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Modelling of *Dictyostelium discoideum* slug migration

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Abstract

The development of most multicellular organisms involves differential movement of cells resulting in the formation of tissues. The principles governing these movements are poorly understood. One exception is the formation of the slug in the social amoebae *Dictyostelium discoideum*. The slug forms by the chemotactic aggregation of up to 10^5 starving cells, it is motile and migrates in response to light and temperature gradients to the surface of the soil to form a fruiting body consisting of a stalk supporting a spore head. Slug migration and behaviour result from coordinated chemotactic movement of the individual cells in the slug. Waves of a chemoattractant, most likely cAMP, are periodically initiated in the tip of the slug and propagate towards the back of the slug resulting in periodic forward movement of individual cells as well as the whole slug. Here we develop a model to investigate how wave propagation and cell movement interacts to result in migration and shape changes of the slug. The slug tissue is modelled as an incompressible liquid, in which waves of chemoattractant are generated in an excitable manner. The liquid is "active", i.e. it is able to generate body forces in response to the gradients of the chemoattractant. These forces lead to the formation of flows (representing chemotactically moving cells) and result in slug movement and shape changes. The model provides a theoretical framework for the understanding of the interactions between cell–cell signalling and cell movement, which govern slug behaviour and tissue morphogenesis.

Keywords: Chemotaxis; Cell movement; Excitable medium; Computational fluid dynamics

1. Introduction

Cell movement plays an essential role in the development and morphogenesis of many organisms. During embryogenesis it is central to processes such as gastrulation, formation of the nervous system and organogenesis, while in the adult it plays a major role in wound healing and function of the immune system. In many cases such as gastrulation, the movement of cells is highly coordinated, however the primary signals and mechanisms controlling movement are largely unknown. Therefore there exist as yet little insight in the principles coordinating cellular movement behaviour at the tissue level. There is one notable exception to this, the multicellular morphogenesis of the cellular slime mould *Dictyostelium discoideum*. *Dictyostelium* morphogenesis is initiated by chemotactic aggregation of free living single amoebae into multicellular aggregates (mounds). In the mound the cells differentiate into a number of prestalk cell-types and prespore cells. These cells are precursors of the stalk cells and spores that make up the final structure, the fruiting body (Kessin, 2001). The fruiting body consists of a stalk made from dead vacuolated cells, which supports a spore head containing thousands of individual spores. The prestalk and prespore cells start to differentiate in the mound in a salt-and-pepper pattern and the prestalk cells then sort out to form a distinct morphological structure, the tip (Williams, 1995). The tip directs the formation of the standing slug, which falls over and migrates away. The slug is photo- and thermotactic and migrates up light and temperature gradients to reach the surface, where it culminates and forms a fruiting body.

Slug movement and shape changes result from differential movement of individual cells in the slug. Prestalk cells move straight forward in the direction of slug migration or more often in a rotational fashion around slug's long axis so that they form a vortex of cell flows, especially when the tip is lifted off the substrate

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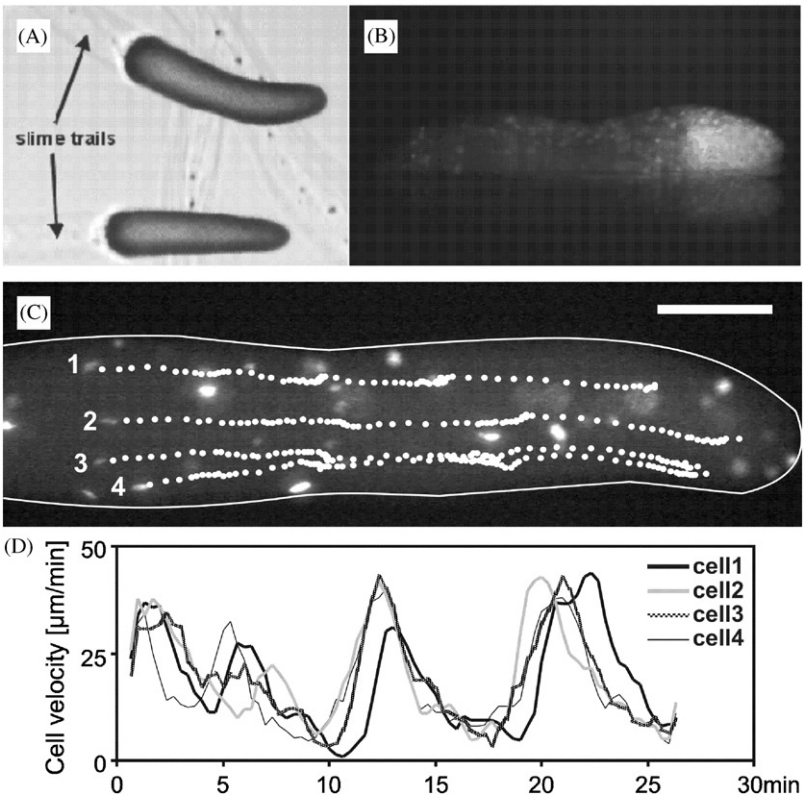

Fig. 1. (A) Photograph of slugs migrating on a 1% agar substrate in a Petri dish. The slime trails, which are left behind, are marked. (B) Photograph of a slug expressing GFP constructs to indicate the prestalk A and prestalk B cells. (C) Tracks of 4 cells in a migrating slug. Locations of cells (white spots) are detected with a constant time interval. Sequential spare and dense areas of detected locations indicate periodical modulations of cells velocities. (D) Velocity profiles for 4 cells given on C. Period of velocity modulations corresponds to period of cAMP waves (Dormann et al., 2000).

(Abe et al., 1994; Siegert and Weijer, 1992). Prespore cells move forward along slug's axis in the direction of the tip. The magnitude of cell velocity in these flows is modulated periodically over time (Fig. 1). The cells still move as individuals in the sense that they can easily change neighbours, however cells in different cross-sectional positions in the slug all seem to move with equal average velocities. This indicates that all the cells gain similar traction independent of their location in the slug. Moving cells form smooth flow patterns very reminiscent of laminar flows in liquids.

A number of mathematical models to study *Dictyostelium* development in general and slug migration particularly have been developed (Bretschneider et al., 1999; Odell and Bonner, 1986; Savill and Hogeweg, 1997; Umeda and Inouye, 1999). Our model is in many ways closely related to the pioneering model developed by Odell and Bonner (1986). They have used the hydrodynamic description of cell flows in a slug and investigated the case of flows caused by a stationary pattern in a concentration field of chemo-attractant with a maximum located at the tip of the slug. In their paper they gave special attention to the forces responsible for slug movement. Their assumption that cells derive traction locally from their neighbours, although quit reasonable from a physical point of view, posed many difficulties. In order to obtain any cell flow at all it had to be postulated that cells produce and brake down an additional substance, which affect their motility. This resulted in a modulation of their velocities from the centre to the periphery of the slug. Using this model they were able to obtain slug migration, however migrating slugs showed a fountain flow cell movement pattern, cells flowing forward in the periphery of the slug and flowing backward through its middle. Although very elegant, this type of movement is not in agreement with recent experimental data on cell movement (Siegert & Weijer, 1992). Furthermore this type of circulating movement cannot be easily reconciled with the axial distribution of cell types observed in the slug.

