

“Buttonin,” a Unique Button-shaped Microtubule-associated Protein (75 kD) That Decorates Spindle Microtubule Surface Hexagonally

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Abstract. A 75-kD protein was purified from sea urchin egg microtubule proteins through gel filtration. It enhanced the polymerization of porcine brain tubulin, but was not heat-stable and did not bind to calmodulin in the presence of calcium as demonstrated by calmodulin affinity column chromatography. Rotary shadowing of the freeze-etched 75-kD protein adsorbed on mica revealed the protein to be a spherical molecule (~ 9 nm in diameter). Quick-freeze deep-etch electron microscopy revealed that the surface of microtubules polymerized with 75-kD protein was entirely covered with hexagonally packed, round, button-

like structures that were quite uniform in shape and size (~ 9 nm) and similar to the buttons observed on microtubules of mitotic spindles in vivo or microtubules isolated from mitotic spindles. Judging from calibration studies of molecular mass by gel filtration, the 75-kD protein probably exists in a dimeric form (~ 150 kD) in its native condition. The stoichiometry of tubulin (dimer) versus 75-kD protein (dimer) in the polymerized pellet was 3–3.4:1. Hence, we concluded that the 75-kD protein was a unique microtubule-associated protein that formed the microtubule button in vivo and in vitro. We propose to name this protein “buttonin”.

MICROTUBULES form one of the main cytoskeletal elements and play a role in various important cellular functions. They are composed of tubulin dimers and a number of microtubule-associated proteins (MAPs).¹ Nerve cells, for example, contain a large number of microtubules and several kinds of MAPs. Because tubulin is a very conserved molecule, the functions of microtubules may be determined by their associated MAPs. Brain microtubules have been most extensively studied. The main MAPs in the brain are composed of high molecular mass MAPs (MAP1 and MAP2) (5, 24) and the low molecular mass protein named tau (35). MAP1 and MAP2 form arm-like structures and cross-link microtubules (7, 20, 29, 33, 34). Recently it has been determined that MAP1 and MAP2 are components of cross-bridges between microtubules in the neuronal cytoskeleton (13, 29, 30).

The mitotic apparatus is also a structure composed mainly of microtubules and related proteins. Molecular dissection of microtubule-associated structures and proteins in the mitotic apparatus has been less frequently performed than with that of nerve cells. Using monoclonal antibodies, Izant et al. found several mitotic apparatus-specific proteins (18). Rebhun

and co-workers have described an 80-kD protein in microtubules extracted from isolated mitotic spindles of sea urchin eggs (19, 25). Vallee and Bloom have identified several mitotic spindle MAPs from sea urchin egg microtubules with monoclonal antibodies (32). In an attempt to understand the structure and molecular composition of mitotic apparatus, we previously investigated the cytoskeletal architecture of the isolated mitotic apparatus by the quick-freeze, deep-etch method (14). We found that the surface of spindle microtubules was densely covered with hexagonally packed, small, round, button-like structures which were quite uniform in shape and size (14). The microtubules were extensively linked by cross-bridges. We found the microtubule reconstituted from the isolated mitotic apparatus to be composed of high molecular mass proteins (245 kD and 250 kD), 75- and 45-kD proteins, and tubulin (14). The unique microtubule buttons attracted our attention, and we attempted to determine their protein composition.

In this study, we purified the 75-kD MAP using gel filtration, and characterized it. The 75-kD protein was found to be a MAP in the sense that it enhanced the polymerization of tubulin. Its molecular mass was close to that of tau, but this protein was not heat-resistant and did not bind to calmodulin, giving it characteristics quite different from tau (22, 31). A quick-freeze deep-etch study demonstrated that the 75-kD protein is a spherical molecule (~ 9 nm in diameter) and forms microtubule buttons when it is polymerized with porcine brain tubulin.

1. *Abbreviations used in this paper:* MAP(s), microtubule-associated protein(s); MAP1 and MAP2, high molecular mass microtubule-associated proteins 1 and 2; PCM, 1 mM MgCl₂, 1 mM CaCl₂, 1 M Pipes; PC tubulin, phosphocellulose column-purified tubulin; PEM, 0.1 M Pipes, 1 mM EGTA, 1 mM MgCl₂.

Materials and Methods

Preparation of Microtubule Proteins and MAPs from Sea Urchin Eggs

Taxol-stabilized microtubules were prepared using modifications of the method of Vallee (31) from eggs of the sea urchins *Pseudocentrotus depressus* and *Hemicentrotus pulcherrimus*.

Unfertilized eggs were dejellied by decreasing the pH of artificial seawater to 5. After being washed with Ca^{++} -free seawater and 0.1 M Pipes, 1 mM EGTA, 1 mM MgCl_2 (PEM) containing 1 M glycerol, the eggs were homogenized in 1.5 vol of PEM buffer containing 1 mM PMSF, 10 $\mu\text{g}/\text{ml}$ leupeptin, and 10 $\mu\text{U}/\text{ml}$ aprotinin at 4°C. They were centrifuged at 16,000 rpm at 4°C for 30 min. The supernatant was further centrifuged at 180,000 g at 4°C for 75 min and the pellets were discarded. Taxol and GTP were added to the supernatant to get final concentrations of 20 μM taxol and 1 mM GTP, respectively. The supernatant was kept warm at 30°C for 10 min and chilled on ice for 15 min, and the microtubules that had formed were centrifuged at 30,000 g for 30 min through a cushion of 10% sucrose in PEM containing 10 μM taxol and 1 mM GTP. The resulting pellets were suspended in PEM containing 0.6 M NaCl, 20 μM taxol, and 1 mM GTP on ice and incubated for 20 min. Then the suspensions were centrifuged at 40,000 g for 30 min at 4°C and the supernatant was collected for gel filtration.

