

Cell-sorting in aggregates of *Dictyostelium discoideum*

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Accepted 6 September; published on WWW 3 November 1999

SUMMARY

When *Dictyostelium* cells are induced to develop between a coverslip and a layer of agarose, they aggregate normally into groups containing up to a thousand cells but are then constrained to form disks only a few cells thick that appear to be equivalent to the three-dimensional mounds formed on top of agarose. Such vertically restricted aggregates frequently develop into elongated motile structures, the flattened equivalent of three-dimensional slugs. The advantage of using this system is that the restricted z-dimension enables direct microscopic visualization of most of the cells in the developing structure. We have used time lapse digital fluorescence microscopy of *Dictyostelium* strains expressing green fluorescent protein (GFP) under the control of either prestalk or prespore specific promoters to follow cell sorting in these flattened mounds. We find that prestalk and prespore cells expressing GFP arise randomly

in early aggregates and then rotate rapidly around the disk mixed with the other cell type. After a few hours, the cell types sort out by a process which involves striking changes in relative cell movement. Once sorted, the cell types move independently of each other showing very little heterotypic adhesion. When a group of prestalk cells reaches the edge of the disk, it moves out and is followed by the prespore cell mass. We suggest that sorting may result from cell type specific changes in adhesion and the consequent disruption of movement in the files of cells that are held together by end-to-end adhesion.

Key words: *Dictyostelium*, Sorting-out, Cell-sorting, Agarose overlay, Green fluorescent protein, Differential adhesion, Time lapse microscopy

INTRODUCTION

Scattered individual amoebae of *Dictyostelium discoideum* aggregate into multicellular mounds and then sort out to generate distinct prespore and prestalk domains in slug shaped structures (Raper, 1940; Bonner, 1967; Loomis, 1982). The relative simplicity of early development in *Dictyostelium* makes it an easily accessible model for the study of cell movement, a process central to all morphogenesis. Within 6 hours following the removal of food sources, *Dictyostelium* cells become chemotactically responsive to cAMP that is propagated in concentric or spiral waves from the center of the emerging field (Devreotes, 1982; Siegert and Weijer, 1995). They also accumulate a surface glycoprotein, gp80, that mediates the EDTA resistant adhesion mechanism termed contact sites A (Beug et al., 1973; Muller and Gerisch, 1978). As cells enter into aggregation streams they form end-to-end adhesions that can be blocked by antibodies to gp80 (Gerisch, 1980). Movement within streams and aggregates is coordinated by contact following in files of cells attached end-to-end (Rappel et al., 1999). Movement of individual cells is to a large extent constrained to that of the file. Once they have entered an aggregate, the files follow a circular path around the mound until a tip containing prestalk cells appears (Reitdorf et al., 1996; Siegert and Weijer, 1995; Doolittle et al., 1995; Durston and Vork, 1979; Kellerman and McNally, 1999). We have

recently shown that such rotational movement can result from a self-organizing vortex state that does not depend on chemotactic responses but only on the motile nature of the individual cells and their mutual adhesion (Rappel et al., 1999). Moreover, we have observed cells rapidly rotating within aggregates formed from a non-signaling strain of *Dictyostelium* in which the gene encoding the aggregation stage adenylyl cyclase is mutationally inactivated and the cells are made independent of cAMP by overexpression of the catalytic subunit of protein kinase A (Rappel et al., 1999). Aggregation in this strain is dependent on having the cells at sufficiently high density on the surface that they randomly collide and adhere to one another (Wang and Kuspa, 1997). Aggregated cells of this mutant strain not only rotate within mounds but subsequently sort out such that prestalk cells become localized to tips that form the anterior of migrating slugs (Rappel et al., 1999; Wang and Kuspa, 1997).

Direct recording of individual cell movements within developing mounds is only possible using technically complex optical sectioning methods such as confocal and deconvolution microscopy. Both methods are severely limited for this work by illumination constraints which increase in proportion to the thickness of the specimen. Bonner (1998) has facilitated the observation of cellular behavior in slugs by describing a method for production of 2-dimensional slugs in which individual cells can be followed by conventional microscopy.

He found that small slugs moving in a drop of mineral oil held beneath a cover slip would occasionally flatten at the interface to form one-cell-thick slugs that continued to migrate. Cells at the anterior of these 2-D slugs were highly motile and moved around chaotically while the posterior cells followed in linear paths. Now and then, cells in the anterior domain coordinated their movement to follow a horizontal circular path similar to what has been observed in 3-D slugs where prestalk cells rotate in a plane perpendicular to the axis of slug migration (Seigert and Weijer, 1995).

We have been able to reliably generate aggregates and slugs only a few cells thick (2-D slugs) by depositing freshly washed cells on coverslips overlaid with a thin film of agarose in a manner similar to that used by Fukui et al. (1986) to view single cells. Under these conditions the cells stream into aggregates but are constrained to disks only a few cells thick. Prespore and prestalk cells can be distinguished soon after they enter aggregates by the expression of cell type specific marker genes. However, the cell types do not sort out until some time later facilitating recording of relative cell movements before and after sorting out. We find that prestalk cells transiently stop moving as they sort out and then resume rotation in a manner independent of the direction of rotation in surrounding prespore cells. These observations suggest to us that prestalk cells acquire side-to-side adhesion as they differentiate such that they leave the files in which they had been moving and form a cell type specific domain that then reorganizes to continue rotating. When a prestalk domain reaches the edge of the aggregate, it moves outwards converting the whole field of cells into a migrating 2-D slug that can be seen to be held together by the extracellular matrix of the surface sheath.

