

Three-dimensional scroll waves of cAMP could direct cell movement and gene expression in *Dictyostelium* slugs

(chemotaxis/morphogenesis/gene expression/*Dictyostelium discoideum*/*Dictyostelium mucoroides*)

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ABSTRACT Complex three-dimensional waves of excitation can explain the observed cell movement pattern in *Dictyostelium* slugs. Here we show that these three-dimensional waves can be produced by a realistic model for the cAMP relay system [Martiel, J. L. & Goldbeter, A. (1987) *Biophys J.* 52, 807–828]. The conversion of scroll waves in the prestalk zone of the slug into planar wave fronts in the prespore zone can result from a smaller fraction of relaying cells in the prespore zone. Further, we show that the cAMP concentrations to which cells in a slug are exposed over time display a simple pattern, despite the complex spatial geometry of the waves. This cAMP distribution agrees well with observed patterns of cAMP-regulated cell type-specific gene expression. The core of the spiral, which is a region of low cAMP concentration, might direct expression of stalk-specific genes during culmination.

The cellular slime mold *Dictyostelium discoideum* is well suited for study of the signals controlling spatiotemporal pattern formation and morphogenesis. The life cycle of *Dictyostelium* is divided into distinct phases of single and multicellularity; therefore it allows a detailed analysis of the principles underlying the formation of a multicellular organism. Multicellularity is achieved by chemotactic aggregation. The chemotactic aggregation process is controlled by a chemical relay system based on the periodic production of the diffusible molecule cAMP (1). Cells in the aggregation center periodically generate and secrete cAMP pulses. The cAMP diffuses away from the aggregation center and stimulates surrounding cells. These cells, which detect cAMP via cell surface receptors, respond in two ways: (i) they amplify the cAMP signal by autocatalytic cAMP production and (ii) they move chemotactically toward higher cAMP concentrations. After relaying the cAMP signal, the cells are for a while refractory to further stimulation. This leads to periodic cAMP waves propagating outward from the aggregation center and at the same time to periodic movement of the cells toward the aggregation center. The cAMP signal can be visualized directly by autoradiography during early development (2) or indirectly as an optical density wave caused by periodic cell shape changes associated with the chemotactic response (3, 4). The cAMP waves appear as spirals or concentric rings. Chemotactic cell movement can be analyzed by digital image processing and was shown to be periodic (4–6).

Once formed, the aggregate elongates upward, falls over, and forms a migratory slug, which behaves as a single organism and responds to environmental signals via photo- and thermotaxis. The foremost 20% of the slug consists of prestalk cells, while the remainder is formed by prespore and 10% randomly scattered anterior-like cells. The prestalk and prespore cells form the stalk and spores in the fruiting body, while the anterior-like cells participate in the formation of the basal disc, which supports the fruiting body on the substratum, and

form the upper and lower cups, which support the spore mass on the stalk (7, 8). The prestalk cells form a distinct morphological structure, the tip, which controls the whole of later morphogenesis (9, 10). Cell type-specific reporter gene constructs have been used to show that there are several distinct populations of prestalk cells within the tip, termed PstA, PstAB, and PstO cells. PstA cells are located in the anterior outer layer of the tip, and PstAB cells are found in the core of prestalk zone and will form the stalk (11). PstO cells are found in the back of the prestalk zone (12).

There is a wealth of data that documents that cAMP is necessary for cellular differentiation of both prespore and prestalk cells. High levels of cAMP are necessary for induction (13) as well as stabilization of prespore cell-specific gene expression (14). cAMP also plays an important role in the differentiation of the various prestalk subclasses. PstA cells need cAMP to express the prestalk cell-specific extracellular matrix gene *ecmA*, whereas expression of another prestalk cell specific gene, *ecmB* in PstAB cells, is inhibited by cAMP (15, 16).

