

Filopodelike Projections Induced with Dimethyl Sulfoxide and Their Relevance to Cellular Polarity in *Dictyostelium*

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ABSTRACT When 5% dimethyl sulfoxide (DMSO) was applied to *Dictyostelium* cells, the cells rounded up in shape and cytoplasmic streaming ceased. The cells resumed both cytoplasmic streaming and locomotion in 20 min. SDS PAGE of isolated plasma membrane fractions showed that actin and myosin apparently became dissociated from the plasma membrane by the action of DMSO.

Scanning electron microscopy revealed that many filopodelike projections formed on the surface of cells treated with 5% DMSO for 5 min. Interestingly, the projections were formed on a restricted portion of the cell surface. The phagokinetic track technique of Albrecht-Buehler (1977, *Cell*, 11: 395-404) showed that the projection region corresponded to the anterior part of a migrating cell.

The possible relationship between the DMSO-induced projection region on the cell surface and intracellular organization of cell organelles was investigated using serial thin sections. The DMSO-induced projections contained arrays of microfilaments; and the microtubule organizing center (MTOC), nucleus, and vesicular structure were usually located in this order from the anterior end of the cell. The indirect immunofluorescent study using monoclonal anti- α -tubulin antibody was performed with a new fixation technique, which greatly improved the phase as well as immunofluorescent microscopy. It was verified that the intracellular positioning of the MTOC and nucleus had significant correlation with the cell polarity.

The results show that DMSO is a powerful tool with which to manipulate the cellular microfilaments and to make visible the differentiation in the cortex layer, which apparently is relevant to the intracellular positioning of cell organelles and cell polarity.

Accumulative data have suggested that nonmuscle cell microfilaments are involved in a variety of cellular motile events such as the determination of cell morphology, cell movements, and cytoplasmic streaming (11, 31). The microfilaments should have some connection with the plasma membrane, assuming that the nonmuscle cell motile activities are regulated by a mechanism analogous to the skeletal muscle contraction system. Ultrastructural as well as biochemical evidence has shown that actin-containing microfilaments terminated at the plasma membrane, and actin and myosin can be isolated in association with plasma membrane preparations (9, 30). Recent studies documented the direct association of actin with the plasma membrane in *Dictyostelium* (7, 19, 22) and erythrocytes (14). These associations must play important roles in cell motility, cytokinesis, determination of cell shape, capping of surface receptors, and phagocytosis.

Previously, we have shown that DMSO acts on *Dictyostelium*

cells to dislocate the cortex microfilaments from the plasma membrane, resulting in cell rounding up and a cessation of cytoplasmic streaming (17). We have also revealed that 5% DMSO impedes the cytokinesis of this organism in a growing condition and that conspicuous multinuclear cells are produced (18). In this study, we used DMSO as a chemical agent to manipulate the microfilament system of the cortex layer to assess its possible roles in cell structure and motile activities. Prominent filopodelike projections containing microfilaments were formed on a restricted portion of the cell surface upon treatment with 5% DMSO for 2.5-5 min. Encouraged by this finding, we studied the relevance of the projection region to the polarity of the cells and found that this region corresponded to the anterior end of migrating cells. The relationship between this differentiation in the cell cortex and the polarized localization of intracellular organelles as well as the polarity of cell locomotion was also demonstrated.

MATERIALS AND METHODS

Cells: *Dictyostelium mucoroides* cells, strain Dm-7 (ATCC 42609), were cultured by liquid shake culture in 17 mM Na/K-Sørensen's phosphate buffer (pH 6.5) with *Escherichia coli* (B/r) at 22°C. The cells were harvested at the preaggregation stage by centrifugation (300 g, 1.5 min), washed and suspended in Bonner's salt solution (BSS) (6) containing 10 mM NaCl, 10 mM KCl, and 3 mM CaCl₂.

Phagokinetic Track Technique: The phagokinetic track technique of Albrecht-Buehler (2) was modified by using *E. coli* cells instead of colloidal gold particles. *E. coli* cells at the exponential growth phase were collected, washed, and suspended in BSS. A drop of the suspension was placed on poly-L-lysine-coated glass coverslips for 10 min. The excess water was removed, and the coverslips were rinsed with distilled water. Then a drop of the *D. mucoroides* cell suspension was put on the coverslips, and the cells were allowed to settle on the bacterial lawn. The coverslips were kept in a moist chamber at 22°C and periodically observed under a phase microscope. Under this condition, cells usually started to form tracks after 30 min.

Membrane Preparations: The membrane fraction was prepared according to Spudich (30), with slight modifications. Preaggregation cells were treated with 5% DMSO in BSS for 0 (control), 5, 15, and 30 min. Immediately after centrifugation, the cell pellet (~1 ml) was frozen in liquid nitrogen. Five milliliters of cold buffer (preparation buffer: 30% [wt/wt] sucrose, 100 mM KCl, 5 mM EGTA, 1 mM dithiothreitol [DTT], 0.1 mM phenylmethylsulfonyl fluoride [PMSF], and 0.1 mM ρ -tosyl-L-lysine chloromethyl ketone hydrochloride [TLCK] in 10 mM Tris-HCl, pH 7.6) was added to the frozen cells, and the cells were thawed at room temperature. This suspension contained plasma membrane ghosts of the disrupted cells, according to observations under a phase-contrast microscope. After two cycles of centrifugation (12,000 g, 40 min), the pellet was suspended in 2 ml of the cold preparation buffer and layered on top of a sucrose stepwise gradient (2.5 ml of 35% [wt/wt], 2.5 ml of 45% [wt/wt], and 2.5 ml of 55% [wt/wt] in the buffer). After centrifugation (12,000 g, 3 h), the 35–45% boundary was collected and suspended in 1 ml of the preparation buffer. Usually, this suspension contained ~2 mg/ml of protein associated with the plasma membrane.

SDS PAGE: The purified membrane preparations were boiled in SDS-sample buffer (20 mM Tris-HCl [pH 6.5], 2% SDS, 20% glycerol, 2% β -mercaptoethanol, and 0.01% bromophenol blue) for a few minutes. SDS PAGE was performed on 5–15% linear gradient polyacrylamide slab gels containing 0.1% SDS according to Laemmli (21). The densitometry was done with the gels stained with Coomassie Brilliant Blue R at 550 nm using a Gelman model DCD-16 densitometer. Protein concentrations were estimated by the method of Lowry et al. (23) after acid precipitation (5).

