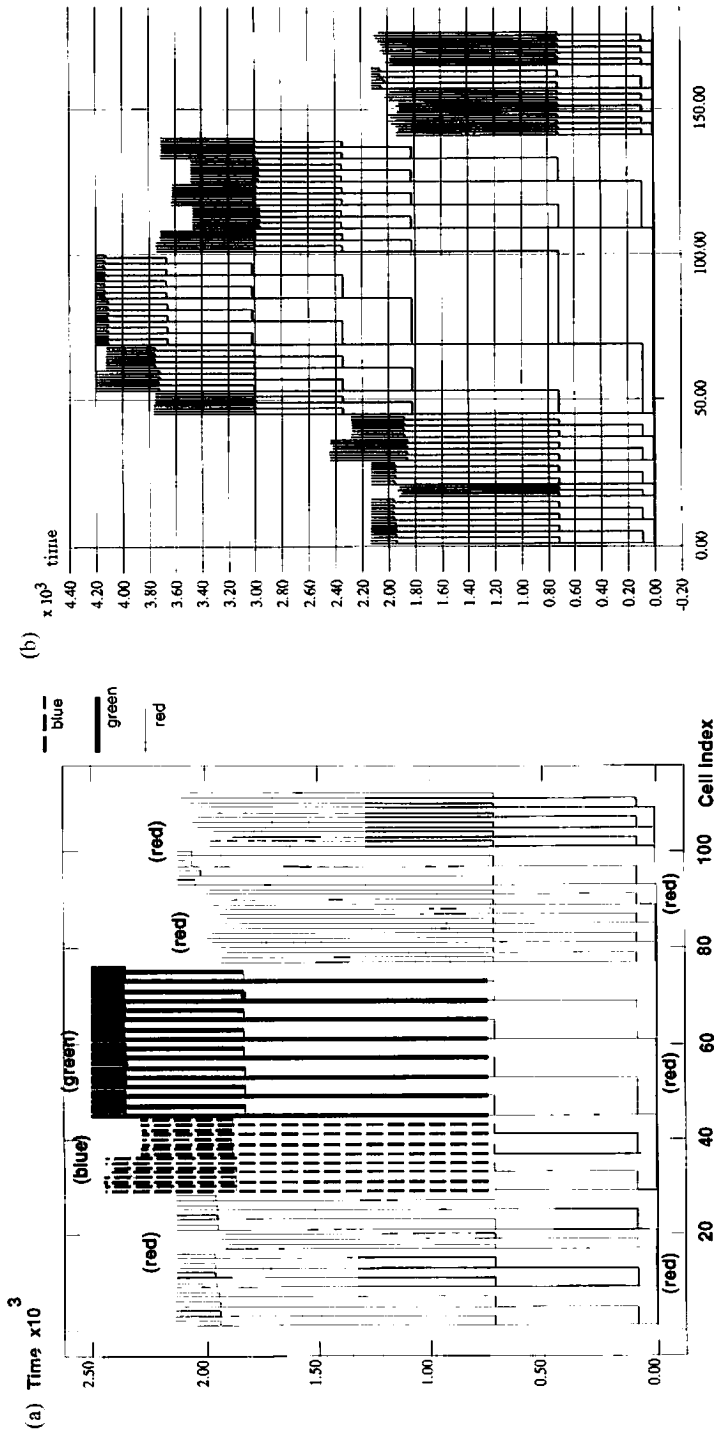


Figure 20. Cell lineage diagram corresponding to simulation in Figs 18-19. Diagram is plotted in same manner as Fig. 13. (a) For $t < 2600$; (b) for $t < 4400$; (c) for $t < 20,000$. In (a), color corresponds to cell's character, defined from average chemical pattern. After differentiation, activity of a cell is in order of green, blue and red, while initial red cells correspond to undifferentiated ones. The "green" cell has $x_i^{(1)} > .12$, while the blue cell has $x_i^{(2)} < .11$ in Fig. 18b.

