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A Model of *Dictyostelium discoideum* Aggregation

O. O. VASIEVA, B. N. VASIEV, V. A. KARPOV AND A. N. ZAIKIN

Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences,
142292 *Pushchino, Moscow Region, Russia*

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The aggregation process in populations of *Dictyostelium discoideum* is mediated by cell communication through cyclic AMP (cAMP) signals and the chemotactic response of the cells to concentration gradients of cAMP. In the present paper several simple algorithms that describe cell behavior are used to create a model that correctly predicts the aggregation patterns of the cells. The formation of the aggregative structures with a definite geometrical characteristics can be studied by using the model.

1. Introduction

The aggregating population of *Dictyostelium discoideum* (Dd) amoebae is one of the simplest developing systems in biology. It is an ideal subject for investigating how the properties of individual cells can define the common geometry of multicellular structure when the cells connect through the space and aggregate.

Dd amoeba in the starving population, which are initially randomly dispersed, move toward foci and form multicellular structures (Durston, 1973; Alcantara & Monk, 1974; Loomis, 1975; Dinauer *et al.*, 1980*a*, *b*; Robertson & Grutsch, 1981; Devreotes *et al.*, 1983). At the same time, this system can be considered as an excitable medium, where chemical waves of cyclic AMP propagate (Durston, 1973; Dinauer *et al.*, 1980; Devreotes *et al.*, 1983). In the starving population, cells appear that spontaneously secrete cAMP pulses. Neighboring cells secrete a pulse of cAMP in response to the increase in concentration to a threshold value (Cohen & Robertson, 1971; Dinauer *et al.*, 1980*a*, *b*; Robertson & Grutsch, 1981; Devreotes *et al.*, 1983). The released cAMP is slowly destroyed by phosphodiesterase, which is also secreted by the amoebae. These processes result in traveling waves of cAMP (Dinauer *et al.*, 1980*a*). On the fronts of these waves amoebae move chemotactically up the cAMP gradient (Alcantara & Monk, 1974; Loomis, 1975; Robertson & Grutsch, 1981; Newell, 1983). In time, the whole population is divided into domains where cells joined in streams move to their center of aggregation, which are the sources of the chemical waves (Fig. 1).

The processes leading to appearance of chemical waves in the *Dictyostelium* population have recently been intensively studied by computer simulation (Rapp *et al.*, 1985; Martiel & Goldbeter, 1987; Tyson *et al.*, 1989; Monk & Othmer 1990). However, the modeling of aggregation initiated by MacKay (1978) and Parnas & Segel (1978) has not been continued. The phenomenological models proposed by these authors are based on the experimental data involving levels of cAMP, receptor affinity, phosphodiesterase activity and other data which are insufficient and limit the future development of such models.

In the present paper we propose a model of amoebae aggregation, where the cell interactions and their motion are defined explicitly without any mediator. In this way we construct a general model of the excitable and the aggregating systems. The model predicts mechanisms of structure formation and the appearance of self-sustaining sources which may have relevance in natural populations.

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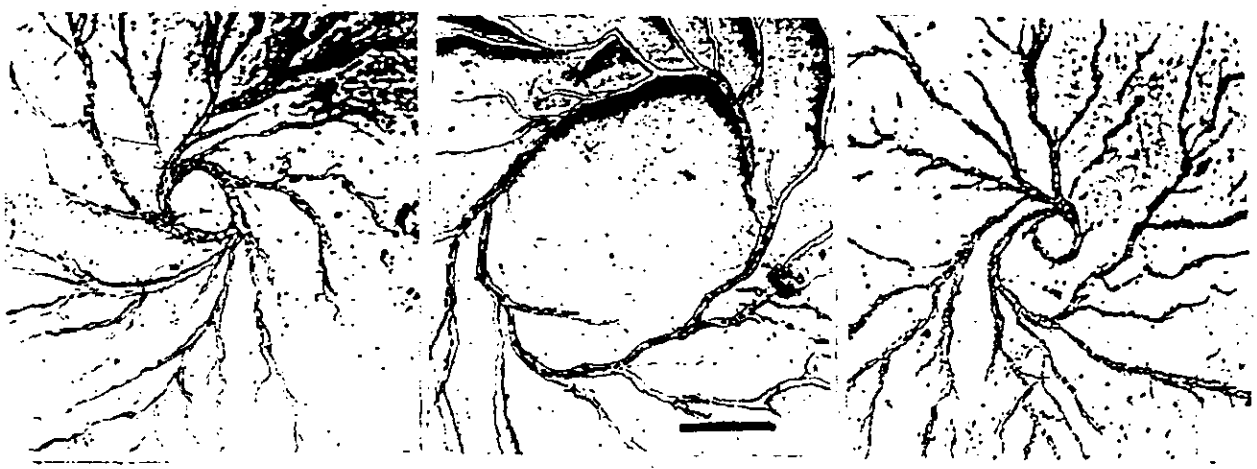

