STRUCTURAL AND BIOCHEMICAL ASPECTS OF CELL MOTILITY IN AMEBAS OF *DICTYOSTELIUM DISCOIDEUM*

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ABSTRACT

Amebas of *Dictyostelium discoideum* contain both microfilaments and microtubules. Microfilaments, found primarily in a cortical filament network, aggregate into bundles when glycerinated cells contract in response to Mg-ATP. These cortical filaments bind heavy meromyosin. Microtubules are sparse in amebas before aggregation. Colchicine, griseofulvin, or cold treatments do not affect cell motility or cell shape. Saltatory movement of cytoplasmic particles is inhibited by these treatments and the particles subsequently accumulate in the posterior of the cell.

Cell motility rate changes as *Dictyostelium* amebas go through different stages of the life cycle. Quantitation of cellular actin by sodium dodecyl sulfate-polyacrylamide gel electrophoresis shows that the quantity of cellular actin changes during the life cycle. These changes in actin are directly correlated with changes in motility rate. Addition of cyclic AMP to *Dictyostelium* cultures at the end of the feeding stage prevents a decline in motility rate during the preaggregation stage. Cyclic AMP also modifies the change in actin content of the cells during preaggregation.

During the early, feeding stages of the life cycle of *Dictyostelium discoideum* the cells are free living and ameboid, feeding upon bacteria. Depletion of the food supply triggers the next phase of the life cycle, the preaggregation phase, leading to aggregation and sporulation. Preaggregation begins immediately after food depletion (42). In another species, *D. mucoroides*, motility is observed to decrease during preaggregation (33), as do phagocytosis and cell division (3, 14, 15, 20).

Cyclic AMP released into the medium by the cells serves as a chemotactic agent in the formation of aggregates of amebas (4, 21). Before aggregation, phosphodiesterase released by the cells breaks down any cyclic AMP in the medium (26).

With the beginning of aggregation, however, the phosphodiesterase activity in the medium decreases and the cells begin migrating in streams toward the source of cyclic AMP release. Within a few hours, aggregates of several thousand cells are formed. During aggregation the cells respond to cyclic AMP flux with an increase in motility (33), but the mechanism by which cyclic AMP stimulates this is unknown.

Dictyostelium cells display ameboid movement which differs from that seen in amebas of the *Chaos* or *Amoeba proteus* type (34). The mechanism of motility in these cells is not fully understood. There is considerable evidence that actin and myosin are involved generally in motility of

amebas (2, 27), as well as in other nonmuscle cells (1, 17, 28, 30). These contractile proteins have been isolated specifically from *Dictyostelium* (8, 37, 46). Microfilaments, presumably consisting of actin, have been observed in association with the cell membrane (7, 36). Microtubules are also present in motile amebas of *Dictyostelium* (27, 22), but their possible role in motility has not yet been defined.

In the present study we characterize the motility of *D. discoideum* in terms of the organizational state of the filamentous and microtubular systems in the cell. We further relate the rate of motility to fluctuations in the quantity of contractile protein in the cell. We have also investigated the role of cyclic AMP as a possible regulator of motility. Our studies have taken advantage of a recently developed method for quantitation of submicrogram amounts of protein using SDS-polyacrylamide slab gel electrophoresis (18). Through the use of this method it has been shown that changes in actin quantity per cell can be detected in another ameba, *Acanthamoeba*, during the process of encystation (31, 32).

MATERIALS AND METHODS

Cell Culture

Stock cultures of *D. discoideum* strain NC-4 (haploid) and *Escherichia coli* strain B/r, obtained from Dr. David Francis, University of Delaware, Wilmington, Del., were maintained on agar slants of either lactose agar (2% agar, 0.1% lactose and 0.1% peptone) or nutrient agar. Slime molds for study were grown on either lactose agar plates or in liquid culture, with *E. coli* as a food source. The liquid medium (39) was composed of 0.05% yeast extract, 0.5% peptone, 2 mM $MgCl_2$ in 10 mM potassium phosphate buffer at pH 6.2. All cultures were grown in the dark.

Light Microscopy

For observation, cells were placed on cover glasses which had been coated on one side with MS-122 dry release agent (Miller and Stephenson Co., Danbury, Conn.) and agar. By gently blowing on the agar as it dried we could produce fine streaks and ripples in the agar surface. After addition of the cells, an agar film was cast on a loop and placed over the cells enclosing them in an agar envelope. The MS-122 allowed easy removal of the cover glass from polymerized resin after fixation and embedding. The agar envelope provided a substrate for the cells as well as a means of holding them in place during experiments. The cover glass was inverted and placed on a slide so that test solutions could be drawn under it with filter paper. In experi-

ments requiring several hours of observation, microperfusion chambers (25) were used. Observations were made with Zeiss Nomarski differential optics. Motility rates were measured by taking photomicrographs at 3-min intervals for 15 min with continuous observation between exposures. Each set of photomicrographs was then traced on paper so that the path of each cell could be followed and measured. These measurements provided rates of movement through the entire path of a cell rather than rates of net movement in a particular direction. Random walk analysis (10) providing directional motility rates was judged to be a less significant parameter for comparison with contractile protein content of the cells (see below).

