INTRANUCLEAR ACTIN BUNDLES

INDUCED BY DIMETHYL SULFOXIDE IN INTERPHASE

NUCLEUS OF *DICTYOSTELIUM*

YOSHIO FUKUI

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

ABSTRACT

Electron microscopic evidence demonstrated that dimethyl sulfoxide (DMSO) induces formation of giant intranuclear microfilament bundles in the interphase nucleus of a cellular slime mold, *Dictyostelium*. These giant bundles are approximately 3 μm long, 0.85 μm wide, and composed of microfilaments 6 nm in diameter.

Studies in which glycerinated cells were used showed that these microfilaments bind rabbit skeletal muscle heavy meromyosin, forming typical decorated "arrowhead" structures, and that this binding can be reverted by Mg-adenosine triphosphate.

These data verify that the intranuclear microfilaments are the contractile protein actin, and that DMSO affects intranuclear actin, inducing the formation of such giant bundles.

The intranuclear actin bundles appear at any developmental stage in two different species of cellular slime molds after treatment with DMSO.

The native form of the intranuclear actin molecules and their possible functions are discussed, and it is proposed that the contractile protein has essential functions in the cell nucleus.

KEY WORDS   actin bundle · arrowhead structure · *Dictyostelium* · dimethyl sulfoxide · intranuclear actin · microfilaments in nucleus

Cumulative data have demonstrated that contractile proteins exist in diverse cell nuclei at specific stages. Ultrastructural (1, 16, 19, 22, 40) as well as immunofluorescence studies (3, 15, 17) have verified that actin and myosin exist in mitotic and meiotic nuclei. The fact that these microfilaments have been observed only in dividing nuclei has suggested that the microfilaments may function in

the chromosome-separating machinery of the spindle apparatus (19).

On the other hand, intranuclear microfilaments or microfibrils were reported to appear as giant bundles in the resting nuclei of chicken sympathetic neurons (32), rabbit hypothalamus neurons (7), and actinomycin D-treated amphibian oocytes (26). The chemical composition of those intranuclear microfilaments has not been fully studied, and only their proteinaceous nature has been suggested. No evidence has been obtained to suggest that these intranuclear microfilaments are a type of contractile protein.

Recent biochemical studies have shown that contractile proteins are present in nuclei as major components of nuclear (18, 27) and chromosomal nonhistone (8, 28, 29) protein fractions. Pederson isolated nonhistone proteins from *Dictyostelium discoideum* and demonstrated that about 35% of the nonhistone chromatin proteins have a molecular weight identical to that of mammalian actin and myosin (37). These data suggest that actin is a major component of the cell nucleus in a variety of organisms. No ultrastructural study has been reported, however, that confirms the presence of intranuclear actin on a large scale in any organisms.

The occurrence of actin in the whole cell of cellular slime molds has been established (4, 5), and quantitation of cellular actin in connection with the life cycle has been reported indicating that as much as 20% of the newly synthesized protein is actin in the preaggregation stage (45). A recent study further demonstrated a direct correlation between the amount of cellular actin and the motility rate (9). However, no ultrastructural evidence exists demonstrating the occurrence of intranuclear actin in this organism, although a recent study verified the involvement of microtubules in the mitotic spindle (34, 39).

The present study showed that dimethyl sulfoxide (DMSO) induces the occurrence of giant bundles of actin in the interphase nucleus, demonstrating the existence of large quantities of the protein in nuclei irrespective of developmental stage. On the basis of the evidence, we discuss the native form of actin and its possible roles, and propose that it functions in the maintenance of nuclear structure, in the morphological changes of nucleus, and possibly in gene expression and replication.

MATERIALS AND METHODS

Cells

Dictyostelium mucoroides, strain DM-7 (11), and *D. discoideum*, strain NC-4 (2), were kindly provided by Dr. Kenneth B. Raper, University of Wisconsin, Madison, Wis. Stock cells were inoculated on agar plates containing 1% glucose and peptone (2) with *Escherichia coli* (B/r) as a food source, and were incubated at 22°C. Cells at the vegetative or aggregation stage were harvested by mild centrifugation (300 g, 2 min) and washed with Bonner's salt solution (10 mM NaCl, 10 mM KCl, 3 mM CaCl₂) (2).

To obtain synchronous cells forming macrocysts, nascent precysts were dissociated by treatment with a mixture of cellulase and macerozyme (14). After the enzymes had been thoroughly removed, the cells were suspended in the salt solution and kept for 5 h at 4°C. The cells were allowed to reconstitute nascent macrocysts engulfing a few peripheral cells by incubation on an 8-shaped shaker at 22°C for 1 h (14).

DMSO Treatment

DMSO was diluted with Bonner's salt solution to make a 10% (vol/vol) solution. Cells were washed with the salt solution, sedimented by centrifugation (300 g, 2 min), then treated with the DMSO solution for 30 min at 20°C. The treated cells were immediately fixed by the method described in the following section.

Electron Microscopy

A modification of Karnovsky's fixation method (24) was employed. Cells were fixed for 1 h at 0°C with 1% paraformaldehyde and 1.25% glutaraldehyde in 25 mM sodium cacodylate buffer, pH 7.2. After the cells had been washed for 1–2 h at 0°C with the buffer, they were postfixed for 1 h at 4°C with 1% OsO₄ in the buffer. After a 2-h wash with the buffer at 0°C, the cells were dehydrated in an ethanol series at 0°C. Cells in absolute ethanol were then transferred to 20°C. After ethanol had been substituted by propylene oxide, the preparations were embedded in Spurr's resin (42). Thin sections were stained with 25% uranyl acetate in methanol for 30 min followed by 10-min staining with Reynolds's lead citrate (38). All preparations were examined under a JEM 100-C electron microscope.

Heavy Meromyosin (HMM) Binding Study

Cells were glycerinated according to the method of Ishikawa et al. (21). Cells embedded in agar were treated with 5, 10, 25, and 50% glycerol in G-solution (50 mM KCl, 5 mM G-EDTA, 2 mM dithiothreitol, 5 mM MgCl₂, 10 mM Tris-HCl, pH 7.0) for 20 min each at 0°C. After the preparations had been kept at 4°C for 24 h, glycerol was removed at 0°C through 50, 25, 10, and 5% glycerol in H-solution (50 mM KCl, 5 mM G-EDTA, 10 mM Tris-HCl, pH 7.0).

