Waves and aggregation patterns in myxobacteria

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Under starvation conditions, a population of myxobacteria aggregates to build a fruiting body whose shape is species-specific and within which the cells sporulate. Early in this process, cells often pass through a “ripple phase” characterized by traveling linear, concentric, and spiral waves. These waves are different from the waves observed during slime mold aggregation that depend on diffusible morphogens, because myxobacteria communicate by direct contact. The difference is most dramatic when waves collide: rather than annihilating one another, myxobacterial waves appear to pass through one another unchanged. Under certain conditions, the spacing and location of the nascent fruiting bodies is determined by the wavelength and pattern of the waves. Later in fruiting body development, waves are replaced by streams of cells that circulate around small initial aggregates enlarging and rounding them. Still later, pairs of motile aggregates coalesce to form larger aggregates that develop into fruiting bodies. Here we present a mathematical model that quantitatively explains these wave and aggregation phenomena.

The morphogenesis of myxobacteria colonies as they form their sporulating fruiting bodies is elaborate and beautiful. Their pattern formation is unlike that of the slime molds whose development is organized by diffusible chemoattractants (1, 2), for the myxobacteria coordinate their movements by direct cell contact (3). In this regard they provide an analog system to study morphogenesis in higher organisms, a process that in many cases involves concentration waves. Still later, pairs of motile aggregates coalesce to form larger aggregates that develop into fruiting bodies. Here we present a mathematical model that reproduces all of the major features of these patterns. The model is based on experimental observations summarized below.

Cell Motility

Individual myxobacteria move by using two types of molecular motors that are concentrated at the cell poles. The two motors are constructed by separate genetic systems. The adventurous (A)-motility motor generates propulsive force by extruding slime from nozzle-like organelles at the posterior pole. The pressure generated as the slime hydrates pushes the cell forwards (5). The social (S)-motility system operates when the cells are in proximity of other cells. Type 4 pili extend from the forward pole, attach to fibrils secreted by nearby cells, and retract, pulling the cell forward (6). Cells change direction not by making a U turn but by exchanging their head and tail motors, so that slime pushing is replaced by pili pulling and vice versa. Because both engines are polar devices, reversal represents a concerted re-polarization of both propulsion systems (3). As fruiting bodies develop, the speed of cell movement and the frequency of reversals change (7, 8).

Cell Signaling

Fruiting body development requires coordinated cell movements. The C-signaling system orchestrates morphogenesis of the fruiting body by modifying the movement behavior of cells. The C-signal is a 17-kDa cell-surface protein that is not diffusible but is transmitted directly by end-to-end contact between pairs of cells (9, 10). Once inserted onto the cell surface, the signal molecule is processed to 17 kDa from the 25-kDa protein product of the csgA gene by a cell-surface protease (11, 12).

In response to a C-signal protein binding to its receptor, the proteins of the act operon increase transcription of the csgA gene. This positive feedback increases the number of C-signal molecules on the cell surface after each signaling event (13, 14). Beginning at 3 h after starvation conditions are initiated the C-signal begins to increase from an average of a few molecules per cell up to several hundred molecules at the time of sporulation (13). The major features of the signal-response circuit are shown in Fig. 1.

The “frizzy” (Frz) system of genes is homologous to chemosensory genes in enteric bacteria (15), and mutations in those genes affect the cell’s reversal frequency (16). C-signal is required to trigger waves and streams at different stages of development via the Frz system. When the density of C-signaling protein on the cells’ surface is low, cells respond to collision and signaling by reversing their gliding direction. This reversal response to the C-signal gives rise to the traveling wave patterns characterizing the ripple phase (Fig. 1) (9, 17–19).

