

Novel Features of the Light Chain of Microtubule-associated Protein MAP1B: Microtubule Stabilization, Self Interaction, Actin Filament Binding, and Regulation by the Heavy Chain

Martin Tögel, Gerhard Wiche, and Friedrich Propst

Institute of Biochemistry and Molecular Cell Biology, Vienna Biocenter, University of Vienna, A-1030 Vienna, Austria

Abstract. Previous studies on the role of microtubule-associated protein 1B (MAP1B) in adapting microtubules for nerve cell-specific functions have examined the activity of the entire MAP1B protein complex consisting of heavy and light chains and revealed moderate effects on microtubule stability. Here we have analyzed the effects of the MAP1B light chain in the absence or presence of the heavy chain by immunofluorescence microscopy of transiently transfected cells. Distinct from all other MAPs, the MAP1B light chain-induced formation of stable but apparently flexible microtubules resistant to the effects of nocodazole and taxol. Light chain activity was inhibited by the heavy chain. In addition, the light chain was found to harbor an actin filament binding domain in its COOH terminus. By

coimmunoprecipitation experiments using epitope-tagged fragments of MAP1B we showed that light chains can dimerize or oligomerize. Furthermore, we localized the domains for heavy chain–light chain interaction to regions containing sequences homologous to MAP1A. Our findings assign several crucial activities to the MAP1B light chain and suggest a new model for the mechanism of action of MAP1B in which the heavy chain might act as the regulatory subunit of the MAP1B complex to control light chain activity.

Key words: microtubule-associated protein 1B • microtubules metabolism • nocodazole • actin stress fibers • cytoskeleton

MICROTUBULE-ASSOCIATED protein 1B (MAP1B)¹ was originally identified as a 320-kD protein that copurified with microtubules from mammalian brain through successive cycles of temperature-dependent polymerization and depolymerization (Greene et al., 1983; Binder et al., 1984; Bloom et al., 1984; Calvert and Anderton, 1985; Herrmann et al., 1985; Riederer et al., 1986). MAP1B is expressed at its highest levels in the brain during early postnatal development (Binder et al., 1984; Calvert and Anderton, 1985; Lewis et al., 1986; Riederer et al., 1986; Safaei and Fischer, 1989; Schoenfeld et al., 1989; Garner et al., 1990). In rodents, expression peaks a few days after birth and then gradually declines to reach adult levels. This pattern of expression is reciprocal to that of the related high molecular mass protein MAP1A, the expression of which is low at birth and increases during

postnatal development (Schoenfeld et al., 1989; Garner et al., 1990). In the adult, MAP1B expression remains elevated in brain regions that retain a high level of axonal growth and synaptic plasticity (Schoenfeld et al., 1989; Tucker et al., 1989; Nothias et al., 1996). Detailed immunohistochemical analysis revealed that MAP1B is present in axons, cell bodies, and dendrites of neurons and in glial cells (Bloom et al., 1985; Calvert and Anderton, 1985; Riederer et al., 1986; Garner et al., 1989; Schoenfeld et al., 1989) and immunoelectron microscopy suggested that MAP1B is a component of a microtubule-associated fibrillar matrix found in axons (Hirokawa et al., 1985). Studies using monoclonal antibodies specific to phosphorylated MAP1B demonstrated that the protein is differentially phosphorylated on several sites depending on its expression in neurons or glial cells, its subcellular localization in neurons, and the developmental stage (Sato-Yoshitake et al., 1989; Gordon-Weeks et al., 1993; Harrison et al., 1993; Riederer et al., 1993; Ulloa et al., 1994; Boyne et al., 1995). Analyses carried out on differentiating neurons in cell culture showed that MAP1B levels and phosphorylation are particularly high in distal axons (Tanaka et al., 1992; Black et al., 1994; Boyne et al., 1995; Bush et al., 1996). Taken together, these findings led to the hypothesis that the protein

Address all correspondence to Friedrich Propst, Institute of Biochemistry and Molecular Cell Biology, Dr. Bohr-Gasse 9, A-1030 Vienna, Austria. Tel.: 43 1 4277 52858. Fax: 43 1 4277 52854. E-mail: friedrich.propst@univie.ac.at

1. *Abbreviations used in this paper:* HA, hemagglutinin; MAP, microtubule-associated protein.

might be involved in neuronal differentiation, regulating microtubule-dynamics in the growing axon.

Cloning of mouse, rat and human MAP1B cDNA revealed highly conserved open reading frames (90% amino acid identity) of ~2,460 codons, the exact length depending on the species (Noble et al., 1989; Zauner et al., 1992; Lien et al., 1994; Liu and Fischer, 1996). Among the highest conserved regions of the protein are the NH₂-terminal 550 and the COOH-terminal 120 amino acids (98 and 100% identity between mouse and human, respectively). In addition, MAP1B contains regions of homology to the distantly related protein MAP1A (Langkopf et al., 1992). The two main stretches of homology comprise 300 and 120 amino acids and are located in the conserved NH₂ and COOH termini, respectively. MAP1B mRNA is translated to give rise to a polyprotein that is subsequently cleaved into two proteins comprising the NH₂-terminal 2,200 amino acids, termed the heavy chain, and the COOH-terminal 250 amino acids, termed the light chain of MAP1B (Hammarback et al., 1991). The exact site where the polyprotein precursor is cleaved is not known but has been narrowed down to a region of ~20 amino acids around amino acid 2,200. Heavy chain and light chain form a complex. The binding site for the light chain has been mapped to a 120 kD region comprising the NH₂-terminal half of the heavy chain (Kuznetsov et al., 1986; Hammarback et al., 1991). A similar post-translational proteolytic processing has been observed for MAP1A (Langkopf et al., 1992).

Consistent with the observed pattern of MAP1B expression during brain development, the protein appears to play an important role in neuronal differentiation. The first such evidence was obtained by antisense oligonucleotide approaches and showed that ablation of MAP1B expression in PC12 cells or cultured cerebellar macroneurons inhibits or reduces neurite outgrowth (Brugg et al., 1993; DiTella et al., 1996). More recently, the phenotypic effects of homozygous MAP1B mutations introduced into the germline of mice by homologous recombination were investigated in two independent studies (Edelmann et al., 1996; Takei et al., 1997). Despite discrepancies concerning the severity of the effects of MAP1B mutations both studies demonstrated that MAP1B mutations can lead to an impairment or retardation of brain development. However, the molecular function of MAP1B during neuronal morphogenesis and its mechanism of action remain unclear and information about functional domains of the protein is limited to the characterization of the two microtubule-binding domains. One of these domains is located on the heavy chain between amino acids 580 to 800 and was identified by expressing MAP1B mutant proteins containing or missing this domain in tissue culture cells (Noble et al., 1989) and by cosedimentation assays *in vitro* (Zauner et al., 1992). The other microtubule-binding domain is located on the light chain in the region spanning amino acids 2,215 to 2,335 (numbering according to the polyprotein precursor) and was identified by analyzing the capacity of recombinant MAP1B fragments synthesized in bacteria to cosediment with microtubules *in vitro* (Zauner et al., 1992).

One approach successfully used to unravel the function of neuronal MAPs has been the expression of these proteins in nonneuronal cells. However, unlike MAP2, which

induces dramatic effects such as microtubule bundling, stiffening and stabilization when expressed in nonneuronal cells (Lewis et al., 1989; Takemura et al., 1992; Weisshaar et al., 1992; LeClerk et al., 1993; Weisshaar and Matus, 1993), MAP1B expression induces only modest effects (Noble et al., 1989; Takemura et al., 1992). MAP1B causes less efficient stabilization of microtubules than MAP2 and does not induce microtubule bundling. Likewise, MAP1B causes microtubule stabilization and nucleation *in vitro* but again, less efficiently than MAP2 (Vandecastelaere et al., 1996). In all these studies the effects of the entire MAP1B protein complex consisting of heavy and light chain were analyzed. Here we attempted to investigate possible effects of the MAP1B light chain on its own to determine which activities, if any, it might have. We chose to express the light chain or light chain deletion mutants in nonneuronal cells expressing little or no endogenous MAP1B to minimize possible interference by the endogenous MAP1B heavy chain. We found that in the absence of heavy chain the light chain has dramatic and unique effects on microtubules. We identified for the first time light chain domains for microtubule stabilization and actin filament binding, obtained evidence for light chain oligomerization and determined the importance of the MAP1A homology domains of MAP1B for oligomerization and heavy chain-light chain interaction. Our findings suggest a new model for MAP1B as a protein complex consisting of a regulatory subunit, the heavy chain, and an active subunit, the light chain.

Materials and Methods

cDNA Constructs

The full-length rat MAP1B cDNA was obtained as described (Zauner et al., 1992; Tögel et al., 1998). Constructs encoding full-length MAP1B polyprotein precursor (amino acids 1–2,459), heavy chain (amino acids 1–2,185), light chain (amino acids 2,210–2,459), NH₂ terminus (amino acids 1–508), COOH terminus (amino acids 2,336–2,459), or the microtubule binding domain of the light chain (amino acids 2,210–2,336; see Fig. 1) all fused in frame to a COOH-terminal myc-tag (amino acid sequence: EQKLISEEDLN; Cravchik and Matus, 1993) by a variable linker of 1–3 amino acids containing unique restriction sites were generated using convenient restriction sites and adapter oligonucleotides. All constructs were cloned into the Tet-Off expression vector pUHD10-3 (Gossen and Bujard, 1992). Hemagglutinin (HA)-tagged constructs were obtained by exchanging the myc-tag against a triple HA-tag (amino acid sequence: ASRYPDVVDYAGYPYDVPDYAGSYDVPDYASR; Field et al., 1988) flanked by appropriate restriction sites. For constructs encoding untagged full-length MAP1B or untagged light chain the myc-tagged COOH terminus was replaced by the untagged COOH terminus from the original cDNA (Zauner et al., 1992; Tögel et al., 1998).

