From Single Cells to a Multicellular Organism: The Development of the Social Amoebae *Dictyostelium Discoideum*

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Abstract. One of the main goals of contemporary biology is to understand the development of multicellular organisms. This is a very complex process that involves cell division, cell death, cell differentiation and cell movement, which are all highly regulated in space and time. The co-ordination of these processes involves extensive cell-cell communication. Development of a vertebrate involves hundreds of cell types and thousands of signalling molecules, all interacting through positive and negative feedback pathways. Due to this complexity, it is very difficult to describe the development of these organisms in a meaningful physical sense. There are however much simpler organisms that show all of the essential characteristics of the development and are more accessible to such a description. *Dictyostelium discoideum* (Dd) is one such a system. Discoideum species are known as social amoebae, and their colonies are among the simplest and best studied objects of developmental biology. Under normal conditions *Dictyostelium* populations consist of groups of independent, single amoebae. However, under unfavourable conditions, when food becomes limiting, these amoebae become "social" and enter a complex developmental program. They collect into multicellular aggregates and differentiate into a number of cell types. These cell types organise themselves to form a fruiting body consisting of (dead) stalk cells and spores. The spores disperse and each spore can start a new colony under favourable conditions. Some of the features of Dd development are beginning to be understood both at the biological and formal level. Here we review *Dictyostelium* development and the progress made in the mathematical modelling of *Dictyostelium* morphogenesis.

1 Introduction

Most multicellular organisms develop from a single cell, the fertilised egg. This involves processes such as regulated cell division, cell death and cell differentiation in a multitude of cell types. These cell types may arise in situ at the right position at the right time of development or they may differentiate first as a precursor cell type and then move to their final destinations, as is the case in the formation of the nervous and immune system. These processes are highly complex and there is not yet any formalised understanding. For these reasons there has been a tendency to study the development of much simpler model organisms which still show some of the essential features of multicellular development. One prominent model organism is the social

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amoebae *Dictyostelium* discoideum. This is a very simple organism that on an evolutionary scale stands on the threshold between unicellular and multicellular life forms. Part of its life cycle it lives as single amoebae in the soil where it feeds on bacteria and multiplies by binary fission. However, upon starvation the cells aggregate to form a multicellular structure, the slug, consisting of up to 10^5 amoebae. The slug is motile and guided by photo and thermotaxis can move away to a place suitable to form a fruiting body. The fruiting body consists of a stalk, formed by dead vacuolated stalk cells, that supports a mass of spore cells. The spores can disperse and germinate, so that each gives rise to a single amoebae. The cells start to differentiate in stalk and spore cells during aggregation and a pre-pattern of prestalk and prespore cells is formed in the slug. The prestalk cells (which will later form the stalk) are localised in the tip of the slug while the prespore cells (transforming later to the spores) are localised in the back of the slug. This organism retains many of the commonly found elements of multicellular development such as differentiation of one cell type, the amoebae, into at least two cells types, the stalk cells and the spores. When the slug is cut into a prestalk and prespore piece both pieces will regulate and form perfectly normal proportioned fruiting bodies. This shows that the proportions of these cell types are strongly regulated by an elaborate feedback mechanism in which the cells signal each other all the time.

During the differentiation phase of the slime moulds, the cells do hardly divide (since there is no food). Therefore, morphogenesis results solely from the co-ordinated movement of individual cells. This co-ordination also requires extensive cell-cell signalling the mechanism of which will be reviewed extensively in this article.

In this article we will give an overview of the essential behaviour and mechanisms involved in the morphogenesis of this simple organism and describe the progress that has been made to understand the essential principles involved in a more theoretical formalism. We will finish by highlighting some unsolved problems, which may direct future research.

2 The life cycle

The life cycle of a developing *Dictyostelium* population is shown schematically in Fig.1. Normally slime moulds live as single amoebae in the soil. They feed on bacteria and divide by binary fission. The population multiplies and at a certain point in time will have depleted the food source. Starvation induces the activation of a developmental program in which the cells aggregate chemotactically to form a multicellular mass of cells containing $10^3 - 10^5$ cells. A few hours after the beginning of starvation cells begin to move synchronously towards the aggregation centre. This is the result of a chemotactic movement of the cells to signals emitted by the cells in the aggregation centre. The cells in the aggregation centre periodically emit a signal, 3'-5' cyclic-adenosine-

monophosphate (cAMP), which is detected and relayed by surrounding cells. This leads to formation of propagating waves of cAMP, which instruct the cells to move towards the centre by chemotaxis. These waves can be seen as target or spiral dark-field waves (Figs.1,4). These dark-field waves are known to reflect changes in the cell shape: elongated moving cells form light areas, round-shaped non-moving cells – dark areas. Cells move during the rising phase of the cAMP waves towards the source of waves. Then the aggregating cells make contacts and form branching streams, in which the cells move towards the aggregation centre. Formation of these streams accelerates aggregation, since cells in streams move faster than individual crawling cells. All cells move towards the wave sources or aggregation centres. The presence of several competing centres divides the Dd population into domains (aggregation territories, Fig. 4), so that all cells in the same domain move to the same aggregation centre. The aggregation stage of Dd finishes when all cells collect in their respective centres and form multicellular aggregates (hemispherical mounds). In the mound, the cells start to differentiate. They transform into several prestalk types which will form different parts (the stalk, basal disk and upper and lower cup) of the fruiting body as well as into prespore cells which will continue to differentiate to form spores [1]. The cells differentiate in random positions in the late aggregate. The prestalk cells sort to the top of the mound to form the tip. 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 thermotactic, 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.

There are several important problems concerning *Dictyostelium* development:

-How do single cells move chemotactically?

-Which signals control the aggregation process?

-Which signals control the differentiation of the cells?

-What controls cell sorting?

-How does the hemispherical mound form a cylindrical slug?

-How does the slug move?

-Which processes are responsible for the formation of the fruiting body?

