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Becoming Multicellular by Aggregation; The Morphogenesis of the Social Amoebae ***Dicyostelium discoideum***

D. DORMANN, B. VASIEV[1] and C.J. WEIJER*
Division of Cell and Developmental Biology, Wellcome Trust Biocentre, University of Dundee, Dundee DD1 5EH, UK
[1]*Present Address: Dept of Mathematics, University of Dundee, Dundee DD1 4EH, UK*
(**Author for correspondence, e-mail: c.j.weijer@dundee.ac.uk*)

Abstract. The organisation and form of most organisms is generated during their embryonic development and involves precise spatial and temporal control of cell division, cell death, cell differentiation and cell movement. Differential cell movement is a particularly important mechanism in the generation of form. Arguably the best understood mechanism of directed movement is chemotaxis. Chemotaxis plays a major role in the starvation induced multicellular development of the social amoebae *Dictyostelium*. Upon starvation up to 10^5 individual amoebae aggregate to form a fruiting body. In this paper we review the evidence that the movement of the cells during all stages of *Dictyostelium* development is controlled by propagating waves of cAMP which control the chemotactic movement of the cells. We analyse the complex interactions between cell-cell signalling resulting in cAMP waves of various geometries and cell movement which results in a redistribution of the signalling sources and therefore changes the geometry of the waves. We proceed to show how the morphogenesis, including aggregation stream and mound formation, slug formation and migration, of this relatively simple organism is beginning to be understood at the level of rules for cell behaviour, which can be tested experimentally and theoretically by model calculations.

Key words: cell sorting, chemotaxis, modelling, morphogenesis, signal relay, wave propagation

1. Introduction

Development of a vertebrate embryo typically involves the generation of millions of cells that differentiate into hundreds of cell types to form a wide variety of 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 celltypes have to move to their final destination. In general more is known about how cells differentiate into different celltypes, but much less is known how they then are organised in specific tissues. The signals controlling cell movement have been difficult to study in higher organisms 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 amoebae *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, they live in the soil and feed on bacteria. When the population size increases, the cells in the centre of the colony will start to starve and starvation is a signal for the cells to enter a multicellular developmental phase. Up to 10^5 cells aggregate to form a multicellular structure, the slug, which migrates under the control of environmental signals such as light and temperature gradients to the soil surface, where low humidity and overhead light trigger the conversion of the slug into a fruiting body. The fruiting body consisting of a stalk of dead vaculoated cells supporting a mass of spores. The spores disperse and each spore can germinate to start a new colony under favourable conditions.

During aggregation the cells start to differentiate into prestalk and prespore cells that are precursors of the stalk and spore cells in the fruiting body. The cells enter the mound in a random order and are initially distributed in a salt and pepper pattern. Then a cell sorting process takes place in which the prestalk cells move to the top of the aggregate to form a distinct morphological structure, the tip, which guides the movement of all the other cells and is also involved in the control of the photo- and thermotactic response [1]. Since the multicellular phase of the development occurs in absence of food there is little cell division during development [2]. The number of cells doubles at most. Morphogenesis therefore results from the co-ordinated cell movement of a few emerging cell types.

During development the cells can communicate over distances of many thousand cell diameters. 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 organise 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 (Figure 1) that govern aggregation are understood at the cellular level to result from the following competencies:

- Periodic cAMP signal generation by cells in the aggregation centre.
- cAMP signal relay by all other cells.
- Chemotaxis up a cAMP gradient during the rising phase of the wave.

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 synthesise 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