Preparation of Microtubule Proteins and MAPs from Isolated Mitotic Apparatus

Microtubule proteins were prepared from the isolated mitotic apparatus using taxol, as described in previous papers (14, 26).

Fractionation of High Molecular Mass and 75-kD MAPs

The supernatant of high salt extracts of the microtubule protein was further fractionated by gel filtration chromatography using a Bio-Gel A-1.5 m column (Bio-Rad Laboratories, Richmond, CA) with PEM buffer containing 1 mM PMSF and 1 $\mu\text{g}/\text{ml}$ leupeptin. The protein composition of each fraction was analyzed by SDS PAGE using 7.5% acrylamide (21). For molecular mass calibration the gel filtration standard (Bio-Rad Laboratories, Richmond, California) was applied through the same column.

Test for Heat Stability of the 75-kD MAP

The 75-kD protein fraction in PEM buffer containing 0.75 M NaCl and 10 mM 2-mercaptoethanol (protein concentration, 0.6 mg/ml) was rapidly pipetted into tubes set in boiling water for 4 min (8). The tubes were then rapidly cooled in ice water and the solution was centrifuged at 4°C for 30 min at 10,000 g. The resulting pellets and supernatant were analyzed by SDS PAGE.

Test for Calmodulin Binding Ability of the 75-kD MAP

Calmodulin was purified from porcine brain and coupled to cyanogen bromide-activated sepharose 4B (16). 75-kD protein was applied in the presence of 1 mM CaCl_2 on a calmodulin-Sephadex 4B equilibrated with 1 mM MgCl_2 , 1 mM CaCl_2 , and 0.1 M Pipes buffer (PCM), pH 6.6. After washing the column with the same buffer (15 ml), and then with PCM containing 0.5 M NaCl (15 ml), the protein adsorbed on the column in a Ca^{++} -dependent manner was eluted with PEM (pH 6.6).

Stimulation of Polymerization of Porcine Brain Tubulin by the 75-kD MAP

Microtubule protein from porcine brains was purified by two cycles of temperature-dependent assembly and disassembly in PEM buffer as described by Shelanski et al. (28). Tubulin was purified by phosphocellulose column chromatography as described by Herzog and Weber (8).

Stimulation of polymerization of phosphocellulose column-purified tubulin (PC tubulin) by 75 kD was assayed by turbidity in a constant tubulin concentration (1 mg/ml) by varying the concentration of 75 kD. The 75-kD protein and porcine tubulin were mixed at 0.57:1, 0.29:1, and 0.14:1 mass ratios in PEM buffer containing 1 mM GTP and incubated at 37°C for 20 min. As a control, tubulin alone was processed similarly. In the case of the sample containing 75-kD protein and tubulin at a 0.57:1 ratio, the sample

was cooled on ice for 5 min and then rewarmed at 37°C to examine the effect of cooling. Microtubule polymerization was also ascertained by negative staining electron microscopy.

To examine the stoichiometry of tubulin versus 75 kD, tubulin was mixed with a large amount of 75-kD protein (75 kD/tubulin was 0.57:1) in PEM buffer containing 1 mM GTP and incubated at 37°C for 20 min. The aliquots of the solutions were diluted 5–10-fold by PEM buffer with 1 mM GTP and dropped on grids coated with Formvar and carbon. They were stained with 2% uranyl acetate and examined with an electron microscope. Other parts of the solutions were centrifuged at 10,000 g for 30 min at 30°C. The resulting pellets and supernatants were analyzed by SDS PAGE and the pellets were concomitantly quick-frozen and deep-etched. The SDS gels were scanned by a densitometer. The area of the peaks of tubulin and the 75-kD protein were measured and the relative ratio of tubulin to 75-kD protein was calculated to determine the stoichiometry.

Analytical Methods

SDS PAGE was performed according to the method of Laemmli using 7.5% acrylamide (21). Gels were stained with Coomassie Brilliant Blue.

Protein concentration was determined according to a method described by Bradford (6) using bovine serum albumin as a standard.

Quick-Freeze Deep-Etch Electron Microscopy of Polymerized Microtubules with 75-kD MAP and 75-kD MAP Adsorbed on Mica

Pellets of porcine brain PC tubulin polymerized with 75-kD MAP in the presence of 1 mM GTP at 37°C were quick-frozen, freeze-fractured, and deep-etched at -95°C for 6 min (9, 11). Then they were rotary shadowed with platinum (at 24°) and carbon as described previously (9, 11). The specimens were dissolved in chromesulfuric acid. The purified 75-kD protein dissolved in PEM ($\sim 100 \mu\text{g}/\text{ml}$) was dropped onto fragmented mica flakes and quick-frozen as described by Heuser (10). Then the flakes were fractured at -95°C and etched for 6 min and rotary shadowed with platinum (at 10°) and carbon. The mica and proteins were dissolved in hydrofluoric acid. After being washed with distilled water, the replicas were viewed with a JEOL 1200 EX or 2000 EX electron microscope at 100 kV with $\pm 10^\circ$ tilt.

Results

Fractionation of 75-kD MAPs

High salt extract from taxol-microtubule proteins was gel-filtrated on Bio-Gel A-1.5 m column. Fig. 1 shows an example of its elution pattern. The 75-kD protein was a main component of the second peak (Fig. 2). As shown in Fig. 2,

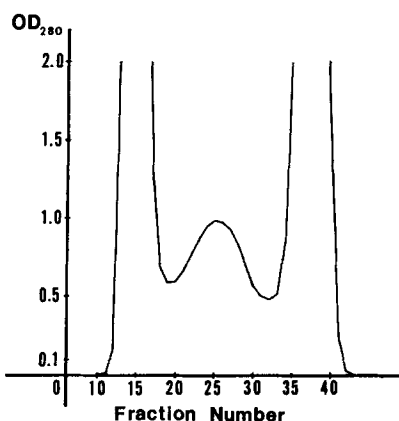


Figure 1. Gel filtration chromatography on a Bio-gel A-1.5 m column of high salt extract. About 2 ml of high salt extract was applied on a 1 × 50-cm column of Bio-gel A-1.5 m preequilibrated and eluted in PEM buffer containing 1 $\mu\text{g}/\text{ml}$ leupeptin at 4°C. Flow rate was 12 ml/h; 1-ml fractions were collected. OD₂₈₀ of the fractions was measured.