All these problems are being addressed in experimental and theoretical studies and some of the mechanisms are beginning to be understood. The best understood part of the developmental cycle is the aggregation stage of development. The cells aggregate by chemotaxis to propagating waves of cAMP (Fig. 3). Cells in the aggregation centre start to produce the chemoattractant cAMP in a periodic fashion. They release the cAMP in the extracellular medium where it diffuses to neighbouring cells. These cells detect the signal

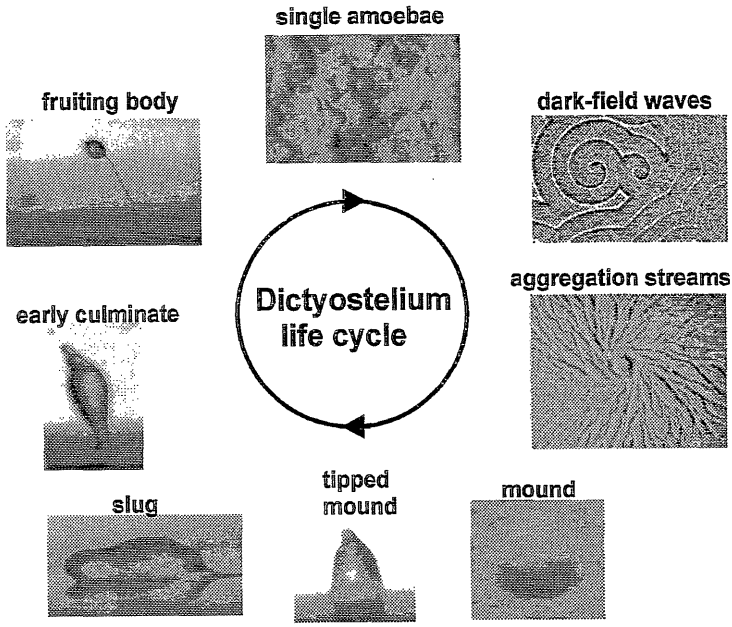

Fig. 1. Dictyostelium life cycle. Starving single amoebae aggregate towards propagating waves cyclic AMP. These waves co-ordinate chemotactic cell movement and can be visualised as darkfield waves. The cells form aggregation streams in which they move towards the aggregation centre to form multicellular aggregates (mounds). Each mound transforms into a migrating slug. The slug migrates to a place suitable to form a fruiting body. The fruiting body consists of a stalk supporting a spore mass. The spores disperse, germinate and each spore releases an amoeba, which can start a new colony.

by specific transmembrane cAMP receptors, which then in a relatively complex cascade of reactions, activate the enzyme adenylate cyclase, which starts to produce cAMP (Fig. 2). This cAMP is secreted to the outside where it can activate neighbouring cells again. After the cells have been stimulated, they become refractory for a while to further stimulation, which leads to a cessation of their relay response. Adaptation ensures the unidirectional wave propagation. Since the cells also secrete an enzyme cAMP phosphodiesterase, which degrades the cAMP, the levels of cAMP are going to fall after a while. This allows the cells to de-adapt to regain sensitivity to a new signal coming from the aggregation centre. This mechanism leads to the initiation of waves by the aggregation centre and their outward propagation (Fig. 3). The biochemical mechanisms underlying this signalling network are quite complex and involve many different proteins [2] (Fig. 2). The most important ones are depicted in the Figure 2. The cAMP signal is detected by transmembrane cAMP receptors, which upon binding of cAMP change their conformation

and activate a hetero-trimeric G protein. This G protein undergoes a complex reaction cycle in which the GDP, which is normally bound to the $G\alpha$ subunit, is exchanged for GTP. It dissociates splits in a $(\beta\gamma)$ complex and an activated (GTP bound) $G\alpha 2$. The γ subunit has a fatty acid modification and stays bound to the membrane where the $(\beta\gamma)$ complex now binds another protein CRAC (cytosolic regulator of adenylate cyclase). This complex then stimulates adenylate cyclase leading to the synthesis of cAMP. It is not yet known how the cAMP is secreted but a major portion gets to the outside. Upon binding of cAMP, the receptor is phosphorylated by a receptor kinase on its cytoplasmic tail. The $G\alpha 2$ subunit also gets phosphorylated. The consequence of phosphorylation is a decrease in the affinity for cAMP. Initially it was thought that the phosphorylation step was the adaptation step, however this does not seem to be so, since cells expressing cAMP receptors, in which the amino acids which are phosphorylated have been changed for ones that cannot be phosphorylated anymore, still adapt [3].

Local binding of cAMP also leads to the rapid local activation of the cell and the extension of pseudopodia in the direction of the cAMP signal [4-6]. This involves a complex biochemical machinery, which stimulates locally actin polymerisation and is catalysed by at least 20 different actin-binding proteins. The activity of these proteins is regulated in part by the cAMP signal associated with second messengers such as calcium, particular lipids and cGMP. It also involves the action of myosin motor molecules. It is not yet clear how this local activation process works. The local activation of the receptors has to be translated in a polarisation of the cell, i.e. in a formation of an extending front end and retracting tail end. This process seems to involve phosphorylation of myosin molecules. Myosin II molecules are preferentially found in the rear retracting end of the cell while several members of the myosin I class of motor protein are found in the extending part of the cell [7-9]. There have been few models for the local activation of the cytoskeleton during chemotaxis, although kinetic models for stimulated actin polymerisation in other chemotactic cells are beginning to appear [10-12].

Initially Dictyostelium cells are not excitable and then as a result of the starvation program the cells start to express essential components of the cAMP relay system such as cAMP receptors, G proteins and the enzyme that synthesises cAMP, adenylyl cyclase. The expression of these genes is under the control of cAMP pulses so that there is a feedback system between the cAMP signal and the components responsible for the cAMP oscillations [14]. This is a very interesting situation and obviously much more complex as that found in physical or chemical systems. This also implies that the system is never the same kinetically at any given moment of time, the consequences of which have not yet been fully investigated. Initially the cells are not excitable, they then become excitable and finally some, or all cells, become autonomous cAMP oscillators. The exact process of wave formation has not yet been studied in great detail experimentally. Since the waves control and direct cell movement,

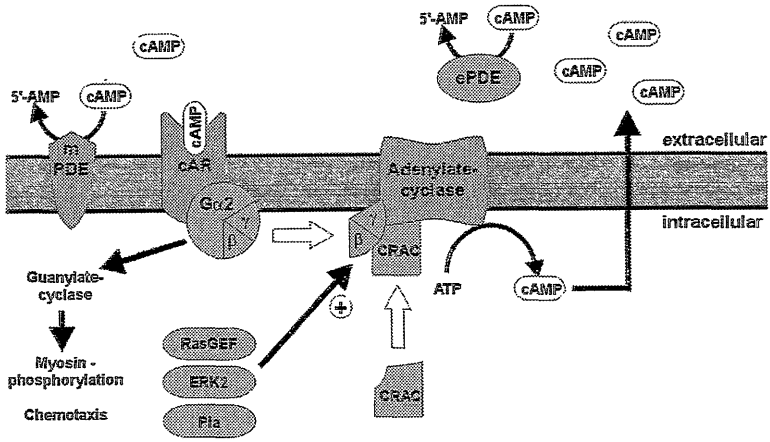

Fig. 2. Figure 2. Diagram of the cAMP signalling system of aggregation competent Dictyostelium cells. Indicated are the biochemical components known to play an essential role in the cAMP relay response. The cyclic AMP receptor (cAR), the G protein Ga2bg, the cytosolic regulator of adenylate cyclase (CRAC), the soluble secreted cAMP phosphodiesterase, the enzyme that degrades cAMP (ePDE), the membrane bound phosphodiesterase (mPDE) as well as the proteins that modulate the activation of cyclase activation (Ras GEF, the map kinase ERK2 and the gene product of the pianissimo gene (Pia) [2,13]. Some of these components may be involved in the adaption step.

the system is in a different state after every passage of a wave, a situation, which is also different from normal chemical and physical excitable systems.

