Microtubule (MT)–associated protein 7 (MAP7) is a required cofactor for kinesin-1–driven transport of intracellular cargoes. Using cryo–electron microscopy and single–molecule imaging, we investigated how MAP7 binds MTs and facilitates kinesin-1 motility. The MT-binding domain (MTBD) of MAP7 bound MTs as an extended α-helix between the protofilament ridge and the site of lateral contact. Unexpectedly, the MTBD partially overlapped with the binding site of kinesin-1 and inhibited its motility. However, by tethering kinesin-1 to the MT, the projection domain of MAP7 prevented dissociation of the motor and facilitated its binding to available neighboring sites. The inhibitory effect of the MTBD dominated as MTs became saturated with MAP7. Our results reveal biphasic regulation of kinesin-1 by MAP7 in the context of their competitive binding to MTs.

Kinesin and dynein are molecular motors that deliver intracellular cargos by walking along microtubules (MTs) (1, 2). Intracellular cargos are differentially regulated by structural MT-associated proteins (MAPs) that decorate the MT surface (3). Distinct cellular localizations of MAPs correlate with their regulatory roles in intracellular traffic (3). Overexpression of tau disrupts kinesin-1 (hereafter kinesin)–driven transport of synaptic vesicles in axons (4, 5), whereas the knockdown of tau rescues defects in axonal transport in Alzheimer’s disease models (6). Unlike tau, MAP7 is a required cofactor for kinesin-driven transport in cells (7–9). The MAP7 projection domain binds to kinesin’s coiled-coil stalk in vitro (8, 10), recruits kinesin to MTs, and activates its motility (9, 11). Transient interactions with the MAP7 projection domain may enable kinesin to hop from one MAP to another, increasing its apparent run length by disfavoring detachment from the MT (9).

To understand how MAP7 regulates kinesin, we determined the cryo–electron microscopy (cryo-EM) structure of MTs decorated with full-length (FL) MAP7 (Fig. 1, A and B; fig. S1A; tables S1 and S2; and movie S1). The best-resolved region (Fig. 1B) forms a half-maximal inhibition constant (IC50) of 111 ± 12 nM (±SE) under physiological salt (Fig. 2, B and C, and fig. S6). As previously reported (9, 11), the addition of 50-nM MAP7 rescued FL kinesin from autoinhibition (17) and substantially increased its run frequency and length (fig. S7). MAP7 also enhanced the motility of constitutively active kinesin (K580, residues 1 to 560) (Fig. 2, D and E; and movie S3) (9, 11). Unlike run length and frequency, kinesin velocity decreases even at low MAP7 concentrations (Fig. 2E), which could be a result of pausing at MTBD obstacles or binding to the projection domain. Previously, dynein has been reported not to be inhibited by 5-nM MAP7 (10); however, we found that it was inhibited by MAP7 with a half-maximal inhibition constant (IC50) of 10 ± 3 nM (Fig. 2, D and E). MAP7 decoration of MTs also switched the direction of an assembly that links FL kinesin to dynein (Fig. 2F) (18). 90% of kinesin-dynein assemblies were minus-end-directed on undecorated MTs, whereas 93% moved toward the plus end upon addition of 10 nM MAP7 (Fig. 2, D, G, and H; fig. S5C, and movie S4).

Unexpectedly, kinesin run frequency started to decrease when MAP7 concentration was increased further (100 to 1000 nM; Fig. 2, D and E, and movie S3). We reasoned that the nonlinear relationship between MAP7 decoration and kinesin motility could arise when the motor is subjected to simultaneous activation and inhibition that dominate at different concentrations (Fig. 2E) (19). To determine the source of these opposing inputs, we disrupted the kinesin-MAP7 interaction by truncating either the MAP7-binding domain of kinesin (K490, residues 1 to 490) or the kinesin-binding domain of MAP7 (MAP7-N and MAP7-MTBD; Fig. 3A). In all cases, MAP7 inhibited kinesin (Fig. 3B, fig. S8, and movies S5 and S6) as strongly as it inhibited dynein (Fig. 2E). Thus, MTBD inhibits whereas the projection domain activates kinesin (10).

