MAP 1C Is a Microtubule-activated ATPase Which Translocates Microtubules In Vitro and Has Dynein-like Properties

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Abstract. We observe that one of the high molecular mass microtubule-associated proteins (MAPs) from brain exhibits nucleotide-dependent binding to microtubules. We identify the protein as MAP 1C, which was previously described in this laboratory as a minor component of standard microtubule preparations (Bloom, G. S., T. Schoenfeld, and R. B. Vallee, 1984, *J. Cell Biol.*, 98:320–330). We find that MAP 1C is enriched in microtubules prepared in the absence of nucleotide. Kinesin is also found in these preparations, but can be specifically extracted with GTP. A fraction highly enriched in MAP 1C can be prepared by subsequent extraction of the microtubules with ATP. Two activities cofractionate with MAP 1C upon further purification, a microtubule-activated ATPase activity and a microtubule-translocating activity. These activities indicate a role for the protein in cytoplasmic motility. MAP 1C coelectrophoreses with the beta heavy chain of *Chlamydomonas* flagellar dynein, and has a sedimentation coefficient of 20S. Exposure to ultraviolet light in the presence of vanadate and ATP results in the production of two large fragments of MAP 1C. These characteristics suggest that MAP 1C may be a cytoplasmic analogue of axonemal dynein.

CYTOPLASMIC microtubules are composed of tubulin and a number of microtubule-associated proteins (MAPs)[1] thought to be involved in microtubule assembly regulation and microtubule function. In brain tissue the most prominent MAPs are a class of five high molecular mass proteins, which we have termed MAP 1A, MAP 1B, MAP 1C, MAP 2A, and MAP 2B (Bloom et al., 1984, 1985; Luca et al., 1986). MAP 2A and MAP 2B appear to be closely related in primary structure (Herrmann et al., 1984; Bloom et al., 1985). The MAP 1 polypeptides are less closely related than the MAP 2 species, as judged by both biochemical and immunological analysis (Bloom et al., 1984, 1985), though some structural relationship appears to exist between MAP 1A and MAP 1B (Luca et al., 1986; Vallee and Luca, 1985; Schoenfeld, T., R. Obar, L. McKerracher, and R. Vallee, manuscript in preparation).

MAP 1C has shown distinct properties from the other MAP species (Bloom et al., 1984), and has remained the least understood of the high molecular mass MAPs. It is typically observed as a trace component of microtubules. It could be distinguished from the other species on the basis of its insensitivity to proteolytic degradation (op. cit.), in contrast to the extreme sensitivity of the other MAPs to either trypsin or chymotrypsin (Vallee and Borisy, 1977; Vallee, 1980).

When the high molecular mass MAPs were first identified, they seemed reasonable candidates for a role in microtubule-associated motility in the cell. They were of a similar subunit size to axonemal dynein, an ATPase understood to provide the motive force for microtubule-associated motility in flagellar and ciliary axonemes (see reviews by Gibbons, 1981; Johnson, et al., 1984). ATPase activity was, indeed, found to be associated with preparations of cytoplasmic microtubules from brain tissue (see, for example, Burns and Pollard, 1974; Gaskin et al., 1974; White et al., 1980; Murphy et al., 1983*a*, *b*). However, extensive biochemical characterization of partially purified MAPs preparations, as well as individual MAPs (see above), showed no apparent evidence in support of a role in microtubule mechanochemistry.

We have now purified and characterized MAP 1C. We find that this protein is a microtubule-activated ATPase and that it can also translocate microtubules in an in vitro assay. Its properties are distinct from all of the other known high molecular mass MAPs, as well as from kinesin (Vale et al., 1985*a*; Kuznetsov and Gelfand, 1986) and the sea urchin egg microtubule-activated ATPase (Collins and Vallee, 1986*a*, *b*), and it may represent the long sought cytoplasmic analogue of axonemal dynein.

Materials and Methods

Purification of Brain Microtubules with Taxol, and Extraction of MAP 1C

Microtubules were purified from calf brain white matter using taxol (Vallee, 1982, 1986*b*). Nucleotide and buffer ion conditions were modified as described below and in Results. For the purification of MAP 1C, the following procedure was used. Calf brain white matter (60–90 g wet wt) from five to six calf brains was dissected away from adjacent gray matter. The tissue

1. *Abbreviation used in this paper*: MAP, microtubule-associated protein.

was homogenized in 1.0 vol of extraction buffer (50 mM Pipes, 50 mM Hepes, pH 7.0, 2 mM $MgCl_2$, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml tosyl arginine methyl ester, 1 μg/ml of pepstatin A, and 0.5 mM dithiothreitol (DTT). The tissue was homogenized by two passes in a 200-ml capacity Teflon pestle homogenizer at 2,000 rpm, and the homogenate was centrifuged in a GSA rotor (Sorvall Instruments Div., DuPont Co., Newton, CT) at 12,000 rpm for 30 min at 2°C. The supernate was recovered and centrifuged at 45,000 rpm in a rotor (60Ti; Beckman Instruments, Inc., Palo Alto, CA) for 60 min. The supernate was again recovered, taxol was added to 20 μM, and the sample was warmed to 37°C for 10 min. Microtubules were sedimented at 18,000 rpm in a rotor (SS-34; Sorvall Instruments) at 37°C for 30 min into a cushion of 10% sucrose in extraction buffer. The microtubules were typically washed and extracted as follows. Buffer wash: The microtubules were resuspended to 0.2 vol in extraction buffer containing 5 μM taxol, and recentrifuged as described above at 30°C. GTP extractions: The pellet was resuspended to volume in extraction buffer containing 5 μM taxol. The microtubules were incubated with 5 mM MgGTP for 10 min at room temperature, and subsequently for 5 min at 37°C, to dissociate kinesin (see Results). The microtubules were again sedimented and resuspended, and the GTP extraction procedure was repeated with 1 mM MgGTP. ATP extraction: Finally, the pellet was resuspended to 0.5 vol (0.1 extract vol), and the microtubules were incubated with 10 mM MgATP for 15–30 min at room temperature to dissociate MAP 1C, and then at 37°C for 5 min to ensure complete assembly of the taxol-microtubules. The microtubules were sedimented at 30°C for 30 min at 150,000 *g* in a rotor (60Ti; Beckman Instruments, Inc.) MAP 1C was recovered in the supernate as 50–75% of total protein. The pellet contained almost all of the tubulin and other MAPs.

Additional Fractionation Techniques

The ATP-extracted proteins, containing MAP 1C as the major polypeptide component, were applied to six 11-ml linear gradients of 5–20% sucrose in 20 mM Tris HCl, pH 7.6, containing 50 mM KCl, 5.0 mM $MgCl_2$, 0.5 mM EDTA (Tris/KCl buffer), or in 10 mM sodium phosphate, 100 mM sodium glutamate, pH 7.0, containing 1 mM $MgSO_4$, 1 mM EGTA, and 0.5 mM DTT (phosphate/glutamate buffer). Centrifugation was for 16 h at 2°C at 31,500 rpm in a rotor (SW41; Beckman Instruments, Inc.). The peak fractions containing MAP 1C at 20S were pooled and stored at 0–2°C for use for up to 3 d. Standards for S-value determination were mouse immunoglobulin, catalase, and thyroglobulin. The MAP 1C peak was usually of sufficient purity at this stage for use in motility and ATPase analysis. Further chromatography of the pooled sucrose density gradient peak fractions was also performed in some instances using a 1-ml column of DEAE-Sepharose CL-6B equilibrated with Tris/KCl buffer and eluted with a 10 ml gradient of 50–550 mM KCl in 20 mM Tris. Alternatively a 2-ml column of hydroxylapatite was used, equilibrated with 20 mM potassium phosphate, pH 6.8, and eluted with a 10 ml linear gradient of 100–500 mM potassium phosphate.

