

Mechanisms of End-tracking by Microtubule-associated Proteins

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Microtubule (MT) end-binding proteins are important for many cellular processes, including regulation of MT dynamics. However, the mechanisms by which these proteins end-track are not well understood. We investigated the end-tracking of the fission yeast proteins Tea2, Mal3, Tip1, and Klp5 using *in vivo* and *in vitro* techniques. Using time-lapse fluorescence microscopy, we examined protein distribution along interphase MTs in fission yeast strains stably expressing GFP-Klp5 or GFP-Mal3. Klp5 accumulated at the plus-end of MTs in contact with the cell wall as well as on depolymerizing MTs, but end-accumulation was not observed on cytoplasmic MTs. To determine whether Klp5 moves processively along MTs we measured the cross-correlation of intensity along the MT over time. Our results are consistent with Klp5 plus-end directed motion of 10 nm/s. Mal3, a homolog of EB1, also end-accumulated *in vivo*, consistent with previous results. We attempted to reconstitute end-tracking *in vitro*, using purified clam tubulin and GFP-Mal3. However, we failed to observe Mal3 end-accumulation in our *in vitro* reconstituted system, possibly as a result of other MT-associated proteins (MAPs) remaining on the clam tubulin. We also measured the intensity of fluorescently-tagged Tea2 and its cargo, Tip1, along growing MTs *in vitro*. Time-averaged intensity profiles showed a gradient-like distribution of Tip1 with increased intensity at the plus-end. Single-molecule analysis of Tea2 revealed plus-end directed movement of $3 \pm 1 \mu\text{m}/\text{min}$, consistent with the *in vivo* results of Browning et al. 2003. Using the parameters obtained *in vivo* and *in vitro*, we modeled end-tracking dynamics using computer simulations. We conclude that proteins may employ distinct strategies to track the ends of MTs including motor activity and differential affinity for the MT plus-end relative to the lattice.