THE REGULATION OF MOTORS BY MICROTUBULE-ASSOCIATED PROTEINS

AHMET YILDIZ

Department of Molecular and Cell Biology, University of California, Berkeley, CA, 94720 USA

Physics Department, University of California, Berkeley CA, 94720 USA

Microtubule-Associated Proteins (MAPs)

Microtubules play critical roles in virtually every cellular process, from cargo transport to cell division, by organizing into unique structures with defined architecture and dynamics. The assembly and dynamics of the microtubule network are regulated by structural MAPs, such as Tau, MAP7, and MAP9. These MAPs are non-catalytic and intrinsically disordered proteins that densely decorate the microtubule surface via a short microtubule binding domain (MTBD) and extend from the microtubule surface through their disordered projection domains (Fig. 1)(*3*).

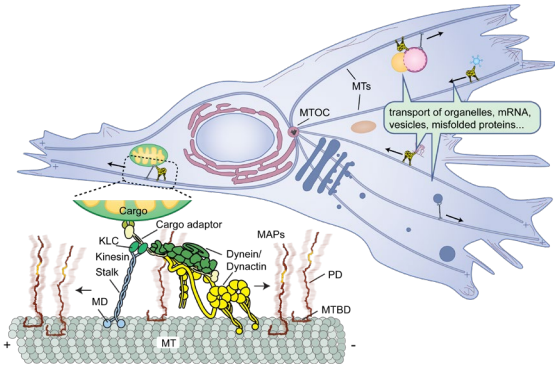

Fig. 1. Intracellular transport. Kinesin and dynein drive intracellular cargos towards the plus- and minus-ends of microtubules, respectively. Cargo transport is regulated by structural MAPs that decorate the MTs (MD: motor domain, PD: projection domain). Figure modified from (*2*).

In neurons, MAPs exhibit distinct microtubule decoration patterns, which correlate with their regulatory role in the control of intracellular traffic (*4, 5*). For example, tau is a neuronal MAP concentrated in axons and synapses, whereas it is absent in dendrites (*6*). Aberrant expression and phosphorylation of tau disrupt the transport of synaptic vesicles along axons, while the knockdown of tau rescues defects in axonal transport (*7, 8*). In comparison, MAP2 sorts cargos at the pre-axonal filtering zone by inhibiting kinesin-1 while allowing kinesin-3-driven transport (*5*).

MAPs were generally considered to serve as obstacles against motor-driven transport. Recent *in vitro* studies altered this view: kinesin-1 is inhibited by tau, DCX, and MAP9 (*9, 10*), but activated by MAP7 (*9, 11*). Conversely, kinesin-3 motility is inhibited by MAP7 and tau but promoted by MAP9 (*9*). Recent studies proposed that MAPs can activate motors through specific molecular interactions (*9-12*), which led to the "MAP code model" postulating that spatial patterning of distinct MAPs routes intracellular cargos to different cellular locations by selectively inhibiting or promoting the motors that transport these cargos (*10*).

The mechanism by which MAPs decorate the microtubule surface and contribute to the inhibition or activation of a motor is not well understood. Previous studies proposed that MTBDs of MAPs inhibit motors by overlapping with their tubulin-binding sites (*9*). It is also possible that MAPs specifically interact with one motor type and promote its motility while inhibiting the motors they do not bind directly (*9*). These models are not mutually exclusive, and their predictions have not been carefully tested in vitro.

Determining the Microtubule-Bound Structure of MAPs

The lack of in-depth mechanistic studies in this field is mainly because MAPs are intrinsically disordered proteins and cannot be directly studied by structural methods. However, when a MAP interacts with the microtubule, a part of it rigidly binds to the microtubule lattice and becomes well-ordered. Together with E. Nogales, we used cryo-EM to solve the structure of MAP-decorated microtubules. MAPs were detected as an extra density on the surface of reconstructed microtubules.

