Proportion Regulation of Biological Cells in Globally Coupled Nonlinear Systems

Tsuyoshi Mizuguchi and Masaki Sano
Research Institute of Electrical Communication, Tohoku University, Sendai 980-77, Japan
(Received 1 February 1995)

Globally coupled activator-inhibitor systems are studied as a model of the regulation of cell proportion in biological differentiation. Formation and destabilization of one- and two-cluster states are predicted analytically. Numerical simulations show that the proportion of units in clusters falls within a finite range determined by the initial conditions.

PACS numbers: 87.10.+e, 87.22.As

The regulation of the proportion of different cell types in a tissue is a general and important aspect of biological development. The proportion of the two different cell types is roughly constant irrespective of the slug size of cellular slime mold *Dictyostelium discoideum* (Dd) [1–4]. Though initially the same Dd cells, when dissociated, randomly mixed, and reaggregated, indifferently differentiate into two cell types (prespore and prestalk cells) without forming a spatial pattern. Cell differentiation is independent of cell position, while later cell sorting forms the two-zoned prestalk-prespore pattern typical of slugs of Dd [3–5]. Similar regulation can be observed in caste populations of social insects such as ants and bees [6,7], where the proportion of worker types is independent of the size of the colony and recovers after manipulation by the experimenter.

No theoretical model exists to describe proportion regulation. Pattern formation models such as the Turing instability with diffusive coupling [8] are incompatible with the observation that the Dd cells start to differentiate independent of their positions. Instead, a large population of identical units interacting identically (a globally coupled nonlinear system) is a good candidate to describe the phenomenon. Global coupling is an idealized model of the case, when the diffusion length of a chemical factor, e.g., differentiation inducing factor (DIF) or pheromone, is very large compared to the cell size, or when the individual units move around to interact with others.

Globally coupled chaotic maps [9,10] and globally coupled oscillators [11–15] display clustering and destabilization. However, a description of the cluster state is difficult because the unit itself is complex. Chaos, oscillations, or excitability probably do not play essential roles in biological proportion regulation in Dd. In this respect, a simpler model of clustering is preferable.

Our globally coupled model is composed of N activator-inhibitor units with two variables u and v. Each quantity is considered as a concentration of morphogen in each cell which activates or inhibits the differentiation process such as cyclic adenosine 3′-monophosphate (cAMP) or ammonia [16]. The dynamics is

$$\begin{aligned}\dot{u}_j &= au_j - bv_j - u_j^3 + K_1(\overline{u} - u_j),\\ \dot{v}_j &= cu_j - dv_j + K_2(\overline{v} - v_j),\end{aligned}$$

here, $\overline{u} \equiv \frac{1}{N}\sum_{i=1}^{N} u_i$ and $\overline{v} \equiv \frac{1}{N}\sum_{i=1}^{N} v_i$. The components u_j and v_j of the jth unit function as activator and inhibitor, respectively, if a, b, c, and d are positive. Each unit couples to all other units through the averaged fields $\overline{u}$ and $\overline{v}$. K_1 and K_2 are the non-negative susceptibilities of each component, since this type of global coupling corresponds to the limit of fast diffusion.

First, we investigate the properties of individual units by setting $K_1 = K_2 = 0$. Depending on the parameter $s \equiv ad - bc$, the number and the stability of the fixed point changes. The linear stability of these fixed points can be analyzed by setting $u = u_0 + \delta u, v = v_0 + \delta v$, $|\delta u|$, $|\delta v| \ll 1$, and $\delta u = \delta u_0 e^{\lambda t}, \delta v = \delta v_0 e^{\lambda t}$. Linearization of (1) leads to the eigenvalue equation

$$0 = \lambda^2 - (a - d - 3u_0^2)\lambda - s + 3du_0^2 . \qquad (2)$$

The fixed point (u_0, v_0) is stable if the conditions $0 > a - d - 3u_0^2$ and $0 < -s + 3du_0^2$ are satisfied. We will assume that $a < d$ and $s < 0$ from now on. In this case, the trivial solution $(0, 0)$ is the unique attractor for a single isolated unit.

