The Morphogenesis of Dictyostelium Discoideum – Pattern Formation in a Biological Excitable System

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1. Introduction

A major goal in the study of development of eukaryotic organisms is to understand the mechanisms of morphogenesis, i.e. how does a complex organism develop from a single cell, the fertilised egg and what determines its final shape. Mechanisms responsible for the development of multicellular organisms involve spatio-temporal control of cell proliferation, cell death and cell differentiation as well as differential cell movement. It is also clear that these processes all have to be precisely controlled in space and time and they have to be stable against external perturbations. This implies that these processes must be precisely regulated. This regulation is mediated via extensive cell-cell communication by extracellular signalling factors. These factors interact in characteristic positive and negative feedback loops to result in spatio-temporal regulation of cell division, cell death, differentiation and movement.

The mechanisms involved are complex and due to the multitude of cell types and signals often difficult to investigate in higher organisms. Since many of the basic properties of these mechanism are essentially conserved during evolution it makes sense to investigate them in simpler model organisms, containing fewer cell types. Furthermore it is advantageous to investigate organisms which are amenable to genetic analysis. The analysis of mutants in these mechanisms allows the study of perturbations on development.

For these reasons we have focused on the study of morphogenesis in a very simple organism the cellular slime mould *Dictyostelium discoideum.* Slime moulds are positioned between uni- and multi-cellular life in the evolutionary tree of life. Dictyostelium undergoes a starvation induced multicellular development (**Figure 1**) which shows many of the characteristic features of the development of higher organisms like controlled cell differentiation and differential chemotactic cell movement.

Normally slime moulds live as single amoebae in the soil. They feed on bacteria and divide by binary fission. Starvation induces the activation of a developmental program in which the cells aggregate chemotactically to form a multicellular mass of 10^4-10^5 cells. Since multicellular development occurs in the absence of food there is essentially no cell division, thus simplifying the analysis of morphogenesis. In the aggregate (mound) the cells start to differentiate into a number of different cell types, i.e. several prestalk types which will form the stalk, basal disk and upper and lower cup in the fruiting body as well as prespore cells which will continue to differentiate to form spores. The cells differentiate in random positions in the late aggregate [1]. The prestalk cells than sort out chemotactically to form the tip of the tipped mound

[2]. The mound erects and extends up in the air to form the standing slug which falls over and migrates away. The slug has a distinct polarity with a tip at the anterior end which guides all its movement. The slug is photo- and thermo-tactic which allows it to move up towards the soil surface. There it transforms into a small fruiting body (up to 4mm high) consisting of a stalk supporting a spore mass. The spores disperse and under suitable conditions germinate to release amoebae and the whole cycle can start all over again. In this article we will give an overview of the mechanisms that control aggregation, mound and slug formation and show that these processes can be viewed as pattern formation in a biological excitable system.

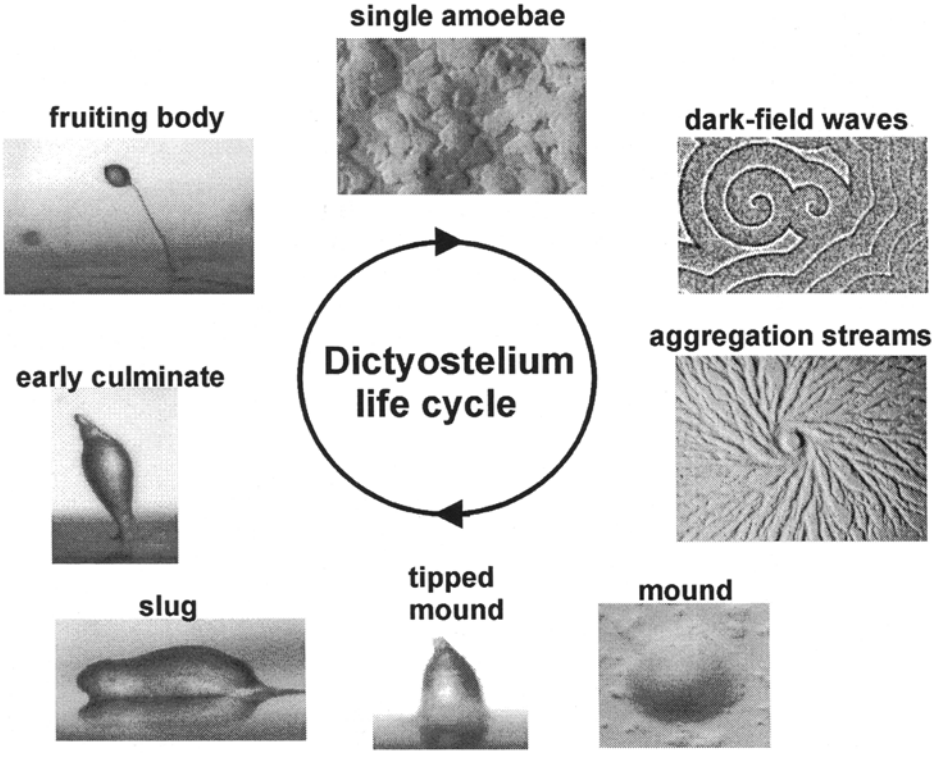

Figure 1: Dictyostelium life cycle. Shown are single amoebae, darkfield waves, aggregation streams, mounds, slug, an early culminate and a fruiting body. Development takes 24 hours at room temperature.