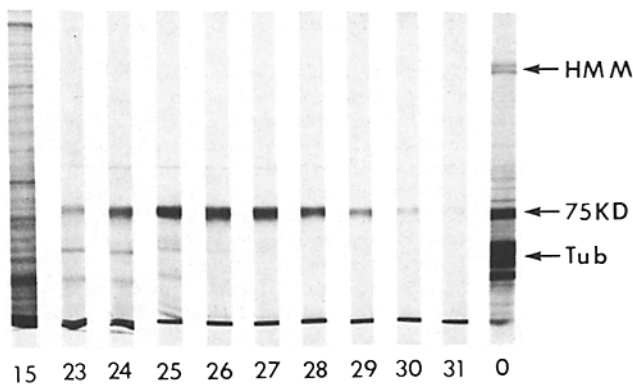


Figure 2. SDS gels of fractions from a Bio-gel A-1.5 m column. Fraction number is indicated at the bottom of the gels. Lane 0, a gel of microtubule proteins obtained from isolated mitotic spindles. HMM, high molecular mass proteins; 75 kD, 75-kD protein; Tub, tubulin.

75-kD proteins were almost purified by this gel filtration alone (see fractions 27 and 28). The peak fractions (25 and 26) contained minor contaminated proteins. Fractions 27 and 28 were composed mainly of 75-kD proteins, and these fractions were used for further experiments. We calibrated this column using the gel filtration standard in order to learn the molecular mass of the native form of 75-kD MAP molecules (Fig. 3). We found that 75-kD MAP was eluted in a position of 150 kD. Because the 75-kD protein was a globular protein, as described below, we assume that it may exist as a dimer in its native condition.

75-kD MAP-stimulated Polymerization of Porcine Brain Tubulin

Purified 75-kD protein was assayed for the polymerization-promoting activity it exerts on PC tubulin using both light scattering analysis (Fig. 4) and electron microscopy (Fig. 5).

PC tubulin purified from porcine brains was mixed with 75-kD protein at various ratios. The suspension was incubated at 37°C for 10–20 min in the presence of 1 mM GTP. Fig. 4 shows the result of light scattering analysis, which demonstrates the polymerization of tubulin by increasing the amount of 75-kD protein. Aliquots of the suspension were examined by the negative staining method, as shown in Fig. 5. Numerous microtubules were formed, but microtubule buttons were not recognizable with the negative staining. We did not find any microtubules in the solution that contained only tubulin. As a result we concluded that the 75-kD protein enhances the polymerization of tubulin, and, thus, this protein can be categorized as a MAP. It was also apparent that microtubules assembled in the presence of the 75-kD protein are cold-labile.

Stoichiometry of Tubulin and 75-kD MAP

The PC tubulin was mixed with a large amount of 75-kD MAP. After incubation at 37°C for 20 min in the presence of 1 mM GTP, the suspension was centrifuged. The resulting pellet and supernatant were subjected to SDS PAGE (Fig. 6). We found that the pellet was mainly composed of 75-kD MAP and tubulin, while in the supernatant we found a 47-kD polypeptide in addition to 75-kD MAP and tubulin. Since

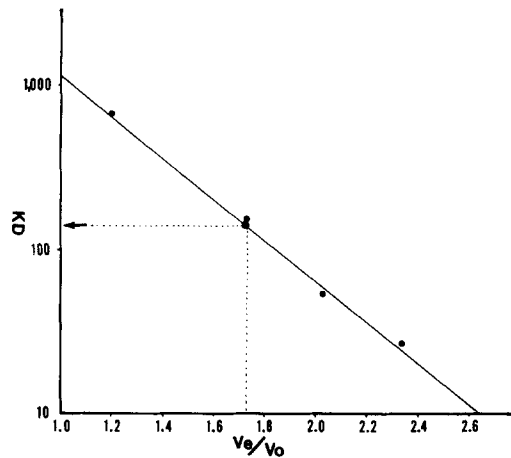


Figure 3. Estimation of the molecular mass of buttonin (75 kD) using the gel filtration standard. Calibration of the Bio-gel A-1.5 m column was carried out under the same conditions as that of the chromatography of 75 kD using Bio-Rad gel filtration standard. V_e , elution volume; V_o , void volume. Small dots indicate marker molecules (from larger to smaller: thyroglobulin [670 kD], gamma globulin [158 kD], ovalbumin [44 kD], myoglobin [17 kD]). A large dot indicates buttonin (75 kD) and an arrow shows its molecular mass.

this 47-kD polypeptide increased in amount after long storage of 75-kD MAP and after heat treatment of 75 kD, we considered this to be a degradation product of 75-kD MAP. Because 75-kD protein was detected in the supernatant and because we found by quick-freeze deep-etch study that the surface of the microtubules was largely covered with microtubule buttons (see Fig. 8), we considered that in these pellets the binding sites of tubulin were essentially saturated by the 75-kD MAP. Therefore, we analyzed the SDS gel with a densitometer to determine the stoichiometry of tubulin versus 75-kD MAP (Fig. 7). We measured the area of peaks of tubulin and 75-kD protein; as a result, the mass ratio of tubulin versus 75-kD MAP was 2.3–2.5:1. Therefore, we estimate that the molar ratio of tubulin (dimer, 110 kD) versus 75-kD MAP (dimer, 150 kD) could be ~ 3 –3.4:1.