Several lines of evidence suggest that cAMP may control cell movement in the postaggregation, multicellular phase of development. When placed in a field of aggregation-competent amoebae, slug tips attracted the amoebae in a periodic fashion, suggesting that periodic cAMP signals were emitted by the tip similar to signals from aggregation centers (10). Prestalk and prespore cells show differential chemotaxis toward cAMP (17–19).

cAMP waves have not been monitored directly in slugs as has been done for aggregation fields. However, it is possible to deduce the pattern of the chemotactic waves by a detailed analysis of cell movement in the slug, under the assumption that the cells move opposite to the direction of wave propagation. Such an analysis in slugs showed that prestalk and prespore cells move along completely different trajectories. While prespore cells move straight forward in the direction of slug movement, prestalk cells rotate around the tip (20). Tracking of individual cells revealed furthermore that cells move in a periodic fashion and show changes in cell shape, which are typical for chemotactically active cells (5, 21, 22). Theoretical considerations have suggested that a three-dimensional spiral wave which rotates around the central axis of the prestalk region (a so-called scroll wave) produces rotational cell movement in the prestalk zone, whereas a twisted scroll wave (a scroll wave with a twist along its long axis) or a series of planar waves splitting off from the scroll wave in the prestalk zone cause the forward movement of prespore cells (20).

Computer simulations which interpreted the slug as a three-dimensional excitable medium helped to understand the complex geometry of signal propagation (23). To obtain the transition from scroll to planar wave fronts, we had to assume a difference in excitability along the long axis of the slug, with prestalk cells being more excitable than cells in the prespore zone. These calculations were performed with a numerically fast, generic model for an excitable system. Here we show that

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similar results can be obtained with a realistic model for cAMP relay. Further, we show that the difference in excitability between the prestalk and prespore zone may be due to a reduction in the fraction of relaying cells in the prespore zone. Our results suggest that the pattern of cAMP waves propagating through the slug could stabilize the expression of prestalk genes in the front of the slug and prespore cells in the back of the slug, as well as control the process of stalk differentiation in the tip of the slug.

MODEL AND NUMERICAL APPROACH

Previous calculations of cAMP wave propagation in slugs (23) were based on the two-variable model of Barkley (24). This model was developed to describe and simulate various properties of a chemical oscillatory system, the Belousov-Zhabotinski reaction. To explain the aggregation-stage cAMP oscillations, we now use a model developed by Martiel and Goldbeter (25), which is more realistic and where the variables are more easily related to known biochemical variables. The three-variable model describes the cAMP relay in the following way (Fig. 1). The behavior of one cell is given by three coupled nonlinear differential equations describing the variation of extracellular cAMP, intracellular cAMP, and the fraction of active receptor over time. Extracellular cAMP binds to a receptor, which is in either an active (R) or an inactive (D) state. The complex of active receptor and cAMP cooperatively activates adenylate cyclase (AC), which produces cAMP from ATP. This step introduces a nonlinearity needed for excitable or oscillatory behavior. Intracellular cAMP will then be released through the membrane in the extracellular space, where it can bind to the receptor and thus autocatalytically amplify the signal. Due to receptor phosphorylation the receptor becomes desensitized (D) and autocatalytic cAMP amplification is stopped. Cells are now insensitive (refractory) to further cAMP stimulation for a certain time period. cAMP is degraded by extra- and intracellular phosphodiesterases. The Martiel-Goldbeter model uses 13 parameters to describe all these processes. Each parameter has been assigned a value derived from experimental data (25) and this can be used to describe the aggregation-stage oscillations in the nanomolar concentration range. All values for the parameters used in the following simulations are equivalent to parameter set C of Tyson *et al* (26) except the rate constant for extracellular phosphodiesterase, which is $k_e = 9.0 \text{ sec}^{-1}$. Coupling of cells through diffusion of extracellular cAMP produces target and spiral patterns in two dimensions, which match the spiral cAMP waves observed during aggregation (27).

In this paper we show that the Martiel-Goldbeter model is also capable of producing the three-dimensional spiral waves that occur in slugs. To simulate signal propagation in slugs, a three-dimensional grid in the shape of a cylinder (diameter, 34; length, 80 grid points) was initialized with a scroll wave over the whole length of the cylinder by applying no-flux boundary

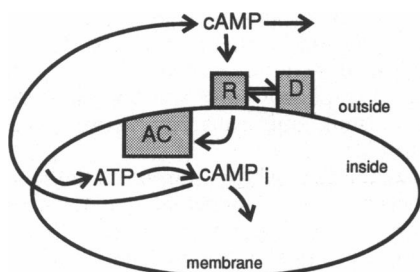


FIG. 1. Biochemical basis of the Martiel-Goldbeter model of the cAMP relay system based on receptor desensitization. R, active cAMP receptor; D, desensitized (inactive) receptor; AC, adenylate cyclase.

conditions. To evaluate the diffusion term, the six nearest neighbors were taken into account. After 10,000 iterations, we reduced the fraction of relaying cells in the prespore zone, which was defined as 75% of the length of the cylinder. The calculations were continued until the wave patterns were stable in time. The positions of the active cells were chosen randomly.