2. Aggregation

The principles that govern aggregation are now relatively well understood at the cellular level. Aggregation of individual *Dictyostelium* amoebae into multicellular aggregates occurs by chemotaxis to 3`-5`cyclic Adenosine monophosphate (cAMP) signals are released in a periodic fashion by cells in the aggregation centre. The cells in the aggregation centre periodically synthesise and secrete cAMP in the extracellular medium. Here it diffuses to neighbouring cells which detect this signal

via highly specific and highly sensitive cAMP receptors. These receptors are transmembrane proteins with an extracellular cAMP binding domain and an intracellular effector domain. Binding of cAMP to the receptor leads to two competing processes, excitation and adaptation (reviewed in [3]). The excitation pathway leads to the activation of the enzyme adenylatecyclase, which produces cAMP from Adenosine-Tri-Phosphate (ATP). This intracellular cAMP is then secreted to the outside where it can bind to the receptors of the same cell leading to autocatalytic feedback and in addition it diffuses away to activate neighbouring cells. The adaptation pathway involves a desensitisation of the receptor, involving phosphorylation of its cytoplasmic tail, resulting in a termination of the autocatalytic relay response. The cells also secrete an enzyme cAMP phosphodiesterase which degrades cAMP. A fall in the extracellular concentration of cAMP then leads to a dephosphorylation and resensitsation of the receptor (**Figure 2A**). The adaptation process is responsible for the outward propagation of cAMP waves, since cells which have just relayed are refractory to further stimulation by cAMP. cAMP also leads to a chemotactic reaction in the direction of higher cAMP concentrations. The cells move up the gradient as long as the cAMP concentration is rising but stop to move as soon as the cAMP concentration starts to fall. This response leads to the periodic movement of the cells towards the aggregation centre guided by outward propagating waves of cAMP (**Figure 2B**). Superimposed on this system there is a complex feedback of the cAMP oscillations on the expression of various components of the signalling system. The cAMP pulses induce the synthesis of cAMP receptors, adenylatecyclase and phosphodiesterase resulting in an increase in excitability during aggregation [4].

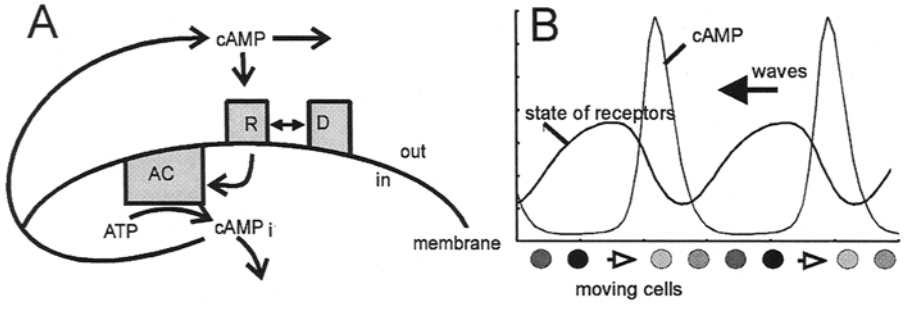

Figure 2: (A) Martiel-Goldbeter model for cAMP oscillations, R - active receptor, D-desensitised receptor, AC-Adenylatecyclase, cAMP and cAMPi - extra- and intra- cellular cAMP. (B) Scheme showing wave propagation and cell movement. The cAMP wave profile and fraction of active receptors are shown as calculated from equations (1-3). Waves propagate from right to left while cells (arrows and dots) move from left to right. Arrows represents moving cells, dark dot - non moving cell.

During early aggregation the cAMP waves can be seen as optical density waves using low-power darkfield optics [5, 6, 7]. These optical density waves are correlated with shape changes which cells undergo upon stimulation with cAMP.

Chemotactically moving cells are elongated and appear brighter than non-moving cells (**Figure 1,2B**). By correlating the cAMP signal via isotope dilution-fluorography with the waves, it was clearly demonstrated that the optical density waves observed during aggregation represent the propagating cAMP signal [8]. Most often waves appear as expanding spirals, in some strains also as concentric ring waves. Waves from neighbouring centres collide and annihilate each other leading to the formation of aggregation territories. Quantitative measurements showed that during aggregation the frequency of the waves increases while the wave propagation speed slows down. This is partly due to the cAMP dependent expression of components of the oscillatory system as well a to the dispersive properties of this excitable medium [9, 10].

