Competing Patterns of Signaling Activity in *Dictyostelium Discoideum*

Kyoung J. Lee,[1] Edward C. Cox,[2] and Raymond E. Goldstein[1]

[1]*Department of Physics, Joseph Henry Laboratories, Princeton University, Princeton, New Jersey 08544*
[2]*Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544*

Quantitative experiments are described on spatiotemporal patterns of coherent chemical signaling activity in populations of *Dictyostelium discoideum* amoebae. We observe competition between spontaneously firing centers and rotating spiral waves that depends strongly on the overall cell density. At low densities, no complete spirals appear and chemotactic aggregation is driven by periodic concentric waves, whereas at high densities the firing centers seen at early times nucleate and are apparently entrained by spiral waves whose cores ultimately serve as aggregation centers. Possible mechanisms for these observations are discussed.

PACS numbers: 87.22.–q, 47.54.+r, 82.40.Ck, 87.10.+e

In a variety of contexts in the biological world we find coherent spatiotemporal patterns of propagating chemical waves [1]. Often, as in cardiac tissue, these waves are triggered and globally controlled by specialized cells termed "pacemakers" [2]. In other systems, traveling chemical waves may arise by a process of self-organization of undifferentiated cells. A well-known biological example is provided by populations of the slime mold *Dictyostelium discoideum* [3], which upon nutrient deprivation sustain waves of cyclic adenosine $3',5'$-monophosphate (cAMP) that drive chemotactic migration of cells. These waves result from a relay mechanism within each cell, and travel by means of the diffusive coupling of nearby cells through the substrate. Despite a large body of experimental and theoretical work on the coupled dynamics of wave propagation and associated chemotaxis leading to cell aggregation [3,4], the means by which a particular pattern of coherent traveling waves emerges from the nonsignaling state has remained

unclear. Many studies have revealed the presence of concentric waves (or "target" patterns) emanating from periodically firing pacemakers [3,5], while others have focused on the rotating spiral waves that also occur [6,7], and are qualitatively similar to those seen in chemical systems such as the Belousov-Zhabotinski (BZ) reaction [8,9]. The distinction between these two types of patterns is significant; targets require an autonomous pacemaker, while rotating spirals do not. Yet, there has been no clear experimental determination of the factors controlling which of these two signaling patterns dominates.

We report here a quantitative experimental study of the competition between autonomous firing centers and rotating spiral waves in *D. discoideum*. Using cell population density as a control parameter, we find that when the cell density is high spiral waves dominate at late times, whereas at low cell density the asymptotic pattern is dominated by circular waves emanating from pacemakers, as shown in Fig. 1. In addition, the spiral
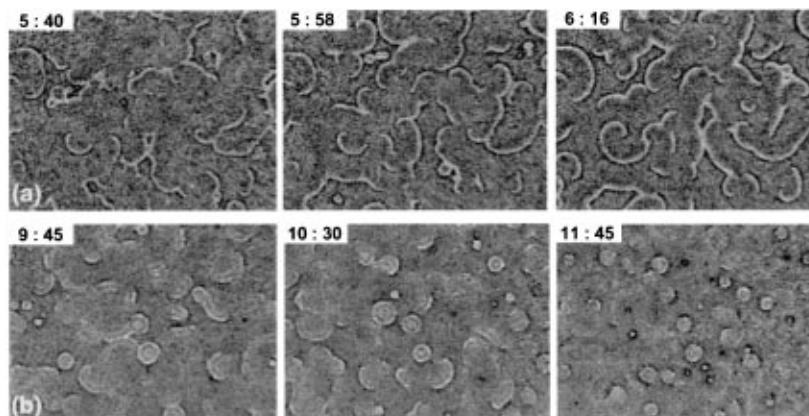

FIG. 1. Dark field images showing the evolution of two kinds of signaling activity. In (a) spirals dominate circular waves at high density ($\rho_H = 21.8 \times 10^5$ cells/cm^2), whereas in (b) circular waves dominate ($\rho_L = 7.3 \times 10^5$ cells/cm^2). Time is indicated in hours and minutes elapsed from the point of food deprivation. Each 24 mm $\times$ 18 mm image was obtained by subtracting two successive images taken 30 sec. apart and rescaling the resulting image over 256 grey levels to enhance the contrast. The bright bands and the accompanying dark shadows indicate the cells in an active state, while the grey background corresponds to inactive cells.

