EBs clip CLIPs to growing microtubule ends

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Proteins that track growing microtubule (MT) ends are important for many aspects of intracellular MT function, but the mechanism by which these +TIPs accumulate at MT ends has been the subject of a long-standing controversy. In this issue, Bieling et al. (Bieling, P., S. Kandels-Lewis, I.A. Telley, J. van Dijk, C. Janke, and T. Surrey. 2008. *J. Cell Biol.* 183:1223–1233) reconstitute plus end tracking of EB1 and CLIP-170 in vitro, which demonstrates that CLIP-170 plus end tracking is EB1-dependent and that both +TIPs rapidly exchange between a soluble and a plus end–associated pool. This strongly supports the hypothesis that plus end tracking depends on a biochemical property of growing MT ends, and that the characteristic +TIP comets result from the generation of new +TIP binding sites through MT polymerization in combination with the exponential decay of these binding sites.

Microtubules (MTs) are dynamic eukaryotic cytoskeletal elements that stochastically switch between phases of growth and shortening. However, it was only when GFP revolutionized cell biology as a tool to observe intracellular protein dynamics that proteins such as EB1 and CLIP-170 were discovered, which associate specifically with growing MT plus ends (Perez et al., 1999; Mimori-Kiyosue et al., 2000). These proteins form a structurally diverse group and are now commonly referred to as +TIPs. Although +TIP function is still poorly understood, a protein complex at the MT plus end is thought to organize and polarize the MT cytoskeleton, and thus contribute to complex aspects of cell architecture, polarity, and behavior (Li and Gundersen, 2008). This idea has evolved largely from experiments in fission yeast in which the homologues of several vertebrate +TIPs are required for polarized growth (Hayles and Nurse, 2001).

On a more fundamental level, it has puzzled cell biologists for the last decade how +TIPs end up at growing MT ends. Initial findings that the originally described +TIP CLIP-170 could bind to nonpolymerized tubulin supported the hypothesis that +TIPs arrive at the MT plus end by copolymerization with tubulin dimers (Diamantopoulos et al., 1999). However, a complication of this copolymerization model is that it also requires an ill-defined mechanism by which the tightly bound +TIP releases from the MT to generate the characteristic exponentially decaying +TIP comet tail observed in cells. Phosphorylation has been proposed as a mechanism involved in +TIP dissociation from the MT, but this has never been demonstrated (Vaughan, 2004).

Two recent articles by the same group now reveal that MT plus end tracking can be explained by a much more straightforward mechanism, at least for end-binding (EB) and CLIP family proteins (Fig. 1; see Bieling et al. on p. 1223 of this issue; Bieling et al., 2007). By using total internal reflection microscopy, Bieling et al. (2008) showed that EB family +TIPs associate with growing MT ends in a purified system consisting only of immobilized dynamic MTs and frog EB1 or its fission yeast homologue Mal3. The comet tails at growing MT plus ends in this minimal system are indistinguishable from EB comets observed in cells, which indicates that no other factors are required for this remarkable dynamic behavior. Both EB1 and Mal3 not only recognized MT plus ends but also tracked slower growing minus ends in vitro. As MT minus ends never grow in cells, this has never been observed before. This indicates that EB proteins have an intrinsic affinity for polymerizing MT ends.

The authors then used the in vitro system to dissect the EB plus end–tracking mechanism and found that the length of the plus end comet generated by either protein only depends on the MT growth rate and not on the concentration of EB1 or Mal3 in the assay (Bieling et al., 2007, 2008). This strongly indicates that EB plus end–tracking and comet shape are determined by the distribution of available high-affinity binding sites at MT ends, which decay at a constant rate and provide a platform for EB binding. Single-molecule experiments further support this idea by demonstrating that individual EB molecules do not remain bound for a long enough time to generate the observed comet tails by copolymerization with tubulin. Such rapid turnover of several +TIPs, which is inconsistent with a copolymerization mechanism, has also been observed in cells (Wittmann and Waterman-Storer, 2005; Dragestein et al., 2008). In addition, neither EB1 nor Mal3 efficiently bind nonpolymerized tubulin.

An important unresolved question is why EB proteins have a higher affinity for growing MT ends as compared with the rest of the MT. MTs do not grow directly as closed cylinders but instead first form open sheets that subsequently close into tubes (Chretien et al., 1995). However, several aspects of EB comet

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tail dynamics strongly argue against the idea that EBs directly recognize such structural intermediates. Sheets at growing plus ends in vitro and in cells are on average much shorter than EB comet tails, and display a wide length variation at a constant MT growth rate (Chretien et al., 1995). In contrast, the length of EB comets at a given MT growth rate is relatively constant (Bieling et al., 2007, 2008). In addition, the exponential decay of EB binding along MT ends in cells and in vitro is not consistent with a distinct transition between sheet and tube or, alternatively, with EB binding along the lateral edges of a tubulin sheet. An exponential decay, however, can readily be explained by first-order reaction kinetics such as the hydrolysis of tubulin-bound GTP. An exponentially decaying GTP-tubulin cap was originally proposed (Mitchison and Kirschner, 1984), and recent nanoscale analysis of MT polymerization dynamics supports the idea of a spatially extended GTP-tubulin cap (Schek et al., 2007).

