Robust and irreversible development in cell society as a general consequence of intra–inter dynamics

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Abstract

A dynamical systems scenario for developmental cell biology is proposed, based on numerical studies of a system with interacting units with internal dynamics and reproduction. Diversification, formation of discrete and recursive types, and rules for differentiation are found as a natural consequence of such a system. "Stem cells" that either proliferate or differentiate to different types stochastically are found to appear when intra-cellular dynamics are chaotic. Robustness of the developmental process against microscopic and macroscopic perturbations is shown to be a natural consequence of such intra–inter dynamics, while irreversibility in developmental process is discussed in terms of the gain of stability, loss of diversity and chaotic instability. © 2000 Elsevier Science B.V. All rights reserved.

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1. Intra–inter dynamics for cell biology

A biological unit has always some internal structure that can change in time. These units, however, are not separated from the outside world completely. For example, consider a cell. In this case, isolation by a biomembrane is flexible and incomplete. When the units are represented by dynamical systems, they interact with each other and with the external environment. Hence, we need a model consisting of the interplay between inter- and intra-unit dynamics [1–3]. Here we will mainly discuss the developmental process of a cell society accompanied by cell differentiation, where the intra–inter

dynamics consist of several biochemical reaction processes. The cells interact through the diffusion of chemicals or their active signal transmission.

In this case (and generally in a biological system), the number of elements is not fixed in time, but they are born (and die) in time. Consider development of cell society with cell divisions. After the division of a cell, if two cells remained identical, another set of variables would not be necessary. If the orbits have orbital instability (such as chaos), however, the orbits of the (two) daughters will diverge. Thus the increase in the number of variables is tightly connected with the internal dynamics.

We have studied several models [4–9] choosing (a) the internal variables and their dynamics, (b) interaction type, and (c) the rule to change the degrees of freedom (e.g., cell division).

As for the internal dynamics, auto-catalytic reaction among chemicals is chosen. Such auto-catalytic reactions are necessary to produce chemicals in a cell, required for reproduction [10]. Auto-catalytic reactions often lead to nonlinear oscillation in chemicals. Here we assume the possibility of such oscillation in the intra-cellular dynamics [11,12]. As the interaction mechanism, the diffusion of chemicals between a cell and its surroundings is chosen.

To be specific, we mainly consider the following model here. First, the state of a cell i is assumed to be characterized by the cell volume and a set of functions $x_i^{(m)}(t)$ representing the concentrations of k chemicals denoted by $m = 1, \ldots, k$. The concentrations of chemicals change as a result of internal biochemical reaction dynamics within each cell and cell–cell interactions communicated through the surrounding medium.

For the internal chemical reaction dynamics, we choose a catalytic network among the k chemicals. The network is defined by a collection of triplets (ℓ, j, m) representing the reaction from chemical m to ℓ catalyzed by j. The rate of increase of $x_i^\ell(t)$ (and decrease of $x_i^m(t)$) through this reaction is given by $x_i^{(m)}(t)(x_i^{(j)}(t))^\alpha$, where α is the degree of catalyzation ($\alpha=2$ in the simulations considered presently). Each chemical has several paths to other chemicals, and thus a complex reaction network is formed. The change in the chemical concentrations through all such reactions, thus, is determined by the set of all terms of the above type for a given network. These reactions can include genetic processes, where $x_i^\ell(t)$ is regarded as expression of a given gene.

Cells interact with each other through the transport of chemicals out of and into the surrounding medium. As a minimal case, we consider only indirect cell–cell interactions through diffusion of chemicals via the medium. The transport rate of chemicals into a cell is proportional to the difference in chemical concentrations between the inside and the outside of the cell, and is given by $D(X^{(\ell)}(t) - x_i^{(\ell)}(t))$, where D denotes the diffusion constant, and $X^{(\ell)}(t)$ is the concentration of the chemical at the medium. The diffusion of a chemical species through cell membrane should depend on the properties of this species. In this model, we consider the simple case in which there are two types of chemicals, one that can penetrate the membrane and one that cannot. With this type of interaction, corresponding chemicals in the medium are consumed. To maintain the growth of the organism, the system is considered to be immersed in a bath of chemicals through which (nutritive) chemicals are supplied to the cells.

