REVIEW ARTICLE

High- M_r microtubule-associated proteins: properties and functions

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INTRODUCTION

Microtubules are one of three principal filamentous elements of the cytoskeleton. They are formed through the self-association of their major constituent protein, tubulin. The work of the past 10–15 years has demonstrated that there is a large and ever-increasing number of proteins that interact and often co-localize with cellular microtubules. The term ‘microtubule-associated proteins’ (MAPs) has more or less become a synonym for these proteins, although ‘microtubule binding proteins’ would probably be a more accurate name, considering that some of the MAPs may interact only transiently with microtubules.

In the absence of well-defined functions for most of the MAPs known to date, they are usually classified according to their size as determined by SDS/polyacrylamide-gel electrophoresis (Table 1). In brain tissue, where MAPs are most abundant, the more prominent and best studied MAP species can be roughly divided into two groups: one comprises proteins of very high M_r ($> 250\,000$), of which MAP 1 and MAP 2, as well as recently identified dynein-like proteins, are the major components (see below); the other represents a set of polypeptides, referred to as τ proteins (Weingarten *et al.*, 1975; Cleveland *et al.*, 1977), with M_r values in the range 55 000–62 000. A third, apparently very heterogeneous, group of MAPs, whose characterization on the molecular level has been recently started, comprises proteins of M_r close to 200 000. Members of this group represent a significant proportion of the MAPs isolated from non-neuronal cells (Bulinski & Borisy, 1979; Parysek *et al.*, 1984; Huber *et al.*, 1985; Goldstein *et al.*, 1986; Kotani *et al.*, 1986, 1988; Murofushi *et al.*, 1986). Other major MAPs include kinesin (M_r 110 000–130 000) (Vale *et al.*, 1985; for a review see Hollenbeck, 1988), a group of proteins called STOPs (M_r 72 000–145 000) (Job *et al.*, 1982; Margolis *et al.*, 1986) and chartins (M_r 69 000–80 000) (Pallas & Solomon, 1982; Magendantz & Solomon, 1985; Aletta & Greene, 1987). A collection of MAPs widely differing in size has also been isolated from sea urchin eggs (Vallee & Bloom, 1983; Vallee & Collins, 1986).

Based on several lines of evidence, the hypothesis has been advanced that high- M_r MAPs are ubiquitous microtubule-connecting links that may be vital in determining the structure of the cytoplasm of a cell or of a particular cellular compartment (Wiche, 1985). In addition, recently identified novel species of high- M_r MAPs may serve other, potentially more dynamic, cytoskeletal functions, such as retrograde vesicle transport in neurons.

In this review I shall try to review the increasingly complex subject of high- M_r MAPs ($M_r > 250\,000$) with a focus on methods of isolation, biochemical properties

and potential functions. Various aspects of high- M_r MAPs were described in several previous review articles on microtubule proteins (e.g., Purich & Kristofferson, 1984; McKeithan & Rosenbaum, 1984), high- M_r MAPs (Vallee & Bloom, 1984; Wiche, 1985), MAPs from brain (Matus, 1988), or MAPs in general (Olmsted, 1986).

STRATEGIES TO ISOLATE AND IDENTIFY HIGH- M_r MAPs

Different methods are in use to isolate and identify high M_r MAPs from various tissues and cells. The choice of method is quite crucial since the type and the yield of high- M_r MAPs obtained depends largely on the method used.

Temperature-dependent assembly cycles

After Weisenberg (1972) succeeded in polymerizing microtubules *in vitro* from cell extracts of mammalian brain, temperature-dependent assembly and disassembly became the standard procedure for isolating tubulin and MAPs for many years. In fact, copurification with microtubules in repeated rounds of temperature-dependent assembly and disassembly was one of the first criteria for the classification of MAPs (Borisy *et al.*, 1975). It became clear, however, that the protein composition of microtubule preparations, particularly with respect to their high- M_r components, was dependent on which protocol was used. When microtubules were polymerized from adult mammalian brain following the procedure of Shelanski *et al.* (1973), in which polymerization is performed in the presence of glycerol to stabilize tubulin (Solomon *et al.*, 1973) and which became a standard procedure in many laboratories, MAP 2 was by far the predominant high- M_r protein obtained; other procedures, such as one described by Murphy & Borisy (1975) yielded similar results. The proportion of MAP 1 obtained is drastically increased if near-iso-osmoticity is maintained during the *in vitro* cycling (Karr *et al.*, 1979) to avoid the release of endogenous proteases from broken vesicles during tissue homogenization. Under these conditions the ratio of MAP 1 to MAP 2 species is close to 1:1. Iso-osmoticity of buffer solutions and control of proteolysis in general turns out to be of great importance for the isolation of high- M_r MAPs, particularly in cases such as non-neuronal cultured cells where the cellular content in microtubule proteins is low compared to brain tissue. Early studies with cultured cell lines indicated that an endogenous factor which was distinct from tubulin decayed rapidly in cell extracts, most likely due to proteolysis (Wiche *et al.*, 1977a, 1979). In fact, in glioma C6 cells the successful isolation of both MAP 1 and MAP 2 by temperature-dependent *in vitro* polymerization was strictly dependent on prevention of

Table 1. Major classes of MAPs

M_r values were determined by SDS/polyacrylamide-gel electrophoresis. For references see the text.

M_r ($\times 10^{-3}$)	Family/species	Primary source	Functions
> 250 (High- M_r MAPs)	MAP1	Brain	Skeletal
	MAP2	Brain	Skeletal
	Dynein	Cilia, flagella, brain	Energy transduction
55–62	τ	Brain	Microtubule stabilization
200	MAP3, MAP4, 210 kDa HeLa MAP, 205 kDa <i>Drosophila</i> MAP, 190 kDa adrenal gland MAP, rat 190 kDa MAP	Neuronal and various non-neuronal cultured cells and tissues	Skeletal
110–134	Kinesin	Brain, sea urchin eggs	Energy transduction
72–145	STOPs	Brain	Microtubule stabilization
69–80	Chartins	Cultured cells	Microtubule stabilization

proteolysis, including the use of iso-osmotic buffered solutions (Koszka *et al.*, 1985).

