

Location of Actin, Myosin, and Microtubular Structures during Directed Locomotion of *Dictyostelium* Amebae

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ABSTRACT During their life cycle, amebae of the cellular slime mould *Dictyostelium discoideum* aggregate to form multicellular structures in which differentiation takes place. Aggregation depends upon the release of chemotactic signals of 3',5'-cAMP from aggregation centers. In response to the signals, aggregating amebae elongate, actively move toward the attractive source, and may be easily identified from the other cells because of their polarized appearance. To examine the role of cytoskeletal components during ameboid locomotion, immunofluorescence microscopy with antibodies to actin, myosin, and to a microtubule-associated component was used. In addition, rhodamine-labeled phallotoxin was employed. Actin and myosin display a rather uniform distribution in rounded unstretched cells. In polarized locomoting cells, actin fluorescence (due to both labeled phallotoxin and specific antibody) is prevalently concentrated in the anterior pseudopod while myosin fluorescence appears to be excluded from the pseudopod. Similarly, microtubules in locomoting cells are excluded from the leading pseudopod. The cell nucleus is attached to the microtubule network by way of a nucleus-associated organelle serving as a microtubule-organizing center and seems to be maintained in a rather fixed position by the microtubules. These findings, together with available morphological and biochemical evidences, are consistent with a mechanism in which polymerized actin is moved into the pseudopod through its interaction with myosin at the base of the pseudopod. Microtubules, apparently, do not actively participate in movement but seem to behave as anchorage structures for the nucleus and possibly other cytoplasmic organelles.

Directed cell locomotion is an example of a highly integrated series of biological reactions. Basically, it requires cellular receptors to sense a tactic signal, a signal-processing system, and a motile apparatus for generation of a motile force. Among the several models employed for the study of directed locomotion in eucaryotic cells, amebae and particularly the ameboid stages of slime mould are of great interest since they offer the possibility of a combined structural, biochemical, and genetic approach. The morphogenetic development of the cellular slime mould *Dictyostelium discoideum* begins at the end of the vegetative growth phase with the aggregation of a sparse population of amebae into masses which will eventually differentiate into spore and stalk cells. Aggregation is an ordered process in which individual cells, dispersed over a territory of several square millimeters, are brought together

to form a multicellular aggregate (28). These aggregating cells respond to chemotactic signals of 3',5'-cAMP by direct active locomotion towards the attractive source (20). Locomoting cells can be easily distinguished from the rest of the cell population by their elongated shape (3). The aggregation of *D. discoideum* thus provides an attractive system for the study of different aspects of ameboid movement during directed cell motility. Several aspects of ameboid movement in *D. discoideum* have already been investigated, including the pattern of movement (31) and the ultrastructural and immunochemical localization of actin (12, 13). Furthermore, the chemotactic system has been extensively studied (see reference 26 for review) and some of the molecular mechanisms involved in the force-generating system for ameboid movement have been characterized (see reference 34 for review). In this paper we

report the distribution of major proteins and structures of the ameba motility system and cytoskeleton including actin, myosin, and microtubules during 3',5'-cAMP-directed locomotion and natural aggregation. For this purpose, indirect immunofluorescence either with specific antibodies against purified *Dictyostelium* actin and myosin or with a rabbit γ -globulin fraction containing antibodies specific for *Dictyostelium* microtubules (8) has been used. F-actin in motile cells has been localized by using rhodamine-labeled phallotoxin (40). In addition the position of the nucleus and the nucleus-associated microtubule-organizing center has been determined in relation to cell locomotion. In this study the generic name of nucleus-associated organelle (NAO)¹ of Girbardt (15) will be used to indicate the nucleus-associated microtubule-organizing center, as proposed in a recent review by Heath (17). The NAO of *D. discoideum* amebae functionally corresponds to the centriole of higher cells.

MATERIALS AND METHODS

Cells: *D. discoideum* amebae (strain Ax2) was grown axenically at 22°C in HL-5 medium containing 86 mM glucose (39). Cells were harvested in the early exponential phase of growth at a concentration of $1-2 \times 10^6$ cells/ml. Cell viability, as determined by plating clonally with *Aerobacter aerogenes* on SM agar plates (33), was >99%.

Aggregation: The natural aggregation of amebae was followed using a glass slide technique (3). A 0.5-ml volume of amebae in HL-5 medium containing 2×10^6 cells/ml was spread over a defined area (2×2 cm) of a standard microscopic slide. Cells were allowed to adhere at 22°C for at least 30 min, the medium was removed, and the slides were gently washed two times in a K_2HPO_4 buffer solution (g/l water: KH_2PO_4 , 2.25; K_2HPO_4 , 0.67; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5; pH 6.1). Excess liquid was drained with absorbent paper, and slides were incubated and inverted in a humid chamber. Under these conditions, aggregation streams are visible after 12–16 h of incubation at 22°C.

3',5'-cAMP-stimulated Chemotaxis: Amebae were collected by low-speed centrifugation (800 g for 10 min), washed twice in Sorensen phosphate buffer (g/l: $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 0.3; KH_2PO_4 , 2.07; pH 6.0), and suspended in the same buffer at a concentration of 1×10^7 cell/ml. To obtain chemotactically competent cells, the suspension was placed in a rotatory shaker (at 100 strokes per min) for 7 h at 22°C. A migration chamber was prepared by attaching a Parafilm ring (internal diameter 1.4 cm, thickness 1 mm) to the surface of a microscope slide, and a glass capillary tube ($\sim 100 \mu\text{m}$ in diameter), connected with a 10- μl microsyringe filled with a 1 mM solution of 3',5'-cAMP (Serva, Heidelberg, W. Germany) in Sorensen buffer, was fixed through one side of the Parafilm ring. A 0.2-ml sample of chemotactically competent cells was then placed into the chamber and subsequently covered with a dialysis membrane. Chemotaxis was induced by pulse-releasing small amounts (0.5–1 μl) of 3',5'-cAMP into the system. Cells were monitored with an inverted Leitz Diavert microscope and fixed for subsequent examination at appropriate times.

Antigens: Actin and myosin from *D. discoideum* amebae were isolated according to Clarke and Spudich (10). The purity of the antigens was assayed by SDS gel electrophoresis in a gradient of 5–20% acrylamide using the method of Laemmli (21). Rabbit actin and myosin were a gift of Dr. Vic Small (Salzburg).