Electron Microscopy

Cells were fixed for 1 h with 3% glutaraldehyde and 1% paraformaldehyde in 0.05 M sodium cacodylate buffer, pH 7.2. All steps were carried out at room temperature. Because of the presence of the agar envelope, the entire cover glass could be handled for dehydration and embedding, allowing relocation of chosen cells after embedding. After a 5-min wash in cacodylate buffer, preparations were postfixed for 45 min with 1% OsO_4 in 0.05 M cacodylate buffer and then stained *en bloc* with 0.5% uranyl acetate in 0.5% NaCl. The cells were dehydrated in ethanol, treated with propylene oxide, and embedded in Spurr's (38) resin by inverting the cover glasses (cell side down) over a glass slide with slide fragments as spacers. The space between the slide and cover glass containing resin was then polymerized into a wafer containing the cells. The cover glass and slide provided two optically flat surfaces and allowed high-resolution observation of the embedded cells. Cells selected before fixation could be relocated and sectioned in any desired orientation.

Glycerination

Cells were glycerinated according to the method of Ishikawa et al. (17). A glycerol series of 5%, 10%, 25%, and 50% in 0.1 M KCl, 0.005 M $MgCl_2$, and 0.006 M potassium phosphate buffer, pH 7.0, was used. The contraction of the cells was elicited by applying solutions of 1 mM ATP, 3 mM $MgCl_2$, and 5 mM EGTA in 0.01 M Tris or 0.006 M phosphate buffer, pH 7.0. Other solutions containing 3 mM $MgCl_2$ alone and 5 mM $CaCl_2$ were also tested. Solutions were applied by being drawn under the cover glass with filter paper. Fixation of glycerinated cells was done as above. Heavy meromyosin (HMM) was prepared according to Szent-Gyorgyi (41) and was applied to glycerinated cells at a concentration of 1 mg/ml for 12 h. HMM was also applied in the presence of ATP (10 mM) as a control.

Microtubules Studies

We found that colchicine and griseofulvin had no effect on slime mold amebas unless administered in the

presence of 0.1% dimethyl sulfoxide (DMSO). When this agent was used, colchicine was effective at 5×10^{-5} M. DMSO alone had no effect on the cells. Cold studies were done by incubating cells for 2–12 h at 2°C. Due to warming on the microscope stage, reliable motility measurements on cold cells were not made. Therefore, cells were fixed in the cold and the morphology of the fixed cells was observed.

Electrophoresis

Samples for electrophoresis were taken from liquid cultures. The cultures were washed repeatedly with Bonner's saline (3) to remove bacteria and culture medium. Cell counts were made in a hemocytometer and the cells were pelleted, drained of excess saline, and frozen at −20°C. Pellets lysed by freezing and thawing were diluted to desired concentrations and aliquots were placed in a sample buffer composed of 10% glycerol, 5% 2-mercaptoethanol, 2% sodium dodecyl sulfate (SDS), 0.0625 M Tris, pH to 6.8 with HCl. The polyacrylamide thin (16 × 12 × 0.08 cm) slab gel system used is described by Kahn and Rubin (18) and Rubin and Maher (32). An acrylamide concentration of 12% and a SDS concentration of 0.1% were used. Gels were run at 15 mA. Staining was done in 0.1% Coomassie brilliant blue R in 25% trichloroacetic acid (TCA) for 25 min. Gels were destained in 7% acetic acid until no background stain was visible, usually for 1 day.

Quantitation

Standards of known concentration of rabbit muscle actin were run on each gel with slime mold samples. The stained bands were cut out of each gel and placed on the inside wall of a Gilford 24.2 rectangular cuvette (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) filled with fresh 7% acetic acid. The gels were scanned at 590 nm with a slit plate of 2.36 × 0.1 mm. Graphs were made by using a Gilson linear transport mechanism (Gilson Medical Electronics, Inc., Middleton, Wis.) to move the gels through the beam. Absorbance was recorded on a Gilford 2400-S chart recorder. Area under the curves was measured with a planimeter for quantitation. Total protein of the slime mold samples was determined by the method of Lowry et al. (23).

A peptide map was made from purified actin and from the actin band eluted from a polyacrylamide gel of Dictyostelium whole cell homogenate. The proteins were labeled with ^{125}I and mapped according to Bray and Brownlee (6). Electrophoresis was done on Whatman 3 mm paper for 90 min at 3 kV. Exposures were made for 6 days on Kodak NS X-ray film.

OBSERVATIONS

Cell Movement and Behavior

Free-living cells of Dictyostelium exhibit ameboid movement different from that of Amoeba

proteus. The streaming of cytoplasm is more localized than in A. proteus, usually seen only in advancing regions of the cell rather than as bulk endoplasmic flow throughout. Hyaline cytoplasm is present at the anterior or advancing end of the cell. In the electron microscope, large organelles are not usually found in the hyaline cytoplasm. The large organelles are restricted to deeper cytoplasm (Fig. 1). In addition to a large anterior pseudopodium, the cells possess small (0.5 μm diam) finger-like projections, or pseudodigits, (34) which extend in many directions away from the cell (Fig. 2). These pseudodigits move actively, often making first contact with other cells (see also reference 13). Occasionally, the pseudodigits expand and fill with cytoplasm, forming a pseudopodium. A more complete description of Dictyostelium ameboid movement may be found in Shaffer (34).