Let us consider a one-cluster state in which every unit has the same value $(u_{(1)}, v_{(1)})$, i.e.,

$$(u_j, v_j) = (u_{(1)}, v_{(1)}), \quad j = 1, \ldots, N .$$

Analyzing the stability of the cluster in the globally coupled system is difficult because we must solve the eigenvalues of a $2N$ dimensional matrix. However, we can determine easily a sufficient condition for cluster destabilization. First, N units are assumed to form an M-cluster state $\{(u_{(i)}, v_{(i)})\}$, $i = 1, \ldots, M$. Next, we consider one additive unit (test unit) $(u(t), v(t))$ in that M-cluster state and approximate the effect on the test unit from the other N units is simply external force. This approximation is justified in the limit $N \to \infty$. The stability of this test unit determines the stability of the original M-cluster state as follows. Noting that the test unit has at least M "entrained" solutions for each

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cluster, i.e., $(u(t), v(t)) = (u_{(i)}, v_{(i)})$, the linear stability of these entrained solutions can be analyzed. If one of the entrained solutions is unstable, we conclude that the original cluster state is unstable. We call this stability analysis a test unit analysis (TUA) [17].

Now, we carry out TUA for a one-cluster state $\{(0,0)\}$, i.e., we consider the stability of a test unit $(u(t), v(t))$ subject to the force created by the N units in the one-cluster state. In this case, both of the average fields $\overline{u}$ and $\overline{v}$ vanish and the equations for the test unit become

$$\begin{aligned} \dot{u} &= (a - K_1)u - bv - u^3, \\ \dot{v} &= cu - (d + K_2)v. \end{aligned} \tag{3}$$

The fixed points of the test unit can be obtained by setting $\dot{u} = \dot{v} = 0$. u satisfies

$$0 = h_1(u) \equiv (d + K_2)u^3 + [(d + K_2)(K_1 - a) + bc]u.$$

Note that $h_1(0) = 0$ because the test unit has the entrained solution $(u, v) = (0, 0)$. To investigate the stability of the one-cluster state, we analyze the test unit's linear stability around the entrained solution $(0, 0)$. Linear stability analysis of (3) yields the eigenvalue equation

$$\begin{aligned} 0 = \lambda^2 &- (a - d - K_1 - K_2)\lambda \\ &- (a - K_1)(d + K_2) + bc. \end{aligned} \tag{4}$$

The stability conditions for the entrained solution of the test unit in the one-cluster state are now given as

$$0 > a - d - K_1 - K_2, \tag{5}$$

$$0 < -s - aK_2 + K_1(d + K_2). \tag{6}$$

From the condition for existence and stability of one-cluster solutions in the uncoupled case, (5) is automatically satisfied. Therefore the critical condition for stability occurs when the right hand side (RHS) of (6) equals 0, where a pitchfork bifurcation occurs. Although this stability condition is for the entrained solution of the test unit, it is obvious that the original one-cluster solution is unstable if (6) is violated. Thus the one-cluster state is linearly unstable when $K_2 > K_{2c}$. Figure 1 shows the result of numerical simulations for $K_2 < K_{2c}$ using a simple Euler method with $dt = 0.01$. Parameters are $N = 100$, $a = 0.4$, $b = 1$, $c = 0.5$, $d = 1$, $K_1 = 0$, and $K_2 = 0.2$. A one-cluster state [Fig. 1(c)] results from a uniform random initial condition Fig. [1(b)]. When $K_2 > K_{2c}$, the one-cluster state becomes unstable and each unit separates into two subpopulations, i.e.,

$$(u_i, v_i) = \begin{cases} (u_{(1)}, v_{(1)}) & \text{for } i = 1, \ldots, N_{(1)}, \\ (u_{(2)}, v_{(2)}) & \text{for } i = N_{(1)} + 1, \ldots, N. \end{cases}$$

Here, $N_{(1)}$ is the number of units which belong to the first cluster. This state is defined as a two-cluster state.

