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Streams of *Dictyostelium*

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When a small number of fluorescently labeled myosin II mutant cells (*mhcA*$^-$) are mixed with wild-type cells and development of the chimeras is observed by confocal microscopy, the mutant cells are localized to the edges of aggregation streams and mounds. Moreover, the mutant cells stick to wild-type cells and become distorted (Shelden and Knecht, 1995). Two independent adhesion mechanisms, Contact Sites A and Contact Sites B, function during the aggregation stage and either one or both might be responsible for excluding the myosin II null cells. We have mixed *mhcA*$^-$ cells with cells in which the appearance of Contact Sites B is delayed (strain TL72) as well as cells which lack Contact Sites A (strain GT10) and double mutants in which both adhesion mechanisms are affected (strain TL73). In all chimeras, the *mhcA*$^-$ cells were distorted by interactions with the adhesion mutant cells, indicating that it does not require significant adhesive interaction to distort the flaccid cortex of *mhcA*$^-$ cells. *mhcA*$^-$ cells were excluded from streams composed of cells lacking either Contact Sites A or Contact Sites B but mixed randomly with cells lacking both adhesion systems. By 10 hr of development, cells of strain TL73 acquire Contact Sites B adhesion. If cells of this strain were mixed with labeled *mhcA*$^-$ cells, allowed to develop for 9 hr, and then dissociated before replating, the myosin II null cells were seen to be distorted and excluded from the reaggregates. Thus the exclusion of *mhcA*$^-$ cells from streams can be accomplished by either Contact Sites A or B. When chimeras of labeled TL73 and wild-type cells were made, the TL73 cells were found to be randomly mixed into aggregation streams. This result indicates that adhesive sorting does not function during aggregation and so cannot account for the exclusion of *mhcA*$^-$ cells from streams. We hypothesize that the flaccid cortex of *mhcA*$^-$ cells cannot generate sufficient protrusive force to break the contacts between adhered cells in aggregation streams but can enter streams where the cells are weakly adherent.

INTRODUCTION

Myosin is an actin-based molecular motor whose biochemical properties are well understood in both muscle and nonmuscle systems (Rayment and Holden, 1994; Taylor, 1979). However, the precise role played by myosin in cortical architecture and motility in nonmuscle cells is still unclear (for review see Spudich, 1989). In *Dictyostelium* strains that lack functional myosin II (*mhcA*$^-$) cytokinesis is completely blocked in suspension while *mhcA*$^-$ cells on a surface sometimes complete division and form a mixed population of giant multinucleated syncytia and normal sized cells (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987). In addition, *mhcA*$^-$ cells are rounder than wild-type cells, extend small pseudopodia, move slowly, and are less efficient than wild-type cells in responding chemotactically to cAMP (Wessels *et al.*, 1988). Due to these defects, *mhcA*$^-$ cells aggregate slowly when starved but eventually form an apparently normal mound. However, morphogenesis is arrested prior to the formation of an apical tip (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987).

Recently, we have shown that *mhcA*$^-$ cells are localized to the edges of aggregation streams when they were developed together with wild-type cells (Knecht and Shelden, 1995). In addition, the *mhcA*$^-$ cells were stretched and distorted by their interactions with wild-type cells. The distortion of *mhcA*$^-$ cells was apparently due to the formation of adhesive interactions and tension between the cells which the weakened cortex of the mutant cells could not resist

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(Shelden and Knecht, 1995). Both of these phenotypes were unexpected and the present work explores the causes of these defects.

Dictyostelium cells that are growing exponentially on a lawn of bacteria do not exhibit cell–cell adhesion. However, a few hours after the initiation of development the cells become sticky and can form clumps when shaken in suspension. The adhesion responsible for this clumping can be blocked by the addition of 10 m*M* EDTA. This early EDTA-sensitive adhesion mechanism has been referred to as Contact Sites B to distinguish it from the later EDTA-resistant adhesion mechanism, Contact Sites A, which is mediated by the surface glycoprotein, gp80 (Gerisch, 1968; Muller and Gerisch, 1978; Noegel *et al.*, 1986). EDTA-sensitive adhesion is mediated by a distinct surface protein, gp24, which is synthesized shortly after the initiation of development and is retained on the surface of the cells throughout the remainder of the developmental cycle (Knecht *et al.*, 1987; Brar and Siu, 1993). Antibodies specific to gp24 can block adhesion during the first 8 hr of development, but thereafter, Contact Sites A can independently mediate cell–cell adhesion. Later in development, a third adhesion system comes into play, the molecular nature of which is still unclear (Loomis *et al.*, 1988).

