Int. J. Dev. Biol. 38: 13-25 (1994)

Review

Regulation of microtubule dynamics by microtubule-associated protein expression and phosphorylation during neuronal development

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0214-6282/94/$03.00

Introduction

Central to brain development in vertebrates is the generation of extremely complicated neuronal morphologies, characterized by the presence and arborization of long cytoplasmic processes (neurites), referred to as axons and dendrites, which eventually form synaptic contacts (Peters *et al.*, 1976). Microtubules are cytoskeletal elements consisting of polymers of α, ß-tubulin heterodimers whose assembly plays an essential role in the formation and maturation of axons and dendrites (Matus, 1988; Mitchison and Kirschner, 1988; Avila, 1990, 1991; Ginzburg, 1991).

Microtubules are present in all eukaryotic cell types, being involved in the regulation of cell shape, in the intracellular distribution of organelles and in cell division. However, they are much more abundant in neurons, where they promote the growth and induce the polarity of axons and dendrites (Matus, 1988; Mitchison and Kirschner, 1988; Avila, 1990, 1991; Tucker, 1990; Ginzburg, 1991). Thus, both axons and dendrites shrink back to the cell body, losing their internal organization after treatment of cultured neurons with microtubule-depolymerizing drugs (Seeds *et al.*, 1970; Yamada *et al.*, 1970; Matus *et al.*, 1986; Matus, 1988). A similar effect is observed when tubulin expression is blocked by specific anti-sense oligonucleotides (Teichmann-Weinberg *et al.*, 1988). The occurrence of severe microtubule dysfunction in some neurodegenerative disorders including Alzheimer's disease also emphasizes the importance of microtubules for normal neuronal function (Matsuyama and Jarvik, 1989).

Microtubules are organized in long bundles within axons and dendrites from mature neurons (Peters *et al.*, 1976; Baas *et al.*, 1988, 1989). It is thought that microtubule bundling results from microtubule stabilization (Lee and Brandt, 1992) and, indeed, neuronal microtubules are more resistant to depolymerization than non-neuronal microtubules (Seitz-Tutter *et al.*, 1988; Lim *et al.*, 1989). As the neuronal-specific organization of microtubules may depend on their high degree of stabilization, a great deal of attention has been paid to the study of factors controlling microtubule dynamics in developing and mature neurons.

Role of microtubule-associated proteins in the regulation of microtubule dynamics

The *in vitro* dynamics of microtubules has been thoroughly studied for the last decade with a focus on possible regulatory factors (Kirschner and Mitchison 1986; Avila, 1990; Caplow, 1992). Among these factors, there is a group of proteins that bind to tubulin in *in vitro* microtubule polymerization assays and are therefore referred to as microtubule-associated proteins or MAPs (Sloboda *et al.*, 1975). Four major families of MAPs have been described: MAP1 proteins (Vallee, 1990), MAP2 proteins (Murphy *et al.*, 1977), MAP3/MAP4 proteins (Olmsted, 1991) and tau proteins (Cleveland *et al.*, 1977). Initially isolated from mammalian brains, these MAPs have also been found in other vertebrate organisms (Tucker *et al.*, 1988; Tucker, 1990). Microtubule-associated proteins are also present in invertebrates, although they have not been characterized in detail. All, except MAP3/MAP4 proteins, are predominantly found in neurons, and are thought to control microtubule dynamics *in vivo*.

There is a major group of microtubule-interacting proteins that have ATPase activity and transiently bind to tubulin. These «motor» proteins are involved in the transport of organelles along microtubules. As these proteins do not show a stable association with tubulin, they will not be considered as MAPs in this review.

A theoretical mechanism to explain the dynamics of microtubule assembly-disassembly has been suggested by Mitchison and Kirschner in their «dynamic instability» model (Mitchison and Kirschner, 1984; Kirschner and Mitchison, 1986). This model assumes that unpolymerized tubulin binds GTP and GTP-bound tubulin has the capacity to polymerize into microtubules (Carlier, 1982). Once tubulin is bound to the polymer, the GTP on tubulin is hydrolyzed to GDP. When the ratio GDP-tubulin: GTP-tubulin at a microtubule end reaches a certain threshold, the microtubule polymer start to rapidly depolymerize (an event known as «catastrophe»). After this «catastrophe», there is the possibility that some microtubules may incorporate GTP-bound tubulin back and, consequently, stop depolymerizing, thus becoming «rescued». Also, depolymerized GDP-tubulin can interchange GDP for GTP, yielding GTP-tubulin that may polymerize into new microtubules again.

Whereas this model may account for the dynamic properties of microtubules assembled from purified tubulin *in vitro* (Mitchison and Kirschner, 1984), some refinement is required to understand the behavior of microtubules directly observed in living cells after microinjection of fluorescently-labeled tubulin (Cassimeris *et al.*, 1988; Walker *et al.*, 1988). In general, microtubules are less dynamic *in vivo* than they are *in vitro*. Furthermore, there are important differences in the behavior of microtubules in distinct cell types (Pepperkok *et al.*, 1990; Shelden and Wadsworth, 1993). Particularly, neuronal microtubules seem to be less dynamic than microtubules in non-neural cells; and this microtubule stabilization is progressively attained during the development and maturation of axons and dendrites (Okabe and Hirokawa, 1988; Seitz-Tutter *et al.*, 1988; Lim *et al.*, 1989; Baas *et al.*, 1991).

These distinctive properties might arise from the presence of specific MAPs, which are notably abundant in neurons (Matus, 1988; Tucker, 1990). Indeed, *in vitro* studies have demonstrated that the addition of neuronal MAPs to purified tubulin leads to a decreased dynamics of the resulting microtubules, which is mainly due to a decrease in the frequency of «catastrophe» and an increase in the frequency of «rescue» events (Pryer *et al.*, 1992).

