

# Cell division, differentiation and dynamic clustering

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## Abstract

A novel mechanism for cell differentiation is proposed, based on the dynamic clustering in a globally coupled nonlinear system. A simple model with metabolic reaction, active transport of chemicals from media, and cell division is found to show three successive stages with the growth of the number of cells; coherent growth, dynamic clustering, and fixed cell differentiation. At the last stage, disparity in activities, germ line segregation, somatic cell differentiation, and homeochaotic stability against external perturbation are found. Our results, providing a simple interpretation of the experiments of the preceding paper, imply that cell differentiation can occur without a spatial pattern. From dynamical systems viewpoint, the new concept of “open chaos” is proposed, as a novel and general scenario for systems with growing numbers of elements, also seen in economics and sociology.

## 1. Introduction

Why do cells differentiate? The orthodox answer to this question is that the mechanism is completely determined by genetic codes. This belief is widely accepted by most molecular biologists. Is it correct though? It is known that genes are not changed in the course of cell differentiation [1]. Cells with identical genes can differentiate, even when in the same environment. Hence it is not a trivial question how identical sets of genes can produce a variety of different cells, not only from a biological but also from a dynamical systems viewpoint.

Experiments on cell differentiation of *E. coli* by one of the authors (TY), however, leads to a serious question to this widely accepted answer. Indeed, as reported in the preceding paper [2], cells with identical genes may split into several

groups with different enzymatic activities. Even prokaryote cells with identical genes can be differentiated there. We note that these cells are under liquid culture, thus are in an identical environment.

Of course, spatial information is also important in differentiation. However, usually one does not seriously discuss whether a spatial pattern is necessary for the cell differentiation. Often, differentiation and pattern formation are discussed together without distinction. The experimental results of the preceding paper, however, suggest that differentiation can occur without such spatial (positional) information. In the present paper we demonstrate theoretically that cell differentiation is possible without spatial information.

Here we note that cells interact with the environment, which is affected by all the other cells.

All cells interact with other cells through the environment. The purpose of the present paper is to present an alternative answer to the top question, with an emphasis on the cell-environment interaction. Our answer is based on dynamic clustering in a globally coupled dynamical system.

One of the simplest examples of such clustering is given by globally coupled dynamical systems [3]. When many identical elements with chaotic dynamics interact globally through a mean field, it is found that the elements differentiate into some clusters. In each cluster, elements oscillate synchronously, while elements in a different cluster oscillate with a different phase, frequency, or amplitude. Thus spontaneous differentiation of elements is possible through interaction among chaotic elements. In the present paper we extend this idea of clustering to spontaneous cell differentiation by introducing a simple metabolic chemical reaction<sup>1</sup> and cell division dynamics.

The clustering mechanism, although it provides a universal key to differentiation, is not enough for the explanation of fixed differentiation in multi-cellular organisms. Indeed, differentiation by clustering is temporal in nature: a group of cells with rich substrates at one time turns to be poor after some time in accordance with the oscillation. By introducing a model with a growing number of cells, we find that the appearance of a new stage after this dynamic clustering, where the differentiation of cells is fixed. Disparity in activities emerges. Thus our result

will provide a novel clustering behavior, also from the viewpoint of dynamical systems.

As a dynamical system, our problem has one important and innovating feature: growth of the dimension of phase space with cell divisions. Since the chemical dynamics in each cell is represented by a set of differential equations of a given number of degrees of freedom, the total number of degrees of freedom of the system increases with the number of cells  $N(t)$ . In our system, the increase is closely associated with chaotic instability.

Our model of cell growth consists of (i) nonlinear dynamics in each cell; (ii) nonlinear and global interaction among cells through a medium; (iii) growth and death of a cell depending on its internal state. We do not claim that our model has a one-to-one correspondence with the experiment in the preceding paper. Rather we present a novel conceptual framework for differentiation. We choose a rather simple dynamics for the above processes, to see the validity of the novel framework.

Indeed, these processes can generally be seen in a system with growth, by reinterpreting a cell as any replicating unit. For example, economic developmental processes have the above three features, by regarding resources and money as the chemicals and activities. Emergence of spontaneous differentiation in our cell society, thus, can be related with the sharing of resources, division of labor, and the formation of classes in an economical or sociological system.

The present paper is organized as follows. A specific model is introduced in Section 2, combining the processes of metabolic reaction, active transport of chemicals into cells, cell division, and death. Numerical results of the model, given in Section 3, show three stages of differentiation; coherent growth, dynamic clustering, and fixation of differentiation. Detailed dynamical aspects are presented. In Section 4, implications of our results to cell differentiation are given. The origin of germ line segregation, differentiation of somatic cells in multicellular or-

<sup>1</sup> We do not assume chaos for metabolic dynamics in each cell, in contrast with the model studied in [3]. Only some nonlinear oscillation is required here. This assumption of the existence of nonlinear metabolic oscillation is realistic from a biological point of view, since there are a number of reports of such oscillations in glycolytic reaction. Even without chaotic dynamics in each cell, some irregular (chaotic-like) dynamics is created through the interaction with other cells. Hence the total dynamics seems to have chaotic instability, although chaos is not mathematically defined here, due to the expansion of dimension of the phase space.

ganisms, a possible mechanism of programmed death, and homeochaotic stability against external perturbation are discussed. In Section 5, we point out a new mechanism of chaotic instability in a system with a growing number of degrees of freedom.

## 2. Model

The biochemical mechanisms of cell growth and division are very complicated, and include a variety of catalytic reactions. The reaction occurs both at the levels of inter- and intra- cells. Hence it is almost impossible to construct a complete descriptive model. Here we introduce a simpler model which captures the essence of these processes (see Fig. 1 for a schematic illustration of our model).

In the model we have to include the following processes:

- metabolic reaction within each cell: intra-cell dynamics;
- interaction with other cells through media: inter-cell dynamics;
- cell division;
- cell death.

