Published in final edited form as:

Math Med Biol. 2006 March ; 23(1): 45–62. doi:10.1093/imammb/dqi016.

Mathematically modelling the effects of counting factor in *Dictyostelium discoideum*

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Abstract

Size regulation is a crucial feature in many biological systems, with misregulation leading to dysplasia or hyperplasia. The recent discovery of counting factor (CF) in *Dictyostelium discoideum* will lead to a greater understanding of how the system regulates the size of a group of cells. In this paper we mathematically model the known effects of CF using two different models: a cellular automata model and a discrete continuum hybrid model. With the use of these models we are able to understand how modulation of adhesion and motile forces by CF can facilitate stream breakup. In addition, the modelling suggests a new possible mechanism for stream breakup involving the frequency of cell reorientation.

Keywords

cellular adhesion; size regulation; cell movement; orientation; group size; cell-number counting

1. Introduction

The cellular slime mould *Dictyostelium discoideum* is a very well-studied organism in developmental biology. This simple eukaryote normally lives as individual amoebae that feed on soil bacteria (for review, see Loomis, 1975; Kessin, 2001). When the cells overgrow their food supply and starve, they aggregate using relayed pulses of cyclic adenosine 3',5'-monophosphate (cAMP) as a chemoattractant. The group of aggregated cells will then form a fruiting body consisting of a thin stalk supporting a mass of spores. Several computer simulations have been performed to understand the mechanisms underlying aggregation (Parnas & Segel, 1977; Levine *et al.*, 1996; Vasiev *et al.*, 1994; MacKay, 1978; Dallon & Othmer, 1997; Palsson & Othmer, 2000; Höfer *et al.*, 1995; Savill & Hogeweg, 1997). Using parameters such as the amount of cAMP secreted by a cell, the diffusion coefficient of cAMP, the distance of a cell from the neighbouring cells and the effect of a cAMP gradient on cell motility, these programs successfully mimicked the aggregation of a field of

cells into aggregation streams and the condensation of the streams into a group. The modelling varies greatly in the methods used, which have included discrete formulations (MacKay, 1978; Dallon & Othmer, 1997; Palsson & Othmer, 2000), continuum formulations (Höfer *et al.*, 1995), cellular automata and energy minimization (Savill & Hogeweg, 1997) and ideas from fluid dynamics (Vasiev *et al.*, 1997). None of this modelling has focused on cells aggregating at high density where the aggregation streams break up into groups to prevent the formation of excessively large fruiting bodies (Shaffer, 1957).

Previously, we identified a 450-kDa protein complex, called counting factor (CF), which regulates stream breakup and thus the size of groups formed by *Dictyostelium* cells. CF is secreted at a moderate level by wild-type cells. Disruption of the *smlA* gene leads to the oversecretion of CF, resulting in the severe fragmentation of aggregation streams, and thus the formation of large numbers of tiny fruiting bodies (Brock *et al.*, 1996; Roisin-Bouffay *et al.*, 2000). When a component (countin or CF50) of CF is disrupted, the cells secrete very little detectable CF activity (Brock & Gomer, 1999; Brock *et al.*, 2002, 2003a). *countin*[−] and *cf50*[−] cells form abnormally large groups, leading to the formation of large fruiting bodies that readily collapse. Streams of *countin*[−] or *cf50*[−] cells seldom break, thus forming few but huge fruiting bodies. The differences in the patterns of breakup seem partly due to altered cell–cell adhesion in these cell lines. The cell–cell adhesion of *smlA*[−] cells is initially low during early development, and then slowly increases at 8 h of development to reach a plateau at 12 h (Roisin-Bouffay *et al.*, 2000). Wild-type cells have a low initial cell–cell adhesion which increases rapidly and reaches a plateau at 6 h of development, whereas *countin*[−] cells have a significantly higher initial cell–cell adhesion than wild-type cells. The adhesion of *countin*[−] cells also reaches a plateau at 6 h. Wild-type cells and *countin*[−] cells have a similar final adhesion, whereas that of *smlA*[−] cells is much lower. Decreasing cell–cell adhesion alone by blocking binding activity of adhesion molecules with antibodies results in a decrease in group size (Siu & Kamboj, 1990; Roisin-Bouffay *et al.*, 2000). These observations suggested that CF regulates group size at least in part by reducing cell–cell adhesion.

The motility of the cell is another factor that determines group size in *Dictyostelium* (Tang *et al.*, 2002). *Dictyostelium* cells depend on myosin and actin for motility (Noegel & Schleicher, 2000). When a cell is not moving, myosin II forms a cortical ring around the cell and is also distributed throughout the cytoplasm. Upon cAMP stimulation, myosin II depolymerizes at the front of a cell (Ogihara *et al.*, 1988; Nachmias *et al.*, 1989; Moores *et al.*, 1996; Clow & McNally, 1999). Cells move by polymerizing actin at the front of a cell to create a pseudopod (Cox *et al.*, 1992; Fukui, 1999). We showed that CF decreases group size in part by decreasing the amount of polymerized myosin II and increasing the amount of polymerized actin (F-actin). This in turn increases the motility of cells (Tang *et al.*, 2002). In addition, decreasing motility by using drugs that interfere with actin polymerization resulted in fewer stream breakups and a concomitant increase in group size (Tang *et al.*, 2002).

