Protein family review

The MAP2/Tau family of microtubule-associated proteins
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Summary

Microtubule-associated proteins (MAPs) of the MAP2/Tau family include the vertebrate proteins MAP2, MAP4, and Tau and homologs in other animals. All three vertebrate members of the family have alternative splice forms; all isoforms share a conserved carboxy-terminal domain containing microtubule-binding repeats, and an amino-terminal projection domain of varying size. MAP2 and Tau are found in neurons, whereas MAP4 is present in many other tissues but is generally absent from neurons. Members of the family are best known for their microtubule-stabilizing activity and for proposed roles regulating microtubule networks in the axons and dendrites of neurons. Contrary to this simple, traditional view, accumulating evidence suggests a much broader range of functions, such as binding to filamentous (F) actin, recruitment of signaling proteins, and regulation of microtubule-mediated transport. Tau is also implicated in Alzheimer's disease and other dementias. The ability of MAP2 to interact with both microtubules and F-actin might be critical for neuromorphogenic processes, such as neurite initiation, during which networks of microtubules and F-actin are reorganized in a coordinated manner. Various upstream kinases and interacting proteins have been identified that regulate the microtubule-stabilizing activity of MAP2/Tau family proteins.

Gene organization and evolutionary history

Several types of microtubule-associated protein (MAP) have evolved in eukaryotes, including microtubule motors, microtubule plus-end-binding proteins, centrosome-associated proteins, enzymatically active MAPs, and structural MAPs. We focus here on the MAP2/Tau family of structural MAPs, which along with the MAP1A/1B family form one of the 'classical', well-characterized families of MAPs. In mammals, the family consists of the neuronal proteins MAP2 and Tau and the non-neuronal protein MAP4 (Table 1).

It has been proposed that the *Escherichia coli* protein ZipA, which interacts with the bacterial tubulin homolog FtsZ [1], might be an ancient prototype of MAP2/Tau family members [2]. ZipA contains a region with limited homology to MAP2/Tau proteins, but this region is neither sufficient nor necessary for FtsZ binding [3]. A single, unambiguous functional ortholog of MAP2/Tau proteins is found in *Caenorhabditis elegans* (alternative splice forms PTL-1A and PTL-1B [4,5]) and in *Drosophila melanogaster* (CG31057 [6]; see Figure 1). Both contain microtubule-binding domains related to those in mammalian MAP2/Tau proteins. In contrast, the genome of the frog *Xenopus laevis* has an ortholog of each member of the family. At least three distinct MAP2/Tau related genes have been identified in the *Tetraodon* (pufferfish) genome: CAF98218 and CAG09246 appear similar to MAP2, whereas CAG03020 appears similar to Tau [7]. Additional MAP2/Tau-related genes appear to be present in *Tetraodon*, but the limited sequence information and lack of mapping data make it difficult to evaluate their significance. No homologs have been found in eukaryotes outside animals. Mammalian MAP2/Tau genes span multiple exons, which are spliced to produce several alternative isoforms [8,9] (Table 1 and see below).

Table 1

Properties of human MAP2/Tau family genes

Gene	Locus	Predicted exons	Splice form	Number of microtubule-binding repeats	Alternatively spliced exons
MAP2	2q34-q35	18	Isoform 1 (MAP2b)	3	+9, +10, +11, -16
			Isoform 2 (MAP2c)	3	-9, -10, -11, -16
			Isoform 3	4	+9, +10, +11, +16
			Isoform 4 (MAP2d)	4	-9, -10, -11, +16
			MAP2a	Unknown	+8, +9, +10, +11, (16?)
Tau	17q21.1	17	Isoform 1 (HMW-tau)	4	+2, +3, +4A, +6, +10
			Isoform 2 (tau 4R/2N)	4	+2, +3, -4A, -6, +10
			Isoform 3 (tau 4R/0N)	4	-2, -3, -4A, -6, +10
			Isoform 4 (tau 3R/0N)	3	-2, -3, -4A, -6, -10
MAP4	3p21	23	Various isoforms	3-5	Various

Chromosomal localization and sequence information about reviewed alternative splice forms were obtained from LocusLink [75]. Commonly used designations for splice forms are indicated in brackets.

