MODELLING *DICTYOSTELIUM DISCOIDEUM* MORPHOGENESIS

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Abstract. Morphogenesis of the social amoebae *Dictyostelium discoideum* results from the aggregation of individual cells to form a multicellular hemispherical cell mass, the mound. In the mound the cells differentiate into several cell types. These cell types arise initially in random location in the mound, but then sort out from one another to form a slug. In the slug these cell types are arranged in a simple axial pattern. The slug can migrate and under suitable conditions transforms into a fruiting body consisting of a stalk supporting a mass of spores. It is well established that cells aggregate in response to propagating waves of the chemoattractant cAMP. There is increasingly good experimental evidence that the later stages of morphogenesis are also controlled by cAMP wave propagation and chemotaxis. Here we present a hydrodynamic model to describe *Dictyostelium* development from early aggregation up to migrating slug. We consider the population of cells as an excitable medium, which supports propagation of waves of the chemoattractant cAMP. To model the chemotactic cell movement we consider the masses of moving cells as a fluid flow. The morphogenesis of this multicellular organism is basically modelled as shape changes occurring in a drop of liquid with a free surface. At the mound stage this liquid consists of two randomly mixed component fluids corresponding to two cell types. Cell sorting can be effectively modelled as the separation of the component fluids driven by differential chemotaxis. Finally, our model calculations show that migration of the slug can result from chemotactic flows inside the slug.

1. Introduction. Morphogenesis, i.e. the generation of form is central to biology. Form is most often generated during the embryonic development of organisms. In higher organisms development starts from a fertilised egg that goes through a great number of cell divisions to generate more cells. These cells differentiate into many different cell types. Sometimes they differentiate *in-situ*, however in many cases they also form in one place, start to differentiate, and then move to their final destination. Besides cell division, programmed cell death plays an important role in the shaping of the embryo. All these processes have to be precisely co-ordinated in space and time to reproducibly result in a functional adult, with its characteristic shape. These processes and their co-ordination are clearly very complex and in most cases not well understood. We have therefore concentrated on understanding the cellular principles governing morphogenesis of a relatively simple organism, the social amoebae *Dictyostelium discoideum*. This organism forms by the chemotactic aggregation of single cells, which are generated during a unicellular growth phase. In *Dictyostelium*, growth and development occur in separate parts of the life cycle and once development is initiated there is no significant cell death until terminal differentiation

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of the stalk cells. Furthermore the cells only differentiate into a limited number of cell types. This implies that development is the result of the co-ordinated movement of individual differentiating cells. All these characteristics make *Dictyostelium* a prime object for the study of the principles controlling simple multicellular morphogenesis (Maeda et al., 1997).

Dictyostelium morphogenesis is initiated by chemotactic aggregation of free living single amoebae (Fig. 1) (Loomis, 1982). During the initial phase of aggregation some cells start to produce and secrete cAMP in a periodic fashion. This cAMP diffuses away to excite neighbouring cells. These cells detect the cAMP via specific transmembrane cAMP receptors and upon stimulation start to produce and secrete cAMP themselves and thereby in turn excite their neighbours. This is the so-called cAMP relay response. cAMP binding to the surface receptor also induces an adaptation process which results in a shutting down of cAMP production. This adaptation process ensures the outward propagation of cAMP waves, since cells which have just relayed are refractory to further stimulation. The cells also secrete an extracellular phosphodiesterase which breaks down cAMP and enables the cells to de-adapt and regain sensitivity to further stimulation. These processes result in the repeated outward propagation of cAMP waves from the place of initiation. Since the cells are also chemotactically sensitive to cAMP and move up gradients as long as the concentration is increasing in time, they move in the direction of the signal source and accumulate at the site of wave initiation. After a while the aggregating cells form patterns of bifurcating streams, in which the cells move in a directed fashion towards the aggregation centre. In the aggregates the cells start to differentiate into prestalk and prespore cells. Differentiated cells sort out so that the aggregate (mound) transforms into a polarised cylindrical shaped structure, the slug. During slug formation the precursor cells of the later stalk, the prestalk cells, sort out to the anterior end of the slug and a sub population of prestalk cells forms the tip which guides all further morphogenesis. The slug falls over and migrates away, guided by signals from its environment such as light and temperature gradients. Under the influence of the right environmental signals (light and low humidity) the slug transforms into a fruiting body consisting of stalk and spore cells. The stalk cells are dead and vacuolated while the spores survive and await favourable conditions to germinate and release single amoebae again.