The next two sections are devoted to the description of the biology and mathematics of the signalling and movement systems. They will lay the foundation for the detailed consideration of all stages of Dd development in later sections of the paper.

3 cAMP signalling system

We already know that aggregating cells communicate by means of travelling waves of cAMP. Such chemical waves are known to occur in oscillatory or excitable chemical systems (see, for example, [15]). At the very early stage of aggregation the waves are concentric and probably initiated by cells, which periodically emit cAMP pulses. We will call such cells "oscillatory" or cells with oscillatory signalling system. There is evidence that all cells are oscillatory [15]. However, frequency of oscillations is different from cell to cell and the concentric waves occurring during early aggregation are presumably initiated by cells having the highest frequencies. Another fact indicating the oscillatory behaviour of cells is that first a few waves in aggregation territories

look very much like phase waves, which have irregular shapes and velocities (Fig. 4A,D). However, after short period of time the shapes and velocities of these waves stabilise, and phase waves transform into slower excitation waves (Fig. 4B,E). Such a transformation is known to happen in inhomogeneous oscillatory media [17,18]. There are many reasons why an aggregation territory can be inhomogeneous, i.e. cells have slightly different signalling systems or cell density varies over an aggregation territory. Spiral waves probably occur when concentric waves break up on these inhomogeneities, although it has been argued recently that small local fluctuation in the level of a phosphodiesterase inhibitor activity can also be responsible for the formation of spiral waves [19].

Over time a number of different mathematical models for the cAMP relay system have been proposed Some are just based on the general properties of chemical oscillators and some try to model the essentials of the biochemical mechanisms in more detail [20-23]. Detailed biochemical models tend to get quickly outdated as new experimental data accumulate. Let us consider in some detail the two most recent models for the cAMP oscillator. The first ("receptor box") model has been suggested by Martiel and Goldbeter [24,25]. It is based on the assumption that cAMP receptors detecting the level of extracellular cAMP can be in two states, active or inactive. Only active receptors after binding of cAMP molecules can activate adenylyl cyclase. Therefore, the transition between the states of receptors plays an essential role in the cells response. Although this assumption has been proven wrong by the most recent experiments (see above) the reduced Martiel-Goldbetter (MG) model remains the most popular among mathematicians modelling the cAMP signalling system. The Martiel-Goldbeter model describes the cAMP relay system of individual cells. It consists of three coupled (there is a two-variable version) non-linear equations which define the level of extracellular and intracellular cAMP, and the activation state of the cAMP receptors:

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

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

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

where

$$\Phi(\rho,\gamma) = \frac{\lambda_1 + Y^2}{\lambda_2 + Y^2};\ Y = \frac{\rho\gamma}{1+\gamma};\ f_1(\gamma) = \frac{k_1 + k_2\gamma}{1+\gamma};\ f_2(\gamma) = \frac{k_{-1} + k_{-2}c\gamma}{1+c\gamma};$$

Equation (1) describes the change in the level of extra-cellular cAMP, c, over time. These changes are due to the secretion of intracellular cAMP, β: cAMP is transported over the cell membrane with the rate defined by k_t and is diluted in the extracellular medium by factor h representing ratio of

the volume of extracellular solution to the total volume of cells. The second is cAMP hydrolysis by phosphodiesterase outside the cell, which is assumed to be proportional (k_e) to cAMP concentration. Equation (2) defines the changes in the level of intracellular cAMP, β as depending on three processes, which are taken into account by the three terms in the right hand side of (2). The first is the synthesis of intracellular cAMP by adenylate cyclase in response to a cAMP stimulus (it depends on the level of extracellular cAMP, c and the state of cAMP receptors, r). The second is a loss of intracellular cAMP due to secretion (same as first term in (1)). The third term denotes the intracellular hydrolysis of cAMP, which differs from second term in (1) by the rate of hydrolysis. Equation (3) reflects changes in the state of the receptors. It defines the change in the relative number of activated (r) and inactivated ($1 - r$) receptors resulting from their transition governed by the level of extracellular cAMP. To describe cAMP waves propagating through a population of cells, a diffusion term for extracellular cAMP has to be included in (1).

A second model has been introduced by Tang and Othmer [26]. This model postulates other pathways for excitation and adaptation of a cell's cAMP signalling system. It is based on the mammalian paradigm for membrane receptor mediated signalling pathways. In this model the activation and adaptation are mediated by stimulatory and inhibitory G proteins. In short, when the receptor binds cAMP it can activate the α subunit of a stimulatory G protein. This in turn activates adenylyl cyclase and causes the production of cAMP. Adaptation involves the slower receptor activation of an inhibitory G protein, which inhibits cyclase activation. Here, as well as in the case of the MG model, there are assumptions, which are not in agreement with newer biochemical data or not confirmed experimentally. For example, the α subunit of stimulatory G protein is not involved in the activation of adenylyl cyclase and there is only little evidence for the involvement of an inhibitory subunit. The final system of equations is more complex as the Martiel Goldbeter model and therefore this model is not used as extensively by other groups. Both models mentioned above are able to capture the essential elements of the cAMP oscillator and describe oscillations in cAMP level in the cell suspensions of isolated cells as well as cAMP wave propagation in a dispersed cell population [27,28]. However, there is a clear scope for improvement, i.e. modelling the detailed biochemical mechanism.

Both models basically describe excitable and/or oscillatory media and are qualitatively similar to the prototype FitzHugh-Nagumo (FHN) system, which is widely used for the study of general properties of excitable, oscillatory or trigger media [15]. Finally, since the MG and TO models are not completely satisfactory, using the FHN model for qualitative description of cell signalling system can also be considered as a good option [29,30]. The FHN model is described by the following equations:

$$\partial g/\partial t = D\Delta g - k_g(g - a)(g - 1) - k_r r \qquad (4)$$

$$\partial r/\partial t = (g - r)/\tau \tag{5}$$

Here g is assumed to define the level of extra-cellular cAMP, and r - the recovery variable responsible for the adaptation step. The last serves the same role as the proportion of active and inactive cAMP receptors (in MG model) or activated $G\alpha_i$ subunits of the inhibitory G- protein (in TO model).