waves themselves originate in the instabilities of finite wave segments [10] and apparently entrain the firing centers. In a low density system, the firing centers also entrain each other. In both cases, the dynamical evolution proceeds from random firings to *periodic* events. The density-dependent competition between signaling patterns is similar to that seen recently in the BZ reaction in the presence of a catalyst imprinted on a lattice of varying density [11]. The appearance of spirals only in a limited range of densities suggests that variations in the diffusive coupling of excitable elements lead to pattern selection. Our results also relate to recent theoretical studies of mechanisms for spiral symmetry breaking [12], specific biochemical origins for pattern evolution [13], and feedback mechanisms [14], as well as the role of stochasticity in pattern selection [15].

First, we briefly review the essential features of the signaling mechanism. Signaling begins with the synthesis of the messenger molecule cAMP from ATP by adenylate cyclase within individual cells [16]. It is also degraded to $5'$-AMP by phosphodiesterase. Both cAMP and phosphodiesterase are excreted through the cell membrane wall. The production of cAMP within individual cells is stimulated and controlled by the state of cAMP receptors in the cell membrane: When the receptors are fully saturated with cAMP, the synthesis of internal cAMP stops. It is now well documented in both experiments and model studies [7] that these chemical reactions can produce rotating spiral waves and circular waves of collective chemical activity in spatially extended systems like those shown in Fig. 1.

In our experiments, cells were grown using standard culturing techniques [17]. This preparation yields a monolayer of cells spread on the surface of an agar layer in a dish. Signaling is generally initiated within several hours after nutrient deprivation, during which time the cells were kept in the dark. Patterns of signaling activity were monitored using a dark field optical setup [18], sensitive to changes in optical properties of the cells in response to chemical waves [19].

Figure 1 shows the evolution of spatiotemporal patterns observed in experiments with two different initial cell densities. In both cases, the spontaneous firing of many cells is observed in the form of spreading circular wave fronts. Spiral waves can form from broken wave segments that naturally arise from inhomogeneities in the medium. When the initial cell density ρ is high (ρ_H), as in Fig. 1(a), competition between circular waves and spirals is quite apparent—spirals eventually suppress all firing events.

A dramatically different situation arises when the initial cell density is low (ρ_L), shown in Fig. 1(b). As in the system with ρ_H, many firing centers appear at an early stage (but with a longer induction period), and, at the same time, wave segments also form from inhomogeneities in the medium. In contrast to high density populations, however, the broken ends of the

waves do not evolve to full spirals, and firings continue to the later cell aggregation phase.

We have located all the firing events occurring during the signaling phase of development. Focusing first on the case of high cell density, Fig. 2(a) shows the positions of firing centers along with those of the spiral cores that ultimately form. We find that most centers fire only once. Given our spatial resolution, the size of the cells (~ 8 μm), and the scale of their random motion, it is not possible for us to determine whether those most closely spaced clusters of data points arise from distinct cells firing independently, or instead from identical cells which have simply moved during the course of observation. Since spirals evolve from the ends of broken waves, the locations of the spiral cores are not correlated with the centers.

A quantification of firing-center and spiral competition is the time evolution of the number of firing events. Figure 3(a) shows this for the same ρ_H as Fig. 2(a). The number N of firing events initially increases in time, reaches a maximum value, then ultimately vanishes as the spirals become more fully formed. Let us now contrast this behavior with our observations at low densities. First, as shown in Fig. 2(b), there are overwhelmingly many more firing centers at ρ_L than ρ_H [compare with Fig. 2(a)]. With the smaller average distance between centers, the ability of broken wave segments to survive as spirals is greatly diminished.