Abbreviations used in this paper: MAP, microtubule-associated protein; LC, light chain; C-terminal; CK II, casein kinase II; PDPK, proline-directed protein kinase; CaMK II, calcium/calmodulin-dependent protein kinase.

Fig. 1. Model for the role of MAPs in favoring the interaction of ß-tubulin subunit with GTP. *The binding of MAPs to the C-terminal region of the ß tubulin subunit interferes with the intramolecular interaction of that domain with the GTP-binding domain. Thus, GTP binding to ß tubulin is facilitated.*

Furthermore, the microinjection of certain neuronal-specific MAPs into non-neural cells (Dubrin and Kirschner, 1986) and the transfection of these cells with cloned cDNAs coding for certain neuronal MAPs (Kanai *et al.*, 1989, 1992; Knops *et al.*, 1991; Chen *et al.*, 1992; Lee and Rook, 1992; Takemura *et al.*, 1992; Weisshaar *et al.*, 1992; Edson *et al.*, 1993; Umeyama *et al.*, 1993) also results in the stabilization and bundling of the cellular microtubules *in situ*. However the molecular mechanisms responsible for the MAP-stimulated microtubule stabilization are still unclear. One possibility is that the binding of MAPs to the microtubule lattice would simply diminish the rate of loss of tubulin from the polymer. Additionally, the binding of MAPs may increase the affinity of tubulin for GTP (Hamel *et al.*, 1983). As MAPs bind to the carboxy terminal region of tubulin subunits (Serrano *et al.*, 1984a,b, 1985), and this domain of the tubulin molecule is also involved in an intramolecular interaction with the GTP-binding site (Padilla *et al.*, 1993), the association of MAPs with tubulin might relieve any restraint for GTP binding to tubulin as indicated in Fig. 1.

MAPs may also influence other properties of the microtubule cytoskeleton. The presence of several closely spaced tubulin-binding motifs on MAP molecules (see below) may link together neighboring tubulin dimers on the microtubule lattice, thus conferring stiffness to the microtubules (Edson *et al.*, 1993). As MAPs are long fibrous molecules that project out of the microtubule surface, MAPs may function as «spacer» molecules controling the distance between microtubules in bundles (Chen *et al.*, 1992; Lee and Brandt, 1992). Finally, MAPs may also serve as anchors for a variety of cytoplasmic proteins, including several protein kinases (Theurkauf and Vallee, 1983; Obar *et al.*, 1989; Rubino *et al.*, 1989; Serrano *et al.*, 1989; Ookata *et al.*, 1993) and other cytoskeletal proteins (Leterrier *et al.*, 1982; Selden and Pollard, 1983; Hirokawa *et al.*, 1988).

Thus, the expression of specific sets of MAPs along neuronal development may partly determine the organization and properties of the microtubule cytoskeleton at distinct developmental stages. In fact, the inhibition of the expression of certain MAPs in cultured neurons by treatments with antisense oligonucleotides blocks neuronal morphogenesis at specific stages (Cáceres and Kosik, 1990; Cáceres *et al.*, 1991; Dinsmore and Solomon, 1991; Hanemaaijer and Ginzburg, 1991; Brugg *et al.*, 1993). Consequently, there is a great interest in the study of the detailed molecular mechanisms controlling neuronal development in which MAPs are implicated.

MAPs are actually a very heterogeneous group of proteins, individual members showing developmental stage-specific expression as well as a subcellular-specific compartmentalization (Matus, 1988; Tucker, 1990). Additionally, MAP functionality may be modulated by post-translational modifications, mainly through phosphorylation and dephosphorylation (Avila and Díaz-Nido, 1991). We will refer to these issues in the following sections, where we will briefly review the MAPs best characterized because of their abundance in mammalian brain.

MAP1 proteins

The MAP1 protein family consists of two distinct but related proteins, MAP1A and MAP1B (Schoenfeld *et al.*, 1989; Garner *et al.*, 1990; Langkopf *et al.*, 1992). MAP1B (Bloom *et al.*, 1985) is also known as MAP1.2 (Greene *et al.*, 1983; Aletta *et al.*, 1988), MAP1X (Binder *et al.*, 1984; Calvert and Anderton, 1985) and MAP5 (Riederer *et al.*, 1986). MAP1A has a molecular mass of 299,000 whereas MAP1B has a molecular mass of 255,000, as calculated from their respective amino acid sequences (Noble *et al.*, 1989; Langkopf *et al.* 1992). However, they show higher apparent molecular masses after denaturing gel electrophoresis (350 and 320 kDa). These MAPs are encoded by two distinct genes (Garner *et al.*, 1990), but they show extensive regional amino acid similarities including a positively-charged segment which is close to the amino terminus and contains multiple repeats of a (Lys/Arg) (Lys/Arg) (Glu/Asp) motif (Noble *et al.*, 1989; Langkopf *et al.*, 1992). These repeats appear to be involved in microtubule-binding (Noble *et al.*, 1989), and might be general tubulin-binding motifs conserved in distinct MAPs from different cell types and organisms. Indeed, they have been found even in one of the major tubulin-binding proteins from yeast (Jiang *et al.*, 1993). However, they are not present in other classes of MAPs (see below).

There are three low molecular weight proteins referred to as light chains: LC1 (34 kDa), LC2 (30 kDa) and LC3 (19 kDa), associated with the microtubule-binding domains of both MAP1A and MAP1B (Vallee and Davis, 1983; Schoenfeld *et al.*, 1989). Interestingly, MAP1A and LC2 (as well as MAP1B and LC1) are coded by single mRNAs which give rise to pre-MAP1A/LC2 and pre-MAP1B/LC1 polyprotein precursors which are proteolytically processed (Hammarback *et al.*, 1991; Langkopf *et al.*, 1992).

