Molecular Cell, Vol. 6, 953–959, October, 2000, Copyright ©2000 by Cell Press

A Precise Group Size in *Dictyostelium* Is Generated by a Cell-Counting Factor Modulating Cell–Cell Adhesion

Céline Roisin-Bouffay,* Wonhee Jang,† David R. Caprette,† and Richard H. Gomer*†‡
*Howard Hughes Medical Institute and
†Department of Biochemistry and Cell Biology
Rice University
Houston, Texas 77005

Summary

A remarkable aspect of *Dictyostelium* development is that cells form evenly sized groups of $\sim 2 \times 10^4$ cells. A secreted 450 kDa protein complex called counting factor (CF) regulates the number of cells per group. We find that CF regulates group size by repressing cell–cell adhesion. In both experiments and computer simulations, high levels of CF (and thus low adhesion) result in aggregation streams breaking up into small groups, while no CF (and thus high adhesion) results in no stream breakup and large groups. These results suggest that in *Dictyostelium* and possibly other systems a secreted factor regulating cell–cell adhesion can regulate the size of a group of cells.

Introduction

Little is known about how cells form multicellular structures of a defined size. An excellent system in which to study processes such as cell counting and size determination is the simple eukaryote *Dictyostelium discoideum*. *Dictyostelium* normally exists as a single cell that eats bacteria on soil surfaces and increases in number by fission. The growing cells secrete a protein called prestarvation factor (PSF), which allows them to sense the density of the cells relative to the amount of available nutrients (Clarke and Gomer, 1995). When a cell starves, it signals that it is starving by slowly secreting a different factor, the glycoprotein CMF (Jain et al., 1992; Yuen et al., 1995). When there is a high density of starving cells and thus a high concentration of CMF, the cells aggregate using relayed pulses of cAMP as a chemoattractant (Parent and Devreotes, 1999). The cells form a dendritic pattern of aggregation streams. If there is a large number of cells in a stream, the stream will break up into groups (Shaffer, 1957). The groups form migrating slugs, each of which in turn forms a fruiting body containing a mass of spore cells supported by a column of stalk cells (see Loomis, 1993; Firtel, 1995, for review).

There is strong selective pressure to have a large fruiting body to hold the spore mass as high off the ground as possible for optimal spore dispersal. However, if the fruiting body is too large, it will collapse. Thus, in a field of starved *Dictyostelium* amoebae, the slugs are usually of the same size, each with around 10^5 cells (Bonner and Hoffman, 1963).

Formation of a multicellular structure requires cell–cell adhesion. During early development, there are two main types of adhesion: EDTA-sensitive (contact sites B) and EDTA-resistant (contact sites A). The EDTA-sensitive adhesion is mediated by the gp24 protein, and blocking this adhesion using anti-gp24 antibodies completely prevented aggregation (Loomis, 1988). gp24 is expressed starting at 3–4 hr after starvation, but under some conditions is expressed in vegetative cells (Garrod, 1972; Loomis, 1988). gp24 expression is induced by high levels of PSF, resulting in its expression in vegetative cells just before starvation (Rathi and Clarke, 1992).

The EDTA-resistant adhesion is mediated by gp80, also referred to as csA (Siu and Kamboj, 1990). gp80 expression is induced typically 6 hr after starvation (Beug et al., 1973). 10-fold overexpression of gp80 under the control of its own promoter causes clumps of cells to form in aggregation streams (Faix et al., 1992). However, expression of gp80 in vegetative cells causes cell–cell adhesion to be high during early development with aggregation streams remaining intact instead of breaking up into groups, resulting in the formation of abnormally large slugs and fruiting bodies (Kamboj et al., 1990). These authors found a linear relationship between the number of gp80 molecules per cell and slug length for four different wild-type strains. Although gp80 null cells starved in suspension culture have abnormally low cell–cell adhesion, under standard laboratory conditions these cells show normal development (Harloff et al., 1989). Wang et al. (2000) found that when the gp80 null cells develop on a substratum, there is a precocious induction of the gp150 adhesion molecule, resulting in approximately wild-type levels of cell–cell adhesion. Siu and Kamboj (1990) showed that by blocking gp80 binding activity with monoclonal antibodies, cells failed to form continuous streams, resulting in broken streams and many small aggregates.