We found that the breakup of aggregation streams can be simulated by varying cell–cell adhesion and motility (Roisin-Bouffay *et al.*, 2000). Starting with a simple model of a stream of cells, if the cell–cell adhesion was decreased and the cell motility was increased,

the stream loosened up and started to break up. At this point, if the cell–cell adhesion was increased and the cell motility was decreased, the fragmented streams condensed into groups. We also found in the simulations that increasing initial cell–cell adhesion alone with the other parameters constant can increase group size (Tang *et al.*, 2002). In addition, although changing adhesion alone or motility alone by the actual amounts of the changes observed in experiments only slightly increased group size in the simulations, changing both adhesion and motility by these amounts increased group size in the simulations approximately to the observed level (Tang *et al.*, 2002). When cell–cell adhesion and motility are regulated by a diffusible factor that is secreted by every cell (such as CF) the computer simulations were able to achieve a more uniform group size (Roisin-Bouffay *et al.*, 2000; Tang *et al.*, 2002).

To further use computer simulations to predict mechanisms that could regulate group size, and whether small changes in parameters such as motility and adhesion affect group size, we investigate the breakup of aggregation streams using a cellular automata model which does not explicitly include cAMP signalling and a discrete continuum hybrid model which includes cAMP signalling. By using two different models which emphasize different aspects of the system, we are able to gain a better understanding. The cellular automata model incorporates random components in the motion and adhesion processes in a more realistic manner than the hybrid model and is simple enough to clearly understand the effects of the simple parameter manipulations. On the other hand, the hybrid model explicitly models the cAMP signal and the forces involved in the motion of the cells. The more sophisticated treatment of the signal and the motion make the model results more realistic and parameter variations more difficult to interpret. By comparing the results of both models we confirm that cell–cell adhesion and motility contribute to regulating the final group size, but the hybrid simulations suggest that they are not the primary cause of breakup.

The paper is organized in the following manner. We first discuss the new cellular automata simulations. There are three sets of simulations for this model. In all simulations the cell–cell adhesion starts at one level and then is increased to another higher level. This mimics previous observations that as aggregation progresses the cell–cell adhesion increases. In the first set of simulations the time at which the increase occurs is fixed but the initial level of adhesion is varied. In the second set of simulations the initial level of the cell–cell adhesion is fixed but the time at which the cell–cell adhesion increases varies. In the third set of simulations the motility distribution is varied to determine its effect on breakup. Next in Section 3, we introduce the new hybrid model and its simulations. The simulations of the hybrid model are grouped into four sets. In the first set, cell–cell adhesion and random cell motility are altered. In the second set, the external cAMP signal is altered by changing the concentration of the external phosphodiesterase (ePDE). In the third set of simulations, the cells are allowed to rapidly reorient and in the final set of simulations a cell's ability to rapidly reorient is combined with cell–cell adhesion variations. Finally, we conclude with a discussion of the results.

2. Cellular automata model without cAMP signalling

2.1 The cellular automata model

The computer program to simulate stream breakup starting with a linear stream of cells was a JAVA version of the program written in BASIC described in Roisin-Bouffay *et al.* (2000). A modification of the program was that the stream started with cells on a 130×2000 2D array, and the aggregation stream was 2000 cells long, and approximately 6–10 cells wide (Fig. 1A). The basic algorithm of the program is shown in Fig. 1(B and C). The model does include a rudimentary chemotaxis towards nearby groups of cells. The two parameters (cell–cell adhesion and motility) were modified in each simulation to test the effect of changing cell–cell adhesion and motility.

2.2 Altering the level or time course of cell–cell adhesion affects group size

In the previous simulations (Roisin-Bouffay *et al.*, 2000; Tang *et al.*, 2002), the motility force of cells was treated as a flat probability distribution, where each cell had an equal probability of having any value between a minimum and a maximum motility (Fig. 2). However, the observed distribution of cell speeds (measured using videomicroscopy of cells that were on a surface and not touching any other cells) is asymmetric and positively skewed as shown in Fig. 2 (Tang *et al.*, 2002). We thus arbitrarily chose a motility force probability distribution function that matched the observed speed distribution. In this system, since the Reynolds number is very small, the sum of the forces acting on a cell is approximately proportional to the cell velocity (see (4)). A key force is the cell substratum adhesion, and we previously observed that CF does not affect this (Roisin-Bouffay *et al.*, 2000). In order to determine the effect of varying cell–cell adhesion on group size, a series of simulations was performed with different cell–cell adhesions. For each cell, cell motility force that ranged from 0 to 20 with a probability distribution corresponding to Fig. 2 was chosen. The adhesion (ranging from 0 to 10) was multiplied by 0.043 times the number of touching cells (Fig. 1), and if the motility was greater the cell moved. In these simulations, group size increased very rapidly as the initial cell–cell adhesion value increased from 50 to 80, suggesting that a slight change in cell–cell adhesion can result in huge differences in group size (Fig. 3). After the initial cell–cell adhesion reached 90, the stream remained unbroken. In our simulations, the maximum group size was 5000 pixels/group and the motility of each cell at each step of the simulation was chosen following the observed distribution of cell speeds as a probability distribution function. The adhesion value is chosen so that 0 would indicate no adhesion and a value of 100 would indicate a very strong cell–cell adhesion.

