

Pattern formation in *Dictyostelium* via the dynamics of cooperative biological entities

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The cellular slime mold *Dictyostelium discoideum* exhibits a variety of spatial patterns as it aggregates to form a multicellular slug. These patterns arise via the interaction of the aggregating amoebae, either via contact or as mediated by chemical signals involving cyclic adenosine monophosphate (AMP). We model this system as a set of reaction-diffusion equations coupled to dynamical biological entities (bions), each of which is endowed with signal receptors and response rules. Simulations of our model reveal a close correspondence with the observed structures. Also, the general framework we propose should be suitable for modeling other biological pattern-forming processes.

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Many important examples of biological pattern formation occur via the interaction of individual cells to create multicellular structures. Cells can interact via direct contact, via elastic forces mediated by an extracellular matrix (or substratum), or via chemical signals. Processes of this class include early embryonic development [1], bacterial colony dynamics [2], and the topic to be studied here, aggregation of amoebae in *Dictyostelium* [3].

The aggregation of *Dictyostelium discoideum* has been extensively studied over the past years as an experimentally accessible example of cell interaction and cell differentiation [4–6]. Roughly, colonies of 10^4 – 10^5 individual amoebae transit to a common point, under the influence of the signaling by an aggregation center. This signal is the organic chemical cyclic adenosine monophosphate (AMP), which binds to a cell surface receptor [7] and causes the cell to both move and relay the “message.” Although some of the detailed biochemistry of the single-cell transduction pathways is not understood [8], enough of the basic features are sufficiently clear so as to enable us to formulate quantitatively testable models of the many-cell dynamics.

Before proceeding to our approach to this specific example of biological pattern formation, we would like to explain the general framework which has guided our thinking. Individual cells are extremely complex “computing” elements; they take in a vast variety of signals from the external world and call into play sophisticated biochemical machinery to respond, both chemically and mechanically. Our approach is to simplify the dynamics at the single cell stage as much as (but not more than) is possible. Specifically, we replace the cell by a simple element (a “bion”) which can measure concentrations and concentration gradients, change internal states, sense the presence of nearby bions, and move [9]. The goal of this type of model is the correlation of the complexity of the pattern with the complexity of the individual element; more directly, we want to learn how much of the final structure is due to simple physics and chemistry and how

much is due to biological sophistication. While we focus in this paper exclusively on the amoebae aggregation system, we believe that this way of simulating biological pattern formation will be of more general use.

The first stage of our *Dictyostelium* model consists of the signaling dynamics. It is well established [7,10] that the cyclic AMP cell-receptor system falls into the general class of excitable chemical dynamics [11]. These are systems which will propagate nonlinear waves in response to an above-threshold perturbation. In this case, the wave is sustained by each cell binding an above-threshold cAMP concentration, turning on a cAMP production and excretion facility, and then relaxing back to the unexcited state over a finite refractory period. Typical numbers for this signaling system are wave speeds of $300 \mu\text{m}/\text{min}$, with cAMP concentration oscillating from $10^{-8}M$ to $10^{-6}M$.

To mimic this behavior, we place bions randomly on a two-dimensional (2D) square lattice with density ρ ; typically ρ should be 5–20%, if we interpret our density as equal to the relative amount of intracellular to total area in an aggregating colony. In addition, cyclic AMP concentration c is taken to obey a (discretized) diffusion equation

$$\frac{\partial c}{\partial t} = a^2 \nabla^2 c - \Gamma c + (\text{sources}), \quad (1)$$

where we have normalized the diffusion constant to a^2 (the square of the lattice size); the decay is due to the degradation of cAMP by phosphodiesterase, here assumed to be at a fixed uniform concentration.

Each bion has an internal state representing the availability of cAMP receptor sites. For the signaling piece of the dynamics, the bion remains in “state” 0 until it detects a concentration at its location greater than c_T , a fixed threshold. Once $c > c_T$, the bion becomes excited (state 1) and emits an amount Δc of cAMP over τ time units. After time τ , the state progresses to quiescent

(state 2) and the bion counts until t_R time units before it reverts to state 0. Until it reverts, it is immune to further excitation.