There have been a number of models describing different stages of *Dictyostelium* development. The streaming phenomenon in aggregation fields has puzzled many researchers for over 30 years (Höfer and Maini, 1997; Höfer et al., 1995; Keller and Segel, 1970; Levine and Reynolds, 1991; Mackay, 1978; Nanjundiah, 1973; Novak and Seelig, 1976). Models devoted to this phenomenon have been studied numerically and analytically and theoretical mechanisms responsible for stream formation have been suggested. It was shown that streams can occur due to an instability in cell distribution due to a dependence of the velocity of the chemoattractant

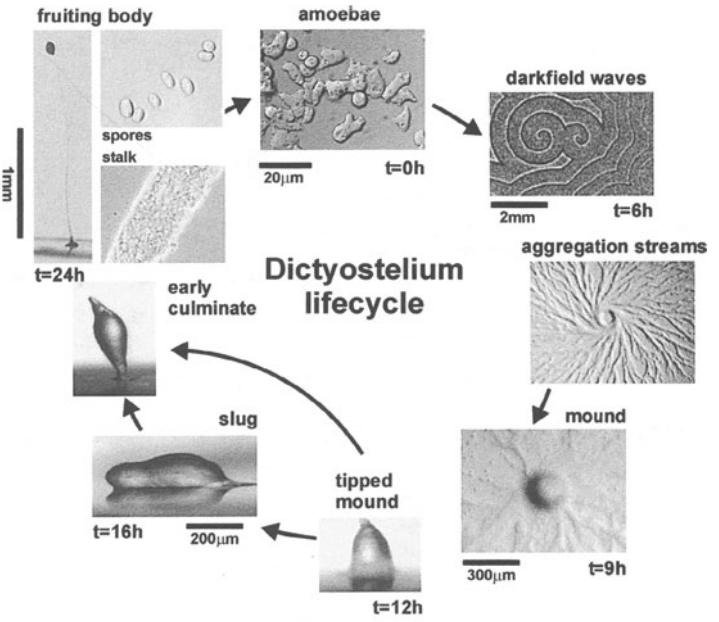

FIG. 1. *The Dictyostelium discoideum life cycle. Shown are in a clockwise order starting at the top, vegetative amoebae, darkfield waves, as observed during aggregation (they reflect the cells in different phases of the movement cycle in response to cAMP waves), aggregation streams, a top view of a mound with incoming streams, a side view of a tipped mound, a side view of a migrating slug and an early culminate and a fruiting body with on its side high magnification images of the stalk cells and spores. This developmental cycle is starvation induced and takes 24 hours at room temperature.*

waves on the density of cells (Vasiev et al., 1994, van Oss et al., 1996;). With the formation of streams and the mound, i.e. when cells get closely packed, mechanical interactions between cells become as important as chemical signalling. Different ways to model these interactions have been proposed in a number of models describing the formation of the mound or migration of the slug (Odell and Bonner, 1986; Bretschneider et al., 1997; Levine et al., 1997; Savill and Hogeweg, 1997). Experimental observations of the mode of cell movement have shown them to be periodic at the aggregation stage when the cells are still single and more continuous at the mound stage (Rietdorf et al., 1996; Siegert et al., 1994; Varnum et al., 1986; Varnum-Finney et al., 1988). These observations suggest that a good way to describe cell movement in the mound is by considering the mound as a drop of liquid, the cells as fluids, and their motion as a flow, which is initiated by chemotactic forces and affected by pressure and viscosity.

We show here that such a hydrodynamic approach can be used to model aggregation, mound formation, cell sorting, slug formation and slug

migration (Vasiev et al., 1997; Vasiev and Weijer, 1999). Our model describes the cAMP relay response and resulting cAMP wave propagation as the propagation of a chemical signal in a generic excitable medium and the chemotactic cell movement of the amoebae in response to the signal as a flow of a fluid. We begin from randomly distributed cells on a plane, which in the course of aggregation form bifurcating aggregation streams and then collect into a three dimensional hemispherical mound. We do not take into account the signals responsible for the differentiation of the cells but we assume that the mound consists of two mixed liquids, corresponding to the two cell types, prestalk and prespore cells. Both liquids are chemotactically responsive to cAMP. They respond with rotational movement to a counter rotating scroll wave of cAMP in the mound. We show that sorting of prestalk cells to the top of the mound (while the prespore cells occupy the rest of the mound) takes place when the excitability of prestalk cells and their chemotactic movement is higher than that of prespore cells. In our model there is a natural transformation from the mound into a cylindrical slug. The slug once fallen over migrates. Migration is driven by internal cell flows, which gain traction from the substrate.