To test whether other +TIPs track growing MT ends by a similar mechanism, the authors next investigated CLIP family proteins (Bieling et al., 2007, 2008). Surprisingly, neither vertebrate CLIP-170 nor its fission yeast homologue Tip1 alone recognized growing MT ends. However, CLIP plus end tracking could be reconstituted in both systems, which revealed an interesting difference between yeast and vertebrate cells. In the yeast system, Tip1 plus end tracking depends on two additional factors. The kinesin motor Tea2 is required to transport Tip1 to the MT plus end, whereas Mal3 is needed to retain Tip1 at the growing end (Bieling et al., 2007). This result is consistent with earlier genetic studies in fission yeast demonstrating that both Mal3 and Tea2 are required for Tip1 plus end accumulation (Browning et al., 2003).

In vertebrate cells, however, no clear Tea2 homologue exists, and kinesin-dependent transport of CLIP-170 particles has not been observed. The current paper resolves this discrepancy by demonstrating that EB1 alone is indeed sufficient to mediate CLIP-170 plus end tracking in vitro and does not require additional motor proteins (Fig. 1). This is backed up by experiments showing that EB1 is necessary for CLIP-170 plus end tracking in frog egg extracts and is also consistent with earlier data that depletion of EB proteins by RNA interference reduces CLIP-170 plus end tracking (Komarova et al., 2005). Because MTs in vertebrate cells grow faster and for much longer distances because of the larger cell size as compared with yeast cells, an intriguing evolutionary explanation for this difference is that kinesin-mediated transport may not be an efficient mechanism to deliver proteins to the plus end in higher eukaryotic cells. It will be interesting to see whether other variations of this mechanism exist.

EB proteins consist of an N-terminal MT-binding domain and a C-terminal domain that mediates dimerization and interacts with almost every other +TIP identified to date (Akhmanova and Steinmetz, 2008). It is intriguing to speculate that EBs are the only bona fide plus end–tracking proteins and function as adaptors to recruit other +TIPs. Interestingly, the extreme C terminus of α-tubulin, with which most MT-associated proteins are thought to interact, is strikingly similar to the C terminus of EB proteins (Komarova et al., 2005). Thus, plus end tracking and binding along MTs may use the same basic domain architecture, recognizing the same sequence motifs in slightly different configurations. An evolutionary reason for this may be that it is easier to modify the arrangement of existing MT-binding domains than inventing a completely novel plus end–tracking mechanism. Indeed, Bieling et al. (2008) find that CLIP-170 plus end tracking requires a composite binding site formed by the C-terminal tyrosines of both EB1 and α-tubulin. Whether plus end tracking of all other +TIPs is EB-dependent remains to be determined, but the in vitro reconstitution approach developed by Bieling et al. (2008) will provide a powerful tool to test this hypothesis.

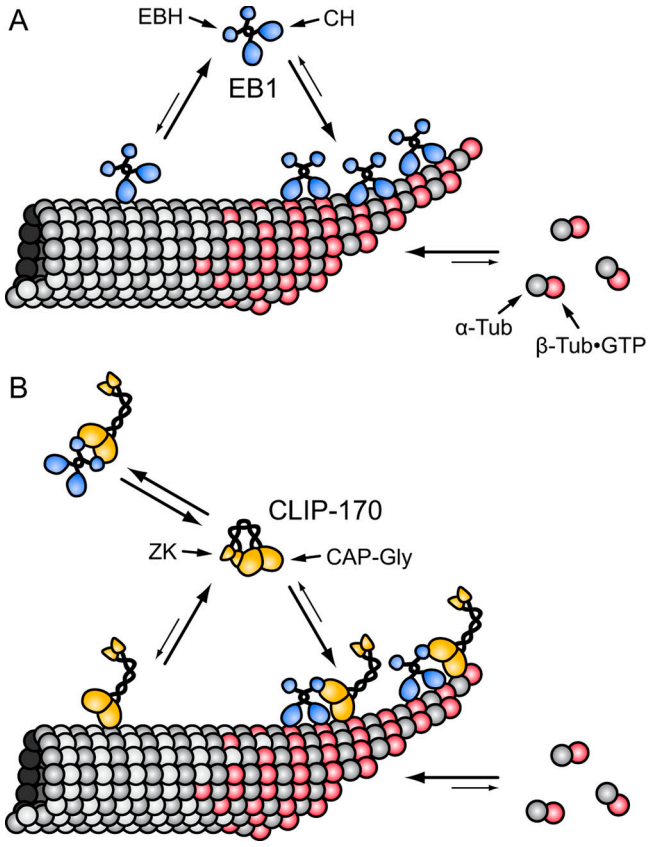

Figure 1. **Equilibrium binding model for vertebrate EB1 and CLIP-170 plus end tracking.** (A) EB1 binds with high affinity to tubulin at the growing MT end that is structurally different from the rest of the MT. CH, N-terminal MT-binding calponin homology domain; EBH, C-terminal +TIP-binding EB homology domain. (B) CLIP-170 binds with high affinity to a composite binding site created by the C termini of EB1 and α-tubulin. CAP-Gly, N-terminal MT-binding domain; ZK, C-terminal zinc knuckle autoinhibitory domain.

Still, very little is known about the intracellular function of +TIPs. Because CLIP-170 was originally identified as a protein involved in endocytosis (Pierre et al., 1992), another paper in this issue (Lewkowicz et al., 2008) investigates the role of +TIPs during complement receptor–mediated phagocytosis. These authors find that CLIP-170 is required for mDia1-mediated actin polymerization at phagocytic cups. Surprisingly, EB proteins appear not to be required for phagocytosis, which indicates that CLIP-170 may have functions independent of its EB-mediated plus end–tracking behavior.

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