

Self-organized Vortex State in Two-Dimensional *Dictyostelium* Dynamics

Wouter-Jan Rappel, Alastair Nicol, Armand Sarkissian, and Herbert Levine

Department of Physics, University of California, San Diego, La Jolla, California 92093-0319

William F. Loomis

Department of Biology, University of California, San Diego, La Jolla, California 92093

(Received 3 November 1998)

We present results of experiments on the dynamics of *Dictyostelium discoideum* in a novel setup which constrains cell motion to a plane. After aggregation, the amoebae collect into round “pancake” structures in which the cells rotate around the center of the pancake. To provide a mechanism for the self-organization of the *Dictyostelium* cells, we have developed a new model of the dynamics of self-propelled deformable objects. In this model, we show that cohesive energy between the cells, together with a coupling between the self-generated propulsive force and the cell’s configuration, produces a self-organized vortex state. The mechanism for self-organization reported here can possibly explain similar vortex states in other biological systems.

PACS numbers: 87.10.+e

Spontaneous organization of self-propelled particles can be found in a variety of systems. Examples include the flocking of birds [1], the movement of traffic [2] and pedestrians [3], and the collective motion of ants [4]. Not surprisingly, the physics of self-propelled particles has recently attracted considerable attention [5]. The systems have been theoretically analyzed using continuum, Navier-Stokes-like, equations and discrete numerical models. The numerical models have in common that the objects are treated as point particles. However, in a variety of systems the actual shape and plasticity of particles play an important role and hence they cannot be modeled in this oversimplified manner. In this Letter we present experimental data in one such system, *Dictyostelium discoideum* cells, and introduce a model that treats cells as deformable objects rather than point particles. Our results provide evidence for a localized vortex state in this biological system.

The developmental dynamics whereby *Dictyostelium discoideum* is transformed from a solitary amoeba state to a functional multicellular organism is of interest to both biologists and nonequilibrium physicists [6]. Past efforts have elucidated the nonlinear chemical wave signals used to guide aggregation [7–9] as well as the chemotactic instability [10] which causes a density collapse to one-dimensional “streams” of incoming cells. However, much less is known regarding subsequent events, especially with regard to organized cell motion in the later, multicellular stages of the day-long developmental process.

Dictyostelium cells are grown in liquid media and plated without nutrients onto a glass surface [11]. An additional thin layer of agarose is then overlaid on the cells. This has the effect of restricting cell motion to the plane; in fact, the multicellular states that form are at most a few monolayers deep. Cells aggregate normally and form round “pancake” structures. In Fig. 1a, we have presented a typical snapshot of the system. In each of the observed structures, the cells have organized their motion into a coordinated vortex

state in which they rotate around the center of the pancake. The rotation can be either clockwise or anticlockwise depending on initial conditions and can persist for tens of hours. Figure 1b shows a closeup of one structure, where now the cells have been illuminated by using a strain in which the gene for green fluorescent protein [12] has been fused to the CAR1 (cyclic AMP receptor) gene [13]; the expression of this gene leads to a membrane-localized fusion protein which causes the cell to be fluorescently outlined, as shown. This new protocol for *Dictyostelium* development allows one to track cell motions in much greater detail than has been possible to date.

It has been previously noted [14] that coherent rotational motion can often be seen in three dimensional *Dictyostelium* mounds, albeit as a short-lived transient prior to cell-type sorting and tip formation at the mound apex. This motion has been attributed [15] to cells moving chemotactically to rotating waves of cyclic AMP. To test this hypothesis, we have repeated our experiments with a nonsignaling strain of *Dictyostelium* [16]. Aside from the need for a higher initial density to overcome the inability of the system to support long-range aggregation, the system behaves in a similar manner and produces rotating vortex structures. Thus, guidance via cyclic adenosine 3′-5′ monophosphate (cAMP) waves is *not* a necessary ingredient for organized rotational motion. Instead, we suspect a self-organization of the system similar to that seen in molecular dynamics simulations of a confined set of particles undergoing dissipative collisions [17,18], albeit with deformable particles and without an artificially imposed box.