Antibodies

A rabbit polyclonal anti-light chain antiserum was raised against the synthetic peptide CSKNVDVEFFKRVR (MAP1B amino acids 2,360–2,373) linked to an NH₂-terminal cysteine for convenient linkage to the affinity matrix; PiChem, Graz, Austria), affinity purified as described (Tögel et al., 1994) and used at a concentration of 5 µg/ml on immunoblots. The mouse anti-light chain MAP1B mAb E-12 (Kuznetsov et al., 1986; Sigma Chemical Co., St. Louis, MO), the mouse anti-heavy chain MAP1B mAb AA-6 (Boehringer Mannheim GmbH, Mannheim, Germany) and the rat anti-HA mAb 3F10 (Boehringer Mannheim GmbH) were used at dilutions of 1:1,000, 1:500 and 1:1,000, respectively. The affinity-purified polyclonal rabbit anti-myc antibody (Tögel et al., 1998) was used at a concentration of 5 µg/ml on immunoblots and 1 µg/ml for immunofluorescence microscopy. Intracellular microtubules were decorated with a mixture of

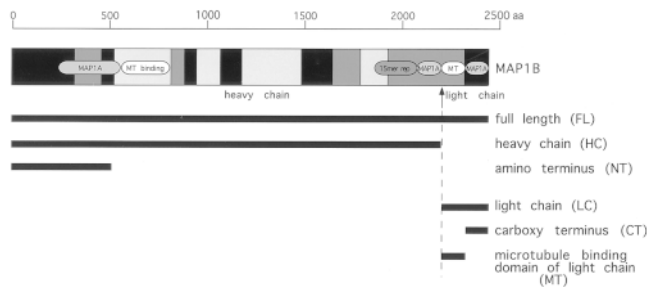


Figure 1. Schematic representation of MAP1B domains and cDNA constructs. Full-length rat MAP1B is depicted as a box with shading ranging from black to grey in distinct areas indicating the degree of conservation of the amino acid sequence between human, rat and mouse in these areas. Black indicates the highest degree of conservation, overall conservation is 90%. The positions of the 12 15mer repeats (*15mer rep*; Noble et al., 1989), the three major areas of amino acid sequence homology to MAP1A (*MAP1A*; Langkopf et al., 1992), and the microtubule binding domains of the heavy chain (*MT binding*; Noble et al., 1989) and the light chain (*MT*; Zauner et al., 1992) are indicated as is the site of cleavage of the polyprotein precursor into heavy and light chain (*arrow*). The line on top indicates amino acid (*aa*) residue positions according to rat full-length MAP1B polyprotein precursor sequence (Zauner et al., 1992; Liu and Fischer, 1996). The bottom part depicts the MAP1B proteins encoded by the cDNA constructs used in this study: the full-length MAP1B polyprotein precursor (*FL*; amino acids 1–2,459), heavy chain (*HC*; amino acids 1–2,185), NH₂ terminus (*NT*; amino acids 1–508), light chain (*LC*; amino acids 2,210–2,459), COOH terminus (*CT*; amino acids 2,336–2,459), and the microtubule binding domain of the light chain (*MT*; amino acids 2,210–2,336). In all cases where the above proteins were epitope tagged by either the myc- or the HA-tag, the tags were attached to the COOH termini of the proteins. The abbreviations given in parentheses correspond to those used in labeling of the subsequent figures.

mouse anti-tubulin mAbs (B-5-1-2 [Sigma Chemical Co.] at 1:1,000, TU-01 [Bioscience Resource Project, Kennebunk, ME] at 1:50, and anti- β -tubulin [Amersham Pharmacia Biotech, Uppsala, Sweden] at 1:25). For the detection of stress fibers the mouse anti- β -actin mAb AC-15 (Sigma Chemical Co.) was used at a dilution of 1:400. As secondary antibodies alkaline phosphatase-conjugated goat anti-rabbit, anti-mouse (both Promega, Heidelberg, Germany, dilution 1:7,500), or anti-rat antibodies (dilution 1:5,000, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) were used in immunoblots. Donkey anti-rabbit, anti-mouse, or anti-rat antibodies (Jackson ImmunoResearch Laboratories, Inc.) preadsorbed against serum proteins from other species (in particular those from which the other primary antibodies used in the same double or triple immunofluorescence experiment originated) labeled with Texas red, FITC or AMCA were used at a dilution of 1:100.

Cell Culture, Transfection, and Analysis

PC12 cells were cultured as described (Greene and Tischler, 1976). NIH3T3 and PtK2 cells were grown at 37°C in an atmosphere containing 7.5% CO₂ in high glucose DME supplemented with 10% FCS in the absence of tetracycline. 18–20 h before transfection cells were seeded onto coverslips or plates at densities of 45% (NIH3T3) and 66% (PtK2). Transient transfection was carried out using Lipofectamin (GIBCO BRL) according to the manufacturer's protocol. The ratio of expression plasmid to transactivator plasmid was 10:1. The transfection mixture was replaced with fresh growth medium after 5 h and the cells were incubated for an additional 24 h (NIH3T3) or 48 h (PtK2) at 37°C.

For immunofluorescence microscopy PtK2 cells were washed in PBS, fixed in methanol (–20°C, 10 min), equilibrated in PBS, blocked for 30

min with 3% BSA, incubated with the primary antibodies in 1% BSA for 1 h, washed extensively in PBS, incubated for 1 h with the secondary antibody and again washed with PBS. Alternatively, cells were fixed with 4% paraformaldehyde and 0.2% glutaraldehyde in a buffer containing 5 mM EGTA, 2 mM MgCl₂, and 0.2 M Hepes, pH 7.2. Cells were washed twice with PBS, incubated for 5 min in 0.1% Triton X-100 in PBS, 3 times for 5 min in 0.5 mg/ml NaBH₄ in PBS and 4 times in PBS. Specimens were analyzed by confocal microscopy (MRC600; Bio-Rad Laboratories, Hercules, CA) or for triple staining with a cooled CCD-Camera (IP-Lab).

For immunoblot analysis (Sambrook et al., 1989), cells were washed with PBS, lysed in buffer A, containing 8 M urea, 4% SDS, 0.125 M Tris-HCl, pH 6.8, 12 mM EDTA, 0.3% DTT, 10 μ M benzamide, 1 mM PMSF, 2 μ M pepstatin, 2 μ M aprotinin, 2 μ M leupeptin, and 0.002% bromophenol blue, sonicated, incubated at 65°C for 10 min and subjected to SDS-PAGE.

For immunoprecipitation experiments all steps starting with the cell lysis were carried out on ice or at 4°C.

Cell Lysis Protocol A. After washing with PBS the cells were lysed on the plates by incubation for 10 min with lysis buffer B containing 0.5% Triton X-100, 100 mM NaCl, 100 mM Tris-HCl, pH 7.5, 10 mM EDTA, 0.1 mM DTT, protease inhibitors as described above, and nucleases (0.5 mg/ml DNase [from bovine pancreas, grade II; Boehringer Mannheim GmbH, Mannheim, Germany] and 0.2 mg/ml RNase [from bovine pancreas; Boehringer Mannheim]). The cell lysates were centrifuged for 10 min at 14,000 rpm and the resulting supernatants were transferred to fresh tubes for further processing.

Cell Lysis Protocol B. The cells were collected by trypsinization and lysed by Dounce homogenization in lysis buffer C containing PBS, 10 mM EDTA, 0.1 mM DTT, and protease inhibitors and nucleases as above. The cell lysates were centrifuged for 10 min at 14,000 rpm and the supernatants were transferred to fresh tubes and supplemented with 1/100 vol of 2.5% Tween 20. The resulting supernatants of either cell lysis protocol were precleared by incubation with 1/10 vol of protein A-Sepharose overnight. After centrifugation for 30 s at 900 rpm in a microcentrifuge the supernatant was transferred into a fresh tube and incubated for 3–5 h with 1/10 vol of protein A-Sepharose containing covalently linked (Harlow and Lane, 1988) affinity-purified anti-myc antibody. The Sepharose-coupled antibody was collected by centrifugation for 30 s at 900 rpm and was washed twice with a buffer corresponding to the buffer in which the immunoprecipitation was performed. After two additional washes with PBS the Sepharose-coupled antibody was collected by centrifugation as above and incubated for 5 min at 95°C in an equivalent volume of sample buffer containing 0.125 M Tris-HCl, pH 6.8, 2 mM EDTA, 4% SDS, and 20% glycerol. After centrifugation the supernatant was supplemented with DTT to a final concentration of 10 mM and incubated for 10 min at 65°C. Samples were analyzed by SDS-PAGE and immunoblotting as described (Sambrook et al., 1989).

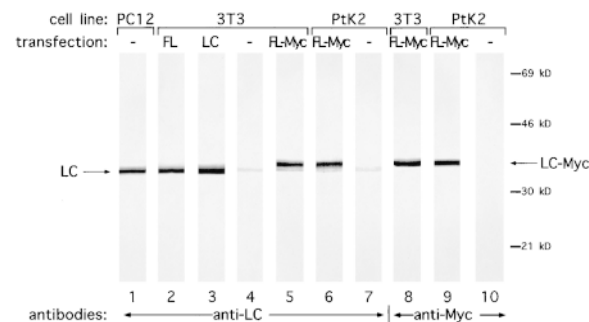


Figure 2. Proteolytic processing of MAP1B ectopically expressed in nonneuronal cells. Total lysates of NIH3T3 (3T3) and PtK2 cells transfected with the indicated constructs and a lysate of non-transfected PC12 cells were fractionated by SDS-PAGE on a 10% gel and analyzed by immunoblotting using antibodies to the light chain (*anti-LC*) or the myc-tag (*anti-Myc*). *FL*, untagged full-length MAP1B; *LC*, untagged light chain; *FL-Myc*, myc-tagged full-length MAP1B; *LC-Myc*, myc-tagged light chain. Molecular mass markers are indicated on the right.

Results

Correct Proteolytic Processing of Ectopically Expressed MAP1B in Nonneuroal Cells

To identify domains of MAP1B that might modify the properties of cellular microtubules or might interact with other cytoskeletal components we chose to express full-length MAP1B, the separate heavy and light chains, and potential subdomains (Fig. 1) in PtK2 and NIH3T3 cells. These cells express little or no endogenous MAP1B and thus permit the investigation of MAP1B domains without the interference of endogenous MAP1B heavy and light chains. To verify that MAP1B is processed correctly into heavy and light chain in these nonneuroal cells we expressed myc-tagged and untagged full-length rat MAP1B in PtK2 and NIH3T3 cells and analyzed the electrophoretic mobility of the resulting light chains on immunoblots (Fig. 2). In NIH3T3 cells the ectopic expression of untagged full-length MAP1B led to the generation of a protein reactive with the light chain-specific antibody that was indistinguishable in its electrophoretic mobility from the endogenous light chain expressed in PC12 cells (Fig. 2, lanes 1 and 2). Expression of myc-tagged full-length MAP1B in NIH3T3 or PtK2 cells yielded a slightly larger

protein that was detected with antibodies to the light chain or the myc-tag (Fig. 2, lanes 5–10). This slight increase in size was expected from the addition of the 14-amino acid long myc-tag. On separate gels we analyzed generation of the heavy chain in these transfected cells using a heavy chain specific antibody (not shown). From these experiments we estimated that at least 50 and 90% of the ectopically expressed full-length MAP1B was processed in PtK2 and NIH3T3 cells, respectively.