Antibodies: Immune γ -globulin was obtained by injecting purified proteins in New Zealand rabbits as previously described (37). Monospecific antibodies against actin and myosin were obtained by affinity chromatography of the γ -globulin fraction on a CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) column to which actin and myosin, respectively, had been covalently attached (14). The monospecific antibodies were eluted with glycine-HCl 0.2 M, pH 2.8, and stored at -20°C in 0.15 M sodium chloride, pH 7.2, at a final concentration of 1–2 mg/ml.

Immunological characterization of antiactin and antimyosin antibodies was obtained by electrophoretic blotting (36). The antigenic mixture was first resolved by SDS PAGE (10% polyacrylamide concentration) followed by electrophoretic transfer of the polypeptides from the polyacrylamide gel onto nitrocellulose membrane (Millipore HAWP 304F0) using the technique described by Byers and Fujiwara (6). After transfer, the membranes were soaked

in 6 M urea and in distilled water and then incubated for 60 min in 20% fetal calf serum in TBS (10 mM Tris, 150 mM NaCl, pH 7.4) to saturate nonspecific binding sites. After extensive washing in TBS, the membrane was incubated with a 1:100 dilution of rabbit antiactin and antimyosin antibodies in TBS for 60 min at 37°C. Immunocomplexes were detected using an immunoperoxidase reaction by adding γ -anti-rabbit horseradish peroxidase-conjugated antibody (Dako, Copenhagen, Denmark) diluted 1:500 in TBS containing 20% fetal calf serum at 37°C for 60 min. After washing, peroxidase was localized by staining the membrane with a freshly prepared solution of 0.3% 4-chloro-1-naphthol and 20% methanol in TBS containing 0.5 $\mu\text{l}/\text{ml}$ of a 30% H_2O_2 solution. The reaction was terminated after 20–30 min by washing with water. The blots were dried between filter papers and stored protected from light.

In addition to monospecific antiactin antibodies, a rhodamine-conjugated phallotoxin preparation was used. This preparation specifically binds to F-actin (40).

To visualize the microtubule system of *D. discoideum*, we used a γ -globulin fraction from a rabbit preimmune serum. This fraction specifically stains *Dictyostelium* microtubules in both interphase and mitosis, giving a pattern indistinguishable from that of monospecific anti-brain tubulin antibodies (8, 30) and of monoclonal anti-yeast tubulin antibodies. Its characterization is given elsewhere (9).

Staining of Cells: Slides containing aggregating cells or 3',5'-cAMP-activated amebae were observed to determine the stage of movement, and selected positions were noted for subsequent relocation. Fixation was accomplished by adding few drops of 3.5% formaldehyde in phosphate-buffered saline, 0.14 M, pH 7.2 (PBS), to the slide for 20 min at 22°C. After extensive washing, slides were postfixed in methanol (10 min) and acetone (7 min) at -20°C and air dried. Cells were then incubated for 60 min at 37°C with antiactin monospecific antibodies, antimyosin monospecific antibodies (both at a final concentration 0.2 mg/ml protein), or antimicrotubule γ -globulin (1–2 mg/ml protein). Fluorescent staining was performed by incubation of slides with fluorescein isothiocyanate-anti rabbit γ -globulin (Behring Werke AG, Marburg-Lahn, W. Germany) diluted 1 in 20 with PBS (pH 7.4) for 60 min at 37°C. For staining with rhodamine-conjugated phallotoxin, amebae were fixed in 3.5% formaldehyde and 0.2% Triton X-100 in PBS. After incubation for 30 min with a solution of labeled phallotoxin (final concentration 0.05 mg/ml), cells were extensively washed in PBS and prepared for microscopic examination.

Giemsa Staining: After immunofluorescence, cells were extensively washed in PBS and treated for 30 min at 37°C with RNase (Biochemia, Mannheim, W. Germany) solution (0.2 mg/ml in phosphate buffer 0.06 M pH 6.8). Staining was performed as described by Brody and Williams (5).

RESULTS

Specificity of Antibodies

The specificity of antiactin and antimyosin antibodies was assessed by electrophoretic blotting (36). As shown in Fig. 1, *c* and *d*, antiactin antibodies react specifically with *D. discoideum* or rabbit muscle actin. Antimyosin antibodies are apparently reacting mainly with the heavy chain of *D. discoideum* myosin, and with rabbit muscle myosin they show little peroxidase reaction product (Fig. 1 *a–b*). No reaction could be detected in the region of myosin light chains. These results are in agreement with our previous characterization of these antibodies using double immunodiffusion test (9).

Antibodies to microtubules were obtained from the preimmune serum of a female New Zealand rabbit, as previously described (8). These antibodies apparently do not recognize tubulin, but react to one or more microtubule-associated proteins (9). This antiserum has been shown to specifically bind to microtubules and to the NAO of *D. discoideum* amebae. We use this serum to localize microtubules, instead of a previously prepared anti-pig brain tubulin antibody (37), because the background staining is much lower and the resolution is higher (8, 9, 29). The microtubule display of *D. discoideum* amebae obtained with this serum is indistinguishable from that obtained with a monoclonal antibody against yeast tubulin (in Fig. 2, cf. *A* and *B* with *D* and *E*).

To demonstrate the specificity of the fluorescence reactions, the following controls were used: (*a*) untreated cells, (*b*) cells

¹ Abbreviations used in this paper: NAO, nucleus-associated organelle; TBS, 10 mM Tris, 150 mM NaCl, pH 7.4.

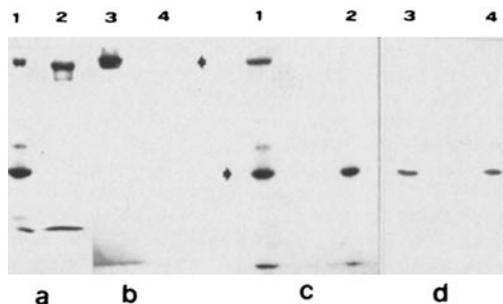


FIGURE 1 Characterization of antimyosin and antiactin monospecific antibodies by electrophoretic-blotting technique. (a) Coomassie Brilliant Blue-stained polyacrylamide slab gel of a crude actomyosin preparation from *D. discoideum* (lane 1) and rabbit muscle myosin (lane 2). (b) Nitrocellulose strip containing electrophoretically transferred proteins from a gel similar to (a) and stained by the peroxidase procedure using antimyosin antibodies. The heavy chain of *D. discoideum* myosin in lane 3 is stained, and a very faint reaction is also present in the heavy chain region of muscle myosin (lane 4). The position of the myosin heavy chain band is indicated by an arrow. (c) Coomassie Brilliant Blue-stained polyacrylamide slab gel of a crude actomyosin preparation from *D. discoideum* (lane 1) and rabbit muscle actin (lane 2). (d) Nitrocellulose strip containing electrophoretically transferred proteins from a gel similar to (c) and stained by the peroxidase procedure using antiactin antibodies. Both actins are stained (lanes 3 and 4). Each position of actin is indicated by an arrow.

stained with anti-rabbit FITC-conjugated antibodies, (c) cells incubated with rabbit pre-immune sera and stained with FITC-conjugated antibodies, and (d) cells incubated with either monospecific antibodies against actin or against myosin, preabsorbed with their respective antigens as described (12) and stained with FITC-conjugated anti-rabbit antibodies. The specificity of the rabbit γ -globulin fraction containing antibodies against *D. discoideum* microtubules was demonstrated as described elsewhere (9). All controls were negative.

