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Impact of the Diffusion of Microtubule-Associated Protein EB1 on Kinesin Translocation in Vitro BENJAMIN LOPEZ, MEGAN VALEN-TINE, Univ of California - Santa Barbara — Using the slowly hydrolyzable GTP analog GMPCPP, we polymerize microtubules that recapitulate the end binding behavior of EB1 along their entire length, and investigate the impact of EB1 on kinesin translocation. Through direct observation of single molecules of EB1 fused to GFP, we find that EB1 diffuses along the microtubule lattice, and that the presence of taxol affects the rate of diffusion. To test whether EB1 presence and diffusion has an effect on kinesin-driven cargo transport, we observe quantum dot labeled kinesins walking on microtubules assembled with GMPCPP and taxol and coated with EB1. We find that the addition of EB1 significantly reduces kinesin speed compared to the no EB1 condition, but when microtubules stabilized by both taxol and GMPCPP are used, the speed reduction is nearly abolished. Our data suggest a new possible mechanism for the regulation of kinesin function by EB1 in which kinesin speed is directly modulated through the interference of EB1 diffusion. Our results also raise important questions about the effects of taxol on microtubule-MAP interactions.

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