We used an explicit Euler time-stepping integration method. We confirmed the numerical accuracy of the results by increasing the number of grid points and reducing the iteration time steps. All calculations were performed on an IBM RS6000 computer (model 350 equipped with a high-speed graphic adapter, Gt4x) with programs written in standard C language. The cAMP isoconcentration surfaces were visualized with the IBM Data Explorer software.

RESULTS

Previously we have shown that we could calculate the transition from a scroll wave in the tip of the slug to planar waves in the prespore region of the slug by assuming a step in excitability between the prestalk and prespore zone (23). The cellular basis for this difference in excitability could be due to a difference in the number of relaying cells in the prestalk and prespore zone; i.e., all cells in the prestalk zone relay the signal, whereas only a fraction of the cells in the prespore zone relay the signal. Relay of the signal in the prespore zone could be the task of the scattered anterior-like cells, which die during the formation of the fruiting body (7, 28).

To test this hypothesis we calculated the pattern of cAMP wave propagation under conditions where only a limited number of randomly distributed cells in the prespore zone relayed the signal. First we determined the conditions to produce scroll waves with the Martiel-Goldbeter model. We have not found conditions where we could find stable scroll waves starting from random initial conditions. This, however, is not necessary since the spiral waves are already present during the early phases of aggregation in the two-dimensional cell layers and then transform into three-dimensional scroll waves at the mound stage (F.S. and C.J.W., unpublished work). Therefore the initial condition in the present simulations is an untwisted scroll wave along the long axis of a slug with a constant excitability. In this homogeneous system the scroll wave stably rotates. After several rotations (10,000 time steps) the fraction of relaying cells in the prespore zone (the right 80% of the slug cylinder) was reduced. At grid points where there were no relaying cells, only the diffusion term was evaluated and there was no amplification of the cAMP signal. For simplicity we assumed that the cells that did not relay also did not bind or break down cAMP. Fig. 2 shows that the transition from scroll waves via twisted scroll waves into planar waves can be readily simulated in the Martiel-Goldbeter model by decreasing the fraction of relaying cells in the prespore zone. Parameters were kept constant for all cells. The fraction of relaying cells in the prestalk zone was always 100%. The photographs show an isoconcentration surface of extracellular cAMP. Note that the wavefronts in the prespore zone are not as smooth as in the prestalk zone. There are discrete maxima at grid points where signal amplification takes place. As the signal propagates in the region with a reduced number of relaying cells, the average cAMP concentration is lowered. This results in a decreased velocity of signal propagation. Fig. 2A shows a transitional stage where the scroll wave begins to transform into a twisted scroll wave. Due to a lower fraction of active cells (90%) in the prespore zone the signal propagation velocity in the prespore zone decreases, resulting in the wave front lagging behind the scroll wave front rotating in the prestalk zone. This leads to a twisting of the wave along the length of the prespore zone. If we simulate 80% relaying cells in the prespore zone, we get twisted scroll waves with a stronger pitch (Fig. 2B). When the number of relaying cells is

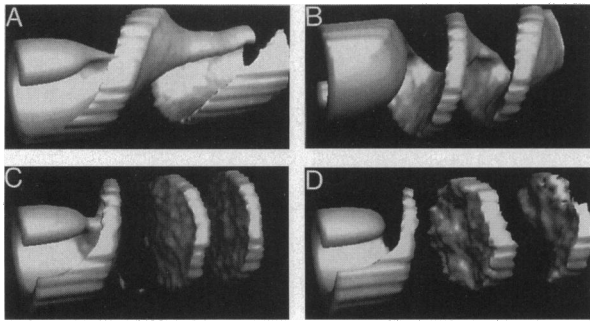


FIG. 2. Wave transitions caused by changing the fraction of relaying cells in the prespore zone of slugs so that 90% (A), 80% (B), 60% (C), or 40% (D) of the cells in the prespore zone actively relay the cAMP signals. The slug is oriented with the 25% prestalk cells to the left and the prespore cells to the right. In the prestalk zone 100% of the cells relay the cAMP signal. Shown are isoconcentration values of propagating waves of extracellular cAMP (extracellular [cAMP]/ K_R , where $K_R = 10^{-7}$ M is the dissociation constant of the cAMP receptor complex). In A–C, concentration = 0.45; in D, concentration = 0.35.