In this paper, we present hydrodynamic model that can explain the experimentally observed movement behaviour. This model is a further development of our previous models used to study aggregation of *Dictyostelium* cells (Vasiev et al., 1997) and cell sorting in the mound (Vasiev and Weijer, 1999). The main model assumptions are following:

1. Similarly to Odell and Bonner we use a hydrodynamic approach to describe cell flow patterns.

2. We assume that communicating cells produce waves of the chemoattractant cAMP, which direct, in turn, cell flows (Dormann et al., 2000; Dormann and Weijer, 2001). We study conditions under which the waves of chemoattractant propagating in a slug can result in cell flows and in slug migration.
3. We treat traction gained by actively moving cells in a global way, assuming that the cells gain traction from the whole body of a slug rather than only from its nearest neighbours. Such an approach allows us to obtain cell flow patterns, which are in good agreement with the experimental observations, i.e. straightforward flows in the prespore zone, and depending on the shape of cAMP waves, straightforward or rotational flows in prestalk zone. Furthermore it avoids the need for a second chemical to locally modulate the chemotactic response of the cells, for which there is no experimental evidence.
4. We consider a slug as a heterogeneous drop of liquid. The liquid consists of two kinds of fluids, corresponding to the prestalk and prespore cell types. Each fluid is chemotactically reacting to waves of cAMP propagating in the slug. We investigate how slug movement depends on the shape of the cAMP waves as well as on the chemotactic properties of its composite cells (fluids). We show numerically that the velocity of the slug depends on its length, the chemotactic properties of the prestalk and prespore cells, and on the shape of cAMP waves.

2. Model

The model is an elaboration of our previous model used to describe aggregation and cell sorting during the mound stage (Vasiev and Weijer, 1999) and is based on the following assumptions:

- We consider the slug as a viscous incompressible fluid, in agreement with the relatively smooth cell flow patterns observed in multicellular *Dictyostelium* tissues (Dormann et al., 1996; Siegert and Weijer, 1995; Siegert et al., 1994).
- The slug is composed of two spatially separated fluids, which correspond to the prestalk and prespore cell-types. The fluids can differ in their chemotactic, excitable and hydrodynamics properties as discussed below.
- The fluids can produce and degrade a diffusible chemo-attractant, cAMP, in an excitable manner.
- Local gradients in the chemo-attractant cAMP result in the generation of local chemotactic movement forces while moving cells experience resistance of the medium. These chemotactic and friction forces are distributed over the slug's volume.

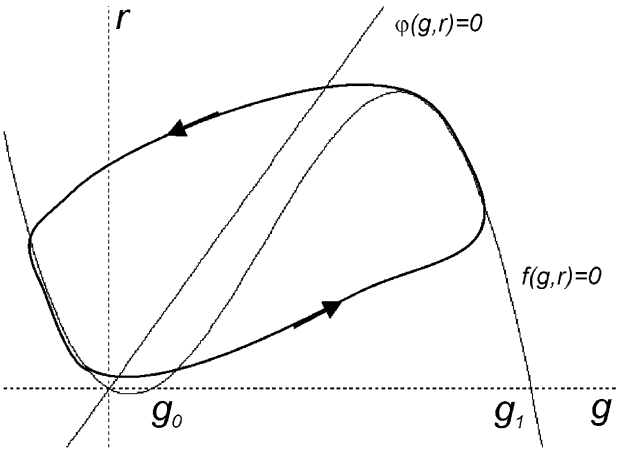

Fig. 2. Null-clines $f(g,r)=0$ and $\varphi(g,r)=0$ of the system (1–2). Here $f(g,r)=k_g g(g-g_0)(g-g_1)+k_r r$ is a cubic function of g and $\varphi(g,r)=g-r$. Nonlinear (cubic) term in (1) allows for an excitable kinetics with a parameter g_0 defining an excitation threshold and g_1—a maximum possible value of chemoattractant concentration. k_g and k_r define the rate of cAMP production and hydrolysis, respectively. Intersection of the null-clines gives a stationary point ($g=r=0$). Phase trajectory corresponding to a train of propagating waves is schematically shown (solid line with arrows).

- The forces are transduced through a network of cytoskeletal elements to the substrate.

To model the cell's excitable cAMP relay system we use the FitzHugh-Nagumo equations, which describe a prototype excitable medium:

$$\partial g/\partial t = D\Delta g - f(g,r), \tag{1}$$

$$\partial r/\partial t = \varphi(g,r)/\tau, \tag{2}$$

where g is the excitation variable in our case extracellular cAMP, and r the recovery variable, i.e. the adaptation of the cAMP relay response (Pitt et al., 1990; Vaughan et al., 1987). D is the diffusion coefficient for cAMP; Δ is the Laplacian operator denoting second derivative of g over space; τ is a time scaling factor for the kinetics of the variables r and g. Null-clines of the system (1–2) is shown on Fig. 2.

Cell movement is described as a flow in a viscous incompressible liquid:

$$\mathbf{F}_{ch} + \mathbf{F}_{fr} + \eta\Delta\mathbf{V} - \mathbf{grad}p = 0. \tag{3}$$

$\mathbf{F}_{ch}$ and $\mathbf{F}_{fr} = -k_{fr}\mathbf{V}$—are chemotactic and friction forces per unit volume. The third term describes cell-cell friction, parameter η is the dynamic viscosity of the liquid and reflects the strength of adhesive forces between cells. The last term defines the forces caused by the hydrostatic pressure, which develops as a result of cell movement. We have neglected inertial forces since it has been determined experimentally that the chemotactic force developed in a slug tissue is in the order of $50\,\mathrm{N/cm^3}$ (Inouye, 1984; Inouye and Takeuchi, 1980), i.e. much higher than the inertial force, which is around

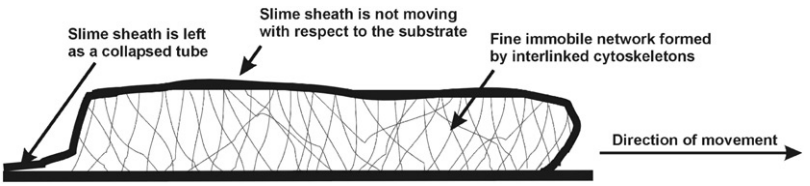

Fig. 3. Model of a migrating slug. The slug is covered by the slime sheath and consists of two components, a liquid and a fine net of actin fibres. The liquid component, the cytoplasm, comprises the bulk of the slug's volume, while the fine meshwork of fibres is formed by the interconnected cytoskeletons of all the slug's cells. The liquid can locally gain forces to move (chemotaxis) and to stop (friction) from the actin network, which is stationary with respect to the substrate but continuously synthesized and degraded all over the slug and which thus moves along with the slug. New slime sheath is produced at the tip of the slug and is left behind as a collapsed tube forming a trail once the slug has moved through it (Fig. 1A).