The isolation of high- M_r MAPs from cells and tissues of non-neuronal origin by temperature-dependent microtubule polymerization is also problematic because in order to reach the critical concentration of tubulin required to drive assembly, extracts of relatively high protein concentration are required (Wiche & Cole, 1976; Wiche *et al.*, 1977b). Another point to consider is that temperature-dependent cycling could be selective for high- M_r MAP species that have a higher affinity to soluble tubulin dimers or tubulin oligomers than to the microtubule polymer. Consequently, certain high- M_r MAP species may preferentially stay associated with cold-stable non-depolymerizing microtubules, which are well-documented for brain tissue (Job *et al.*, 1982). Finally, assuming that there is only a limited number of binding sites for interacting proteins on the surface lattice of microtubules, temperature-dependent polymerization, like any other method based on coassembly, probably selects for high- M_r MAPs with the relatively highest binding affinities.

Taxol-driven assembly

New avenues in the isolation of high- M_r and other MAPs were opened by the discovery of Horwitz and coworkers that taxol, a plant alkaloid, efficiently drives tubulin polymerization both *in vitro* and *in vivo* and stabilizes the polymer (Horwitz *et al.*, 1982). In this way microtubules became accessible to *in vitro* polymerization even from sources with very low levels of microtubule proteins and, more importantly, assembly became independent of MAPs. As first demonstrated by Vallee (1982), taxol-assembled microtubules can be used as a kind of affinity matrix for microtubule binding proteins that, once isolated, can be released from the microtubule surface by salt without disassembling the microtubule. In addition, taxol promotes assembly even at low temperature where proteolytic breakdown of high- M_r MAPs is less favoured. However, as in the case of temperature-dependent cycling, the yield and type of high- M_r MAPs obtained in taxol-driven assembly is strongly dependent on the conditions prevailing during isolation. For instance, although it has been demonstrated that a MAP 2-related protein copurifies with HeLa cell microtubules in

temperature-dependent assembly (Weatherbee *et al.*, 1982), when microtubules were prepared by taxol-driven assembly from hypo-osmotic extracts of HeLa cells, no proteins corresponding in size to high- M_r MAPs from brain tissue were observed (Vallee, 1982). In our laboratory, the most reliable and efficient procedure for the isolation of high- M_r MAPs from sources containing low levels of microtubule proteins has been taxol-driven assembly combined with stringent control of proteolysis, which includes the use of iso-osmotic solutions (Koszka *et al.*, 1985). Under these conditions it was possible to isolate high- M_r MAPs, some of which were immunologically related to mammalian brain MAP 1 and MAP 2, from various non-neuronal cell lines and tissues (Wiche *et al.*, 1984; Koszka *et al.*, 1985). Careful control of proteolysis seems to be of particular importance in the isolation of MAP 2-related proteins from cultured cells. As shown for C6 cells, the successful isolation of a proteolytically undegraded 300 kDa protein immunoreactive with anti-MAP 2 was strongly dependent on the presence of EGTA in order to inhibit Ca^{2+} -activated proteases. The presence of EGTA was important even before cell lysis, i.e. during dislodging of the cells from the vessel walls. Without these precautions, proteolytic breakdown of the C6 cell MAP 2 variant was inevitable. In consistence with this, no immunoreactive MAP 2 components of high M_r were found in C6 cells in a recent study by Garner *et al.* (1988), in which cell lysis was performed under less controlled conditions.

Taxol-treated microtubules resist depolymerization under a variety of conditions that readily disassemble untreated microtubules. Recently, the depolymerization and subsequent repolymerization of taxol-treated microtubules was achieved at low temperature in the presence of millimolar levels of Ca^{2+} (Collins & Vallee, 1987). It was noted that cytoplasmic dynein, in contrast to other high- M_r MAP species, partitioned preferentially into the microtubule fractions which remained polymerized, indicating its higher affinity for tubulin polymers than to soluble tubulin. Thus, as in the case of temperature-dependent cycling, the composition of high- M_r MAPs recovered in Ca^{2+} /taxol-cycled microtubule preparations is clearly dependent on the extent to which microtubule proteins are converted into the insoluble polymeric or the more soluble oligomeric state.

Selective isolation based on ATP-mediated microtubule binding

Since both temperature-dependent cycling and taxol-driven assembly are selective for the MAPs with the highest microtubule binding affinities under the particular conditions of assembly, a number of procedures were devised to either selectively increase the affinity of some MAPs or to provide microtubule surface templates with sufficient MAP binding sites to reduce losses due to competitive binding. These approaches were particularly useful for the isolation of high- M_r MAPs with ATP-dependent binding characteristics. Lye *et al.* (1987) observed enhanced binding of a 400 kDa polypeptide to taxol-stabilized endogenous microtubules after depletion of ATP levels in cell extracts of the nematode *C. elegans*. Purification of this protein revealed properties characteristic for both dynein (Gibbons, 1965), the energy-transducing ATPase from cilia and flagella, and the presumptive anterograde transport vehicle, kinesin (Vale *et al.*, 1985). Similarly, Paschal *et al.* (1987) found that cytoplasmic dynein from brain tissue, originally termed MAP 1C, was enriched for, when microtubules were prepared with taxol and no triphosphate nucleotides were added. In a slightly different approach, Euteneuer *et al.* (1988) used taxol-stabilized microtubules from an exogenous source (brain) to selectively absorb a 440 kDa polypeptide from the great amoeba, *Reticulomyxa*, with properties expected for an organelle motor. Other high- M_r proteins shown to bind to taxol-treated microtubules in an ATP-dependent fashion are a MAP 2-like protein extracted from squid axoplasmic vesicles (Gilbert & Sloboda, 1986) and a 315 kDa protein, termed HMW4, in spinal nerve roots (Hollenbeck & Chapman, 1986).

A different strategy aimed at the isolation of high- M_r MAPs with low microtubule-binding affinity from brain tissue was to selectively destroy MAP 1 and MAP 2 in soluble cell extracts prior to taxol-driven microtubule assembly by exploiting the sensitivity of these proteins to Ca^{2+} -activated proteases. The idea was to facilitate the coassembly of presumptive low-affinity MAPs, such as dynein, which normally have to compete with high-affinity MAP 1 and MAP 2 for microtubule binding sites. Following this strategy, a Ca^{2+} -protease-resistant 300 kDa polypeptide with properties compatible with a cytoplasmic dynein variant was isolated from mammalian brain (Koszka *et al.*, 1987).

Other procedures

Selective co-extraction of cellular MAPs with cold- or drug-depolymerized microtubule proteins, another established MAP isolation procedure (Solomon *et al.*, 1979; Pillus & Solomon, 1986), does not yield high- M_r MAPs. This may reflect that these proteins remain bound to less soluble tubulin or non-tubulin structures. The latter case would be consistent with the presumptive role of high- M_r MAPs as linkers between microtubules and other cytoplasmic elements (see below).