Preparation of Pure Tubulin Microtubules and Axonemal Dynein

Calf brain tubulin was obtained from reversible assembly-purified microtubules by DEAE-Sephadex ion exchange chromatography (Vallee, 1986*a*). For use in ATPase assays, tubulin (4 mg/ml) was exposed to taxol (80 μM) at 37°C for 5 min and centrifuged at 18,000 rpm for 30 min at 37°C in a rotor (SS-34; Sorvall Instruments). The microtubules were twice resuspended in Tris/KCl buffer and recentrifuged. Axonemal outer arm dynein was prepared as a crude KCl extract from *Chlamydomonas* flagella (King et al., 1986), and kindly provided by Drs. Steven King and George Witman.

Other Biochemical Methods

ATPase assays were conducted at 37°C in a 50 μl vol, with 10–20 μl of enzyme sample, 20 μl of Tris/KCl buffer, with or without added microtubules as noted, and 10 μl of 2 mM [γ-^{32}P]ATP (Collins and Vallee, 1986*a*). Protein concentration was determined by the method of Lowry et al. (1951). Gel electrophoresis was performed according to the method of Laemmli (1970) using either 4 (where noted) or 7% acrylamide (if not noted) in the separating gel, generally at 65 V for 4% gels and 150 V for 7% gels. Quantitative analysis of electrophoretic samples was performed by densitometry as described previously (Vallee and Davis, 1983). MAP 1C was compared to dynein using silver-stained SDS/urea gels (Pfister et al., 1983).

Chymotrypsin Digestion and Vanadate Cleavage

Chymotrypsin digestion was performed as described in Bloom et al. (1984). Vanadate cleavage was performed according to the method described by Gibbons et al. (1987) and King and Witman (1987).

Motility Assay

The assay of Vale et al. (1985*c*) was used. 5 μl of sample ± 1 mM MgATP was applied to a glass coverslip and allowed to adsorb for 5 min at room temperature. 2 μl of taxol-stabilized microtubules in Tris/KCl buffer composed of purified calf brain tubulin (see above) was applied to a glass slide. The coverslip was applied to the slide, and the edges of the coverslip were sealed with Valap (a 1:1:1 mixture of petrolatum/lanolin/beeswax). Observations were made at ambient temperature, determined to be 30°C. The microtubules were visualized by video-enhanced differential interference contrast microscopy, using a Zeiss IM35 microscope equipped with a 63× Planapochromat lens and a DAGE MTI 67m video camera (transfer factor to camera = 6.25×). Digitization, contrast enhancement, and background subtraction were accomplished using an image processor (model 794 Image Sigma; Hughes Aircraft Co., El Segundo, CA). The final magnification to the video screen was ~1:8,500, and was determined precisely using a stage micrometer (model No. 1400; American Optical Corp., Buffalo, NY). Rates of gliding were determined by tracing microtubules from the video monitor onto clear plastic sheets. Microtubules judged to show uninterrupted motion were used. In most preparations, >90% of the microtubules showed gliding motility.

Biochemical Reagents

Taxol was obtained as a gift from Dr. Matthew Suffness (National Cancer Institute, Bethesda, MD). Protease inhibitors, nucleotides, and sedimentation standards were obtained from Sigma Chemical Co. (St. Louis, MO), vanadate from Fisher Scientific Co. (Pittsburgh, PA), and electrophoretic standards from Bio-Rad Laboratories (Richmond, CA).

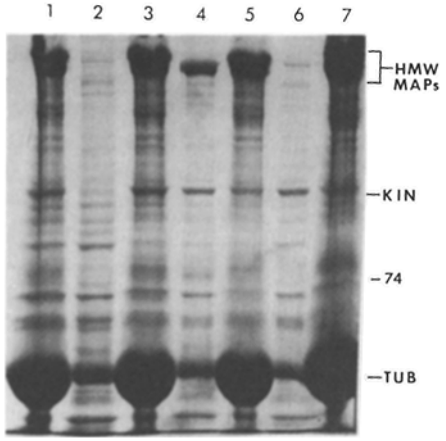

Figure 1. Extraction of microtubules with nucleotides. Microtubules were prepared from calf brain white matter by the taxol method, but without added nucleotide. The microtubules were washed in extraction buffer. The sample was split, and then extracted with either ATP or GTP. Lane *1*, first microtubule pellet; lanes *2* and *3*, supernate and pellet after wash in extraction buffer; lanes *4* and *5*, supernate and pellet after ATP extraction; lanes *6* and *7*, supernate and pellet after GTP extraction. High molecular weight (*HMW*) MAPs were too heavily loaded to be resolved into individual bands. *KIN*, kinesin; *TUB*, tubulin; *74*, 74,000-D protein that cofractionates with MAP 1C (see text).

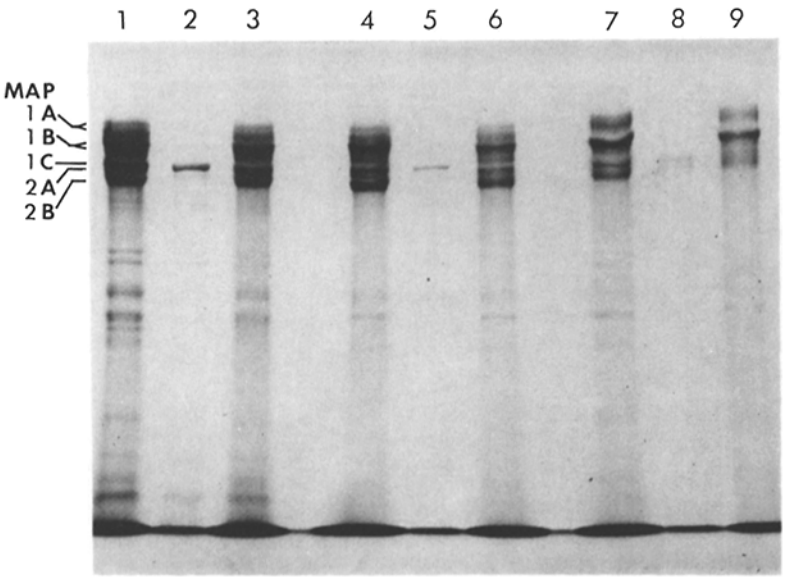

Figure 2. ATP extraction of MAP 1C from microtubules prepared under different nucleotide conditions. Microtubules were prepared in the absence of added nucleotide (lanes *1–3*), the presence of 5 mM GTP (lanes *4–6*), or the presence of 5 mM ATP (lanes *7–9*). Groups of three lanes represent first microtubule pellets (lanes *1*, *4*, and *7*); and supernates (lanes *2*, *5*, and *8*) and pellets (lanes *3*, *6*, and *9*) after extraction of the microtubules with ATP (10 mM). Some of the MAP bands were observed to split, complicating the identification of individual MAP species. The identification of subbands as shown is based on reactivity with monoclonal anti-MAP 1A, anti-MAP 1B, and anti-MAP 2 antibodies. The MAP 1C band ran almost exactly at the position of MAP 2A (see also Fig. 3), though in lane *4* it could be distinguished just above MAP 2A. The position of MAP 1C is variable depending on electrophoretic conditions.[2] A 4% polyacrylamide gel was used.

