EB1 Proteins Regulate Microtubule Dynamics, Cell Polarity, and Chromosome Stability

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The EB1 family represents a highly conserved group of proteins, present in yeast through humans, that localize to spindle and cytoplasmic microtubules, especially at their distal tips. The budding yeast homologue of EB1, Bim1p, regulates microtubule stability and is important for positioning the mitotic spindle, anchoring it to the bud through astral microtubule attachment to the cortical protein Kar9p. Bim1p interacts functionally with dynactin in a late mitotic cell cycle checkpoint. EB1 proteins in human cells interact physically with the adenomatous polyposis coli (APC) tumor suppressor protein, targeting APC to microtubule plus ends, and with members of the dynactin complex. Here, we review recent studies in yeast and human cells that suggest the involvement of EB1 in promoting microtubule search and capture and in maintaining chromosome stability.

The EB1 Family

Human EB1 is a 35-kD, mildly acidic, leucine zipper protein cloned as an interacting partner with the APC COOH terminus in a yeast two-hybrid screen (see Fig. 1A; Su et al., 1995). Because loss of heterozygosity at the APC tumor suppressor locus is an early event in colon cancers, and germline APC mutation leads to the familial adenomatous polyposis coli (FAP) colon cancer syndrome (Knizler and Vogelstein, 1996), this interaction raises questions about the role of EB1 in facilitating APC functions. However, EB1 proteins are also found in unicellular organisms that lack APC, suggesting a more primitive role that predates the evolution of APC. In this review, we first focus on the functions of EB1 proteins in yeasts, and then we speculate on how these functions may have been adapted in multicellular eukaryotes.

The full diversity of EB1 family members remains unknown at this time. To date, EB1 has been found in every organism and nearly every cell type examined, including neuronal, lymphocytic, and epithelial cells. Genome sequencing projects have revealed that budding yeast possess a single EB1 sequence homologue, called BIM1 (binding to microtubules 1), because the protein interacted with α-tubulin in a two-hybrid screen (Schwartz et al., 1997). The fission yeast homologue ma3+ was isolated in a screen for mutations that caused chromosome loss (Benz et al., 1997). Caenorhabditis elegans has two EB1 related genes (GenBank accession numbers VW02B12L3 and Y59A8B.P; these data were produced by the C. elegans Sequencing Group at the Sanger Centre and can be obtained from http://www.sanger.ac.uk/Projects/C_elegans/), and Drosophila melanogaster has at least three EB1 family members (GenBank accession numbers [Benson et al., 2000] AAD27859, AAF46575, and AAF57623). The number of EB1 proteins in humans is unknown, but to date EB1, EB2, EB3, and EB3 have been reported, along with the highly related RP1, RP2, and RP3 proteins (Su et al., 1995; Renner et al., 1997; Su, L.K., and Qi, Y., Association of APC with EB1 family proteins. 1998. 4193 (Abstr.); Juwana et al., 1999; Nakagawa et al., 2000). Recently, EB3 was shown to be neuronally expressed and to interact with a neuron-specific homologue of APC, A PCL (Nakagawa et al., 2000). RP1 was identified by its induction upon T lymphocyte activation, and it shares APC binding and subcellular localization with EB1 (Renner et al., 1997; Juwana et al., 1999). The sequence relationships of selected EB1 proteins are shown in Fig. 1B.

EB1 Proteins Localize to Microtubule Tips

In living yeast cells, overexpressed GFP-Bim1p localizes to the entire microtubule cytoskeleton, including the mitotic spindle, the spindle pole body (the budding yeast centrosome), and cytoplasmic microtubules (Schwartz et al., 1997). However, when native levels of expression are driven from the BIM1 promoter, fluorescence on cytoplasmic microtubules is limited to the microtubule distal tips (presumed to be plus ends) and the spindle pole body (Tirnauer et al., 1999). Together, these studies suggest that Bim1p is capable of binding along the length of microtubules, but that at endogenous levels, it binds preferentially to microtubule ends. Fluorescence at the spindle pole body may represent the physical association of Bim1p with centrosomal proteins, Bim1p binding to the microtubule proximal (minus) ends, or Bim1p binding to the distal (plus) ends of extremely short microtubules. Bim1p may bind to more than one of these structures, as it was present in a partially purified yeast spindle pole body preparation (Wigge et al., 1998).

In the cells of higher organisms, EB1 is found in a similar distribution, albeit on a more spatially extensive micro-
By indirect immunofluorescence in tissue culture cells, EB1 has been localized to the centrosome, the mitotic spindle, and the distal tips of cytoplasmic microtubules (Berrueta et al., 1998; Morrison et al., 1998). EB1 and RP1 staining also have been visualized at what appear to be microtubule ends within the mitotic spindle, possibly at or near kinetochores (Juwana et al., 1999). Thus, EB1 proteins are found on microtubule plus ends throughout the cell cycle in diverse cell types, a location ideally suited for linking the microtubule cytoskeleton to other structures within the cell. Although EB1 was named before its end-binding properties were known, end binding aptly labels the protein.

