DYNAMICS OF NUCLEAR ACTIN BUNDLE INDUCTION BY DIMETHYL SULFOXIDE AND FACTORS AFFECTING ITS DEVELOPMENT

YOSHIO FUKUI and HIRONOBU KATSUMARU

From the Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

ABSTRACT

We have reported in a previous paper that dimethyl sulfoxide (DMSO) induces the formation of huge bundles of actin filaments in the nuclei of *Dictyostelium mucoroides*. The present study was performed to provide electron microscope data on the induction of nuclear actin bundles, illustrating both their formation and their reversion, as well as on the effects of various factors on the induction.

The large nuclear bundles of actin appeared after 20–30 min of treatment with 10% DMSO. A DMSO concentration of 5 or 10% was optimal for the induction of the bundles. The nuclear actin bundle reverted to the original morphology within 5 min after removing DMSO. Induction of nuclear actin bundles was inhibited by Mg^{++} and low temperatures, but not by Na^{+}, K^{+}, Ca^{++}, ATP, 3′,5′-cyclic adenosine monophosphate (cAMP), phosphate buffer, or cytochalasin B. Neither NaN_3 nor cycloheximide totally inhibited the induction of the bundles.

KEY WORDS actin bundles in nucleus · cortical microfilaments · *Dictyostelium mucoroides* · DMSO induction of actin bundles

Cellular actin is a major contractile protein which may be involved in the contractile as well as cytoskeletal functions in nonmuscle cell cytoplasm (5, 28). In *Dictyostelium*, the actin is similar to skeletal muscle actin, and possible supramolecular forms of this actin were demonstrated (7, 34, 35). *Dictyostelium* actin seems to be involved with contraction of cell extracts (6), and structural studies (9, 10) suggest a role for actin in cell motility.

Ryser (31) was the first to find the involvement of microfilaments in the cell nucleus, and he speculated that those nuclear microfilaments might be contractile. There have been a few studies demonstrating the occurrence of microfilaments in the dividing nuclei of various organisms, i.e., in the oömycete fungus *Achyla* by Ellizey et al. (11), in the alga *Cryptomonas* by Oakley and Heath (26), in the higher plant *Haemanthus* by Forer and Jackson (13, 14), in crane fly testis by Behnke et al. (2), in locust testis by Gawadi (18), in rat kangaroo cells by Sanger (32), in mouse neuroblastoma cells by Hinkley and Telser (20), and in human HeLa cells by Herman and Pollard (19). There is less evidence for the existence of microfilaments in interphase nuclei (1, 29), possibly because of the difficulties in fixation and/or permeability of antibodies through the nuclear envelope. There is biochemical evidence that actin is a component of the nonhistone proteins of chromatin, i.e., LeStourgeon et al. (23) in *Physarum*, Pederson (27) in *Dictyostelium*, and Douvas et al. (8) in rat liver nuclei. We have found that dimethyl sulfoxide (DMSO) induces the formation of microfilament bundles of actin in the interphase

nucleus of *Dictyostelium* (16). We further demonstrated that the induction of the nuclear actin bundles also occurred in *Amoeba proteus* and human HeLa cells (17), and *Tetrahymena pyriformis* and Friend's leukemic cells of mouse (Katsumaru and Fukui, manuscript in preparation). Together, these findings, using different methods, provide conclusive evidence for the existence of actin molecules in cell nucleus in a variety of organisms.

There has been some speculation on the possible function of the nuclear actin. Forer (12) proposed that nuclear microfilaments might be involved in the chromosome-separation, side by side with microtubules of the spindle bodies. The possible involvement of microfilaments in the movement of chromosomes to the poles during mitosis was also suggested in rat kangaroo cells by Sanger and Sanger (33) and by Cande et al. (4). Another major suggestion is that actin might function in the regulation of gene transcription and/or replication, by virtue of its contractile nature (22). Finally, we have proposed that nuclear actin might play essential roles in diverse functions, such as the maintenance of nuclear structure, changes in nuclear shape, and possibly the alteration of the structure of chromatin (16). For an ultimate understanding of the function of nuclear actin it would be desirable to study its chemical properties in terms of the dynamics of the molecules in association with cofactor proteins.

In the present study, we found that the induced nuclear actin bundles are preceded by changes in the structure of nuclear matrix material, and that factors such as Mg^{++}, low temperatures, and metabolic inhibitors can modify the effect of DMSO on nuclear shape and the bundling of the nuclear microfilaments.

MATERIALS AND METHODS

Cells and Cultures

Spores of *D. mucoroides*, strain Dm-7, were incubated at 22°C on nutrient agar plates (3) with *Escherichia coli* (B/r). After the bacterial food supply had been exhausted, the cells were washed with Bonner's salt solution (3) containing 10 mM NaCl, 10 mM KCl, and 3 mM $CaCl_2$. Then the cells were either spread on nonnutrient agar plates to obtain cells at various stages of development, or suspended in the salt solution in liquid shake culture to obtain nascent macrocysts (15).

Treatment with DMSO

Cells were harvested, washed with the salt solution by centrifugation (150 *g*), and suspended in DMSO solution. The cell suspension (4.5 ml) was placed in a plastic Petri dish (6 cm in diameter), and the dishes were kept at 22°C.

Viability Test

Cells which had been treated with DMSO were washed with the salt solution, and 100 cells were inoculated on an agar plate with the bacteria. The numbers of plaques were counted under a dissecting microscope after 2 d. The survival rate was estimated by the ratio of the number of plaques of treated to untreated samples.

