Signal Propagation and Failure in Discrete Autocrine Relays

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(Received 28 January 2004; published 10 September 2004)

A mechanistic model of discrete one-dimensional arrays of autocrine cells interacting via diffusible signals is investigated. Under physiologically relevant assumptions, the model is reduced to a system of ordinary differential equations for the intracellular variables, with a particular, biophysically derived type of long-range coupling between cells. Exact discrete traveling wave and static kink solutions are obtained in the model with sharp threshold nonlinearity. It is argued that the considered mechanism may be used extensively for transmission of information in tissues during homeostasis and development.

DOI: 10.1103/PhysRevLett.93.118101 PACS numbers: 87.17.Aa, 47.54.+r, 87.18.Pj

Understanding the mechanisms of information flow in living matter is, perhaps, the most fundamental problem in biophysics. In multicellular organisms, cell-to-cell communication plays a key role in an organism's development, homeostasis, and function [1–3]. Cell communication relies on a variety of alternative biophysical mechanisms, which may involve electrical activity, direct mechanical contact, exchange of chemical messages, etc., and is tightly regulated on both the biophysical and the genetic levels [1–3].

A classical example of a well-characterized cell communication system is the nerve axon [2,3]. Over half a century ago, on the basis of careful quantitative experimental studies, Hodgkin and Huxley came up with a mechanistic model of signal transmission in squid giant axon [4]. This model was able to quantify the functional properties of the axon in terms of the biophysically measurable quantities and brought in one of the first major success stories in quantitative biology. Since then, it has become a paradigm for modeling cell communication via action potentials generated by excitable cell membranes [2].

On the other hand, while some of the mechanisms of cell communication are currently well understood, many are being characterized only now. A large fraction of information exchange between cells in multicellular organisms is encoded by diffusible peptide growth factors [5]. Biochemical and genetic approaches have identified the key molecular components for signal generation, transmission, detection, and processing in these systems [5,6]. Importantly, these mechanisms are often autocrine in nature; that is, the signal generated by one cell can affect the same cell and involve positive and negative feedback [7]. The growing amount of available biochemical information makes these systems ready for the development of quantitative models capable of giving insights into their functional capabilities [6].

In this Letter, we introduce a mechanistic model of cell-to-cell communication by diffusible signals in autocrine relays. This model provides a biophysical framework for studying growth factor-mediated signal transmission in tissues [6,7]. Under biophysically reasonable assumptions, the model can be simplified to yield exact closed form solutions in the form of discrete traveling waves, enabling a complete characterization of signal propagation and failure in the model.

At the core of our model is the mechanism of ligand-induced ligand release [Fig. 1(a)]. There is an increasing amount of biochemical, cellular, and genetic evidence that this kind of positive feedback plays a key role in tissue homeostasis and development and in disease states such as cancer [8–12]. In the case of the epidermal growth factor receptor (EGFR) system, ligand-receptor binding at the cell surface can induce the activation of the signal transduction cascades (most notably the mitogen activated protein kinase pathway) and upregulate the transcription of the EGFR ligands or ligand-releasing proteases [8–10,13]. While the feedback itself is well established, its physiological and developmental functions are still unclear. Here, we propose that it can serve as a module for long-range signal transmission.

We begin by considering a monolayer of cells covered by a layer of extracellular medium of thickness H. Diffusible chemical signals (ligands) can move within the layer and be secreted or absorbed through the surfaces of a linear array of identical cells of size L at the bottom of the layer [Fig. 1(b)]. For simplicity, we assume that

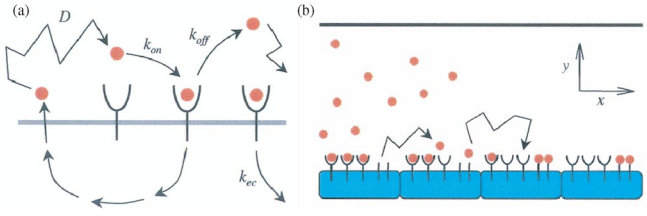

FIG. 1 (color online). The schematics of an autocrine relay.

there is only one ligand, whose concentration within the extracellular space is denoted by s. The uniform secretion of s by the nth cell at the bottom of the layer is controlled by an intracellular species (protease) with concentration p_n. After s has been secreted, it diffuses in the extracellular space and binds to the receptors uniformly distributed on the cell surfaces. The total number of ligand-receptor complexes on the cell surface regulates the downstream processes, such as intracellular signal transduction, which, in turn, determine the rate of production of the intracellular protease p_n [6,8–10,13]. The complexes, whose density is denoted by c, can then either dissociate, releasing s back into the layer, or be internalized and removed from the layer, Fig. 1(a) [6].