reduced to 60%, the scroll wave breaks and transforms into planar wave fronts (Fig. 2C). For this parameter set, wave propagation can occur down to 40% relaying cells (Fig. 2D). Below 35% no relay is possible with the present parameter set (see *Discussion*), as the overall cAMP production of the cells is not sufficient to stimulate neighboring cells, and the waves die out in the prespore zone.

Time–space plots of these waves allow the analysis of the velocity and period of the propagating cAMP signals. The time–space plots in Fig. 3A–D correspond to the wave patterns in Fig. 2A–D. At regular time intervals a two-dimensional section was made through the long axis of the cylinder. These sections were placed next to each other, resulting in a cube with two space axes and one time axis. This summarizes wave propagation along the section over time. A view on the top of this cube shows the propagation of the waves along the long axis of the cylinder for 11 rotations. Each section through the prestalk–prespore axis corresponds to a horizontal line from left to right. Successive time points are shown as lines below each other. The extracellular cAMP concentration is color coded: black represents high cAMP concentration, and white represents low cAMP concentrations. The rotating scroll wave in the prestalk zone (Fig. 3) appears as a succession of black horizontal lines. The distance between the lines represents the period of rotation. In the prespore zone the wave cannot keep

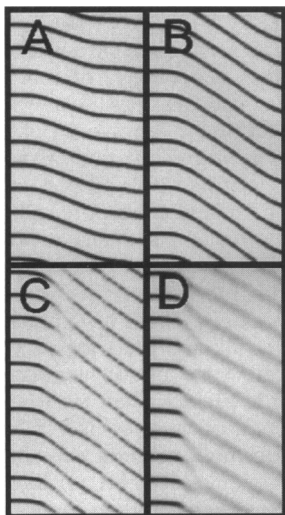


FIG. 3. Time–space plots of cAMP wave propagation. The regular pattern of black and white bands shows cAMP wave propagation along the long axis of a slug. White indicates low cAMP, and black, high cAMP. Wave propagation is shown for a period of 11 rotations of the scroll wave (50 min). The stripes bend downward when the scroll wave becomes twisted due to lower cell density of relaying cells in the prespore zone. As in Fig. 2, 90% (A), 80% (B), 60% (C), or 40% (D) of the cells in the prespore zone actively relay the cAMP.

up with the velocity of the wave in the prestalk region, due to the reduced excitability. The stripes are bent down as the scroll wave becomes twisted; the slope indicates wave propagation velocity. The period in the prespore zone does not change as long as twisted scroll waves are maintained. Under these conditions the number of waves initiated in the prestalk zone is the same as the number of waves reaching the end of the prespore zone (Fig. 3A and B). However, when the difference between wave propagation speed in the prestalk and prespore zone becomes too large, some of the twisted wave fronts run into refractory tissue and die out, leading to a frequency difference between the prestalk and prespore zone (Fig. 3C and D). The remaining waves then propagate as planar waves. Planar waves can therefore only occur when some of the waves vanish while running through the prespore zone. In Fig. 3C and D it can be seen that while 11 waves were initiated in the prestalk zone, only 10 and 9 waves, respectively, arrive at the end of the prespore zone. The time–space plots also show that for scroll waves (Fig. 3A and B) the velocity of wave propagation decreases with decreasing fraction of relaying cells. This phenomenon is what causes scroll waves to convert to twisted scroll waves.