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

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

Of course, there can be a variety of choices on the chemical reaction network. The observed results do not depend on the details of the choice, as long as the network allows for the oscillatory intra-cellular dynamics leading to the growth in the number of cells. Note that the network is not constructed to imitate an existing biochemical network. Rather, we try to demonstrate that important features in a biological system are the natural consequence of a system with internal dynamics, interaction, and reproduction. From the study we extract a universal logic underlying this class of models.

In this sense, our approach is constructive [13,14]. We first construct a simple 'world' by combining fundamental procedures, and clarify universal class of phenomena. Then we try to reveal the underlying universal logic that the life process should obey. With this we try to provide a new look at the present organisms from our discovered logic.

We believe that this 'constructive' approach is essential to understand the logic of life. As long as we study only the present organisms in nature, it is hard to distinguish the logic that organisms necessarily should obey, from frozen accident through evolution. The logic and universality class can be clarified only by constructing some world. The present organisms, then, are understood as one representative of a universal class, to which the life-as-it-could-be also belongs.

1.1. Remarks

In his pioneering study, Turing proposed a pattern formation mechanism through spatial symmetry breaking induced by dynamic instability [15,16]. On the other hand, existence of multiple attractors in genetic networks are found that could be corresponded to different cell types [17]. In our approach the generation of differentiation rules is studied by introducing a rich internal dynamics (including chaos), and the reproduction of units. With this reproduction, initial conditions are selected to form discrete cell types and rules for differentiation.

2. Scenario for cell differentiation

From several simulations of the models starting from a single-cell initial condition, Yomo and the authors proposed the following scenario ("isologous diversification"), as
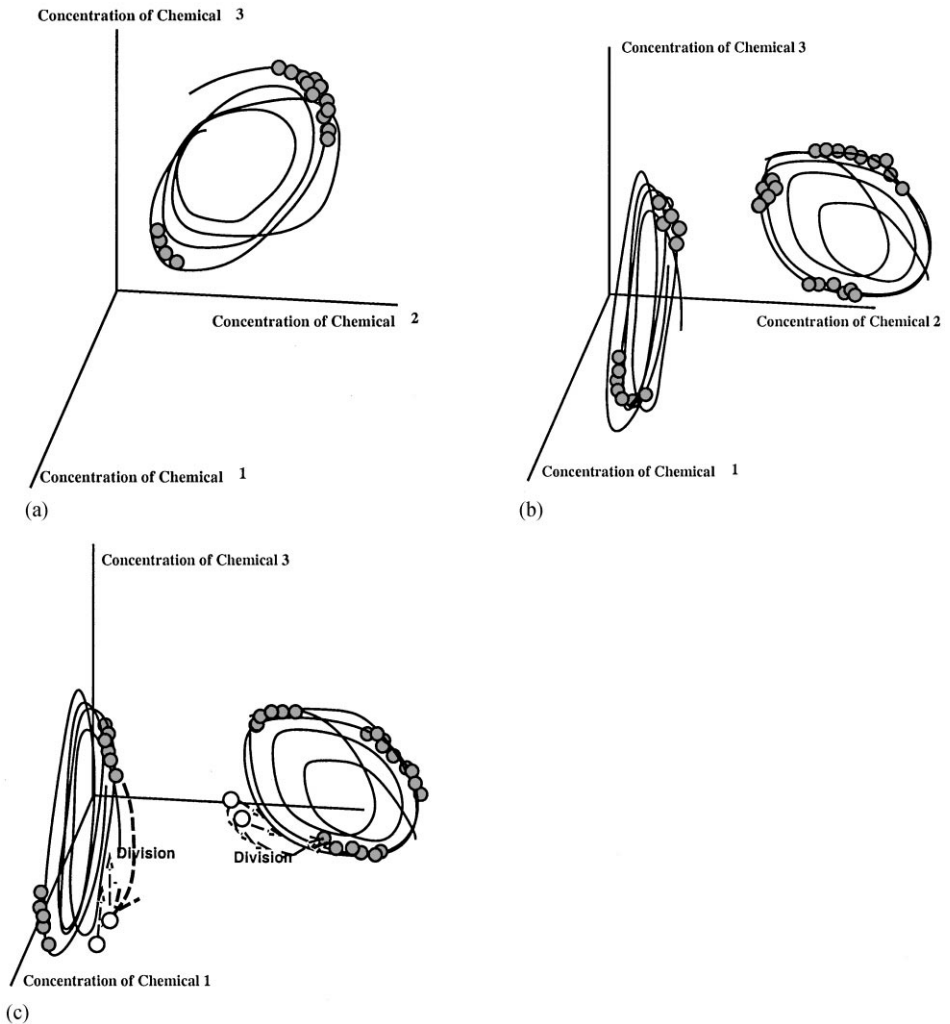
Fig. 1. Schematic representation of the stages of our differentiation scenario. These results are supported by numerical simulations of several models. See Refs. [5,8] for direct numerical results.

