THE ROYAL SOCIETY

doi 10.1098/rstb.2000.0634

The control of chemotactic cell movement during *Dictyostelium* morphogenesis

Dirk Dormann, Bakhtier Vasiev and Cornelis J. Weijer*

Department of Anatomy and Physiology, University of Dundee, Medical Sciences Institute/Wellcome Trust Biocentre Complex, Dow Street, Dundee DD1 5EH, UK

Differential cell movement is an important mechanism in the development and morphogenesis of many organisms. In many cases there are indications that chemotaxis is a key mechanism controlling differential cell movement. This can be particularly well studied in the starvation-induced multicellular development of the social amoeba *Dictyostelium discoideum*. Upon starvation, up to 10^5 individual amoebae aggregate to form a fruiting body. The cells aggregate by chemotaxis in response to propagating waves of cAMP, initiated by an aggregation centre. During their chemotactic aggregation the cells start to differentiate into prestalk and prespore cells, precursors to the stalk and spores that form the fruiting body. These cells enter the aggregate in a random order but then sort out to form a simple axial pattern in the slug. Our experiments strongly suggest that the multicellular aggregates (mounds) and slugs are also organized by propagating cAMP waves and, furthermore, that cell-type-specific differences in signalling and chemotaxis result in cell sorting, slug formation and movement.

Keywords: chemotaxis; signal relay; morphogenesis; cell sorting; wave propagation

1. INTRODUCTION

Development of a vertebrate embryo typically involves the generation of hundreds of cell types from the zygote to form all the different tissues and organs. Some cell types may arise and differentiate *in situ* at the right position at the right time of development; however, many cell types have to move to their final destination. This is difficult to study and in most cases little is known about the mechanisms involved, although it is often suspected that chemotaxis may play an important role. Chemotactic cell movement is a key mechanism in the multicellular development of the social amoeba *Dictyostelium discoideum*. Its development is in many respects much simpler and more amenable to analysis than that of vertebrates. The cells proliferate in the vegetative stage as single amoebae. Under starvation conditions up to 10^5 cells aggregate chemotactically to form a multicellular organism. During aggregation the cells start to differentiate. The cells arrange themselves by differential movement into a simple axial pattern in the slug. The movement of the slug, which is photo- and thermotactic, is guided by an apical tip. The slug forms a fruiting body after a facultative period of migration of up to several days. The fruiting body consists of a stalk, formed by dead vacuolated stalk cells, that supports a mass of spore cells. The spores disperse and each spore can germinate to start a new colony under favourable conditions (Loomis 1982). Since the multicellular phase of the development occurs in the absence of food there is little cell division during development (Weeks & Weijer 1994). The number of cells doubles at most. Morphogenesis therefore results from coordinated cell movement of a few emerging cell types.

During development the cells have to communicate over distances large compared with the size of the cell (5–10 μm). An aggregation territory can be a few centimetres wide and slugs can be up to 5 mm long. During aggregation the cells communicate by relaying a cAMP signal from cell to cell. They use this signal to aggregate by chemotaxis. We have investigated whether the same mechanisms, cAMP signal relay and chemotaxis towards cAMP gradients, also organize the later multicellular stages of *Dictyostelium* development. Since the aggregation phase has been most thoroughly investigated we will review the key facts first and then discuss what is known for the later stages of development.

2. EARLY AGGREGATION

The principles that govern aggregation are understood at the cellular level to result from the following cellular competencies: (i) periodic cAMP signal generation by cells in the aggregation centre, (ii) cAMP signal relay by all other cells, (iii) adaptation of the cAMP relay response, and (iv) chemotactic movement of cells up-rising cAMP gradients.

A few hours after the initiation of starvation, cells become sensitive to cAMP due to the expression of cell-surface cAMP receptors. They gain the ability to synthesize and secrete cAMP in response to a cAMP stimulus, i.e. they become excitable. The mechanism of cAMP excitability involves two different feedback loops, a fast autocatalytic cAMP-induced cAMP amplification and a slightly slower negative feedback loop (Devreotes 1989; Devreotes *et al*. 1987). The molecular mechanisms underlying these feedback loops are rapidly being elucidated

*Author for correspondence (c.j.weijer@dundee.ac.uk).

