The major microtubule-associated protein in echinoderms is a 77-kDa, WD repeat protein, called EMAP. EMAP-related proteins have been identified in sea urchins, starfish, sanddollars, and humans. We describe the purification of sea urchin EMAP and demonstrate that EMAP binding to microtubules is saturable at a molar ratio of 1 mol of EMAP to 3 mol of tubulin dimer. Unlike MAP-2, MAP-4, or tau proteins, EMAP binding to microtubules is not lost by cleavage of tubulin with subtilisin. In addition to binding to the microtubule polymer, EMAP binds to tubulin dimers in a 1:1 molar ratio. The abundance of EMAP in the egg suggests that it could function to regulate microtubule assembly. To test this hypothesis, we examined the effects of EMAP on the dynamic instability of microtubules nucleated from axoneme fragments as monitored by video-enhanced differential interference contrast microscopy. Addition of 2.2 μM EMAP to 21 μM tubulin results in a slight increase in the elongation and shortening velocities at the microtubule plus ends but not at the minus ends. Significantly, EMAP inhibits the frequency of rescue 8-fold without producing a change in the frequency of catastrophe. These results indicate that EMAP, unlike brain microtubule-associated proteins, promotes microtubule dynamics.

Microtubules are essential structural and functional components of the mitotic apparatus, the machinery responsible for chromosome movements to the daughter cells in all eukaryotic cells. The transition from interphase to mitosis (G2/M) requires a rapid reorganization of the microtubule array and imposes compulsory changes in microtubule dynamics. Longer interphase microtubules are replaced by shorter, less stable, mitotic microtubules (1, 2). This microtubule behavior is best described by dynamic instability, a phenomenon where microtubules in a population exist in both growing and shortening phases (3). Dynamic instability is driven by GTP hydrolysis and exchange, such that microtubules with ends composed of GTP-bound tubulin preferentially elongate whereas microtubules with GDP-tubulin rapidly shorten (3–5). In addition to the kinetics of subunit addition, the morphology of the microtubule ends may contribute significantly to the dynamic properties of microtubules (6). Measurable parameters of dynamic instability include the number of nucleation sites, the growth and shortening rates, the frequency of transition from growing to shortening (catastrophe), and the frequency of transition from shortening to growing (rescue) (7, 8). It is likely that many factors interact to regulate microtubule dynamics (1, 9, 10).

Factors known to regulate microtubule dynamics include microtubule-associated proteins (MAPs). 1 Neuronal brain MAPs, the most abundant of which are MAP-2 and tau, stimulate microtubule assembly by promoting nucleation and, more specifically, by decreasing the rate of microtubule depolymerization (11, 12). When the assembly of individual microtubules is monitored in the presence of MAP-2 or tau, dynamic instability is suppressed through the reduction of catastrophes, the promotion of rescue events, and a reduction in the rate of microtubule shortening (13–17). Other neuronal MAPs, such as MAP1B and the 3-repeat, immature form of tau, do not suppress dynamic instability (18, 19). Therefore, the dynamic properties of neuronal microtubules may be controlled in part by their MAP composition.

In actively dividing cells, much less information is available regarding the effects of specific MAPs on microtubule dynamics. Within the mitotic apparatus, the turnover rates of individual microtubules vary such that kinetochore microtubules have longer half-lives than non-kinetochore microtubules (20, 21). It is likely that the stability of individual microtubules in the mitotic apparatus is controlled by individual MAPs. MAP-4, from HeLa cells, appears to stabilize microtubules by enhancing rescue events at microtubule plus ends (22). This 30-fold enhancement of the rescue frequency is abolished when MAP-4 is phosphorylated by the p34cdc2 kinase, demonstrating that MAP function can be regulated further by post-translational events (22). In Xenopus, the thermostable, MAP-4-like XMAP230 stabilizes microtubules by suppressing catastrophes and decreasing the rate of microtubule shortening (23). In contrast, the unrelated XMAP215 increases the rate of microtubule elongation and shortening and decreases the rescue frequency (24). A third Xenopus MAP, XMAP310, decreases the rate of shortening and promotes rescue (25). Thus, within the same cell type, there are MAPs with unique effects on microtubule dynamics.