There have been several attempts to model the early aggregation process. Two main questions need to be addressed: how do the cells produce cAMP waves and how do they move in response to these waves? Essentially two types of models were developed to describe mathematically the cAMP relay kinetics of Dictyostelium amoebae. The first model has been suggested by Martiel and Goldbeter [11, 12, 13]. It is based on the assumption that activation/inactivation of the cAMP receptors plays a key role in the response. A second model has been introduced by Tang and Othmer [14] in this model activation of activating and inhibitory G proteins control the periodic production of cAMP by the cells. Both models are able to describe oscillations in cAMP level in the cell suspensions as well as a cAMP wave propagation in a dispersed cell population [9, 15]. These models basically describe excitable and/or oscillatory media. For example the reduced Martiel-Goldbeter model [12] describing the cAMP relay system of individual cells, consists of three coupled non-linear equations which define the level of extra cellular and intracellular cAMP, and the activation state of the cAMP receptors (**Figure 2A**):

$$\frac{\partial c}{\partial t} = \left(\frac{k_t}{h}\right)\beta - k_e c \tag{1}$$

$$\frac{\partial \beta}{\partial t} = q'\Phi(r,c) - k_t\beta - k_i\beta \tag{2}$$

$$\frac{\partial r}{\partial t} = -f_1(c)r + f_2(c)(1-r) \tag{3}$$

Equation (1) describes the change in the level of extra-cellular cAMP (c), over time. These changes occur due to the secretion of intracellular cAMP β in the extracellular medium and hydrolysis by phosphosphodiesterase. Secretion is taken into account by the first term in right hand side of (1): cAMP is transported over the cell membrane with the rate defined by k_t. It is diluted in the extracellular medium by factor h which represents the ratio of extracellular to cell volume. Hydrolysis is taken into account by the second term and is assumed to be proportional (k_e) to extra cellular cAMP concentration. Equation (2) defines the level of intracellular cAMP (β). It takes into account the synthesis of intracellular cAMP by adenylatecyclase, its secretion and

hydrolysis. The rate of cAMP synthesis (first term) is dependent on the level of extracellular cAMP, *c*, and the state of cAMP receptors (r) and represented by an allosteric non linear function (Φ(r,c)). Equation (3) reflects changes in the state of the receptors. It defines the relative number of activated (*r*) and inactivated (*1-r*) receptors whose (slow) interconversion is dependent on the level of extracellular cAMP. To describe cAMP waves propagating through a population of cells a diffusion term has to be included in (1). Finally the model becomes very similar to the prototype FitzHugh-Nagumo system.

cAMP waves not only propagate through the cell population but also co-ordinate their movement. cAMP orients the direction of otherwise randomly moving cells. There is strong evidence that the cells detect the gradient of cAMP over their length [16, 17, 18]. However there is also evidence that cells use the temporal derivative of cAMP and only move up the gradient as long as the cAMP level is rising [19, 20]. This allows cells to move chemotactically on the wave front rather than on the wave back. A number of mathematical models have been proposed for chemotactic cell movement. The best known one is the Keller-Segel model [21] describing a cell flux, J, as a function of cell density, ρ, and concentration of cAMP, c.

$$J = -D(c)\nabla\rho + \chi(c)\rho\nabla c \tag{4}$$

The first term on the right hand side describes random cell movement (the velocity can depend on the level of cAMP) and the second term describes the directed motion of the cells along the cAMP gradient. There are also a number of models where chemotactic cell motion is described in axiomatic way as rules for motion of units (cells) in a concentration field of cAMP [22, 23, 24, 25].

Much effort has been directed towards understanding the physical principles leading to the formation of streaming patterns by amoebae responding chemotactically to propagating cAMP waves. It has been proven analytically and numerically that streams form due to an instability caused by a coupling between the velocity of signal propagation and density of cells. Local accumulation of cells will, due to the dependence of the rate of cAMP accumulation on cell density, result in a local speeding up of the wave propagation at regions of high cell density. This local deformation of the wave front will lead to the attraction of even more cells to this region and finally to the formation of bifurcating aggregation streams [26, 25, 27, 28]. in which the cells move towards the aggregation centre. There they pile on top of each other to form a three dimensional hemispherical structure, the mound. In the streams the cells are elongated and connected by rather characteristic end to end contacts. The movement of individual cells however is still periodic, but somewhat faster than that of isolated cells, possibly suggesting a co-operative effect on their movement [29].

3. Wave propagation and cell movement in mounds

Special image processing techniques allowed to visualise faint optical density waves