Results

Identification of MAP 1C as a Nucleotide-dependent Microtubule-binding Protein

In earlier work we found that the composition of the high molecular mass MAPs depends on a variety of factors, most notably the region of the brain used as a biochemical source (Vallee, 1982; Bloom et al., 1985). In the present study we sought to explore the effect of nucleotides on MAP composition. Our aim was to gain further insight into the basis for the complexity of the MAPs, and to identify possible candidates for a role in microtubule mechanochemistry.

Fig. 1 shows microtubules prepared from calf brain white matter without the use of nucleotides. The overall composition of the microtubules (Fig. 1, lanes *1* and *3*) is similar to that in our standard preparation (Vallee, 1982; Bloom et al., 1984), which includes GTP. The most prominent proteins in the preparation other than tubulin are the high molecular mass MAPs. In addition, an electrophoretic species at the position of kinesin can be seen in the microtubule pellet, as well as other minor species.

To examine further the effect of nucleotide on MAP composition, the microtubules were resuspended in 10 mM MgATP and recentrifuged. Three proteins were specifically solubilized by this procedure (Fig. 1, lane *4*): a high molecular mass species, which was most prominent, kinesin, and a polypeptide of M_r 74,000. Several other polypeptides were observed, which had also been solubilized when microtubules were washed without nucleotide (Fig. 1, lane *2*). When the microtubules were extracted with GTP instead of ATP (Fig. 1, lane *6*), neither the high molecular mass nor the M_r 74,000 polypeptide were solubilized. However, kinesin was as efficiently solubilized with GTP (Fig. 1, lane *6*) as with ATP (lane *4*).

To determine whether the ATP-extractable high molecular mass species was related to any of the previously identified MAPs, we prepared microtubules using no added nucleotide, GTP, or ATP (Fig. 2). Each preparation was then extracted with ATP and examined on a low percent polyacrylamide gel. A single high molecular mass electrophoretic species was observed in the no-nucleotide preparation (Fig. 2, lanes *1–3*). The same species was found in the GTP preparation (Fig. 2, lanes *4–6*), suggesting that it corresponded to one of our previously identified MAPs. Less of the protein was obtained in the GTP preparation, and virtually none was detectable when microtubules were prepared with added ATP (Fig. 2, lanes *7–9*).

The ATP-extractable protein migrated just at or slightly above the electrophoretic position of MAP 2A (Fig. 2). This suggested that it was MAP 1C,[2] which was originally identified as a protease-resistant MAP in our standard GTP microtubule preparations (Bloom et al., 1984). Fig. 3 shows the effect of chymotrypsin on microtubules prepared without nucleotide, with GTP (our standard condition), and with ATP. A single chymotrypsin-resistant species corresponding to MAP 1C was observed in the standard GTP preparation (Fig. 3, lanes *4–6*). What appeared to be the same species was, indeed, enriched in the no-nucleotide preparation (Fig. 3, lanes *1–3*), and was almost absent in the ATP-preparation (lanes *7–9*).

Additional tests confirmed that the protein was distinct from MAP 1A, MAP 1B, and MAP 2. Using immunoblotting, it showed no reaction with monoclonal antibodies to MAP 1A and MAP 1B (Bloom et al., 1984, 1985), or with three separate monoclonal and one polyclonal antibodies to MAP 2 (Vallee and Luca, 1985). The protein precipitated when microtubules were incubated at elevated temperature,

2. A curious property of MAP 1C is that its electrophoretic mobility relative to the other MAPs increases with increasing electrophoresis rate, as first pointed out to us by Dr. J. De Mey. We have observed this behavior both in our standard microtubule preparations and with the ATP-extractable high molecular mass MAP. This property further identifies the latter as MAP 1C, though it makes observation of the protein in unfractionated microtubule preparations somewhat difficult. At slow electrophoretic rates, MAP 1C runs between MAP 1B and MAP 2A (Bloom et al., 1984; 1985). However, at rapid rates, it has a higher mobility, and can even appear below MAP 2B. Under the conditions used in the present report, MAP 1C may be found just above MAP 2A, or at the same electrophoretic position.

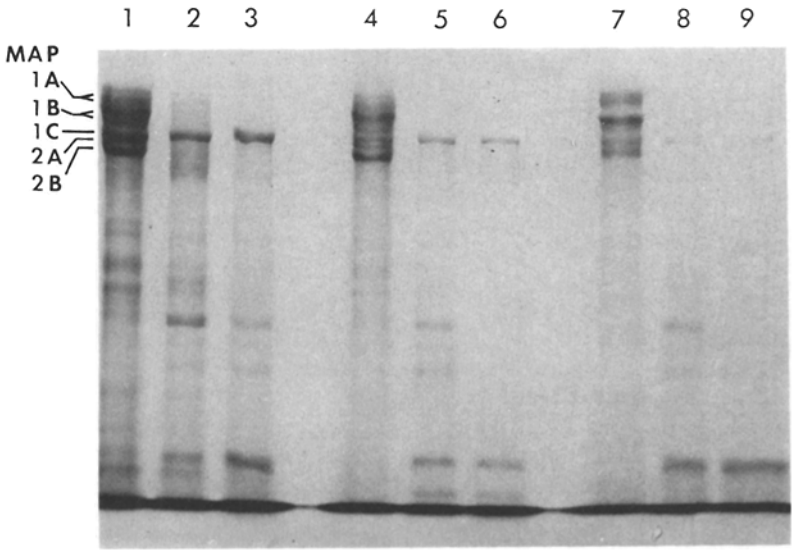

Figure 3. Chymotryptic digestion of microtubules prepared under different nucleotide conditions. As in Fig. 2, microtubules were prepared in the absence of added nucleotide (lanes *1–3*), the presence of 5 mM GTP (lanes *4–6*), or the presence of 5 mM ATP (lanes *7–9*). In each case the microtubules were exposed to chymotrypsin (0.33 μg/ml) for 0 (lanes *1*, *4*, and *7*), 3.5 (lanes *2*, *5*, and *8*), and 28 min (lanes *3*, *6*, and *9*). A single protease resistant band, judged to be MAP 1C, was observed. A 4% polyacrylamide gel was used.

further distinguishing it from the MAP 2 polypeptides (Kim et al., 1979).