2. Model. To model propagation of cAMP waves during *Dictyostelium* development we use the FitzHugh-Nagumo equations, which are widely known as describing a prototype excitable medium:

$$\frac{\partial g}{\partial t} = D\Delta g + \rho\Big(k_g g(g-0.05)(g-1) - k_r r\Big) \tag{1}$$

$$\frac{\partial r}{\partial t} = \frac{(g-r)}{\tau}\,. \tag{2}$$

Here g is assumed to define the level of extra-cellular cAMP, and r is the fraction of active cAMP receptors (Martiel and Goldbeter, 1987) or activated α subunits of the inhibitory G-proteins (Tang and Othmer, 1995; Tang and Othmer, 1994). D is the diffusion coefficient for cAMP; τ is a time scaling factor for the variables r and g. k_g and k_r define the rate of cAMP production and hydrolysis respectively. Locally, the rate of cAMP production and decay is proportional to the density of cells ρ (Levine and Reynolds, 1991; Vasiev et al., 1994).

Cell movement is described by the Navier-Stokes equation:

$$\rho\left[\frac{\partial \mathbf{V}}{\partial t} + (\mathbf{V}\,\mathrm{div})\mathbf{V}\right] = \mathbf{F}_{ch} + \mathbf{F}_{fr} + \eta\Delta\mathbf{V} + \left(\xi + \frac{\eta}{3}\right)\mathbf{grad}\,\mathrm{div}\,\mathbf{V} + \mathbf{F}_{ad} - \mathbf{grad}\,p \tag{3}$$

This equation defines the acceleration of the cells under the influence of various forces given in its right hand side. $\mathbf{V}$ is the velocity of the cells. $\mathbf{F}_{ch}$ is the chemotactic force, which is active on the rising front of the cAMP waves, $\mathbf{F}_{fr}$ is a friction force responsible for slowing down cell movement,

The third and fourth terms on the right hand side describe cell-cell friction: η and ξ are, respectively, the first and second viscosity coefficients. $\mathbf{F}_{ad}$ takes into account cell-cell and cell-substrate adhesion forces; ρ is the pressure due to the chemotactic accumulation of the cells. Equation (3) is given here in the most common, full notation. While modelling different stages of *Dictyostelium* morphogenesis we will use different modifications (reductions in complexity) of the right hand side.

We assume that chemotactic force is proportional to gradient of cAMP:

$$\mathbf{F}_{ch} = K_{ch}\left(\frac{\partial g}{\partial t}\right)\mathbf{grad}\, g \tag{4}$$

where K_{ch} is equal to zero when $\frac{\partial g}{\partial t} \leq 0$ and to a positive constant when $\frac{\partial g}{\partial t} \geq 0$. This step-wise function allows to distinguish a front of chemoattractant wave where cells are chemotactically active from its back where cells do not exhibit chemotactic response (Futrelle, 1982; Futrelle et al., 1982). The friction force is assumed to be proportional to velocity:

$$\mathbf{F}_{fr} = K_{fr}\mathbf{V} \tag{5}$$

where K_{fr} is a negative constant. In some computations adhesion was needed to keep the aggregate/slug attached to the substrate. It was treated as a force directed towards the substrate. This force should be considered as adhesion and is not a gravitational force. When plates with aggregates or slugs are turned upside down they keep developing/migrating in a manner indistinguishable from normal.

The last term in the right hand side of (3) is a force generated by a pressure field in the mound. This force is responsible for limiting the increase in cell density caused by chemotaxis in case of a compressible liquid. Here the pressure is assumed to be proportional to cell density. In the case of an incompressible liquid, it keeps the density constant. Pressure allows cells to reorient the direction of their motion so that they not only move towards the source of chemotactic signal, resulting in mound formation and its shape changes.

While dealing with a compressible liquid we calculate density of cells using the equation of conservation of mass:

$$\frac{\partial \rho}{\partial t} = D_\rho \Delta \rho - \operatorname{div}(\rho \mathbf{V}) \tag{6}$$

The first term on the right hand side of the equation describes the random motion of the cells, while the second term describes co-ordinated (chemotactic) cell movement.

Cell sorting and slug migration can be best described as flows in a heterogeneous incompressible liquid, consisting of two kinds of fluids. These fluids correspond to prestalk and prespore cells and each fluid is characterised by its volume fraction, α_1 and α_2:

$$\alpha_1 + \alpha_2 = 1 \text{ inside the mound;} \qquad \alpha_1 + \alpha_2 = 0 \text{ outside the mound} \tag{7}$$

To model differences in excitability between prestalk and prespore cells we assume that they differ in their rate of cAMP production:

$$k_g = k_1\alpha_1 + k_2\alpha_2 \tag{8}$$

where k_1 and k_2 define the rate of cAMP production by each cell type.