We used shotgun antisense to isolate *smlA*, a transformant that develops at a normal speed but that forms very small fruiting bodies (Spann et al., 1996). *sml*A is a novel cytosolic protein that appears to regulate the secretion of some proteins (Brock et al., 1996). Conditioned starvation medium (CM) from *smlA* cells causes wild-type cells to form large numbers of small aggregates, suggesting that the *smlA* phenotype is due to oversecretion of a factor that regulates group size (Brock et al., 1996). During the development of wild-type cells, the cells form long streams that flow in toward a common center. If the cell density is high, the streams break up into groups of 1×10^4 to 1×10^5 cells, depending on environmental conditions (in our standard laboratory conditions, the group size is 2×10^4 cells). The CM from *smlA* cells causes aggregation streams to break up into groups of $\sim 5 \times 10^3$ cells and thus form very small fruiting bodies. We purified the counting factor (CF) and found that it behaves as a complex of polypeptides with an effective molecular mass of 450 kDa (Brock and Gomer, 1999). One of the polypeptides is a 40 kDa hydrophilic protein we named countin. In transformants with a disrupted *countin* gene, there was no

‡To whom correspondence should be addressed (e-mail: richard@bioc.rice.edu).

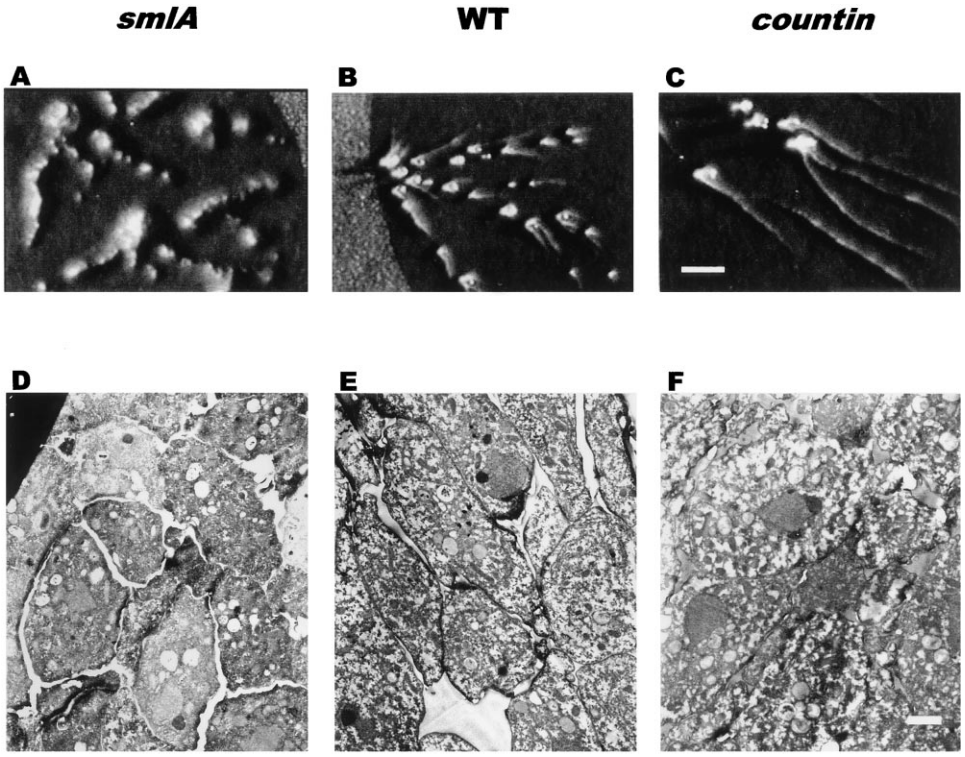

Figure 1. Streams of *smlA*, Wild-Type Ax4, and *countin* Cells

(A–C) Cells were developed in filter pads and photographed after 7 hr of development. Bar in (C) is 0.5 mm. (D–F) Similar streams of cells were fixed, stained, and sectioned for electron microscopy. Bar in (F) is 2 μm.

detectable secretion of CF activity, and the aggregation streams did not break up, resulting in huge (up to 2×10^5 cell) fruiting bodies. Like *smlA* cells, *countin* cells begin aggregation, and form tipped mounds, slugs, and fruiting bodies at the same time as wild-type cells. Addition of crude CF caused the *countin* cells to form normal size aggregates. Preincubation of CF with anti-countin antibodies neutralized the CF activity. Using diffusion calculations, we found that as a general principle such a factor can be used to sense the approximate number of cells in a group (Clarke and Gomer, 1995; Brock and Gomer, 1999; Gomer, 1999).

There are several ways group size can be regulated in *Dictyostelium*. The first is by altering the size of the aggregation territories, for instance by altering the range of the relayed pulses of cAMP that mediate aggregation (Thadani et al., 1977; Faure et al., 1988). The second mechanism is to regulate the breakup of the aggregation streams into groups as was observed for wild-type cells, and which we found to be regulated by CF (Shaffer, 1957; Brock et al., 1996; Brock and Gomer, 1999). Each group then forms a tipped mound. The tip secretes a signal with a limited range, and if the mound is too large, several tips form. A third mechanism then causes the excessively large group to fragment into smaller groups (Kopachik, 1982). We previously found that CF does not affect the size of aggregation territories, and that cells lacking functional CF form large groups that will fragment if they exceed a certain size (Brock et al., 1996; Brock and Gomer, 1999). This suggests that of the three size regulation mechanisms, CF affects only the stream breakup.