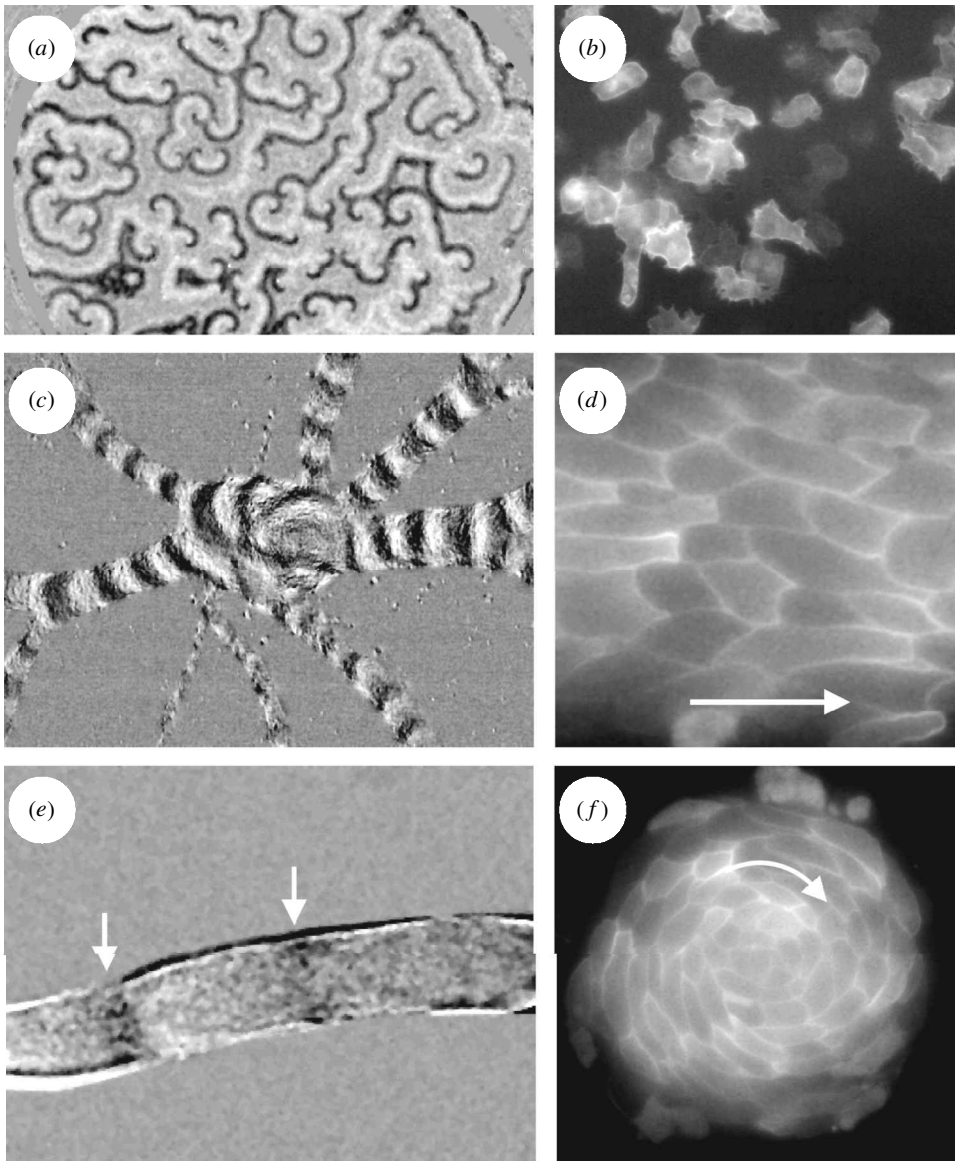

Figure 1. Optical density (OD) waves and cells at different stages of development. (*a*) Spiral OD waves during early aggregation in strain Ax3. (*b*) Ax2 cells expressing a car2-GFP (green fluorescent protein) fusion protein at a density typical for early aggregation. Note the large spaces where the cAMP signal propagates by diffusion. (*c*) OD waves in a streaming aggregate of the strain NP377, the multi-armed spiral waves in the mound separate in single wave-trains that propagate down the streams towards the periphery. The cells move inward. (*d*) Cells in an aggregation stream of Ax2, expressing a GFP fusion protein of the PH domain of GRP1 (a mammalian PIP_3-binding protein, which binds predominantly to the plasma membrane). The cells are closely packed. The cells move in the direction of the white arrow. (*e*) A slug of the strain NP377 migrating from right to left, showing two dark OD waves, indicated by the white arrows that travel from left to right. (*f*) An Ax2 mound expressing the GFP-GRP1-PH fusion protein; note again the close packing of the cells. The cells move in the direction of the white arrow. The images in (*a*,*c*,*e*) have been digitally processed by image subtraction of successive images and contrast enhancement to improve the visualization of the waves.

(Parent & Devreotes 1996; Aubrey & Firtel 1999). cAMP is detected via highly specific and sensitive serpentine cAMP receptors, which triggers two competing processes, excitation and adaptation. The excitation pathway leads from the receptor, via the activation of a heterotrimeric G protein, G2, to the liberation of a βγ complex. This complex activates directly or indirectly one or several phosphatidylinositol-3 kinases, which transiently synthesize phosphatidylinositol-3,4,5-trisphosphate (PIP_3). This results in the transient translocation of the PH-domain-containing protein CRAC (cytosolic regulator of adenylate cyclase) from the cytosol to the membrane (Parent & Devreotes 1999; Parent *et al*. 1998). This, in combination with other factors such as pianissimo, aimless, a ras guanine nucleotide exchange factor and another ras-interacting protein, RIP3, results in the activation of the membrane-bound adenylate cyclase ACA, which starts to produce cAMP (Chen *et al*. 1997; Insall *et al*. 1996; Lee *et al*. 1999; Wu *et al*. 1995). cAMP is constitutively secreted to the outside, where it can bind to the receptor again. This is a positive feedback loop leading to the production of cAMP. cAMP is degraded continuously by an intracellular phosphodiesterase regA and a secreted extracellular phosphodiesterase ePDE (Franke & Kessin 1992; Shaulsky *et al*. 1998; Thomason *et al*. 1999). Binding of cAMP to the receptor also triggers an adaptation process,

which results in an inhibition of the cyclase activation. The molecular basis of adaptation is still unknown but may involve a Gi-type G protein. After adaptation has set and the cyclase is no longer activated the extracellular cAMP levels are going to fall, due to the action of the secreted phosphodiesterase.

The secreted cAMP diffuses away to activate neighbouring cells, which now in turn start to produce cAMP and stimulate their neighbours. These signals form initially small wave fragments that start to travel through the population of cells. Adaptation ensures the unidirectional propagation of cAMP waves, since cells which have just relayed are refractory to further stimulation by cAMP. The waves will interact and form spiral wave centres (figure 1*a*). There is a feedback of the cAMP signal on the expression of the components necessary for signal detection and amplification, such as cAMP receptors, G proteins, CRAC and adenylate cyclase itself (Firtel 1996; Gerisch 1987). As a result the cells become more excitable and presumably all start to oscillate autonomously being entrained by cells in the aggregation centre.

The cells show a chemotactic reaction in the direction of higher cAMP concentrations. The cells move up the gradient, as long as the cAMP concentration is rising. This involves the detection of small differences ($< 10\%$) in the number of occupied receptors between the front and the back end of the cells, since the cells are small compared with the wavelength of the cAMP waves (see § 4). This difference has to be amplified to result in directed chemotactic movement. This polarization process can be visualized by the localized translocation of PH-domain-containing proteins such as CRAC and protein kinase B (Parent & Devreotes 1999; Parent *et al*. 1998; Meili *et al*. 1999). The cells stop moving as soon as the cAMP wave passes and the concentration starts to fall (Futrelle 1982; Varnum-Finney *et al*. 1987, 1988). This chemotactic response leads to the periodic inward movement of the cells to the aggregation centre guided by outwardly propagating waves of cAMP.

During early aggregation the cAMP waves can be seen as optical density (OD) waves using low-power darkfield optics (Alcantara & Monk 1974; Gross *et al*. 1976; Siegert & Weijer 1989). These OD waves are correlated with shape changes which cells undergo upon stimulation with cAMP. Chemotactically moving cells are elongated and groups of moving cells appear brighter than groups of non-moving cells. This results in the appearance of dark and light bands in fields of aggregating cells. By correlating the cAMP signal via cAMP isotope dilution fluorography with the darkfield waves, it was shown that the OD waves observed during aggregation faithfully represent the propagating cAMP signal (Tomchik & Devreotes 1981). Most often waves appear as expanding spirals, in some strains they also form target wave patterns. Spiral waves can arise in excitable media and do not need autonomously oscillating cells, concentric waves need autonomously oscillating cells in the aggregation centre.