Characteristic structural features

All MAP2/Tau family proteins have microtubule-binding repeats near the carboxyl terminus [10], each containing a conserved KXGS motif that can be phosphorylated (Figure 2) [11,12]. In addition, each family member contains an amino-terminal projection domain of varying size. In MAP2 and Tau, this domain has a net negative charge and exerts a long-range repulsive force as shown by atomic force microscopy [13]. Each protein has several isoforms, with variation in the length of the projection domain and the number of microtubule-binding repeats [8,9]. The main forms of MAP2 are MAP2c, which is relatively short, and MAP2a and MAP2b, which have longer projection domains.

MAP2/Tau family members are natively unfolded molecules and, like other proteins in this class, are thought to adopt specific conformations upon binding to their targets (microtubules, F-actin and potentially other molecules) [14]. Most regions of MAP2/Tau proteins seem to be devoid of secondary structure. The only region of MAP2 that appears to form a secondary structure is an amino-terminal domain (residues 86-103), which is found in all isoforms and interacts with the regulatory subunit of protein kinase A (PKA). Like the related domain in the A-kinase anchoring protein AKAP79/150, this region is predicted to form an amphipathic helix [15].

MAP2 also can interact directly with F-actin [16]; interestingly, the F-actin-binding site is located within the domain containing the microtubule-binding repeats. Although the MAP2 repeat region is highly similar to that of Tau, neither wild-type Tau nor MAP2 chimeras containing the Tau microtubule-binding repeats can bind to F-actin directly. However, F-actin binding is conferred on Tau if its microtubule-binding domain is exchanged for the corresponding region of MAP2 [16].

Localization and function
Developmental and regional expression

Mammalian MAP2 is expressed mainly in neurons, but MAP2 immunoreactivity is also detected in some non-neuronal cells such as oligodendrocytes. Its expression is very weak in neuronal precursors and then becomes strong about 1 day after expression of neuron-specific tubulin isoform βIII [17]. MAP2c is the juvenile isoform and is downregulated after the early stages of neuronal development [18], whereas MAP2b is expressed both during development and adulthood. MAP2a becomes expressed when MAP2c levels are falling and is not detected uniformly in all mature neurons [19]. In the brain, smaller splice forms of Tau (of 50-65 kDa) are differentially expressed during early development. Specifically, Tau isoforms with three microtubule-binding repeats are predominantly expressed during early development, whereas isoforms with four repeats are expressed during adulthood [20,21]. High-molecular-weight variants of Tau (110-120 kDa) are expressed in peripheral neurons and also at a much lower level in the brain [22]. MAP4 is expressed in various organs, including brain, adrenal gland, lung and liver [23], but it is not ubiquitously expressed: in the brain, for example, MAP4 is expressed only in non-neuronal cells and is absent from neurons [24].

Shortly after axonogenesis in developing cortical and hippocampal neuronal cultures, Tau gradually segregates into axons, while MAP2 segregates into the nascent dendrites (at this stage dendrite precursors are called 'minor neurites') [25]. It is believed that a combination of protein stability
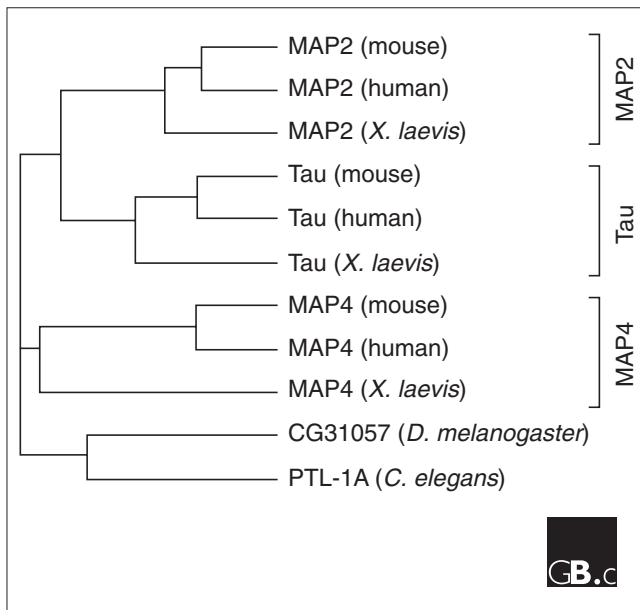
Figure 1
Phylogenetic analysis of MAP2/Tau family proteins. Homologous protein sequences of the microtubule-binding repeats of MAP2 (using splice forms (with three microtubule-binding repeats), Tau (four-repeat isoforms), MAP4 (five-repeat isoforms) and the invertebrate MAPs CG31057 and PTL-1A (five-repeat isoforms) were analyzed using the program Phylip [76]; gaps were ignored. The available *Tetraodon* sequences are incomplete and were therefore not included in the analysis.

