Microtubule-associated Proteins from Cultured HeLa Cells

ANALYSIS OF MOLECULAR PROPERTIES AND EFFECTS ON MICROTUBULE POLYMERIZATION

(Received for publication, May 12, 1980)

Jeannette Chloe Bulinski$ and Gary G. Borisy

From the Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisconsin 53706

We have attempted a biochemical characterization of the microtubule-associated proteins (MAPs) of cultured HeLa cells. The HeLa MAPs consist of a group of three polypeptides of 200,000 to 220,000 molecular weight (the 210K MAP) and a protein of 125,000 molecular weight (the 125K MAP). The solution properties of the HeLa MAPs were examined using molecular sieve chromatography and sucrose gradient sedimentation. With both analytical procedures, the 125K and 210K MAPs behaved independently of one another. The effects of each of the MAPs on microtubule polymerization were also studied. Both the 125K and 210K MAPs stimulated the polymerization of pure tubulin. The effect of high levels of MAPs on microtubule polymerization was also examined. Increasing the concentration of MAPs at a constant tubulin concentration increased both the rate and extent of microtubule polymerization. The 125K and 210K MAPs showed independent behavior with respect to binding to microtubules. The 210K MAP saturated its binding sites at a level of 14.0% (210K MAP:tubulin in polymer, w/w), while the 125K MAP showed no saturation even at a level of 18.3%. These results demonstrate that the 125K and 210K MAPs are distinct molecular species, differing in their solution properties and their binding to microtubules. The 210K MAP showed some similarities and some differences when compared to the porcine brain high molecular weight MAP. The HeLa MAPs, although showing some properties similar to brain MAPs, are nevertheless distinctive in several respects and may best be considered as separate though possibly related species.

Microtubules isolated from mammalian brain tissue are composed of tubulin subunits and various nontubulin proteins which bind to and copurify with the microtubules. Several microtubule-associated proteins have been described as components of brain microtubules. These MAPs fall roughly into two groups according to molecular weight, high molecular weight MAPs (280,000 molecular weight, Murphy and Borisy, 1975) and MAPs of 55,000 to 62,000 molecular weight, identified and named \( \tau \) protein by Weingarten et al. (1975). The brain high molecular weight MAPs have been characterized with respect to their solution behavior (Murphy et al., 1977b) and their stimulation of polymerization of pure tubulin (Sloboda et al., 1976a, 1976b; Murphy et al., 1977a, 1977b; Herzog and Weber, 1978). The solution properties of \( \tau \) proteins have also been examined (Cleveland et al., 1977a, 1977b), and the behavior of \( \tau \) proteins in stimulation of tubulin polymerization has been reported (Penningroth et al., 1976; Witman et al., 1976; Cleveland et al., 1977b; Herzog and Weber, 1978).

Another categorization of brain MAPs has been made on the basis of the degree to which they copurify with microtubules (Murphy et al., 1977b; Berkowitz et al., 1977). Berkowitz et al. have introduced the terms quantitative and nonquantitative to designate proteins that do or do not copurify over multiple cycles with constant stoichiometry to tubulin. According to the designation, only the HMW species and a species of 30,000 to 35,000 daltons which is a putative tubule-binding fragment of HMW (Vallee and Borisy, 1978) were found to be quantitative MAPs.

The \( \tau \) and HMW MAPs have been reported in a variety of cultured cells using immunofluorescence methods (Connolly et al., 1977, 1978; Sherline and Schiavone, 1977, 1978; Shetler, 1978), and they have also been extracted in isotopic amounts from mouse fibroblasts using copolymerization with carrier hog brain microtubules (Cleveland et al., 1979).

However, in no case other than for brain tissue have MAPs been purified directly and analyzed biochemically. We have recently isolated microtubule protein from cultured HeLa cells (Bulinski and Borisy, 1979). The HeLa microtubules contained two principal species of MAPs in addition to tubulin. In the previous report, we established that the HeLa MAPs copurify in multiple cycles of assembly-disassembly in constant stoichiometry to tubulin. In addition, we established their molecular weights according to SDS gels and demonstrated their ability to stimulate the assembly of purified HeLa or porcine brain tubulin. In a subsequent study on the mechanism for the temporal control of spindle and cytoplasmic microtubule assembly (Bulinski et al., 1980), we demonstrated that the amount and type of MAPs were similar in both mitotic and log phase HeLa cells.