Effects of DMSO on the Development

Cells were harvested and suspended at a density of 10^8 cells/ml in salt solution containing various concentrations of DMSO. A drop (0.5 ml) of cell suspension was placed evenly on a black Millipore filter (AABG 047 SO) which was placed on an absorbent pad (AP 10047 SO) saturated with buffered salt solution (25) containing 20 mM KCl, 2.5 mM $MgCl_2$, 10 mM Na/K-phosphate buffer (pH 6.4), and DMSO at various concentrations. The cells were incubated at 22°C in the dark, and their development was examined under a dissecting microscope.

Isolation of Nuclei

Nuclei were isolated according to the method of Pederson (27) with a modification that spermidine (1 mM) was added to the lysis buffer. The final pellet of nuclei was treated with 10% DMSO in salt solution, or placed in 10 mM Tris-HCl, pH 7.2, and 50 mM KCl, or was immediately fixed.

Glycerination and Heavy Meromyosin Binding

Cells were subjected to glycerination and binding with rabbit skeletal muscle heavy meromyosin (HMM) according to the method of Ishikawa et al. (21) with a modification as described in Fukui (16). Finally the glycerinated cells were rinsed with the ice-cold H-solution (50 mM KCl, 5 mM EGTA, and 10 mM Tris-HCl, pH 7.0) for 20 min and then treated with 10% DMSO in Bonner's salt solution (3) for 30 min at 20°C, or were immediately fixed.

Electron Microscopy

Cells were fixed with a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde in ice-cold 25 mM Na-cacodylate buffer (pH 7.2) for 1 h. After being washed with the ice-cold buffer for 1 h, the cells were postfixed with 1% OsO_4 in the buffer for 1 h at 4°C. Then the cells were washed with the cold buffer for 2 h, and were subjected to dehydration through an ice-cold ethanol series. The preparation in the final absolute alcohol was transferred to room temperature, propylene oxide was substituted for the alcohol, and then the sample was infiltrated with Spurr's low viscosity embedding medium. The resin was cured for 20 h at 70°C. Thin sections were stained for 30 min with 25% uranyl acetate in methanol, followed by a 10-min staining with Reynolds' lead citrate. Electron micrographs were taken under a JEM-100 C electron microscope at 80 kV.

Chemicals

Cytochalasin B (Aldrich Chemicals Co., Inc., Milwaukee, Wisc.); glutaraldehyde (Ladd Research Industries, Inc., Burlington, Ver.); OsO_4 (Merck and Co., Ltd., Rahway, N. J.); DMSO (Nakarai Chemicals, Kyoto, Japan, or Merck); Spurr's resin (Polyscience, Inc., Warrington, Penn.); spermidine trihydrochloride (Sigma Chemical Co., St. Louis, Mo.); paraformaldehyde (Taab

Laboratories, Reading, Scotland); dimethyl acetamide and tetramethyl urea (Wako Pure Chemicals Co., Ltd., Osaka, Japan) were used.

RESULTS

Effects of DMSO on Survival and Aggregation

When *Dictyostelium* cells were treated with 10% DMSO in Bonner's salt solution, they rounded up and did not move. The survival of cells treated with DMSO decreased exponentially, resulting in the death of 50% of the cells in 3 h. Nearly 80% of the cells, however, were alive after the treatment with DMSO for 30 min; under those conditions, the large bundles of nuclear actin were induced in 50–75% of the cells.

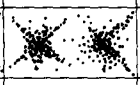
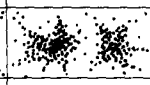
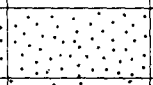
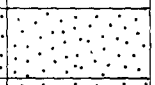
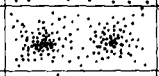
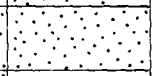
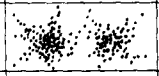
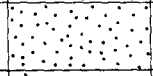
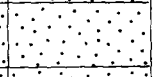
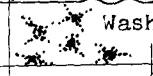
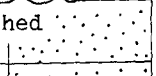
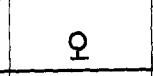
As shown in Table I, when 5% DMSO was applied the cells could not aggregate at all. At lower concentrations, DMSO delayed aggregation and migration.

Effects of DMSO on Cytoplasmic Microfilaments

The postvegetative cells suspended in Bonner's salt solution are very adhesive to each other and to the substratum. The average diameter of cells was 8.1 μm and each cell had thin hyaline layer in the cortex. In 10% DMSO in the salt solution, the cells lost their adhesiveness. The most prominent change observed under a transmission electron microscope was that the average cell diameter increased to 10 μm, and an empty space appeared between the plasma membrane and cytoplasmic microfilaments. This suggested to us that the cy-

TABLE I

Effects of DMSO on the Development of D. mucoroides

DMSO / Hour	0 %	0.5 %	1.0 %	2.5 %	5.0 %	10 %
3.0 h						
4.5 h						
6.0 h						
8.0 h						
24.0 h						
30.0 h						
48.0 h						
Inhibition of Aggregation	-	++	++	++++	+++++	Toxic Effect
Inhibition of Migration	-	++	++	++++	Not Tested	Not Tested
Inhibition of Fruiting	-	+	+	++++	Not Tested	Not Tested

Cells were inoculated on black Millipore filter paper saturated with the lower pad solution of Newell et al. (26) containing various concentrations of DMSO, and the development of the cells was observed under a dissecting microscope. The illustrations show stages in the development and should be read from top to bottom.

toplasmic microfilaments were dislocated from the plasma membrane. Evidence for this possibility was obtained from study of glycerinated cells. In control cells, the cytoplasmic microfilaments were located just beneath the plasma membrane (Fig. 1 *a*), while in cells treated with 10% DMSO for 30 min before the glycerination, the cortex microfilaments were obviously dislocated from the plasma membrane by the action of DMSO (Fig. 1 *b*). Nuclear microfilament bundles could be induced in 50–75% of the cells treated with 10% DMSO for 30 min before the glycerination, as reported in our previous paper (16). We show such nuclear bundles of microfilaments to be compared with the cytoplasmic microfilaments (Fig. 1 *c* and *d*).

