Destabilization of cell aggregation under nonstationary conditions

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A simplified model describing the early stage of *Dictyostelium discoideum* aggregation is developed and applied to study the spatiotemporal evolution of the cell density pattern under the condition of a nonuniform initial cell distribution or quasiperiodical parametric forcing. A sufficiently large gradient and a parametric forcing result in destabilization of the aggregation due to a continuous drift of spirals of cyclic adenosine 3′-5′-monophosphate controlling the chemotactic cell movement. In a small colony consisting of only one aggregation domain the spiral wave can disappear while the aggregation has not yet been completed. In the case of many aggregation domains the drift leads to the disappearance of some aggregation centers, emergence of multiple new centers, displacement of cell colony domains, and washing out of the boundaries between them. [S1063-651X(98)11310-7]

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I. INTRODUCTION

There are numerous examples of self-organization phenomena observed in nature, among the most impressive being morphogenesis in higher organisms and pattern formation in communities of micro-organisms, such as bacteria and slime moulds. All of them include complex rearrangements of the spatial cell distribution, influenced by chemical and mechanical interactions between cells. Mathematical descriptions of these nonlinear active systems are commonly based on reaction-diffusion models. Then the observed spatiotemporal patterns can be explained as a result of a Turing instability (e.g., some morphogenetic processes [1–3]) or of wave phenomena (e.g., aggregation of *Dictiostelium discoideum* amoebae [4–9]).

In the majority of these models the parameters of the active medium are fixed and a feedback between the emerging pattern and the properties of the medium is neglected. However, in many cases a feedback really exists and influences the patterning process significantly. An example is the early stage of *D. discoideum* aggregation, where chemical waves of cyclic adenosine 3′-5′-monophosphate (cAMP) are established by active membrane processes of cells of the mould. In this active medium steadily rotating spiral waves and expanding target patterns are commonly observed under the assumption of a constant and uniform cell density [4,5]. In reality, however, the cell density is influenced by the propagating cAMP waves, since the cells move towards the direction of the cAMP gradients. A nonuniform distribution of the cell density thus arises, which drastically changes the conditions of wave propagation such that wave fronts may even break up in regions with low density [10–12]. Several models for *Dictyostelium* aggregation were proposed that take this additional feedback into account: chemical waves of cAMP, described either by the Fitzhugh-Nagumo equations [7] or by equations based on the Martiel-Goldbeter scheme of cAMP signalling [8,13], were coupled to the corresponding chemotactic cell response. These models describe early stages of *Dictyostelium* aggregation successfully, but they postulate a uniform initial cell distribution throughout the medium and disregard possible external influences during the aggregation, whereas nonstationary conditions are quite natural for biological objects. Such conditions may significantly change the process of self-organization and the formation of the final pattern, as was demonstrated for chemical waves in the Belousov-Zhabotinsky (BZ) reaction [14–16].

The kinematical theory of waves in excitable media [17] predicts that under nonstationary conditions rotating spiral waves should undergo a drift in space. On the other hand, one knows both from experiments and mathematical models that local inhomogeneities can stabilize spiral rotation [18–20]. In the case we consider here such inhomogeneities naturally appear due to the chemotactic cell aggregation (i.e., near spiral wave core). Thus, a competition between two opposite effects may occur in the system that essentially complicates its behavior as compared with a spatially uniform active medium (like a petri dish containing a layer of the BZ reaction).

In this work we study numerical pattern formation in a model of a colony of micro-organisms exhibiting chemotaxis under conditions of initially nonuniform cell distribution or external forcing. The model equations are designed to describe *D. discoideum* aggregation, but the results obtained should be considered in a more general context and applied to other systems, when a feedback exists between the emerging spatiotemporal pattern and the properties of the active medium.

II. THE MODEL

The numerical simulations are performed with a model especially developed to study the spatiotemporal evolution of *Dictyostelium* aggregation under nonstationary conditions. The part of the model describing the local reaction kinetics is motivated by the model of Martiel and Goldbeter [13], while the description of the spatial dynamics is similar to the model proposed by Höfer *et al.* [8].

In accordance with the Martiel-Goldbeter model, cAMP is produced by the cells with a rate proportional to the amount of active cAMP receptors in the cell membranes. The num-

ber of the active receptors is in turn a function of the cAMP concentration in the extracellular space. We preserve the main properties of the model proposed in [13], which are essentially determined by the shape of the null-clines of the variables in phase space. However, we did not assume a uniform distribution of the cell density, which can change due to the chemotactic motion of the cells.

