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CYTOSKELETON

Getting to the TIP

Microtubule plus end-tracking proteins (+TIPs) are a diverse group of proteins involved in numerous cell activities, including the regulation of cell shape. Recently, it was discovered that end-binding protein 1 (EB1; also known as MAPRE1) regulates the interaction of +TIPs with microtubule ends, but the mechanism by which this is achieved was unclear. Honnappa *et al.* have now delineated this mechanism by identifying a microtubule tip localization signal that guides +TIPs to bind EB1 and, ultimately, track microtubule tips.

To investigate the interaction between +TIPs and EB1, the authors carried out sequence analysis of +TIPs and identified a motif (SxIP, in which x denotes any amino acid) that was conserved among several +TIPs. Full-length +TIPs and fragments containing SxIP were found to interact with EB1 in pull-down assays and to track microtubule ends in cells. Changes in the amino acid sequence of SxIP abrogated the process, indicating that this motif forms the basis of the +TIP–EB1 interaction. Furthermore, in *in vitro* reconstitution experiments using purified components, +TIPs localized to microtubule tips only in the presence of EB1, suggesting that EB1 is necessary and sufficient for microtubule tip tracking.

Proteins (such as EB1) with an EB homology domain are known to contain a highly conserved hydrophobic cavity, and the substitution of hydrophobic residues in SxIP with polar ones abolished the interaction between +TIP and EB1. Based on this, the authors proposed that SxIP and the hydrophobic cavity of EB1 are the major interacting sites. Indeed, examination of the crystal structure of a complex of EB1 and the +TIP microtubule-actin crosslinking factor (MACF) identified contact sites that correspond to the SxIP of MACF and the hydrophobic cavity of EB1. This finding was extended to other +TIPs using nuclear magnetic resonance studies.

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> “… + TIPs bind to the hydrophobic cavity of EB1 through the SxIP motif, and this interaction directs them to microtubule tips…”

Taken together, these data reveal that +TIPs bind to the hydrophobic cavity of EB1 through the SxIP motif, and this interaction directs them to microtubule tips, where they can carry out their functions. Future studies interfering with this microtubule tip localization signal through amino acid substitutions will enable further dissection of the role of microtubule tip tracking in cellular processes.

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ORIGINAL RESEARCH PAPER Honnappa, S. *et al.* An EB1-binding motif acts as a microtubule tip localization signal. *Cell* **138**, 366–376 (2009)
FURTHER READING Akhmanova, A. & Steinmetz, M. O. Tracking the ends: a dynamic protein network controls the fate of microtubule tips. *Nature Rev. Mol. Cell Biol.* **9**, 309–322 (2008)