Simulations of these dynamics reveal the typical behavior of an excitable system. For example, in Fig. 1, we see a steady-state rotating spiral wave for $\rho=0.2$, $\Delta c/c_T=300$, $\Gamma=0.5$, $\tau=2$, and $t_R=20$. One can similarly find target patterns as the response to a periodic “pacemaker” at the origin.

One important and realistic element of our first stage model is the fact that signals will only propagate for high enough “excitability”; in our case, this translates to a minimum density ρ necessary to sustain the signal relay network. To get an analytic handle on this phenomenon, we use a simple mean-field theory and replace the bion density ρ with release amount Δc by an averaged system with density 1 and release $\rho\Delta c$. A planar pulse solution to (1) can be represented by

$$\begin{aligned} c &= c_T e^{k_- z}, \quad z > 0, \\ c &= \frac{\rho\Delta c}{\Gamma\tau} + A e^{k_- z} + B e^{k_+ z}, \quad -v\tau < z < 0, \\ c &= D e^{k_+ z}, \quad z < -v\tau, \end{aligned} \quad (2)$$

where v is the velocity, $k_{\pm} = -(v/2) \pm \sqrt{v^2/4 + \Gamma}$, $z = (x - vt/a)$, and A , B , and D are constants. Imposing continuity at $z=0$ and $-v\tau$, we find the nonlinear consistency condition

$$c_T = \frac{k_+}{k_+ - k_-} \frac{\rho\Delta c}{\tau\Gamma} (1 - e^{k_- v\tau}). \quad (3)$$

For $\tau=2$, $\Gamma=0.5$, and $\Delta c/c_T=300$, a graph of $v(\rho)$ is given in Fig. 2. The saddle-node bifurcation at $\rho \sim 0.015$ signifies the end of the (upper) stable plane pulse branch. One can also calculate the excitation width, defined as the length over which $c > c_T$, as compared to the refractory width vT_R ; also, this calculation can be easily extended to deal with waves with finite wavelength. One should note,

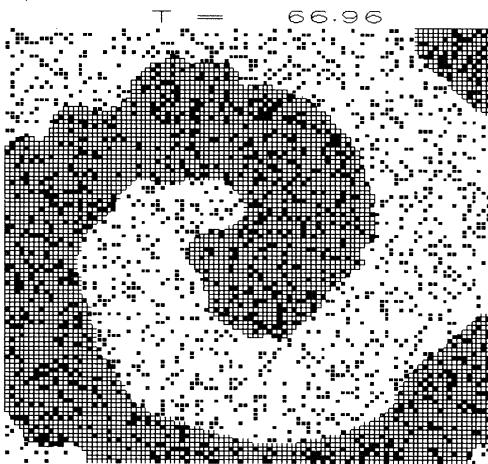


FIG. 1. Spiral in box of size 100 with no flux boundary conditions. Black boxes have cells and boxes with black borders have $c > c_T$.

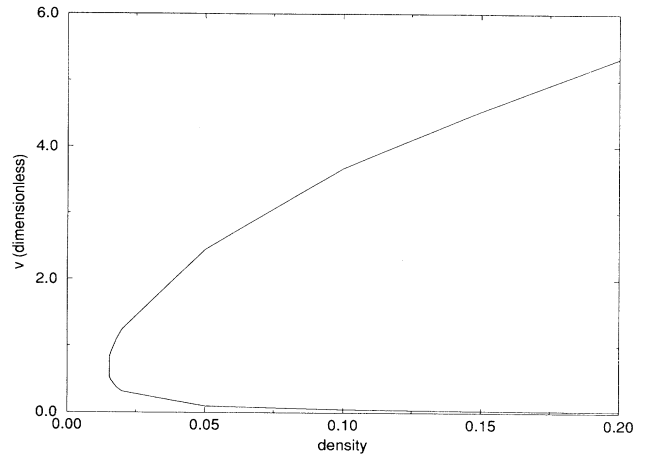


FIG. 2. Velocity vs density as predicted by signaling mean-field theory.

however, that propagation from an excitation center during a typical simulation usually requires a value of ρ higher than the estimate given by the plane-wave analysis. This is due both to curvature effects (retarding the wave propagation) and also to the fact that fluctuations are not negligible at densities of several percent.