Actin, Myosin, and Microtubules during Locomotion

When amoebae are subjected to pulses of 3',5'-cAMP they migrate toward the attractive source. During locomotion, modification of cell shape takes place; amoebae elongate and emit a pseudopod from the anterior end (13, 31). Such locomoting cells can easily be identified (Fig. 3, D and E) and the distribution of actin, myosin, and microtubules can be compared to that of nonlocomoting cells. Fig. 4, A-G shows actin fluorescence in unspread, spread, and locomoting cells, respectively. Small, rounded cells (as seen by phase-contrast microscopy) show a bright fluorescence almost uniformly distributed (Fig. 4, A and F). In more enlarged and flatter cells, however, fluorescence is less uniform, and bright patches along the cell membrane are more concentrated where filopods are visualized (Fig. 4D). Locomoting cells display a more intense fluorescence particularly at the leading edge of the cell, while diminished fluorescence is noted in the tail region. Bright areas between the cell poles are seen mainly along lines corresponding to the cell borders (Fig. 4, E and F). These results are similar to those obtained by Eckert and Lazarides (12) using an antiactin antibody prepared with chicken gizzard actin. Cells stained with rhodamine-conjugated phallotoxin show a similar pattern (Fig. 4G).

Rounded amoebae stained with monospecific antibodies against *Dictyostelium* myosin show a strong bright fluorescence similar to that seen when antibodies against actin are used (Fig. 5A). Stretched cells, however, present diffuse fluorescence with a typical "mottled" appearance including large unstained holes corresponding to nuclei and smaller unstained areas probably corresponding to smaller vacuoles or organelles (Fig. 5, A-D). This pattern cannot be correlated with pseudopod or filopod emission. A similar mottled distribution of fluorescence is also evident in locomoting cells, along with brighter areas corresponding to the tail region where amoeba thickness is greater (Fig. 5, E and F). Fluorescence can not be detected at the tip of the large frontal pseudopods of locomoting cells (Fig. 5F).

Microtubule patterns in 3',5'-cAMP-stimulated amoebae and control cells are shown in Fig. 2. In unstimulated cells, microtubules originate from a microtubule-organizing center located approximately in the middle of the cell and show a random distribution throughout the cytoplasm (Fig. 2, A and D). Locomoting cells have an elongated appearance, with the microtubule network aligned along the cells longitudinal axis (Fig. 2, B and E). Few microtubules are seen in pseudopods and appear to terminate near the leading edge. The pattern of microtubules in motile amoebae can be more clearly seen during the streaming phase of natural aggregation (Fig. 3D). These amoebae have a very elongated shape, reaching as much as 50 μ m in length, and are flat enough to allow immunofluorescence examination. Fig. 3, A-C shows three areas at increasing distances from the center of a streaming aggregate. As in the case of chemotactic response to pulses of 3',5'-cAMP, the microtubules of moving amoebae are longitudinally aligned and their absence from the leading edge of the cell is even more clear. This pattern is more evident in cells located at the periphery of the streaming aggregate where cell locomotion is faster (Fig. 3C). In fact, the diffuse fluorescence seen in pseudopods of these cells cannot be due to individual microtubules or bundles of microtubules which, under these conditions, should appear as fluorescent lines. Immunofluorescence also shows that the NAO of moving amoebae are preferentially located in the central region of the cell. This observation is confirmed by the examination of Giemsa-stained locomoting amoebae (Fig. 6). More than 80% of these amoebae show the NAO in the central area, while only some cells with longer pseudopods have their NAO in the posterior third (Fig. 6A). In locomoting amoebae, nuclei can be found in the frontal region of the cell but more preferentially follow the NAO, and in the longer cells the majority of the nuclear chromatin is seen several micrometers behind it. In binucleated amoebae, the two nuclear NAO complexes are either paired or seen one behind the other along the axis of the cell (Fig. 6, E and F). The general impression obtained from examining thousands of Giemsa-stained motile cells is that NAO seems to indicate the orientation to the cell. It is not possible to establish, however, whether it is the NAO itself that mandates the direction of locomotion to the cell, as proposed for the centriole of higher cells (1, 2, 15, 16). The basis for NAO staining with Giemsa stain is not completely understood but is probably dependent on its deoxyribonucleoprotein content (29).

DISCUSSION

The generation of movement during amoeboid locomotion is thought to be largely dependent on the interaction between