10^{-10} N/cm^3. Furthermore, we skip effect of gravity since it is negligible compared to the other forces involved and it is well known experimentally that gravity does not influence the migration behaviour of *Dictyostelium* slugs, i.e. they can develop and migrate perfectly in a Petri dish turned upside down.

Among the most important and difficult problems concerning the migration of cells into multicellular tissues is to give an adequate description of the origin of forces developed by moving cells. Odell and Bonner (1986) have shown that when the slug is treated as a pure liquid, no net movement forces can be generated inside the slug (unless some additional assumptions are postulated) and therefore no cell flows can be observed. On the other hand considering each cell as a solid body, would result in the addition of velocities of cells moving on top of other cells and therefore the cells on top would move faster, a mechanism that can only be sustained when the cells circulate continuously through the slug. This is not in agreement with the experimental observations, which show that all cells inside the slug move with roughly the same average speed. This means that slug cannot be described as pure liquid or solid and most likely has both kinds of properties. This "dualism" in mechanical properties of the slug becomes evident after detailed consideration of the events resulting in movement of cells in the slug.

First we consider the chemotactic movement of a single cell on a substrate. A cell moves by extending pseudopodia in the direction of the cAMP gradient, while at the same time inhibiting pseudopod extension in other parts of the cell (Wessels and Soll, 1996; Stites et al., 1998; Soll, 1999). Pseudopod extension at the front is coupled with an active retraction of the rear end of the cell. This requires the cell interacting with the substrate via specific adhesion sites, which are continuously formed in the front end of the cell while being dissolved in its rear end. Every cell has an internal actin-myosin cytoskeleton, which is linked to specific adhesion molecules that protrude through the cell membrane and make contact with the substrate (Niewohner et al., 1997; Weber et al., 1995). These connections thus transduce the traction forces from the cytoskeleton to the substrate. The process of pseudopod extension is driven by localized actin polymerization in the leading edge of the cell in response to external cAMP gradients (Fukui et al., 1989, 1999; Nachmias et al., 1989; Westphal et al., 1997; Yumura and Fukui, 1998). This process is beginning to be understood in considerable detail but will not be discussed here any further.

Extension in the front of the cell and contraction in the back result in a hydrostatic pressure and thus in a cytoplasmic flow from back to the front of the cell. This flow transports the bulk of the cell's mass. Our observations of cell movement suggest that all cells in a slug move in a manner similar to that of individual cells on a substrate. We propose therefore that all the cells contact each other via adhesion sites, which effectively link their internal cytoskeletons together. The cells in contact with the slime sheath adhere to the slime sheath and the slime sheath is stationary with respect to the substrate. This interconnectivity allows the cells to gain traction from each other and finally from the substrate. At any time the slug can thus be considered to have one heavily inter-linked dynamical cytoskeletal structure. This structure is continuously being modified everywhere in the slug under the influence of the cAMP waves, which direct actin polymerization and depolymerization as myosin filament assembly and localization in the individual cells as well as the dynamic changes in cell-cell contact.

All this can be summarized in the following physical model of a slug (Fig. 3). The slug is covered by a membrane (slime sheath) and consists of two components, a liquid and a fine net of actin fibres. The liquid component, the cytoplasm, comprises the bulk of the slug's volume, while the fine meshwork of fibres is formed by the interconnected cytoskeletons of all the cells of the slug. The liquid can locally gain forces to move (chemotaxis) and to stop (friction) from the actin meshwork, which is stationary with respect to the substrate, but continuously being synthesized and degraded in all cells in the slug and thus moves along with the slug. New slime sheath is produced at the tip of the slug and is left behind as a collapsed tube forming a trail once the slug has moved through it.

Now after this general justification of Eq. (3) we can describe the forces in detail. We assume that

the chemotactic force is proportional to the gradient of cAMP:

$$\mathbf{F}_{ch} = K_{ch}(\partial g/\partial t)\mathbf{grad}\ g. \quad (4)$$

Cells move up the gradient of chemo-attractant during the rising phase of the waves only. When the wave passes and the concentration falls they are desensitized and as a result do not turn round and follow the passing wave. To distinguish the wave front from the wave back we use the time derivative of the chemo-attractant level ($\partial g/\partial t$), which is positive on the wave front and negative on its back. We assume that K_{ch} equals to zero when $\partial g/\partial t \leqslant 0$, and to a positive constant otherwise, i.e. an increase in the level of chemo-attractant triggers the chemotactic activity of the cell, which starts to generate a force to move along the gradient.

The slug is heterogeneous, i.e. it consists of two kinds of fluids, which correspond to prestalk and prespore cells. Each fluid characterized by the volume fractions, α_{pst} and α_{psp}:

$$\alpha_{pst} + \alpha_{psp} = 1 \text{ inside the slug},$$
$$\alpha_{pst} = \alpha_{psp} = 0 \text{ outside the slug}. \quad (5)$$

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

$$k_g = k_{pst}\alpha_{pst} + k_{psp}\alpha_{psp}, \quad (6)$$

where k_{pst} and k_{psp} define the rate of cAMP production by each cell type. In the same way (making other parameters in Eqs. (1)–(2) cell type dependent) we can model other kinds of differences in the cAMP relay systems of the prestalk and prespore cells.

Similar to the differential excitability we model differential chemotactic movement by introducing parameters, K_{pst} and K_{psp}, which define the chemotactic force developed by prestalk and prespore cells:

$$K_{ch} = K_{pst}\alpha_{pst} + K_{psp}\alpha_{psp}. \quad (7)$$

One of the important parameters in our model is viscosity, η. According to our physical model of the slug the viscosity of the liquid representing the slug summarizes two factors: the viscosity of cytoplasm and ability of individual cells to move past each other. Stronger adhesive contacts between cells will reduce the ability of cells to move past each other and effectively increase the viscosity of liquid phase in the slug. Strong adhesive contacts, for instance caused by the over-expression of adhesion molecules reduce ability of cells to move i.e. they "freeze" cells and therefore add to solid nature of the liquid. Furthermore it is known that prespore cells are more adhesive than prestalk cells. This would imply that sub-liquid representing prespore cells has larger viscosity and differential adhesion can be modelled by differential viscosity, i.e. similarly to (6) and (7):

$$\eta = \eta_{pst}\alpha_{pst} + \eta_{psp}\alpha_{psp}. \quad (8)$$

To calculate volume fractions of prestalk and prespore cells we have distributed massless particles inside the slug (MAC method, Harlow and Welch, 1965). A part of these particles represent prestalk cells while another part represent prespore cells. The relative number of these two types of particles represents the volume fractions of the prestalk and prespore cells. These particles were also used to calculate the position of the slug boundary.

