Review

Microtubule-associated protein 1B, a growth-associated and phosphorylated scaffold protein

Beat M. Riederer

Abstract

Microtubule-associated protein 1B, MAP1B, is one of the major growth associated and cytoskeletal proteins in neuronal and glial cells. It is present as a full length protein or may be fragmented into a heavy chain and a light chain. It is essential to stabilize microtubules during the elongation of dendrites and neurites and is involved in the dynamics of morphological structures such as microtubules, microfilaments and growth cones. MAP1B function is modulated by phosphorylation and influences microtubule stability, microfilaments and growth cone motility. Considering its large size, several interactions with a variety of other proteins have been reported and there is increasing evidence that MAP1B plays a crucial role in the stability of the cytoskeleton and may have other cellular functions. Here we review molecular and functional aspects of this protein, evoke its role as a scaffold protein and have a look at several pathologies where the protein may be involved.

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Keywords: Microtubules; Actin; Cytoskeleton; Scaffold; MAP1B

Contents

1. Introduction ............................................................................................................ 542
   1.1. MAP1B discovery .......................................................................................... 542
2. Molecular aspects ....................................................................................................... 542
   2.1. Molecular particularities ............................................................................... 543
   2.2. Turnover ....................................................................................................... 543
   2.3. Axonal transport .......................................................................................... 544
   2.4. MAP1B upstream regulation ......................................................................... 544
   2.5. Structural homologies and similarities ......................................................... 544
   2.6. Genetically modified animals ...................................................................... 544
   2.7. Behavior of mutants ..................................................................................... 545
3. MAP1B distribution ..................................................................................................... 545
   3.1. MAP1B detection with antibodies ................................................................. 545
   3.2. MAP1B isolation .......................................................................................... 545
   3.3. MAP1B earliest expression .......................................................................... 545
   3.4. Location and species .................................................................................... 545
   3.5. MAP1B differences in the nervous system and in a variety of cells ............ 546
   3.6. Subcellular particularities ............................................................................. 546

* Correspondence address: Département de Biologie Cellulaire et de Morphologie (DBCM), Université de Lausanne, 9 rue du Bugnon, CH-1005 Lausanne, Switzerland. Tel.: +41 21 692 5154; fax: +41 21 692 5105.
E-mail address: Beat.Riederer@unil.ch.

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1. Introduction

In neurons, the cytoskeleton is essential for the organization and maintenance of shape and cellular function. This includes axonal transport, transport of proteins and cellular elements and we will highlight its role in several diseases [79,88,224].

1.1. MAP1B discovery

In the mid eighties MAP1B was discovered as one of three high molecular weight MAP1 forms. It is considered a major cytoskeletal protein and essential for neurite outgrowth. Independent studies named MAP1B also MAP1.2, MAP1x or MAP5 [14,15,33,90,177]. It is the earliest embryonic brain MAP and is associated with MAP1B and named MAP1B light chain, MAP1B-LC1 [111]. At birth, MAP1B is highly abundant in brain and decreases in concentration with progression of postnatal development. The essential role of MAP1B in neuritogenesis has been proven in different in vitro and in vivo models, as reviewed previously [79]. Very soon, it was shown that MAP1B has the ability to promote microtubule assembly [164,177], that it is one of the first MAPs to appear in neuroblasts and essential for differentiation and growth of neuronal processes [24,176,224]. MAP1B is involved in the early axonal outgrowth and therefore essential in establishing the neuronal polarity [78,87,126]. Furthermore, MAP1B, MAP2 and tau play some synergistic role in neurite outgrowth. MAP1B controls directionality of growth cone migration, while neurons that lack MAP1B are characterized by increased terminal and collateral branching and impaired turning of growth cones [21,67,78,208]. Phosphorylation of MAP1B is involved in modulation of microtubule stability and turning of growth cones [124,129] and interacts with microfilaments [163,217].

2. Molecular aspects

MAP1B is a large protein with an apparent molecular weight (Mr) between 320 and 330 kDa and a calculated molecular mass of 255,534 Da [145]. MAP1B is composed of 2464 amino acids. The microtubule binding domain of MAP1B contains a repeat sequence motif unrelated to that of tau and MAP2 with short motifs of KKEE or KKEVI and a set of 12 imperfect repeats of 15 amino acids each [145]. As outlined in Fig. 1, MAP1B has binding sites for microtubules and microfilaments. Cloning
Fig. 1. Restriction sites in the MAP1B genome (A) and the molecular organization of the full-length mouse MAP1B is represented (B) with its separation at around amino acid sequence 2100 into heavy and light chain. The restriction sites in panel A are essential to produce different MAP1B fragments, since it is difficult to work with the full-length molecule. In panel B different functional sites are indicated: MAP1B is composed of 2464 amino acids. Actin binding (AB) sites, localize at the MAP1B-LC (2′336–2′459) and in the N-terminal domain of MAP1B-HC1-517. The tubulin binding site (MTB) of MAP1B is characterized by 21 KKEK repeats near the N-terminus (517–848) and comprises also a MTB in the MAP1B LC (2′210–2′336). In addition, a assembly-helping site MTA is found in the flanking region 9981–14401 helping to increase microtubule assembly [17]. A hydrophilic region (1866–2071) near the C-terminus contains 12 imperfect repeats (YSYETXEXTTXXPXX) as indicated as xxx in panel B. It is believed that not only the tubulin-binding site is involved in binding to microtubules. In addition, the MAP1B light chain has a self-interaction and can dimerize or oligomerize [217] as does the MAP1B-HC [38].

of cDNA encoding rat MAP1B and regulation of mRNA during development in different brain areas mRNA and protein levels pattern are the same. Molecular cloning of MAP1A and MAP1B, revealed a single mRNA of 11kb for MAP1B. The MAP1B locus was located to the mouse chromosome 13, while the MAP1A locus was found on chromosome 2; this clearly indicated that MAP1A and 1B are structurally different proteins and that they are expressed from separate genes [53,70]. The human gene was localized to the long arm of chromosome 15 with sequence homologies between mouse rat and human at 90% identity [114–116].

2.1. Molecular particularities

Several upstream consensus sequences include two TATA boxes that independently direct neuron-specific expression of a reporter gene [114]. There are two alternative and overlapping promoters that direct neuron-specific expression of the rat MAP1B gene and regulate the temporal and tissue-specific expression, one inducible and the other one constitutive [114–116]. This may also explain some of the differences in the temporal, cellular and subcellular distribution.