MATERIALS AND METHODS

Cells and culture

Strains JS24, JS30 and JS39 (Stege, 1998) were the kind gifts of Justin Stege. Strain JS24 was derived from wild-type strain AX4 cells following stable transformation with a construct which confers resistance to geneticin (G418^R) and expresses GFP under the control of the prespore specific promoter of *cotB* (Fosnaugh and Loomis, 1993). Likewise, strains JS30 and JS39 were derived from wild-type strain AX4 cells stably transformed with a G418^R construct that expresses GFP under the control of the prestalk specific promoters of *ecmA* and *tagB*, respectively. Cells of each of these strains were grown in shaken flasks containing HL5 liquid medium with 10 µg/ml G418 (Gibco BRL) (Sussman, 1987; Nellen et al., 1984).

Development of 2-D slugs

Exponentially growing cells were collected from liquid medium and washed 3× with buffer containing 2 mM MgSO₄, 20 mM KHPO₄/K₂PO₄, pH 6.3. Cells were collected by centrifugation after each wash and the final pellet was resuspended at 5×10⁶/ml in buffer. 15 µl droplets of cells were placed on ethanol washed 18 mm square no.1 glass coverslips and maintained in humidified Petri dishes. After allowing 5-15 minutes for cell attachment, excess buffer was withdrawn using a strip of filter paper and the spot of cells immediately covered with a thin agarose sheet. Sheets containing 2% agarose in buffer were freshly cast between two microscope slides using no.1 coverslips as spacers and cut into 64 mm² squares using single edge razor blades in the manner of Fukui et al. (1986). Addition of 1% w/v activated carbon to the agarose was found to increase the frequency of slug development and was used when transmitted light

microscopy was not required. The coverslip assemblies were inverted over square holes cut in the lids of 50 mm diameter plastic Petri dishes in which the humidity was maintained by wet filter paper.

Time lapse microscopy

After 10 hours of development under agarose, epifluorescence or phase contrast images of fields containing up to a thousand cells were captured on a cooled digital camera (Photometrics SenSys) mounted on a Nikon Photomicroscope microscope controlled by IP-Lab Spectrum software. Epifluorescence illumination and image recording conditions were chosen to minimize total irradiation of the specimens. Typically we used a 16× neutral density filter in the light path of a 50 W mercury arc lamp and collected 2 second exposures with 2× binning every 2 minutes. Standard fluorescein filter sets were used. Sequential images were saved as 8 bit TIFF files and subsequently displayed using the stacking facility of the public domain NIH Image program available on the Internet at <http://rsb.info.nih.gov/nih-image/>.

RESULTS

Development under agarose

Washed vegetative amoebae streamed into aggregates about 8 hours after being deposited in both the flattened 2-D preparations under an agarose overlay and unconstrained 3-D preparations on top of agarose. Sorting out of the cell types occurred a few hours later in the 3-D mounds as evidenced by the formation of tips. Sorting was delayed a few hours in the 2-D preparations but was often observed within 16 hours of the initiation of development under an agarose overlay. Sorting of the prespore marked JS24 cells in the flattened preparations was characterized by the establishment of a coherent domain of non-fluorescent cells that could be seen in the light scattered from surrounding fluorescent cells. This group of cells could be easily distinguished from the fluorescently labelled prespore cells and were likely to be prestalk cells. They moved around as a coherent group within the mass of rotating cells for periods ranging from 1 to 4 hours before encountering the edge of the aggregate, whereupon they moved out to form a migrating 2-D

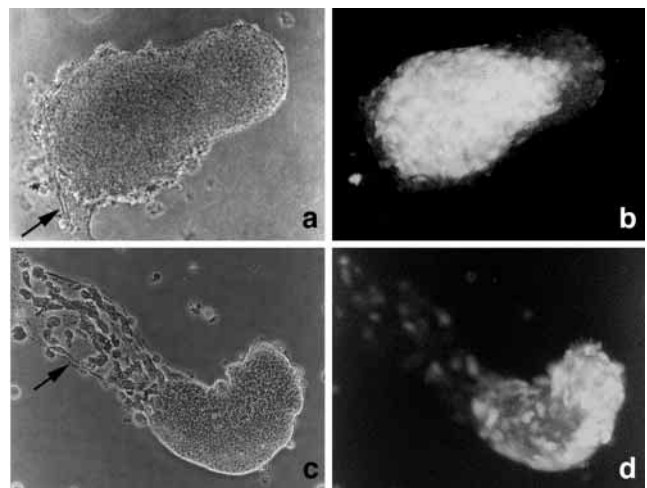


Fig. 1. 2-D Slugs. Strains JS24 [*cotB*::GFP] (a,b) and JS30 [*ecmA*::GFP] (c,d) were allowed to develop to the slug stage under an agarose sheet. Phase contrast images (a,c) show the trails (arrows) left behind the migrating cell masses. Fluorescence images show the position of prespore cells (b) and prestalk cells (d). Bar, 100 µm.