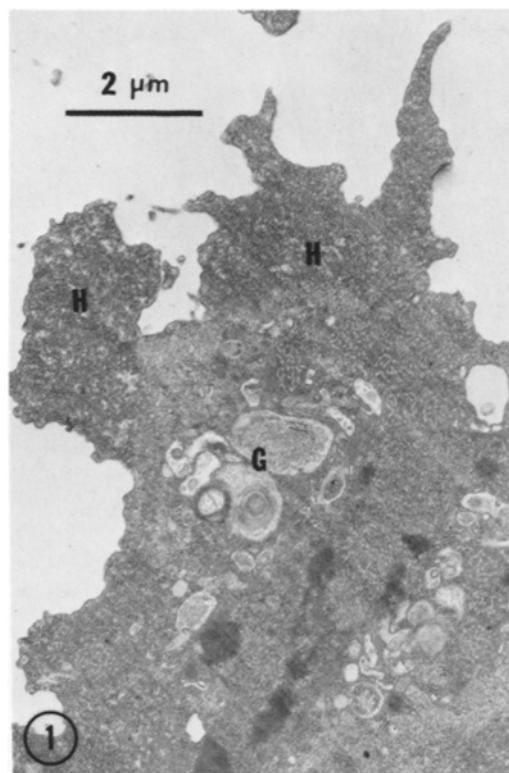The nucleus usually remains in the posterior half of the cell. Numerous mitochondria, crystal-

FIGURE 1 Electron micrograph of a vegetative ameba of Dictyostelium. Hyaline pseudopodia (H) contain ribosomes while larger organelles (G), which appear as granules in the light microscope, are restricted to deeper cytoplasm. × 9,300.

line inclusions, and bacteria in various stages of digestion are distributed throughout the cell and move freely within the cytoplasm. These particles also undergo saltatory movement, often against the flow of cytoplasm.

Dictyostelium amebas display contact guidance. The term contact guidance is used when "a cell on a given oriented substratum assumes a corresponding orientation and moves along that line" (45). On a smooth, agar-coated cover glass the cells move at random, without any apparent orientation. On a similar cover glass whose agar film has been prepared with 2-5-μm wide grooves and ripples in it, the amebas move at random until they encounter one of the grooves. The cells orient along the grooves (Fig. 3), often becoming more elongate. The grooves may become filled with cells. If the cells are approaching aggregation phase, they form end-to-end files in the grooves.

Microfilaments

Dictyostelium amebas contain both microtubules and microfilaments (see reference 22). A cortical network of filaments, 6-7 nm in diameter, is found around the entire cell but is most prominent at the anterior, or advancing end (Fig. 4). The cortical filaments also become more prominent around areas of phagocytosis. Pseudodigits contain a bundle of 6-7-nm filaments which are continuous with the cortical filament layer (see also reference 13). Occasional bundles of filaments, arising from the cortical layer, may also penetrate the deeper cytoplasm.

The distribution of microfilaments is not altered by contact guidance. Analysis of serial sections of entire amebas shows that the cortical filament layer does not change in size or configuration in contact-guided cells.

When glycerinated amebas of 10 μm diameter (SE = 0.14) are treated with ATP (1 mM) and Mg^{++} (3 mM), they contract to a diameter of 7.9 μm (SE = 0.12) in 1-1.5 min (Fig. 5). Ca^{++} concentration in all solutions was less than 10^{-7} M and was not required for contraction. After 10 min in Mg-ATP, the cells relax to their original diameter. ATP, Mg^{++}, or Ca^{++} alone does not
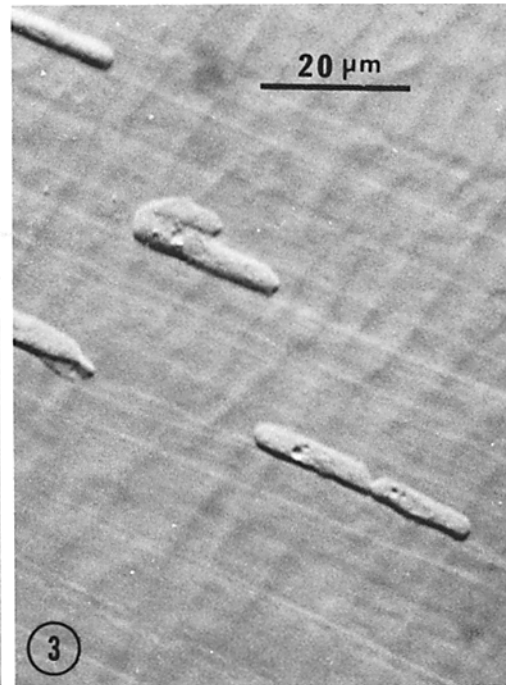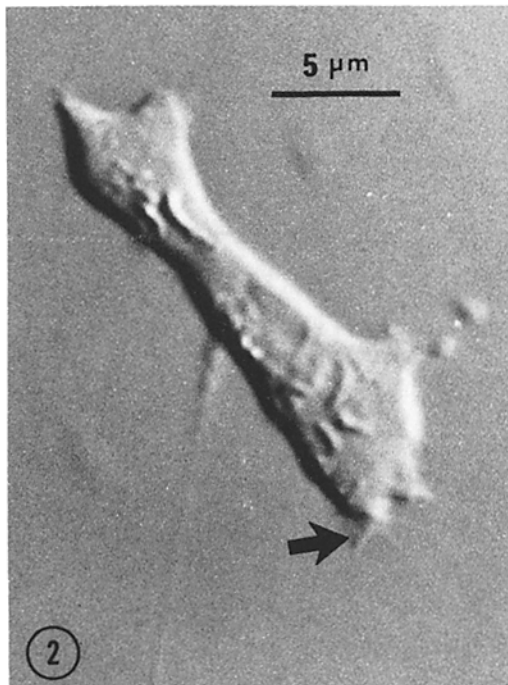

FIGURE 2 Nomarski interference light micrograph of a vegative ameba. Arrow indicates small projections, or pseudodigits, which are found at the leading edge of the cell. × 3,500.

