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CYTOSKELETON NEWS

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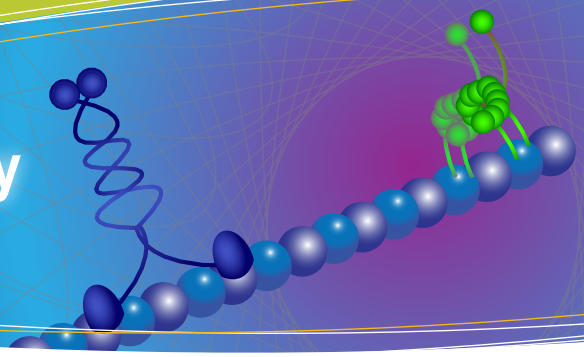

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MAPping Tau's Future

Tau is a structural microtubule-associated protein (MAP) predominantly (but not exclusively) localized to microtubules (MTs) within neuronal axons. Tau is arguably the most studied MAP, primarily due to its central role in neurodegenerative tauopathies (e.g., Alzheimer's Disease, corticobasal degeneration, frontotemporal dementia)[1,2]. Importantly, tau is as essential in normal cellular physiology as in neurodegeneration. First described as a MAP which promotes assembly of MTs[3-5], tau's roles now also include MT stabilization, MT bundling, modulation of MT-dependent axonal transport, and regulation of neurite outgrowth[1,2,6-10]. However, the complete physiological understanding of how tau regulates MT functional organization remains unknown. Four recent studies of novel means by which tau interacts with MTs and other MAPs are discussed below.

Technological advances, especially in microscopy, provide unparalleled direct, single molecule insights into how tau binds MTs[9-11]. A recent cryo-electron microscopy study[12] reveals that tau's repeat MT binding regions adopt extended structures and bind to the MT surface along a protofilament to stabilize interactions between tubulin heterodimers (Fig. 1). The extended conformation of each repeat region spans intra- and interdimer interfaces, allowing connections between tubulin heterodimers[12] (Fig. 1). These analyses at the near-atomic level have led some researchers[10] to suggest that tau is ideally situated to promote MT assembly, perhaps in the absence of any stabilization given tau's rapid on-off rate[13]. Tau's rate was determined through fast single molecule tracking experiments. In living neuronally-derived cells and primary neurons, tau dynamically binds, dissociates, and binds neighboring MTs rapidly (termed "kiss and hop") with an on-off rate of 40 milliseconds[13]. Despite this unexpectedly rapid MT dwell time (shorter by two orders of magnitude than previously reported[14]), tau was still a potent promoter of tubulin polymerization in neurites. These findings contrast with the dogma that structural MAPs adhere to the MT surface in a static fashion to prevent disassembly.

Although tau is a long-studied structural MAP, novel findings continue to emerge regarding tau-mediated regulation of other MAPs. Recent studies describe novel spatial and functional heterogeneities in tau localized on MTs[15-17]. Dense islands of bound tau (a.k.a. condensates) assemble in a physiological and reversible manner and distribute along discrete regions of individual MTs to compartmentalize the MT and form selectively MAP-permeable barriers[15-17] (Fig. 1). Although an earlier paper described tau "patches" on MTs[18], Siahaan et al.[16] posit that the tau islands are "fundamentally distinct" from these patches. The MT lattice itself regulates physiological self-assembly of tau into condensates/islands in a reversible manner. The condensates form at areas of high MT curvature, are controlled by the nucleotide state of the MT lattice, and rely upon the C-terminal tails of tubulin[17]. Despite tau's rapid on-off rate, it regulates the binding and functionality of motile (e.g., plus-end-directed kinesin motors and minus-end-directed dynein motor)[18-22] and non-motile MAPs (e.g., MT severing enzymes such as spastin and katanin, MAP6, MAP7)[16,17] (Fig. 1). Tau condensates inhibit kinesin motility in an isoform-specific manner and recent single molecule imaging studies[15-17] provide additional insights into how tau condensates interact with various MAPs. The condensates/islands halt kinesin-1 and kinesin-3 motility, while kinesin-8 traverses the tau condensates which triggers their disassembly[15-17]. Similarly, the majority of dynein motors pass through the condensates/islands after a pause[16]. A shortened, but active form, of the MT severing enzymes spastin and katanin were mostly excluded from condensates, thus protecting the MT from cleavage[15-17]. Tau expression is inversely related to the expression and function of MAP7

