# On the origin of spiral waves in aggregating *Dictyostelium*.

Eirikur Palsson
*Departments of Molecular Biology and Applied and Computational Mathematics Princeton University, NJ, USA epalsson@math.utah.edu*
Edward C. Cox
*Department of Molecular Biology Princeton University, Princeton, NJ,USA ecox@molbiol.princeton.edu*

February 17, 1997

**Introduction.**

Shortly after the onset of starvation, *Dictyostelium* cells begin to signal to each other with cAMP, marking the beginning of the aggregation stage. As is well known, cells respond to the initial stimulus by first secreting a pulse of cAMP, which gives rise to waves of cAMP propagating outwards from the source, and by moving towards the origin of the cAMP signal. The movement of the cells in response to cAMP waves can be visualized under dark-field optics, and one can easily see bands of moving and stationary cells. The bands of moving cells correspond to high cAMP levels (1). The usual patterns observed are spirals, double spirals and target patterns. The initial conditions, such as cell density, and the strain, influence which patterns will dominate (Fig. 1).

Aggregating *Dictyostelium* cells have all of the properties of an excitable medium: When cells are perturbed above a certain level, they respond by sending out discrete pulses of cAMP; these pulses can be relayed from one cell to another; after perturbation, the original cell enters a refractory period; and in two dimensions the diffusing and relayed pulses spread as circular and spiral wave forms. Excitable systems have been widely studied, and a great deal is known about their essential features. However, why (and how) one wave form is chosen over another in a 2D system is not well understood.

newell.ps

kyoung2.ps

Figure 1: Patterns seen during *Dictyostelium* aggregation. The bright bands correspond to moving cells. Left field of view $60 \times 40$ mm$^2$. Taken from (2). Right field of view $24 \times 18$ mm$^2$, density $2 \times 10^6$ cells per cm$^2$. Courtesy Kyoung Lee.

We have recently shown, both in theory (3) and experimentally (4), that it is possible to select spiral over circular wave forms in aggregating *Dictyostelium* by triggering the cAMP relay system with pulses of phosphodiesterase inhibitor (PDI), the 26 kD protein (5) that inhibits external 3', 5' cAMP phosphodiesterase. The PDI pulses are distributed randomly, both in space and time, and supply the "noise" that initiates the system by driving it from an excitable, meta-stable state, to one in which temporary or permanent oscillating relay centers form.

In this essay we discuss some of the evidence that led us to this view by focusing on how spiral waves might form. This will involve a discussion of the literature, with particular emphasis on how waves propagate in excitable media, why spirals tend to dominate circles once formed, and, particularly, ways in which spirals do not form. Much of what we have to say may be found in a scattered literature that we here bring together for the interested biological reader.

**Introduction to waves in excitable media.**

As we have noted, the response of the it Dictyostelium signaling system to a cAMP stimulus above a threshold is a substantial amplification of the stimulus, after which the system relaxes back to the resting state. This behavior is characteristic of excitable media (6).

The reasons for pulse initiation and relay, followed by a refractory period, are in detail complex. However, the essential features may be summarized by the model outlined below, after the pioneering work of Martiel and Goldbeter (7).

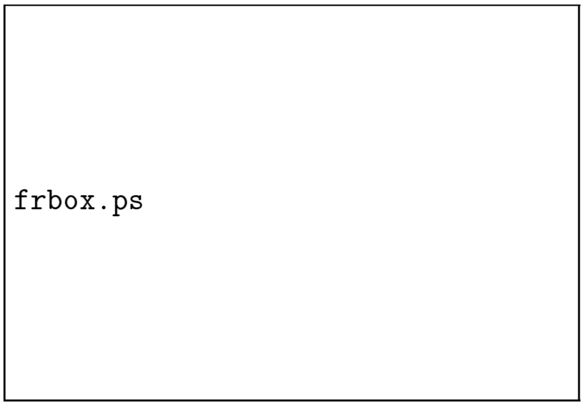


Figure 2: The Receptor Box. R = Active and unphosphorylated cAMP receptor. D = Inactive and phosphorylated cAMP receptor. RP and DP indicate receptor bound to cAMP. P = external cAMP.

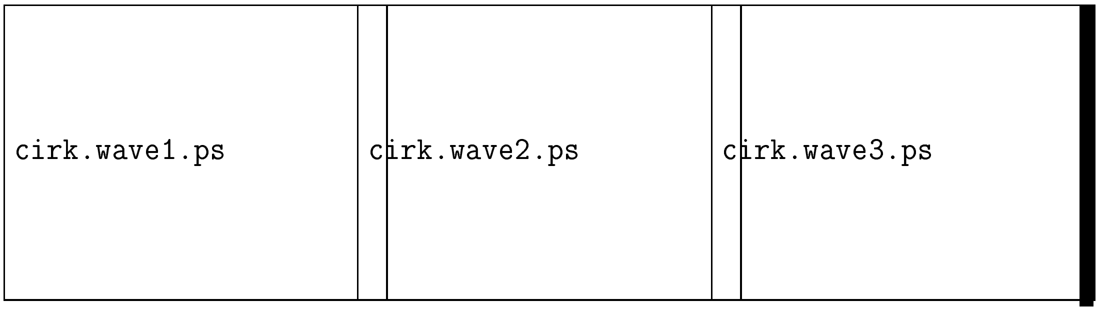


Figure 3: The initiation of a circular wave. Parameters are as given in (7) and we include spatial variations so cAMP diffuses. Diffusion constant, D=$4 \times 10^{-6}$cm$^2$sec$^{-1}$. A cAMP pulse is initiated in the center. The time between each frame is 10 min. $dx = 0.1$mm, $dt = 0.04$ min. Each grid unit is $dx \times dx$ and the grid is $100 \times 100$.