Figure 2
The domain organization of MAP2/Tau family proteins. Selected isoforms of the human members of the family are shown, as well as the nematode homolog PTL-1. All family members have alternative splice forms with varying numbers of carboxy-terminal microtubule-binding repeats and amino-terminal projection domains of varying lengths. PKA (RII) indicates a domain interacting with the RII subunit of protein kinase A. Repeats that are not present in all major isoforms are shown lighter.

[26], differential protein sorting [27], and dendrite-specific transport of *MAP2* mRNA [28] are responsible for this spatial segregation of the two MAPs. Thus, in mature neurons Tau is present mainly in axons whereas MAP2 is restricted to cell bodies and dendrites (Figure 3).

Functions of MAP2 and Tau in neurons
MAP2/Tau family proteins were originally discovered for and characterized by their ability to bind and stabilize microtubules. Ultrastructural analyses revealed the presence of these MAPs along the sides of microtubules [29-31]. MAP2 and Tau also increase microtubule rigidity [32] and induce microtubule bundles in heterologous cell systems [33-35]. Microtubule bundle formation induced by MAP2 was suggested to be an indirect effect of its stabilization of microtubules within the confinement of cell borders [36], but more recent results suggest that MAP2-induced bundles can form even within the interior of the cell [37], indicating the existence of crosslinks. Evidence for direct crosslinking of microtubules by MAP2/Tau family proteins is lacking, leaving open the possibility that additional proteins are necessary.

As described above, MAP2 can bind both microtubules and F-actin, and both activities have been mapped to its microtubule-binding-repeat domain. It is not yet known wh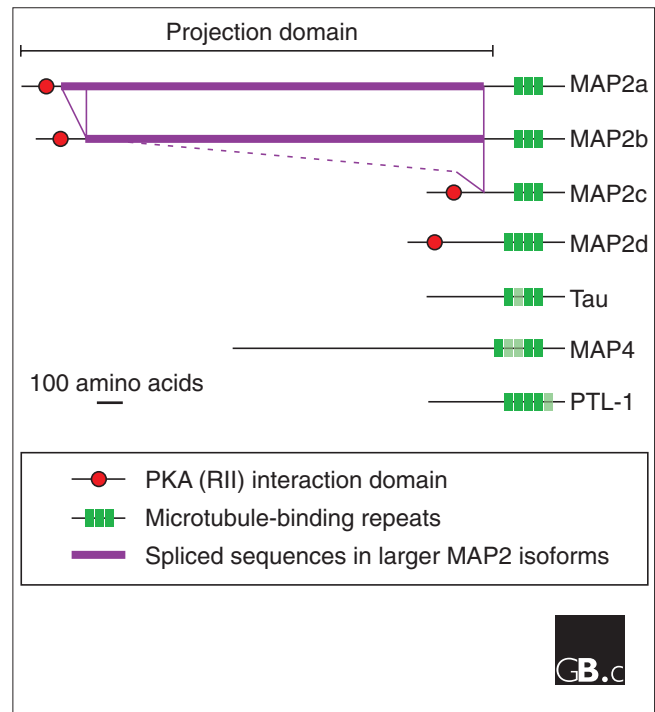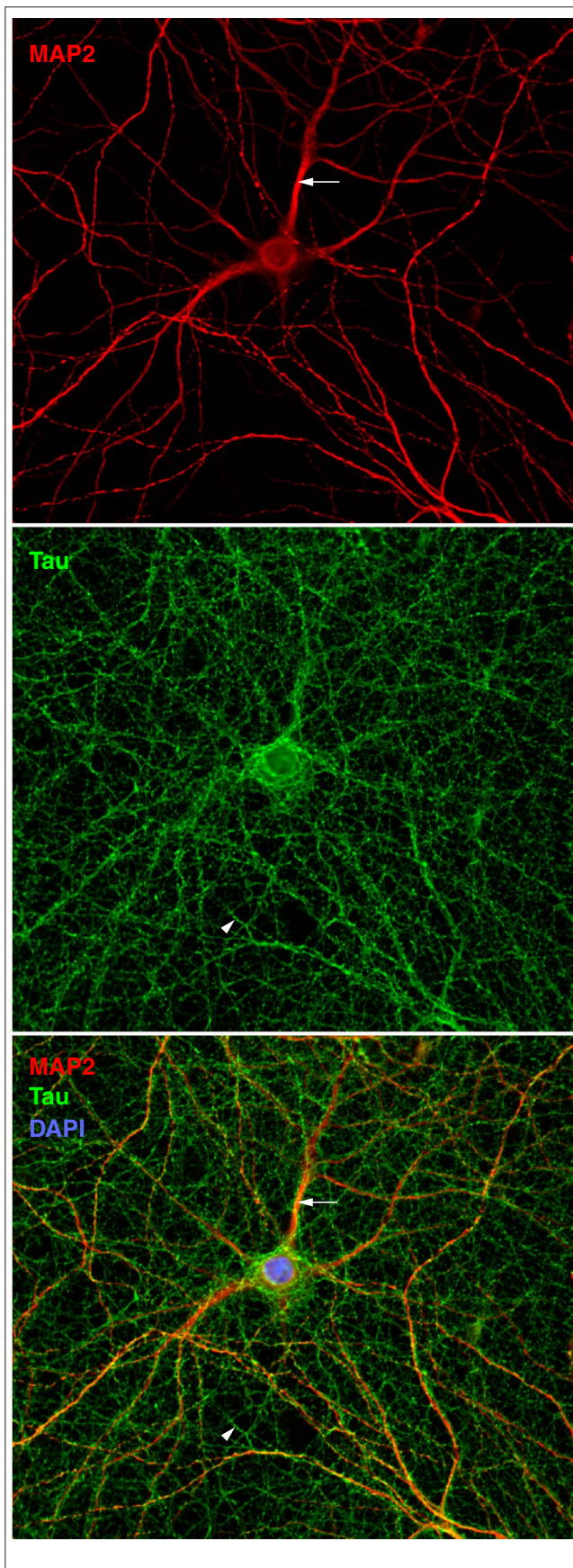ether a single molecule can crosslink an actin filament to a microtubule. MAP2 can bundle actin filaments *in vitro* [16]. MAP2c by itself can induce neurites in Neuro-2a neuroblastoma cells; its microtubule-stabilizing activity is necessary for this effect but is not sufficient, and F-actin dynamics also need to be altered [38]. MAP2's ability to interact with F-actin appears to be key to this specific biological function. Unlike MAP2c, neither Tau nor chimeric MAP2c containing the Tau microtubule-binding domain can trigger neurite initiation, an observation that correlates with their lack of F-actin binding *in vitro* [16]. This suggests that MAP2c's ability to interact with both microtubules and F-actin is essential for its neurite-initiation activity.