Dynamics of the Nuclear Bundle Induction

Drastic changes in the nucleus occurred during the treatment with 5 or 10% DMSO in the salt solution. The most prominent change occurring during the first 5 min was that the electron density of the nuclear matrix decreased (Fig. 2 *b*). This change was followed during the next 5 min by the formation of the meshwork-like structure of microfilaments (Fig. 2 *c* and *e*). This meshwork structure turned into microfilament bundles in the next 10 min (Fig. 2 *d*), and the bundles reached their maximal size within 30 min in DMSO (Fig. 2 *e*). No further development of the bundles could be induced by continuation of the DMSO treatment, and the cells gradually changed in their fine structure as if they were degenerating. We could not find any specific interaction between the bundles and nucleoli.

No structural changes were apparent after the treatment with 0.5 or 1.0% DMSO. Changes in the electron density of nuclear matrix materials were observed after the treatment with 2.5% DMSO resulting in the occurrence of electron-transparent regions in the nucleus. However, nuclear shape was still irregular and no microfilaments were induced.

We found that virtually all of the cellular structures reverted to their original morphology within 5 min after washing with the salt solution, and the cells recovered their ability of adhesion as well as locomotion (Fig. 3). Furthermore, the nuclear bundles had disappeared, and the normal electron-opaque amorphous structure of the nuclear matrix returned.

Effects of Various Factors on the Induction of Nuclear Actin Bundles

Bundles could be induced in cells treated with DMSO diluted with distilled water, but the induction was most prominent when cells were treated with DMSO dissolved in Bonner's salt solution. Neither Na^+ (20 mM), K^+ (20 mM), Ca^{++} (3 mM), ATP (5 mM), cAMP (5 mM), nor phosphate buffer (10 mM, pH 6.4) affected the efficiency of the induction; in all cases the bundles were induced in >50% of the cells. It was most interesting that the frequency as well as the size of the bundles could be reduced by the administration of magnesium ions (2.5 or 5 mM).

The nuclear bundle formation was not blocked by cytochalasin B (10 mM), although microfilament bundles in the cytoplasmic cortex layer were disorganized and formed abundant bird's nest-like aggregates of microfilaments. When the cells were treated with DMSO at 0° or 10°C, the meshwork-like structure of the nuclear microfilaments similar to that of the early stage of the formation (Fig. 2 *c*) was induced within 30 min. The bundling of the microfilaments, however, was totally inhibited by the low temperatures.

The effects of NaN_3 (20 mM) and cycloheximide (250 μg/ml) were indistinguishable, and quite different from the effects of low temperatures. When cells were pretreated with the inhibitors, and then treated with the inhibitors and DMSO at the same time, the bundles could be induced in the nucleus although their size and their frequency was smaller than for those treated with DMSO alone. A significant morphological feature of such cells was that the nuclear shape remained as irregular as in the control cells (Fig. 4).

Effects of DMSO on Glycerinated Cells and Isolated Nuclei

DMSO did not induce nuclear bundles in glycerinated cells or isolated nuclei.

The treatment of *Dictyostelium* cells with other aprotic solvents, such as dimethyl acetamide (DMAc) and tetramethyl urea (TMU), also failed to induce nuclear bundles. Both DMAc and TMU were highly toxic to the cells at concentrations as low as 0.1%.

DISCUSSION

Dynamics of the Nuclear Bundle Formation

The present study showed that the transitional changes in the structure of nuclear matrix always precede the bundle formation of microfilaments. The process of the bundle formation can be separated into four steps as follows: (*a*) The nuclear matrix is composed of electron-opaque amorphous