Although these MAPs, or related proteins, are widely distributed in different cell types, they are mainly found in neurons (Vallee *et al.*, 1986; Díaz-Nido and Avila, 1989; Tucker *et al.*, 1989). The expression of these proteins in the mammalian brain is under strong developmental control. MAP1B has been shown to be the first MAP which is expressed in neurons *in situ* (Tucker *et al.*, 1988, 1989; Tucker, 1990). The expression of MAP1B is down-regulated during brain development (Binder *et al.*, 1984; Bloom *et al.*, 1985; Riederer *et al.*, 1986; Tucker *et al.*, 1989; Garner *et al.*, 1990), whereas the expression of MAP1A is up-regulated (Binder *et al.*,1984; Tucker *et al.*, 1989; Garner *et al.*, 1990). Immunohistochemical analyses have shown that MAP1B is highly concentrated in developing neurons, particularly within their growing axons, and exhibits a more moderate expression both in axons

and dendrites of mature neurons (Riederer *et al.*, 1986; Schoenfeld *et al.*, 1989). MAP1A is mainly abundant in dendrites of mature neurons (Bloom *et al.*, 1984; Huber and Matus, 1984; Shiomura and Hirokawa, 1987; Schoenfeld *et al.*, 1989). In view of these data, it is tempting to speculate that MAP1B is important in neurite growth in the developing brain and in neurite plasticity in the adult brain, whereas MAP1A may be required for dendrite maintenance in the adult brain. An essential role for MAP1B in the initiation of neurite growth is supported by recent experiments using antisense oligonucleotides which inhibit MAP1B expression in cultured neuronal-like cells (Brugg *et al.*, 1993). Likewise, an up-regulation of MAP1B has been correlated with neuritic regeneration in adult retinal explant cultures (Bates *et al.*, 1993).

MAP1 phosphorylation

Both MAP1A and MAP1B are extensively phosphorylated in the living rat brain (Díaz-Nido *et al.*, 1990). Phosphorylation of MAP1B has been more thoroughly studied, as it occurs during neurite growth in a variety of cell lines of neuronal origin (Aletta *et al.*, 1988; Díaz-Nido *et al.*, 1988).

The existence of at least two major modes of MAP1B phosphorylation which can be distinguished by the use of different antibodies to phosphorylation-sensitive epitopes has been recently described (Ulloa *et al.*, 1993a,b,c). The mode I of MAP1B phosphorylation induces an important upward shift in the electrophoretic mobility of the protein and might be catalyzed by proline-directed protein kinases (cyclin-dependent kinases and/or MAP kinases), whereas the mode II of MAP1B phosphorylation hardly modifies the electrophoretic mobility of the protein and is presumably catalyzed by casein kinase II (Ulloa *et al.*, 1993a,c). Mode I-phosphorylated sites on MAP1B can be readily dephosphorylated by calcineurin (protein phosphatase 2B) and protein phosphatase 2A, whereas mode II-phosphorylated sites are dephosphorylated by protein phosphatases 2A and 1 (Ulloa *et al.*, 1993c).

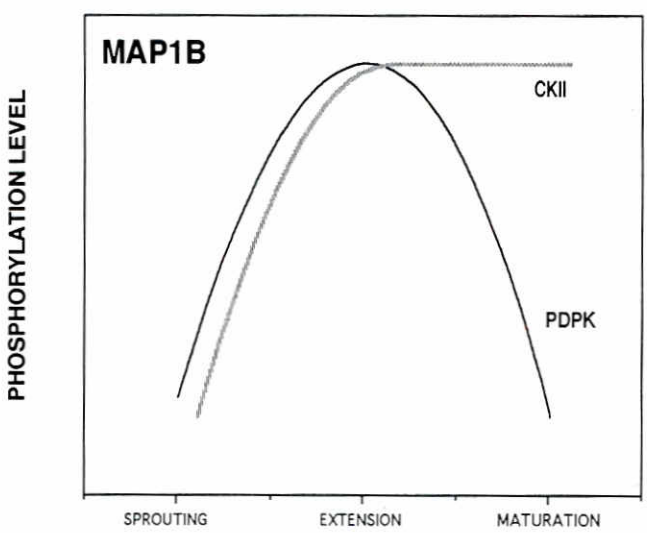


Fig. 2. Scheme for possible changes in the phosphorylation state of MAP1B during neuronal development. *During neurite sprouting, there is an increase in the level of phosphorylation of MAP1B which may be due to casein kinase II (CK II) and proline directed protein kinase (PDPK). Phosphorylation of MAP1B by PDPK is axonal-specific and notably decreases after axonal maturation. Phosphorylation of MAP1B by CK II remains in mature neurons.*

Interestingly, these two modes of MAP1B phosphorylation are independently regulated during brain development and show a differential subcellular distribution. The mode I of MAP1B phosphorylation strongly diminishes during development in most adult brain regions (Viereck *et al.*, 1989; Fischer and Romano-Clarke, 1990; Ulloa *et al.*, 1993b) (Fig. 2). Mode I-phosphorylated MAP1B is localized to the distal growing segments of developing axons, and axonal maturation is accompanied by dephosphorylation (Mansfield *et al.*, 1992; Gordon-Wecks *et al.*, 1993; Riederer *et al.*, 1993; Ulloa *et al.*, 1994). No dephosphorylation of mode I-phosphorylated MAP1B occurs in the olfactory system, where there is a persistent growth of axons from sensory neurons of the olfactory epithelium. This supports the view that mode I-phosphorylated MAP1B can be considered as a marker for active axonal growth. In contrast, the mode II of MAP1B phosphorylation is maintained in the adult brain (Ulloa *et al.*, 1993b) and is present both in axons and dendrites (Ulloa *et al.*, 1994).

Evidence obtained from neuroblastoma cells suggests that phosphorylation by casein kinase II at mode II sites on MAP1B may favor its binding to microtubule and may be essential for neurite growth (Díaz-Nido *et al.*, 1988; Ulloa *et al.*, 1993c).