Assume $K_1 = 0$. Consider a two-cluster state $(u_{(1)}, v_{(1)})$,$(u_{(2)}, v_{(2)})$ with proportion $p : 1 - p$, where

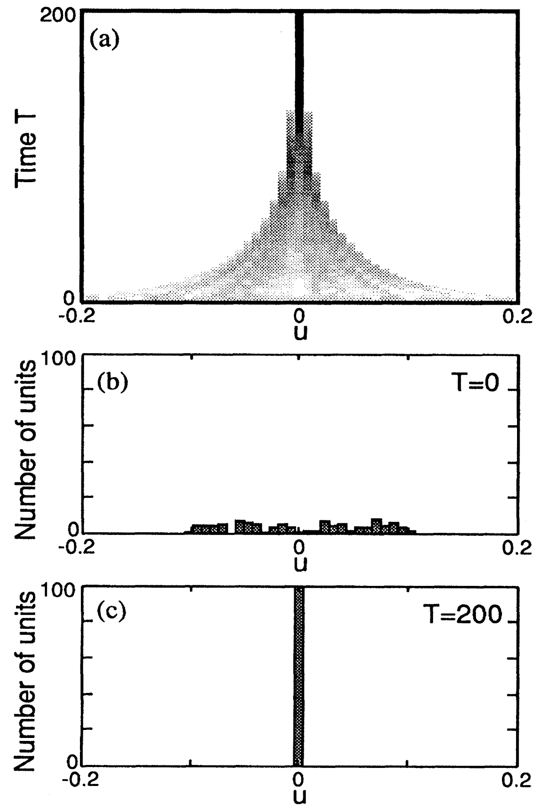

FIG. 1. (a) Temporal evolution of the distribution of units with respect to u. The gray scale shows the number of units: white representing 0 and black N. Randomly distributed units aggregate into the origin, and a one-cluster state is formed. (b) Initial distribution. Each unit has a uniform random number between -0.1 and 0.1 for u and v, respectively. (c) Snapshot of a one-cluster state at $T = 200$.

$p \equiv N_{(1)}/N$ and $0 < p < 1$. The averaged fields $\overline{u}$ and $\overline{v}$ become $pu_{(1)} + (1 - p)u_{(2)}$ and $pv_{(1)} + (1 - p)v_{(2)}$, respectively. Eliminating $v_{(1)}$ and $v_{(2)}$ and transforming from $(u_{(1)}, u_{(2)})$ to $(u_{(1)}, \phi)$, where $\phi \equiv u_{(2)}/u_{(1)}$, yields an equation for ϕ:

$$\begin{aligned} (\phi - 1)[bcK_2(1 - p)\phi^3 &- s(d + K_2)\phi^2 \\ &- s(d + K_2)\phi + bcK_2p] = 0. \end{aligned}$$

Note that $\phi = 1$ implies $u_{(1)} = u_{(2)}$, i.e., a one-cluster state. The solution must satisfy the inequality $u_{(1)}^2 > 0$. Therefore the condition for the existence of a two-cluster state is

$$K_2 > K_{2c} \equiv -s/a. \tag{7}$$

Under the condition (7), we use TUA again to analyze the stability of the two-cluster state, i.e., we consider the stability of a test unit entrained solution to the external force created by the N units in the two-cluster state. The test unit equations are

$$\dot{u} = au - bv - u^3,$$
$$\dot{v} = cu - dv + K_2[pv_{(1)} + (1-p)v_{(2)} - v].$$

There exist two entrained solutions for the cluster, $(u_{(1)}, v_{(1)})$ and $(u_{(2)}, v_{(2)})$. To investigate the stability of the two-cluster state, we analyze the test unit's linear stability around the entrained solution $(u_{(1)}, v_{(1)})$. Linearization around $(u_{(1)}, v_{(1)})$ leads to the eigenvalue equation

$$0 = \lambda^2 - (a - d - K_2 - 3u_{(1)}^2)\lambda - (a - 3u_{(1)}^2)(d + K_2) + bc. \qquad (8)$$

The entrained solution becomes unstable if the constant term in (8) becomes 0. The stability condition is

$$0 > \{(aK_2 + s)[(2ad - 3bc)K_2 + 2ds]^2(d + K_2p)\} \times \{-9bcK_2p + 2(ad + 3bc)K_2 + 2ds\}. \qquad (9)$$