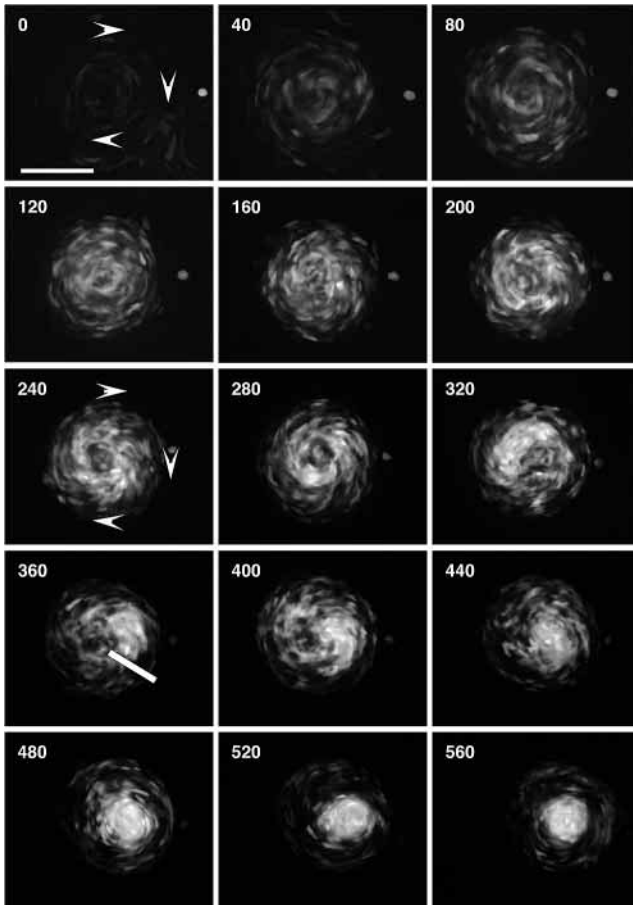


Fig. 2. Sorting out in prestalk marked cells. Cells of strain JS39 [tagB::GFP] were developed under agarose for 10 hours before the start of time lapse microscopy. The numbers on the frames indicate the subsequent time elapsed in minutes. Arrows indicate the direction of movement of the cells. The bar on the 360 minute frame indicates the point where the clockwise rotation of prestalk cells was arrested. Bar, 100 μm . Videos of these and other fields of cells are available at either <http://glamdring.ucsd.edu/others/dsmith/anp2v3.html> or <http://herbie.ucsd.edu/~levine/dicty.html>

slug (Fig. 1b). Sorting of the prestalk marked cells of strains JS30 and JS39 was characterized by the accumulation of fluorescent cells at a site within the 2-D disk. These fluorescent cells form the anterior of migrating 2-D slugs (Fig. 1d). While these strains were seen at times to sort out within 6 hours of forming aggregates, they were often delayed for up to 15 hours in the 2-D preparations.

Phase contrast images of migrating 2-D slugs (Fig. 1a,c) clearly show that they are surrounded by an extracellular matrix that forms the surface sheath and is left behind as the cells advance. The sheath keeps most of the cells together in 2-D slugs but a few cells are left behind in the trails.

Cells differentiate at random and then sort out

Time lapse recordings of development under a thin agarose layer showed that prestalk and prespore cells expressing the fluorescent cell type marker GFP first arose in a spatially random manner throughout the aggregates (Figs 2, 3, 4). Thus,

under our conditions, divergence of the cell types is clearly independent of any positional information.

In most of the recorded series cells entered the aggregate from tangential streams and continued to move in circles within the enlarging disk. The contact following of end-to-end adherent cells so clearly seen within aggregation streams (Gerisch, 1980) appears to be maintained as the cells rotated within aggregates (Rappel et al., 1999). In a few cases where streams approached the center radially, the entering cells encountered a mass of cells that was not uniformly rotating but was directionally confused. Within an hour regions of consistent directionality were established by an unfolding of swirling files of cells until uniform rotation, either clockwise or counterclockwise, could be seen throughout the aggregate. Video clips of this movement are available on the Internet at <http://glamdring.ucsd.edu/others/dsmith/anp2v3.html> or <http://herbie.ucsd.edu/~levine/dicty.html>

After aggregation was complete and the cells were rotating in a coordinated manner, they frequently flattened out thereby increasing the area of the 2-D aggregates. However, the total number of fluorescent cells did not increase significantly during this process or thereafter. This can be most clearly seen in Fig. 3. In some cases the outward spreading tendency of the constituent cells resulted in the formation of toroidal structures with the central hole devoid of cells (Fig. 4). Throughout this period the cells were in constant movement following each other round in endless circles in both the compact and doughnut shaped structures.

