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ARTICLE *in* CELL MOTILITY AND THE CYTOSKELETON · JULY 2008

Impact Factor: 4.19 · DOI: 10.1002/cm.20284 · Source: PubMed

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HMWMAP2: New Perspectives on a Pathway to Dendritic Identity

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Neuronal polarity is established by the differentiation of two types of cytoplasmic processes: dendrites and the axon. These processes can be distinguished by their composition in microtubule-associated proteins, the high molecular weight MAP2 proteins (HMWMAP2) being found in the dendrites and tau proteins in the axon. It is believed that the main contribution of HMWMAP2 to the acquisition and maintenance of dendrites is to promote microtubule assembly and stability. However, recent studies force us to enlarge our view on how HMWMAP2 might contribute to defining the role of the dendritic microtubules. The purpose of this article is to convey our view that HMWMAP2 are important players in defining the contribution of microtubules to dendritic identity by anchoring membranous organelles and signaling proteins to the dendritic microtubules and by being a receptor for neurosteroids. Cell Motil. Cytoskeleton 65: 515–527, 2008.

Key words: MAP2; microtubules; the endoplasmic reticulum; neurosteroids

INTRODUCTION

The respective function of the dendrites and the axon is conferred to them by: (1) their distinct morphology: dendrites are relatively short, have a tapering diameter and are multiple whereas the axon can extend many centimetres from the cell body, presents a uniform diameter along most of its trajectory and is unique in most neurons [Bartlett and Banker, 1984a,b; Dotti et al., 1988; Hillman, 1988], (2) their content in membranous organelles: the rough endoplasmic reticulum (RER) is found in the somato-dendritic compartment but not in the axon and the number of free ribosomes is a lot higher in dendrites than in the axon [Bartlett and Banker, 1984a,b; Peters et al., 1991] and (3) the molecular composition of their cytoskeletal elements: HWMMAP2 are found in the dendrites whereas tau proteins are enriched in the axon [Caceres et al., 1984; Hirokawa, 1991; Ludin and Matus, 1993]. HWMMAP2 have been shown to be important for dendritic differentiation. Their suppression in primary neuronal cultures blocks the differentiation of dendrites and their overexpression in non-neuronal cells induces the formation of cytoplasmic processes similar to dendrites [Caceres et al., 1992; Edson et al., 1993; Langkopf et al., 1995; Leclerc et al., 1993, 1996;

Contract grant sponsor: National Sciences and Engineering Research council of Canada (NSERC); Contract grant sponsor: Canadian Institute of Health Research (CIHR); Contract grant number: MOP-53218; Contract grant sponsor: Fonds de la recherche en santé du Québec (FRSQ).

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Received 4 September 2007; Revised 18 December 2007; Accepted 27 March 2008

Published online 12 May 2008 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/cm.20284

Kalcheva et al., 1998; Boucher et al., 1999; Belanger et al., 2002]. In the adult brain, the role of HMWMAP2 in the maintenance of dendrites is supported by the fact that their suppression or degradation is correlated either to dendritic loss or remodelling [Faddis et al., 1997; Sanchez et al., 2000]. This role was further confirmed in MAP2-deficient mice which present a reduction of dendritic length [Harada et al., 2002]. Khuchua and colleagues also reported that the deletion of the first 158 a.a. from the projection domain of HWMMAP2 by gene targeting disrupts dendritic morphology of the CA1 area of mice hippocampus [Khuchua et al., 2003].

The role of HMWMAP2 in dendritic differentiation and maintenance is believed to depend mainly on their stabilizing effects on microtubules. However, recent data demonstrated that the interaction of HWMMAP2 with microtubules has an enlarged role in the maintenance of dendritic integrity by specifying the binding of membranous organelles to microtubules and by acting as receptors for neurosteroids, factors that exert protective effects on dendritic microtubules. In the present article, we will integrate these new functions of HMWMAP2 with the old ones to reveal the extented contribution of these proteins to dendritic identity. We will firstly review the data on HMWMAP2 structure and isoforms. Secondly, the observations leading to the conclusion that HMWMAP2 act as microtubule stabilizing proteins will be presented. Thirdly, the pivotal role of HMWMAP2 in the segregation of signalling proteins in dendrites by their anchorage to microtubules will be discussed. In particular, we will focus on their role as neurosteroid receptors. Fourthly, the contribution of HMWMAP2 to the segregation of RER membranes in the somato-dendritic compartment will be discussed. Finally, a model that will integrate the new with the old functions of HMWMAP2 will be presented in regard to the role of HMWMAP2 in dendritic differentiation and maintenance.