Rabbit skeletal muscle HMM was isolated according to Szent-Györgyi (44) by Dr. Akio Inoue, Department of Biology, Faculty of Science, Osaka University, Toyonaka, Japan. The glycerinated preparations were treated with H-solution containing HMM (8 mg/ml) for 24 h at 0°C, then washed with H-solution for 7 h at 0°C. For a control, samples were similarly treated with H-solution only or with A-solution (50 mM KCl, 5 mM adenosine triphosphate (ATP), 5 mM MgCl₂, 10 mM Tris-HCl, pH 7.0) for 30 min after the HMM treatment. Thin sections of the glycerinated cells were prepared by the technique described above.

RESULTS

General Ultrastructure of Control Nucleus

The interphase nucleus of a postaggregative cell displayed an electron-opaque granular matrix (Fig. 1a). Nucleoli (Nu) were always located adjacent to the nuclear envelope. The surrounding cytoplasm contained typical mitochondria (Mi), rough endoplasmic reticulum (Er), and microfilaments arranged in the cortex layer inside the plasma membrane (not shown in this figure). No 10-nm filaments were observed.

General Ultrastructure of Cells Treated with DMSO

Cells were fixed after treatment with 2.5, 5, 10, or 20% DMSO at 20°C for 30 min. Here we refer only to data on cells treated with 10% DMSO, as these preparations gave the best results. A low-power electron micrograph of postaggregative cells treated with 10% DMSO for 30 min demonstrated that their ultrastructure was well preserved (Fig. 1b). The most prominent structures unique to cells treated with the solvent were bundles of microfilaments (If) which specifically appeared in the nucleus but not in the cytoplasm. Such intranuclear microfilament bundles were observed in the nuclei of a vegetative cell (Fig. 1c), aggregating or macrocyst-forming cells (Fig. 1b) of D. mucoroides, as well as vegetative or aggregating cells of D. discoideum. A more precise study should be designed to fully elucidate the frequency and size of the bundles in connection with life cycle. However, at present it is more essential to know their chemical nature, origin, and development. With experiments repeated 10 times, we obtained conclusive evidence demonstrating that DMSO induces the occurrence of such intranuclear microfilament bundles in two species of cellular slime molds, irrespective of the developmental stage.

The giant intranuclear bundles occurred most frequently in cells during macrocyst formation (Figs. 1b, 2). Consequently, we employed such cells as material for further studies.

Origin and Development of Intranuclear Microfilament Bundles

Intranuclear microfilament bundles were examined in detail in several hundred nuclei. The bundles displayed various structures and dimensions (Fig. 3 a-i). The nuclei generally were surrounded by a nuclear envelope with crevices between the inner and outer membrane (Figs. 1b, c, 2, 3 c, f, g, h, i). Also, microtubules were frequently observed in the neighboring cytoplasm and were arranged parallel to the nuclear envelope (Figs. 2, 3 c, i).

Some nuclei (Fig. 3 a-c) contained small bundles at several locations. In such nuclei, the direction of the bundles was random, and both longitudinal and cross sections appeared in the same plane (Fig. 3 a, c, e, f). Note also that many randomly oriented microfilaments could be observed in these nuclei (Figs. 3b, 4b). Fig. 4b demonstrates that the microfilaments composing the intranuclear bundles originated from such unorganized microfilaments.

Apparently, the intranuclear bundles were gradually unified (Fig. 3d, e, f) and finally became a single giant bundle stretching between opposite sides of the nuclear envelope (Figs. 2, 3 g-i). In its extreme form, the intranuclear bundle reached perpendicularly to the inner membrane of the nuclear envelope. However, no evidence was obtained which suggested that the microfilaments of the bundle passed through the membrane. Conclusively, the giant bundle seems to be formed by unification of small bundles which develop through elongation and side-by-side assembly of unorganized microfilaments originating from amorphous electron-opaque nuclear matrices.

Dimensions of Intranuclear Microfilaments and Bundles

The mean diameter of the microfilaments of the intranuclear bundle was 6 nm, according to examination of 10 different cross sections. The random distribution of the microfilaments in the bundle was evident (Fig. 3 a, c, e, f, 4b), although one rare section displayed a spherical cross section of the bundle (Fig. 4a). In maximum dimensions, the bundles were 3 μm in length and 0.85 μm in width (Fig. 3i).