Thus the description of cell aggregation process is formulated in terms of cAMP concentration $v(x,y,t)$, the fraction of active cAMP receptors per cell $g(x,y,t)$ and the cell density $u(x,y;t)$. The model in nondimensional form is

$$\dot{v} = \gamma u\left(g\,\frac{v^2+A^2}{v^2+1} - \delta v \right) + D_v \Delta v,$$

$$\dot{g} = B - (1+Hv)g, \tag{1}$$

$$\dot{u} = D_u \Delta u - \nabla(\chi(g-g_0)^4 u \nabla v).$$

The first equation in (1) describes the autocatalytic synthesis of cAMP depending on the fraction of active cAMP receptors g (in the membrane) and the degradation of cAMP that occurs due to hydrolysis by the enzyme phosphodiesterase, produced by the cells. It is important that the local production of cAMP is proportional to the cell density u. The last term describes the spatial diffusion of cAMP in extracellular space with a constant D_v. The second equation is an expression for the fraction of active cAMP receptors per cell g, which depends on the cAMP concentration.

The last equation for the spatial rearrangement of cells includes two terms describing a random cell motion and the chemotactic response to a cAMP gradient, respectively. In order to simplify the model we disregard the possible dependence of the random cell motility coefficient D_u on cell density due to cell-cell adhesion. Such a dependence is important for cell streaming during aggregation [8], but does not essentially affect the initial stage of aggregation we are interested in. Note that the term describing the chemotactic velocity cannot be simply proportional to the cAMP gradient. Indeed, such an assumption would result in the well known ''chemotactic wave paradox'' [12]: within the wave front cells move in the direction opposite to that of the wave propagation, whereas within the wave back they move in the same direction. Since more time is spent in the wave back than in the wave front, the resulting shift in the direction of wave propagation should be expected, which contradicts the observations. To solve this paradox one has to explain why cells practically do not react to the cAMP gradient on the wave back. Several different solutions have been suggested (for a review see [8]). In our model we assume that the chemotactic cell motility is large, when the cAMP receptors are in the active state. Due to this dependence on g the motility is large on the wave front and sufficiently small on the wave back.

The parameters used in the numerical simulations are the following: $A=0.12$, $\delta=0.24$, $\gamma=100$, $B=1.85$, $H=8.0$, $g_0=0.3$, $\chi=0.3$, $D_v=1$, $D_u=0.01$. Integration of the last equation in (1) over the region shows that the mean cell density u_0 is constant for zero-flux boundary conditions. In all computations u_0 was taken equal to unity unless indicated otherwise. The choice of parameter values reflects the case of a weakly excitable active medium, in which the core of a spiral wave has a pronounced size, as frequently found in *Dictyostelium* aggregation [10,11].

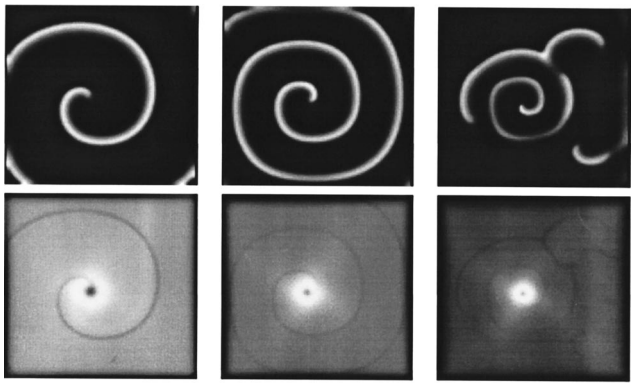

FIG. 1. Results of numerical simulations of *Dictyostelium* aggregation under stationary conditions. Evolution of the system for three successive moments of time: $T=21$, 39, and 52. The top row of images show cAMP concentration, the bottom row, cell density. The dark shaded area corresponds to low concentration and cell density.

The equations (1) were solved numerically on a square domain with zero-flux boundary conditions. A finite-difference approximation with a five-point Laplacian and central differences for the first derivatives was used. Simulations were performed either on a grid with 93×93 mesh points for the region of the nondimensional size 27×27, or on a grid with 363×363 points for the region 108×108. The total cell number never deviated more than 0.2% from the initial value, which is an important indication for the correctness of the computational scheme.

III. RESULTS

In the first series of numerical simulations we investigated the aggregation process in one domain formed in a square region of 27×27. This corresponds to about 30 mm^2 in the original dimensional model, a reasonable value for the size of one domain of aggregating *Dictyostelium* amoebae [10,11].