FIG. 1. View of aggregative structures formed by a starving population of *Dictyostelium discoideum* (Potapova *et al.*, 1986). Bar is 150 μm.

2. Model

The model is described by the following axioms:

(1) A number of individual cells are randomly spread out in a square. Square sizes are $N \times N$, cell localization is defined by real co-ordinates, and the number of cells is K. Each cell is presumed to have zero size.

(2) Each cell may be in one of three states: (i) resting, (ii) excited and (iii) refractory. Cells which are in the excited state during one time step go to the refractory state for a *Refr* number of time steps, and then to the resting state.

(3) One of these cells is excited either periodically or only once. All the resting cells around an excited one at a distance that does not exceed R become excited at the next time step.

(4) The new excited cells cover a distance $S < R$ toward the nearest exciting cell. If the distance between these cells is less than S, the moving cell stops at the position of the excited one.

Figure 2(a) demonstrates the propagation of excitation and cell motion in a model medium described by the rules given above. In our consideration an excited cell is a cell reacting to cAMP signal. It moves and releases a cAMP pulse. After this the cell becomes refractory and temporarily loses the ability to react to cAMP signals. In time, refractory cells regain this ability and achieve the resting state.

One can see that model time step is equal to the mean time of signal relay between cells, i.e. it equals 1 minute. The unit of length used in the model was given the arbitrary value of 1 micron. The parameters of the model were chosen so that it would be possible to compare the results obtained with those of natural experiments (Cohen & Robertson, 1971; Alcantara & Monk, 1974; Robertson & Grutsch, 1981). The model parameters were given the following values: $R = 60$ microns; $S = 10$–20 microns; the duration of the excited state of cells was 1 min; the period of the refractory state was 3 min; the value of the cell's density ($\rho = K/(N \times N)$) varied from $0{\cdot}3$–$1{\cdot}6 \times 10^5$ cells cm^{-2}.

3. Results

The evolution of a multicellular structure when the stimulated cell is in the center of the computing model region is presented in Fig. 3. The excitation waves propagate from the stimulated cell. The propagation of such waves leads to an appearance of branching cell streams, as occurs in natural populations. These streams disappear gradually during the development of the aggregative structure. In time the size of the structure decreases and, finally, it contracts to the point of the stimulated cell position. This happens because the cells in the model have no area and behave as points.

The processes leading to appearance of the aggregation structure are as follows. The excitation from the central cell passes through the cells, transferring the excitation. These excited cells lie on the knots of a broken line binding the central cell with any other. Figure 4 shows how each cell, once excited, joins with the excited cell for the first, second and third excitation waves in the medium shown in Fig. 3. The lines of the first excitation transfer determine the cells' trajectory during the propagation of succeeding waves of excitation. These lines are fixed by the following waves, and the structure becomes visible owing to the increase of cell density on these lines. The number of waves necessary to create the structure can be estimated by the formula $M = R/S$ giving the