Since the exact NH₂ terminus of the light chain is not known, we initially expressed three candidate constructs in NIH3T3 cells, encoding amino acids 2,185–2,459, 2,210–2,459, or 2,222–2,459 (numbers referring to the amino acid position in the full-length polyprotein precursor). The construct encoding untagged amino acids 2,210–2,459 gave rise to a protein that reacted with the light chain-specific mAb E-12 (Kuznetsov et al., 1986) and was indistinguishable in size from the endogenous light chain expressed in PC12 cells (Fig. 2, lanes 1 and 3). In addition, the protein encoded by this construct corresponds in its NH₂ terminus to the NH₂ terminus of the MAP1B light chain as predicted by amino acid sequence data (Hammarback et al., 1991). Therefore, this protein comprising amino acids 2,210–2,459 is henceforth referred to as the light chain of MAP1B in this report. The construct encoding amino ac-

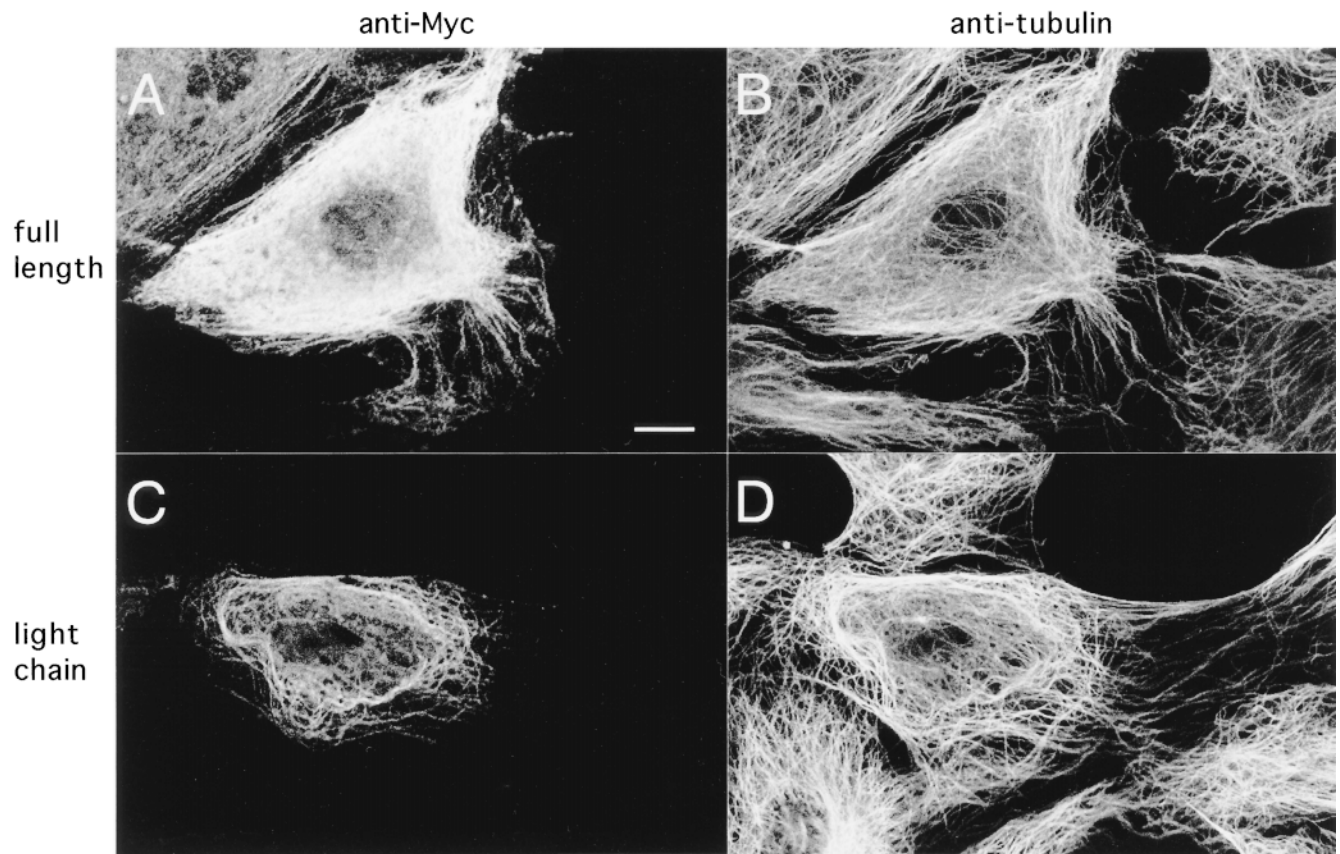


Figure 3. The MAP1B light chain binds to microtubules in vivo and induces changes in the microtubule network. PtK2 cells transfected with myc-tagged full-length MAP1B (A and B) or myc-tagged light chain (C and D) were analyzed by double immunofluorescence microscopy using antibodies to the myc-tag (A and C) and tubulin (B and D). The cellular arrangement of microtubules remained unchanged in cells expressing full-length MAP1B. Cells expressing light chain only showed binding of the light chain to microtubules and displayed wavy microtubules concentrated in areas about halfway between the nucleus and the periphery. Bar, 10 μ m.

ids 2,185–2,459 gave rise to a protein larger than the endogenous light chain of PC12 cells, and the construct encoding amino acids 2,222–2,459 gave rise to a protein that did not react with the light chain-specific mAb E-12 (data not shown), indicating that the E-12 epitope is localized at the NH₂ terminus of the light chain between amino acids 2,210 and 2,222. The faint band detected with anti-light chain antibody in untransfected NIH3T3 and PtK2 cells (Fig. 2, lanes 4 and 7) may have represented endogenous light chain or a related protein expressed in these cells at low levels (Hammarback et al., 1991; Domínguez et al., 1994).

The Light Chain of MAP1B Has Microtubule Stabilizing Activity

It has previously been shown by *in vitro* cosedimentation assays that one of the two microtubule binding domains of MAP1B is located between amino acids 2,210 and 2,331 (Zauner et al., 1992). This region of the full-length polypeptide precursor constitutes the NH₂-terminal half of the light chain. Expression of the light chain in PtK2 cells revealed that it can bind to microtubules *in vivo* and that it can induce a change in cellular microtubule arrangement (Fig. 3, C and D). Many microtubules assumed a wavy ap-

pearance with a relatively high density of closely spaced wavy microtubules about halfway between the nucleus and the periphery. This altered microtubule arrangement was characteristic for the MAP1B light chain and has not been observed with other MAPs such as MAP1A (Cravchik et al., 1994), MAP2 (Weisshaar et al., 1992), or MAP4 (Olson et al., 1995; Nguyen et al., 1997). Full-length MAP1B consisting of heavy and light chain did not induce this change in microtubule appearance (Fig. 3, A and B). Full-length MAP1B displayed diffuse and fibrillar localization, the latter colocalizing with microtubules, consistent with published observations (Noble et al., 1989).

We next analyzed whether the MAP1B light chain can induce microtubule stabilization by conducting experiments with nocodazole. Nocodazole prevents the *de novo* polymerization of tubulin heterodimers and thus leads to depolymerization of microtubules owing to their dynamic instability (Hoebeke et al., 1976). In nontransfected cells and in cells transfected with full-length MAP1B comprising heavy and light chain nocodazole treatment at 10 μg/ml for 1 h before fixation caused the depolymerization of most microtubules and led to a diffuse cytoplasmic tubulin staining with the occasional detection of remaining microtubules (Fig. 4, A and B). Only ~5% of cells displayed stable microtubules. In contrast, when the light chain on its

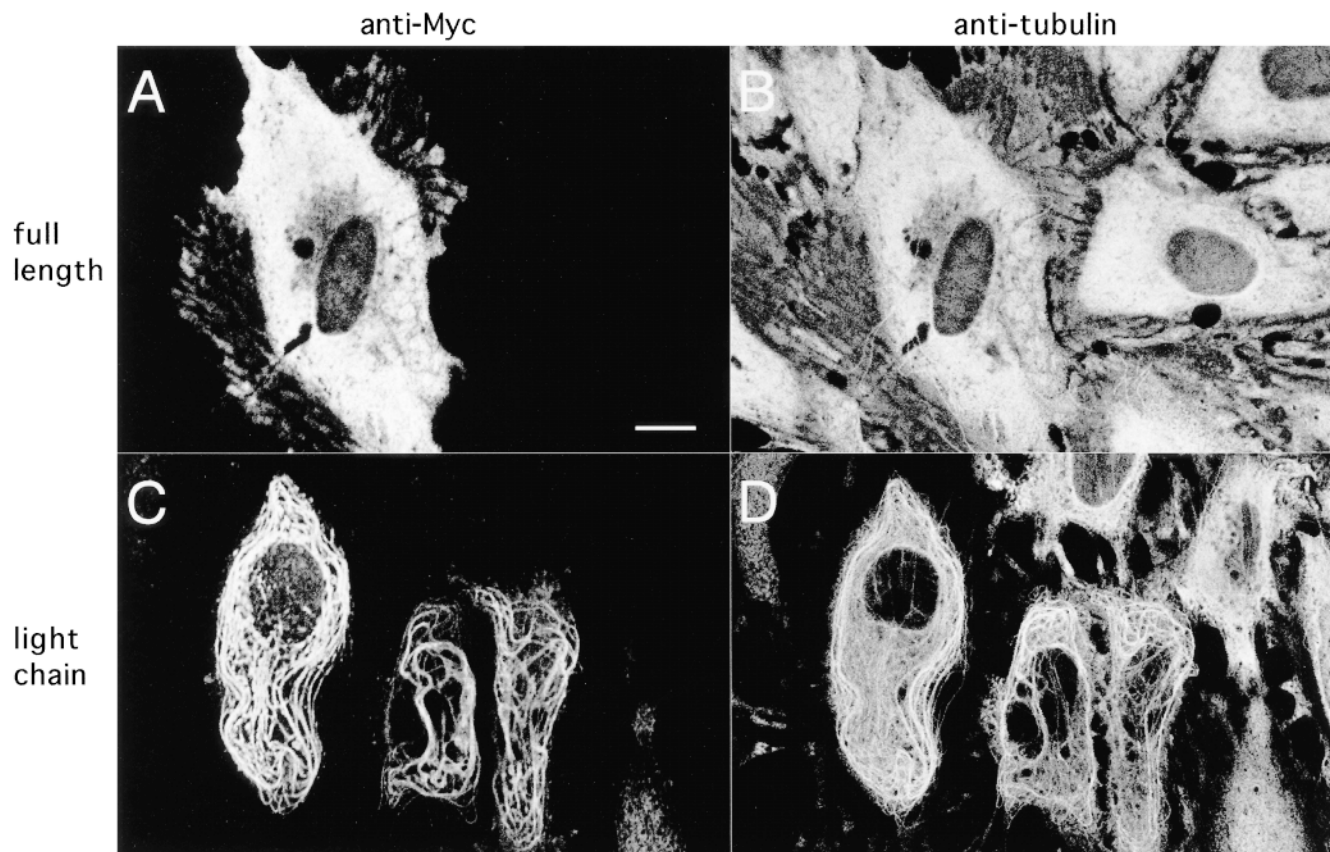


Figure 4. The MAP1B light chain protects microtubules against the effects of nocodazole. PtK2 cells transfected with myc-tagged full-length MAP1B (A and B) or the myc-tagged MAP1B light chain (C and D) were treated with nocodazole (1 h, 10 μg/ml) and then analyzed by double immunofluorescence microscopy using antibodies to the myc-tag (A and C) and tubulin (B and D). Microtubules were depolymerized in nontransfected cells and in cells expressing full-length MAP1B. In cells expressing the light chain, microtubules with the characteristic light chain-induced appearance were present despite treatment with nocodazole. Bar, 10 μm.

own was ectopically expressed, stabilization of microtubules against the effects of nocodazole was observed in about 60% of the cells (Fig. 4, *C* and *D*). In addition to stabilization, the light chain induced the characteristic changes of the microtubule network with many microtubules assuming a wavy, sometimes looped appearance. These results show that the light chain is capable of inducing microtubule stabilization and that this light chain activity is inhibited in the presence of the heavy chain.

We also tested the effect of taxol in cells expressing MAP1B light chain. Taxol reduces the critical concentration of tubulin polymerization and induces de novo polymerization of centrosome-independent microtubules (de Brabander et al., 1981). Treatment of cells with 5 μ M taxol for 7 h before fixation induced multiple centrosome-independent microtubule bundles at the periphery of nontransfected cells, and expression of full-length MAP1B did not prevent this effect of taxol (Fig. 5, *A* and *B*). In contrast, in cells expressing the light chain only taxol treatment failed to induce formation of centrosome-independent, peripheral microtubule bundles. Instead, microtubules again displayed the characteristic light chain-induced wavy and looped appearance (Fig. 5, *C* and *D*).