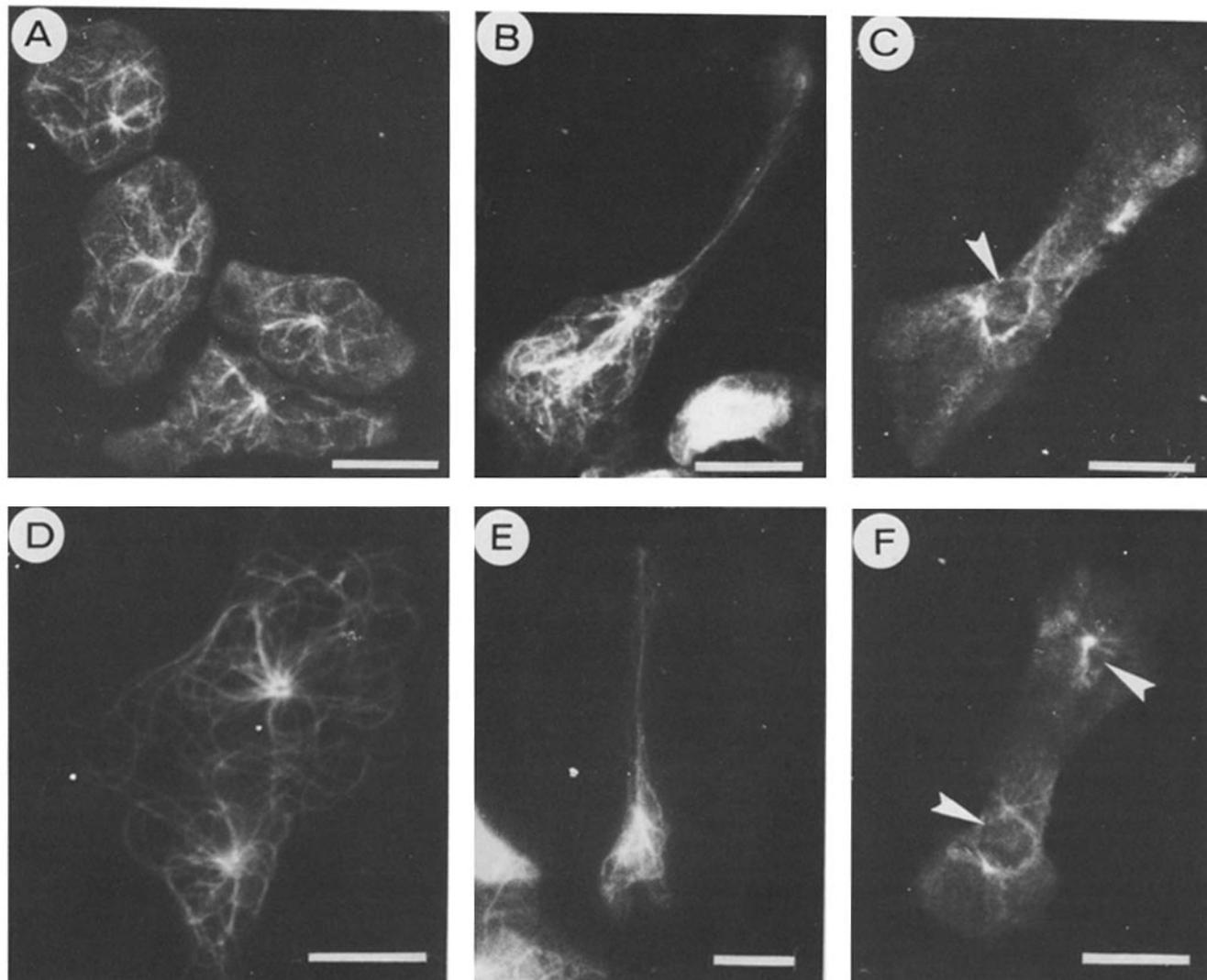


FIGURE 2 Fluorescent staining of microtubules in *D. discoideum* with a rabbit antibody against a microtubule-associated component (A, B, C, and F) and rat monoclonal anti-yeast tubulin antibodies (D and E). Unstimulated cells showing microtubules originating from a fluorescent microtubule-organizing center corresponding to the NAO. (B and E) Locomoting cells after stimulation by pulses of 3',5'-cAMP are represented. (C and F) Two amoebae depleted of microtubules after incubation for 24 h with 7 $\mu\text{g}/\text{ml}$ of the microtubule inhibitor nocodazole. Arrows show position of nuclei. Direction of movement from bottom to top. Bars, 10 μm .

structured forms of actin and myosin (see reference 35). In *D. discoideum*, actin is present in both filamentous (F) and soluble form (G) and seems to undergo cycles of gelation and contraction in the cell cytoplasm (35). A large amount of filamentous actin is membrane associated (11) and co-purifies with the membrane fraction (23, 32). Furthermore, in the electron microscope, bundles of actin filaments have been seen preferentially associated with pseudopods (13, 24). Our immunofluorescence results on the distribution of actin in resting and motile cells are in agreement with these data, showing a rather uniform distribution of actin in rounded cells and a preferential association of the fluorescence with the membrane and with pseudopod emission in both stretched and locomoting cells. Actin fluorescence is always intense in the leading pseudopod of locomoting cells. This is consistent with the frontal contraction theory of Allen (4), according to which the leading pseudopod corresponds to the area of contraction where a large amount of gelled actin should be present. The preferential staining of the leading pseudopod

with rhodamine-labeled phallotoxin indicates that most of the actin in this area is indeed in the F form.

Generally, myosin seems to be randomly distributed in the cytoplasm of *D. discoideum* amoebae. In addition, myosin fluorescence is absent or only slightly visible in pseudopods in both standing and locomoting cells. This situation is similar to that observed in spreading HeLa cells in which myosin seems to be absent from the margins of ruffling membranes and the interior cytoplasm (19). Therefore, if myosin has a role in moving the pseudopod, it must do so at the base of the pseudopod itself. However, the apparent lack of fluorescence in selected areas of the cell does not signify an absolute absence of the antigen, since, as evidenced with myosin in stress fibers, the antigen may be present in small amount (18). Therefore, we can conclude that myosin is not prominent in the pseudopods of *D. discoideum*. In this regard, the pseudopods appear to be different from those of other motile cells such as polymorphonuclear leukocytes in which a large amount of myosin has been demonstrated (38).