The core of their model is a so-called receptor box (Fig. 2). The receptor box relies on the fact that the cAMP receptor exists in two different states, an active [R] form and an inactive [D] form that can not activate adenylyl cyclase. The receptor is predominantly in the [R] state at low cAMP levels, but at high cAMP levels it is predominantly in the bound, inactive state [DP]. We use this model because it reproduces the essential properties of the signaling system, such as excitability, oscillation, and adaptation, even though the kinetic expressions of the Martiel and Goldbeter scheme do not correspond exactly to the complex biochemistry of the signaling system, still not completely understood (8, 9).

When the properties of such an excitable system are coupled by diffusion on a surface, circular (Fig. 3) and spiral waves form (10, 3).

It is often thought that spiral waves arise from colliding circular waves. However, as Fig. 4 shows, colliding waves annihilate each other. This is easily understood when one realizes that the cells on the back side of two waves about to collide are in a refractory phase, and thus the waves extinguish one another.

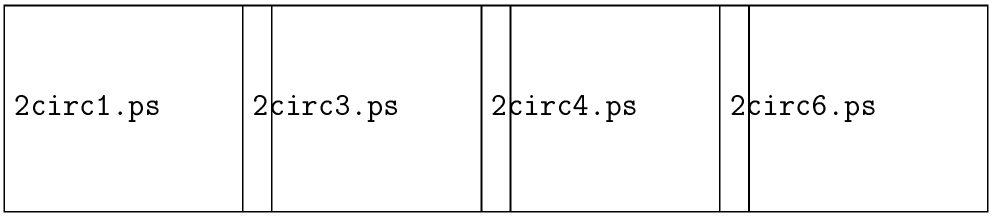


Figure 4: Two colliding circular waves. Parameters as before. The time between each frame is 13 min. $dx = 0.1$mm, $dt = 0.04$ min, $100 \times 100$ grid.

The reasons for this, and therefore the reasons for looking elsewhere for spiral wave genesis, become clear when we examine in greater detail the theoretical properties of waves in excitable media.

**Some theoretical background on waves in excitable media.**

Some analytical explorations have been done on propagating waves in excitable systems (11, 12, 13). Tyson and Keener (6) applied singular perturbation analysis to a pair of general reaction-diffusion equations of the form:

$$\varepsilon \frac{\partial u}{\partial t} = \varepsilon^2 \nabla^2 + f(u, v) \tag{1}$$

$$\frac{\partial v}{\partial t} = \varepsilon \delta \nabla^2 + g(u, v) \tag{2}$$

where $\varepsilon << 1$ is a small positive parameter, and $\delta = D_v/D_u$ is the ratio of the two diffusion coefficients of the 2 components. The smallness of $\varepsilon$ allows us to assume that changes in $u$ occur on a much faster time scale than changes in $v$. The functions $f(u,v)$ and $g(u,v)$ describe the nonlinear kinetics of the system. $u$ and $v$ are often referred to respectively as the propagator and the controller species, since the autocatalytic production of $u$ and its diffusion are responsible for the wave fronts, while the level of $v$ in the wave controls the speed. For our system of equations, $\delta = 0$, since we are assuming the cells are stationary, and $u$ and $v$ would roughly correspond to external [cAMP], and the number of active receptors, respectively.

For equations 1, 2 Tyson and Keener (1988) showed that the speed $c$ of a single planar wave is given by :

$$c \approx c(v_0)\sqrt{kD} \tag{3}$$

where $c(v_0)$ is a dimensionless quantity $\mathcal{O}(1)$, $D$ is the diffusion coefficient of the propagator species, and $k$ is a first order rate constant for the autocatalytic production of the propagator in response to supra-threshold stimuli.

For periodic wave trains the dispersion relation for the speed $c$ of the wave *vs.* the period $T$ can be determined (6)

$$c = \sigma(T), \;\; \lambda = cT, \;\; \lambda \text{ is the wavelength.} \tag{4}$$

In general, for long $T$ the wave speed of the wave train approaches the speed of a solitary wave. However as the period becomes shorter the wave speed decreases. This is because the system has not fully recovered from the previous stimulation, and therefore its response is weaker, and the wave propagates more slowly. If the time between successive stimuli becomes too short, the system will be fully refractory when the next stimulus is applied, and it won't respond *e.g.* the wave will not propagate. Thus there exists a critical $T_{crit}$, below which wave propagation is impossible. This reduction of wave speed with shortening periods has in fact been observed for cAMP waves in *Dictyostelium* (14, 15).