This leads to the following mechanistic model that takes into account ligand processing, transport, binding, and endocytosis:

$$\frac{\partial s}{\partial t} = D\left(\frac{\partial^2 s}{\partial x^2} + \frac{\partial^2 s}{\partial y^2}\right), \qquad \left.\frac{\partial s}{\partial y}\right|_{y=H} = 0, \tag{1}$$

$$\left.\left(D\frac{\partial s}{\partial y} - k_{\mathrm{on}} r s\right)\right|_{y=0} = -k_{\mathrm{off}} c - g_s \sum_n \chi_n p_n, \tag{2}$$

$$\frac{\partial c}{\partial t} = -(k_{\mathrm{off}} + k_{\mathrm{ec}})c + k_{\mathrm{on}} r \bar{s}, \qquad \bar{s} = s|_{y=0}, \tag{3}$$

$$\frac{dp_n}{dt} = -k_p p_n + g_p \sigma\left(L \int \chi_n c dx\right). \tag{4}$$

Here, k_{on} and k_{off} are the rate constants for forward and backward ligand-receptor binding, k_{ec} is the rate of receptor-mediated endocytosis, r is the number of receptors per unit area, D is the ligand diffusion constant, g_s is the rate constant for ligand release, and $\chi_n(x) = \theta(Ln + L - x)\theta(x - Ln)$ is the characteristic function of the surface of the nth cell [here and everywhere below $\theta(x)$ is the Heaviside step]. Equation (1) describes ligand diffusion in the extracellular medium with an impermeable barrier at the top of the layer, Eq. (2) specifies the boundary condition on cellular surfaces, and Eq. (3) governs the kinetics of the complexes. Also, Eq. (4) describes the cellular response to receptor activation by ligand-receptor binding. In this equation, k_p is the first order degradation rate constant of the protease, g_p is the maximum protease production rate, and $\sigma(C)$ is a sigmoidal function, whose argument is the total number of ligand-receptor complexes on the cell surface [14,15].

Since the protease expression is a slow process [16,17], on the time scale k_p^{-1} the diffusion of s may have enough time to equilibrate. This occurs on the length scale of a single cell, if $k_p L^2/D \ll 1$. If also $k_{\mathrm{off}}, k_{\mathrm{ec}} \gg k_p$, which is typically true [6], we can use a quasi-steady-state approximation for the s and c variables and set the time derivatives in Eqs. (1) and (3) to zero. Then, after rescaling lengths and times with the cell size L and the protease degradation time constant k_p^{-1}, respectively, and suitably rescaling the dependent variables, we obtain the following reduced system of equations:

$$0 = \frac{\partial^2 s}{\partial x^2} + \frac{\partial^2 s}{\partial y^2}, \qquad \left.\frac{\partial s}{\partial y}\right|_{y=h} = 0, \tag{5}$$

$$\left.\left(\frac{\partial s}{\partial y} - ks\right)\right|_{y=0} = -\sum_n \chi_n p_n, \tag{6}$$

$$\frac{dp_n}{dt} = -p_n + \sigma(c_n), \qquad c_n = kC_0 \int \chi_n \bar{s} dx, \tag{7}$$

where c_n is the total number of ligand-receptor complexes on the surface of the nth cell, $C_0 = g_s g_p L^2/(k_p k_{\mathrm{ec}})$ is the maximum number of complexes on the surface of an individual cell, and

$$h = \frac{H}{L}, \qquad k = \frac{k_{\mathrm{ec}} k_{\mathrm{on}} R_0}{DL(k_{\mathrm{off}} + k_{\mathrm{ec}})}, \tag{8}$$

where $R_0 = rL^2$ is the total number of receptors on the surface of an individual cell (assumed constant). The obtained linear boundary value problem for c_n can be solved by standard Fourier transform techniques. After some algebra, the resulting solution is

$$c_n = C_0 \sum_{m=-\infty}^{+\infty} M_{n-m} p_m, \tag{9}$$

where the coupling coefficients matrix M_m are given by

$$M_m(k, h) = \int_{-\infty}^{+\infty} \frac{2k \sin^2(\frac{1}{2}q)\cos(qm)dq}{\pi q^2(k + q\tanh qh)}. \tag{10}$$

Thus, we get a system of ordinary differential equations for p_n [Eqs. (7) and (9)] coupled through M_m which is obtained from the original first-principles-based spatially distributed model (compare with [18–21]).