Two-dimensional (2-D) Gel Electrophoresis: The membrane preparations were solubilized by boiling for 2 min in 1% SDS and 5% β -mercaptoethanol, and then urea and ampholine (LKB, pH 3.5–10) and NP-40 were added to make 8.5 M, 1.6% and 8% (4) in concentrations, respectively. The isoelectric focusing electrophoresis (first dimension) was done using glass tubes (0.15 mm in diameter, 16 cm in length) according to O'Farrell (26). The first-dimension gels were equilibrated in the SDS-sample buffer, and the second dimension was done in 10% polyacrylamide slab gels and stained with Coomassie Brilliant Blue R.

Indirect Immunofluorescent Microscopy: To prepare aggregation centers, we harvested cells and allowed them to settle on a cover glass for 10–12 h at 22°C. A thin agarose sheet (0.15-mm thick made of 2% agarose in 15 mM Na/K-phosphate buffer, pH 6.5) was put on the cells. This "sandwich" was fixed in -20°C methanol for 5 min. After a brief rinse in PBS, mouse ascites fluid (1:500) containing monoclonal antibody against chick brain α -tubulin (kindly provided by Dr. Steve Blose, Cold Spring Harbor Laboratory, New York) was applied onto the agarose sheet and the preparation was incubated for 30 min at 36°C. The "sandwich" was rinsed with PBS for 45 min and treated with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG for 30 min at 36°C. After washing with PBS for 45 min, the agarose sheet was removed by agitating carefully in distilled water, and the stained cells on the cover glass were embedded in Gelvatol (28) and observed under an Olympus epifluorescence microscope (BH-RFL). All photomicrographs were taken with a $\times 100$ oil immersion lens.

Electron Microscopy: The cells were allowed to spread on Thermanox plastic cover slips (Lux Scientific Corporation, Newbury Park, CA) at a density of $1-2 \times 10^6$ cells/ml for 20 min at 20°C. Then the cells were treated with DMSO for various periods and fixed with a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde in 25 mM cacodylate buffer (pH 7.2) for 1 h (15). After postfixation with 1% OsO₄ in the buffer for 1 h, they were dehydrated through an ethanol series. For the SEM preparations, the samples were extracted with isoamylacetate and dried at critical point, then the gold-coated samples were observed under a Hitachi S-430 scanning electron microscope.

For the TEM preparations, the samples were embedded in Spurr's resin (Polysciences, Inc., Warrington, PA), and thin sections were observed under a JEM 100-C electron microscope after staining with uranyl acetate and lead citrate.

RESULTS

Filopodelike Projections Induced with DMSO

We previously reported the effect of DMSO in the dislocation of cortical microfilaments from the plasma membrane which eventually results in the rounding up of the cells (17). In the present study, the initial process of this effect of DMSO on *Dictyostelium* cells was carefully studied with special reference to the surface architecture and cytoplasmic streaming.

Under phase-contrast or differential interference phase microscopy, the cells treated with 5% DMSO formed numerous projections immediately upon treatment (arrows in Fig. 1b), and the cytoplasmic streaming, judging from the movement of the granules, abruptly ceased. By 5 min of treatment, the cells rounded up in shape and the projections free from the substratum shortened (small arrows in Fig. 1c). At the same time, the number of the projections had markedly decreased. The cells resumed their cytoplasmic streaming in 20 min, and soon after this formed motile filopodes as well as lobopodia (large arrow in Fig. 1f) and started to migrate.

Because of the small size (~7 μ m in diameter), neither small projections nor the fine surface architecture of the cells could be identified under the light microscopy. This led us to perform the SEM studies to find that dramatic changes occurred on the surface of cells treated with 5% DMSO. The untreated cells migrated on the substratum at ~400 μ m/h (18) forming several to tens of long filopodes (Fig. 2a), which apparently serve as

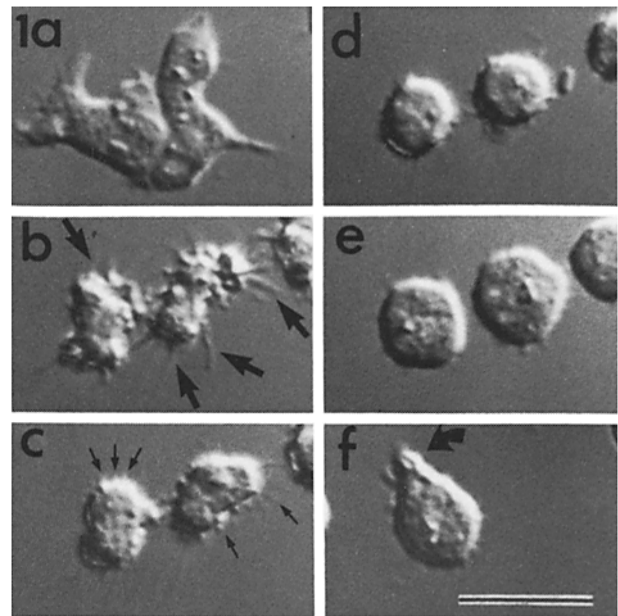


FIGURE 1 Differential interference micrographs showing the sequential changes in the morphology of *D. mucoroides* cells treated with 5% DMSO (same micrographic field). (a) Control cells, not treated with DMSO, (b) 1 min, (c) 2.5 min, (d) 5 min, (e) 30 min, and (f) 45 min after treatment. Note that thin projections formed immediately after the treatment (arrows in b), and these projections reduced in length at the same time with the rounding-up of the cells (small arrows in c). In 20–30 min, the cells resumed the cytoplasmic streaming and locomotion, and started to migrate forming a lobopodium (large arrow in f). Bar, 10 μ m. $\times 1,900$.