We model differential chemotactic movement by introducing parameters, K_1 and K_2, which define the chemotactic force developed by prestalk and prespore cells:

$$K_{ch} = K_1\alpha_1 + K_2\alpha_2 \tag{9}$$

Consequently the velocities of prestalk, $\mathbf{V}_1$, and prespore, $\mathbf{V}_2$, cells are different. They are obtained from the momentum balance equation for each component-liquid:

$$\rho\alpha_i\left(\frac{\partial \mathbf{V}_i}{\partial t} + (\mathbf{V}_i \operatorname{div})\mathbf{V}_i\right) = \mathbf{F}_i + \eta\alpha_i\Delta\mathbf{V}_i - \alpha_i \mathbf{grad}\, p \tag{10}$$

where the index $i = 1, 2$ denotes prestalk or prespore cells, $\mathbf{F}_i$ – corresponds to chemotactic forces $\mathbf{F}_i = \alpha_i K_i\left(\frac{\partial g}{\partial t}\right)\mathbf{grad}\, g$. The effects of viscosity and pressure are proportional to their volume fractions. Volume fractions for prestalk and prespore cells are found using the equation for the conservation of mass:

$$\frac{\partial \alpha_i}{\partial t} = -\operatorname{div}(\alpha_i \mathbf{V}_i) \qquad \text{where } i = 1, 2 \tag{11}$$

We do not include a diffusion term in (11) since random motion at this stage of development is small compared to chemotactic movement. In addition in (6) it was necessary for the stability of the computations but we find that (11) is stable without this term.

In principle the velocity obtained from (3) and the velocities from (10) and volume fractions from (11) should satisfy the following equation:

$$V = \alpha_1 V_1 + \alpha_2 V_2\ . \tag{12}$$

Our calculations showed that this is true during the early stages of the computations but that the difference between the left and right side of the equation increased over the course of the simulations up to 20% for Figure 3 and up to 10% for simulations shown in Figure 4 and 5. The inaccuracy stems from (10), which is a simplified version of the full equation from which we removed all terms involving derivatives of volume fractions, which result in instability of the computations.

All calculations were performed in three-dimensional domains using the finite volume method. Equations (1), (2) were integrated by the Euler explicit method using a forward time centred space method for the diffusion term. Equation (3) was integrated explicitly using forward time centred

space method for diffusion term and the upwind method for the convective term (Press et al., 1988). In the case of an incompressible liquid it was integrated by the two-step projection method (Kothe et al., 1991) using a simultaneous over-relaxation scheme (SOR) for the pressure Poisson equation (PPE). Equations (10), (11) were integrated explicitly, using the upwind method for the convection terms and taking values for pressure, p, from solution of equation (3). The location of the free surface was determined by the level $\rho = 0.5$ in the compressible liquid model or detected by tracking massless particles distributed in the volume of the mound (MAC method (Harlow and Welch, 1965)) in the incompressible liquid model.

For the cAMP concentration (1), density (6) and volume fraction (11) fields we have used Neumann "no flux" boundary conditions at the boundary of the medium as well as at the free boundary of the aggregate. For the velocity fields (3), (10) we used both no flux (Neumann) and no slip (zero value) boundary conditions (on the free boundary of the aggregate and free-slip (zero value for the normal component and Neumann condition for the tangential components) conditions on the boundaries of the medium. For pressure in (3) we used zero value boundary conditions on the free boundary of the aggregate.

3. Results.

Aggregation streams and mound formation. We simulate *Dictyostelium* morphogenesis up to the mound stage treating the population of cells as an inviscid compressible liquid (Vasiev et al., 1997). At this stage of development the cells move towards each other and thereby increase in density. Therefore compressibility is essential. Since most of cells are separated from each other and do not interact mechanically, we neglect viscosity in a first approximation. We initiate a spiral cAMP wave in the two- dimensional field of randomly distributed cells (Fig. 2). This wave causes periodic changes in cell movement and results in the formation of aggregation streams. Bifurcating aggregation streams form due to the dependence of wave propagation speed on the cell density (Höfer and Maini, 1997; Levine and Reynolds, 1991; Vasiev et al., 1994). As more cells move towards the centre, a hemispherical mound forms. The pressure p between the cells is responsible for the mound formation. It increases during aggregation and forces the cells up into the third dimension. The aggregation patterns observed in the simulations are remarkably similar to those from real experiments (compare aggregation patterns and mound in Figs. 1 and 2). Experiments have shown that during aggregation there is a decrease in the period and propagation speed of the cAMP waves which results in a decrease in the wavelength of the spiral wave (Gross et al., 1976; Siegert and Weijer, 1989). This behaviour is also observed in the model calculations (wavelength in Fig. 2 decreases from A to D). Including a viscous term in the computations basically does not alter the overall phenomena but results

in wider aggregation streams that look less similar to the experimentally observed aggregation patterns.

