

# The Evolution of Microtubule End-Binding Protein 1 (EB1) and Roles in Regulating Microtubule Behavior

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## Abstract

All organisms must transmit genetic information to offspring through cell division, and mitotic spindle participates in the process. Spindle dynamics through depolymerization or polymerization of microtubules generates the driving force required for chromosome movements in mitosis. To date, studies have shown that microtubule arrays control the directions of cell division and diverse microtubule-associated proteins regulate cell division. But a clear picture of how microtubules and microtubule-associated proteins modulate cell division remains unknown. Depletion of end-binding protein 1 by RNA-mediated inhibition shows that one of the microtubule-associated proteins, end-binding protein 1, plays a crucial role in mitotic spindle formation and promotes microtubule dynamics and is needed for the proper segregation of mitotic chromosomes during anaphase in *Drosophila* cells. Here, we review the properties of end-binding protein 1 and the roles of end-binding protein 1 in regulating microtubule behavior and in cell cycle.

## Keywords

Microtubules, End-Binding Protein 1, Chromosome Segregation

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## 1. Introduction

The microtubule cytoskeleton is essential for a variety of essential processes such as intracellular organization, intracellular transport, cell motility, and mitosis of eukaryotic cells. This is possible because of the intrinsic dy-

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dynamic properties of microtubules. Microtubules (MTs) are dynamic hollow tubes comprising  $\alpha$ ,  $\beta$ -tubulin dimers that disassemble and reassemble at two ends: the slow-growing (minus) and fast-growing (plus) ends. It is now widely known that MT behavior is modulated by a number of MT-associated proteins (MAPs), which can influence dynamic instability parameters and consequently impact on mitotic progression and fidelity. Many of these MAPs share the ability to recognize only the distal part of a polymerizing MT, known as the MT plus end. For this reason, these MAPs are currently known as MT plus-end-tracking proteins (+TIPs) [1] [2]. Recently, +TIPs have emerged as regulators of MT dynamics. The plus end explores the cell periphery and shows dynamic instability, switching rapidly between the two phases of growth and shrinkage. Thus this dynamic scaffold performs a variety of very different functions. Genetic and biochemical studies have shown that +TIPs interact with each other and form protein complexes [3].

End-binding protein 1 (EB1) promotes MT polymerization and interacts directly with many other +TIPs and cytoskeletal proteins such as cytoplasmic linker protein 170 (CLIP-170) and the dynactin large subunit p150<sup>Glued</sup>, and mitotic centromere-associated kinesin, microtubule-actin crosslinking factor and adenomatous polyposis coli (APC) [4] [5]. Thus, EB1 has been proposed to form the core of the microtubule plus-end complex and act as a hub in interactions with +TIPs [6] [7].

Division of one cell into two genetically identical daughter cells occurs through two coordinated processes which are known as mitosis (division of the nucleus) and cytokinesis (division of the cytoplasm). The transition from interphase to mitosis involves a dramatic reorganization of the MT cytoskeleton. In fact, there is an increase in MT dynamics which occurs concomitantly with NEB that could be important for spindle morphogenesis [8] [9]. Studies have shown that accurate segregation of the replicated genome during cell division depends on dynamic attachments between kinetochores, proteinaceous structures assembled on the centromeric regions of chromosomes, and spindle microtubules. Kinetochores harness the forces generated by microtubule dynamics to drive chromosome segregation and ensure chromosome biorientation [10]. EB1 localizes to the plus ends of polymerizing MTs, suggesting that it may regulate MT dynamics during mitosis [11]. Depletion of Dm EB1 affects microtubule dynamics but causes minimal perturbation of microtubule organization in interphase cells. This result indicates that Dm EB1 promotes microtubule dynamics in *Drosophila* cells. Moreover, Dm EB1 is necessary for proper chromosomal segregation and spindle elongation during anaphase [12]. And studies show that together with APC, EB1 regulates chromosomal stability during mitosis [13]. So, EB1 is important for driving chromosome segregation by promoting microtubule dynamics. But a clear picture of how EB1 regulate microtubule behavior to drive chromosome segregation has not still emerged.

## 2. Microtubule Structure and Dynamics

Microtubules are intrinsically dynamic structures. In cells they are usually found in states of either growth or shrinkage and they exhibit rapid transitions between the two phases. This feature, termed dynamic instability, underlies many aspects of microtubule function including the ability to rearrange into different arrays [14] [15]. Microtubules are polymeric cylinders composed of  $\alpha$  and  $\beta$  tubulin dimers. Rows of tubulin subunits, protofilaments, associate with each other laterally to form the microtubule lattice. Because they are arranged head to tail into 13 protofilaments that are aligned longitudinally in the tubule wall, microtubules are intrinsically polar: the plus-end where  $\beta$ -tubulin monomer is exposed, is the fast-growing end *in vitro* and the only end that grows in cells. The opposite (minus) end can slowly grow *in vitro*, while in cells it is usually stabilized or serves as the site of disassembly [16]. Microtubules are structurally polar and dynamic filaments that grow by addition of guanosine triphosphate (GTP)-loaded tubulin subunits to their end. After complex, largely unknown structural rearrangements at the nanoscale, GTP hydrolysis and phosphate release lead to the formation of a guanosine diphosphate (GDP)-loaded microtubule lattice. The matured microtubule lattice is protected from depolymerization by a stabilizing structure at the growing microtubule end. Stochastic loss of this end structure leads to depolymerization (catastrophe) [17].