However, the ripple phase is self-limiting because with each C-signaling event, more C-signal molecules are exported to the cell surface. When the number of C-signal proteins on the surface exceeds a threshold, the responding cell decreases its reversal frequency and increases its speed, and streaming commences (7, 8). Streaming behavior can be observed inside nascent fruiting bodies by tracking individual cells. Within an aggregation, cells circulate clockwise and counterclockwise in roughly equal proportions. Within each stream cells do not reverse, but cells outside the aggregate, where C-signaling is less intense, do reverse (20, 21). That an increased C-signal concentration is necessary for streaming is shown by ΔactA and ΔactB mutants that fail to raise the C-signal level to the streaming threshold (13). These mutants continue in wave mode for several days. Cells in a stream have many more opportunities to C-signal each other as they move, and there is more positive feedback (22). With repeated signaling several hundred molecules of C-signal accumulate on the cell surface; the responding cell phosphorlyates most of its FruA, which in turn induces expression of sporulation genes and eventually sporulation itself (13, 23).

Waves and Aggregation in Submerged Cultures

Fruiting body development of Myxococcus xanthus can be followed by using time-lapse photography in submerged agar cultures (3, 17). Fig. 2a shows a frame from a movie showing waves and aggregations (Movie 1, which is published as supporting information on the PNAS web site). As waves counterpropagate along the colony edge, the cells begin to form stationary clusters spaced one ripple wavelength apart along the edge of the culture.

Where each pair of countermigrating wave crests intersect at the high-density edge of the culture, the total cell density is much higher than elsewhere (17). These clusters have roughly equal numbers of cells entering from opposite directions. This creates “traffic jams” of oppositely oriented cells which stop as both...
Isolated bacteria glide for a few body lengths, then reverse direction and retrace their original path. The frequency of reversals is not exponentially distributed, indicating randomly timed reversals; instead, it displays a broad, but peaked, distribution about a mean of \( \approx 8 \) min.

2. C-signaling between cells takes place by cell contact only and is most efficient if the contacts are end-to-end. The frequency of reversals increases with the frequency of cell collisions.

3. Ripple-phase waves cannot exist unless there is a “refractory” period after each signal-induced direction reversal wherein the cells are insensitive to C-signal; during this time, reversal of cell polarity takes place. Also, the response of a cell to C-signaling must be a nonlinear function of the local cell density.

To these characteristics of 1D ripples, we add the following properties to describe aggregation and 2D rippling patterns based on the experimental observations summarized in the Introduction.

4. Cells gliding in the direction of their long axis tend to align for steric reasons. The degree of alignment increases with cell density. The aligned polymers of the slime trails record the cells’ orientation field even when no cells are present. This is consistent with the micrograph shown in Fig. 2b (rightmost panel).

5. Cells tend to jam in regions of high density and at orientation field singularities. The jamming probability goes up with cell density early in fruiting body development.

6. Cells turn perpendicular to a cell density gradient as they enter into, or back out of, high-density traffic jams. This is consistent with the micrograph shown in Fig. 2b (rightmost panel). Experimental evidence for turning is presented in ref. 29.

7. The cell-density dependence of reversal frequency changes to promote streaming as the level of C-signal rises during aggregation, and these streams do not create jams but flow smoothly.

For simplicity, we begin with all cells aligned along the x axis, and we focus on the behavior of a single bacterium. During one reversal cycle, the cell passes through four states (Fig. 3a). A cell sensitive to C-signal moves to the right (s - ). When a cell reverses in response to C-signal, and begins moving to the left, it enters a refractory state (r - ). It emerges from the refractory state and continues to move to the left in a C-signaling-sensitive state (s - ). When it reverses to move to the right once again, it enters a refractory state (r - ).
Transitions between states are denoted by arrows; only the constant speed, orientation of the slime and cells. We assume that cells move at a speed of \( v_0 \) in all states:

\[
\frac{\partial}{\partial t} \mathbf{n}(x, y, t) = \nabla \left( J_{\text{Diffusion}} + J_{\text{Gliding}} \right) + K \cdot \mathbf{n}
\]

where \( \mathbf{n} = (n^r_0, n^s_0, n^r_s, n^s_r, n_0) \) is the vector of cell states shown in Fig. 3b. The transition rates between the states are described by the transition matrix \( K \). The local average cell motion is described by the sum of a convective gliding flux, \( J_{\text{Gliding}} \), and a random (diffusive) flux, \( J_{\text{Diffusion}} \). The exact form of these terms is given in the supporting information.