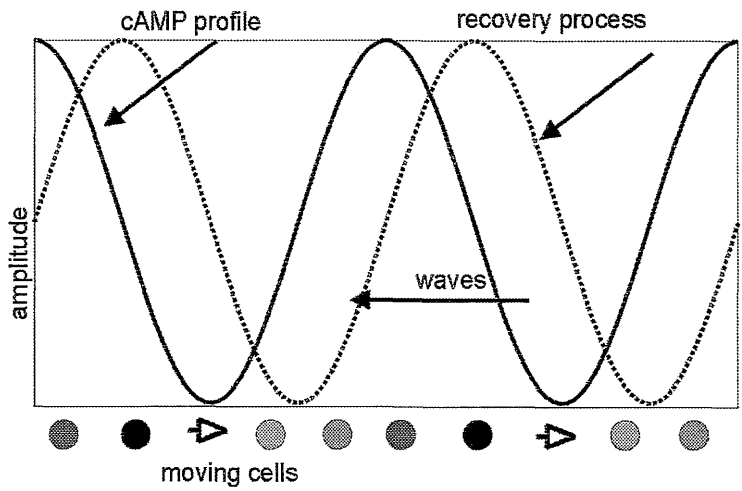

Fig. 3. Dictyostelium cells communicate over long distances by chemical (cAMP) waves. The wave propagation in a one-dimensional medium is schematically shown to occur from right to left, the cells (circles and arrows) move from left to right. A black spot is a resting cell and arrow indicates a fast moving cell. The x-axis represents distance, while y-axis corresponds to the level of cAMP (solid line) and adaptation (dotted line). According to experimental data [31] cAMP profiles should be very close to sinusoidal. At the early stage of aggregation the level of cAMP varies between 10^{-10} and 10^{-6} M. During development both these values presumably increase. This happens due to accumulation of cells in densely packed aggregates and is accompanied by switching of cells to lower affinity cAMP receptors [2]. Cells move chemotactically during the rising phase of the cAMP waves, in the direction opposite to that of cAMP wave propagation.

4 Chemotactic cell movement

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 cells detect the gradient of cAMP over their length [32,33,34]. 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 [35,36]. This is a result of adaptation of the chemotactic response

and inhibits the cells from turning around and chasing the waves outwards again once they have passed. There is a good experimental evidence for an adaptation step in the chemotactic response. It appears to be mediated by an adaptation of the cGMP signalling system that leads to chemotactic cell movement [2].

A number of mathematical models have been proposed to describe chemotactic cell movement. The oldest and best known one is the Keller-Segel model [37] describing a cell flux, J, as a function of cell density, ρ , and concentration of cAMP, c.

$$J = -D(c)\nabla\rho + \chi(c)\rho\nabla c \tag{6}$$

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 an axiomatic way, as rules for motion of cells in a concentration field of cAMP. A few sets of such rules have been used successfully to model cell aggregation [23,38-40,41-43]. Most recently chemotactic cell motion in multicellular aggregates was successfully modelled by assuming that a group of cells can be considered as a fluid whose behaviour can be described by the Navier-Stokes equations, [44,30]. The equation for the velocity of cells then assumes the following form:

$$\rho[\partial \boldsymbol{V}/\partial t + (\boldsymbol{V}\nabla)\boldsymbol{V}] = \boldsymbol{F}_{ch} + +F_{fr} + \eta\Delta\boldsymbol{V} + \xi \mathrm{grad} div\boldsymbol{V} + F_{ad} - \mathrm{grad}p \tag{7}$$

where $\boldsymbol{V}$ is the velocity and r is a density of cells; p is a pressure in an aggregate developing due to differences in the velocities of cells, $\boldsymbol{F}_{ch}$ is a force exerted by the cells in response to the chemoattractant gradient; the second and third terms in the right hand side of (7) take into account the mechanical (viscous) cell-cell interactions. Once we know the cell flow velocities (equation 6 or 7) we can compute the evolution of cell density in an aggregation territory using the equation for the conservation of mass:

$$\partial\rho/\partial t = D_\rho\Delta\rho - div(\rho\boldsymbol{V}) \tag{8}$$

Here we assume that the coefficient for random cell movement is constant (Fig. 5,6).

All models developed so far for chemotaxis are phenomenological i.e. they describe the phenomenology of chemotactic motion rather than the mechanisms of chemotaxis. To obtain a more mechanistic model of chemotaxis it will be necessary to develop more detailed models for amoeboid cell motility first [45] and then incorporate these in the description of the behaviour of populations of cells. This is a clear goal for the near future.

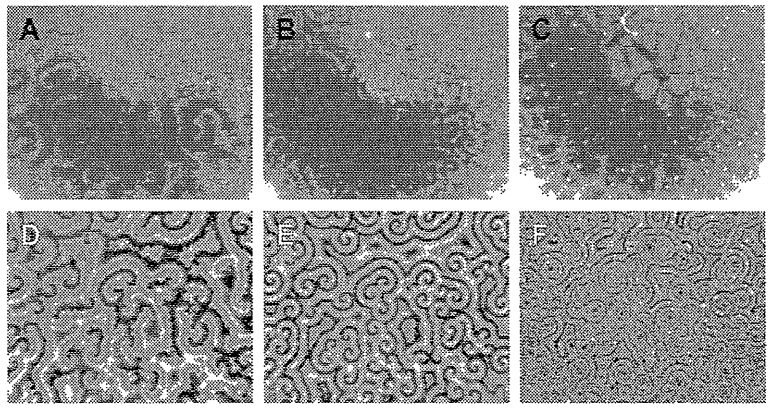

Fig. 4. Development of optical density waves during the aggregation of Dictyostelium cells strain DH1. A-C show original images as recorded under a macroscopic darkfield [46] at three successive times in development 4,5 and 6 hrs of development. A, D show the early darkfield waves, during the time that the phase waves are about to disappear. The wave fronts are still ragged. B,D show images when the excitation waves are fully developed, the wave fronts are smooth. C,F show images at a time where the cells start to organise themselves into streams and aggregation territories start to develop. The sections shown are 2.5 cm wide. The lower series of images are obtained after enhancement of the images shown in A-C by a rolling subtraction of three successive images taken 10 seconds apart.