While numerous proteins localize along the lengths of microtubules, specific localization to microtubule plus ends is limited to relatively few. These include the KIN I kinesins, which bind to both plus and minus ends of microtubules in vitro and induce catastrophes (see Appendix; Desai et al., 1999); members of the dynactin complex, a 20S multiprotein complex important for activating cytoplasmic dynein (Pfarr et al., 1990; Steuer et al., 1990; Vaisberg et al., 1993; Echeverri et al., 1996; Busson et al., 1998; Skop and White, 1998; Vaughan et al., 1999); and the cytoplasmic microtubule-vesicle linker protein CLIP170. GFP-CLIP170 has been imaged in living tissue culture cells and shown to treadmill specifically along the plus ends of growing (but not shrinking) microtubules (Perez et al., 1999). While the mechanism remains unknown, proteins that bind to microtubule ends rather than along their lengths could do so by using dynamic or structural cues; for example, as diagrammed in Fig. 2, proteins that specifically recognize growing plus ends could copolymerize with tubulin (as proposed for CLIP170; Diamantopoulos et al., 1999), or they could recognize a specific conformation (such as the GTP cap or the unrolled sheet) at the growing microtubule end. It will be interesting to see whether EB1 binds microtubule plus ends directly or via another protein, and whether the microtubule tip localization shared by these proteins translates into physical and functional interactions among them.

**Role(s) of Budding Yeast EB1 in Spindle Positioning**

Once bound, what do EB1 proteins do to microtubules? The first studies of EB1 function have come from yeast mutants. Yeast lacking the BIM1 gene are viable, but their cytoplasmic microtubules are shorter than those in wild-type cells and they show abnormalities in parameters of dynamic instability (see Appendix). In bim1Δ cells, microtubules were found to depolymerize more slowly and to undergo fewer transitions and longer pauses, at the expense of growing, than in wild-type cells (Tirnauer et al., 1999). Thus, even though Bim1p increases the depolymerization rate, it promotes net polymerization by increasing both the time spent growing and the rescue frequency, resulting in microtubules that are longer as well as more dynamic. In wild-type budding yeast, microtubules are most dynamic during the G1 phase of the cell cycle, which is the phase when the spindle pole body moves toward the presumptive bud site in preparation for mitosis (Carminati and Stearns, 1997). Interestingly, microtubules in the bim1Δ mutant are most abnormal during G1, and bim1Δ cells are defective in mitotic spindle positioning (Tirnauer et al., 1999). While spindle alignment across the bud neck is unique to budding yeast, the mechanisms used to achieve it are relevant to more universal processes, i.e., the capture of kinetochores by microtubules within the spindle.

In this issue, Adames and Cooper use time-lapse sequences from living yeast cells to dissect the spindle positioning process into two sequential steps (Adames and Cooper, 2000). The first step consists of spindle movement to the bud neck, resulting from cytoplasmic microtubule
capture and end-on depolymerization at the bud tip (or at the presumptive bud site, earlier in the cell cycle). Consistent with its localization to the microtubule tip, Bim1p is a central component of this first step. Recent studies by Lee et al. (2000) and Korinek et al. (2000) have revealed that Bim1p acts at this stage not just by increasing microtubule dynamicity, but in the capture event itself. By interacting with Kar9p, which is a cortical protein that appears as a dot at the bud tip (Miller and Rose, 1998), Bim1p forms a physical link between the microtubule end and the bud, as depicted in Fig. 3. Bim1p and Kar9p coimmunoprecipitate from yeast cells and cofractionate by sucrose gradient sedimentation; in cells lacking Bim1p, microtubules fail to contact the Kar9p dot (Lee et al., 2000), demonstrating the functional importance of this interaction. Proteins with homology to Kar9p have not been found in multicellular eukaryotes, but mechanistically similar interactions are likely to occur.

In the second step in spindle positioning, the cytoplasmic microtubule binds laterally and slides along the concavity of the bud, pulling the nucleus into the narrow bud neck through dynein-dependent forces.