For the description of its molecular structure, its localization and phosphorylation-dependent expression in developing neurons, two monoclonal antibodies were helpful [191]: MAP1B appears as long filamentous molecule of 186+38 nm, it forms cross-bridges between microtubules with mainly phosphorylated forms in axons and unphosphorylated forms (or differently phosphorylated forms) in soma and dendrites. Its abundance in developing axons suggests that it plays an essential role in axonal elongation. MAP1B is encoded as a polyprotein (full length MAP1B) and comprises the MAP1B heavy chain (MAP1B-HC) and light chain 1 (MAP1B-LC1), with a cleavage site located near amino acid sequence 2100. A microtubule binding domain (MTB) is localized near the N-terminal end of MAP1B-HC and a MTB is also present in the MAP1B-LC1 [91]. This indicates that the full length protein has the capacity to cross-link microtubules as previously mentioned [191]. Nevertheless, cells transfected with the full length MAP1B do not reveal excessive bundling of microtubules [209]. LC3 is a subunit of MAP1A and variation in LC composition may control the binding activity of MAP1A and MAP1B to microtubules [127,128]. The MAP1B-LC1 is involved in microtubule stabilization, self-interaction and actin filament binding. MAP1B-LC1 stabilizes microtubules but such microtubules remain flexible. The LC1 harbors an actin-binding site that is homologous to the binding site found in MAP1A [217]. Differences in the composition of MAP1A, MAP1B and LCs exist as different protein complexes and determine distinct functional properties, in that MAP1B and LCs are essential during neuritogenesis, while MAP1A and LCs are more important in mature neurons [146].

2.2. Turnover

The turnover of cytoskeletal proteins in vivo was determined in 10-day-old rats by using [35S]methionine and by measuring the fast and slow decay rates, for MAP1B (5.8 days ± 0.7/12.0 ± 1.3), for MAP2 (6.9 ± 0.3/12.4 ± 1.7), for tubulin (4.8 ± 0.5/15.0 ± 0.5) and for spectrin (4.9 ± 0.4/16.0 ± 1.2), while the detergent insoluble NF-H showed only a monophasic rate (18.5 days ± 1.5). The detergent insoluble MAP1B showed also a monophasic decay of 29.0 days ± 2.3 [186]. This suggests that part of MAP1B may be strongly associated with the detergent insoluble cytoskeleton. Proportions between MAP1B-LC1 and MAP1B–HC
varies greatly between cells. The MAP1B-LC1: MAP1B-HC ratio in postnatal brain homogenates is between 6:1 and 8:1. Therefore, the MAP1B-LC1 has a greater half life than the MAP1B-HC, given that they are synthesized at a 1:1 ratio [135,136].

2.3. Axonal transport

MAP1B moves along the axon together with slow component a and b, at a speed of 7–9 mm/day. This transport rate comprises phosphorylated MAP1B, interestingly different phosphorylation states move at different velocity with the slow axonal transport [122]. MAP1B is also involved in retrograde transport of mitochondria [101]. During axonal development, MAP1B is characterized by a phosphorylation gradient that is increasing towards the growth cone shaft [78,87,126]. Since the phosphorylation of MAP1B influences MAP1B binding to microtubules it is feasible that differences in phosphorylation modify the transport rate of MAP1B and variations may exist according to the phosphorylation state and location of MAP1B within the axon or dendrites.

2.4. MAP1B upstream regulation

The expression of MAP1B is under control of homeoprotein transcription factors [64] and as already mentioned under differential transcription [114,116]. The MAP1B promoter is regulated by the Engrailed homeoprotein in vivo [140]. A joint regulation of the MAP1B promoter by two overlapping homeoproteins HNF3beta/Foxa2 and Engrailed indicates a highly conserved mechanism for a direct interaction of homeoprotein transcription factors. Transfection experiments in primary cultures demonstrated that Foxa2 antagonizes the Engrailed driven regulation of MAP1B [64]. The Hmob3 brain-specific sequence is part of the human MAP1B gene 3’UTR and encoded by a portion of the exon 7. Furthermore, two transcripts were identified, one for brain and one for kidney, while skeletal muscle comprises both transcripts [43]. The Dlx-2 homeobox gene controls neuronal differentiation and immature cells that contain Dlx genes do express MAP1B and MAP2 but not the glial fibrillar acidic protein, GFAP [50]. This underlines, that an expression of several MAPs, including MAP1B, is important for neuronal polarity and for setting the stage for synaptic connectivity. A differential transcription may also explain some of the discrepancies in MAP1B distribution in neurons and glia, differences in phosphorylation or between genetically modified mice.

2.5. Structural homologies and similarities

A sequence and structural homology to MAP1B has been identified in claustrin and neuraxin [29,145,183,252] and more recently in the Drosophila Futsch/22C10 [98]. Claustrin, an anti-adhesive neural keratan sulfate proteoglycan is structurally related to MAP1B an inhibits cell adhesion. Claustrin has a high sequence homology with an alternatively spliced 5’ truncated MAP1B fragment. It is expressed in chick brain, in cardiac and smooth muscles and is found at high levels in astrocytes [29,30]. However, MAP1B and claustrin are structurally and functionally not identical since only the N-terminal 1022 codons are utilized and encode the core protein of the extracellular proteoglycan claustrin and when the corresponding N-terminal part of MAP1B was expressed it bound to microtubules but did not localize in the extracellular matrix [216]. The Drosophila FUTSCH/22C10 is a MAP1B-like protein required for dendritic and axonal development. It regulates synaptic microtubule organization and is necessary for synaptic growth and structure [184]. The N- and C-terminal domains of Futsch are homologous to vertebrate MAP1B, while the central domain is highly repetitive and shows sequence homologies to neurofilament proteins. The Futsch/22C10 is regulated by the fragile X-related gene, a gene responsible for mental retardation [254]. C190RF5 is a homologue of MAP1B that interacts with a microtubule-stabilizer and candidate tumor suppressor involved in the generation of cancer cells [117,118]. Coronin (Crm1) promotes rapid assembly and cross-linking of actin filaments and actin to microtubules. Crm1 contains sequences homologous to MAP1B [82] that may explain binding to microtubules. VCY2 is a testis-protein that locates in the Y chromosome. An interacting partner was identified by two-hybrid screen to be VCY2IP-1, a protein of 1059 amino acids. Its sequence has 59.3% and 41.9% homology to MAP1B and MAP1A respectively, with large homologies with MAP1B at the N- and C-terminal ends [246]. The stroma membrane-associated protein 1 is involved in erythropoiesis and contains similar repeats (KKD/E) as found in MAP1B [190]. Dynein interacts with microtubules with an ATP-sensitive linkage. Three clusters of contact of amino acids, two of which are regions sharing sequence homology with MAP1B have been identified. It is noteworthy that point mutations within these regions weaken an interaction with microtubules [109].