FIGURE 3 Nomarski micrograph of contact-guided amebas showing their alignment parallel to the straight grooves in the agar substrate. × 2,000.
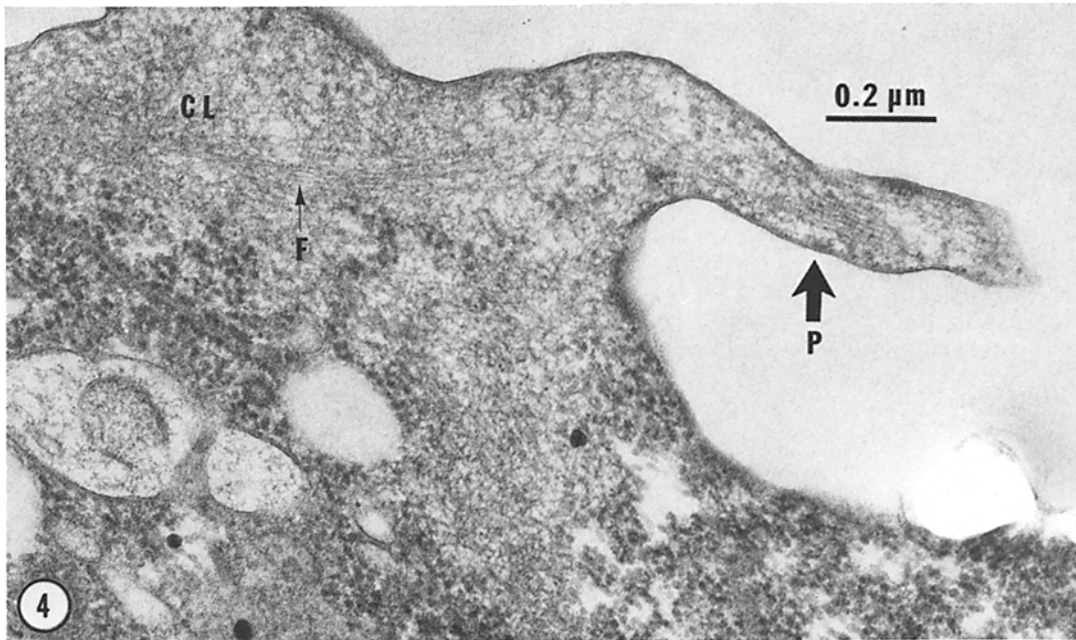
FIGURE 4 Electron micrograph of the anterior region of a vegetative ameba. The cortical layer (CL) is prominent and corresponds to the network of microfilaments in glycerinated cells (see Fig. 6). Some parallel filaments (F) are visible, particularly in the pseudodigit (P). Ribosomes and larger organelles are excluded from the cortical layer. × 74,600.

stimulate contraction. These observations correspond to those originally made on *Amoeba proteus* (35). Electron microscopy of glycerinated cells demonstrates microfilaments at the periphery of the cells which correspond to the cortical filament network. In the contracted model, these filaments are found in bundles (Fig. 6a).

When glycerinated cells are treated with HMM, decorated filaments are observed in the cortical filament layer (Fig. 6b). This decoration appears as additional thickness and gives the filaments a fuzzy appearance. A few distinct arrowheads are apparent. The bound HMM is released by ATP. Filament networks in glycerinated cells become more prominent and numerous after HMM treatment. At regions where the filament networks contact the membrane, the latter may be pulled inward, or puckered (Fig. 6c).

Microtubules

Microtubules are very sparse in feeding and preaggregation amebas. The few that are present usually are associated with the large organelles in deeper cytoplasm of the cell, although an occasional microtubule may appear in the cortical (or

hyaline) cytoplasm. In randomly moving cells the microtubules tend to orient in the long axis of the cell. The number and configuration of the microtubules do not appear to change as cells become contact guided.

The role of microtubules was assessed by using the antimitotic agents colchicine (5×10^{-5} to 2.5×10^{-4} M), griseofulvin (2×10^{-5} M), and cold. All of these agents affected cell behavior similarly: saltations of particles within the cells stopped after 2 h and the particles accumulated in the posterior of the cell. A rather prominent hyaline region remained around the edges and anterior portion of the cell (Fig. 7). It is important to note that ameboid movement is unaffected by antimitotic agents and that the cells continue to move with no change in rate or directionality. It can be seen in Fig. 8 that cells of various shapes and degrees of elongation can be found after treatment with colchicine. Drug-treated cells are slightly smoother in contour than normal cells, although overall cell shape is unaffected. Pseudodigits are still observed (Fig. 7).