3. AGGREGATION STREAMS

After around 15–20 waves have passed the cells become organized in aggregation streams. These streams radiate from the aggregation centre outward and branch towards the periphery. Based on theoretical considerations it has been suggested that stream formation results from a deformation of the cAMP wavefronts caused by random variations in distribution of cells in the aggregation territory. A small local accumulation of cells will, due to the autocatalytic nature of the cAMP relay reaction, result in a local increase in the rate of cAMP production. This will locally speed up wave propagation, resulting in a slightly outwardly bulging wavefront. This then will result in the further attraction of cells to this region (Hofer & Maini 1997; Levine & Reynolds 1991; Vasiev *et al*. 1994). Stream formation is an autocatalytic process and will rapidly spread through the aggregation territory. Cells in streams are highly polarized and elongated and very closely packed (figure 1*d*). Propagating OD waves can readily be observed in aggregation streams. Most late-aggregation centres are organized by spiral waves, which break up into individual wavefronts that propagate down the streams (figure 1*c*). The wave propagation speed can be as low as $50\,\mu m\,min^{-1}$, which implies that the signal propagates around one cell diameter every 10–15 s. The cAMP signal hops from cell to cell, as can be visualized by the translocation of CRAC in aggregation streams (D. Dormann and C. J. Weijer, unpublished data). Cell movement is still periodic (Rietdorf *et al*. 1996). The cells make specific end-to-end contact via homophilic interactions of an ethylenediaminetetraacetic acid-resistant cell adhesion molecule, contact site A (CSA). Its expression requires exposure of the cells to nanomolar pulses of cAMP. CSA-null mutants can still aggregate, but lack, however, the clear stream morphology, implying a role for these sites in stream preservation (Harloff *et al*. 1989; Ponte *et al*. 1998; Stadler *et al*. 1989). When the cells reach the body of the aggregate, they start to become more amoeboid in shape again, suggesting a change in the adhesion mechanism operative during later development (Rietdorf *et al*. 1996).

4. MOUNDS

The cells entering the aggregation centre start to pile on top of each other and form a three-dimensional hemispherical structure. We could visualize propagating OD waves in the later stages of *Dictyostelium* development (Rietdorf *et al*. 1996; Siegert & Weijer 1995). Continuous measurements of the OD waves from aggregation until tip formation showed that the frequency of the waves increases during aggregation while the wave propagation speed slows down (Gross *et al*. 1976; Rietdorf *et al*. 1996; Siegert & Weijer 1989). During early aggregation the period of the signals may be as long as 6–7 min while later in development the period decreases to 1–2 min. Wave propagation speed decreases simultaneously from $600\,\mu m\,min^{-1}$ to around $50–100\,\mu m\,min^{-1}$ in aggregation streams and mounds. As a result of this the chemical wavelength decreases from 3000–4000 μm to 50–200 μm. Therefore the spiral waves can initially organize the several centimetre large aggregation territories during early aggregation and the much smaller mounds (100–500 μm diameter) during later development. This change in signalling properties is partly due to the cAMP-dependent expression of components of the oscillatory system, described above, as well as to the dispersive properties of

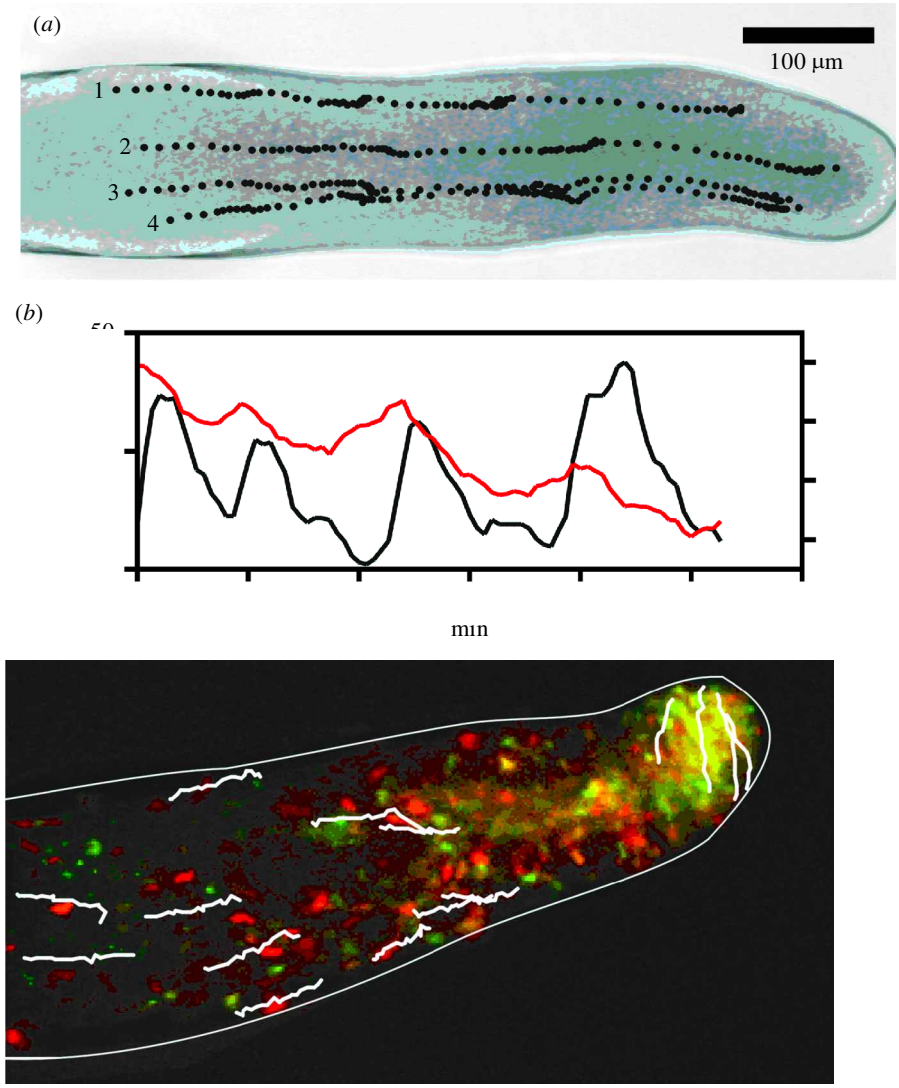

Figure 2. Cell movement in slugs. (*a*) An image of an NP377 slug indicating the position of four different GFP-expressing cells at the start of the experiment. The slug moves from left to right. The positions of centres of mass of the cells have been marked at 20s intervals (dotted lines). (*b*) The instantaneous velocity of cell number 1 versus time (black trace) and the local OD (red) measured in a 25 × 25 pixel window tracking the position of cell number 1. (*c*) An image of an Ax2 slug expressing a green (wild-type) GFP under the control of the full ecmA promoter and a red-shifted GFP construct under the ecmO promoter. The motion of a number of pstA cells in the tip was followed for ten successive time-points. The cells in the tip show a clear upward directed rotational motion. The pstO cells in the back move straight forward in the direction of slug migration.

this excitable medium (Foerster *et al*. 1990; Gerisch *et al*. 1987; Siegert & Weijer 1989).