MTBD may inhibit kinesin by competing for the same tubulin binding site, because superimposing the MT-bound structure of kinesin (20) onto our model reveals an apparent clash between kinesin and segment II of MAP7 (fig. S9A). Alternatively, the flexible segment II may accommodate kinesin binding by shifting away from the intradimer interface, as proposed for DCX and MAP4 (14, 21).
To distinguish between these possibilities, we determined the structure of MTs incubated with rigor kinesin [K350, residues 1 to 350, E236A mutation (K350 E236A)] and MAP7 (Fig. 3C). Because distinct binding sites of MAP7 and kinesin can be artifactually averaged during reconstruction (Fig. S9B), we performed focused 3D classification around the putative shared binding site of MAP7 and kinesin (Fig. S10; see methods). The classification resulted in two distinct maps of the binding site, one occupied by MAP7 only, and the other occupied by kinesin only (Fig. 3D). Thus, kinesin and MAP7 cannot simultaneously bind to the same tubulin dimer.

Consistent with cryo-EM, MTBD was unable to bind MTs predecorated with K350E236A (Fig. 3E). However, FL MAP7 or a construct lacking the MTBD (∆MTBD; fig. S11, A to C) still bound to K350E236A-decorated MTs, presumably through the P123 domain (residues 175 to 316) (8, 9). MT binding of ∆MTBD was nearly abolished upon cleaving the flexible tails of tubulin (Fig. 3E and fig. S11D), explaining why P123 was invisible in our structure. To verify that kinesin is inhibited at high levels of MAP7 decoration as a result of binding site overlap, we also replaced the MT-binding regions of MAP7 with those of tau, which also overlaps with the tubulin binding site of kinesin and inhibits kinesin motility (Fig. S12) (13, 22). Similar to FL MAP7, binding of this chimeric MAP to the MT resulted in biphasic regulation of kinesin motility (Fig. 3, F and G).

To reveal how kinesin “walks” along MT7-decorated MTs despite competing for the same binding site, we fluorescently labeled the motor domain of K560 and tracked kinesin stepping with nanometer precision under limiting adenosine triphosphate (ATP) conditions (Fig. 4A). On undecorated MTs, kinesin took 16-nm steps in the forward direction (23), whereas steps...
in the sideways and backward directions were rare (9 and 3%, respectively; Fig. 4, A and B, and fig. S13A) (24). At 36 nM MAP7, we observed a modest increase in the probability of sideways and backward stepping (Fig. 4, A and B). At nearly saturating MAP7 concentrations, we observed 16- to 64-nm displacements in our trajectories, as well as increased stepping in sideways (35%) and backward (26%) directions (Fig. 4, A and B). Because kinesin cannot take such large steps on its own (Fig. 4, A and B), these large displacements likely represent transient detachment and reattachment of the motor to the MT.

In the absence of the projection domain, kinesin was stuck on MTs decorated with 36-nM MTBD and dissociated from MTs at increased ATP concentrations (fig. S13, B and C). Furthermore, the addition of the C-terminal half of MAP7 (MAP7-C) was unable to stimulate kinesin motility on MTs decorated with the N-terminal half of MAP7 (MAP7-N, fig. S14). Thus, the projection domain is needed to be tethered to the MTBD to enable to bypass MTBD obstacles.

On the basis of our results and previous reports (9, 11), we propose a model for kinesin and dynein motility. (A) Schematic of kinesin and dynein motility on MTs coated with MAP7. (B) MT decoration of fluorescently labeled MAP7 under different concentrations. (C) Fluorescence intensity (mean ± SD) of MAP7 was fit to the Langmuir isotherm (solid curve) to calculate $K_D$ (± SE). From left to right, $n = 40, 44, 50, 68, and 109$ MTs (two technical replicates). (D) Kymographs of K560 and dynein motility in the presence of MAP7. Assays were performed in 150 mM potassium acetate (KAc) and 0.1% methylcellulose. (E) Run frequency, run length, and velocity of K560 and dynein at different MAP7 concentrations (mean ± SEM). K560 run frequency was fit to the biphasic Hill equation (solid black curve) to reveal the half-maximal activation (blue dashed curve) and inhibition (red dashed curve) concentrations (± SE). Dynein run frequency was fit to the Langmuir equation (not shown) to calculate $IC_{50}$ (± SE). From left to right, $n = 281, 463, 836, 233, 386, 235, 213, and 146$ for dynein; two technical replicates). (F) Schematic of kinesin and dynein assembled onto BicDR1. The addition of MAP7 switches the active motor. (G) Schematic of kinesin-dynein assembled with and without 10 nM MAP7. Yellow arrows show kinesin-dynein colocalizers. (H) Directionality of kinesin-dynein assemblies (mean ± SD, $n = 356$ and 580 for 0- and 10 nM MAP7, respectively, three replicates).
stepping on MAP7-decorated MTs. MAP7 recruits kinesin-1 to the MT and activates subsequent motility \((9, 11)\). MT binding of MAP7 also obstructs kinesin stepping along the protofilament, which results in kinesin dissociation from the MT. However, the MAP7 projection domain tethers kinesin to the MT surface, allowing it to rebind the MT at nearby sites not blocked by its MTBD. This “tethered diffusion” of kinesin appears as large forward, sideways, or backward displacements in our trajectories. When the MT surface is nearly saturated with MAP7, the frequency and length of kinesin runs are reduced because of the scarcity of empty tubulin sites to which the motor can rebind after it dissociates (Fig. 4C). Unlike kinesin-1, MAP7 inhibits dynein motility because its MTBD overlaps with the dynein binding site (fig. S15) but its projection domain does not tether dynein to MTs.