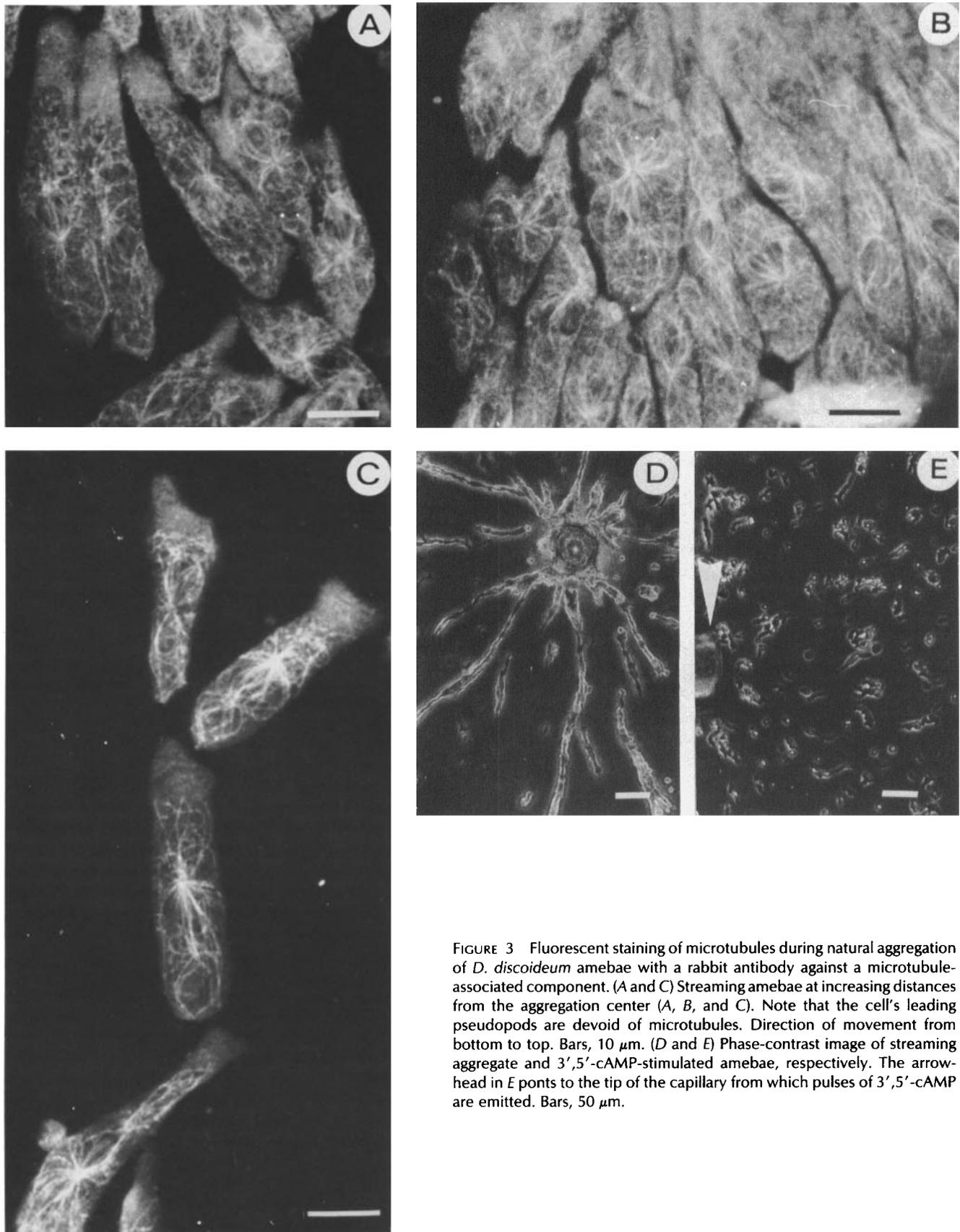


FIGURE 3 Fluorescent staining of microtubules during natural aggregation of *D. discoideum* amebae with a rabbit antibody against a microtubule-associated component. (A and C) Streaming amebae at increasing distances from the aggregation center (A, B, and C). Note that the cell's leading pseudopods are devoid of microtubules. Direction of movement from bottom to top. Bars, 10 μm . (D and E) Phase-contrast image of streaming aggregate and 3',5'-cAMP-stimulated amebae, respectively. The arrowhead in E points to the tip of the capillary from which pulses of 3',5'-cAMP are emitted. Bars, 50 μm .

D. discoideum amebae are thought to contain few microtubules (22). This impression, probably due to the difficulty in reconstructing the microtubule pattern of a cell by electron

microscopy, is not supported by our immunofluorescence results that show a well-developed microtubule network in both resting and motile amebae. Although microtubules can