In this paper, we present experimental evidence that CF regulates stream breakup and thus group size by regulating cell–cell adhesion. Computer simulations of cells in a stream show that a feedback loop involving CF levels (indicating the local number of cells) repressing cell–cell adhesion is sufficient to cause the stream to break up into groups of a specific size.

Results and Discussion

We observed that in the process of forming small fruiting bodies *smlA* cells formed broken streams whose appearance was very loose compared to wild-type streams, whereas *countin* cells formed unbroken streams (Figures 1A–1C). Electron microscopy of streaming cells showed the presence of gaps between *smlA* cells, some gaps between wild-type cells, and very few gaps between *countin* cells (Figures 1D–1F). Compared to wild-type cells, the loose streams and intercellular gaps in *smlA* cells suggested that these cells might have a decreased amount of cell–cell adhesion. Because altering cell–cell adhesion can alter group size in *Dictyostelium* (Kamboj et al., 1990), we examined whether *countin* or *smlA* cells have altered cell–cell adhesion. Cells were starved for various times on filter pads, and cell–cell adhesion was measured following Desbarats et al. (1994). As previously observed, the adhesion of the Ax4 parental cells was initially low and then increased,

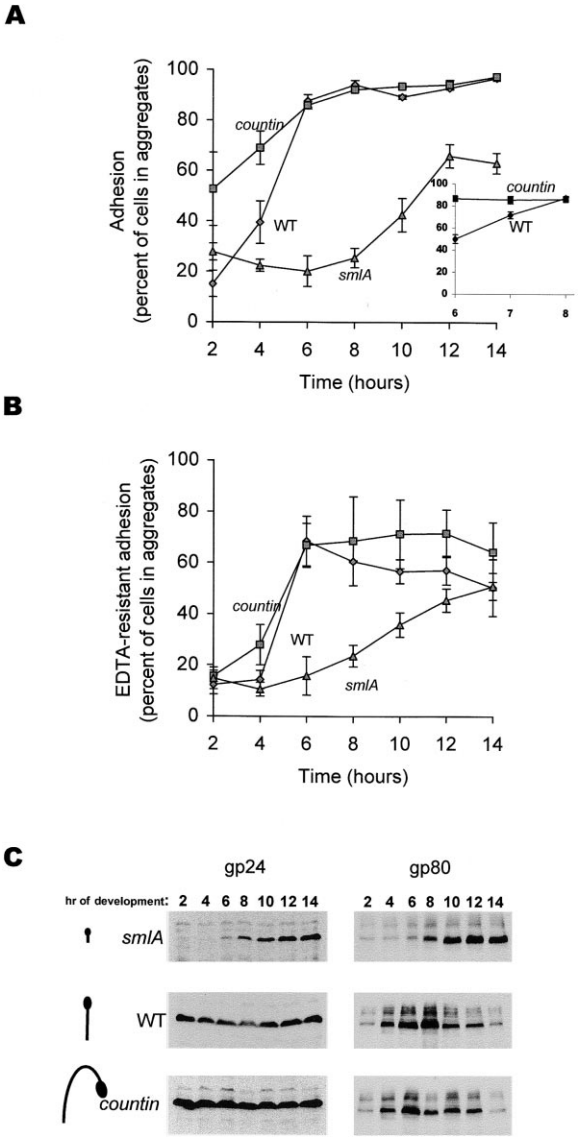

Figure 2. Cell–Cell Adhesion in Wild-Type Ax4, *smlA,* and *countin* Cells during Development

At the indicated times of development on filters, cells were collected, and cell–cell adhesion was assayed following Desbarats et al. (1994). (A) Total adhesion; (B) EDTA-resistant adhesion. Insert in (A) shows the results of assaying for the percentage of WT and *countin* cells in aggregates at 2 min after disaggregation rather than 20 min after disaggregation for cells at 6, 7, and 8 hr of development. (C) Protein samples from cells developing as in (A) were separated by SDS-PAGE, and protein blots were stained with anti-gp24 antibodies (left panels) or anti-gp80 antibodies (right panels).

reaching a plateau at 6 hr (Figure 2A; Loomis, 1988; Siu et al., 1997). During early development, *countin* and *smlA*/*countin* cells had significantly higher adhesion than wild-type cells (Figure 2A and data not shown). Since the adhesion assay involves dissociating cells and then allowing the cells to adhere to one another for 20 min, we were concerned that the ~90% of cells in aggregates seen for wild-type and *countin* cells at 6 hr might represent the assay saturating. We thus did the assays for wild-type and *countin* cells at 6, 7, and 8 hr with a 2 min incubation time after dissociating the cells. In this less sensitive assay, we observed that at 6 and 7 hr of development, *countin* cells showed a higher adhesion than wild-type cells (Figure 2A, insert). Interestingly, Streamer F cells, which like *countin* form large unbroken streams, also had a high adhesion similar to that of *countin* (data not shown). *smlA* cells had a low adhesion during the first 8 hr, which then increased, reaching a rough plateau at 12 hr; however, this adhesion remained lower than that of control cells (Figure 2A). Levels of cell–substratum adhesion were the same in *smlA,* wild-type, and *countin* cells (data not shown).