At ρ_L, no complete spirals form, and firing centers survive until the later aggregation stage (at ≈ 11 h), as shown in Fig. 3(b). Figure 3 illustrates the fact that the fractional subpopulation of firing centers (N/ρ) *decreases* with density. This suggests that the formation of a center is not simply a characteristic of a subpopulation of cells, but rather is determined in part by cell-cell interactions [20]. Likewise, the lack of correlation between the positions of the centers and the spiral cores suggests that the latter are determined primarily by the positions
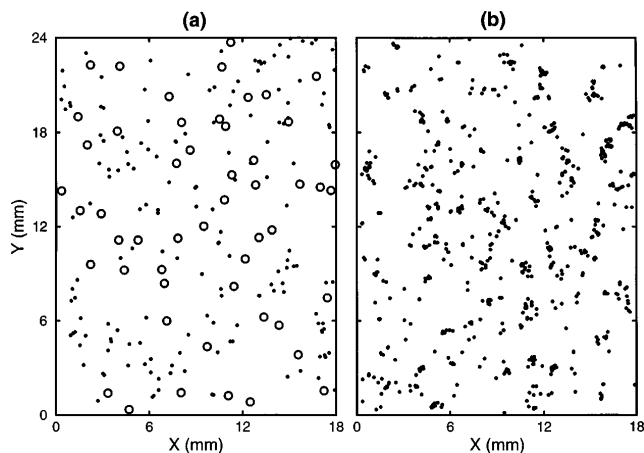
FIG. 2. Composite diagrams showing the location of firing centers (filled circles) and spiral cores (empty circles): (a) $\rho = \rho_H$; (b) $\rho = \rho_L$.
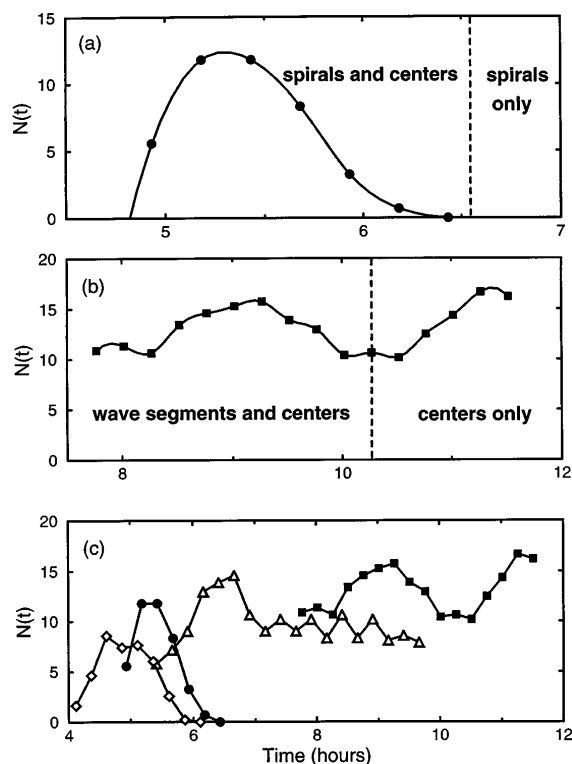
FIG. 3. Dynamical evolution of the number of firing centers per unit time (min) and unit area (cm^2): (a) $\rho = \rho_H$; (b) $\rho = \rho_L$; (c) composite of (a) and (b) and $1.5\rho_L$ (triangles), and $3.5\rho_L$ (diamonds). The plots were obtained by counting the number of firing centers in 15 min intervals.

FIG. 4. Enlarged images showing suppression of a low-frequency center by a higher-frequency spiral wave at ρ_H.

and dynamics of wave segments, rather than intrinsic properties of the cells at the core.

Systems with several different ρ in addition to the two already discussed have also been studied. In a system with $\rho = 1.5\rho_L$, similar entrainment dynamics appear between spirals and centers and between different centers. In this case, N initially increases, reaches a maximum, decreases gradually as before, but to a nonzero value, as shown in Fig. 3(c). In systems with higher values of ρ (toward and beyond ρ_H), spirals gradually extinguish firing events with the same entrainment dynamics as ρ_H.