In Fig. 1 the result of a simulation supposing an initially homogeneous cell distribution and without any external forcing is shown. As initial conditions the variable u is taken equal to 1 in the whole region, while the distribution of the variables v and g is nonuniform in order to create a spiral wave of cAMP. In the upper half of the simulated region this distribution corresponds to a plane wave of cAMP propagating to the right. The initial condition in the lower half of the medium is the steady state of the medium. Thus a half-wave with an open end is initiated that evolves into a single rotating spiral with its core located approximately in the center of the domain. In the course of time cells move to the core of the spiral as a response to the rotating cAMP spiral wave. With the increase of cell density in the central region the time period of the spiral rotation and its wavelength decrease in agreement with experimental observations [21]. The large core specific for the low excitability of medium results in the appearance of a characteristic loop of cells around the rotation center. In the middle of this loop the cell density is low,

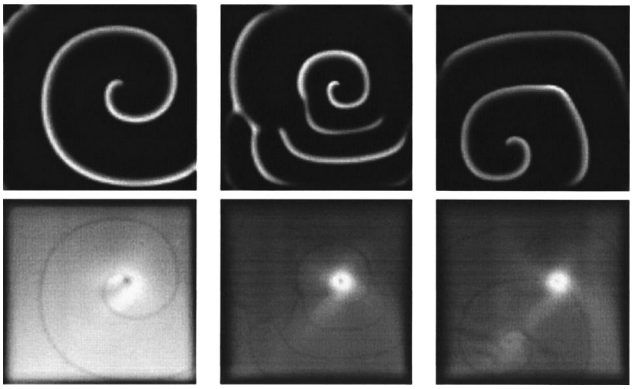

FIG. 2. Simulation of *Dictyostelium* aggregation in the presence of an initial gradient in cell distribution: initial cell density changed linearly from 0.83 at the top to 1.17 at the bottom of the domain. $T=36$, 63, and 72.

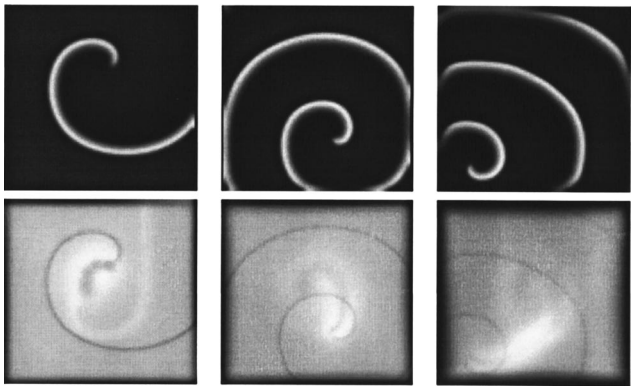

FIG. 3. Simulation of *Dictyostelium* aggregation under pulsatory decrease of excitability of the medium synchronized with the cAMP spiral rotation. The parameter γ controlling the excitability was decreased synchronously with the spiral rotation to 90% of the initial value for one-fifth of the previous period of spiral rotation. $T=19$, 48, and 82.

which is clearly seen as a black hole within the bright ring in the left bottom picture of Fig. 1. However, the size of the central hole slowly vanishes with time due to random cell motion (see the bottom row of Fig. 1). In later stages most of the cells concentrate near one aggregation center, whereas on the periphery of the domain the cell density drastically decreases and no waves of cAMP concentration can propagate here. For this reason the spiral waves break, as shown in the upper right picture of Fig. 1. Thus, our simplified model describes well the creation of a single aggregation center.

In the next computation we started with the same initial conditions as in the previous one, with the exception that initially the cells were distributed nonuniformly along the Y axis (the density u changes from 0.83 on the upper edge of the domain to 1.17 on the lower one). The result obtained in the presence of such an initial gradient of cell density is shown in Fig. 2. First the cAMP spiral drifts to the right across the gradient of cell density (compare locations of the spiral wave cores in Figs. 1 and 2). The cells continue to aggregate around this moving spiral core. Subsequently, due to cell gathering, there appears a loop of cells that anchors the spiral rotation for some time. However, in a later stage the central hole of the loop vanishes (similar to Fig. 1) and the spiral core begins to drift rapidly. It finally disappears at the lower edge of the domain. Obviously, in this case the destabilizing effect of the spiral drift caused by the initial cell gradient dominates over the stabilizing effect due to cell gathering. It turns out, however, that a slightly smaller initial gradient (from 0.85 on one edge to 1.15 on the other) does not suffice to destabilize the aggregation, and then the process follows the scenario of Fig. 1. Thus the system exhibits a bifurcation phenomenon.