In *Dictyostelium* and other systems, cell–cell adhesion can be subdivided into EDTA-sensitive and EDTA-resistant adhesion. As previously observed in wild-type cells, EDTA-resistant cell–cell adhesion is initially low and then sharply increases at 6 hr (Figure 2B and Desbarats et al., 1994). The *countin* cell EDTA-resistant adhesion began to increase slightly earlier than control cells and at 6 hr reached a similar plateau. *smlA* cells had a initial EDTA-resistant adhesion similar to that of control cells, but the adhesion took a much longer time to increase, reaching a value similar to that of control cells after 14 hr.

Two proteins mediate much of the cell–cell adhesion during stream formation. The EDTA-sensitive adhesion is mediated by gp24, and the later EDTA-resistant adhesion is mediated by gp80 (Loomis, 1988). There is an essentially constant level of gp24 in wild-type cells throughout early development (Figure 2C and Sesaki and Siu, 1996). In *smlA* cells, gp24 expression begins later than in wild-type and *countin* cells, and the amount is lower. This correlates with the low adhesion observed in *smlA* cells. *countin* cells expressed more gp24 than wild-type and *smlA* cells. This high gp24 level correlates with the high adhesion in *countin* cells, especially at the beginning of development.

As previously observed, gp80 levels were maximal at ~6 hr for wild-type cells (Siu et al., 1985). This correlates with the high EDTA-resistant adhesion observed at 6 hr. For *smlA* cells, gp80 levels began to increase much later (Figure 2C). We observed no significant difference in gp80 levels in *countin* and control cells. Thus, during the first 8 hr of development there is a rough agreement between the gp80 levels and the EDTA-resistant adhesion seen in *smlA*, wild-type control, and *countin* cells.

To determine whether a secreted factor affects adhesion, cells were starved for 6 hr on filter pads soaked with CM from wild-type, *smlA,* and *countin* cells. Cells were collected and adhesion was measured as described above. A significant decrease in the adhesion of wild-type cells was observed when cells were starved in the presence of *smlA* CM as opposed to wild-type CM (Figure 3A). The adhesion of wild-type cells increased when they were starved in *countin* CM. These effects could be seen with a 30 min exposure of cells to CM's (data not shown). To determine whether CF itself regulates adhesion, cells were starved for 3 hr on filters in PBM buffer to allow aggregation to begin, and the filters were then transferred to pads soaked with purified CF. Three hours later, the cells were collected and adhesion was measured. An ~25% decrease in adhesion was observed for 100 ng/ml of CF (Figure 3A). This concentration of CF is about the same as CM from

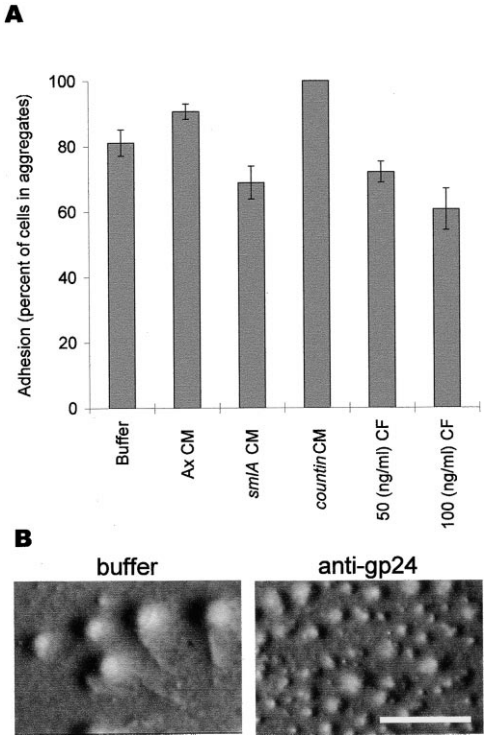

Figure 3. Counting Factor Affects Adhesion and Adhesion Affects Group Size

(A) The effect of development in the presence of CM's from Ax4 wild-type, *smlA*, *countin* cells, and different concentrations of purified CF on the adhesion of Ax4 cells. Adhesion was measured at 6 hr of development. (B) The effect of anti-gp24 antibodies on the development of Ax4 cells. Pictures show cells after 10 hr of development. Bar is 1 mm.

smlA cells and reduces wild-type adhesion by an amount similar to that caused by *smlA* CM. No statistically significant change in adhesion was observed for 20 ng/ml CF (data not shown), and some effect was seen for 50 ng/ml (Figure 3A).