Binding of Intranuclear Microfilaments to HMM

Intranuclear as well as cytoplasmic microfilaments of glycerinated cells displayed reversible alterations of their structure, depending on the treatment for "rigor" or "relaxation" states. Low-power electron micrographs (Fig. 5a, b) demonstrated that both nuclear and cytoplasmic microfil-
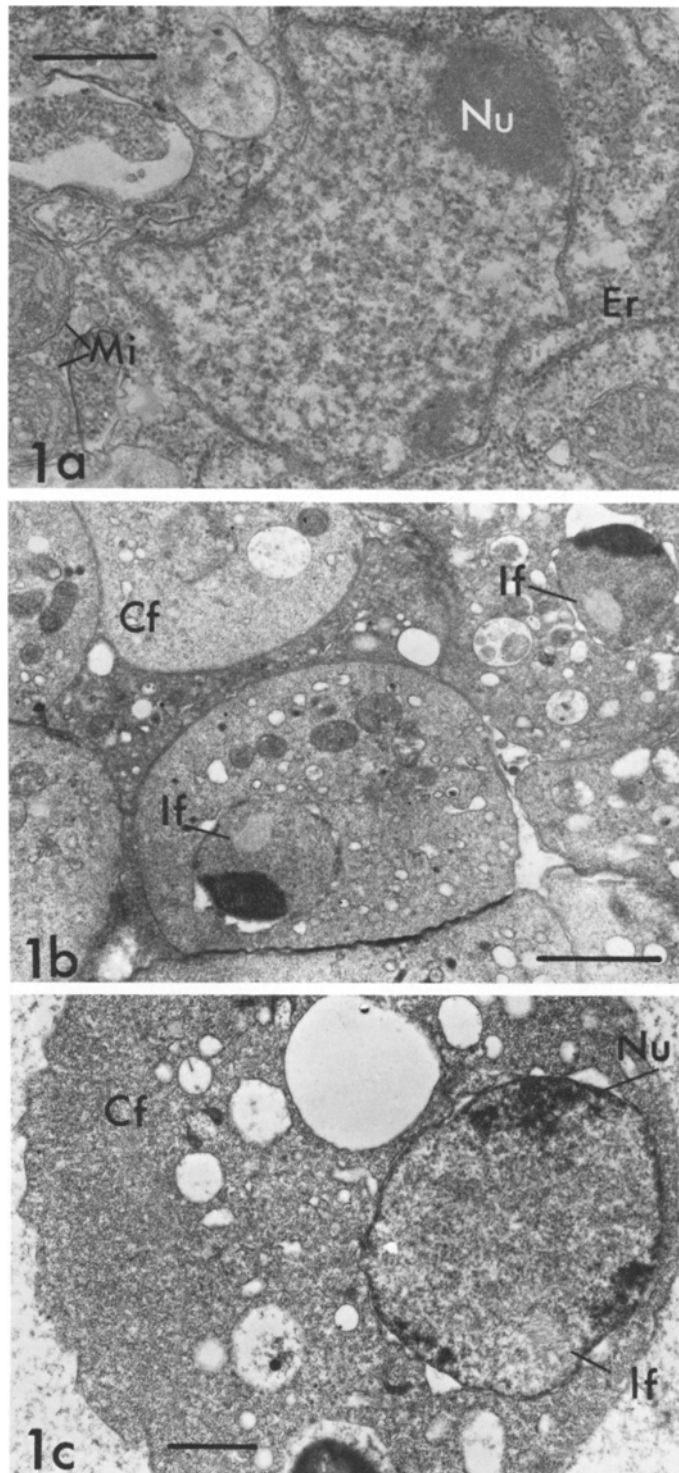
FIGURE 1 Electron micrographs of *D. mucoroides* cells. (*a*) Nucleus of a control postaggregative cell which was not treated with DMSO before fixation. No filamentous structure is visible inside the nucleus. × 33,000. Bar, 0.5 µm. (*b*) A low-power electron micrograph of macrocyst-forming cells treated with DMSO showing intranuclear microfilament bundles. Cytoplasmic microfilaments in the cortex layer are also visible. × 8,250. Bar, 2 µm. (*c*) A vegetative cell treated with DMSO containing a small intranuclear bundle. *Nu*, nucleolus, *Mi*, mitochondrion, *Er*, endoplasmic reticulum, *If*, intranuclear microfilament bundle, *Cf*, cytoplasmic microfilaments. × 12,000. Bar, 1 µm.
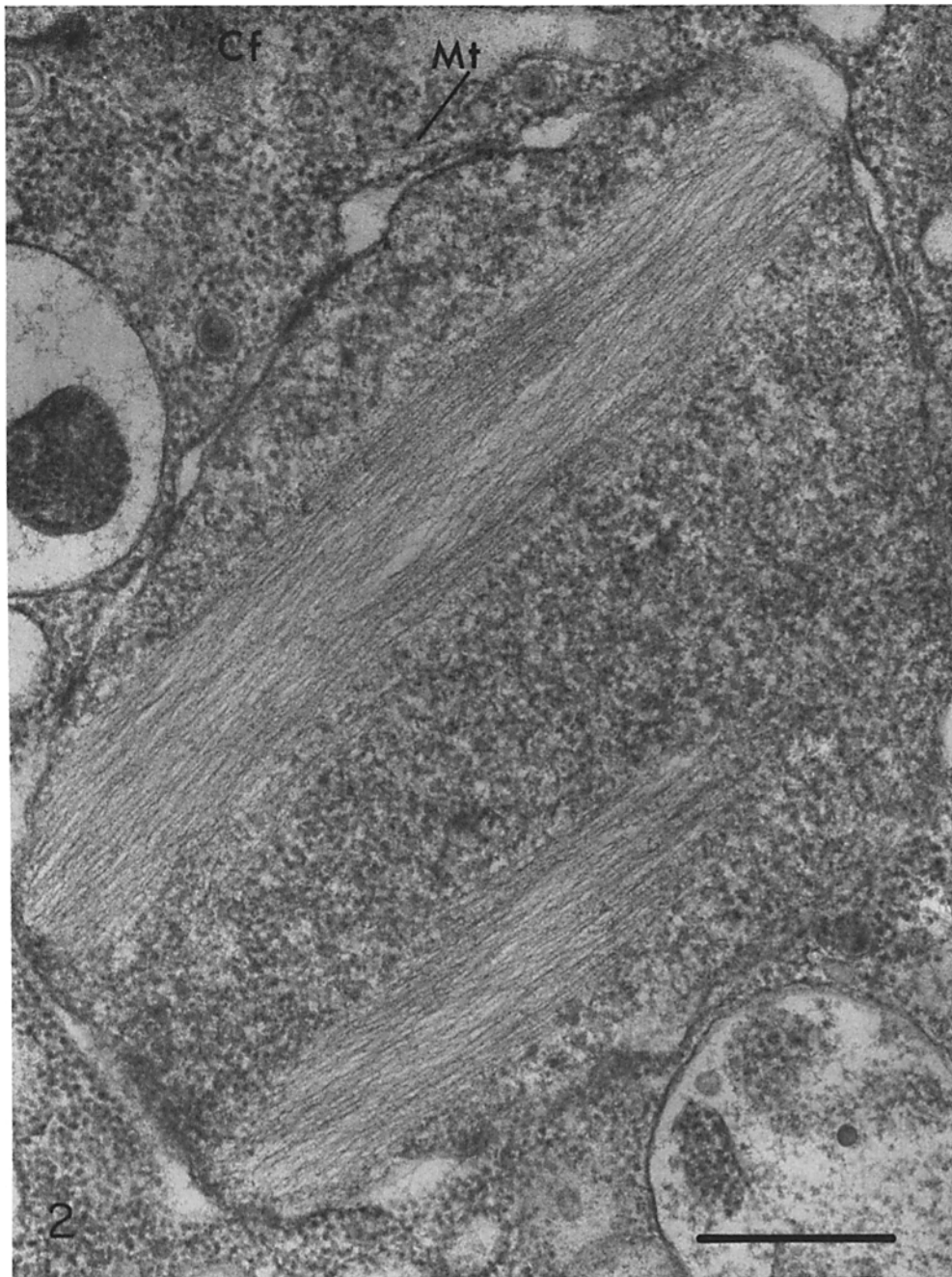