a general mechanism of spontaneous differentiation of replicating biological units [5,6]. First, we briefly review this scenario. (see Fig. 1 for schematic representation):

(1) *Synchronous divisions with synchronous oscillations of the chemicals.* Up to a certain number of cells (which depends on the model parameters), dividing cells from a single cell have the same characteristics. Although each cell division is not exactly identical due to the accompanying fluctuation in the biochemical composition, the phase of oscillation in the concentrations, and as a result, the timing of cell division, remains synchronous for all cells. The synchronous cell division is also observed with the cells in the embryo-genesis of mammals up to the eight cells.

(2) *Clustering in the phases of oscillations.* When the number of cells rises above a certain (threshold) value, the state with identical cells is no longer stable. Small differences introduced by the fluctuation start to be amplified, until the synchrony of the oscillations is broken. Then the cells split into a few groups, each having a different oscillation phase. The cells belonging to each group are identical in phase. This diversification in the phases, however, cannot be called cell differentiation, because the time average of the biochemical concentrations reveals that the cells are almost identical. The change of phases at the second stage is due to dynamic clustering, studied in coupled nonlinear oscillators [1,18]. Clusters are formed through the balance between the two tendencies, and the number ratio of the clusters satisfies some condition, to have stability against perturbations.

(3) *Differentiation in chemical composition.* With the further increase of the cell number, the average concentrations of the biochemicals over the cell cycle become different. The composition of biochemicals as well as the rates of catalytic reactions and transport of the biochemicals become different for each group. The orbits of chemical dynamics plotted in the phase space of biochemical concentrations, lie in a distinct region within the phase space, while the phases of oscillations remain different by cells within each group (see Fig. 1b).

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

(4) *Determination of the differentiated cells.* After the formation of cell types, the chemical compositions of each group are inherited by their daughter cells. In other words, chemical compositions of cells are recursive over divisions. The biochemical properties of a cell are inherited by its progeny, or in other words, the properties of the differentiated cells are stable, fixed or determined over the generations (see Fig. 1c). After several divisions, such initial condition of units is chosen to give the next generation of the same type as its mother cell.

(5) *Generation of rules for hierarchical organization.* As the cell number increases, further differentiation proceeds. Each group of cells differentiates further into two (or more) subgroups (see Fig. 2).Thus, the total system consists of units of diverse behaviors, forming a heterogeneous society.

The most interesting example here is the formation of stem cells, given in Fig. 2 [8]. This cell type, denoted as 'S' here, either reproduces the same type or forms different cell types, denoted for example as type A and type B. Then after division events $S \rightarrow S, A, B$ occur. Depending on the adopted chemical networks, the types A and B replicate, or switch to different types. For example $A \rightarrow A, A1, A2, A3$ is observed in some network. This hierarchical organization is often observed when the internal dynamics have some complexity, such as chaos.
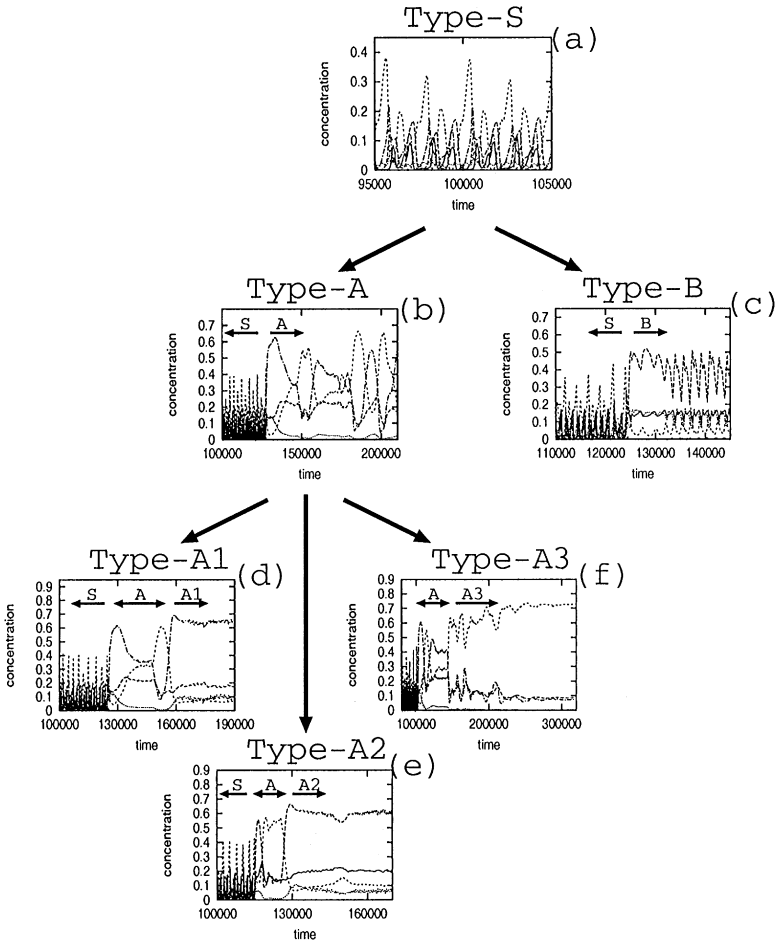
Fig. 2. An example of hierarchical differentiation observed in our model: (a) represents the time series of chemical concentration of the type-S (stem cell). (b)–(f) represent the course of differentiation to the corresponding cell type. In this example, we use a network with 20 chemicals and the time series of five chemicals are plotted, with different line styles. Starting from a single cell, the type S appears initially. As the number increases the differentiations represented by arrows progress successively. The type-S has a potential to differentiate to type-A, B and type-A can further differentiate to type-A1–A3. See also Ref. [8].