Decreasing cell–cell adhesion by exposing cells to anti-gp80 antibodies results in the formation of small groups (Siu and Kamboj, 1990). To determine whether modulating gp24 levels also affects stream breakup, we starved wild-type cells on filter pads and after 3 or 5 hr transferred the filters to pads soaked with anti-gp24 antibodies. When added at high concentrations immediately after starvation, these antibodies completely blocked adhesion and prevented aggregation (Loomis, 1988); when added at a 1:50 dilution after streams have formed, the antibodies repressed adhesion by approximately 20% and caused increased stream breakup (Figure 3B).

To test the hypothesis that altering cell–cell adhesion can cause a thick column of cells to break into groups, we made a computer simulation of cells in a stream and examined the effect of adhesion coefficient on stream breakup. We modeled a stream of cells where each cell had a random motility force F_m and an adhesion force F_{AS} between each pair of touching cells, which we set as a function of developmental time. The total adhesion force holding a cell in place due to its touching and adhering to n cells would then be $F_A = n \times F_{AS}$. We

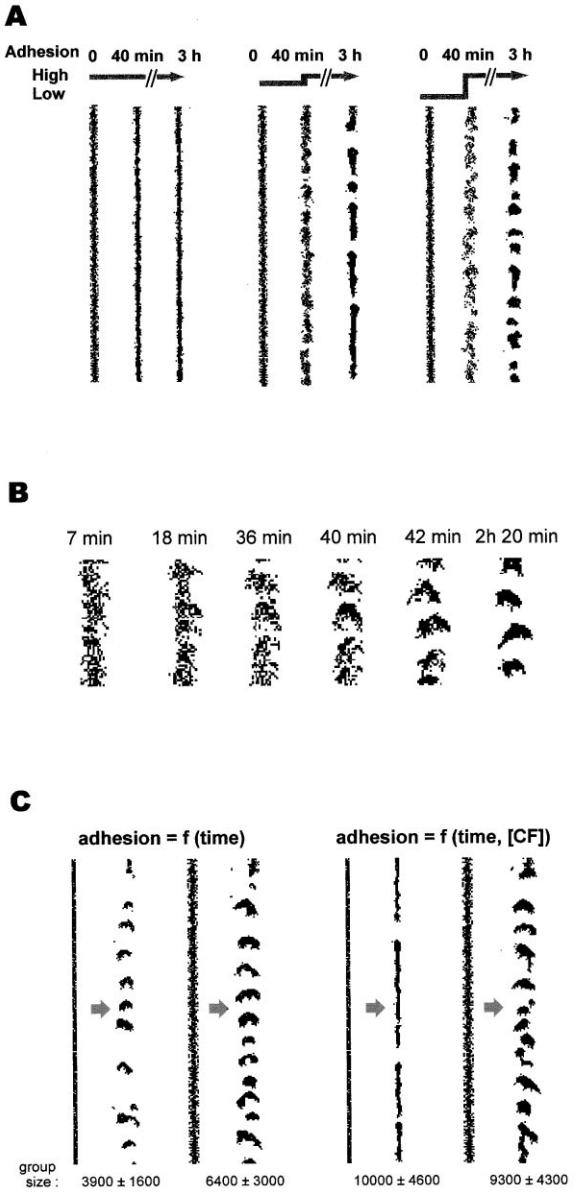

Figure 4. Computer Simulations of Stream Breakup

(A) A column or stream of cells where the initial adhesion is either high (left panel), moderate (middle panel), or low (right panel). (B) A section of a stream at different times in the simulation showing the progress of the stream breakup. (C) Having the adhesion slowly increase with time also causes stream breakup (left panel), but the group size is dependent on the initial stream thickness. The right panel shows the effect of a diffusible factor slightly repressing the adhesion that increases with time.

defined the dimensionless ratio of the two forces as $R = F_M/F_A$, so that if $R > 1$, the cell moved; otherwise, the cell remained in place. In addition, for cells that had moved away from the stream there was a chemotaxis toward the stream or nearby group of cells.

When the cells had a high constant adhesion (R ranging from 0 to 2.28 for $n = 2$, 0 to 1.52 for $n = 3$, etc.), the aggregation stream remained unbroken and even tightened (Figure 4A, left panel). When there was no

adhesion, or adhesion was initially high and then decreased to zero, the stream would randomly break down and fall apart (data not shown). When the adhesion force was moderate (R ranging from 0 to 4 for n = 2, 0 to 2.67 for n = 3, etc.) and then became high, the stream would break up into groups (Figure 4A, middle panel). A lower adhesion force F_{AS} during the first phase of the simulation (corresponding to the first hour after the stream formed) resulted in more groups with fewer cells per group (Figure 4A, right panel). Snapshots of the stream at different times during the remodeling show the loose stream gathering into discrete clumps. The results of the simulations suggest that an elongated group of cells will fragment into subgroups if the cell–cell adhesion is low relative to cell motility, and then increases. In addition, the simulations indicate that the initial adhesion to motility ratio determines the average size of the resulting subgroups.