Our data indicate that MAP 1C, uniquely among the high molecular mass MAPs, binds to microtubules in a nucleotide-dependent fashion. The protein can, therefore, be obtained in highest yield when microtubules are prepared in the absence of added nucleotide, and can be extracted from microtubules by ATP, but not by GTP.

Cofractionation of ATPase Activity with MAP 1C

A purification strategy for MAP 1C was developed based on its nucleotide-dependent microtubule-binding characteristics. Microtubules were assembled in the absence of added nucleotide to induce the binding of MAP 1C (Fig. 4, lanes *1–3*). The microtubules were then washed successively in extraction buffer without nucleotide, extraction buffer with GTP, and, finally, extraction buffer with ATP. The first wash step (Fig. 4, lanes *4* and *5*) removed some residual cytosolic contaminants. GTP solubilized most of the kinesin and another band at M_r 100,000, but had no effect on MAP 1C and an M_r 74,000 species (Fig. 4, lanes *6–9*). ATP extraction resulted in a fraction that contained MAP 1C as its major component (Fig. 4, lane *10*). The M_r 74,000 band was also specifically extracted in this fraction. This species proved to copurify with MAP 1C through all subsequent steps (see below). A variable level of tubulin was also present in the ATP extract. In addition, a low level of the other high molecular mass MAPs was often found in this material, along with low levels of kinesin and other minor proteins that were incompletely desorbed in the GTP extraction steps.

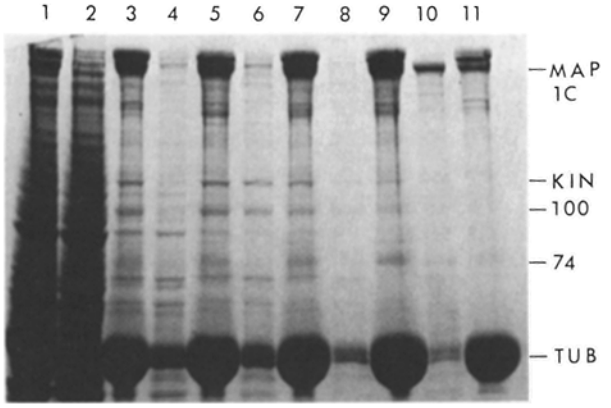

Figure 4. Preparation of microtubules and ATP extraction of MAP 1C. Microtubules were prepared by the taxol method without added nucleotide, washed to remove contaminating proteins and kinesin, and then MAP 1C was specifically extracted with ATP. Lane *1*, white matter cytosolic extract; lanes *2* and *3*, supernate and pellet after taxol-assisted microtubule assembly; lanes *4* and *5*, supernate and pellet after wash with extraction buffer; lanes *6* and *7*, supernate and pellet after first GTP extraction; lanes *8* and *9*, supernate and pellet after second GTP extraction; lanes *10* and *11*, supernate and pellet after final ATP extraction. MAP 1C is the major band in the ATP extract (lane *10*). A cofractionating M_r 74,000 band was also seen in that fraction (see text). Kinesin (*KIN*) and an M_r 100,000 band were specifically removed in the GTP extraction steps (lanes *6–9*).

To purify MAP 1C further, the ATP extract was applied to a 5–20% sucrose density gradient (Fig. 5 *left*). MAP 1C was observed to sediment as a single symmetric peak at 20S. The other MAPs (in this preparation MAP 1B and MAP 2A and B) were found at ~3–5S, and kinesin was found at ~10S. Several polypeptides were found to cosediment with MAP 1C, including a prominent species of M_r 74,000 and other minor species (see below). Polypeptides just below MAP 1C may be proteolytic fragments of the MAP, and they are not usually observed in the MAP 1C sucrose density gradient peak (cf. Figs. 6, 8, and 10).

ATPase assays of the gradient fractions (Fig. 5 *right*) revealed low levels of activity throughout the gradient, with a peak of activity coincident with the MAP 1C fractions. Addition of microtubules dramatically increased the ATPase activity. Typically, as seen in Fig. 5 *right*, almost all of the activity resided in a peak coincident with the peak of MAP 1C. In the experiment shown, stimulation of the MAP 1C peak activity was fourfold, and resulted in a specific activity in the peak fraction of 356 nmol/min/mg protein.

The sucrose density gradient peak fractions were pooled and subjected to DEAE-Sepharose chromatography (Fig. 6). MAP 1C ran as a single peak. The M_r 74,000 polypeptide,

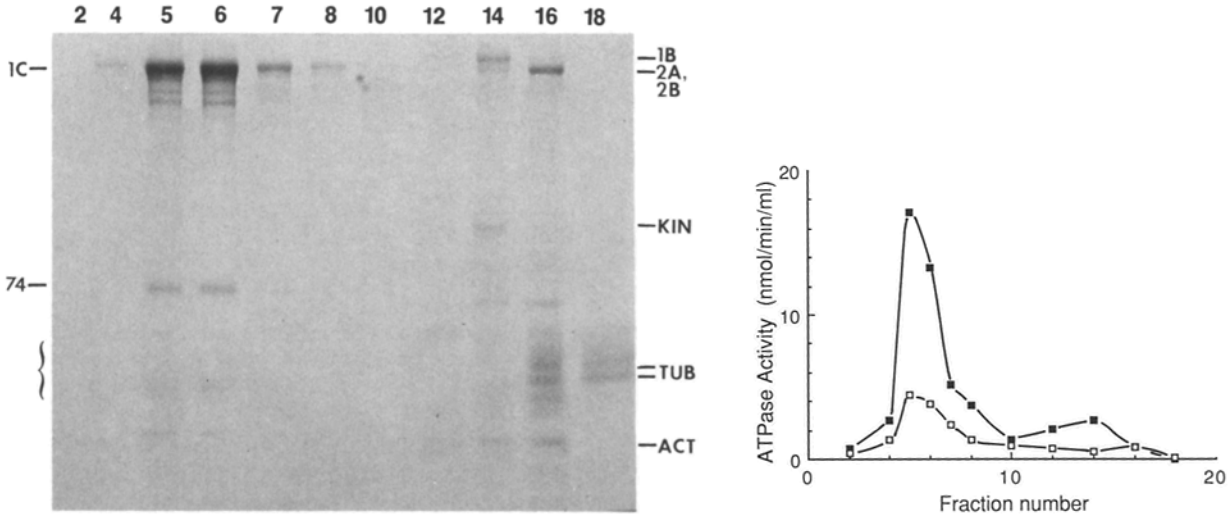

Figure 5. Cofractionation of microtubule-activated ATPase activity with MAP 1C by sucrose density gradient centrifugation. An ATP extract of microtubules prepared without nucleotide (as in Fig. 4, lane *10*) was applied to a 5–20% sucrose gradient and centrifuged. (*Left*) Gel electrophoresis of gradient fractions (numbers shown at top of lanes). (*Right*) ATPase activity in the absence (□) and the presence (■) of added microtubules (2.8 mg/ml). The protein concentration of the MAP 1C peak fraction was 48 μg/ml, giving a specific activity of 356 nmol/min/mg. This particular preparation showed some evidence of proteolysis, as indicated by the bands just below MAP 1C (cf. Figs. 8 and 10). The M_r 74,000 band, and several weak bands in the M_r 50,000–60,000 range (*bracket*) appear to be integral components of MAP 1C (see text). Abbreviations as in previous figures. *ACT*, actin.

as well as four polypeptides in the M_r 50,000–60,000 range (cf. Fig. 10) continued to copurify with the high molecular mass species. A small amount of contaminating actin eluted after the MAP 1C peak. ATPase activity was found to cochromatograph with MAP 1C (Fig. 6 *right*). Microtubule activation was not assayed because of the inhibitory effect of elevated ionic strength on this activity (Shpetner, H. S., B. M. Paschal and R. B. Vallee, manuscript in preparation). The yield of MAP 1C ATPase activity is estimated in Table I.