2.6. Genetically modified animals

A lack of MAP1B in MAP1B null mutants suggests that the protein is implicated in the locally coordinated assembly of cytoskeletal components required for branching and straight directional axon growth [21]. The protein seems not to be essential for neuronal survival, but a disruption of the MAP1B gene leads to a delayed development of the nervous system in mice [207] or abnormal morphology of individual cells such as Purkinje cells and phenotypes with slower axonal growth rates, a lack of visual acuity, motor system abnormalities and variations in several brain areas such as olfactory bulb, hippocampus and retina [54]. In a homozygous mutant mice with MAP1B deletion, suggest a role of MAP1B in axon guidance and polarity in the central and peripheral nervous system [75,78,137]. Homozygous MAP1B −/− mice were viable but displayed absence of corpus callosum and misguided myelinated fiber bundles and reduced number of myelinated larger axons [137]. Functional redundancy of MAP1B and tau suggests some common action in the growth cone shaft, while MAP2 and MAP1B knock-out mice point to a similar redundancy in dendritic growth [21,51,67,207,208,214], while MAP2-deficient mice behaved without apparent abnormalities. A different result was obtained when MAP1B expression was deficient; mice died in their peri-
natale period, showing fiber tract malformation, disrupted cortical patterning due to retarded neuronal migration and disrupted laminar organization in the hippocampus and the cerebellum [74,77]. Cultured neurons from these mutant mice were characterized by a significant inhibition of axon formation, a delay in neurite outgrowth, a reduced neurite elongation rate and inhibited microtubule bundling [75,80,214].

2.7. Behavior of mutants

Gene targeting study revealed neuronal abnormalities and phenotypes with slower growth rates, lack of visual acuity, motor system abnormalities and variations in several brain areas including the olfactory bulb, hippocampus and retina [54]. The full length MAP1B is encoded by seven exons. Alternative transcripts contain either exon 3A or 3U, and give rise to N-terminal truncated MAP1B (resembling those of MAP1A). Differences may explain some of the conflicting results obtained in MAP1B knockout mice [110]. The MAP1B gene has a highly conserved 4.3 kb 3′ untranslated region [138]. Mice deficient of MAP1B altered performance in behavioral phenotype. Impaired locomotor activity, correlated with lack of physical endurance, but without significant differences in cognitive function and memory impairment [160]. An impaired hippocampal long-term potentiation (LTP) in MAP1B deficient mice and a rapid dephosphorylation after induction of LTP in MAP1B +/- mice, may suggest some role in activity-dependent synaptic plasticity in LTP [253].

3. MAP1B distribution

3.1. MAP1B detection with antibodies

Many monoclonal antibodies were used, differing in their reactivity with either phosphorylated epitopes, with a specific conformational state of MAP1B, with modification independent epitopes or with a species-specific sequences of MAP1B [15,33,124,129,177,181,191,231]; in addition many polyclonal antibodies have been raised against specific recombinant fragments of MAP1B. This variety of antibodies, with subtle or great differences in their reactivity with MAP1B, in combination with molecular and species differences, with differences in post-translational modification and temporal changes in expression, with variations in location and modification make it very difficult to compare and generalize results. The RT97 and SMI31 antibodies with a crossreactivity with phosphorylated MAP1B were used to identify a stabilizing function of neurofilaments and an interaction between neurofilaments and MAPs [199]. The SMI31 monoclonal antibody reacts with a phosphorylated epitope comprised within a 20 amino acids region of MAP1B from amino acids 1244–1264 [102]. The RT97, as also the SMI31 antibodies react with NF-H, NF-M, MAP1B and tau [200], therefore makes an interpretation of immunohistochemical data rather difficult. The distribution of phosphorylated epitopes on MAP1B in the developing rat spinal cord were studied with antibody 150 that is reacting with mode I phosphorylated MAP1B and that detects MAP1B from E13 to the third postnatal week, while SMI31 staining persisted into adulthood [31]. This persistence in staining is explained by a crossreactivity of this antibody with neurofilament and tau proteins, that increases or persists, respectively, during maturation of brain tissue. Immunologically related proteins to MAP1B and MAP1A with Mr in the range of 160–220 kDa were identified during mouse spermatogenesis [71]. However, a sequence homology of these proteins with brain MAPs was not established.

3.2. MAP1B isolation

MAP1B was first isolated by affinity purification by using the monoclonal antibody AA6, actually the same antibody that was used for the identification of MAP5 [177]. In a large scale purification of bovine brain MAP1B, by using a two step ion exchange, a yield of 25–30 mg/kg MAP1B with a 95% purity was obtained [165]. The isolation of recombinant MAP1B proved difficult due to the large size of MAP1B [54]. Therefore, recombinant MAP1B need to be used. This is useful to define specific functions for parts of the molecule or to produce sequence specific polyclonal antibodies.

3.3. MAP1B earliest expression

Although MAP1B is the earliest brain MAP in comparison to MAP1A, MAP2, or tau; none of these proteins was present in the two cell stage of rat embryos [132]. Expression of MAP1B occurred during the initial pathfinding of olfactory receptor neurons in the mouse, by E10 [6]. MAP1B is also strongly expressed in cells of the outer granular layer of the developing cerebellum, where neuroblasts undergo a last cell division before migrating towards the inner granular layer and forming the parallel fibers [181]. MAP1B is concentrated in the distal region of growing axons, by several fold excess compared to proximal parts of axons [13]. Many reports suggest that MAP1B and MAP1A are complementary in that MAP1B is essential during initial growth and during the maturation process is replaced by MAP1A [223]. However, this is not always the case. The expression of MAP1A in the human fetal brain was found in two transient structures in the ganglion eminence of the telencephalic proliferative zone and the periretalicular nucleus within the internal capsule. MAP1A was present between 18 and 22 weeks of gestation while MAP1B was found from the 26th week to the 33 week in the internal capsule [229]. MAP1B and MAP2 are present in early embryonic spinal cord [154], this suggests that several brain structures differ in their maturation rate [155]. This complementarity in function could also be explained by the formation of protein complexes with the same MAP1 light chains [146].