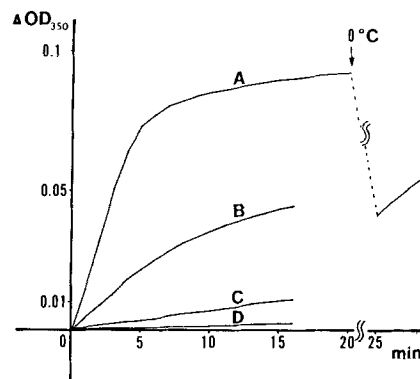


Figure 4. Polymerization-promoting activity of purified buttonin (75-kD protein) on PC tubulin measured by light scattering analysis. The tubulin concentrations are 1 mg/ml. Concentrations of buttonin (mg/ml) are (A) 0.57; (B) 0.29; (C) 0.14; and (D) 0. In the case of A the solution was cooled at 0°C for 5 min after a 20-min incubation at 37°C and then rewarmed at 37°C.

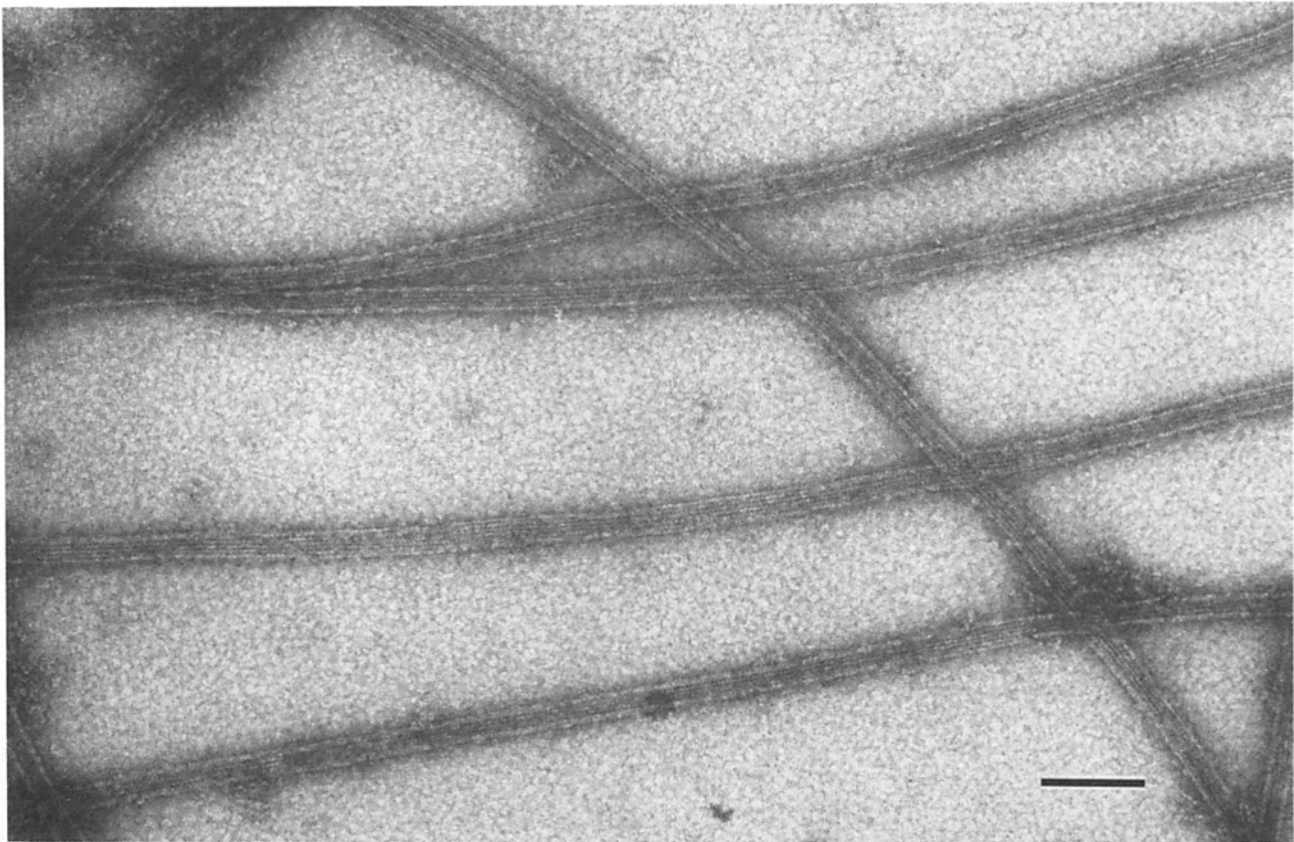


Figure 5. Negative stained microtubules reassembled from porcine brain tubulin plus buttonin (75 kD) (tubulin, 0.35 mg/ml; buttonin, 0.35 mg/ml) incubated at 37°C for 10 min in the presence of 1 mM GTP. Numerous microtubules are formed. Bar, 0.1 μ m.

The 75-kD Protein Was Not Heat-Stable and Did Not Bind to Calmodulin in the Presence of Calcium

Heat stability of 75-kD protein was examined by boiling followed with analysis on SDS PAGE. SDS PAGE of the pellets and supernatant after boiling showed that the 75-kD protein had sedimented into the pellet. This proved that the 75-kD protein was heat-labile under our conditions.