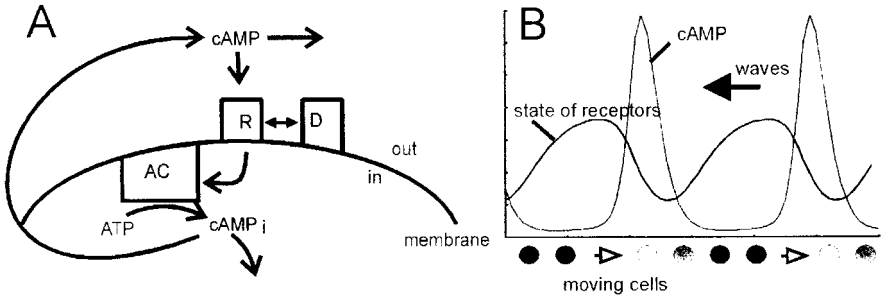

Figure 1. (**A**) Martiel-Goldbeter model for cAMP oscillations. cAMP binds to the receptor (R) and activates the adenylylcyclase (AC) to produce cAMP in the cell ($cAMP_i$) part of this cAMP is secreted to the outside (cAMP) where it binds to the receptor again, thus forming a positive feedback loop. Binding of cAMP to the receptor also uncouples the receptor form the adenylylcyclase (D) resulting in a cessation of cAMP production. Extracellular and intracellular cAMP are being degraded all the time by extracellular and intracellular phosphodiesterase thus allowing the system to return to a basal state where it be activated again resulting in cAMP oscillations. (**B**) Scheme showing wave propagation and cell movement. The cAMP wave profile and fraction of active receptors are shown as calculated from the model. Waves propagate from right to left, while cells (arrows and circles) move from left to right. Arrows represents moving cells, black circles resting cells, grey circles cells unable to move as a result of adaptation.

autocatalytic cAMP induced cAMP amplification and a slightly slower negative feedback loop [3, 4]. cAMP binds to highly specific and sensitive serpentine cAMP receptors, which triggers two competing processes, excitation and adaptation. The complex signalling pathways underlying these processes are being rapidly elucidated and are reviewed in detail elsewhere [5]. The difference in activation and adaptation is measured and results in the activation of the aggregation stage adenylylcyclase the enzyme that produces cAMP inside the cell from ATP. cAMP is secreted to the outside, where it can bind to the receptor again. This positive feedback loop leads to the production of cAMP. cAMP is degraded continuously by an intracellular phosphodiesterase regA and a secreted extracellular phosphodiesterase (ePDE) (Figure 1A). The molecular basis of adaptation is still unknown but may involve an inhibitory g protein (Gi). After adaptation has set in adenylylcyclase is no longer activated and the extracellular cAMP levels are going to fall, due to the action of the secreted phosphodiesterase, which degrades 3′–5′cAMP to the inactive 5′AMP.

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 2). There is also a feedback of the cAMP signal on the expression of the components necessary for signal detection