The major microtubule-associated protein in the sea urchin is a 75–80-kDa protein called EMAP, which was first identified by its ability to co-polymerize with tubulin isolated from the mitotic apparatus (26). EMAP is not restricted to the mitotic apparatus; immunolocalization studies also reveal EMAP along interphase microtubule arrays in adult coelomocytes (27, 28). It is likely that EMAP function is regulated by phosphorylation, since EMAP is phosphorylated in vivo in a cell cycle-dependent manner (29). In addition to microtubules, EMAP may interact with components of the translational machinery,

* 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.

† To whom correspondence should be addressed: Dept. of Biochemistry, Cell and Molecular Biology, 4010 Hawthor Hall, University of Kansas, Lawrence, KS 66045. Tel.: 785-864-4580; Fax: 785-864-5321; E-mail: ksupre@kuhub.cc.ukans.edu.

\[1\] The abbreviations used are: MAP, microtubule-associated protein; Pipes, piperazine-N,N′-bis(2-ethanesulfonic acid); VE-DIC, video-enhanced differential interference contrast microscopy; Hu, human; PAGE, polyacrylamide gel electrophoresis.
such as ribosomes, that are associated with sea urchin microtubules in vitro and in vivo (28, 30, 31). Translation of a cDNA sequence for EMAP reveals that EMAP does not share significant sequence homology with other MAPs (32). In particular, a microtubule-binding domain has not been identified, although the very basic (pI 10) amino terminus of the protein is a candidate domain.

Recently, a human gene for an EMAP-related protein was identified by positional cloning (33). Human EMAP (HuEMAP) is a 79-kDa protein that is very similar to EMAP, sharing both a very basic amino terminus and a large WD-40 repeat domain (32, 33). Remarkably, the HuEMAP gene appears to be located at the Usher syndrome type 1a locus at position 14q32 (33, 34). Usher syndrome is a recessive genetic disorder that is characterized primarily by deafness accompanied by retinitis pigmentosa. Another variant of Usher syndrome, type 1b, results from mutations in the myosin VIIa gene (35). It is possible that HuEMAP may be an important candidate domain.

To understand how EMAP affects microtubule behavior in dividing cells, we have purified EMAP to homogeneity from unfertilized sea urchin eggs. For this study, we have used video-enhanced differential interference contrast microscopy (VE-DIC) to examine the effects of EMAP on microtubule dynamics. Our results indicate that EMAP promotes microtubule turnover by dramatically decreasing the frequency of rescue. These results suggest that EMAP may play an important role in regulating microtubule dynamics.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—The following reagents were purchased from Research Organics Inc. (Cleveland, OH); ultrapure acrylamide, 5-bromo-4-chloro-3-indolyl phosphate, dithiothreitol, glycine, Pipes, and Tris. Paclitaxel was purchased from Molecular Probes Inc. (Eugene, OR). All other reagents were from Sigma or Fisher unless otherwise specified in the text.

**Microtubule Protein**—Microtubule protein was purified from unfertilized sea urchin eggs (Strongylocentrotus purpuratus) as described previously (30, 40). Porcine tubulin and sea urchin sperm flagellar axonemes were also purified as described previously (8, 24, 41).

**Electrophoresis and Western Blotting**—SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on discontinuous gels (3% stacking and 8% separating) as described in Laemmli (43). For immunoblotting, proteins were transferred to nitrocellulose using the procedure of Towbin et al. (44), probed with anti-EMAP antisera (AS8), and developed with alkaline phosphatase-conjugated secondary antibodies, as described previously (28, 45).

**EMAP Purification**—Thirty to 40 mg of purified sea urchin egg microtubule protein was thawed on ice. Microtubules were induced by incubation at 30 °C for 20 min, and the microtubules were pelleted (50,000 rpm, 10 min, 30 °C, TLA100.3 rotor). The pellet was resuspended in 2 ml of ice-cold column buffer (10 mM Pipes/KCl, 1 mM MgCl₂, 0.1 mM GTP, 0.2 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, and 1 μg/ml pepstatin A), homogenized, incubated on ice for 20 min, then centrifuged (50,000 rpm, 10 min, 2 °C). The supernatant was applied to a DE52 cellulose (Whatman) column. A 1.5 × 6-cm DE52 cellulose column was equilibrated in ≥10 column volumes of degassed column buffer at 4 °C. After equilibration, 2 ml of 10–15 mg/ml cold depolymerized microtubule proteins (50,000 rpm supernatant) were loaded on the column. The column was washed with ~80 ml of column buffer and run by gravity with ~5 cm pressure. Proteins bound to the column were eluted with 160 ml of a 0 to 6 M KCl linear gradient in column buffer. Two-milliliter fractions were collected throughout the gradient and analyzed by gel electrophoresis.