3.4. Location and species

In one of the first comparative studies, several MAPs were compared, and differences in distribution were highlighted for MAP1A, MAP2, MAP3 and MAP5 alias MAP1B [133]. MAP1B was identified in many vertebrates as rat, mice, human, baboon, hamster, guinea pig, felines, bovine, sheep, chicken, xenopus and fish and invertebrates such as flies.
and in trypanosomes [1,4,30,106,150,153,165,179,184,196,224,229,240]. Interestingly the MAP1B expression in continuously growing fish follows the continuous development [1], suggesting that MAP1B expression is closely related to differentiation. MAP1B is expressed in the visual system of the tench during optic regeneration (fish) especially the phosphorylated form of MAP1B [239]. MAP1B (MAP5) is expressed and present as a phosphorylated form in the amphibian CNS of Xenopus, and was identified in the optic tectum to remain at high levels in adult animals [240]. During layer formation in the chicken optic tectum, MAP1B and NF-M appear at embryonic day 3, at a time of neuroblast production, while MAP1A and MAP2A&B are expressed later, at day E5 [251]. In a teleost fish, several MAPs were characterized: MAP1A and MAP2 were found of similar Mr, while MAP1B was about 25% of its mammalian homologue [218]. A 60% homology with MAP1B was found in a 110 kDa protein of Trypanosoma brucei, and it was speculated to be one of the parasite molecules associated to molecular mimicry.

3.5. MAP1B differences in the nervous system and in a variety of cells

A differential regulation of MAP1B was found between the rat central and peripheral nervous system development, in that MAP1B mRNA is highest in early stages, and decreases several fold in CNS but remained high in doral root ganglia of the PNS [123]. It is evident that MAP1B is a developmentally regulated phospho-protein, with a pronounced decrease during postnatal development [185]. In adult brain, phosphorylated MAP1B remained high only in a few areas including primary afferents and motor neurons, olfactory tubercles, habenular and raphe projections to interpeduncular nuclei, septum and hypothalamus. Furthermore there was an overlap with N-CAM suggesting that MAP1B may play a role in the structural remodeling in adult brain tissue [147,148].

An expression and upregulation of MAP1B in retinal pigment epithelial cells corresponds to the time when cells start to lose epithelial characteristics and change their morphology [57]. MAP1B is present in cultured oligodendrocytes and co-localizes with tubulin but is absent from astrocytes [61]. Nevertheless, in glial cells of type I astrocytes, a specifically phosphorylated MAP1B was found [234]. MAP1B expression precedes the morphological differentiation of oligodendrocytes, as tested by development-specific oligodendrocyte antigens A2B5, O4 and O1. One of the low Mr forms of MAP2 was also transiently expressed in pre-oligodendrocytes [241]. MAP1B expression was induced in Schwann cells during nerve regeneration, interestingly phosphorylation-dependent antibodies do not react with this glial form of MAP1B [5]. This confirms that neurons and glia cells have different phosphorylation modes [234]. A human cell line with a phenotype resembling committed CNS neuronal precursor cells (NT2/D1) expressed nestin, vimentin, MAP1B, NCAM and N-cadherin and only small amounts of NF-L, alpha internexin, NF-M and MAP2c indicating neuronal fate [171]. MAP1B antisense treatment by oligonucleotides inhibits initiation of neurite outgrowth in NGF-treated PC12 cells with a recovery after antisense removal [25,176]. A chronological expression of MAPs was observed in embryonal carcinoma P19 cells, induced by retinoic acid, in that MAP1B and MAP2c appeared 12 h after induction, while MAP1A and MAP2A/B appeared between 3 and 5 days [212].

3.6. Subcellular particularities

A MAP1B-related antigen localized to centrosomes seems involved in the organization of microtubule organizing centers [52]. Interestingly, a mitotic protein MPM-2, that increases the microtubule nucleation capacity of centrosomes contains sequences of the topoisomerase II and MAP1B [238]. Immunoreactivity with monoclonal antibody G10 is lost during brain homogenization [94], suggesting a reactivity with a specific conformational state of MAP1B. Localization of MAP1B in postsynaptic densities of the rat cerebral cortex is found in about half of the total synapses [103]. MAP1B is a neuronal plasma membrane glycoprotein and co-localizes with the peri-axonal region [213]. A specific binding of acidic phospholipids such as phosphatidylserine but not phosphatidylcholine to MAP1B regulates its interaction with tubulin pointing to a regulatory role in the MAP1B-microtubule interaction with biological membranes [250]. MAP1B is a typical phosphoprotein of growth cones [68,129], its location in growth cones of PC12 cells is also demonstrated in the insert of Fig. 2. One should also note that part of MAP1B is not co-localizing with microtubules and may constitute a soluble pool of proteins or may relate to the subcortical actin cytoskeleton (Fig. 3).

4. MAP1B function and development

4.1. Functional aspects

MAP1B antisense oligonucleotides inhibits initiation of neurite outgrowth in NGF-treated PC12 cells with recovery after antisense removal [25]. By injecting polyclonal antibodies
MAPs, in that MAP1A microtubules were short and straight and MAP1B microtubules were longer and bendy [162]. Several studies suggest an overlapping in vivo role of tau with MAP1B in axonal stability, axon elongation and axon structure, suggesting some common functions of brain MAPs [21,67]. Expression of MAP1B was identified in actively myelinating oligodendrocytes in the adult rat brain [247]. A contact with astroglial membranes induces axonal and dendritic growth of CNS neurons with a common distribution of GAP43 and MAP1B [170], that also points to an interaction between glial and neuronal cells. The myelin associated glycoprotein, MAG, modulated expression and phosphorylation of MAP1B, NF-H and NF-M and increased activity of ERK1/2 and cdk5, while in mice lacking MAG the expression of MAP1B was decreased [40].