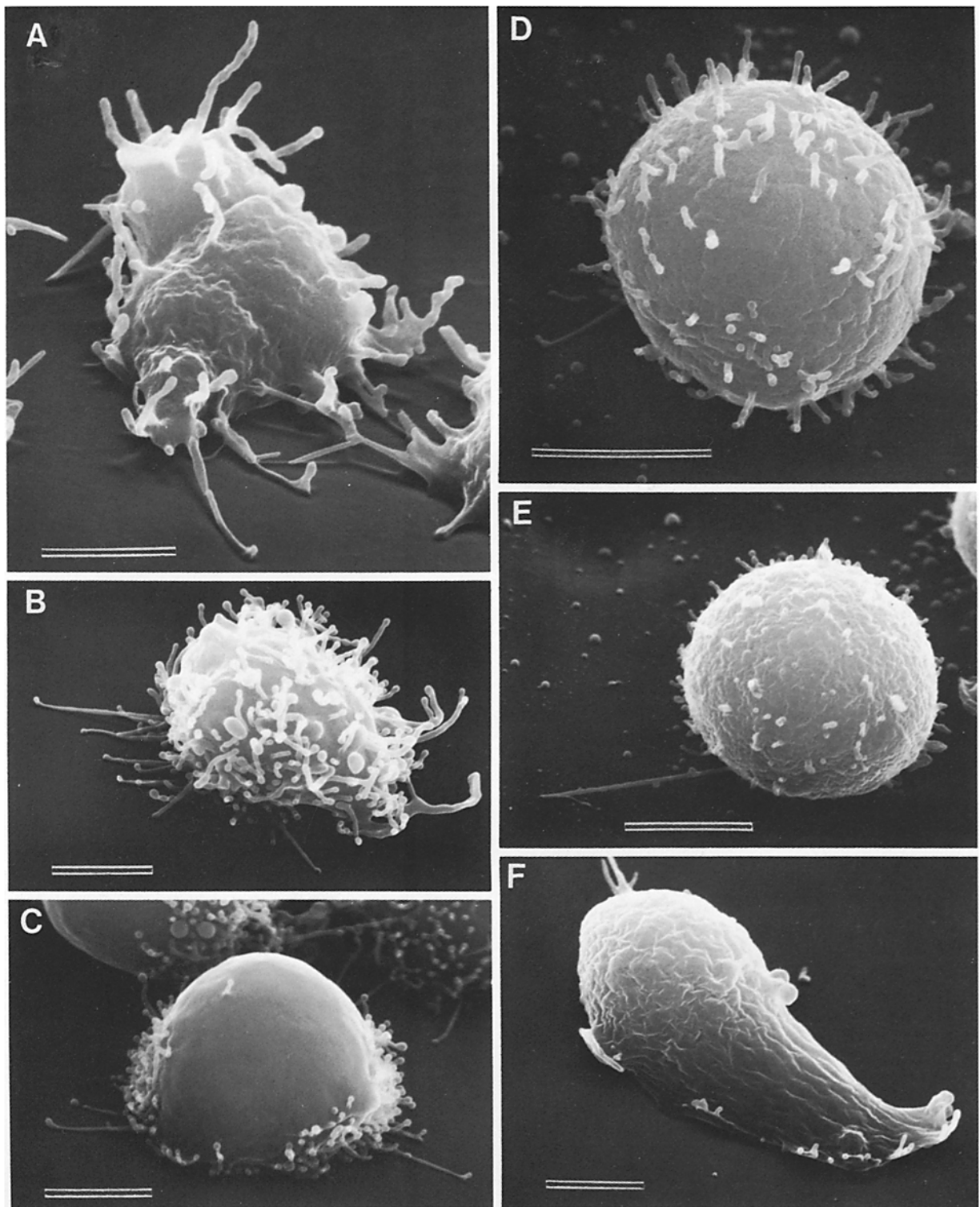


FIGURE 2 Scanning electron micrographs showing the sequential changes in the surface architecture of *D. mucoroides* cells treated with 5% DMSO. (a) A control cell on the plastic substratum, extending several to tens of long filopodia. (b) A cell treated for 2.5 min, showing numerous thin projections as well as a few blebs. (c) The cells treated for 5 min, showing the smooth architecture of the surface and the decrease in length of the projections. (d) A cell treated for 10 min, showing the rounding-up of the overall morphology and the decrease in length and number of the projections. (e) A cell 30 min after the treatment, showing that most projections had disappeared, and the surface displayed a wrinkled texture again. (f) A cell treated for 60 min. It extended a lobopodium and apparently started to migrate. Bar, 2.5 μm . Tilt angle: a, b, c, e, and f, 60°. d, 45°.

cellular exploring organs as suggested by Albrecht-Buehler (1). By 2.5 min of treatment, numerous thin projections as well as a few large blebs were formed and the surface became very smooth in texture (Fig. 2b). Between 5 and 10 min of treatment,

the extended long projections shortened and decreased in number (Fig. 2c and d). After 30 min, virtually all of the projections had disappeared and the surface displayed a wrinkled texture again (Fig. 2e). In 60 min, the cells had reformed