To verify that such a self-organized state is indeed possible, we turn to a new model of cell motion. Following Glazier and Graner [19,20], we assume that cells move via (roughly) volume-preserving fluctuations of their shape. These fluctuations are driven by an effective free energy [21] which incorporates specific biophysical mechanisms

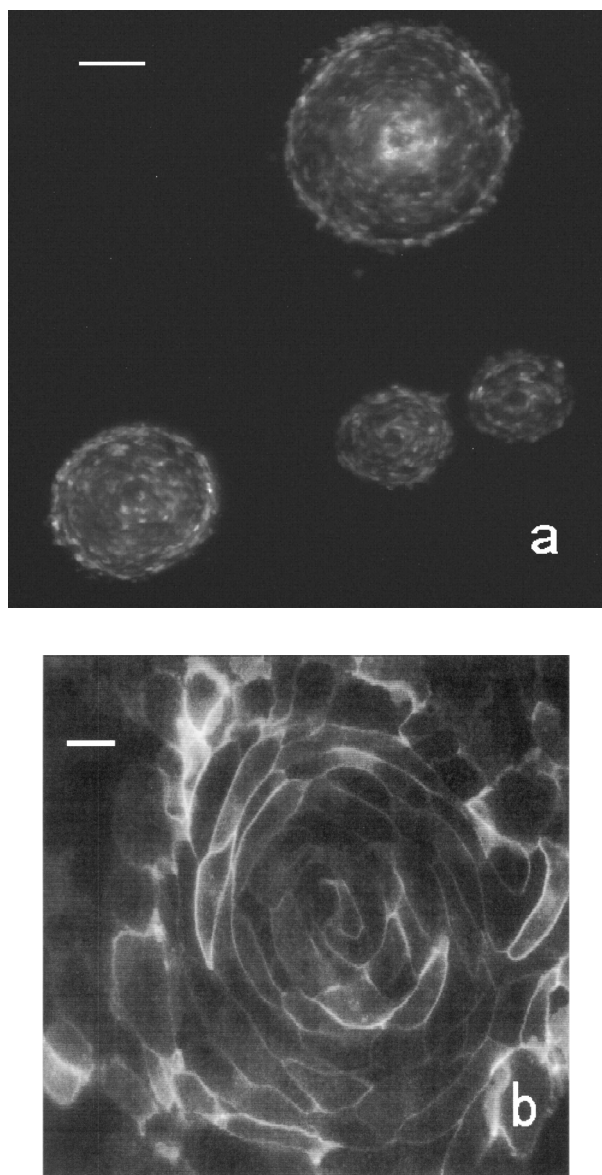


FIG. 1. Pictures of the vortex state in the experiments. In (a) the entire cell body is fluorescent while in (b) only the cell membrane is fluorescent. The bar represents $100\ \mu\text{m}$ in (a) and $10\ \mu\text{m}$ in (b).

appropriate to *Dictyostelium* cells. First, cells stick to each other and tend to move in a manner which maximizes cell-cell boundaries over cell-medium ones [22]. In addition, each cell contains an active cytoskeleton which can generate forces by cycles of front protrusions and back retractions [23]. This force appears in our model as a potential which results in cell movement in the direction of the propulsive force. The simulated motion of a single such particle clearly exhibits the type of deformable amoeboid motion familiar from observations of *Dictyostelium* cells.

In the aforementioned previous work on flocking and related systems, a key insight is that local interactions can cause local velocity correlations which then causes a

macroscopically ordered state to emerge. In our model, this suggests that one requires that a cell adjust its own propulsive force based on its interactions with its neighbors. We have investigated several mechanisms whereby this could be incorporated in our model. The most biologically plausible assumption is that the direction of the force is updated so as to better match the forces exerted by neighboring cells—essentially a minimal frustration hypothesis, which is in accord with what one observes directly in the videomicroscopy. The results that follow were derived for this specific assumption, but depend very weakly on any details beyond the basic correlation idea.

To investigate the possibility of coherent vortex motion we started simulations with 100 square cells stacked in a square surrounded by medium. The force direction of each cell was chosen at random. A snapshot of a typical final state is shown in Fig. 2 which shows the boundaries of the cells and the force direction as a line which starts at the center of mass (CM) of the cell and which points in the direction of the force. The cells form a roughly circular patch and are rotating around the center of the patch. The final state is typically reached after a transient of 100–1000 Monte Carlo steps (MCS) [21]. Depending on the initial conditions, the cells will rotate either clockwise or counterclockwise. Other than the sense of rotation and the duration of the transient phase, nothing depends on the starting state. Similar results were obtained for different numbers of cells. The existence of a localized rotating state is a robust consequence of the model, present as long as the cells are sufficiently cohesive ($J_{\text{cm}} > J_{\text{cc}}$). Hence, we have proven that simple models based directly on the observed cell motions can indeed account for the self-localized vortex.