### 2.1. Chemicals

As a set of dynamical variables we need some chemicals' concentrations in each cell, and also those of the medium surrounding the cells. Here we use the following variables; a set of concentrations of chemical substrates  $x_i^{(m)}(t)$ , the concentration of  $m$ th chemical species at the  $i$ th cell, at time  $t$ . The corresponding concentration of the species in the media is denoted as  $X^{(m)}(t)$ . We assume that the medium is well stirred, and neglect the spatial variation of the concentration. Furthermore we regard the chemical species  $x^{(0)}$  (or  $X^{(0)}$  in the media) as playing the role of the source for other substrates. Another assumption we make here is the existence of enzymes (for convenience and simplicity we assume that there

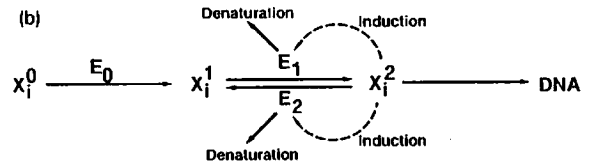
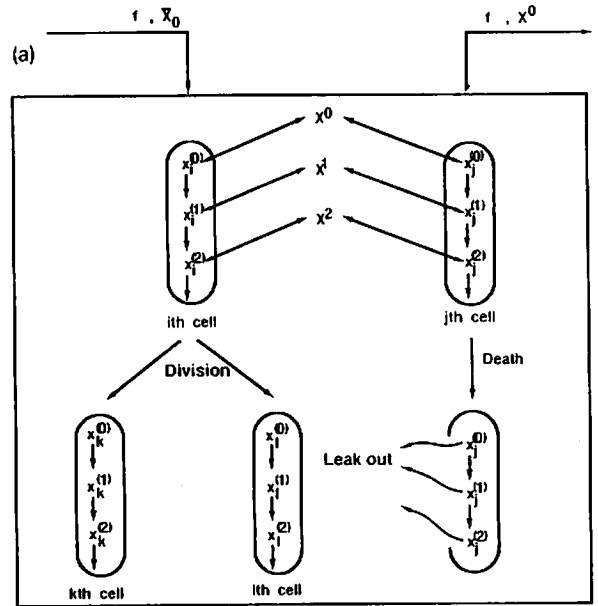


Fig. 1. Schematic representation of our model: (a) the whole dynamics of our system; (b) metabolic reaction within each cell.

is a corresponding enzyme  $E^{(m)}$  for each chemicals  $x^{(m)}$ ).

### 2.2. Metabolic reaction

The metabolic reaction here is schematically shown in Fig. 1b.

In the present paper we make further simplifications:

- (1) Only three chemicals including the source  $x^{(0)}$  are considered. In other words, each cell contains the variables  $x_i^{(0)}(t)$ ,  $x_i^{(1)}(t)$ , and  $x_i^{(2)}(t)$ . Of course, this is a vast simplification. There can be several orders of cascade reactions

in the course of synthesis of DNA [5]. We will see, however, that our simple reaction scheme is enough for our new scenario of cell differentiation.

(2) Simplification of enzyme dynamics. To be specific, we take the reaction scheme shown in Fig. 1b. Here the enzyme  $E^0$  is constitutive while the others,  $E^1$  and  $E^2$ , are inductive [6]. First we assume that the concentration of the enzyme  $E^{(0)}$  is constant, given by  $E^0$ , for simplicity. Here, the generation of the enzymes  $E^{(1)}$  and  $E^{(2)}$  are activated by the chemical  $x^{(2)}$ . The rate equation within each cell (including the enzymatic dynamics) is written as

$$dx_i^{(1)}(t)/dt = E_i^{(2)}(t)x_i^{(2)}(t) - E_i^{(1)}(t)x_i^{(1)}(t) + E^0x_i^{(0)}(t), \quad (1)$$

$$dx_i^{(2)}(t)/dt = E_i^{(1)}(t)x_i^{(1)}(t) - E_i^{(2)}(t)x_i^{(2)}(t) - \delta \times x_i^{(2)}(t). \quad (2)$$

The dynamics of enzymes  $E_i^{(m)}(t)$  can be highly complex and nonlinear, by gene expression, transcription, translation, and modification [5]. Following the scheme in Fig. 1b, we adopt here

$$dE_i^{(1)}(t)/dt = a_1(x_i^{(2)}(t) - d_{e1}E_i^{(1)}(t)),$$

$$dE_i^{(2)}(t)/dt = a_2(x_i^{(2)}(t) - d_{e2}E_i^{(2)}(t)).$$

as one of the simplest choices. Each enzyme is created from chemicals by reactions within a cell. We neglect the possibility of transportation of enzymes across cells (since the sizes of enzymes are much larger). For simplicity, we adiabatically solve the equations to get  $E_i^{(1)}(t) = e_1x_i^{(2)}(t)$ ; and  $E_i^{(2)}(t) = e_2x_i^{(2)}(t)$ , with constants  $e_1$  and  $e_2$ . Throughout the paper we adopt this elimination. Further simplification we make here is the neglect of the reaction  $x^{(2)} \rightarrow x^{(1)}$ . Thus the terms with  $E^{(2)}$  are neglected, although some simulations with these terms lead to qualitatively identical results.

### 2.3. Active transport and diffusion through membrane

A cell takes chemicals from the surrounding medium. The rates of chemicals transported into a cell are proportional to their concentrations outside. Further we assume that this transport rate also depends nonlinearly on the internal state of a cell. Since the transport here requires energy [4], the transport rate depends on the activities of a cell. The rate can depend nonlinearly on the chemicals in the cell. To be specific, we choose the following form;

$$\text{Transp}_i^{(m)}(t) = p \left( \sum_{k=1}^2 x_i^{(k)}(t) \right)^\nu X^{(m)}(t), \quad (3)$$

where  $\nu$  is taken to be 3 throughout the paper, although other nonlinear dependences ( $\nu > 1$ , i.e., with positive feedback effect) lead to qualitatively similar results. The summation ( $\sum_{k=1}^2 x_i^{(k)}(t)$ ) is introduced here to mean that a cell with more chemicals is more active. This form, again, is rather arbitrarily chosen, but similar forms with "active" transport can lead to the same result. Besides the above active transport, the chemicals spread out through the membrane with normal diffusion by

$$\text{Diff}_i^{(m)}(t) = D(X^{(m)}(t) - x_i^{(m)}) \quad (4)$$

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

$$dx_i^{(0)}(t)/dt = -E^0x_i^{(0)}(t) + \text{Transp}_i^{(0)}(t) + \text{Diff}_i^{(0)}(t), \quad (5)$$

$$dx_i^{(1)}(t)/dt = E^0x_i^{(0)}(t) - e_1x_i^{(2)}(t)x_i^{(1)}(t) + \text{Transp}_i^{(1)}(t) + \text{Diff}_i^{(1)}(t), \quad (6)$$

$$dx_i^{(2)}(t)/dt = e_1x_i^{(2)}(t)x_i^{(1)}(t) - \delta \times x_i^{(2)}(t) + \text{Transp}_i^{(2)}(t) + \text{Diff}_i^{(2)}(t). \quad (7)$$