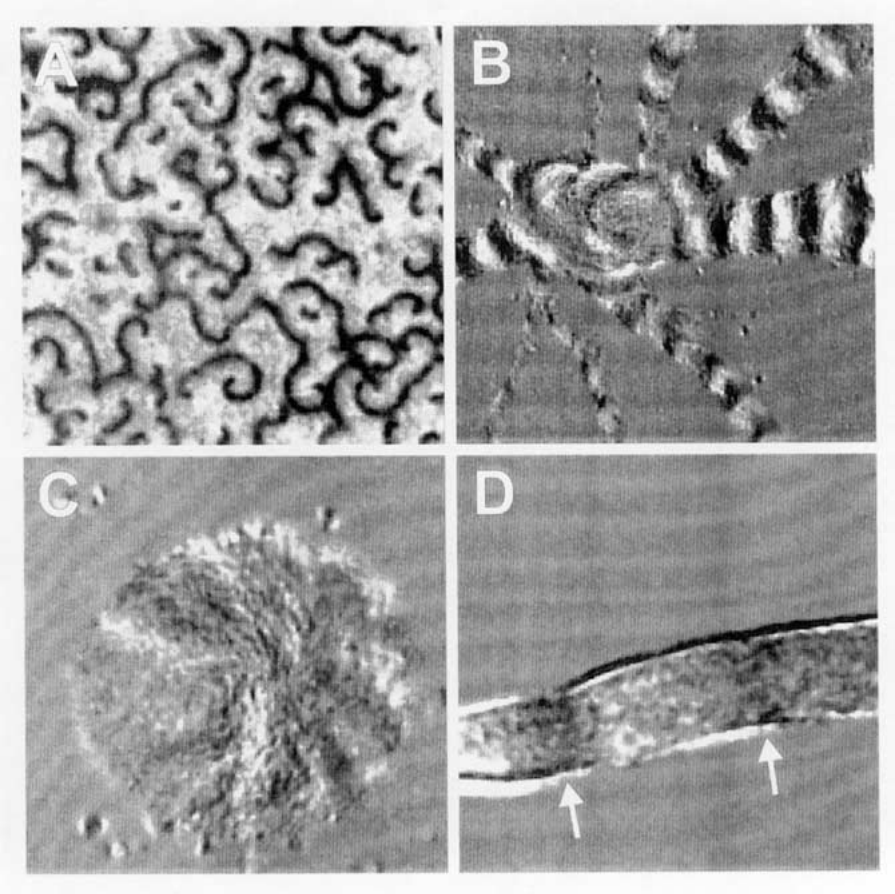

Figure 2. Optical density waves at different stages of development. **A**: Spiral optical density waves during the early aggregation phase, when the cells are still in a monolayer on agar. **B** Optical density waves in a streaming aggregate. In the body of the aggregate multi-armed spiral waves rotates counter clockwise throwing off individual wavesfronts that propagate down the streams to the periphery of the aggregate. **C**: Multi-armed waves in the body of a mound after the streams have reached the aggregate. The waves rotate counter clockwise directing the clock wise movement of the cells **D**: A slug of the strain NP377 migrating from right to left, showing two dark optical density waves that travel from left to right, indicated by the white arrows.

and amplification, such as cAMP receptors and adenylate cyclase itself [6, 7]. 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 to the wavelength of the cAMP waves. This difference has to

be amplified to result in directed chemotactic movement. This polarisation process can be visualised by the localised translocation of PH domain containing proteins such as CRAC and protein kinase B (PKB) [8, 9]. The cells stop moving as soon as the cAMP wave passes and the concentration starts to fall [10]. This chemotactic response leads to the periodic inward movement of the cells to the aggregation centre guided by outward propagating waves of cAMP.

During early aggregation the cAMP waves can be seen as optical density waves using low-power darkfield optics [11]. These optical density 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 optical density waves observed during aggregation faithfully represent the propagating cAMP signal [12]. Most often waves appear as expanding spirals, in some strains they also form target wave patterns. Spiral waves can arise spontaneously in excitable media and do not need autonomously oscillating cells, unlike concentric waves, which require oscillating cells in the aggregation centre.

3. Aggregation Streams

After around 15–20 waves have passed the cells become organised 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 wave fronts 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 speed up wave propagation locally, resulting in a slightly outward bulging wave front, which will result in the further attraction of cells to this region [13, 14]. Stream formation is an autocatalytic process and will rapidly spread through the aggregation territory. Cells in streams are highly polarised and elongated and very closely packed. Propagating optical density waves can readily be observed in aggregation streams. Most late aggregation centres are organised by spiral waves, which break up into individual wave fronts that propagate down the streams. The wave propagation speed can be as low as 50 μm/minute, which implies that the signal propagates around one cell diameter every 10–15 seconds. The cAMP signal hops from cell to cell, as can be visualised by the translocation of CRAC in aggregation streams (Dormann and Weijer, unpublished observations). Cell movement is still periodic [15]. The cells make specific end-to-end contact via homophilic interactions of an EDTA resistant contact molecule, contact site A (CSA). Expression of these cell-cell contact molecules requires exposure of the cells to nano-molar pulses of cAMP. CSA null mutants can still aggregate, lack however the clear stream morphology,

implying a role for these sites in stream preservation [16, 17]. 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 [15].

Mounds

The cells entering the aggregation centre start to pile on top of each other and from a three dimensional hemispherical structure. We could visualise propagating optical density waves in the later stages of *Dictyostelium* development [15, 18]. Continuous measurements of the optical density waves from aggregation until tip formation showed that the frequency of the waves increases during aggregation while the wave propagation speed slows down [11, 15, 19]. During early aggregation the period of the signals may be as long as 6–7 minutes while later in development the period decreases to 1–2 minutes. Wave propagation speed decreases simultaneously from 600 μm/minute to around 50–100 μm/minute in aggregation streams and mounds. As a result of this the chemical wavelength decreases from 3000–4000 μm to 50–200 μm. Therefore large spiral cAMP waves initially organise the several centimetre large aggregation territories during early aggregation and much smaller spiral waves organise the mound (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 this excitable medium [11, 20].