Thus, the results of both, nocodazole or taxol treatment of cells indicated that the MAP1B light chain can inhibit microtubule depolymerization.

The MAP1B Light Chain Binds to the Heavy Chain and Can Oligomerize via Its COOH-terminal MAP1A Homology Domain

The results presented above demonstrated that the MAP1B light chain had distinct effects on the morphological appearance and stability of microtubule networks as long as it was expressed in the absence of the heavy chain. Coexpression of heavy and light chains by transfecting cells with a construct encoding the full-length polyprotein precursor that is cleaved to give rise to heavy and light chains had little or no effect on microtubules, suggesting that the heavy chain might inhibit light chain activity. Similar results were obtained when heavy and light chain were coexpressed in the same cells from two separate constructs, one encoding a myc-tagged heavy chain and the other encoding an HA-tagged light chain. Cells coexpressing both proteins displayed both diffuse and fibrillar localization of heavy and light chain (Fig. 6, *A* and *B*), reminiscent of

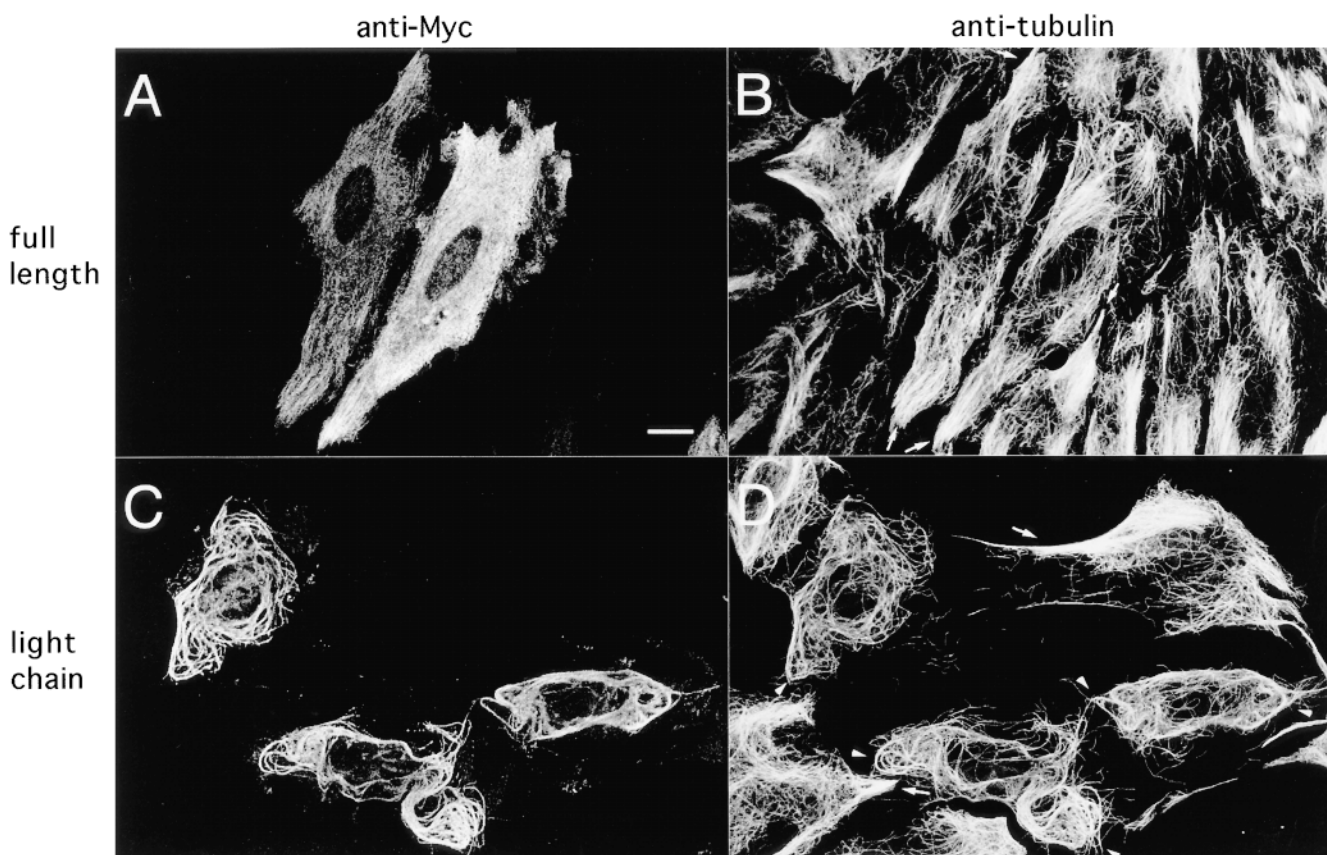


Figure 5. The MAP1B light chain protects microtubules against the effects of taxol. PtK2 cells transfected with myc-tagged full-length MAP1B (*A* and *B*) or the myc-tagged MAP1B light chain (*C* and *D*) were treated with taxol (7 h, 5 μ M) and then analyzed by double immunofluorescence microscopy using antibodies to the myc-tag (*A* and *C*) and tubulin (*B* and *D*). Microtubules formed short, centrosome-independent bundles in nontransfected cells and in cells expressing full-length MAP1B (*B* and *D*, arrows). As was observed in the absence of taxol (Fig. 3 *A*) full-length MAP1B displayed diffuse cytoplasmic localization in addition to colocalization with taxol-induced microtubule bundles indicative of its weak association with microtubules. In cells expressing the light chain microtubules displayed the characteristic light chain-induced appearance despite treatment with taxol (*D*, arrowheads). Bar, 10 μ m.

what had been observed in cells expressing heavy and light chains generated by precursor cleavage (Fig. 3 *A*). Triple immunofluorescence analysis revealed that the intracellular fibers decorated by anti-myc and anti-HA antibodies were microtubules (Fig. 6 *C*). Thus, heavy and light chain colocalized with each other and with microtubules. When the heavy chain was expressed in the absence of the light chain, only diffuse staining was observed (Fig. 6 *D*), indicating that the light chain is necessary for efficient microtubule binding of the heavy chain–light chain complex. Furthermore, no significant changes of microtubule appearance or stability were observed in cells expressing the heavy chain only (not shown). In populations of cells coexpressing heavy and light chain from separate constructs (cotransfection at a molar ratio of 2:1) the number of cells displaying changes in microtubule arrangement or stability against nocodazole or taxol was reduced to 40% or less compared with cells expressing the light chain only. These results provided further evidence that the heavy chain has

little or no effect on its own but can inhibit the effect of the light chain on microtubule appearance and stability.

An analogous cotransfection experiment was carried out using a construct encoding an NH₂-terminal fragment of the heavy chain instead of the entire heavy chain. This fragment contained the NH₂-terminal MAP1A homology domain of the MAP1B heavy chain but lacked its microtubule binding domain (Fig. 1). As with the entire heavy chain, we observed that the NH₂ terminus, when expressed on its own, showed diffuse cytoplasmic distribution (Fig. 7 *D*) and had no effect on microtubules (not shown). In contrast, when the NH₂ terminus was coexpressed with the light chain, NH₂ terminus (Fig. 7 *A*) and light chain (Fig. 7 *B*) colocalized on microtubules (Fig. 7 *C*). In addition, both proteins displayed diffuse cytoplasmic distribution (Fig. 7, *A* and *B*) but apparently to a lesser extent than when the entire heavy chain and the light chain were coexpressed (compare Fig. 7, *A* and *B* with Fig. 6, *A* and *B*). In populations of cells coexpressing

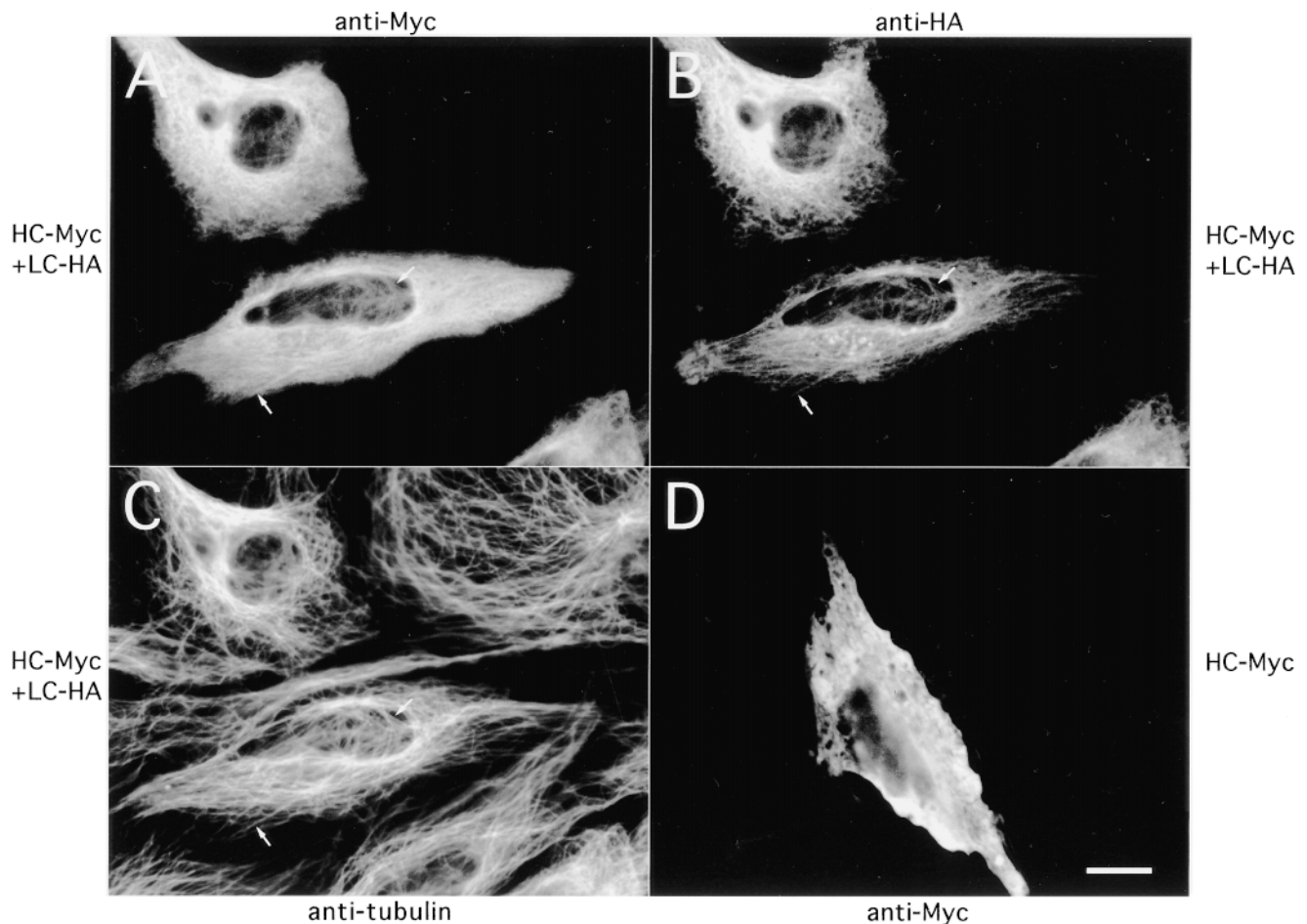


Figure 6. The light chain mediates microtubule binding of the MAP1B heavy chain–light chain complex. PtK2 cells cotransfected with cDNA constructs encoding the myc-tagged heavy chain (HC-Myc) and the HA-tagged light chain (LC-HA; *A–C*) at a molar ratio of 0.8:1, or transfected with a single construct encoding the myc-tagged heavy chain (*D*) were analyzed by triple immunofluorescence microscopy using antibodies to the myc-tag (*A* and *D*), the HA-tag (*B*), and tubulin (*C*). In cells expressing only the heavy chain, the protein showed diffuse cytoplasmic distribution (*D*). In contrast, in cells coexpressing heavy and light chain, both proteins displayed not only diffuse cytoplasmic distribution but also fibrillar staining that colocalized with microtubules (*A–C*, arrows). This pattern of intracellular distribution reflects the weak microtubule association of the MAP1B heavy chain–light chain complex. Bar, 10 μ m.