Another method to study MAP-tubulin interaction, namely binding of soluble tubulin to solid phase-immobilized proteins, seems suited for the identification rather than the isolation of high- M_r MAPs. Using hog brain α - and β -tubulin individually eluted from SDS/polyacrylamide gels or phosphocellulose-purified tubulin, it was recently demonstrated that tubulin binds to nitrocellulose-immobilized MAP 1 and 2 from hog brain (Furtner & Wiche, 1987), to cytoplasmic dynein from chicken brain and to a MAP 2 analogue from chicken erythrocytes (unpublished results from the author's laboratory). Thus, solid phase binding assays could become a valuable new tool for the identification and characterization of high- M_r microtubule-binding proteins in various sources. Similar assays may also lead to the identification of MAP genes from cDNA expression libraries.

BIOCHEMICAL PROPERTIES, GENES AND OCCURRENCE OF HIGH- M_r MAPs

Of the high- M_r MAPs identified to date, only a few have been thoroughly characterized biochemically; the majority has not yet been purified. Information concerning the molecular properties of high- M_r MAPs from sources other than brain is particularly lacking. Work aimed at identifying and characterizing the genes of high- M_r MAPs has started only recently and just a few reports bearing on this subject have appeared.

MAP 1

Variety. When analysed on high-percentage polyacrylamide gels, MAP 1 migrates as a single band with an apparent M_r of 350 000 (Sloboda *et al.*, 1975). However, on low-percentage gels MAP 1 splits into a number of bands. A somewhat confusing situation arose lately

Fig. 1. Terminology of high- M_r MAPs

The scheme shows high- M_r species isolated in different laboratories as originally designated and displayed by polyacrylamide-gel electrophoresis. The position of bands within a column is roughly as documented, but the standardization of electrophoretic mobilities among the columns is arbitrary because different gel systems were used. (a) PC 12 cells (Greene *et al.*, 1983); (b) calf brain white matter (Bloom *et al.*, 1984a), the vertical lines indicate the variable position of MAP 1C (=cytoplasmic dynein; Paschal *et al.*, 1987); (c) hog brain (Herrmann *et al.*, 1985), the dynein variant isolated in this laboratory comigrates with MAP 2A (Koszka *et al.*, 1987); (d) rat brain (10 days) (Binder *et al.*, 1984); (e) rat brain (adult) (Calvert & Anderton, 1985); (f) rat brain (Riederer & Matus, 1986).

regarding the terminology of these bands (Fig. 1). Greene *et al.* (1983) first referred to two MAP 1 components in pheochromocytoma (PC) 12 cells as MAP 1.1 and 1.2, in order of increasing electrophoretic mobility. Bloom *et al.* (1984a) designated three MAP 1 bands obtained after purification of microtubule proteins with taxol from calf *corpus callosum* as MAP 1A, 1B and 1C, again, in order of decreasing apparent M_r . The same terminology was used for three major MAP 1 components copurifying with microtubules assembled from hog brain (Herrmann *et al.*, 1985) by the procedure of Karr *et al.* (1979), which is based on temperature-dependent polymerization; a fourth, minor, component exhibiting an electrophoretic mobility slightly lower than that of MAP 1A was called MAP 1A'. MAPs with similar electrophoretic mobilities include MAPX (Binder *et al.*, 1984), MAP 1(x) (Calvert & Anderton, 1985) and MAP 5 (Riederer *et al.*, 1986).

To what extent all these proteins classified as MAP 1 are actually related in structure is an open question that in most cases will be settled only when DNA or protein sequence data become available. On the basis of biochemical and immunological data at hand one can predict, however, that some of these proteins have certain structural features in common. For instance, in the case of MAP 1A, 1A', 1B and 1C isolated from hog brain by temperature-dependent polymerization (Fig. 1, column c), thorough analyses by one- and two-dimensional peptide mapping strongly suggested that all four protein species are related in structure to some extent (Herrmann *et al.*, 1985; Wiche *et al.*, 1986). Particularly when the proteins had been radiolabelled with [32 P]phosphate using endogenous kinases, peptide maps obtained were of striking similarity, arguing for a strong conservation of MAP 1 phosphorylation sites. This conclusion was confirmed by the identification of a monoclonal antibody that was reactive with phosphorylated epitopes on both MAP 1A and MAP 1B isolated from calf brain by taxol-driven microtubule assembly (Luca *et al.*, 1986). On the other hand, structural divergencies of these two MAP 1 species were revealed by silver-stained one-dimensional peptide maps and by several monoclonal antibodies that only react with one of the two (Bloom *et al.*, 1985a). A confusing situation pertains to MAP 1C, because two apparently different proteins are referred to by the same name. Peptide mapping analyses strongly suggested that MAP 1C isolated from hog brain by temperature-dependent *in vitro* polymerization of microtubules has structural features in common with the other major MAP 1 components present in such preparations (see above). Furthermore, this protein, together with MAP 1A, is the predominant MAP 1 species of repeatedly cycled microtubule preparations (Herrmann *et al.*, 1985), it is readily susceptible to proteolysis, and it represents a major substrate for endogenous kinases. None of these characteristics seems to apply to the protein referred to as MAP 1C by Bloom *et al.* (1984a), which was later identified as cytoplasmic dynein (see below). In line with this, a Ca^{2+} -protease-resistant dynein variant isolated from mammalian brain in the authors's laboratory (Koszka *et al.*, 1987) which is potentially homologous to the dynein variant identified by Vallee and coworkers, is clearly distinct from the MAP 1C which copurifies with microtubules in repeated cycles of temperature-dependent microtubule assembly.

Proteins MAPX, MAP 1(x) and MAP 5 are apparently

all subject to strong developmental regulation, since they appear much more abundantly during development than in adult tissue. Their relationship to the other major MAP 1 species present in microtubule preparations from adult tissues is unclear. At least two of them, MAP 1(x) and MAP 5, seem to be closely related (see discussion in Tucker & Matus, 1987). The issue of MAP 1 identification is further complicated by the use of different electrophoresis systems in various laboratories for analysing high- M_r MAPs. The electrophoretic mobilities of some high- M_r MAPs are quite sensitive to the conditions of electrophoresis, as described, for instance, for cytoplasmic dynein (Paschal *et al.*, 1987; Collins & Vallee, 1987). Moreover, the mobility of high- M_r MAPs is affected by their state of phosphorylation. Increased phosphorylation apparently shifts MAP 1.2 from PC 12 cells (Aletta *et al.*, 1988) as well as MAP 2A and MAP 2B from brain (Binder *et al.*, 1984; Herrmann *et al.*, 1985; Wiche *et al.*, 1986) to more slowly migrating positions on SDS/polyacrylamide gels.