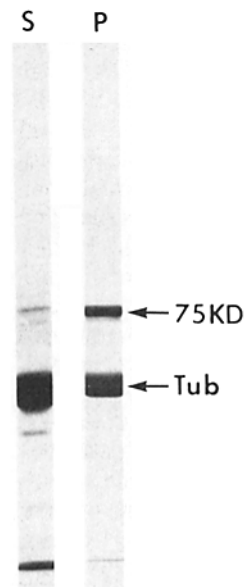


Figure 6. SDS gels of supernatant (lane S) and pellet (lane P) of the PC tubulin (Tub) mixed with an excess amount of buttonin (75 kD), incubated at 37°C for 20 min in the presence of 1 mM GTP, and centrifuged.

The binding of the 75-kD protein to calmodulin was assayed using a calmodulin-Sephrose 4B column. The 75-kD protein was washed out with PCM containing 0.5 M NaCl. Very little protein was eluted with PEM. Therefore, although the 75-kD might weakly interact with calmodulin or might remain in the calmodulin column nonspecifically, it did not bind to calmodulin in a Ca^{++} -dependent manner. These results indicated that the 75-kD MAP was different from tau, which is known to be a neuronal MAP with a similar molecular mass and is heat-resistant and binds to calmodulin in the presence of Ca^{++} .

Molecular Structure of 75-kD MAP

The suspension containing tubulin and 75-kD protein was centrifuged after incubation for 20 min at 37°C. The pellets were quick-frozen and deep-etched.

We found that the surfaces of the microtubules were entirely covered with hexagonally packed, round, button-like structures quite uniform in shape and size (~9 nm) (Figs. 8, 9, and 10). The structure and arrangement of this molecule looked quite like those of the microtubule buttons seen on

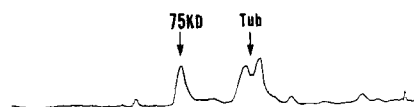


Figure 7. A densitometric scanning of an SDS gel of tubulin-buttonin pellet equivalent to the gel shown in Fig. 6. (Left) Top of the gel; (right) bottom of the gel. 75 kD, buttonin; Tub, tubulin.

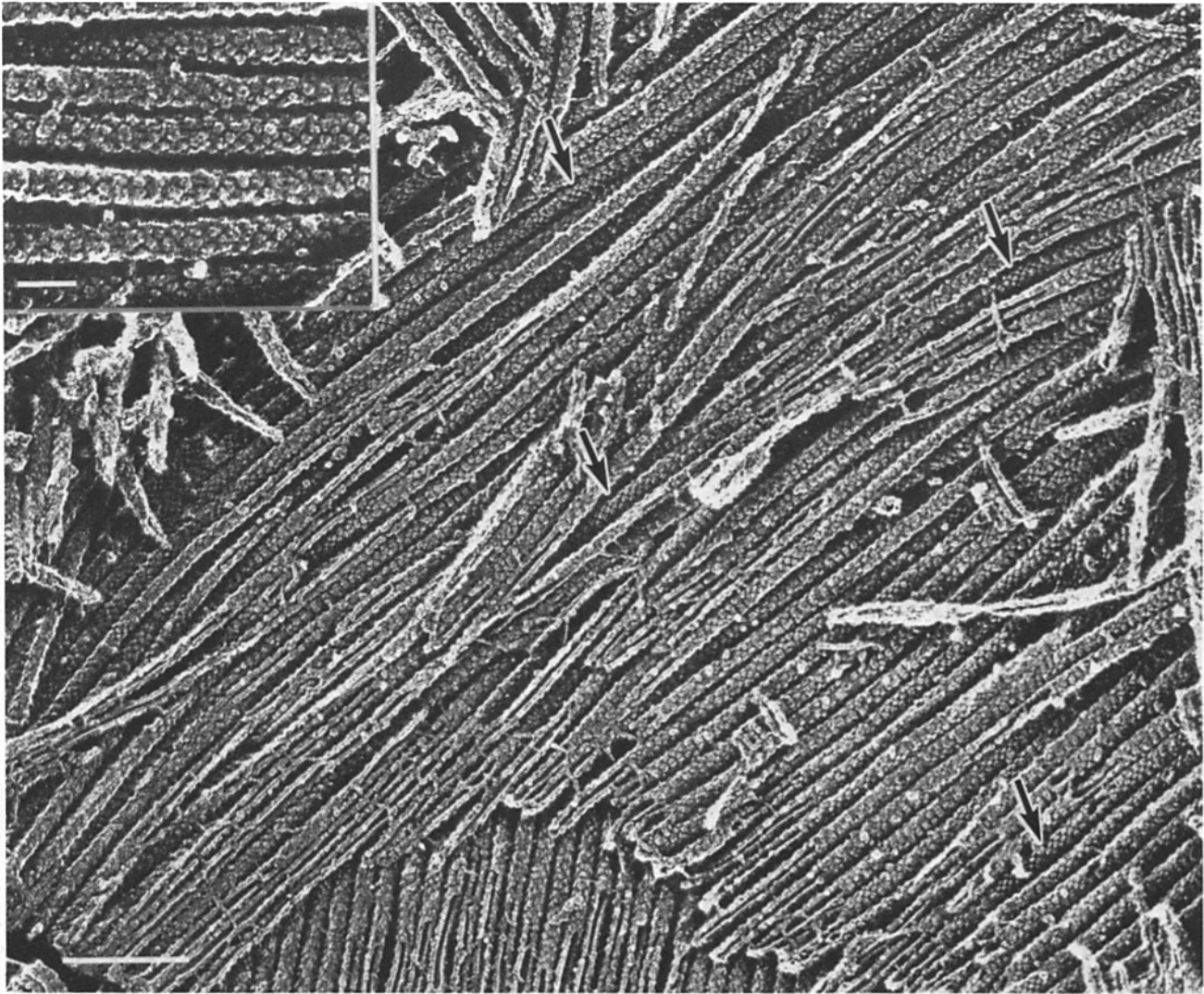


Figure 8. A quick-freeze deep-etch view of the pellet of porcine brain tubulin polymerized with butonin (75 kD) (tubulin/75 kD = 1). Numerous microtubules are formed. The surface of microtubules is mostly covered with round button-shaped molecules that tend to be packed hexagonally (*arrows*). Bar, 0.1 μm . (*Inset*) A higher magnification of microtubules covered with 75-kD MAP. Bar, 0.1 μm .

the surfaces of microtubules in the mitotic apparatus (Fig. 10). These button-like structures were arranged in a line rising at an angle of 11° to the horizontal axes of the microtubules (Figs. 9 and 10).