Because myxobacteria are cylindrical, steric alignment of cells strongly influences gliding motility and dominates dense swarms (30). In addition, cells align with and follow slime trails; thus, our description of cell motion should account for alignment (5, 31). We do this by introducing a vector field, \( \mathbf{T} \), describing the orientation of the slime and cells. We assume that cells move at constant speed, \( \pm v_0 \), so that the cell velocity is given by \( \mathbf{v} = \pm v_0 \mathbf{T} \), and obeys the equation

\[
\frac{\partial T(x, y, t)}{\partial t} = \alpha \nabla^2 T(x, y, t) + \tau \nabla \times (\mathbf{v} \times \mathbf{T})
\]

where both \( \alpha \) and \( \tau \) are functions of \( n_{\text{tot}} \), the local density of cells in all states: \( n_{\text{tot}} = n^r_0 + n^s_0 + n^r_s + n^s_r + n_0 \). The first term on the right side describes the tendency of cells to align with one another when crowded (a steric effect analogous to aligning pencils in a confined space). The second term accounts for the turning torque experienced by cells when moving in a region of impenetrably high cell density such as the edge of a traffic jam.

In our earlier model the reversal cycle was modeled as a continuous phase variable on the circle. The relationship to the current formulation is given in Supporting Methods, which is published as supporting information on the PNAS web site.

The functional form of \( \alpha \) and \( \tau \) are given in Supporting Methods. The relationship of the model to other models (32, 33) describing the motion of oriented objects is also given in Supporting Methods. Table 1, which is published as supporting information on the PNAS web site, gives the numerical values of the model parameters used in the simulations.

**Results and Discussion**

**Waves and Aggregation Patterns at the Colony Boundary.** Time-lapse videos of aggregation in the submerged agar culture show several important features (supplemental materials of refs. 3 and 17). Those features are compared with numerical simulations of Eqs.
1 and 2 in Fig. 4 (corresponding movies are published as supporting information on the PNAS web site).

**Waves.** The density waves in the ripple phase of myxobacteria differ in a fundamental way from those observed aggregating slime molds [e.g., Dictyostelium discoideum (34)]; counterpropagating waves do not annihilate one another but appear to interpenetrate and pass through each other, analogous to (but physically different from) soliton waves in fluids. In our previous analysis of these waves we explained how this phenomenon arises from two features of myxobacteria motion and communication (28). First, cell signaling is by direct contact exchange of C-signal protein, rather than diffusible chemotactic chemicals. Second, the periodic reversal cycle that characterizes individual cell motility manifests itself in collective waves of cell density wherein each cell shuttles back and forth between wave crests. The wavelength, \( \lambda \), is given by

\[
\lambda = 2vT_{rev}
\]

where \( v \) is the mean gliding velocity and \( T_{rev} \) the mean time between reversals (28). The qualitative description of the cells behavior in the waves is given below.

Near the edge of the submerged culture (Fig. 2a), cells are predominately aligned parallel to the boundary, so we can simplify the model in this region by fixing the cell orientation vector to be parallel to the boundary: \( T = (1, 0) \). Most of the cells move in the high-density crests separated by the less dense troughs. Two effects maintain the stability of the waves. Cells in the crests encounter and signal counterpropagating cells entering the crests from the trough, causing them to reverse and join the crest. As two crests traveling in opposite directions collide, all of the sensitive cells receive enough C-signal to eliciting reversal, and the two waves exchange cells (10). Cells that were in a refractory state continue through the collision with their parent wave, so that the outgoing waves are populated by cells from both incoming waves. This effect refocuses the wave at each collision, countering the dispersive effect of randomizing diffusive motion of the cells. The curvature of the boundary produces a wave tilt equivalent to a small (\( \approx 5\% \)) velocity gradient along the y axis. This is because cells closer to the colony center move with higher angular velocities than those near the edge of the colony. Collisions between counterpropagating crests stabilize the tilt at \( \approx 45^\circ \) (Fig. 2).