Our development of a microtubule self assembly system in HeLa cells (Bulinski and Borisy, 1979) has permitted for the first time the preparation of cultured cell MAPs in sufficient amounts to undertake a physicochemical analysis. The purpose of this paper is to report a characterization of the HeLa MAPs as other workers have characterized the MAPs isolated from brain tissue. Through an examination of the similarities and differences between the brain and HeLa MAPs it should be possible to more fully define properties characteristic of this class of molecules.

1 This study was supported by National Institutes of Health Grants GM-00066 and GM-25062 to G. G. B. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 National Institutes of Health Predoctoral Trainee during this study.

3 The abbreviations used are: MAP, microtubule-associated protein; 125K, 210K, 255K, proteins of 125,000, 210,000, and 255,000 molecular weight, respectively; HMW, high molecular weight; SDS, sodium dodecyl sulfate; EGTA, ethylene glycol bis(\( \beta \)-aminoethyl) ether\( N,N',N''\)-tetraacetic acid.\n
11570
Characterization of HeLa Microtubule-associated Proteins

MATERIALS AND METHODS

Preparation of HeLa Microtubule Protein—HeLa cells were grown and microtubule protein prepared by cycles of assembly and disassembly, as described previously (Bulinski and Borisy, 1979). For all the experiments reported here thrice-cycled protein (C, S, see terminology of Borisy et al., 1975) was used.

Molecular Sieve Chromatography on 4% Agarose—Thrice-cycled HeLa microtubule protein, C, S, in polymerization buffer containing 0.1 M piperazine-N,N'-bis(2-ethanesulfonic acid), 1 mM MgSO₄, 1 mM dithiothreitol, 1 mM EGTA, 0.1 mM GTP, and 0.02% sodium azide was applied to a column (0.9 × 16 cm) which contained 10.6 ml of Bio-Gel A-15m pre-equilibrated in the same buffer. Samples of 0.5 ml were chromatographed at a flow rate of 7.5 ml/h, and 0.25-ml fractions were collected. Gel electrophoresis was used to analyze each column fraction. Elution volumes for each species were used to measure distribution coefficients (K_d). The column was monitored at 280 nm; GTP was used to mark the bed volume. To determine diffusion coefficients from K_d values, the following standards were used: collagen, myosin, brome mosaic virus, and fibrinogen. Collagen and brome mosaic virus were applied to the column in a buffer containing 50 mM sodium acetate, pH 4.5, 1 mM GTP. Myosin and fibrinogen were applied in 0.1 M piperazine-N,N'-bis(2-ethanesulfonic acid) with 1 mM MgSO₄, 1 mM GTP, and 0.5 mM NaCl. Values for the diffusion coefficients were obtained from Tanford (1961) except the value for brome mosaic virus which was obtained from Bochtahl and Kaesberg (1962).

Sucrose Gradient Sedimentation—HeLa C, S, microtubule protein in polymerization buffer, as above, was sedimented through a gradient of 5 to 20% sucrose in the same buffer. The 3.6-ml gradient was centrifuged in a Beckman SW 60 Ti rotor at 60,000 rpm for 6 h. Parallel gradients were checked for linearity by refractometry. Parallel gradients contained catalase, phosphorylase a, chymotrypsinogen A, cytochrome c, and either bovine serum albumin or DEAE-purified HeLa tubulin as sedimentation standards. An internal standard in the HeLa microtubule protein gradients consisted of 3H-labeled catalase, obtained from the laboratory of R. R. Rueckert, Laboratory of Biophysics, University of Wisconsin. Gel electrophoresis was used to analyze each 0.15-ml fraction of the gradient.