In order to address the question of which adhesion system is responsible for affecting *mhcA*$^-$ cell behavior in wild-type streams, the *mhcA*$^-$ cells were developed in chimerical streams with mutant strains lacking specific adhesion systems. The results show that either Contact Sites A or Contact Sites B adhesion is sufficient to exclude *mhcA*$^-$ cells from streams, but that in the absence of both adhesion systems, *mhcA*$^-$ cells are randomly distributed throughout the streams. Therefore, either adhesion system is sufficient to exclude *mhcA*$^-$ cells from streams. In addition, *mhcA*$^-$ cells were distorted by their interactions with all of the adhesion mutant cell lines. Thus even the residual adhesion present in the double mutant cell line is sufficient to allow cells to apply distorting force to each other. Surprisingly, *mhcA*$^+$ cells lacking both adhesion systems are not mislocalized when chimerical streams are formed with wild-type cells. This result argues that *mhcA*$^-$ cells are not sorted out of wild-type streams because they are less adhesive; rather we hypothesize that the flaccid cortex of the *mhcA*$^-$ cells is unable to generate sufficient protrusive force to penetrate between the adhered wild-type cells.

MATERIALS AND METHODS

Cell Lines

Dictyostelium strain HK321 is a myosin II heavy chain null mutant (Knecht and Shelden, 1995). Strain GT10 carries a deletion of *csaA* which encodes gp80 and was the kind gift of Drs. A. Noegel and G. Gerisch. TL72 was constructed by gene replacement in *ura*$^-$ strain HL330 (Kuspa and Loomis, 1992). A transformation vector was constructed for the replacement of all three linked *csb* genes by the *pyr5-6* gene following selection for uracil independence. A 2.3-kb fragment from the region upstream of the *csb* cluster was positioned on one side of the *pyr5-6* gene while a 170-bp fragment from the carboxy-terminal portion of the *csbC* gene was positioned on the other side (Fig. 1). The three *csb* genes were shown to be deleted in strain TL72 by Southern analyses of restriction enzyme digests of the genome (data not shown). The wild-type cell line in all cases is AK156, a control transformed HL330 derivative in which the *csb* knockout vector did not target the *csb* locus. TL73 is a derivative of strain TL72 in which the *csaA* gene was disrupted. A *ura*$^-$ derivative of strain TL72 was transformed with a construct in which the *pyr5-6* gene was inserted into the *csaA* gene (Noegel *et al.*, 1986). Southern analysis of uracil-independent transformants showed that the *csaA* gene was disrupted in strain TL73 while Northern analyses of this strain showed that the 1.8-kb mRNA encoded by *csaA* failed to accumulate during aggregation (data not shown). All strains were grown either on plastic petri dishes in HL5 medium or on lawns of *Klebsiella aerogenes* on SM agar plates (Sussman, 1987).

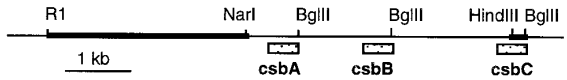

FIG. 1. The csb cluster of genes. The highlighted portions of the gene were used to construct the gene knockout vector. Bar, 1 kb.

Cell Labeling

Fluorescent labeling of *Dictyostelium* cells was performed as described by Knecht and Shelden (1995) with minor modifications. Cells (2×10^6) were harvested from HL-5 medium, washed once with cold 20 m*M* KPO_4 buffer (pH 6.5), and resuspended in 200 μl of buffer containing 50 m*M* 5-chloromethyl-fluorescein diacetate (CMF) (Molecular Probes, Inc). After staining for 30 min at room temperature, the cells were pelleted, washed twice with KPO_4, and resuspended in a final volume of 100 μl. Unlabeled cells were harvested from HL-5 medium and washed twice with MCPB buffer (10 m*M* Na_2HPO_4, 10 m*M* KH_2PO_4, 2 m*M* $MgCl_2$, 0.2 m*M* $CaCl_2$, 0.5 mg/ml dihydrostreptomycin, pH 6.5). In some experiments, unlabeled cells were harvested from lawns of *K. aerogenes* on SM agar plates and washed five times with MCPB buffer. The unlabeled cells were resuspended to a final density of 8×10^6 cell/ml. For most experiments, 1.6×10^7 unlabeled cells were mixed with 2–3% labeled cells. Observation chambers were made by drilling a 25-mm hole in a 60-mm glass petri dish and gluing in a 30-mm round cover slip. A 2-ml layer of 1% Noble agar melted in MCPB buffer was added to the dish and then 2 ml of cells in MCPB buffer were pipetted onto the agar surface. After the cells attached, excess buffer was removed and the plates were incubated in a humid chamber at 23°C until aggregation began. The chamber was then placed in a stage temperature controller (20/20 Technologies, Inc.) set to 23°C, mounted on the stage of a Zeiss Axiovert microscope. For disaggregation experiments, cells were washed from the agar plates during streaming using 6 ml of MCPB buffer. The aggregates were dissociated by vortexing, centrifuged, and then resuspended into a final volume of 200 μl. The cells were then quickly dispersed dropwise onto the agar surface. After the cells attached, excess buffer was removed.