The second stage of our model endows each bion with a new sensor and a motion rule. To wit, the gradients of cAMP in the $\pm\hat{x}$ and $\pm\hat{y}$ directions are computed for each bion in state 1 and compared to a motion threshold c'_T/a . If any directional gradient is above threshold, the bion tries to move one lattice spacing either in that direction. It will not move onto a site already occupied [12] and will continue attempting to move either until it succeeds or until it progresses back to state 2. A bion can move only once in each direction per chemical signal. If we compare our rule to the fact that individual amoeba typically move one cell diameter ($\sim 10 \mu\text{m}$) per pulse, we can roughly associate $a \sim 10 \mu\text{m}$. Then if we assume that our t_R of 20 reflects an actual refractory time of about 20 sec, we get a time unit of 10 sec and a wave speed (at $\rho=0.2$) of $5 \mu\text{m}/\text{sec}$. This is well within the range of observed velocities.

In Fig. 3 we present a series of three snapshots of the bion configuration at times 0, 100, and 200; the signaling was due to a central pacemaker operating with a signal interval $\Delta t=30$ and with $c'_T=2c_T$. Note the dramatic rearrangement of the density from a uniform pattern to a highly ramified network. The aggregation on this time scale has produced a set of dense streams along which the bions move in (almost) single file. The transition from chemical wave patterns with uniform azimuthal density to network behavior is a familiar feature of experimental studies. The azimuthal clumping is due to an instability of the combined signaling-chemotaxis system [13] which does not get restabilized until the local density reaches $O(1)$. It is hence difficult to imagine a continuum treatment of the streaming pattern which is so naturally produced by our cooperative bion dynamics.

One simple aspect of our streaming pattern is the focusing of the chemical wave along the stream with a

concomitant speed up of the wave motion. To understand this, we can crudely think of the stream as a constant density $\bar{\rho}$ source of width Na ; a typical stream is fairly dense and rather narrow, say $\bar{\rho}=0.5$, $N=2$. We can modify the previous calculation by using a square

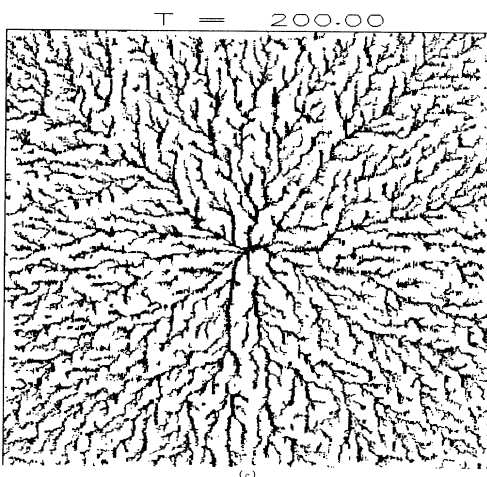
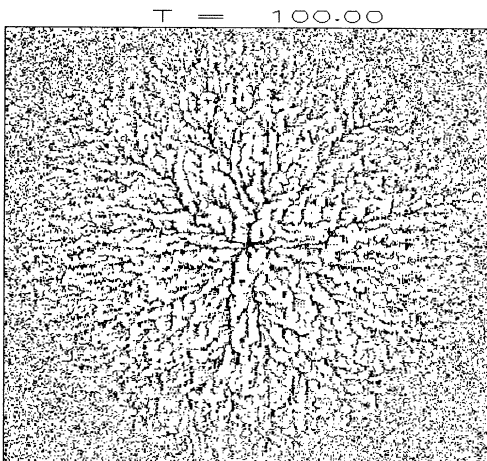
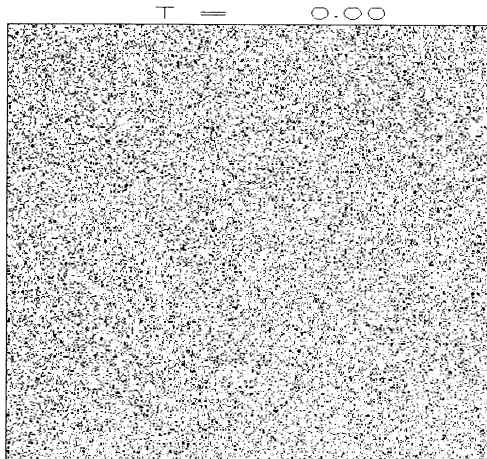