The EMAP containing fractions were pooled and dialyzed against several changes of ice-cold PME buffer, pH 6.9 (100 mM Pipes/KCl, 1 mM MgSO₄, 1 mM EGTA), over a 12–15-h period. EMAP was concentrated either by dialysis against solid polyvinylpyrrolidone (Sigma) or by concentration in a Slide-a-Lyzer (Pierce) according to the manufacturer’s instructions. The concentration of EMAP was limited to approximately 0.4 mg/ml in PME buffer; above this concentration, EMAP precipitated out of solution. The concentrated proteins were frozen in liquid nitrogen and stored at −80 °C.

The egg tubulin-containing fractions were concentrated by ammonium sulfate precipitation (50% saturation, 4 °C, 20 min) and centrifuged at 27,000 × g for 20 min at 4 °C. Tubulin precipitates were resuspended in 1–2 ml of PME buffer, pH 6.9, containing 0.1 mM GTP and were desalted on Sephadex G-25 spin columns. Tubulin in the flow-through fractions was drop frozen in liquid nitrogen and stored at −80 °C.

**Protein Determination**—Sea urchin microtubule proteins were quantitated by the enhanced bichinchoninic assay (Pierce) with bovine serum albumin as the protein standard. Porcine tubulin concentration was determined as described by Hyman and Karsenti (6).

**Size Exclusion Chromatography**—A 300 × 7.8-mm gel filtration high pressure liquid chromatography column (BioSep-SEC-S2000; Phenomenex, Torrence, CA) was used to determine the native molecular mass of EMAP. The column was equilibrated and run in 100 mM potassium phosphate, pH 7, with a flow rate of 1 ml/min. Gel filtration standards for size exclusion chromatography were thyroglobulin (670,000), bovine gamma globulin (155,000), chicken albumin (44,000), equine myoglobin (17,000), and vitamin B-12 (1,350) from Bio-Rad. Protein elution profiles were monitored by absorbance at 280 nm. A linear relationship was obtained from a plot of the logarithms of the molecular mass standards versus their elution time. The relative elution time for EMAP was used to determine the Mr for EMAP.

**EMAP Saturation**—Purified sea urchin egg tubulin was assembled into microtubules (24 °C, 20 min) in the presence of increasing amounts of purified EMAP in a microtubule assembly buffer (100 mM Pipes, pH 6.9, 1 mM MgSO₄, 1 mM EGTA, 0.5 mM GTP, 20 μM taxol). The microtubules were pelleted (100,000 × g, 24 °C, 10 min), and the supernatants and resuspended pellets were analyzed on Coomassie Blue-stained SDS gels. Densitometric analysis of the gels were carried out with a Hewlett-Packard Scan Jet Iicx and quantitated using NIH Image software.

**Molar Stoichiometry Calculations**—The molar stoichiometry of EMAP bound to tubulin dimers in microtubules and the stoichiometry of EMAP:tubulin dimers:37-kDa polypeptide in DE52 cellulose column fractions were estimated from densitometric analysis of Coomassie Blue-stained gels. Areas under each protein peak were converted to total mass units and divided by the appropriate molecular mass of each polypeptide to determine the relative number of moles of each polypeptide to the relative molecular masses used in these calculations were 100,000 for the tubulin dimer (α and β subunit), 75,000 for the EMAP monomer, and 37,000 for the unknown polypeptide.

**Subtilisin Cleavage**—Bovine brain tubulin (1.5 mg/ml) was polymerized in the presence of 20 μM paclitaxel, in the presence or absence of 1.5% (w/w) subtilisin (Boehringer Mannheim). After 25 min at 30 °C, the reaction was stopped by placing the sample on ice and adding 2 mM phenylmethylsulfonyl fluoride (46). Purified EMAP (5 μg) was added to both tubulin samples, along with an additional 1 mM GTP. The proteins were incubated at 37 °C for 30 min and then the microtubules were pelleted (100,000 × g, 30 °C, 10 min). The supernatants and resuspended pellets were analyzed by SDS-PAGE.