For simulations with the adhesion strength increasing gradually with time, the stream broke into aggregates as observed for wild-type cells (Figure 2), but the aggregate size varied with stream thickness (Figure 4C, left panel). To simulate the effect of CF, we modeled every cell secreting 3×10^{-10} ng of CF per second (Brock and Gomer, 1999). At each step in the simulation, if a cell could move and was touching two or more cells, the local concentration of CF was then calculated by summing the local concentrations of CF due to CF diffusing away from every cell within 150 μm of itself. The diffusion calculations done by Yuen and Gomer (1994) were adopted in the simulation. Cells farther apart from the possibly moving cell were not considered since the CF contribution of those cells is trivial (data not shown). If the concentration of CF was below 20 ng/ml in the initial phase, the adhesion was not affected. If the concentration of CF was higher than this threshold value, the cell–cell adhesion was decreased proportionally to the level of counting factor so that at 100 ng/ml CF the adhesion would be decreased by 25%. As the number of cells in a stream increases, the concentration of the counting factor increases. If there are too many cells, they sense an excessively high concentration of counting factor and in response become less adhesive to each other. Under these conditions, the number of cells per group was unaffected by the stream thickness (Figure 4C, right panel). This suggests that having a secreted diffusible factor decrease the ratio of adhesive force to random motility force can cause a variable thickness column of cells to break up into evenly sized groups.

We have found that the secreted factor CF, which regulates group size in *Dictyostelium,* induces a decrease in cell–cell adhesion during early development. Computer simulations indicate that by decreasing cell–cell adhesion, such a factor can regulate group size. To directly test this hypothesis, we altered adhesion during early development using antibodies against the gp24 adhesion molecule and, as with similar experiments with anti-gp80 antibodies (Siu and Kamboj, 1990), found that a decreased adhesion causes the formation of smaller groups. We hypothesize that a decreased adhesion causes stream breakage in the following manner. For any two cells along the length of the stream, at some point the cell in front tries to move before the cell in back moves, and there will thus be tension forces in the stream. These would be counteracted by adhesive forces. If a decreased adhesion causes the cell in front to pull away from the cell in back, there is a possibility that the break would tend to propagate, much like the propagation of a crack in a material under tension.

The most striking differences in adhesion between the *smlA*, wild-type, and *countin* cells occur during early development, as the streams are forming. However, the electron microscopy (Figure 1) and adhesion assays (Figure 2) indicate that as the streams are forming and breaking up at 6 and 7 hr of development there is a noticeable difference in the adhesion of *smlA*, wild-type, and *countin* cells. The computer simulations indicate that small groups can form as a result of either forming a dissipated stream or causing a tight stream to dissipate, and then increasing the cell–cell adhesion to cause the dissipated stream to form clumps. Our data suggest that CF acts by causing the initial formation of loose streams rather than dissipating an already tight stream (Brock et al., 1996).

Computer simulations as well as experiments indicated that changing adhesion by 20% could have a strong effect on group size, and that an increased adhesion causes an increased group size. These results are in agreement with those of Kamboj et al. (1990), who overexpressed gp80 2-fold from the beginning of development and observed increased group sizes. However, when gp80 was overexpressed 10-fold starting during stream formation, the streams broke into clumps, causing a decreased group size (Faix et al., 1992). This suggests that as adhesion increases, group size increases, but when adhesion increases beyond a certain point, group size actually starts to decrease.

In higher eukaryotes, there are a wide variety of diffusible signals secreted by cells, and both motility and cell–cell adhesion can be regulated by extracellular signals. It is thus possible that in other systems a diffusible signal increasing cellular motility and/or decreasing adhesion could be used to regulate the subdivision of primordia into groups and the number of cells in a group, and thus regulate structure size.

Experimental Procedures

Cell Culture

Dictyostelium discoideum Ax4 wild-type, *smlA* antisense, and *countin* knockout cells were grown axenically in HL5 media as previously described (Brock et al., 1996; Brock and Gomer, 1999). Conditioned medium (CM) was made following Jain et al. (1992). Starvation in the presence of exogenous CM followed (Brock et al., 1996). Counting factor (CF) was purified as previously described (Brock and Gomer, 1999). To examine the effect of altering gp24 adhesion, 30 μl of Ax4 cells at a density of 5×10^6 cells/ml was starved on filters placed on pads soaked with PB (3 mM Na_2HPO_4, 7 mM KH_2PO_4 [pH 6.5]) for 5 hr. The filters were then transferred to pads soaked with different anti-gp24 dilutions.