Periodic Signals Can Also Control Position-Dependent Differentiation. Prespore gene expression can be as efficiently induced by pulses of cAMP as by steady concentrations of cAMP, as long as the total dose is comparable (29). To investigate the action of cAMP, it is therefore important to determine the total amount of extracellular cAMP experienced by cells in the various regions of the slug and whether the distribution of total cAMP shows a pattern which could be used to stabilize cell type-specific gene expression. We therefore investigated the average concentration of extracellular cAMP generated by the signal system at different positions in the slug. Fig. 4 shows the changes in extracellular cAMP for three different volume elements in the prestalk and prespore zone respectively. The dynamic behavior of the elements is shown for 1000 sec. We used the same parameters as shown in Fig. 3C, where 60% of the cells in the prespore zone relay the signal and planar waves are formed. Fig. 4A shows the location of the elements. Elements 1 and 1' are located at the central axis of the cylinder in the prestalk and prespore zone, respectively, whereas 2, 3, and 2', 3' lie at different distances from the center toward the periphery. Elements in different positions in the prestalk zone oscillate with different phases and amplitudes. The amplitude of the extracellular cAMP concentration in the element in the center of the cylinder (element 1) is about half of that of the element in the periphery (elements 2 and 3) because element 1 lies in the core of the scroll wave. Here the extracellular cAMP concentration is low because the tissue is always partly refractory. The three elements in the prespore zone (1', 2', and 3') all oscillate in phase and show only marginal differences in their amplitude. This is expected for the propagation of planar waves. These observations suggest that on average there is less cAMP in the prespore zone, but more than in the core of the prestalk zone (element 1).

To visualize the total amount of cAMP produced over time, the extracellular cAMP concentration in every grid point in a longitudinal section through the slug was added over a 5.5-hr simulation time (80 rotations). A cross section through the symmetry axis of the slug is shown (Fig. 5A). The cAMP concentration is color coded: blue represents the lowest, green and yellow the intermediate, and red the highest cAMP levels. The parameter set used to calculate Fig. 5A produces planar waves in the prespore zone and corresponds to 60% relaying cells (Fig. 2C). There is a clear difference in the total amount of extracellular cAMP seen by cells in the prespore and prestalk zone. High amounts of cAMP are found in the prestalk zone and intermediate amounts in the prestalk zone (40% of the peak values in the prestalk zone). As expected from Fig. 4, we find the lowest levels of cAMP (blue) in the

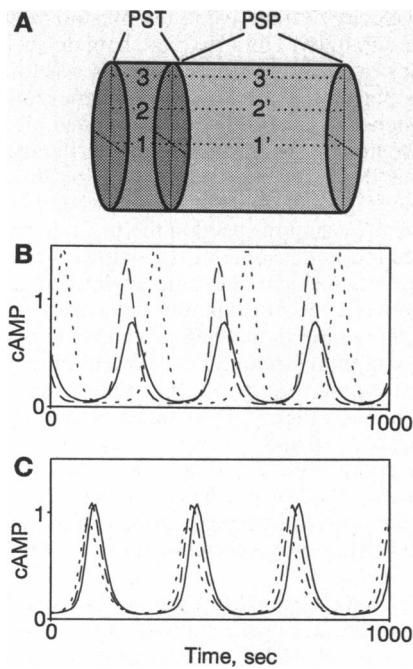


FIG. 4. Cells in different regions of the slug sense different cAMP signals. (A) Location of three cells in the prestalk zone (1, 2, and 3) and three cells in the prespore zone (1', 2', and 3'). (B and C) Time course of the changes in extracellular cAMP in the prestalk and prespore zone, respectively. Due to the propagation of planar waves, cells in the prespore zone (1', 2', and 3') are all in phase. Cells in the core of the prestalk zone where a scroll wave rotates (cell 1) show markedly lower peaks in extracellular cAMP as compared with cells 2 and 3. The parameter set corresponds to that used in Fig. 2C, where planar waves propagate through the prespore region due to a step in cell density (60% relaying cells). —, Cells 1 and 1'; — — —, cells 2 and 2'; - - - - -, cells 3 and 3'.

core region of the prestalk zone. Fig. 5B shows a three-dimensional iso surface representation of the region of low cAMP in the slug. The core bends to the surface of the slug cylinder at the prespore–prestark boundary (see Fig. 5C). Grid points belonging to the core were calculated as the region in which the fraction of active receptor stays under a certain threshold ($r < 0.6$) during 1.5 periods of rotation—i.e., as the region where a significant number of receptors are desensitized. The bent part of the core is not fixed in space but describes a rotational movement in the plane between the prestalk and prespore zone, resulting in the disc-shaped region of low cAMP seen at the prestalk–prespore boundary in Fig. 5B. Fig. 5C shows a composite figure made by combining the filament in red calculated over 1.5 periods of rotation as described with a snapshot of a propagating cAMP wave taken at one time point. The core region extends straight through the prestalk zone and bends at the border between prestalk and prespore zone toward the outer circumference of the cylinder.