5 Aggregation

Dictyostelium aggregation has been the subject of many modelling attempts. There are two main problems associated with the early aggregation phase of Dd development. The first concerns the mechanism of the formation, interaction, and geometry of the cAMP waves. The second is the mechanism responsible for the formation of aggregation streams. The modelling of the aggregation waves is mostly dealt with as purely being a consequence of the cell signalling system. The formation of streaming patterns is clearly more complicated and depends on properties of both, the signalling system and the chemotactic response of the cell.

One of the first models devoted to Dd aggregation was developed by Novak and Seelig [38]. It describes the cAMP signalling system and chemotaxis by simple rules and they were able to obtain simple aggregation of patterns of discrete cells in computer simulations. However, no clear aggregation streams were observed in their model. Streams were obtained a few years later in simulations by MacKay [23] using a more complex discrete model. These and other model simulations [47,48] showed that accumulation of cells in aggregation centres is easy to understand and to simulate, i.e. the cells simply collect at the location of waves source. However, the mechanisms of stream formation remained unclear for a much longer period. The first theoretical

papers on the mechanisms responsible for aggregation and stream formation were devoted to the analytical estimation of the stability of equation (6), see for instance [37]. "Chemotactic instability" introduced in this ref. could be used to explain accumulation of cells in aggregation centres but not to explain the formation of streaming patterns. A large step forward was made by Nanjundiah [49] and later by Levine [50] who showed the existence of a "streaming instability" which results from the influence of cell density on the effectiveness of the cell signalling system. This has been shown in more detail in [29] where it was stated that streams form when the velocity of the cAMP waves increases with an increase in the local cell density. This statement contradicted experimental data that failed to show a dependence of the velocity of cAMP waves on cell density [51] as well as some theoretical considerations [52,53]. However, an increase in the velocity of cAMP waves with an increase in cell density agrees with numerical results obtained from a variety of models (convection-reaction-diffusion model [29] cellular-automata model [40] and hybrid model [39]) and newer experiments [39]. Stream formation can be explained qualitatively as following. 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. However, many researchers are still trying to formulate new explanations [54-56,57].

One area of research concerns itself with the formation of wave patterns in aggregation territories. There is little doubt that an aggregating population can be viewed as an inhomogeneous oscillatory medium where the sources of concentric waves are cells whose frequencies of oscillation are higher than frequencies of other cells (see, for example [18,58]). Describing population of aggregating Dictyostelium cells as an excitable system and the waves propagating through fields of cells as excitation waves also works well [27] and can easily describe the formation of spiral waves. While concentric waves of cAMP initiate at spots of local high frequency in aggregation territories, the mechanisms of spiral waves formation are less clear. In both [40] and [29] it was suggested that concentric waves can result from breakpoints due to inhomogeneities of the system (i.e. in areas of lower local density of cells) and spirals develop from these break points. In [59] a more complicated approach (positive feedback between the cAMP level and local excitability of the medium) to get formation of spirals in homogeneous medium was developed. The problem of the interaction between concentric and spiral waves has been studied in [60,61]. The main result of these papers can be formulated in general terms of excitable media. Spirals are dominant over concentric waves and remove the latter from the medium when the excitability of the medium is high. Under these conditions spiral will run at their maximal frequency in agreement with early experimental data [62-64]. A decrease in the exci-

tability of the medium can alter the situation so that, concentric waves will become dominant and will replace spirals in the medium. Another interesting observation is that the spiral wavelength decreases continuously during development. This is the reason that initially during aggregation the spiral waves can organise aggregation territories of several centimetres in diameter and later in development also much smaller mounds, which are typically a few hundred microns in diameter. This change in spiral size has been partially attributed to the change in cell density during aggregation leading to an increase in oscillation frequency and a dispersion mediated decrease in wave propagation velocity [65,66], which has also been found in model calculations [30,39]. However in reality, this effect is quite large, the decrease in spiral wavelength has been estimated to be a factor of 20 [67] and dispersion is not enough to account for this. It is most likely caused by the feedback of the cAMP pulses on the increase of the expression of the components of the cAMP oscillator (cAMP receptors, adenylate cyclase etc [14]). One model has recently tried to incorporate this feedback [68] and found that it can control the dominance of established spiral centres.

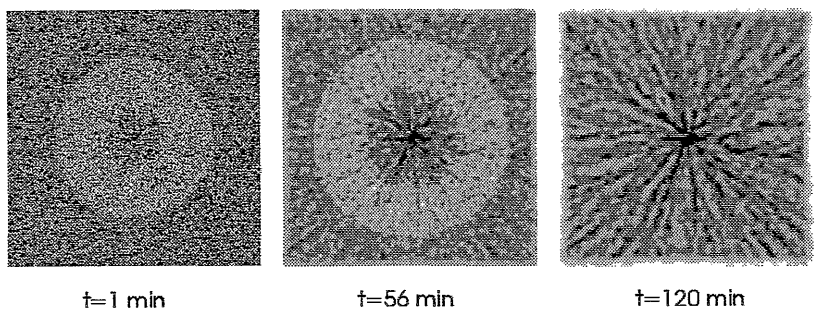

Fig. 5. Formation of streaming patterns in convection-reaction-diffusion system. Two- variable version of Martiel-Goldbeter model has been used two simulate cAMP waves and equation of mass conservation based on the Keller-Segel equation for chemotactic cell flows – to describe cell motion. Random numbers between 0 and 1 in each grid point gives the initial distribution of cell density. Concentric waves of cAMP were initiated every 6 minutes by stimulating the central area of medium. The cAMP wave (white) is superimposed on the pattern of amoebae density (various shades of grey).

6 Mound stage of development

When all the cells accumulate at the aggregation centre they form a mound and development enters a new phase. The most important problems concerning the mound are the following: How do the cells in the mound signal each

other? How do the cells move? What is responsible for the formation and shape of the mound? What controls the differentiation of cells and how do the differentiated cells sort out? Finally, which mechanisms are responsible for the transformation of the mound into a slug?

There is good evidence, that the Dictyostelium mound is still organised by cAMP waves [69]. In the mound, clear optical density (OD) waves can be seen to propagate and these waves can be interfered with by periodic microinjection of cAMP pulses with a glass microelectrode. This suggests that the OD waves are carried by cAMP. Furthermore, mutants expressing cAMP receptors of different affinities show different wave patterns (Weijer et al, unpublished observations). The geometry of the waves is strain dependent and can be either concentric, single- or multi-armed spiral waves. Multi-armed spirals are often observed in mounds and the mechanisms for their formation and their significance are not yet clear [67,69]. These diverse geometry's of the signals lead to a variety of complex cell motion patterns. Since cell movement is always opposite to the direction of signal propagation [67,70] cell movement in mounds organised by concentric waves is directed towards the organising centre and slow. In the case of spiral waves, cell movement is rotational and fast.