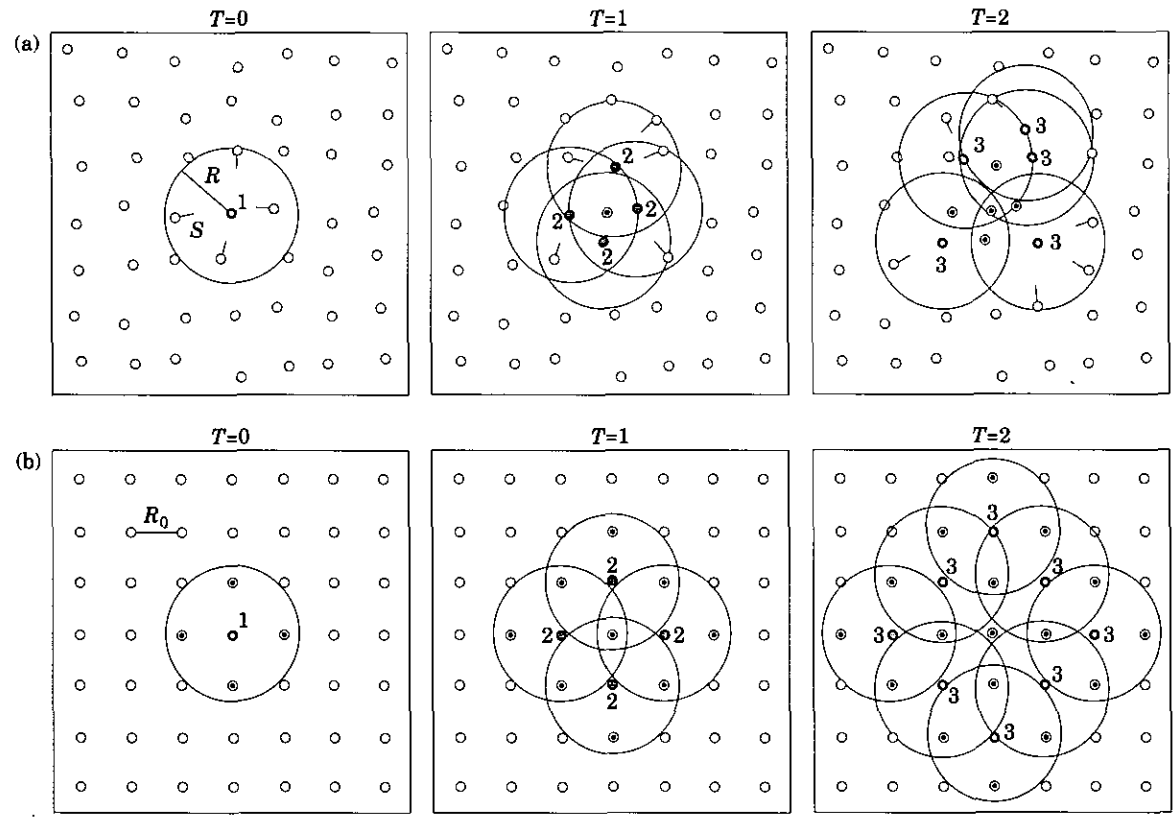

FIG. 2. (a) The transfer of excitation and cell motion in the model medium. A stimulated cell (1) excites all resting cells (2) in its neighboring R-region. Excited cells (2) move a distance S to the stimulated cell (1). In the next time step, cells (2) excite resting cells (3) and cells (3) move to the nearest of cells (2). As a result of such processes, the excitation wave is propagated and cells are displaced. (b) When cells spread out along the square mesh and do not move, our model acts in the same way as that of Wiener & Rosenblueth (1946). The circles depict the cells: (⊖) excited cells; (⊙) refractory; (○) rest. The model parameters are $R = 60$, $N = 320$, $K = 49$; $S = 16$ for (a) and 0 for (b).

maximal number of cell shifts before a cell flows into one of the streams.

The structure shown in Fig. 3 results from the external periodic stimulation of the medium. Under suitable conditions one stimulation of any cell can be enough to fire the process of aggregation. This happens when the propagation of a single wave leads to the appearance of self-sustaining sources of excitation. Two examples of such aggregation are shown in Fig. 5.