A significant difference in cell–cell adhesion between wild-type cells and *smlA*[−] cells was that the cell–cell adhesion of *smlA*[−] cells reached a maximum level much later than wild-type cells, suggesting that the length of time cells have low cell–cell adhesion can affect group size (Roisin-Bouffay *et al.*, 2000). In order to find out how the length of time cells have a low cell–cell adhesion affects group size, we varied the length of time during which cells have a low cell–cell adhesion (Fig. 4). At the beginning of the simulation, cells have moderate (50) cell–cell adhesion. At the transition point indicated on the graph (the step size corresponds to 1 min), the cell–cell adhesion of cells increased to 100. In this simulation, 500 stages correspond to 9 h in real life. Since the transition point indicates the time when

the adhesion of cells switches from low to high, a low transition point indicates that cells spend a short time with a low adhesion and then switch to a high adhesion. When the cell–cell adhesion changed from moderate to high at simulation stages 50–100, the streams remained unbroken. As the time cells stay with a moderate cell–cell adhesion increases, streams become fragmented and group size decreases very rapidly, indicating that small changes in the amount of time cells have a moderate cell–cell adhesion can lead to large changes in group size. When the transition point occurred at stage 500, cell–cell adhesion never reached the maximum level and streams were severely fragmented (Fig. 4).

2.3 Varying the observed motility distribution affects group size

We previously observed that CF increases motility and increases the number of cells with very high motility and to a first approximation this appears as a shift of the cell motility probability distribution along the motility axis (Gao *et al.*, 2002; Tang *et al.*, 2002; Brock *et al.*, 2003a,b). To determine the effect of motility on group size, we varied the cell motility distribution while the cell–cell adhesion was fixed at 50 for 400 stages with an increase to 95 at stage 400. We shifted the motility by shifting either the minimum or the maximum motility. Shifting the total motility was performed by moving the frequency polygon along the x -axis. Since there is no negative motility in cells, any negative values obtained by negatively shifting the frequency polygon were treated as no motility (0) (Fig. 5A). As shown in Fig. 5(B), the group size decreases as the motility of cells increases. Group size increased rapidly at a $-2 \mu\text{m}/\text{min}$ shift, reaching a plateau at a $-3 \mu\text{m}/\text{min}$ shift. To test the hypothesis that cells with very high motility play a major role in stream breakup, we used computer simulations to mimic the condition where the cells have equal probabilities of having a speed within a given range (a flat distribution), or where cells have the observed distribution of speeds (Fig. 6). In the observed distribution, even though the average speed is the same as in the flat distribution, the skewing causes some cells to have a motility higher than any cells in the flat distribution (Fig. 2). The flat distribution produced significantly larger groups, confirming our hypothesis that cells with high motility play crucial roles in stream breakup.

3. The hybrid model with cAMP signalling

We now describe a more detailed model which will allow us to verify some results obtained from the cellular automata model. Since adhesion is treated differently in the two models, we can gain insight by comparing their results.

3.1 The hybrid model

The hybrid model combines modelling efforts dealing with cAMP signalling and efforts in cell–cell interaction models. Before giving a brief explanation of the details of the model, we give the basic ideas. In the hybrid model, spherical cells move in response to their external cAMP signal and the forces acting on them. Their motion is determined by solving force equations involving adhesion, drag and the force they exert based on the cAMP signal to which they are responding. The cAMP signal is determined by a diffusion equation and each cell's output of cAMP. Each cell's output of cAMP is determined by a model of the signal transduction pathway which determines how much cAMP a cell will release based on

the external cAMP signal detected by the cell. Thus, when the cell responds to the cAMP stimuli, it may output cAMP and exert force in response to the stimuli in an attempt to move. By outputting cAMP and moving, the cell will change the external stimuli.

The hybrid model uses the Tang & Othmer (1995) model for signal transduction of cAMP. The implementation of the signal transduction model is the same as that described in Dallon & Othmer (1997), but there the cell description is inadequate to address effects of CF on parameters such as cell adhesion. Therefore, we used a simplified version of the cell–cell interaction model introduced in Palsson & Othmer (2000) and modified in Dallon & Othmer (2004). We now briefly explain the model.

The signal transduction pathway for the cAMP signal is modelled by postulating two major pathways in the transduction and adaptation to an extracellular cAMP signal. In both pathways cAMP binds with receptors cAR1, and the resulting complex activates a G protein. In the stimulatory pathway a subunit of the activated G protein binds with inactive adenylyl cyclase causing the production of cAMP internally. In the inhibitory pathway, the activated G protein inhibits the stimulatory pathway by binding with the cAMP–cAR1 complex and preventing activation of the G protein in the stimulatory pathway. Although it is now known that the competitive interference does not take place at the level of receptors as hypothesized in the Tang & Othmer model (Othmer & Schaap, 1998), the model is useful as it reproduces the excitation, adaptation and relay quantitatively correctly. The version of the model developed in Tang & Othmer (1994) leads to a system of seven differential equations and auxiliary algebraic equations for the time evolution of the intracellular species. However, as was shown in Tang & Othmer (1995) the scheme can be reduced to four primary species for the intracellular dynamics without affecting the input–output behaviour significantly. The four equations are:

$$\begin{aligned}\frac{dw_1^i}{d\tau} &= \alpha_4 u_2^i - w_1^i - \alpha_4 u_2^i w_1^i, \\ \frac{dw_2^i}{d\tau} &= \beta_2 \beta_3 c_2 u_4^i - \beta_5 w_2^i + \beta_6 c_3 w_3^i - c_3 \beta_4 u_1^i w_2^i - \beta_2 \beta_3 c_2 u_4^i (w_2^i + c_3 w_3^i), \\ \frac{dw_3^i}{d\tau} &= -(\beta_5 + \beta_6) w_3^i + \beta_4 u_1^i w_2^i, \\ \frac{dw_4^i}{d\tau} &= \gamma_1 \gamma_2 w_1^i + \Gamma_5 (1 - \Gamma_7 w_1^i) - \gamma_4 \frac{w_4^i}{w_4^i + \gamma_3} - sr (w_4^i),\end{aligned}\quad (1)$$

where

$$u_1^i = \frac{\alpha_0 w_5(\mathbf{x}_i) + (\beta_5 - \alpha_0 w_5(\mathbf{x}_i)) w_3^i}{\alpha_1 + \alpha_0 w_5(\mathbf{x}_i) + \beta_4 w_2^i}, \quad u_2^i = \frac{\alpha_2 \alpha_3 c_1 u_1^i (1 - w_1^i)}{1 + \alpha_4 + \alpha_2 \alpha_3 c_1 u_1^i - \alpha_4 w_1^i}, \quad u_4^i = \frac{\beta_0 w_5(\mathbf{x}_i)}{\beta_1 + \beta_0 w_5(\mathbf{x}_i)}.$$

Here the superscript i designates the i th cell, whose position in the plane is denoted $\mathbf{x}_i$, and sr is the function denoting the dimensionless secretion rate of cAMP. The parameter values used are given in Table 1 and are the same as those used in Dallon & Othmer (1997) unless otherwise stated. The variables represent intracellular chemical complexes with w_1 representing activated adenylyl cyclase (adenylyl cyclase and a subunit of the G protein), w_2 the activated subunit of an inhibitory G protein, w_3 the cAMP–cAR1 complex in the stimulatory pathway (represented by u_1) bound with w_2 and w_4 the intracellular cAMP. In the algebraic equations, u_1 represents the cAMP–cAR1 complex in the stimulatory pathway,

u_2 represents the activated subunit of the G protein, which will activate the adenylyl cyclase forming w_1 , u_4 represents the cAMP–cAR1 complex in the inhibitory pathway and w_5 represents the extracellular cAMP.

The evolution of extracellular cAMP is governed by the partial differential equation

$$\begin{aligned} \frac{\partial w_5(\mathbf{x})}{\partial \tau} = & \overbrace{\Delta_1 \nabla^2 w_5(\mathbf{x})}^{\text{diffusion of cAMP}} \\ & - \overbrace{\hat{\gamma}_9 \frac{w_5(\mathbf{x})}{w_5(\mathbf{x}) + \gamma_8}}^{\text{degradation due to ePDE}} \\ & + \sum_{i=1}^N \frac{V_c}{V_o} \delta(\mathbf{x} \\ & - \mathbf{x}_i) \left(\overbrace{sr(w_4^i)}^{\text{secretion of cAMP}} - \overbrace{\frac{\gamma_7 w_5(\mathbf{x})}{w_5(\mathbf{x}) + \gamma_6}}^{\text{degradation due to mPDE}} \right). \end{aligned} \quad (2)$$

Here $\hat{\gamma}_9 = \gamma_9 \frac{NV_c}{V_o}$, γ_9 is a dimensionless variable which is proportional to the concentration of ePDE, N is the number of cells, $V_c = 696.9 \mu\text{m}^3$ is the volume of a cell (which corresponds to a spherical cell with radius of about $5 \mu\text{m}$), $V_o = 1.944 \times 10^8 \mu\text{m}^3$ is the volume of the extracellular medium (assuming it to be 0.5 cm^2 and about $7.8 \mu\text{m}$ thick), $\mathbf{x}$ is a generic point in the plane and δ is the Dirac distribution. The terms on the right-hand side of the partial differential equation represent, in order, a diffusive contribution, the degradation due to ePDE, which is assumed to be constant in space and time unless otherwise stated, the secretion of cAMP and the degradation of cAMP by membrane-bound phosphodiesterase (mPDE). The cAMP signal is solved numerically as described in Dallon (2000). With the exception of the cell motion described below, the algorithms are the same.

The cells move according to a modified version of the model described by Dallon & Othmer (2004). In that 3D model, cells are assumed to be oriented ellipsoids, are viscoelastic in nature and are free to move in space according to Newton's second law. Our model considers forces due to three types of cell interactions: cell adhesion, active cell motion and interactions due to the physical presence of other cells (or the substrate) including their viscoelastic nature. We also include reactive forces which are the equal and opposite forces from Newton's third law. Because cell motion is determined by force equations, including adhesional forces, many of the questions we want to investigate are easily accessible with this model framework. In this paper we modified the model by constraining the cells to be spherical in shape. They maintain their viscoelastic nature in the sense that when two cells collide, the forces they experience are determined by the viscoelastic nature of the cells, even though the cells maintain their spherical shape. This simplification is motivated by computational constraints and the belief that allowing the cells to take on an ellipsoidal shape will not alter the results of our investigation.