In the mound stage the geometry of the waves can be very variable. The pattern of wave propagation seems to be a characteristic of the strain used (Siegert & Weijer 1995; Dormann *et al*. 1998). Some strains predominantly show concentric ring waves, which originate from one or more centres in the mound; other strains show simple and multi-armed spirals waves. The multi-armed spirals rotate around a common core. We have not yet been able to observe exactly how these multi-armed spiral waves form from the single-armed spirals present during aggregation. From theoretical considerations, however, it is evident that in a multi-armed spiral the de-adaptation process has to be much faster than the period of rotation of one wavefront (Vasiev *et al*. 1997*a*). The molecular basis for the formation of multi-armed spirals is as yet unknown.

There is good evidence that the OD waves in mounds still reflect propagating waves of cAMP. Periodic micro-injection of pulses of cAMP into the extracellular space in mounds initiated OD waves which propagated from the electrode tip outwards. These elicited waves annihilated endogenous waves upon collision, showing a common propagation mechanism (Rietdorf *et al*. 1998). Our recent experiments using a temperature-sensitive adenylate cyclase mutant (tsaca2), whose activity is inhibited at 28 °C, show that wave propagation can be reversibly inhibited by shifting mounds of the temperature-sensitive mutant to the restrictive temperature. At the restrictive temperature the waves disappear and the cells loose their coordination of movement. Upon lowering the temperature the waves come back. These experiments clearly show that the OD waves in mounds are still carried by cAMP (Patel *et al*. 2000).

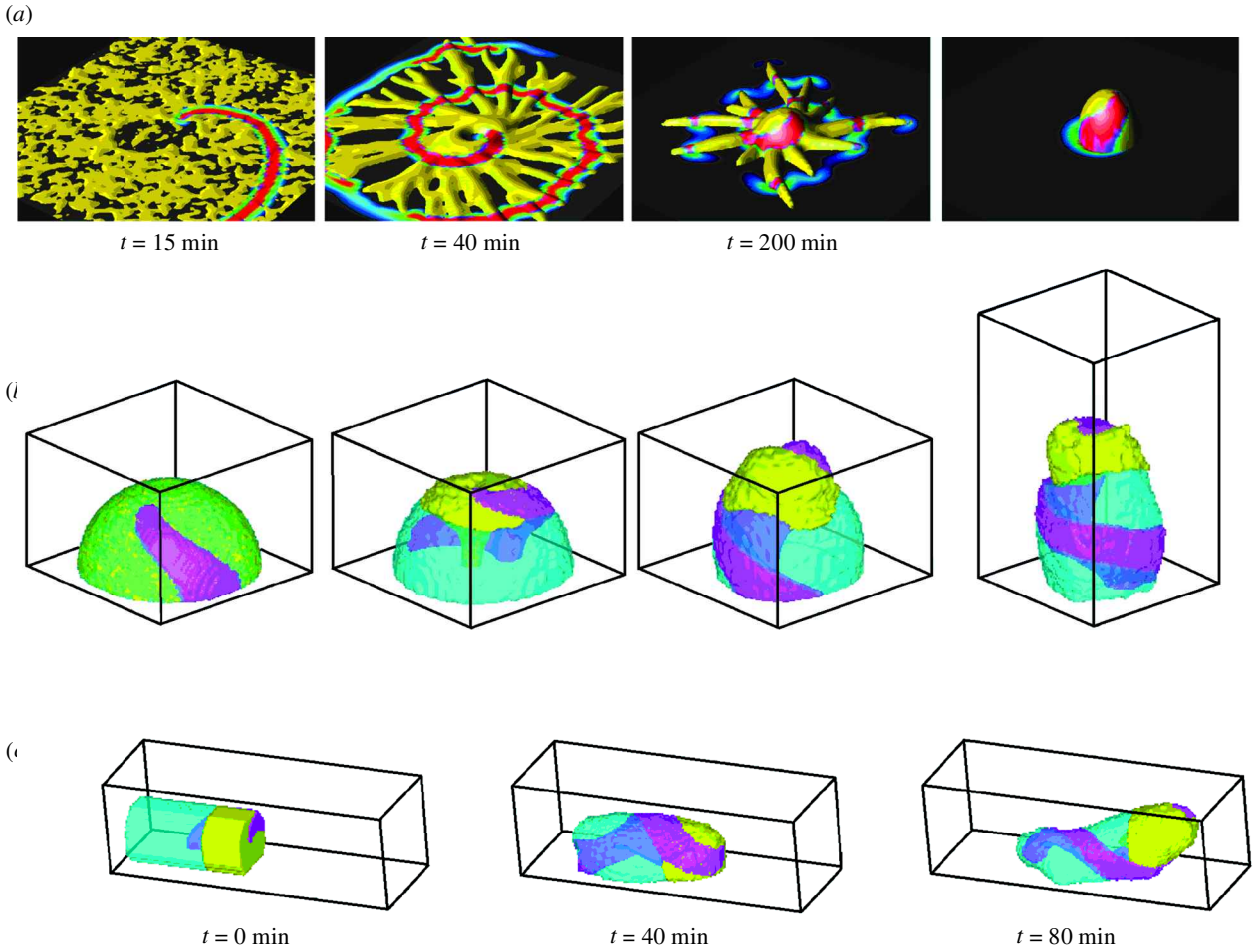

Figure 3. Model calculation of wave propagation and cell movement from aggregation to slug migration using a hydrodynamic model. (*a*) The aggregation up to the mound stage. The first image starts with the randomly distributed cells (yellow) which are organized by a spiral wave of cAMP (red). They form aggregation streams and finally a hemispherical mound (Vasiev *et al.* 1997*b*). (*b*) Cell sorting and the formation of a slug. The mound consists of two cells types, 20% yellow prestalk cells and 80% green prespore cells. They are initially randomly mixed. The cAMP waves (purple) organizes the movement of the cells. The prestalk cells are more excitable and move more strongly in response to a cAMP wave. They move towards the centre of the mound and up to form the tip. The separation of the cells feeds back on the signal propagation resulting in the formation of a twisted scroll wave. This leads to an intercalation of the cells and an upward extension of the slug (Vasiev & Weijer 1999). (*c*) Shows that a slug organized by a scroll wave can move (Vasiev & Weijer 2000).