We analyzed the structure of single molecules of the 75-kD MAP using the low angle shadowing technique combined with the quick-freeze deep-etch method. The 75-kD MAP solution was adsorbed on mica flakes. Then it was quick-frozen and deep-etched. We frequently found spherical structures ~ 9 nm in diameter on the mica (Fig. 10). These structures appeared similar to the microtubule buttons on the surfaces of the microtubules polymerized with 75-kD MAP (Fig. 10). Occasionally, smaller granules were also found on the mica. Because a ~ 9 nm structure can be the dimeric form (~ 150 kD) of 75-kD MAP, this smaller one could very well be a monomer.

Discussion

In the previous study we found that the surfaces of spindle microtubules were covered quite regularly with hexagonally

packed button-like spherical structures 8–9 nm in diameter (14). The surfaces of microtubules polymerized in vitro from isolated mitotic spindles using taxol were also entirely covered with similar kinds of spherical molecules. The usual cross-bridges were also found between the polymerized microtubules. The microtubule fractions contained proteins of 250, 245, 75, and 45 kD as the main components besides tubulin. High salt treatment (0.6 M NaCl) removed both microtubule buttons and cross-bridges from the surfaces of the microtubules and concomitantly released high molecular mass proteins, the 75- and 45-kD proteins. Therefore, we were able to conclude that the microtubule buttons could very well be composed of one of these proteins. However, our previous studies could not ascertain which protein was a main component of the microtubule button.

In the present study we purified the 75-kD protein from sea urchin eggs, characterized it, and examined its molecular structure. A single molecule of 75-kD MAP (probably existing as a dimer [150 kD]) assumed a spherical shape ~ 9 nm in diameter, and looked quite like the microtubule buttons. Furthermore, the 75-kD protein enhanced the polymeriza-