All calculations were performed in three-dimensional domains using the finite volume method. Eqs. (1)–(2) were integrated by the Euler explicit method using forward time centred space method for the diffusion term (Press et al., 1988). Eq. (3) was integrated implicitly (using a simultaneous over-relaxation scheme, (Press et al., 1988) by a two-step projection method (Kothe et al., 1991): to find the velocity $\mathbf{V}$ we first find the velocity $\tilde{\mathbf{V}}$ satisfying $\mathbf{F}_{ch} + \mathbf{F}_{fr} + \eta\Delta\tilde{\mathbf{V}} = 0$; then we use $\tilde{\mathbf{V}}$ to find a field ψ: $\nabla\tilde{\mathbf{V}} = \Delta\psi$ and finally use $\tilde{\mathbf{V}}$ and the field ψ to find V: $V = \tilde{\mathbf{V}} - \Delta\psi$. Note that the pressure $p = -\eta\Delta\psi$ to satisfy (3). Eq. (3) was integrated using staggered grids. For the cAMP concentration field (Eq. (1)) we used von Neumann's "no flux" boundary conditions at the boundary of the medium as well as at the free boundary of the slug. For the velocity fields (Eq. (3)) we used von Neumann's boundary conditions on the free boundary of the slug and free-slip condition (zero value for the normal component and Neumann condition for the tangential components) on the boundaries of the medium. For the pressure we used zero value boundary conditions on the free boundary of the slug and Neumann's boundary conditions on the boundaries of the medium.

Model parameters: $g_0 = 0.05$; $g_1 = 1, \tau = 4$; $k_r = 1.5$; $k_{fr} = 0.1$; $D = 1$; k_g was varied between 5.4 and 7.0; K_{ch} between 1 and 4, η between 1 and 100. The velocity of the cAMP waves measured in our model was 1.2 space units per time unit, the period of waves about 20 time units. The velocity of the fluid flows inside the slug varied between 0.2 and 0.5 space units per time unit (depending on K_{ch}). The initial diameter of slug and its length in our computations were varying from 10 to 15 and from 30 to 40 space units respectively. Assuming that a time unit is equal to 0.5 min and the space unit to 15 μm these parameters we have: slug's size up to 600 μm; period of cAMP waves, 10 min; velocity of cAMP waves, 36 μm/min; velocity of cell flows, 6–15 μm/min. All these numbers are close to those measured in experimental conditions (Rietdorf et al., 1998; Siegert et al., 1994). Assuming that average chemotactic force per volume in simulations corresponds to 50 N/cm^3 (Inouye, 1984; Inouye and Takeuchi, 1980) we have for the kinematic viscosity of the slug

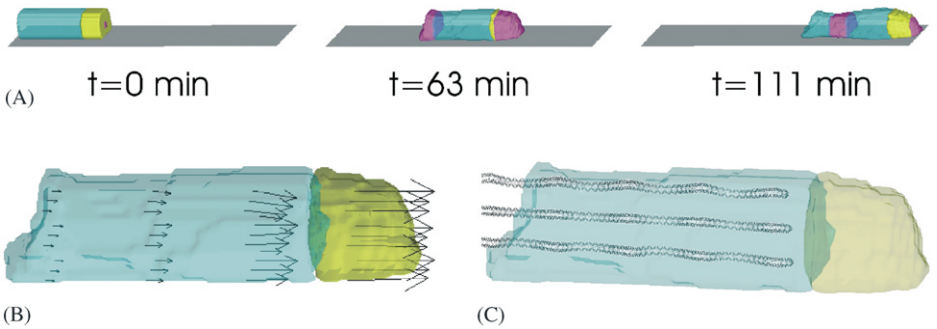

Fig. 4. Slug organized by a pacemaker located in its tip. The slug consists of 25% prestalk cells (yellow) and 75% prespore cells (blue). There is no difference in the chemotactic or relay properties between the prestalk and prespore cells. Waves of chemoattractant are initiated by periodical stimulation of a spot ($4 \times 4 \times 4$ grids) of cells located in the centre of the slug tip (A). The waves are represented by a red isosurface (B, C). (A) The slug is migrating in the direction of the pacemaker, while waves of the chemoattractant propagate through the slug in the opposite direction from the tip to the back. (B–C) Velocity field (B) and tracks of three cells (C) are shown for a slug at $t = 63$ min (A). Velocities range from 6 to 17 μm/min (see Fig. 6). Medium size: $40 \times 40 \times 200$ grids. Model parameters: $k_{psp} = k_{pst} = 6$ (in Eq. (6)) and $K_{psp} = K_{pst} = 2$ (in Eq. (7)).

$5 \times 10^3\,\mathrm{m}^2/\mathrm{s}$ (corresponds to $\eta = 10$ units in the model) which is the value of a cell's viscosity as measured experimentally (Bausch et al., 1998, 1999).

3. Results

In this paper we investigate whether wave propagation and chemotaxis can result in a stable slug, that can migrate and whether the characteristic shape changes frequently observed experimentally can be understood in terms of this model. We have performed a systematic series of computations varying signalling and movement properties of the cells to study their effect on migration. To simplify the calculations we have assumed that in the slug the cell types have already sorted and that their proportions are being kept constant by a cell type proportioning mechanism the details of which we do not further consider here. We started all the computations with a cylindrical slug, consisting of two cell types: 25% prestalk cells located in the anterior part and 75% of prespore cells occupying the posterior.

3.1. Migration of a slug organized by a pacemaker located in the anterior tip

Experimental observations show that in many cases cell flows inside migrating slugs are directed forward without a rotational component (Fig. 1). Such flows can arise in response to the planar waves propagating throughout slug and originated at the pacemaker in the tip of the slug. We have checked this in model simulations assuming that some cells in the tip of the slug act as a pacemaker while all other cells can relay the cAMP signal and show a chemotactic response to the signal. The pacemaker is simulated by a periodic stimulation of small region in the leading edge of slug. It causes the initiation of waves of the chemo-attractant, which are relayed by surrounding cells and result in wave propagation from the tip towards the rear. Cells develop chemotactic force in the direction of the wave source during the rising phase of the waves. Therefore at any given point in time there will be areas in the slug where the cells are actively moving, while cells in other areas are pushed and pulled by the adhesive (viscous) interactions and hydrodynamic pressure. In sum this results in slug migration in the direction of the signal source (Fig. 4A). All cells in this slug move straightforward forming laminar and parallel cell flows (Fig. 4B–C). The velocity of the slug is about 10 μm/min, the velocity of cAMP waves, 35 μm/min, their period, 10 min. The shape of the slug changes slightly over time, however it remains more-or-less cylindrical and shows a shape that is rather similar to that observed under experimental conditions (Figs. 1 and 4). The migration velocity of the slug is modulated over time with a period corresponding to the period of the pacemaker.

3.2. Slug migration speed

One of the important questions related to crawling slug is what affects its speed. Correlations between slug's sizes and its speed were observed experimentally. Those observations were generalized by Bonner who concluded that slug's speed depends on its volume (Bonner et al., 1953). This conclusion was later corrected by Inouye, who showed that the speed of the slug correlates with the length of slug rather than with its volume (Inouye, 1984). Our model permits checking correlations between slug's speed and its geometry as well as with other model parameters. The average velocity of the slug depends on the model parameters in the following way:

- It is proportional to the motile force ($\mathbf{F}_{ch}$) generated by cells in response to the chemoattractant and inversely proportional to its intrinsic resistance, $\mathbf{F}_{fr}$ (see Fig. 5A).