During later development the cells start to express at least four different cAMP receptors, which can all couple to the same cAMP signalling pathways. They differ in their affinity for cAMP and cell-type specificity of their expression pattern (Chen *et al.* 1996; J. Y. Kim *et al.* 1998). During early aggregation a high-affinity receptor (car1) is expressed and deletion of this receptor leads to a loss of the ability to aggregate. During aggregation the cells start to express car3, a cAMP receptor of slightly lower affinity, in low copy number (Johnson *et al.* 1992, 1993). Deletion of this receptor has no clear morphological consequences. During tip formation, a third low-affinity receptor car2, is expressed by prestalk cells. Deletion of this receptor leads to a block of tip formation and the strain arrests at the mound stage of development (Saxe *et al.* 1993). Our investigation of mutants expressing only one receptor of known affinity has shown that receptor affinity determines the frequency of wave initiation. The lower the affinity the lower the frequency of the waves. Receptor affinity does not influence wave propagation speed. This again is good evidence that cAMP waves are involved in organizing cell movement at the mound stage (Dormann *et al.* 2000).

The patterns of cell movement observed in mounds are dependent on the geometry of the waves coordinating their behaviour. Cell movement in mounds organized by concentric waves is directed towards the pacemaker centre, and is slow. In mounds organized by spiral waves, cell movement is counter-rotational to the direction of wave propagation, and is fast (Rietdorf *et al.* 1996; Sukumaran *et al.* 1998). The rotational movement of the cells is rather continuous and less periodic as during early aggregation where the cells do not yet make strong cell–cell contacts. Their movement is almost fluid like. Each cell moves clearly as an individual, but the behaviour of all cells is coordinated, as evident from the observation of propagating OD waves, which are a measure of the synchrony of the response of the cells to the chemotactic

signal. The cells still make close cell–cell contacts and the low wave propagation speed suggests that the cells signal only their immediate neighbours.

5. SLUGS

During slug formation the hemispherical mound transforms into a long cylinder, the 'standing slug'. This transformation happens by an intercalation of the cells. The first sign of this intercalation is the formation of a nipple-shaped structure, the tip. The tip forms by the sorting of prestalk A cells towards the tip of the mound (Williams 1995; Williams & Morrison 1994; Williams & Jermyn 1991). The details of the sorting mechanism are still unknown but may involve differential chemotaxis to cAMP signals (Matsukuma & Durston 1979; Sternfeld & David 1981; Early *et al*. 1995) as well as the development of cell-type-specific difference in motive force. The difference in motive force may arise from differences in the function of the cytoskeleton (De Lozanne & Spudich 1987; Rivero *et al*. 1996; Springer *et al*. 1994), or cell-type-specific differences in adhesion (Siu & Kamboj 1990). The tip forms on top of the mound and seems to direct all subsequent morphogenesis. The tip has the properties of an organizer. When a tip is transplanted into the side of a slug, it will organize the tissue more posterior to the side to form a secondary slug (Raper 1940; Rubin & Robertson 1975). When the tip is cut off a slug the tip continues to move forward but the prespore zone stops movement immediately and rounds up. The scattered anterior-like cells sort out to reform a new tip. This new slug will then continue to migrate. The tip effectively controls the behaviour of cells up to hundreds of cell diameters away. This is also evident during the directed motion up light and temperature gradients, which are all guided by the tip. This requires long-range signalling.

We have studied the patterns of cell movement in the slug. A good description of movement of individual cells in the slug should help to understand the signals responsible for the control of their movement (Abe *et al*. 1994; Siegert & Weijer 1991, 1992). Cells in the back of the prespore zone move forwards in the direction of slug migration in a periodic fashion (figure 2). The movement velocity of neighbouring cells is modulated in a similar manner, suggesting that they react to a common periodic signal. They do not strictly keep their neighbours; however, all the cells in the back of the slug move on average with slug speed. The cells in the prestalk zone show a very different movement pattern. Often their movement is rotational around the long axis of the prestalk zone, slightly slanted to the direction of slug migration. This rotational movement is especially strong in the pstO zone when the tip is lifted up from the substrate in the air (Abe *et al*. 1994). Due to their twisted tracks the speed of movement of the individual prestalk cells is greater then the forward movement speed of the slug.

This movement pattern can be most easily explained by chemotaxis to a cAMP signal that takes the form of a rotating three-dimensional spiral (scroll) wave in the tip and single planar waves of cAMP in the back of the slug (Siegert & Weijer 1992). We have shown using model calculations that a difference in oscillation frequency of the cells in the prestalk and prespore zone can give rise to these wave forms (Bretschneider *et al*. 1995; Steinbock *et al*. 1993). If the prestalk cells oscillate faster they can sustain a scroll wave which twists to form a twisted scroll in the prespore zone, a region of lower oscillation frequency. This wave pattern could result from all the cells in the prestalk zone relaying the cAMP signal, while only the anterior-like cells relay the signal in the prespore zone. They have a prestalk-like character, make up 10–15% of the cells in the prespore zone and are randomly scattered. Upon culmination they will form parts of the basal disk, upper and lower cup (Bretschneider *et al*. 1995).

We observed OD wave propagation in the back end of the prespore zone of *Dictyostelium mucoroides* slugs (Dormann *et al*. 1997). Furthermore, we found that the motion of the tip is clearly periodic with a periodicity, which is similar to the period of the OD waves. More recently we have also found propagating planar OD waves in the prespore zone of *D. discoideum* slugs of wild-type strains (D. Dormann & C. J. Weijer, unpublished observations). It has been shown that strains lacking the aggregation stage-specific adenylate cyclase gene ACA can be made to develop if pulsed with cAMP during the early stages of development (Pitt *et al*. 1992, 1993). This can be improved by constitutively overexpressing the catalytic subunit of the cAMP-dependent protein kinase (Wang & Kuspa 1997). Our initial experiments have shown that we cannot see any OD wave propagation in either mounds or slugs of this mutant. Furthermore, there is little evidence for periodic cell movement in slugs of this strain. However, cells in mounds and slugs of this strain can show extensive rotational cell movement (D. Dormann and C. J. Weijer, unpublished data). These new data seem to suggest that in the absence of ACA another mechanism can take over the control of cell movement.

The rotational movement observed in this strain reminds one of the rotational movement of cells in colonies of some bacterial strains (BenJacob 1997). The rotational movement there has been postulated to result from chemotaxis of the cells towards a chemoattractant secreted by all cells, which keeps them together with a persistent cell autonomous movement. It is not difficult to imagine that a similar mechanism could result in circular cell movement in mounds. One possibility is that a newly discovered adenylate cyclase (ACB) produces extracellular cAMP under these conditions (H. J. Kim *et al*. 1998; Meima & Schaap 1999). In slugs the situation is much less clear since the movement of all the cells is clearly controlled by the slug tip. This is especially evident during changes in slug shape, photo- and thermotaxis and culmination (Fisher *et al*. 1997; Dormann *et al*. 1996). Therefore the tip must be able to exercise some long-range control over the movement of all the other cells in the slug to do this.