STRUCTURE AND ISOFORMS OF MAP2

MAP2 proteins are rod-like proteins that can form anti-parallel dimers [Wille et al., 1992a,b]. They can be divided in two major functional domains: the projection domain located at the N-terminal extending at the surface of microtubules and the microtubule-binding domain located at the C-terminal containing 3–4 imperfect repeats of 18 amino acid (a.a.) responsible for its binding to microtubules [Lewis et al., 1988, 1989]. Between the microtubule-binding domain and the projection domain lies a proline-rich region which is thought to regulate microtubule binding and assembly activities through intramolecular interactions with the repeat regions of the microtubule-binding domain [Sanchez et al., 1996; Felgner et al., 1997; Goode et al., 1997]. Human MAP2 sequences are contained within 19 exons found on chromosome 2q33-35 [Neve et al., 1986; Goedert et al., 1991; Kalcheva et al., 1995; Shafit-Zagardo and Kalcheva, 1998]. A diversity of MAP2 isoforms is generated by alternative splicing in the mammalian central nervous system [Neve et al., 1986; Shafit-Zagardo and Kalcheva, 1998]. There exists at least four MAP2 isoforms that can be classified into two groups in mammalian brain: (1) HMWMAP2: MAP2a (280kDa) and MAP2b (270kDa) and (2) LMWMAP2: MAP2c (70kDa) and MAP2d (75kDa). The main difference between HMW and LMWMAP2 isoforms is the insertion of 1372 a.a. encoded by exons 9–11 in the projection domain of HMWMAP2 [Lewis et al., 1988; Kindler et al., 1990]. MAP2a differs from MAP2b by the insertion of an additional sequence of 82 a.a. encoded by exon 8 in its projection domain [Chung et al., 1996; Kalcheva et al., 1995, 1998]. MAP2d contains 4 repeats in the microtubule-binding domain whereas MAP2c contains 3 [Ferhat et al., 1994]. The first, second and third repeats are encoded by exons 15, 17 and 18 respectively and the fourth repeat by exon 16 [Doll et al., 1993; Ferhat et al., 1994]. In human fetal spinal cord, six additional MAP2 transcripts generated by alternative splicing of exons 8 and 13 have been identified [Kalcheva et al., 1997].

In mammalian brain, MAP2a and MAP2b are neuron-specific while MAP2c and MAP2d can also be found in glial cells [Vouyiouklis and Brophy, 1995; Richter-Landsberg and Gorath, 1999]. In neurons, HMWMAP2 isoforms are present in the cell body and dendrites whereas LMW isoforms are uniformly distributed in all neuronal compartments [Caceres et al., 1984, 1986; Tucker and Matus, 1988; Meichsner et al., 1993; Albala et al., 1995; Chung et al., 1996].

The expression of MAP2 isoforms is developmentally regulated in the mammalian brain. The LMW-MAP2c isoform is only expressed during development while HMWMAP2 isoforms remain expressed in the adult brain [Tucker et al., 1988; Viereck et al., 1989; Tucker, 1990; Doll et al., 1993; Ferhat et al., 1994; Chung et al., 1996]. However, MAP2c is found in regions of the adult brain presenting neuritogenesis such as the olfactory bulb [Tucker and Matus, 1988; Viereck et al., 1989; Tucker, 1990].

STABILIZATION AND ORGANIZATION OF MICROTUBULES BY MAP2 ISOFORMS

MAP2 proteins bind longitudinally along the outer ridges of the microtubule protofilaments [Al-Bassam et al., 2002]. In vitro, they promote the assembly and stabilization of microtubules by reducing the rate of rapid shortening of microtubules and increasing the elongation

rate [Hirokawa, 1991]. The suppression of all MAP2 isoforms in primary neuronal cultures was correlated to a decreased assembly of microtubules [Caceres et al., 1992]. In neuronal cultures, MAP2c was shown to induce the assembly and bundling of microtubules during initiation of neurite outgrowth [Dehmelt et al., 2003]. In non-neuronal cells, the overexpression of either MAP2c or MAP2b induces the formation and stabilization of microtubules [Weisshaar et al., 1992; Leclerc et al., 1993; Matus, 1994]. Overexpression of MAP2c induces the formation of multiple thin bundles of microtubules in Sf9 cells whereas that of MAP2b results in the formation of a single thick bundle [Belanger et al., 2002].

MAP2 proteins also determine the spacing between microtubules [Chen et al., 1992; Cunningham et al., 1997; Belanger et al., 2002]. MAP2b and MAP2c induce a spacing between microtubules of ~60 nm and ~20 nm, respectively. Several studies indicated that the length of the projection domain was the main determinant of the spacing between microtubules [Chen et al., 1992; Cunningham et al., 1997; Belanger et al., 2002]. To further investigate this point, we generated truncated forms of MAP2b deleted from different portions of the projection domain and expressed them in Sf9 cells to measure their effects on microtubule spacing [Belanger et al., 2002]. This study led us to conclude that the length of the MAP2b projection domain is not the sole determinant of the spacing between microtubules in dendrites. The projection domain of HMWMAP2 is highly unstructured and flexible and the flexibility properties of this domain might vary along its length [Voter and Erickson, 1982; Wille et al., 1992a]. This could explain why the expression of MAP2b truncated forms affected differently the spacing between microtubules.

Although the above observations strongly suggest that MAP2 proteins contribute to the stabilization of microtubules within a neuron, adult MAP2-deficient mice only showed a moderate reduction of microtubule density (23% less than in wild-type mice) in the dendrites [Harada et al., 2002]. From these data, one can conclude that HMWMAP2 might possess additional functions to that of stabilizing proteins at the surface of microtubules. These functions will be discussed in the following sections.