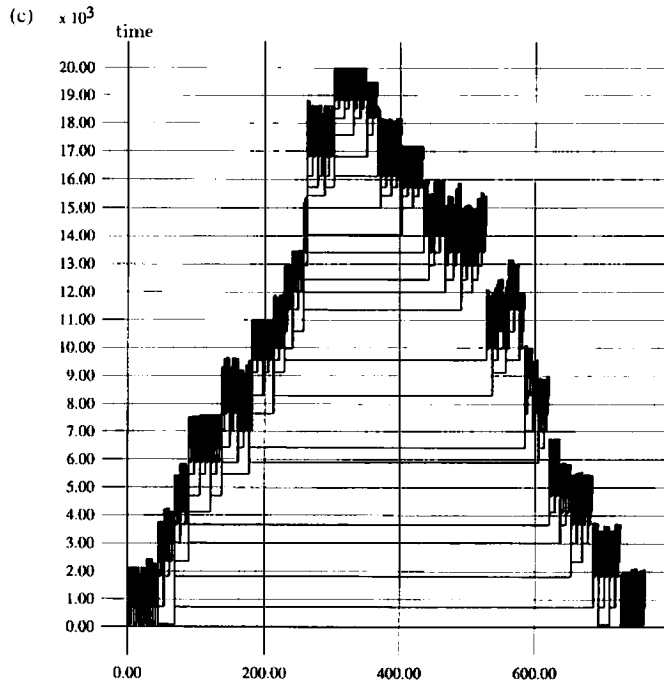


Figure 20. (Continued).

oscillatory dynamics. As for paths to the division factor ($P(I)$) and paths from the source chemical ($S(I)$), there is a tendency that the differentiation process is enhanced when these connections are not full ($= k$).

6.6. *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 cell, 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 cell is decreased (e.g. by external removal), the distribution is recovered through further divisions.

This kind of robustness at an ensemble level is indeed expected from our isologous diversification theory, since the stability of macroscopic characteristics is attained in coupled dynamical systems (Kanku, 1992, 1994a; Yomo

and Kaneko, 1994). This robustness gives a key to understanding 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 characteristics 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 section 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.

7.1. Starting only from a few differentiated cells of the same type in addition to undifferentiated cells. Figure 21a gives the evolution of average chemical concentrations by cells starting from four determined cells (whose cell index is from 1 to 4) and four undifferentiated cells. The former cells are sampled from later stages ($t = 691$) in the simulation of section 3, while the latter ones come from the former stages ($t = 205$). In Fig. 21a, cells 14–17 are the first daughters of cells 1–4 and the cells from 25 to 32 are the daughters of the above eight 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 seen in Fig. 21a.

This robustness of cell memory is kept as long as the ratio of initial determined cells to undifferentiated cells is not too much. (see 7.3 for the case otherwise).