FIGURE 2 Well-developed intranuclear bundles of microfilaments appearing in a macrocyst-forming cell treated with DMSO. The upper bundle stretches between opposite sides of the nuclear envelope, and the end of the bundle finishes at the inner membrane of the envelope (upper right). The microfilaments run parallel without visible cross-bridges, and no nuclear matrices are involved in the bundle. *Mt*, microtubule, *Cf*, cytoplasmic microfilaments. × 54,000. Bar, 0.5 μm.
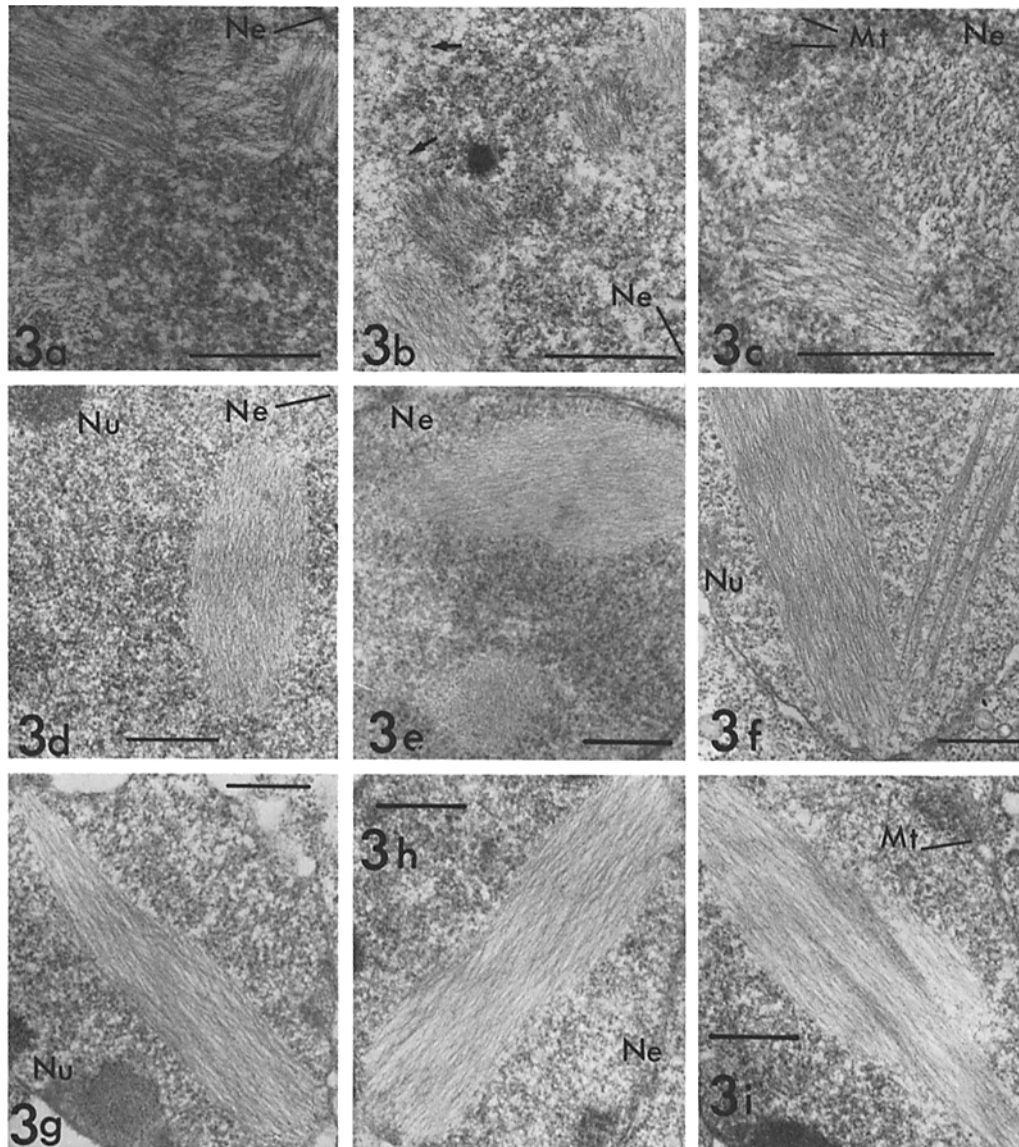
FIGURE 3 Electron micrographs arranged to show the possible development of intranuclear microfilament bundles. In some nuclei, longitudinal, oblique, and cross sections of filament bundles appear in several locations (a-c). Large bundles are probably produced by unification of the small bundles (d, e). Finally, a single or a few bundles reach the nuclear envelope (f-i). The largest bundle reached 3 μm in length and 0.85 μm in width (i). Nu, nucleolus, Ne, nuclear envelope, Mt, microtubule. Arrows in (b) indicate the points where the microfilaments are combined with granular matrix material. Bar, 0.5 μm. Magnifications: (a) × 36,000, (b) × 27,000, (c) × 54,000, (d) × 23,000, (e) × 24,000, (f) × 24,000, (g) × 22,000 (h) × 24,000, (i) × 24,000.