4.2. MAP1B in regeneration

MAPs plays an essential role in both, PNS development and regeneration and MAP1B undergoes a similar expression and modification as during postnatal development [149]. Its expression is induced in Schwann cells during nerve regeneration [121]. After lesions (peripheral and central) of trochlear motoneurons MAP1B expression was increased, this may reflect initial recapitulation of early development [19]. Partial regeneration and long-term survival of rat retinal ganglion cells after optic nerve crush, by increased MAP1B expression and phosphorylation with a gradient in phosphorylation towards the soma of ganglion cells [48]. After percussion brain injury, MAP1B and GAP43 are reexpressed and MAP1B phosphorylation is increased, suggesting that these proteins are part of a regeneration potential of neurons [56]. The two modes of MAP1B phosphorylation (as will be explained below in more detail) are differentially regulated during PNS regeneration in that mode I phosphorylation was concentrated at distal region of nerves, while mode II phosphorylation showed an overall decrease in distal parts [174,175]. Target deprived afferent fibers, after a kainic acid lesion in the adult rat brain, induced MAP1B expression and its phosphorylation. MAP1B phosphorylation seems essential since it allows modulation of microtubule dynamics [202]. Phosphorylated MAP1B is induced in central sprouting of primary afferents in response to peripheral injury but not in response to rhizotomy. MAP1B was proposed as rapidly expressed, axon-intrinsic marker associated with plasticity of myelinated fibers [203].

5. MAP1B post-translational modifications

5.1. MAP1B polyglutamylation and glycosylation

By blot overlay it was measured that polyglutamylation of tubulin influences its binding to MAP1B, MAP2 and tau [18]. MAP1B was identified to be a membrane glycoprotein and was localized as integral membrane protein in vesicles and the plasma membrane of neurons [213]. Yet, most of the protein may be associated with microtubules, actin filaments or may be present in soluble form.
5.2. MAP1B phosphorylation

There is no doubt that phosphorylation of MAP1B plays a crucial role for the modulation of MAP1B function as already suggested by the first observations on (NGF) induced neurite outgrowth in PC12 cells [24,189,222]. MAP1B is strongly present in growing axons in developing brain tissue, in primary neuronal cultures and in various cell lines with an increasing gradient of phosphorylated MAP1B towards the growth cones [13,23,32,86,89,105,232]. However, an outgrowth of neurites may not necessarily depend on MAP1B phosphorylation [105,113].

5.3. Kinases using MAP1B as substrate

The research group of Jesus Avila identified two phosphorylation types, a proline-dependent kinase (PDK) or mode I phosphorylation in outgrowing axons and a Casein Kinase II (CKII) or mode II phosphorylation in axons and dendrites remaining into adulthood [7,47,79,230]. The two modes regulate also MAP1B phosphorylation during PNS regeneration, in cultured neurons [175,232] or in cell lines [93,166,210]. Mode I phosphorylation is development dependent and is reduced after the critical period of brain development, while mode II phosphorylation is present throughout postnatal development [175,178,179,224]. Phosphorylation of MAP1B by mode I is involved in the local stabilization of turning growth cones [124]. In glial cells of type 1 astrocytes, only mode II phosphorylation was found [234]. Cytosolic MAP1B is highly phosphorylated by mode I and mode II during development, suggesting a lesser binding of MAP1B to microtubules [230] and also influencing its actin binding [163].

Several kinases have already been tested to phosphorylate MAP1B such as CKII [141,231], cyclin-dependent kinases as cdc2 and glycogen synthase kinase 3 [68,69,83,86,120] or cdk5, cdk5/p25 and JNK [104,158]. Glycogen synthase kinase 3β (GSK3β) induced PC12 cell differentiation and modulates microtubule stability in growth cones [83,86,156], while inhibitors of this kinase such as WNT7a, lithium and SB-216763 induced axonal spreading and branching, a reduced axon elongation rate, a change in growth cone morphology as characterized by an altered filopodia dynamic and microtubule bundle distribution [120,156], and altered F-actin motility [13,84,86,156]. Reelin is involved in the signaling cascade that leads to the MAP1B mode I phosphorylation [76] and MAP1B is required for Nurtl 1 signaling in neuronal migration and axonal guidance via the signaling pathways including GSK3β and cdk5 [42]. The GSK3β phosphorylates mouse MAP1B at ser1260 and thr 1265 and is spatially restricted to growing axons in a gradient that is highest distally towards the growth cone [221]. The MAPK pathway is upstream of the activation of GSK3β that enables MAP1B phosphorylation and axonal growth [85] and is induced by growth factors and phorbol esters [97]. NGF activates the phosphorylation of MAP1B by GSK3β through the TrkA receptor while an inhibition of the TrkA receptor inhibits neurite elongation [84]. The central role of GSK3β, its signalling cascade and its control of microtubule dynamics has recently been discussed [255]; several upstream regulators such as semaphorins, and phosphatidylinositol 3-kinase, or neurotrophins and ERKs have been recognized. MAP1B phosphorylation is differentially regulated by cdk5, cdk5/p25 and JNK [104,158,169]. JNK1 is required for the maintenance of neuronal microtubules and for the control of MAP phosphorylation. Interestingly, Jnk−/− mice exhibit disrupted anterior commissure, a progressive loss of MTs within axons and dendrites, and hypophosphorylated MAP2 and MAP1B [34]. A disrupted anterior commissure in Jnk−/− mice or a disrupted corpus callosum in Mab1b−/− mice may suggest that the presence of MAP1B and a specific phosphorylation type seem necessary for normal development of cortico-cortical projections. NMDA-glutamate receptors were found to regulate phosphorylation of dendritic cytoskeletal proteins in the hippocampus, including MAP1B and MAP2, thus confirming that they are involved in NMDA-dependent dendritic plasticity [188]. A phosphorylated MAP1B was found in neuroblast mitosis [181]. A M-Phase kinase in isolated spindles was identified to use MAP4 and MAP1B as substrates. This kinase may be related p34cdc2 [219]. Mitotic spindles contain also a CKII-like enzyme that uses MAP1B as major substrate [46]; however this kinase has a rather subplasmamembrane location and may therefore relate more to process outgrowth than to mitosis [141]. Phosphorylation of MAP1B by an ecto-kinase suggests that MAP1B has a transmembrane domain and that MAP1B phosphorylation may relate to synaptogenesis between cortical neurons [144]. Yet, most data suggest that the large majority of MAP1B is localized in the cytoplasm and is associated with microtubules and/or microfilaments.