At all densities, a peak in the number of firing centers occurs at intermediate times. (The gradual increase in N during the period 10–12 hours at ρ_L is due to the increase in the firing *frequency* of the surviving centers.) In the high-density systems, the decrease in N beyond the peak appears to be due to a suppression of firing centers by spiral waves. Some direct evidence for this is presented in Fig. 4. Over the course of three periods of firing activity, a pair of nearby spiral waves repeatedly passes through a center (indicated by an arrow). Because the firing frequency of the center is slower than the rotation frequency of spiral waves [21], the collision zone at which waves annihilate gradually moves toward the center (first three frames in Fig. 4), and ultimately the firing center is suppressed (last frame in Fig. 4). This observed behavior can
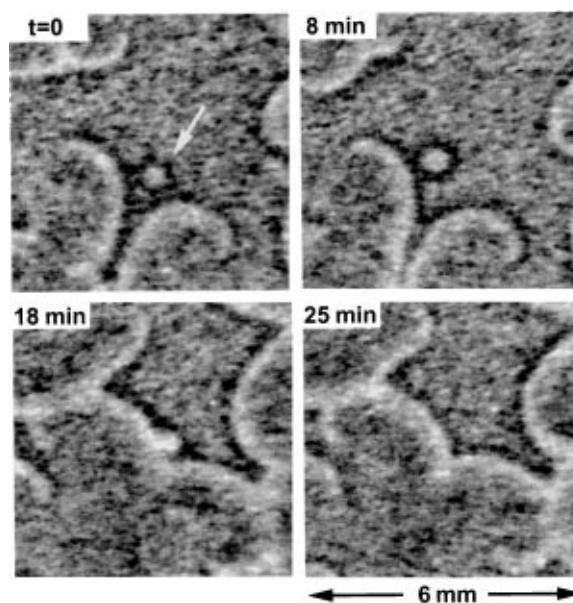
be viewed as an *entrainment* of slow centers by faster periodic spiral waves. A corollary to this observation is that since the spiral waves all have very similar rotation frequencies they are not effective in suppressing one another. However, we have observed some examples of the breaking of symmetry between members of a spiral pair, leading to dominance of one over the other (as seen left of center in the first frame of Fig. 1). This is consistent with theoretical arguments based on the existence of certain ultraslow chemical dynamics [12], or from chemical inhomogeneities in the medium [13]. Similar observations on the suppression of slow autonomous centers by spirals were reported without quantification in earlier experiments on the Belousov-Zhabotinskii reaction in a Petri dish [22] and in cellular automaton models [23]. The decrease in N in the low-density systems appears to arise from the competition *among* firing centers, rather than from the influence of incomplete spirals (wave segments). For instance, a center that fires with a higher frequency may suppress a slowly firing neighbor. Taken together, these earlier observations and the present ones suggest that this entrainment phenomenon is a generic feature of excitable media.

Among the possible mechanisms for firing center suppression, several appear quite likely on the basis of known experimental and/or theoretical observations. First, from the general phenomenology of excitable media, we may expect the passage of cAMP waves past a center to act as a kind of phase resetting event, leading to synchronization. This would be a *local* version of oscillator entrainment, mediated by the passage of waves, complementary to that studied recently in systems with *global* coupling [24,25]. It may also be an example of "spatiotemporal stochastic resonance" [15], in which a wave of excitation entrains noisy excitable elements (e.g., the firing centers). Second,

since the thickness of the agar substrate may play a role in the diffusive coupling of neighboring cells [3], gradual changes in the local chemical environment of a cell by the repeated passage of waves may alter its excitability. Third, gradual changes in the internal properties of the cells (e.g., density of membrane receptors, etc.) during the signaling stage may also play a role [12,13].

Future experiments to elucidate the mechanism of selection of signaling activity will focus on controlled perturbations of the system. One avenue of interest involves external stimulation through changes in background levels of chemicals such as cAMP in the agar substrate (both static and periodic in time). A second, complementary to our use of cell density, involves the use of different wild-type and mutant strains with variations in biochemical properties associated with signaling [26]. Experiments along these lines are currently in progress. Finally, the interplay between chemotaxis and pattern selection is an important area for further experimental and theoretical study, perhaps along the lines of recent work on bacterial systems [27].

[1] *Oscillations and Morphogenesis*, edited by L. Rensing (Marcel Dekker, New York, 1993).

[2] *Theory of Heart*, edited by L. Glass, P. Hunter, and A. McCulloch (Springer-Verlag, New York, 1991).

[3] J. J. Bonner, *The Cellular Slime Molds* (Princeton Univ. Press, Princeton, NJ, 1967); W. F. Loomis, *Dictyostelium Discoideum, A Developmental System* (Academic Press, New York, 1975).

[4] H. Levine and W. Reynolds, Phys. Rev. Lett. **66**, 2400 (1991); D. A. Kessler and H. Levine, Phys. Rev. E **48**, 4801 (1993); B. N. Vasiev, P. Hogeweg, and A. V. Panfilov, Phys. Rev. Lett. **73**, 3173 (1994).

[5] A. J. Durston, Dev. Biol. **37**, 225 (1974); **38**, 308 (1974).