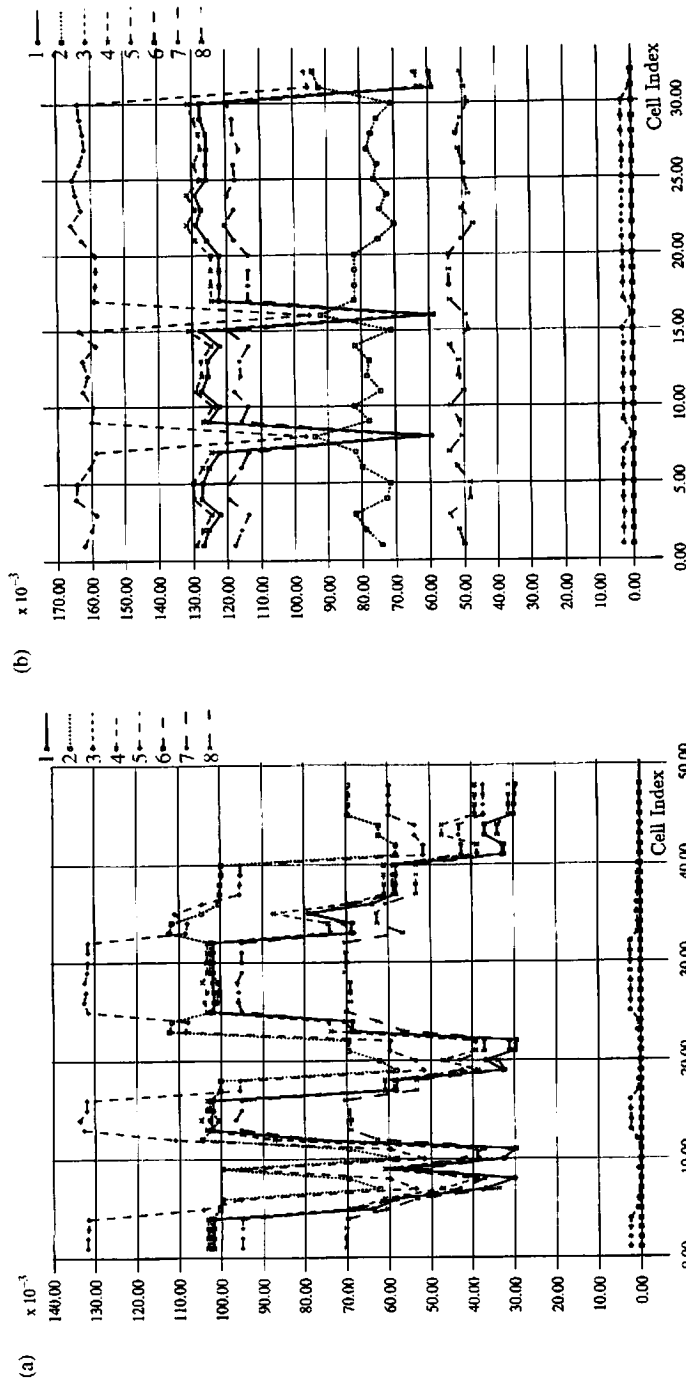
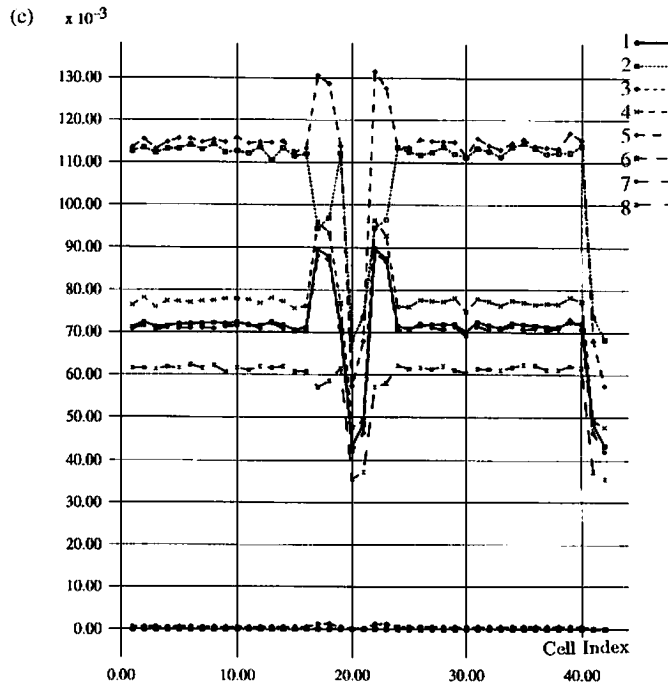


Figure 21. Average chemical concentrations of $x_i^{(m)}$. Average is taken over time steps from latest cell division (or since its birth, when it has not experienced division yet). Network and parameters are same as for Fig. 6. (a) $t = 48$, when cell number $N = 48$, starting from four determined cells and four undifferentiated cells; (b) $t = 30$ and $N = 32$, starting from seven 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.



7.2. *Starting from the mixture of different determined cells.* Again, the cell memory is preserved, and daughters of a cell keep the same characteristics. In Fig. 21b, two types of determined cells are initially taken, seven cells for one type (with the index from 1 to 7), and one cell (the 8th cell in the figure) of another type, obtained from section 3 at $t = 691$. Figure 21b shows the average chemical pattern after two divisions. The 16th, 31st, and 32nd cells, which are offsprings of the 8th cell, keep the characteristics, while other cells remain as 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 7.3 for the case otherwise).