Microscopy

Streams of cells were photographed with a Nikon microphot microscope with a 4× lens. Electron microscopy was performed following Brazill et al. (2000) with the exception that to fix the cells, filter pads with streaming cells were placed on a filter soaked with 2.5% glutaraldehyde, and then after 1 hr the filters with cells were submerged in glutaraldehyde. Instead of centrifugation to collect cells, the filters with cells were transferred from one solution to another.

Motility assays on cells moving in toward a stream were performed as described in Yuen et al. (1995).

Cell–Cell Adhesion Assay and Western Blots

To measure adhesion, cells were dissociated by placing the filter pad with developing cells in a 50 ml conical tube, adding 2 ml of PBM, and vortexing for 20 s. A 500 μl sample of cells was placed in an Eppendorf tube and gently rotated with a Labquake rotator (Labindustries, Berkeley, CA) for 20 min. In some experiments, EDTA was added for a final concentration of 10 mM. Adhesion was estimated by counting the number of single cells and the number of cells in aggregates with a hemacytometer following Desbarats et al. (1994). Doublets were counted as aggregated cells. Cell–substratum adhesion was measured following Barondes et al. (1987). Western blots were done following Lindsey et al. (1998).

Computer Simulations

The computer program to simulate stream breakup starts with an aggregation stream 1200 cells in length, approximately 15–20 cells in width, and ~4 cells thick. The cells are on a 130 × 1200 two-dimensional array, with a 5 μm spacing between points. This represents an approximately 6 mm long aggregation stream. Starting at one corner, the program searched for cells. Identical results were obtained by either scanning the array systematically or randomly choosing positions in the array (data not shown). For each cell that it found, a random movement direction was selected. If the movement was blocked by another cell, the cell did not move and the next cell on the array went through the algorithm. If the cell could move, it then counted the neighboring cells. If the cell was touching two or more cells, it then selected a random motility force. The adhesion force per cell was a function we supplied. The total adhesion was assumed to be the adhesion force per cell times the number of neighboring cells. In some cases, we used an adhesion that increased stepwise with time; in others, the adhesion increased gradually with time. The program compared the adhesion force to motility force, and if the motility force was greater, the cell moved; otherwise, it stayed in place.

If the cell was touching one or fewer cells, it possibly chemotaxed toward nearby cells. To do this, the program determined the local gradient of a chemoattractant being secreted from any cell within 80 μm. This determined the direction of cell movement, and a movement was made if the steepness of the gradient was greater than a randomly chosen value. The number of cells per aggregate was measured by using the NIH Image program, discarding aggregates with less than 200 cells.

Acknowledgments

We thank Bill Loomis for the kind gift of anti-gp24 antibodies, Chi-Hung Siu for the kind gift of anti-gp80 antibodies, and Debra Brock for the purified CF. R. H. G. is an associate investigator of the Howard Hughes Medical Institute.

Received May 8, 2000; revised August 28, 2000.

References

Barondes, S.H., Cooper, D.N.W., and Springer, W.R. (1987). Discoidins I and II: endogenous lectins involved in cell-substratum adhesion and spore coat formation. Methods Cell Biol. *28*, 387–409.

Beug, H., Katz, F.E., Stein, A., and Gerisch, G. (1973). Quantitation of membrane sites in aggregating *Dictyostelium* cells by use of tritiated univalent antibody. Proc. Natl. Acad. Sci. USA *70*, 3150–3154.

Bonner, J.T., and Hoffman, M.E. (1963). Evidence for a substance responsible for spacing pattern of aggregation and fruiting bodies in the cellular slime mold. J. Embryol. Exp. Morphol. *11*, 571–589.

Brazill, D.T., Caprette, D.R., Myler, H.A., Hatton, R.D., Ammann, R.A., Lindsey, D.F., Brock, D.A., and Gomer, R.H. (2000). A protein containing a serine-rich domain with vesicle-fusing properties mediates cell-cycle dependent cytosolic pH regulation. J. Biol. Chem. *275*, 19231–19240.

Brock, D.A., and Gomer, R.H. (1999). A cell-counting factor regulating structure size in *Dictyostelium*. Genes Dev. *13*, 1960–1969.

Brock, D.A., Buczynski, G., Spann, T.P., Wood, S.A., Cardelli, J., and Gomer, R.H. (1996). A *Dictyostelium* mutant with defective aggregate size determination. Development *122*, 2569–2578.

Clarke, M., and Gomer, R.H. (1995). PSF and CMF, autocrine factors that regulate gene expression during growth and early development of *Dictyostelium*. Experientia *51*, 1124–1134.

Desbarats, L., Brar, S.K., and Siu, C.H. (1994). Involvement of cell–cell adhesion in the expression of the cell cohesion molecule gp80 in *Dictyostelium discoideum*. J. Cell Sci. *107*, 1705–1712.