ROLE OF HMWMAP2 IN DEFINING DENDRITIC IDENTITY BY LINKING SIGNALING PATHWAYS TO MICROTUBULES

Segregation of Signaling Proteins in the Somato-Dendritic Compartment

Microtubules contribute to the elaboration of the distinct function of a cellular compartment by interacting with signaling proteins that determine its response to external cues. In this context, the enrichment of a distinct pool of MAPs such as HMWMAP2 and tau in dendrites and axon, respectively, is likely to be involved in the segregation of signaling proteins in these two types of processes. Signaling proteins involved in dendritic plasticity were shown to interact with HMWMAP2. This is the case of the c-AMP dependent protein kinase (PKA) [Obar et al., 1989; Rubino et al., 1989; Davare et al., 1999]. The absence of HMWMAP2 leads to a reduced amount of dendritic and total PKA and reduced activation of CREB, a transcription factor activated by PKA, in MAP2-deficient mice [Harada et al., 2002]. More recently, expression of the kinase-associated phosphatase (KAP) was shown to be reduced in the cortical dendrites as well as its amount bound to microtubules in MAP2-deficient brains [Iriuchijima et al., 2005]. This study suggested an indirect association between KAP and HMWMAP2 through its interaction with PKA [Iriuchijima et al., 2005]. HWMMAP2 were also recently shown to link the signaling pathway of the small GTPase Ras to microtubules through an interaction with very-KIND (v-KIND), a novel brain-specific RasGEF preferentially localized in the somato-dendritic compartment [Huang et al., 2007].

Receptors for Neurosteroids

MAP2 proteins have the most intriguing interaction with neurosteroids. So far, MAP2 proteins are considered the sole brain specific receptor of neurosteroids. Neurosteroids refer to steroids which can be synthesized in the brain independently of gland secretion system [Plassart-Schiess and Baulieu, 2001]. The major steroids in the brain include 3 beta-hydroxy-delta 5-compounds such as pregnenolone (PREG), pregnenolone-sulfate (PREG-S), dehydroepiandrosterone (DHEA) and dehydroepiandrosterone-sulfate (DHEA-S) [Robel and Baulieu, 1995; Tsutsui et al., 2000]. They can all be synthesized from cholesterol de novo in various types of brain cells such as Purkinje cells and glial cells [Robel and Baulieu, 1995; Tsutsui et al., 2000] or they can be synthesized from steroidal precursors imported from peripheral sources [Baulieu, 1997]. Neurosteroids have several beneficial effects on neurons. For example, they were shown to enhance neuronal survival and long-term memory [Roberts et al., 1987; Flood et al., 1992], to exert neuroprotective effects [Cardounel et al., 1999; Marx et al., 2000] and to stimulate neurogenesis in rat hippocampus [Karishma and Herbert, 2002].

Neurosteroids act mostly via binding to specific nuclear receptors and through modulation of neurotransmitter receptor function at the plasma membrane level [Parsons et al., 1982; Monnet et al., 1995]. For example, PREG-S and DHEA-S are excitatory neurosteroids that

antagonize inhibitory γ-aminobutyric acid ($GABA_A$) receptors and potentiate the excitatory N-methyl-D-aspartate (NMDA) receptors [Morrow et al., 1987; Purdy et al., 1990; Irwin et al., 1994; Monnet et al., 1995; Bergeron et al., 1996; Imamura and Prasad, 1998; Hige et al., 2006; Wang et al., 2006]. No specific neurosteroid receptors were identified in the brain until recently when MAP2 proteins were shown to play such a role. The binding domain of HWMMAP2 to PREG has not been characterized yet. The LWMMAP2 isoform, MAP2c, was identified as the first brain specific receptor for DHEA [Laurine et al., 2003]. The N-terminal region of MAP2c mediates its binding to DHEA. MAP2c shows sequence homologies with 17β-hydroxysteroid dehydrogenase 1, an enzyme required for estrogen synthesis. Based on these sequence homologies, the binding of DHEA to MAP2c was modeled [Laurine et al., 2003]. The binding of DHEA to MAP2c would involve hydrophobic residues that would form a hydrophobic pocket with highly specific hydrogen bonds that orient DHEA into the pocket. HWMMAP2 most likely also interact with DHEA since all of the six regions forming the DHEA binding pocket are found in these proteins.

The functional significance of the binding of PREG to HMWMAP2 was investigated in vitro and in vivo assays. Both types of assays demonstrated that the binding of PREG increases the HMWMAP2 driven microtubule assembly and stability [Murakami et al., 2000; Fontaine-Lenoir et al., 2006]. Moreover, the binding of PREG to HMWMAP2 is increased more than 8-fold when HMWMAP2 is bound to microtubules. In nerve growth factor (NGF)- pretreated PC12 cells, the enhancement of neurite outgrowth induced by PREG and its chemically synthesized analog 3β-methoxypregnenolone (MePREG) is correlated to an increase of HMWMAP2 immunostaining [Fontaine-Lenoir et al., 2006]. Furthermore, the pretreatment of PC12 cells with PREG or MePREG has a protective effect against nocodazole-induced retraction of neurites. The suppression of HMWMAP2 expression by RNA interference confirmed that the stimulatory effects of PREG and MePREG on neurite extension occured through HMWMAP2 [Fontaine-Lenoir et al., 2006]. PREG induces HMWMAP2 expression in the hippocampus and nucleus accumbens in adult rat brain when it is administered to rats during the neonatal period [Iwata et al., 2005]. So far, no functional significance was demonstrated for the binding of DHEA to MAP2c. In primary cortical neurons, DHEA was shown to selectively increase the length of tau-positive neurites whereas DHEA-S selectively increases the length of MAP2-positive neurites. The contribution of MAP2 isoforms to these effects of DHEA and DHEA-S on neurite outgrowth has not been explored yet. The PKA-binding domain of MAP2 (residues 82–113) partially overlaps with its DHEA-binding domain (residues 108–119). It is possible that DHEA binding to MAP2 proteins interferes with the binding of PKA to these proteins. This could have protective effects on microtubules and prevent excessive and aberrant dendritic remodeling when PKA, a kinase known to phosphorylate MAP2 proteins and to decrease their binding to microtubules, is abnormally activated in pathological conditions.