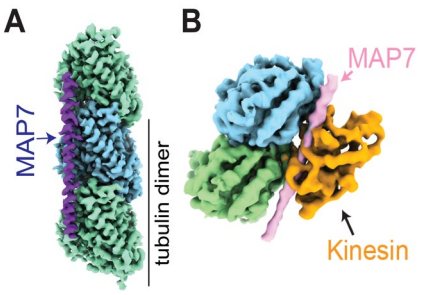

Fig. 2. MT-bound structure of MAP7. A. Cryo-EM MAP of MAP7 on tubulin at 3.3 Å resolution. **B.** Superimposing of kinesin-1 motor domain onto the cryo-EM map shows an overlap. Figure modified from (*1*).

We first performed structure-function studies on MAP7, which binds to microtubules virtually everywhere in interphase cells and neurons and serves as a required cofactor for kinesin-1-driven transport (*11, 13, 14*). The projection domain of MAP7 binds to kinesin-1's stalk (*9, 11*), recruits the motor to microtubules, and activates its motility in vitro (*10, 11*). The cryo-EM reconstruction revealed a single α-helix that runs parallel to the microtubule axis about the length of a tubulin dimer (*1*) (Fig. 2A). Unlike tau, which binds along the outer ridges of the protofilaments (*15*), MAP7 ran halfway between the outer ridge and the site of lateral contacts.

Superimposing the microtubule-bound structure of kinesin-1 onto our model revealed an apparent clash between kinesin-1 and MAP7 (Fig. 2B). This raised the question of how MAP7 activates kinesin-1 despite competing for the same tubulin binding site. Previous studies on DCX and MAP4 proposed that a MAP can accommodate kinesin binding by shifting away from its tubulin binding site (*12, 16*). To test this possibility for MAP7, we determined the structure of microtubules incubated with kinesin-1 and MAP7. While standard methods to obtain the high-resolution structure of microtubules showed that kinesin-1 and MAP7 can bind to the same tubulin, we noticed that the density of MAP7 and kinesin might be averaged during reconstruction. To avoid this artifact, we developed a new approach in structural analysis of MAPs by performing focused 3D classification around the putative shared binding site of MAP7/kinesin-1. This approach revealed two distinct maps of the binding site, one occupied by MAP7 only, and the other occupied by kinesin-1 only (*1*). Thus, kinesin-1 and MAP7 cannot bind to the same tubulin.

Single-molecule studies of MAP7-kinesin-1 interactions

Despite an overlap, we observed that the addition of MAP7 rescued kinesin-1 from autoinhibition (*17*) and substantially boosted its motility (*9, 11*). Unexpectedly, kinesin-1 run frequency decreased at high MAP7 concentrations. The nonlinear relationship between MAP7 decoration and kinesin-1 motility arises because the motor is subjected to activating and inhibiting effects that dominate at different concentrations. We showed that MAP7 MTBD inhibits, while the projection domain activates kinesin-1 (*1, 9*). To reveal how the projection domain allows kinesin-1 to bypass MTBD obstacles on microtubules, we tracked kinesin-1 stepping with nanometer precision (Fig. 3A). While kinesin-1 rarely takes sideways and backward steps

on undecorated microtubules, it took large (30-64 nm) displacements, as well as more frequent sideways and backward stepping on MAP7-decorated microtubules (*1*) (Fig. 3B). Because kinesin cannot take such large steps on its own, these results indicate that the motor dissociates from microtubules when it encounters an MTBD obstacle but remains tethered to the microtubule by the projection domain of MAP7 (Fig. 3C). This enables kinesin-1 to efficiently rebind to a nearby empty site and keep walking on another protofilament (*18*). The tethered diffusion mechanism no longer works at high MAP7 concentrations due to the scarcity of empty tubulin sites for the motor to rebind after dissociation (*1*).