**Role of Fission Yeast EB1 in Chromosome Segregation**

Proteins involved in aligning the spindle to the cortex are expected to overlap with those used within the spindle, during the comparable process of kinetochore capture. Both processes make use of connections between microtubules and a specialized capture site, and in both cases, successful capture is likely to be enhanced by increased microtubule dynamicity as well as by stabilized interactions between the microtubule end and the capture site. The critical events in chromosome segregation are monitored by the highly regulated spindle assembly checkpoint machinery. In fission yeast, the EB1 homologue mal3+ was cloned in a screen for novel mutants that failed to accurately segregate a nonessential minichromosome (Behnauer et al., 1997). In addition to chromosome loss, mal3Δ cells also showed similar changes to bim1Δ cells in microtubule length and nuclear position, as well as abnormalities in cell shape and septum placement (Behnauer et al., 1997). Whether chromosome loss in the mal3Δ mutant is due to defective kinetochore capture or to these other abnormalities is currently unknown, but the localization of EB1 proteins to spindle microtubules in diverse organisms suggests that they may act to regulate dynamic instability or microtubule end-on attachments in the spindle. Interestingly, human EB1 substituted functionally for Schizosaccharomyces pombe mal3+ in this study, highlighting the remarkable degree of conservation within the EB1 family.

**Interaction of EB1 with APC**

Chromosomal instability is a major defect in the cancer of the colon. Could the EB1–APC interaction play a role in regulating chromosome segregation? A large body of work has shown that by regulating β-catenin levels, APC affects the transcription of several oncogenic proteins including c-myc, cyclin D1, and PPARβ (He et al., 1998, 1999; Tetsu and McCormick, 1999; for reviews see Polakis, 1997; Barker et al., 2000; Nathke, 2000). These transcriptional effects alone may account for the role of APC in preventing cancer development, but the question remains whether the interaction of APC with the cytoskeleton contributes to its tumor suppressor role. Transgenic mice, bearing an APC truncation distal to the β-catenin binding region (designed to address the function of the COOH-terminal, EB1-binding portion of APC; Fig. 1 A), showed no evidence of colon cancer (Smits et al., 1999), and a study of human colon tumors failed to detect somatic mutations in the EB1 coding sequence by reverse transcriptase single strand conformational polymorphism (Jais et al., 1998). However, the possibility remains that disruption of EB1–APC binding may play a role in later, accelerating events once tumorigenesis has been initiated, or it may be permissive for tumorigenesis only in the presence of other abnormalities. Understanding the role of EB1 in colon cancer, cell cycle progression, and chromosome stability is a significant objective for future studies.

One important aspect of the EB1–APC interaction may be its role in directing protein localization. Like EB1, APC localizes to the microtubule cytoskeleton, as well as to the leading edges of migrating epithelial cells (Nathke et al.,...
EB1 and R P1 colocalize with A PC in the membrane protrusions of fibrosarcoma cells (Juwana et al., 1999). Recently, GFP–APC protein has been imaged in living cells and shown, like CLIP170, to associate specifically with the plus ends of growing microtubules, but, unlike CLIP170, to drop off the ends of shrinking microtubules as a preserved granular structure (M imori-K iyosue et al., 2000). In colon cancer cell lines that exclusively contain truncated A PC (unable to bind E B1), endogenous E B1 localization is unchanged, suggesting that E B1 binds to microtubule tips independent of A PC (B errueta et al., 1998; M orrison et al., 1998). Transfection of these cells with G FP–A PC constructs containing or lacking the E B1 binding region demonstrated that the E B1 binding region directed A PC localization to microtubule tips; G FP–A PC constructs lacking the E B1 binding region (but still able to bind microtubules) localized nonspecifically to microtubules, whereas A PC constructs containing the E B1 binding region localized to the tips (A skham et al., 2000). This result is consistent with the earlier observation that A PC possesses a microtubule binding domain and is capable of bundling microtubules in vitro (M unemitsu et al., 1994). Taken together, these findings suggest that while E B1 is not required for the binding of A PC to microtubules, it may target A PC to the ends, facilitating delivery or interaction of A PC with specific sites at the cell membrane. In colon polyps, abnormalities in A PC targeting could synergize with rapid cell proliferation to disrupt cell migration and/or accelerate aneuploidy.

Could the E B1–A PC interaction have a role outside of the colon? F A P patients exhibit a variety of extracolonic manifestations, including skin and central nervous system tumors, suggesting a role for A PC in other polarized tissues. Moreover, the finding of additional A PC isoforms in D rosophilia and the neuronal specific interaction between E B3 and A PCl leaves open the possibility that other members of the E B1 or A PC families may play an as-yet-undiscovered role in normal development and possibly in other disorders (H ayashi et al., 1997; H amada et al., 1999; M Cartney et al., 1999; Y u et al., 1999; N akagawa et al., 2000). Binding between E B1 and A PC is downregulated during mitosis, possibly because of mitotic phosphorylation of A PC (Trzepacz et al., 1997; A skham et al., 2000). How cell cycle changes affect E B1, and how E B1 interacts with A PC in other cell types are also open questions.