The functional consequences of the mode I of MAP1B phosphorylation are not yet understood, although it can be speculated that this specific mode of phosphorylation of MAP1B might contribute to the dynamic configuration of microtubules which has been observed in growing axon terminals. The dephosphorylation of mode I sites on MAP1B may therefore lead to more stabilized and tightly packed microtubule bundles during axonal maturation.

It is interesting to note the presence of hyperphosphorylated MAP1B (particularly at mode I sites) associated with dystrophic neurites and neurofibrillary tangles within the brains of patients with Alzheimer's disease (Hasegawa *et al.*, 1990). This fact may be correlated with the aberrant neurite growth characteristic of this disorder (Ihara, 1988; Masliah *et al.*, 1991).

Other brain MAPs

MAP2, MAP3/MAP4 and tau proteins are protein families sharing the presence of a carboxy-terminal microtubule-binding domain consisting of a proline-rich cationic region followed by three or four imperfect tandem repeats containing homologous 18-amino-acid motifs which are completely different from those present in MAP1 proteins (Lee *et al.*, 1988; Lewis *et al.*, 1988; Kindler *et al.*, 1990; Aizawa *et al.*, 1991; Goedert *et al.*, 1991; West *et al.*, 1991).

MAP2, MAP3/MAP4 and tau are each encoded by a single-copy gene, but considerable heterogeneity is generated by alternative splicing of primary transcripts.

MAP2

There are several forms of MAP2 that arise from an alternative splicing which is under developmental control in neurons. In this way, there is a high molecular weight protein known as MAP2B (1828 amino acids, with an apparent molecular mass of 270,000 by denaturing gel electrophoresis; Lewis *et al.*, 1988) and a smaller form referred to as MAP2C (467 amino acids, with an apparent

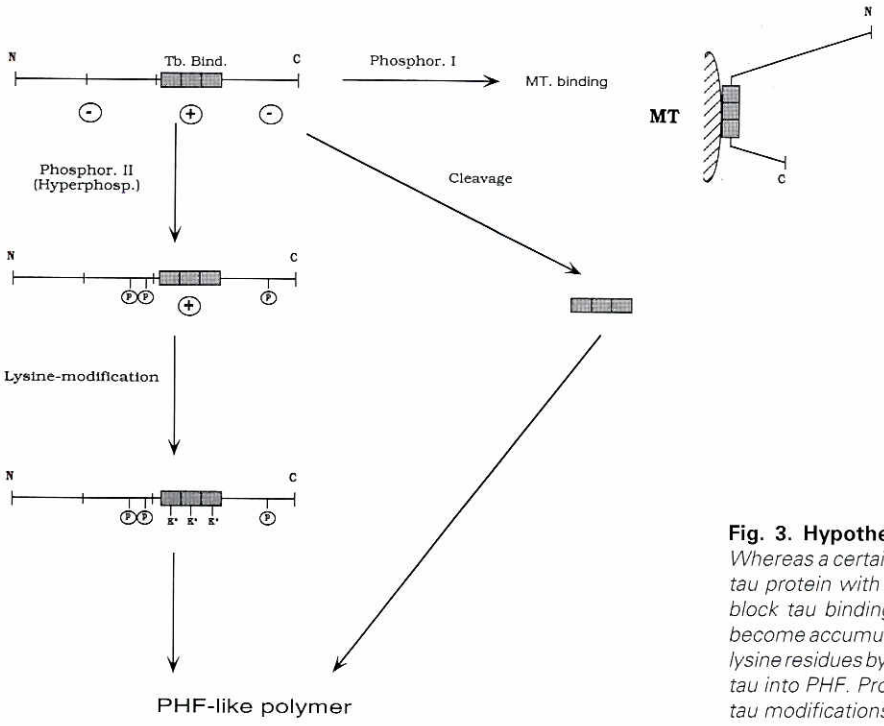


Fig. 3. Hypothetical model to explain tau aggregation into PHF. *Whereas a certain type of phosphorylation (I) may allow the association of tau protein with microtubules, another type of phosphorylation (II) may block tau binding to microtubules. Hyperphosphorylated tau may thus become accumulated in the cytosol. Further modification of tau protein on lysine residues by glycation may lead to aggregation of hyperphosphorylated tau into PHF. Proteolytic cleavage of tau protein and other still-unknown tau modifications may also result in tau aggregation into PHF.*

molecular mass of 70,000 by denaturing gel electrophoresis; Kindler *et al.*, 1990). MAP2C consists of amino-terminal and carboxy-terminal domains of high molecular weight MAP2 joined together and lacking the 1372-amino-acid intervening sequence. The amino-terminal domain contains a binding site for the regulatory subunit of cyclic AMP-dependent protein kinase, whereas the carboxy-terminal segment contains the microtubule-binding domain (Obar *et al.*, 1989; Rubino *et al.*, 1989; Kindler *et al.*, 1990). Three tubulin-binding motifs have been found in MAP2B and MAP2C. Recently, a new MAP2 form named MAP2D has been described. This form contains four repeats of the tubulin binding motif and is abundant in glial cells (Doll *et al.*, 1993).

MAP2C is expressed in the developing brain and it is strongly down-regulated during brain maturation, whereas high molecular weight MAP2B is expressed in both developing and adult brain. Additionally, a high molecular weight form of MAP2 with a slower electrophoretic mobility (MAP2A) appears only after brain maturation (Matus, 1988; Nunez, 1988; Tucker, 1990). MAP2C appears in postmitotic neuroblasts and has a widespread distribution, being present in neuronal cell bodies, dendrites and axons as well as in glial cells (Tucker, 1990; Charriére-Bertrand *et al.*, 1991). In contrast, high molecular weight MAP2 is a neuronal-specific protein selectively localized in dendrites and neuronal cell bodies (Cáceres *et al.*, 1984; De Camilli *et al.*, 1984; Huber and Matus, 1984; Tucker, 1990; Charrière-Bertand *et al.*, 1991). The specific compartmentalization of high molecular weight MAP2 into dendrites may be due to the selective transport of the corresponding mRNA into dendrites (Garner *et al.*, 1988).