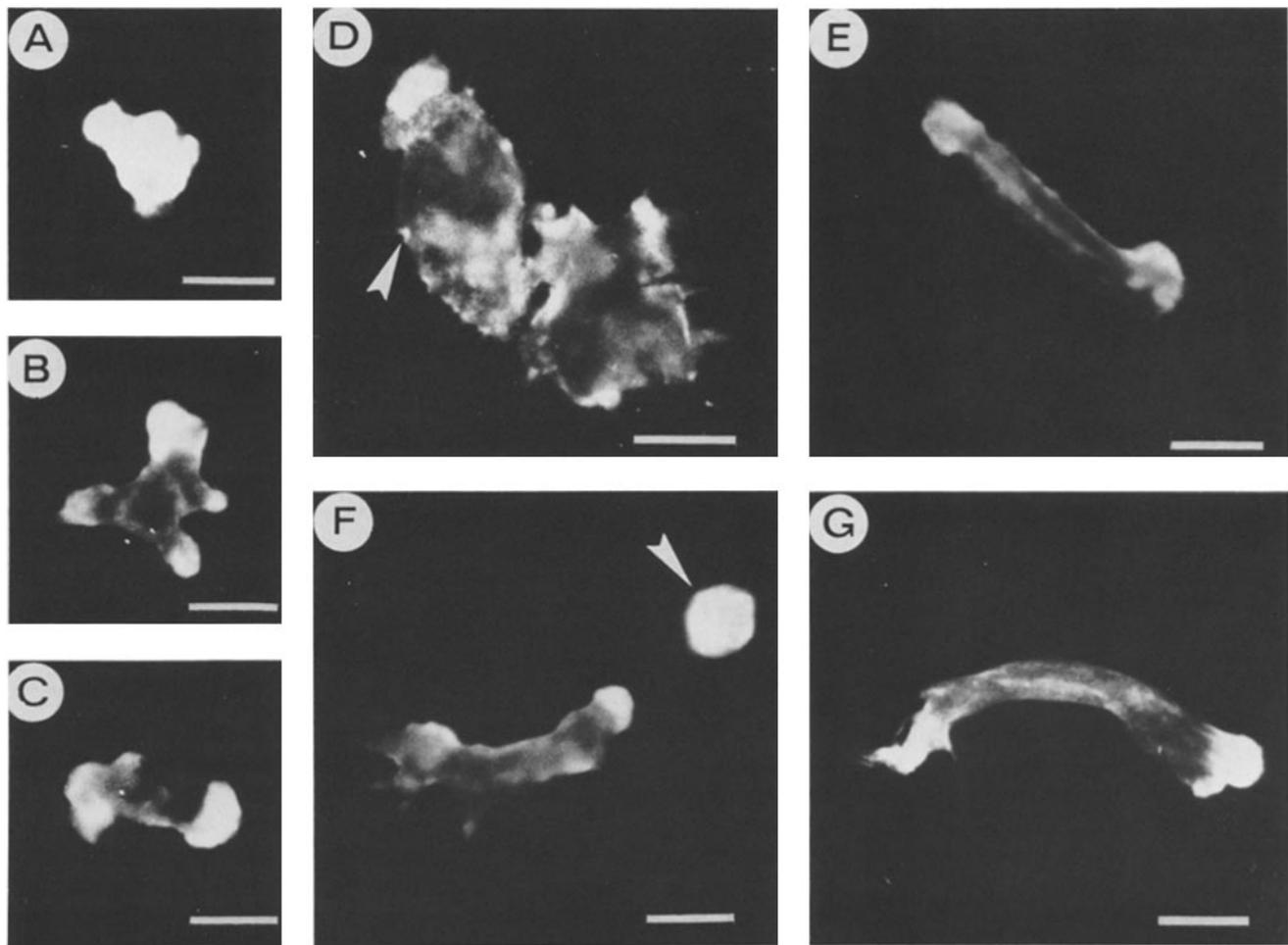


FIGURE 4 Fluorescent staining of *D. discoideum* amebae with monospecific antiactin antibodies (A–F) and rhodamine-conjugated phalloidin (G). (A and F, arrowheads) unstretched cells with bright, diffuse fluorescence. (D) Two stretched cells with patches of fluorescence distributed mainly along the cell periphery; the arrow shows a spot of intense fluorescence at the base of a filopod. (B, C, E, F, and G) locomoting cells after stimulation by pulses of 3',5' cAMP; actin fluorescence is clearly associated with pseudopod emission (B and C) and in oblongated cells is prominent in the leading pseudopod (E, F and G). The striking similarity between staining due to antiactin antibodies and that due to labeled phalloidin is apparent in E and G. In all moving cells, direction is from left to right. Bars, 10 μ m.

be seen in pseudopods, the leading pseudopod of fast-moving amebae is largely devoid of these structures. This suggests that microtubules are not necessary for the formation of the leading pseudopod although they might support its formation by functioning as an anchoring point of attachment for actin or myosin filaments. However, amebae treated with a variety of microtubule inhibitors can still undergo an almost normal aggregation phase (7, 27), implying that microtubules do not play an essential role in ameboid locomotion. In addition, cells in which microtubules have been disarranged by inhibitors have the same elongated appearance as normal cells, suggesting that microtubules are not necessary for determining polarity. These results, however, must be interpreted with caution, because all treated cells show at least some microtubules radiating from the NAO that may be sufficient for the establishment of polarity (cf. Fig. 4, C and F).

Regarding the arrangement of nucleus and NAO in the ameboid cells of *D. discoideum*, our data support the hypothesis that the centriole or equivalent organelles may be involved in coordinating cell movements as reported by Swanson and Taylor (34) for *Dictyostelium* amebae and by other investi-

gators for various cell types (2, 16, 25). However, a clear demonstration of the coordinating role of these structures is still lacking.

Our results can be summarized using a model of a motile ameba in which the relative locations of the nucleus-microtubular complex and the actomyosin system overlap (Fig. 7). A standing cell shows a fairly uniform distribution of actin and myosin with microtubules filling the cytoplasm, and the nucleus connected to them through the NAO (Fig. 7A). During locomotion, polymerized actin rapidly protrudes into the leading pseudopod (Fig. 7B). Although the actin displacement is probably due to its interaction with myosin, this phenomenon seems to occur only at the base of the pseudopod since little, if any myosin, can be detected in the advancing pseudopod. Microtubules also do not follow actin into in the leading pseudopod and seem not to be involved, even indirectly, in movement generation since, as indicated, microtubule-depleted amebae demonstrate an apparently normal locomotion. During movement, by forces generated in the cytoplasm, the nucleus appears to be driven either backward or forward. In normal cells the nucleus usually remains at-

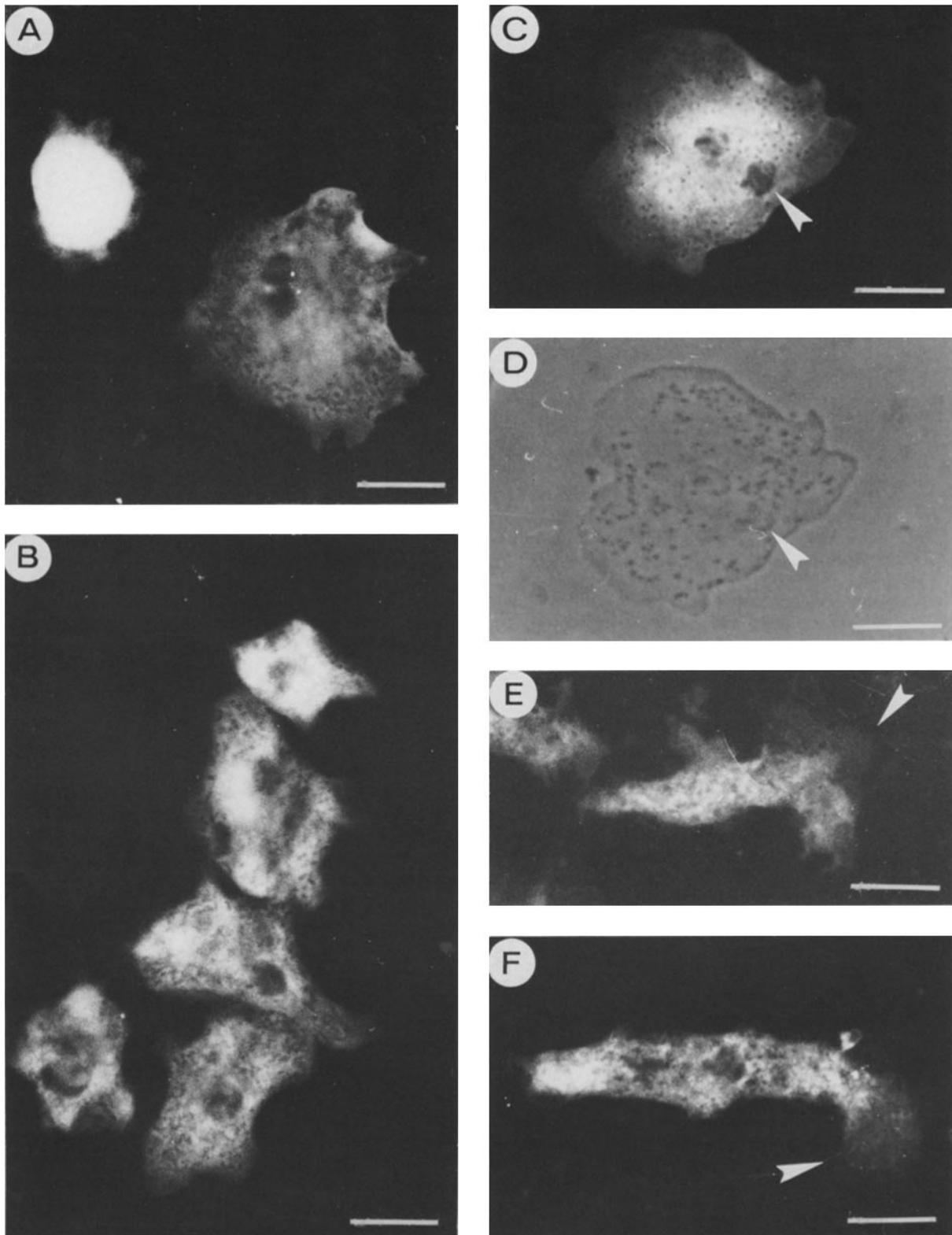


FIGURE 5 Fluorescent staining of *D. discoideum* amoebae with monospecific antimyosin antibodies. (A) Small unstretched cell (left) with bright fluorescence and a large flat cell with a diffuse "mottled" distribution of the stain. (C and D) The same cell is compared using fluorescence (C) and phase-contrast microscopy (D). Large unstained areas (arrow) probably correspond to nuclei while the small speckles correspond to cytoplasmic organelles (mitochondria?). (B, E, and F) $3',5'$ -cAMP activated cells; note the large frontal pseudopod (arrow) of locomoting amoebae in E and F, with almost complete absence of fluorescence. Direction of movement from left to right. Bars, 10 μ m.