While aggregation patterns were an object of intensive theoretical investigations for almost 30 years, formation of mound wasn't considered in any detail until recently. Last year however 4 publications dealing with the simulation of mound formation appeared [71,30,43,41]. All of them are very interesting and treat the problem from different points of view. The model by Savill and Hogeweg [71] is based on the assumption that the main reason for cell motility is the tendency to decrease a cell's free surface energy, which is defined by its adhesive properties to other cells and to the extra cellular matrix is strongly effected by the level of cAMP. Using this model authors were able to simulate all stages of Dd development from single cells to a crawling slug. According to this model, the main force responsible for mound formation is cell-cell adhesion. Another important outcome is that differential cell-cell adhesion results in cell sorting in the mound.

The model developed by Vasiev et al [30] was based on the assumption that the chemotactic movement of the cells can be viewed as a fluid flow and described by the Navier-Stokes equations. Using this hydrodynamic approach, it was possible to simulate the formation of streaming patterns and mound formation (Fig.6). It was found that hemispherical mounds form even in absence of cell-cell adhesion. The forces maintaining the mound are an inward directed force (accumulation by chemotaxis) and a pressure, which prevents too close compaction of the chemotactically moving cells. A further two papers [41,43] deal with hybrid models, in which a number of cells were modelled to move according to a number of rules (different rules in each model) in a continuum field of cAMP. The model by Bretschneider et al [43] reinforces the importance of pressure in the process of mound formation,

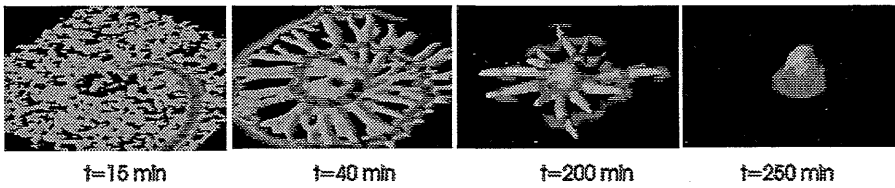

Fig. 6. Formation of the streaming patterns and the mound in hydrodynamic model [30]. Cell density is shown as a white iso-surface ($\rho = 0.5$) and the cAMP concentrations are mapped on this surface from low cAMP (white) to high cAMP (black). The initial density of cells was zero everywhere in 3d-space except for the bottom plane. A random number varying between 0 and 1 represented the cell density in each grid of this plane so that average density in this plane was equal to 0.5. In response to cAMP spiral wave cells move and form aggregation streams (t =40-200 min) and then mound (t =250 min) which represents a stable solution of the system.

while another [41] stresses the importance of adhesive forces in maintaining the mound. There is however an important advantage in the results obtained using the pressure based models. In these models the cells continue to move inside the mound (i.e. rotate along mound's vertical axis in response to spiral wave), while in both adhesion based models the cells stop to move after the formation of the mound. In reality, both adhesion and pressure affect the formation of the mound. A further development of the hybrid model by Bretschneider et al [72] incorporates both pressure and adhesion.

Probably the most important events during the mound stage of Dd development are cell differentiation and sorting [73]. In the mound cells differentiate in prestalk and prespore cells. The differentiation of cells happens at random positions, but then prestalk cells sort towards the top of the mound to form a tip [74]. The mound then contracts at the base while extending up in the air to form a standing slug.

The control of differentiation has been the object of several theoretical studies [75-78]. Since cell differentiation in a mound happens independently from their location, the theory of dissipative structures developed by Gierer and Meinhard, and applied for the process of cell differentiation in hydra [79,80] does not work here. Let us briefly introduce two of the most recent models for cell differentiation in mound as they look to be most promising. Schaap and co-authors [81] developed a model where detailed biological information about the interactions of the morphogens cAMP, ammonia, DIF (differentiation-inducing factor) on differentiated cells are taken into account to estimate the dynamics of the different cell types over time. Different cells in the population produce these factors and different cell types show different responsiveness to these factors (morphogens). By variation of model parameters, they were able to get experimentally observed ratio of fractions of differentiated cells with reasonable kinetics. A completely different model has been proposed by Mizuguchi and Sano [82]. The units (cells) are

each considered to be described by a system of coupled activator-inhibitor (FitzHugh-Nagumo) equations. By introducing a global coupling between equations describing each unit, the authors where able to get a separation of all units into two groups representing the two different cell types. When the coupling is strong enough, there are two stationary solutions of the system describing the state of cells, and a portion of cells get one solution while others adopt the second. This description does however not correspond to any known biochemical mechanism.

Once the cell types have formed they have to sort to form the axial pattern of cell types found in the slug. Sorting of cells in mound or slug has also been the subject of extensive theoretical considerations [83-85]. The main problems to be solved are what mechanism is responsible for cell sorting and how is the final pattern stabilised? According to several theoretical investigations, the force that drives cell sorting is differential adhesion and the final pattern of cell sorting is defined by the relative adhesion forces between different cell types and the cells and the substratum [66,80]. In order to sort out, the different cell types should move differentially inside the mound. This differential motion can be caused by cell type specific differences in the motive force generated by prestalk and prespore cells, caused by differential chemotaxis (different cell types exert different forces due to cell type specific differences in their cytoskeleton) or by differential adhesion. We have shown that cell sorting can be driven only by differential chemotaxis [86]. According to these model calculations sorting can be achieved if the following conditions are satisfied:

1. A scroll wave of cAMP is rotates in the mound and the filament coincides with the vertical axis of a mound
2. Prestalk and prespore cells have different excitability: amount of cAMP in each pulse produced by prestalk cells is higher than by prespore cells.
3. Prestalk and prespore cells move chemotactically to cAMP signals as during aggregation. However, prestalk cells move faster in response to the cAMP waves than prespore cells.
4. Movement of cells can be treated as a fluid flow and described by the Navier-Stokes equations.