Towards the latter part of the period of strong rotation some of the prestalk cells started to associate with each other while still circling round with the cell mass. Then a point was reached when a significant number of prestalk cells quite suddenly stopped circling round the aggregate. This occurred at 360 minutes in the prestalk marked cells shown in Fig. 2, at 260 minutes in the prespore marked cells shown in Fig. 3 and at 160 minutes in Fig. 4. When the arrested group of prestalk cells was centrally located, surrounding prespore cells transiently slowed their rotation but quickly resumed circulating leaving the overall shape of the aggregate minimally perturbed. When the prestalk cells stopped near the edge of the disk, as occurred in the fields shown in Figs 3 and 4, cells in front moved away while cells behind piled up. The consequences were most dramatic in the counterclockwise rotating toroid shown in Fig. 4. The aggregate transformed into a comet shaped structure before forming a compact disk with a central domain of prestalk cells.

Slug formation

Soon after a distinct prestalk domain was formed, it developed its own swirling movement. The direction of net rotation was sometimes the same as that followed before sorting out but was just as often the opposite. In these cases internal prestalk cells were moving unhindered past surrounding prespore cells that were going the other way. Prespore cells initially maintained their rotational direction but could reverse it if necessary to match that of the prestalk cells. The first prespore cells to reverse direction were those closest to the contra-rotating prestalk region. The reversal of direction of the prespore cells then spread outwards until all the prespore cells had adopted the direction of the prestalk cells (Fig. 4).

Observations on a considerable number of flattened

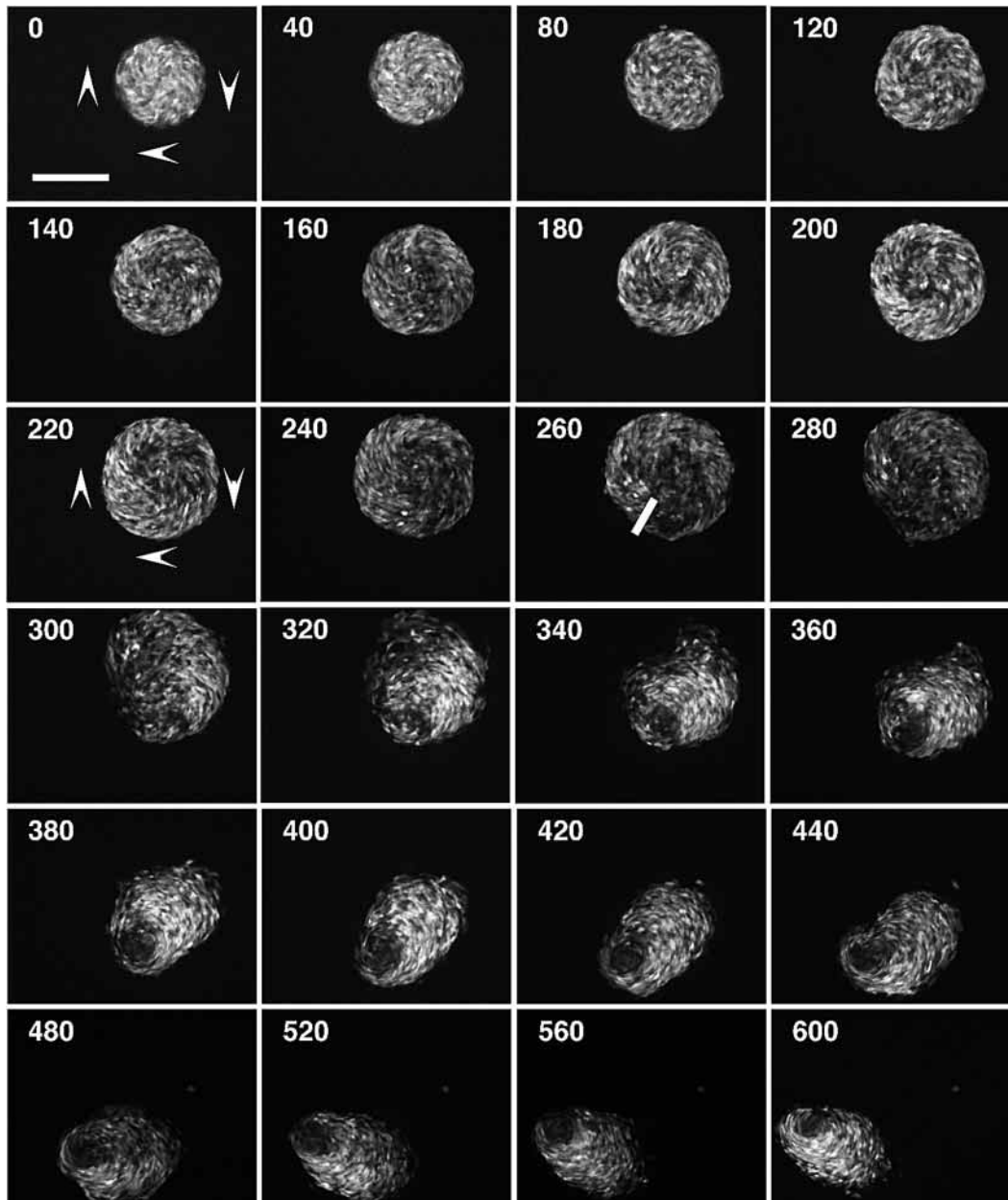


Fig. 3. Sorting out in prespore marked cells. Cells of strain JS24 cells (*cotB::GFP*) were developed under agarose for 11 hours 30 minutes before the start of time lapse microscopy. Frame numbers indicate the subsequent time elapsed in minutes. Arrowheads indicate the direction of movement of the cells. The bar on the 260 minute frame indicates the point where the clockwise rotation of prestalk cells was arrested. Bar, 100 μ m.

aggregates showed that neither the position at which prestalk cells sort out nor the subsequent path of movement of the prestalk domain was determined. However, when the prestalk domain reached an edge, it invariably moved rapidly outward. The prestalk domain then led the posterior prespore cells off in the direction of the bleb.