Interaction of E B1 with Dynactin Components

In higher eukaryotes, E B1 interacts with components of the dynactin complex, the activator for cytoplasmic dynein. Cytoplasmic dynein is a minus end–directed microtubule-based motor that, with the dynactin complex, participates in Golgi dynamics, vesicle transport, and focusing the poles of the mitotic spindle (for review see K ark and H olzbaur, 1999). E B1 has been shown to communoprecipitate the dynactin components p150\textsuperscript{glued} , p50/dynamitin, and the intermediate chain of dynein, from lymphocytes and epithelial cells (B errueta et al., 1999). This interaction occurred independently of microtubules, because it was preserved in cells treated with the microtubule-depolymerizing agent nocodazole, and independently of A PC, as it occurred in cells lacking the COOH terminus of A PC. Like E B1, dynactin has been localized to the plus ends of cytoplasmic microtubules at cortical sites in epithelial cells (B usson et al., 1998). A s with A PC, E B1 might regulate dynactin localization, or, reciprocally, dynactin may help to load E B1 onto the microtubules. A role for dynactin analogous to that in yeast is suggested by a study of the early spindle rotations of the developing C. elegans embryo (S kop and W hite, 1998). Dynactin was shown to localize to the cortical sites and to be required for these spindle rotations. It will be interesting to see whether the C. elegans and D. melanogaster E B1 family members interact with dynactin and whether E B1 proteins play a role in spindle rotations in these organisms.

Effect of Paclitaxel on E B1 Localization

E B1 is a microtubule end–binding protein with several potential roles in normal cellular processes. Is it possible that anticancer therapies might affect E B1 function? Paclitaxel is a chemotherapeutic agent that reduces microtubule dynamics without depolymerizing the microtubule cytoskeleton, and it is thought to cause malignant cells to arrest in mitosis because of spindle damage and, subsequently, to undergo apoptotic cell death. In tissue culture cells, paclitaxel treatment disrupted the localization of E B1 to the microtubules (M orrison et al., 1998). The mechanisms of this effect could include a change in the structure of the microtubule polymer (along its length or at its tip), or a requirement of dynamic microtubules to confer E B1 binding. The idea that the antitumor effects of paclitaxel could be mediated through E B1 must remain speculative, as this drug has shown little activity against colon cancer in clinical trials. Alternatively, the lack of clinical efficacy of paclitaxel in colon cancer may result from the absence of the E B1–A PC interaction specifically in this setting. Both situations highlight the important possibility that natural products that affect the microtubule cytoskeleton might interact with or perturb endogenous microtubule end-binding proteins.

Summary

Functional studies of yeasts deficient in the microtubule end–binding E B1 proteins demonstrate their roles in several aspects of microtubule search and capture, cell polarization, and chromosome stability. In cells that require a cell cycle delay to correct spindle position abnormalities, Bim\textsuperscript{1p} may participate in the checkpoint machinery as well. The E B1–A PC interaction in higher eukaryotes could have arisen evolutionarily to take advantage of specific targeting to the microtubule tip. In multicellular organisms, the interaction between E B1 and dynactin components may have been adapted to exert force on microtubule structures in complex cellular behaviors important for development and cell migration. Many questions about E B1 function remain: how does E B1 influence chromosome segregation? A re E B1 proteins in multicellular organisms important for both spindle and cytoplasmic microtubule behaviors? Wa hat role does the loss of the E B1–A PC interaction play in the pathogenesis of colon cancer? A re E B1 proteins important for dynactin or dynein func-
tion? A might drugs that specifically target EB1 play a role in cancer therapy? Future studies to address these questions are eagerly awaited.

Appendix

Dynamic instability, usually measured at the microtubule plus (distal) end, is defined by four parameters: the rates of transitions between growth and shrinkage (catas-

trophes) and between shrinkage and growth (rescues) (reviewed in Desai and Mitchison, 1997). Two other terms used in reference to microtubule dynamics are pauses, peri-

dods during which length appears constant, reflecting ei-

ther a non dynamic state or a state such as treadmilling in which polymerization at the plus end and depolymeriza-

tion at the minus end are coupled; and dynamicity (Toso et

al., 1999), a composite measurement of the total tubulin

dimers gained or lost per unit time. Dynamicity is in-

creased by faster growth or shrinkage or more frequent

transitions. Microtubule length is likewise affected by all

four parameters. Classically, greater dynamicity correlates

with shorter length, and EB1 is unusual but not unique in

increasing both dynamicity and microtubule length.

Measurements of dynamic instability in living cells can

be affected by multiple factors; in addition to biological differences, methodological variables include cell temper-

ature, choice of fluorescent protein marker, and imaging

and software configurations. As an example, two studies of microtubule dynamics in the bim1Δ mutant (Tirnauer et

al., 1999; A dames and Cooper, 2000) both showed as the major effect reduced dynamicity and increased pausing,

but changes in individual parameter differed somewhat between the studies.

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