**Microtubule Dynamics**—Microtubule assembly assays were performed as described in Walker et al. (8) and Vasquez et al. (24) using pig brain tubulin and sea urchin flagellar axonemes. Briefly, individual microtubules assembled from axoneme fragments were observed using video-enhanced differential interference contrast light microscopy (VE-DIC). Porcine brain tubulin (21 μM) was incubated in the presence or absence of purified EMAP (2.2 μM). The slides were maintained at 23 °C and examined for 30–60 min. The concentration of tubulin was low enough to prevent spontaneous microtubule assembly under these conditions, and therefore, all assembly was off the axoneme fragments. For all samples, the plus and minus ends were distinguished by the faster rate of elongation on the plus ends.

Individual microtubules were visualized using VE-DIC microscopy as described previously (24, 47, 48). Microtubule assembly and shortening rates were determined from video tapes using PC-based analysis software (49, 50). For each microtubule tracked, the change in microtubule length was plotted versus time. The average rate of microtubule growth or shortening was determined using least squares regression analysis. The frequencies of catastrophe and rescue were determined as described in Walker et al. (8). Standard deviations for transition frequencies were determined from the catastrophe or rescue frequency divided by the square root of the number of transitions observed (8). This
calculation assumes a Poisson distribution of growth or shortening times (8). Dynamicity, as a measure of tubulin subunit addition or loss per unit time, was calculated as described previously (24, 51).

RESULTS

Purification of EMAP—The most abundant microtubule-associated protein in sea urchins is a 77-kDa polypeptide called EMAP that copurifies stoichiometrically with microtubules assembled from sea urchin eggs. To examine the effect of EMAP on microtubule dynamics, EMAP was purified from third cycle egg microtubule protein. Two-dimensional gel electrophoresis demonstrated that the pI of EMAP in microtubule protein was 6.9; therefore, EMAP was purified from the more acidic tubulin subunits on a DE52 cellulose, anion exchange column (Fig. 1). EMAP eluted from the column in two distinct peaks. The first EMAP fraction was greater than 98% pure and eluted from the column at approximately 0.17M KCl (fractions 22–27). In this manner, over 400 mg of purified EMAP was obtained from a typical microtubule preparation starting with 100 ml of unfertilized sea urchin eggs (Figs. 1 and 2). The second EMAP fraction came off the column at approximately 0.25M KCl (fractions 34–38) in a peak that contained tubulin dimers and a prominent 37-kDa polypeptide (Figs. 1 and 2). The molar ratio of the 37-kDa polypeptide to EMAP to tubulin dimers in this complex was estimated from the apparent mass ratios in the stained gels. A comparison of five separate experiments indicates that the molar ratio of EMAP:tubulin dimers appears to be constant at 1:1 in these complexes. In contrast, the ratio of the 37-kDa polypeptide to EMAP or tubulin appears to vary from 1:1, 1:2, and 1:3. On average, the molar ratio of the 37-kDa polypeptide/tubulin dimer-EMAP is 1:1.9:2.0 (n = 5). Finally, the last proteins to elute off the column, at approximately 0.35 to 0.5 M KCl, are tubulin dimers (fractions 43–48).

The native molecular mass of purified EMAP (Fig. 2A, fractions 22–27) was determined to be 75.7 kDa (n = 2) by high pressure liquid size exclusion chromatography. This is in close agreement with its molecular mass, 75,488 daltons, calculated from a translated cDNA sequence (32) and with the protein’s estimated mass of 77 kDa on SDS-polyacrylamide gels. These results indicate that EMAP is probably a globular protein and is a monomer in its purified form.

EMAP Binding to Microtubules—The acidic COOH terminus of tubulin has been postulated to be important for MAP binding to microtubules (46, 52–54). To determine if this region of tubulin was necessary for purified EMAP to bind to tubulin, we cleaved the COOH terminus of \( \beta \)-tubulin with subtilisin and assayed for EMAP binding (Fig. 3). The shift in the mobility of \( \beta \)-tubulin on the gel is consistent with subtilisin digestion. That all the EMAP pellets with subtilisin-digested microtubules indicate that EMAP does not bind to the COOH terminus of \( \beta \)-tubulin. EMAP alone does not pellet under these conditions.