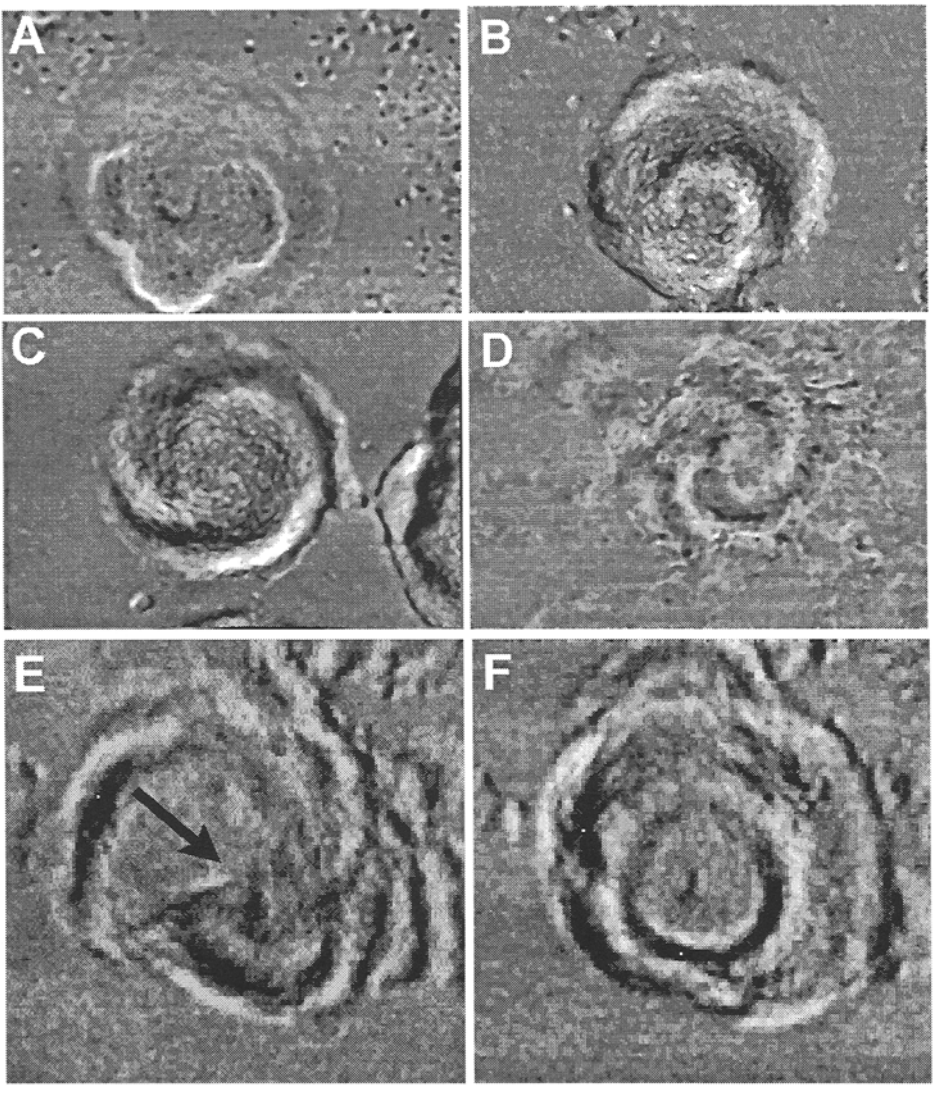

Figure 3: Different modes of signal propagation in mounds .(A) Centre organised by three pacemakers in strain XP55,(B) a single-, (C) Double- and (D) a triple- armed spiral centre in strain AX3. (E,F) Micro-injection of cAMP in mounds. Micro-injection of periodic cAMP pulses from a fine glass micro electrode can change the mode of signal propagation. A spiral centre (E) transforms to a concentric ring centre after periodic stimulation by exogenous cAMP through an electrode (F) The location of the tip of the needle is indicated by the arrow in (E).

in multicellular structures. These investigations showed that *Dictyostelium* mounds are organised by periodic signals displaying a whole range of different wave geometry's [30]. The exact pattern of the propagating cAMP signal seems to be strain specific. Some mounds are organised by single or by several coexisting pacemakers,

others by single- or multi-armed spirals (**Figure 3A,B,C,D**). The conversion of one type into another was also observed [30].

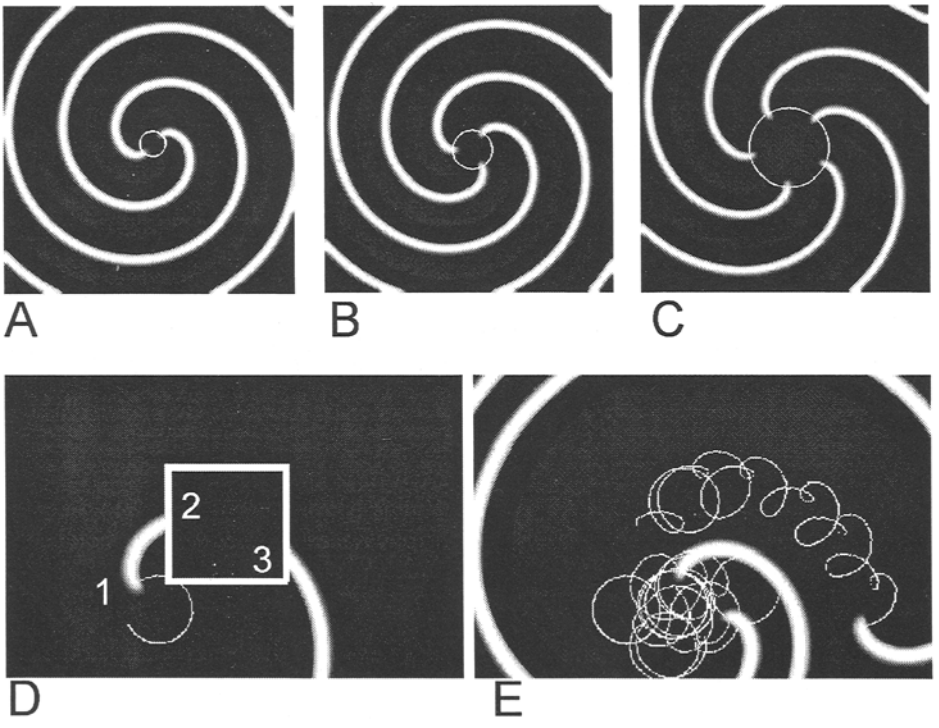

Figure 4: Formation of multi-armed spirals in a two-dimensional excitable medium described by the FitzHugh-Nagumo model. (A) Two- three- and five-armed spirals are shown in media with different excitability. The possible number of arms increases with a decrease in the excitability of the medium. Cycles in the centre of patterns indicate cores of the spirals. (B) A break of a single spiral can result in formation of a two-armed spiral. It happens when the break is located not too far from the spiral tip. We assume that multi-armed spirals in the mound occur by the same way.