The essential role of MAP2 in the growth of dendrite-like processes has been demonstrated using antisense oligonucleotides in certain cultured neuronal-like cells (Dinsmore and Solomon, 1991).

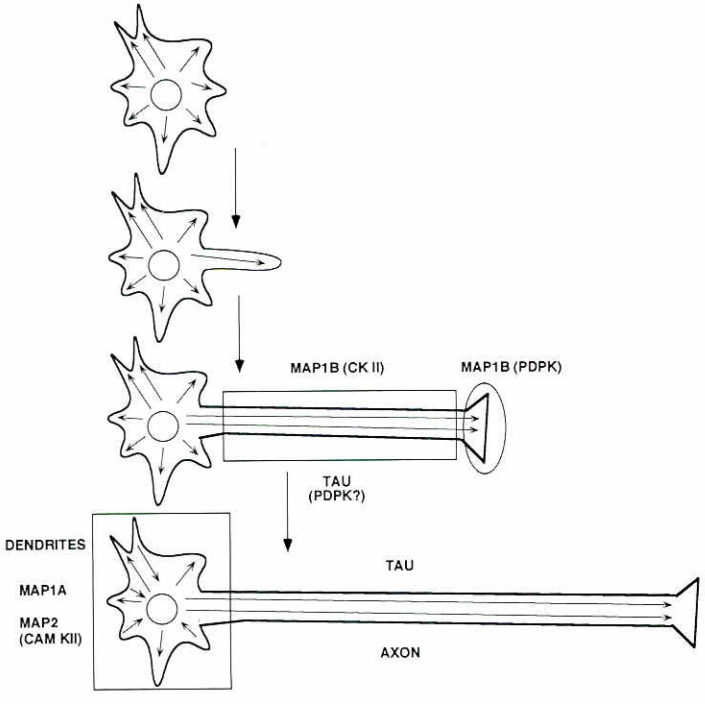
In addition to its association with microtubules, MAP2 is co-localized with actin and associated with actin microfilaments, membrane organelles and the post-synaptic density in dendritic spines (Cáceres *et al.*, 1983; Morales and Fifkova, 1989). In view of these data, a role for MAP2 in the organization of both dendrite and dendritic spine cytoskeletons can be expected.

Regulatory factors controlling MAP2 expression remain to be determined. However, some preliminary evidence emphasizes the role of neuronal-glial interactions in promoting both MAP2 expression and dendrite arborization (Chamak *et al.*, 1987).

MAP2 phosphorylation

MAP2 has been identified as one of the preferred *in vitro* substrates for cAMP-dependent protein kinase (Sloboda *et al.*, 1975; Theurkauf and Vallee, 1983), calcium/calmodulin-dependent protein kinase type II (Yamamoto *et al.*, 1983; Schulman, 1984), protein kinase C (Hoshi *et al.*, 1988) and proline-directed kinases such as MAP kinase (Hoshi *et al.*, 1992) and cdc2 kinase (Faruki *et al.*, 1992). MAP2 can be *in vitro* dephosphorylated by protein phosphatases 1, 2A (Yamamoto *et al.*, 1988) and calcineurin (Goto *et al.*, 1985).

Interestingly, current evidence suggests that some of these phosphorylation and dephosphorylation events may also occur *in vivo* (Tsuyama *et al.*, 1987; Díaz-Nido *et al.*, 1990; Brugg and Matus, 1991; Riederer, 1992; Arias *et al.*, 1993; Díaz-Nido *et al.*, 1993; Montoro *et al.*, 1993). Highly phosphorylated MAP2 containing up to 46 phosphates per molecule binds less efficiently to tubulin than underphosphorylated MAP2 containing up to 16 phosphates per molecule (Tsuyama *et al.*, 1987). However, completely dephosphorylated MAP2 seems to be the least efficient in tubulin binding (Brugg and Matus, 1991).



STAGE	MAPs
Neurite outgrowth	MAP1B
Axonal elongation	MAP1B TAU
Dendrite arborization	MAP1A MAP2

Fig. 4. Hypothetical outline of the contribution of MAP modifications to neuronal morphogenesis. *The initiation of neurite extension is correlated with the phosphorylation of MAP1B. Phosphorylation by a proline-directed protein kinase (PDPK) appears to occur only in growing axons. Phosphorylation of MAP1B by casein kinase II (CK II) may favor microtubule assembly. Axonal elongation is correlated with the expression of tau protein. The balance between microtubule dynamics and stabilization may be controlled by tau phosphorylation and dephosphorylation. The extension and arborization of dendrites is correlated with the expression of MAP1A and high molecular weight MAP2 and with their phosphorylation. One of the putative MAP2 kinases is calcium/calmodulin-dependent protein kinase II (CaMK II).*

In vitro studies have shown that extensive phosphorylation of MAP2 with purified protein kinases decreases its binding to tubulin (Jameson *et al.*, 1980; Jameson and Caplow, 1981; Murthy and Flavin 1983; Hoshi *et al.*, 1988, 1992). At least in the cases of protein kinase C and MAP kinase, this has been correlated with the phosphorylation of sites on the microtubule-binding domain of the MAP2 molecule (Hoshi *et al.*, 1988, 1992). However, nothing is known about the protein kinases responsible for the phosphorylation at other sites which can stimulate the binding of MAP2 to microtubules (Brugg and Matus, 1991).