Microtubule-translocating Activity of MAP 1C

In view of the ATP dependence of microtubule binding, and the microtubule-activated ATPase activity, it seemed reasonable to expect that MAP 1C might have a mechanochemical function. This possibility was assayed by application of protein to a glass substratum, and observation of microtubule gliding in the presence of ATP, as has been done with kinesin (Vale et al., 1985*a*, *c*).

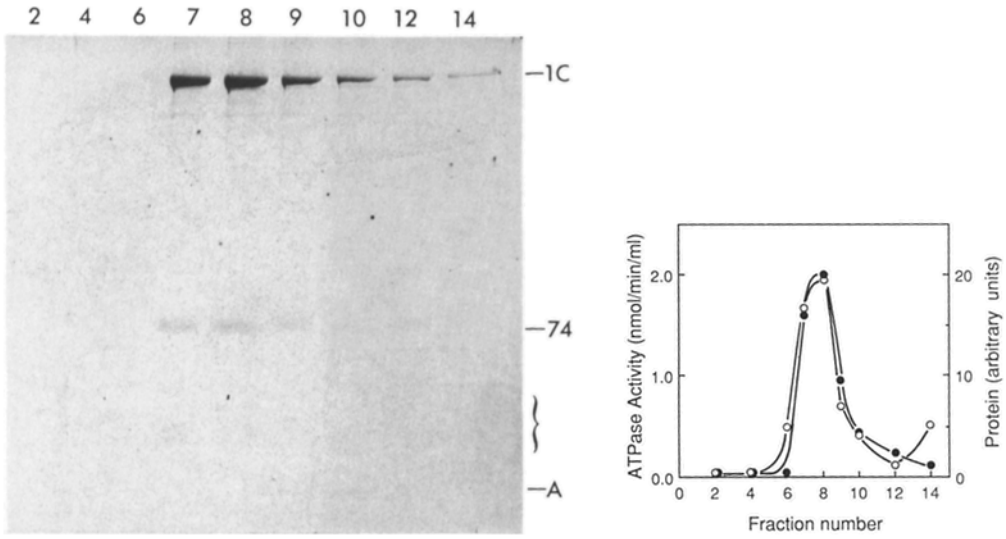

Figure 6. Cofractionation of ATPase activity with MAP 1C by DEAE-Sepharose chromatography. The starting material was a sucrose density gradient MAP 1C peak. MAP 1C was eluted from the DEAE-Sepharose at ~250 mM KCl, using a linear gradient of 50 (fraction *2*) to 500 mM KCl (fraction *15*). (*Left*) Gel electrophoresis of column fractions. (*Right*) ATPase activity (○), and concentration of MAP 1C (●). The concentration of MAP 1C was determined by densitometric scanning of the MAP 1C electrophoretic band, and is expressed in arbitrary units. In this experiment a trace of residual actin (lane *10*) was removed from the MAP 1C. The M_r 74,000 species, and several minor bands in the range M_r 50,000–60,000 (*bracket*) continued to copurify with MAP 1C and are barely visible.

Table I. Yield of ATPase Activity

Preparative stage	ATPase Activity		
	Specific activity	Total activity	Estimated yield
	nmol/min/mg	*nmol/min*	*%*
Microtubules (−ATP)*	10.9	491	100
Microtubules (+ATP)*	3.2	144	
Difference		347	71
Sucrose gradient peak (+MTs)	356	169	34
Sucrose gradient peak (−MTs)	91	56	
DEAE-Sepharose peak (−MTs)	80	30	18

Activities are based on 90 g of brain tissue, which typically yields ~45 mg of microtubules. Only one-sixth of the total sucrose density gradient purified MAP 1C was applied to DEAE-Sepharose. Data for the latter step are normalized to a full-sized preparation, and yield for this step is based on activity in the absence of microtubules. MT, microtubule.

* Data of Dr. C. Collins (The Worcester Foundation for Experimental Biology) (cf. Brady, 1985).

Table II. Relative Amounts of Polypeptides Copurifying with MAP 1C

Polypeptide	Concentration (arbitrary units)					
	Experiment No.					
	1	2	3	4	Average	Molar ratio
MAP 1C	100	100	100	100	100	1.0
74K	30	28	27	26	28 ± 2	1.1
59K	4	4	5	5	5 ± 1	0.2
57K	4	4	6	5	5 ± 1	0.3
55K	8	6	7	6	7 ± 1	0.4
53K	8	6	8	9	8 ± 1	0.4

Concentrations of polypeptides were determined by densitometry of electrophoretic gels, and are expressed in arbitrary units. Calculation of molar ratios was based on a value of M_r = 300,000 for MAP 1C, though choice of this value is of necessity inaccurate in view of variable electrophoretic mobility,[2] and possible dynein-like molecular mass (see Results). MAP 1C samples for experiments 1–3 were from sucrose density gradient peaks, and for experiment 4 from a subsequent hydroxylapatite purification.

In our standard MAP 1C preparative procedure, we selectively remove much of the kinesin by GTP extraction of microtubules (Fig. 4, lanes *6–9*). Nonetheless, we found that the final ATP-extracted protein fraction (Fig. 4, lane *10*), of which MAP 1C was the major component, showed motile activity. The ATP-extracted fraction was subjected to sucrose density gradient centrifugation, and the gradient fractions were assayed for motile activity (Fig. 8). Motility was detected only in the MAP 1C peak fractions. Microtubule gliding occurred in a continuous, unidirectional fashion (Fig. 7), and lasted for as long as 6 h in undisturbed preparations. A narrow distribution for the rates of gliding was observed, with the average = 1.25 ± 0.11 μm/s (Fig. 9). In preliminary experiments, we found that motility could be completely inhibited by addition of 1 mM *N*-ethylmaleimide. No motility was detected at 10S, the approximate sedimentation position of kinesin, reflecting the low level of residual kinesin in the preparation.

Subunit Size and Composition

We noted throughout this study that smaller polypeptides copurified with the high molecular mass MAP 1C polypeptide. The most prominent was a species of M_r 74,000. This species could be detected in the preparation as early as the stage of ATP desorption of MAP 1C from microtubules (Fig. 4, lane *10*). It was seen to cofractionate with MAP 1C by sucrose density gradient centrifugation, (Figs. 5 and 8), DEAE-Sepharose (Fig. 6), hydroxylapatite (Table II), and Bio-gel A-5m chromatography (not shown). Four additional, less prominent polypeptides of M_r 59,000, 57,000, 55,000, and 53,000 were also observed to copurify with MAP 1C under all of these conditions. These species may be detectable in Figs. 5, 6, and 8, but are more clearly shown in Fig. 10.