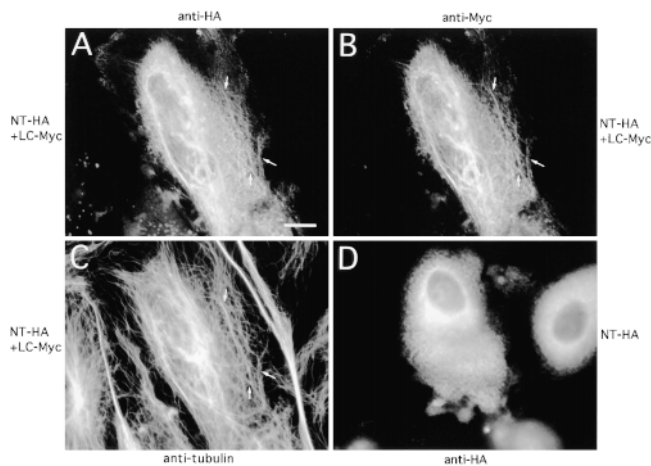


Figure 7. The light chain binds in vivo to the NH₂-terminus of the heavy chain. PtK2 cells cotransfected with constructs encoding the HA-tagged NH₂ terminus of the heavy chain (*NT-HA*) and the myc-tagged light chain (*NT-HA + LC-Myc*; *A–C*) at a molar ratio of 2:1, or transfected with a single construct encoding the HA-tagged NH₂ terminus (*D*) were analyzed by triple immunofluorescence microscopy using antibodies to the HA-tag (*A* and *D*), the myc-tag (*B*), and tubulin (*C*). In cells expressing only the NH₂ terminus of the heavy chain, the protein showed diffuse cytoplasmic distribution (*D*). In cells coexpressing NH₂ terminus and light chain both proteins displayed fibrillar staining that colocalized with microtubules (*A–C*, arrows) in addition to diffuse cytoplasmic distribution. Bar, 10 μm.

NH₂ terminus and light chain (cotransfection at a molar ratio of 4:1) the number of cells displaying stabilization of microtubules against nocodazole was reduced to 66% compared with cells expressing the light chain alone. Taken together these results indicated that the light chain can form a complex with the NH₂-terminal 508 amino acids of the heavy chain and that this complex can bind to microtubules via the microtubule binding domain of the light chain. Binding of the NH₂ terminus led to a reduction of the light chains activity to induce changes in microtubule appearance and stability but did not appear to block its binding to microtubules.

To confirm heavy chain–light chain interactions observed by immunofluorescence microscopy and to more precisely define the interacting domains we performed coimmunoprecipitation experiments using lysates of NIH3T3 cells cotransfected with constructs encoding the myc-tagged NH₂ terminus of the heavy chain and the HA-tagged COOH terminus of the light chain. The latter comprises the most COOH-terminal MAP1A homology domain of MAP1B (Fig. 1). Lysates of transfected cells were subjected to immunoprecipitation using anti-myc antibodies and the precipitates were analyzed by immunoblotting for the presence of NH₂ terminus and COOH terminus using antibodies directed against the myc- and the HA-tag, respectively. Precipitation of the NH₂-terminal fragment with anti-myc antibodies (Fig. 8, lane 1) led to the coprecipitation of the COOH terminus of the light chain that was detected on immunoblots using anti-HA antibodies (Fig. 8, lane 3). From lysates of control cells ex-

pressing only the COOH terminus of the light chain (Fig. 8, lane 4) this fragment could not be precipitated with the anti-myc antibody (Fig. 8, lane 5) demonstrating the specificity of precipitation. Equivalent results were obtained when the experiment was performed with HA- instead of myc-tagged NH₂ terminus and myc- instead of HA-tagged COOH terminus. Thus, we have narrowed down the location of MAP1B heavy chain–light chain interaction domains to the NH₂-terminal 508 amino acids of the heavy chain and the COOH-terminal 125 amino acids of the light chain.

We also investigated whether individual light chain molecules can directly or indirectly associate with each other, a feature that could have important implications for their mechanism of action. NIH3T3 fibroblasts were cotransfected with two constructs both encoding the COOH terminus of the light chain either in its HA-tagged or in its myc-tagged (Fig. 9). After transient expression of the constructs the cells were lysed and the lysates were subjected to immunoprecipitation using the anti-myc antibody. The precipitates were analyzed on immunoblots using either the anti-myc antibody (Fig. 9, lane 1) or the anti-HA antibody (Fig. 9, lane 2). This analysis showed that both proteins were expressed and that precipitation of the myc-tagged COOH terminus with anti-myc antibodies led to coprecipitation of the HA-tagged COOH terminus. For controls, cells were cotransfected with constructs encoding the HA-tagged COOH terminus and the myc-tagged microtubule binding domain of the light chain. Immunoprecipitation with the anti-myc antibody led to the precipitation of the myc-tagged microtubule binding domain (Fig. 9, lane 4) but not the HA-tagged COOH terminus (Fig. 9, lane 5) despite the presence of this protein in the extracts (Fig. 9, lane 3). This demonstrated the specificity of coprecipitation as the anti-myc antibody precipitated the HA-

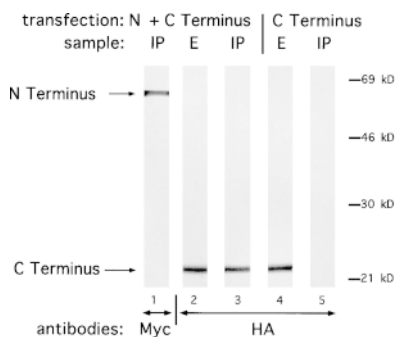


Figure 8. Domains responsible for the mutual interaction of MAP1B heavy and light chains. NIH3T3 cells were cotransfected with constructs encoding the myc-tagged NH₂ terminus (*N Terminus*) and the HA-tagged COOH terminus (*C Terminus*; lanes 1–3), or were transfected with a single construct encoding the HA-tagged COOH terminus (lanes 4 and 5) as indicated. Lysates of transfected cells were prepared by protocol A (see Materials and Methods) and analyzed by immunoblotting, either directly (*E*) or after immunoprecipitation (*IP*) using the anti-myc antibody. The blot was probed with antibodies directed against the HA-tag (*HA*) or the myc-tag (*Myc*) as indicated. Positions of the NH₂ and COOH terminus are indicated. Molecular mass markers are shown on the right.

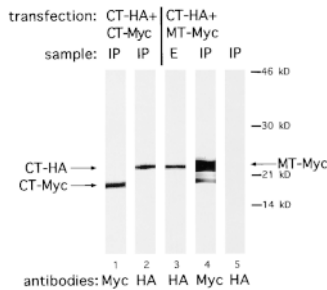


Figure 9. MAP1B light chains can interact through a domain in the COOH terminus. NIH3T3 cells were cotransfected with constructs encoding the COOH terminus in its HA-tagged (*CT-HA*) or myc-tagged (*CT-Myc*) form (lanes 1 and 2) or were cotransfected with constructs encoding the HA-tagged COOH terminus and

the myc-tagged microtubule binding domain (*MT-Myc*) of the light chain (lanes 3–5). Lysates of transfected cells were prepared by protocol B (see Materials and Methods) and analyzed on an immunoblot either directly (*E*) or following immunoprecipitation (*IP*) using the anti-myc antibody. The blot was probed with antibodies directed against the HA-tag (*HA*) or the myc-tag (*Myc*) as indicated. The positions of the COOH terminus and the microtubule binding domain on the blot are indicated. Molecular mass markers are shown on the right.

tagged COOH terminus only when cells had been cotransfected with myc-tagged COOH terminus (Fig. 9, lane 2) but not with other myc-tagged proteins (Fig. 9, lane 5). At the same time it showed the specificity of the interaction of the COOH terminus with itself but not with other coexpressed proteins. Similar results were obtained when coprecipitation experiments were performed using cells coexpressing the entire untagged light chain and the myc-tagged COOH terminus, ruling out that light chain oligomerization is artificially induced by truncation or epitope tagging of the protein (not shown). Moreover, we observed colocalization of the untagged light chain and the myc-tagged COOH terminus by immunofluorescence microscopy (not shown) providing independent evidence for light chain oligomerization. Thus, our experiments demonstrated that light chain molecules can form complexes with each other by specific interaction through a domain located in their COOH-terminal 125 amino acids.

Binding of the MAP1B Light Chain COOH Terminus to Actin Stress Fibers

Amino acid sequence comparison of MAP1B and MAP1A revealed two remarkable features about the COOH terminus of the MAP1B light chain. First, this region of MAP1B is among those highest conserved between human, mouse and rat (Fig. 1) and secondly, this region comprises one of the three longer stretches of homology between MAP1B and MAP1A (Fig. 1; Langkopf et al., 1992). To investigate whether this part of the light chain contains functional domains in addition to those identified above we transiently expressed the COOH terminus in PtK2 cells and analyzed its intracellular localization by immunofluorescence microscopy (Fig. 10). In addition to diffuse intracellular distribution we observed colocalization of the myc-tagged COOH terminus with actin stress fibers (Fig. 10, *A* and *B*). Decoration of the stress fibers by the anti-myc antibody occurred in a punctate manner, regardless of whether the cells had been fixed with methanol or paraformaldehyde before incubation with the antibody. Anti-myc staining

was not detected at the most peripheral segments of actin stress fibers indicating that binding of the COOH terminus was reduced or absent in these regions.

Since the COOH terminus of the light chain harbors the domain for the binding of the light chain to the NH₂ terminus of the heavy chain (Fig. 8) we examined whether binding of the COOH terminus to the heavy chain or to stress fibers interfere with each other. PtK2 cells were cotransfected with constructs encoding the HA-tagged NH₂ terminus of the heavy chain and the myc-tagged COOH terminus of the light chain and analyzed by triple immunofluorescence microscopy using anti-HA and anti-myc antibodies (Fig. 10, *C* and *D*), and anti-actin antibodies (not shown). The COOH terminus again displayed punctate localization on stress fibers in addition to diffuse cytoplasmic distribution. Remarkably, the NH₂ terminus of the heavy chain, which when expressed on its own displayed diffuse cytoplasmic distribution (Fig. 7 *D*), was found to colocalize with the COOH terminus on stress fibers. This observation confirmed our results obtained by coimmunoprecipitation analysis (Fig. 8) and showed that the COOH terminus of the light chain can bind to the NH₂ terminus of the heavy chain *in vivo*. Furthermore, these results demonstrated that binding of the COOH terminus to the heavy chain and to stress fibers were not mutually exclusive and that the COOH terminus can target the otherwise diffusely distributed MAP1B NH₂ terminus to stress fibers.