Different phenotypes of aggregation patterns observed in experiments can be simulated by variation of model parameters. For example, decreasing the excitability of the medium in the computer simulations by varying the rate of cAMP production leads to a large cell free region in the centre of the aggregates. This is very similar to the effect of caffeine seen in experimental conditions, which is known to decrease the excitability of the cells by inhibiting their cAMP production (Brenner and Thoms, 1984; Siegert and Weijer, 1989). To explain this phenomenon we have to take into account that the cell free region in the centre of the aggregate represents the core of spiral wave of chemoattractant. Decrease in the excitability of the medium results in an increase of the core so that the latter can become enormously large. Simulations show that, in addition, excitability effects the behaviour of the mound. A mound made of a very low excitability is not stable and exhibits oscillatory motion (meandering), which is also observed in experiments (Vasiev et al., 1997).

Cell sorting in the mound. Contrary to early aggregation, cells in mounds and slugs constitute a compact body, therefore we treat them as an incompressible liquid in which viscosity plays an important role. To study cell sorting we have performed computations starting with a hemispherical mound (drop of incompressible viscid liquid) consisting of two cell types that are initially randomly distributed. The most realistic cell sorting patterns are obtained when the cell types differ in velocity and excitability, i.e. such that prestalk cells are faster and more excitable compared to prespore cells (Vasiev and Weijer, 1999). An example of cell sorting using these conditions is shown in Fig. 3. A scroll wave of cAMP rotating in a hemispherical mound causes cell movement in the mound. Cells tend to move inward towards the core of the scroll. There is competition for the space in the middle of the mound between cells of different type. Faster cells, which are able to move more effectively win this competition and accumulate in the middle of the mound. In the middle of the mound there is an upward flow and most of the faster cells move further up and finally form a plume-like structure pointing to the top surrounded by slower cells. If the cell types only differ in their velocity, cell sorting stops at this stage. If the cell types differ, in addition, in excitability, the structure formed by highly excitable cells deforms the shape of the scroll wave. The plume-like structure formed by prestalk cells results in an anisotropy in the mound, i.e. the top of the mound becomes more excitable than its bottom. As a result the scroll wave becomes twisted and gets a new downward component. Waves propagate from the top down, which leads to further accumulation of the fast moving cells on top and elongation of the mound upwards causing further upward cell flows in the mound. There is again the competition

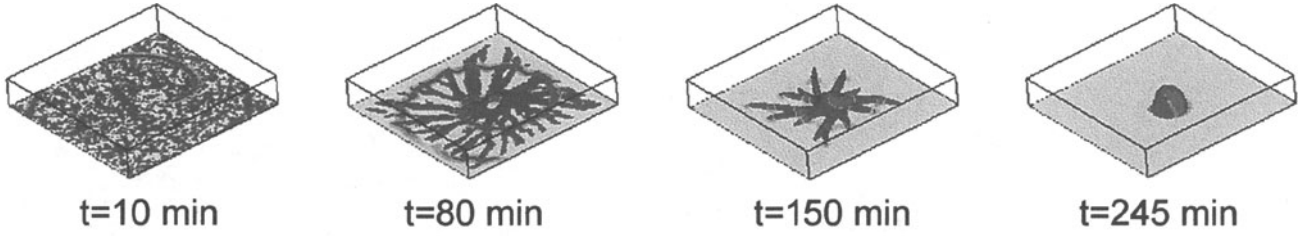

FIG. 2. Development of *Dictyostelium* from single cells to the mound stage. *Successive images of aggregation as calculated by the model. The cell density is shown as an iso-surface* ($\rho = 0.5$) *and the cAMP concentrations are mapped on this surface from low cAMP (blue) to high cAMP (red). The initial density of cells was zero everywhere in 3d-space except for the bottom plane. A random number varying between 0 and 1 represented the cell density in each grid of this plane so that average density in this plane was equal to 0.5. In response to cAMP spiral waves cells move and form aggregation streams* ($t = 40$–200 *min) and then mound* ($t = 250$ *min) which represents a stable solution of the system. Model parameters:* $\tau = 5$; $k_g = 10.5$; $k_r = 3; \eta = 1$; $D = 1$; $\xi = 0.5$; $D_\rho = 0.005$; $K_{ch} = 0.2$; $\mathbf{F}_{fr} = -\mathbf{V}$; $\mathbf{grad}\, p = 0.01\mathbf{grad}\, \rho$; *space and time steps* $h_x = 1.2$; $h_t = 0.01$; *size of the medium:* $100 \times 100 \times 20$ *volume elements. Scaling to real time is made assuming that a time unit corresponds to 15 sec.*

for the space on the top of the mound. Finally all the faster cells collect at the top of the mound and form a tip.