aments were "decorated" after treatment with HMM. The ultrastructure of the decorated intra-nuclear microfilaments was evident in longitudinal and oblique sections (Fig. 6a, b), which showed the "arrowhead" structure typical of actin fila-ments decorated with HMM (1, 4, 9, 19–21). This binding with HMM was sensitive to Mg-ATP, and, when the decorated intranuclear mi-
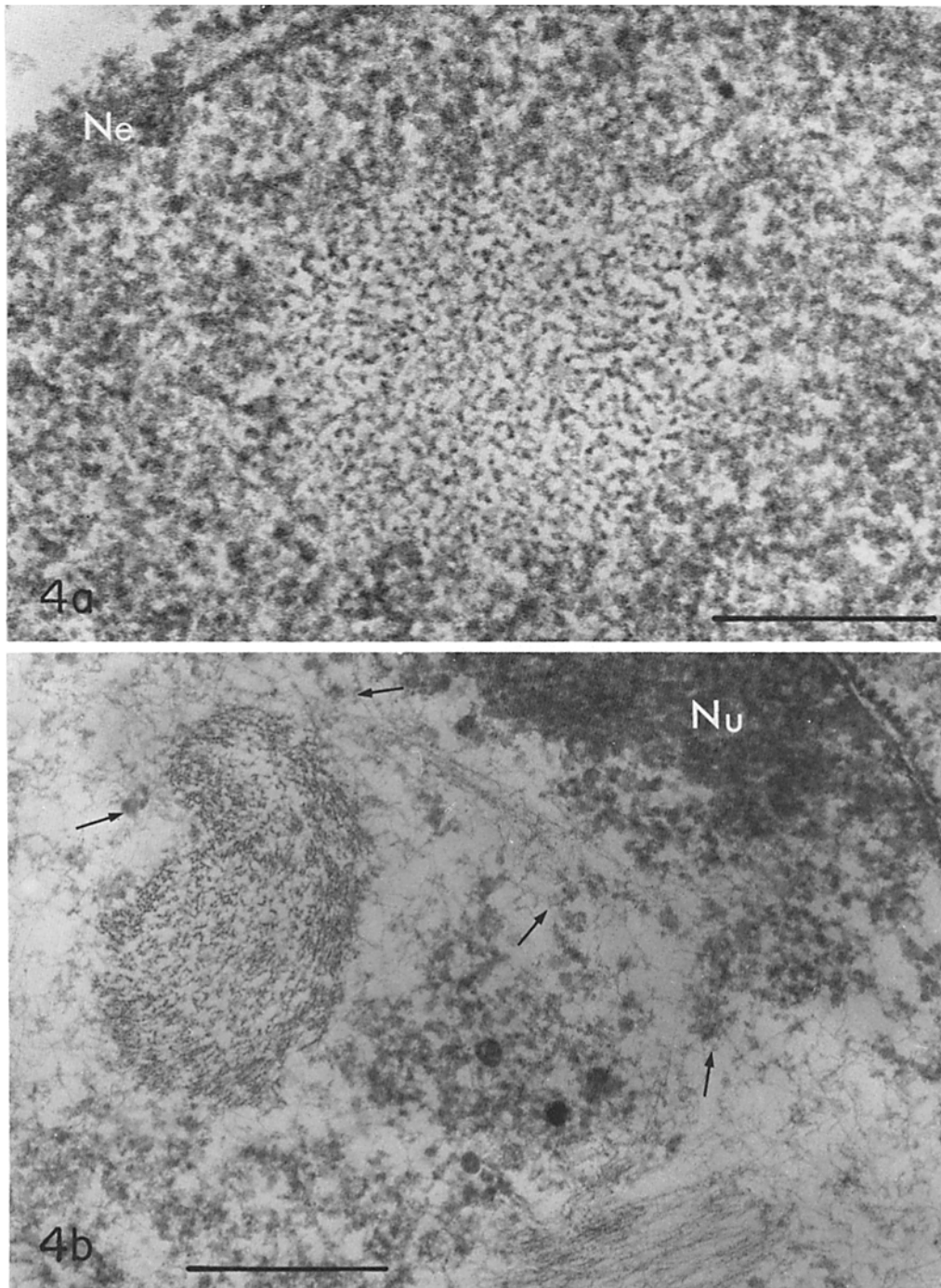
FIGURE 4 Cross sections of intranuclear bundles. (a) A spherical outline of a bundle showing random
arrangement of the microfilaments involved. × 130,000. Bar, 0.25 μm. (b) Cross as well as longitudinal
sections of bundles. Unorganized microfilaments are prominent. Microfilaments composing the bundles
apparently originate from these randomly oriented microfilaments. The unorganized microfilaments
appear to have originated from granular matrix material (arrows). Nu, nucleolus, Ne, nuclear envelope.
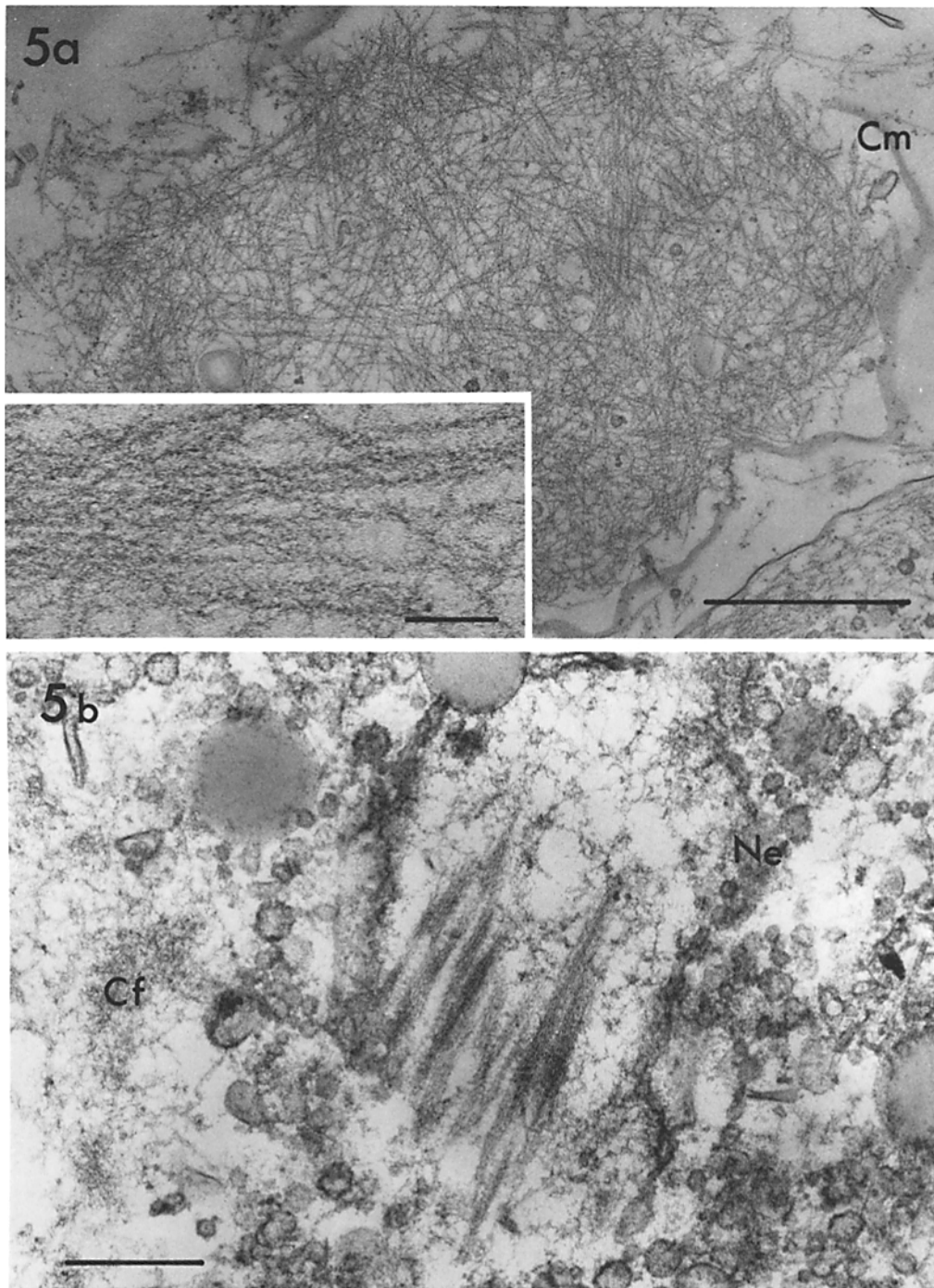× 66,000. Bar, 0.5 μm.