7.3. *Starting only from a few differentiated cells of the same type.* Some cells start to de-differentiate 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 7.1 and 7.2, again some of them start to de-differentiate and become a different type of cell. In Fig. 21c, the average chemical pattern is plotted starting from 20 cells of one type of determined cell of section 3 at $t = 691$. Cells with the index from 1 to 16 and 19 preserve their character, while cells 17 and 18 become a different type, and cell 20 is trans-differentiated to another type. Here, their offsprings again

keep their characteristics: cells 22 and 23 are daughters of 17 and 18, while cell 21 is a daughter of 20, and cells 41–42 are those of 20 and 21. Thus, determination again occurs after the process of de-differentiation.

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 7.1–3, we can conclude that cell memory is preserved mainly in each cell, but cellular interactions are also important to sustain it. The recursivity achieved in section 4 is understood as the choice of internal dynamics through cellular interactions.

Thus, 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.

7.4. Differentiation of “tumor” cells. Another interesting initial condition is the use of “tumor” cells. Starting only from tumor cells, their offsprings remain the same type initially. As the divisions are repeated, some of the 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 $x^{(m)}$ large and $x^{(l)} = 0$ for $l \neq m$. Such a 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, $x^{(l)}$ remains zero for the cell, whose offsprings keep the same type. In this extreme case, the cellular society continues 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 an 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 intra-cellular 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 non-linear oscillators.
- Chemicals with tiny amounts in cells are relevant to trigger differentiations.
- With further divisions, cells lose totipotency, and offsprings preserve the same characteristics. This recursive division of cells appears only after some stage of cell division.
- 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 a 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 small range of cell types successively.
- Determined cell types formed at the later stage are preserved by their transplantation to other parts of cell society as long as there are not too many cells of the same type.
- It is possible to de-differentiate cells by putting them in conditions such as overcrowded cells of an identical type.
- Spontaneous multiple deaths appear through the interaction of cells, after cells are differentiated.
- A type of tumor-like cell is formed, depending on the cellular interaction. These cells destroy the ordered use of resources attained in the cell society. For example, formation is enhanced by the cell density or the diffusion coupling. Transplantation of cells of the same type may enhance 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 amounts of chemicals for triggering 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 differentiation. Here, such a switching-type expression appears naturally through cellular interactions.

As described in section 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 stated, 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 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 on the laboratory scale.

Furthermore, the theory can be extended for medical application. As mentioned in section 6.4, the tumor cells in our model are in the extreme case of loss of variety, where cells lose some metabolites, or start 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 a 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 the metabolites or macromolecules that the cancers lack.

8.2. Isologous diversification. It should be noted that the introduction of tiny differences at the division is not essential to our differentiation scenario. A system composed of identical cell states is unstable when the interaction is strong. On the other hand, 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, 1994a). 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 the same type with other cells, while some cells with identical parameters or close initial conditions become a different type. From this perspective, it is expected that most somatic mutations are irrelevant to differentiation, unless it brings about a drastic change in the parameter for the interaction and intra-cellular dynamics.

Our proposal here is that the differentiation and diversification are not due to variations by reproducing errors but by 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 multi-cellular organism is directly related to 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 differentiation reaches the stage that only one group of cells brings about its offsprings. According to our results, this feature of a multi-cellular organism spontaneously emerges as a consequence of strongly coupled reproducing units. It is not a product of chance, but of necessity in the course of 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 to the extension of our modeling, while the latter three are of a more fundamental issue.

8.3.1. *Universality and simpler models.* Our results are rather universally observed as long as individual dynamics allow for some oscillations. Since globally coupled dynamical systems are known to show spontaneous differentiation as clustering (Kaneko, 1989, 1990), we may expect that our differentiation is universally observed in a large class of coupled system non-linear reproducing units.