FIG. 3. $L=400$ simulation with central pacemaker. Only the cells are depicted. (a)–(c) correspond to $t=0, 100, 200$.

wave source in the transverse direction and Fourier decomposing everything in y . This leads to the approximate solution

$$c(y,z) = \int e^{iqy} \frac{dq}{2\pi} \frac{\bar{k}_+}{\bar{k}_+ - \bar{k}_-} \frac{\Delta c}{\tau(\Gamma + q^2)} \times \bar{\rho}(q) e^{\bar{k}_- z} (1 - e^{-\bar{k}_- v \tau}) \quad (4)$$

(for $z > 0$), where $\bar{k}_\pm = (-v/2) \pm \sqrt{(v^2/4) + \Gamma + q^2}$. Here, $\bar{\rho}(q)$ is the Fourier transform of the square wave $\rho = \bar{\rho}$ for $N/2 \leq y \leq N/2$ and 0 otherwise. (There are similar expressions for $z < 0$). The system is closed by demanding that $c(y=z=0) = c_T$, where we have used the approximation that c varies slowly over the width Na of the stream. For the typical values given above, this leads to about a factor of 2 increase in wave speed; this is mostly due to the increased bion density in the stream, i.e., the two dimensionality seems not to affect the speed to a large extent. This speed up is in qualitative accord with direct simulation results.

One interesting experimental observation [14] concerns the existence of a ring of bions surrounding a low-density hole; this structure occurs when the chemical wave occurs as a spiral and is presumably due to the low cAMP concentration at the spiral core repelling bions from the center. We have performed a simulation where we established a rotating spiral wave and then switched on the chemotaxis. The resultant pattern after $t=200$ is shown in Fig. 4 and clearly exhibits both the small central ring and the expected bending of the streams.

To summarize, we have introduced a flexible simulation approach to early stage aggregation pattern in *Dictyostelium*. There is much work to be done in exploring this model's parameters, analyzing the resultant patterns (perhaps by using methods from the study of river networks [15]), and understanding the model behavior on an analytic basis. More crucially, there is much work to be done in using this model to see how much of the vast literature of detailed experimental measurements can be

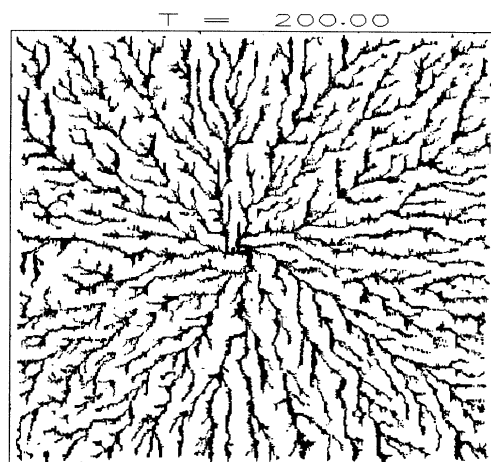


FIG. 4. Same as Fig. 3, except that the central pacemaker has been replaced by a self-sustaining spiral.

explained with only the simplest dynamics used here and what modifications might be necessary for more quantitative comparisons. (For example, it is known [16] that the decay rate due to phosphodiesterase degradation is not constant but is instead coupled to the basic signaling system). A much longer term goal would be to incorporate more sophisticated cell-cell interactions into the coopera-

tive bion simulation so as to enable us to proceed to later stages in the aggregation process such as slug formation and migration.

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