Faix, J., Gerisch, G., and Noegel, A.A. (1992). Overexpression of the csA cell adhesion molecule under its own cAMP-regulated promoter impairs morphogenesis in *Dictyostelium*. J. Cell Sci. *102*, 203–214.

Faure, M., Podgorski, G.J., Franke, J., and Kessin, R.H. (1988). Disruption of *Dictyostelium discoideum* morphogenesis by overproduction of cAMP phosphodiesterase. Proc. Natl. Acad. Sci. USA *85*, 8076–8080.

Firtel, R.A. (1995). Integration of signaling information in controlling cell-fate decisions in *Dictyostelium*. Genes Dev. *9*, 1427–1444.

Garrod, D.R. (1972). Acquisition of cohesiveness by slime mould cells prior to morphogenesis. Exp. Cell Res. *72*, 588–591.

Gomer, R.H. (1999). Cell density sensing in a eukaryote. ASM News *65*, 23–29.

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

Jain, R., Yuen, I.S., Taphouse, C.R., and Gomer, R.H. (1992). A density-sensing factor controls development in *Dictyostelium*. Genes Dev. *6*, 390–400.

Kamboj, R.K., Lam, T.Y., and Siu, C.H. (1990). Regulation of slug size by the cell adhesion molecule gp80 in *Dictyostelium discoideum*. Cell Regul. *1*, 715–729.

Kopachik, W.J. (1982). Size regulation in *Dictyostelium*. J. Embryol. Exp. Morphol. *68*, 23–35.

Lindsey, D.F., Amerik, A., Deery, W.J., Bishop, J.D., Hochstrasser, M., and Gomer, R.H. (1998). A deubiquitinating enzyme that disassembles free polyubiquitin chains is required for development but not growth in *Dictyostelium*. J. Biol. Chem. *273*, 29178–29187.

Loomis, W.F. (1988). Cell–cell adhesion in *Dictyostelium discoideum*. Dev. Genet. *9*, 549–560.

Loomis, W.F. (1993). Lateral inhibition and pattern formation in *Dictyostelium*. Curr. Top. Dev. Biol. *28*, 1–46.

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

Rathi, A., and Clarke, M. (1992). Expression of early developmental genes in *Dictyostelium discoideum* is initiated during exponential growth by an autocrine-dependent mechanism. Mech. Dev. *36*, 173–182.

Sesaki, H., and Siu, C. (1996). Novel redistribution of the Ca(2+)-dependent cell adhesion molecule DdCAD-1 during development of *Dictyostelium discoideum*. Dev. Biol. *177*, 504–516.

Shaffer, B.M. (1957). Variability of behaviour of aggregating cellular slime moulds. Quart. J. Micr. Sci. *98*, 393–405.

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

Siu, C.-H., Lam, T.Y., and Choi, A.H.C. (1985). Inhibition of cell–cell binding at the aggregation stage of *Dictyostelium discoideum* development by monoclonal antibodies directed against an 80.000-Dalton surface glycoprotein. J. Biol. Chem. *260*, 16030–16036.

Siu, C.H., Harris, T.J.C., Wong, E.F.S., Yang, C., Sesaki, H., and Wang, J. (1997). Cell adhesion molecules in *Dictyostelium*. In Dictyostelium - a model system for cell and developmental biology. Y. Maeda, K. Inouye, and I. Takeuchi, eds. (Tokyo, Japan: Universal Academy Press), pp. 111–121.

Spann, T.P., Brock, D.A., Lindsey, D.F., Wood, S.A., and Gomer, R.H. (1996). Mutagenesis and gene identification in *Dictyostelium* by shotgun antisense. Proc. Natl. Acad. Sci. USA *93*, 5003–5007.

Thadani, V., Pan, P., and Bonner, J.T. (1977). Complementary effects of ammonia and cAMP on aggregation territory size in the cellular slime mold Dictyostelium discoideum. Exp. Cell Res. *108*, 75–78.

Wang, J., Hou, L., Awrey, D., Loomis, W.F., Firtel, R.A., and Siu, C.-H. (2000). The membrane glycoprotein gp150 is encoded by the *lagC* gene and mediates cell–cell adhesion by heterophilic binding during *Dictyostelium discoideum* development. Dev. Biol., in press.

Yuen, I.S., and Gomer, R.H. (1994). Cell density-sensing in *Dictyostelium* by means of the accumulation rate, diffusion coefficient and activity threshold of a protein secreted by starved cells. J. Theor. Biol. *167*, 273–282.

Yuen, I.S., Jain, R., Bishop, J.D., Lindsey, D.F., Deery, W.J., Van Haastert, P.J.M., and Gomer, R.H. (1995). A density-sensing factor regulates signal transduction in *Dictyostelium*. J. Cell Biol. *129*, 1251–1262.