Since the processes (B) here are just the transportation of chemicals through membranes, the sum of the chemicals must be conserved. The dy-

namics of the chemicals in the medium is then obtained by converting the sign, i.e.,

$$dX^{(m)}(t)/dt = -\sum_{i=1}^N \{\text{Transp}_i^{(m)}(t) + \text{Diff}_i^{(m)}(t)\}. \quad (8)$$

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 of the container. Here we assume that only the source chemical  $X^0$  is supplied by a flow into the container. By denoting the external concentration of the chemicals by  $\bar{X}^0$  and its flow rate per volume of the tank by  $f$ , the dynamics of source chemicals in the media is written as

$$dX^{(0)}(t)/dt = f(\bar{X}^0 - X^0) - \sum_{i=1}^N \{\text{Transp}_i^{(0)}(t) + \text{Diff}_i^{(0)}(t)\}. \quad (9)$$

#### 2.4. Cell division

Through chemical processes, cells can replicate. For the division, accumulation of some chemicals is required. In our model the final product from the chemical species “2” is assumed to act as the chemical for the cell division (note the term  $-\delta \times x_i^{(2)}(t)$  in Eq. (2)). For example assume that chemical 2 is a mononucleotide. Then the DNA synthesis process occurs through the reaction  $2 \rightarrow \text{DNA}$  in Fig. 1; thus  $d\text{DNA}/dt \propto x^{(2)}$ . Accordingly we impose the following condition for cell division: the  $i$ th cell (born at time  $t = t_0(i)$ ) divides into two at a time  $T$  such that

$$\int_{t_0(i)}^T dt x_i^{(2)}(t) > R \quad (10)$$

is satisfied, where  $R$  is the threshold for cell replication. Here again, choices of other similar division conditions can give qualitatively similar results as those to be discussed. The essential part

is that the division condition satisfies an integral form representing the accumulation of DNA as in Eq. (10).

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  $\frac{1}{2}x_i^{(m)} + \epsilon$  and  $\frac{1}{2}x_i^{(m)} - \epsilon$ , respectively, with a small “noise”  $\epsilon$ , a random number with small amplitude, say over  $[-10^{-3}, 10^{-3}]$ . We should note that this inclusion of imbalance is not essential to our differentiation. Indeed any tiny difference is amplified to yield a macroscopic differentiation.

#### 2.5. Cell death

To avoid infinite growth, a condition for cell death is further imposed. Here we choose either a deterministic or a probabilistic death mechanism. In the former case, we choose the condition for the death as

$$\sum_{j=1}^2 x_i^{(j)}(t) < S, \quad (11)$$

where  $S$  is a threshold for “starvation”. This choice is again rather arbitrary. We have assumed that a cell dies when the chemicals included therein are too little. Again, a choice of similar other forms is expected to give the same results. Here, the chemicals inside the dead cells are released into the medium. Thus there is a jump in  $X^{(j)}(t)$  at every cell death.

In the probabilistic case, a number of randomly chosen cells (together with the chemicals included therein) are removed per given time steps (decimation). This choice is closer to experimental situations, since incubated cells are decimated per some time steps in order to avoid the divergence of the number of cells (see the preceding paper). In fact, both the deterministic and the probabilistic deaths give qualitatively the same behavior with regard to the three stages to be discussed.

### 3. Results: three stage differentiation

A typical example for the growth of the number of cells is plotted in Fig. 2, as well as the overlaid oscillation of chemicals  $x_i^{(2)}(t)$  over all cells in Fig. 3. In Fig. 2, the number of cells doubles at certain time during the first stage, while the number increases almost linearly with time at the last stage. As can be seen in Fig. 3, we have observed the following three stages of evolution, with the growth of the number of cells, for a wide range of parameters.

(a) *Stage I: coherent growth.* All the metabolic reactions of cells oscillate coherently. Thus cells grow coherently. Starting from a single cell, the number increases as  $1 \rightarrow 2 \rightarrow 4 \rightarrow 8 \rightarrow 16 \dots$  at the stage I.

(b) *Stage II: dynamic clustering.* If there is intra- and/or inter-cell nonlinear dynamics, the coherent oscillation can lose its stability as the cell number increases. As can be seen in Fig. 3 of the overlaid time series of  $x_i^{(2)}(t)$ , the variance of the cells' oscillations is enhanced with time. Then dynamic clustering sets in. Depending on the parameters, the number of clusters can be different. As the diffusion constant  $D$  is decreased, the number of clusters increases, and the oscillation gets more complicated. The projected orbit of  $(x_i^{(1)}(t), x_i^{(2)}(t))$  is given in Fig. 4a. The phases of oscillations, as well as their amplitudes vary by cells. Some cells start to have large concentrations of chemicals, which prepares them for the differentiation at the next stage.

The origin of the clustering (i.e., temporal differentiation) lies in the instability of the reaction and transport dynamics. Any tiny difference between two cells is amplified to a macroscopic difference. The mechanism of clustering has been investigated in globally coupled maps [3], although here there exists a novel feature in dynamical systems, as will be discussed in Section 5. We note that the clustering here is possible by the interaction among cells through the chemicals in the medium, whose concentrations can also oscillate in time.