Measurement of Assembly Kinetics—HeLa microtubule protein, DEAE-purified tubulin, and MAP fractions were prepared as described previously (Bulinski and Borisy, 1979), except that thrice-cycled microtubule protein was used. Solutions containing the separated 125K MAP and 210K MAP were obtained by sucrose density gradient sedimentation of microtubule protein as described above. These fractions contained some residual tubulin, but the concentration of active tubulin molecules was sufficiently low as not to contribute significantly to polymerization of the samples. After fractions were collected, they were pooled and dialyzed twice against 100 volumes of polymerization buffer at 4°C for 3 days. Gel electrophoresis was used to examine their composition and to verify the absence of cross-contaminating 125K or 210K MAPs. Kinetics of microtubule polymerization was determined by measuring the change in optical density at 320 nm (Johnson and Borisy, 1977).

Quantitation of Microtubule Polymer—Aliquots of microtubules were polymerized from various mixtures of HeLa MAP fraction and tubulin for 30 min at 37°C. Microtubules were sedimented at 48,000 × g for 30 min at 37°C. Lowry protein assays (Lowry et al., 1951) and gel electrophoresis using the system of Laemmli (1970) were performed on MAP-tubulin mixtures. The proportions of tubulin and MAPs in pellet and supernatant fractions were measured using densitometry of photographic negatives of stained polyacrylamide slab gels. All MAP quantitations were made at protein loadings in the linear range of Coomassie brilliant blue staining (Borisy et al., 1975) and in the linear range of the film used. Using purified tubulin to construct a standard curve, densitometric scans of electropherograms were used to measure absolute amounts of each MAP species in the MAP-tubulin mixtures.

RESULTS

Molecular Properties and Interaction of the Native MAP Species—An electropherogram of HeLa microtubule protein containing tubulin and MAPs is shown in Fig. 1. The thrice-cycled HeLa microtubule protein, C, S, which we used in all of our experiments, contained 94% tubulin, 2.5% of the 210K MAP, and 2.2% of the 125K MAP. Although for convenience we shall refer to the HeLa MAPs as the 210K and 125K species, the 210K MAP is actually resolved into a triplet of bands of 220,000, 198,000, and 200,000 molecular weight, as shown in Fig. 1. The remaining 1 to 2% of the protein was distributed over a number of minor species detectable at the higher loadings. Of these, we shall consider only the 255,000 molecular weight species (see Fig. 1), because immunological evidence (Bulinski and Borisy, 1980) indicates that it shares antigenic determinants with the 210K MAP. Also shown in Fig. 1 is an electrophoretic profile of porcine brain HMW MAP, which may be compared with the 210K HeLa MAP. The HMW-MAP consists of a doublet of proteins of 271,000 and 286,000 molecular weight and is readily distinguished in gel mobility from both the HeLa 210K or 255K MAPs in SDS polyacrylamide gels.

In order to characterize the native 125K, 210K, and 255K HeLa MAP species, we turned to methods suitable for analyzing mixtures of proteins in solution under nondenaturing conditions. Molecular sieve chromatography was used to obtain an estimate of the diffusion coefficient, D (Andrews, 1965), and sucrose gradient sedimentation was used to determine the sedimentation coefficient, s.

A thrice-cycled preparation of HeLa microtubule protein, C, S, was applied to a Bio-Gel A-15m column. An electrophoretic analysis of the material applied and each fraction of the agarose column is shown in Fig. 2. Although the 255K, 210K, and 125K MAPs were eluted with overlapping distributions, it is evident from Fig. 2 and from additional determinations that the peak of the distribution for the 255K species was significantly earlier than for the other two species, which were also slightly separated from each other. Tubulin eluted later and was largely separated from all three MAP species. Three or four closely spaced bands which resemble the porcine brain MAP, T, in their electrophoretic behavior can be seen toward the center of the A-15 column profile. However, these proteins are of higher molecular weight than T (all have molecular weights greater than 80,000), and their significance is not known. The brackets in Fig. 2 estimate our uncertainty in estimating the peaks of the distributions. Although the patterns were overlapping, the lack of identity in the distributions suggests that the MAP species were eluted as separate mole-
Characterization of HeLa Microtubule-associated Proteins

Fig. 2. Molecular sieve chromatography of HeLa microtubule protein on 4% agarose. Electrophoretic elution profile of HeLa microtubule protein on A-15. Lane at left is material applied. Brackets indicate peak fractions in elution distributions of individual MAPs. See under "Materials and Methods" for details of chromatography.