**Waves in two Dimensions.**

In two dimensions the effect of curvature on the wave speed for equations 1, 2 is (6):

$$v_\perp = c_0 + D\,\kappa \tag{5}$$

Here $v_\perp$ is the normal velocity of the wave front, and $c_0$ is the speed of a planar wave. $\kappa$ is the curvature of the wave front, defined such that when the wave curves away from the direction of propagation, which is the case for a circular wave traveling outwards, $\kappa < 0$ and the normal velocity is slower than for a planar wave. When the wave curves towards the direction of propagation, $\kappa > 0$ and the wave moves faster. Therefore if a cusp with positive curvature forms, it catches up with the rest of the wave.

The curvature relation can be understood by examining the effective diffusion at the wave front. When the wave curves away from the direction of propagation, the propagator species $u$ must effectively diffuse both forward and sideways. Therefore the effective diffusion of $u$ is slower than for a plane wave, where lateral concentration is the same and diffusion can only be forward. An extreme case would be the difference between diffusion in 1-dimension and 2-dimensions. Similarly, when $\kappa > 0$, the region where $u$ is low is surrounded by regions where $u$ is high, so the amount of $u$ that diffuses to the region of low $u$ is higher than for a planar wave, and therefore the speed is faster.

For rotating spirals both the dispersion relation, equation 4, and the curvature relation, equation 5, must be satisfied, and this imposes 2 conditions:

$$c = \sigma(2\pi/\omega;\varepsilon),\ \ \omega = \varepsilon\Omega(c/\varepsilon; r_0) \tag{6}$$

Here $\omega = \frac{2\pi}{T}$ is the frequency of rotation of the spiral, and $r_0$ is the radius of the core that the spiral rotates around. $\Omega$ is determined to a first order approximation by considering the effect of curvature on wave speed (6). Notice that the frequency of the spiral is uniquely determined by the radius of the core that it rotates around, and by the dispersion relation, which depends on the excitability of the system. This relation seems to hold in *Dictyostelium*, where target period varies significantly, whereas all spirals tend to rotate with the same frequency (15).

Equation 5 has been used to make statements about the minimum area, and therefore the minimum number of cells required to initiate a target pattern (16). The argument is based on the fact that when $-\kappa = c_0/D$, the normal velocity equals zero and the wave fails to spread outwards (recall that the curvature is negative for an outgoing circular wave). For *Dictyostelium*, the wave speed is about $300\mu$m min$^{-1}$ (14, 15), so from the curvature relation 5, estimates of the minimum radius are $r_{min} = \frac{1}{\kappa} \approx \frac{4\times10^{-6} cm^2\, sec^{-1}}{5\times10^{-4} cm\, sec^{-1}} = 80\mu$m (about 50 cells if plated at $10^6$ cells per cm$^2$).

However, one should be careful not to take these conclusions too literally, for two reasons. First, the curvature relation 5 was derived from a pair of differential equations with a small parameter $\varepsilon$, and these equations may not adequately represent the properties of the *Dictyostelium* cAMP signaling system; therefore the curvature relation may not be an accurate representation of a real system. Second, the "influence" of one cell is much larger than the size of the cell, *i.e.* cAMP produced by one cell spreads to neighboring cells, and excites them without forming a wave. Consequently, if the cell produces enough cAMP, it can excite a region large enough to initiate a circular wave from the edges. Since the amount of cAMP required to excite the cells depends on how low their threshold for cAMP stimulus is, the region that a single cell can excite depends critically on the excitability of the system. None of these factors are taken into account in the curvature relation. Thus, if the system is very excitable, one firing cell may be all that is necessary to initiate a wave.

**Target Patterns.**

In the equations from (7) (see also equations 7-11) the parameter $k_e$ represents the activity of phosphodiesterase and $\epsilon$ is the receptor density. we find that if locally the value of $k_e$ is low or $\epsilon$ is high, the cells in that region become oscillatory and secrete cAMP pulses periodically. Cells that oscillate like this are often called pacemakers. If the rest of the field is excitable, cAMP pulses can propagate and these pacemakers give rise to target patterns (Fig. 5). The period of the pacemaker is determined solely by local parameter values.

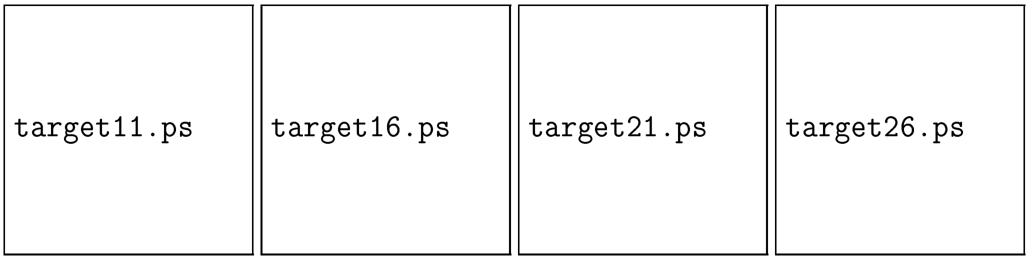


Figure 5: Formation of a target pattern. Parameters as in Fig. 3 but here $\epsilon = 1.48$ in a region $4dx \times 4dx$ in the center. The time between each frame is 20 min. $dx = 0.1$mm, $dt = 0.04$ min, $200 \times 200$ grid.