A physiological role for MAP2 phosphorylation and dephosphorylation in triggering cytoskeletal changes in response to certain neurotransmitters has been suggested. In fact, a rapid and selective MAP2 dephosphorylation after activation of NMDA-type glutamate receptors has been described in rat hippocampus (Halpain and Greengard, 1990Díez-Guerra and Avila, 1993a; Montoro *et al.*, 1993). Hippocampal MAP2 dephosphorylation may be catalyzed by the calcium/calmodulin-dependent phosphatase calcineurin (Montoro *et al.*, 1993) and might lead to a stabilization of the microtubule cytoskeleton (Bigot *et al.*, 1991). On the other hand, the presence of a high concentration of extracellular potassium, which leads to membrane depolarization, results in an up-phosphorylation of MAP2 in which protein kinase C may be implicated (Díaz-Nido *et al.*, 1993). Additionally dendrite arborization has been correlated with an increase in the phosphorylation of hippocampal MAP2 in which calcium/calmodulin-dependent protein kinase may be implicated (Díez-Guerra and Avila, 1993b).

MAP3/MAP4

Similarly to MAP2, there are at least five forms of MAP4 generated by alternative splicing (West *et al.*, 1991). These include proteins previously identified as MAP3 (Huber and Matus, 1990). In contrast to other MAPs, MAP4 proteins are predominantly expressed in non-neuronal tissues. In the brain, MAP4 is only expressed in glial cells and in immature neuroblasts (Bulinski and Borisy, 1980; Olmsted *et al.*, 1986; Huber and Matus, 1990).

Tau

There are also a large number of tau protein isoforms generated by alternative splicing of a primary transcript (Himmler, 1989; Kosik *et al.*, 1989; Lee, 1990; Goedert *et al.*, 1991, 1992; Couchie *et al.*, 1992; Montejo de Garcini *et al.*, 1992). Several tau proteins with apparent molecular masses ranging from 55,000 to 68,000 (as determined from denaturing gel electrophoresis) are found in the central nervous system. Two classes of tau isoforms have been described. One class containing three tubulin binding motifs and another containing four motifs. Isoforms containing three tubulin-binding repeated motifs are predominantly expressed in the developing brain, whereas isoforms containing four tubulin-binding repeats are expressed in the adult brain (Kosik *et al.*, 1989; Lee, 1990; Goedert *et al.*, 1991). These latter tau isoforms are the most efficient in *in vitro* microtubule binding (Goedert and Jakes, 1990; Butner and Kirschner, 1991). Additional tau isoforms with an apparent molecular mass of 110,000 have been identified in the peripheral nervous system (Georgieff *et al.*, 1991). This high molecular weight tau contains four repeated motifs in its microtubule-binding domain, similarly to adult brain tau proteins, but it has an additional 254-amino-acid insertion in the amino-terminal region of the molecule (Couchie *et al.*, 1992; Goedert *et al.*, 1992). A modulatory role of high molecular weight tau in neuritogenesis in cells of the peripheral nervous systems has been suggested (Montejo de Garcini *et al.*, 1992; Teng *et al.*, 1993).

In brain, tau proteins are mainly localized to axons (Binder *et al.*, 1985; Brion *et al.*, 1988), although the presence of some tau

proteins within neuronal cells bodies and dendrites has also been reported (Papasozomenos and Binder, 1987). The accumulation of tau proteins (particularly of the adult isoforms) into axons may partly depend on the prior sorting of mRNA in the proximal segment of the axon (Litman *et al.*, 1993).

Interestingly, the selective inhibition of tau protein expression by treatment of cultured neurons with antisense oligonucleotides leads to a block in the elongation of axon-like neurites (Cáceres and Kosik, 1990; Cáceres *et al.*, 1991; Hanemaaijer and Ginzburg, 1991). Thus, a specific role for tau protein in the microtubule stabilization which occurs during axonal elongation may be hypothesized.

Tau phosphorylation

Tau proteins are modified *in vitro* by several protein kinases, including cyclic AMP-dependent protein kinase (Pierre and Nunez, 1983; Johnson, 1992; Scott *et al.*, 1993a), calcium/calmodulin-dependent protein kinase II (Yamamoto *et al.*, 1983; Steiner *et al.*, 1990; Johnson, 1992), protein kinase C (Baudier *et al.*, 1987; Hoshi *et al.*, 1987; Correas *et al.*, 1992), casein kinase I (Pierre and Nunez, 1983), casein kinase II (Correas *et al.*, 1992) and proline-directed protein kinases such as MAP kinases (Drechel *et al.*, 1992; Drewes *et al.*, 1992; Ledesma *et al.*, 1992), cyclin-dependent kinases (Ledesma *et al.*, 1992; Mawan-Dewal *et al.*, 1992; Vulliet *et al.*, 1992; Liu *et al.*, 1993; Scott *et al.*, 1993b), glycogen synthase kinase-3 (Hanger *et al.*, 1992; Mandelkow *et al.*, 1992) and tau I and II protein kinases (Ishiguro *et al.*, 1992a,b; Arioka *et al.*, 1993). Recently, the identity of glycogen synthase kinase 3 and tau protein kinase I has been demonstrated (Ishiguro *et al.*, 1993) and tau protein kinase II has been identified as the neural-specific cyclin-dependent kinase cdk5 (Hellmich *et al.*, 1992; Lew *et al.*, 1992; Meyerson *et al.*, 1992; Xiong *et al.*, 1992; Hisanaga *et al.*, 1993; Shetty *et al.*, 1993).

Some of the residues modified by these protein kinases have been identified. A serine residue located downstream of the repeats in the carboxy terminus of the molecule can be phosphorylated by calcium/calmodulin-dependent protein kinase (Steiner *et al.*, 1990) and cyclic AMP-dependent protein kinase (Scott *et al.*, 1993a). The functional consequences of phosphorylation at this site are not clear (Drechsel *et al.*, 1992; Johnson, 1992). Serine residues located on the repeats can be phosphorylated by protein kinase C (Correas *et al.*, 1992) and cyclic AMP-dependent protein kinase (Scott *et al.*, 1993a). Phosphorylation at these residues may decrease the binding of tau to tubulin (Correas *et al.*, 1992; Johnson, 1992; Scott *et al.*, 1993a).