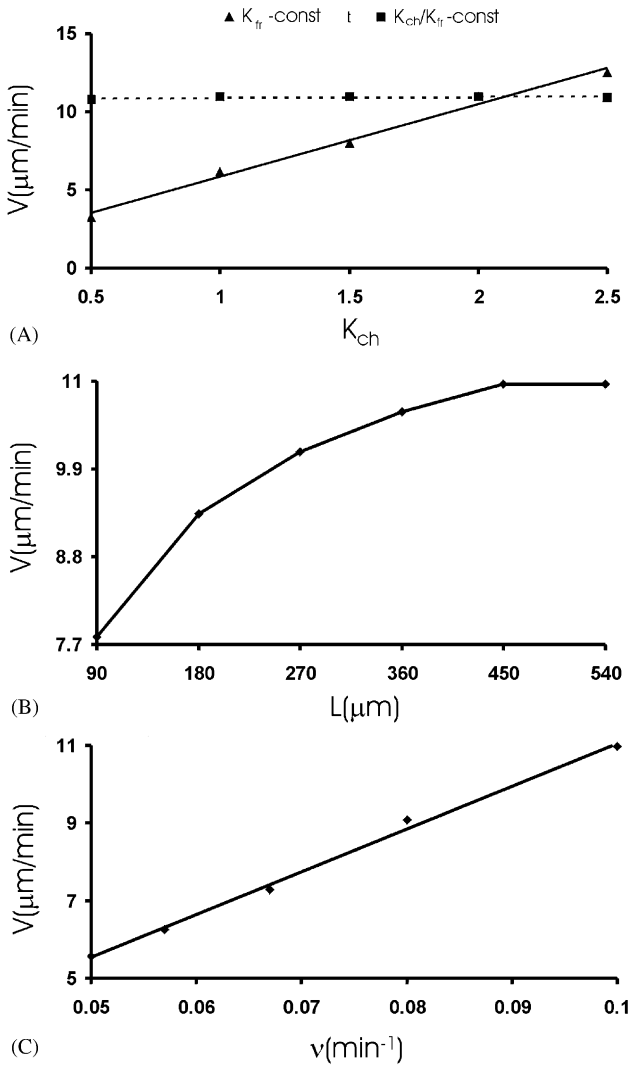

Fig. 5. The velocity of slug organized by a pacemaker is proportional to K_{ch} (solid) and constant when the ratio K_{ch}/K_{fr} is constant (dashed) (A). It increases with slug length (L) and saturates when the slug length exceeds the chemoattractant wavelength (B). It is proportional to the frequency of pacemaker (ν), i.e. inverse proportional to the wavelength of the chemotactic signal (C).

- It increases with the length of the slug as long as the slug is smaller that the wavelength of the chemotactic signal, when the slug gets longer, its velocity saturates (Fig. 5B). The effect of the radius of slug on its velocity is negligible.
- It is inverse proportional to the chemoattractant wavelength (Fig. 5C).

These observations can be generalized in the following way. The velocity of the slug is defined by the average motile force (averaged over time and the slug's volume) divided by the friction constant:

$$\mathbf{V}_{av} = \mathbf{F}_{av}/k_{fr}. \quad (9)$$

The average velocity is derived by integration of Eq. (3) over the slug's volume and time. The average value of the viscous term over the slug's volume is zero. As a result of the free-slip boundary conditions for velocity the pressure does not accumulate and therefore also does not effect the slugs velocity. Thus the velocity of a stationary migrating slug only depends on chemotaxis and friction as defined in Eq. (9). Obviously, $\mathbf{F}_{av}$ is proportional to the motive force developed by the cells as shown in Fig. 5A. To explain the effect of the slug's length on its velocity (Fig. 5B) let us consider a small slug, i.e. a slug whose length is smaller than the chemo-attractant wavelength. In this case the slug moves during the time interval when there is a wave propagating through it and it slows down or stops when there are no waves. Since $\mathbf{F}_{av}$ is averaged over time, it increases with an increase of the ratio of time intervals during which the slug accelerates and slows down. The last is proportional to the ratio of the slug length to wavelength up to the point when the slug length is equal to the wavelength (ratio is equal to one). From this point $\mathbf{F}_{av}$ is not changing anymore and the dependence of the slug velocity on its length saturates. To explain Fig. 5C we should take into account that $\mathbf{F}_{av}$ is averaged over slug's volume and should be proportional to ratio of chemotactically moving cells to the total amount of cells. The latter is inversely proportional to the wavelength. Note that velocity of chemoattractant waves is practically constant in a range of frequencies used to plot Fig. 5C, which means that the frequency of pacemaker and the wavelength are inversely proportional.

The velocity of individual cells in a slug changes periodically according to the periodicity of chemotactic signal (Fig. 6). It is responsible for the stop-and-go motion described for the slug tip as frequently observed in experiments (Dormann et al., 1997; Durston et al., 1976). The slug's velocity, defined as the velocity of its tip, is also modulated over time with amplitude that is

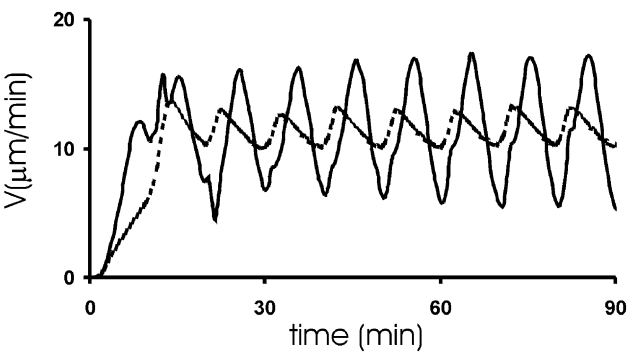

Fig. 6. The velocity of each cell in a slug as well as the velocity of a slug (defined as a velocity of slug's tip) are oscillating over time. The velocity profile of a cell in the middle of a slug (its track is shown in Fig. 4C) is represented by the solid line. The velocity of the slug's tip is shown by the dashed line.

much smaller than that of individual cells (Fig. 6). Periodic changes in slug velocity correspond to that in total chemotactic force generated by the cells, which in turn reflects the periodicity of cAMP waves propagating through the slug.

An important parameter affecting the slug's behaviour is its viscosity. Although it does not influence the velocity of the slug very much, it has a big impact onto the velocity profiles of its cells and the slug's shape (Fig. 7). An increase in the viscosity makes the slug more rigid in the sense that the velocity profiles of individual cells become smoother and the slug's shape less flexible. The velocities of cells in a low viscous slug (Fig. 7A, dashed line) change rapidly, the cells show a pronounced stop and go type of motion. On the other hand the velocity of cells in a highly viscous slug (Fig. 7A, stippled line) almost don't change. As a result of this the shape of a low viscous slug is very flexible, its shape changes during each passage of a wave, while on the other hand the shape of a highly viscous slug does not change (Figs. 7B and D). The comparison of the velocity profiles of cells obtained in our model simulations (Fig. 7) and experimentally observed (Fig. 1) shows that the profiles in the low viscous slug (Fig. 7A, dashed line) match experimental observations the best. However, the shape of low viscous slug (Fig. 7B) is too dynamic (every single wave propagating through the slug causes significant deformations in slug's shape) and concerning the shape the best match to experimental observations would be the slug with an intermediate viscosity (Fig. 7C). All these indicate that viscosity of real slug must be between viscosities of slug's in Figs. 7B and C, i.e. between 5×10^2 and 5×10^3 m^2/s.