tached at the center of the cell by its NAO which in turn remains fixed to the microtubule network. However, in cells in which microtubules have been destroyed by inhibitors, the

nucleus frequently falls to the posterior end (data not shown). Although connections between microtubules and cytoplasmic organelles are at present unidentified, indirect evidence for

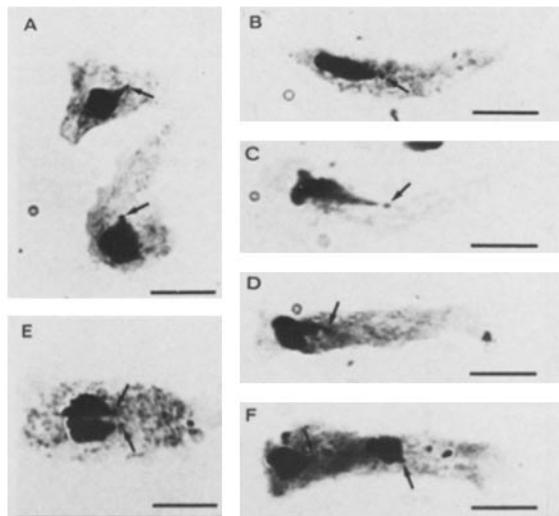


FIGURE 6 Giemsa staining of 3',5'-cAMP-stimulated amoebae in different stages of locomotion. Note the position of the nucleus and the NAO (arrows). (E and F) Two binucleated cells. Direction of movement from left to right. Bars, 10 μ m.

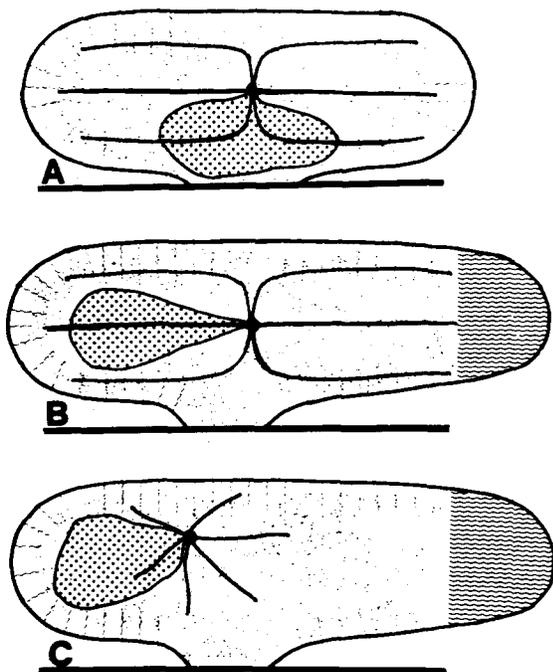


FIGURE 7 Schematic representation of cytoskeleton orientation during pseudopod emission in *D. discoideum*. (A) Resting amoeba, (B) pseudopod emission (left to right), (C) pseudopod emission in a microtubule-depleted amoeba. Large dots, nucleus; fine dots, myosin; bold intracellular lines, microtubules emanating from the NAO (large round spot); wavy lines (horizontal and vertical), actin.

their existence comes from the accumulation of cytoplasmic particles at the posterior end of colchicine-treated, locomoting amoebae (13). This model stresses the importance of the actomyosin system in cell motility and relegates to microtubules the limited role of anchorage of the nucleus and possibly other intracellular organelles in the cytoplasm, during amoeboid locomotion of *D. discoideum*.

Many thanks are due to Dr. Chandler Fulton and Dr. Vic Small for their much appreciated advice and suggestions, to Prof. Th. Wieland

for kindly providing the rhodamine-conjugated phallotoxin, and to Dr. J. Kilmartin for the generous gift of monoclonal antitubulin antibodies.

This work was supported by a grant of Ministry of Education.

Received for publication 24 June 1982, and in revised form 15 August 1983.