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 [18, 21]. 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 [22]. 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 optical density waves which propagated from the electrode tip outwards. These elicited waves annihilated endogenous waves upon collision, showing a common propagation mechanism [23]. Furthermore we have used a temperature sensitive adenylylcyclase mutant (tsaca2), whose activity is normal at the permissive temperature of 22°C but inhibited at 28°C. Wave propagation at the mound stage can be reversibly inhibited by shifting mounds of the temperature sensitive mutant from the permissive to the restrictive temperature. At the restrictive temperature the waves disappear quickly (within 5 minutes), cells loose

their co-ordination of movement and as a result the mounds start to disperse again. Upon lowering the temperature the waves are reinitiated, initially from many different centres, which compete until one or a few dominant centres are left that will organise the mound again and development continues. These experiment clearly show that the OD waves in mounds are still carried by cAMP [24].

cAMP is detected by at least four different cAMP receptors (cAR1-cAR4), which are expressed at different stages of development and in different cell types. These receptors are closely related in structure and seem mainly to differ in their affinity for cAMP. Biochemical experiments in knockout mutants in which only one receptor is expressed at any given time have shown that all receptors can couple to cAMP relay and chemotaxis when stimulated with the appropriate cAMP concentration [25]. 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 [26, 27]. Deletion of this receptor has no clear morphological consequences in that null mutants can form normal looking fruiting bodies. There are some indications that the cAR3 receptor is necessary for the activation of GSK3, a protein kinase involved in the activation of prespore specific gene expression. 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 [28]. 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 organising cell movement at the mound stage [29].

The patterns of cell movement observed in mounds are dependent on the geometry of the waves co-ordinating their behaviour. Cell movement in mounds organised by concentric waves, is directed towards the pacemaker centre and slow. In mounds organised by spiral waves, cell movement is counter rotational to the direction of wave propagation and fast [15]. 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 co-ordinated, 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. Recently we have been able to see signal transduction in individual cells in aggregation fields and in mounds by looking at the translocation of a series of Pleckstrin Homology domains, which have a highly specific affinity for particular phosphatidyl-inositides such as PIP2 and PIP3. Upon receptor activation these lipids are rapidly generated locally in the plasma membrane and act as docking sites for proteins such as CRAC (Cytosolic

Regulator of Adenylylcyclase) which then form signalling complexes resulting in the activation of adenylylcyclase as well as other protein kinases such as AKT/PkB which are involved in regulation of the actin-myosin cytoskeleton [30, 31]. We can follow the stimulus dependent translocation from the cytoplasm to the membrane in individual cells in aggregates, mounds and slugs and thereby for the first time study the dynamics of signalling at the single cell level in a multi-cellular organism (Dormann et al., in prep).

4. Slugs

During late aggregation the cells start to differentiate into prestalk and prespore cells, which arrive in a random order in the mound. However as a result of cell differentiation the cells start to differ in their signalling and movement properties, which will ultimately result in the sorting out of the prestalkA cells to form the tip on top of the mound [32–34]. The tip continues to act as a signalling source and will direct the movement al all other cell during slug formation, migration and behaviour. The details of the sorting mechanism are still unkown but may involve differential chemotaxis to cAMP signals [35] as well as the development of celltype specific difference in motive force. The difference in motive force may arise from differences in the function of the cytoskeleton [36, 37] or celltype specific differences in adhesion [38]. The tip forms on top of the mound and seems to direct all subsequent morphogenesis. The tip has the properties of an organiser. When a tip is transplanted in the side of a slug, it will organise the tissue more distal from it to from a secondary slug [39, 40]. 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 [41, 42]. Cells in the back of the prespore zone move forwards in the direction of slug migration in a periodic fashion (Figures 2, 3). 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