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csbA    MVDLKITLVNEDGESTISGKGHPLPAPLIFPPIYIFRFTQYQTEGKLWDKNEFQIK
csbB    MTDLKITLVNEDGESTISGKGHPLPAPLIFPPIYCFCFIQYKTEGKLWDKNDFQIK
csbC    MAELKITLINEDGESTISGKAHPAPTPRILPTPYFMSFTEYKIEGKLWDKKEFHIK
csbψ    MAELKITLINEDGESTISGKASQIPAPPLFHHHILCILPNTKLMVSLGTKMNFK*

csbA    SGKIEFDGEEYDIPESKGTWSKDDEENAIDVNLHLFRPPEKFFPKN
csbB    SGKIEFGGEEYDITESKGTWSKDDEENHIKVSLHLIVPPKKIFQKNF
csbC    SGKIEFNGKEFDIPESQGTWIKDNVEIIIRIFLSQQANKPFSLDF
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FIG. 2. Comparison of the products of csbA, B, C, ψ. The protein sequences encoded by the *csbA*, *csbB*, and *csbC* genes are >80% identical to each other. The pseudogene, *csbψ*, is truncated.

Image Collection

Cells were imaged using a Bio-Rad MRC-600 confocal laser scanning microscope (CLSM) equipped with a 25-mW krypton–argon laser operated on its low power setting. Fluorescence and phase-contrast images were collected with a 10× 0.30 NA or 20× 0.50 NA Zeiss Plan neofluar phase-contrast objective. The brightness and contrast of images were adjusted and in some cases the data were smoothed. The fluorescence data were then subtracted from the phase-contrast data and then the two images were merged and pseudocolored using locally written software. In some experiments, in order to assess the dynamic behavior of *mhcA*$^-$ cells, images were collected at 30-sec intervals using the slowest direct scan rate, and recorded in time-lapse mode using a Panasonic $\frac{1}{2}$-in. S-VHS video recorder. Videotapes were played back in real time to assess cell behavior.

RESULTS

Construction of the csb Genes Mutant Cell Lines

In order to study the behavior of myosin mutant cells developing with cells lacking one or both of the early adhesion systems, two new mutant strains, TL72 and TL73, were constructed (see Materials and Methods). The *csbA*, *csbB*, and *csbC* genes, which may play auxiliary roles in the establishment of Contact Sites B, were deleted in strain TL72 (Fig. 1). These three genes are linked within 4 kb and encode protein products that are >80% identical to each other (Fig. 2). A more distant member of this family is a pseudogene in which a deletion results in premature termination (Fig. 2). Cells of strain TL72 aggregate normally, forming loose aggregates by 8 hr and mounds by 10 hr of development. In cell adhesion assays, clumps first started to form at 4 hr of development and the adhesion was found to be EDTA resistant as soon as it could be observed (Fig. 3). Strain TL73 is a derivative of TL72 in which the *csaA* gene which encodes gp80 was disrupted and so the cells cannot express Contact Sites A. Cells of this strain showed no evidence of mutual adhesion during the first 8 hr of development (Fig. 3). Cell–cell adhesion first appeared between 8 and 10 hr of development and was sensitive to EDTA as well as to antibody which is specific to gp24 (R851). At 12 hr of development, cell–cell adhesion showed partial resistance to both EDTA and anti-gp24 antibody, indicating that the late adhesion mechanism had come into play at this time. In our shaking suspension assay, it is difficult to measure EDTA-sensitive adhesion in the presence of EDTA-resistant adhesion. In TL72, the presence of EDTA resistant adhesion at 4 hr of development meant that the presence of EDTA sensitive adhesion could not be measured. Thus it is possible that EDTA-sensitive adhesion could be present in these cells. However, the fact that TL73 cells show no measurable EDTA-sensitive adhesion before 8 hr of development indicates that this is not the case.

Cells of strain TL73 form loose aggregates by 8 hr of development and mounds by 10 hr of development. The fact that cells of this strain were able to form loose aggregates before showing any indication of clumping in the shaking suspension adhesion assay indicates that the initial stages of aggregation are independent of cell–cell adhesion that can be measured by this assay. TL73 mounds form normal-looking slugs at the normal time; however, culmination was aberrant in both strain TL72 and strain TL73. Reduced numbers of fruiting bodies were formed and those that did form had short thick stalks (data not shown and Fig. 4). Thus, the *csb* genes appear to play an essential role in culmination.

The fact that EDTA-sensitive adhesion could be observed at 10 hr of development in cells lacking the *csb* and *csaA* genes and that this adhesion was sensitive to anti-gp24 antibodies shows that cell–cell adhesion was mediated by gp24 at this stage. Western analyses of extracts taken at various times of development and stained with R851 antibody specific to gp24 showed that gp24 protein began to accumulate in wild-type and mutant cells by 6 hr of development (data not shown). Thus the *csb* genes do not appear to encode gp24, but the appear-

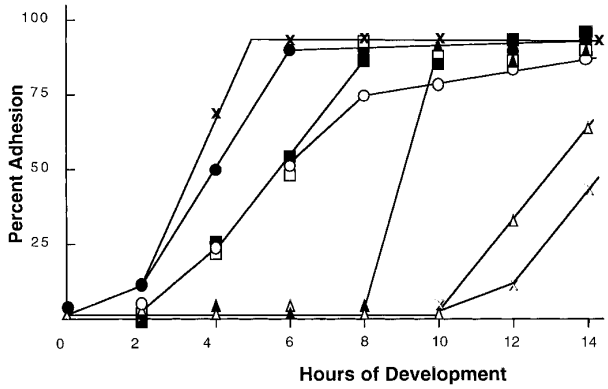


FIG. 3. Cell–cell adhesion in wild-type and mutant cell lines. Cells of the wild-type strain AX4 (○–○), strain GT10 (×–×), TL72 (□–□), and TL73 (△–△) growing exponentially on a bacterial food source, were collected, washed, and deposited for development on filters. At the indicated times, samples were assayed for cell–cell adhesion in the presence of 10 m*M* EDTA (open symbols) or in the absence of EDTA (closed symbols).