In a few cases, such as the one shown in Fig. 4, two migrating slugs collided head on. They then rapidly fused much as 3-D slugs have been seen to do (Raper, 1940). The prestalk regions of the colliding structures coalesced into a single domain indicating a ready mutual affinity. The collision generated an initially complex pattern of cell movement

suggesting that files of cells retained their directional momentum for some time.

DISCUSSION

During the first 6 hours of development *Dictyostelium* cells acquire the ability to signal and respond to each other using cAMP as a chemoattractant (Bonner, 1967; Devreotes, 1982). They also become mutually adhesive (Beug et al., 1973; Gerisch, 1980; Loomis, 1988). When certain cells spontaneously release cAMP, surrounding cells respond by

relaying the signal and moving towards the source. As a result they stream together to form aggregates where the cells stick together and subsequently sort out to form discrete prestalk and prestalk domains. While the mechanism of relay of cAMP signals and chemotactic aggregation is fairly well understood (Devreotes, 1982; Reitdorf et al., 1996; Sukumaran et al., 1998), the processes that result in sorting out are still unclear. It has been suggested that prestalk cells sort by preferential chemotaxis to cAMP utilizing the components that function during the aggregation phase (Durstion and Vork, 1979; Siegert and Weijer, 1995; Early et al., 1995). Optical waves have been observed in mounds that have been interpreted as cellular responses to relayed cAMP signals (Siegert and Weijer, 1995; Sukumaran et al., 1998; Kellerman and McNally, 1999).

However, at least one of the components essential for chemotactic aggregation, the adenylyl cyclase encoded by *acaA*, appears to be dispensible for sorting when PKA is over-expressed (Wang and Kuspa, 1997). Since PKA acts in a cell-autonomous manner, it is unlikely that chemotaxis to cAMP is essential for sorting although it may continue to play a role.

By using prestalk and prestalk promoters to drive GFP expression we have been able to follow the movement of the

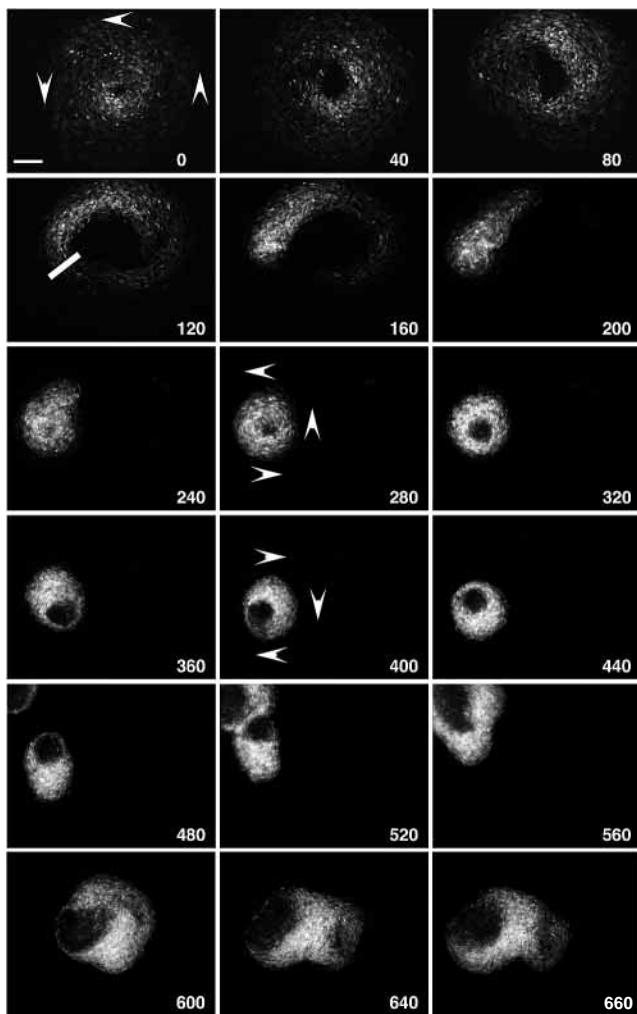


Fig. 4. Sorting out in a toroidal flattened mound. Cells of strain JS24 cells (*cotB::GFP*) were developed under agarose for 12 hours 9 minutes before the start of time lapse microscopy. Frame numbers indicate the subsequent time elapsed in minutes. Arrowheads indicate the direction of movement of the cells. The bar on the 120 minute frame indicates the point where the counterclockwise rotation was arrested. Notice that the outer cells change direction to match the clockwise rotation of the prestalk cells at 400 minutes. At 520 minutes two slugs collided. The field of view was re-centered after the 560 minute frame so as to visualize movement in the coalesced slug. Bar, 100 μ m.