Fig. 5D–F show the distribution of total cAMP and the form of the filament for the case of propagating twisted scroll waves (80% relaying cells; see Fig. 2B). Again a difference in the amount of extracellular cAMP between the prespore and prestalk zone is seen, but the difference is less pronounced than in the case of planar waves (Fig. 5A). Very low cAMP levels are found in the core region, which extends through the whole slug under these conditions. The blue core region seems to be interrupted in the prespore zone. This is artificial, since the core meanders through the plane of section. A three-dimensional representation of the filament (Fig. 5E) clearly demonstrates that the core extends through the whole prespore zone. Fig. 5F shows a composite picture of the filament with

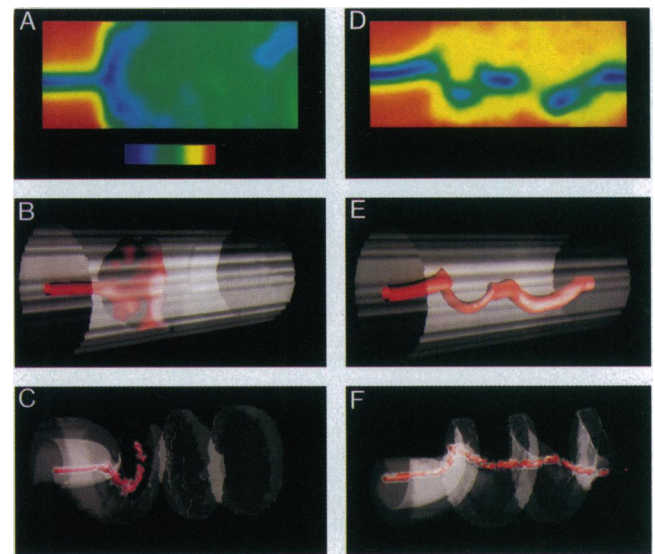


FIG. 5. Integral cAMP distribution in a longitudinal slug section. (A) Longitudinal section through the slug cylinder with 60% relaying cells in the prespore zone. The cAMP concentration is color coded: low total amounts of cAMP appear blue; high total amounts of cAMP are red. The conditions used produce planar waves in the prespore zone. (B) cAMP isosurface representation of the region of low cAMP (red). The slug boundary is shown in gray. (C) Illustration of the core relative to a propagating cAMP wave (gray isosurface of 0.45). (D–F) Same representations as in A–C but with 80% relaying cells in the prespore zone, conditions which produce twisted scroll waves.

a snapshot of a propagating cAMP wave as seen at one time point.

DISCUSSION

We have used the Martiel–Goldbeter (25) model, a biochemical model of the *Dictyostelium* cAMP relay response, to simulate wave propagation in *Dictyostelium* slugs. Our aim was to test two hypotheses. (i) Can this more realistic model produce the chemotactic waves required to direct the observed cell movement in slugs? Specifically we investigated the possibility that the signal is relayed by all cells in the prestalk zone and only by a subpopulation of cells in the prestalk zone. (ii) Can such a wave propagation mechanism produce the spatial information necessary to stabilize prespore and prestalk cell-specific gene expression?