TABLE 1
Comparison of the Percentage of Labeled Cells Found at the Edge of Aggregation Streams

	WT in WT	*mhc A*$^-$ in WT	*mhc A*$^-$ in AK156	*mhc A*$^-$ in GT10	*mhc A*$^-$ in TL72	*mhc A*$^-$ in TL73	*mhc A*$^-$ RA in TL73	TL73 in AK156
Edge/total	21.6% *N* = 127	81.7% *N* = 97	84.0% *N* = 61	73.9% *N* = 138	83.0% *N* = 62	21.0% *N* = 240	83.4% *N* = 65	20.0% *N* = 88

Note. Cells were counted as being at the edge of the stream if all or part of a labeled cell was at the edge. Several sets of images were counted and totaled together for each strain combination listed above. RA, reaggregation experiment; WT, wild-type cell; N, number of labeled cells counted.

ance of Contact Sites B is delayed in mutants that lack these genes (see Discussion).

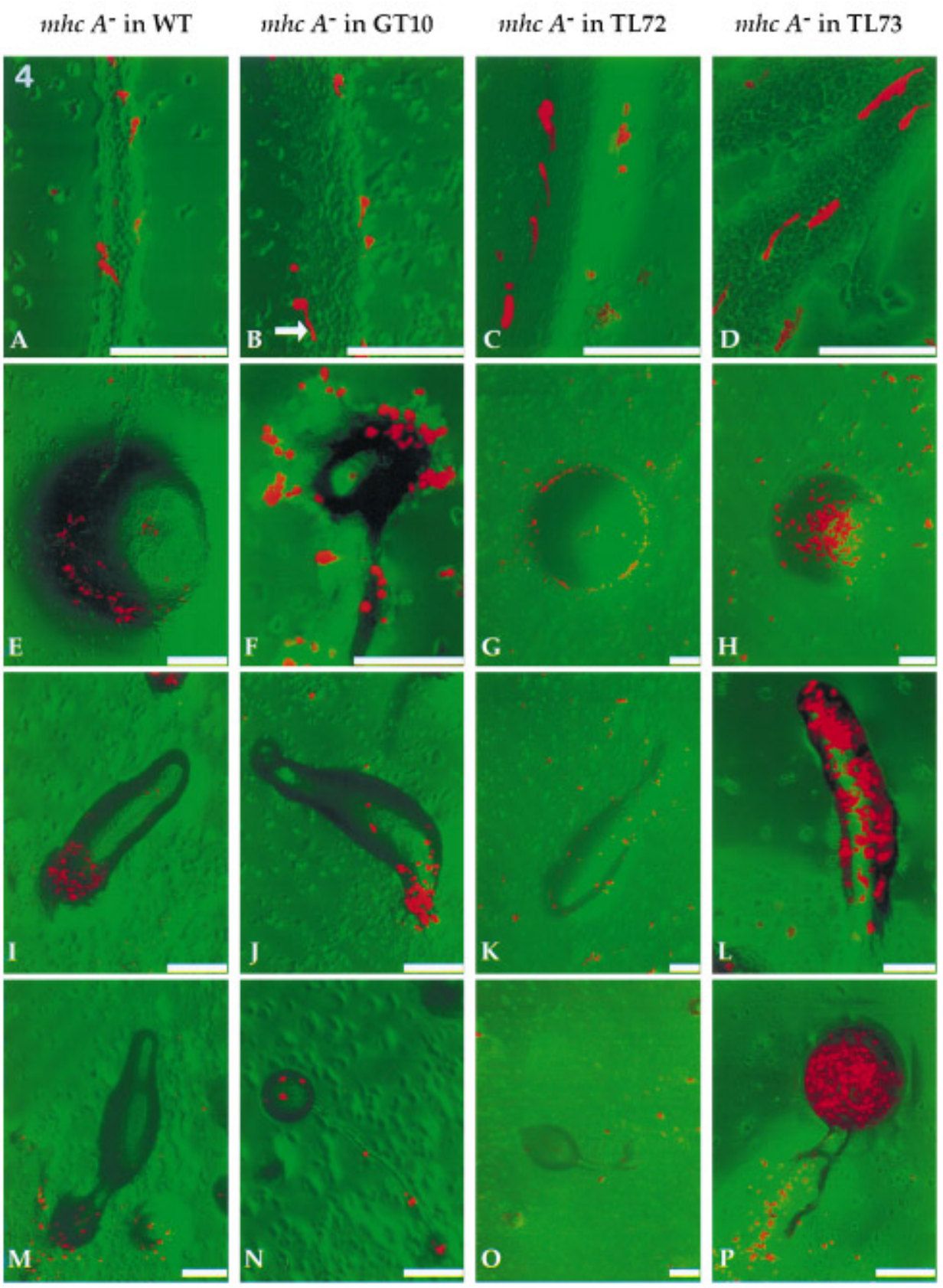
Development of mhcA$^-$ Cells Together with Contact Site A Mutants