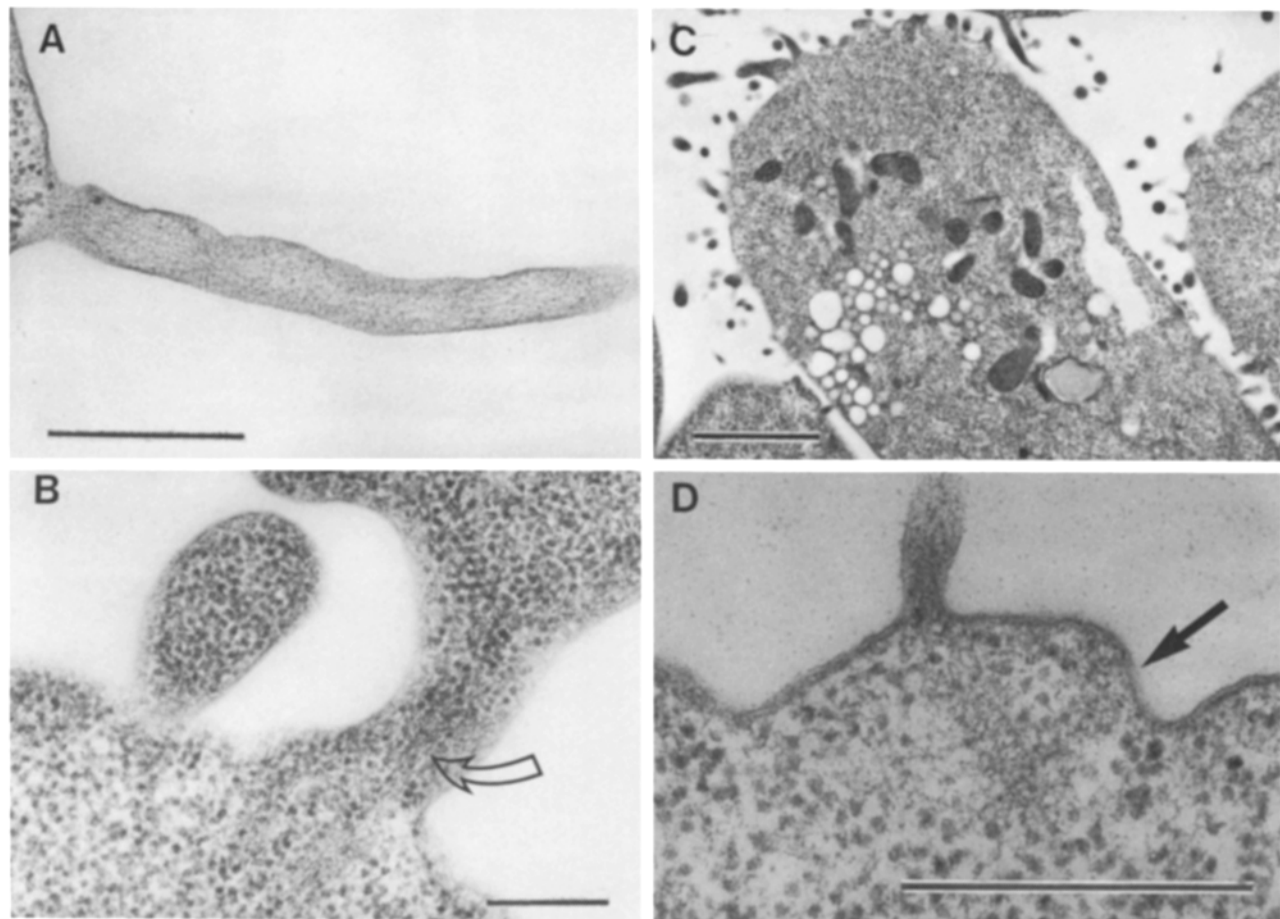


FIGURE 3 Transmission electron micrographs showing the fine structure of surface projections of control and DMSO-treated cells. (a) A filopodium of a control cell, showing arrays of microfilaments inside of it. (b) The DMSO-induced projections of the cell treated with 5% DMSO for 5 min. Note that the microfilaments, as well as numerous ribosomes, were involved in the projections (arrow). (c) A low-magnification micrograph of the cells treated with 5% DMSO for 5 min, showing many projections with high electron density formed on the surface. (d) A high magnification micrograph of the cortex layer of the cell treated for 30 min. Note that the cortex microfilaments returned to their location just beneath the plasma membrane and were apparently associated with the concavities of the membrane (arrow). Bar, (a, b, and d) 0.5 μm . Bar (c), 2 μm .

large lobopodia and looked as though they were in locomotion (Fig. 2f). Under a light microscope these cells could be clearly seen migrating. Interestingly, apparent exocytosis of vesicles as well as endocytosis of bacteria was prominent in cells which had regained their ability of locomotion, which was observed under a differential interference phase microscopy.

The DMSO-induced projections of cells treated for 5 min contained microfilament bundles similar to those observed in the native filopodes in thin sections (Fig. 3). The unique feature of the projections was that they contained numerous ribosomes (Fig. 3c). It should be noted that the cortex microfilaments running parallel to the plasma membrane could not be observed in these cells. Instead, in cells treated for 10 min, the microfilament bundles were located at the circumference of nuclei in the deep cytoplasm and surrounded most cell organelles, thus confirming the results of our previous study (17). After 30 min, the cortex microfilaments returned to their location just beneath the plasma membrane and were apparently associated with the concave surfaces of the membrane (Fig. 3d).

Dissociation of Microfilaments from the Plasma Membrane

To examine the effects of DMSO on the membrane-bound microfilaments, we performed the SDS PAGE analysis on isolated plasma membrane fractions. The 42 and 230 kdalton proteins obviously became dissociated from the membrane by treatment of the cells with 5% DMSO for 5–15 min (Fig. 4). The densitometry analysis showed that ~80% of the 42 kdalton protein was released from the membrane by the treatment with DMSO. These protein species most likely represented actin and myosin heavy chains, respectively, since they co-migrated with rabbit skeletal muscle actin and chicken gizzard myosin heavy chains; and both were dissociated from the membrane by Mg-ATP (data not shown), confirming the data of Spudich (30) and Condeelis (9). Furthermore, the 2-D gel electrophoresis (Fig. 5) showed that ~85% of the 42 kdalton protein had an isoelectric point identical to that of *Dictyostelium* actin (pI = 5.6) (8).

The amount of membrane-bound actin and myosin increased

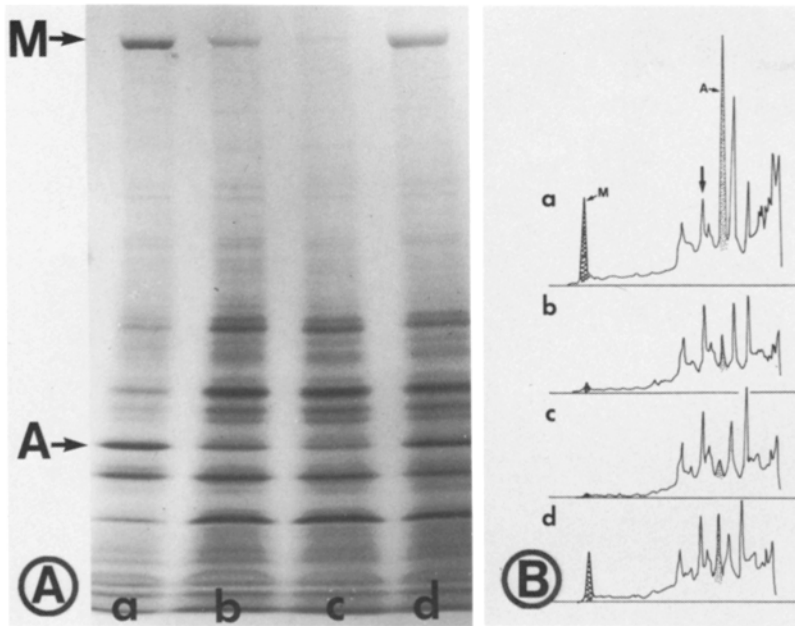


FIGURE 4 The SDS PAGE of *D. mucoroides* membrane fractions. [A] The membrane fractions were prepared according to Spudich (25), followed by the SDS PAGE on 5–15% linear gradient gels containing 0.1% SDS according to Laemmli (18). 50 μ g of the proteins was charged on each slot. The membranes were isolated from cells treated with 5% DMSO for (a) 0 min, (b) 5 min, (c) 15 min, and (d) 30 min. A, actin (42 kdaltons); B, myosin heavy chain (230 kdalton). [B] The densitometry traces of the gels in panel A, which were standardized by the surface glycoprotein peak (arrow). a–d represent the traces of the gels a–d in [A].