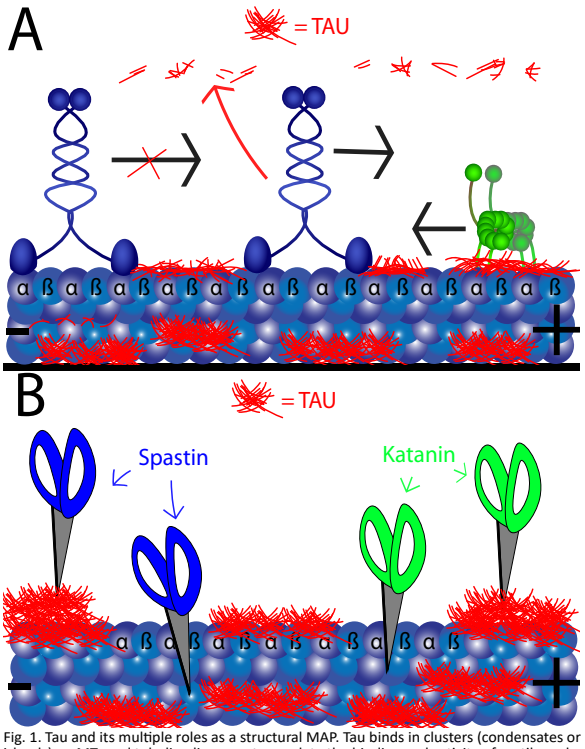

Fig. 1. Tau and its multiple roles as a structural MAP. Tau binds in clusters (condensates or islands) on MTs and tubulin oligomers to regulate the binding and activity of motile and non-motile MAPs. Tau binds at the inter-dimer interface via an interaction with tubulin heterodimers which links the dimers. (A) Tau condensates block plus-end-directed motility of kinesin-1 and kinesin-3, but not kinesin-8. Kinesin-8 triggers disassembly of the condensates. Minus-end-directed motility of dynein is primarily unimpeded. (B) Tau condensates block the MT severing enzymes spastin and katanin. Green motor, dynein; Blue motors, kinesins.

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and MAP6. MAP7 competes with tau for the same MT binding sites and can displace the tau condensates/islands[15]. Also, MAP7 positively regulates kinesin-based cargo transport and kinesin-1 binding to MTs, respectively, *in vivo* and *in vitro*[15]. After MAP7-mediated disruption of bound tau, MAP7 recruits kinesin-1 for binding at MT sites previously occupied by tau[15]. The two MAPs are not always antagonistic; both MAP7 and tau inhibited kinesin-3 motility[15]. The relationship between MAP6 and tau is discussed below. Notably, Yuan et al.[23] find that changes in tau expression levels (either elevated or reduced) in retinal ganglion cell axons did not affect axonal transport rates *in vivo*. These seemingly disparate data regarding tau modulation of axonal transport require further analyses, although the "kiss and hop" mechanistic model of tau/MT interactions is compatible with the *in vivo* results[13].

Arguably the most thought-provoking tau results challenge the very dogma that has guided tau research for decades. The majority of tau research, both physiological and pathological, starts with the premise that tau is a stabilizer of MTs under physiological conditions and that upon pathological hyperphosphorylation, tau dissociates from MTs which compromises their stability and results in disassembly[1,2,10]. Normal axonal MTs consist of labile and stable domains[24-26] with tau primarily decorating the former regions[10,27]. In contrast, MAP6 is predominantly on the stable domains of axonal MTs[28,29]. Upon depletion of tau in cultured rat neurons, labile MT domains were lost, while the stable domains increased. Furthermore, MAP6's expression increases and its distribution on axonal MTs expands[10,27]. Conversely, loss of MAP6 increases the degree of lability in the labile MT domains and results in an increase in tau expression levels and its distribution along axonal MTs[10,27]. These findings were the basis for the conclusion that tau is in fact not a stabilizer of MTs but instead promotes the assembly of long labile domains and prevents MAP6-mediated stabilization, conferring flexibility to MTs[10,27].

Summary

Despite tau's discovery decades ago and its central role in tauopathies, much remains to be discovered about how tau regulates MT structure, function, and binding to other MAPs in healthy and diseased neurons. This dearth of knowledge likely extends beyond tau to other MAPs. To assist researchers in studies of MT and MAP functions and interactions, Cytoskeleton offers tubulin polymerization and binding assay kits, MT live cell imaging probes, purified tubulins, and Signal-Seeker Enrichment kits which allow detection and measurement of endogenous levels of various post-translational modifications – all of which target tubulin and MTs.

Tubulin Biochem Kits

Product	Assays	Cat. #
Tubulin Polymerization Assay Biochem Kit™ Turbidometric-based, >97% pure tubulin	24-30	BK004P
Tubulin Polymerization Assay Biochem Kit™ Turbidometric-based, >99% pure tubulin	24-30	BK006P
Tubulin Polymerization Assay Biochem Kit™ Fluorescence-based, >99% pure tubulin	96	BK011P
Microtubule Binding Protein Spin-Down Assay Biochem Kit™	30-100	BK029
Microtubule / Tubulin In Vivo Assay Biochem Kit™ Quantitates *in vivo* ratio of tubulin polymers & monomers	30-100	BK038

Purified Tubulins and MAPs

Product	Amount	Cat #
Tubulin protein (97% pure) Source : porcine brain	1 x 4 mg 1 x 40 mg	HTS03-A HTS03-B
Microtubule associated protein rich fraction Source : porcine brain	1 x 100 µg 5 x 100 µg	MAPF-A MAPF-C
Tau protein Source : bovine brain	1 x 50 µg 3 x 50 µg	TA01-A TA01-B
Tubulin protein (>99% pure) Source : porcine brain	1 x 1 mg 5 x 1 mg	T240-A T240-B

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Tubulin Live Cell Imaging Products

Product	Ex / Em	Amount	Cat #
SiR-Tubulin™ Kit Includes SiR-Tubulin, and Verapamil	630 / 680 nm	50 nmol	CY-SC002
SiR700-Tubulin Kit 35 nmol SiR700-Tubulin and 1 µmol verapamil	680 / 720 nm	50 nmol	CY-SC014
Cytoskeleton Kit Includes SiR-Actin, SiR-Tubulin and Verapamil	630 / 680 nm	50 nmol each	CY-SC006