The differentiation here is "stochastic", arising from chaotic intra-cellular chemical dynamics. The choice for a stem cell either to replicate or to differentiate looks like stochastic as far as the cell type is concerned. Since such stochasticity is not due to external fluctuation but is a result of the internal state, the probability of differentiation can be regulated by the intra-cellular state. This stochastic branching is accompanied by a regulative mechanism. When some cells are removed externally during the developmental process, the rate of differentiation changes so that the final cell distribution is recovered.

In some biological systems such as the hematopoietic system, stem cells either replicate or differentiate into different cell type (s). This differentiation rule is often hierarchical [19,20]. The probability of differentiation to one of the several blood cell types is expected to depend on the interaction. Otherwise, it is hard to explain why the developmental process is robust. For example, when the number of some terminal cells decreases, there should be some mechanism to increase the rate of differentiation from the stem cell to the terminal cells. This suggests the existence of interaction-dependent regulation of the type we demonstrated in our results.

2.1. Remarks

Of course the behavior of our cell dynamics depends on the specific choice of biochemical networks.[1] Indeed the network with stem cells (with chaotic dynamics) is not so common. However, such cell system with stem cells and differentiation generally has a higher growth speed as an ensemble of cells [21]. In this sense, it is natural to expect that a multicellular system with such biochemical network that allows for chaotic stem cells has been selected through evolution.

3. Stability

In a developmental system, there are four kinds of stability, which are mutually interrelated.
- Microscopic: The developmental process is robust against molecular fluctuations.
- Macroscopic: The process is robust against macroscopic perturbations such as the removal of some cells or somatic mutations.
- Path: Not only the final states but also the developmental path resulting in such states is stable against microscopic and macroscopic perturbations.
- Generation: The developmental process is recursive to the next generation. In a multicellular organism, the dynamical process from a single germ cell to adult organism is almost preserved to the next generation, in spite of a long-term process amidst all the fluctuations involved therein.

3.1. Microscopic stability

The developmental process is stable against molecular fluctuations. First, intra-cellular dynamics of each cell type are stable against such perturbations. Then, one might think that this selection of each cell type is nothing more than a choice among basins of attraction for a multiple attractor system. If the interaction were neglected, a different type

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[1] In some examples with a larger diffusion coupling, cells that may be termed 'tumor-type cell' appear. These cells differentiate in an extraordinary way, destroy the cooperativity attained in the cell society, and grow faster in numbers in a selfish way. Their chemical configuration loses diversity, and the ongoing chemical pathway there is simpler than other cell types [5].

of dynamics would be interpreted as a different attractor. In our case, this is not true, and cell–cell interactions are necessary to stabilize cell types. [2] Given cell-to-cell inter-actions, the cell state is stable against perturbations on the level of each intra-cellular dynamics.