SEGREGATION OF RER MEMBRANES IN THE SOMATO-DENDRITIC COMPARTMENT

The somato-dendritic compartment is characterized by the presence of RER membranes. The mechanisms underlying the segregation and/or the maintenance of the RER membranes in this neuronal compartment seem to involve HMWMAP2. In a recent study, we reported that HMWMAP2 are associated with the rough endoplasmic reticulum (RER) [Farah et al., 2005] and that this association is mediated by the interaction of the HMWMAP2 projection domain with the RER protein termed cytoskeleton-linking membrane protein-63 (CLIMP-63). CLIMP-63 is an integral membrane protein that links RER membranes to microtubules in non-neuronal cells [Klopfenstein et al., 1998]. The function of HMWMAP2 on the surface of RER membranes is most likely to anchor these membranes to microtubules and thereby to contribute to their segregation in the somato-dendritic compartment [Bartlett and Banker, 1984a,b; Peters et al., 1991]. We showed that the interaction of HMWMAP2 with CLIMP-63 is necessary for the association of RER membranes with microtubules in an in vitro assay [Farah et al., 2005]. Several studies reported that the ER membranes are associated with microtubules [Terasaki et al., 1986; Terasaki, 1990; Terasaki and Reese, 1994; Hirokawa, 1998; Aihara et al., 2001]. Single point attachments were visualized by electron microscopy [Baumann and Walz, 2001]. This association is either dynamic for trafficking of ER membranes or stable for the positioning of these membranes within a cell [Terasaki, 1990; Cole and Lippincott-Schwartz, 1995; Hirokawa, 1998]. Depolymerization of microtubules using the drug nocodazole affects both the trafficking and positioning of ER membranes [Terasaki et al., 1986; Terasaki and Reese, 1994; Waterman-Storer and Salmon, 1998; Aihara et al., 2001].

Proteins called CLIPs (cytosolic linker proteins) establish a link between microtubules and membranous organelles. As such, CLIP-170 mediates the interaction of endocytic carrier vesicles to microtubules [Pierre et al., 1992]. CLIP-115 is responsible for the polarized distribution of the dendritic lamellar bodies (DLB) in neurons by anchoring them to the dendritic microtubules

[De Zeeuw et al., 1997; Hoogenraad et al., 2000]. Recently, a new class of proteins termed CLASPs (CLIP-associated proteins) was identified [Akhmanova et al., 2001]. These proteins bind CLIPs and microtubules and have a microtubule-stabilizing effect. A family of proteins named Hooks also mediates the interaction between microtubules and membrane organelles. More specifically, Hook3 links the Golgi membranes to microtubules [Walenta et al., 2001]. We decided to classify MAP2 proteins in a category of their own since: 1- the microtubule-binding domain of MAP2 has no sequence homology with that of known CLIPs, CLASPs, Hooks and CLIMPs and 2- all the linker proteins identified so far bind to growing end of microtubules whereas MAP2 proteins bind to microtubules along their length [Al-Bassam et al., 2002].

The transport of ER membranes by microtubules can be generated by three strategies: 1- Motor proteins can drag membranes along microtubules 2- Motor proteins drive the sliding of membrane-associated microtubules and 3- ER membranes are bound to the growing tip of microtubules and the movement of ER membranes is generated by the polymerization of microtubules. From these data, one can speculate that proteins that attach membranes at the growing end of microtubules could mediate the transport of membranous organelles to a specific cellular compartment whereas proteins like HMWMAP2 that link membranous organelles along the length of microtubules could be responsible for the long-term anchorage of these organelles to microtubules.

The binding of microtubules to RER was shown to decrease the mobility of the translocon and thereby to create domains of protein synthesis in the RER in non-neuronal cells [Nikonov et al., 2007]. The association of HMWMAP2/microtubules with RER could contribute to define such domains in the somato-dendritic compartment.