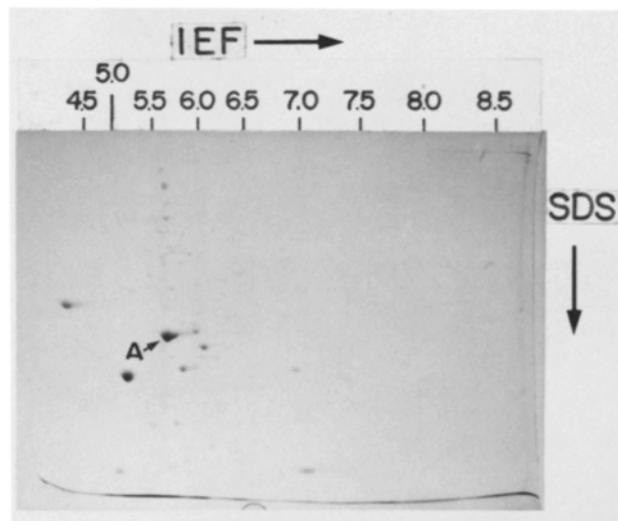


FIGURE 5 Two-dimensional gel electrophoresis of the membrane fraction from *D. mucoroides* cells. The membrane was solubilized with 1% SDS and analyzed by two-dimensional electrophoresis according to O'Farrell (26). The pH gradient of the first-dimension isoelectric focusing (IEF) is indicated at the top of the figure. The major spot (A) had an isoelectric point of \sim 5.6 and a mobility identical to that of the 42-kdalton protein, indicating that it was actin (8).

by the 30-min treatment with DMSO (Fig. 4d), supporting the data described above, namely, the cessation and resumption of the cytoplasmic streaming and the reversible changes in cell surface architecture. The application of DMSO to the membrane preparation was ineffective for dissociating actin and myosin from the plasma membrane (data not shown).

Restricted Localization of the DMSO-induced Projections and Its Correlation with Cellular Polarity

The most dramatic feature observed in cells treated with 5% DMSO for 2.5–5 min was that the filopodelike projections

formed in a restricted portion of the cell surface (Fig. 6). The SEM observation that a great number of the cells displayed such surface differentiation led us to speculate on the possible correlation of the projection region to cellular polarity. The finding that colloidal gold particles attached to the cell surface accumulated on the tail end of migrating cells (our unpublished observation) encouraged us to assess this idea by using the phagokinetic track technique of Albrecht-Buehler (2). In *Dic-tyostelium*, the application of *E. coli* cells, instead of go. particles, on coverslips provided the best results.

The cells engulfed the bacteria, digested them, and performed cell divisions forming branched phagokinetic tracks (Fig. 7a). In many cases, formation of the DMSO-induced projection region was observed on the head portion of a migrating cell (Fig. 7b). The apparently immotile cells which had no tracks sometimes formed the projections on their lower part facing the substratum as shown in Fig. 2c. The frequency of the formation of the projection region was quantitatively estimated in relation to the direction of cell locomotion and diagrammatically represented in Fig. 8. The Student's *t* test of the probability ($P < 0.01$) strongly suggested that the formation of the DMSO-induced projection region at the head portion of the cells was significant.

DMSO-induced Projections and Intracellular Polarity

The possible correlation between the projection region and the direction of cell locomotion next led us to study the relevance of surface topography to the intracellular polarity of cell organelles. Seven migrating cells were studied as serial thin sections, and the three-dimensional structure was reconstituted from 65–80 serial thin sections 100-nm thick. In five out of the seven sections, the DMSO-induced projection region, MTOC (20), nucleus and vesicular structures were obviously located in this order from the anterior end of the cell (Fig. 9). The evidence suggested that the intracellular polarity represented by the positioning of the MTOC, nucleus, and vesicular structures also has a positive correlation with the direction of cell locomotion.