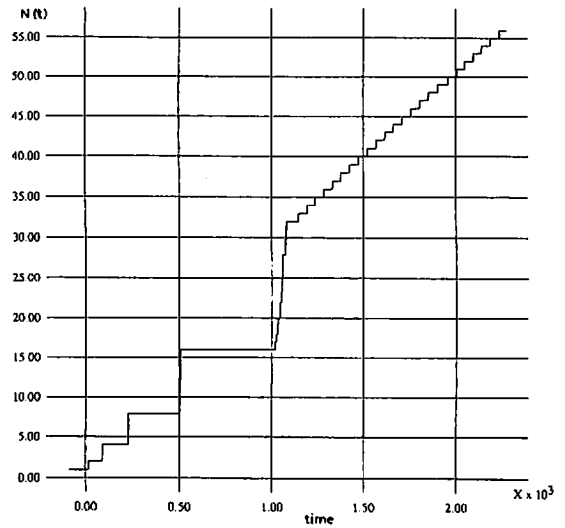


Fig. 2. Temporal change of the number of cells  $N$ . Throughout the present paper we use  $p = 1$ ,  $f = 0.1$ ,  $E^0 = e_1 = 1$ , and  $R = 50$ . Other parameters are set at  $D = 0.1$ ,  $\delta = 0.5$ ,  $\bar{X}^0 = 15$ ,  $S = 0.1$ . All figures of the present paper are plotted per  $\Delta t = 0.5$ .

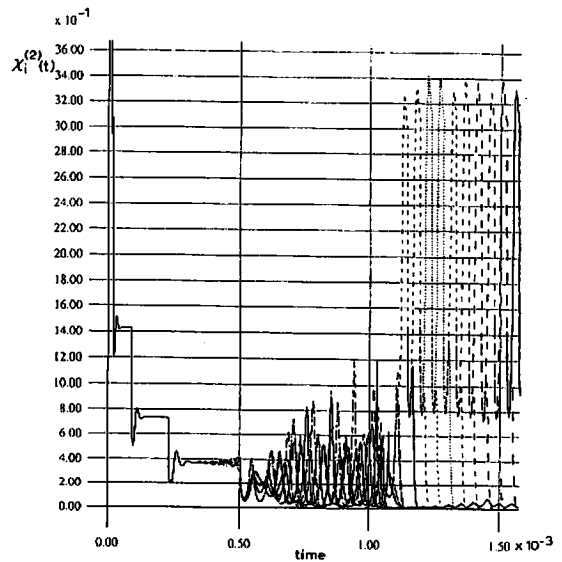


Fig. 3. Overlaid time series of  $x_i^{(2)}(t)$ , corresponding to Fig. 2.

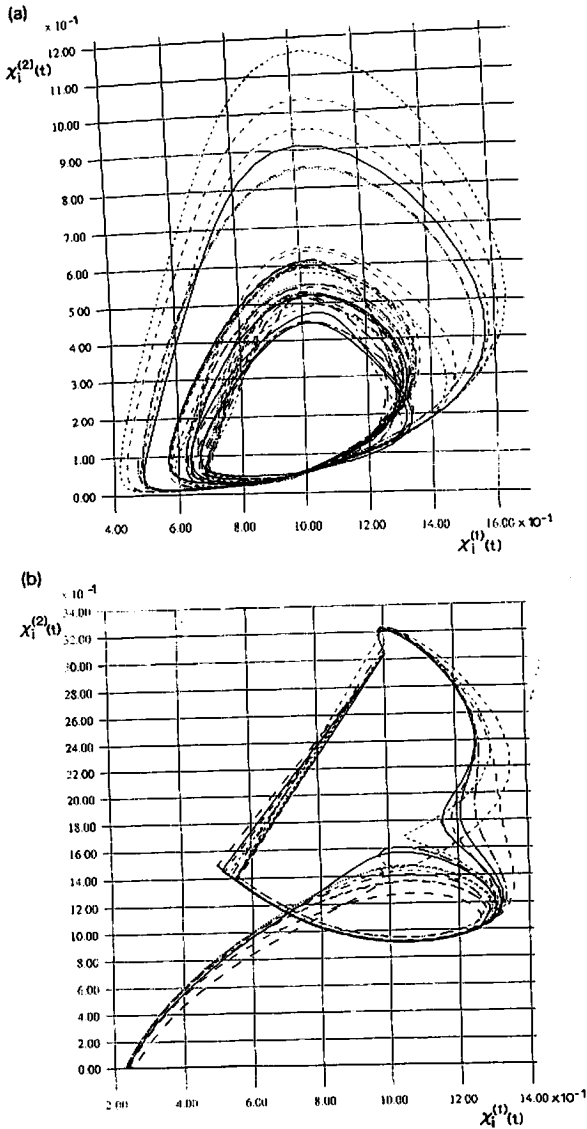


Fig. 4. (a) Plots of  $(x_i^{(1)}(t), x_i^{(2)}(t))$  over the time steps 800–1000 (stage II). (b) The plots over the time steps 1900–2200 (stage III). Corresponding to Fig. 3.

(c) *Stage III: Fixation of cell differentiation.* As the number of cells increases further, our cell society enters into a new stage. An example of overlaid time series is given in Fig. 3, as well as the orbit in phase space  $(x_i^{(1)}(t), x_i^{(2)}(t))$  in Fig. 4b. We note that the cells are classified into two completely distinct groups; the population

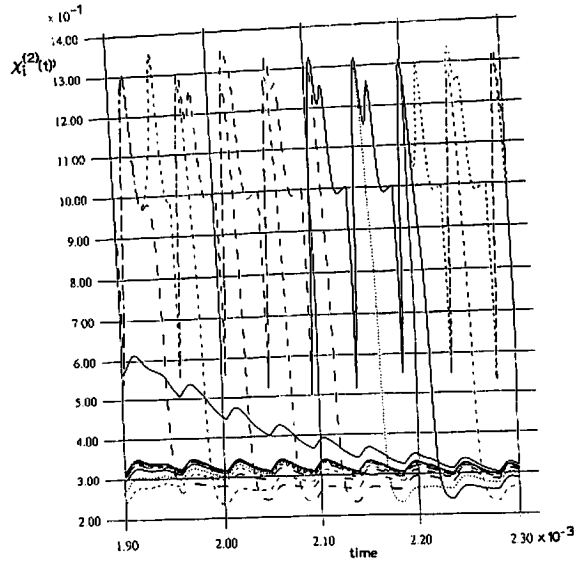


Fig. 5. Magnified plot of the time series  $x_i^{(2)}(t)$ , for stage III (corresponding to Figs. 2–4).