To search for a simpler model, we have also checked a model only with a phase variable (Kaneko, 1994a). 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 disparity and fixed differentiation. For this 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 clustering in the 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 for the dynamical systems theory.

8.3.2. *Introduction of spatially local interaction and development.* In the paper, we have assumed that the chemical medium is well stirred, and all cells interact with all others uniformly through the medium. In the developmental process of a multi-cellular organism, spatially local interactions among cells are, of course, important, as 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 a later stage, due to local interaction, spatial organization of differentiated cells occurs, leading to pattern formation, as in the pioneering study by Turing (1952).

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

Since distant cells do not interact directly with each other, differentiation as well as its determination is often enhanced. Another consequence of 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 inter-cellular force to study cellular rearrangements leading to 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 be directly extended to this “batch”-type simulation, by cutting the flow from the outside, i.e. by setting $D_{\text{out}} = f = 0$ and taking a higher density of nutrition initially. Since non-linear 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 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.

8.3.3. *Cellular memory.* The emergence of 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 to co-existing attractors corresponding to different basins for initial conditions, while the stability of differentiation is sustained by the interaction. 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 intra-cellular 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 the immune system or neural networks.

8.3.4. *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 keep the same characteristics. 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 completely identical. We note that the initial condition itself after each division does not fall onto 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 the roles of egg (DNA) and chicken (protein) will be reconsidered along this line.

8.3.5. *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 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 the 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 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 intra-cellular dynamics should be studied in the future.

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 his hospitality during their stay at Santa Fe Institute.

APPENDIX A

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:

$$\begin{aligned} dx^{(1)}/dt &= x^{(2)}x^{(1)}/(1+x^{(2)}) - x^{(1)}x^{(2)}/(1+x^{(1)}), \\ dx^{(2)}/dt &= x^{(1)}x^{(2)}/(1+x^{(1)}) - x^{(2)}x^{(1)}/(1+x^{(2)}). \end{aligned}$$

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 bi-directional connection tends to lead to competition of chemical species in the present model. Indeed, selection of few chemicals is seen when there are many bi-directional pathways.

As an extreme, 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 non-linearity regime (i.e. with large e_1). Here, the dynamical process is just the selection of one chemical species through competition for resources.