The appearance of circular sources can be described in the following general terms. The cell's displacement by the propagating excitation wave can change the excitability of any region of a medium and open new ways of excitation transfer. Both in Fig. 5(a) and (b) the wave propagating along the boundary of unexcitable region (with low cell density) makes a turn owing to an appearance of new ways of excitation translation.

The processes leading to the appearance of the source are shown in Fig. 6. This figure shows the translation of excitation in the region depicted by the frame in Fig. 5(a). The excitation cannot be transferred from cell 1 to cell 2 directly because the distance between these cells is larger then R and therefore several intermediate cells are required. Cell 2, once excited, can excite cell 1 because the distance between these cells, after the motion of cell 2, becomes less than R. Therefore, the loop of excitation rotation appears.

The main condition for the appearance of self-sustaining sources concerns the stability of the propagation of excitation waves. This stability is defined by the ratio d/R_0 where d is the mean shift of excitation for each time step, $d = R - S - R_0/2$, and R_0 is the average distance between cells, $R_0 = N/\sqrt{K}$. If $d/R_0 < 1$, the excitability of the medium is very low and the waves cannot propagate. On the other hand, if $d/R_0 > 2$, the wave of excitation is stable and propagates in all directions without breaks, which is essential for the appearance of self-sustaining sources. In the intermediate range $1 < d/R_0 < 2$ the propagating wave is sensitive to the cells' distribution and displacement so that it can make a loop of