cules, and this suggestion was corroborated in the sucrose density gradient. The volumes at which each of the HeLa MAPs eluted at greatest concentration from the A-15 column were used to calculate partition coefficients, \( K_d \), which were 0.27, 0.31, and 0.39, for the 255K, 125K, and 210K species, respectively. Using a standard plot of the reciprocal of the diffusion coefficients \( 1/D_{20, w} \) versus \( K_d \) for proteins of known diffusion coefficients, as shown in Fig. 3, we converted \( K_d \) values for the HeLa MAPs into diffusion coefficients, \( D_{20, w} \). The diffusion coefficients of the HeLa MAPs were all small (-1 \( \times 10^{-7} \) cm/s). This may be either because the MAPs are asymmetric molecules or because they exist as oligomers, or both.

To distinguish between these possibilities and to test further whether the MAPs were separate molecules or existed together in a complex, we examined their solution behavior by an independent method, namely, sucrose density gradient sedimentation. A C8 preparation of HeLa microtubule protein was sedimented through a 5 to 20% gradient. Gel electrophoresis of each fraction of the gradient, as shown in Fig. 4, served to locate the peak position of each MAP. The sucrose gradient data clearly showed a large difference in sedimentation behavior for the 125K and 210K species, confirming the suggestion that they are separate molecules in solution. The sedimentation behavior of the 255K species was similar but not identical with that of the 210K MAP. A parallel gradient containing various protein standards as well as an internal standard of \([14C]\)-catalase was used to assign apparent sedimentation coefficients, \( s_{20, w}^o \), values, to the MAPs. The values obtained were 5.5 \( \pm \) 1.1 S, 7.8 \( \pm \) 1.0 S, and 14.0 \( \pm \) 1.0 S for the 210K, 255K, and 125K MAPs, respectively.

With a high sedimentation coefficient and low diffusion coefficient, the 125K MAP behaved in solution as though it had a molecular weight of 1,380,000. These data suggest that the 125K MAP aggregates with itself or other molecules. The 125K MAP also failed to saturate its binding sites on microtubules, as discussed later in this report. Because the 210K MAP showed neither of these experimental problems, we chose to concentrate on a full characterization of its solution properties.

Knowledge of the diffusion and sedimentation coefficients of the 210K MAP permitted us to calculate molecular properties for the native molecule in solution. Table I lists the molecular properties of 210K MAP and compares them to the properties of porcine brain HMW. The 210K MAP apparently exists as a very large molecule in solution. Comparison of its calculated molecular weight with the chain weights determined from polyacrylamide gel electrophoresis suggests that the 210K MAPs exist as dimers in solution. This is in contrast to brain HMW which has a smaller solution molecular weight and which apparently exists as a monomer in solution. However, we note that the assignment of the number of subunits should only be considered as provisional, due to the uncer-
Characterization of HeLa Microtubule-associated Proteins

11573

Fig. 4. Sucrose gradient sedimentation of HeLa microtubule protein. Sedimentation direction is from left to right. Lane at left is the material applied. Brackets indicate peaks in distributions of individual MAPs.

TABLE I
Molecular properties of MAPs

<table>
<thead>
<tr>
<th>MAP</th>
<th>HeLa 210K</th>
<th>Brain HMW*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{20,w} \times 10^3$ (cm² s⁻¹)</td>
<td>1.19 ± 0.10</td>
<td>1.46 ± 0.16</td>
</tr>
<tr>
<td>$s_{20,w} \times 10^{-13}$ (s)</td>
<td>5.3 ± 1.15</td>
<td>4.4 ± 0.15</td>
</tr>
<tr>
<td>Stokes radius (nm)</td>
<td>18 ± 2</td>
<td>15.0 ± 1.7</td>
</tr>
<tr>
<td>Molecular weight, native (calculated from s, D)</td>
<td>409,000 ± 66,000</td>
<td>266,000 ± 26,000</td>
</tr>
<tr>
<td>Molecular weight, chain (from SDS gels)</td>
<td>210,000</td>
<td>280,000</td>
</tr>
<tr>
<td>Ratio of molecular weight (native) to molecular weight (chain)</td>
<td>1.9 ± 0.3</td>
<td>0.95 ± 0.12</td>
</tr>
</tbody>
</table>

* Figures in this column are from unpublished data of D. B. Murphy and G. G. Borisy.