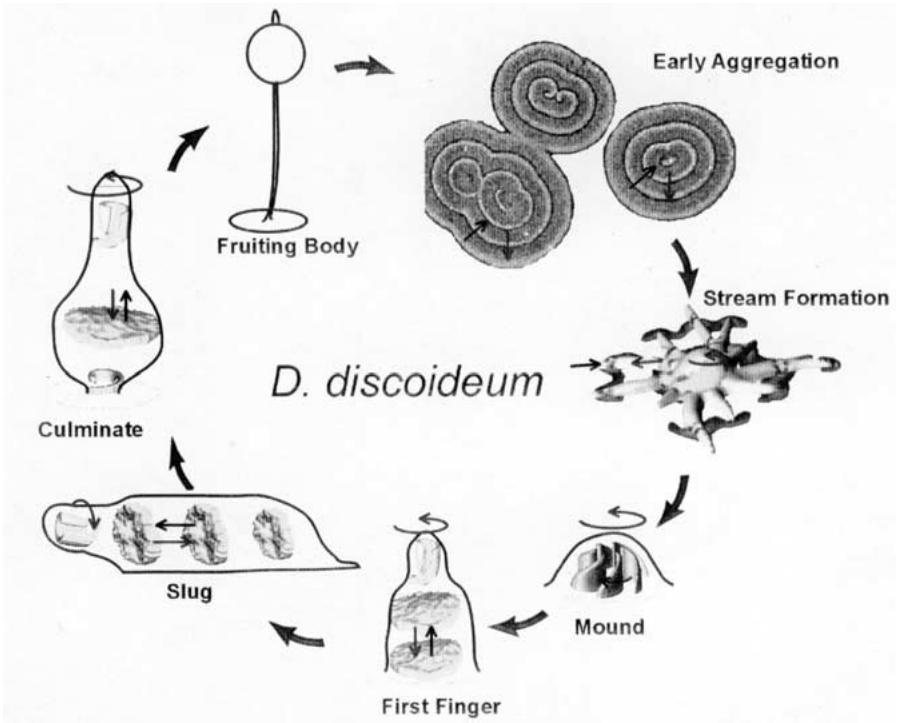

Figure 3. Diagram of the various cAMP wave controlling cell movement at different stages of development. During aggregation the cells communicate by propagating waves of the chemo-attractant cAMP, which spread as spiral waves. Typically wave ends form counter rotating spiral centres. Small random changes in the cell density during aggregation result in the formation of aggregation streams. The aggregation is organised by a spiral wave that throws off simple wave fronts that propagate down the aggregation streams resulting in inward directed cell movement. Once the cells pile up to form the mound the spiral waves form three dimensional scroll waves, which convert into multi-armed spiral waves in the mound. In the mound the cells start to differentiate and the prestalk cells move to the top of the mound to form the tip which functions as a pacemaker. The tip supports a scroll wave which converts in twisted scroll or planar waves in the body of the slug. This directs rotational movement in the tip and periodic forward movement in the body of the slug. Under suitable conditions the slug stands up again to form the fruiting body. The signals controlling fruiting body formation not completely known. Red arrows indicate direction cAMP wave propagation, blue arrows indicate direction of the cell chemotactic cell movement in response to the waves.

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 [41]. 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 [43]. 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 [43].

Recently we have been able to observe optical density waves in the prespore zone that propagate from the region behind the tip towards the back. We have shown that the waves originate in the tip, when the tip is cut off from a slug the prespore zone stops moving immediately and the waves disappear, cell movement is disorganised until a new tip is regenerated. The tip on the other hands continues to migrate while producing OD waves that control its movement. Periodic microinjection of cAMP pulses of the right duration and amplitude can act as a competing signalling centre in the prespore zone of the slug and bring all the cells posterior to the site of injection under its control while most of the cells anterior to the site of injection will stay under the control of the tip giving additional strong evidence that the tip, the *Dictyostelium* organiser, is a centre of cAMP signalling. We have also shown that the tip is a region of high average cAMP concentration using the cAMP dependent translocation of a STATA-GFP fusion protein form the cytoplasm to the nucleus as marker. STATA is normally found in the nucleus of cells in the tip and not in cells in the prespore zone. By injecting cAMP in the prespore zone we could induce the rapid translocation of STATA to the nucleus of prespore cells suggesting that they sense a lower average cAMP signal than prestalk cells *in vivo* [44]. This finding is in good agreement with the idea that all the prestalk cells relay the cAMP signal and only some cells in the prespore zone, the anterior like cells, relay the cAMP signal.

It has been shown that strains lacking the aggregation stage specific adenylylcyclase gene ACA can be made to develop if pulsed with cAMP during the early stages of development [45]. This can be improved by constitutively overexpressing the catalytic subunit of the cAMP dependent protein kinase [46]. Our initial experiments have shown, that we cannot see any optical density 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 (Dormann and Weijer, unpublished observations). 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 [47]. 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 adenylyl cyclase (ACB) produces extracellular cAMP under these conditions [48]. 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 [49]. 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.