of cells in one group is very few (one or two in the example) but they contain large concentrations of chemicals, while the population of the other group is large but the concentrations of the chemicals are much smaller. We call the former cells *active* and the latter as *sleeping*. The active cells replicate much faster than the other (sleeping) ones. In Fig. 4b, orbits with a large amplitude are those of the active group. The segment of straight lines at the upper middle is a result of the cell division. (Here  $x^{(2)}$  of the sleeping cells is so small that their orbits in the  $(x_i^{(1)}(t), x_i^{(2)}(t))$  plane are hardly visible in Fig. 4b.

In the extreme, and here, typical case, the number of cells in the active group is just one. It divides almost periodically in time. After each division, one of the two created cells takes more chemicals than the other. The difference between the two cells is enhanced, till the weaker one belongs to the sleeping group. Thus the number of cells increases by one per some period as long as the "cell death" condition is not satisfied. The sleeping cells, on the other hand, are not identical. They are again weakly differentiated by the concentrations of chemicals

(see Fig. 5). This differentiation depends on the ordering of the time of birth of the cell. Temporally there are weak oscillations in the chemicals of a sleeping cell.

The above regular growth is seen if  $\delta$  (production rate of DNA from  $x^{(2)}$ ) and  $D$  (diffusion coupling with a medium) are large (say  $\delta > .2$ ,  $D > .08$ ). When the parameters  $\delta$  and/or  $D$  are smaller, the dynamics is more complicated: the number of active cells is larger than one (stage “complex-III”). Their oscillations are not periodic, and the division cycle is not regular either. See Fig. 6a for the irregular growth of cells, while the overlaid time series of  $x_i^{(2)}(t)$  are given in Fig. 6b, as well as the plot of the  $(x_i^{(1)}, x_i^{(2)})$  in Fig. 6c. Here, the oscillations of active cells are chaotic<sup>2</sup> and display dynamic clustering.

By a division of a cell in either group, the balance of the number of cells between the two groups may be destroyed. After this (rare) occurrence, one (or few) of the cells in one group switch to the other group. In Fig. 7, sleeping cells “wake up” around  $t = 4620$  and get active by taking chemicals from the medium, while active ones get inactive. Such waking-up of sleeping cells has also been observed in the experiments of the preceding paper.

Before closing the paragraph on stage III, we note that for some parameter regimes (e.g., much larger  $D$ ) this stage has not yet been observed, and the cell society remains at stage II.

(d) *Cell death.* After the number of cells gets large enough, the external source term is not sufficient to support any more cells. The number saturates at the maximal number and fluctuates around it. When there is just one active cell, the cell still divides periodically in time. However, one of the divided cells dies within the same period. Thus the number of cells does neither increase nor decrease. Cell lineage for this case is shown in Fig. 8. The cell lineage diagram shows

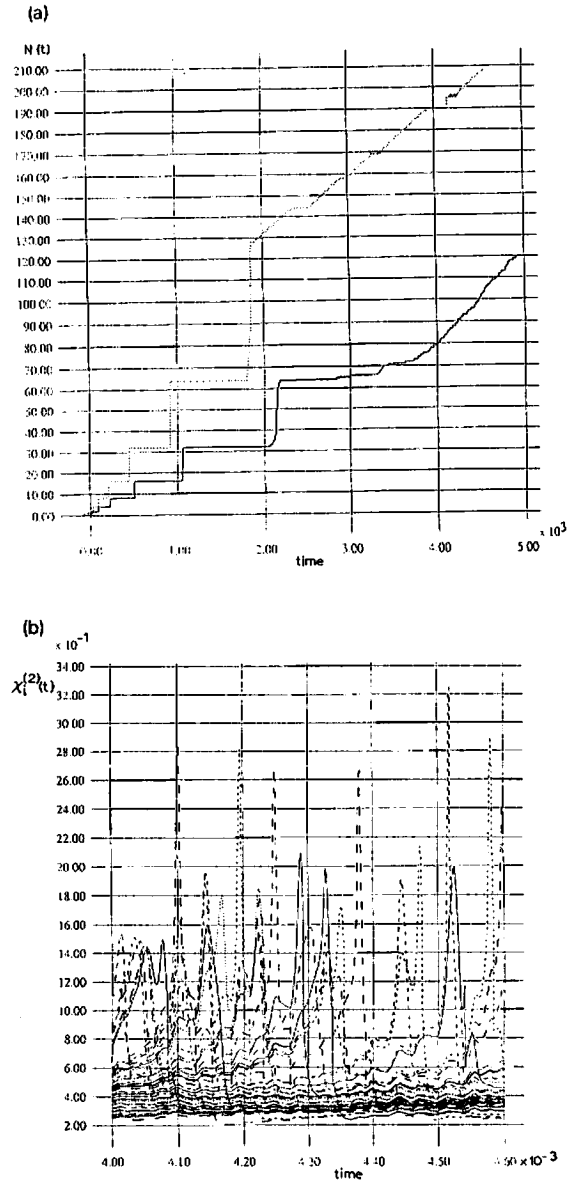


Fig. 6. (a) Temporal change of the number of cells  $N$ , for the parameters  $D = 0.05$ ,  $\delta = 0.5$ ,  $\bar{X}^0 = 15$ ,  $S = 0.1$ . (for reference the evolution with  $\bar{X}^0 = 35$  is plotted as a dotted line). (b) Overlaid time series of  $x_i^{(2)}(t)$ , corresponding to Fig. 6a. (c) Plots of  $(x_i^{(1)}(t), x_i^{(2)}(t))$  over the time steps 4000–4600.

from which cell a new cell is born at time  $t$  (given by the vertical axis). In Fig. 8, we can clearly distinguish active and sleeping cells (where the cell index  $\ell$  (horizontal axis) satisfies  $\ell \leq 28$  or

<sup>2</sup> The term “chaos” is not used in a rigorous sense here. Since the degrees of freedom are varying, the conventional definition of chaos is not applicable in its rigorous sense.