**Spiral waves.**

In a 2-dimensional excitable medium spiral waves can also form. Spirals are unique in a sense that they regenerate. Once a spiral is formed, it will survive as long as conditions don't change. The reason for this is that the spiral rotates around a central core, which remains in a refractory state. By the time the spiral has completed one rotation, the cells outside the core, where the spiral started, have recovered and can be excited again. Thus the spiral continues to rotate around the core, re-exciting itself. This should be contrasted to a target pattern which needs a pacemaker that periodically excites the system.

Spirals generally originate from broken waves or wave fragments. The ends of these broken waves have a larger negative curvature than a plane wave. Thus, from the curvature equation 5, they propagate more slowly than the rest of the wave. In consequence, the ends begin to curve inwards. The ends continue to curve until a stable spiral is formed, and both relationships in 6 are satisfied. These broken waves do not originate from colliding waves, however, since as mentioned earlier, colliding waves annihilate each other.

**How spirals form.** A standard way to generate a spiral is to break up a planar wave by cutting it in half (10). This can be achieved by initiating a planar wave and then resetting the system to the steady state values on one half of the field perpendicular to the wave (Fig. 6). This leaves a planar wave with a broken end that begins to curve inwards and form a spiral, as described above. This cannot be how waves are broken in *Dictyostelium*, since it is impossible for the cells to reset all of their their parameters instantaneously back to steady state values; nor can only half of them do so.

Another way a wave can break up is when it encounters a region that is not excitable. If this non excitable region is small, the 2 broken ends from either side will immediately join on the other side of the region rather than form a spiral. This joining of the 2 ends creates a cusp that quickly catches up with the rest of of the wave, since it has positive curvature and travels faster. If the non excitable region is made larger, 2 spirals begin to form; but since they cannot propagate into the non excitable region, they are forced to move parallel to the boundary; thus they collide and as before form a cusp which catches up with the rest of the wave (Fig. 7a).

If the region becomes excitable shortly after the wave has passed, the two newly formed spirals can propagate into that region and survive, eventually forming a double spiral. Fig. 7b shows what happens when the wave passes a $2 \times 6$ mm$^2$ region; initially $\epsilon = 0.05$, but increases 0.0003 at each time step,

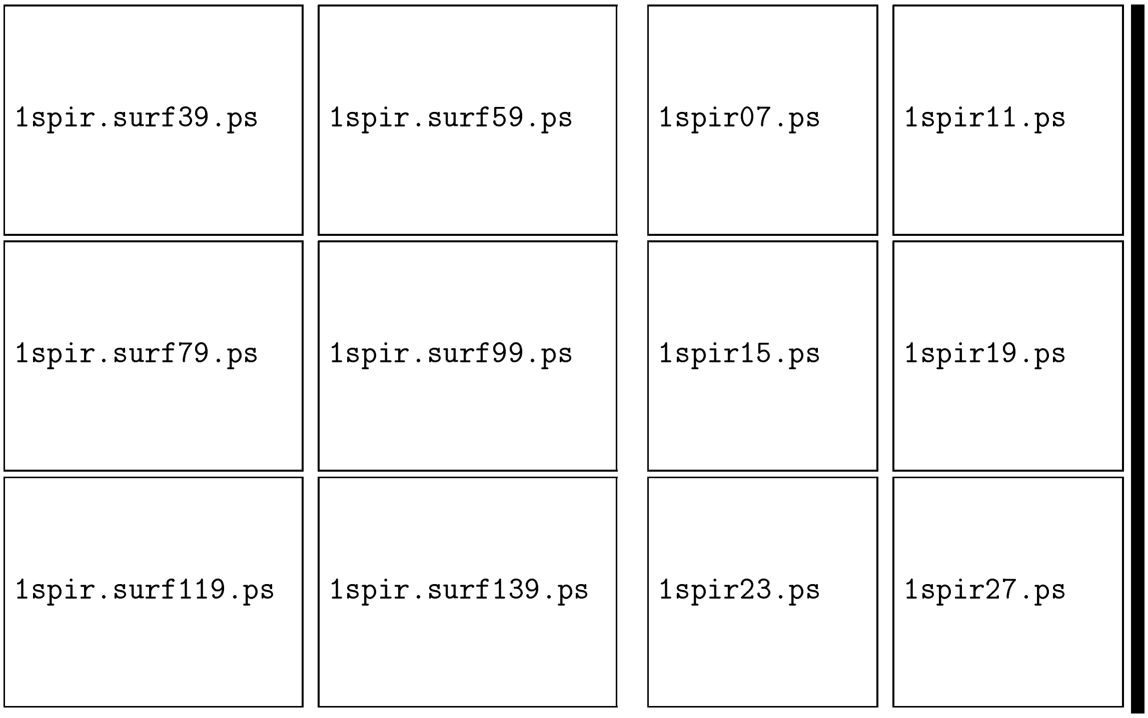


Figure 6: Formation of a spiral. The Z-axis indicates cAMP concentration. We initiate a plane wave from the side. Later we reset one half of the plane back to the steady state values, breaking the wave. The end curves inwards and forms a spiral, as described in the text. Parameters as in Fig. 3. The time between each frame is 20 min. $dx = 0.1$mm, $dt = 0.01$ min. $200 \times 200$ grid. The period for the spiral $T \approx 60$ min. To the right is a contour plot, where the cAMP concentration is represented in gray scale.