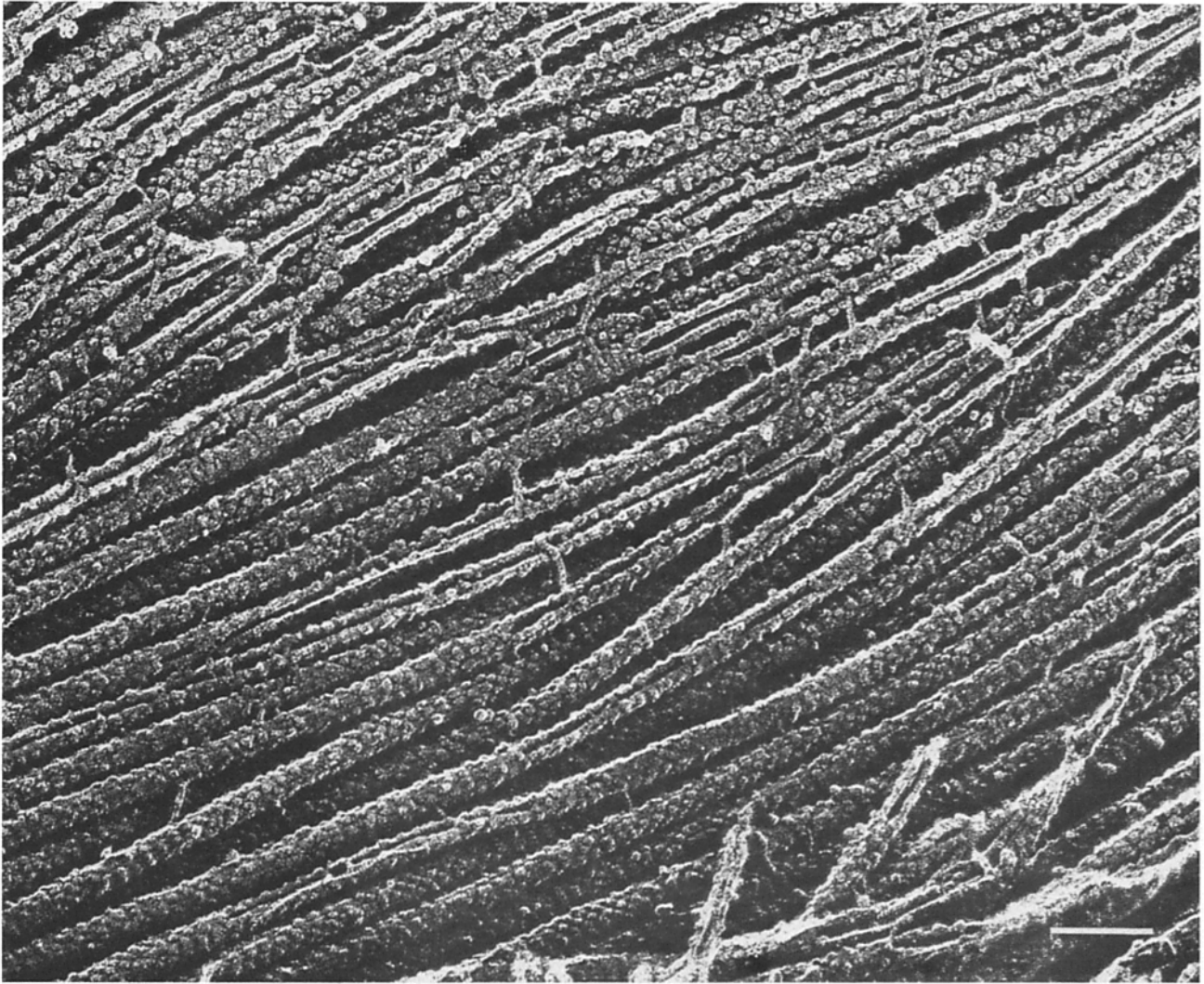


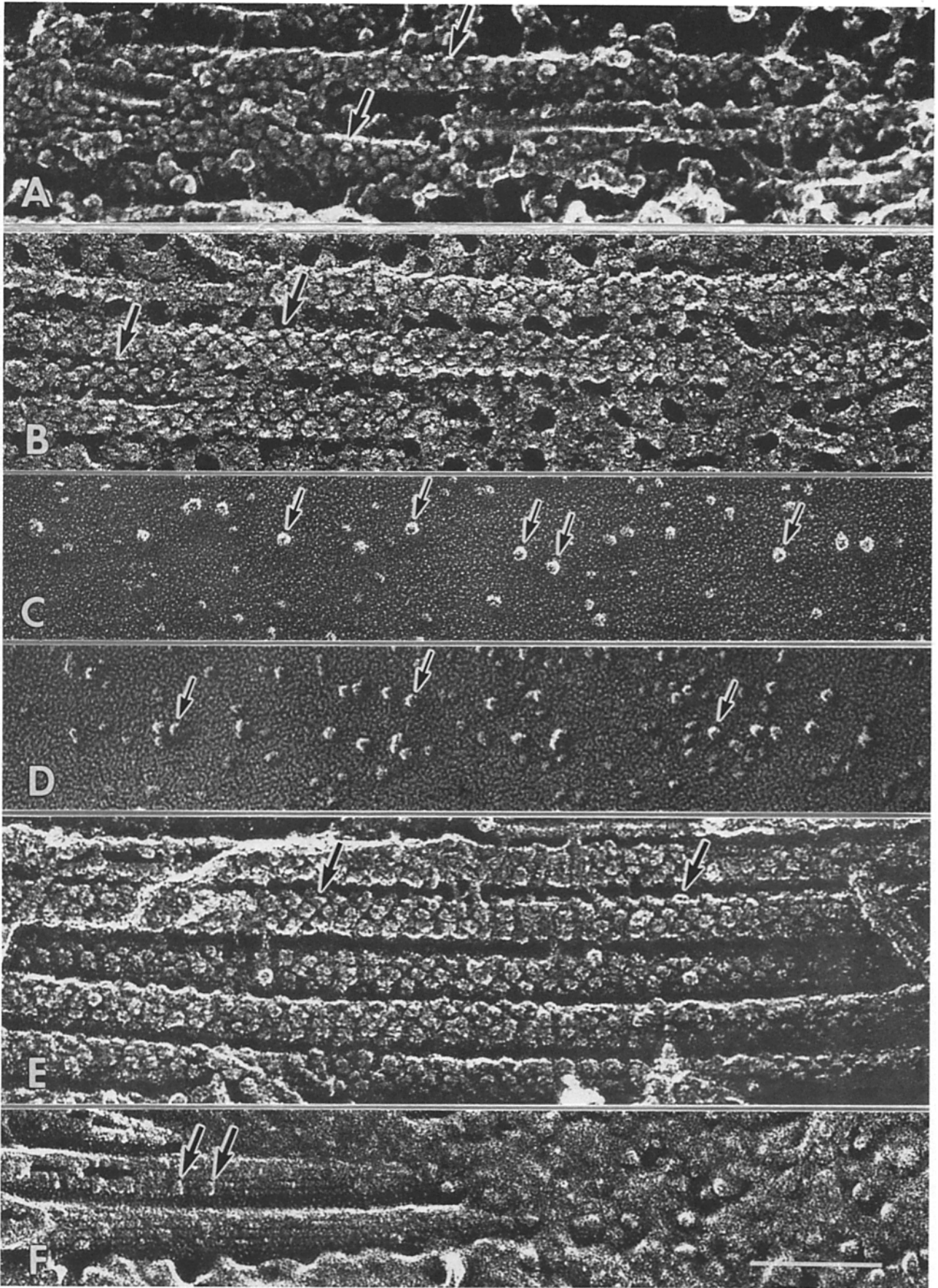
Figure 9. A higher magnification view of microtubules polymerized with buttonin (75 kD) (tubulin/75 kD = 1). Although at some portions the knife hit the surface of microtubules and removed the microtubule buttons, it is obvious at this magnification that most of the microtubule surfaces are decorated with hexagonally packed microtubule buttons. Bar, 0.1 μ m.

tion of porcine brain tubulin, and the surface of the formed microtubules was entirely covered with hexagonally packed spherical structures (\sim 9 nm in diameter), again quite similar in appearance to the microtubule buttons. Therefore, we conclude that the microtubule buttons are composed of 75-kD MAP.