In our earlier study [17] we demonstrated the possibility of autocrine signal transmission in the continuum model of the considered mechanism. While the continuum setup may be well suited for experiments in cell tissue cultures [22], it ignores the essential discreteness of cells on the level of tissues. On the other hand, it is well known that discreteness often results in a failure of the system's signal propagating ability (see, e.g., [19]). Therefore, in the following we study the feasibility of signal transmission in the fully discrete model. To proceed, we introduce two more approximations. First, we assume that the thickness H of the extracellular layer is much smaller than the cell size L, which is often true in tissues [16], and expand M_m with $h \ll 1$. Retaining only the leading order term in the Taylor expansion of the integrand in Eq. (10) and performing the integration, we obtain that in this limit

$$M_m = \frac{(\cosh\kappa - 1)e^{-\kappa|m|}}{\kappa}, \qquad m \neq 0, \tag{11}$$

$$M_0 = \frac{\kappa + e^{-\kappa} - 1}{\kappa}, \qquad \kappa = \sqrt{\frac{k_{ec}k_{on}R_0}{DH(k_{off} + k_{ec})}}, \tag{12}$$

where κ is a dimensionless parameter, characterizing the range of cell-cell coupling. Note that the above expressions for the coupling coefficients remain valid when $\kappa h \ll 1$.

Second, we assume that the sigmoidal response of the protease production to receptor activity is characterized by a sharp threshold behavior, which is supported by biochemical measurements [14,15]. This means that we can set $\sigma(C) = \theta(C - C_T)$, where C_T is the critical number of complexes needed for protease production activation. Combining this with the results above and Eq. (7) yields

$$\frac{dp_n}{dt} = -p_n + \theta\Big[\frac{\kappa + e^{-\kappa} - 1}{\kappa}p_n + \frac{\cosh\kappa - 1}{\kappa} \times \sum_{m=1}^{\infty} e^{-\kappa m}(p_{n-m} + p_{n+m}) - a\Big], \tag{13}$$

where $a = C_T/C_0$ is the dimensionless threshold.

We are now going to use Eq. (13) to study signal propagation and failure in autocrine relays. To do that, we look for the discrete traveling wave solutions of the form $p_n(t) = p(t - n\tau)$ [19,20], so that $v = Lk_p/\tau$ gives the (dimensional) propagation speed and is to be determined. This leads to a differential-delay equation which can be solved exactly. For monotone solutions the argument of the Heaviside function is also a monotone function, so the threshold is crossed only once. Therefore, with no loss of generality we may assume that this happens at $t = 0$. Then the traveling wave solution advancing the "on" state of signaling from left to right is $p(t) = (1 - e^{-t})\theta(t)$. From this, the self-consistency condition at $t = 0$ reads

$$a = \frac{\cosh\kappa - 1}{\kappa}\sum_{m=1}^{\infty} e^{-\kappa m}(1 - e^{-\tau m}). \tag{14}$$

Summing up the geometric series in Eq. (14) and then solving for τ, we obtain explicitly

$$\tau = \log\Big(\frac{1 - e^{\kappa} + 2a\kappa}{1 + e^{\kappa}(2a\kappa - 1)}\Big). \tag{15}$$

This expression can be used to obtain the speed of the wave as a function of the physiological parameters. A plot of the propagation velocity (together with that of a back-propagating front; see below) for a particular value of the cell-cell coupling parameter κ is presented in Fig. 2.

The analysis of Eq. (15) shows that the discrete traveling waves exist for all values of $a < a_{cr}^{+}(\kappa) = (1 - e^{-\kappa})/(2\kappa)$. At $a = a_{cr}^{+}$ we have $v = 0$, and the waves fail to propagate forward, signifying the propagation threshold. Note that the phenomenon of propagation failure is typical for discrete reaction-diffusion systems [18–20,23–25]. Observe that when $\kappa \ll 1$, i.e., when the coupling between cells is long range, the value of a_{cr}^{+} approaches 1/2, the propagation threshold that can be obtained from the continuum approximation to Eq. (13). On the other hand, when $\kappa \gg 1$, that is, in the regime of effectively nearest-neighbor coupling, propagation is still possible for very small thresholds $a < 1/(2\kappa) \ll 1$.