Continuous measurements of the optical density waves from late aggregation until tip formation over a period of 3 hours demonstrated that there was a clear evolution in the dynamics of the waves [31]. Initially the waves propagate fast at low frequency but in the course of aggregation the wave frequency increased, while wave propagation speed decreased. Although we can observe a continuous succession of optical waves from aggregation to the mound stage it is not proven that the waves in the mound are caused by chemotaxis to propagating cAMP signals. In order to investigate this possibility we tested whether we could initiate optical density waves in mounds by periodic injection of cAMP in between the cells. Periodic micro-injection of 0.01nl pulses of 0.5mM cAMP into mounds initiated optical density waves which propagated from the electrode tip outwards and which interacted with endogenous waves (**Figure 3E,F**) shows, that periodic injection of cAMP pulses can

override the endogenous multi-armed spirals and induce concentric ring waves emanating from the electrode tip. These observations clearly show, that optical density waves in mounds can be induced by cAMP oscillations and furthermore that induced waves annihilate endogenous waves upon collision, showing a common propagation mechanism.

The different patterns of wave propagation observed in mounds of different strains most likely are a consequence of strain specific differences in their ability to generate and relay the cAMP signal. This change in wave geometry observed within one strain, i.e. the change from one armed spirals to multi armed spirals in mounds of strain AX-3, are most likely caused by a switch from high affine cAR1 receptors to the less affine cAR2 and cAR3 receptors which are newly expressed at the end of aggregation [3]. Interestingly the period length increased from 2 minutes to 4 minutes at a certain time point during tip formation. This increase may also be attributed to a switch from cAR1 to newly expressed cAR2 receptors in prestalk cells. This switch to a different receptor type will change the excitability of the system resulting in different wave patterns and could explain the formation of multi-armed spirals from single armed spirals observed during aggregation [32]. Model calculations showed that this can happen in low excitable media via breaks in the spiral with one chemical wavelength from the core of the original spiral (**Figure 4**).

The diverse geometry of the signals leads to variety of complex cell motion patterns. Since cell movement is always opposite to the direction of signal propagation [5, 31] cell movement in mounds organised by concentric waves is directed towards the organising centre and slow. In the case of spiral wave cell movement is rotational and fast.

4. A model for mound formation

Observation of cells moving in mounds prompted theoretical investigations where the cell movement is considered as a flow of a viscous compressible liquid. This approach has been successfully used in a hydrodynamic model describing the whole process of aggregation until mound formation (**Figure 5**). In this model cell velocity has been defined by the Navier-Stokes equation:

$$\rho[\partial \mathbf{V} / \partial t + (\mathbf{V}\nabla)\mathbf{V}] = \mathbf{F}_{ch} + \mathbf{F}_{fr} + \eta\Delta\mathbf{V} + \xi\,\mathbf{grad}\,div\mathbf{V} + \mathbf{F}_{ad} - \mathbf{grad}\,p \qquad (5)$$

The left hand side of the equation describes the acceleration of cells under the influence of various forces described in the right hand side of the equation. **V** is the velocity of the cells. $\mathbf{F}_{ch}$ is the chemotactic force which is active on the front of cAMP waves, $\mathbf{F}_{fr}$ is a friction force responsible for slowing down cell movement, The third and fourth terms on the right hand side describe cell-cell friction: η and ζ are viscosity coefficients. $\mathbf{F}_{ad}$ takes into account cell-cell and cell-substrate adhesion forces, p is the pressure between the cells caused by the chemotactic accumulation of the cells (see [33] for details).