Contact guidance is also unaffected by antimitotic drugs. Cells on textured substrates, treated
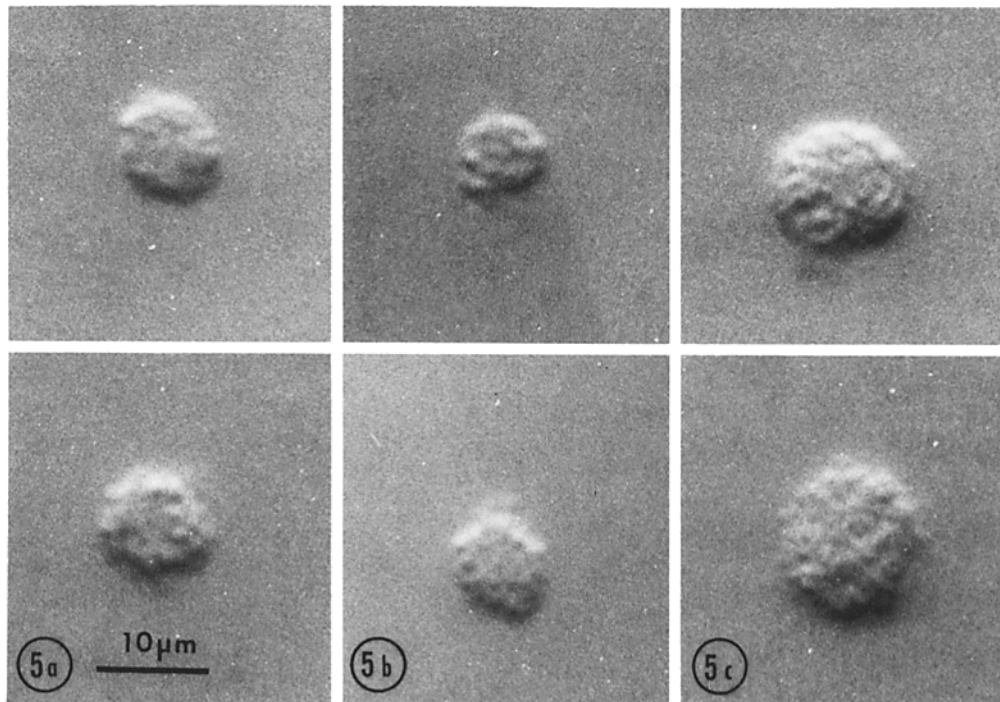
FIGURE 5   Glycerinated amebas of *Dictyostelium*, × 1,500: (*a*) before treatment; (*b*) contracted 1.5 min after addition of Mg-ATP; (*c*) relaxed 10 min after addition of Mg-ATP. Each micrograph is of a different cell. All cells are from the same sample.

with either colchicine or griseofulvin, remain contact guided although the distribution of cytoplasmic particles shows the characteristic response. This response is found if the drugs are applied before or after contact guidance is established.

In the electron microscope, the hyaline region of drug-treated cells consists mainly of ribosomes, rough endoplasmic reticulum, and the cortical filament layer. This region is comparable to the hyaline cytoplasm of normal cells (Fig. 1) but is more extensive. Colchicine- and cold-treated cells contain no microtubules. In cells treated with griseofulvin, on the other hand, microtubules accumulate in the posterior region of the cells along with the larger organelles. These results are summarized in Table I.

Actin Quantitation

Actin was identified in gels of *Dictyostelium* whole cell homogenate by coelectrophoresis with purified muscle actin. Peptide maps of the actin band eluted from *Dictyostelium* gels were compared to peptide maps of purified muscle actin (Fig. 8). This comparison confirmed the identity of the *Dictyostelium* actin band and established that no significant impurities were present to cause error in quantitation of the *Dictyostelium* actin band.

Cells were harvested every 2 h, from 16 to 36 h, for actin quantitation on polyacrylamide slab gels. Fig. 9*b* shows changes in cellular actin content expressed as percent total cell protein. Actin content increases in the early part of the feeding phase and peaks at the end of feeding. As the cells enter preaggregation, the actin content of the cells drops and stays at a low level until aggregation begins. As aggregation commences, cellular actin content again rises and remains high as aggregation is completed (36 h).

Fig. 9*a* shows motility rates of amebas from the same cultures used in actin quantitation. The motility rate during feeding shows much variability but is generally higher than in preaggregation. The large standard error indicates greater variability in motility rate among individuals during feeding than in later stages. When the cells enter preaggregation their motility rate declines, as noted previously in *D. mucoroides* (33). The motility rate
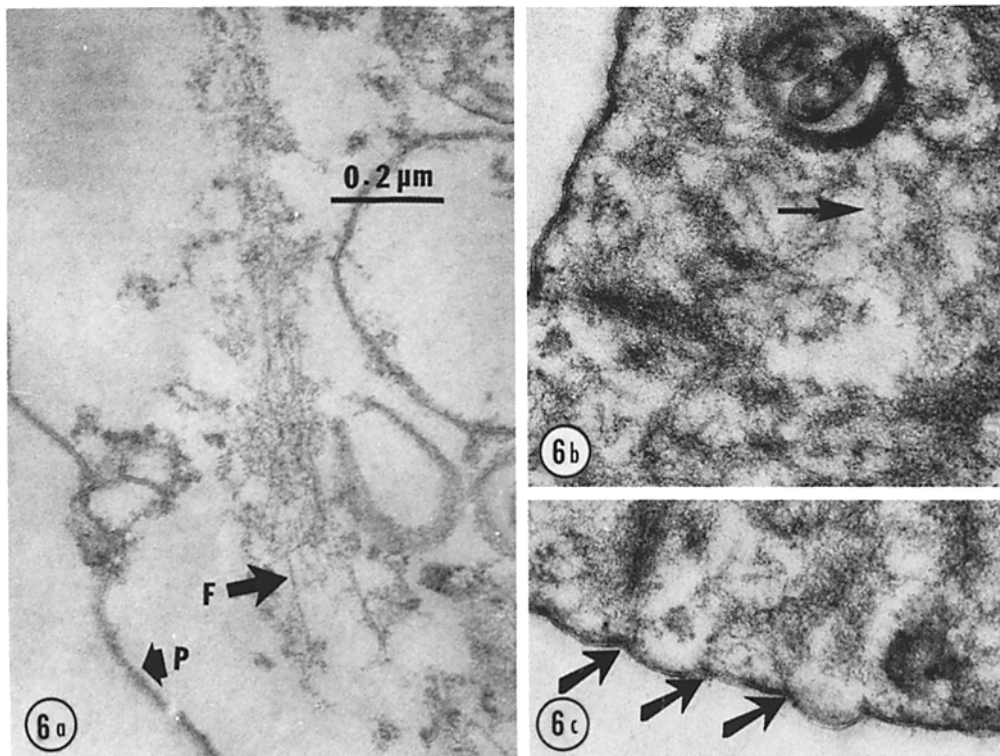
FIGURE 6 Glycerinated amebas of *Dictyostelium*, × 74,000. (*a*) Contracted cell identical to those in Fig. 5*b*. A bundle of filaments (*F*) is found in the cortical layer. *P* = plasmalemma. (*b*) Cell after treatment with HMM. Filaments have become decorated, appearing thicker and fuzzy. A few arrowheads are visible where indicated. (*c*) Cell after treatment with HMM. Arrows indicate areas where filaments appear associated with the plasmalemma and may cause it to pucker.