5.4. MAP1B phosphorylation sites

MAP1B contains many potential phosphorylation sites with a downstream proline as targeting amino acid for PDKs, and many CKII target sites. In a proteomic study phosphorylated sites of many synaptic proteins were identified. MAP1B, is phosphorylated at 33 sites in vivo [36]. In summary, the GSK3β is phosphorylating seven sites (S829, S1247, S1347, S1395, S1793, S1911, S2094), the cdk5 is phosphorylating eight sites (S829, S1260, S1317, S1334, S1610, S1621, S1775, S1793), the cdc2 is phosphorylating two sites (S1768, S1775), the p38MAPK phosphorylates six sites (S1307, S1373, S1384, S1391, S1781, T1784) including one of the two threonine sites, the CKII phosphorylates five sites (S828, S1307, S1382, S1768, S1877). Several kinases phosphorylate one or two sites, such as ERK1 (S1255) and PKG (T1806) with one site each and the PKA with two sites (S1371, S1778). Most interestingly, a thyrosine site (Y1331) is phosphorylated by INSR, src. In this study five predicted sites were mentioned including a DNA PK. Among phosphorylated sites only two sites localized in the microtubule binding region (S17–848). No phosphorylation site of the C-terminal end and corresponding to MAP1B-LC1 was picked up in this study [36]. One wonders, whether the MAP1B-LC1 is not present in growth cones or is not phosphorylated. This may also signify that MAP1B is cleaved into HC and LC and the LC is metabolized before arriving into the growth cone or synapse. Despite the fact that many phosphorylated sites have been iden-
tified, most functions of the phosphorylated sites remain largely unknown. In addition, there must be more sites that are phosphorylated since the SMI31 epitope is phosphorylated by GSK3β at ser1260 and thr 1265 of mouse MAP1B [221]. Furthermore, several kinases that have been previously identified to phosphorylate MAP1B were not picked up in this study. One has to consider that the identification of phosphorylated sites depends on the starting material, since MAP1B phosphorylation may change during development, and depend on various cues. Phosphorylation of MAP1B changes during rat brain development and a structural microheterogeneity of MAP1B was speculated to be due to differences in phosphorylation [62]. One needs now to connect the specific phosphorylation sites to specific functions. Why has MAP1B that many phosphorylated sites?

5.5. Protein phosphatases

Dephosphorylation of distinct MAP1B sites such as mode I are dephosphorylated effectively by PP2A and PP2B but not by PP1, while mode II are dephosphorylated by PP1 and PP2A but not by PP2B [233]. An inhibition of PP2A and 2B results in inhibition of microtubule binding activity [72,73]. Ocadia acid (OA) was used to inhibit PP2A/PP1 and modulate MAP1B phosphorylation in neuroblastoma cells SY5Y. OA induced cell death and reduced microtubule stability [211].

6. MAP1B regulation by various factors

Angiotensin II and NGF influence MAP expression in PC12 cells, while NGF increases MAP1B expression, Angiotensin II is reducing it [205]. Activity-driven dendritic remodeling requires MAP1B in postsynaptic densities and modulates ion channel activity [206]. Agrin induced an increase in MAP1B expression, dendritic elongation and dendritic branching, while in agrin depleted cultured hippocampal neurons extended longer but non-branched axons and shorter dendrites [130]. Methylazoxymethanol causes a long-term inhibition of axonal outgrowth in cultured rat hippocampal neurons with a depletion of the soluble MAP1B pool [96]. This substance causes microencephaly and kills neuroblasts. Glucocorticoid at the dose used clinically alters cytoskeletal protein expression in that MAP1B expression was increased by 30% [4]. Betamethasone, a drug that is used to accelerate fetal lung maturation has acute effects on cytoskeletal proteins, for example: MAP1B was diminished in the frontal cortex of sheep [196]. The convulsant pentylenetetrazole leads to a MAP1B upregulation in rat [242]. The substance panaxynol, a polyacetylene with neurotrophic effects resulted in reduced cell division of PC12 cells and in an upregulation of MAP1B [245].

7. Is MAP1B a scaffold protein?

It has become clear by now that MAP1B is involved in a variety of cell functions. Therefore, the name microtubule-associated protein may be misleading. MAPs may be the missing link between the actin and microtubule cytoskeleton in that they link the microtubule bundles and organize actin filaments, this especially for MAP1B and MAP2 [41,163]. MAP1B may bind a variety of proteins either directly, or via tubulin and actin. Furthermore, binding of proteins may differ between MAP1B HC and LC1.

Other proteins may interact with microtubule stability in that they counteract the stabilizing effect of MAP1B, such as SCG10 a neuron specific protein of the stathmin family [182]. MAP1B interacts with other proteins, such as gigaxonin, a protein that links microtubules and intermediate filaments and is involved in giant axonal neuropathy [16,49]. It may link a GABA receptor via the rho1 subunit to the cytoskeleton [12,92]. The glycine transporter GLYT-1 associates with this protein complex and is essential in neurotransmission in the retina [92,161]. An interaction of MAP1B-LC1 with the glutamate receptor-interacting protein 1 (GRIP1) was identified by the yeast two hybrid assay and suggests a novel mechanism for the AMPA receptors [197]. A G-protein coupled receptor is upregulated by erythropoietin, interacts with MAP1B and colocalizes with the 5-hydroxytryptamine 2a receptor [134]. MAP1B may bind myelin-associated glycoprotein (MAG) and seems to modulate expression and phosphorylation of MAP1B, NF-H and NF-M [40,65]. Given the large size of MAP1B one can expect to find novel binding partners that add to the variety of its function as outlined in preliminary results [38]. Binding of heatshock protein 70 (hsp70) to the tubulin C-terminal sequence 431–444 was measured. The putative tubulin binding motif in the hsp70 protein contains a sequence related to the motif described in MAP1B [187]. MAP1B-LC1 binds to and enhances Rap1 activation and it is attributed a chaperone activity [20]. Several proteins interact with MAP1B and modulate microtubule dynamics: RASSF1A is active in the cell cycle and involved in growth inhibition of cancer cells [39]; the end binding protein-1 (EB1) complements MAP1B during axogenesis, overexpression of EB1 and facilitates axogenesis in MAP1B −/− cells [100]; semaphorins function in the signaling cascade of growth cone collapse, in chemorepulsion, in neuronal apoptosis during early development and in an upregulation of MAPs [142,143]. Isolation of a multiprotein complex that contained MAP1B, CRMP-2, plexins A1 and A2 and Sema3A is suggested to play a potential role in neurodegeneration and Alzheimer’s disease [81]. Cororinin, Cnr1 promotes rapid assembly and cross-linking of actin filaments, links actin and microtubules and contains sequences that are homologous to MAP1B [82]. The Mapmodulin/leucine-rich acidic nuclear protein binds the MAP1B-LC and modulates neurotogenesis [151]. MAP1B binds to lissencephaly-related protein 1 (LIS1), an interaction that interferes with LIS1-binding to dynein and is regulated by the phosphorylation of MAP1B [101]. This could be explained by the fact that dynein interacts with microtubules via an ATP-sensitive linkage, as mentioned before, via contacts that share some sequence homology with MAP1B [109]. An overexpression of the receptor tyrosine kinases Ror1 and Ror2 enhance extension of short and highly branched processes, possibly regulated via MAP1B [157]. The nuclear export factor (NFX1) of mRNA is able to bind MAP1B and UNRIP in vitro and may be linked to microtubules via MAP1B [220].
8. MAP1B in pathology