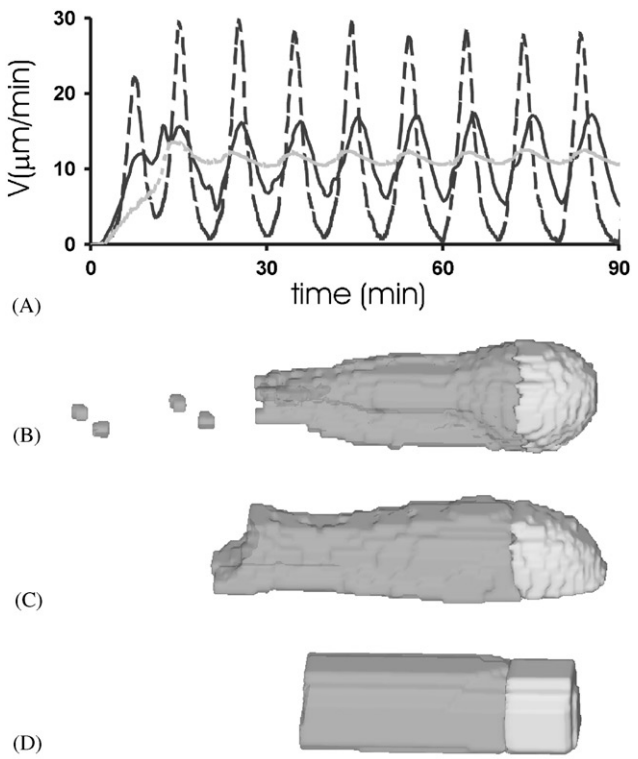

Fig. 7. The velocity profiles of cells get smoother and slug's shape gets more rigid with an increase in slug's viscosity. (A) Velocity profiles for a cell in the middle of a slug when viscosity of slug is 10^2 (dashed) 10^3 (solid) and 10^4 (stippled) m^2/s. (B–D) Shapes of slugs having different viscosities: B: 10^2,C: 10^3 and D: 10^4 m^2/s. Prestalk (lighter) and prespore (darker) zones are marked with different shadows of grey.

3.3. *Effects of differential excitability and chemotaxis and viscosity on slug shape*

In reality prestalk and prespore cells differ in many respects: prestalk cells are more excitable (produce more cAMP), move faster (seem to generate stronger chemotactic forces), less adhesive (show lower viscosity) and so on. We have performed a set of simulations to investigate the effects of differential excitability, chemotaxis, and viscosity (Fig. 8). Simulations where differences in excitable properties of cell types are taken into

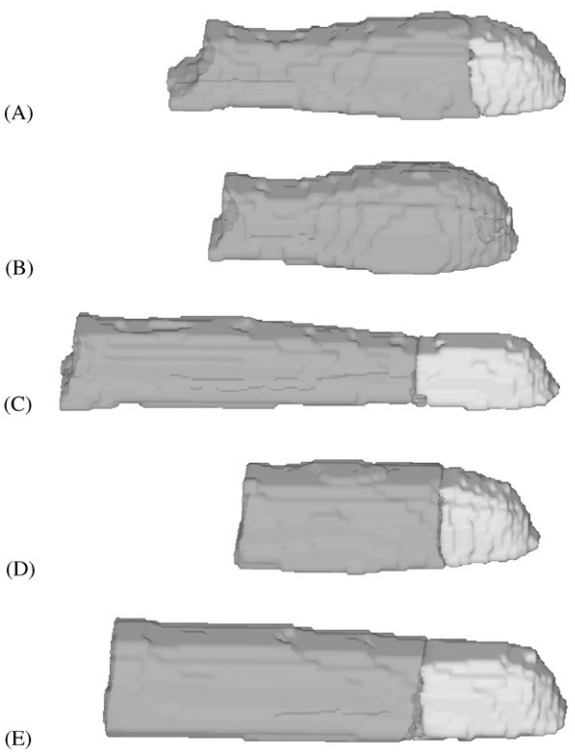

Fig. 8. Slug shapes observed due to differences in cAMP signal relay, chemotactic force and viscosity between prestalk and prespore cells. (A) No difference between prestalk and prespore cells (from calculation presented in Fig. 4). (B) Slug movement is controlled by a small number of oscillatory cells ($5\times5\times5$ grids) located at its anterior tip; there is no difference in relay kinetics between prestalk and prespore cells. (C) The chemotactic force developed by prespore cells is twice smaller than that of prestalk cells ($K_{psp}=1$; $K_{pst}=2$). (D) The viscosity of prespore cells is higher than that of prestalk cells ($\eta_{psp}=100$; $\eta_{pst}=10$). (E) Combination of the conditions shown in C and D, i.e. chemotactic force developed by prespore cells is small while their viscosity is high ($\eta_{psp}=100$; $\eta_{pst}=10$, $K_{psp}=1$; $K_{pst}=2$).

account (prestalk cells are more excitable than prespore cells) showed that the behaviour of the slug does not change in any detectable way as a result of this difference in excitability. We have also performed simulations in which we use a group of oscillating cells in the tip to initiate waves instead of the external simulation of the slug's tip. These calculations showed that the slugs' behaviour did not change a lot, although its velocity decreased slightly and its length shortened (Fig. 8B). This may relate to the observation that the pacemaker cells moved into a central core in the slugs tip. We have also investigated the effect of differential chemotaxis on the shape of the slug. When the prespore cells develop lower chemotactic force and as a result move slower than the prestalk cells this results as expected into an elongation of the slug (Fig. 8C) and a decrease of its speed. This suggests that the spontaneous shape changes often observed experimentally may be caused by the local modulation of the chemotactic movement response. We have also investigated the effect of differential viscosity on slug migration and shape. We assumed the prespore cells to be more viscous in line with the experimental observation that the prespore cells are more adhesive (Bozzaro and Ponte, 1995; Lam et al., 1981). This results in a slug, which is shortened in length and shows a slightly decreased speed of migration (Fig. 8D). In the last simulation of these series we tested what effect the assumption that prespore cells are slower and more viscous than prestalk cells, which is most in line with the available experimental facts, would have on slug migration and shape. This assumption resulted in slugs with a shape, which shows the best similarity with the shape of "typical slugs" observed experimentally (Fig. 8E).