6. MODELLING MORPHOGENESIS

A population of *Dictyostelium* cells behaves as a biological excitable medium, in which the cells communicate by propagating waves of cAMP. These waves interact with the dynamics of the medium on at least two time-scales. On a short time-scale they induce motion of the

excitable elements, the cells. On a longer time-scale they control the gene expression of signalling molecules, which in turn changes the signalling and movement dynamics of the cells. Since these interactions are highly nonlinear it is very difficult just to imagine how they may work even qualitatively. We therefore have attempted to model the morphogenesis of *Dictyostelium* to test our understanding of the basic principles involved. We model the excitable cAMP kinetics by considering the cells as a generic excitable medium that is capable of producing waves of cAMP in two and three dimensions. To describe cell movement we either consider them as a composite viscous fluid (figure 3), or as a collection of discrete cells (Bretschneider *et al*. 1997, 1999; Vasiev *et al*. 1997*b*; Vasiev & Weijer 1999), that move in response to the chemotactic gradients created by the cells. Using these descriptions we can model wave initiation during early aggregation, stream and mound formation. By taking into account two different cell types with different signalling and movement properties we can model cell sorting, tip formation and slug migration. Cell sorting will feedback on the signalling patterns in the tipped mound, since prestalk and prespore cells differ in their excitability (see above). Cell sorting affects the signalling system in the following way: the collection of fast oscillating prestalk cells in the tip leads to an increase in excitability in the tip. This will result in a loss of spiral arms to form a simple scroll wave in the tip (Bretschneider *et al*. 1997; Vasiev *et al*. 1997*a*). The removal of the highly excitable prestalk cells from the body of the mound will result in a decrease in local excitability of the prespore cell mass, and a conversion of the scroll wave in the tip into a twisted scroll wave in the mound (Bretschneider *et al*. 1995; Steinbock *et al*. 1993). This will then direct a twisted rotational cell movement in the base of the mound. As a result the mound contracts, i.e. the cells intercalate and at the same time the mound elongates and extends up into the air (Vasiev & Weijer 1999). The slug can fall over and migrate away.

The work described here shows that we have started to understand some of the cellular principles involved in the morphogenesis of *Dictyostelium*. It seems most likely that *Dictyostelium* morphogenesis results from the propagation of waves of a chemoattractant, cAMP, which coordinates a differential chemotactic movement response. The geometry of the signal controls the movement patterns of the cells and therefore the shape of the organism. The proposed mechanism of cell sorting needs to be tested further by investigation of wave propagation and cell movement patterns in various signalling and cell motility mutants. Understanding the cellular events leading to culmination will be the next major challenge.

This work was supported by the Wellcome Trust.

REFERENCES

Abe, T., Early, A., Siegert, F., Weijer, C. & Williams, J. 1994 Patterns of cell movement within the *Dictyostelium* slug revealed by cell type-specific, surface labeling of living cells. *Cell* **77**, 687–699.

Alcantara, F. & Monk, M. 1974 Signal propagation during aggregation in the slime mould *Dictyostelium discoideum*. *J. Gen. Microbiol.* **85**, 321–334.

Aubrey, L. & Firtel, R. 1999 Integration of signalling networks that regulate *Dictyostelium* differentiation. *A. Rev. Cell. Dev. Biol.* **15**, 469–517.

BenJacob, E. 1997 From snowflake formation to growth of bacterial colonies. II. Cooperative formation of complex colonial patterns. *Contemp. Phys.* **38**, 205–241.

Bretschneider, T., Siegert, F. & Weijer, C. J. 1995 Three-dimensional scroll waves of cAMP could direct cell movement and gene expression in *Dictyostelium* slugs. *Proc. Natl Acad. Sci. USA* **92**, 4387–4391.

Bretschneider, T., Vasiev, B. & Weijer, C. J. 1997 A model for cell movement during *Dictyostelium* mound formation. *J. Theor. Biol.* **189**, 41–51.

Bretschneider, T., Vasiev, B. & Weijer, C. J. 1999 A model for *Dictyostelium* slug movement. *J. Theor. Biol.* **199**, 125–136.

Chen, M. Y., Insall, R. H. & Devreotes, P. N. 1996 Signaling through chemoattractant receptors in *Dictyostelium*. *Trends Genet.* **12**, 52–57.

Chen, M. Y., Long, Y. & Devreotes, P. N. 1997 A novel cytosolic regulator, pianissimo, is required for chemoattractant receptor and G protein-mediated activation of the 12 transmembrane domain adenylyl cyclase in *Dictyostelium*. *Genes Dev.* **11**, 3218–3231.

De Lozanne, A. & Spudich, J. A. 1987 Disruption of the *Dictyostelium* myosin heavy chain gene by homologous recombination. *Science* **236**, 1086–1091.

Devreotes, P. 1989 Cell–cell interactions in *Dictyostelium* development. *Trends Genet.* **5**, 242–245.

Devreotes, P., Fontana, D., Klein, P., Sherring, J. & Theibert, A. 1987 Transmembrane signaling in *Dictyostelium*. *Meth. Cell Biol.* **28**, 299–331.

Dormann, D., Siegert, F. & Weijer, C. J. 1996 Analysis of cell movement during the culmination phase of *Dictyostelium* development. *Development* **122**, 761–769.

Dormann, D., Weijer, C. & Siegert, F. 1997 Twisted scroll waves organize *Dictyostelium* mucoroides slugs. *J. Cell Sci.* **110**, 1831–1837.

Dormann, D., Vasiev, B. & Weijer, C. J. 1998 Propagating waves control *Dictyostelium discoideum* morphogenesis. *Biophys. Chem.* **72**, 21–35.

Dormann, D., Kim, J. Y., Deureotes, P. N., Weijer, C. J. 2000 cAMP receptor affinity controls the frequency of cAMP wave initiation and *Dictyostelium* morphogenesis. *Development*. (Submitted.)

Early, A., Abe, T. & Williams, J. 1995 Evidence for positional differentiation of prestalk cells and for a morphogenetic gradient in *Dictyostelium*. *Cell* **83**, 91–99.

Firtel, R. A. 1996 Interacting signaling pathways controlling multicellular development in *Dictyostelium*. *Curr. Opin. Genet. Dev.* **6**, 545–554.