Molecular shape. MAP 1 molecules have been visualized on the ultrastructural level as filamentous arms extending from the surface of *in vitro* polymerized microtubules, using embedded and thin-sectioned samples (Vallee & Davis, 1983). Similarly, rotary-shadowed samples of purified MAP 1 reveal thin, filamentous and apparently highly flexible molecules (Shiomura & Hirokawa, 1987a). Furthermore, the association of MAP 1 with fuzzy structures extending from axonal and dendritic microtubules has been demonstrated by immunoelectron microscopy (Hirokawa *et al.*, 1985; Shiomura & Hirokawa, 1987b).

Phosphorylation. The subcomponents of MAP 1, like those of MAP 2, are phosphoproteins of pI around 5.0. Early experiments *in vivo* indicated that in brain tissue MAP 1 may be an even more prominent target of kinases than MAP 2 (see Fig. 5 in Sloboda *et al.*, 1975), the major target of cyclic AMP-dependent kinases. However, endogenous kinases copurifying with microtubules prepared by temperature-dependent polymerization from hog brain phosphorylate MAP 1 subcomponents at a much lower rate than MAP 2 proteins and their phosphorylation is much less stimulated by cyclic AMP (Herrmann *et al.*, 1985). On the other hand, there is recent evidence that MAP 1 subcomponents, in contrast to MAP 2, are substrates of casein kinase II, both *in vitro* (Avila *et al.*, 1988) and *in vivo* (Diaz-Nido *et al.*, 1988). Thus, kinases of different types may use different sub-species of high- M_r MAPs, including individual MAP 1 subcomponents, as their targets. Moreover, a calcium-dependent phosphorylation of MAP 1-related proteins has been observed upon mitogenic stimulation of fibroblast cells (Sato *et al.*, 1985a).

Binding partners. Bulk separation of MAP 1 from MAP 2 components under non-denaturing conditions revealed that brain MAP 1, like MAP 2, stimulates *in vitro* polymerization of microtubules (Kuznetsov *et al.*, 1981; Vallee & Davis, 1983). It was also found that low- M_r components, referred to as light chains 1–3 (Berkowitz *et al.*, 1977; Vallee & Davis, 1983; Kuznetsov *et al.*, 1986; Kuznetsov & Gelfand, 1987) are tightly associated with the 300 kDa heavy chains of MAP 1. Whether there is a preferential interaction between certain light and

heavy chains has not been worked out to date. It is also unclear whether MAP 1-driven microtubule polymerization requires light chains or not. Using solid phase binding assays, bulk, unfractionated, preparations of MAP 1 were shown to bind to nitrocellulose-immobilized proteins such as β -tubulin, all three neurofilament proteins, and MAP 2 (Furtner & Wiche 1987). In addition, MAP 1 possesses a binding site for the cytomatrix protein plectin (Herrmann & Wiche, 1987).

Relationship to other proteins. Several observations suggest that MAP 1 subcomponents have certain structural features in common with MAP 2. First, there are polyclonal antibodies which recognize both MAPs (Wiche *et al.*, 1983; Sato *et al.*, 1985a). Second, extensive proteolytic peptide mapping analyses revealed several common fragments to be generated under a variety of conditions (Herrmann *et al.*, 1984; Wiche *et al.*, 1986). Particularly, when individual [32 P]phospholabelled subcomponents of both species were compared, striking similarities were revealed, indicating strong conservation of phosphorylation sites on both protein species. Conservation of functional molecular domains is indicated also by the identification of as many as six common interaction partners using solid phase binding assays (see above). On the other hand, since several monoclonal as well as polyclonal antibody preparations reported (for details see Olmsted, 1986) do not react with both MAP species, and no hybridization between cDNA clones specific for substantial portions of MAP 2 and a MAP 1 subcomponent was observed (see below), any structural homology must be limited to certain parts of the molecules. More insight into the question can be expected once the complete nucleotide sequences of the various MAP genes are known. A number of other proteins have been shown to share epitopes with MAP 1. These include a widely distributed nuclear protein that is associated with the mitotic spindle (Bonifacio *et al.*, 1985), nuclear antigens that are distributed in a speckled fashion (Sato *et al.*, 1985b), a stress fibre component (Asai *et al.*, 1985), a 205 kDa sulphoglycoprotein of the extracellular matrix (Briones & Wiche, 1985), ankyrin (Bennett & Davis, 1981) and components of microtubule organizing centres (Mascardo *et al.*, 1982). It is unknown whether all these cases reflect structural similarities of the crossreactive proteins that are functionally relevant.

Genes. The first MAP 1-specific DNA sequences were recently isolated by Cowan and coworkers using an expression library prepared from mouse brain mRNA (Lewis *et al.*, 1986a). The overlapping clones isolated covered 6.6 kB of MAP 1-specific cDNA in total, half of which were derived from the noncoding region of the mRNA. Genomic Southern experiments indicated that there was one single gene coding for the cloned MAP 1. However, it was not established which of the MAP 1 subcomponents had been cloned. Since antibodies raised against a fusion protein derived from one of the clones crossreacts with MAP 1.2, the lower of the two MAP 1 bands found in PC 12 cells (Fig. 1, column a), it was later suggested that the cDNAs isolated are specific for MAP 1B (Aletta *et al.*, 1988). The identity of MAP 1.2 with MAP 1B has still to be demonstrated, however. By Northern hybridization MAP 1 transcripts were found in several non-neuronal tissues, confirming previous immunological data. Interspecies reactions were confined

to rat, human and chicken; no reaction was seen with frog, sea urchin or *Drosophila* DNA.