To assay the stoichiometry of EMAP binding to microtubules, increasing amounts of purified EMAP were added to a constant amount of purified sea urchin egg tubulin in the
EMAP inhibits microtubule rescue

EMAP binding to microtubules is saturable. Purified sea urchin egg tubulin was assembled into microtubules in the presence of increasing amounts of purified EMAP. The microtubules were pelleted, and the supernatants (A) and pellets (B) were analyzed by SDS-PAGE. Lane 1, no tubulin, 1.25 μg of EMAP. Lanes 2–7 contain 2.5 μg of tubulin with EMAP concentrations as follows: lane 2, 0; lane 3, 0.31 μg; lane 4, 0.62 μg; lane 5, 1.25 μg; lane 6, 2.5 μg; lane 7, 5 μg. C shows the amount of EMAP bound to microtubules in the pellet (solid diamonds) and the amount of EMAP in the supernatant (open boxes) at each of the EMAP concentrations used.

Microtubule assembly was initiated by the introduction of 21 μM tubulin in the presence or absence of 2.2 μM EMAP. These EMAP and tubulin concentrations were chosen for two reasons. First, this ratio of EMAP to tubulin approximates the concentration of EMAP and tubulin in the unfertilized egg. Second, EMAP precipitates out of Pipes-containing solutions at concentrations greater than 5 μM. EMAP stocks («5 μM) were diluted with tubulin, GTP, and casein in the presence of 0.1 M Pipes, pH 6.9, 2 mM EGTA, 1 mM MgSO4, and 0.5% Nonidet P-40 (24). In this assay, the concentration of microtubules is low, and therefore, EMAP is present at a concentration sufficient to saturate the microtubule polymer.

Microtubules assembled from tubulin alone exhibited characteristic phases of dynamic instability with alternating periods of growth and rapid shortening (Fig. 5 and Table I). In the presence of EMAP, microtubules were also dynamic; however, EMAP only had a slight effect on the growth rate of microtubules: the rate of elongation (Ve) increased slightly at the plus ends (p < 0.01) and decreased slightly at the minus ends (p < 0.05) (Fig. 5, Table I). EMAP had no statistically significant effects on the rate of rapid shortening (Vrs) at either the plus or minus ends of microtubules (Table I). Qualitatively similar results were obtained in four additional experiments using two different tubulin preparations and three EMAP preparations. For some experiments, microtubule assembly was monitored at 35 °C (12–18 μM tubulin). In general, plus end elongation rate was slightly faster in the presence of EMAP (4 of 5 experiments) and minus end elongation rate was slightly slower (3 of 5 experiments).

EMAP did not inhibit the transition from elongation to rapid shortening (catastrophe) at either the plus or minus ends (Table I). For example, plus end catastrophes were observed approximately once every 163 s in the presence of EMAP (40 catastrophes in 6600 s of elongation) and once in 116 s in the absence of EMAP (25 catastrophes in 2903 s of elongation).

Although EMAP had little to no effects on the catastrophe frequency, EMAP significantly reduced the plus end rescue frequency. At a tubulin concentration of 21 μM, rescues (transition from rapid shortening to elongation) occurred once every 50 s (16 rescues in 812 s of shortening) at the same tubulin concentration, in the presence of 2.2 μM EMAP, rescues were
EMAP Inhibits Microtubule Rescue

**TABLE I**
Parameters of dynamic instability with and without EMAP*

| Parameters                  | Plus ends | Minus ends |
|-----------------------------|-----------|------------|
|                            | 21 μM tubulin | 2.2 μM EMAP | 21 μM tubulin | 2.2 μM EMAP |
| Ve (μm/min) S.D. ± (n)       | 0.95 ± 0.29 (29) | 1.26 ± 0.31 (58) | 0.3 ± 0.12 (27) | 0.22 ± 0.14 (28) |
| Vrs (μm/min) S.D. ± (n)      | 6.26 ± 3.54 (24) | 15.7 ± 9.8 (42) | 5.94 ± 4.58 (17) | 9.45 ± 7.34 (14) |
| Catastrophe frequency (s⁻¹) ± S.D. | 0.0066 ± 0.0017 | 0.0061 ± 0.001 | 0.003 ± 0.0008 | 0.0028 ± 0.0008 |
| Elongation time (min), (n)   | 48.38 (25) | 110.0 (40) | 87.98 (16) | 76.29 (13) |
| Rescue frequency (s⁻¹) ± S.D.| 0.02 ± 0.005 | 0.0026 ± 0.0003 | 0.034 ± 0.019 | 0.024 ± 0.01 |
| Shortening time (min), (n)   | 13.54 (16) | 25.5 (4) | 5.76 (12) | 4.96 (6) |