When analyzing brain tissue, one need to consider post mortem effects, this is even more essential when the delay between death and the collection of tissue depasses several hours. Postmortem loss of MAP2 and MAP1B occurs within few hours after death, while tau proteins remained constant over a period of 8h [195]. This becomes even more important when analyzing human brain tissue. One needs to consider that it is impossible to obtain tissue form age-matched controls and patients with the same pharmacological background, disease, postmortem delay, age or cognitive evaluation. This means also that studies for MAP1B in human diseases need to be studied by other studies than protein analysis of autopsy tissue. Naturally occurring human autoantibodies against MAP1B were identified in sera of healthy persons, yet the clinical relevance is unclear [168].

8.1. The fragile X mental retardation gene

MAP1B is implicated in fragile X mental retardation, in giant axonal neuropathy and in ataxia type 1 [152]. Fragile X syndrome (mental retardation) mRNA co-localizes with MAP1B mRNA [3]. The fragile X protein (FMRP) controls MAP1B translation and microtubule stability in brain neuron development. A lack of FMRP represses the translation of MAP1B, while in FMRP KO mice microtubules are extremely stable, FMRP plays a critical role in controlling cytoskeleton organization and normal development and microtubule dynamics is a conceivable underlying factor for the pathogenesis of fragile X mental retardation [119]. The drosophila fragile X gene negatively regulates neuronal elaboration and synaptic differentiation. The disease is caused by silencing the fmr1 gene; in turn this gene acts a negative translational regulator of Futsch/MAP1B. In conclusion, the fmr1 gene is central for neuronal architecture and synaptic differentiation in the CNS and PNS of flies [159].

8.2. The giant axonal neuropathy

(GAN) is an autosomal recessive disorder, caused by mutations in GAN and characterized by cytoskeletal abnormalities. Yeast-two hybrid screening identified MAP1B-LC1 as binding partner. Gigaxonin and MAP1B LC1 co-localized. GAN patients with two specific mutations in gigaxonin looss this interaction and consequently show axonal degeneration and neuronal death [49]. Gigaxonin overexpression results in degradation of MAP1B-LC1, giant axonal neuropathy and neurodegeneration [2]. Intermediate filament aggregation in giant axonal neuropathy patients crosstalk with intermediate filaments and microtubules [16].

8.3. Tuberous sclerosis

Tuberous sclerosis is one of the most common neurocutaneous syndromes and characterized by mental retardation and early-onset seizures. Lesions in the central nervous system consist of cortical tubers, subependymal glial nodules and subependymal giant cell tumors. The neuron-like giant cells expressed strong immunoreactivity of neurofilament and MAP1B and occasionally nestin and vimentin and rarely GFAP. These cells show inconsistent neuronal and astroglial characteristics, implying aberrant cellular differentiation [249].

8.4. MAP1B, aging and neurodegeneration

Calpain-mediated proteolysis of MAP1B and MAP2 in developing brain is calcium-dependent and may be related to neurodegeneration [63]. The MAP1B signal was more abundant in early postnatal stages compared with mature animals. In 24-months-old rats it was 1.7 times that of 6-months-old rats [243]. In 18-month-old rats, MAP1B mRNA was very present and aberrant immunolabeling occurred in cortical layer VI. A loss of MAP1B and progressive loss of coordination of gene activity could explain in parts the limited plasticity of the aging brain [244]. Fragments of phosphorylated MAP1B are bound to neurofibrillary tangles in Alzheimer’s disease, while other studies did not find MAP1B in tangles [108] and one has to be careful in the interpretation due to some sequence homology of other proteins, or due to the presence of similar phosphorylation sites [95]. A fetal MAP1B phosphorylation pattern is present in neurofibrillary degeneration in brains of Alzheimer’s disease. Both, MAP1B and tau proteins, may be subject to common phosphorylation, this during development and in disease, as was also shown for tau proteins [180] and may control microtubule stability [235]. Alzheimer’s disease neurofibrillary tangles contain mitosis-specific phosphoepitopes, however MAP1B was not present in the PHF fraction [108] and a dysfunction of tau is much more prevalent [99]. Over expression of MAP1B full-length but not the N-terminal truncated MAP1 accelerates apoptosis of cultured cortical neurons. Aβamyloid is presumed pathogenic in Alzheimer’s disease and induces aggregation especially of the 3U MAP1B transcript [227]. Estrogen-induced changes are beneficial in Alzheimer’s disease with a neuroprotective effect, an increased pool of unstable microtubules and expression of juvenile MAPs such as MAP2C and MAP1B [198]. MAP1B and other MAPs (1A and 2) proteolysis is induced by amyloid β peptide and involves calpain and caspase 3 both related to mechanisms in cell death [59]. The Drosophila homologue: FUTSCH/22C10 is a MAP1B-like protein required for dendritic and axonal development. The N- and C-terminal domains of Futsch are homologous to vertebrate MAP1B, while the central domain is highly repetitive and shows sequence homologies to neurofilament proteins [98] and regulates synaptic organization and synaptic growth [184]. The MAP1B homologue in flies, FUTSCH, is widely expressed in flies and in MAP1B knockout animals resulted in neurodegeneration and its relation to the drosophila fragile X mental retardation gene needs to be mentioned again [11]. MAP1B (MAP5) was found in Lewy bodies and Lewy neurits in the brain stem and forebrain regions from Parkinson’s disease patients [66].
8.5. Ischemia and stroke