Generating Planar Waves by Lowering the Fraction of Relaying Cells. The results show that the Martiel–Goldbeter model can produce scroll waves that convert to planar wave fronts at the prestalk–prespore boundary (Fig. 2) if we assume that only a fraction of the cells in the prespore zone participate in the relay reaction. There is a clear dependence of wave propagation speed on the fraction of relaying cells. A lower fraction of relaying cells leads to a decrease in wave propagation speed and to a scroll wave with a greater pitch. One attractive hypothesis is that only anterior-like cells propagate the cAMP signal in the prespore zone, while the prespore cells just show chemotaxis to the cAMP wave fronts. There is no direct experimental evidence for this hypothesis, since it has not been possible to measure the relay properties of prestalk, anterior-like, and prespore cells separately. There have been some measurements on enriched prestalk and prespore populations, which show that prestalk cells have a stronger relay response. It was not clear whether the prespore cell fraction was contaminated with a large fraction of anterior-like cells (30). However, anterior-like cells resemble prestalk cells in their biochemical properties (31). In many ways anterior-like cells are like late-aggregation-stage cells and can therefore be

expected to relay the signal. The hypothesis that anterior-like cells relay the signal is attractive, since this would provide a good explanation for their existence and more or less random distribution in the prespore zone. The concept that only a fraction of the cells relays the signal is not even ruled out for aggregation-stage cells, since it has not been possible to measure cAMP relay at the single-cell level.

In our present calculations, wave propagation ceased when the fraction of relaying cells in the prespore zone dropped below 35%. This is significantly more than the fraction of anterior-like cells. We do not think that this presents a serious objection to our hypothesis. The model and the parameter values we used were developed to explain the relay behavior of aggregation-competent cells (31). The situation in the slug, however, is much more complex. There are different cell types that express at least three different cAMP receptor genes (cAR genes) encoding proteins with different affinities for cAMP (32). cAR3 has a high affinity for cAMP ($K_d \approx 5$ nM), whereas cAR2 and cAR4 have much lower affinity for cAMP ($K_d > 5$ μ M; ref. 32). Some of these receptors show a cell type-specific expression pattern. However, detailed information on expression of these receptors on different cell types and their possible function is not available. We expect that it will be possible to decrease the number of actively relaying cells to the real number of anterior-like cells (10–15%) in the prespore zone (18) by using receptors of different affinities and cell-type localization. The present calculations demonstrate that the principle could work and needs to be seriously considered.

Wave Propagation Can Control Cell Type-Specific Gene Expression in the Slug. Since cell type-specific gene expression is influenced by cAMP, an attractive possibility would be to use the spatial information provided by the cAMP signal propagation system to direct gene expression. Our simulations show that the cAMP relay system, despite its complex modes of wave propagation, produces striking differences in the total amount of cAMP in different positions (Fig. 5A and D). In simulations which form planar waves, there is a high cAMP concentration in the outer region of the prestalk zone and low levels of cAMP in the central core region of the prestalk zone and in the border region between the prestalk and prespore zone. The prespore zone has intermediate cAMP levels. This distribution of cAMP agrees very well with the distribution of cell types in the slug and with the data available for the cAMP requirement for expression of their cell type-specific genes. PstA cells are found in the anterior outer part of the prestalk zone; PstO cells are found at the boundary between prestalk and prespore cells, and PstAB cells, which will form the stalk, are found in the central core of the prestalk zone. Prespore genes need cAMP for their induction and stabilization (13, 28). Expression of the stalk-specific gene *ecmB* by the PstAB cells is inhibited by high concentrations of extracellular cAMP, whereas *ecmA* expression by PstA cells requires high concentrations of cAMP (15, 16). PstO cells are exposed to low levels of cAMP, consistent with the fact that these cells express *ecmA* at a lower level than the anteriorly located PstA cells (8). The cells in the prespore zone are exposed to intermediate levels of cAMP, presumably sufficient to direct expression of prespore-specific genes.

Different species of slime molds show different modes of stalk formation. In some species the stalk is formed along culmination, as in *D. discoideum*. In other species, the stalk is formed continuously during slug migration, as in *Dictyostelium mucoroides* (33). In both cases the stalk is formed as a tube in the middle of the slug. Our simulations of cAMP signal

propagation and the resulting steady-state cAMP levels give a simple explanation for these different modes of stalk formation. Conditions that lead to the formation of twisted scroll waves with cores that extend through the prespore zone could produce a stalk that extends throughout the whole slug (see Fig. 5E and F). This could explain the *D. mucoroides* phenotype. The conversion of scroll waves to planar waves, which produces a core that stops at the prespore prestalk boundary, would explain the *D. discoideum* slug phenotype (Fig. 5B and C).

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