so that by the time the wave has passed by, the region has become excitable and the newly formed spirals can propagate. Here the spirals survive and form a double spiral, which regenerates. Again, if the non excitable region is too small the two ends join rather than form a spiral (Fig. 8).

How does a region that is temporarily non-excitable, and large enough to initiate spirals, form? This region must be larger than $2.0 \times 2.0$ mm$^2$, which is hundreds of cells wide, and it is therefore very unlikely that it could form due to random initial variations in density or other cell properties. However, a large region may be rendered temporarily non-excitable if a cAMP wave passes by. Therefore if a cAMP pulse is secreted in the wake of a passing wave (see Fig. 9), where the cells are still refractory, a spiral can form. Here the

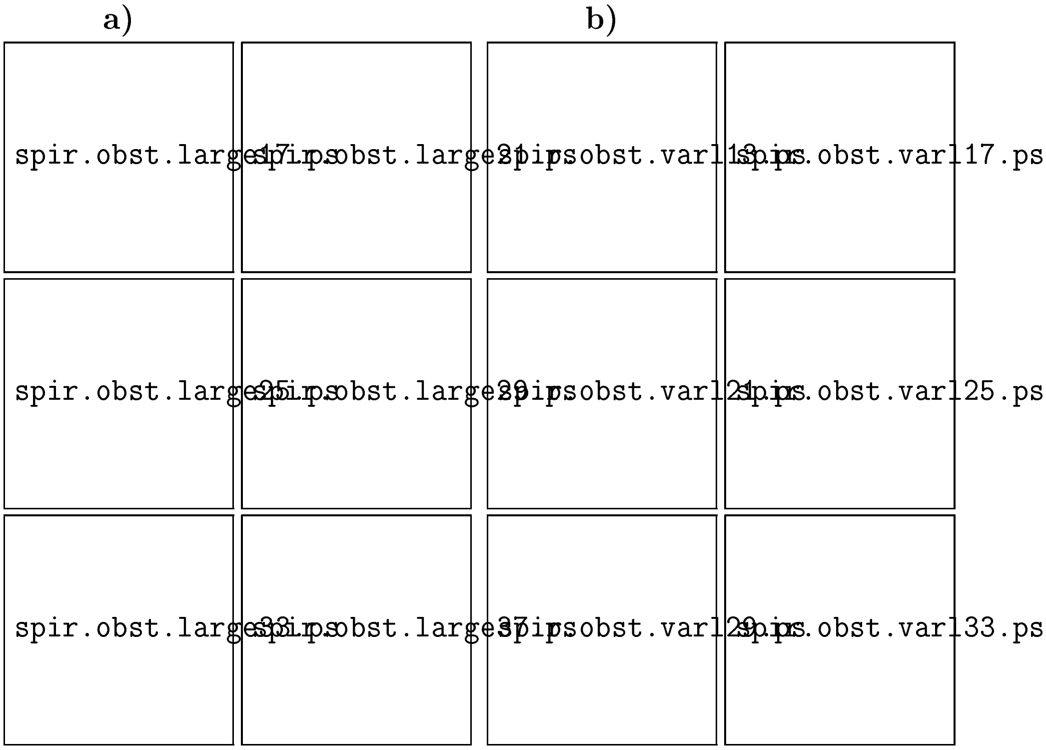


Figure 7: a) Wave propagating past a non-excitable region. The size of the region is $2\times 10$ mm$^2$, 8 min. between frames. b) Wave propagating past a temporarily non-excitable region, $2 \times 6$ mm$^2$, $\epsilon = 0.05$ initially, increasing by 0.0003 each time step. 16 min between frames. Other parameters as in Fig. 3 $dx = 0.1$mm, $dt = 0.04$ min, $200 \times 200$ grid.

new pulse begins to initiate a circular wave, but since the cells closer to the passing wave are refractory, the wave does not propagate in that direction. Thus the result is a wave in the shape of a half circle propagating away from the first wave. The two ends of the half circle then begin to curl inward and form a double spiral. We believe this mechanism is largely responsible for spiral formation in *Dictyostelium.*

Another possibility occurs when a stimulus triggers a wave that does not propagate but instead decays. This incomplete wave formation can give rise to a large region of cells that are temporarily non excitable. If a new wave passes over this region it can break up and a spiral can form, since the region slowly becomes excitable again.