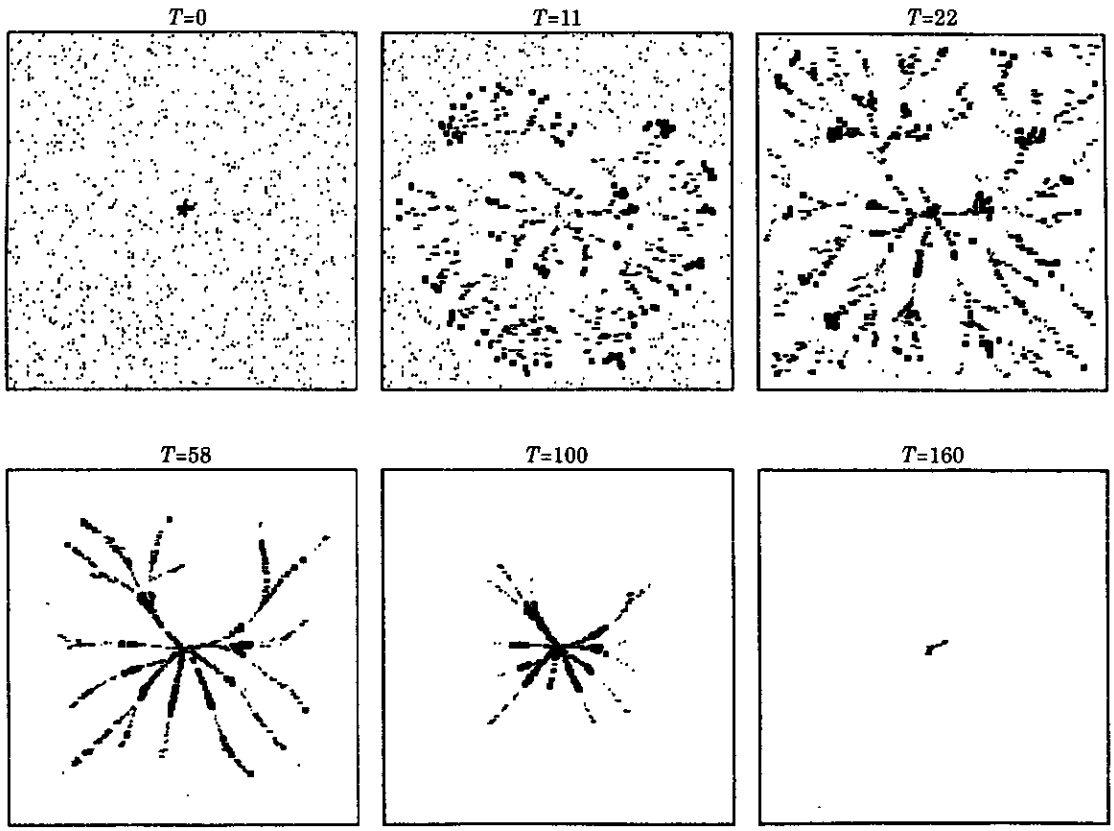

FIG. 3. Formation of the structure in the model field. The cell in the center of a field is stimulated periodically (depicted in the first frame, $T = 0$). The points of various intensity mark the cells in various states: points at peak intensity mark the excited cells, points of intermediate intensity depict the cells in the refractory state, and points of minimum intensity depict the resting cells. The model parameters are: $R = 60$, $Refr = 3$, $N = 800$, $K = 1089$, $S = 20$. The period of stimulation of the central cell is $T_s = 6$.

rotation. In fact, the occurrence of the source becomes random (it depends on the cell's initial displacement) and the noted range is a range of its higher probability.

The initial size of a source and its initial period of wave emission can vary over a wide range [compare Fig. 5(a) and (b)]. But, in time, both decrease. Finally, the size of the source becomes zero and the period of wave emission becomes equal to the minimal period of excitation of any cell, such that $T_{\min} = \tau^*(Refr/\tau + 2)$, where τ is the value of the time step.

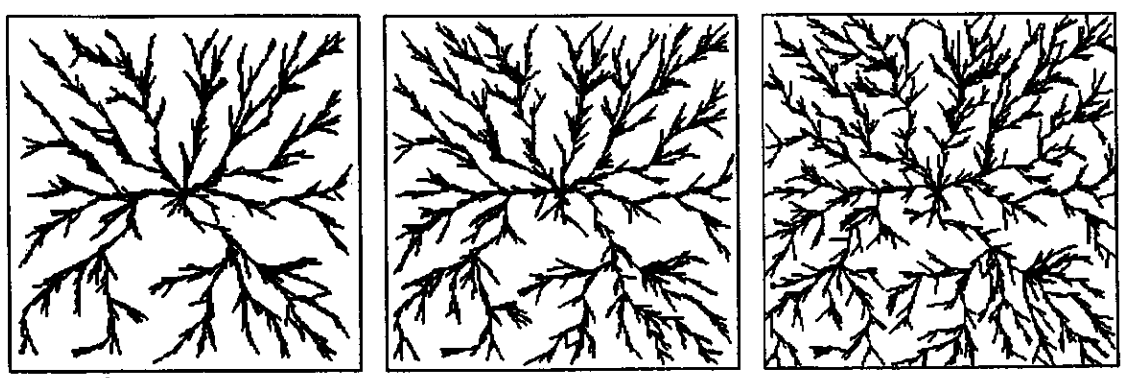

FIG. 4. The lines of the excitation transfer between cells. The lines for the three first waves of excitation, propagating through the model medium in Fig. 3, are presented. These lines are formed randomly for the first wave. However, they are fixed by successive waves and become visible owing to the collection of cells along these lines (compare the view of the structure at the time $T = 22$ (Fig. 3) with the picture formed by the lines for the third wave).