CONTRIBUTION OF HMWMAP2 TO DENDRITIC DIFFERENTIATION AND MAINTENANCE: INTEGRATIVE MODEL OF HMWMAP2 OLD AND NEW FUNCTIONS

Several external cues that modulate dendritic outgrowth and remodeling affect HMWMAP2 expression and/or phosphorylation indicating that HMWMAP2 could be effectors of these pathways. During differentiation, sculpting of the dendritic arborization requires several external cues such as neurotrophins, neurosteroids, guidance molecules and neuronal activity [Compagnone and Mellon, 1998; Parrish et al., 2007]. All these external signals have to exert an effect on the architectural elements of the dendrites, mainly microtubules and actin microfilaments (F-actin), to induce dendritic outgrowth and stabilization. The organization of microtubules and F-actin within a cell is highly regulated by the Rho GTPases. These are convergent and integrative signaling molecules of external cues [Van Aelst and Cline, 2004; Charest and Firtel, 2007]. In recent years, the respective roles of the Rho GTPases, RhoA, Rac1 and Cdc42 was examined during dendritic differentiation and it was found that RhoA controls branch length whereas Rac1 and Cdc42 regulate branch dynamics [Van Aelst and Cline, 2004]. Rho GTPases are activated by guanine nucleotide exchange factors (GEFs) such as Kalirin which were shown to be necessary for maintenance of hippocampal pyramidal dendrites and dendritic spines [Luo, 2002; Quilliam et al., 2002; Ma et al., 2003]. Kalirin have multiple Rho GEF domains, allowing them to interact with multiple Rho proteins. Several isoforms of Kalirin generated by alternative splicing exist within a neuron, Kal-7 and Kal-12 being concentrated in the soma and dendrites in hippocampal neurons [Quilliam et al., 2002]. The suppression of all Kalirin isoforms using antisense methods results in a reduction of dendritic spines and a simplification of the dendritic tree [Ma et al., 2003].

Phosphorylation regulates the binding of HMWMAP2 to microtubules. An increase of HMWMAP2 phosphorylation is correlated to their reduced binding to microtubules and an increase of microtubule dynamics and dendritic outgrowth [Sanchez et al., 2000]. Kinases known to phosphorylate HMWMAP2 are effectors of Rho GTPases. HMWMAP2 were reported to be a substrate of Rho-kinase and myosin phosphatase, two effectors of Rho [Amano et al., 2003]. The effect of HMWMAP2 phosphorylation by these Rho effectors on their ability to bind to microtubules and F-actin has not yet been characterized. The phosphorylation of HMWMAP2 by the c-Jun NH_2-terminal protein kinase-1 (JNK-1), a downstream kinase of Rac1, was recently shown to be essential for the formation of dendrites. HMWMAP2 are hypophosphorylated in JNK-1 (-/-) mice compromising their ability to promote microtubule assembly and this appeared to be detrimental to the elaboration of dendritic morphology [Chang et al., 2003]. A subsequent study confirmed these results and reported that the activation of JNK-1 was necessary for HMWMAP2 to induce process formation in COS cells [Bjorkblom et al., 2005]. However, in a recent study, the phosphorylation of HMWMAP2 by JNK-1 was correlated to the impairment of dendritic formation when the Ras signaling pathway was activated by the overexpression of v-KIND [Huang et al., 2007]. Using a specific inhibitor for JNK-1, HMWMAP2 phosphorylation was reduced when v-KIND was overexpressed in COS cells. The authors proposed that v-KIND increases HMWMAP2 phosphorylation by JNK-1 and this induces

their detachment from microtubules and consequently a reduction of dendrites. Several reasons could explain these contradictory results on the effects of HMWMAP2 phosphorylation by JNK-1 on dendritic formation. In the first study carried out in JNK-1 (-/-) mice, although it is clear that suppression of JNK-1 activity results in a decrease of HMWMAP2 phosphorylation and an alteration of dendritic morphology, no experiment was performed to demonstrate the direct involvement of the reduced HMWMAP2 phosphorylation state in the aberrant dendritic morphology in these mice [Chang et al., 2003]. In the second study reporting that HMWMAP2 phosphorylation by JNK-1 was necessary for process formation in COS cells it was not demonstrated that the increase number of processes was caused by an increase binding of phosphorylated HMWMAP2 to microtubules [Bjorkblom et al., 2005]. JNK-1 seems to be able to phosphorylate several sites of HMWMAP2 [Bjorkblom et al., 2005]. From the above studies, one can conclude that some sites can decrease HMWMAP2 binding to microtubules whereas others can increase it. JNK-1 can be activated by several signaling pathways. For example, in the study on v-KIND, it is possible that the activation of the Ras pathway leads to the activation of a pool of kinases inducing a conformation of HMWMAP2 that renders possible the phosphorylation of certain sites by JNK-1 that decrease their microtubule binding activity. Finally, none of the above studies has clearly demonstrated that the alteration of HMWMAP2 phosphorylation is caused by the direct phosphorylation of HMWMAP2 by JNK-1.