In the third numerical experiment, starting again from the initial conditions of Fig. 1, we considered the system under parametrical forcing synchronous with the spiral rotation, in a way similar to that discussed in Ref. [16]. Namely, the parameter γ was abruptly decreased from 100 to 90 as the wave passed through an arbitrarily chosen fixed point in the domain. The decrease lasted for a time interval equal to 20% of the previous period of spiral rotation. This can be interpreted as a temporal decrease of excitability. As shown in Fig. 3, this relatively weak forcing completely disorganized the aggregation process. The spiral core continuously drifts in the domain and finally collapses at the boundary. Unlike in the previous case, no bifurcation effect was noticed as the spiral starts to drift for any small parametric forcing amplitude.

In the next series of computations the more realistic case was considered, when several aggregation domains are formed due to several initial organizing centers in a larger region of the size 108×108. These centers are created with initial conditions for the distributions of the variables g and v corresponding to several fragments of plane cAMP waves either with one or with two open ends. Here one encounters a somewhat higher level of self-organization in *Dictyostelium* in that spatial patterning of aggregation domains and aggregation of cells in each of domains occurs. Such domain structures are observed in real living systems [11] but have not yet been reproduced with any model. In Fig. 4 the initial stage of cell aggregation and the final state of the system are shown. This figure reveals the mechanism of how the bound-

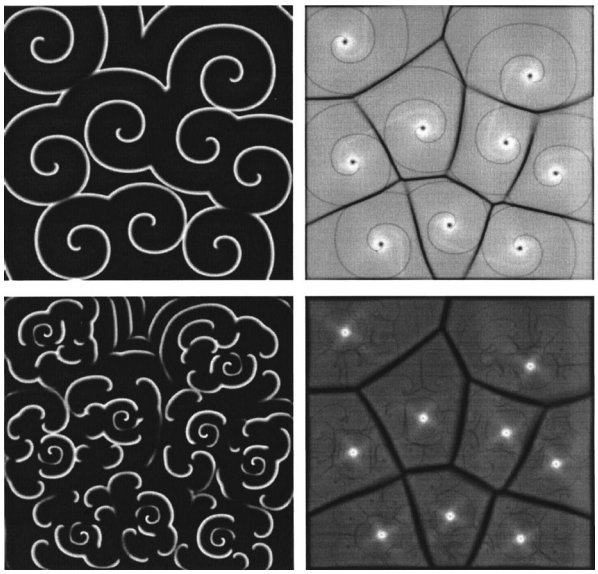

FIG. 4. Results of simulation of multiple *Dictyostelium* aggregation domains under stationary conditions. The evolution of the system from the initial ($T=18$) to the final ($T=54$) stage is shown.

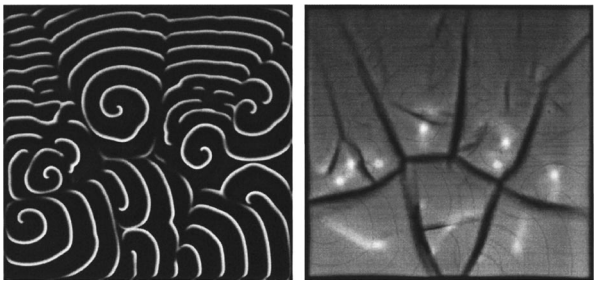

FIG. 5. The final stage of multiple *Dictyostelium* aggregation ($T=63$) in the presence of an initial gradient in cell distribution obtained numerically. Cell density changed from 0.9 on the top to 1.3 on the bottom of the domain; the mean cell density u_0 was equal to 1.1.

aries between domains are formed. Apparently, their location is determined by the lines of adjacent spirals colliding with each other. Note that the amoebae always move in the direction perpendicular to the wave front towards the center of the spiral. As in the points of spiral collision wave fronts have a cusplike shape, cells separate and move to one center of aggregation or to the other (see upper-right picture of Fig. 4). Therefore, the location of the boundaries depends not only on the position of the spiral cores (like for Voronoy diagrams), but also on the relative phases of spiral rotation.

When introducing an initial gradient of the cell distribution while keeping the same initial conditions for the other variables (as in Fig. 4), the pattern of the domain boundaries changes drastically (Fig. 5). This is obviously the result of the drift of spiral cores, as was observed in a single colony (Fig. 2). This drift changes the phase relations between adjacent spirals and shifts the locations of their collision points. As a consequence the boundaries between the domains are washed out. Another interesting phenomenon in the final stage is the appearance of a number of secondary centers of aggregation as seen in Fig. 5.