EMAP Inhibits Microtubule Rescue

Dynamic instability was quantitated by calculating the “dynamicity,” a measure of dimer exchange per unit time (51), indicates that EMAP inhibits microtubule turnover. EMAP appears to have a unique microtubule-binding domain. A comparison of the amino acid sequence of EMAP with other MAPs (32) reveals that EMAP does not share the microtubule-binding domain of MAP2 (58), tau (59, 60), MAP61 (61, 62), MAP1 (63, 64), E-MAP-115 (65), or CLIP-170 (66). Furthermore, unlike the brain MAPs, MAP-2, MAP-4, and tau, EMAP does not appear to bind to the acidic COOH terminus of β-tubulin (52, 56). Proteolytic cleavage of the COOH terminus with subtilisin did not affect the binding of EMAP to paclitaxel-stabilized microtubules. These results suggest that EMAP may bind to a different tubulin domain and that the effects of EMAP on microtubule assembly probably differ significantly from those neuronal MAPs.

DISCUSSION

The 77-kDa EMAP is the most abundant microtubule-associated protein in echinoderms. The protein is present in both embryonic and somatic cells, and it localizes to interphase and mitotic microtubule arrays (26–28, 40, 55, 56). The function of EMAP is unknown. The abundance of EMAP in the mitotic apparatus suggests that EMAP might be involved in the regulation of microtubule assembly during the cell cycle.

**EMAP Purification and Binding to Microtubules**—We describe the purification of EMAP from sea urchin microtubule protein using anion exchange column chromatography (Fig. 1). EMAP elutes off the DE52 cellulose column in two distinct fractions. The earlier fraction is usually quite pure. In the second EMAP fraction, there is an equimolar amount of tubulin dimers. The EMAP-tubulin complex also has an abundant 37-kDa polypeptide. Although the identity of this polypeptide is not yet known, its abundance indicates it may be a functionally important component of these complexes.

EMAP binding to microtubules is saturable with approximately 1 mol of EMAP binding per 3 mol of tubulin dimer. The stoichiometry of EMAP binding to microtubules is similar to that reported for buttonin, a 75-kDa sea urchin microtubule-associated protein that may be related to EMAP (57). Size exclusion chromatography indicates that buttonin is a homodimer of approximately 150 kDa and forms very distinct spherical structures along the walls of microtubules (57). EMAP appears to be a monomeric globular protein with a molecular mass approximately 75 kDa.

EMAP appears to have a unique microtubule-binding domain. A comparison of the amino acid sequence of EMAP with other MAPs (32) reveals that EMAP does not share the microtubule-binding domain of MAP2 (58), tau (59, 60), MAP61 (61, 62), MAP1 (63, 64), E-MAP-115 (65), or CLIP-170 (66). Furthermore, unlike the brain MAPs, MAP-2, MAP-4, and tau, EMAP does not appear to bind to the acidic COOH terminus of β-tubulin (52, 56). Proteolytic cleavage of the COOH terminus with subtilisin did not affect the binding of EMAP to paclitaxel-stabilized microtubules. These results suggest that EMAP may bind to a different tubulin domain and that the effects of EMAP on microtubule assembly probably differ significantly from those neuronal MAPs.

EMAP Decreases the Rescue Frequency—Microtubules in vivo are more dynamic than purified microtubules in vitro indicating that MAPs or other cellular factors increase microtubule dynamics (9). Until recently it appeared that MAPs primarily stabilize microtubules and decrease dynamic instability, meaning that the microtubules undergo fewer transitions from growing to shrinking (7, 13–15). We examined the mechanism by which EMAP modulates the assembly of tubulin by observing the growth of single microtubules in real time by VE-DIC. We found that EMAP promoted a small net increase in polymer turnover at the plus end nearly 3-fold, whereas EMAP has no detectable effect on turn-
EMAP Inhibits Microtubule Rescue

...able to be modulated by phosphorylation events (49). Treatment of interphase extracts with the phosphatase inhibitor okadaic acid converts the extract to a mitotic-like state. Plus end elongation and shortening velocities increased slightly, and the frequency of catastrophe approximately doubled, and most notably, rescue was abolished (49). Significantly, EMAP is phosphorylated in a cell cycle-dependent manner, and the appearance of a mitosis-specific phosphopeptide suggests that EMAP function is regulated at the onset of mitosis (29). Whether EMAP’s inhibition of rescue is regulated by phosphorylation remains to be determined.