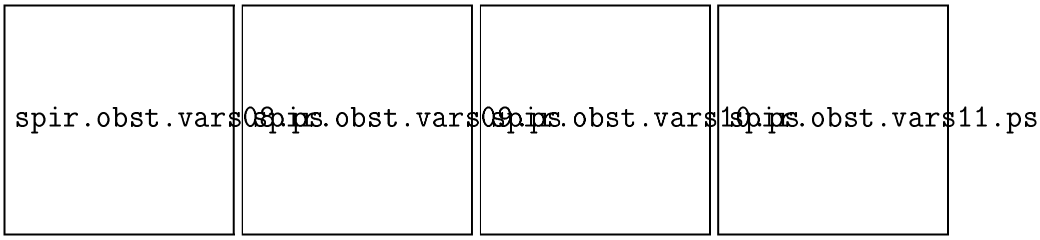


Figure 8: Wave propagating past a temporarily non excitable region. Same as Fig. 7b except now size of region is $2 \times 2$ mm$^2$, 8 min. between frames.

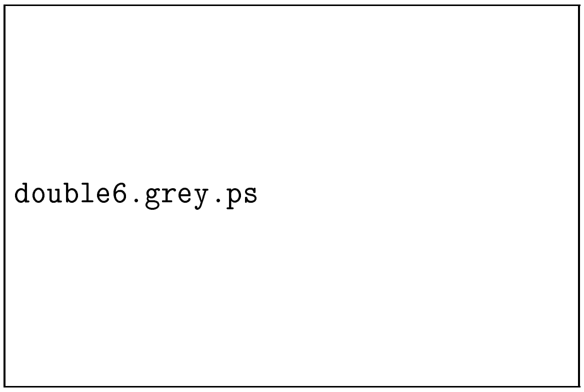


Figure 9: Formation of a double spiral. A second pulse is initiated off-center and shortly after a circular wave has passed by. Parameters as in Fig. 3. The numbers indicate time, $dx = 0.1$mm, $dt = 0.04$ min, $100 \times 100$ grid.

**Initial conditions.**

In this section we discuss the effect various initial conditions have on pattern formation. To do this we modified the equations from Martiel and Goldbeter (7). The resulting equations are:

$$\begin{array}{lclll} \dfrac{d\beta(\mathrm{x,y,t})}{dt} & = & \Phi(\rho,\gamma) & -\beta k_i & -\beta k_t \\ \text{Rate of change in intracellular [cAMP]} & = & \text{Production of cAMP} & - \text{Intracellular hydrolysis} & - \text{Secretion of cAMP} \end{array} \quad (7)$$

$$\begin{array}{lclll} \dfrac{d\gamma(\mathrm{x,y,t})}{dt} & = & \frac{\mathrm{k_t}}{\mathrm{h}}\beta & -k_e\gamma & +D_{cAMP}\nabla^2\gamma\blacksquare \\ \text{Rate of change in extracellular [cAMP]} & = & \text{Secretion of cAMP} & - \text{Extracellular hydrolysis} & - \text{Diffusion of cAMP} \end{array} \quad (8)$$

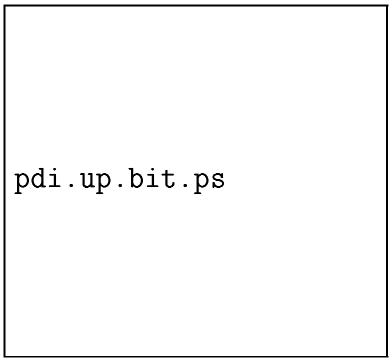


Figure 10: *Dictyostelium* cells begin to secrete PDI shortly after the onset of starvation. From (17).

$$\begin{array}{rcll} \dfrac{d\rho(\mathrm{x,y,t})}{dt} & = & -f_1(\gamma)\rho & +\, f_2(\gamma)(1-\rho) \\ \text{Rate of change in fraction of active receptor} & = & \text{Dephosphorylation of receptor} & -\ \text{Phosphorylation of receptor} \end{array} \tag{9}$$

$$\begin{array}{rcll} \dfrac{d\mathrm{PDI}(\mathrm{x,y,t})}{dt} & = & S(\mathrm{x,y,t}) & +\ D_{PDI}\nabla^2\mathrm{PDI} \\ \text{Rate of change in PDI concentration} & = & \text{Secretion of PDI} & -\ \text{Diffusion of PDI} \end{array} \tag{10}$$

$$\begin{array}{rcl} k_e & = & \frac{k_{e\ max}}{1+PDI} \\ \text{Activity of phosphodiesterase} & = & \text{Effect of phosphodiesterase inhibitor} \end{array} \tag{11}$$

Diffusion terms for external cAMP ($\gamma$) and PDI, and the term $S(x,y,t)$, which represents PDI secretion, are new features added to the Martiel and Goldbeter model. The latter term is made up of random delta functions in time and space, and is critical for spiral formation.