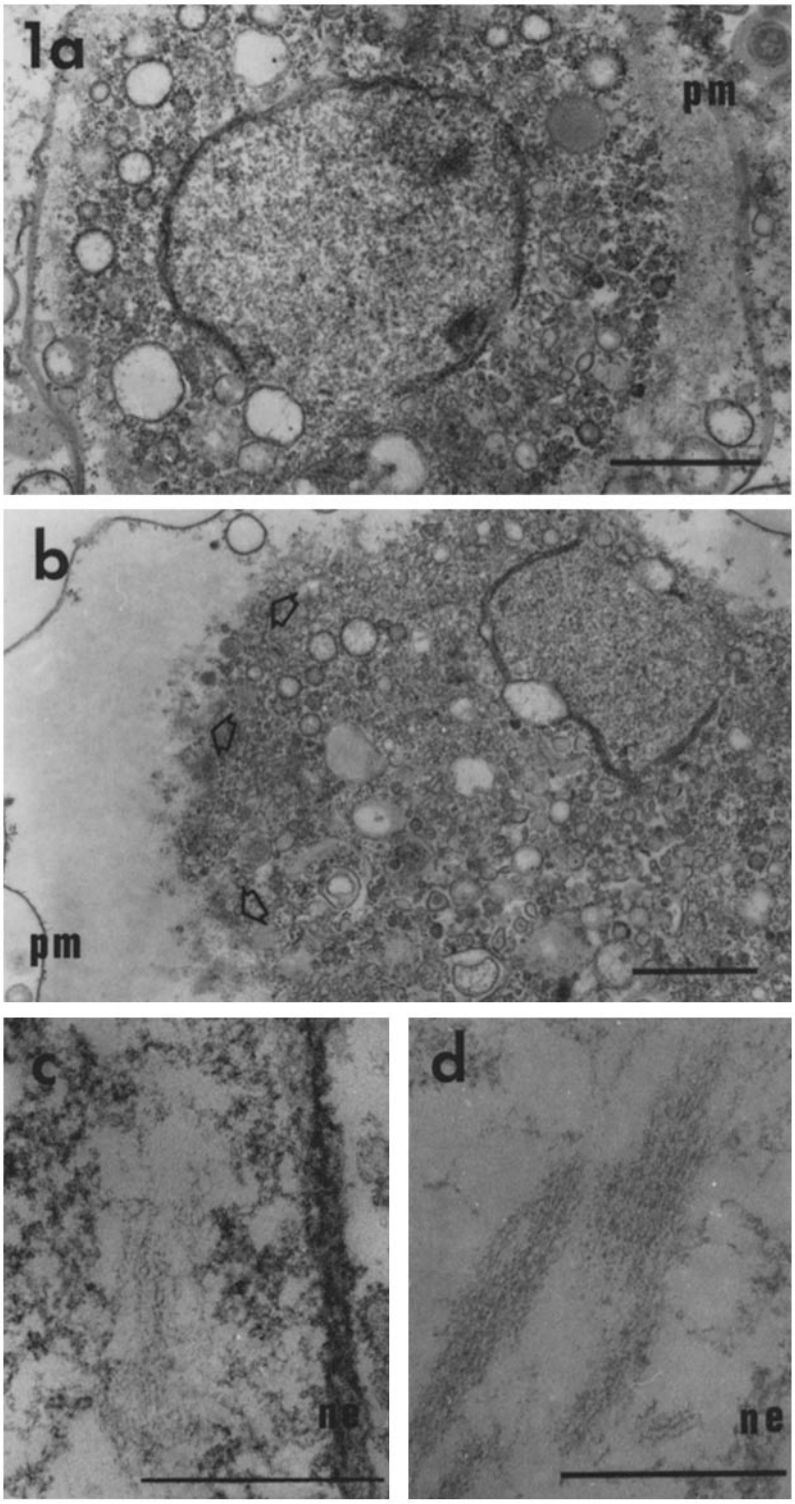


FIGURE 1 The effect of DMSO on nuclear and cytoplasmic microfilaments demonstrated in glycerinated cells. (*a*) Control glycerinated cell showing the spacial arrangement of nucleus, cytoplasm, and cortical microfilaments in the cytoplasm. × 20,000. (*b*) Glycerinated cell treated with 10% DMSO for 30 min, demonstrating that cytoplasmic microfilaments were dislocated from plasma membrane forming aggregates (arrows), and empty space was formed between the membrane and the microfilament aggregates. × 17,000. (*c*) Nuclear microfilament bundles in a glycerinated cell, which was induced by the treatment with 10% DMSO for 30 min. × 64,000. (*d*) Nuclear microfilaments decorated with rabbit skeletal muscle HMM, demonstrating that those nuclear microfilaments are actin. *pm*, plasma membrane; *ne*, nuclear envelope. × 30,000. Bars, (*a*, *b*, and *d*) 1 μm (*c*) 0.5 μm.