3.4. *Migration of a slug organized by a twisted scroll wave of cAMP*

We have experimental evidence that cell movement in the tip of a slug can be controlled by a scroll wave of cAMP in the prestalk zone, which can convert to a twisted scroll wave and in the extreme case to planar waves in the prespore zone (Abe et al., 1994; Bretschneider et al., 1995; Siegert and Weijer, 1992). It was shown numerically that this conversion takes place when the prespore cells are less excitable than prestalk cells (Bretschneider et al., 1995). We now show that such a twisted scroll wave can also direct slug migration.

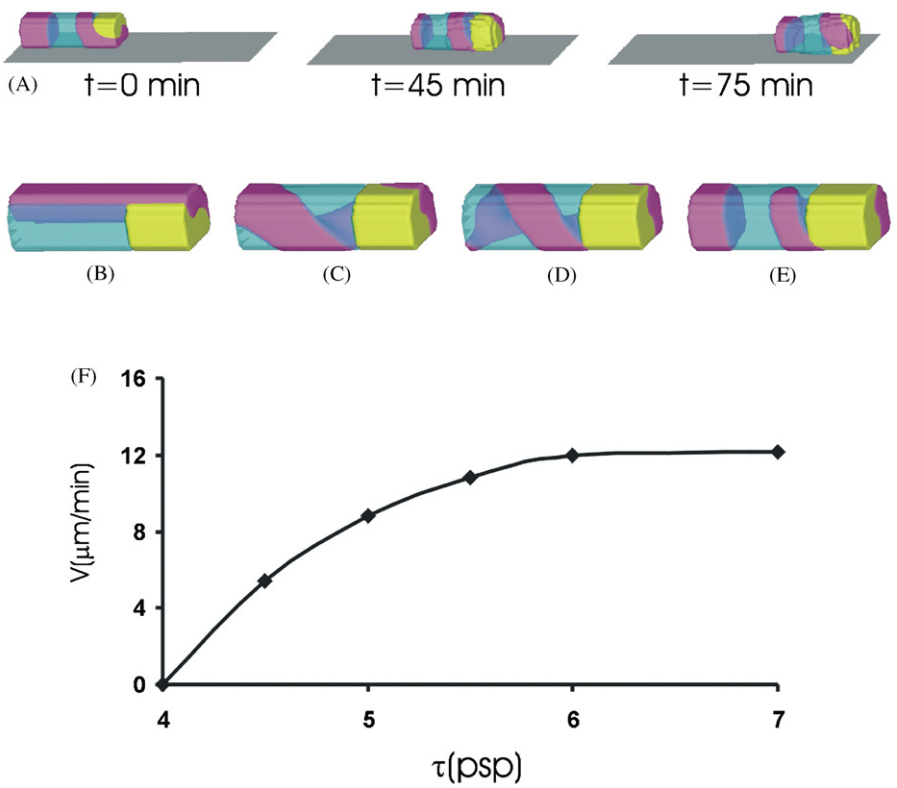

Fig. 9. Slug organized by a twisted scroll of chemoattractant in the slug's tip and planar waves in its tail. This wave pattern forms due to a difference in excitable properties (refractoriness) of prestalk and prespore zones. (A) The slug is migrating in the direction of the more excitable (less refractory) prestalk zone, while the twisted scroll wave has a velocity component directed towards the back of the slug. The size of the medium is: $40 \times 50 \times 160$ grids. Model parameters: $\tau_{psp} = 7$, $\tau_{pst} = 4$ (in Eq. (2) $\tau = \tau_{pst}\alpha_{pst} + \tau_{psp}\alpha_{psp}$). The colours are described in Fig. 4. (B–E) The scrolls obtained by varying the relaxation parameter τ, which defines the refractoriness, for prespore cells: B: $\tau_{psp} = 4$; C: $\tau_{psp} = 5$; D: $\tau_{psp} = 6$; E: $\tau_{psp} = 8$. The relaxation parameter for prestalk cells, $\tau_{pst} = 4$ in all cases. (F) The velocity of slug migration increases with an increase of the twist of the scroll.

Images from a simulation of a slug with a twisted scroll in the prestalk zone and planar waves in the prespore zone are shown in Fig. 9A. It can be seen that this type of wave pattern also results in slug migration the direction of which is guided by the tip.

The velocity of a slug organized by a scroll wave also depends on chemotactic and friction force, as was the case for slugs organized by a point source. Calculations show that the velocity increases and saturates with an increase of slug's length also similar to what is shown in Fig. 5B. However the velocity of a slug organized by a twisted scroll wave saturates when slug's length is much longer compare to the chemical wavelength. Our calculations show that the velocity of a slug organized by a scroll wave depends on the degree of twist of the scroll rather than on the wavelength. If the scroll is not twisted the slug will not move. The twist of the scroll determines the effective front to back propagation of the waves along the slug's axis and, therefore, determines the migration of slug. The twist of scroll wave is determined by the axial difference in the excitable properties of the prestalk and prespore cells and an increase in this difference results in a stronger twist (Fig. 9B–E). This difference in excitability therefore determines the velocity of slug migration (Fig. 9F).

4. Discussion

4.1. The mechanism of slug migration

The model presented here is a further development of our previous continuous models, which were used to describe aggregation, stream formation, mound formation and cell sorting (Vasiev & Weijer, 1999; Vasiev et al., 1997). In the previous models we used the Navier–Stokes equation (i.e. we had an inertial term in Eq. (3)) and the effective viscosity of the slug tissue was small. The inertial term described a persistence of motion after the cells were chemotactically stimulated. The present model contains physically more realistic description that there is no real inertia, but that the medium has a high viscosity. This high viscosity as described in Section 2 reflects both the cytoplasmatic viscosity of individual cells and local coupling of the movement behaviour of neighbouring cells. Viscosity results in a dampening out of very abrupt changes in cell velocity in response to periodic waves of cAMP, in agreement with the experimentally observed data (Figs. 1 and 7). Another important difference between current and previous models concerns the implementation of boundary conditions for the velocity field on the free boundary of the slug. In previous models we used zero value boundary conditions for velocities, which effectively reduced the rate of boundary relocation. This allowed the cell sorting process so that cell sorting was taking place before transformation of a hemispherical mound into a slug. However zero value boundary conditions turned out not to be capable of describing slug migration and resulted only on deformation of the shape of the slug. We found that von Neumann's boundary conditions for the velocity field are much more appropriate to model slug migration. This switch in boundary conditions most likely reflects the formation of the slime sheath during tip formation. The slime sheath surrounds the slug and allows cells on the surface to get better traction.

We show that this model can effectively describe slug migration and we have used it here to investigate the interplay between signalling dynamics and slug morphology. Considering the slug as a drop of "active liquid", capable of generating volume forces, allowed us to obtain patterns of internal cell flows in migrating slugs very similar to those observed experimentally (Figs. 1 and 5). Variation of the model parameters showed that the model is robust: the output quantities, such as velocity of slug (Figs. 5 and 8) or amplitude of cells speed modulations (Fig. 8) change smoothly with changes in parameters. All this indicates that the model gives a good framework to analyse the slug behaviour in a systematic way.