Shortly after cells begin to starve, they secrete PDI into the medium (Fig. 10) (18, 17, 19). At the onset of starvation cells are caught at different stages in their cell cycle, and therefore exist in different biochemical states. It is also known that the stage they are in at the onset of starvation has a significant effect on where the cells sort out in the final fruiting body (20, 21).We assume therefore that all of the cells do not secrete PDI at the same time, and that time of secretion may depend on where they are caught in the cell cycle upon starvation.

pdi.dif8.grey.ps

Figure 11: Random spatial PDI secretions can account for the establishment and evolution of wave patterns. Time is given in min. Increasing PDI levels correspond to increasing background intensity, cAMP levels are in grayscale. Probability of PDI secretion $p = 0.5 \times 10^{-6}$ / timestep / cell). The diffusion constants for cAMP and PDI were $4 \times 10^{-6} cm^2 sec^{-1}$ and $8.3 \times 10^{-7} cm^2 sec^{-1}$ . Grid $400 \times 400$, with $dx, dy = 0.1$ mm, $dt$= 0.04 min.

In a field of aggregation competent cells, this leads to random temporal and spatial PDI secretion. But since a reduction in $k_e$, the rate constant for PDE activity (equation 11), causes the cell to begin emitting periodic pulses of cAMP, the net effect of PDI secretion is random cAMP pulses in both space and time. At the same time, PDI diffuses away, so that cells eventually return back to their excitable but non oscillatory state.

Given these observations, we decided to explore the effect of random PDI secretion in an excitable medium with nonuniform initial conditions. In Fig. 11 each cell had a probability of secreting PDI per time step of $p = 5 \times 10^{-6}$. Notice how circular waves break up and form double spirals, which then later turn into spirals.

The double-armed spirals are formed when a cAMP pulse is emitted in the wake of a passing wave, as discussed earlier (Fig. 9). These double spirals regenerate and continue to produce waves. They are created at random locations, and therefore the field becomes strongly asymmetric. Because of this asymmetry, one end of a double spiral may experience a different

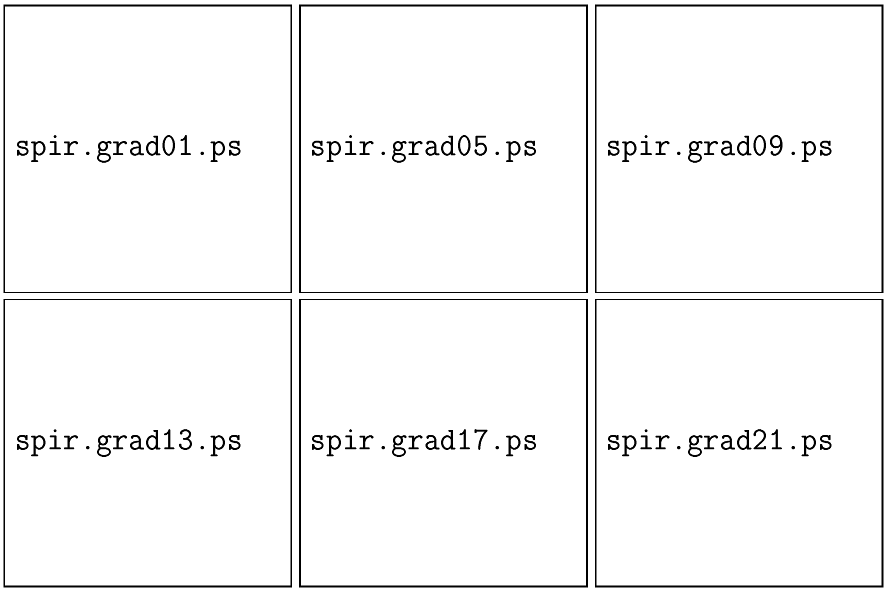


Figure 12: We begin with random cAMP pulses. The probability of a cAMP pulse was $p = 2\times10^{-6}$ per grid unit per time step. We stopped initiating pulses after 2000 time steps. Parameters as before. The time between each frame is 8 min. $dx = 0.2$ mm, $dt = 0.04$ min, $200 \times 200$ grid and 4 cone shaped gradients located at $x = 100 \pm 50$, $y = 100 \pm 50$ given as $k_e = 3.58 - 0.02 \times (20 - r)$. Here $r$ is the distance away from the center of the cone.

environment than the other end, and therefore rotate faster. When this occurs the more slowly rotating end is pushed outward, leaving the faster rotating end as a single armed spiral. This effect is obvious when the double spiral forms on top of a gradient of some parameter of the signaling system, *e.g.*, Fig. 12, which is a simulation with random cAMP pulses, but this time there is a cone-shaped gradient of $k_e$ in the 4 corners of the field ($k_e$ is lowest at the center of the cone). Here we clearly see that one end of the double spiral rotates faster than the other; eventually the double spiral forms a one armed spiral.

When amoeba are grown on a bacterial plate, they form a feeding front. The region behind the front has been cleared of bacteria while there are still bacteria in front of it. The front slowly moves outward until all of the bacteria have been eaten. The further away a cell is from a feeding front, the longer it has starved, and so there is a gradient in time of starvation from the center to the feeding front. Presumably this gives rise to a gradient of excitability of the signaling system, and from Fig. 12 should enhance the formation of large single armed spirals. This effect can be seen if we compare the two pictures in Fig. 1. On the right the cells were harvested and then resuspended without bacteria on a plate (effectively randomized), and on the left the cells were left undisturbed where we can clearly see much larger single armed spirals.