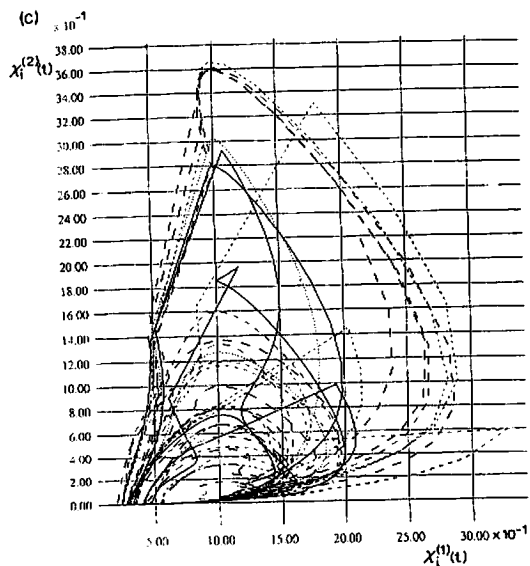


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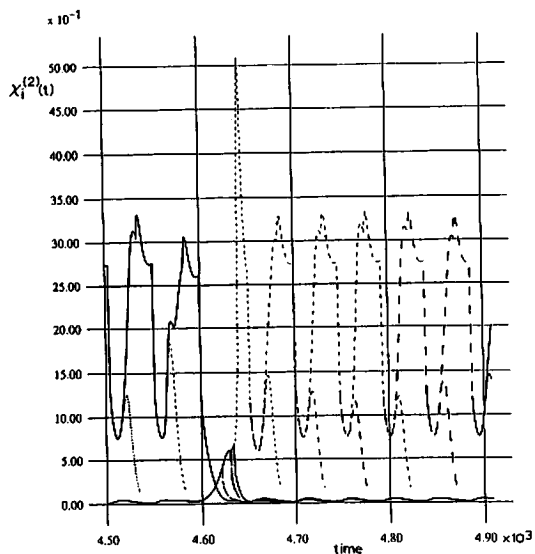


Fig. 7. Overlaid time series of  $x_i^{(2)}(t)$ , for the parameters  $D = 0.2$ ,  $\delta = 0.5$ ,  $\bar{X}^0 = 15$ , and  $S = 0.5$ . At the time step around 4620, switching between active and sleeping cells is observed.

$\ell \geq 103$ ). Successive division and death is seen for the cells with the index  $28 < \ell < 103$ .

If we wait for a very long time, the division condition is satisfied for sleeping cells also (since the condition is of an integral form). Then divi-

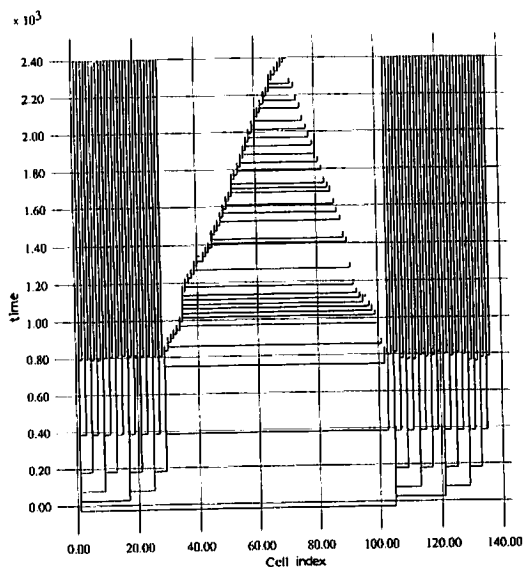


Fig. 8. Cell lineage diagram for  $D = 0.1$ ,  $\delta = 0.5$ ,  $\bar{X}^0 = 40$ , and  $S = 0.3$ . The vertical axis shows the time step, while the horizontal axis shows the cell index. (Only for practical purpose of keeping trace of the branching tree, we define the cell index as follows: when a daughter cell  $j$  is born from a cell  $i$ 's  $k$ th division, the value  $s_j = s_i + 2^{-k}$  is attached to the cell  $j$  from the mother cell's  $s_i$ . The cell index for the cell  $j$  is the order of  $s_j$ , sorted with the increasing order.) In the diagram, the horizontal line shows the division from the cell with index  $n_i$  to  $n_j$ , while the vertical line is drawn as long as the cell exists (until it dies out).

sions of many cells occur successively within a short time scale, but many of the divided cells die right after the divisions. An almost simultaneous death of many cells occurs. Thus the number returns to the level before the multiple division.

If the number of active cells is larger than one (“complex-III stage”), the death process is irregular, while the total number of cells is constant on average. Here switching between active and sleeping cells can occur through cell death. When the number of active cells is reduced, the cell society goes back to the stage II, where many cells with similar activities compete for resources, and show dynamic clustering. After some duration of stage II, few cells become active, and the society comes back to the complex-III stage. Accordingly temporal oscillation is observed in the ratio between active and sleeping

cells.

#### 4. Implications to cell differentiation

The results in the previous section have many implications for cell growth and differentiation. At first we present a possible interpretation of the “differentiation-without-gene” experiment in the preceding paper, based on our results here. Besides this interpretation, we can provide novel viewpoints to cell differentiation in general. They include mechanisms of germ line segregation, somatic cell differentiation, programmed death, stability of complex cell lineage.

##### 4.1. *Explanation of the results in the preceding paper*

Since the present toy cell with simple metabolic reaction systems can show dynamic clustering, it is rather natural to assume that the present clustering can appear in an experimental system. In the experiment of the preceding paper, differentiation of cells and dynamic clustering of oscillations of E-coli are observed. The possibility of fixation of differentiation corresponding to our stage III is also suggested experimentally. Two distinct groups of bacteria are found there, one with high enzymatic activity, and the other with low activity, where the populations of the two groups oscillate in time. This oscillation is explained by assuming that the cell society is at the complex-III stage in our simulation. Indeed, populations of active and sleeping cells oscillate in time in the stage.

