TIP maker and TIP marker; EB1 as a master controller of microtubule plus ends

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The EB1 protein is a member of the exciting and enigmatic family of microtubule (MT) tip-tracking proteins. EB1 acts as an exquisite marker of dynamic MT plus ends in some cases, whereas in others EB1 is thought to directly dictate the behavior of the plus ends. How EB1 differentiates between these two roles remains unclear; however, a growing list of interactions between EB1 and other MT binding proteins suggests there may be a single mechanism. Adding another layer of complexity to these interactions, two studies published in this issue implicate EB1 in cross-talk between mitotic MTs and between MTs and actin filaments (Goshima et al., p. 229; Wu et al., p. 201). These results raise the possibility that EB1 is a central player in MT-based transport, and that the activity of MT-binding proteins depends on their ability or inability to interact with EB1.

EB1 is a MT tip-tracking protein
EB1 was first described as an adenomatous polyposis coli (APC)–interacting protein whose binding domain was affected by APC mutations implicated in colon cancer (Su et al., 1995). Subsequent localization revealed that EB1 binds to and labels a subset of the total microtubule (MT) population and that it displays some preference for the very plus end of these MTs (Morrison et al., 1998). Live-cell imaging of transfected GFP fusion proteins further revealed specificity for the tips of MTs undergoing elongation (Mimori-Kiyosue et al., 2000). This tendency to bind elongating MT plus ends and to appear to “track” with these ends as long as they extend is known as “tip tracking,” and an impressive number of MT-binding proteins display this tip-tracking activity in live-cell imaging assays (for review see Vaughan, 2004). In most cases, high resolution imaging suggests that these proteins “treadmill” at the elongating plus ends rather than translocate or “surf” with the growing tip. This contrasts, to some extent, the behavior of homologues in yeast and filamentous fungi that appear to translocate toward the plus end via a kinesin motor or other mechanisms (Xiang et al., 2000; Liakopoulos et al., 2003). In mammalian cells, tip tracking is best characterized in transfection assays where fluorescent fusion proteins are overexpressed at some level. Often the degree of tip specificity is influenced by the amount of expressed protein. Although tip specificity is evident at low levels of expression, a transition to decoration along the length of MTs becomes prominent as the level of expression increases. For some tip trackers, this tendency is thought to reflect a regulatory cycle at the MT plus end (Vaughan et al., 2002). However, MTs can tolerate high levels of EB1 better than the other tip trackers, suggesting something unique and intriguing about EB1.

Tip tracking and EB1 function
Despite an explosion of recent work on EB1, the precise function and location of endogenous EB1 at native levels remains unclear. The MT-associated population of EB1 represents a small subset of total EB1, but this subset has received the most attention. The remainder is thought to be largely soluble, similar to the form that dominates the GFP-EB1 expression studies. Immunofluorescence microscopy images suggest that native EB1 is punctate, resembling vesicles or large protein complexes (Morrison et al., 1998; Faulkner et al., 2000; Mimori-Kiyosue et al., 2000). This would be consistent with the extensive colocalization of other membrane-associated proteins such as CLIP-170 and dynactin. However, nonmembranous protein complexes including binding partners such as APC are also described, and the function of these is under investigation (Mimori-Kiyosue et al., 2000; Wen et al., 2004). In these settings, direct interactions between EB1 and other proteins (p150Glued, CLIP-170, and CLASPs) have been interpreted as recruitment mechanisms (Fig. 1). The fact that these binding partners can also bind tubulin directly suggests some transition or sequential loading process at plus ends that will require more work to resolve.

Related to the recruitment function of EB1, another model is focused on the ability of EB1 to regulate MT growth. A series of elegant biochemical, immunodepletion, and small interfering RNA (siRNA)/rescue assays indicate that EB1 can stimulate growth at MT plus ends (Fig. 1) and that this is critical in situations where search-capture requires long and dynamic MTs (Tirnauer and Bierer, 2000; Rogers et al., 2002; Tirnauer et al.,

Abbreviations used in this paper: APC, adenomatous polyposis coli; C-MT, centrosomal MT; K-fibers, kinetochore fibers; MT, microtubule; siRNA, small interfering RNA.
that this behavior requires EB1. This association of melanophillin with EB1 does not require either myosin V or rab27a, because tip tracking is evident in melanocytes from ashen and dilute mice, and interaction-disrupting melanophillin mutants tip-track as well. Although melanophillin depends on EB1 for MT binding, EB1 does not appear to require melanophillin for normal function. Only a subset of EB1 cometson contains detectable melanophillin in melanocytes, and EB1 is expressed in many cell types that do not express melanophillin.