The relative abundance of the copurifying polypeptides in several preparations was determined and is shown in Table II. As suggested by inspection of the density gradient and

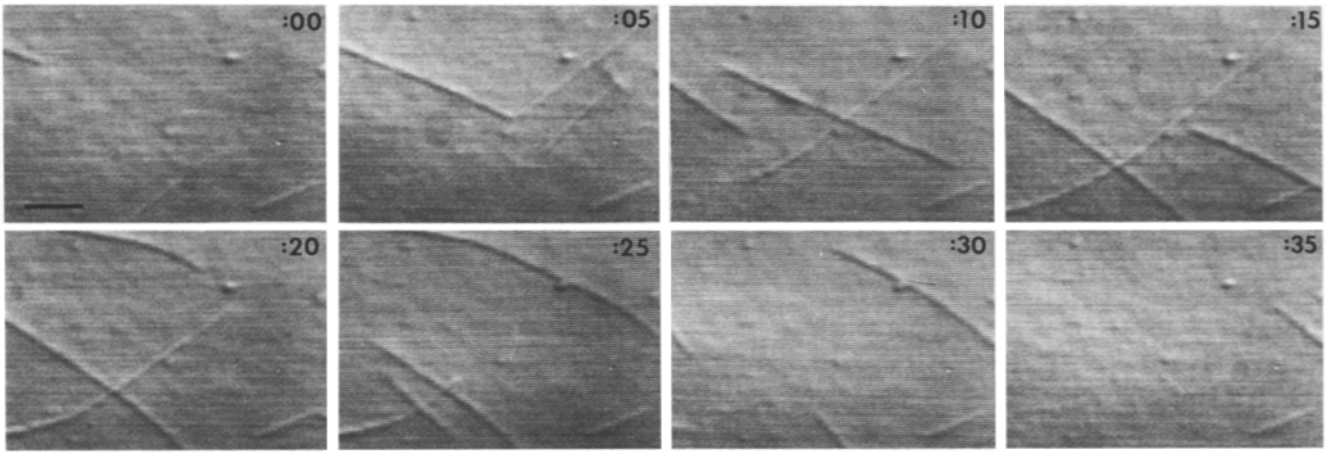

Figure 7. Motility of microtubules mediated by purified MAP 1C. MAP 1C was purified by sucrose density gradient centrifugation. The peak fractions were pooled (gel is shown in Fig. 8), dialysed into Tris/KCl buffer, and adsorbed to a coverslip. Microtubules were applied in the presence of 1 mM added MgATP. Most of the microtubules in the preparation showed gliding motility. The sequence presented here shows a typical field in the preparation. Seven microtubules can be observed to cross the field in the 35-s interval shown. One immobile microtubule may be seen at the lower right of the field. The sequence shown was taken 3 h after the preparation had been mounted for microscopic examination, and the average rate of movement had decreased from 1.25 (Fig. 9) to 0.97 μm/s. Bar, 2 μm.

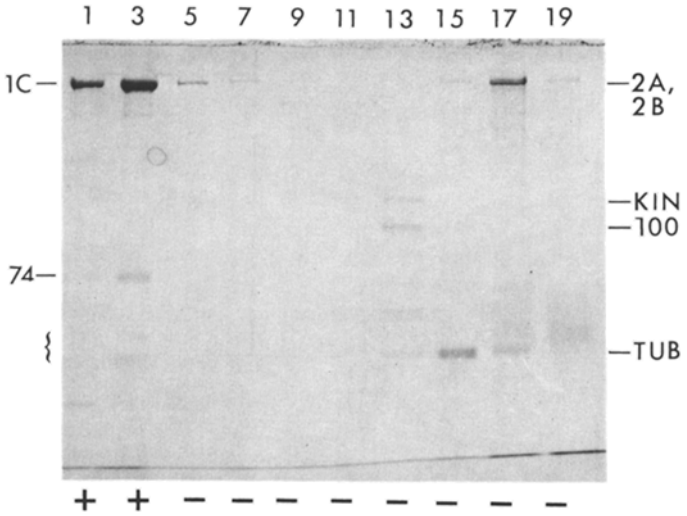

Figure 8. Cofractionation of motile activity with MAP 1C by sucrose density gradient centrifugation. ATP extract of microtubules prepared without nucleotide (as in Fig. 4, lane *10*) was applied to a 5–20% sucrose gradient and centrifuged. Gel electrophoresis of gradient fractions is shown. Motile activity is indicated at the bottom of the lanes (+ or −). Motility was seen only in the MAP 1C peak fractions. No motility was observed in fraction 13, which contained the peak of residual kinesin (*KIN*) and a M_r 100,000 protein which also eluted from microtubules during the ATP extraction step (Fig. 4, lane *10*). No motility was observed in fraction 17, which contained the peak of contaminating MAP 2 in this preparation.

chromatographic profiles, the ratio of the five polypeptides to the high molecular mass MAP 1C polypeptide was constant from preparation to preparation and during purification. The stoichiometry of the M_r 74,000 species to MAP 1C was close to 1:1, while the other species were present at lower levels.

The four smaller polypeptides were similar in electrophoretic mobility and composition to the tau MAPs (Cleveland et al., 1977), and the lower two bands migrated very close to alpha- and beta-tubulin. However, immunoblotting of these polypeptides with antibodies to tau and to tubulin were negative. Thus, these appear to be distinct polypeptide species from the known microtubule components.

Relationship to Axonemal Dynein

In view of the ATPase activity of MAP 1C and its large native size (20S), it was of interest to examine the relationship of this protein to axonemal dynein. Fig. 11 shows that MAP 1C coelectrophoresed with the beta heavy chain of *Chlamydomonas* axonemal dynein outer arms. As a further means of comparison, MAP 1C was subjected to ultraviolet light irradiation in the presence of ATP and vanadate. This treatment has been observed to cleave axonemal dynein heavy chains specifically at a single site, yielding two large polypeptides (Lee-Eiford et al., 1986; Gibbons et al., 1987; King and Witman, 1987). Irradiation of MAP 1C (Fig. 12) yielded two large fragments. The molecular masses for the fragments determined by comparison with electrophoretic standards were M_r ~185,000 and 225,000.