Contradictory results exist for other kinases such as calcium/calmodulin dependent protein kinase II (CaMKII) and extracellular signal regulated kinases (ERKs). External signals also activate calcium-dependent intracellular signaling pathways that regulate dendritic outgrowth. Neuronal activity is accompanied by an increase in intracellular calcium $[Ca^{2+}]_i$ levels which triggers the signaling pathway of the CaMKII [Lohmann and Wong, 2005]. In sympathic neurons, Vaillant and colleagues demonstrated that dendritogenesis was induced by the activation of both CaMKII and the extracellular signal regulated kinases (ERKs) [Vaillant et al., 2002]. The activation of these kinases resulted in increased HMWMAP2 association with microtubules. This contradicts previous in vitro studies demonstrating that the phosphorylation of HMWMAP2 by CaMKII and ERKs decreased their binding affinity for tubulin and its tubulin assembly ability [Sanchez et al., 2000]. However, it is important to note that Vaillant and colleagues reported an increase of HMWMAP2 binding to microtubules and an increase of HMWMAP2 protein levels but they did not report an increase of HMWMAP2 phosphorylation [Vaillant et al., 2002]. This last point, crucial to conclude that phosphorylation of HMWMAP2 by CaMKII and ERKs was responsible for their binding to microtubules, was not investigated in this study. The increased binding of MAP2 proteins to microtubules could merely be explained by the increase of HMWMAP2 protein levels. The accumulation of contradictory results in regard of HMWMAP2 phosphorylation and dendritic formation is mainly caused by the paucity of tools to investigate the phosphorylation state of MAP2. Studies on the axonal homologue of MAP2, tau, have revealed that it is not the level of phosphorylation but rather the sites that are phosphorylated that influence its binding to microtubules. It could also be the case for HMWMAP2. HMWMAP2 can be phosphorylated by kinases other than the ones mentioned above. These kinases are members of the Ser/Thr kinases, Proline-directed kinases, Tyrosine kinases and the microtubule-affinity regulating kinases (MARKs) families respectively [Drewes et al., 1997; Drewes et al., 1998; Sanchez et al., 2000; Zamora-Leon et al., 2001, 2005]. In the present review, we focused on the most recent studies on HMWMAP2 phosphorylation and dendritogenesis.

From the above observations, one can conclude that HMWMAP2 are effectors of the main signaling pathways that lead to dendritic differentiation. We present below a model in which we speculate on the respective role of HMWMAP2 at each step of dendritogenesis and we integrate in this model the newly-discovered interactions of HMWMAP2 to the previously described ones:

Step 1- Breaking the neuronal sphere. After their exit from cell division, neurons are spherical with surrounding lamellipodia. The first event towards the acquisition of dendrites and an axon by a neuron is the formation of minor neurites with no dendritic and axonal identity. This is achieved by the breaking of the neuronal sphere (Fig. 1A). This step was reviewed by Da Silva and Dotti [2002] and involves the relaxation of the actin cytoskeleton by actin-severing proteins (ex. Profilin) at the sites where neurites will emerge. MAP2 proteins are known to bind and cross-link actin filaments in vitro and in vivo [Cunningham et al., 1997; Boucher et al., 1999; Ozer and Halpain, 2000; Belanger et al., 2002; Dehmelt and Halpain, 2004]. A recent study reported that the actin-binding domain of MAP2 proteins is located in the microtubule-binding domain [Roger et al., 2004]. As noted for their binding to microtubule, MAP2 proteins binding to F-actin is regulated by phosphorylation. We speculate that during this step the phosphorylation state of MAP2 isoforms will not favor their association with F-actin to allow the severing proteins to bind to this cytoskeletal element.

During this initial step, microtubules have to be released from the centrosome for their transport into the future neurites. The microtubule-severing protein, kata-