Fig. 5. Polymerization of purified porcine brain tubulin stimulated by 125K and 210K MAPs. Change in $A_{320}$ was plotted against time as a measure of microtubule polymerization. At $t = 0$, samples were incubated at 37°C. At the arrow, depolymerization was induced by cooling samples to 4°C. a) 125K MAP fraction. The fraction contained 0.1 mg/ml of 125K MAP and 0.25 mg/ml of residual tubulin. b) 210K MAP fraction. The fraction contained 0.08 mg/ml of 210K MAP and 0.2 mg/ml of residual tubulin. c) DEAE-purified tubulin from porcine brain, 1.2 mg/ml. d) Mixture of 125K MAP fraction and 1.0 mg/ml of added tubulin. e) Mixture of 210K MAP fraction and 1.0 mg/ml of added tubulin.

- Stimulation of Microtubule Polymerization by 125K and 210K MAPs—We have previously demonstrated (Bulinski and Borisy, 1979) that a HeLa MAP fraction which contains 125K, 210K, and various minor MAP species is capable of stimulating the polymerization of purified tubulin. Since it was not clear in the previous study which species were causing the stimulation of microtubule polymerization, we wished to analyze the effect of the 125K and 210K MAPs individually. Using sucrose gradient centrifugation, we were able to effect a separation of the 125K and 210K MAPs. By combining fractions from near the bottom of a sucrose gradient (e.g., Fractions 21 to 23 as shown in Fig. 4) we were able to obtain solutions which contained 125K MAP, tubulin and minor species, but no detectable 210K MAP. Similarly, by mixing fractions from near the top of a sucrose gradient (e.g., Fractions 4 to 6 as shown in Fig. 4) we obtained solutions which contained 210K MAP, some tubulin, and minor species, but no detectable 125K MAP. As shown in Fig. 5 (curves a and b), neither of these mixtures was capable of spontaneous microtubule polymerization. Although some assembly might have been expected in these mixtures because of the residual tubulin, the concentration of active tubulin following the 8-h preparative procedures was apparently too low to support detectable amounts of polymerization in either mixture. However, when DEAE-purified tubulin from porcine brain was added (at a concentration too low for spontaneous polymerization to occur in the absence of MAPs (Fig. 5c)), each mixture showed significant temperature-reversible turbidity (Fig. 5, d and e). Electron microscopic examination of the turbid solutions showed only normal microtubules. Thus, each of the HeLa MAPs, the 125K and the 210K species, is capable of stimulating the assembly of purified tubulin.

- HeLa MAPs Promote both Nucleation and Elongation of Microtubules—MAPs obtained from mammalian brain tissue stimulate both the nucleation and elongation of purified tu-
bulin polymerization (Murphy et al., 1977a; Sloboda et al., 1975; Cleveland et al., 1977a; Herzog and Weber, 1978). We wished to determine if the cultured cell MAPs displayed similar properties. In order to monitor the effects of various amounts of HeLa MAP on the polymerization of a constant amount of tubulin we used preparations containing all of the HeLa MAPs. In this way, we were able to obtain sufficient amounts of MAPs at high enough concentrations to be able to examine the polymerization of several mixtures containing high MAP:tubulin ratios. We prepared DEAE-purified tubulin and MAPs by a method described previously (Bulinski and Borisy, 1979). The tubulin used was greater than 99.5% pure. The MAP fraction used contained <5% by weight of a protein with a gel mobility similar to tubulin and was composed of 125K MAP (19.4% by weight) and 210K MAP (35.3% by weight).