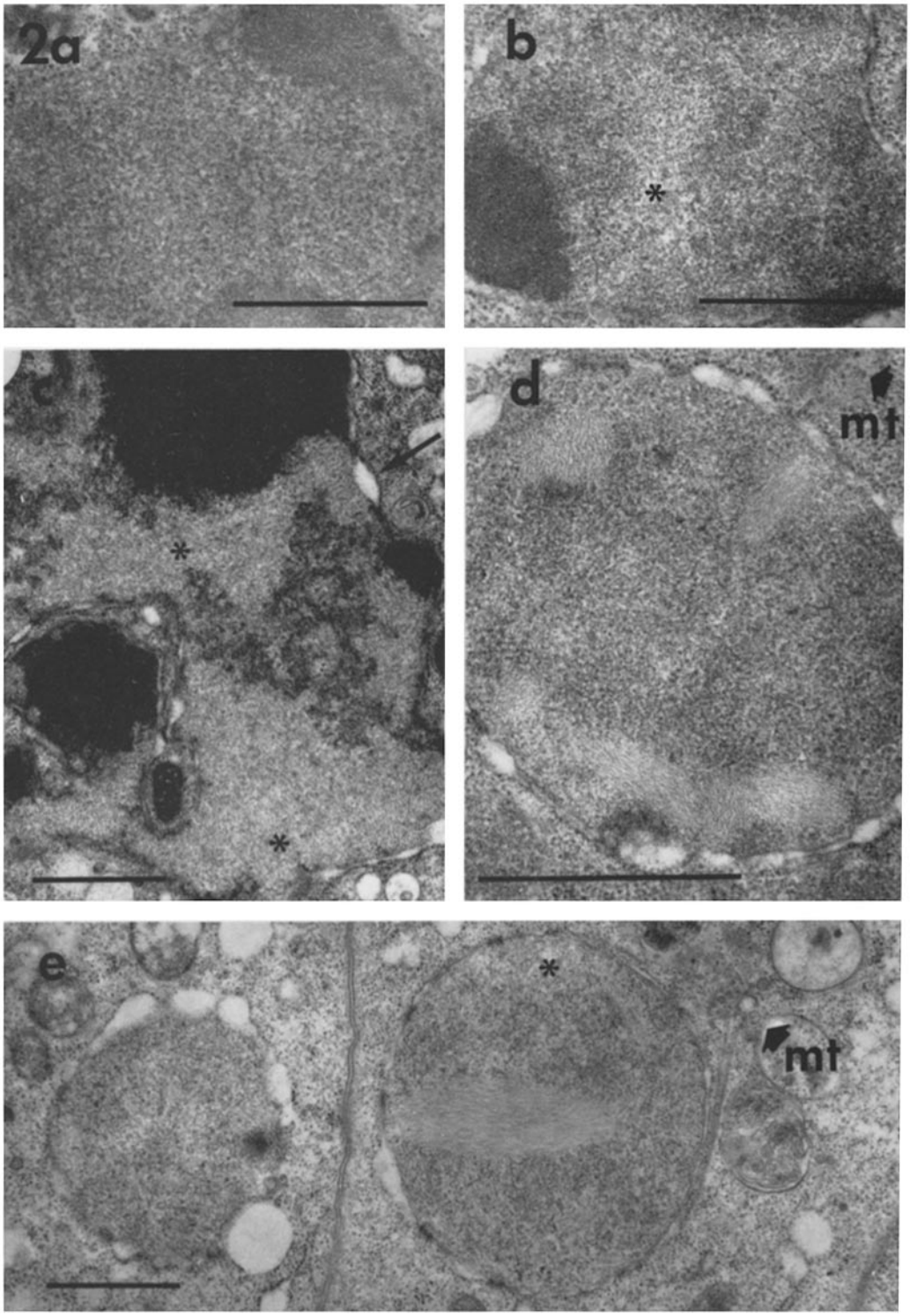


FIGURE 2 Electron micrographs showing the stages in the formation of nuclear actin bundles. (*a*) Control nucleus not treated with DMSO showing the nuclear matrix materials with high electron density. × 30,000. (*b*) Nucleus treated with 10% DMSO in Bonner's salt solution for 5 min, showing the occurrence of electron-transparent regions (*). × 30,000. (*c*) Nucleus treated with 10% DMSO for 10 min, showing microfilament meshworks in the electron-transparent regions (*). Note that the nucleus is still irregular in shape and small scale blebs are evident in the nuclear envelope (arrow). × 20,000. (*d*) Nucleus treated with 10% DMSO for 20 min, showing the formation of nuclear actin bundles. × 40,000. (*e*) Nucleus treated with 10% DMSO for 30 min. Nuclear bundles are at their most advanced stage of the development. Occasionally microfilament meshwork still remains in some regions (*). *Mt*, microtubules. × 20,000. Bar, 1 μm.