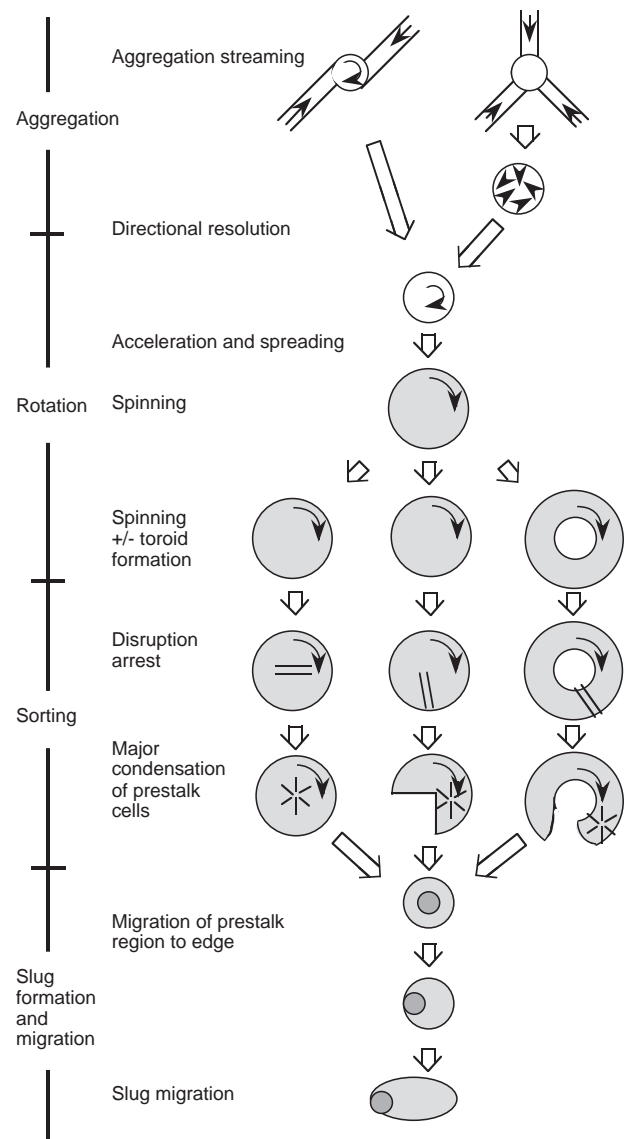


Fig. 5. Patterns of movement during sorting out in flattened mounds. Solid arrows indicate the direction of the coordinated cell movements. Although only clockwise rotation of the cells in flattened mounds is shown, the direction of rotation was equally frequently counter-clockwise. Prestalk cells are represented in the shadowed domain. Once coordinated rotation is established the aggregates spread and may leave a clearing in the center. When a group of prestalk cells slows, prestalk cells that are behind pile up while prestalk cells that are ahead proceed around until they reach the prestalk domain. When the prestalk domain reaches the perimeter, it leads the mass of cells away in much the same way as in three-dimensional slugs.

separate cell types using time lapse fluorescence microscopy. As has been previously observed under standard developmental conditions (Williams et al., 1989; Fosnaugh and Loomis, 1993), prespore and prestalk genes are first expressed in a few cells spatially dispersed in early 2-D aggregates. The pattern of locomotory behavior of the cells in the flattened mound is similar to that observed for the normal mound (Seigert and Weijer, 1995; Sukumaran et al., 1998; Kellerman and McNally, 1999). There is a period of fast rotational movement which suddenly slows immediately prior to sorting out. An unexpected advantage of developing cells under an agarose sheet is that it delays sorting out for several hours during which time cells express either the prespore or the prestalk marker. During this time the cells continue to rotate rapidly and show no indication of being oxygen deprived. Sorting may be delayed as a consequence of mechanical differences in cellular motility or generation of the extracellular matrix imposed by the agar overlay. In any case, it greatly facilitates observations on the relative movements of the cell types as they sort out. Cells expressing the prestalk specific construct are found at the anterior of migrating 2-D slugs while cells expressing the prespore specific construct are found in the posterior just as in normal 3-D slugs. As 2-D slugs migrate they leave an easily observable trail indicative of being enclosed in a surface sheath of extracellular material in a manner similar to normal 3-D slugs (Fig. 1). Thus, while it is possible that certain mechanisms for sorting are amplified in our 2-D preparations, we believe our observations and interpretations are applicable to sorting out in the normal 3-D situation.

The range of our observations on dozens of 2-D aggregates with strains carrying either prespore or prestalk markers are summarized in Fig. 5. Whether cells rotate around in the disk immediately upon entering or go through a directionally confused period appears to depend on the number of entering aggregation streams and the angles at which they enter. Radial streaming usually results in uncoordinated movement for a period of an hour or so. During this period the cells are not moving independently but appear to be moving in twisted files. Uniform rotation results from a swirling unwinding of the convoluted files and is accompanied by flattening of the disk such that the area increases. Files of cells can slip past each other as they move in concentric circles (Rappel et al., 1999). We observed about an equal number of 2-D aggregates that rotated clockwise as those that rotated counterclockwise.