Discussion

Previous studies on the effects of MAP1B on microtubule organization and function have used the MAP1B protein complex consisting of heavy and light chains. In these studies the observed effects of MAP1B on microtubule stabilization were weak when compared with those of MAP2 (Lewis et al., 1989; Noble et al., 1989; Takemura et al., 1992; Weisshaar et al., 1992; LeClerk et al., 1993; Weisshaar and Matus, 1993; Pedrotti and Islam, 1995) and assay systems for a molecular analysis of MAP1B function based on induction of dramatic phenotypic changes in cellular microtubule networks have not been described previously. In the present study we attempted to identify functional domains and possible activities of the MAP1B light chain without the interference by the heavy chain. For this purpose we chose to use as the experimental system cultured nonneuronal cells that have a low background of endogenous MAP1B. This enabled us to study effects of the light chain when it is not complexed by the heavy chain. In this system we were able to show for the first time that the light chain of MAP1B indeed has function(s) on its own. It bound to microtubules *in vivo* and effectively stabilized microtubules in a manner distinct from other microtubule-stabilizing proteins or drugs. We have also demonstrated that light chains can oligomerize and have narrowed down the regions of MAP1B necessary for light chain–heavy chain interaction. In addition, we have identified a novel functional domain of the MAP1B light chain that mediates binding of the COOH terminus of the light chain to actin stress fibers. Our results show that many activities of MAP1B previously observed in *in vivo* and *in vitro* assays using the entire MAP1B protein complex can be attrib-

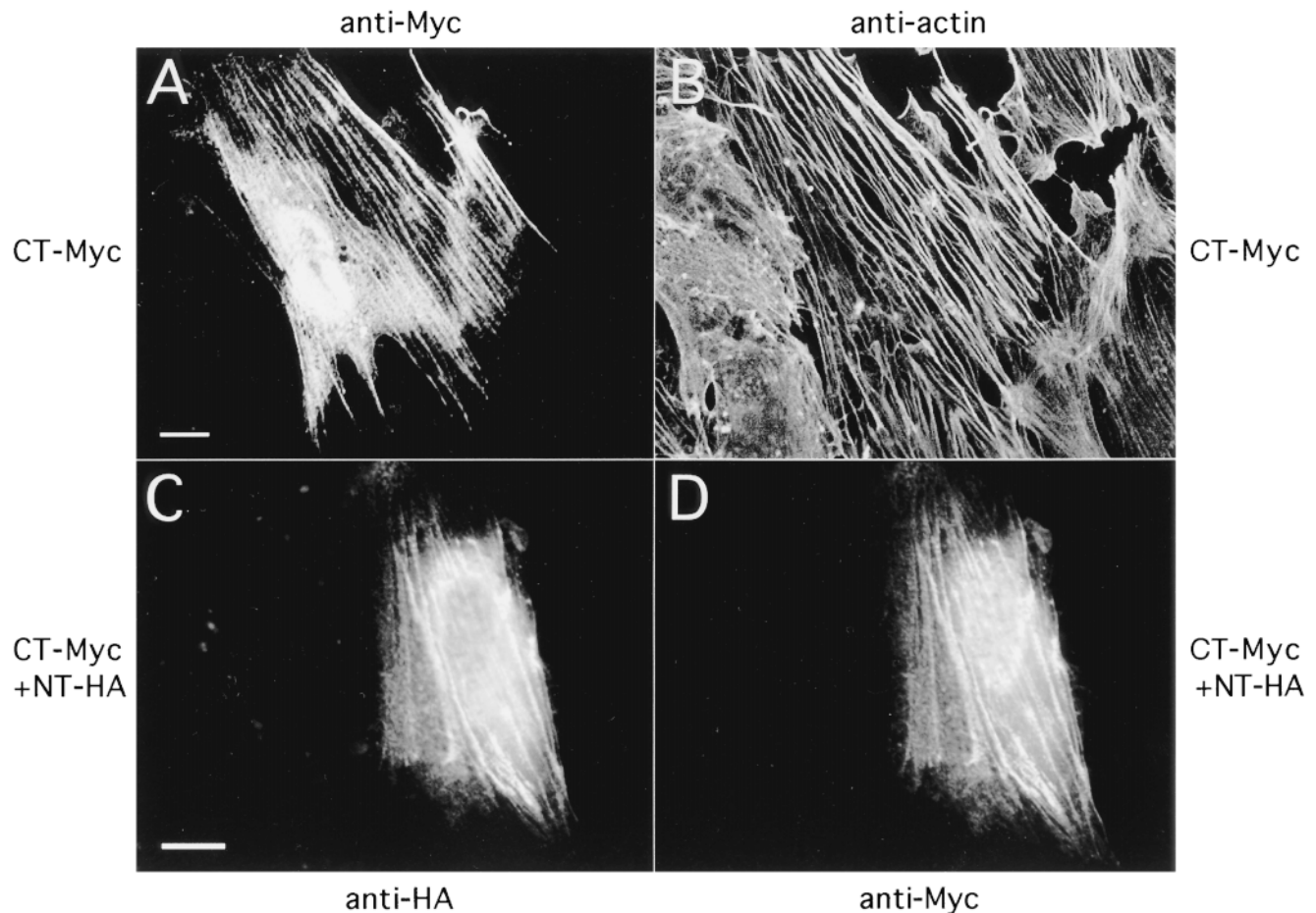


Figure 10. Interaction of the MAP1B light chain COOH terminus with actin stress fibers. PtK2 cells were transfected with a construct encoding the myc-tagged COOH terminus of the light chain (*CT-Myc*; *A* and *B*) and were analyzed by double immunofluorescence microscopy using antibodies to the myc-tag (*A*) and actin (*B*). In a separate experiment cells were cotransfected with constructs encoding the myc-tagged COOH terminus of the light chain and the HA-tagged NH₂ terminus of the heavy chain (*CT-Myc + NT-HA*; *C* and *D*) and were analyzed by triple immunofluorescence microscopy using antibodies to the HA-tag (*C*), the myc-tag (*D*), and actin (not shown). The COOH terminus when expressed on its own or in the presence of the NH₂ terminus was found to localize on stress fibers in a punctate manner (*A* and *D*). In cells coexpressing NH₂ terminus and the COOH terminus both proteins displayed stress fiber localization (*C* and *D*) in addition to diffuse cytoplasmic distribution. Bar, 10 μ m.

uted to the light chain and are unmasked and enhanced by expressing the light chain in the absence of the heavy chain. Apart from the microtubule binding domain, all light chain activities identified here were found to be localized in the COOH terminus that is among those regions of MAP1B most highly conserved in evolution. This region of \sim 120 amino acid residues is also found in the COOH terminus of the light chain of the related protein MAP1A (Langkopf et al., 1992). On the other hand, the NH₂-terminal half of the MAP1B light chain that contains the microtubule binding domain (Zauner et al., 1992) has no corresponding counterpart in the light chain of MAP1A. These results suggest that the light chains of MAP1B and MAP1A might have overlapping but distinct sets of activities.

Expression of the MAP1B light chain induced a unique change in the appearance of microtubules that had not been observed previously with any other MAP including MAP2 (Weisshaar et al., 1992), MAP4 (Olson et al., 1995;

Nguyen et al., 1997), and MAP1A (Cravchik et al., 1994). This distinctness of MAP1B light chain effects is reflected by the primary structure of its microtubule binding domain, a basic region without pronounced repetitive motifs, lacking sequence homology to the microtubule binding domains of other MAPs (Zauner et al., 1992). Microtubules in light chain-expressing cells were stabilized against the effects of nocodazole (Fig. 4), but did not form the straight peripheral bundles seen in the presence of MAP2, indicating that MAP1B does not have microtubule nucleating activity. This interpretation is consistent with results obtained in *in vitro* assays that demonstrated that MAP1B has a weaker nucleating activity than MAP2 (Pedrotti et al., 1996a). Instead of forming peripheral bundles, many microtubules in MAP1B light chain-expressing cells displayed a wavy appearance, perhaps due to excessive length. This observation suggests that microtubules were stabilized but retained considerable flexibility. The wavy appearance of microtubules in the presence of the MAP1B

light chain is of particular interest for two reasons. First, it has been demonstrated that MAP1B induces the formation of long, “bendy” microtubules *in vitro* and that this property of MAP1B distinguishes it from MAP1A and MAP2 (Pedrotti et al., 1996a). Our results obtained *in vivo* in PtK2 cells are consistent with these findings. More importantly, bent microtubules and microtubule loops have been detected in growth cones of extending neurites *in vivo* (Tsui et al., 1984; Sabry et al., 1991; Tanaka and Kirschner, 1991; Challacombe et al., 1996) where they are believed to be instrumental in growth cone migration and guidance. The detection of phosphorylated MAP1B at high concentrations in distal axons and growth cones has led to the suggestion that growth cones might be an important site of action of MAP1B (Tanaka et al., 1992; Black et al., 1994; Boyne et al., 1995; Bush et al., 1996). Taken together, these observations raise the possibility that MAP1B, acting in growth cones, might be involved in the induction of bent microtubules and microtubule loops *in vivo*. Our finding that the MAP1B light chain can induce wavy microtubules in PtK2 cells lends support to this hypothesis. MAP1B light chain effects on microtubules also differed from those of the microtubule stabilizing drug taxol (de Brabander et al., 1981). Expression of the MAP1B light chain prevented taxol-induced formation of centrosome-independent microtubule bundles, indicating that in the presence of the light chain the concentration of free tubulin heterodimers was lowered below the critical concentration for taxol-induced tubulin polymerization. It will be interesting to investigate whether the MAP1B light chain-induced stabilization of microtubules differs from taxol stabilization in that it might be resistant to changes in microtubule dynamics taking place when cells enter mitosis.

We have obtained evidence that MAP1B light chains can form dimers or oligomers either directly or through binding to additional factors (Fig. 9). Our results have been obtained in the absence of the MAP1B heavy chain and the question arises whether light chain oligomerization can at all occur in neurons where the light chain is expressed together with the heavy chain. However, it has been shown previously that the MAP1B protein complex purified from rat or calf brain contains a two- to threefold molar excess of light chains over the heavy chain (Schoenfeld et al., 1989; Pedrotti et al., 1996b). This is consistent with the interpretation that light chain oligomerization could occur in the presence of heavy chain in neurons and glial cells. Light chain dimers or oligomers could have microtubule cross-linking activity, leading to the formation of microtubule bundles. However, at this point we have no clear indication that microtubules, which sometimes appeared closely spaced in light chain-expressing cells, form bundles. Although it has been demonstrated that MAP1B can cross-link microtubules *in vivo* and *in vitro* (Hirokawa et al., 1985; Sato-Yoshitake et al., 1989), in previous studies using full-length MAP1B no microtubule bundling activity was observed in NIH3T3, HeLa or COS cells (Noble et al., 1989; Takemura et al., 1992) in agreement with the results shown here. It is tempting to speculate that oligomerization of the light chain might have an alternative purpose. Bringing together two or more microtubule binding domains could mimic the arrangement found in other

MAPs, where three or more microtubule binding domains are found in tandem on a single polypeptide chain. If light chain oligomerization were necessary for efficient binding to single or multiple microtubules through closely spaced binding domains, binding of the light chains to microtubules should be reduced under conditions where oligomerization is inhibited. In preliminary experiments we found that binding of the light chain to microtubules can be inhibited by simultaneous overexpression of the COOH terminus of the light chain (not shown). The COOH terminus contains the oligomerization domain but lacks the microtubule binding site and thus might prevent the formation of light chain oligomers containing multiple microtubule binding sites by replacing one or more light chains in the oligomers.