REFERENCES

- Albrecht-Buehler, G. 1979. The angular distribution of directional changes of guided 3T3 cells. *J. Cell Biol.* 80:53-60.
- Albrecht-Buehler, G., and A. Bushnell. 1979. The orientation of centrioles in migrating 3T3 cells. *Exp. Cell Res.* 120:111-118.
- Alcantara, F., and M. Monk. 1974. Signal propagation during aggregation in slime mould *Dictyostelium discoideum*. *J. Gen. Microbiol.* 85:321-334.
- Allen, R. D. 1961. Amoeboid movement. In *The Cell*. J. Brachet and A. E. Mirsky, editors. Academic Press, Inc., New York. 2:135-216.
- Brody, T., and K. L. Williams. 1974. Cytological analysis of the parasexual cycle in *Dictyostelium discoideum*. *J. Gen. Microbiol.* 82:317-338.
- Byers, H. R., and K. Fujiwara. 1982. Stress fibers in cells in situ: immunofluorescence visualization with antiactin, antimyosin, and anti-alpha actinin. *J. Cell Biol.* 93:804-811.
- Cappuccinelli, P., and J. M. Ashworth. 1976. The effect of inhibitors of microtubule and microfilament function on the cellular slime mould *Dictyostelium discoideum*. *Exp. Cell Res.* 103:387-393.
- Cappuccinelli, P., E. Unger, and S. Rubino. 1981. Immunofluorescence of microtubular structures during the cell cycle of *Dictyostelium discoideum*. *J. Gen. Microbiol.* 124:207-211.
- Cappuccinelli, P., S. Rubino, M. Fighetti, and E. Unger. 1982. Organization of the microtubular system in *Dictyostelium discoideum*. In *Microtubules in Microorganisms*. P. Cappuccinelli, and N. R. Morris, editors. Marcel Dekker, New York. 71-98.
- Clarke, M., and J. A. Spudich. 1974. Biochemical and structural studies of actomyosin-like proteins from non-muscle cells. Isolation and characterization of myosin from amoebae of *Dictyostelium discoideum*. *J. Mol. Biol.* 86:209-222.
- Clarke, M., G. Schatten, D. Mazia, and J. A. Spudich. 1975. Visualization of actin fibers associated with the cell membrane in amoebae of *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. USA.* 72:1758-1762.
- Eckert, B. S., and E. Lazarides. 1977. Localization of actin in *Dictyostelium* amoebae by immunofluorescence. *J. Cell Biol.* 77:714-721.
- Eckert, B. S., R. H. Waren, and R. W. Rubin. 1977. Structural and biochemical aspects of cell motility in amoebae of *Dictyostelium discoideum*. *J. Cell Biol.* 72:339-350.
- Fuller, G. M., B. R. Brinkley, and M. J. Boughter. 1975. Immunofluorescence of mitotic spindles by using monospecific antibody against bovine brain tubulin. *Science (Wash. DC)*. 187:948-950.
- Girbardt, M. 1979. Verteilung und experimentelle Beeinflussung cytoplasmatischer Mikrotubuli in der Spitzenzelle von *Trametes (= Polystictus) Versicolor*. In *System-Fungizide*. Abhandlungen der Akademie der Wissenschaften, Technik. H. Lyr and C. Polter, editors. Akademie Verlag, Berlin. 265-276.
- Gotlieb, A. I., L. McBurnie-May, L. Subramanyan, and V. I. Kalnins. 1981. Distribution of microtubule organizing centers in migrating sheets of endothelial cells. *J. Cell Biol.* 91:589-534.
- Heath, I. B. 1980. Variant mitoses in lower eukaryotes: indicators of the evolution of mitosis. *Int. Rev. Cytol.* 64:1-79.
- Herman, I. M., and T. D. Pollard. 1981. Electron microscopic localization of cytoplasmic myosin with ferritin-labeled antibodies. *J. Cell Biol.* 88:346-351.
- Herman, I. M., N. J. Crisona, and T. D. Pollard. 1981. Relation between cell activity and the distribution of cytoplasmic actin and myosin. *J. Cell Biol.* 90:84-91.
- Konijn, T. H., J. G. C. Vande Meene, J. T. Bonner, and D. S. Barkley. 1967. The acrasin activity of adenosine-3',5'-cyclic phosphate. *Proc. Natl. Acad. Sci. USA.* 58:1152-1154.
- Laemmli, V. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage 14. *Nature (Lond.)*. 227:680-685.
- Loomis, W. F. 1975. *Dictyostelium discoideum*. A developmental system. Academic Press, Inc., New York.
- Luna, E. J., V. M. Fowler, J. Swanson, D. Branton, and D. L. Taylor. 1981. A membrane cytoskeleton from *Dictyostelium discoideum*. I. Identification and partial characterization of an actin-binding activity. *J. Cell Biol.* 88:396-409.
- Maeda, Y., and G. Eguchi. 1977. Polarized structures of cell in the aggregating cellular slime mould *D. discoideum*. An electron microscope study. *Cell Struct. Funct.* 2:159-161.
- Malech, H. L., R. K. Root, and J. I. Gallin. 1977. Structural analysis of human neutrophil migration. Centriole, microtubule and microfilament orientation and function during chemotaxis. *J. Cell Biol.* 91:822-826.
- Mato, M. J., and T. M. Konijn. 1979. Chemosensory transduction in *Dictyostelium discoideum*. In *Biochemistry and Physiology of Protozoa*. M. Levandowski and S. H. Huntner, editors. Academic Press, Inc., New York. 181-219.
- O'Day, D. H., and A. J. Durston. 1978. Colchicine induces multiple axis formation and stalk cell differentiation in *Dictyostelium discoideum*. *J. Embryol. Exp. Morphol.* 47:195-206.
- Raper, K. B. 1940. Pseudoplasmodium formation and organization in *Dictyostelium discoideum*. *J. Elisha Mitchell Sci. Soc.* 56:241-282.
- Roos, U. P. 1982. Morphological and experimental studies on the cytocenter of cellular slime molds. In *Microtubules in Microorganisms*. P. Cappuccinelli and N. R. Morris, editors. Marcel Dekker, New York. 51-69.
- Rubino, S., E. Unger, G. Fogu, and P. Cappuccinelli. 1982. Effect of microtubule inhibitors on the tubulin system of *Dictyostelium discoideum*. *Z. Allg. Mikrobiol.* 22:127-131.
- Shaffer, B. M. 1964. Intracellular movement and locomotion of cellular slime mould amoebae. In *Primitive Motile Systems in Cell Biology*. R. D. Allen and N. Kaniva, editors. Academic Press, Inc., New York. 387-405.
- Spudich, J. A. 1974. Biochemical and structural studies of actomyosin like proteins from non-muscle cells. *J. Biol. Chem.* 249:6013-6022.
- Sussman, M. 1966. Biochemical and genetic methods in the study of cellular slime mold

- development. In *Methods in Cell Physiology*. Prescott, D. editor. Academic Press, Inc., New York, 2:397-410.
34. Swanson, J. A., and D. L. Taylor. 1982. Local and spatial coordinated movements in *Dictyostelium discoideum* amoebas during chemotaxis. *Cell*. 28:225-232.
35. Taylor, D. L., and J. S. Condeelis. 1979. Cytoplasmic structure and contractility in amoeboid cells. *Int. Rev. Cytol.* 56:57-144.
36. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. *Proc. Natl. Acad. Sci. USA*. 76:4350-4354.
37. Unger, E., S. Rubino, T. Weinert, and P. Cappuccinelli. 1979. Immunofluorescence of the tubulin system in cellular slime moulds. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett.* 6:317-320.
38. Valerius, N. H., O. Stendahl, J. M. Hartwig, and T. P. Stossel. 1981. Distribution of actin-binding protein and myosin in polymorphonuclear leukocytes during locomotion and phagocytosis. *Cell*. 24:195-202.
39. Watts, D. J., and J. M. Ashworth. 1970. Growth of myxamoebae of the cellular slime mould *Dictyostelium discoideum* in axenic culture. *Biochem. J.* 119:171-174.
40. Wulf, E., A. Deboen, F. A. Bautz, H. Faulstich, and Th. Wieland. 1979. Fluorescent phallotoxin, a tool for the visualization of cellular actin. *Proc. Natl. Acad. Sci. USA*. 76:4498-4502.