At this time we can only speculate on how EMAP could decrease the rescue frequency. One possibility is that EMAP sequesters tubulin dimers and prevents them from reassociating with the microtubule polymer. That EMAP binds tubulin dimers is indicated by the DE52 cellulose columns where a considerable amount of EMAP is found in a complex with soluble tubulin dimers (Fig. 1). However, it is unlikely that EMAP sequesters tubulin dimers in the nucleated assembly assay because EMAP has only a slight effect on the elongation rate, so it is not expected that EMAP would alter the free tubulin concentration. Moreover, there is not sufficient EMAP to sequester most of the 20 μm tubulin dimers estimated to be present in the egg (67). Another possibility is that EMAP has a higher affinity for GDP-tubulin than for GTP-tubulin. It is difficult to reconcile this possibility with the observation that EMAP does not reduce the elongation rate or increase the shortening velocity. The elongation rate depends upon the GTP-tubulin concentration which is in huge excess in these in vitro experiments. A more attractive hypothesis is that EMAP stabilizes protofilament “peels” during microtubule disassembly. During microtubule shortening, protofilaments curl back from the microtubule ends similar to a banana peel (68–71). Although the molecular events required for a rescue are not known, the peeling events must stop before microtubule elongation can resume. Stabilization of the protofilament peels through EMAP binding could reduce the frequency of rescue without affecting the shortening velocity.

**EMAP Function in Vivo**—By using a computer simulation of mitotic and interphase microtubule dynamics, Glicksman et al. (72) have postulated that a modest decrease in the frequency of rescue would rapidly lead to shorter microtubules. Theoretically, EMAP could contribute to the increase in microtubule dynamics at the G2/M transition. EMAP is the only MAP known whose principle effects are in decreasing the frequency of rescue. XMAP215 decreases the frequency of rescue in addition to dramatically increasing the elongation rate and shortening velocity (24). The major effect of XMAP310 is on the frequency of rescue, but in contrast to EMAP, XMAP310 promotes rescue (25). Other proteins such as Op18 and XKCM1 may be involved in the targeting of mRNAs and ribosomes to sea urchin microtubules in vivo and in vitro (28, 30, 31, 75). EMAP also appears to interact with the p34cdc2 kinase, perhaps targeting the kinase to the mitotic spindle poles (29). EMAP may target the translational machinery and possibly other regulatory proteins to the microtubule cytoskeleton through its β-transducin-like WD-40 repeat domains (32). WD repeat motifs are approximately 40 amino acids long, beginning in glycine-histidine (GH) and ending in tryptophan-aspartate (WD) (76). WD repeat proteins belong to a large family of proteins with diverse cellular functions including signal transduction, vesicular trafficking, and cytoketal assembly (76, 77). Each WD repeat folds into a structure of β-strands and β-turns that takes on the appearance of an airplane propeller (76, 78–81). The variable regions of the WD repeats are predicted to be exposed on the surface of the propellers where they can present a changeable surface for protein-protein interaction (76). EMAP with its 10 divergent WD repeats may fold into a compact structure with up to 10 individual propellers and the capacity to interact with up to 10 different partner proteins (77). Identification of the binding partners of EMAP will be significant for understanding the function of EMAP in embryonic and somatic cells.

The gene for a human EMAP-related protein is located on the long arm of chromosome 14 at a locus, 14q32, implicated as a tumor suppressor locus in a variety of cancers (36–39) and as the Usher syndrome type Ia locus (33, 34). HuEMAP is 58% identical and 78% similar to the sea urchin EMAP and appears to have 9 WD repeat motifs (33). Recently, we cloned and sequenced two additional human EMAP-related proteins that are approximately 55% identical and 75% similar to EMAP, suggesting that HuEMAP belongs to an important multigene family.2 Future experiments are directed toward understanding EMAP and HuEMAP function in somatic cells.