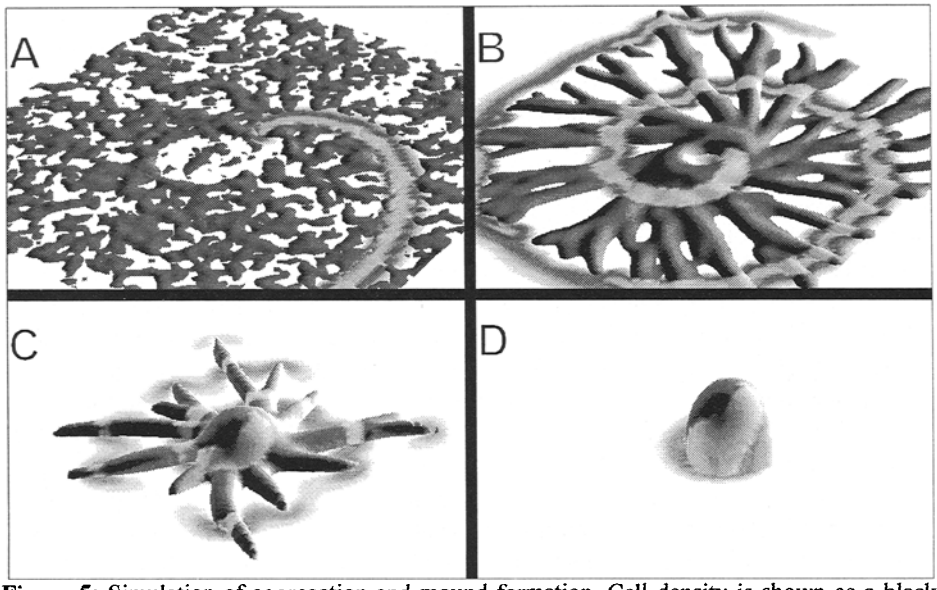

Figure 5: Simulation of aggregation and mound formation. Cell density is shown as a black iso-surface (ρ=0.5) and the cAMP spiral is mapped on this surface. The initial density of cells was zero everywhere in 3d-space except for the bottom plane (A). The cell density in each grid of this plane was represented by a random number varying between 0 and 1 so that average density in this plane was equal to 0.5. In response to cAMP spiral wave cells move and form aggregation streams (B-C) and then mound (D) which represents a stable solution of the system.

The evolution of the shape of the aggregate into a mound in this model has been obtained by solving an equation for the cell density field, i.e. by the equation for the conservation of mass:

$$\partial \rho / \partial t = D_\rho \Delta \rho - div(\rho \mathbf{V}) \qquad (6)$$

The first term on the right hand side of the equation describes the random motion of the cells, while the second term describes co-ordinated chemotactic movement. Aggregation patterns found as solutions of these equations (5-6) in combination with a FitzHugh-Nagumo to describe the excitable cAMP kinetics are shown in **Figure 5**.

This model although rather qualitative shows some remarkable similarities with the formation and appearance of aggregates observed in real life **(Figure 1,5).** Furthermore it is able to describe some mutant phenotypes frequently observed [33].

5. Wave propagation in slugs

Up to the mound stage cAMP wave propagation can be seen as optical density wave using darkfield optics and digital image processing techniques [6, 30]. During slug migration and culmination an extra cellular slime sheet, which is secreted continuously, surrounds the slug. This gives the slug some mechanical stability but

also impedes the observation of optical density waves. There are however many experimental results, which indicate a role for extra cellular cAMP during later development in cellular communication as well as in cell differentiation (reviewed in [34, 35]. To find out, if periodic cAMP signals also control slug migration and culmination, we investigated single cell behaviour and cell movement in multicellular structures, assuming, that periodic signals should cause periodic cell movement. It was shown, that indeed cells in the prespore zone of slugs go through periodic velocity and shape changes typical for chemotactically moving cells [36, 37]. Further investigations showed, that there exists a characteristic pattern of cell movement in *D. discoideum* slugs: cells in the prestalk zone show vigorous rotational movement around the central core of the tip, while cells in the prespore zone move straight forward in the direction of slug migration (**Figure 6A**) [38]. From these observations the geometry of the propagating signal was deduced: a three-dimensional scroll wave (spiral wave) produces rotational cell movement in the tip and planar wave fronts produce the straight forward movement observed in the prespore zone (**Figure 6B**).

Computer simulations showed, that the conversion of a scroll into a series of planar waves occurs, if there is a substantial difference in excitability between the prestalk and prespore cell population or if not all but only a part of the prespore population is actively relaying the cAMP signal (**Figure 6B**) [39, 40].

During the slug stage there is a further specification of cell types. pstA cells are formed in the anterior outer part of the prestalk zone. pstO cells are found at the boundary between prestalk and prespore cells, while pstB cells, which will form the stalk, are found in the central core of the prestalk zone. The prespore cells are located in the back two thirds of the slug [41]. Prespore genes need cAMP for their induction and stabilisation. Expression of the prestalk specific ecmB gene by the pstB cells is inhibited by high concentrations of extra cellular cAMP while ecmA expression by pstA cells requires high concentrations of cAMP [42, 43, 44]. Computer simulations using the Martiel-Goldbeter model for cAMP oscillations showed, that the core of the scroll wave in the prestalk zone is a region of low average extracellular cAMP, exactly the condition which facilitates the expression of the stalkspecific ecmB gene in the central core of the prestalk zone (**Figure 6C**) [40]. Despite the complex mode of wave propagation it gives rise to a relatively simple spatial pattern of average cAMP, which can be read out by the cells in different positions in the slug to keep the differentiation state of the cells in the slug stable (**Figure 6D**). These simulations suggest that the wave propagation pattern not only is responsible for the control of cell movement but also might be involved in the differentiation of the prestalk cell types [40].

6. Tip formation, cell differentiation and sorting

One of the most interesting but also most complicated phases of development is slug formation. During aggregation the cell density increases dramatically and cells start to move up on top of each other in the third dimension. In the mound cells begin to differentiate in prestalk and prespore cells. The prestalk cells differentiate at random positions, but then sort towards the top of the mound to form a tip [45]. The mound then contracts at the base while extending up in the air to form a standing slug. Slug

formation can be seen as a two step process, i.e. sorting of prestalk cells to form a tip followed by a tip induced contraction and elongation of the mound to form a standing slug (**Figure. 1**).