[6] F. Siegert and C. J. Weijer, Physica (Amsterdam) **49D**, 224 (1991), and references therein.

[7] J. J. Tyson and J. D. Murray, Development **106**, 421 (1989).

[8] H. L. Swinney and V. I. Krinsky, *Waves and Patterns in Chemical and Biological Media* (MIT/North-Holland, Cambridge, MA, 1992).

[9] E. Meron, Phys. Rep. **218**, 1 (1992).

[10] A. T. Winfree, Science **175**, 634 (1972); A. J. Durston, J. Theor. Biol. **42**, 483 (1973).

[11] O. Steinbock, P. Kettunen, and K. Showalter, Science **269**, 1857 (1995), and references therein.

[12] I. Aranson, H. Levine, and L. Tsimring, Phys. Rev. Lett. **76**, 1170 (1996).

[13] E. Palsson and E. C. Cox, Proc. Natl. Acad. Sci. U.S.A. (to be published).

[14] I. Aranson, H. Levine, and L. Tsimring, report, 1995 (to be published).

[15] P. Jung and G. Mayer-Kress, Chaos **5**, 458 (1995); Phys. Rev. Lett. **74**, 2130 (1995).

[16] A. Goldbeter, Nature (London) **253**, 540 (1975); J. Martiel and A. Goldbeter, Biophys. J. **52**, 807 (1987).

[17] *D. discoideum* strain AX-2 was grown on nutrient agar with *E. coli B/r* [A. J. Warren, W. D. Warren, and E. C. Cox, Genetics **83**, 27 (1976)]. Cells were harvested by centrifugation (1,600 RPM, 5 min) twice and resuspended at the desired density in PB buffer (8.4 mM KH_2PO_4, 8.4 mM $Na_2HPO_4 \cdot 7H_2O$, Baker), pH 7.0. A 5 ml portion of this solution was transferred onto an optically transparent nonnutrient agar bed (thickness, 1 mm; diameter, 87 mm; volume content, 1%; Bacto agar, Difco). The supernatant was removed after 5 min.

[18] See, e.g., J. D. Gross, M. J. Peacey, and D. J. Trevan, J. Cell. Sci. **22**, 645 (1976). Images were acquired with a CCD camera (Cohu, 4910 series) equipped with a zoom objective (Titan Tool Supply, TZOVA). Using an electronically-shuttered light source, ten successive images (480×640 pixels, 256 grey levels, 1/30 sec. interval) were acquired every 30 sec., digitized (Bit-Flow, Raptor board), averaged in real time, and further processed for image enhancement.

[19] The low concentrations ($10^{-6} - 10^{-9}$ M) [as determined by fluorographic means; K. J. Tomchik and P. N. Devreotes, Science **212**, 443 (1981)], and thin optical path length preclude imaging cAMP by light absorption. Previous studies have shown that the cAMP waves map to the bands seen in dark field images, except for small shifts in amplitude and phase.

[20] P. M. Glazer and P. C. Newell, J. Gen. Microbiol. **125**, 221 (1981).

[21] J. D. Gross, M. J. Peacey, and D. J. Trevan, J. Cell. Sci. **22**, 645 (1976).

[22] G. R. Ivanitskii, V. I. Krinsky, A. N. Zaikin, and A. M. Zhabotinskii, Sov. Sci. Rev. **2D**, 280 (1980).

[23] A. S. Mikhalov and A. Engel, Phys. Lett. A **117**, 257 (1986); A. S. Mikhailov, *Foundations of Synergetics I* (Springer-Verlag, New York, 1994).

[24] J. Buck, Q. Rev. Biol. **63**, 265 (1988); G. B. Ermentrout, J. Math. Biol. **22**, 1 (1985); *ibid.* **29**, 571 (1991); S. M. Strogatz and R. Mirollo, SIAM J. Appl. Math. **50**, 1645 (1990).

[25] S. H. Strogatz and R. E. Mirollo, Phys. Rev. E **47**, 220 (1993).

[26] L. Wu, J. Franke, R. L. Blanton, G. J. Podgorski, and R. H. Kessin, Dev. Bio. **167**, 1 (1995).

[27] E. O. Budrene and H. C. Berg, Nature (London) **376**, 49 (1995); L. Tsimring *et al.*, Phys. Rev. Lett. **75**, 1859 (1995).