To further test our model, we consider the angular velocity of cells as a function of radial location within the

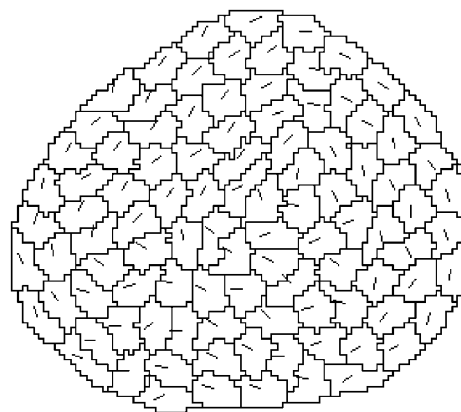


FIG. 2. Snapshot of a typical final state of the model. The solid lines within each cell start at the CM of the cell and point in the direction of the force. The parameter values for this and all other figures in this Letter are $N = 100$, $A_{\text{target}} = 100$, $\lambda = 10$, $J_{\text{cc}} = 5$, $J_{\text{cm}} = 15$, $C = 1.0$, and effective “temperature” $T = 5$. The lattice contains 200×200 sites and the force direction is updated every 2.5 MCS.

pancake. In the experiments, the angular velocity was obtained by direct tracking of cells. This tracking is greatly facilitated by the use of the cell-membrane outlining approach as shown in Fig. 1b. In detail, we have taken six separate sequences of 15 min each and measured the angular velocity every 8 s. During each sequence the radial location of the individual cells changed little. Next, we grouped the data in radial location intervals of $4 \mu\text{m}$ and calculated the average velocity and the standard deviation for each interval. The data are shown in Fig. 3 where the vertical bars represent 1 standard deviation. As expected, the data are noisier at small radii where small errors in the radius dramatically alter the angular speed. The overall time scale corresponding to a MCS was adjusted to provide the best fit of the model (solid line) to the data; this yields 1 MCS as 0.006 min. As a consistency check, we note that this gives an isolated cell velocity of $8 \mu\text{m}/\text{min}$, which is very close to the experimentally reported value of $10 \mu\text{m}/\text{min}$ [24]. Although there is some way to go for a fully quantitative theory, our simple model does surprisingly well in capturing both the tendency of the cell-cell interaction to speed up the motion and the tendency of cells to slip as they move past each other, thereby limiting the angular velocity at large radii. The decrease in angular velocity for small radii in Fig. 3b can be explained by the time scale of the update rule which prevents cells from moving in very small circles. The experiments seem to indicate a similar behavior.

What additional predictions does our model make? As the model proposes that cell-cell adhesion is the cause of the localized coherent state, mutant strains with reduced adhesion should not be able to form this structure. Also, mutants with weakened cytoskeletons would not be able to organize their motion. As chemical signaling is not necessary, disrupting the *external* cAMP concentration should

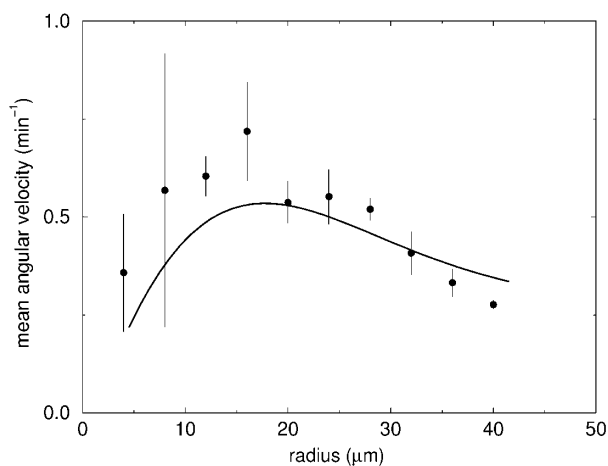


FIG. 3. The angular velocity as a function of the radial location measured in the experiments (solid circles) and calculated using the model (solid line). The overall time scale in the simulations was adjusted to provide the best fit of the model to the data.

have minimal effects. Finally, our model suggests that the time scale for organization should roughly be tens of minutes for aggregates with hundreds of cells; this is in qualitative agreement with our observations (data not shown).