To observe the kinetics of microtubule formation, mixtures of HeLa tubulin and various concentrations of the HeLa MAP solution were incubated at 37°C, and polymerization was again monitored by turbidity at 320 nm. The results are shown in Fig. 6. Neither the MAP fraction (a) nor the purified tubulin (b) showed any turbidity development alone. Mixture (c) contained a MAP:tubulin proportion similar to the proportion in cycled HeLa microtubule protein. The mixtures (d to f) contained increasing proportions of MAP:tubulin which were greater than that present in cycled material. Turbidity traces of the MAP and tubulin mixture (c) demonstrated that the HeLa MAPs stimulated microtubule polymerization. Addition of greater concentrations of HeLa MAP increased both the rate and extent of the microtubule polymerization. Previous analyses (Johnson and Borisy, 1977) have indicated that the initial rate of turbidity increase is proportional to the number of microtubules present and, hence, to the extent of the nucleation reaction, whereas the level of the plateau is a measure of the mass of polymer formed. Electron microscopic examination of the polymer formed from each mixture revealed microtubules with normal morphology. The kinetics of cold-induced depolymerization of the various MAP-tubulin mixtures is also shown in Fig. 6. Depolymerization was rapid and complete. The HeLa MAPs, therefore, promoted both nucleation and elongation of HeLa tubulin in a temperature-reversible manner.

**Stoichiometry of MAP Binding to Tubulin**—In order to analyze in greater detail the effects of the HeLa MAPs on microtubule polymerization, we used quantitative sedimentation assays and SDS polyacrylamide gel electrophoresis. To determine how efficiently the MAPs promoted assembly of pure tubulin, we measured the proportion of tubulin which polymerized in each of the mixtures. Fig. 7A shows that tubulin polymerization increased from 0% in the absence of MAPs to a near plateau level of 90% in the presence of 0.41 mg/ml of HeLa MAP protein. This corresponds to an equilibrium monomer concentration of 0.07 mg/ml. We previously showed that the equilibrium monomer concentration in the cycled HeLa microtubule protein was 0.5 mg/ml. Therefore, addition of increasing amounts of HeLa MAP fraction increased the effective equilibrium constant (equal to the reciprocal of the equilibrium monomer concentration) approximately 7-fold.

We next determined the ratio of each MAP to tubulin in the polymer sedimented from each MAP-tubulin mixture by quantitative densitometry of the SDS gel electrophorograms. The results are shown in Fig. 7B. The ratio of 210K MAP:

![Fig. 6. Kinetics of polymerization of HeLa tubulin with various concentrations of HeLa MAPs.](image)

![Fig. 7. Quantitation of HeLa tubulin polymerization in the presence of enriched MAP concentrations. A, effect of added MAP concentration on percentage of tubulin polymerized. Quantitative sedimentation assays and densitometry of stained gels of microtubule polymer were used to measure the percentage of tubulin which was incorporated into microtubule polymer in each MAP-tubulin mixture; B, effect of added MAP concentration on the ratio (w/w) of each major HeLa MAP group to tubulin in polymer: ⋄, 210K MAP; ○, 125K MAP. Small symbols at right, --○--; --□--; --□□--, indicate the percentages of the 210K and 125K MAPs, respectively, in microtubule protein isolated from HeLa extracts by three cycles of polymerization and depolymerization. Tubulin concentration in all samples summarized in panels A and B, 0.68 mg/ml.)
Characterization of HeLa Microtubule-associated Proteins

11575

tubulin in sedimented microtubules increased to a plateau level of 14.0% (w/w). This represents a 6-fold enrichment over the ratio of 210K MAP to tubulin in microtubule protein prepared by cycling the HeLa extracts, and, using the native molecular weight for 210K MAP, (Table I) implies a ratio of one 210K MAP to 26 ± 5 tubulin dimers at the saturation level of 210K MAP. However, it should be emphasized that this value was obtained in a mixture of MAPs. We have not tested whether the different species of MAPs compete for the same sites on the tubule. Were competition to occur, then the binding stoichiometry of one MAP in the presence of others might be anomalously low. The minor species of MAP of molecular weight 255,000 also saturated its microtubule binding sites at the same concentration of added MAP fraction at which the 210K attained its plateau level. The ratio of 125K MAP:tubulin did not attain a plateau at the highest concentration of added MAP used in this experiment, corresponding to a weight ratio of 18.3% in the polymer. This is an 8-fold enrichment over the 125K MAP:tubulin ratio which obtains in cycled microtubules. Thus, the two principal groups of HeLa MAPs, the 125K and the 210K, seem to be independent molecular species with regard to binding to microtubules, as well as in their solution properties.