**Aggregations.** Cells in high-density regions have a tendency to form temporary traffic jams wherein their motion is arrested. The probability of a jammed state increases with cell density. Therefore, jamming is more likely at loci where waves intersect. Experiments show that the initial jams only occur at the boundary of the colony, that is, at “triple points” where wave intersections meet the boundary. As the waves counterpropagate around the periphery of the colony, the intersection points are spaced one-half wavelength apart and move vertically downwards (Fig. 4b and c). Because the cell density is higher where waves intersect, the intersection loci periodically deposit a bolus of cells at the boundary spaced one-half wavelength apart. Each such deposition increases the probability of a traffic jam at these loci of high density.

However, not every triple intersection jam forms an initial aggregate. Cells in a jam are temporarily stalled but, with some probability, can extricate themselves by their back-and-forth motions. If they do succeed in leaving the jam, they glide parallel to the boundary until they encounter another aggregation. Because cells travel a distance \( \approx \lambda / 2 = vT_{rev} \) before reversing, jams that are separated by less than a wavelength can exchange cells. When a cell enters an aggregate, its mean residence time before escaping increases with the size of the aggregate. Thus, cells in larger aggregates tend to accumulate cells at the expense of smaller neighboring aggregates (a process analogous to “rippling” in colloids). Initially, aggregates form where each wave overlap intersects the boundary. However, smaller aggregates fade away and the eventual aggregate spacing tends to be one wavelength. Because this process is stochastic, the spacing is not perfectly regular: the dominant spacing is \( \lambda \) but may be interspersed with aggregates \( \approx \lambda / 2 \) apart. Further ripening proceeds as jams are resolved and aggregates turn into swirls capable of moving along the boundary. We address these phenomena below.

**2D Patterns in the Ripple Phase.** The waves discussed above are essentially 1D near the culture periphery. However, in Kunert-type submerged cultures the ripple phase develops both parallel as well as concentric and spiral waves, as shown in Fig. 5 (see also ref. 10). In these complex patterns, waves appear to pass through one another as in the planar waves discussed above. Myxobacterial cells tend to align with one another, and with the slime trails on which they glide. The tendency to align is modeled by diffusion of orientation in Eq. 2. That is, diffusion of density tends to make the density homogeneous; similarly, diffusion of orientation tends to make cell orientations homogeneous. This is equivalent to assuming that each cell’s orientation relaxes to the average orientation of the surrounding cells (see Supporting Methods and ref. 33). Because the orientation field is remodeled by gliding cells, the time for orientation relaxation increases with total cell density (see supporting information for details). Fig. 5 shows that the model accurately captures these features, along with the interpenetration of counterpropagating waves.

**Streaming and Swirling During Aggregation.** As described in the Introduction, the increased number of C-signal molecules per cell changes the cells’ responses from rippling to streaming. In streams, reversals are suppressed and wave behavior ceases (7, 35). Streams in the myxobacterium Chondromyces crocatus are frequently seen to coalesce into rotating swirls, as shown in Fig. 6a (18, 27). To model this behavior we modify the reversal frequency so that it is a decreasing function of the density of cells moving in the same direction, producing a decrease in reversal.
cells streaming into larger aggregates can be seen. As time progresses aggregates grow and absorb smaller ones.

To summarize, to model fruiting body formation in the colony center (in Kuner-type submerged culture) the model must combine (i) initiation by jamming in regions of high density, (ii) orientation field singularities, (iii) suppression of reversals in streams, and (iv) a dynamic orientation field. Rounded (convex) aggregates require that cells turn perpendicular to a density gradient as they enter into, or back out of, high-density aggregates.

Conclusions

We have constructed a computational model that accurately describes the 2D waves and aggregation morphogenesis of myxobacteria. The model accounts for the observed planar, concentric, and spiral rippling fields. Experimentally, aggregation commences at the very high cell density regions of the culture periphery where two wave crests interpenetrate. The model explains the position and spacing of the aggregates of *M. xanthus*. It accounts for the enlargement of the initial aggregates, and for their conversion to the observed motile aggregates that merge and organize circumferentially. The model also explains the swirling motions that are pronounced in *C. crocatus*. Finally, it is consistent with the 3D spherical shape of *M. xanthus* fruiting bodies.

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