The direction of rotation was usually maintained until the prestalk cells sorted out to form an interior domain. Most of the cells moved at about the same average rate that ranged from 5 to 15 $\mu\text{m}/\text{minute}$ depending on the developmental stage. Cells maintained their positions in the circulating files and appeared to be attached in an end-to-end fashion, following the cell in front in an endless circular path. When some 2-D mounds expanded, a central region clear of cells was left in the center and the cells followed a toroidal path. It is not unusual to see such doughnut shaped aggregates during early development of wild-type strains under normal 3-D conditions.

Quite abruptly a domain enriched in prestalk cells could be seen to stop rotating and remain in a fixed position blocking the movement of cells behind it. The position of this rotational arrest is marked by a bar in Figs 2-5. The process can be more dramatically seen in the video clips available at <http://glamdring.ucsd.edu/others/dsmith/amp2v3.html>. Soon

after prestalk cells have sorted out they once again develop coordinated movements within their domain. The direction of rotation appeared to be independent of that in the surrounding prespore cells.

It has previously been suggested that sorting involves either differential chemotaxis, relative cell movement, adhesion or a combination of these processes (Bonner, 1967; Early et al., 1995; Kellerman and McNally, 1999; Siegert and Weijer, 1995; Siu et al., 1983; Sukumaran et al., 1998). While it is clear that cAMP mediated chemotaxis is essential for controlling the direction of cell movement towards aggregates, evidence for cAMP chemotaxis in sorting out within mounds is more indirect. In the 2-D preparations, cells stream into aggregates in a manner similar to chemotactic streaming seen in 3-D preparations and is likely to be cAMP directed. In both cases, once cells have entered into an aggregate they go through a period of rapid rotational movement. While propagated waves of cAMP could organize such rotation, we have shown that cohesive energy between the cells and self-generated propulsive force is sufficient to account for such a vortex state (Rappel et al., 1999). Moreover, we have directly shown that cells lacking the aggregation stage adenylyl cyclase, ACA, take on rotational movement when they form aggregates if they are genetically engineered to over-express PKA (Rappel et al., 1999). Since the levels of cAMP are below detection in such cells (Wang and Kuspa, 1997), it is unlikely that they are responding to external cAMP. While it is possible that chemotaxis to some other relayed signal could account for rotational movement, such a system has not been defined. For the first few hours of rotational movement both prespore and prestalk cells circulate at the same rate as surrounding cells and show no evidence of differential cell motility (Figs 2-4). A group of prestalk cells then abruptly slows down, allowing prestalk cells behind them to pile up. Prestalk cells ahead of them proceed around the vortex until they join the prestalk domain. Such behavior is inconsistent with a chemotactic mechanism to a signal released by the prestalk cells that slow down but could result from changes in differential adhesion.

If, as a part of their program of differentiation, prestalk cells acquired surface properties that made them mutually adhesive, it could provide a mechanism to account for the patterns of cell movement as prespore and prestalk cells sort out. When they enter an aggregate, all cells appear to be adhesive at their ends where they contact the cells ahead and behind them in their file (Rappel et al., 1999). This end-to-end adhesion can account for the unwinding of the convoluted files in directionally confused aggregates as well as the maintenance of nearest neighbor relationships as the cells rotate around in flattened mounds. If prestalk, but not prespore cells, express new homotypic adhesion molecules evenly distributed over the cell surface, the resulting overall increase in lateral cell adhesion would pull them out of their files when they encountered other prestalk cells. Prespore cells would be able to reestablish contact with other cells and continue to rotate almost uninterrupted. As the prestalk cells continue to leave their coordinating files they would cease rotating and establish a prestalk specific domain. Our observation that the area of the 2-D mound shrinks following sorting out is consistent with the establishment of a new adhesion mechanism that holds the cells more tightly (Figs 2-5). The cell type specificity of this adhesion can be inferred from the apparently low drag of counter-rotating masses of

prespore and prestalk cells where the boundaries pass by each other in opposite directions.

Since prestalk cells make up less than 20% of the total number of cells in mounds, a prestalk specific adhesion molecule could easily have escaped detection in previous experiments aimed at understanding the molecular basis of cell-cell adhesion in *Dictyostelium* (Gerisch, 1980; Loomis, 1988). Moreover, differential adhesion could result from prestalk cells acquiring a common adhesion system earlier than prespore cells. The cell adhesion molecule gp150 is a candidate for the sorting mechanism since it has been shown that antibodies to gp150 are able to block sorting of prestalk and prespore cells (Siu et al., 1983).

We are proposing that sorting out results from differential adhesion and the consequent disruption of cell files where end-to-end adhesion predominates. Similar suggestions have been raised to account for sorting in a variety of contexts (Steinberg, 1970). Lateral adhesion would initially lead to clumping of prestalk cells and might subsequently transform into end-to-end adhesion among prestalk cells as the result of capping. Although this mechanism would reduce adhesion between prespore and prestalk cells, it need not abolish it. Moreover, the extracellular matrix that forms when prestalk cells reach the edge of a mound could help to direct movement of the mass of prespore cells such that they follow along in the posterior.

We thank Justin Stege for the GFP expressing strains. This work was supported by the National Science Foundation (grant nos 9728463 and 9512809).