It has previously been shown that the MAP1B light chain binds to a 120-kD fragment of the NH₂ terminus of the heavy chain that includes the NH₂-terminal microtubule binding site (Kuznetsov et al., 1986). Here we have further narrowed down the sites of interaction between heavy and light chains. Coexpression of the light chain and a fragment of the NH₂ terminus of the heavy chain (amino acids 1–508) that lacks the NH₂-terminal microtubule binding domain revealed that the light chain can bind to this fragment *in vivo* (Fig. 7). Further experiments showed that the domain on the light chain responsible for interaction with the heavy chain is located in its COOH-terminal 120 amino acids (Figs. 8 and 10). Both regions of MAP1B shown here to be sufficient for heavy chain–light chain interaction contain long stretches of homology to MAP1A (Fig. 1). Our findings raise the possibility that the MAP1A homology domains in the NH₂ terminus of the heavy chain and the COOH terminus of the light chain of MAP1B are in fact the heavy chain–light chain interaction domains. This interpretation is supported by reports locating the light chain binding domain on the MAP1A heavy chain in the corresponding NH₂-terminal region of MAP1A and it has been shown that the heavy chain of MAP1A can bind the light chain of MAP1B (Schoenfeld et al., 1989).

Earlier studies using purified MAP1B in *in vitro* experiments have shown that MAP1B and in particular the MAP1B light chain can bind to actin filaments (Fujii et al., 1993; Pedrotti and Islam, 1996). Here we were able to confirm and extend these results by demonstrating that binding to actin stress fibers can occur *in vivo* (Fig. 10). In addition, we found that the COOH terminus of the light chain can efficiently target the NH₂ terminus of the heavy chain to stress fibers, suggesting that heavy chain–light chain interaction per se might not be incompatible with binding of MAP1B to actin filaments. Our results indicate that the domain responsible for binding to stress fibers is located in the 120 COOH-terminal amino acids of the light chain. As pointed out above, the COOH terminus of the MAP1B light chain corresponds to one of the three long MAP1A homology domains of MAP1B. Interestingly, actin binding has also been reported for MAP1A and its light chain (Fujii et al., 1993; Pedrotti and Islam, 1996). At this point it is not clear whether binding of the COOH terminus to stress fibers is due to an interaction with actin or with actin filament-associated proteins. The amino acid sequence of the COOH terminus does not contain an actin binding site of the type found in spectrin and dystrophin (Matsudaira,

1991). On the other hand, we observed that the COOH terminus did not bind to the most peripheral segments of stress fibers, suggesting that stress fiber-associated proteins might be involved in mediating or inhibiting binding.

Our experiments revealed that the COOH-terminal fragment of the MAP1B light chain can associate with stress fibers whereas the entire light chain consisting of the NH₂-terminal microtubule binding domain and the COOH terminus could not be detected on stress fibers and was found to colocalize with microtubules instead. One possible explanation for this observation is that the COOH-terminal actin filament binding domain is masked in the full-length protein and as a consequence the protein binds to microtubules through the microtubule binding domain. If the NH₂-terminal half of the light chain is removed, the COOH-terminal domain could be exposed leading to association with stress fibers. Such results have been obtained, for example, for ezrin, an 80-kD actin binding protein that mediates plasma membrane-actin cytoskeleton interactions (Algrain et al., 1993). Subsequently it has been shown that masking of the COOH-terminal actin binding site in ezrin by the NH₂ terminus is part of the regulatory mechanism controlling ezrin activity. In vivo, unmasking of the actin binding domain of ezrin is achieved by phosphorylation. A similar mechanism could be involved in MAP1B light chain action. It has recently been demonstrated that phosphorylation of MAP1B on sites yet to be identified influences its binding to microfilaments in vitro (Pedrotti and Islam, 1996).

The comparison of MAP1B light chain activities in the absence and presence of the heavy chain revealed that binding to microtubules and microtubule stabilization were greatly reduced in the presence of the heavy chain (Figs. 3–5). This inhibition was not due to incomplete processing of the polyprotein precursor that was used in these experiments as a convenient means to express both heavy and light chain in the same cell, because similar results were obtained when heavy and light chain were coexpressed in the same cell from separate constructs. We hypothesize that one function of the heavy chain might be to act as a regulatory subunit to control light chain activity. We found that heavy chain–light chain interaction does not per se inhibit light chain microtubule binding capacity and only partially reduces its microtubule stabilization activity. Thus, a separate regulatory domain could be present in the heavy chain and might act on bound light chains. In our experiments we were able to observe light chain activity, because we expressed the light chain in the absence of this hypothetical regulatory domain. Under physiological conditions, when heavy and light chains are present at similar levels other regulatory mechanisms would need to be involved. There is ample evidence in the literature that in brain and cultured neurons there is a large soluble cytoplasmic pool of MAP1B (Bloom et al., 1985; Díaz-Nido et al., 1990; Tanaka et al., 1992; Ulloa et al., 1993a; Keating and Asai, 1994) and that binding of MAP1B to microtubules in vivo (Díaz-Nido et al., 1990; Ulloa et al., 1993a,b) and to microfilaments in vitro (Pedrotti and Islam, 1996) is regulated by phosphorylation. It is conceivable that phosphorylation of MAP1B at specific sites leads to a conformational change that liberates the light chain from the inhibitory influence of the

regulatory domain. This would provide neurons with a mechanism to regulate a transient association of soluble MAP1B with microtubules and thus transient microtubule stabilization and perhaps interaction with actin filaments in individual cellular and axonal compartments in response to extra- and/or intracellular signals (Pigino et al., 1997).

We are grateful to L. Greene for kindly providing PC12 cells and to W. Zauner for providing helpful technical suggestions.

M. Tögel is recipient of a Ph.D. fellowship from the Vienna Biocenter Ph.D. Program funded by the Austrian Science Fund (Fonds zur Förderung der Wissenschaftlichen Forschung [FWF]). This research was supported by grants from the Austrian Science Fund (Project No. F607).

Received for publication 4 June 1998 and in revised form 6 August 1998.

References

- Algrain, M., O. Turunen, A. Vaheri, D. Louvard, and M. Arpin. 1993. Ezrin contains cytoskeleton and membrane binding domains accounting for its proposed role as a membrane-cytoskeletal linker. *J. Cell Biol.* 120:129–139.
- Binder, L.I., A. Frankfurter, H. Kim, A. Caceres, M.R. Payne, and L.I. Rebhun. 1984. Heterogeneity of microtubule-associated protein 2 during rat brain development. *Proc. Natl. Acad. Sci. USA.* 81:5613–5617.
- Black, M.M., T. Slaughter, and I. Fischer. 1994. Microtubule-associated protein 1b (MAP1b) is concentrated in the distal region of growing axons. *J. Neurosci.* 14:857–870.
- Bloom, G.S., T.A. Schoenfeld, and R.B. Vallee. 1984. Widespread distribution of the major polypeptide component of MAP1 (microtubule-associated protein 1) in the nervous system. *J. Cell Biol.* 98:320–330.
- Bloom, G.S., F.C. Luca, and R.B. Vallee. 1985. Microtubule-associated protein 1B: identification of a major component of the neuronal cytoskeleton. *Proc. Natl. Acad. Sci. USA.* 82:5404–5408.
- Boyne, L.J., K. Martin, S. Hockfield, and I. Fischer. 1995. Expression and distribution of phosphorylated MAP1B in growing axons of cultured hippocampal neurons. *J. Neurosci. Res.* 40:439–450.
- Brugg, B., D. Reddy, and A. Matus. 1993. Attenuation of microtubule-associated protein 1B expression by antisense oligonucleotides inhibits initiation of neurite outgrowth. *Neuroscience.* 52:489–496.
- Bush, M.S., R.G. Goold, F. Moya, and P.R. Gordon-Weeks. 1996. An analysis of an axonal gradient of phosphorylated MAP 1B in cultured rat sensory neurons. *Eur. J. Neurosci.* 8:235–248.
- Calvert, R., and B.H. Anderton. 1985. A microtubule-associated protein (MAP1) that is expressed at elevated levels during development of the rat cerebellum. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:1171–1176.
- Challacombe, J.F., D.M. Snow, and P.C. Letourneau. 1996. Actin filament bundles are required for microtubule reorientation during growth cone turning to avoid an inhibitory guidance cue. *J. Cell Sci.* 109:2031–2040.
- Cravchik, A., and A. Matus. 1993. A novel strategy for the immunological tagging of cDNA constructs. *Gene.* 137:139–143.
- Cravchik, A., D. Reddy, and A. Matus. 1994. Identification of a novel microtubule-binding domain in microtubule-associated protein 1A (MAP1A). *J. Cell Sci.* 107:661–672.
- de Brabander, M., G. Geuens, R. Nuydens, R. Willebrords, and J. de Mey. 1981. Taxol induces the assembly of free microtubules in living cells and blocks the organizing capacity of the centrosomes and kinetochores. *Proc. Natl. Acad. Sci. USA.* 78:5608–5612.
- Díaz-Nido, J., L. Serrano, M.A. Hernández, and J. Avila. 1990. Phosphorylation of microtubule proteins in rat brain at different developmental stages: comparison with that found in neuronal cultures. *J. Neurochem.* 54:211–222.
- DiTella, M.C., F. Feiguin, N. Carri, K.S. Kosik, and A. Cáceres. 1996. MAP-1B/TAU functional redundancy during laminin-enhanced axonal growth. *J. Cell Sci.* 109:467–477.
- Domínguez, J.E., B. Buendia, C. López-Otín, C. Antony, E. Karsenti, and J. Avila. 1994. A protein related to brain microtubule-associated protein MAP1B is a component of the mammalian centrosome. *J. Cell Sci.* 107:601–611.
- Edelmann, W., M. Zervas, P. Costello, L. Roback, I. Fischer, J.A. Hammarback, N. Cowan, P. Davies, B. Wainer, and R. Kucherlapati. 1996. Neuronal abnormalities in microtubule-associated protein 1B mutant mice. *Proc. Natl. Acad. Sci. USA.* 93:1270–1275.
- Field, J., J. Nikawa, D. Broek, B. MacDonald, L. Rodgers, I.A. Wilson, R.A. Lerner, and M. Wigler. 1988. Purification of a RAS-responsive adenyllyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method. *Mol. Cell Biol.* 8:2159–2165.
- Fujii, T., M. Watanabe, Y. Ogoma, Y. Kondo, and T. Arai. 1993. Microtubule-associated proteins, MAP1A and MAP1B, interact with F-actin *in vitro*. *J. Biochem.* 114:827–829.
- Garner, C.C., A. Matus, B. Anderton, and R. Calvert. 1989. Microtubule-asso-