In a similar fashion, one can construct the solutions in the form of retracting traveling waves of the on state of signaling. Observe that Eq. (13) is invariant with respect to the transformation $p \to 1 - p$ and $a \to 1 - a$, so the speed of the backward-propagating waves can be obtained from Eq. (15) by replacing a by $1 - a$ there. These waves exist as long as $a > a_{cr}^{-}(\kappa) = (2\kappa - 1 + e^{-\kappa})/(2\kappa)$. Furthermore, it is not difficult to show that for $a_{cr}^{+} \leq a \leq a_{cr}^{-}$ stationary kink solutions, in which $p_n = \theta(n)$, exist. Therefore, traveling waves become arrested when the parameter a is changed into this range. This is supported by the direct numerical simulations of Eq. (13). Let us point out that when a approaches a_{cr}, the

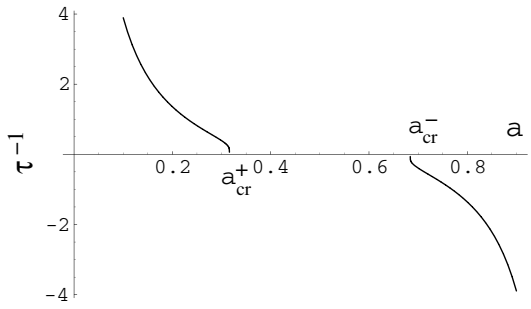

FIG. 2. Velocities of advancing and retracting waves as functions of the threshold at $\kappa = 1$.

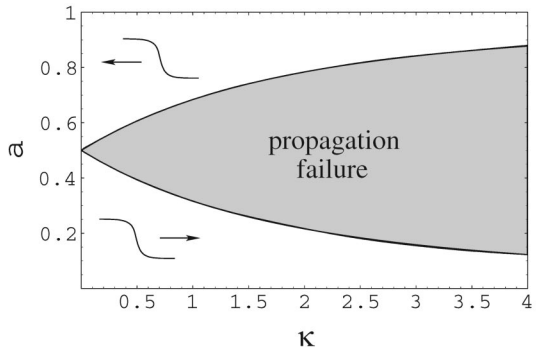

FIG. 3. Propagation-failure diagram for the discrete traveling waves in the model.

speed of the wave depends singularly as $1/\log(|a - a_{\rm cr}|^{-1})$ on the distance to the threshold; see Eq. (15). This is typical for threshold nonlinearities [23], in contrast to discrete systems with smooth nonlinearities [18,24,25].

The propagation-failure diagram obtained from these arguments is presented in Fig. 3. Let us give a simple intuitive interpretation to this figure. In order for a wave to advance, the threshold a must be sufficiently low, so that the cell ahead of the wave can sense the incoming signal and switch on its positive feedback. The shorter the range of the signal (larger κ), the more sensitive (smaller a) the cell should be to be able to relay the signal. On the other hand, for high thresholds the role of the on and "off" states is reversed.

We now demonstrate how our results translate to a realistic biophysical situation [17,26]. Consider a representative set of parameters [6], in which $L = 5\ \mu$m, $H = 1\ \mu$m, $C_0 = 2 \times 10^3$ complexes/cell, $C_T = 500$ complexes/cell, $R_0 = 10^4$ receptors/cell, $k_{\rm off}^{-1} = k_{\rm ec}^{-1} = 5$ min, $k_p^{-1} = 30$ min, $k_{\rm on} = 0.1\ {\rm nM}^{-1}\,{\rm min}^{-1}$, and $D = 10^{-7}\ {\rm cm}^2\,{\rm s}^{-1}$. This translates into the dimensionless parameters $h = 0.2$, $\kappa = 1.18$, and $a = 0.25$. Using these values and Eq. (15), we obtain the speed $v = 0.1\ \mu$m/min, or 1.25 cells/h. Let us also point out that the simplifying assumptions that went into the derivation of Eq. (15) are satisfied, since $h \ll 1$, $\kappa h \ll 1$; $k_{\rm ec}^{-1}, k_{\rm off}^{-1} \ll k_p^{-1}$, and $k_p L^2/D = 1.4 \times 10^{-3} \ll 1$.

To summarize, we have analyzed a new mechanism for long-range signal transmission in tissues. The mechanism is mediated by a well-established positive feedback between growth factor-mediated receptor activation and receptor-mediated ligand release. We have used the molecular and cellular parameters of the EGFR system to estimate the characteristic rates of wave propagation and suggest that these slow waves are indeed feasible *in vivo*. In reality, such positive feedback is always under the control of additional mechanisms. For example, multiple intracellular negative feedback has been described in the EGFR system [6,11]. Clearly, the addition of slow local negative feedback may lead to a possibility of transmissions of pulses of autocrine activity, not unlike the action potentials in neural systems (compare, e.g., with [19,27]), but on the time scale of days rather than seconds. This opens up an intriguing possibility of autocrine relays being actively used for information processing in tissue homeostasis and repair. Feedback can also modulate the traveling waves and produce complex spatial patterns in various developmental contexts [28]. The analytical results reported in this Letter can be extended to account for these processes.

This work is supported by NSF Grant No. DMS-0211864.

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