REFERENCES

- Beug, H., Katz, F. E. and Gerisch, G. (1973). Dynamics in antigenic membrane sites relating to cell aggregation in *Dictyostelium discoideum*. *J. Cell Biol.* **56**, 647-658.
- Bonner, J. T. (1967). *The Cellular Slime Molds*. Second edition. Princeton Univ. Press, Princeton, NJ.
- Bonner, J. T. (1998). A way of following individual cells in the migrating slugs of *Dictyostelium discoideum*. *Proc. Nat. Acad. Sci. USA* **95**, 9355-9359.
- Devreotes, P. (1982). Chemotaxis. In *Development of Dictyostelium* (ed. W. F. Loomis), pp. 117-168. Acad. Press, San Diego, CA.
- Doolittle, K. W., Reddy, I. and McNally, J. G. (1995). 3D analysis of cell movement during normal and myosin-II-null cell morphogenesis in *Dictyostelium*. *Dev. Biol.* **167**, 118-129.
- Durston, A. J. and Vork, F. (1979). A cinematographical study of the development of vitally stained *Dictyostelium discoideum*. *J. Cell Sci.* **36**, 261-279.
- Early, A., Abe, T. and Williams, J. (1995). Evidence for positional differentiation of prestalk cells and for a morphogenetic gradient in *Dictyostelium*. *Cell* **83**, 91-99.
- Fosnaugh, K. L. and Loomis, W. F. (1993). Enhancer regions responsible for temporal and cell-type-specific expression of a spore coat gene in *Dictyostelium*. *Dev. Biol.* **157**, 38-48.
- Fukui, Y., Yumura, S., Yumura, T. K. and Mori, H. (1986). Agar overlay method: High-resolution immunofluorescence for the study of the contractile apparatus. *Meth. Enzymol.* **134**, 573-580.
- Gerisch, G. (1980). Univalent antibody fragments as tools for the analysis of cell interactions in *Dictyostelium*. In *Current Topics in Developmental Biology* (ed. M. Friedlander), pp. 243-269. Academic Press, New York.
- Kellerman, K. A. and McNally, J. G. (1999). Mound cell movement and morphogenesis in *Dictyostelium*. *Dev. Biol.* **208**, 416-429.
- Loomis, W. F. (1982). *The development of Dictyostelium discoideum*. Academic Press, New York.
- Loomis, W. F. (1988). Cell-cell adhesion in *Dictyostelium discoideum*. *Dev. Genet.* **9**, 549-559.
- Muller, K. and Gerisch, G. (1978). A specific glycoprotein as the target site of adhesion blocking Fab in aggregating *Dictyostelium* cells. *Nature* **274**, 445-449.
- Nellen, W., Silan, C. and Firtel, R. A. (1984). DNA-mediated transformation in *Dictyostelium discoideum*: regulated expression of an actin gene fusion. *Mol. Cell. Biol.* **4**, 2890-2898.
- Raper, K. B. (1940). Pseudoplasmodium formation and organization in *Dictyostelium discoideum*. *J. Elisha Mitchell Sci. Soc.* **56**, 241-282.
- Rappel, W.-J., Nicol, A., Sarkissian, A., Levine, H. and Loomis, W. F. (1999). Self-organized vortex state in two-dimensional *Dictyostelium* dynamics. *Phys. Rev. Lett.* **83**, 1247-1250.
- Rietdorf, J., Siegert, F. and Weijer, C. J. (1996). Analysis of optical density wave propagation and cell movement during mound formation in *Dictyostelium discoideum*. *Dev. Biol.* **177**, 427-438.
- Siegert, F. and Weijer, C. J. (1995). Spiral and concentric waves organize multicellular *Dictyostelium* mounds. *Curr. Biol.* **5**, 937-943.
- Siu, C. H., Des Roches, B. and Lam, T. Y. (1983). Involvement of a cell-surface glycoprotein in the cell-sorting process of *Dictyostelium discoideum*. *Proc. Nat. Acad. Sci. USA* **80**, 6596-6600.
- Stege, J. (1998). The role of *tip* genes in parallel pathways of *Dictyostelium* cell sorting and tip formation. PhD thesis, University of California San Diego, CA.
- Steinberg, M. S. (1970). Does differential adhesion govern self-assembly processes in histogenesis? Equilibrium configurations and the emergence of a hierarchy among populations of embryonic cells. *J. Exp. Zool.* **173**, 395-433.
- Sukumaran, S., Brown, J. M., Firtel, R. A. and McNally, J. G. (1998). lagC-null and gbf-null cells define key steps in the morphogenesis of *Dictyostelium* mounds. *Dev. Biol.* **200**, 16-26.
- Sussman, M. (1987). Cultivation and synchronous morphogenesis of *Dictyostelium* under controlled experimental conditions. *Meth. Cell Biol.* **28**, 9-29.
- Wang, B. and Kuspa, A. (1997). *Dictyostelium* development in the absence of cAMP. *Science* **277**, 251-254.
- Williams, J. G., Duffy, K. T., Lane, D. P., McRobbie, S. J., Harwood, A. J., Traynor, D., Kay, R. R. and Jermyn, K. A. (1989). Origins of the prestalk-prespore pattern in *Dictyostelium* development. *Cell* **59**, 1157-1163.