remains low during preaggregation, corresponding to the low actin content during the same period. As aggregation begins, there is an increase in motility rate as amebas respond to the cyclic AMP stimulus and form aggregates. In liquid culture, aggregation is signaled by the presence of clumps of cells corresponding to the pinwheel-shaped aggregates that form in cultures grown on substrates.

At 36 h in our cultures motility declines again, whereas on the substrate the motility will remain high as migratory slugs formed (12). In liquid culture the aggregates of cells will develop no further unless provided with a substrate and air-liquid interface.

Comparison of the graphs of actin quantity and motility rate (Fig. 9*a* and *b*) shows close correlation between these data. Actin content and motility rate increase together (feeding and aggregation) and decrease together (preaggregation). Statistical tests (analysis of variance) show that differ-

ences between high and low values of motility and actin are significant at 95% confidence levels.

Effect of Exogenous Cyclic AMP

We observed that the times of high actin content corresponded to the times that the amebas are chemotactic. To examine the effect of cyclic AMP on cellular actin and motility rate, we applied an exogenous cyclic AMP stimulus at the end of the feeding stage. To do this, phosphodiesterase in the medium must be inhibited since it will break down cyclic AMP added to the cultures. Malchow and Gerisch (24) have shown that cyclic GMP can be used as an effective inhibitor of slime mold phosphodiesterase. A stock solution containing both cyclic nucleotides was added every 2 h to liquid cultures, giving final concentrations of 0.1 mM cyclic GMP and 1.5 mM cyclic AMP.

If the cyclic AMP stimulus is given before preaggregation begins, the typical drop in motility
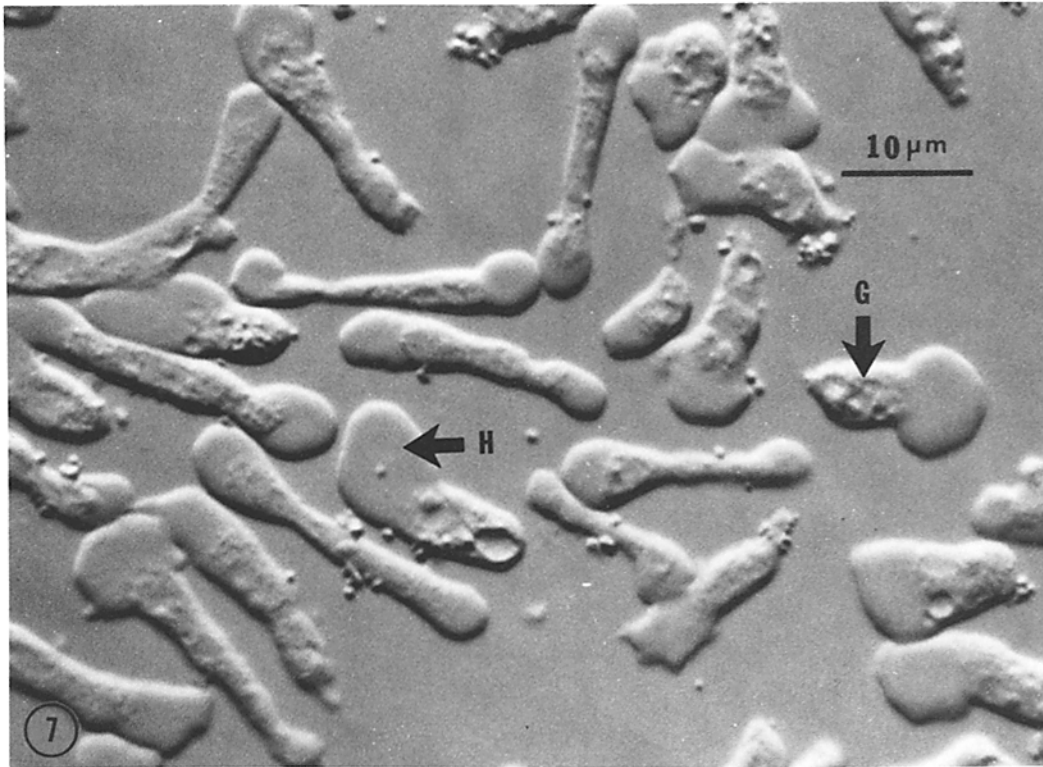
FIGURE 7   Amebas of *Dictyostelium* after 2 h in 2.5 × 10⁻⁴ M colchicine. The cells show a large hyaline region (*H*) while all of the cytoplasmic granules (*G*) have accumulated in the posterior of the cells. × 1,750.