In order to determine whether the adhesion molecule gp80 is responsible for the sorting of *mhcA*$^-$ cells to the outside of aggregation streams, *mhcA*$^-$ cells were fluorescently labeled with CMF and mixed with a 50-fold excess of cells of the Contact Sites A null mutant strain, GT10. After 9 hr of development the *mhcA*$^-$ cells were found at the edges of the GT10 streams just as they were when mixed with wild-type cells (Figs. 4A and 4B). The percentage of labeled *mhcA*$^-$ cells found on the edge of the streams is very similar to *mhcA*$^-$ cells in wild-type streams (Table 1). In addition, cells lacking myosin II became distorted just as they were by wild-type cells (arrow in Fig. 4B). The *mhcA*$^-$ cells stayed on the edges of the mounds and ended up in the back of slugs (Figs. 4F and 4J). Only a few *mhcA*$^-$ cells were found in the sori (Fig. 4N). Many *mhcA*$^-$ mutant cells never became part of streams or were left behind on the agar surface as the streams entered the aggregate and slugs crawled away from the site of aggregation (data not shown). The similarity in the response of *mhcA*$^-$ cells to wild-type cells and cells lacking gp80 shows that Contact Sites A are not essential for the sorting behavior.

Development of mhcA$^-$ Cells with Contact Sites B Mutants

Strain TL72 shows adhesion by 4 hr of development and all of this adhesion is EDTA resistant (Fig. 3). When *mhcA*$^-$ cells were mixed with a 50-fold excess of cells of strain TL72, *mhcA*$^-$ cells were also excluded from the streams and aggregates (Table 1; Figs. 4C and 4G). As development progressed, most *mhcA*$^-$ cells were left behind and very few labeled cells were visible in slugs or sori (Figs. 4K and 4O). It appears that Contact Sites B is also not essential for mislocalization. However, if *mhcA*$^-$ cells were mixed with excess cells of strain TL73, which lacks gp80 and only expresses Contact Sites B adhesion after the formation of loose aggregates, *mhcA*$^-$ cells were found to mix randomly in the aggregation streams (Fig. 4D). The percentage of labeled *mhcA*$^-$ cells found on the edge of the streams is very similar to wild-type cells in wild-type streams (Table 1). The labeled *mhcA*$^-$ cells were also found randomly distributed in the multicellular structures throughout the remainder of development (Figs. 4H, 4L, and 4P). This result indicates that either of the two early adhesion systems is sufficient to exclude *mhcA*$^-$ cells from the aggregation streams; however, when both systems are removed, *mhcA*$^-$ cells are able to enter streams. In all cases, distortion of the *mhcA*$^-$ cells by adhesion mutant cells was evident (Figs. 4A–4D). Presumably, there is some residual adhesion that is holding the streams together and is sufficient to distort the *mhcA*$^-$

FIG. 4. Distribution of labeled myosin mutant cells in wild-type and adhesion mutant multicellular structures. Two percent of myosin mutant cells were labeled with CMF, mixed with unlabeled wild-type cells, and plated for development. In most cases, fluorescence images and phase-contrast images were captured simultaneously in a single focal plane which has the brightest fluorescence signal with a confocal microscope. The images were merged and pseudocolored to show the location of fluorescent *mhcA*$^-$ cells (red) within the streams (green). In panels M, O, and P the fluorescence data were projected from multiple optical sections and then merged with phase-contrast data. (A) Labeled *mhcA*$^-$ cells in a wild-type stream. Most of the labeled cells stay at the outside edges of the streams and are distorted. (B) Labeled *mhcA*$^-$ cells in GT10 streams. The arrow shows the position and distortion of *mhcA*$^-$ cells in GT10 streams. (C) Labeled *mhcA*$^-$ cells in TL72 streams. (D) Labeled *mhcA*$^-$ cells in TL73 streams. The *mhcA*$^-$ cells are found throughout the TL73 streams. (E) Labeled *mhcA*$^-$ cells in a wild-type aggregate. (F) Labeled *mhcA*$^-$ cells in a GT10 aggregate. (G) Labeled *mhcA*$^-$ cells in a TL72 aggregate. (H) Labeled *mhcA*$^-$ cells in a TL73 aggregate. (I) Labeled *mhcA*$^-$ cells in a wild-type slug. (J) Labeled *mhcA*$^-$ cells in a GT10 slug. (K) Labeled *mhcA*$^-$ cells in a TL72 slug. (L) Labeled *mhcA*$^-$ cells in a TL73 slug. (M) Labeled *mhcA*$^-$ cells in a wild-type sorus. (N) Labeled *mhcA*$^-$ cells in a GT10 sorus. (O) Labeled *mhcA*$^-$ cells in a TL72 sorus. (P) Labeled *mhcA*$^-$ cells in a TL73 sorus. Bar, 100 μm.