The viscoelastic nature of the cell is modelled by a Kelvin body which is a spring in parallel with a Maxwell element (Fig. 7). A Maxwell element is a spring in series with a dashpot. Kelvin bodies deform and exert forces according to the following ordinary differential equation,

$$f_a(t) + \nu_a f'_a(t) = f_2(u_a(t)) + \nu_a [f'_2(u_a(t)) + k_a] u'_a(t) \quad (3)$$

(Fung, 1981), where $\nu_a = \frac{\mu_a}{k_a}$, f_2 is the force from the spring in parallel and for this paper is $k_2 u_a$, μ_a is the viscous coefficient of the dashpot, k_a is the spring constant for the spring in the Maxwell element, u_a is the change in the length of the element or in our case the overlap distance of two cells, and f_a is the magnitude of the force applied at each end in opposite directions. Knowing the shape and position of the cells we can determine u_a , the amount the cell is being compressed. Thus, in this equation we solve for $f_a(t)$ which is used as the rheological force.

The force equation which describes the position of cell i is given by

$$\frac{A_{if}\mu_f}{A\mu_{\text{cell}}} \mathbf{v}_i + \frac{A_{is}\mu_s}{A\mu_{\text{cell}}} \mathbf{v}_i + \sum_{j \neq i} \frac{A_{ij}}{A} (\mathbf{v}_i - \mathbf{v}_j) + \frac{1}{6\pi r \mu_{\text{cell}}} \left(\mathbf{M}_{s,i} + \mathbf{R}_{s,i}^* + \sum_{j \neq i} \mathbf{T}_{j,i} + \sum_{j \neq i} \mathbf{A}_{j,i} + \sum_{j \neq i} \mathbf{R}_{j,i} + \sum_{j \neq i} (\mathbf{M}_{j,i} + \mathbf{R}_{j,i}^*) \right) = 0. \quad (4)$$

The first three terms are drag terms and the rest represent forces on cell i . The surface area of a cell is denoted A and is equal to the area of a sphere with the appropriate radius, A_{i*} is the contact area of the i th cell with the fluid, the substrate or the j th cell, depending on whether $*$ is f , s or j , respectively. The parameters μ_{cell} , μ_s and μ_f are determined by the degree of adhesiveness between cells, between the substrate and cells and the fluid viscosity. Finally, r is the radius of a cell, and $\mathbf{v}_i$ denotes the velocity of cell i . The first subscript on the force terms indicates the source and the second indicates the target of the force. The active force a cell generates is denoted $\mathbf{T}$ (tractional force), and its corresponding reactive force is denoted $\mathbf{M}$ (motive force), thus $\mathbf{T}_{ij} = -\mathbf{M}_{ji}$ and similarly $\mathbf{T}_{is} = -\mathbf{M}_{si}$; the adhesive forces are denoted $\mathbf{A}$ and the forces due to the viscoelastic nature of the cell are denoted $\mathbf{R}$ (rheological force) and their corresponding reactive forces are denoted $\mathbf{R}^*$. The tractional force is ramped up and down to constant values depending on whether a cell is actively moving or randomly moving and its direction is determined in response to the cAMP stimuli. The adhesion forces are determined by the amount of overlap of cells, or the amount of cell-cell contact. The magnitude of $\mathbf{R}$ in the direction of each of the axes of the cell, $\|\mathbf{R}\| = f_a$, is found by solving (3) for f_a assuming that the axis has been compressed by the amount of cell overlap in one time step (see Dallon & Othmer, 2004, for more details). Due to the low Reynolds number of the system (Dallon & Othmer, 2004), we neglect the term due to acceleration. When forces stop acting on a cell, the cell does not continue to move, i.e. inertial terms are very small.

Thus, to determine the cell positions we rewrite (4) as a system of first-order differential equations of the following form:

$$\mathcal{M}(\mathbf{u}) \mathbf{u}' = \mathbf{b}(\mathbf{u}), \quad (5)$$

where $\mathcal{M}(\mathbf{u})$ is a $3N \times 3N$ real matrix with N as the total number of cells, $\mathbf{u}$ and $\mathbf{b}$ are vectors with $3N$ real components, $\mathbf{u}$ is the location of the cells and $\mathbf{u}' = \mathbf{v}$ is the velocity of the cells. We solve this system using an Adams fourth-order predictor corrector method (Kincaid & Cheney, 2002). The linear solves at each time step are approximated using a sparse iterative linear system solver which uses a generalized minimum residual method (Saad, 2003).

The cell motion model requires two stimuli, one to determine the direction a cell should move and the other to determine when the cell should actively move. In the simulations for this paper these stimuli are given by:

1. The cell moves if the dimensionless concentration of u_2 is greater than 0.004.
2. The cell moves either in the direction of the gradient of cAMP when the motion is started, or in some random perturbation of that direction.

The first rule is based on the assumption that the cell uses some component in the signal transduction pathway to control when it should move. The variable u_2 has the appropriate time response, i.e. the u_2 variable increases sharply at the same time after stimulation as the cell begins to move. It is chosen as a convenient stand-in for a variable which is most likely in the cyclic guanosine-5'-monophosphate pathway (Dallon & Othmer, 1997). The other rule is chosen as a simple representation of empirical observations.