rate and actin quantity during preaggregation is eliminated (Fig. 10*a* and *b*). The cells maintain their high motility rate until the end of aggregation. The actin quantity peaks at the normal time (corresponding to the cessation of feeding), but rather than falling off quickly to a low, preaggregation level, it declines only gradually. The time of aggregation is not altered by this treatment. Neither cyclic AMP nor cyclic GMP has any effect when added alone.

DISCUSSION

It now seems clear that the cortical region of *Dictyostelium* amebas contains much of the actin-based contractile machinery of the cell. Our observations support the suggestion by Spudich (36) and by Clark et al. (7) that actin filaments are associated with plasma membranes of amebas. The general cortical distribution of filament networks in amebas has been demonstrated by our serial thin sections, and the identification of these networks as actin has been shown by HMM label-

ing. That these networks are contractile is indicated by our studies of glycerinated, ATP-contracted amebas, in which overall cortical contraction and an increase in density of the actin filament bundles have been produced.

We have noted in these studies that HMM treatment of glycerinated amebas also results in more prominent actin filament bundles in cell cortices. Recent work by Szamier et al. (40) suggests that actin filaments may be protected from destruction during osmium fixation if tropomyosin is bound to the filaments. HMM may provide similar protection in our cells.

We believe that the cortical contractile machinery of *Dictyostelium* functions in cell locomotion and may serve as a cytoskeletal system in maintaining cell shape. For example, localized contractions of the cortical filaments could result in formation of pseudopodia at the advancing end of the cell. The cytoskeletal role, as well as motility, could involve controlled gelation of the cytoplasmic proteins. Kane (19) and Pollard (29) have
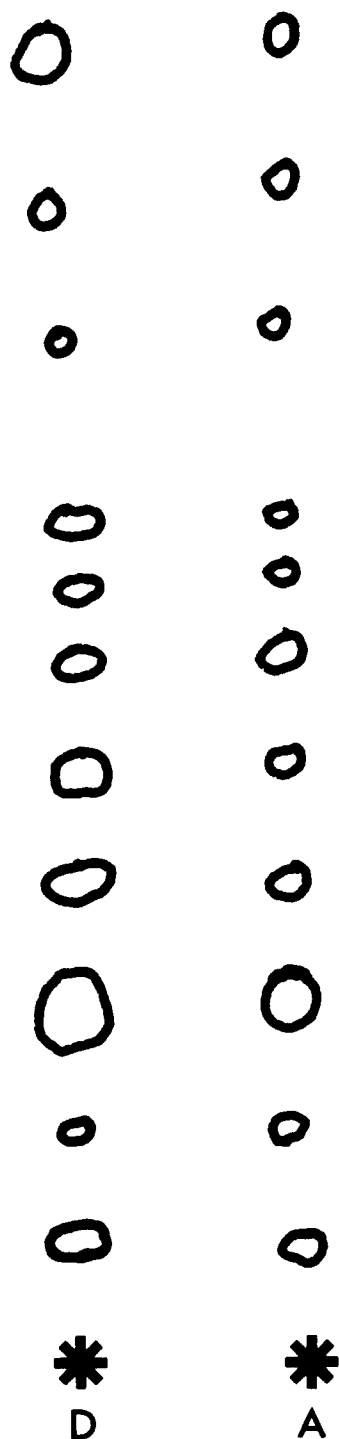
FIGURE 8 Tracing of one-dimensional peptide maps of actin from Dictyostelium polyacrylamide gels (D) and of purified rabbit muscle actin (A). The positions of the spots are identical in each map.

shown that cytoplasmic extracts containing contractile proteins will form rigid gels under certain conditions. Formation of gelled arrays of filaments under physiological conditions has been demonstrated in extracts of Amoeba proteus (43). These properties may provide a basis for cytoskeletal and motility functions of the cortical filaments.

Our results indicate that microtubules in Dictyostelium play only a minor role in cell movement since the number of microtubules in these cells is small and motility and maintenance of cell shape are not appreciably affected by antimitotic agents. Microtubules do appear to be involved in saltatory particle movements. This conclusion rests on the observation that colchicine and griseofulvin block these movements. It should be noted, however, that localized cytoplasmic streaming is not blocked by these agents, and that in their presence the particles appear to accumulate passively in the posterior region of the amebas.

Although the behavior responses of amebas to colchicine and griseofulvin are similar, the cytological effects of these agents are different in that the latter does not disrupt the visible microtubules in the cell. The mechanism of action of this drug is yet to be defined but its ability to disrupt microtubule-associated functions in the mitotic spindle (16) without disrupting significant numbers of microtubules has been observed.

Our observation of colchicine-treated Dictyostelium amebas differs significantly from observations of similar treatments on fibroblasts. In mouse fibroblasts, for example, cell polarity, directionality, and motility rate are all altered by colchicine treatment (11). In Dictyostelium none of these changes occur. This may indicate different cytological organization of these cell types and may reflect differences in the relative importance of microtubules to motility.