mhc A⁻ in WT
mhc A⁻ in GT10
mhc A⁻ in TL72
mhc A⁻ in TL73
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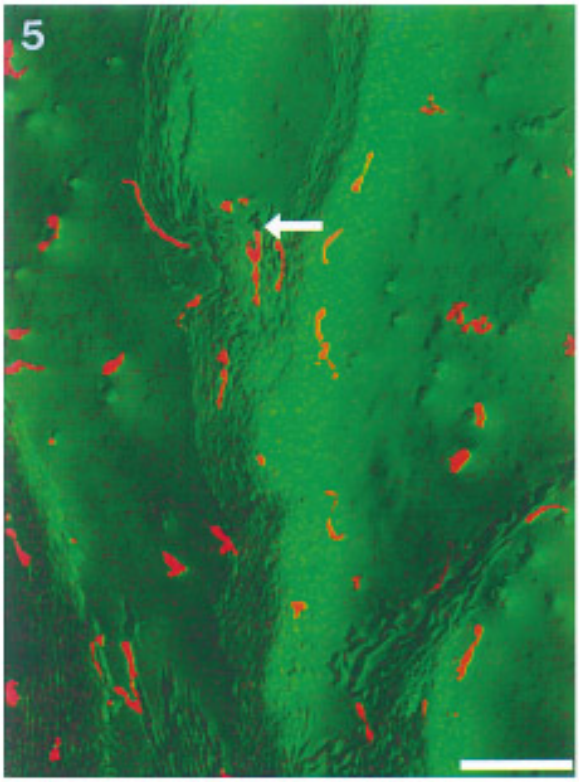

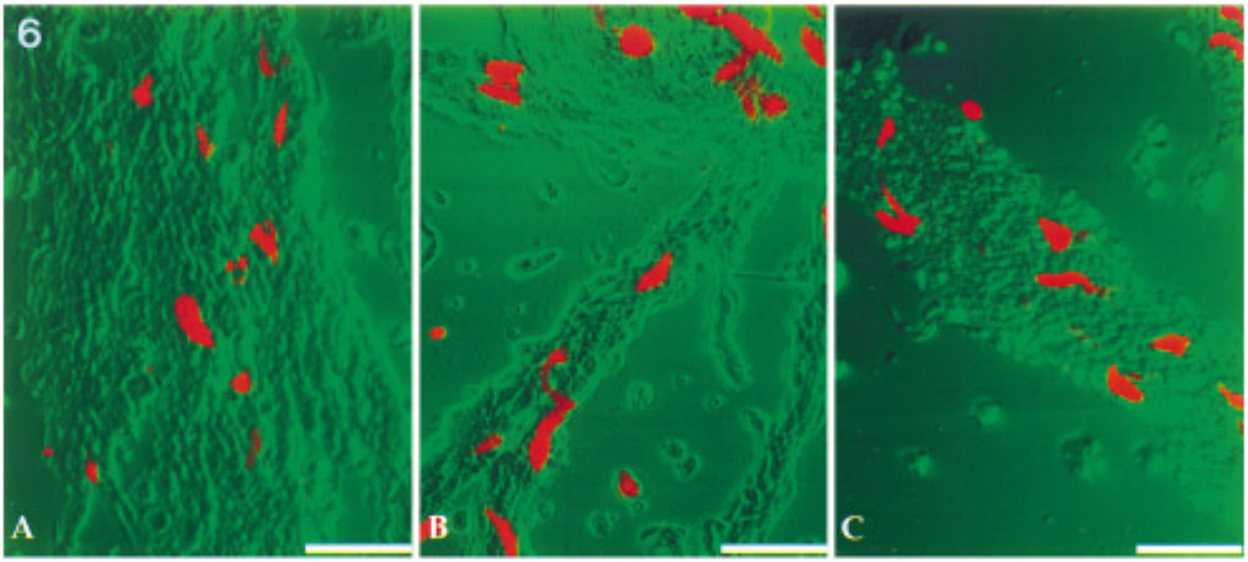

FIG. 5. Distribution of labeled myosin mutant cells in adhesion mutant streams during reaggregation. CMF labeled *mhcA*$^-$ cells were developed with TL73 cells for 8 hr and then disaggregated and allowed to reaggregate after gp24 expression was induced. The *mhcA*$^-$ cells are now found at the edges of the streams. The arrow shows the junction of two streams where *mhcA*$^-$ cells were seen in the center of the streams. Bar, 100 μm.

FIG. 6. The distribution of labeled adhesion mutant cells in wild-type streams. (A) Labeled GT10 cells in AK156 streams. (B) Labeled TL73 cells in AK156 streams. (C) Labeled TL73 cells in AK156 streams. The labeled adhesion mutant cells were randomly distributed in the wild-type streams. Bar, 50 μm.

cells but cannot be measured by the shaking suspension assay.