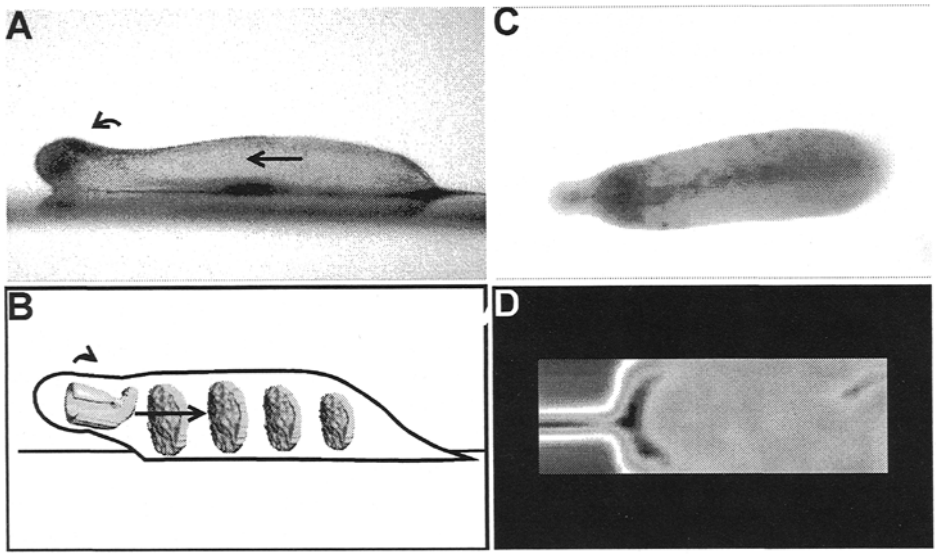

Figure 6: Wave propagation, cell movement and differentiation in slugs. (A) Photograph of a neutral red stained slug. The dark stained region on the left hand side is the prestalk region. The arrows indicate the direction of cell movement. (B) model for waves in the slug. The arrows indicate direction of cAMP wave propagation. (C) Photograph of slug showing expression of the prestalk specific gene ecmB (dark region in slug), note the expression in the slug middle and at the prestalk-prespore boundary. (D) average cAMP levels after integration over 10 periods of wave rotation. Note the close correspondence between average cAMP (D) and cell type differentiation (A,B).

Cell sorting most likely results form differential chemotactic cell movement towards cAMP. Experiments showed that prestalk cells preferentially sort towards an artificial cAMP source [46]. Furthermore mutants which overexpress cAMP phosphodiesterase are blocked at the mound stage of development and defective in cell sorting [47]. The difference in effective movement speed towards a cAMP source could be caused by cell type specific differences in the motive force generated by prestalk and prespore cells. Differences in motive force could result from differences in the cytoskeleton or cell-cell adhesion, i.e. prespore cells being more adhesive than prestalk cells. There is experimental evidence for both types of mechanisms: Isolated pstA cells, which will sort to the top of the aggregate, are able to move faster to an artificial cAMP source as isolated prespore cells [45]. Furthermore several mutants with defects in components of the cytoskeleton are arrested at the mound stage [48, 49]. Using a cold sensitive myosin mutant it has been shown, that there are two stages in development where myosin II is absolutely required for morphogenesis, at the mound stage during tip formation and during culmination [50]. Secondly in multi cellular tissues cell-cell interactions are likely to play an important role in the control of cell movement. It is

known that prespore cells are more adhesive as prestalk cells [51]. Prestalk cells may therefore move more efficiently in a multicellular aggregate consisting of prespore and prestalk cells. We therefore suspect that cell sorting involves all these mechanisms, i.e. differential chemotaxis towards cAMP, cell type specific differences in the generation of motive force as well as cell type specific differences in cell-cell interactions and cell-substrate interactions.

Cell sorting will feedback on the signalling patterns in the tipped mound since prestalk and prespore cells differ in their excitability. Many experiments suggest that prestalk cells are more excitable than other cells in the mound. Prestalk cells express higher amounts of the enzymes involved in the synthesis and degradation of cAMP, adenylatecyclase and phosphodiesterase [52, 53, 54] and they express a specific subset of low affinity cAR2 receptors, which will allow them to relay high amplitude cAMP signals, while the expression of the high affinity cAR3 receptor becomes restricted to prespore cells [55, 56].

Taken together cell sorting should affect the signalling system in the following way: the collection of fast oscillating prestalk cells in the tip will lead 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 [32]. The removal of the highly excitable prestalk cells from the body of the mound will result in a decrease in local excitability and to conversion of the scroll wave in the tip to a twisted scroll wave in the mound [39, 40]. This will lead to a twisted rotational cell movement in the mound. As a result the mound contracts and extends up into the air.

We are now testing this possibility by an extension of the model from mound formation by incorporation of different cell types (fluids) with different chemotactic and relay properties. We consider the mound to be a drop of liquid consisting of two kinds of fluids and use a two-field description of this drop to model cell sorting. The velocity of the liquid, $\mathbf{V}$ defined in (5), is assumed to have two components corresponding to velocities of prestalk, $\mathbf{V}_1$, and prespore, $\mathbf{V}_2$, cells:

$$V = (\rho_1 V_1 + \rho_2 V_2) / (\rho_1 + \rho_2) \tag{7}$$

Since the liquid is incompressible: $\rho_1 + \rho_2 = 1$ in the mound. The chemotactic forcing, $\boldsymbol{F}_{ch}$, in (5) is also assumed to consist of two components corresponding to chemotactic forcing of prestalk and prespore cells.