The direct relationship between cellular actin quantity and rate of motility suggests that Dictyostelium could limit its motility rate in different phases of the life cycle by regulation of the quantity of actin and of other contractile proteins as well. During the feeding stage, when actin increases, cells may utilize actin in a number of ways. Their overall motility rate is high as the cells feed upon bacteria. An increase in thickness of the cortical filament layer also appears to be associated with phagocytosis (22). Cells actively divide at this stage, and actin may play a role in cytokinesis.

Our observation that exogenous cyclic AMP

TABLE I
Response of Slime Mold Amebas to Antimitotic Agents

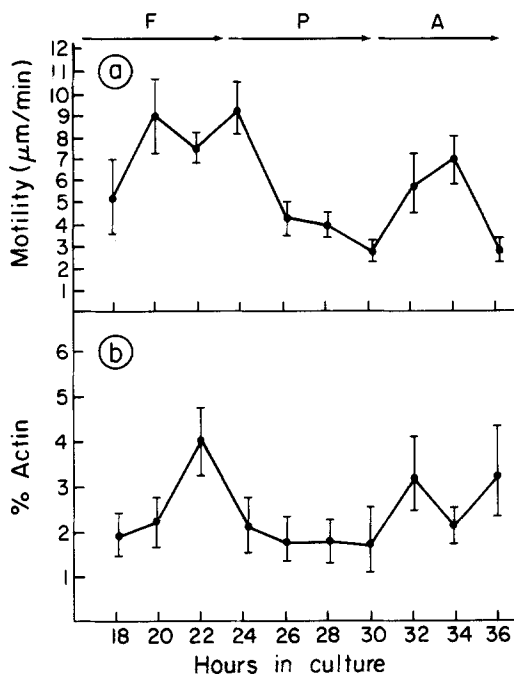| Drug	Motility	Cell shape	Contact guidance	Cytoplasmic particles	Microtubules
Colchicine 2.4 × 10⁻⁴ M 0.1% DMSO	No effect	No effect	No effect	Clustered	Absent
Griseofulvin 2 × 10⁻⁵ M 0.1% DMSO	No effect	No effect	No effect	Clustered	Present
Cold (2°C)	—	No effect	No effect	Clustered	Absent
DMSO (0.1%)	No effect	No effect	No effect	Dispersed	Present

FIGURE 9 A comparison of motility rate (a) and cellular actin quantity (b), expressed as percent of total cellular protein. The stages of the life cycle are indicated at the top: F, feeding stage; P, preaggregation stage; and A, aggregation stage. Bars indidate standard error.
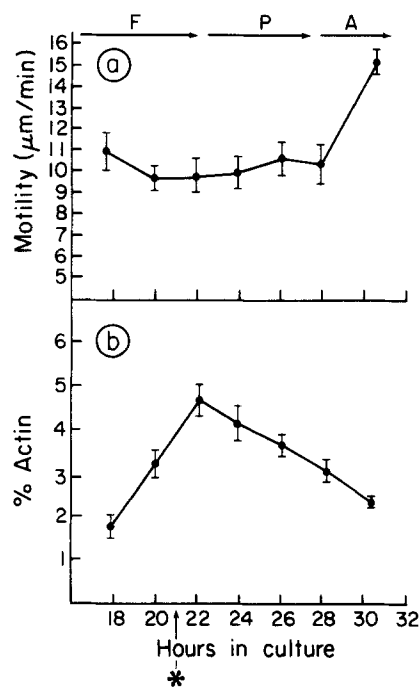
FIGURE 10 A comparison of cell motility rate (a) and cellular actin quantity (b), expressed as percent of total cellular protein. Stages are indicated as in Fig. 9. Cyclic AMP was added in the presence of cyclic GMP at the time indicated by (*).

inhibits the normal decrease of motility rate and actin content during preaggregation suggests that cyclic AMP levels could play a role in the control of these variables during development. Our experiments do not tell us, however, whether variation in actin content is linked to changing rates of actin synthesis or degradation, or both. Tuchman et al. (44) have shown that synthesis of actin is greatly enhanced during the first few hours of development. This time corresponds to late preaggrega-

tion or early aggregation phases of our study. This increase in synthesis occurs just before the times when we find the highest levels of actin in aggregating cells. Comparing two stages having high motility rates, feeding and early aggregation, Tuchman et al. (44) show a great difference in actin synthesis with a dramatic increase in early aggregation. The high actin content that we demonstrate at aggregation correlates with the high synthesis rates that Tuchman et al. demonstrate.

However, at the end of feeding we also demonstrate high actin content while they show a low synthesis rate. The reason for this is unclear.

Preparation for aggregation and spore formation in *Dictyostelium* involves a number of cellular changes. Among these are the rise in cyclic AMP levels in the medium and the cellular response to it (9). We have demonstrated changes in actin levels and motility rates. These events are presumably initiated in concert with the overall developmental program. When we alter the level of exogenous cyclic AMP after the developmental program has begun, the levels of motility rate and actin content remain abnormally high but the timing of aggregation is not affected. This suggests that the developmental events may be programmed in such a manner that once the overall program has begun, the motility rate can be altered independently of the other aspects of the program. Thus, it appears that although cyclic AMP level in the medium may control parts of the developmental program, it does not totally control development.

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