The first term on the RHS of (9) is positive definite, and the second term determines its stability. The bifurcation line is given by solving it for p, i.e.,

$$p = p_c(K_2) \equiv \frac{2}{9bc}\left(\frac{ds}{K_2} + (ad + 3bc)\right), \qquad (10)$$

where a transcritical bifurcation occurs. For example, $p_c = 7.6/9 - 0.2/4.5K_2$ for the case $a = 0.4$, $b = 1$, $c = 0.5$, $d = 1$. A typical phase diagram is shown in Fig. 2. For $K_2 < K_{2c}$, no two-cluster solution exists. For $K_2 > K_{2c}$, one of the two-cluster solutions with a proportion within region B is realized. The two-cluster state in region C is linearly unstable and is not realized. The bifurcation diagram

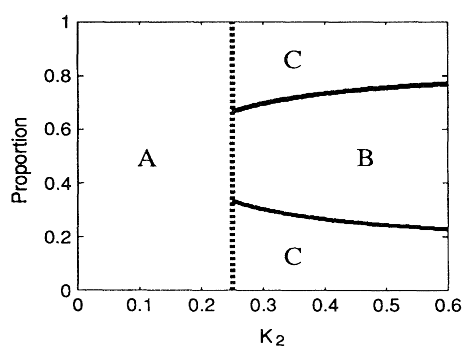

FIG. 2. Typical phase diagram of one- and two-cluster states. The upper line is given by (10) and the lower line denotes $1 - p_c$. The dotted line is $K_2 = K_{2c}$. In the region A, there is no two-cluster solution. In B and C, a linearly stable and an unstable two-cluster solution exists, respectively.

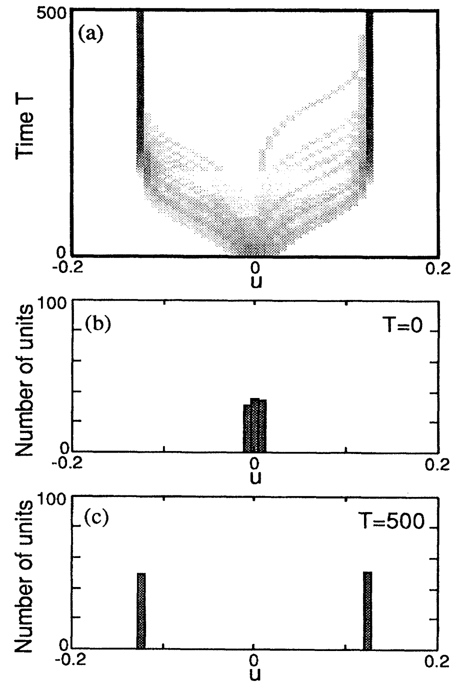

FIG. 3. (a) Evolution of the distribution with respect to u. Units around the origin separate into a two-cluster state. (b) Initial distribution. Each unit has a uniform random number between -0.01 and 0.01 for u and v, respectively. (c) Snapshot of the two-cluster state at $T = 500$.

is symmetric around $p = 0.5$ because the proportion of the other cluster is $1 - p$. Therefore the possible proportion has a minimum $p_{\min} = 1 - p_c(K_2)$ and maximum $p_{\max} = p_c(K_2)$ for a given K_2. To investigate the dynamics of destabilization we perform numerical simulations. Figure 3 shows the formation of a two-cluster state from a uniform random initial condition with $K_2 = 0.3 > K_{2c}$. Other parameters are as in Fig. 1. At $T = 500$, a two-cluster state is selected with proportion $p : 1 - p = 49 : 51$. In Fig. 4, the proportion regulation under artificial partial extinction is shown. We start with a relaxed state of the previous simulation [shown in Fig. 3(c)] removing the 49 units which belong to the cluster with negative u value [hatched in Fig. 4(a)]. The remaining 51 units reform a two-cluster state with the proportion $p : 1 - p = 23 : 28$ falling in region B. Three or more cluster states have not been observed.