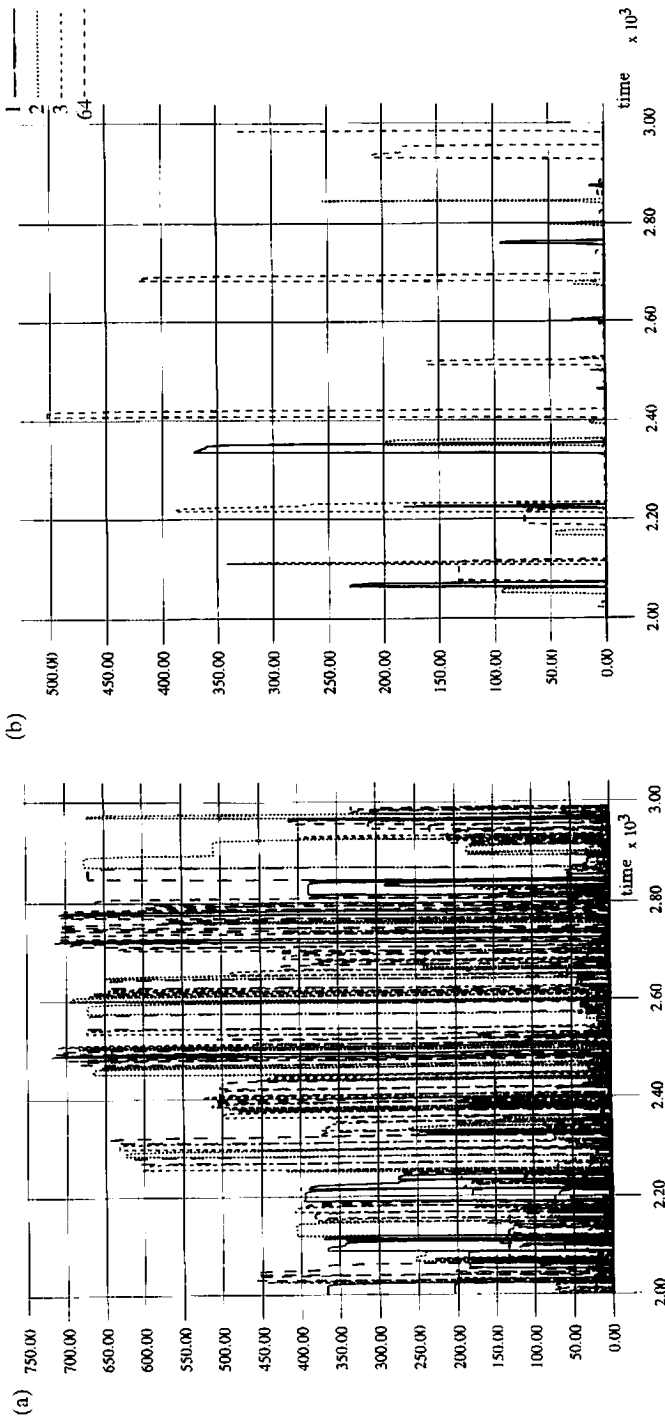


Figure 22. Overlaid time series of $x^{(m)}(t)$ of a single cell in medium. Network is given by four (randomly chosen) autocatalytic connections from 64 chemicals. The Dynamics of four chemicals from (a) is given in (b). Parameters are set as $p = 10.0$, $e_0 = e_1 = 1$, $\bar{X}_0 = 5$, $\gamma = 0.2$,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

APPENDIX B

Choice of Internal Chemical Dynamics. When the number of autocatalytic paths is large, the mechanism mentioned in Appendix A works, and only one or a few chemicals are activated. When the number of autocatalytic paths is small, on the other hand, many chemicals are generated, but the dynamics are stabilized and go to a fixed-point state. With a medium number of autocatalytic paths, non-trivial metabolic reactions appear, as mentioned in section 2¹⁰. 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 and b, where the dynamics are possibly aperiodic.

If there are a few non-autocatalytic (i.e., $\text{Con}(m, j, l)$ with $j \neq l$) paths, fixed-point states are stabilized, and oscillations are hardly observed. We adopt the intra-cellular 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 hold for $x^J > 0$. Hence the condition $\partial/\partial x^J(dx^J/dt) > 0$. Thus, it is expected that $(dx^J/dt) \propto (x^J)^\alpha$ with $\alpha > 0$. Thus, some kind of autocatalytic processes 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 section 2, the term "autocatalytic path" is not necessarily taken strictly, but may be assumed to represent chemicals autocatalytic "as a set" (see Fig. 23 schematic). In such a case, one may approximately represent the set of chemicals by one variable $x^{(j)}$, and adopt an autocatalytic reaction for $x^{(j)}$. Thus, our chemical reaction may be interpreted to represent the network composed of a set of autocatalytic networks, expected from the evolutionary process.

¹⁰ Besides the number of autocatalytic connections, there is further dependence on each pathway of the network. We have examined several random networks of two autocatalytic paths per chemical 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.

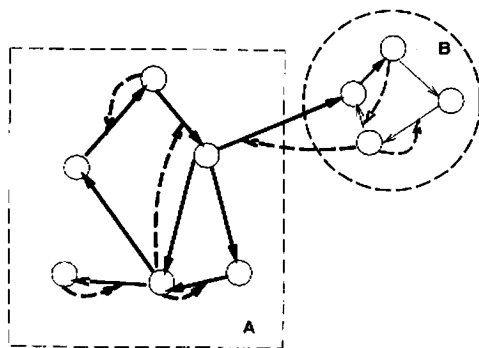


Figure 23. Schematic representation of evolutionary process of metabolic networks. Network (b) is added to (a). Note that part (b) is autocatalytic as a set.

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Received 12 February 1996

Revised version accepted 21 May 1996