Discussion

MAP 1C was previously observed as the least prominent of the high molecular mass MAP species in both gray and white

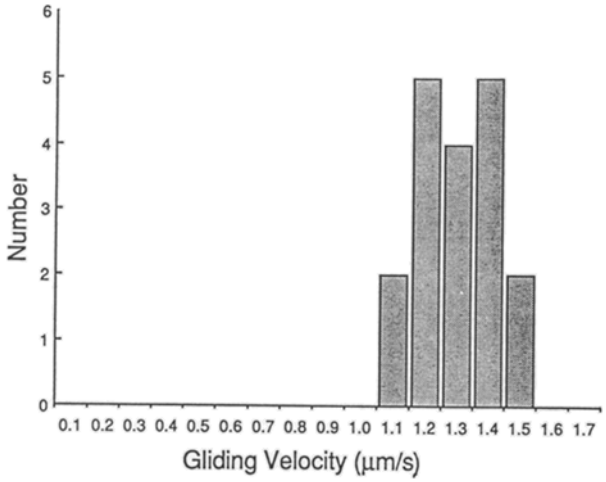

Figure 9. Histogram of velocities of gliding microtubules. Motility of microtubules along coverslips to which sucrose density gradient–purified MAP 1C had been applied (sample of Fig. 7) was recorded on videotape. Number of microtubules per rate class is shown. Almost all of the microtubules in the preparation showed unidirectional gliding motility. The average rate of translocation of individual microtubules was 1.25 μm/s.

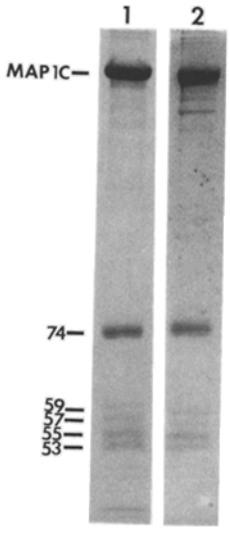

Figure 10. Polypeptides copurifying with MAP 1C. MAP 1C peak fractions are shown from (lane *1*) a sucrose density gradient, and (lane 2) sequential sucrose density gradient and DEAE-Sepharose purification (reprint of peak fraction of Fig. 6). Gel was stained with Coomassie Brilliant Blue. Molecular masses are in thousands.

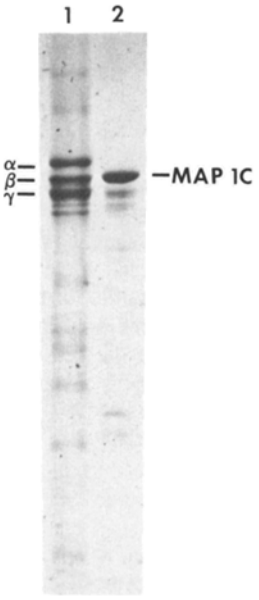

Figure 11. Electrophoretic comparison of MAP 1C and *Chlamydomonas* flagellar dynein outer arm heavy chains. Sucrose density gradient-purified MAP 1C (lane *2*) was run alongside a crude KCl extract of *Chlamydomonas* axonemes (lane *1*). MAP 1C ran close to the position of the beta heavy chain of *Chlamydomonas* outer arm dynein. 3–5% polyacrylamide gel was used.

matter microtubule preparations (Bloom et al., 1984, 1985). The present study has indicated that its yield in microtubule preparations is affected by nucleotides, and that it is more abundant than we had previously realized. MAP 1C could be extracted from microtubules by ATP, but not by GTP (Figs. 1 and 4). These results have been confirmed in purified MAP 1C preparations (Shpetner, H. S., B. M. Paschal, and R. B. Vallee, manuscript in preparation). We infer, therefore, that the decreased level of MAP 1C in GTP preparations probably reflects generation of ATP as the result of nucleoside diphosphokinase activity present in brain cytosol. It should be mentioned that incubation of calf brain cytosolic extracts with hexokinase and glucose to specifically hydrolyse ATP had no obvious effect on the final yield of MAP 1C relative to that obtained when nucleotides were simply omitted from the procedure. This suggests that the endogenous level of ATP in our calf brain cytosolic extracts is sufficiently low to allow complete MAP 1C binding.

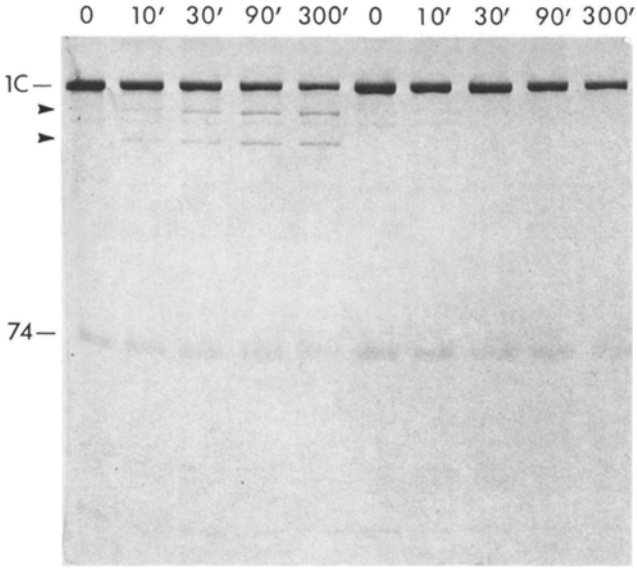

Figure 12. Ultraviolet-induced cleavage of MAP 1C in the presence of Vanadate and ATP. MAP 1C purified by sucrose density gradient centrifugation in Tris/KCl buffer containing 0.5 mM DTT was exposed to 365 nm ultraviolet light for the series of times shown at the top. (*Left*) Samples that contained 100 μM sodium vanadate and 100 μM MgATP; (*right*) samples that contained 100 μM MgATP alone. *Arrowheads* mark positions of fragments.

The effect of ATP on microtubule binding suggested a role in microtubule mechanochemistry. A microtubule-activated ATPase activity was, indeed, found to copurify with MAP 1C by sucrose density gradient centrifugation (Fig. 5). Our data suggest that the ATPase active site resides with the high molecular mass MAP 1C polypeptide rather than the smaller associated polypeptides (Fig. 10; Table II). Exposure to ultraviolet light in the presence of vanadate and ATP resulted in the specific cleavage of this polypeptide. Cleavage was not seen in the absence of vanadate. As discussed below, this reaction, previously observed only for axonemal dynein, is understood to occur at the gamma-phosphate position of the dynein ATP-binding site (Lee-Eiford et al., 1986). These data and other similarities to axonemal dynein (see below) are all consistent with ATP hydrolysis occurring in association with the high molecular mass MAP 1C polypeptide.

Microtubule-translocating activity was observed during MAP 1C purification as early as the ATP extraction of microtubules. At this stage, MAP 1C was already the major nontubulin protein in the preparation (Fig. 4, lane *10*). While kinesin had been almost completely extracted from microtubules by GTP (Fig. 1, lane *6*), low levels of the kinesin polypeptide species could still be detected in the subsequent sucrose density gradient step (Fig. 5 *left*, lane *14*; Fig. 8, lane *13*). However, the kinesin band was clearly absent from the MAP 1C fractions, which contained almost all of the ATPase activity, and all of the microtubule-translocating activity.

The translocating activity attributed to MAP 1C could be further distinguished from that of kinesin by the sensitivity of the MAP 1C to *N*-ethylmaleimide. In preliminary experiments 1 mM *N*-ethylmaleimide abolished the translocating activity of MAP 1C, in contrast to the noteworthy insensitivity of kinesin to high levels of this sulfhydryl alkylating agent (Vale et al., 1985*a*). The rate of movement mediated by MAP 1C, 1.25 μm/s, was considerably higher than that reported for squid and calf brain kinesin under similar solvent conditions (0.3 and 0.2 μm/s, respectively; Vale et al., 1985*a*). Thus, MAP 1C moved microtubules at a rate about four to fivefold higher than kinesin. It is worth noting that the rate for MAP 1C is more nearly comparable to rates reported for vesicle motility in mammalian systems (Breuer et al., 1975) or in squid (Allen et al., 1982).