Occurrence in non-neuronal cells. MAP 1 species are widespread in neuronal cells and tissues and their cellular localization has been studied extensively (for reviews see Wiche, 1985; Olmsted, 1986; Matus, 1988). Based on the results from several laboratories, there is now also no doubt about the occurrence of proteins related to brain MAP 1 outside of brain tissues. Using affinity-purified polyclonal antibodies that recognize epitopes on several MAP 1 subcomponents or a monoclonal antibody to MAP 1A, MAP 1 antigens were localized on typical microtubule structures of cultured cells, including the spindle apparatus, the midbody and primary cilia (Wiche *et al.*, 1984; Bloom *et al.*, 1984b). MAP 1 antigens were also localized in frozen sections of several tissues. Co-distribution with tubulin was observed in most, but not all, cases; notably Z-line structures of cardiac and skeletal muscle were MAP 1-positive, but tubulin-negative (Wiche *et al.*, 1984; Zernig & Wiche, 1985). The association of MAP 1 antigens with non-neuronal microtubules was also shown by the immunodecoration of *in vitro* polymerized microtubules from glioma C6 cells (Koszka *et al.*, 1985). The widespread occurrence of MAP 1 species revealed by immunocytochemistry was fully confirmed in immunoblotting analyses. Proteins with electrophoretic mobilities of MAP 1 subcomponents from brain were identified in cell extracts and microtubule preparations from many different cultured cell lines (Bloom *et al.*, 1984b; Wiche *et al.*, 1984; Sato *et al.*, 1985a; Bonifacio *et al.*, 1985) as well as from tissues, including pituitary gland (Bloom *et al.*, 1985b; Wiche *et al.*, 1984), adrenal gland (Kotani *et al.*, 1986), heart and testis (Wiche *et al.*, 1984). In addition, MAP 1-specific mRNA species were identified by Northern blot analysis in liver, kidney, stomach, spleen and thymus, although at levels significantly lower than in brain (Lewis *et al.*, 1986a).

MAP 2

Isomers. Brain MAP 2 is the best studied of all high- M_r MAPs. It migrates as a single band with an apparent M_r of 300000 upon electrophoresis on SDS/high-percentage polyacrylamide gels, but its actual M_r may be much lower (Hernandez *et al.*, 1986). On low-percentage gels the MAP 2 band splits into two closely spaced and equally proportioned bands, MAP 2A and 2B (Kim *et al.*, 1979) (Fig. 1), which have very similar structure as judged from peptide mapping (Herrmann *et al.*, 1985, and citations therein) and from immunoreactivity with monoclonal antibodies (Binder *et al.*, 1984). Furthermore, genomic DNA blots were indicative of only a single gene coding for both MAPs (Lewis *et al.*, 1986b; Garner & Matus, 1988). A third, much smaller isoform, MAP 2C (M_r 70000), apparently is derived from the same gene by differential splicing of its transcript (Garner & Matus, 1988).

Molecular shape and domain structure. MAP 2 is a thermostable, thread-like molecule. Length estimates vary between 90 (Gottlieb & Murphy, 1985) and 185 nm (Voter & Erickson, 1982) in its microtubule-detached form and up to 90 nm (Voter & Erickson, 1982) in its microtubule-bound form. The spatial organization of MAP 2 projections on brain microtubules has recently been analysed by microdensitometer computer correlation

techniques and a predominant (12-dimer) superlattice has been proposed (Jensen & Smaill, 1986). Limited proteolysis of MAP 2 produces two major domains of roughly 30–40 kDa and 240 kDa (Vallee, 1980; Flynn *et al.*, 1987; Aizawa *et al.*, 1987) thought to represent the microtubule binding and projection domains of the molecule, respectively. However, studies with domain-specific antibodies indicate that the binding domain may occupy as much as one-third of the molecule's length (Gottlieb & Murphy, 1985). MAP 2 fragments containing the tubulin binding domain have been suggested to contain the *N*-terminal of the molecule (Hernandez *et al.*, 1987) and they appear to be relatively basic compared to the projection domain or to undigested MAP 2 (Sattilaro, 1986; Flynn *et al.*, 1987).

Phosphorylation and other modifications. Like MAP 1, MAP 2 has a *pI* of around 5.0 and it is a phosphoprotein. More than 30 mol of phosphate per mol of MAP 2 were incorporated *in vitro* after previous dephosphorylation of the protein (Tsuyama *et al.*, 1986). Recent evidence suggests that two kinds of MAP 2, one with 46 phosphates and not bound to tubulin and the other with 10–16 phosphates and bound to tubulin, are present in living rat brain (Tsuyama *et al.*, 1987). Several different types of kinases have been identified that phosphorylate brain MAP 2 *in vitro* on serine and threonine residues, including cyclic AMP-dependent (Sloboda *et al.*, 1975), cyclic AMP-independent (Theurkauf & Vallee, 1983; Singh *et al.*, 1984), Ca^{2+} /calmodulin-dependent (Goldenring *et al.*, 1983; Schulman, 1984), and Ca^{2+} /phospholipid-dependent (Akiyama *et al.*, 1986a; Tsuyama *et al.*, 1986) kinases. A serine/threonine kinase that phosphorylates brain MAP 2 *in vitro* has also been identified in 3T3 adipocytes (Ray & Sturgill, 1987, 1988). In addition, purified insulin receptor kinase (Kadowaki *et al.*, 1985), epidermal growth factor receptor kinase and Rous sarcoma virus *src* kinase (Akiyama *et al.*, 1986b) were shown to phosphorylate MAP 2 on tyrosine residues. Whether all of these reactions are of physiological significance remains to be seen. It has been shown that the extent of MAP 2 phosphorylation influences microtubule assembly and disassembly *in vitro* (Jameson & Caplow, 1981; Burns *et al.*, 1984) and that extensive phosphorylation inhibits the binding of MAP 2 to formed microtubules (Murthy & Flavin, 1983). Furthermore, both phosphorylation of MAP 2 by Ca^{2+} /phospholipid-dependent kinase and tyrosine phosphorylation markedly reduce its ability to interact with actin (Akiyama *et al.*, 1986a; Nishida *et al.*, 1987) and inhibit its ability to induce tubulin polymerization (Nishida *et al.*, 1987; Hoshi *et al.*, 1988). There is also evidence that a Ca^{2+} /phospholipid-dependent kinase phosphorylates MAP 2 *in vivo* and that the sites affected are essentially confined to the projection domain of the molecule, whereas cyclic AMP-dependent phosphorylation is likely to affect primarily the microtubule binding domain (Murthy *et al.*, 1985; Tsuyama *et al.*, 1986).

Besides phosphorylation, very little is known about other post-translational protein modifications that may possibly affect the interaction of MAP 2 with microtubules or other cellular structures. Modulation of microtubule assembly and stability by phosphatidylinositol acting on MAP 2 has been reported (Yamuchi & Purich, 1987), but it was not determined whether the compound becomes covalently attached to the protein.