**When do spirals form?**

In order to form a double spiral we must have a pulse in the wake of a wave. The timing of the second pulse is important, since if it is secreted too early, no wave can form, and if it is secreted too late, a second circular wave will just form. The probability of forming a double spiral thus depends on two events: 1) That a wave passes by, and 2) that a pulse is secreted in the wake of the wave and at the appropriate time. Consequently the probability of double spiral formation is very low; given a high frequency of cAMP pulses, however, a double spiral will form now and then. When the density of the cells is lowered, the probability of a cAMP pulse per unit area goes down, and therefore the number of double spirals formed drops even faster. At very low cell densities, no double spirals will be initiated; and since single armed spirals form from double spirals, no spirals will form. Cells continue to develop, however, since they are not being entrained by wave centers; eventually some cells will become oscillators, and form target patterns. This effect has been observed in our lab (22) where cells plated at high densities form spirals while cells plated at low densities form only target patterns.

**Summary**

In this essay we have tried to explain how the various patterns in *Dictyostelium* form. We reviewed a theory for waves in excitable media, and introduced analytical expressions for wave speed, a curvature relation, and the dispersion relation, all derived from a pair of reaction-diffusion equations of a general form (6). We compared the analytical expression to the numerical results from the model, and showed what effect different initial conditions, randomness in parameter values, temporal fluctuations of some parameters, and nonuniform distributions, would have on the resulting patterns. We showed how target patterns might arise, and suggested mechanisms that might be responsible for spiral formation. We indicated that the formation of spirals in *Dictyostelium* is caused by the random secretion of PDI. Although not discussed here, experiments with PDI deletion mutants show that these strains cannot form spirals, supporting these arguments (4). On the one hand understanding pattern formation in excitable media has helped us understand how part of the cAMP signaling system works, and on the other hand, studying pattern formation in *Dictyostelium* has given us greater insight into pattern formation in other excitable media.

**Acknowledgments** We want to thank the National Science Foundation for finacial support and Kyoung Lee for Fig.1.


1. Tomchik, K. J & Devreotes, P. N. (1981) *Science* **212**, 443–446.
2. Newell, P. C. (1983) in *Fungal differentiation: a contemporary synthesis (Mycology series)*, ed. Smith, J. (M. Dekker, New York), pp. 43–71.
3. Palsson, E & Cox, E. C. (1996) *Origin and evolution of circular waves and spirals in Dictyostelium discoideum territories.* Vol. 93, pp. 1151–1155.
4. Palsson, E. (1996) *The cAMP Signaling System in Dictyostelium discoideum.* (Princeton University, Princeton).
5. Franke, J & Kessin, R. H. (1981) *J. Biol. Chem.* **256**, 7628–7637.
6. Tyson, J. J & Keener, J. P. (1988) *Physica D* **32**, 327–361.
7. Martiel, J. L & Goldbeter, A. (1987) *Biophys. J.* **52**, 807–828.
8. Caterina, M. J, Hereld, D, & Devreotes, P. N. (1995) *J. Biol. Chem.* **270**, 4418–4423.
9. Lilly, P. J & Devreotes, P. N. (1995) *J. Cell Biol.* **129**, 1659–1665.
10. Tyson, J. J & Murray, J. D. (1989) *Development* **106**, 421–426.
11. Keener, J. P. (1980) *SIAM J. APPL. MATH* **39**, 528–548.
12. Zykov, V. S. (1980) *Biophysics* **25**, 906–911.
13. Fife, P. C. (1984) in *Non-Equilibrium Dynamics in Chemical Systems*, eds. Vidal, C & Pacault, A. (Springer, Berlin), pp. 76–88.
14. Alcantara, F & Monk, M. (1974) *J. Gen. Microbiol.* **85**, 321–334.
15. Gross, J. D, Peacey, M. J, & Trevan, D. J. (1976) *J. Cell Sci.* **22**, 645–656.
16. Foerster, P, Muller, S, & Hess, B. (1990) *Development* **109**, 11–16.
17. Kessin, R. H, Orlow, S. J, Shapiro, J. R, & Franke, J. (1979) *Binding of inhibitor alters kinetic and physical properties of extracellular cyclic AMP phosphodiesterase from Dictyostelium discoideum.* Vol. 76, pp. 5450–5454.
18. Yeh, R. P, Chan, F. K, & Coukell, M. B. (1978) *Dev. Biol.* **66**, 361–374.
19. Franke, J, Faure, M, Wu, L, Hall, A. L, Podgorski, G. J, & Kessin, R. H. (1991) *Dev. Genet.* **12**, 104–112.
20. McDonald, S. A & Durston, A. J. (1984) *J. Cell Sci.* **66**, 195–204.
21. Ohmori, R & Maeda, Y. (1987) *Cell Differ.* **22**, 11–18.
22. Lee, K, Goldstein, R, & Cox, E. C. (1996) *Phys. Rev. Letters* **76**, 1174–1177.