The microtubule buttons were aligned at an angle of 11° to the horizontal axes of the microtubules. The center-to-center distance between adjacent buttons that were side by side and parallel to the horizontal axes of the microtubules

was \sim 15 nm. The characteristic striations observed on the inner luminal walls of the microtubules were three start helices and were separated by \sim 4 nm. They were also arranged at an angle of 11° to the horizontal axes of the microtubules. Because these striations probably reflect the arrangement of tubulin monomers, we can suppose that the 75-kD MAP is arranged in close relationship to the tubulin lattice. The center-to-center distance between adjacent buttons aligned at an angle of 11° to the horizontal axes of the microtubules was \sim 15 nm (the distance between oblique stri-

Figure 10. (A) Quick-frozen deep-etched spindle microtubules in isolated mitotic apparatus. Microtubules are covered with microtubule buttons (arrows) that tend to be packed hexagonally. (B) Quick-frozen deep-etched microtubule proteins that were isolated from mitotic spindles and polymerized in vitro. Surface of microtubules is decorated with microtubule buttons (arrows). (C and D) Quick-frozen freeze-etched, low angle rotary-shadowed buttonin (75 kD) absorbed on mica. Most spherical molecules (arrows) are \sim 9 nm in diameter, while smaller ones also exist. These spherical molecules are similar in shape and size to those on the microtubules polymerized with buttonin (75 kD) (E and F) and also to the microtubule buttons observed on spindle microtubules (A) or microtubule proteins polymerized in vitro (B). (E and F) Quick-frozen deep-etched microtubules polymerized with buttonin (75 kD) MAP. In E (75 kD/tubulin = 1) the entire surface of the microtubules is covered with spherical buttons (\sim 9 nm in diameter) that are packed hexagonally (arrows). In F (75 kD/tubulin = 1/20), microtubule buttons are observed sporadically on the surfaces of microtubules (arrows). In this case (F), microtubules were polymerized in the presence of taxol (10 μ M). Bar, 0.1 μ m.



ations on the inner surface was ~ 4 nm), and the buttons were 8–9 nm in diameter. Therefore, it is reasonable to assume that each button mainly associates with two tubulin monomers (alpha, beta or beta, alpha) and tends to span the immediately adjacent two protofilaments arranged side by side. Thus, a single button is related to about three tubulin dimers. This configuration was depicted in Fig. 9 in our previous paper (14). Theoretically if 75 kD fully saturated binding sites on microtubules, the molecular ratios of 75 kD versus tubulin could be 1:2.2–2.4. However, the stoichiometry of tubulin versus 75-kD MAP examined in the present study is 3–3.4:1. This is probably because either the binding sites on microtubules have not yet been fully saturated by 75-kD MAP in spite of the excess amount of 75-kD proteins, or the densitometric measurement underestimated the amount of tubulin.

Among the MAPs present in nerve cells and other cells, high molecular mass MAP1 and MAP2 and low molecular mass tau are the species that have been studied most extensively (1, 2, 3, 13, 17, 23). The molecular mass of 75-kD MAP was close to that of tau, but this protein was heat-labile and did not bind to calmodulin in the presence of Ca^{++} . In this regard 75-kD MAP is different from tau. This 75-kD protein may be similar to the protein that was reported by Keller and Rebhun (19), Vallee and Bloom (32), and Scholey et al. (27), who referred to it as 80-kD protein (19, 27) or 77-kD protein (32). However, although the 80-kD protein of Keller and Rebhun cycled with tubulin, it was not shown to have any effect on the rate or extent of spindle tubulin assembly under the conditions employed (19). Further, the microtubules assembled from microtubule protein that contained the tubulins, the 80-kD protein, and little else were connected by bridges; thus the 80-kD protein was assumed to act as a cross-bridge (25). Difference between species of tubulins, i.e., spindle tubulin (19) versus brain tubulin (this study), may explain different effects of these proteins on the tubulin assembly.

In terms of molecular structure, MAP2 was revealed to consist of arm-like projections from the microtubule surface (20, 24). The quick-freeze deep-etch technique showed brain high molecular mass MAPs as cross-bridges between microtubules in vitro (12, 13). MAP1 was also reported to consist of arm-like projections from the surfaces of microtubules (29, 33), and recently it has been found that it comprises a family of polypeptides, namely MAP1A, 1B, and 1C (2, 3). Rotary shadowing revealed that both MAP1A and MAP2 take the form of long, thin, flexible structures (7, 29, 34). 270-kD MAP from crayfish peripheral nerve axons that cross-reacted with anti-MAP2 antibodies also revealed a similar kind of structure (15). Thus, many high molecular mass MAPs have appeared as thin rod-like structures (15, 29, 34). Recently we have identified both MAP1 and MAP2 as components of cross-bridges between microtubules in neuronal cytoskeletons (13, 29, 30). In this regard the 75-kD MAP is unique in that it is spherical in shape and quite regularly and densely arranged on the microtubules in vivo and in vitro. Because we have previously identified unique microtubule buttons on the spindle microtubules in mitotic apparatus in vivo and in pellets of microtubule proteins from isolated mitotic spindles that contained 75-kD protein as a major protein (14), and because we observed identical microtubule buttons on microtubules formed from purified tubulin

and 75-kD protein in this study, this molecule is the first MAP whose molecular structure has been clearly identified at a single molecular level both in vivo and in vitro.

Because in the present study it becomes clear that the 75-kD MAP takes a unique button-like shape, we propose to call this MAP "buttonin." The physiological function of buttonin is still unknown. However, we found that microtubule buttons densely cover the surface of spindle microtubules and that the buttonin stimulates polymerization of tubulin. In addition, because Bloom and Vallee recently reported that the anti-77-kD antibody stained the mitotic spindle specifically (4), we can readily suppose that buttonin may play an important role in spindle formation and may as well be involved in somehow stabilizing spindle microtubules.

We thank the Misaki Marine Biology Institute of the University of Tokyo for help in collecting the sea urchins. We are grateful to Ms. Y. Kawasaki for her secretarial assistance and to Mr. Y. Fukuda for his photographic expertise. We thank the monitoring editor and reviewers of our paper for their valuable suggestions.

This work was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science, and Culture, and a grant from the Muscular Dystrophy Association of America to N. Hirokawa.

Received for publication 23 June 1986, and in revised form 11 February 1987.

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