During this process the period of the scroll wave decreases from 48 to 21 time units (or from 4.8 to 2.1 min according to our scaling). The mound's shape also changes over time: the hemispherical mound elongates and gradually transforms into a cylindrical slug.

Slug migration. To study slug migration we have performed computations starting with a cylindrical slug, which consists of two cell types: 20% of prestalk cells located at the anterior end of a cylinder and 80% of prespore cells occupying the more posterior positions. We have checked the modes of slug migration driven by a pacemaker located at leading edge of a slug and a twisted scroll wave rotating inside the slug.

Migration of a slug controlled by pacemaker at its anterior end. Let us assume that the movement of the slug is controlled by waves of a chemoattractant, which are initiated by pacemaker located in the tip of the slug. For simplicity we will first consider a case where there is no difference between cell types or, in other words, a slug consisting of only one cell type. The pacemaker is simulated by the repeated external stimulation of a small area in the tip of the slug. The behaviour of such a slug is shown in Fig. 4. Waves of chemoattractant originate in the tip and propagate along the slug axis backward, while the slug migrates forward in the direction of the pacemaker. The shape of the slug is gradually changing: it remains more- or-less cylindrical, however it gets narrower at the anterior and posterior ends and becomes more similar in shape to experimentally observed slugs.

Simulations where differences in the excitable properties of the cell types are taken into account show that all the results described above do not change. Variations of excitability as described in the model section do not affect the motive forces. However, when motive force generated by prestalk and prespore cells differ from each other, the behaviour of the slug changes dramatically. The tip and the tail of the slug are moving at different speeds. The slug is gradually elongating until the prestalk and prespore zones are completely separated. After this occurs, only the prestalk zone is moving since it is the only part containing a pacemaker. Such a phenotype has also been observed experimentally, when slugs are placed on cAMP containing agar. The prestalk zone keeps moving, while the prespore zone is immobilised (Weijer et al., unpublished observations). This effect is most likely due to the quantitative differences in phosphodiesterase produced by prestalk (more) and prespore cells (less), the higher amounts of adenylate cyclase, the enzyme that produces cAMP, and the lower affinity of the cAMP receptors in prestalk cells (Firtel, 1996; Parent and Devreotes, 1996). This could result in higher amplitude cAMP oscillations in the prestalk zone of the slug compared to the prespore zone and make the cAMP signalling in the prespore zone more sensitive to inhibition by external cAMP.