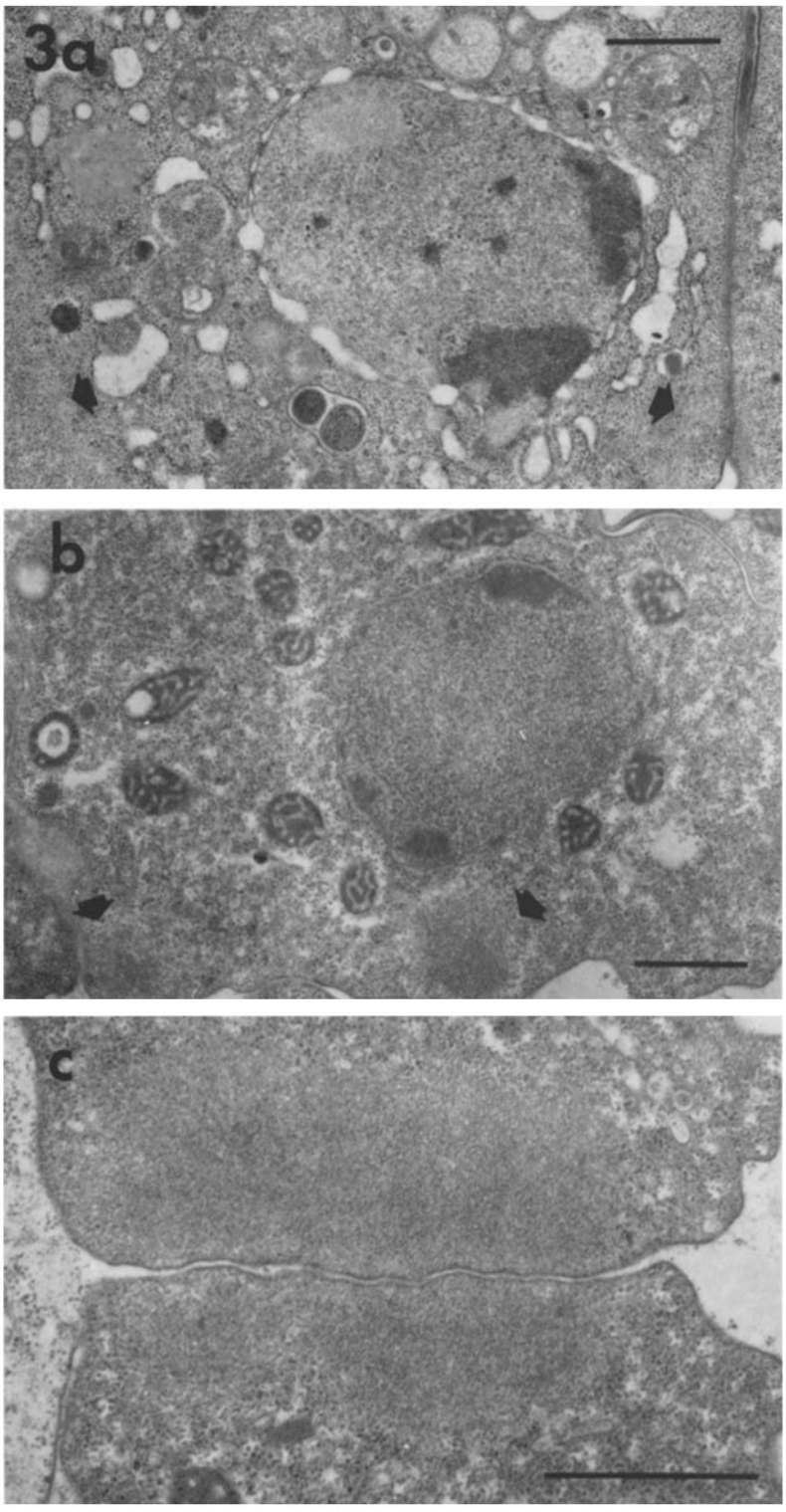


FIGURE 3 Electron micrographs showing the process of the recovery from the ultrastructural changes induced by DMSO. (*a*) Cell treated with 10% DMSO for 30 min, showing the rounding of the cell, dislocation of the cortical microfilament bundles (indicated by arrows), blebs in the nuclear envelope, and the nuclear actin bundles. × 16,000. (*b*) Fine structure of the cell 5 min after the washing with Bonner's salt solution, showing the irregular cell surface, cortical microfilament aggregates (arrows), and the recovery of the high electron density in the nuclear matrix materials. No more nuclear bundles are evident. × 15,000. (*c*) Cells have recovered their adhesiveness, 10 min after the washing, and huge microfilament aggregate is apparent in the cortical layer of the contact site, × 28,000. Bar, 1 μm.

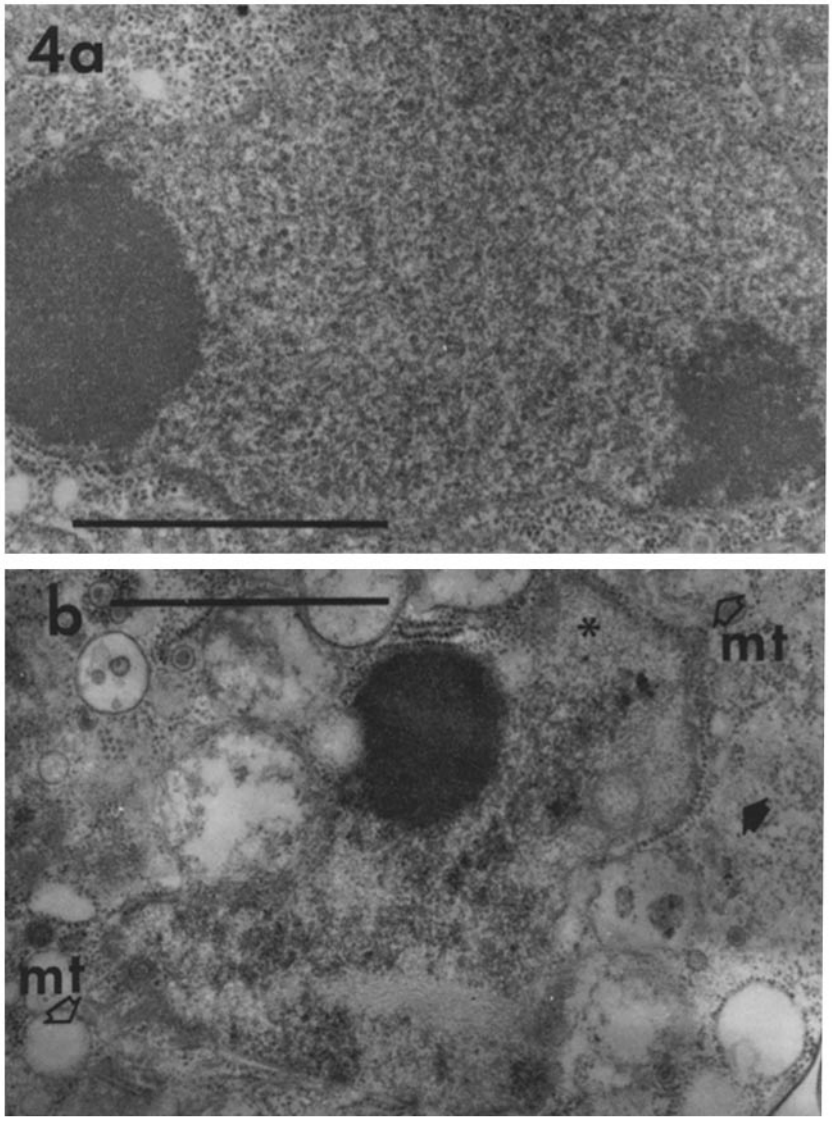


FIGURE 4 The effect of NaN_3 on the induction of nuclear bundles by DMSO. Effect of cycloheximide is indistinguishable from the effect of NaN_3. (*a*) Electron micrograph of cell treated with 10 mM NaN_3 dissolved in Bonner's salt solution for 30 min. The nucleus is irregular in shape, and no filamentous structure is evident in the nucleus. × 42,000. (*b*) Electron micrograph of cell treated with 10 mM NaN_3 and 10% DMSO dissolved in the salt solution. The cell is rounded whereas the nucleus remains irregular. Microfilament bundles as well as meshworks (*) are evident in electron-transparent regions of the nucleus. Note that cytoplasmic microfilaments are located apart from the plasma membrane (arrows). *Mt*, microtubules. × 37,000. Bar, 1 μm.