We note that the differentiation in the experiment is performed with the use of liquid culture, where the coupling is global (through a medium) as in our model. Thus cell differentiation occurs even without a spatially local interaction, in strong contrast with conventional models for differentiation and pattern formation (e.g., reaction-diffusion equation model of Turing-type or a cellular automaton model).

One might think that the deterministic death condition is different from the previous experimental situation. In the experiments, some cells are randomly removed to avoid overgrowth in the medium. To take the experiments into account, we have also simulated a model with stochastic decimation. The results are essentially the same as those given so far, obtained by the deterministic death condition. Of course, death is not periodic, thus the growth is not completely periodic in the regular regime of the deterministic case. Still the dynamical behavior is the same except for some effects of noise. Statistically the ratio of active to sleeping cells is kept at the same level. When an active cell is eliminated, for example, one of the sleeping cells starts getting more chemicals and becomes an active cell.

Of course it is very difficult to check the validity of the present scenario to the experiment completely. However, the present scenario for cell differentiation, due to its simplicity and plausibility, provides an important conceptual framework, in general. As long as the genotypes are homogeneous in the bacteria society, no clearer scenario is known than the present one.

##### 4.2. *From time sharing to emergence of disparity in activities*

In the present model, many cells compete with each other for finite resources (the source term  $X^{(0)}$ ). Generally speaking, coherent growth may not be advantageous in a system with finite resources, since all elements need the same amount of resources at the same time. There can be two remedies to this problem; the time sharing system and disparity of activities by elements. In the former case, time sharing (also often adopted in computer systems,) is accomplished here through temporal differentiation (clustering). Through clustering, elements use resources successively, thus avoiding strong competition for the resources. In the latter case, elements split into (two) fixed groups. One

group is “rich” and uses the resources more and grows much faster than the other (poor) group. In our system, these two types of differentiation appear as successive stages.

We should note that the active and sleeping cells coexist. Sleeping cells, although they may look like a “loser” for the competition for resources, can live together with active ones. Indeed this coexistence is important for the stability of cell history and society to be discussed.

When there are many active cells, they compete with each other for resources. In this case, the oscillations are chaotic and show dynamic clustering. Thus time sharing of resources is still adopted among active cells.

#### 4.3. Germ line segregation

To have a stable cell society, cells, once differentiated, often must be fixed in time. As found at stage III, such fixation occurs in our simulation. This splitting into active and sleeping cells reminds us of the germ line segregation. Germ cells are distinguished from somatic cells by their very high division ability. The emergence of the germ line segregation in our simple model is rather striking.

#### 4.4. Somatic cell differentiation

At stage III, concentrations of chemicals in the sleeping group (somatic cells) are again differentiated into smaller groups. This differentiation is according to the slight difference of the amount of chemicals, in our model. We should note that a very simple metabolic network with three chemicals has led to this differentiation. It is expected that the inclusion of more chemicals can easily provide a larger variety of differentiated cells as observed in a real cell society.

In our model, the growth speed of somatic cells is very low. This observation agrees rather well with the well-known fact in biology that the division speed of cells gets much slower when they are differentiated [7].

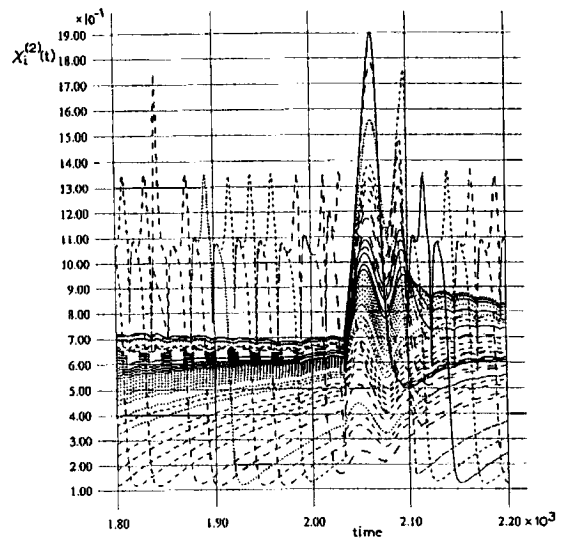


Fig. 9. Overlaid time series of  $x_i^{(2)}(t)$ , for the parameters  $D = 0.1$ ,  $\delta = 0.5$ , and  $X^0 = 40$ . Instead of the deterministic death process, the stochastic decimation of a cell is adopted. A cell is randomly removed with a probability of  $10^{-5}$  per 0.01 second. Around step 2050, the active cell is removed.

#### 4.5. Sleep/active switch and homeochaos

Switching between sleeping and active cells is observed in our simulation when the balance between the numbers of active and sleeping cells is destroyed. There are two origins for this destruction: (a) internal dynamics (typically seen at the “complex-III” stage where the dynamics of many active cells is chaotic); (b) external perturbation.

The second case is seen, when, for example, some cells are eliminated externally. Then some sleeping cell becomes active and the balance is restored, implying that our system has stability against external perturbation. In Fig. 9, the active cell is removed around  $t = 2030$ . After this removal, sleeping cells get active and compete for resources. After competition over few periods of oscillations, one of the sleeping cells wins and remains active. Thus the original state is restored. This stability might remind one of “homeostasis”. However, the stability here

is sustained not by a static (fixed-point) state, but by a dynamical state. Such dynamic stability with the use of high-dimensional chaos is noted as homeochaos [8].

#### 4.6. Stability of cell lineage

Since chaotic dynamics underlies our cell system, one might be afraid that the scenario here is unstable, sensitive to initial conditions. This is not the case. The scenario here is, for example, invariant under changes of initial conditions (except for a variation of the time required for the first division), and also under slight changes of parameters. The oscillation itself can be chaotic (in stage II), and the time for the division is not exactly identical by the initial conditions. Still the division time is statistically invariant, and the cell lineage is completely identical.