4.2. Factors controlling slug movement

The migration of a slug was studied in response to two different modes of wave propagation, planar waves and scroll waves. In both cases waves of the chemoattractant cAMP propagating through the slug cause formation of cell flows, which in turn result in slug migration. Waves initiated by a pacemaker located in the tip of slug result in cell flows in the direction of the tip along the slug's long axis (Fig. 4B). Twisted scroll waves (Fig. 8) cause more complex cell flows. The cells form rotating flow patterns around the slug's axis, which also result in net forward motion of the slug in the direction of the tip. The velocity of the slug in this case is smaller (compared to a slug with a pacemaker, described by the same set of model parameters), since only the component of the cell flow along slug's long axis contributes to slug migration. The speed of migration is therefore dependent on the pitch of the scroll wave. The pitch of the spiral is controlled by the difference in excitability of the prestalk and prespore cells. By increasing the difference between excitable properties of prestalk and prespore cells the twisted scroll wave can be transformed into a piece of twisted scroll wave rotating in prestalk zone and planar waves propagating in prespore zone (Fig. 9). In this case we observe rotational movement of cells in anterior part of slug and forward movement of cells in its posterior part, very similar to results obtained experimentally (Siegert and Weijer, 1992; Abe et al., 1994).

We have investigated the effect of variations in the basic model parameters on slug migration velocity. For small slugs the velocity of the slug increases with its length, but saturates when the length of the slug exceeds the chemotactic wavelength (Fig. 5). This corresponds with experimental observations (Inouye, 1984; Inouye and Takeuchi, 1979, 1980) and with model simulations in (Maree et al., 1999a). The slug's velocity–length dependence was explained in terms of average chemotaxis for a slug organized by a pacemaker (Eq. (9)). However a similar dependence was observed for a slug driven by twisted scroll and this case cannot be explained by Eq. 9. If the twist of the scroll is constant along the slug it must result in the same value for average chemotaxis independent of the slug's length. We have found that in a framework of our model an increase in slug's length (while keeping the properties for prestalk and prespore cells constant) results in a decrease in the wavelength of twisted scroll and therefore affects the velocity of slug (Fig. 9). Why slug length affects wavelength of the twisted scroll is not clear and should be addressed as a problem related to heterogeneous excitable media in a further study.

4.3. Comparison with other models

The model that is closely related to our model is that proposed by Umeda and Inouye (1999). Although they have modelled migration of slug in response to stationary pattern of chemotactic signal, their basic physical model describing the underlying biology is rather similar to ours. In their model the cell surface is considered to be immobile and in order to migrate the cell produces new membrane at its front and degrades it in the back of the cell. The components are then recycled internally from the back to the front. This model therefore also allows the chemotactic forces to be treated in a global way, i.e. cells gain traction to move from the stationary system formed by cell membranes. However their model does not describe the slug tissue as a viscous medium. In their simulations this has no effect since they only study the steady movement of cells in a static gradient of cAMP. Furthermore their model is at present only two-dimensional. It is to be expected that when they start to include periodic signalling in their model and extend it into three dimensions they also will have to include additional interactions to describe movement behaviour of cells in slugs correctly.

Models developed in (Bretschneider et al., 1999; Odell and Bonner, 1986; Savill and Hogeweg, 1997; Umeda and Inouye, 1999) are all the so-called "hybrid" models, where cells are treated as discrete entities while dynamics of chemotactic agent—in a continuous manner. In these models it was assumed that chemotactic movement of cells is triggered by propagating waves of chemoattractant, and chemotactic forces where treated in a global way without further justification. The model developed (Bretschneider et al., 1999) was successfully used to reproduce modulations in cell velocities as well as rotational movement of cells in moving slug, while the model in (Maree et al., 1999a, b) was used to study chemo- and thermo-taxis of the moving slug. Our model simulations are in agreement with some of the results obtained in other models. Cell flow patterns obtained in our model are very similar to those in (Bretschneider et al., 1999; Maree et al., 1999). These models predict cell flows directed towards the source of the chemoattractant. However the pulsatile character of cell movement is too strong in these models. According to our results viscous interactions between moving cells are essential to smooth temporal modulations in their velocity so that this would correspond to experimental observations (Dormann et al., 2000).

In agreement with (Maree et al., 1999a) we conclude that the primary reason for the cylindrical shape of the slug is a convex shape of the chemoattractant waves especially at the tip of the slug. Most of prestalk cells move not straightforward, but rather towards the centre of the slugs' tip, thereby pushing it forward and causing the elongation of the slug. An increase in the size of the pacemaker as well as in the core of scroll wave reduces curvature of the waves in the tip of the slug and consequently slug becomes less elongated and thereby thicker. We propose that an alternative mechanism for the slug elongation is a reduced velocity of prespore cells compared to that of prestalk cells. The slug increases in length when this difference in velocities gets larger.

The most important discrepancy between our model and the model developed in (Maree et al., 1999a, 1999b) concerns the differential adhesion of prestalk and prespore cells. In the latter model it is assumed that adhesion of prestalk cells is stronger. This assumption is critical for the prestalk cells to move faster and to be able to sort to the tip of the slug. However there is strong experimental evidence that adhesion of prespore cells is stronger compared to that of prestalk cells although prestalk cells move faster (Casademunt et al., 2002; Lam et al., 1981; Ratner and Borth, 1983; Siu and Kamboj, 1990). In the model we describe differential adhesion by differential viscosity of the sub-liquids corresponding to prestalk and prespore cells. The assumption that the sub-liquid corresponding to more adhesive prespore cells is more viscous results in much more realistic shapes of the slug (Figs. 1 and 8) and we therefore take this to be an important factor in determining slug shape.

4.4. Outlook

There are still many open questions about the behaviour of migrating slugs, many of which could be further investigated using our model. Slugs vary

significantly in their sizes under experimental conditions although they are always cylinder-shaped. It is not clear what is responsible for slug's particular proportions and it would be interesting to explain their variations based on cellular signalling and movement properties. According to our preliminary computations a slug's diameter increases (correspondingly its length decreases) with an increase of the size of pacemaker. The diameter of slug organized by a twisted scroll wave may be very sensitive to many things like degree of scroll twist, or difference between velocities of prestalk and prespore cells. However, as we saw both a pacemaker and a twisted scroll conserve the slug's cylindrical shape. The model can easily be extended to study the changes in behaviour of the slug in response to external signals such as thermo- and phototaxis. There is some recent evidence that light affects the cAMP relay properties of cells in the slug (Miura and Siegert, 2000). This could result in local differences in cAMP wave propagation speed and thus in local changes in cell movement resulting in a reorientation of the migrating slug.

We started to explain the morphogenesis of a simple multicellular organism, the social amoebae *Dictyostelium discoideum* on the basis of relatively simple cellular properties such as chemotaxis and cell-to-cell relay of a chemotactic signal. We have shown that the geometry of the waves and the resulting flow patterns of the cells producing these waves, subject to relatively simple chemical and physical constrainsts, can explain the basic morphogenesis of *Dictyostelium*. A further improvement of our model in the future will require a more explicit description of the cytoskeletal elements possibly in first instance by treating the tissue as a viscoelastic rather that a purely viscous medium, which is now under further investigation.

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