The interaction of MAP 2 with microtubules is affected also by estramustine (Friden *et al.*, 1987; Stearns & Tew, 1988). In this context it is worth noting that microtubules assembled from detyrosinated tubulin bind MAP 2 less efficiently than those polymerized from tyrosinated tubulin (Kumar & Flavin, 1985).

Binding partners. MAP 2 apparently possesses binding sites for several different proteins. The regulatory subunit of cyclic AMP-dependent kinase binds with high affinity to the projection domain (Vallee *et al.*, 1981; Lohmann *et al.*, 1984), whereas calmodulin binds to the tubulin-binding region (Lee & Wolff, 1984). Using solid phase binding assays, a number of proteins have been shown to interact with MAP 2. These include the 68 kDa (Heimann *et al.*, 1985; Furtner & Wiche, 1987) as well as the 160 kDa and the 200 kDa neurofilament proteins (Furtner & Wiche, 1987), intact and fragmented tubulin subunits (Littauer *et al.*, 1986; Furtner & Wiche, 1987), plectin (Herrmann & Wiche, 1987) and MAP 2 itself (Furtner & Wiche, 1987), the latter indicating self-association of MAP 2 (see also Foisner & Wiche, 1985). Furthermore, it was shown in previous studies that MAP 2 binds to neurofilaments (Leterrier *et al.*, 1982) and to actin filaments (see Pollard *et al.*, 1984, and references therein), the latter being affected by Ca^{2+} /phospholipid-dependent kinase (see above) and Ca^{2+} /calmodulin (Kotani *et al.*, 1985; Sobue *et al.*, 1985). Interestingly, the relatively basic tubulin binding domain seems to be involved in the interactions with both neurofilaments (Flynn *et al.*, 1987) and actin filaments (Sattilaro, 1986); but MAP 2 may have more than one actin binding site (Sattilaro, 1986). Considering MAP 2-dependent interaction *in vitro* of microtubules with coated vesicles and endocrine secretory granules (Suprenant & Dentler, 1982; Sattilaro & Dentler, 1982) it is likely that MAP 2 also possesses binding sites for some unidentified surface components of these organelles which could be, however, membrane-bound tubulin (Kelly *et al.*, 1983). Furthermore, satellite DNA (Wiche *et al.*, 1978) and metaphase chromosomes (Wandosell *et al.*, 1984) have been identified as interaction partners of MAP 2 *in vitro*, and Ca^{2+} -binding sites have been located on the projection domain of MAP 2 (Hernandez *et al.*, 1988).

Relationship to other proteins. Aside from a certain structural homology to MAP 1 subcomponents (see above), MAP 2 was found to share epitopes with spectrin, the major component of the erythrocyte subplasma membrane protein skeleton (Davis & Bennet, 1982). This homology may be related to common binding sites of both proteins for actin and plectin. Anti-MAP 2 crossreactivity was also observed in the case of a 110000- M_r protein associated with membranes of the Golgi apparatus (Allan & Kreis, 1986) and with a high- M_r MAP from *Trypanosoma brucei* (Schneider *et al.*, 1988).

Genes. Partially overlapping MAP 2-specific cDNA clones encoding two-thirds of the molecule were recently isolated from a mouse brain expression library (Lewis *et al.*, 1986b). In RNA blot transfer experiments with MAP 2 cDNA probes, no corresponding sequences were detected in RNA prepared from liver, kidney, spleen, stomach or thymus, suggesting that the expression of MAP 2 is brain-specific or the levels were too low to be

detected. Genomic Southern blot transfer experiments indicated the presence of a single gene encoding MAP 2 in mouse. The microheterogeneity of MAP 2A and MAP 2B was, therefore, ascribed to either alternative splicing within a single gene or to post-translational modifications. Based on the weak, if at all detectable, cross-hybridization of the MAP 2 cDNA probes with genomic sequences from rat, man, chicken, frog, *Drosophila* and sea urchin, an extensive interspecies divergence of MAP 2 sequences was proposed. Such divergence would require that the biological function of MAP 2 remain intact in spite of considerable neutral drift, so that the same function could be provided by molecules encoded by weakly or non-hybridizing genes. Alternatively, the observed lack of interspecies conservation may reflect selective constraints on small, discrete regions of the molecule or regions not encoded by the set of MAP 2-specific clones used (Lewis *et al.*, 1986b). Similar phenomena may account for the observed lack of hybridization of the cDNA probes to the RNA blots. In the only other study reported to date in which cDNA probes specific for brain MAP 2 were isolated, Garner & Matus (1988) described three partially overlapping cDNA clones covering in total about 4.3 kb. Using these clones in RNA and DNA blot experiments, the conclusion was reached that one MAP 2 gene codes for three distinct protein species, namely MAP 2A, MAP 2B and a 70 kDa protein, termed MAP 2C. Since two mRNA species 9 kb and 6 kb long were identified in Northern blot experiments, it was suggested that the two transcripts arose by differential splicing of a primary nuclear MAP 2 gene transcript.

Occurrence outside of brain tissue. The widespread distribution and the localization of MAP 2 in cells and tissues of neuronal provenance have been well documented and thoroughly reviewed (Vallee, 1984; Wiche, 1985; Olmsted, 1986; Matus, 1988). The subject of non-neuronal MAP 2, however, is less transparent and still controversial. It has clearly been shown in several laboratories that polypeptides with the apparent M_r of brain MAP 2 and with immunoreactivity towards MAP 2-specific antibodies occur in a variety of non-neuronal tissues and cells. These include pituitary gland (Wiche *et al.*, 1984; Bloom *et al.*, 1985b), skeletal muscle (Wiche *et al.*, 1984), adrenal gland (Kotani *et al.*, 1986), squirrel fish erythrocytes (Stearns & Binder, 1987), squid axoplasm and optic lobe (Do *et al.*, 1988), chromaffin cells from adrenal medulla (Burgoyne & Norman, 1985), nucleated erythrocytes (Sloboda & Dickerson, 1980, and see below), tracheal epithelium cells (Kuznetsov *et al.*, 1980), Hela cells (Weatherbee *et al.*, 1982), pig kidney cells (Valdivia *et al.*, 1982), and C6 cells (Koszka *et al.*, 1985). However, in other studies (Izant & McIntosh, 1980; Garner *et al.*, 1988), carried out, notably, with monoclonal antibodies, immunoreactive antigens were not found in some of these and a few other cell types. A likely explanation for these disparate results is the existence of common as well as distinct epitopes on brain MAP 2 and on its non-neuronal counterparts. Particularly if the common epitopes are less immunogenic, as could in fact be expected, and/or less frequent than the unique epitopes, there are high chances that monoclonal antibodies may create the impression of a more restricted antigen distribution than there actually is. The existence of tissue-specific variations in the primary structures of