Since Contact Sites B adhesion can be observed after 8 hr of development in the strain TL73, it was possible to correlate the appearance of this adhesion system with the sorting effect on *mhcA*$^-$ cells. *mhcA*$^-$ cells were mixed with an excess of cells of strain TL73 and allowed to develop for 9 hr. At this time the *mhcA*$^-$ cells could be seen to be randomly distributed throughout the streams. Then, the streams were disaggregated into single cells and replated on an agar surface to recapitulate aggregation (see Materials and Methods). Within a few hours, streams reformed, and this time *mhcA*$^-$ cells could be seen mostly at the edges of the streams (Fig. 5 and Table 1). Some of the *mhcA*$^-$ cells can be seen in the center of the larger stream. Viewing time-lapse movies of aggregation confirms that these cells came from the inside edges of the two smaller streams, which necessarily places them at the center of the larger stream after joining. Many *mhcA*$^-$ cells were left on the agar surface and so few are found incorporated into slugs or fruiting bodies formed from the reaggregated cells (data not shown). Therefore, there is a clear correlation between the time of appearance of Contact Sites B and the exclusion of *mhcA*$^-$ cells from aggregation streams.

Previous results showed that only a small proportion of *mhcA*$^-$ cells differentiate into spores when developed together with wild-type cells (Knecht and Loomis, 1988). This result can now be attributed primarily to the fact that the *mhcA*$^-$ cells never end up in the sorus due to their exclusion from streams and aggregates (Knecht and Shelden, 1995). When developed in chimeras with TL73, the *mhcA*$^-$ cells might be able to form normal numbers of spores. To investigate this question, *mhcA*$^-$ cells were mixed with TL73 and allowed to form fruiting bodies. Either sori or entire plates of developed cells were collected and treated with 1% Triton-100 in phosphate buffer to lyse amoebae without killing spores. Cells were then plated in HL-5 medium to assess the number of survivors. Samples taken from sori of strains TL72 and TL73 alone or chimeras of these strains with *mhcA*$^-$ cells contained no viable spores. Therefore, the *csbB* mutant cell lines are unable to form spores, and *mhcA*$^-$ cells cannot autonomously form spores in this environment.

Adhesive Sorting Does Not Occur in Aggregation Streams

To test whether differential adhesiveness plays a role in the distribution of cells in chimerical aggregation streams, CMF-labeled cells of the nonadhesive strain TL73 were developed in chimerical streams with wild-type cells. The TL73 cells were found randomly distributed in the streams and aggregates (Fig. 6C). Similar results were found when TL72 and GT10 were mixed with excess wild-type cells (Figs. 6A and 6B). The percentage of labeled TL73 cells found on the edge of wild-type streams is very similar to that of wild-type cells in wild-type streams. These results show that differential adhesion is not sufficient to account for the exclusion of the *mhcA*$^-$ cells from streams. We hypothesize that in order to enter streams, cells must be able to move between adhered cells and in so doing, break the adhesions between cells. The *mhcA*$^-$ cells may have such an elastic or flaccid actin cortex that they cannot generate sufficient force to break the contacts holding the wild-type cells together.

DISCUSSION

Previous analysis of myosin mutant cell behavior during development has demonstrated that myosin II is required for normal development of *Dictyostelium* (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987; Springer *et al.*, 1994). When 20% *mhcA*$^-$ cells were mixed with 80% wild-type cells, mutant spores were found, but at a frequency 1000 times lower than expected from the mixing ratio (Knecht and Loomis, 1988). These results demonstrated that cells lacking myosin II were capable of spore differentiation, even though they normally differentiated only to the mound stage. The lower than expected number of spore cells in chimerical development now appears to be due at least in part to the fact that *mhcA*$^-$ cells are mislocalized such that many never make it into aggregates; those that do integrate into aggregates become localized to the back of slugs and the base of fruiting bodies and seldom become part of the sorus (Eliott *et al.*, 1993; Traynor *et al.*, 1994; Knecht and Shelden, 1995). Time-lapse movies show that the later arriving wild-type cells can become mixed into streams (data not shown), indicating that the edge effect is not due simply to the slow movement of *mhcA*$^-$ cells and the time of their arrival at the stream. We have now shown that the exclusion of *mhcA*$^-$ cells from the normal developmental process in chimerical aggregates is due to the adhesion of the wild-type cells.