Vortex structures have been seen in other microorganism systems, namely, the nutrient-limited spreading of a newly found bacterium [25], and in *Bacillus circulans* [26]. The motion of bacteria occurs through flagella and is thus fundamentally different from the amoeboid motion of *Dictyostelium*. However, it is tempting to speculate that in these cases as well velocity correlations induced by cell-cell interactions as well as cohesive forces may be enough to account for these structures [27]. The fact that these disparate systems exhibit such strikingly similar nonequilibrium structures offers comfort to the physicist proposing a simplified model for an inscrutably complex biological process.

In summary, we have documented the existence of a localized, coherent vortex state in *Dictyostelium*. Furthermore, we have argued both experimentally and via construction of a new model that the coherent motion is self-organized and not the result of a rotating chemical wave guiding the motion. The advantage of this deformable-cell model is that it allows for the direct comparison of simulation with observation. In the future, we plan to extend our calculations to the later stage processes of cell sorting and slug formation.

We thank A. Kuspa and P. Devreotes for providing some of the *Dictyostelium* strains used in the experiments. Also, one of us (H. L.) acknowledges useful conversations with E. Ben-Jacob. This work was supported in part by NSF DBI-95-12809.

- [1] B. L. Partridge, *Sci. Am.* **246**, No. 6, 114–123 (1982).
- [2] K. Nagel, *Phys. Rev. E* **53**, 4655 (1996).
- [3] D. Helbing, J. Keltsch, and P. Molnar, *Nature (London)* **387**, 47 (1997).
- [4] E. M. Rauch, M. M. Millonas, and D. R. Chialvo, *Phys. Lett. A* **207**, 185 (1995).
- [5] T. Vicsek *et al.*, *Phys. Rev. Lett.* **75**, 1226 (1995); J. Toner and Y. Tu, *Phys. Rev. Lett.* **75**, 4326 (1995); E. Albano, *Phys. Rev. Lett.* **77**, 2129 (1996); H. J. Bussemaker, A. Deutsch, and E. Geigant, *Phys. Rev. Lett.* **78**, 5018 (1997).
- [6] For a general introduction, see W. Loomis, *The Development of Dictyostelium discoideum* (Academic, New York, 1982); J. T. Bonner, *The Cellular Slime Molds* (Princeton University Press, Princeton, NJ, 1967).
- [7] P. N. Devreotes, *Science* **245**, 1054 (1989).
- [8] The easiest way of detecting the nonlinear waves is to detect the cell “cringe” response via dark field microscopy; see F. Alacantha and M. Monk, *J. Gen. Microbiol.* **85**, 321 (1975); P. C. Newell, in *Biology of the Chemotactic Response*, edited by J. M. Lackie (Cambridge University Press, Cambridge, 1981); K. J. Lee, E. C. Cox, and R. E. Goldstein, *Phys. Rev. Lett.* **76**, 1174 (1996).