The model used in this investigation is an expansion of the hydrodynamic model used in [40]. Here we consider a mound as consisting of a mixture of two liquids which, correspond to prestalk and prespore cells. The motion of each liquid is defined by the momentum balance equation:

$$(\partial/\partial t + \boldsymbol{V}_i \cdot \nabla)(\alpha_i \boldsymbol{V}_i) = \boldsymbol{F}_i + \eta \nabla(\alpha_i \nabla \boldsymbol{V}_i) - \alpha_i \mathrm{grad} p + (-1)^i \psi \alpha_1 \alpha_2 (\boldsymbol{V}_1 - \boldsymbol{V}_2) \tag{9}$$

where $i = 1$ or 2 is an index representing prestalk and prespore cells, α_i are the corresponding volume fractions, $\boldsymbol{V}_i$ the velocities and $\boldsymbol{F}_i$ the chemotactic forces. The second term on the right hand side defines the cell-cell interactions between the cells of the same type; the last term defines the

interactions between the different cell types. Pressure is defined by the condition of total incompressibility of the mound. We neglect terms containing density derivatives and use the reduced version of equation (9):

$$\partial \boldsymbol{V}/\partial t + (\boldsymbol{V}\nabla)\boldsymbol{V} = \boldsymbol{F}_{ch} + \boldsymbol{F}_{fr} + \eta \Delta \boldsymbol{V} - \mathrm{grad} p \qquad (10)$$

We assumed that the force exerted by prestalk cells in response to the chemoattractant cAMP is stronger than that of prespore cells. The FitzHugh-Nagumo equations describe the cell signalling system, where we assume that the excitability of prestalk cells (k_g in (4)) is higher that for prespore cells, in agreement with previous experimental data.

Our computations show that starting from a random distribution of cell types in the mound one can obtain a spatially separated pattern of cell types as well as tip formation. In response to scroll waves rotating along the vertical axis of the hemispherical mound, the liquid begins to rotate in the opposite direction. The faster moving fluid (prestalk cells) accumulates in the centre and top of the mound (the pressure developing in the centre of the mound drives the prestalk cells to the top of the mound and gives rise to asymmetry along vertical axis). Since the faster fluid is more excitable, separation of the fluids leads to the mound becoming inhomogeneous with respect to its excitability so that the topside of the mound is more excitable than the bottom. This in turn results in a change of the cAMP wave shape i.e. the scroll becomes twisted and gets a component of velocity directed downwards to the substratum, along the vertical mound's axis. Therefore, the cells experience a chemotactic force with an upward component, 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, very similar to what happens in real mounds.

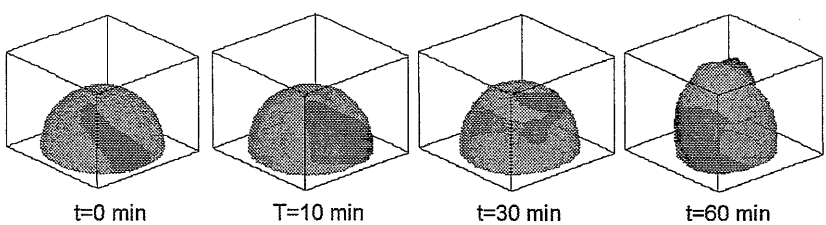

Fig. 7. Cell sorting in the hydrodynamic model. The mound consists of 20% of prestalk cells (light grey) and 80% prespore cells (grey). The cell types differ in their chemotactic response and excitability [86]. The prestalk cells move faster and are more excitable than prespore cells. Initially the mound is a hemisphere in which a cAMP scroll wave (dark grey) rotates clockwise, and both cell types are mixed randomly. Affected by the cAMP waves the cells move and sort, such that the prestalk cells collect at the top of the mound and form a tip.

We found that sorting of cells in the mound results in a transformation of the hemispherical mound into an elongated slug. According to our numerical simulations, the mechanisms responsible for this transformation are the following: 1. The chemotactic cell flow in a mound occurring in response to a scroll wave of cAMP always transforms the hemispherical aggregate into cylindrical one. 2. The geometry of this cylinder depends on the excitability of the cells: cylinders formed by cells that are more excitable are thinner. 3. Since the more excitable prestalk cells form the tip, it differs in geometry from the rest of the mound and has a smaller cross-section. 4. Since the twisted scroll originates in the tip and prestalk cells move to the top of the mound, the mound becomes elongated to form a standing slug (Fig.8).

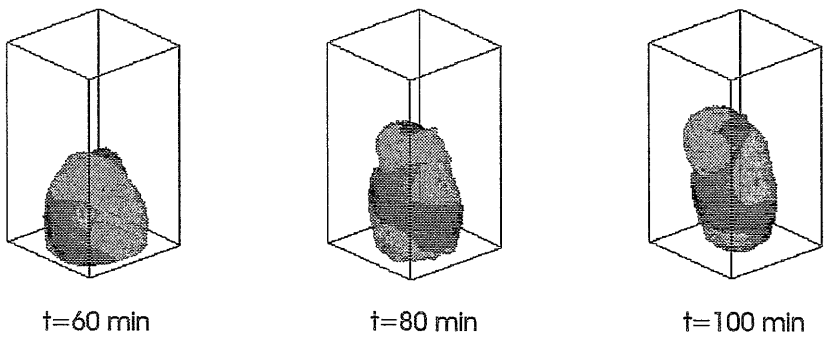

Fig. 8. Transformation of the mound into the standing slug in hydrodynamic model. The mound shown in Fig.7 has been placed in larger medium and allowed to evolve further.

7 Slug stage of development

The standing slug falls over and starts to crawl to form a migrating slug [87,88]. The anterior of the slug (about 20% of its volume) consists of prestalk cells while the rest of its volume consists of the prespore cells. A slime sheath consisting of 50% cellulose and 50% glycoproteins surrounds the slug, which gives the slug some mechanical stability.

The mechanism of slug movement is an interesting problem since it represents one of the simplest kinds of motion exhibited by any multicellular organism. According to experimental data, cells in the back of the slug move forward in the direction of slug migration in a periodic manner [89,90,91,92]. This indicates that they, most probably, move chemotactically in response to propagating waves of cAMP (or other chemoattractant). Tracking of cells shows that cells in the slug tip rotate around the slug's long axis. Assuming

that cells move in response to propagating wave of chemoattractant one can conclude that this wave should have a shape of scroll at the top of the mound and be shaped as planar waves in the back of the slug (Fig.9). Computer simulations showed that the conversion of a scroll into a series of planar waves could occur when there is a substantial difference in excitability between the prestalk and prespore cell zones of the slug [93,94]. If this difference is not very large there is no transformation of scroll to planar waves, instead the scroll wave is only twisted, rotating around slug's axis. The waves propagate from the tip to the back. Cells in the back should exhibit a rotational forward motion in response to these waves. This is the case observed in another slime mould strain Dictyostelium mucoroides [88].

A further important point is the differentiation of the cells in the slug [1,95]. New cell types occur in the slug stage of Dd morphogenesis: pstA cells form in the anterior outer part of the prestalk zone; pstO cells at the boundary between prestalk and prespore cells; and pstB cells in the central core of the prestalk zone. The assumption about twisted scroll of cAMP rotating inside the slug helps in understanding this differentiation pattern. It is known that 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 [96]. Computer simulations show, that the core of the scroll wave in the prestalk zone is a region of low average extracellular cAMP [94], exactly the condition which facilitates the expression of the stalk specific ecmB gene in the central core of the prestalk zone. 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 stabilise the differentiated state of the cells in the slug.