MAP 2 molecules would also be consistent with the detection of no or very few transcripts in tissues other than brain using a MAP 2-specific cDNA probe from mouse brain in Northern blot analysis (Lewis *et al.*, 1986b). A more complete understanding of the structural relationship between the various MAP 2 species will probably be reached once their genes have been sequenced. It would not be unexpected to find that structural conservation of MAP 2 species is confined to a few, maybe relatively small, molecular domains with common functions, such as binding sites for tubulin or other cytoplasmic structures. If so, one interesting question remaining would be which factors dictate the large size common to MAP 2 molecules. As long as the structure of the various presumptive MAP 2 species is not better understood, I consider similar apparent M_r in combination with immunological crossreactivity as sufficient criteria in the classification of a protein as MAP 2. A protein that fulfils these criteria has recently been purified from chicken erythrocytes (unpublished results from the author's laboratory). Data already available show that this protein co-localizes with microtubules at different stages of erythrocyte development and shares a number of properties with brain MAP 2. It will be interesting to learn more about the structure of this putative erythrocyte MAP 2 or of any other non-neuronal MAP 2 yet to be purified, in order to assess their homology with brain MAP 2.

Cytoplasmic brain dynein

There were early hypotheses (Burns & Pollard, 1974; Gaskin *et al.*, 1974) that high- M_r MAPs copurifying with microtubules polymerized *in vitro* from brain were cytoplasmic counterparts of dynein, the force-generating enzyme for microtubule motility in cilia and flagella (Gibbons, 1965), but neither a chemical nor a functional relationship of these proteins has been revealed. Although the occurrence of ATPases related to axonemal dynein in the cytoplasm of different cell types, including brain tissue, was reported by Pallini *et al.* (1983), microtubule binding proteins with dynein-like properties were only recently purified from brain tissue, independently in two laboratories. In the approach used in our laboratory, a high- M_r protein with an electrophoretic mobility indistinguishable from MAP 2A was isolated from the cytosol of hog brain after selective proteolysis of MAP 1 and MAP 2 by Ca^{2+} -activated proteases and subsequent assembly of microtubules with taxol (Koszka *et al.*, 1987). This protein was shown to be immunologically related to the *Tetrahymena* dynein 14 S heavy chain and to exhibit, in purified form, an ATPase activity that, characteristic of dynein-like ATPase, is inhibited by EHNA. Furthermore, the microtubule binding of this protein is ATP-dependent, its sedimentation coefficient is 20 S, similar to that of axonemal dynein, and it possesses binding sites for ATP and tubulin as demonstrated by ATP-affinity labelling and gel overlay techniques (our unpublished results). In addition, the immunolocalization of this protein in primary cultures of chicken embryo cells revealed a cytoplasmic distribution. A few properties, for instance its vanadate-insensitive ATPase activity, distinguish, however, the protein from axonemal dyneins. Moreover, preliminary experiments indicate that this protein may have only limited activity in motility assays based on the gliding of microtubules on glass.

Vallee and coworkers identified MAP 1C, the least

prominent high- M_r MAP in their standard taxol microtubule preparation from calf brain, as cytoplasmic dynein (Paschal *et al.*, 1987). [As discussed above, this protein has to be distinguished from the MAP 1C described as a major component of microtubules polymerized from hog brain by temperature-dependent polymerization (Herrmann *et al.*, 1985).] The initial enrichment for this protein by taxol-driven microtubule assembly was shown to be strongly nucleotide-dependent. In a series of papers (Paschal & Vallee, 1987; Vallee *et al.*, 1988a,b; Shpetner *et al.*, 1988) it was convincingly demonstrated that MAP 1C from calf brain possesses all the properties qualifying it as a cytoplasmic form of dynein: sedimentation coefficient, M_r , morphology (two heads with associated stalks and base), total particle mass determined by STEM, susceptibility to cleavage by u.v. light in the presence of vanadate, microtubule-stimulated and vanadate-, EHNA- as well as *N*-ethylmaleimide-sensitive ATPase activity, and retrograde force production in microtubule gliding assays. Whether the dynein-like proteins isolated from brain in the two laboratories represent homologous species is unknown. There are quite noticeable differences in both preparations with respect to proteins of lower M_r that copurify with the high- M_r polypeptide chains, but the significance of this has to be determined.

FUNCTIONS

The possible functions of high- M_r MAPs shown to generate force, such as brain dynein or the recently described ATPases from *C. elegans* and *Reticulomyxa* (see above), are relatively easy to imagine. Since motility assays *in vitro* showed that they cause gliding of microtubules or latex bead movement in the anterograde (Lye *et al.*, 1987), retrograde (Paschal & Vallee, 1987) or both directions (Euteneuer *et al.*, 1988), it is likely that they are involved in one- or bi-directional movement and sorting of organelles along cytoplasmic microtubules, including retrograde transport in neurons. Whether these molecules play a role in other motility phenomena involving microtubules, such as spindle movement, remains a challenging task for future research. Anyway, by propelling organelle movement along cytoplasmic microtubules, this type of high- M_r MAP functionally resembles kinesin, another ATP-sensitive MAP which, however, is considerably smaller (for a recent review see Hollenbeck, 1988).

The potential function of the other, ATP-insensitive class of high- M_r MAPs is less clear. Based on the evidence in hand it is very likely, however, that these MAPs are involved in interlinking microtubules with other cellular structures, including other cytoskeletal elements. First, several of their properties, especially their molecular shape, would be consistent with this idea. As thin, filamentous molecules protruding from the surface of microtubules polymerized *in vitro*, high- M_r MAPs seem fit to connect microtubules with themselves and with other cytoplasmic structures by forming bridges that potentially span over relatively long distances. It has been demonstrated that high- M_r MAPs are indeed components of such bridges in axons and dendrites (Hirokawa *et al.*, 1985; Shiomura & Hirokawa, 1987b). Secondly, given the definition of MAP 2 species as immunologically related proteins with an apparent M_r of roughly 300 000 (see above), members of the MAP 1 as

well as the MAP 2 family can be considered to be of widespread occurrence not only in neuronal but also in non-neuronal cell types and tissues. Finally, the observed structural heterogeneity of both neuronal and non-neuronal high- M_r MAPs, together with their proposed tissue-specific primary structure, would allow for a wide spectrum of structural variability. This, in turn, could provide the basis for molecular domains of distinct binding specificity, not only for various cellular structures and non-tubulin components, but also for cell type-specific tubulin isoforms. Based on the work from several laboratories (Bond *et al.*, 1986; Lewis *et al.*, 1987; Lopata & Cleveland, 1987) it is becoming increasingly clear that tubulin isoforms alone hardly can be responsible for the sorting of microtubules within the cell and for specifying their diverse cellular functions. There have to be additional determinants, very likely including high- M_r MAPs.