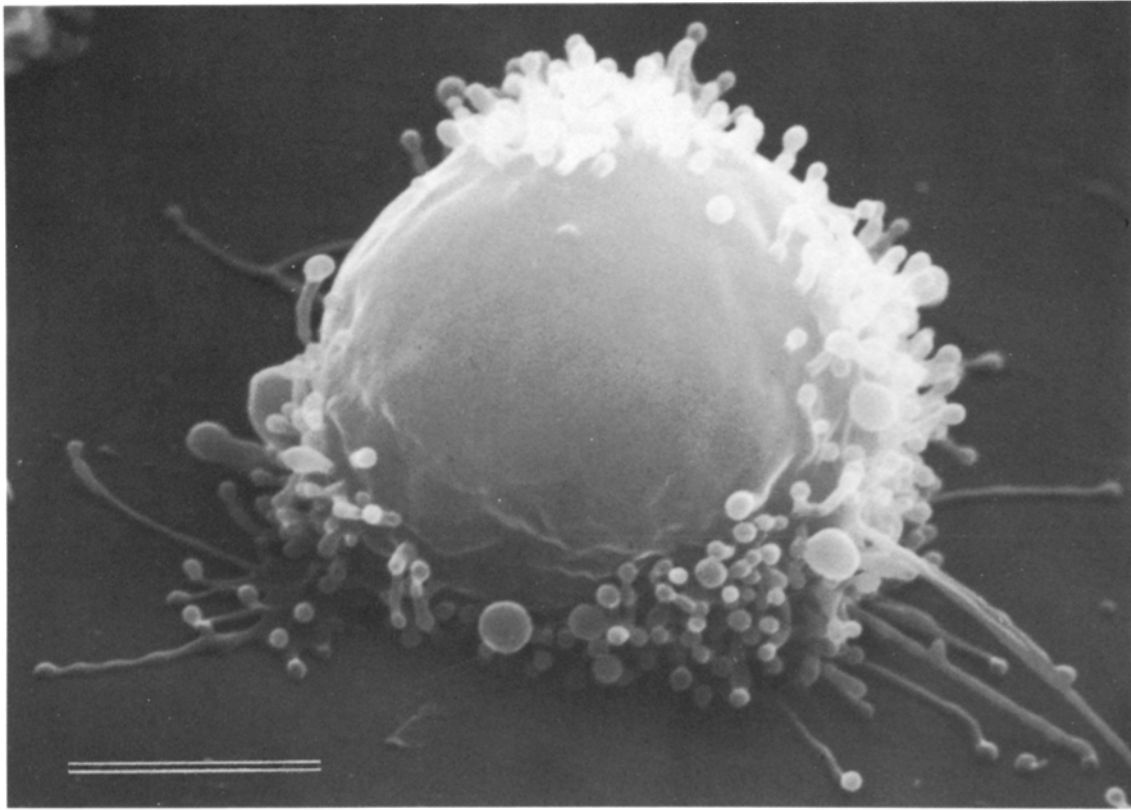


FIGURE 6 A scanning electron micrograph of a cell treated with 5% DMSO for 5 min at 20°C. Note that the filopodlike projections were induced to form on a restricted region of the cell surface. It is also noteworthy that the surface architecture is very smooth on the portion excluded from the projections. Bar, 2.5 μm .

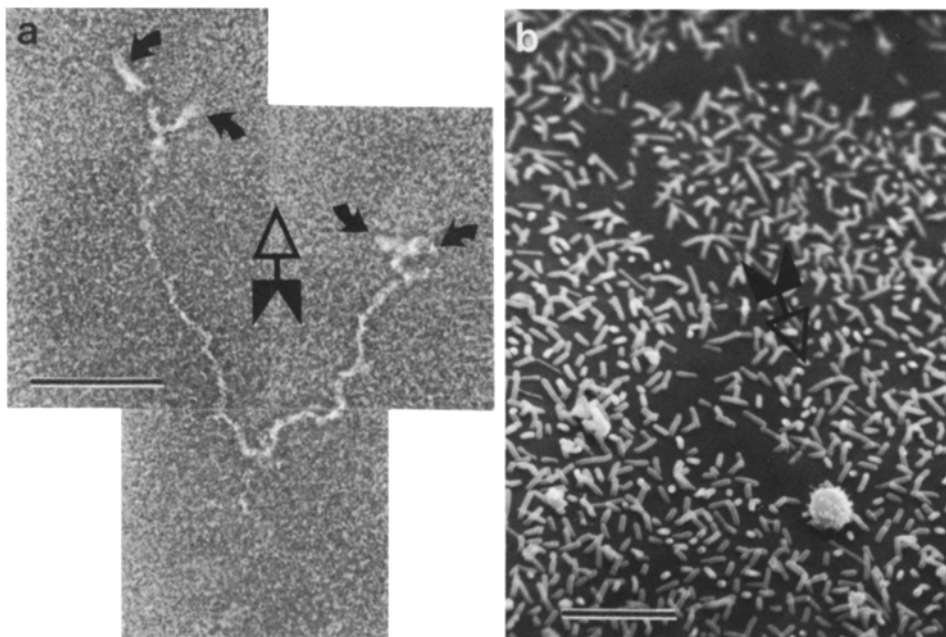


FIGURE 7 The phagokinetic tracks of *D. mucoroides* cells. (a) A composite phase-contrast micrograph showing a branched track representing two successive cytokineses, of producing four daughter cells (small arrows). The large arrow indicates the direction of the movement of the cells. Bar, 50 μm . (b) A scanning electron micrograph of a migrating cell treated with 5% DMSO for 5 min subsequent to the formation of the phagokinetic track. It is apparent that the projections were primarily induced to form on the anterior end of the cell. Tilt angle, 60°. Bar, 10 μm .

To assess the possible correlation between the direction of cell locomotion and the organization of intracellular organelles, we performed the quantitative study using indirect immunofluorescence on aggregating cells (Fig. 10). At this stage of devel-

opment, the cells migrate to the aggregation center forming streams, and we can inevitably recognize their polarity of locomotion. Furthermore, the "sandwich" method greatly improved the fixation of cells and made it possible to recognize

MTOC's as well as mitochondria under phase-contrast microscopy. By comparing the phase and immunofluorescent micrographs, the positions of MTOC's and nuclei could be clearly determined in the total 693 cells. The frequency that the MTOC's were present in the anterior side of the nuclei was 52.5% and the probability ($P < 0.01$) of the Student's t test suggested that the specific localization was very significant

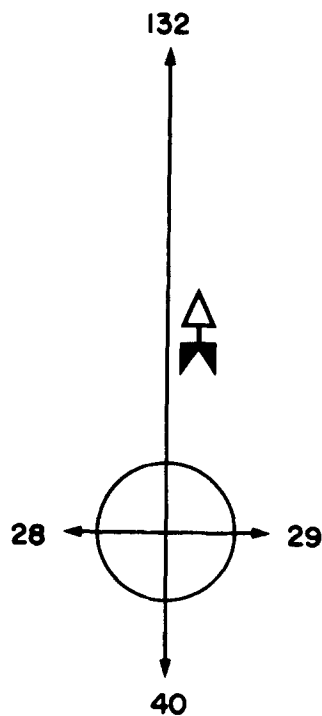


FIGURE 8 The diagram showing the frequency of the formation of the DMSO-induced projection regions with reference to the direction of cell locomotion. The frequency was estimated according to the SEM observations. The large arrow indicates the direction of cell migration, and the numbers represent the frequency at which the DMSO-induced projection regions formed on each side of the cells. The Student t test shows that the probability of the formation of the projection region at the anterior end of the cells is significant ($P < 0.01$).