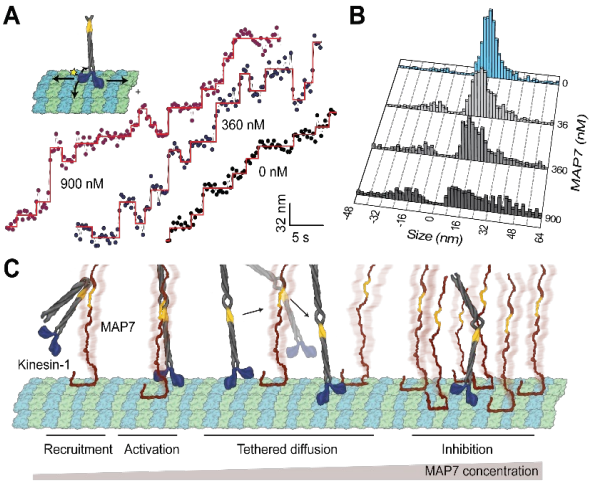

Fig. 3. Kinesin-1 stepping on MAP7 decorated MTs. A. Kinesin-1 stepping on MTs decorated with different MAP7 concentrations. **B.** Step size histograms reveal large jumps and backward stepping of kinesin-1 on MAP7-decorated MTs (*1*). **C.** Tethered diffusion model. Figure modified from (*1*).

MAP9 binds to the circumference of microtubules

We next investigated a relatively understudied MAP9, which plays critical roles in mitosis, cilia, and neurite outgrowth (*5*). Compared to tau and MAP7, the molecular mechanism of how MAP9 interacts with microtubules is unknown. Our study reveals that MAP9 engages adjacent protofilaments via a 300 Å α-helix that encircles the microtubule. The interaction of MAP9 with β-tubulin involves a newly identified sequence present five times along MAP9 MTBD, with 35 or 36 residue spacers that allow the repeats along the helix to reach across neighboring protofilaments. This unprecedented binding mode enables MAP9 to “staple” protofilaments together, dramatically enhancing microtubule stability by suppressing catastrophes in vitro.

Unlike MAP7, MAP9 emerged as a specific cofactor of kinesin-3 KIF1A while inhibiting kinesin-1 (*10*), but it remained unclear how MAP9 distinguishes between these two motors that are so closely related. To address this question, we obtained the cryo-EM structure of microtubules co-decorated with MAP9 MTBD and the kinesin-3 motor domain. We show

that MAP9 distinguishes between kinesin-1 and kinesin-3 via a divergent loop (loop-8) within the kinesin motor domain. Unlike MAP7 or tau, MAP9 does not overlap with the kinesin binding interface on tubulin. Instead, it positions itself adjacent to loop-8, selectively permitting kinesin-3 motility while blocking kinesin-1. Remarkably, inserting loop-8 from kinesin-3 into kinesin-1 renders kinesin-1 resistant to MAP9. These results demonstrate that MAP9 distinguishes between kinesins through divergent loop sequences, revealing a new principle by which MAPs control transport driven by motor proteins.

MAP9 is an essential factor for neuronal development

The traditional view of MAPs is that they control the structural integrity and dynamics of microtubules (*5*). While knockdown of neuronal MAPs induces axonal branching and dendritic phenotypes, neurons were still able to develop without major perturbations to their overall morphology *(19)*. These observations led to the proposal that MAPs have overlapping functions in stabilizing microtubules, such that the loss of one MAP can be compensated for by the others. However, it remains unclear whether there are MAPs that play indispensable, non-redundant roles in driving early axonal extensions.

We identified MAP9 as a structurally and functionally distinct MAP that stabilizes microtubules. A unique circumferential binding mode of MAP9 effectively “staples” the protofilaments together, dramatically promoting microtubule growth and suppressing microtubule catastrophe compared to tau and MAP7. MAP9 binding likely prevents protofilament curvature and peeling, thereby protecting microtubules against depolymerization more effectively than those MAPs that stabilize longitudinal contacts within single protofilaments (*1, 15*).

Consistent with this mechanistic difference, we show that depletion of MAP9 results in complete arrest of neurite outgrowth, suggesting that not all MAPs contribute equally to lattice stability. These results challenge the long-held view that MAPs act as functionally interchangeable stabilizers and instead point to a division of labor in which the specific binding geometry of individual MAPs dictates their ability to preserve axonal and dendritic microtubules. These insights position MAP9 as a key regulator of neuronal architecture and a promising target for future studies into the mechanisms of neuronal development.