Fisher, P. R., Noegel, A. A., Fechheimer, M., Rivero, F., Prassler, J. & Gerisch, G. 1997 Photosensory and thermosensory responses in *Dictyostelium* slugs are specifically impaired by absence of the F-actin cross-linking gelation factor (ABP-120). *Curr. Biol.* **7**, 889–892.

Foerster, P., Muller, S. & Hess, B. 1990 Curvature and spiral geometry in aggregation patterns of *Dictyostelium discoideum*. *Development* **109**, 11–16.

Franke, J. & Kessin, R. H. 1992 The cyclic nucleotide phosphodiesterases of *Dictyostelium discoideum*—molecular genetics and biochemistry. *Cell. Signal.* **4**, 471–478.

Futrelle, R. P. 1982 *Dictyostelium* chemotactic response to spatial and temporal gradients. Theories of the limits of chemotactic sensitivity and of pseudochemotaxis. *J. Cell. Biochem.* **18**, 197–212.

Gerisch, G. 1987 Cyclic AMP and other signals controlling cell development and differentiation in *Dictyostelium*. *A. Rev. Biochem.* **56**, 853–879.

Gerisch, G., Noegel, A., Schleicher, M., Segall, J. & Wallraff, E. 1987 Signal transduction and chemotaxis in *Dictyostelium discoideum*. *Biol. Chem. H.-S.* **368**, 1045–1046.

Gross, J. D., Peacey, M. J. & Trevan, D. J. 1976 Signal emission and signal propagation during early aggregation in *Dictyostelium discoideum*. *J. Cell Sci.* **22**, 645–656.

Harloff, C., Gerisch, G. & Noegel, A. A. 1989 Selective elimination of the contact site A protein of *Dictyostelium discoideum* by gene disruption. *Genes Dev.* **3**, 2011–2019.

Hofer, T. & Maini, P. K. 1997 Streaming instability of slime mold amoebae: an analytical model. *Phys. Rev.* E **56**, 2074–2080.

Insall, R. H., Borleis, J. & Devreotes, P. N. 1996 The aimless RasGEF is required for processing of chemotactic signals through G-protein-coupled receptors in *Dictyostelium*. *Curr. Biol.* **6**, 719–729.

Johnson, R. L., Van Haastert, P. J. M., Kimmel, A. R., Saxe III, C. L., Jastorff, B. & Devreotes, P. N. 1992 The cyclic nucleotide specificity of three cAMP receptors in *Dictyostelium*. *J. Biol. Chem.* **267**, 4600–4607.

Johnson, R. L., Saxe III, C. L., Gollop, R., Kimmel, A. R. & Devreotes, P. N. 1993 Identification and targeted gene disruption of cAR3, a cAMP receptor subtype expressed during multicellular stages of *Dictyostelium* development. *Genes Dev.* **7**, 273–282.

Kim, H. J., Chang, W. T., Meima, M., Gross, J. D. & Schaap, P. 1998 A novel adenylyl cyclase detected in rapidly developing mutants of *Dictyostelium*. *J. Biol. Chem.* **273**, 30 859–30 862.

Kim, J. Y., Borleis, J. A. & Devreotes, P. N. 1998 Switching of chemoattractant receptors programs development and morphogenesis in *Dictyostelium*: receptor subtypes activate common responses at different agonist concentrations. *Devl Biol.* **197**, 117–128.

Lee, S., Parent, C. A., Insall, R. & Firtel, R. A. 1999 A novel Ras-interacting protein required for chemotaxis and cyclic adenosine monophosphate signal relay in *Dictyostelium*. *Mol. Biol. Cell* **10**, 2829–2845.

Levine, H. & Reynolds, W. 1991 Streaming instability of aggregating slime mold amoebae. *Phys. Rev. Lett.* **66**, 2400–2403.

Loomis, W. F. 1982 *The development of* Dictyostelium discoideum. New York: Academic Press.

Matsukuma, S. & Durston, A. J. 1979 Chemotactic cell sorting in *Dictyostelium discoideum*. *J. Embryol. Exp. Morphol.* **50**, 243–251.

Meili, R., Ellsworth, C., Lee, S., Reddy, T. B. K., Ma, H. & Firtel, R. A. 1999 Chemoattractant-mediated transient activation and membrane localization of Akt/PKB is required for efficient chemotaxis to cAMP in *Dictyostelium*. *EMBO J.* **18**, 2092–2105.

Meima, M. E. & Schaap, P. 1999 Fingerprinting of adenylyl cyclase activities during *Dictyostelium* development indicates a dominant role for adenylyl cyclase B in terminal differentiation. *Devl Biol.* **212**, 182–190.

Parent, C. A. & Devreotes, P. N. 1996 Molecular genetics of signal transduction in *Dictyostelium*. *A. Rev. Biochem.* **65**, 411–440.

Parent, C. A. & Devreotes, P. N. 1999 A cell's sense of direction. *Science* **284**, 765–770.

Parent, C. A., Blacklock, B. J., Froehlich, W. M., Murphy, D. B. & Devreotes, P. N. 1998 G protein signaling events are activated at the leading edge of chemotactic cells. *Cell* **95**, 81–91.

Patel, H., Guo, K., Parent, C., Gross, J., Deureotes, P. N. & Weijer, C. J. 2000 A temperature sensitive adenylyl cyclase mutant of *Dictyostelium*. *EMBO J.* **19**, 1–11.

Pitt, G. S., Milona, N., Borleis, J., Lin, K. C., Reed, R. R. & Devreotes, P. N. 1992 Structurally distinct and stage-specific adenylyl cyclase genes play different roles in *Dictyostelium* development. *Cell* **69**, 305–315.

Pitt, G. S., Brandt, R., Lin, K. C., Devreotes, P. N. & Schaap, P. 1993 Extracellular cAMP is sufficient to restore developmental gene expression and morphogenesis in *Dictyostelium* cells lacking the aggregation adenylyl cyclase (ACA). *Genes Dev.* **7**, 2172–2180.

Ponte, E., Bracco, E., Faix, J. & Bozzaro, S. 1998 Detection of subtle phenotypes: the case of the cell adhesion molecule csA in *Dictyostelium*. *Proc. Natl Acad. Sci. USA* **95**, 9360–9365.

Raper, K. B. 1940 Pseudoplasmodium formation and organization in *Dictyostelium discoideum*. *J. Elisha Mitchell Sci. Soc.* **56**, 241–282.

Rietdorf, J., Siegert, F. & Weijer, C. J. 1996 Analysis of optical-density wave-propagation and cell-movement during mound formation in *Dictyostelium discoideum*. *Devl Biol.* **177**, 427–438.