Knockout experiments in mice suggest that neither MAP2 nor Tau is essential by itself, but each single knockout leads to detectable morphological phenotypes. Tau expression was undetectable after targeted deletion of the first *Tau* exon, which includes the protein start codon [39]. Homozygous animals showed no major defects in brain morphology, but the microtubule density in small-caliber axons was reduced. Similarly, MAP2 expression was undetectable after deletion of one exon encoding a portion of the MAP2 microtubule-binding domain [40]. Again, homozygous

Figure 3
A neuron from a culture of rat brain hippocampus, showing the distinct subdomains of MAP2 and Tau enrichment in mature neurons. MAP2 is found specifically in dendrites (arrow), whereas Tau is mainly axonal (arrowhead). Note the fine meshwork of axons from neighboring cells outside the field of view that make numerous synaptic connections among the neurons in the culture.

animals showed no major defects in brain morphology, but microtubule density in dendrites was reduced. In addition, dendrite length in cultured neurons was reduced, suggesting a role for MAP2 in supporting dendrite elongation.

The phenotypes of single knockouts suggest specific but nonessential roles for Tau and MAP2 in the morphogenesis of the nervous system. However, these proteins probably have multiple roles in other pathways and can be compensated for by other proteins with redundant functions. Interestingly, the structurally unrelated microtubule-associated protein MAP1B appears to have some redundant roles with both Tau [41,42] and MAP2 [43]. Simultaneous inhibition of either MAP1B and Tau or MAP1B and MAP2 resulted in more severe phenotypes than those seen in single knockouts. Taken together, these experiments suggest a role for Tau, MAP2 and MAP1B in both neuronal migration and outgrowth of neurites. Redundancy among MAP2, Tau and MAP4 has not been adequately tested in mammalian systems. It is also possible that other classes of MAP such as stable tubule only protein (STOP), adenomatous polyposis coli (APC), doublecortin, or spectraplakins might provide additional redundancy with MAP functions.