**REFERENCES**

1. Goland, V. I., and Berlshtash, A. D. (1991) Annu. Rev. Cell Biol. 7, 93–116

2. Inoue, S., and Salmon, E. D. (1995) Mol. Biol. Cell 6, 1619–1640

3. Mitchell, T., and Kirschner, M. (1984) Nature 312, 237–242

4. Carlier, M., and Pantaloni, D. (1981) Biochemistry 20, 1918–1920

5. Hill, T., and Carler, M. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 7234–7238

6. Hyman, A. A., and Karsenti, E. (1996) Cell 84, 401–410

7. Horio, T., and Hotani, H. (1986) Nature 321, 605–607

8. Walker, R. A., O’Brien, E. T., Pryer, N. K., Soboeiro, M. F., Voter, W. A., Erickson, H. P., and Salmon, E. D. (1988) J. Cell Biol. 107, 1437–1448

9. Cassimeris, L. (1993) Cell Motil. Cytoskeleton 26, 275–281

10. McNally, F. J. (1996) Curr. Opin. Cell Biol. 8, 23–29

11. Sobeda, R. D., Dentler, W. L., and Rosenbaum, J. L. (1976) Biochemistry 15, 4479–4505

12. Murphy, D. B., Johnson, K. A., and Borisy, G. G. (1977) J. Mol. Biol. 1178, 33–52

13. Dreschel, D. N., Hyman, A. A., Cobb, M. H., and Kirschner, M. W. (1992) Mol. Biol. Cell 3, 1141–1154

14. Pryer, N. K., Walker, R., Sreek, V. P., Bourns, B. D., Soboeiro, M. F., and Salmon, E. D. (1992) J. Cell Sci. 103, 965–976

15. Walski, R. J., and Williams, R. C. (1993) Cell Motil. Cytoskeleton 26, 282–290

16. Itoh, T. J., and Hotani, H. (1994) Cell Struct. Funct. 19, 279–290

17. Panda, D., Goode, B. L., Feinstein, S. C., and Wilson, L. (1995) Biochemistry 34, 11117–11127

18. Trinczek, B., Bierhart, J., Baumann, K., Mandelkow, E. M., and Mandelkow, E. (1993) Mol. Biol. Cell 6, 1887–1902

19. Vandeveerdere, A., Pedrotti, B., Utton, M. A., Calvert, R. A., and Bayley, H. (1995) Cell Motil. Cytoskeleton 35, 134–146

20. Saxton, W. M., Stemple, D. L., Salmon, E. D., Zavarontin, M., and McIntosh, R. J. (1984) J. Cell Biol. 99, 2175–2186

21. Zhai, Y., Kromenber, P. J., Simon, P. M., and Borisy, G. G. (1996) J. Cell Biol. 135, 201–214

22. Okata, K., Hisanaga, S., Bulinski, J. C., Murofushi, H., Aizawa, H., Itoh, T. J., Hotani, H., Okumura, E., Tachibana, K., and Kishimoto, T. (1995) J. Cell Biol. 126, 849–862

23. Andersen, S. S. L., Buendia, B., Dominguez, J. E., Sawyer, A., and Karsenti, E. (1994) J. Cell Biol. 127, 1289–1299

24. Vasquez, R. J., Gard, D. L., and Cassimeris, L. (1994) J. Cell Biol. 127, 965–993

25. Andersen, S. S. L., and Karsenti, E. (1997) J. Cell Biol. 139, 975–983

26. Keller, T. C. S., III, and Rebhun, L. I. (1982) J. Cell Biol. 93, 275–281

27. Hamill, D., Davis, J., Drawbridge, J., and Suprenant, K. A. (1995) J. Cell Biol. 127, 973–984

28. Li, Q., and Suprenant, K. A. (1994) J. Biol. Chem. 269, 31777–31784

29. Eddy, J. D., Ma-Edmonds, M., Yen, S., Talmadge, C. B., Kelley, P. H., Weston, M. D., Kimberling, W. J., and Sumegi, J. (1997) Genomics 43, 104–106

30. Kaplan, J., Gerber, S., Bonneau, D. B., Desroz, J., Deliroux, B., Briard, M., Dollfus, H., Ghazi, I., Frezal, J., and Munich, A. (1992) Genomics 14, 979–987

31. Weil, D., Banchard, S., Kaplan, J., Guilford, P., Givson, F., Walsh, J., Mburu, P., Varela, A., Levilliers, J., Weston, M., Kelly, P. M., Kimberling, W. J.,

2 D. Lepley and K. Suprenant, unpublished observations.