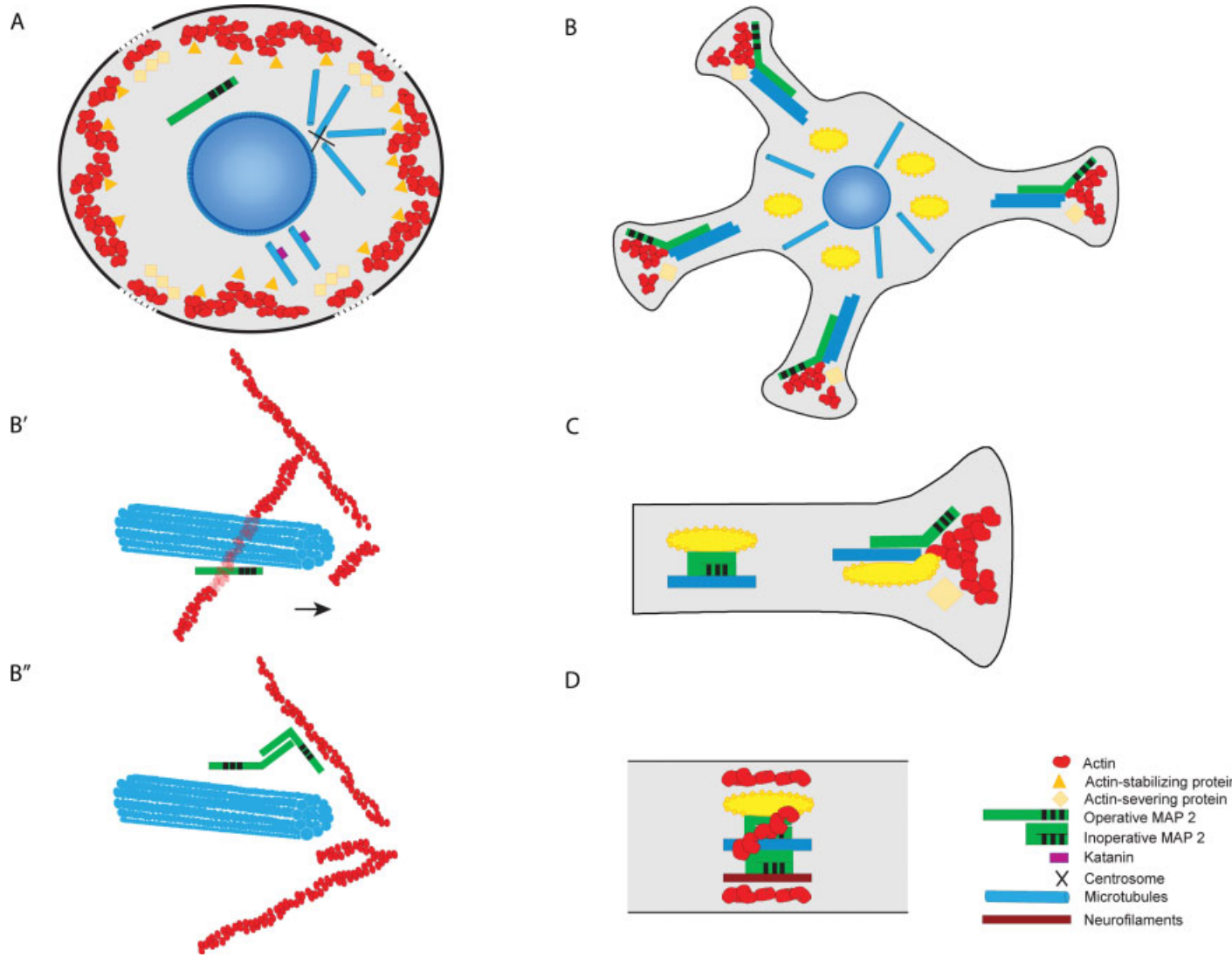

Fig. 1. Schematic representation of the steps involved in dendritogenesis: (**A**) After plating, neurons are round cells with surrounding lamellipodia. Actin- and microtubule-severing proteins are active during this step. The dashed lines indicate the sites of emergence of the minor neurites. (**B**) The interaction of MAP2 with F-actin and microtubules appears to be necessary for the formation of minor neurites. A single MAP2 molecule (**B′**) or MAP2 antiparallel dimers (**B″**) could link together F-actin and microtubules. MAP2 would be in an operative conformation. (**C**) During the elongation and stabilization of dendrites, HMWMAP2 would stabilize microtubules and would mediate their association with RER membranes in the proximal region of the growing dendrites. HMWMAP2 would be in an inoperative conformation in this region. In the distal region of the dendrites, HMWMAP2 would be in an operative conformation. MAP2c would play similar roles in the axon. (**D**) For the maintenance of dendrites, MAP2 could act as a cytoskeletal integrator by consolidating the interactions between the three cytoskeletal elements, F-actin, microtubules, and neurofilaments. MAP2 would be in an inoperative conformation.

nin was shown to be responsible for the detachment of microtubules from the neuronal centrosome [Ahmad et al., 1999]. The severing of microtubules by katanin is essential to shorten microtubules in the cell body and to facilitate their transport in the neurites. MAP2c was shown to compete with katanin for binding to microtubules during neurite formation [Qiang et al., 2006]. We speculate that during this initial step of dendritic formation, the phosphorylation state of MAP2 isoforms will prevent their binding to microtubules to allow Katanin binding. In conclusion, MAP2 proteins would not play an active role during this step.

Step 2- Formation of minor neurites: During this step, F-actin is reorganized from lamellipodia to growth cones and microtubules are stabilized and invade the lamellipodia [Dehmelt et al., 2003]. The juvenile isoform, MAP2c was shown to contribute to the reorganization of F-actin and to the stabilization of microtubules during this step [Dehmelt et al., 2003] (Fig. 1B). MAP2c has to be phosphorylated by PKA to exert these effects. It remains unclear whether MAP2c acts separately on microtubules and F-actin or whether it mediates the interaction between these two cytoskeletal elements during this step. Indeed, the coordination of F-actin and microtubules during neurite outgrowth is believed to necessitate proteins able to simultaneously interact with both of these cytoskeletal elements. An in vitro binding assay demonstrated that HMWMAP2 bound to microtubules had a reduced ability to bind to F-actin whereas HMWMAP2 bound to F-actin was able to bind to microtubules [Pedrotti et al., 1994]. These data indicate that MAP2 proteins could contribute to the cross-talk between F-actin and microtubules at the initial step of neurite outgrowth.

Our work in Sf9 cells revealed that the projection domain of MAP2b regulates its process outgrowth activity through intramolecular interactions with the microtubule-binding domain [Belanger et al., 2002]. We speculate that these intramolecular interactions only exist under certain conformational states of MAP2b that are related to its phosphorylation. By activating kinases and phosphatases, external cues would cause a modification of the phosphorylation state of MAP2b and of its conformation leading to the existence of the protein in either an operative form where the projection domain is not folded back and does not interact with the microtubule-binding domain (open conformation) or an inoperative form where the projection domain is folded back and interacts with the microtubule-binding domain (closed conformation). We propose that MAP2b mostly exists in an operative form during dendritic differentiation. The existence of MAP2b in an operative form would: (1) allow its binding to F-actin and microtubules and (2) allow binding of signalling proteins to MAP2b since the interacting sites would be accessible in this conformation. MAP2c that lacks the 1372 a.a. insert in its projection domain, would only exist in an operative form (open conformation). As mentioned above, this isoform remains expressed in adult brain regions where neurite outgrowth is observed.