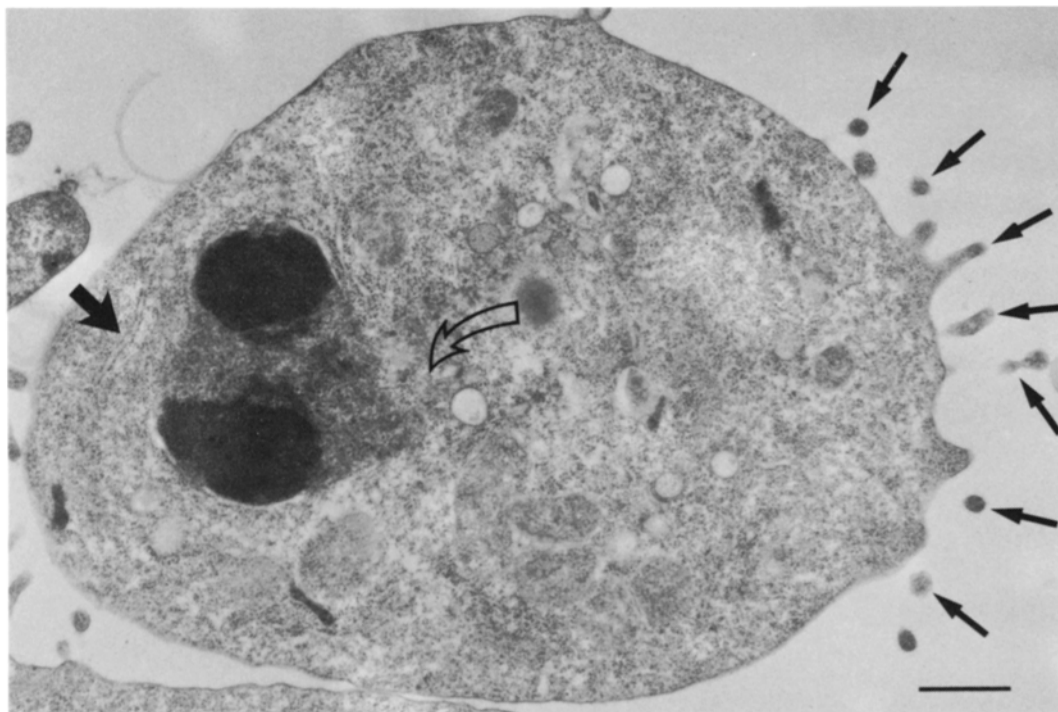


FIGURE 9 A transmission electron micrograph of one of the 65 serial thin sections ~ 100 -nm thick, showing the spatial distribution of the DMSO-induced projections (small arrows), the MTOC (open arrow), nucleus, and membrane system (large arrow). The cell was treated with 5% DMSO for 5 min before the fixation. The location of the MTOC could be determined by the presence of the electron-dense nuclear associated body (20) in the neighboring section. Bar, 0.5 μm .

(Fig. 10a). Thus, the above data, side by side with those of the phagokinetic track experiment, showed that the surface topography has some correlation with the intracellular positioning of MTOC and nucleus in relation to the cell polarity.

DISCUSSION

Since finding the prominent effect of DMSO of inducing nuclear actin bundle formation (15, 16), we have performed some studies on cell motility by using this agent as a chemical probe with which to manipulate the cellular microfilaments (17, 18). Our preliminary finding that the cortical microfilaments became dislocated from the plasma membrane by the action of DMSO encouraged us to do the present study to gain insight into the supramolecular mechanism of cell motile activities. Thus we started on SDS PAGE studies of the purified plasma membrane fractions and found that the major microfilament components, actin and myosin, became dissociated from the plasma membrane upon treatment of the cells with DMSO (Fig. 4).

Treatment with 5% DMSO for a short period (2.5–5 min) caused the cells to round up and induced the formation of filopodelike projections on the surface. In these cells, the two different classes of cortical microfilaments could be obviously seen in thin sections: those of the first class had become dissociated from the plasma membrane and formed circumferential bundles inside the deep cytoplasm (we designate these class I microfilaments), whereas those of the other class remained at the membrane and were eventually involved in the induced projections (class II). Treatment of the cells with NaN_3 or DNP further provided evidence for the presence of the two classes of microfilaments. With pretreatment with one of these respiratory inhibitors, no projections were formed in the presence of DMSO, whereas the class I microfilaments inevitably