As ubiquitous, but structurally varied microtubule linking components, high- M_r MAPs may thus specify the interaction partners of microtubules in addition to promoting microtubule polymerization (Wiche, 1985). This MAP-mediated interaction of microtubules with structures in their vicinity may stabilize the microtubules themselves and, in turn, the whole cytoplasmic environment where this linkage occurs. The presence of one type of MAP along the length of single or bundled microtubules, for example, could facilitate the lengthwise association of other structures specified by this MAP species. The arrangement in parallel of microtubules and neurofilaments over long distances in axonal processes of neurons would be an extreme example of such a situation. However, for certain key connections between microtubules and other cellular structures to be established or maintained, for instance at microtubule-organizing centres, competent high- M_r MAPs would not necessarily have to make contact with microtubules along their entire length, but only at spatially defined areas or points. This would require the expression of a relatively low level of high- M_r MAPs of this type compared to tubulin. On the other hand, high- M_r MAPs may be present all along the microtubule surface, but their interaction potential with non-tubulin structures could be locally regulated by secondary protein modification, such as phosphorylation. At any rate, in specifying the interaction partner of microtubules, high- M_r MAPs may play a decisive role as morphogenic control elements in the cellular cytoplasm. Moreover, the differential expression of high- M_r MAPs during cell development and differentiation could lead to changes in the cytoarchitecture that are of importance for dynamic alterations in cell morphology and function.

The experimental evidence in support of this concept is mounting, but most of it is still indirect. It is encouraging that in brain virtually all MAPs tested, including those of high M_r , have been found to be developmentally regulated and that most of them show a differential distribution within neurons (for review see Matus, 1988). This may imply that neurons require microtubules with different interaction specificities, depending on the stage of neuronal development or the neuronal cell compartment in question. Further data supporting a morphogenic role of high- M_r MAPs is the enhancement of the steady-state level of MAP 1.2 as well as of its phosphorylation in nerve growth factor-treated PC 12 cells (Aletta *et al.*, 1988; Drubin *et al.*, 1988), a model for process outgrowth

Fig. 2. Working model of high- M_r MAP-ligand interaction specificity

Three hypothetical high- M_r MAP species, A (binding)-, B- and C-MAP are depicted. Each of them possesses a unique binding site for the ligand (A, B or C) indicated by the prefixes. Different structural features of MAP molecules give rise to their unique binding sites. The molecular domains specifying binding sites for tubulin/microtubules or any other common interaction partners, however, are similar in structure. Prefixes indicating ligand specificities could be useful in distinguishing between MAP species.

by intact neuronal cells. Specific modifications *in vivo* of high- M_r MAPs have also been observed in cell systems of non-neuronal provenance. Upon growth stimulation of fibroblast cells, there was an increase in the phosphorylation and intracellular appearance of cytoskeleton-associated MAP 1-related polypeptides (Sato *et al.*, 1986). Non-neuronal cell systems where high- M_r MAPs have been shown to be major components of microtubule preparations, such as adrenal gland (Kotani *et al.*, 1986) and nucleated erythrocytes (Sloboda & Dickersin, 1980; P. Feick & G. Wiche, unpublished work), seem to be good candidates for future studies on morphology-related effects of high- M_r MAPs.

If high- M_r MAPs are to play a major role in specifying the interaction partners of microtubules, they ought to have binding sites for polypeptides other than tubulin. As discussed above, to date this has been demonstrated on the molecular level only in the cases of brain MAP 2 and bulk preparations of brain MAP 1. Questions of particular interest to solve will be whether high- M_r MAPs from sources other than brain possess binding sites that differ from those of their neuronal counterparts, on the one hand, and whether different species of high- M_r MAPs within a single tissue have different binding specificities, on the other. A working model which could be relatively easy to test is presented in Fig. 2. In this model, every high- M_r MAP species has at least one specific ligand binding site that is unique and, thus, distinct from those of other MAPs.

OUTLOOK

To prove their putative role as mediators between microtubules and other cellular structures and, in par-

ticular, to test the hypothesis of high- M_r MAPs being involved in determining the specific associations of microtubules (Wiche, 1985; Wiche *et al.*, 1987), work on several levels needs to be done. Protein chemistry will be required to identify the molecules interacting with high- M_r MAPs and to determine which MAP domains are involved in a particular binding reaction. Assuming a receptor/ligand relationship between high- M_r MAP molecules and their interacting molecules, the goal is to narrow down the interaction site to the essential amino acid sequences. In this approach it should also be possible to establish whether certain high- M_r MAP species show preferences in binding to particular tubulin isoforms and their peptides (Littauer *et al.*, 1986; Maccioni *et al.*, 1988). Major advances in the understanding of MAP functions are expected to be made using experimental strategies based on recombinant DNA technology. Once the genes for all high- M_r MAPs have been cloned and their complete nucleotide sequences have been established, several open questions are likely to be solved. These pertain to the number, structural relationship and regulation of MAP genes. It will be of particular interest to learn whether any common structural motifs revealed at the DNA level give rise to MAP domains serving similar functions, such as binding to microtubules or other cytoplasmic structures. It would not be surprising to find that high- M_r MAPs of diverse binding specificity are derived from single genes through differential, cell-type-specific splicing, as has been demonstrated for other multifunctional cross-linking proteins, for instance fibronectin (Schwarzbauer *et al.*, 1987). In efforts to associate gene regions with binding domains of high- M_r MAPs, deletion analyses combined with functional assays of fusion proteins seem a promising approach. Another approach may be the transfection of cells with vectors containing partially deleted or otherwise altered cDNA sequences of high- M_r MAP species. Experiments of this kind have been performed successfully in recent studies on cytokeratins (Albers & Fuchs, 1987).

I am grateful to colleagues who sent preprints of their work to be included in this review and I thank my colleagues in Vienna for reading the manuscript. The work described from the author's laboratory was supported by grants from the Austrian Science Research Fund (Österreichischer Fonds zur Förderung der Wissenschaftlichen Forschung). This review covers work published through July 1988.

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