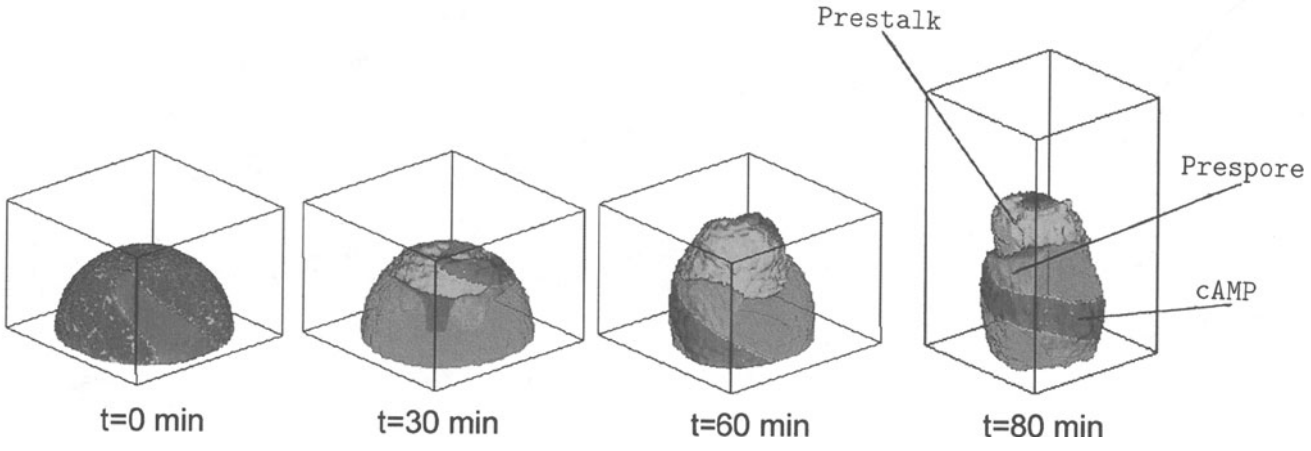

FIG. 3. *Cell sorting in the mound. The mound consists of 20% of prestalk cells (yellow) and 80% prespore cells (blue). The cell types differ in chemotactic velocity ($K_1 = 2$ and $K_2 = 1$ in Equation (9)) and in excitability $k_1 = 6.0$ and $k_2 = 5.4$ in Equation (1)). Initially the mound is a hemisphere in which a cAMP scroll wave (purple) is initiated and rotates clockwise, and both cell types are randomly mixed. Affected by the cAMP waves the cells move and sort, so that the prestalk cells collect at the top of the mound and form a tip. In the course of time hemispherical mound elongates and transforms into a cylindrical standing slug. The model parameters:* $\tau = 4$; $k_r = 1.5; K_{ch} = 0.1$; $D = 1$; $\rho = 1$; $\eta = 1$; $\mathbf{F}_{fr} = 0$; *(friction is delivered by zero value boundary conditions) space and time steps* $h_x = 0.6$; $h_t = 0.06$. *Size of the medium is* $70 \times 70 \times 50$ *volume elements. Scaling to real time is made assuming that a time unit is equal to 6 sec.*

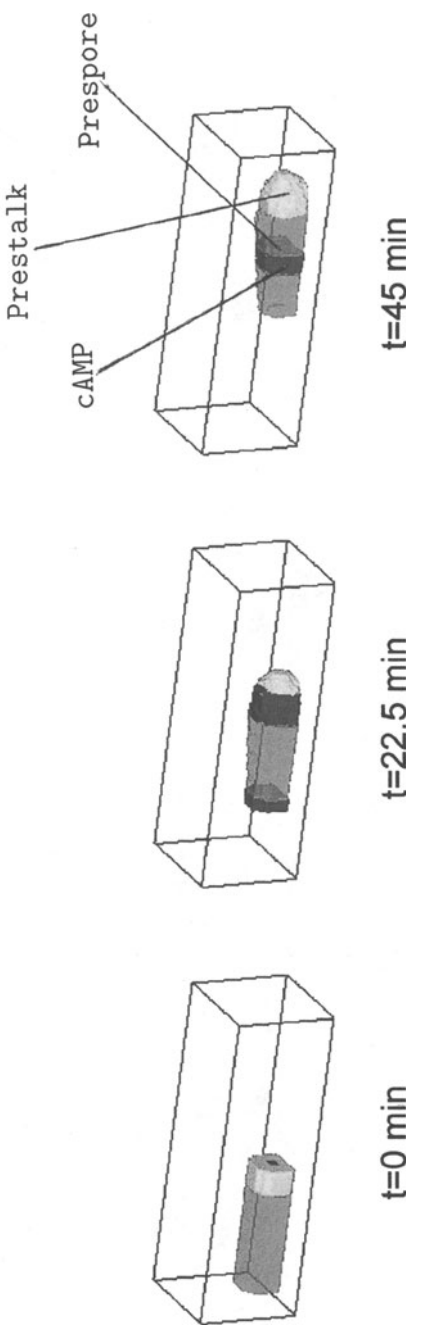

FIG. 4. *Slug organised by a pacemaker located in its tip. The slug is homogeneous, i.e. there are no differences between prestalk (yellow) and prespore (blue) cells:* $k_1 = k_2 = 5.4$ *in (1) and* $K_1 = K_2 = 2$ *in (9). Initially the slug is represented by a cylinder with diameter 180μm (20 volume elements) and length 540μm. Waves (represented by a purple isosurface) are initiated by external stimulation of a small group of cells in the slug tip. The medium is* $40 \times 40 \times 150$ *volume elements. Model parameters are the same as in Fig. f3, except* $\mathbf{F}_{fr} = 0.1$ *and Neumann boundary conditions are used for velocity on the free surface of the slug.*

Migration of a slug organised by a scroll wave of cAMP. There are many indications that the cell flows in a mound result from a rotating scroll wave of cAMP. As it was shown above (Fig. 3) such a scroll wave twists during the course of slug formation, so that it has a component of velocity directed from the tip to the back. This twisted scroll can persist in a migrating slug since a slug is axially asymmetric with respect to its excitability: anterior prestalk cells are more excitable than posterior prespore cells. Such a twisted scroll wave can also organise the motion of a slug. Results of simulations with a twisted scroll wave initiated in a slug are shown in Fig. 5. Since the scroll originates in the tip, the slug migrates in the direction pointed at by the tip. The slug's shape changes over time in a way similar to that observed for a slug organised by a point source. The tip of the slug lifts off the substrate, similar to what is often observed in experiments. In these simulations the chemotactic force for prestalk and prespore cells is the same. In further computations we found that a decrease in chemotactic force (50%) for prespore cells resulted in an elongation of the slug but does not result in it breaking. This suggest a mechanism for the regulation of the slug shape which can be vastly different under different experimental conditions and between different strains.