All the simulations discussed in this paper are run with the equivalent of 15000 cells on a 5×5 mm domain. Unless otherwise stated, the force a moving cell exerts is set to be 40 nN (Oliver *et al.*, 1999; Usami *et al.*, 1992) and the force due to random motion is 4 nN. This causes an isolated cell to move at a maximum speed of 35.5 $\mu\text{m}/\text{min}$ when actively moving. In addition, the simulations all start from the same initial conditions which were obtained by running a simulation with random initial cell positions, no adhesion and no random motion to time 300 (Fig. 8). This allows the cells to aggregate in dense streams despite the fact that the density of cells in the numerical simulation is not as high as that in the experiments.

3.2 Altering adhesion and motility strength in the hybrid model does not affect breakup

Several simulations were designed using different functions of time for the adhesion and various values for the force generated during random motion. All the simulations resulted in a large aggregation around the location of the strong pacemaking cells set at the start of the simulations. These simulations did not depict the fragmentation of the streams which naturally occurs at high densities (results not shown). Although in some of the simulations the streams would start to break up, the streams or the aggregates would rejoin due to the cAMP signalling from the major pacemaking region.

In view of the results from the cellular automata model, these results were unexpected. A likely explanation for this discrepancy is due to the cellular automata model not explicitly modelling the cAMP signal. One can think of these simulations as being in a moving frame of reference. The stream breakups but the fragmented stream is still moving towards an

aggregation centre and the fragmented stream may ultimately join together with the main aggregation. Furthermore, the purpose of the cAMP signal is to draw the cells towards an aggregation centre and it does so by drawing the cells into streams. Thus, it is not surprising that by explicitly modelling the cAMP signal, the streams are more cohesive and any subgroups formed due to streams breaking are more likely to merge together as they migrate towards the main aggregate. In fact our simulations suggest that the cAMP signal overpowers any fragmenting tendencies changes in adhesion or motility may cause.

3.3 Altering ePDE in the hybrid model does not affect breakup

Since the hybrid simulations which varied only adhesion and motility did not mimic the breakup of the streams, we began exploring other options which would disrupt the dominance of the cAMP signal originating at the large aggregate. Thus, we allowed the ePDE and mPDE to vary with time (Sucgang *et al.*, 1997). Again we tried numerous simulations and found it very difficult to successfully reproduce the shattering of the streams by varying the adhesion, motility and phosphodiesterase (PDE) (Fig. 9A). For all the simulation results shown in this paper, unless otherwise stated, the ePDE determines the parameter γ which was set to be 959.3 until time 300 and then rises linearly to three times that amount. It seems that by altering these three characteristics of the system one can cause the streams to shatter, but only when the ePDE is set to extremely high levels when the cells form streams (somewhere between 100 and 200 times 959.3). Our numerical experiments suggest that this type of mechanism is not very robust and therefore unlikely.

3.4 Rapid reorientation can induce stream breakup

In previous modelling work, the authors introduced a theoretical mutant cell called 'jittery' (Dallon & Othmer, 1997). This cell chooses a new orientation every 20 s. The justification for modelling this type of mutant is the observations of experiments where a cell is stimulated to move with a pipette filled with cAMP. The pipette is then moved to stimulate the cell in another direction. These experiments indicated that the cell can change its response to the stimulus within 20 s (Futrelle *et al.*, 1982). For a typical cAMP wave causing aggregation, the 'jittery' mutant would orient roughly three times during the time that cAMP levels were high due to a passing wave. The waves of cAMP are due to secretion from each cell. At a macroscopic scale this can produce spiral waves or target pattern waves. At a microscopic scale (the cell level) the chemical profile is very rough due to all the pulses from neighbouring cells and the 'jittery' mutant would get lost and start moving towards locally more dense locations in the stream rather than the originating pacemaker. This previous modelling indicated that these theoretical mutants would not successfully aggregate unless other features such as cell polarization are included in the simulations. With the other observed features combined with the 'jittery' feature, the simulations successfully aggregated giving realistic results. Because of this, we devised simulations where the cells have the 'jittery' feature which means that they orient every 20 s. In all simulations where the 'jittery' feature started at 300 min, the streams shattered. Altering adhesion or motility then affects the breakup caused by the 'jittery' feature (Fig. 9B and C). It is clear from these simulations that the dominant feature of the system causing the breakup of the streams is a frequent reorientation of the cells.

3.5 Altering adhesion in reorienting cells affects group size

By using the ‘jittery’ feature in the simulations and then varying adhesion and cell speed we were able to partially reproduce the breakup behaviour of two mutants: *smlA*[−] cells and *countin*[−] cells (Fig. 9D–F). *smlA*[−] cells form many small aggregates and *countin*[−] cells form a small number of large aggregates when compared to wild-type cells (Roisin-Bouffay *et al.*, 2000). The adhesion protocols for the simulations shown in Fig. 9 are as follows: for wild type the adhesion starts at $60a$ at 300 min and rises linearly to $2000a$ at 500 min, rises linearly to $4000a$ at 600 min and then remains constant; for *smlA*[−] the adhesion is fixed at $40a$ until time 550 and then it rises linearly to $3000a$ and for *countin*[−] the adhesion starts at $180a$ at 300 min, rises linearly to $2000a$ at 500 min, rises linearly to $4000a$ at 600 min and then remains constant. Here $a = 1.207 \times 10^4$ nN/mm². The adhesion force is multiplied by the contact surface between cells. The functional forms for the adhesion are motivated by experimental data (Roisin-Bouffay *et al.*, 2000). In addition to the high adhesion, *countin*[−] cells do not have the ‘jittery’ feature.