Rietdorf, J., Siegert, F. & Weijer, C. J. 1998 Induction of optical density waves and chemotactic cell movement in *Dictyostelium discoideum* by microinjection of cAMP pulses. *Devl Biol.* **204**, 525–536.

Rivero, F., Koppel, B., Peracino, B., Bozzaro, S., Siegert, F., Weijer, C. J., Schleicher, M., Albrecht, R. & Noegel, A. A. 1996 The role of the cortical cytoskeleton—F-actin cross-linking proteins protect against osmotic-stress, ensure cell-size, cell-shape and motility, and contribute to phagocytosis and development. *J. Cell Sci.* **109**, 2679–2691.

Rubin, J. & Robertson, A. 1975 The tip of *Dictyostelium discoideum* pseudoplasmodium as an organizer. *J. Embryol. Exp. Morphol.* **33**, 227–241.

Saxe III, C. L., Ginsburg, G. T., Louis, J. M., Johnson, R., Devreotes, P. N. & Kimmel, A. R. 1993 CAR2, a prestalk cAMP receptor required for normal tip formation and late development of *Dictyostelium discoideum*. *Genes Dev.* **7**, 262–272.

Shaulsky, G., Fuller, D. & Loomis, W. F. 1998 A cAMP-phosphodiesterase controls PKA-dependent differentiation. *Development* **125**, 691–699.

Siegert, F. & Weijer, C. 1989 Digital image processing of optical density wave propagation in *Dictyostelium discoideum* and analysis of the effects of caffeine and ammonia. *J. Cell Sci.* **93**, 325–335.

Siegert, F. & Weijer, C. J. 1991 Analysis of optical density wave propagation and cell movement in the cellular slime mold *Dictyostelium discoideum*. *Physica* D **49**, 224–232.

Siegert, F. & Weijer, C. J. 1992 Three-dimensional scroll waves organize *Dictyostelium* slugs. *Proc. Natl Acad. Sci. USA* **89**, 6433–6437.

Siegert, F. & Weijer, C. J. 1995 Spiral and concentric waves organize multicellular *Dictyostelium* mounds. *Curr. Biol.* **5**, 937–943.

Siu, C. H. & Kamboj, R. K. 1990 Cell–cell adhesion and morphogenesis in *Dictyostelium discoideum*. *Devl Genet.* **11**, 377–387.

Springer, M. L., Patterson, B. & Spudich, J. A. 1994 Stage-specific requirement for myosin II during *Dictyostelium* development. *Development* **120**, 2651–2660.

Stadler, J., Keenan, T. W., Bauer, G. & Gerisch, G. 1989 The contact site A glycoprotein of *Dictyostelium discoideum* carries a phospholipid anchor of a novel type. *EMBO J.* **8**, 371–377.

Steinbock, O., Siegert, F., Muller, S. C. & Weijer, C. J. 1993 Three-dimensional waves of excitation during *Dictyostelium* morphogenesis. *Proc. Natl Acad. Sci. USA* **90**, 7332–7335.

Sternfeld, J. & David, C. N. 1981 Cell sorting during pattern formation in *Dictyostelium*. *Differentiation* **20**, 10–21.

Sukumaran, S., Brown, J. M., Firtel, R. A. & McNally, J. G. 1998 lagC-null and gbf-null cells define key steps in the morphogenesis of *Dictyostelium mounds*. *Devl Biol.* **200**, 16–26.

Thomason, P. A., Traynor, D., Stock, J. B. & Kay, R. R. 1999 The RdeA–RegA system, a eukaryotic phospho-relay controlling cAMP breakdown. *J. Biol. Chem.* **274**, 27 379–27 384.

Tomchik, K. J. & Devreotes, P. N. 1981 Adenosine 3′,5′-monophosphate waves in *Dictyostelium discoideum*: a demonstration by isotope dilution-fluorography technique. *Science* **212**, 443–446.

Varnum-Finney, B., Edwards, K. B., Voss, E. & Soll, D. R. 1987 Amebae of *Dictyostelium discoideum* respond to an increasing temporal gradient of the chemoattractant cAMP with a reduced frequency of turning: evidence for a temporal mechanism in ameboid chemotaxis. *Cell Motil. Cytoskel.* **8**, 7–17.

Varnum-Finney, B., Schroeder, N. A. & Soll, D. R. 1988 Adaptation in the motility response to cAMP in *Dictyostelium discoideum*. *Cell Motil. Cytoskel.* **9**, 9–16.

Vasiev, B. & Weijer, C. J. 1999 Modeling chemotactic cell sorting during *Dictyostelium discoideum* mound formation. *Biophys. J.* **76**, 595–605.

Vasiev, B. & Weijer, C. J. 2000 A hydrodynamic model for *Dictyostelium* slug migration. *Biophys. J.* (Submitted.)

Vasiev, B. N., Hogeweg, P. & Panfilov, A. V. 1994 Simulation of *Dictyostelium discoideum* aggregation via reaction–diffusion model. *Phys. Rev. Lett.* **73**, 3173–3176.

Vasiev, B. N., Siegert F., Weijer C. J. 1997*a* Multiarmed spirals in excitable media. *Phys. Rev. Lett.* **78**, 2489–2492.

Vasiev, B. N., Siegert, F. & Weijer, C. J. 1997*b* A hydrodynamic model for *Dictyostelium discoideum* mound formation. *J. Theor. Biol.* **184**, 441.

Wang, B. & Kuspa, A. 1997 *Dictyostelium* development in the absence of cAMP. *Science* **277**, 251–254.

Weeks, G. & Weijer, C. J. 1994 The *Dictyostelium* cell cycle and its relationship to differentiation. (Minireview.) *FEMS Microbiol. Lett.* **124**, 123–130.

Williams, J. 1995 Morphogenesis in *Dictyostelium*—new twists to a not-so-old tale. *Curr. Opin. Genet. Dev.* **5**, 426–431.

Williams, J. G. & Jermyn, K. A. 1991 Cell sorting and positional differentiation during *Dictyostelium* morphogenesis. In *Cell–cell interactions in early development* (ed. J. Gerhart), pp. 261–272. New York: Wiley-Liss.

Williams, J. G. & Morrison, A. 1994 Prestalk cell-differentiation and movement during the morphogenesis of *Dictyostelium discoideum*. *Progr. Nucl. Acid Res. Mol. Biol.* **47**, 1–27.

Wu, L. J., Valkema, R., Van Haastert, P. J. M. & Devreotes, P. N. 1995 The G protein beta subunit is essential for multiple responses to chemoattractants in *Dictyostelium*. *J. Cell Biol.* **129**, 1667–1675.