Step 3- Elongation and stabilization of the dendritic processes: During this step, the proximal region of the dendritic process is stabilized whereas the distal portion is motile (Fig. 1C). HMWMAP2 would stabilize microtubules in the proximal region of the dendritic process whereas MAP2c would help to coordinate F-actin and microtubules in the distal region of the neurite. HMWMAP2 binding to microtubules would be enhanced by their interaction with neurosteroids during this step. Most importantly, HMWMAP2 would also mediate the interaction between microtubules and RER membranes in the proximal region whereas in the distal one, RER membranes would be attached to the plus end of microtubules to be transported in the growing dendrites. Calcium release by the RER was shown to be necessary to stabilize dendrites [Lohmann et al., 2002]. Thus HMWMAP2 would contribute to the maintenance of dendrites by stabilizing microtubules and by anchoring the RER to microtubules. ER membranes have to be transported from the dendritic shaft to dendritic spines. This necessitates the transfer of ER membranes from microtubules to the actin cytoskeleton, the main cytoskeletal element in spines. The ability of HMWMAP2 to interact with F-actin, microtubules and ER membranes indicates that they might be involved in coordinating this transfer. The presence of HMWMAP2 in dendritic spines was the first in vivo observation indicating that HMWMAP2 can interact with F-actin [Caceres et al., 1983; Morales and Fifkova, 1989]. However, the localization of HMWMAP2 in dendritic spines remains controversial to this date. A study reported that the detection of HMWMAP2 in spines was an artifact due to the fixation procedure [Kaech et al., 1997]. Another major difference in the experimental approach used to detect HMWMAP2 in spines could account for the controversy. The two first studies reporting HMWMAP2 in spines were done in intact tissue from rat brain whereas the last one was performed in primary neuronal cultures. HMWMAP2 might exist in a different phosphorylation state in vivo and in vitro. Phosphorylation regulates the F-actin binding activity of HMWMAP2 [Sanchez et al., 2000]. It is possible that the phosphorylation state of HMWMAP2 in neuronal cultures significantly decreases their association with F-actin rendering it difficult to detect. Another possibility is that the presence of HMWMAP2 in spines is transitory and is only detectable in specific conditions that do take place in vivo but not in vitro.

Step 4- Maintenance of the dendrites: HMWMAP2 would adopt an inoperative conformation to stabilize the dendrites (Fig. 1D). They would act as an integrator of the cytoskeleton by interacting with the three elements of the cytoskeleton. Indeed, besides interacting with microtubules and F-actin, HMWMAP2 were shown to interact with the 70kDa neurofilament subunit in vitro [Leterrier et al., 1982; Heimann et al., 1985; Hirokawa et al., 1988]. Although no study has demonstrated that this interaction exists within a neuron, immunogold labeling experiments revealed that HMWMAP2 were present in the cross-bridges found between microtubules and neurofilaments [Hirokawa et al., 1988]. Finally, HMWMAP2 would also contribute to the maintenance of the dendritic identity by the compartmentalization of signaling proteins and by the anchoring of RER membranes to dendritic microtubules.

CONTRIBUTION OF HMWMAP2 TO NEURODEGENERATION IN ALZHEIMER DISEASE

In Alzheimer disease (AD), the microtubule-associated protein, tau accumulates in the somato-dendritic compartment and becomes hyperphosphorylated [Duff, 2006]. It was reported that HMWMAP2 detache from microtubules by being sequestered by hyperphosphorylated tau in AD brain [Alonso et al., 1997]. The removal of HMWMAP2 from the dendritic microtubules could contribute to increase microtubule dynamics and thereby to induce the aberrant remodeling of dendrites noted at an early stage of the disease [Arendt, 2001]. Tau is also a substrate for several kinases that phosphorylate HMWMAP2 such as PKA, CaMKII, ERKs and JNK1 [Avila, 2006]. By accumulating in the somato-dendritic compartment, tau could compete with HMWMAP2 for these kinases and alter HMWMAP2 phosphorylation level. This alteration could lead to the aberrant remodeling of dendrites and ultimately to their retraction in AD brain.

Recent studies have suggested that neurosteroids could be neuroprotective by their stabilizing effects on dendritic microtubules through the increase of HMWMAP2 binding to microtubules. A decrease of neurosteroids was noted in AD brain [Weill-Engerer et al., 2002]. This decrease and the sequestration of HMWMAP2 by hyperphosphorylated tau could weaken the dendritic microtubule network in AD brain. Any therapeutic strategy based on the increase of neurosteroids and their binding to HMWMAP2 could be employed to maintain a functional microtubule network in dendrites and ultimately to prolong neuronal survival in AD brain.

CONCLUSIONS

HMWMAP2 proteins are common effectors of several signaling proteins that contribute to sculpt the dendritic arborization. Their new protein interactions force us to conclude that HMWMAP2 proteins are much more than simple microtubule-stabilizing proteins. Indeed, by connecting the RER membranes to microtubules and acting as receptors for neurosteroids, HMWMAP2 could define the interpretation and responses of dendrites to external cues. Several MAP2 isoforms exist within a neuron. The current most challenging task for cell biologists is to elucidate the respective role of each MAP2 isoform in the acquisition and maintenance of dendrites.

ACKNOWLEDGMENTS

The authors would like to thank Manon Moreau-Gauthier for her excellent work on the graphical illustrations included in Fig. 1.

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