There are at least two adhesion systems functioning during aggregation: Contact Sites A which are mediated by the surface protein gp80 (Gerisch, 1968; Muller and Gerisch, 1978; Noegel *et al.*, 1986) and Contact Sites B which are mediated by the surface protein gp24 (Knecht *et al.*, 1987; Brar and Siu, 1993). The *csaA* gene was previously isolated and mutants were analyzed (Noegel *et al.*, 1986; Harloff *et al.*, 1989). *csb* genes were identified from an expression library using antibodies specific to gp24 (Loomis and Fuller, 1990). The complete *csb* genes were isolated from genomic libraries by probing with the cDNA clone (DF9) (Loomis and Fuller unpublished data). When DF9 was used to probe Northern blots of RNA isolated at various times in development, it recognized a 0.65-kb mRNA that was absent in growing cells but started to accumulate after 4 hr of development and reached a peak at 10 hr before declining (Loomis and Fuller, 1990). All three *csb* genes are predicted to encode 12-kDa proteins with no signal sequence for membrane translocation nor any significant hydrophobic region for membrane anchoring. However, the protein recognized by

adhesion blocking antibodies migrates on SDS acrylamide gels as a protein of 24 kDa and is found on the outer face of the cell membrane. Several other inconsistencies of the predicted *csb* products and the characteristics of gp24 suggest that the *csb* genes do not encode gp24 although they appear to be involved in the mechanism of early adhesion which is sensitive to EDTA (Loomis and Fuller, 1990). Recently, the gene that encodes gp24 has been identified (Wong *et al.*, 1996). Nevertheless, the delayed appearance of Contact Sites B adhesion in the *csb* mutants has made them a valuable tool for addressing the relative importance of the different adhesion systems in early development.

Our results demonstrate that either Contact Sites B adhesion or Contact Sites A adhesion is sufficient to exclude myosin mutant cells from aggregation streams. It is only when both adhesion systems are removed that *mhcA*$^-$ cells are capable of entering streams. Once *mhcA*$^-$ cells become part of TL73 aggregates, they continue to be localized throughout the multicellular slug and fruiting body. This is in spite of the fact that by later stages, both gp24 and the late adhesion systems are induced. The *mhcA*$^-$ cells do not form spores in *csb* mutant sori, but this appears to be due to the local environment since the TL73 cells also do not form spores.

The presence of the *mhcA*$^-$ cells at the edge of early aggregation streams was initially presumed to be due to a differential adhesive sorting effect. Randomly mixed cells of different adhesive capabilities could theoretically sort from each other over time. This phenomenon does not appear to account for the localization of the *mhcA*$^-$ cells. When cells that are clearly deficient in adhesive capability (TL73) are mixed in chimerical streams with wild-type cells, they are not sorted to the edge (Fig. 6C). It is known that the cortex of *mhcA*$^-$ cells is flaccid both in terms of an inwardly directed ''poking'' force (Pasternak *et al.*, 1989) and an outwardly directed distention force (Shelden and Knecht, 1995). We therefore favor a model in which the lack of myosin has removed the structural integrity of the actin cortex of the cells. While the actin filaments are still presumably cross-linked, the cortex is now extremely flexible and elastic. Without the structural support of the cortex, when an outward protrusion encounters a resisting object, it would recoil rather than push the object. Therefore, when an *mhcA*$^-$ cell encounters a stream, it would not be able to insert itself between the adhered cells.

Two other recent experiments argue that *mhcA*$^-$ cells have difficulty moving when their environment becomes restrictive. Jay *et al.* (1995) recently characterized the motility of wild-type and *mhcA*$^-$ cells on substrates of increasing adhesiveness. They found that wild-type cells slowed down, but continued to move on adhesive surfaces, but *mhcA*$^-$ cells ceased movement completely. Doolittle *et al.* (1995) showed that *mhcA*$^-$ cells in aggregates did not move significantly, while wild-type cells moved in spiral patterns. Both of these results can be interpreted as indications of the inability of *mhcA*$^-$ cells to generate sufficient force to break adhesive contacts, either with the substrate or with each other.

The other phenomenon that occurs when *mhcA*$^-$ cells are developed in chimeras with wild-type cells is that the *mhcA*$^-$ cells are distorted through their interactions with neighboring cells. Wild-type cells can be observed to change direction and neighbors frequently during movement in multicellular streams (Shelden and Knecht, 1995). Therefore, it appears that cells frequently make and break contacts with their neighbors during streaming. Myosin mutant cells appear to have a cortex which is so flexible that the cell shape becomes distorted before the contact is released. This distortion can be observed when *mhcA*$^-$ cells are present in streams containing the adhesion mutant cells (Fig. 4) or even in streams composed solely of *mhcA*$^-$ cells (X. Xu, unpublished observations). It is unclear what adhesive interaction causes this phenomenon in mixtures with TL73 cells. It seems likely that there is some residual adhesion in these cells. The fact that streams form and that cells adhere to the agar surface argues that some adhesive capability is present. While it is possible that Contact Sites B adhesion or other adhesion mechanisms (Fontana, 1993), may be functioning, we cannot measure any cell–cell adhesion in our shaking suspension assay. This question will be more clearly resolved when mutants lacking the gene encoding gp24 become available.

Taken together, the data argue that the acto-myosin cortex of cells plays a critical role in multicellular development. Cells lacking myosin can neither perform morphogenetic movements, nor can they control their shape when forced into mixtures with wild-type cells. Recently we have found that *mhcA*$^-$ cells are also incapable of generating a 3-dimensional shape independent of the substrate (Shelden and Knecht, submitted for publication). Dissecting how myosin controls cell shape, motility, and adhesion will be critical for understanding the mechanism of morphogenesis.

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