MAP2/Tau family proteins have been shown to interact with numerous proteins; Table 2 provides an overview of identified interaction partners and briefly describes the proposed function of each interaction. Binding of MAP2 to the RII regulatory subunit of PKA is one of the best-characterized examples of a classical MAP functioning as an adaptor protein. The interaction site was mapped to the amino terminus of MAP2 and is present in all common MAP2 splice forms in mammals [44] but absent in Tau. Knockout mice show that MAP2 is essential for linking PKA to microtubules in various brain regions [40]. Interestingly, the absence of MAP2 affects the phosphorylation of cAMP-responsive element binding protein (CREB), suggesting a role for the MAP2-PKA interaction in CREB-mediated signal transduction [40]. Deletion of the PKA-binding site in MAP2c reduces its ability to induce neurites in neuroblastoma cells [38].

Tau has been studied extensively for its involvement in neurofibrillary tangle formation in Alzheimer's Disease and in frontotemporal dementias associated with chromosome

Table 2

Selected interaction partners of MAP2/Tau family proteins

Family member	Interacting protein	Proposed function of the interaction	Reference
MAP2	Microtubules	Stabilization of microtubules; inhibition of depolymerization (catastrophes); increase in microtubule rigidity, neurite initiation	[77]
	F-actin	Modulation of neurite initiation	[16]
	Regulatory subunit RII of PKA	Localization of PKA to hippocampal dendrites; facilitation of cAMP-responsive element binding protein (CREB) phosphorylation; modulation of neurite initiation	[44]
	Tyrosine kinase Src	Signal transduction and integration	[78]
	Adapter protein Grb2	Signal transduction and integration	[78]
	Tyrosine kinase Fyn	Signal transduction and integration	[79]
	Neurofilaments	Crossbridges between microtubules and neurofilaments	[80]
	Class C L-type calcium channels	Linking PKA to channels	[81]
	MAP2-RNA trans-acting proteins MARTA1 and MARTA2	Interaction with MAP2 mRNA: targets MAP2 mRNA to dendrites	[82]
Tau	Microtubules	Stabilization of microtubules; inhibition of depolymerization (catastrophes); increase in microtubule rigidity	[83]
	Fyn	Modulation of microtubule organization; pathogenesis of Alzheimer's disease	[84]
	Src	Unknown	[84]
	Presenilin 1	Links Tau to glycogen synthase kinase 3β; pathogenesis of Alzheimer's disease	[85]
	Apolipoprotein E	Regulation of Tau metabolism; pathogenesis of Alzheimer's disease	[86]
	Calmodulin	Regulation of microtubule assembly	[87]
	Calmodulin-related protein S100b	Regulation of Tau phosphorylation by protein kinase C	[87]
MAP4	Microtubules	Stabilization of microtubules; inhibition of depolymerization (catastrophes)	[49]
	Cyclin B	Links p34[cdc2] kinase to microtubules; regulation of M-phase microtubule dynamics	[51]

17 (FTDP-17); see several excellent discussions of Tau pathology [45-48].

Functions of MAP4 and non-neuronal functions of MAP2 and Tau

The widely expressed non-neuronal member of the MAP2/Tau family, MAP4, shares many features with other members of the family, including the presence of microtubule-binding repeats [49] and microtubule-stabilizing activity [50]. MAP4 has been proposed to play a role in regulating mitotic microtubule dynamics during metaphase [51]. However, using function-blocking antibodies that interfere with the MAP4-microtubule interaction, a more recent study [52] failed to detect an obvious phenotype in mitosis or during interphase, suggesting that MAP4 might be a component of a functionally redundant system. Muscle-specific MAP4 isoforms have been shown to be required for myogenesis [53], but the exact role of MAP4 is not known in this process.

Although MAP2 is primarily neuronal, some isoforms are also present in certain astrocytes [54], oligodendrocytes [55], as well as in the testis [56]. The testicular isoform of MAP2 contains a functional nuclear localization sequence [56] and is enriched in nuclei of germ cells. Like MAP2, the primarily neuronal Tau is also expressed in oligodendrocytes [57]. Interestingly, alternative splicing of MAP2 [55] and Tau [58] is similar during the maturation of oligodendrocytes and neurons. In oligodendrocytes, Tau and its regulation by the Fyn tyrosine kinase are proposed to be involved in process outgrowth [59].