FIGURE 5 Glycerinated cells treated with HMM. (a) General appearance of cortical layer microfilaments decorated with rabbit skeletal muscle HMM, shown for comparison with intranuclear microfilaments. × 30,000. Bar, 1 μm. The insert shows the "fuzzy" structure typical of actin microfilaments decorated with HMM. × 135,000. Bar, 0.1 μm. (b) A low-power electron micrograph showing an intranuclear bundle decorated with HMM. The nuclear envelope was partially dissolved, and the nuclear material was eluted except for the bundle and the granular electron-opaque material of the matrix. Ne, nuclear envelope, Cf, cytoplasmic microfilaments, Cm, cell membrane. × 40,000. Bar, 0.5 μm.
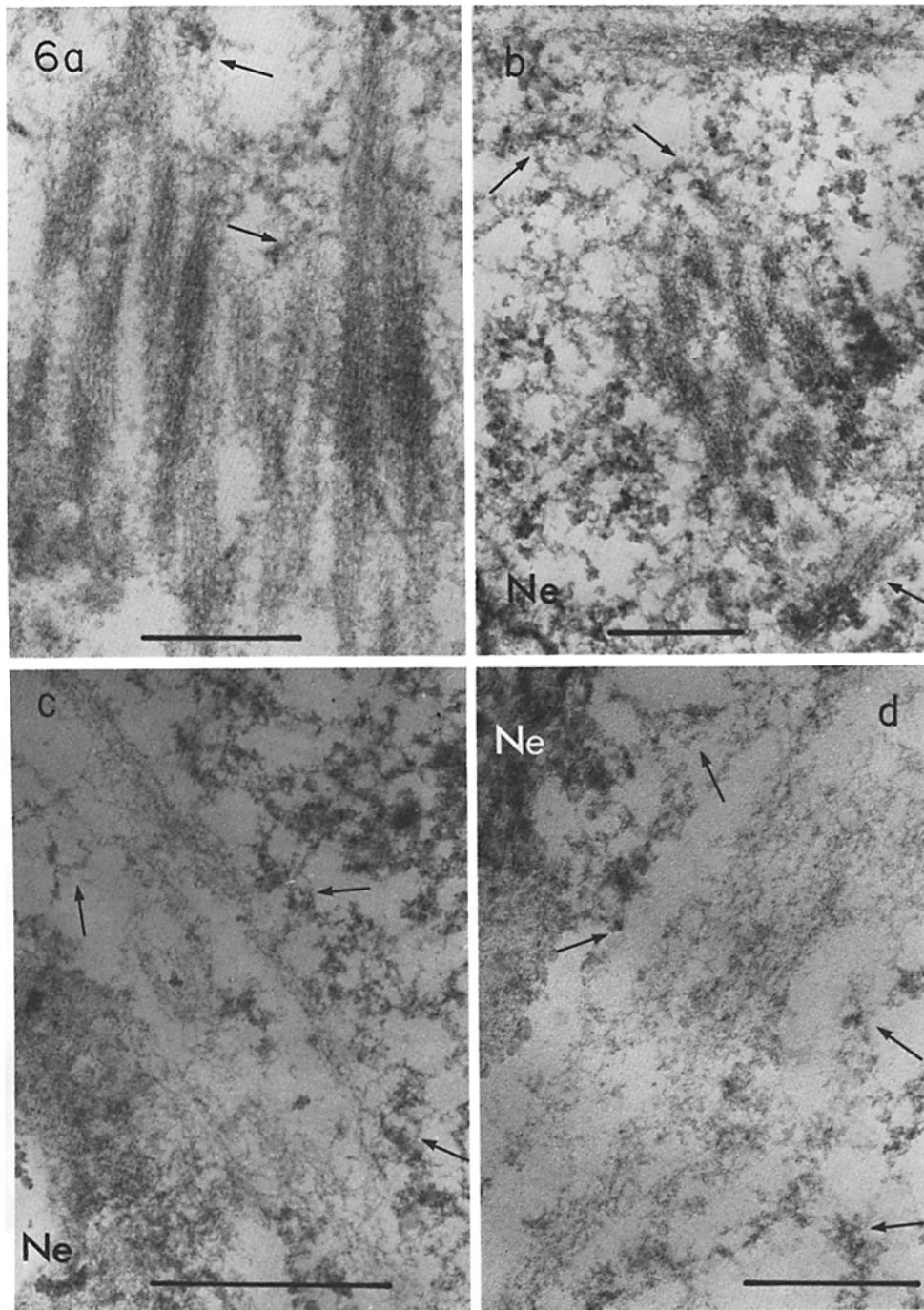
FIGURE 6 High-magnification electron micrographs showing intranuclear microfilament bundles in glycerinated cells. Bar, 0.25 μm. (a) A longitudinal section showing microfilaments decorated with HMM forming striated spindle-shaped bundles. × 90,000. (b) An oblique section showing the decorated structure in detail. × 76,000. (c) Intranuclear microfilaments after glycerination for the control. × 135,000. (d) Intranuclear microfilaments treated with Mg-ATP after HMM reaction. × 100,000. Arrows indicate the points where the microfilaments are combined with granular matrix material. Ne, nuclear envelope.