Of course, the scenario depends on genetic and environmental parameters, such as the external supply of resources and internal parameters characterizing chemical reactions in the cell. Since the supply of chemicals from the medium cannot be genetically determined, the genetic information is not enough for the characterization of cell differentiation. We should again emphasize here that spatial information is not necessarily important for differentiation, either.

Examples of cell lineage diagrams are shown in Fig. 10. In Fig. 10a, coherent division (stage I) is observed up to 32 cells (at around 500 steps), while the cell society enters into the stage III at around 600 steps. Here cells with an index larger than 107 get inactive (sleeping cells). Cells with the index less than 108 are active and divide frequently, although one of the created cells dies. Around the time step 2200, divisions of many sleeping cells occur leading to the multiple deaths. Then the system goes back to stage II, till new grouping into active and sleeping cells is organized around 2700 steps. On the other hand, the lineage of Fig. 10b, corresponding to Fig. 7, shows the switching between active and sleeping

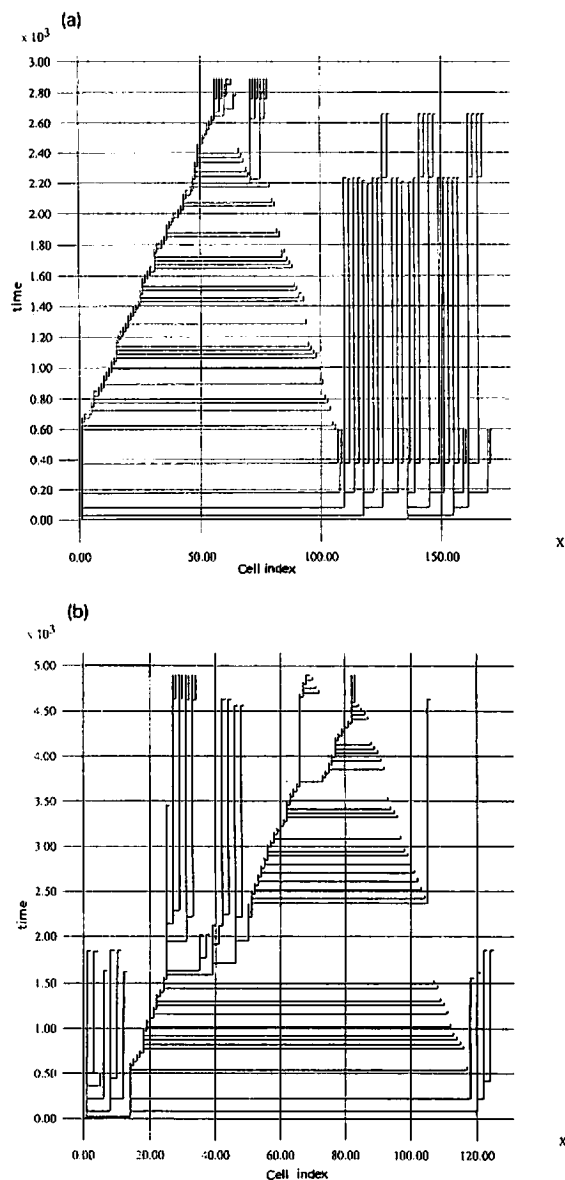


Fig. 10. Cell lineage diagram (a)  $D = 0.05$ ,  $\delta = 0.3$ ,  $\bar{X}^0 = 15$ , and  $S = 0.5$ , up to time step 2910. (b)  $D = 0.2$ ,  $\delta = 0.5$ ,  $\bar{X}^0 = 15$ , and  $S = 0.5$ , corresponding to Fig. 7; up to time step 4910.

cells around time steps 2000 and 4600.

#### 4.7. Cell death

In our simulations with the deterministic cell death condition, some cells are programmed to

die. This history of cell death is stable against changes of initial conditions. We have often observed simultaneous deaths of multiple cells. The process eliminates the overgrowth of inactive cells in our simulation. Such programmed death is also known in cell biology and immunology. Indeed some of the cell lineages obtained (see Fig. 10) from our simulations agree with those found for some multicellular organisms (such as the *C. elegans*) in the following points.

(i) Not all of the cells divide (emergence of sleeping cells). The number of such cells and the cell lineage diagram are insensitive to a wide-range change of initial condition. Many cells are derived from few active cells.

(ii) The timing of divisions and deaths of cells is independent of initial conditions.

If our scenario is true for the programmed death, we have to conclude that programmed death is due to the interaction among cells. It is predicted that the cell lineage by a single cell (with repetitive removal of one of divided cells) does not show the programmed death as seen in a cell society. Experimental check for this conjecture is strongly requested.

### 5. Open chaos; novel mechanism of unstable and irregular dynamics

Although the mechanism in our differentiation is based on dynamic clustering in a network of chaotic elements, there is a novel dynamic instability in our system with “growing” phase space.

By the active transport dynamics of chemicals, the difference between two cells can be amplified, since a cell with more chemicals is assumed to get even more. Tiny differences between cells can grow exponentially if parameters satisfy a suitable condition. The grown cell is divided into two, with an (almost) equal partition of the contained chemicals. The process is quite analogous with chaos in Baker’s transformations; stretching (exponential growth) and folding (division).

One difference between our cell division mechanism and chaos is that phase space itself changes after a division in the former, while the orbit comes back to the original phase space in the stretching-folding mechanism of chaos.

Thus conventional quantifiers (such as Lyapunov exponents) in “closed” chaos may not be applicable to our problem, since they require a stationary measure in the closed phase space. A quantifier to measure the instability along the increase of phase space is required just as the co-moving Lyapunov exponent was introduced to measure the instability along a flow [9].

Our “open chaos” here provides a general scenario of instability and irregular dynamics in a system with growing phase space. Such systems with replicating units for the competition of finite resources are often seen in economics and sociology.

In open chaos, disparity of elements in activities emerges through time sharing by clustering. Still, “poor” cells are not extinct but coexist. Such coexistence of very active and inactive elements is also seen in economics and sociology; coexistence of very big and small firms, or the very rich and the poor. Spatial information is not essential to the differentiation or class formation here, as in our cell differentiation. It is interesting to extend the idea of the present paper to economics and sociology, and to discuss the origin of differentiation, diversity, and complexity there.

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