Mechanism and regulation

Microtubules exhibit dynamic instability, an intrinsic behavior characterized by alternating phases of growth, shortening, and pausing. The switch from growth to shortening and the switch from shortening to growth are called catastrophes and rescues, respectively. MAP2/Tau proteins bind along the length of microtubules and stabilize microtubules by altering

this dynamic behavior [31,60,61]. The small isoform MAP2c stabilizes microtubules primarily by reducing the frequency and duration of catastrophes [60]. Under conditions where its concentration is non-saturating, MAP2 can also form clusters on microtubules, and microtubule catastrophes stop at such clusters [62]. Interestingly, isoforms of Tau containing three or four microtubule-binding repeats have distinct effects on microtubule dynamics, with four-repeat isoforms protecting microtubules from depolymerization much more robustly than three-repeat isoforms [61]. In cells, microtubules still exhibit dynamic behavior even when stabilizing MAPs are highly expressed [63], perhaps because their binding is regulated by phosphorylation and other factors.

A detailed cryo-electron microscopy (cryo-EM) analysis has suggested a possible mechanism by which MAP2/Tau might reduce catastrophes and thus stabilize microtubules. This study revealed that the microtubule-binding repeats interact in an elongated fashion on the outer microtubule lattice, spanning two tubulin dimers along a single protofilament rather than bridging adjacent protofilaments [31]. Tau appeared to show a similar pattern. Several other experiments confirm that MAP2 binds to the outside of microtubules *in vivo*. First, the projection domain of MAP2 can regulate microtubule spacing [64]. In addition, an EM study that compared wild-type to knockout animals suggested that electron-dense structures on the outer surface of microtubules contain MAP2 [40]. Another cryo-EM analysis suggested that Tau binds to the inner surface of microtubules [65], but the role of this binding is not yet clear. Tau might be able to bind to multiple sites, both inside and outside the microtubule lattice. This idea is consistent with the observation that Tau has different kinetic properties when bound to pre-polymerized microtubules than when co-polymerized with microtubules [66].

MAP2/Tau family proteins can inhibit kinesin- and dynein-dependent transport along microtubules [67-71]. Observations *in vitro* suggest that this inhibition of microtubule motor activity occurs by direct competition of MAP2/Tau proteins with dynein and kinesin for microtubule binding and also suggest a major role for the projection domain of the MAP2/Tau proteins in this competition [69,71]. In cells, overexpression of Tau interferes with kinesin-based transport and alters the balance of plus-end- versus minus-end-directed transport [67,68]. *In vivo*, the MAP2 and Tau projection domains appear to be involved in regulating microtubule spacing [64]. Such control over microtubule spacing might facilitate efficient organelle transport.

Binding of MAP2/Tau family proteins to microtubules can be regulated by phosphorylation of the KXGS motif within each microtubule-binding repeat. For both MAP2 and Tau, these motifs are phosphorylated by multiple protein kinases, including PKA [11] and the microtubule affinity regulating kinase (MARK) [12], and phosphorylation leads to decreased affinity for microtubules. Recent evidence also links the Jun kinase (Jnk) pathway to phosphorylation of MAP2 [72]. Many other protein kinases can phosphorylate MAP2/Tau proteins *in vitro*, but for most the identity of the targeted residues *in vivo* and the functional consequences of phosphorylation remain to be determined. For example, in the olfactory bulb, a site in the amino-terminal domain of MAP2 is phosphorylated *in vivo* in a manner that is regulated by sensory-driven neural activity; the function of this phosphorylation is not yet known, however [73]. The regulation of MAPs, including the MAP2/Tau family, has been summarized in a comprehensive review [74].

Frontiers

Since their original identification over 20 years ago, classical structural MAPs of the MAP2/Tau family have been extensively characterized *in vitro* and *in vivo*. A major challenge for further illuminating their function is the vast number of interaction partners and protein kinases predicted and confirmed to phosphorylate MAP2/Tau proteins. Although some key pathways controlling their activity have been elucidated, a broader and more precise analysis of phosphorylation and other post-translational modifications is needed to fully understand MAP2/Tau protein function in signaling networks controlling the morphogenesis of neurons. Recent progress in understanding the molecular mechanisms underlying MAP-microtubule and MAP-actin interactions *in vitro* is promising, but biological functions remain elusive. Future studies will need to correlate the effects of MAP2/Tau proteins *in vivo* with molecular knowledge gained from *in vitro* analyses. The apparent functional redundancies and cross-talk with other MAPs and cytoskeletal regulators are challenges that will require creative experimental strategies if we are to elucidate the specific functions of MAP2/Tau family proteins in cytoskeletal organization and morphological change.

Acknowledgements

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