How do cells get traction ? We will now address the following problem: how do cells inside mounds and slugs gain traction to move in a way described in the model section. When a cell has a contact with a substrate there are no difficulties with traction, it is derived from the substrate. However in mounds or in slugs most cells have no direct contact with the substrate. Odell and Bonner many years ago made the appealing assumption that cells gain traction locally from their direct neighbours (Odell and Bonner, 1986). Since the cells were all motile, a cell moving forward pushed back other cells. This would result in no net forward movement. Therefore they introduced a second factor produced by all cells, which modulated their motility. This resulted in cells in the centre moving slower than in the periphery. This assumption led to circulating cell flows in the slug, which are not in agreement with our successive experimental work in which we showed that these flows do not occur (Abe et al., 1994; Siegert and Weijer, 1992). Furthermore a fountain-like motion would require continuous cell sorting to keep the axial distribution of cell types in a slug upright, which is also not in agreement with experimental observations.

One way to avoid these problems is to assume that cells can gain traction also from distant neighbours. For example, to assume that long ranging cell-cell interactions exist. This links up cells over larger distances and introduces solid-like properties in the slug. Via such a mechanism accelerating cells can develop traction from a significantly larger area. As an extreme case we can assume that each cell gains traction from the whole slug, which delivers the reaction forces to the substrate. This assumption would perfectly agree with our formal description of chemotaxis. In addi-

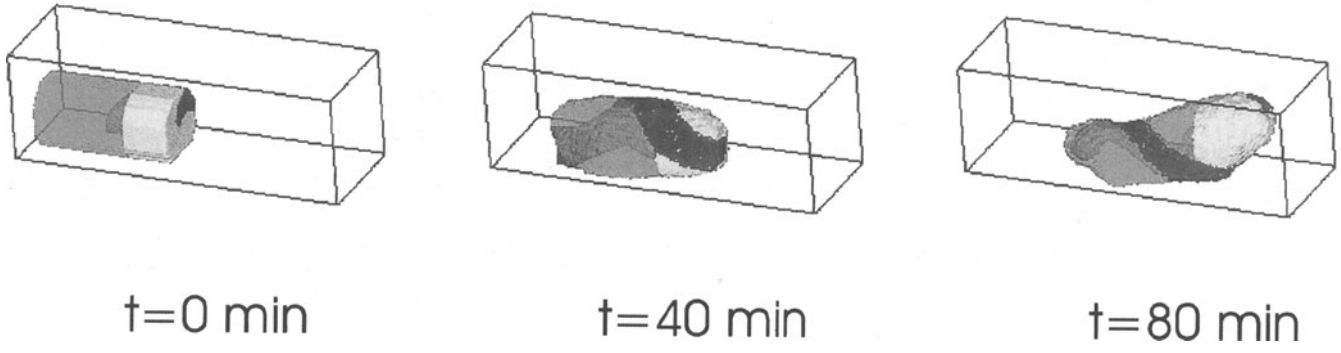

FIG. 5. *Slug organised by a twisted scroll wave of chemoattractant. Model parameters are the same as in Fig. 4 except:* $k_1 = 6.0$ *and* $k_2 = 5.4$ *in (1) the same values used in Fig. 3. The diameter of the slug is 270μm. A twisted scroll wave is initiated in the slug, it twists because there is a difference in excitability between the prestalk and prespore cells. The adhesive force* $\mathbf{F}_{ad}$ *is directed towards the substrate and equal to 0.01.*

tion, it would mean that no matter whether a cell is isolated or in contact with other cells, it exerts the same force in response to the same chemotactic signal. In reality we think that cells are made up of a "stiff" internal actin cytoskeleton which links to the substrate and neighbouring cells via specific adhesion molecules. A cell moves by locally extending its internal actin cytoskeleton in the direction of a chemotactic signal. This extended part now becomes stiff and new contacts are made with neighbouring cells. Extension obviously has to be co-ordinated with actin depolymerisation, release of adhesive contacts and retraction at the back end of the cell. These processes are co-ordinated over many cells by the propagating waves of cAMP and therefore result in waves of co-ordinated motion.

4. Conclusion. Slime mould morphogenesis results from propagation of cAMP waves, which control the chemotactic movement of individual amoebae. The main assumption made in this paper is that we can consider cell movement as the flow of a liquid. We have shown that this approach can be used successfully to describe formation of aggregation pattern and mound, cell sorting, transformation of mound into the slug, and migration of slug. A hydrodynamic description of the cell-cell interactions by pressure and viscosity terms seems to work as a good approximation. Unfortunately a quantitative comparison of our model parameters with experimental values is not yet possible since it has not yet been possible to measure chemotactic and adhesive forces produced by the cells quantitatively. Likewise viscosity of slug tissue and pressure inside the slug are unknown. Performing quantitative measurements of these parameters clearly has to be a prime experimental research objective.

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