Several serine and threonine residues corresponding to (Ser/Thr)-Pro motifs can be phosphorylated by proline-directed protein kinases (Drewes *et al.*, 1992; Hanger *et al.*, 1992; Ishiguro *et al.*, 1992a,b; Ledesma *et al.*, 1992; Mandelkow *et al.*, 1992; Vulliet *et al.*, 1992; Arioka *et al.*, 1993; Liu *et al.*, 1993; Scott *et al.*, 1993b). Phosphorylation at some of these sites also decreases the affinity of tau for tubulin (Drechsel *et al.*, 1992; Gustke *et al.*, 1992), thus reducing the ability of tau to stabilize microtubules (Drechsel *et al.*, 1992). This type of phosphorylation may favor microtubule dynamics during axonal growth in developing neurons (Drechsel *et al.*, 1992; Arioka *et al.*, 1993; Bramblett *et al.*, 1993; Goedert *et al.*, 1993; Pope *et al.*, 1993). Consequently, the dephosphorylation of these sites may contribute to microtubule stabilization during axonal maturation. Both protein phosphatase 2A (Goedert *et al.*, 1992a) and calcineurin (Gong *et al.*, 1993) can readily dephosphorylate these sites *in vitro*. Accordingly, tau phosphorylation by proline-directed kinases has some similarities to the mode I of MAP1B phosphorylation discussed above. It is important to note that the abnormal hyperphosphorylation of tau proteins at these sites in mature neurons may contribute to the microtubule dysfunction which is found in certain neurodegenerative disorders, including Alzheimer's disease (Drewes *et al.*, 1992; Hanger *et al.*, 1992; Ishiguro *et al.*, 1992a,b; Ledesma *et al.*, 1992; Mandelkow *et al.*, 1992; Vulliet *et al.*, 1992; Arioka *et al.*, 1993; Bramblett *et al.*, 1993; Goedert *et al.*, 1993; Liu *et al.*, 1993; Pope *et al.*, 1993).

Similarly to MAP2, an efficient binding of tau to tubulin appears to require the presence of some phosphorylated sites on tau protein (García de Ancos *et al.*, 1993). However, neither the modified sites nor the protein kinases implicated in promoting tubulin-binding have been identified yet.

Phosphorylation and dephosphorylation events at certain sites on the tau molecule may contribute to the generation of axonal and dendritic polarity. Thus, some phosphorylated tau isoforms are mainly localized in neuronal cell bodies and dendrites, whereas other phosphorylated tau isoforms are restricted to axons (Papasozomenos and Binder, 1987; García de Ancos and Avila, 1993; Pope *et al.*, 1993).

Finally, phosphorylation of tau can also modulate the self-association and aggregation of tau (García de Ancos *et al.*, 1993). Tau aggregation may result in the assembly of the Alzheimer's disease-specific paired helical filaments (PHF). Hyperphosphorylated tau is the major PHF component (Grundke-Iqbal *et al.*, 1986; Kosick *et al.*, 1986; Nukina and Ihara, 1986; Wood *et al.*, 1986; Wischick *et al.*, 1987; Ihara *et al.*, 1989; Nieto *et al.*, 1990; Lee *et al.*, 1991; González *et al.*, 1992). However, tau hyperphosphorylation does not directly result in PHF formation (Köpke *et al.*, 1993), so an additional tau modification seems to be required. Recent results suggest that a modification (glycation) in lysine residues on tau protein may contribute to PHF formation (Ledesma *et al.*, unpublished observations). Alternative post-translational modification of tau protein may also lead to tau aggregation into PHF-like polymers (Montejo de Garcini *et al.*, 1986, 1988; Montejo de Garcini and Avila, 1987). In particular, proteolysis may be one of these modifications, as the ability of a tau fragment containing the tubulin-binding domain to self-associate into PHF-like polymers at low pH has been described (Crowther *et al.*, 1992; Wille *et al.*, 1992). A hypothetical model with the putative post-translational modifications which are required to allow tau assembly into PHFs is shown in Fig. 3.

Conclusions and perspectives

Current evidence supports the view that neuronal MAPs determine the microtubule rearrangements underlying neuronal morphogenesis. This can be achieved through the regulation of the expression of particular MAP isoforms at specific cell locations and at distinct developmental stages, as well as through the modification of MAPs by phosphorylation and dephosphorylation.

There are, however, several unresolved issues in this respect. First, it is not clear how subtle structural differences among distinct MAP isoforms may be responsible for different effects on microtubule dynamics and organization. The study of simple models such as *in vitro* assays using recombinant proteins (Brandt and Lee, 1993)

and transfection assays of non-neuronal cells (Chen *et al.*, 1992; Edson *et al.*, 1993; Montejo de Garcini *et al.*, 1993) may be useful to clarify this point. Second, the molecular factors controlling the developmental stage-specific expression of genes coding for MAPs, the alternative splicing of primary transcripts and the subcellular-specific sorting of the mature mRNAs and proteins are entirely unknown. Presumably these topics can only be addressed using either primary cultures of neurons or certain cell lines which exhibit a high degree of neuronal differentiation (McBurney *et al.*, 1988; Tanaka *et al.*, 1992; Pleasure and Lee, 1993). Finally, much more research is required to identify all the sites on MAP molecules which are modified by phosphorylation/dephosphorylation, to determine the functional consequences of these site-specific modifications, to identify the protein kinases and phosphatases responsible for these modifications, and to understand the physiological regulation of these phosphorylation and dephosphorylation events. These studies require the use of the different systems already mentioned (recombinant proteins, transfection assays and neuronal cultures).

Notwithstanding these limitations, available data allow the drawing of a tentative model which may serve as a provisional working hypothesis (see Fig. 4).

Thus, MAP1B is implicated in the initiation of neurite outgrowth (Brugg *et al.*, 1993), which is the first stage of neuronal morphogenesis. Phosphorylation of MAP1B is important in controlling its function; the mode II of phosphorylation may favor the association of MAP1B with microtubules (Díaz-Nido *et al.*, 1988; Ulloa *et al.*, 1993c) whereas the mode I of MAP1B phosphorylation might contribute to the specific dynamics of axonal growth (Mansfield *et al.*, 1992; Ulloa *et al.*, 1993b, 1994).