materials in their native state (Fig. 2*a*). (*b*) The electron density of those materials decreases and they become electron-transparent regions in the nucleus (Fig. 2*b*). (*c*) Microfilaments appear singly in the electron-transparent regions to form a mesh-work-like structure (Fig. 2*c* and *e*). (*d*) And lastly, those microfilaments come together forming bundles of microfilaments (Fig. 2*d*). Previously, we suggested that the bundles formed by elongation of the microfilaments and their assembly into bun-

dles (16). The electron-opaque matrix material might be a storage form of actin corresponding to nonfilamentous form of actin in *Thyone* acrosomes, which has been shown by Tilney (36). The fact that magnesium ions inhibit the bundle formation supports this idea, since Mg^{++} possibly works on the nonfilamentous form of actin to keep the complex of actin-high molecular weight proteins intact in the acrosomes (37).

Differences in the Properties of Nuclear and Cytoplasmic Actin

Then we must ask why are the bundles induced specifically in the nucleus? It might be possible that nuclear and cytoplasmic actins are different in their chemical properties. If this is the case, this difference might explain the reason for the presence of multiple actins from a single cellular source (24, 28). It might also explain the difference in the degree of polymerization of nuclear and cytoplasmic actin (30).

The present study showed that low temperature totally inhibited the bundling of nuclear microfilaments, although it permitted the occurrence of meshwork-like formation in the electron-transparent regions. This suggests that the bundling process of the nuclear microfilaments is specifically sensitive to low temperature. This inhibition of the bundling by low temperature was probably caused by a general decrease in the cell metabolism, since metabolic inhibitors such as NaN_3 or cycloheximide could not prevent the bundle formation (Fig. 4).

We found that cytochalasin B had virtually no effect on the induction of nuclear actin bundles, although it aggregated cytoplasmic microfilaments. One possible explanation for this differential effect of cytochalasin B is that the nuclear microfilaments, but not the cytoplasmic microfilaments, are associated with accessory proteins which retard the effects of cytochalasin (see Pollard and Weihing [28]).

Nuclear Actin and Nuclear Shape

It is interesting that the shape of the nuclei in cells treated simultaneously with DMSO and NaN_3 or cycloheximide remained irregular as in control cells (Fig. 4*b*), whereas the nuclei of cells treated only with DMSO became round. This means that the formation of the bundles in the nucleus and the rounding of the nucleus are not necessarily coupled, and that the changes in the nuclear shape may require active cell metabolism. It should be noted that the morphology of the whole cell was affected by treatment with DMSO, resulting in rounding of the cell, in spite of the presence of metabolic inhibitors (Fig. 4*b*). This suggests to us that the elements involved in the cellular and nuclear skeletal activities must be different, at least in their energy requirements.

We express our sincere gratitude to Dr. John T. Bonner for critically reviewing the manuscript. This paper was prepared and submitted to the *Journal of Cell Biology* by Y. Fukui during his stay at Princeton University, and the authors are indebted to a grant from Yamada Science Foundation that has made it possible. We are grateful to the Department of Biology, Princeton University, for its hospitality. Thanks are also due to Mrs. Ruth Bonner, Will Kopachic, and Joel Swanson for reviewing, and to Mrs. Elizabeth Drotar for typing the manuscript.

Received for publication 28 March 1979, and in revised form 20 August 1979.

REFERENCES

1. ALLEN, E. D., R. J. LOWRY, and A. S. SUSSMAN. 1974. Accumulation of microfilaments in a colonial mutant of *Neurospora crassa*. *J. Ultrastruct. Res.* **48**:455–464.
2. BEHNKE, O., A. FORER, and J. EMMERSON. 1971. Actin in sperm tails and meiotic spindles. *Nature (Lond.)* **234**:408–410.
3. BONNER, J. T. 1947. Evidence for formation of cell aggregation by chemotaxis in the development of *Dictyostelium discoideum*. *J. Exp. Zool.* **106**:1–26.
4. CANDE, W. Z., G. LAZARIDES, and J. R. MCINTOSH. 1977. Comparison of the distribution of actin and tubulin in the mammalian mitotic spindle as seen by indirect immunofluorescence. *J. Cell Biol.* **72**:552–567.
5. CLARKE, M., and J. A. SPUDICH. 1977. Nonmuscle contractile proteins: the role of actin and myosin in cell motility and shape determination. *Ann. Rev. Biochem.* **46**:797–822.
6. CONDEELIS, J. S., and D. L. TAILOR. 1977. The contractile basis of amoeboid movement. V. The control of gelation, solation, and contraction in extracts from *Dictyostelium discoideum*. *J. Cell Biol.* **74**:901–927.
7. COOKE, R., M. CLARKE, R. J. VON WEDEL, and J. A. SPUDICH. 1976. Supramolecular forms of *Dictyostelium* actin. *In* Cell Motility. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y. **3**:575–587.
8. DOUVAS, A. S., C. A. HARRINGTON, and J. BONNER. 1975. Major nonhistone proteins of rat liver chromatin: preliminary identification of myosin, actin, tubulin, and tropomyosin. *Proc. Natl. Acad. Sci. U. S. A.* **72**:3902–3906.
9. ECKERT, B. S., and E. LAZARIDES. 1978. Localization of actin in *Dictyostelium* amoebas by immunofluorescence. *J. Cell Biol.* **77**:714–721.
10. ECKERT, B. S., R. H. WARREN, and R. W. RUBIN. 1977. Structural and biochemical aspects of cell motility in amoebas of *Dictyostelium discoideum*. *J. Cell Biol.* **72**:339–350.
11. ELLIZEY, J., E. HUIZAR, and D. YANG. 1976. Microfilament bundles in a anteridial nuclei of *Achyla ambisexualis* E 87. *Arch. Microbiol.* **107**: 113–114.
12. FORER, A. 1976. Actin filaments and birefringent spindle fibers during chromosome movements. *In* Cell Motility. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor N. Y. **3**:1273–1293.
13. FORER, A., and W. T. JACKSON. 1975. Actin in the higher plant *Hemanthus katherinae* BAKER. *Cytobiology* **10**:217–226.
14. FORER, A., and W. T. JACKSON. 1976. Actin in the endosperm spindles of the higher plant *Haemanthus katherinae* BAKER. *Cytobiology*. **12**: 199–214.
15. FUKUI, Y. 1976. Enzymatic dissociation of nascent macrocysts and