**DISCUSSION**

Our analysis of the solution properties of the HeLa MAPs has revealed that all three species studied, the 125K, 210K, and 255K, behave independently. In addition a detailed comparison of the molecular properties of the 210K MAP with those of the brain HMW-MAP has demonstrated both similarities and differences. Both are highly asymmetric, but the oligomeric states of the native molecules appear to be different (see Table I).

The effects of HeLa MAPs on tubulin polymerization resembled the effects of the brain MAPs. Both the 125K and 210K MAPs stimulated the polymerization of tubulin, as has previously been shown for the brain MAPs τ (Penningroth et al., 1976, Witman et al., 1976; Cleveland et al., 1977b; Herzog and Weber, 1978) and HMW (Sloboda et al., 1976a, 1976b; Murphy et al., 1977a, 1977b; Herzog and Weber, 1978).

Experiments to determine the effects of large amounts of HeLa MAPs on microtubule polymerization largely showed similarities between the HeLa MAPs and their brain counterparts. Increasing concentrations of HeLa MAPs increased both the rate and extent of polymerization by lowering the equilibrium monomer concentration for polymerization. The same effects have been demonstrated for the HMW MAPs (Murphy et al., 1977a; Sloboda et al., 1976a, 1976b; Herzog and Weber, 1978) and τ MAPs (Cleveland et al., 1977a).

We observed an additional similarity between brain and HeLa MAPs when we examined the polymer formed from mixtures with various MAP:tubulin ratios. There was a limit to the amount of 210K MAP which would bind to the polymer, and the MAP concentration at which the 210K-binding sites were saturated coincided with the MAP concentration at which tubulin in polymer reached its plateau value. The 255K species also saturated its binding sites at the same concentration of added MAP fraction. This coincidence may not be fortuitous, since the 255K MAP appears to be antigenically related to the 210K MAP (Bulinski and Borisy, 1980).

However, the 125K MAP behaved differently. At higher levels of added MAP fraction, turbidity continued to increase and the 125K MAP continued to bind. We did not observe a saturation of 125K-binding sites. Perhaps the concentrations used were not sufficient to demonstrate a plateau, or, possibly, sedimentable aggregates which contribute turbidity were formed at the high ratios of 125K MAP to tubulin. Evidence for sedimentable aggregates of 125K MAP and tubulin was presented in the sucrose gradient experiment. Association of 125K MAP with tubulin in vitro could be viewed as a nonspecific reaction, because the binding was apparently not saturable. However, the presence of 125K MAP on microtubules in fixed cells (Bulinski and Borisy, 1980) implies that the association of 125K MAP with microtubules in vitro is specific.

Since the HeLa MAPs share a number of features with the brain MAPs, the two groups might be closely related molecules. Both the 125K and 210K HeLa MAPs are large and asymmetric molecules although they differ in their exact solution properties, and the 125K appears to self aggregate while the 210K does not. Both stimulate the rate and extent of microtubule polymerization, and one of the HeLa MAPs (the 210K species) shares with the brain HMW MAP the property of saturable binding sites on the microtubule. However, antibodies to the HeLa 125K and 210K MAPs do not cross-react with each other or with brain HMW or τ MAPs, and antisera to HMW does not cross-react with any of the HeLa MAPs (Bulinski and Borisy, 1980). Thus by immunological criteria, the various MAP species are distinct. Nonetheless, the similarities between the molecular properties of HeLa MAPs and brain MAPs and their similar role in microtubule polymerization in vitro suggest that an analogous if not homologous relation may exist between some of their functions in vivo.

**Acknowledgments**—We thank John G. Peloquin for assistance in the experimental design and calculations presented in this paper and for critical reading of this manuscript. We also thank Douglas B. Murphy for access to his unpublished data and manuscript.

**Note Added in Proof:**—As this paper went to press, a study by Weatherbee, J. A., Luftig, R. B., and Weihring, R. R., ((1980) Biochemistry 19, 4116-4123) appeared, also reporting that a MAP of molecular weight slightly greater than 200,000, isolated from HeLa cells, could promote tubulin polymerization.

**REFERENCES**

Murphy, D. B., Vallee, R. B., and Borisy, G. G. (1977b) Biochem. 16, 2958-2965
Characterization of HeLa Microtubule-associated Proteins