$$\mathbf{F}_{ch} = (\rho_1 \varphi_1 (\partial g / \partial t) + \rho_2 \varphi_2 (\partial g / \partial t))\mathbf{grad} g \tag{8}$$

The difference in cAMP signalling between prestalk and prespore cells is also taken into account by two different sets of parameters for the FitzHugh-Nagumo model. To find the velocities of prestalk and prespore cells we put expressions (7-8) for velocity and chemotactic force into equation (3), put coefficient $\rho_1 + \rho_2$ to the last term in (3) and by separating terms consisting ρ_1 and ρ_2 we get two equations for $\mathbf{V}_1$ and $\mathbf{V}_2$:

$$\partial \mathbf{V}_i / \partial t = \mathbf{V}_i \nabla(\mathbf{V}_i \rho_i) / \rho_i - (\mathbf{V}_i \nabla)\mathbf{V} + \mathbf{F}_i + \eta \Delta(\mathbf{V}_i \rho_i) / \rho_i - \mathbf{grad} p \quad \text{where i=1,2} \tag{9}$$

The two equations (9) each coupled with the equation of conservation of mass (5) (used to define the densities of both fluids) give the evolution of the cell density fields over time. Our preliminary computations show that starting from a random distribution of cell types in the mound one can obtain spatially separated patterns of cell types as well as tip formation. In response to scroll waves rotating along the axis of hemispherical mound

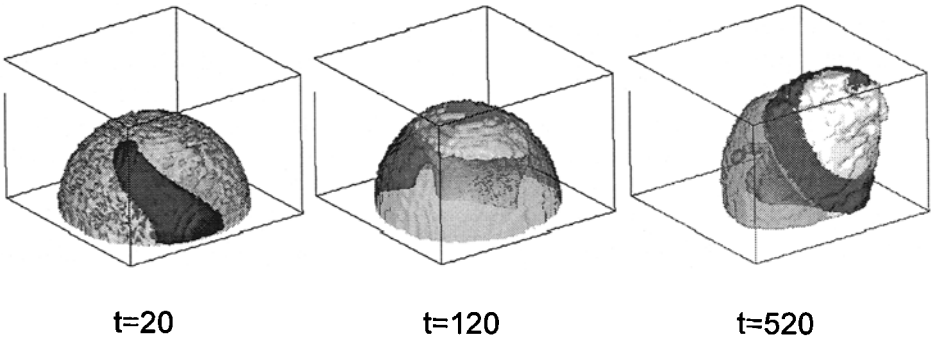

Figure 7: Simulation of cell sorting and tip formation. To describe cell movement we used equations (5,7,8,9) and to describe cAMP signalling we used the FitzHugh-Nagumo equations. A sequence of images is shown depicting sorting of highly excitable fast moving prestalk cells (white) from less excitable slow moving prespore cells (light grey(transparant)) as well the as the cAMP signal (dark grey). A scroll wave was initiated in the mound with an initially random distribution of prestalk and prespore cells. In the course of time the cells sort which result in a twisting of the scroll wave. The period of the scroll wave decreases from 38 to 21 time units.

the liquid begins to rotate in the opposite direction. The faster moving fluid (prestalk cells) accumulates in the centre and top of the mound. Since the faster fluid is more excitable their separation leads to the mound becoming inhomogeneous with respect to its excitability. This in turn results in a change of the cAMP wave shape. Since the density of the excitable cells is higher on the top of the mound the velocity of wave propagation there is higher as in the base of the mound and the scroll becomes twisted. As a consequence the cells experience a vertical chemotactic force which results in the further collection of faster moving cells on the top. Finally all the faster cells collect at the top of the mound and form a tip, exactly as it happens in real mounds (**Figure 7**).

7. Conclusions

It is now becoming clear that periodic signals not only control aggregation but also all later stages of morphogenesis. Multicellular mounds are organised by either concentric ring waves emanating from one or more centres or by spiral waves with up to 10 arms. These signals are used to regulate the process of cell sorting, in which the most excitable cells move on top of the aggregate. This sorting process leads to a highly excitable tip and a less excitable main body. This spatial separation feeds back

on the signal geometry. We propose that the cells in the tip are organised by a rotating scroll wave of cAMP with the core of the scroll wave coinciding with the long axis of the tip. The scroll wave converts into a twisted scroll wave and planar waves in the body of the mound. This pattern of wave propagation leads to a rotational movement of the prestalk cells in the tip and a periodic upward movement of the cells in the base of the mound. Furthermore it results in a contraction of the tip and an elongation of the mound into a standing slug which becomes unstable and topples over. The slug now moves away, while the movement of the cells is still being controlled by a scroll wave in the tip and twisted scroll or planar waves in the prespore zone. This pattern of cAMP wave propagation is also used to stabilise prestalk cell type specific gene expression and to initiate stalk differentiation.

Dictyostelium is possibly the first organism whose morphogenesis is beginning to be understood at the cellular level. During early development morphogenesis is based on wave propagation in a two dimensional excitable medium which becomes three dimensional by chemotactic aggregation of the cells. More complexity is brought into the system as the cells differentiate into several types with different excitable and chemotactic properties. Due to these additional levels of regulation and feedback complicated wave forms such as multi-armed spirals, twisted scroll waves etc. arise.

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8. References

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