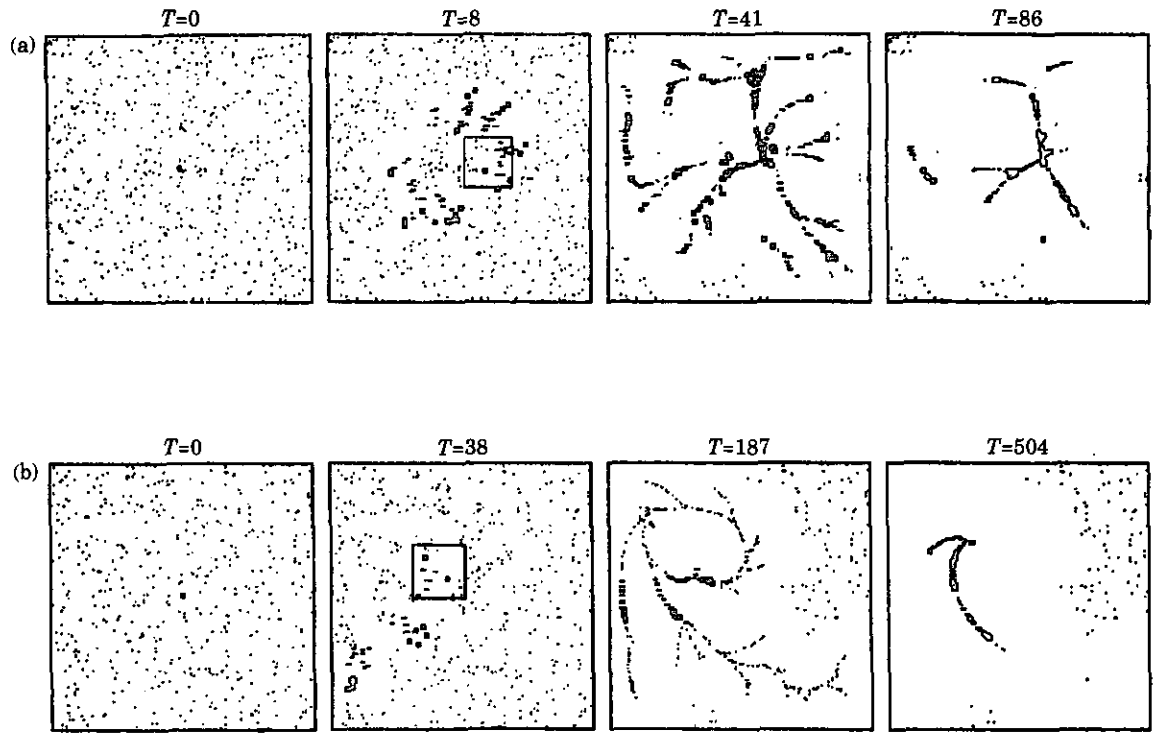

FIG. 5. Appearance and evolution of the sources of excitation. The places where sources appear are framed. Comparison of (a) and (b) shows that initial sizes of sources can vary over a wide range. In (a) four sources occur, and on (b) only one does. Note the effect of the cell density on the probability of source occurrence. The model parameters are $R = 60$, $Refr = 3$, $N = 800$, $S = 20$; $K = 60$ in (a) and 500 in (b).

4. Discussion

The results show that, from the rules of the proposed model, aggregation occurs and the features of this process mimic those of living cells (Durston, 1973; Alcantara & Monk, 1974; Loomis, 1975; Potapova *et al.*, 1988).

We do not consider the dynamic of cAMP release by cells or the cAMP diffusion processes (cf. MacKay, 1978). However, such calculations do not seem to be