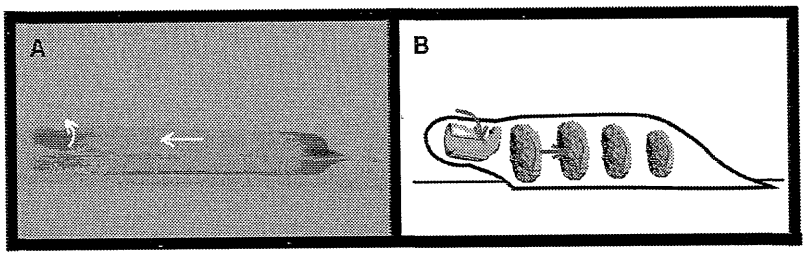

Fig. 9. Model for wave propagation in slugs. A: neutral red stained slug. Cells in the anterior tip exhibit rotational movement around slug's axis, while all other cells move progressively along this axis. B: model for waves in the slug.

Probably the most important property of the slug is its ability to crawl. There were a few attempts to model migration of the slug [44,72,97]. Slug migration must result from the co-ordinated motion of its constitutive cells and most models assume that this motion is chemotactic. Odell and Bonner [44] were the first to propose that the flow of tissue in a slug could be modelled as the flow of a viscous fluid under the influence of a chemoattractant. In addition, one main assumption was that all cells produced and destroyed a chemical, which modulated the chemotactic response of all cells, resulting in a gradient from the centre to the outside. This results in fountain like cell flow patterns along the long axis of the slug, which are not in agreement with experimental observations [91].

Fig. 10. Migrating slug in a model system [72]. Side view of a migrating slug at three successive points of time is shown. Slug is moving from the right to the left; its tip (formed by prestalk cells) is depicted in grey. The track of one (arbitrarily chosen) prestalk cell is shown. The spiral-shaped trajectory indicates that the cell is moving in rotational fashion. Slug migration is driven by cAMP waves of the shape shown in Fig.9B.

More recent attempts to simulate slug migration [72] are more successful, since they show reasonable patterns of cell movement inside the moving slug. However, all these models fail to give a satisfactory description of the mechanism of cell movement inside the slug, i.e. it is not clear how the cells gain traction. Obviously this traction comes from the substrate, and therefore might be proportional to the area of slug- substrate contact. There are experimental data that are in agreement with this notion [98]. However, a better understanding of the force transmission by cells during slug migration can only be achieved based on a good model for individual cell motion.

8 Culmination

The ability of the slug to crawl is very important. Under natural conditions, it migrates up from the leave litter in the soil to the surface before to enter a culmination phase when the slug transforms into a fruiting body. The cellular basis of culmination is not yet well investigated. During culmination the prestalk cells from the tip of the slug start to move downward through the middle of the slug in an inverse fountain like process [99,100]. As soon as the prestalk cells touch the substrate they undergo the final differentiation

step into stalk cells. These cells are highly vacuolated cells that have a rigid cell wall and resemble plant cells in structure. During the culmination process cells move on top of the stalk where they also vacuolate. By doing so, the stalk extends from the top end since more and more cells crawl on top of it. The other cells move upward along the stalk as it extends. By this mechanism all prespore cells are lifted in the air. These cells perform a final differentiation step into spore cells. They are attached to the stalk by structures made from prestalk cells called the upper and lower cup. The spores can then disperse and wait for favourable conditions to germinate and release amoebae again so that the life cycle can start all over again. Since the cellular basis of the culmination process is not yet understood, i.e. there is especially a lack of understanding of the signals that control this process there have been no serious attempts to model this stage of development. This clearly has to be seen as a future goal but has to await further experimentation first.

9 Conclusions and outlook

Except for the culmination, all stages of Dictyostelium development have been intensively studied using mathematical methods and physical concepts. Since during all parts of the Dictyostelium life cycle the cells communicate by propagating chemical waves of cAMP, a population of these cells can be considered as forming a non-linear dynamical system, which can be described as an excitable medium. Due to the chemotactic movement of cells, the geometry of this excitable medium changes over time. It transforms from a 2-dimensional to a 3- dimensional medium. The change of shape of the different three-dimensional media is accompanied by complex transformations of the chemical waves in the system. In the course of the development phase waves transform into excitation waves, concentric waves give rise to spirals, single-armed spirals transform into multi-armed spirals and scrolls, which in turn twist and even give rise to planar waves. These waves co-ordinate cell movement and the combination of both processes leads to morphogenesis.

To conclude let us note here some of the main unsolved problems concerning Dictyostelium development: -We know that cells communicate by means of chemical waves of cAMP, which result from co-ordinated cell-cell signalling. The detailed biochemical network of this process is still not yet completely known. This clearly has to be the subject of further experimentation and modelling efforts. For instance, it is now well known that there are different cAMP receptors of different affinity that are expressed in different phases of the life cycle [2]. Their precise role is still the subject of both further experimental and theoretical investigations. –The detailed process of chemotaxis at the single cell level is still poorly understood. We know that Dictyostelium cells react chemotactically to cAMP, i.e. can move up a cAMP gradient. Many questions remain unanswered. How do the cells detect these gradients and translate them into cell movement? Where and how are the traction forces

generated? How are contacts with the substrate made and released how are all these processes regulated and coordinated in a precise spatio-temporal manner? The physical basis of force generation by individual cells in multicellular tissues is very important but as yet also only poorly understood and investigated. This is clearly a very important area of investigation. –The interaction between the wave propagation system and the regulation of the gene expression of the components involved is also a very important area of investigation and has only been explored to a limited extent. This introduces further feedbacks into the system and can give rise to new types of behaviour. Furthermore, the fact that all cells are different and express different numbers of important macromolecules leads to a large heterogeneity, i.e. no two cells are alike. This again is very different from most chemical and physical excitable systems and will give rise to further heterogeneity and new solutions to the underlying dynamical systems. –Most models for cell sorting have dealt only with two cell types but there are likely to be more which all differ in the movement and signalling parameters. This has to be explored further as well. The cell type proportioning mechanism has to be integrated in the models for cell movement in mounds and slugs. – Finally the genome of Dictyostelium will soon be sequenced and it is to be expected that all of it approximately 10.000-15.000 genes will be known. The big challenge will be to understand this very complex highly integrated and compartmentalised dynamical system.

Acknowledgements

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