The conceptual challenge of this work is to determine the function of EB1 and MT binding for melanophillin. Is this a nuance of overexpression or an important clue into the role melanophillin plays in melanosome transport? Interestingly, the authors point out that melanosomes do not tip-track normally, and that the movements of melanosomes are very different from tip-tracking proteins. Furthermore, expression of the tagged melanophillin constructs reveals both MT- and actin-associated structures in the cell periphery. Perhaps this dichotomy is the crucial finding for melanophillin. The authors propose the enticing possibility that melanophillin uses a combination of MT binding (via EB1) and actin binding (via myosin V) to build a transient transfer station in the cell periphery where melanosomes can be efficiently handed from MTs to microfilaments (Fig. 2). This is consistent with the known behaviors of melanosomes (Rogers and Gelfand, 1998) and uncovers a functional aspect of melanophillin that would be difficult to examine due to the transient nature of these intermediates. In common with other EB1 studies, it remains unclear if EB1 serves a role as a marker for MT plus ends that have reached the cell periphery, or if EB1 actively preserves particular MT plus ends long enough to allow the hand-off. However, this work provides compelling evidence that EB1 plays a larger role than previously anticipated.

Adding further weight to the possibility that EB1 coordinates transfer between cytoskeletal systems, this issue also includes a study from Goshima et al. (p. 229) that reports a new role for EB1 in mitotic spindle function. Using Drosophila S2 cells as a model together with siRNA-driven depletion, this group dissects the contribution of ncd and cytoplasmic dynein in the formation and motility of kinetochore-linked (K-fibers) and centrosome-linked (C-MTs) MT bundles during spindle pole focusing. Depletion of cytoplasmic dynein primarily impacted the ability of K-fibers to move toward the spindle poles, whereas depletion of ncd affected the focusing of the k-fibers into a tight spindle. Although these motors share some functional redundancy, time-lapse sequences highlight the phenotypic distinction between k-fiber focusing and transport.

To better understand the specific function of each motor, imaging of ncd-GFP was accomplished through depletion of endogenous ncd by siRNA coupled with expression of ncd-GFP. Focusing on cells with almost normal levels of functional ncd, Goshima et al. (2005) report accumulation of ncd-GFP at spindle poles and along K-fibers. This finding is consistent with the bivalent nature of ncd-MT interactions, potentially playing a role in MT cross-linking in the spindle. However, FRAP analysis revealed that ncd-GFP is highly dynamic on these MTs and displays enrichment at the plus ends of MTs.
emanating from the spindle poles similar to tip tracking. Expression of ncd-GFP during interphase also revealed tip tracking, adding ncd to the list of plus end–binding proteins. Although it was unclear how ncd (a minus end–directed motor) would target to plus ends, previous work on EB1 (Rogers et al., 2002, 2004) suggested a mechanism. siRNA-mediated depletion of EB1 induced a transition from tip-specific binding to more uniform labeling of MTs. This was coupled with a reduction in MT dynamics that correlated with a loss of K-fiber focusing similar to ncd depletion.

As a tool to predict how MT plus end binding of ncd could contribute to K-fiber focusing, Goshima et al. (2005) use molecular modeling of a minimal spindle and compare the outcomes of two scenarios. The first scenario assumes that an ncd–EB1 complex targets the motor domain of ncd to K-fibers and the tail of ncd (NH2-terminus) to the plus ends of C-MTs via the EB1 interaction. The second scenario has the opposite orientation with the ncd motor binding C-MTs and the tail binding K-fibers via EB1. Simulations support the second model with the ncd motor associating with the plus ends of C-MTs and moving toward the poles (Fig. 2). In this arrangement, the ncd tail and EB1 attach to K-fibers, allowing ncd to draw the K-fibers toward the poles. Given the proposed role of EB1 in imparting plus end specificity to ncd, this outcome is somewhat counterintuitive. However, EB1 could contribute by ensuring that the K-fiber/ncd/C-MT interaction occurs only at the plus ends of C-MTs. Similar to the melanophillin story above, EB1 could represent a marker for transient interactions between distinct filament systems.

**Insights into EB1 function**

Although the studies of melanophillin and ncd reported in this issue appear to focus on very different questions, the overlapping contributions of EB1 to both stories suggests a fundamentally new model for EB1 function and the potential role of tip tracking. If one adds the other locations where EB1 is thought to function, a theme emerges that supports a more central role for EB1 in interactions between large multi-subunit complexes. Although not completely understood, in interphase cells MT plus ends contain EB1 and other tip-trackers where they appear to mark locations of search-capture between MTs and membranes (Pierre et al., 1992; Valetti et al., 1999; Vaughan et al., 2002; Wen et al., 2004). An overlapping class of EB1-associated proteins are found at kinetochores during prometaphase where search-capture of chromosomes occurs (Dujardin et al., 1998; Faulkner et al., 2000; Tirnauer et al., 2002a). If we add transfer of melanosomes from MTs to actin filaments (Wu et al., 2005) and linkage of K-fibers and C-MTs (Goshima et al., 2005) to the list, one could propose that EB1 is the master integrator of protein complex assembly on MTs. It remains unclear if this function is related to the impression conveyed by tip-tracking assays, or if tip tracking simply reflects the fact that EB1–MT interactions are tightly regulated (Vaughan, 2004). Future work and the identification of new EB1-binding partners will shed light on this intriguing question.

**Conclusion**

The growing list of EB1-interacting proteins and functions suggests that our understanding of EB1 and MT tip tracking is incomplete. The addition of two new candidates to the plus end–binding protein family implies that EB1 plays a fundamental role in coordinating movement along MTs by defining locations where specific conditions have been met for transport. This model represents a conceptual advance for analysis of tip-tracking proteins and provides the framework for further dissection of their function.
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