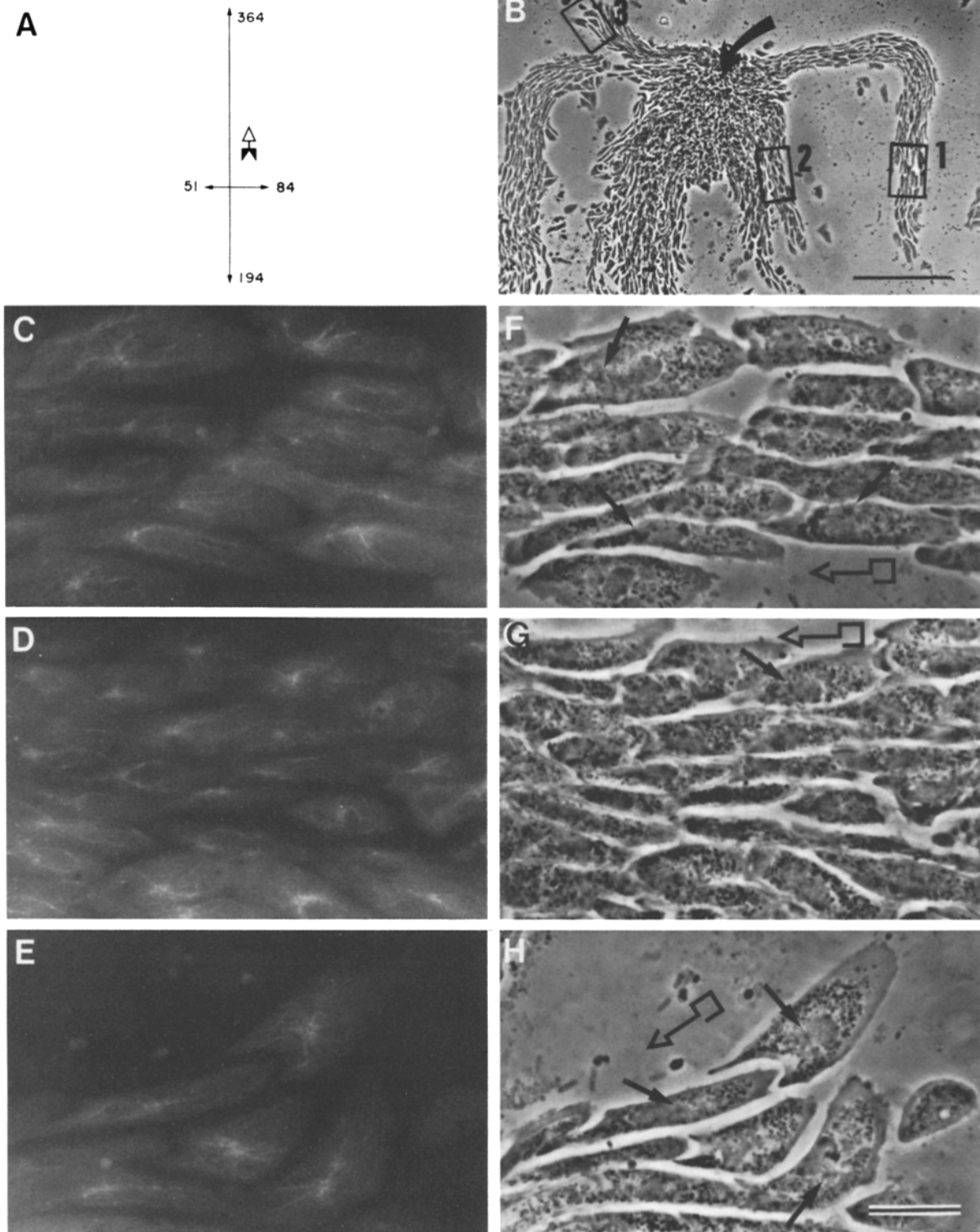


FIGURE 10 (a) The diagram showing the frequency of the position of MTOC with reference to that of nucleus. A total of 693 cells was investigated, and the Student *t* test ($P < 0.01$) indicated that the positioning of the MTOC in the anterior side of the nucleus was very significant. (b) A low-magnification phase-contrast micrograph of aggregating cells prepared for indirect immunofluorescence by the "sandwich" method described in the text. The cells were migrating toward the center of the aggregate (arrow). Bar, 50 μm . (b1, b4), (b2, b5), (b3, b6), The pairs of immunofluorescence and phase-contrast micrographs of the aggregating cells corresponding to those enclosed by the frames 1, 2, and 3 in *b*. The large arrows indicate the direction of cell migration, and the small arrows show the dark spots obviously indicating the MTOC's. Bar, 5 μm .

became dislocated from the plasma membrane (our unpublished data). These results suggested to us that the possible actin-myosin interaction in the formation of the filopodelike projections might be dependent on intracellular ATP. In rela-

tion to this, the requirement of ATP in the contraction of isolated intestinal epithelial microvilli (25) is very much suggestive to us.

Recently, Sanger et al. (29) and Osborn and Weber (27)

have reported that stress fiber microfilaments are dissolved and translocated into nuclei resulting in the formation of nuclear actin bundles in DMSO-treated PtK2 cells. *Dictyostelium* is an amoeboid cell which has only a primitive motile apparatus compared to those of mammalian cells and has neither stress fibers nor intermediate filaments. This implies that the effects of DMSO may differ in these two cell types. Actually, until now, we have found no evidence suggesting the overall dissolution of the cytoplasmic microfilaments by the action of DMSO in *Dictyostelium*. Instead, the ultrastructural studies have shown that the cortical microfilaments become dislocated from the plasma membrane by the treatment with DMSO (17). An indirect immunofluorescent study has also suggested the dislocation of these cortical microfilaments to form circumferential bundles of actin filaments inside the deep cytoplasm (Taki and Fukui, manuscript in preparation). We realize that this evidence does not deny the possible dissolution of some of the cortical microfilaments and their translocation into nuclei to form bundles. Such dynamic translocation of actin must usually occur between the cytoplasm and the nucleus, as shown by DeRobertis et al. (10), and might be reinforced by the action of DMSO.

The present study showed that resumption of the cytoplasmic streaming occurred concomitant with the shortening of the DMSO-induced projections 20 min after the treatment. This temporal identity suggests that class I and class II microfilaments regained their association with each other and that some of their functions could be recovered by the reorganization of the cortical microfilament system. The cells resumed their cytoplasmic streaming, cytokinesis, and locomotion in 30 min. However, this recovery was not free of defects; incomplete cytokinesis resulting in prominent multinuclear cells during long-term incubation of cells in 5% DMSO has been reported (18).

This study showed that the DMSO-induced projections were formed in a restricted region of the cell surface. The uneven distribution of the projections probably did not result from their lateral movement, because our study demonstrated that the capping of surface receptors was reversibly inhibited by DMSO (13). Thus, the unevenness seems to reflect an *in situ* distribution of the actin-containing microfilament system on the plasma membrane. This result was not unexpected since a local increase in the amount of actin at the leading edge of a migrating cell has been supported by Eckert and Lazarides (12). This presumption was supported by the results of the "phagokinetic track" experiment, which verified the obvious positive correlation between the projection region and the direction of cell movement. Furthermore, the differential distribution of the projections could be observed in suspended nonmotile cells. This observation encouraged us to see whether the projection region has any correlation with the intracellular polarity of cell organelles, which has been demonstrated to have significant correlation with cell polarity (3, 24). Using serial thin sections, we found that the projection region, MTOC, and nucleus were arranged in this order from the anterior end of the cell. Furthermore, the Student's *t* test on the immunofluorescent study showed that the positioning of the MTOC in front of the nucleus was very significant in relation to cell polarity.

We recognize that we cannot tell any causal relationship between the intracellular polarity and the direction of cell movement simply because of their topographical correlations. However, we believe that the intracellular organization of the

motile apparatus should be studied as the first step in assessing the significance of cellular behavior in normal development of the organism. The mechanism for transmitting extracellular information into the cell to determine the direction of movement remains to be elucidated.

We are greatly indebted to Prof. H. Shibaoka for his encouragement during the course of this study, and to Prof. T. Hara for his generosity in allowing us to use the Hitachi S-430 scanning electron microscope. Our special thanks are due to Dr. S. Blose of the Cold Spring Harbor Laboratory for kindly providing monoclonal anti- α -tubulin antibody. We are also grateful to Dr. M. Ikebe and Mr. K. Furukawa for kindly providing chicken gizzard myosin and rabbit skeletal muscle actin for protein standard.

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