Next, the number distribution of cell types is stable against fluctuations. Indeed, we have carried out simulations of our model, by adding a noise term, considering finiteness in the number of molecules. The obtained cell type as well as the number distribution is hardly affected by the noise as long as the noise amplitude is not too large [6]. When the noise amplitude is too large, distinct types are no longer formed. Cell types are continuously distributed. In this case, the division speed is highly reduced, since the differentiation of roles by differentiated cell types is destroyed.

3.2. Macroscopic stability

Each cellular state is also stable against perturbations of the interaction term. If the cell-type number distribution is changed within some range, each cellular dynamics keep its type. Hence discrete, stable types are formed through the interplay between intra-cellular dynamics and interaction. The recursive production is attained through the selection of initial conditions of the intra-cellular dynamics of each cell, so that it is rather robust against the change of interaction terms as well.

The macroscopic stability is clearly shown in the spontaneous regulation of differ-entiation ratio in the last section. How is this interaction-dependent rule formed? Note that depending on the distribution of the other cell types, the orbit of internal cell state is slightly deformed. For a stem cell case, the rate of the differentiation or the repli-cation (e.g., the rate to select an arrow among $S \rightarrow S, A, B$) depends on the cell-type distribution. For example, when the number of "A" type cells is reduced, the orbit of an "S"-type cell is shifted towards the orbits of "A", with which the rate of switch to "A" is enhanced. The information of the cell-type distribution is represented by the in-ternal dynamics of "S"-type cells, and it is essential to the regulation of differentiation rate [8].

But why is the regulation oriented to keep the stability, instead of the other direction? In the example of Fig. 2, the differentiation from S to A is enhanced when the type A cell is removed. Assume that the regulation worked in the other way (i.e., the decrease of the rate $S \rightarrow A$ by the removal of A). Then, at the initial stage when type-A cells are produced, their number would decrease to zero, since there are fewer type-A cells at the moment. Hence, the type-A cell would not appear from the beginning. In other words, only the cell types that have a regulation mechanism to stabilize their coexistence with other types can appear in our developmental process.

It should be stressed that our dynamical differentiation process is always accompanied by this kind of regulation process, without any sophisticated programs implemented in

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[2] Importance of cell–cell interaction is discussed in the study of equivalent cells [22] and community effect [23].

advance. This autonomous robustness provides a novel viewpoint to the stability of the cell society in multicellular organisms.

4. Irreversibility

Since each cell state is realized as a balance between internal dynamics and interaction, one can discuss which part is more relevant to determine the stability of each state. In one limiting case, the state is an attractor as internal dynamics, which is sufficiently stable and not destabilized by cell–cell interaction. In this case, the cell state is called 'determined' following the terminology in cell biology. In the other limiting case, the state is totally governed by the interaction, and by changing the states of other cells, the cell state in concern is destabilized. In this case, each cell state is highly dependent on the environment or other cells.

Each cell type in our simulation generally lies between these two limiting cases. To see such intra–inter nature of the determination explicitly, one effective method is a transplantation experiment. Numerically, such experiment is carried out by choosing determined cells (obtained from the normal differentiation process) and putting them into a different set of surrounding cells, to make a cell society that does not appear through the normal course of development.

When a differentiated and recursive cell is transplanted to another cell society, the offspring of the cell keeps the same type, unless the cell-type distribution of the society is strongly biased when a cell is transplanted into a biased society, differentiation from a 'determined' cell occurs. For example, a homogeneous society consisting only of one determined cell type is unstable, and some cells start to switch to a different type. Hence, the cell memory is preserved mainly in each individual cell, but suitable inter-cellular interactions are also necessary to keep it.

Following the above argument, cell types can be classified into groups according to the degree of determination (Fig. 3):

- (U):'Undifferentiated cell class'; Cells change their chemical compositions by generations.
- (S):'Stem cell class'; Cells either reproduce the same type or switch to different types with some rate.
- (D) 'Determined cell class'; Chemical compositions are preserved by the division.