partition of the liberated cytophagic giant cells in *Dictyostelium mucoroides*. *Dev. Growth Differ.* **18**:145–155.
16. FUKUI, Y. 1978. Intranuclear actin bundles induced by dimethyl sulfoxide in interphase nucleus of *Dictyostelium*. *J. Cell Biol.* **76**:146–157.
17. FUKUI, Y., and H. KATSUMARU. 1979. Nuclear actin bundles induced by dimethyl sulfoxide in *Amoeba proteus*, *Dictyostelium* and human HeLa cells. *Exp. Cell Res.* **120**:451–455.
18. GAWADI, M. 1971. Actin in the mitotic spindle. *Nature (Lond.)*. **234**: 410.
19. HERMAN, I. M., and T. D. POLLARD. 1978. Actin localization in fixed dividing cells stained with fluorescent heavy meromyosin. *Exp. Cell Res.* **114**:15–25.
20. HINKLEY, R., and A. TELSER. 1974. Heavy meromyosin-binding filaments in the mitotic apparatus of mammalian cells. *Exp. Cell Res.* **86**: 161–164.
21. ISHIKAWA, H., R. BISHOFF, and H. HOLTZER. 1969. Formation of arrowhead complexes with heavy meromyosin in a variety of cell types. *J. Cell Biol.* **43**:312–328.
22. LESTOURGEON, W. M., R. TOTTEN, and A. FORER. 1974. The nuclear acidic proteins in cell proliferation and differentiation. *In* Acidic Proteins of the Nucleus. I. L. Cameron and J. R. Jeter, Jr., editors. Academic Press, Inc., New York. 159–187.
23. LESTOURGEON, W. M., A. FORER, Y. YANG, J. S. BERTRAM, and H. P. RUSCH. 1975. Major components of nuclear and chromosome nonhistone proteins. *Biochim. Biophys. Acta.* **379**:529–552.
24. MAKEON, M., W. C. TAYLOR, K. L. KINDLE, R. A. FIRTEL, W. BENDER, and N. DAVIDSON. 1978. Multiple, heterogeneous actin genes in *Dictyostelium*. *Cell.* **15**:789–800.
25. NEWELL, P. C, A. TELSER, and M. SUSSMAN. 1969. Alternative developmental pathways determined by environmental conditions in the cellular slime mold *Dictyostelium discoideum*. *J. Bacteriol.* **100**:763–768.
26. OAKLEY, B. R., and I. B. HEATH. 1978. The arrangement of microfilaments in serially sectioned spindles of the alga *Cryptomonas*. *J. Cell Sci.* **31**:53–70.
27. PEDERSON, T. 1977. Isolation and characterization of chromatin from the cellular slime mold, *Dictyostelium discoideum*. *Biochemistry.* **16**: 2771–2777.
28. POLLARD, T. D., and R. R. WEIHING. 1974. Actin and myosin and cell movement. *CRC Crit. Rev. Biochem.* **2**:1–65.
29. PUCK, T. T. 1977. Cyclic AMP, the microtubule-microfilament system, and cancer. *Proc. Natl. Acad. Sci. U. S. A.* **74**:4491–4495.
30. RUBIN, R. W., L. GOLDSTEIN, and C. KO. 1978. Differences between nucleus and cytoplasm in the degree of actin polymerization. *J. Cell Biol.* **77**:698–701.
31. RYSER, U. 1970. Die Ultrastruktur der Mitosekerne in den Plasmodien von *Physarum polycephalum*. *Z. Zellforsch.* **110**:108–130.
32. SANGER, J. W. 1975. Presence of actin during chromosomal movement. *Proc. Natl. Acad. Sci. U. S. A.* **72**:2451–2455.
33. SANGER, J. W., and J. M. SANGER. 1976. Actin localization during cell division. *In* Cell Motility. R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor N. Y. **3**: 1295–1316.
34. SPUDICH, J. A. 1974. Biochemical and structural studies of actomyosin-like proteins from non-muscle cells. II. Purification, properties, and membrane association of actin from amoebae of *Dictyostelium discoideum*. *J. Biol. Chem.* **249**:6013–6020.
35. SPUDICH, J. A., and R. COOKE. 1975. Supramolecular forms of actin from amoebae of *Dictyostelium discoideum*. *J. Biol. Chem.* **250**:7485–7491.
36. TILNEY, L. G. 1976. The polymerization of actin. II. How nonfilamentous actin becomes nonrandomly distributed in sperm: evidence for the association of this actin with membranes. *J. Cell Biol.* **69**:51–72.
37. TILNEY, L. G. 1976. The polymerization of actin. III. Aggregates of non-filamentous actin and its associated proteins: storage form of actin. *J. Cell Biol.* **69**:73–89.