This biphasic regulation mechanism may enable precise control of kinesin-1–driven transport by varying MAP7 density on cellular MTs. Although MAP7 is required for kinesin-1-driven processes in many cell types \((10, 25, 26)\), dense MAP7 localization was shown to slow and pause organelle transport at brunch junctions \((8)\). Increased accumulation of MAP7 may reroute cargos to their destinations by facilitating the detachment of kinesin from its MT track and rebinding to neighboring MTs at these junctions \((8)\).

**Fig. 3.** MT tethering enables kinesin to move on MAP7-decorated MTs despite their overlapping binding sites. (A) Schematic of kinesin, MAP7, and tau constructs. (B) Kymographs showing kinesin motility in the presence of FL and truncated MAP7. (C) Average cryo-EM map of an MT decorated with both MAP7 and rigor kinesin. Atomic model of MAP7 and previously reported structure of kinesin on MT (PDB code: 4HNA) \((20)\) were fitted into the cryo-EM map. (D) Focused 3D classification resulted in two distinct density maps showing either MAP7-bound (left) or kinesin-bound (right) tubulin, indicating competitive binding. (E) Fluorescent signal (top) and normalized intensity (bottom) of 1 μM LD655-labeled MAP7 constructs on MTs that are either untreated (control), subtilisin-treated, or predecorated with 1 μM rigor kinesin (mean ± SEM; n values are given for each bar). (F) Kinesin motility in the presence of chimeric MAPs. (G) Run frequency and velocity of kinesin in the presence of Tau-MTBD/MAP7-projection (mean ± SEM, n = 27, 112, 211, 102, and 55 from left to right, two technical replicates).
Fig. 4. Kinesin bypasses MTBD obstacles through tethered diffusion. (A) (Inset) K560 was labeled with LD655 at its N terminus, and its stepping was tracked in parallel (straight arrows) and perpendicular (curved arrows) axes of MTs under different MAP7 concentrations. Representative traces of K560 motility along parallel (top) and perpendicular (bottom) directions. Horizontal lines represent a fit to a step-finding algorithm. (B) Histograms reveal the percentage of instantaneous jumps in backward and perpendicular directions under different MAP7 concentrations (bar graphs). From top to bottom, n = 437, 580, 502, and 331 for parallel and 43, 145, 195, and 181 for perpendicular directions. (C) Model for regulation of kinesin by MAP7. The MAP7 projection domain rescues kinesin from autoinhibition and tethers the motor to the MT. When kinesin encounters an MTBD obstacle, it dissociates from the MT, remains tethered to MAP7, and rebinds to an available tubulin site on another protofilament. Kinesin is inhibited at high MAP7 concentrations due to the scarcity of available binding sites.

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Data and materials availability: All data are available in data S1. Materials are available from A.Y. under a material agreement with the University of California, Berkeley. The coordinates for MAP7 bound to tubulin are available at the Protein Data Bank (PDB) with accession code 1SGS. All cryo-EM maps are available at the EMD repository with accession codes EMDB-25120 (FL MAP7), EMDB-25119 (MAP7-MTBD and FL tau), EMDB-25118 (MAP7 and kinesin), EMDB-25117 (MAP7-MTBD and FL tau).

SUPPLEMENTARY MATERIALS

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Materials and Methods

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