Next, we tried to reproduce results from two different sets of experiments. In the first set of experiments, cells were exposed to antibodies reducing the adhesion by about 20% (Siu & Kamboj, 1990; Roisin-Bouffay *et al.*, 2000) and they formed smaller aggregates. When the adhesion was completely blocked, the cells failed to aggregate. From the experiments we expected to find a decreasing relationship between number of aggregates and adhesion. As the adhesion is varied from no adhesion between cells to high adhesion between cells for those with the ‘jittery’ feature, the number of aggregates indeed decreases (Fig. 10).

In another set of previous experiments, wild-type cells were treated with actin-destabilizing drugs (Tang *et al.*, 2002). The drug-treated cells moved slower but formed larger aggregates compared to the control cells. As mentioned previously, both motility and cell–cell adhesion are factors in breakup. It is really the ratio of the motile force and the adhesion force which is important. By decreasing the motile force and maintaining the same adhesion, this ratio is decreased. Thus, it should have the same effect as increasing the adhesion, which our simulations show (see Fig. 10). Together, our results suggest that when cells rapidly reorient, changing adhesion and/or motility affects group size in the same way seen in the experiments and in the cellular automata simulations.

4. Discussion

The process of aggregation in *Dictyostelium discoideum* is a crucial step in the developmental cycle of the organism. Failure at this stage could be catastrophic. Thus, it is not surprising that the models suggest that the aggregation process is somewhat robust. On the other hand, an aggregate that is too large will result in a fruiting body whose stalk cannot support the spore mass, and the resulting collapse would be very disadvantageous. In order to regulate the size of the aggregate, the mechanisms designed to break up an aggregate once it has started must be strong enough to overcome the natural tendency to aggregate.

Breaking the streams must be accompanied by a new aggregation process causing the broken streams to aggregate into several smaller mounds. Our simulations suggest that a

rapid reorientation of cells to the local chemotactic gradient allows all three processes (aggregation, breakup and reaggregation) to occur.

As in any model, there are several parameters which we have not considered. We have not examined the effect of changing the motility force probability distribution function with time. CF alters both the size of the cAMP-induced cAMP pulse to which the cell responds and the manner in which the cell responds to cAMP. Another factor which we have not investigated is the observation that adenylyl cyclase (which makes the cAMP signal) is located at the rear of a cell, and that the manner in which the cell secretes the cAMP affects streaming (Kriebel *et al.*, 2003). The effect of CF on this process has not been studied and our models are not designed to consider this type of result. Thus, the effect of CF on cAMP relay is another possible place where the system could be altered to change the chemotactic signal and disrupt the aggregation process.

In this paper we suggest two different mechanisms for the breakup. In the first mechanism, aggregation starts, and as part of the natural process the extracellular PDE starts to increase. If there is a high density of cells, the associated high density of CF causes more random motion and less adhesion. The combination of very high levels of PDE and the effect of CF on adhesion and motility leads to the breakup of the streams. Once the streams break up, the natural process of cells becoming pacemakers starts (since they are no longer being stimulated by waves of cAMP), and the new aggregation centres restart the aggregation of the small broken streams. Without the increase in the extracellular PDE, the random motion and low adhesion are not sufficient to stop the original aggregation. And without the ability of cells to become pacemakers, the stream fragments will not aggregate anew. Our simulations suggest that this mechanism is unlikely to be the primary cause for the breakup of the streams. Despite many attempts, we could not find reasonable parameters which gave hybrid simulation results similar to biological experiments. Although we cannot rule out this type of mechanism, our simulations suggest that something else is happening.

In the second mechanism, aggregation begins but when local cell density is great enough, the cells are allowed to follow the cAMP gradient more closely. As was shown in Dallon & Othmer (1997), if the cells follow the cAMP gradient too closely they will aggregate towards local regions of high density and not necessarily to the global site of aggregation. The effects of CF on adhesion and motility reinforce the breakup but they are not the dominant cause. In this scenario, the breakup and reaggregation are partially due to the same cause—the cells moving towards a higher density region in the stream. Our simulations thus predict that the cells may be able to reorient more frequently when CF is present, and that this is the dominant and more robust factor in stream breakup. It should be possible to test this prediction by examining the effect of CF on cell reorientation using videomicroscopy of cells at low cell density. The use of a secretion factor to regulate cell–cell adhesion, cell motility and cell reorientation could potentially be a general mechanism to regulate group size, especially the breakup of a primordium into groups, during morphogenesis, but whether this is used in other systems remains to be elucidated.

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Fig. 1.

Aggregation stream and schematic drawings of simulation algorithm and cell movement. (A) The starting aggregation stream is 2000 cells long and approximately 6–10 cells wide. (B) Simulation algorithm for one cell. (C) Arrows show three of the eight possible directions the cell can move. The indicated cell is touching five other cells. If it chooses a direction corresponding to one of the three arrows and if the randomly chosen motility strength is greater than five times the fixed cell–cell adhesion strength for the n th cycle of the simulation, the cell will move. Otherwise it will remain in place.

Fig. 2.