Future Challenges

Although most MAPs are predicted to span multiple tubulin subunits on a microtubule protofilament, the current cryo-EM image processing pipeline cannot determine the microtubule footprint of a MAP longer than the length of a tubulin heterodimer due to the averaging and helical reconstruction of

microtubules. It is possible to determine the full microtubule footprint of a MAP by further advancing the focused 3D classification methods in cryo-EM or by using a fiducial mark on the microtubule footprint of a MAP.

While the microtubule binding mode of MAPs can be characterized using cryo-EM, the rest of these proteins remain disordered and cannot be studied by structural methods. How disordered projection domains contribute to microtubule binding, microtubule stability, and motor regulation remain to be studied. Biochemical reconstitution and protein engineering studies are poised to reveal how these modifications affect MAP binding to surface-immobilized microtubules and the polymerization of dynamic microtubules in vitro and determine how the decoration of microtubules by MAPs modulates the ability of motors to walk along these microtubules.

References

1. L. S. Ferro *et al.*, Structural and functional insight into regulation of kinesin-1 by microtubule-associated protein MAP7. *Science* **375**, 326-331 (2022).
2. A. Yildiz, Y. Zhao, Dyneins. *Current biology : CB* **33**, R1274-R1279 (2023).
3. W. A. Voter, H. P. Erickson, Electron microscopy of MAP 2 (microtubule-associated protein 2). *J Ultrastruct Res* **80**, 374-382 (1982).
4. L. F. Gumy *et al.*, MAP2 Defines a Pre-axonal Filtering Zone to Regulate KIF1- versus KIF5-Dependent Cargo Transport in Sensory Neurons. *Neuron* **94**, 347-362 e347 (2017).
5. S. Bodakuntla, A. S. Jijumon, C. Villablanca, C. Gonzalez-Billault, C. Janke, Microtubule-Associated Proteins: Structuring the Cytoskeleton. *Trends in cell biology* **29**, 804-819 (2019).
6. Y. Wang, E. Mandelkow, Tau in physiology and pathology. *Nat Rev Neurosci* **17**, 5-21 (2016).
7. A. Ebneth *et al.*, Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. *J Cell Biol* **143**, 777-794 (1998).
8. T. Ishihara *et al.*, Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron* **24**, 751-762 (1999).
9. B. Y. Monroy *et al.*, Competition between microtubule-associated proteins directs motor transport. *Nature communications* **9**, 1487 (2018).
10. B. Y. Monroy *et al.*, A Combinatorial MAP Code Dictates Polarized Microtubule Transport. *Developmental cell* **53**, 60-72 e64 (2020).
11. P. J. Hooikaas *et al.*, MAP7 family proteins regulate kinesin-1 recruitment and activation. *J Cell Biol* **218**, 1298-1318 (2019).
12. J. S. Liu *et al.*, Molecular basis for specific regulation of neuronal kinesin-3 motors by doublecortin family proteins. *Molecular cell* **47**, 707-721 (2012).

13. K. Barlan, W. Lu, V. I. Gelfand, The microtubule-binding protein ensconsin is an essential cofactor of kinesin-1. *Current biology : CB* **23**, 317-322 (2013).
14. S. R. Tymanskyj, B. H. Yang, K. J. Verhey, L. Ma, MAP7 regulates axon morphogenesis by recruiting kinesin-1 to microtubules and modulating organelle transport. *eLife* **7**, 36374 (2018).
15. E. H. Kellogg *et al.*, Near-atomic model of microtubule-tau interactions. *Science* **360**, 1242-1246 (2018).
16. H. Shigematsu *et al.*, Structural insight into microtubule stabilization and kinesin inhibition by Tau family MAPs. *J Cell Biol* **217**, 4155-4163 (2018).
17. A. Yildiz, Mechanism and regulation of kinesin motors. *Nature reviews. Molecular cell biology*, (2024).
18. L. S. Ferro *et al.*, The mechanism of motor inhibition by microtubule-associated proteins. *bioRxiv*, 2020.2010.2022.351346 (2020).
19. Y. D. Ke *et al.*, Lessons from tau-deficient mice. *Int J Alzheimers Dis* **2012**, 873270 (2012).