- ciated proteins MAP5 and MAP1x: closely related components of the neuronal cytoskeleton with different cytoplasmic distributions in the developing brain. *Mol. Brain Res.* 5:85–92.
- Garner, C.C., A. Garner, G. Huber, C. Kozak, and A. Matus. 1990. Molecular cloning of microtubule-associated protein 1 (MAP1A) and microtubule-associated protein 5 (MAP1B): identification of distinct genes and their differential expression in developing brain. *J. Neurochem.* 55:146–154.
- Gordon-Weeks, P.R., S.G. Mansfield, C. Alberto, M. Johnstone, and F. Moya. 1993. A phosphorylation epitope on MAP 1B that is transiently expressed in growing axons in the developing rat nervous system. *Eur. J. Neurosci.* 5:1302–1311.
- Gossen, M., and H. Bujard. 1992. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci. USA.* 89:5547–5551.
- Greene, L.A., and A.S. Tischler. 1976. Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma which respond to nerve growth factor. *Proc. Natl. Acad. Sci. USA.* 73:2424–2428.
- Greene, L.A., R.K.H. Liem, and M.L. Shelanski. 1983. Regulation of a high molecular weight microtubule-associated protein in PC12 cells by nerve growth factor. *J. Cell Biol.* 96:76–83.
- Hammarback, J.A., R.A. Obar, S.M. Hughes, and R.B. Vallee. 1991. MAP1B is encoded as a polyprotein that is processed to form a complex N-terminal microtubule-binding domain. *Neuron.* 7:129–139.
- Harlow, E., and D. Lane. 1988. *Antibodies: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 726 pp.
- Harrison, L., M.E. Cheetham, and R.A. Calvert. 1993. Investigations of the changes in neuronal distribution and phosphorylation state of MAP1X during development. *Dev. Neurosci.* 15:68–76.
- Herrmann, H., J.M. Dalton, and G. Wiche. 1985. Microheterogeneity of microtubule-associated proteins, MAP-1 and MAP-2, and differential phosphorylation of individual subcomponents. *J. Biol. Chem.* 260:5797–5803.
- Hirokawa, N., G.S. Bloom, and R.B. Vallee. 1985. Cytoskeletal architecture and immunocytochemical localization of microtubule-associated proteins in regions of axons associated with rapid axonal transport: the β , β '-iminodipropionitrile-intoxicated axon as a model system. *J. Cell Biol.* 101:227–239.
- Hoebeke, J., G. Van Nijen, and M. de Brabander. 1976. Interaction of oncodazole (R 17934), a new antimitotic drug, with rat brain tubulin. *Biochem. Biophys. Res. Commun.* 69:319–324.
- Keating, H.H., and D.J. Asai. 1994. Distribution of phosphorylated microtubule-associated protein 1B during neurite outgrowth in PC12 cells. *Dev. Biol.* 162:143–153.
- Kuznetsov, S.A., V.I. Rodionov, E.S. Nadezhkina, D.B. Murphy, and V.I. Gelfand. 1986. Identification of a 34-kD polypeptide as a light chain of microtubule-associated protein-1 (MAP-1) and its association with a MAP-1 peptide that binds to microtubules. *J. Cell Biol.* 102:1060–1066.
- Langkopf, A., J.A. Hammarback, R. Müller, R.B. Vallee, and C.C. Garner. 1992. Microtubule-associated proteins 1A and LC2. *J. Biol. Chem.* 267:16561–16566.
- LeClerk, N., K.S. Kosik, N. Cowan, T.P. Pienkowski, and P.W. Baas. 1993. Process formation in Sf9 cells induced by the expression of a microtubule-associated protein 2C-like construct. *Proc. Natl. Acad. Sci. USA.* 90:6223–6227.
- Lewis, S.A., P. Sherline, and N.J. Cowan. 1986. A cloned cDNA encoding MAP1 detects a single copy gene in mouse and a brain-abundant RNA whose level decreases during development. *J. Cell Biol.* 102:2106–2114.
- Lewis, S.A., I.E. Ivanov, G.-H. Lee, and N.J. Cowan. 1989. Organization of microtubules in dendrites and axons is determined by a short hydrophobic zipper in microtubule-associated proteins MAP2 and tau. *Nature.* 342:498–505.
- Lien, L.L., C.A. Feener, N. Fischbach, and L.M. Kunkel. 1994. Cloning of human microtubule-associated protein 1B and the identification of a related gene on chromosome 15. *Genomics.* 22:273–280.
- Liu, D., and I. Fischer. 1996. Isolation and sequencing of the 5' end of the rat microtubule-associated protein (MAP1B)-encoding cDNA. *Gene.* 171:307–308.
- Matsudaira, P. 1991. Modular organization of actin crosslinking proteins. *Trends Biochem. Sci.* 16:87–92.
- Nguyen, H.-L., S. Chari, D. Gruber, C.-M. Lue, S.J. Chapin, and J.C. Bulinski. 1997. Overexpression of full- or partial-length MAP4 stabilizes microtubules and alters cell growth. *J. Cell Sci.* 110:281–294.
- Noble, M., S.A. Lewis, and N.J. Cowan. 1989. The microtubule binding domain of microtubule-associated protein MAP1B contains a repeated sequence motif unrelated to that of MAP2 and tau. *J. Cell Biol.* 109:3367–3376.
- Nothias, F., I. Fischer, M. Murray, S. Mirman, and J.-D. Vincent. 1996. Expression of a phosphorylated isoform of MAP1B is maintained in adult central nervous system areas that retain capacity for structural plasticity. *J. Comp. Neurol.* 368:317–334.
- Olson, K.R., J.R. McIntosh, and J.B. Olmsted. 1995. Analysis of MAP4 function in living cells using green fluorescent protein (GFP) chimeras. *J. Cell Biol.* 130:639–650.
- Pedrotti, B., and K. Islam. 1995. Microtubule associated protein 1B (MAP1B) promotes efficient tubulin polymerisation in vitro. *FEBS Lett.* 371:29–31.
- Pedrotti, B., and K. Islam. 1996. Dephosphorylated but not phosphorylated microtubule associated protein MAP1B binds to microfilaments. *FEBS Lett.* 388:131–133.
- Pedrotti, B., M. Francolini, F. Cotelli, and K. Islam. 1996a. Modulation of microtubule shape in vitro by high molecular weight microtubule associated proteins MAP1A, MAP1B, and MAP2. *FEBS Lett.* 384:147–150.
- Pedrotti, B., L. Ulloa, J. Avila, and K. Islam. 1996b. Characterization of microtubule-associated protein MAP1B: phosphorylation state, light chains, and binding to microtubules. *Biochemistry.* 35:3016–3023.
- Pigino, G., G. Paglini, L. Ulloa, J. Avila, and A. Cáceres. 1997. Analysis of the expression, distribution and function of cyclin dependent kinase (cdk5) in developing cerebellar macroneurons. *J. Cell Sci.* 110:257–270.
- Riederer, B., R. Cohen, and A. Matus. 1986. MAP5: a novel brain microtubule-associated protein under strong developmental regulation. *J. Neurocytol.* 15:763–775.
- Riederer, B.M., F. Moya, and R. Calvert. 1993. Phosphorylated MAP1b, alias MAP5 and MAP1x, is involved in axonal growth and neuronal mitosis. *NeuroReport.* 4:771–774.
- Sabry, J.H., T.P. O'Connor, L. Evans, A. Toroian-Raymond, M. Kirschner, and D. Bentley. 1991. Microtubule behavior during guidance of pioneer neuron growth cones in situ. *J. Cell Biol.* 115:381–395.
- Safaei, R., and I. Fischer. 1989. Cloning of a cDNA encoding MAP1B in rat brain: regulation of mRNA levels during development. *J. Neurochem.* 52:1871–1879.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Sato-Yoshitake, R., Y. Shiomura, H. Miyasaka, and N. Hirokawa. 1989. Microtubule-associated protein 1B: molecular structure, localization, and phosphorylation-dependent expression in developing neurons. *Neuron.* 3:229–238.
- Schoenfeld, T.A., L. McKerracher, R. Obar, and R.B. Vallee. 1989. MAP 1A and MAP 1B are structurally related microtubule associated proteins with distinct developmental patterns in the CNS. *J. Neurosci.* 9:1712–1730.
- Takei, Y., S. Kondo, A. Harada, S. Inomata, T. Noda, and N. Hirokawa. 1997. Delayed development of nervous system in mice homozygous for disrupted microtubule-associated protein 1B (MAP1B) gene. *J. Cell Biol.* 137:1615–1626.
- Takemura, R., S. Okabe, T. Umeyama, Y. Kanai, N.J. Cowan, and N. Hirokawa. 1992. Increased microtubule stability and alpha tubulin acetylation in cells transfected with microtubule-associated proteins MAP1B, MAP2 or tau. *J. Cell Sci.* 103:953–964.
- Tanaka, E.M., and M.W. Kirschner. 1991. Microtubule behavior in the growth cones of living neurons during axon elongation. *J. Cell Biol.* 115:345–363.
- Tanaka, Y., K. Kawahata, T. Nakata, and N. Hirokawa. 1992. Chronological expression of microtubule-associated proteins (MAPs) in EC cell P19 after neuronal induction by retinoic acid. *Brain Res.* 596:269–278.
- Tögel, M., B. Mossier, K. Fuchs, and W. Sieghart. 1994. γ -Aminobutyric acid_A receptors displaying association of γ ₃-subunits with β _{2/3} and different α -subunits exhibit unique pharmacological properties. *J. Biol. Chem.* 269:12993–12998.
- Tögel, M., G. Wiche, and F. Propst. 1998. Evidence against structural and functional identity of microtubule-associated protein MAP1B and proteoglycan claustrin. *FEBS Lett.* 423:254–258.
- Tsui, H.T., K.L. Lankford, H. Ris, and W.L. Klein. 1984. Novel organization of microtubules in cultured central nervous system neurons: formation of hairpin loops at ends of maturing neurites. *J. Neurosci.* 4:3002–3013.
- Tucker, R.P., C.C. Garner, and A. Matus. 1989. In situ localization of microtubule-associated protein mRNA in the developing and adult rat brain. *Neuron.* 2:1245–1256.
- Ulloa, L., J. Avila, and J. Díaz-Nido. 1993a. Heterogeneity in the phosphorylation of microtubule-associated protein MAP1B during rat brain development. *J. Neurochem.* 61:961–972.
- Ulloa, L., J. Avila, and J. Avila. 1993b. Depletion of casein kinase II by antisense oligonucleotide prevents neurogenesis in neuroblastoma cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:1633–1640.
- Ulloa, L., N. Ibarrola, J. Avila, and F.J. Díez-Guerra. 1994. Microtubule-associated protein 1B (MAP1B) is present in glial cells phosphorylated different than in neurones. *Glia.* 10:266–275.
- Vandecastelaere, A., B. Pedrotti, M.A. Utton, R.A. Calvert, and P.M. Bayley. 1996. Differences in the regulation of microtubule dynamics by microtubule-associated protein MAP1B and MAP2. *Cell Motil. Cytoskel.* 35:134–146.
- Weisshaar, B., and A. Matus. 1993. Microtubule-associated protein 2 and the organization of cellular microtubules. *J. Neurocytol.* 22:727–734.
- Weisshaar, B., T. Doll, and A. Matus. 1992. Reorganisation of the microtubular cytoskeleton by embryonic microtubule-associated protein 2 (MAP2c). *Development.* 116:1151–1161.
- Zauner, W., J. Kratz, J. Staunton, P. Feick, and G. Wiche. 1992. Identification of two distinct microtubule binding domains on recombinant rat MAP 1B. *Eur. J. Cell Biol.* 57:66–74.