Degeneration and regeneration after ischemia in aged rats was found with an upregulation of MAP1B [9]. After experimental ischemia in rats, induced by photothrombic induction and with postischemic intravenous BDNF application, improves functional motor recovery. A BDNF treatment induced MAP1B expression in the ischemic border zone [192]. A delayed recovery of MAP1B following stroke in aged rats was related to MAP1B as a plasticity factor [8]. An upregulation of MAP1B and MAP2 was measured after occlusion of the rat middle cerebral artery [173]. This suggest that MAP1B is upregulated in an attempt to repair or to regenerate the tissue.

8.6. Convulsion and substances that influence MAP1B

Pentylenetetrazole-induced seizure upregulates MAP1B in the hippocampus of the rat [60]. MAP1B expression is increased in hippocampuss. subiculum and peroforant path with high doses of pentylenetetrazole [172]. This convulsant substance leads to robust long-term changes and upregulation in MAP1B [242] and increased seizure susceptibility [194]. However, electroconvulsive shock induces an increase of MAP2 but nor of tau nor of MAP1B [167].

8.7. Dysplasia

MAP1B and nestin are enriched in neural progenitors and abundant in neurons that populate the dysplastic regions with cytoarchitectonic abnormalities [37]. Early forms of MAPs are strongly expressed in cortical dysplasia, especially MAP1B and MAP2c. Furthermore, an upregulation of the NMDA receptor R1 suggests that activated excitatory structural remodeling of neuronal processes is activated by epileptic conditions in cortical dysplasia [248].

8.8. Schizophrenia

Postmortem olfactory mucosa of elderly schizophrenia patients and controls revealed both dystrophic neurites, without differences in immunoreactivity of synaptophysin, MAP1B and neurofilament proteins [201]. An increase in MAP2 and MAP1B in schizophrenia and cytoarchitectonic abnormalities suggest some relation to the disease [10]. However, a decrease of MAP1B and MAP2 was measured in bipolar disorders, but not in schizophrenia [22].

8.9. MAP1B and cancer

RASSF1A interacts with MAP1B, modulates microtubule dynamics in cell cycle and is involved in growth inhibition of cancer cells [39]. C19orf5 is a homologue of MAP1B that interacts with a microtubule-stabilizer and candidate tumor suppressor RASSF1A [117,118]. Analysis of candidate genes included in the mammary cancer susceptibility 1 (Mcs1) region in chromosome 2 (a region that expresses centromeric proteins), revealed among other proteins also MAP1B to be expressed in the mammary glands of rats [112]. An accelerated neuronal differentiation with premature induction of MAP1B and dephosphorylation of tau as a result of P53−/− KO might prevent neuronal terminal differentiation in neuroblasts and may be related to malignant cell growth [58].

8.10. Congenital dyserythropoietic anemia

Congenital dyserythropoietic anemia (an inherited red blood cell disorder) is associated to changes in late erythroid precursors. The CDAN1 gene is encoding codanin-1 and is a putative o-glycosylated protein with sequence homology in its N-terminal part with collagens, with MAP1B (neuraxin) and with synapsin. Codanin-1 seems involved in nuclear envelope integrity [44].

8.11. Spinal muscular atrophy (SMA)

In the fine-mapping of the spinal muscular atrophy (SMA) locus, one uses the MAP1B intron sequence at the distal flanking region on chromosome 5q13. A closest linkage of the SMA and MAP1B locus is possible by using 5q13 microsatellite markers. However, MAP1B is not related to the disease [26–28,35,55,125,193,204,236]. Prenatal diagnosis of SMA, via microsatellites diagnosis is useful also for the Werdnig–Hoffmann disease [131].

8.12. Vitreoretinopathy

Vitreous treatment of cultured human retinal pigment epithelia results in reduced expression of several proteins, including MAP1B. A contact of epithelia cells with vitreous liquid may cause proliferative vitreoretinopathy [107]. Therefore, the evaluation of the MAP1B level may be important in the treatment of the disease.

9. Conclusion and outlook

MAP1B has first been identified with different antibodies, and it was soon recognized as major cytoskeletal protein during neurite outgrowth. The molecular characterization revealed several forms of expression and post-translational modifications, with phosphorylation as an effective means to change binding characteristics to structures such as microtubules or microfilaments and influence cellular dynamics. As growth-associated protein it takes an important part during neural development and in regeneration events. MAP1B is also involved in a variety of diseases, but apparently in itself is not the cause for a specific disease. As a large molecule with many phosphorylation sites and separation into heavy and light chain, it provides many possibilities to interact with a variety of proteins. Despite the fact that MAP1B has many phosphorylated sites, we still know little on the specific function of individual phosphorylation sites. Indeed, from the recent literature it is emerging that MAP1B can bind many other proteins and it seems therefore plausible that MAP1B forms a scaffold protein, between cytoskeletal structures such as microtubules and microfilaments.
and membranes and may link functional proteins eventually in a phosphorylation-dependent manner. There is also much to learn how MAP1B is involved in the etiology of different pathologies.

References


I. Foucher, M.L. Montesinos, M. Volovitch, A. Prochiantz, A. Trembleau, Joint regulation of the MAP1B promoter by HNF3beta/Foxa2 and Engrailed is the result of a highly conserved mechanism for direct interaction of homeoproteins and Fox transcription factors, Development 130 (2003) 1867–1876.


J.A. Hammaback, R.A. Obar, S.M. Hughes, R.B. Vallee, MAP1B is encoded as a polypeptide that is processed to form a complex N-terminal microtubule-binding domain, Neuron 7 (1991) 129–139.


M. Hasegawa, T. Arai, Y. Ibara, Immunoochemical evidence that fragments of phosphorylated MAP5 (MAP1B) are bound to neurofilbrillary tangles in Alzheimer’s disease, Neuron 4 (1990) 909–918.