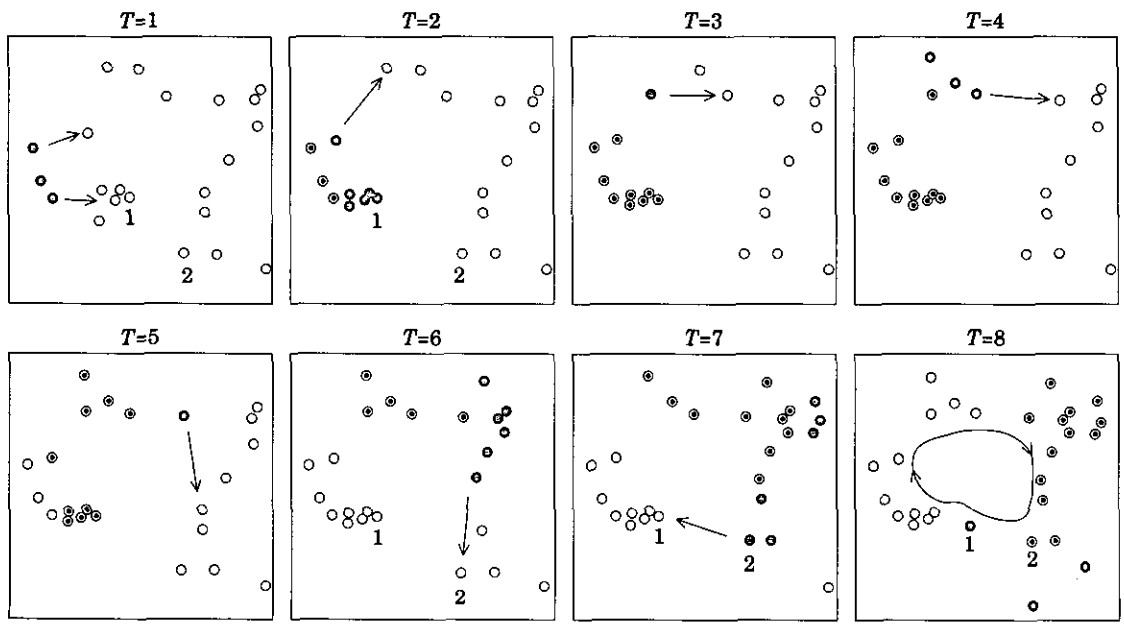

FIG. 6. Mechanisms of the appearance of self-sustaining sources of excitation. The occurrence of the source depicted by the frame in Fig. 5 is shown in detail. Excitation arrives at cell 1 after transmission from cell 2 and begins to circulate.

necessary for the investigation of the common features of aggregative structures. Models based on biochemical criteria are difficult because detailed conditions of cell excitation and movement are unknown, and it is thus necessary to use hypothetical assumptions (MacKay, 1978; Parnas & Segel, 1978). The absence of such data is one of the reasons why, in the present model, we use the axiomatic rules of excitation relay and cell motion. A second reason concerns the simplicity of the model that makes it more general and more useful as a prototype model of an excitable media formed by movable elements. Our model can be considered as a new modification of the axiomatic model proposed by Wiener & Rosenblueth (1946). If cells spread out along the square net and do not move ($S = 0$), our model becomes similar to this classic model [see Fig. 2(b)].

It should be noted that the present model can be easily modified to describe fully aggregation of *Dictyostelium discoideum*. Here we give two examples.

(i) All the interesting processes occur with excited cells. To consider these processes in more detail, we can use time steps smaller than the duration of the excited state, τ. In this way we can take into account the latent period of cAMP secretion, and duration of such secretion.

(ii) The direction of cell motion, defined by the fourth rule, is simple to calculate but not very exact. In real population, cells move in the direction of the cAMP gradient, but this direction does not always coincide with that to the nearest excited cell. It is possible to modify the fifth rule, defining exactly the average direction from one excited cell to all the excited cells, in proportion to the square of the distances between them.

(iii) The model predicts different types of aggregation dynamics owing to a time shift between the beginning of cell-directed movement and its secretion. It has shown to be an important parameter determining the common flexibility of structure during aggregation (O. O. Vasieva *et al.*, unpublished data). These data seem to correlate with recent experimental observations.

The model can be considered as a new cellular automaton model of an excitable medium (Wiener & Rosenblueth, 1946; Winfree *et al.*, 1985; Markus & Hess, 1990; Gerhard *et al.*, 1990). On account of the mobility of elements forming the medium, the following peculiarities arise:

(i) Excitability is determined by spatial characteristics. The local excitability in a field at each point is defined by the cell distribution in the neighborhood of that point and in time may be changed as a result of cell movement.

(ii) In this model system, besides ordinary waves of excitation, there are complicated spatial structures formed by moving cells.

(iii) The appearance of new types of self-sustaining sources of excitation is possible in the medium and mechanisms leading to this appearance differ from those already known (Krinsky, 1968).

The model is constructed to describe local processes of self-organization in aggregating populations of *Dictyostelium discoideum* amoebae. This model seems to be one of the simplest that can be constructed and functions without direct biological data. We hope that it may be helpful for modeling the dynamics of developmental and excitable tissues, membrane processes, spatial displacements in plants and animal societies and other systems with similar behavior.

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