- [9] Theoretical studies of the temporal evolution of the cAMP wave field include E. Palsson and E. Cox, Proc. Natl. Acad. Sci. U.S.A. **93**, 1151 (1996); H. Levine, I. Aranson, L. Tsimring, and T. V. Truong, Proc. Natl. Acad. Sci. U.S.A. **93**, 8803 (1996); J. Lauzeral, J. Halloy, and A. Goldbeter, Proc. Natl. Acad. Sci. U.S.A. **94**, 9153 (1997); M. Falcke and H. Levine, Phys. Rev. Lett. **80**, 3875 (1998).
- [10] H. Levine and W. N. Reynolds, Phys. Rev. Lett. **66**, 2400 (1991); B. N. Vasiev, P. Hogeweg, and A. V. Panfilov, Phys. Rev. Lett. **73**, 3173 (1994); T. Hofer, J. A. Sherratt, and P. K. Maini, Proc. R. Soc. London B **259**, 249 (1995); Physica (Amsterdam) **85D**, 425 (1995); T. Hofer and P. K. Maini, Phys. Rev. E **56**, 2074 (1997); J. C. Dallon and H. G. Othmer, Philos. Trans. R. Soc. London B **352**, 391 (1997).
- [11] More details of the protocol and the genetic constructs are given in A. Nicol, W.-J. Rappel, H. Levine, and W. Loomis (to be published).
- [12] W. Ward, in *Photochemical and Photobiological Reviews*, edited by K. Smith (Plenum, New York, 1979).
- [13] Z. Xiao, N. Zhang, D. B. Murphy, and P. N. Devreotes, J. Cell Biol. **139**, 365–374 (1997).
- [14] J. Rietdorf, F. Siegert, and C. J. Weijer, Dev. Biol. **177**, 427 (1996), and references therein.
- [15] F. Siegert and C. J. Weijer, Curr. Biol. **5**, 937 (1995); B. Vasiev, F. Siegert, and C. J. Weijer, J. Theor. Biol. **184**, 441 (1997).
- [16] We use the construct of B. Wang and A. Kuspa, Science **277**, 251 (1997) which bypasses the need for internal cAMP and thereby allows for development without the presence of adenylyl cyclase (ACA), the enzyme which manufactures cAMP.
- [17] Y. L. Duparcmeur, H. Herrmann, and J. P. Troadec, J. Phys. I (France) **5**, 1119 (1995).
- [18] J. Hemmingson, J. Phys. A **28**, 4245 (1995).
- [19] J. A. Glazier and F. Graner, Phys. Rev. E **47**, 2128 (1993).
- [20] For previous applications of the Glazier-Graner approach to *Dictyostelium*, see N. Savil and P. Hogeweg, J. Theor. Biol. **184**, 229 (1997); Y. Jiang, J. Glazier, and H. Levine, Biophys. J. **75**, 2615 (1998).
- [21] Each cell is treated as a finite number of sites on a two-dimensional square lattice with a spacing of $1 \mu\text{m}$; each site is given a Potts spin σ , the value of which specifies

to which cell the site belongs. Empty sites, belonging to the medium, are assigned $\sigma = 0$. Cell motion is then implemented via a Monte Carlo update of spins using a Metropolis algorithm (at temperature T) with the effective energy $\mathcal{H}_{\text{area}} + \mathcal{H}_{\text{coh}} + \mathcal{H}_{\text{prop}}$ where

$$\mathcal{H}_{\text{area}} = \lambda \sum_{\sigma} [A(\sigma) - A_{\text{target}}]^2$$

ensures that cells maintain a more-or-less constant area,

$$\mathcal{H}_{\text{coh}} = \sum_{\sigma, \sigma'} J_{\sigma\sigma'} (1 - \delta_{\sigma, \sigma'})$$

describes the cohesive energy ($J_{\sigma\sigma'} = J_{\text{cc}}$ for $\sigma\sigma' \neq 0$ and $J_{\sigma\sigma'} = J_{\text{cm}}$ for $\sigma = 0$ or $\sigma' = 0$), and

$$\mathcal{H}_{\text{prop}} = -C \sum_{\sigma} U(\sigma)$$

models the propulsive force \mathbf{F} . Here, C is a force constant which determines the strength of the propulsive force and $U(\sigma)$ is the potential of cell σ that has its origin at the center of mass (CM) of the cell and that is linearly decreasing in the direction of the force

$$U_{\sigma} = \sum \hat{\mathbf{F}} \cdot \mathbf{r},$$

where the sum is over all sites of the cell and where \mathbf{r} is the vector pointing from the CM to the site. We define one Monte Carlo step (MCS) to be as many trial steps as there are lattice points. Further details of the basics of the model can be found in Ref. [19].

- [22] S. Bozzaro and E. Ponte, *Experientia* **51**, 1175 (1995).
- [23] T. P. Stossel, *Am. Sci.* **78**, 408 (1990).
- [24] R. Escalante *et al.*, *Mol. Biol. Cell* **8**, 1763 (1997).
- [25] E. Ben-Jacob, A. Tenenbaum, O. Shochet, and O. Avidan, *Physica (Amsterdam)* **202A**, 1 (1994).
- [26] R. N. Smith and F. E. Clark, *J. Bacteriol.* **35**, 59 (1938).
- [27] Ben-Jacob and collaborators have proposed in [28] that a “rotational chemotaxis” term, arising via a correlation between the concentration of some chemical and the speed of the individual cell, can account for self-localization. At least in *Dictyostelium*, there is no apparent need for such a mechanism.
- [28] E. Ben-Jacob, I. Cohen, A. Czirók, and T. Vicsek, *Physica (Amsterdam)* **238A**, 181 (1997).