crofilaments were treated with Mg-ATP, their structure reverted to that of the control (Fig. 6c, d), providing definitive evidence that the intranuclear microfilaments were actin.

Physiological Effects of DMSO on Cellular Slime Mold Cells

Our preliminary study showed that DMSO treatment did not inhibit the cells to proliferate or differentiate when the solvent was removed after the treatment. However, cells in the solvent became round in shape, and their locomotion and aggregation were completely inhibited.

DISCUSSION

DMSO is an aprotic solvent widely used to preserve cells in liquid nitrogen (12, 25, 43). Its chemical nature suggests that it may enhance membrane permeability. It is known to induce hemoglobin synthesis in murine leukemic cells, resulting in erythroid differentiation (13, 23). This induction of hemoglobin synthesis may be related to changes in the membrane fluidity of the cells (31). On the other hand, the solvent directly affects protein-protein and protein-nucleotide hydrogen bonds, resulting in the release of coat protein from tobacco mosaic virus (35, 36). These facts suggest that DMSO interferes with the chemical structure of cellular macromolecules in situ.

Factors affecting the monomer-polymer transformation of cytoplasmic actin or the formation of actin filament bundles was recently reviewed by Clarke and Spudich (6). The present study demonstrated that DMSO specifically acts upon intranuclear actin, resulting in the bundle formation. How then does DMSO cause the formation of the giant intranuclear actin bundle? At present, the following two mechanisms can be suggested: (a) DMSO affects intranuclear actin molecules directly and interferes with their hydrogen bonds, resulting in conformational changes of the molecules, or (b) DMSO enhances the permeability of the nuclear envelope and allows some cytoplasmic factor(s) to act upon actin molecules in the nucleus, resulting in the formation of the giant bundles. The fact that DMSO specifically acts upon intranuclear actin and causes the formation of actin bundles is probably due to the different mode of existence of actin molecules between the nucleus and the cytoplasm. To elucidate the mechanism, we must await the results of studies on isolated nuclei.

Until now, ultrastructural studies have not demonstrated the occurrence of actin filaments in the nucleus of the cellular slime mold (10, 11, 30, 34, 39). The next question is, what is the native form of intranuclear actin molecules? Our present data showed that the intranuclear bundle is formed through the assembly of randomly oriented actin filaments (Figs. 3b, 4b). Such unorganized microfilaments might originate from the amorphous electron-opaque matrices of the nucleus (indicated by arrows in unglycerinated and glycerinated cells in Figs. 4b, 6a-d). Thus, it is highly plausible that the intranuclear actin exists in the nuclear matrix in its native state and that the G → F transformation as well as the formation of filament bundles might be induced by DMSO. In this sense, the present study may present a helpful tool for determining the mechanism of G → F transformation and bundle formation with respect to actin molecules in vivo.

Although many possible roles have been proposed for intranuclear contractile proteins, there is virtually no evidence showing their function(s). The present study provides confirmatory evidence for the previous studies demonstrating the existence of actin molecules in the nuclei of rat liver (8), Physarum (29), and Dictyostelium (37). According to the concept that the contractile proteins are major components of nonhistone proteins of nuclear chromatin, LeStourgeon et al. (28) proposed a model in which cell proliferation and chromatin function may be regulated by actin, myosin, and a Mg^{2+}-dependent actomyosin-binding protein through a condensation and a concomitant inactivation of chromatin. This idea is attractive because the nonhistone proteins may have a role in regulating gene expression (41).

However, the appearance of the intranuclear actin bundles in the present study cannot be fully explained by the involvement of only the actin in the nonhistone protein fractions of chromatin, as the quantity of molecules in the nuclei appears to be on a larger scale (Fig. 2). If actin is a major component of the nuclear matrix, as discussed in the foregoing section, it is reasonable to speculate that the molecules may play an essential role in maintaining nuclear structure and transforming nuclear shape through their contractile nature. In this connection, it is interesting that Escherichia actin-like protein may function in regulating cell hydration through "cytotonus" (33).

According to the data and suggestions previously described, we propose that actin occurs in large quantities in the nucleus, and that through its contractile properties plays essential roles in

diverse functions, such as the maintenance of nuclear structure, the dynamic transition in nuclear shape, and probably the alteration of chromatin structure (28, 29). Elucidation of the precise mechanism of the organization and functions of native intranuclear actin molecules must await the results of future studies. The present study offers a powerful probe for such investigations.

REFERENCES

1. BEHNKE, O., A. FORER, and J. EMMERSON. 1971. Actin in sperm tails and meiotic spindles. *Nature (Lond.).* **234:**408-410.

2. BONNER, J. T. 1947. Evidence for formation of cell aggregation by chemotaxis in the development of *Dictyostelium discoideum. J. Exp. Zool.* **106:**1-26.

3. CANDE, W. Z., G. LAZARIDES, and J. R. MACINTOSH. 1977. A comparison of the distribution of actin and tubulin in the mammalian mitotic spindle as seen by indirect immunofluorescence. *J. Cell Biol.* **72:**552-567.

4. 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. U. S. A.* **72:**1758-1762.

5. 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.

6. CLARKE, M., and J. A. SPUDICH. 1977. Nonmuscle contractile proteins: the role of actin and myosin in cell motility and shape determination. *Annu. Rev. Biochem.* **46:**809-815.

7. CLATTENBERG, R. E., R. P. SINGH, and D. G. MONTEMURRO. 1972. Intranuclear filamentous inclusions in neurons of the rabbit hypothalamus. *J. Ultrastruct. Res.* **39:**549-555.

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., 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.