Tau protein seems to be specifically involved in axonal elongation (Cáceres and Kosik, 1990; Cáceres *et al.*, 1991; Hanemaaijer and Ginzburg, 1991). Phosphorylation of tau protein may constitute an additional mechanism to control the balance between microtubule dynamics and stabilization in developing axons (Drechsel *et al.*, 1992; Arioka *et al.*, 1993; Bramblett *et al.*, 1993; Goedert *et al.*, 1993; Pope *et al.*, 1993).

The dephosphorylation of mode I sites on MAP1B (Ulloa *et al.*, 1993b, 1994) and of similar sites on tau protein (Arioka *et al.*, 1993; Bramblett *et al.*, 1993; Goedert *et al.*, 1993; Pope *et al.*, 1993) may contribute to microtubule stabilization and bundling during axonal maturation.

Both high molecular weight MAP2 (Cáceres *et al.*, 1984; De Camilli *et al.*, 1984; Huber and Matus, 1984; Dinsmore and Solomon, 1991) and MAP1A (Shiomura and Hirokawa, 1987; Schoenfeld *et al.*, 1989) may be implicated in the growth and maturation of dendrites. Likewise, phosphorylation and dephosphorylation may also be important in modulating the function of these proteins in dendrites (Díaz-Nido *et al.*, 1990, 1993; Díez-Guerra and Avila, 1993a,b; Montoro *et al.*, 1993).

This hypothetical model scheme of neuronal morphogenesis emphasizes the major roles performed by phosphorylation and dephosphorylation of MAPs. Indeed, reversible phosphorylation of proteins is the best-established molecular mechanism for the rapid and efficient regulation of intracellular events by extracellular signals (Fischer and Krebs, 1989; Walaas and Greengard, 1991). In this context, MAP phosphorylation and dephosphorylation may induce changes in the cytoskeletal organization in response to those extracellular signals (neurotrophic factors, hormones, neurotransmitters, neuromodulators, adhesion molecules, extracellular matrix proteins, extracellular proteases) which control neuronal morphogenesis through the modulation of the activity of different proteins kinases and phosphatases (Keegan and Halegoua, 1993; Lauder, 1993; Wood and Roberts, 1993).

However, cytoskeletal rearrangements should not be considered restricted to developing neurons. There seems to be a high degree of neuronal plasticity not only throughout development but also in the adult brain. Synaptic connections are presumably capable of modification by activity during the entire life of an organism. Large-scale rearrangements of synaptic contacts occur during development to generate the patterns of connectivity underlying the representations of sensory systems in the brain (Montague, 1993). Similar remodelings of synaptic junctions may reflect some records of learning in the adult brain (Chang and Greenough, 1984; Greenough and Bailey, 1988; Rose, 1991; Bailey and Kandel, 1993). Cytoskeletal modifications may contribute to such synaptic changes, which generally involve the distal portions of neurites. A role for MAPs in synaptic plasticity may therefore be hypothesized. Indeed, preliminary evidence has shown a correlation between some modifications in the expression and/or phosphorylation of MAP2 and certain examples of synaptic plasticity (Aoki and Siekevitz, 1985; Cáceres *et al.*, 1988; Hendry and Bhandari, 1992; Montoro *et al.*, 1993). The study of the MAP modifications related to synaptic remodeling is a promising area for future research.

It is also important to note that aberrant synaptic plasticity has been correlated with Alzheimer's disease (Ashford and Jarvik, 1985; Ihara, 1988; Di Patre, 1991; Masliah *et al.*, 1991, 1992; Masliah and Terry, 1993), a neurodegenerative disorder which is accompanied by an abnormal hyperphosphorylation of some MAPs including MAP1B and tau (Hasegawa *et al.*, 1990; Bramblett *et al.*, 1993; Goedert *et al.*, 1993). It is thus plausible that the disregulation of the phosphorylation systems controlling MAPs and synaptic plasticity may lead to neurodegeneration. Some studies with phosphatase inhibitors are consistent with this possibility (Arias *et al.*, 1993). Furthermore, it has been speculated that reduced expression or abnormally post-translationally modified forms of some MAPs (MAP1B and MAP2) may affect the ability of dendrites to maintain and remodel synaptic junctions in certain neurons of the hippocampal formation in schizophrenic patients (Arnold *et al.*, 1991).

In summary, a better understanding of the molecular properties of MAPs and of their modulation by reversible phosphorylation is not only relevant to the study of neuronal morphogenesis but may also provide important insights into the mechanisms of synaptic plasticity and several neuropathological conditions.

Summary

Neuronal morphogenesis is driven by cytoskeletal changes in which microtubules play a leading role. A very heterogeneous group of microtubule-associated proteins (MAPs) seems to control the dynamics and contribute to the organization of the microtubule cytoskeleton. Of great importance in this regard is the developmental regulation of the expression of certain MAPs in specific neuronal compartments. Furthermore, MAP functionality is also modulated by phosphorylation and dephosphorylation events. A correlation between the expression and/or phosphorylation of distinct MAPs and definite stages of neuronal development may be established. A putative role in synaptic plasticity for MAP modifications similar to those occurring during development can be anticipated. Inter-

estingly, gross alterations in microtubule-associated proteins are found in several neuropathologies including Alzheimer's disease. In this review we focus on recent advances in the understanding of the molecular properties of major neuronal MAPs which may be relevant to these issues.

KEY WORDS: *neurons, axons, dendrites, cytoskeleton, microtubules, phosphorylation, kinases, phosphatases, neurodegeneration, Alzheimer's disease, synaptic plasticity*

Acknowledgments

This work has been supported in part by grants from the Spanish CICYT, the EEC and an institutional grant from Fundación R. Areces.

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Accepted for publication: January 1994