Relationship to Dynein and Other Proteins

MAP 1C has some similarities to flagellar and ciliary dynein, but there are differences as well. MAP 1C, like dynein, is a high molecular mass ATPase that interacts with microtubules. The electrophoretic mobility of the brain protein was, in fact, observed to be equivalent to that of *Chlamydomonas* beta-dynein (Fig. 11). Under native conditions MAP 1C behaved as a large particle, with a sedimentation coefficient (20S) much greater than the other MAPs (~3–5S), but similar to undissociated or partially dissociated forms of axonemal dynein (see reviews by Johnson et al., 1984; Gibbons,

1984). The effect of ATP on the binding of MAP 1C to microtubules corresponds to the commonly observed behavior of axonemal dynein in vitro; whether MAP 1C has an additional nucleotide-independent microtubule-binding site as is the case for axonemal dynein must await further work. Perhaps the most striking similarity is that exposure of MAP 1C to ultraviolet light in the presence of vanadate and ATP resulted in specific, limited cleavage of the molecule. This effect was reported to be specific for dynein alone (Lee-Eiford et al., 1986; Gibbons et al., 1987; King and Witman, 1987).

In several important respects MAP 1C is distinguishable from axonemal dynein. MAP 1C was isolated from the cytosolic fraction of brain homogenates. Its ATPase activity (~50 nmol/min/mg unactivated) is lower than that reported for axonemal dyneins (Bell et al., 1982), though the specific activity of MAP 1C in the presence of microtubules (~350 nmol/min/mg) is in the range reported for unactivated axonemal dynein. While the ATPase of axonemal dynein is stimulated by microtubules (Omoto and Johnson, 1986), stimulation of the MAP 1C ATPase occurs at ~100-fold lower concentration of microtubules (Fig. 5; Shpetner, H. S., B. M. Paschal, and R. B. Vallee, manuscript in preparation). Whether these differences are inherent to the different molecular species remains to be seen. Additional similarities and differences in the enzymatic and pharmacological properties of MAP 1C and axonemal dynein will be explored in a subsequent paper (Shpetner, H. S., B. M. Paschal, and R. B. Vallee, manuscript in preparation).

Several recent reports have appeared regarding other high molecular mass proteins that bound to microtubules in an ATP-dependent fashion. Hollenbeck and Chapman (1986) described a high molecular weight protein (HMW 4) in spinal nerve roots that could be released from microtubules by incubation with ATP. A low level of ATPase activity was ascribed to this species, which, like MAP 1C, sedimented at 20S. In other respects the protein differed from MAP 1C. In particular, HMW 4 bundled microtubules, behavior not seen with MAP 1C. Vale et al. (1985*a*) found a high molecular mass protein in their kinesin preparations, though the protein was not seen in calf brain. The squid protein did not show microtubule-translocating activity. Gilbert and Sloboda (1986) extracted a high molecular mass protein from cytoplasmic vesicles that were competent to translocate along microtubules in vitro. The protein was found to cosediment with microtubules in an ATP-dependent fashion. It showed reactivity with an anti-mammalian MAP 2 antibody, suggesting that it was not MAP 1C. We note that our polyclonal anti-MAP 2 antibody and three anti-MAP 2 monoclonals all failed to react with MAP 1C. Pratt (1986) also identified a high molecular mass microtubule-binding protein in squid axoplasm, though binding was enhanced, rather than diminished by ATP. Finally, Lye et al. found a high molecular mass protein in *Caenorhabditis elegans* that showed microtubule-translocating activity. This protein sedimented at 20S like MAP 1C, and showed a low level of ATPase activity. Microtubule activation of the ATPase activity was not observed. While this protein could be related to MAP 1C, it also shared some properties with kinesin, which was not detected in *C. elegans*.

An extensive body of work has appeared regarding "cytoplasmic dyneins". Most of these studies have been performed in marine eggs (Weisenberg and Taylor, 1968; Pratt et al., 1980; Pratt, 1980; Hollenbeck et al., 1984; Asai and Wilson, 1985), in which dyneins may be stockpiled for subsequent use in ciliogenesis (see, e.g., Asai, 1985). Description of a 20S CTPase in brain tissue, referred to as a "dynein", has appeared, though this activity was not characterized sufficiently to allow comparison with MAP 1C (Pallini et al., 1983).

In a previous study, our laboratory described a microtubule-activated ATPase in sea urchin eggs that copurified with egg microtubules (Collins and Vallee, 1986*a*, *b*). The molecular identity of the particular MAP responsible for the activity could not be determined. Could it be an egg homologue of MAP 1C? Our present evidence does not support this possibility. The sedimentation coefficient of the egg enzyme (10S) is different from that of MAP 1C, the egg enzyme was not extractable from microtubules by ATP, and some important characteristics of the enzymatic activities were distinct, though common features were identified as well (Shpetner, H. S., B. M. Paschal, and R. B. Vallee, manuscript in preparation). Further work will be required to resolve this question completely, though we favor the hypothesis that the egg enzyme represents yet an additional molecular species.

Conclusion

Our data indicate that MAP 1C, the fifth of the high molecular mass MAPs to be described in detail, has properties of a mechanochemical enzyme. Thus, it appears to be totally distinct from the other proteins. In some ways its properties are comparable to those of axonemal dynein. We are at present attempting to resolve the relationship of the two species more fully.

The biological function of MAP 1C is unknown. Like kinesin, it may play a role in the transport of vesicular organelles. Alternatively, by analogy with axonemal dynein, which mediates microtubule sliding, it could be involved in the reorganization of cytoplasmic microtubules. Such behavior has not been extensively described, but could be involved in the growth or the reorganization of the neuronal cytoskeleton. In either case, understanding the function of MAP 1C should provide fundamental insight into the workings of neuronal cells and, perhaps, other types of cell as well.

We thank Dr. Christine Collins for her help in identifying MAP 1C in the early stages of this project, and for providing her expertise throughout the project in the analysis of the MAP 1C ATPase activity. We thank Drs. George Witman and Stephen King at the Worcester Foundation for continued advice and support, for sharing their expertise and equipment for the vanadate cleavage experiments and other aspects of dynein biochemistry, for electrophoretic comparison of MAP 1C and *Chlamydomonas* dynein heavy chains, and for critical reading of parts of this manuscript. We thank Dr. Jan De Mey for sharing with us his observations on the erratic electrophoretic behavior of MAP 1C. We thank Dr. Kenneth Kosik for his generous gift of monoclonal anti-tau antibody, and Drs. J. Chloe Bulinski and Greg Gundersen for their generous gift of site-directed anti-tubulin antibodies. Finally, we thank Nancy Kravit and Christine Collins for their critical reading of this manuscript.

Received for publication 21 April 1987, and in revised form 23 June 1987.

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