The degree of determination increases as (U) → (S) → (D). In the class (U), the cell is in a transient state as a dynamical system, and changes its character (e.g., average chemical composition) in time (and accordingly by division). The class (S) is a stable state, and is regarded as a kind of "attractor" *once the interaction term is specified.* Still, the type is weak against the change of interaction, and can switch to a different state, as mentioned. In the case of (ideally) determined type, it is an attractor by the internal dynamics itself. In fact, when the interaction term is changed to a large extent, it loses the stability, and switches to (U) or goes to a dead state.
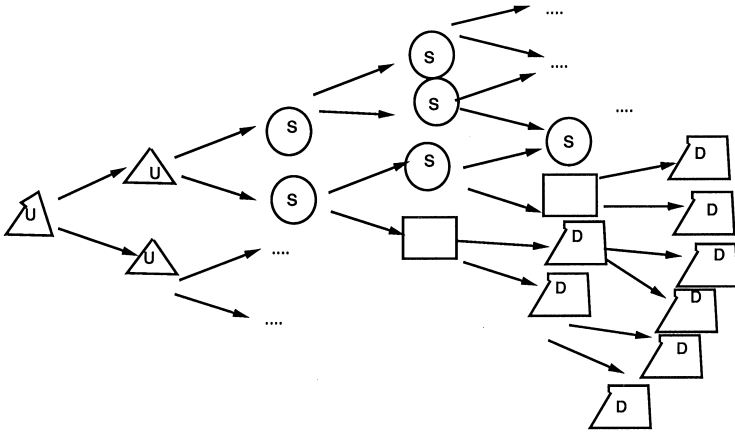
Fig. 3. Schematic representation of differentiation of cell types.

In a real organism, there is a hierarchy in determination, and a stem cell is often over a progenitor over only a limited range of cell types. In other words, the degree of determination is also hierarchical. In our model, we have also found such hierarchical structure. So far, we have found only up to the second layer of hierarchy in our model with the number of chemicals $k = 20$, as shown in Fig. 2.

In a real organism and in our model, there is a clear temporal flow, as (U) → (S) → (D), as the development progresses. The degree of determination increases in the normal course of development. Then how can one quantify such irreversibility? Can we define some quantity similar to thermodynamic entropy? So far no decisive answer is available. In our model simulation, with the process of (U) → (S) → (D) in time

- (I) Stability of intra-cellular dynamics increases
- (II) Diversity of chemicals decreases
- (III) Dynamics become less chaotic.

The degree of (I) could be determined by a minimum change in the interaction to switch a cell state, by properly extending the 'attractor strength' introduced in Refs. [24,25]. A cell of (U) spontaneously changes the state without the change of the interaction term, while the state of (S) can be switched by tiny change in the interaction term. The degree of determination in (D) is roughly measured as the minimum perturbation strength required for a switch to a different state.

The diversity of chemicals (II) can be measured, for example, by $-\sum_{j=1}^{k} p(j) \log p(j)$, with

$$p(j) = \left\langle \frac{x(j)}{\sum_{m=1}^{k} x(m)} \right\rangle,$$

with $\langle \cdots \rangle$ as temporal average. For the example of Fig. 2, this index of diversity is 2.8 for type S, 1.4 for A, 2.4 for B, 1.0 for A3 and A4, 0.9 for A5. Successive decrease as S → A, and → A1, A2, and A3 is numerically expressed.

The tendency (III) is numerically confirmed by the subspace Kolmorogorv–Sinai (KS) entropy of the internal dynamics for each cell. Here, this subspace KS entropy is measured as a sum of positive Lyapunov exponents, in the tangent space restricted only to the intracellular dynamics for a given cell. In the example of Fig. 2, the KS entropy is positive (~ 0.001) for S, while for others it is almost zero.

5. Discussion

In the present paper we have provided a new look at developmental cell biology, based on the intra–inter dynamics, and production. Differentiated cell types are given as stable states realized as a balance between internal dynamics and interaction. Hierarchical differentiation starting from a stem cell is a general consequence of our scenario. Stochastic differentiation with spontaneous regulation of differentiation probabilities is found to be supported by intra-cellular chaotic dynamics. In the present paper we claim that cell differentiation and the formation of cell societies are a natural consequence of reproducing interacting units with internal dynamics. It is just a universal phenomenon in a system having such intra–inter dynamics.

Stability and irreversibility in development are stressed as a universal feature in a developmental system with intra–inter dynamics. The irreversibility is proposed to be expressed as the increase of stability of each cellular state, and decrease of diversity in chemicals and KS entropy.

We have also applied the idea of the isologous diversification to evolution, which shows that the interaction-induced phenotypic differentiation is later fixed to genes. Distinct species with distinct genotypes and phenotypes are formed from a population of organisms with identical genotypes. This leads to a scenario for robust sympatric speciation [26].

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