To clarify what selects the final proportion, we perform numerical simulations of Eq. (1) with changing initial conditions. Parameters are the same as in Fig. 1 except that $K_2 = 0.5$. We start from 500 initial conditions which are uniform random numbers between -0.1 and 0.1 with different seeds. Figure 5 shows the distribution of final proportions, which peaks at $p = 0.5$, showing that the initial condition determines the proportion.

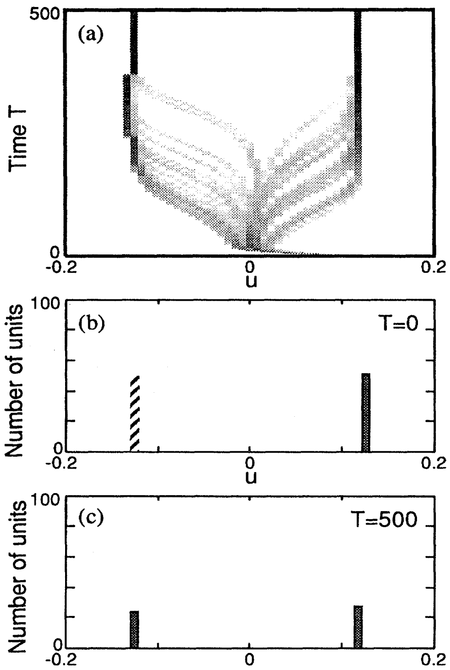

FIG. 4. (a) Evolution of the distribution. After a sudden decrease for u of each unit to zero till $T \sim 10$, 51 units separate into two clusters again. (b) Initial distribution. 49 units in the hatched cluster are removed. (c) Snapshot at $T = 500$. The final proportion $p : 1 - p = 23 : 28$.

Finally, we check the structural stability of these results by adding a small positive constant term ϵ, i.e.,

$$\dot{u}_j = au_j - bv_j - u_j^3 + K_1(\overline{u} - u_j),$$
$$\dot{v}_j = cu_j - dv_j + K_2(\overline{v} - v_j) + \epsilon .$$

Numerical simulation shows that the distribution of proportion also has a finite width. The most probable proportion, however, moves from 0.5 because the added term ϵ breaks the symmetry of (1) [18]. For example, the most probable value is about 0.25, $p_{\min} \sim 0.1$, and $p_{\max} \sim 0.4$, respectively, when $a = 0.6$, $b = 1$, $c = 2$, $d = 1$, $K_1 = 0$, $K_2 = 3$, and $\epsilon = 0.2$. From this result we conclude that the proportion regulation phenomenon discussed in this Letter is generic.

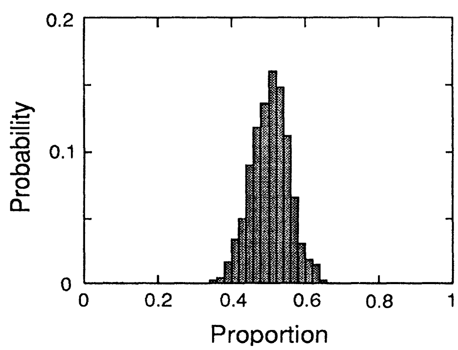

FIG. 5. Probability distribution of proportions of two-cluster states for 500 randomly chosen initial conditions.

The authors wish to thank N. Nakagawa, Y. Kuramoto, and Y. Sawada for many fruitful discussions.

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[17] The condition given by the TUA is only a sufficient condition for the destabilization of the original cluster state and is not a necessary condition. There is an example in which the original cluster state is unstable even if the TUA is stable. Consider the following globally coupled system: $\dot{u}_j = u_j - u_j^3 + K(\overline{u} - u_j)$. Although the fixed point $u_j = 0$ is unstable to the perturbation that every unit moves in the same direction, the solution entrained to 0 of the test unit is stable so as to $K > 0$. The relation between the stabilities of the entrained test unit solution and the stability of the original cluster state remains a further problem.

[18] The added term ϵ also changes the bifurcation type between the one- and two-cluster states. Coexistence of the one-cluster solution and the two-cluster solution occurs to some range of K_2.