5. Culmination

Culmination is the final and most complex transformation that occurs during the Dictyostelium developmental cycle. During the final stages of migration the slug the cells on the top half of the slug slow down their movement speed while the cells close to the substrate keep on moving. This results in a displacement of the tip to the centre top of the slug to form the "mexican hat" stage. During this process the cells in the tip start to form a tube like structure, from extracellular matrix molecules, a main component being cellulose. This is the beginning of the stalk. During culmination some of the prestalk cells start to move into the stalk tube and move down until they make contact with the substrate. After this has happened they differentiate in mature highly vacuolated stalk cells containing stiff cellulose walls. The stalk forms a mechanical structure along which the other cells can move up. During this process more cells enter the stalk tube and the stalk elongates. This process is very complex and requires precise co-ordinated cell movement in combination with a precisely controlled differentiation of the amoebae in stalk cells. We have observed that culmination is initiated by a local aggregation of anterior-like cells at the base of the slug at the prestalk-prespore boundary, where they form a stationary mass of cells. The mass of cells forms by directed aggregation of a selective subclass of cells to this region [50]. The majority of the cells follow the tip, moving over this pile but as a consequence are lifted up in the air. The stationary group of cells eventually forms the basal disk and part of the lower cup of the fruiting body. These cells are characterised by vigorous rotational cell movement. We think it likely that these cells are involved in attracting the cells from the tip downward to the substrate. However the molecule by which these cells communicate has not yet been identified. It has been shown that prestalk cells are motors for the culmination process. Mutants in the cytoskeleton that disrupt the ability of prestalk cells to move properly result normally in a failure of culmination [36, 37, 51, 52].

6. Modelling Morphogenesis

The development of *Dictyostelium* presents a prime example of the complex interactions and feedbacks that exist between the signals being generated by cells and their responses to these signals. 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 and rearrangement 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 non-linear it is very difficult just to imagine how they may work even qualitatively These interactions are far more complex than those found in any physical or chemical excitable system and in order to understand them it will be necessary to model these interactions to test our understanding of the basic principles involved. We model the excitable cAMP kinetics by considering the cells as an generic excitable medium that is capable of producing waves 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 [53–56], 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 with all cell having the same properties. By taking into account two different cell types with different signalling and movement properties we can model cell sorting, tip formation and slug migration. To model cell sorting we have to assume that the prestalk cells that will sort eventually to the tip of the mound have to develop more effective chemotactive force than prespore cells. The mound is initially organised by a scroll wave, which directs all the cells to the centre. The cells that can exert most effective chemotactic forces are able to displace the other cells from the central position where they will be pushed up by the other cells trying to move towards the centre. The cell sorting will feedback on the signalling patterns in the tipped mound, since prestalk and prespore cells differ in their excitability, prestalk cells as a result of their differentiation are more excitable as prespore cells (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 [22, 55]. 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 to a conversion of the scroll wave in the tip, into a twisted scroll wave in the body of the mound [43]. This will then direct a twisted counter rotational cell movement of the cells in the mound. As a result the cells in the mound experience an inward and upward directed chemotactic gradient when they move and this will result in the intercalation of the cells and extension of the mound in the direction of the tip into the air [54]. The structure that forms is a slug, which will fall over and migrate away (Vasiev and Weijer, submitted). These elements can all be caught quite well by any model that describes cells as excitable units and requires the existence of at least two different cell populations which differ in their ability to move in response to a cAMP signal and furthermore differ in their ability to relay the signal. The part which still has to be included is the mechanism responsible for the cell type differentiation and the stabilisation of the cell types once they have formed and are spatially separated in the slug. It is well known from experimental observations that cAMP plays a major role as signalling molecule in controlling not only the expression of the early genes but it is also involved in the induction and

stabilisation of prespore gene expression. We have proposed in the past that cAMP, which is produced in a prestalk dependent manner acts by inhibiting the conversion of prespore to prestalk cells. Another factor such as DIF, which is produced by prespore cells could result in the stabilisation of prestalk cells [57, 58].

The work described here shows that we start 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 chemo-attractant, cAMP, which co-ordinates 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 further tested 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.

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