Frequency polygon of flat distribution (squares) and the observed distribution of cell speeds (diamonds). The relative frequency and speed of cells were obtained from frequency tables of wild-type (Ax2) cells. The average speeds of the observed distribution and the flat distribution were the same.

Fig. 3.

The effect of varying initial cell–cell adhesion. The adhesion of cells was increased while the motility of cells had the observed probability distribution function (Fig. 2). The drawing inside the graph represents the concept of varying the initial cell–cell adhesion with the lower levelling corresponding to the value on the horizontal axis in the main figure. Cell–cell adhesion initially starts with low (dotted line) or moderate adhesion (blue line) and reaches a high level at simulation step 400. Values are means $\pm$ SEM from four independent simulations. The absence of an error bar indicates that the bar was smaller than the plot symbol.

Fig. 4.

The effect of varying the adhesion transition point. The transition point was increased while the initial cell–cell adhesion was fixed at 50 and the motility of cells was mimicking that of actual wild-type cells. The drawing inside the graph represents the concept of varying the transition point at the indicated simulation stage or step. At the transition point, the cell–cell adhesion jumps from 50 to 100. Values are means $\pm$ SEM from four independent simulations. The absence of an error bar indicates that the bar was smaller than the plot symbol.

Fig. 5.

The effect of shifting the total motility. (A) The black line designated as 0 represents the original frequency polygon. The curves designated as -4 or +4 represents how the frequency polygon moves as motility shifts positively or negatively. (B) The effect of shifts on group size. Values are means $\pm$ SEM from four independent simulations. The absence of an error bar indicates that the bar was smaller than the plot symbol.

Fig. 6.

The effect of reducing the number of cells having high motility. We simulated stream breakup using a flat distribution and the observed distribution of cell speeds. Both distributions had the same average speeds and both simulations used the same adhesion values and adhesion transition point. Values are means $\pm$ SEM from four independent simulations.

Fig. 7.

A schematic of a Kelvin body used to model the deformation forces of a cell. It consists of a non-linear spring in parallel with a Maxwell element, which comprises a linear spring in series with a dashpot.

Fig. 8.

All of the hybrid simulations start from this initial condition which is obtained by running the simulations, starting with a random distribution of cells on the surface, with no adhesion and no random motion for 300 min.

Fig. 9.

Density plots at 600 min are shown for several different hybrid simulations. Plots of results of simulations showing the effects of changing various parameters and mimicking various mutants are shown. In plot (D) wild-type cells are simulated, in (E) *smlA*⁻ cells are simulated and in (F) *countin*⁻ cells are simulated. For these three simulations the results are consistent with experiments. The remaining plots show results from simulations which differ from that shown in (D) only in the following manner: in (A) there is no 'jittery' feature, in (B) there is no adhesion and in (C) there is no random motion. Notice that without the 'jittery' features there is no fragmentation of the streams and with no random motion the mini aggregates are closer together. Wild-type cells are simulated with the 'jittery' feature turned on at 300 min and the adhesion varies according to the wild-type protocol. The *countin*⁻ cells are simulated with no 'jittery' feature and the adhesion varies according to the *countin* protocol. The *smlA*⁻ cells are simulated with the 'jittery' feature and the adhesion varies according to the *smlA*⁻ protocol. Unless otherwise stated, all simulations have ePDE which varies with time and random motion starting at time 300.

Fig. 10.

The effect of varying adhesion in the hybrid model. The diamonds indicate the number of aggregates plotted against the initial adhesion strength between cells for several simulations. The box and the 'x' represent the number of aggregates for a simulation with no adhesion and a simulation mimicking an experiment which decreased $\times$ cell motility, respectively. The circle represents wild-type simulations. All the simulations are run to time 600 except for the motility simulation which is run to time 750. The point indicated by the box is determined from the image shown in Fig. 9(B), and the point for the wild-type simulation is determined from the image shown in Fig. 9(D).

Table 1

Parameter values. See Tang & Othmer (1995) for a full description of dimensionless parameters

$\alpha_0 = 312.0$	$\beta_0 = 61.0$	$\gamma_1 = 323.2$	$\gamma_6 = 11.6$	$\tau_1 = 4.0 \times 10^{-5}$
$\alpha_1 = 0.8$	$\beta_1 = 16.0$	$\gamma_2 = \text{varied}$	$\gamma_7 = 36.7$	$\text{sr}_1 = 0.02$
$\alpha_2 = 2.67$	$\beta_2 = 0.48$	$\gamma_3 = 57.7$	$\Gamma_7 = 1.09$	$\text{sr}_2 = 0.65$
$\alpha_3 = 1.0$	$\beta_3 = 1.0$	$\gamma_4 = 350.0$	$\gamma_8 = 750.0$	$sw = 0.5$
$\alpha_4 = 26.7$	$\beta_4 = 2.0 \times 10^4$	$\Gamma_5 = 2.4$	$\gamma_9 = 959.3$	$c_1 = 1.0$
$k_2 = 14750 \text{ nN/mm}$	$\beta_5 = 0.4$	$\mu_{\text{cell}} = 4250 \text{ nN min/mm}$	$\mu_s = 2250 \text{ nN min/mm}$	$c_2 = 1.0$
$k_a = 83800 \text{ nN/mm}$	$\beta_6 = 204.0$	$\mu_f = 425 \text{ nN min/mm}$	$\mu_a = 63000 \text{ nN min/mm}$	$c_3 = 0.668$