10. ERDOS, G. W., A. W. NICKERSON, and K. B. RAPER. 1972. Fine structure of macrocysts in *Polysphondylium violaceum. Cytobiologie.* **6:**351-366.

11. FILOSA, M. F., and R. E. DENGLER. 1972. Ultrastructure of macrocyst formation in the cellular slime mold, *Dictyostelium mucoroides:* extensive phagocytosis of amoebae by a specialized cell. *Dev. Biol.* **29:**1-16.

12. FREED, J. J., and L. MEZGER-FREED. 1970. Culture methods for anuran cells. *Methods Cell Biol.* **4:**20-47.

13. FRIEND, C., W. SHER, J. G. HOLLAND, and T. SATO. 1971. Hemoglobin synthesis in murine virus-induced leukemic cells *in vitro:* stimulation of erythroid differentiation by dimethylsulfoxide. *Proc. Natl. Acad. Sci. U. S. A.* **68:**378-382.

14. 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.

15. FUJIWARA, K., and T. POLLARD. 1976. Fluorescent antibody localization of myosin in the cytoplasm, cleavage furrow, and mitotic spindle of human cells. *J. Cell Biol.* **71:**848-875.

16. GAWARDI, N. 1971. Actin in the mitotic spindle. *Nature (Lond.).* **234:**410.

17. HAUSER,, M., G. BEINBRECH, U. GRÖSCHEL-STEWARD, and B. M. JOCKUSCH. 1975. Localization by immunological techniques of myosin in nuclei of lower eukaryotes. *Exp. Cell Res.* **95:**127-135.

18. HITCHCOCK, S. E., L. CARLSON, and U. LINDBERG. 1976. Depolymerization of F-actin by deoxyribonuclease I. *Cell.* **7:**531-542.

19. HINKLEY, R., and A. TELSER. 1974. Heavy meromyosin-binding filaments in the mitotic apparatus of mammalian cells. *Exp. Cell Res.* **86:**161-164.

20. HUXLEY, H. E. 1963. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* **7:**281-308.

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. JOCKUSCH, B. M., U. RYSER, and O. BEHNKE. 1973. Myosin-like protein in *Physarum* nuclei. *Exp. Cell Res.* **76:**464-466.

23. KABST, D., C. C. SHERTON, L. H. EVANS, P. BIGLEY, and R. D. KOLER. 1975. Synthesis of erythrocyte-specific proteins in cultured Friend leukemia cells. *Cell.* **5:**331-338.

24. KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J. Cell Biol.* **27**:137-138 a. (Abstr.).

25. KATSUTA, H., and T. TAKAOKA. 1973. Cultivation of cells in protein- and lipid-free synthetic media. *Methods Cell Biol.* **6**:1-42.

26. LANE, N. J. 1969. Intranuclear fibrillar bodies in actinomycin D-treated oocytes. *J. Cell Biol.* **40**:286-291.

27. LAZARIDES, E., and U. LINDBERG. 1974. Actin is the naturally occurring inhibitor of deoxyribonuclease I. *Proc. Natl. Acad. Sci. U. S. A.* **71**:4742-4746.

28. 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.

29. LESTOURGEON, W. M., A. FORER, Y. YANG, J. S. BERTRAM, and H. P. RUSCH. 1975. Major components of nuclear and chromosome non-histone proteins. *Biochim. Biophys. Acta.* **379**:529-552.

30. LOOMIS, W. F. 1975. Amoeboid Stage. *Dictyostelium discoideum:* A Developmental System. Academic Press, Inc., New York. 12-28.

31. LYMAN, G., H. D. PREISLER, and D. PAPAHADJO-POULOS. 1976. Membrane action of DMSO and other chemical inducers of Friend leukemic cell differentiation. *Nature (Lond.).* **262**:360-363.

32. MASUROVSKY, E. B., H. H. BENITEZ, S. U. KIM, and M. R. MURRAY. 1970. Origin, development, and nature of intranuclear rodlets and associated bodies in chicken sympthetic neurons. *J. Cell Biol.* **44**:172-191.

33. MINKOFF, L., and R. DAMADIAN. 1976. Actin-like proteins from *Escherichia coli:* concept of cytotonus as the missing link between cell metabolism and the biochemical-ion-exchange resin. *J. Bacteriol.* **125**:353-365.

34. MOENS, P. B. 1976. Spindle and kinetochore morphology of *Dictyostelium discoideum. J. Cell Biol.* **68**:113-122.

35. NICOLAIEFF, A., G. LEBEURIER, and L. HIRTH. 1974. Electron microscopy and infectivity studies of the action of dimethyl-sulfoxide on two strains of tobacco mosaic virus. *J. Gen. Virol.* **22**:43-53.

36. NICOLAIEFF, A., G. LEBEURLER, M. C. MOREL, and L. HIRTH. 1975. The uncoiling of native and reconstituted TMV by DMSO: the polarity of stripping. *J. Gen. Virol.* **26**:295-306.

37. PEDERSON, T. 1977. Isolation and characterization of chromatin from the cellular slime mold, *Dictyostelium discoideum. Biochemistry.* **16**:2771-2777.

38. REYNOLDS, E. S. 1963. The use of lead citrate of high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208-212.

39. ROOS, U.-P. 1975. Mitosis in the cellular slime mold *Polysphondylium violaceum. J. Cell Biol.* **64**:480-491.

40. RYSER, U. 1970. Die Ultrastruklur der Mitosekerne in den Plasmodien von *Physarum polycephalum. Z. Zellforsch. Mikrosk. Anat.* **110**:108-130.

41. STEIN, G. S., J. S. STEIN, and L. J. KLEINSMITH. 1975. Chromosomal proteins and gene regulation. *Sci. Am.* **234**:64-79.

42. SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**:31-43.

43. THOMPSON, L. H., and R. M. BAKER. 1973. Isolation of mutants of cultured mammalian cells. *Methods Cell Biol.* **6**:210-281.

44. SZENT-GYÖRGYI, A. G. 1953. Meromyosin, the subunits of myosin. *Arch. Biochem. Biophys.* **42**:305-320.

45. TUCHMAN, J., T. ALTON, and H. F. LODISH. 1974. Preferential synthesis of actin during early development of the slime mold *Dictyostelium discoideum. Dev. Biol.* **40**:116-128.