When an external force is applied to the system in the same way as described for a single colony (cf. Fig. 3), the locations of the domain boundaries continuously change due to the spiral drifts and are again washed out, some spirals disappear. The size of surviving domains becomes larger, but the boundaries between them are not so pronounced and the whole process of aggregation is obstructed to a great extent (Fig. 6). The low contrast between the bright spots and the background indicates that the process of aggregation is yet far from complete in this system (cf. Fig. 4). On the other hand, the pattern shown in Fig. 6 remains practically the same during a long time interval and can be considered as the final one.

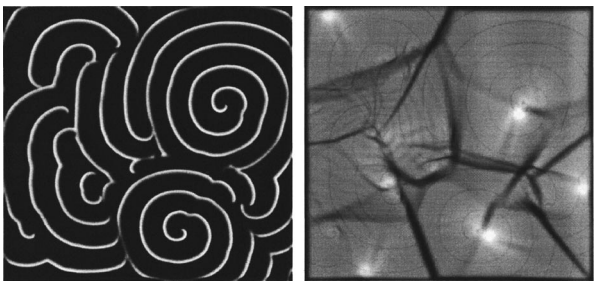

FIG. 6. The final stage of multiple *Dictyostelium* aggregation ($T=54$) under external forcing conditions. The parameter γ controlling the excitability was decreased synchronously with the spiral rotation to 90% of the initial value for one-fifth of the previous period of spiral rotation.

IV. DISCUSSION

A simplified model of *D.discoideum* aggregation has been presented as an example of an excitable medium with specific feedback properties. In this system the properties of the medium, and first of all its excitability, depend on the local cell density, which itself is influenced by the waves of cAMP due to the chemotactic response of the cells. Under stationary conditions, as was shown here and elsewhere (see, e.g., [7,8]), the process of cell aggregation is quite stable: once the center of aggregation appears, it does not change its location, cells concentrate near this point, and the cAMP wave rotates around it. Later on, the spiral wave breaks into segments and outside the central region it disappears because of low cell density and inhomogeneities in cell distribution.

The situation changes when the conditions of aggregation are nonstationary. This means that either the initial cell distribution is nonuniform or an external forcing is applied that changes the excitability of the medium quasiperiodically. In both cases the excitability near the spiral wave tip varies with time. This leads to a drift of the spiral corresponding well to theoretical predictions [17] and experimental results obtained with the BZ reaction [14,15]. In the considered system where the local excitability of the medium associated with the cell density is not constant, the consequence of this drift is much more dramatic: centers of aggregation change their location in space and the whole process of aggregation is disorganized. The spiral drift itself also becomes rather irregular, as the core of the spiral, while moving through the domain, traverses regions with different excitability due to the evolving inhomogeneity. When the initial gradient of cell density becomes smaller than some critical value, the spiral rotation is stabilized after an initial drift due to cell aggregation, while for larger gradients the spiral drift never stops. The finding of this bifurcation phenomenon is different from spiral wave behavior in excitable media without a feedback. In the considered case the chemotactic motion changes the initially given nonuniform distribution of the cells in the course of time. As a result the local gradient of the cell density can vanish or even change its sign after one or several revolutions of the spiral cAMP wave. Thus, the system can overcome the destabilizing effect of the primary nonuniformity if the latter was not pronounced enough. When quasiperiodic parametric forcing is applied such bifurcation behavior is not observed: The spirals continue to drift for any value of the forcing amplitude used in the computations. However, only a more rigorous analysis can verify that the drift still occurs for arbitrarily small amplitudes and for any other choice of parameters.

Another important aspect of the problem is the interaction of several organizing centers. Our simplified model reproduces the phenomenon of the appearance of separate aggregation domains as known from experimental data [11]. The very first simulation of this phenomenon clarifies the mechanism of the formation of the domain boundaries.

The macroscopic pattern of these boundaries undergoes

pronounced changes under nonstationary conditions. There are some differences between the effects of initial nonhomogeneity and that of periodic external forcing. For example, in the first case multiple new centers of aggregation may appear, while in the second one a large portion of the initial centers die out. However, in both cases spirals cannot maintain a stationary rotation near centers of aggregation and inevitably drift away.

We see two possible applications of the present investigation: The first is a fundamental one, as we study the general problem of the stability of wave processes in active media including a feedback between wave propagation and excitability of the medium. A feedback of this kind exists, for instance, between the electrical activity and the contraction of heart tissue and is assumed as one of the factors leading to cardiac arrhythmias [22]. The second application is the particular process of *Dictyostelium* aggregation. The model we have considered here does not describe all the details of the real process. In particular, in its simple form it does not describe cell streaming. Nevertheless, our simulations show that nonstationary conditions always disorganize the process of cell aggregation and reveal new phenomena that are to be verified in future experiments.

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