In cells, MT dynamics are not only regulated by the intrinsic dynamic instability of the polymers, but also by stabilizing/destabilizing structural MT-associated proteins (MAPs) that play a role in MT organization [18]. Among these, a large number of MAPs specifically recognize the terminal portion of MT. These are collectively known as MT plus-end-tracking proteins or +TIPs [1]. These +TIPs appear as comets in the MT tip, moving throughout the cell as MT grows and disappearing when MT shrinks [19]. One of these +TIPs, EB1 recruits several other proteins to growing microtubule ends and EB1 as a microtubule maturation factor and provide a

mechanistic explanation for its effects on microtubule growth and catastrophe frequency, which cause microtubules to be more dynamic. EB1 binding accelerates conformational maturation in the microtubule, most likely by promoting lateral protofilament interactions and by accelerating reactions of the guanosine triphosphate (GTP) hydrolysis cycle. The microtubule maturation time is directly linked to the duration of a growth pause just before microtubule depolymerization, indicating an important role of the maturation time for the control of dynamic instability [20].

### 3. The Microtubule Plus-End Binding Protein 1 (EB1) and Localization of EB1 in Cells

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 [6]. EB1 was the first member identified in a yeast two-hybrid screen as an interactor of the C-terminus of the adenomatous polyposis coli (APC) tumor suppressor protein [21]. EBs are relatively small, elongated proteins (around 32 kDa) with conserved structural features. All members have at the N-terminal region an MT-binding portion containing a calponin homology (CH) domain with a highly conserved fold [2]. It was shown that this CH domain was both required and sufficient for binding to MT plus ends [22] [23]. The C-terminal portion of EB1, on the other hand, contains a coiled-coil region which is necessary for EB dimerization. The EEY/F motif at the flexible tail region provides a binding site for CAP-Gly domains found in select cytoskeleton-associated proteins, including a +TIP p150<sup>Glued</sup> [4] [24]. Members of the EB1 family (EBs) are mostly known for recruiting a variety of other plus-end-tracking proteins through interactions with their C-terminal EB homology domain [4] [25] [26]. The N-terminal microtubule binding domains bind to the outer microtubule surface in the grooves between adjacent protofilaments, close to the exchangeable GTP binding site [27]. End binding (EB) proteins are part of a highly conserved family which, in mammals, comprises three members encoded from three different genes: EB1, EB2 (RP1), and EB3 (EB3F). Both EB1 and EB3 seem to be ubiquitously expressed, whereas EB2 expression is restricted to only certain cell types/tissues [28]. Normally, EB1 is expressed in higher levels when compared to other EBs. However, EB3 is also highly expressed in specific cell types. EB3 was originally reported in neurons, where it was shown to interact with a brain-specific form of APC (APC2), but it is also highly abundant in muscle cells [29]. EB1 homologs are also found in plant lineages [30] [31], but interestingly, the EEY/F motif is not present in plant EB1 proteins, which are divided in two subgroups. Arabidopsis has three EB1s: EB1a, EB1b, and EB1c [32]. EB1a and EB1b are similar in sequence and localize to MT plus ends in all MT structures throughout cell cycle. EB1c, which is conserved only in vascular plants, localizes to nucleoplasm during interphase and colocalizes with MT only in the mitotic spindle and phragmoplast [11] [33] [34]. Both immunofluorescence analyses and live imaging using GFP tagging showed that EB1 is able to localize to the growing ends of MTs throughout mitosis [8] [35]. More in-depth observations demonstrated that EB1 can target to kinetochores with attached growing MTs [36]. Besides its plus end localization, EB proteins were also shown to bind other subcellular structures either directly (centrosome) or indirectly (F-actin and membranes). The functions for the CH and CC domains are conserved in the Arabidopsis EB1 proteins. The C-terminal tail of the EB1a and EB1b subgroup is autoinhibitory in the MT assembly capacity of EB1, despite the absence of the EEY/F motif; instead, the EB1c tail has a nuclear localization signal (NLS). EB1a and EB1b form heterodimers with each other, but not with EB1c. Furthermore, the EB1 genes are expressed in various cell types of Arabidopsis, but the expression of EB1c is particularly strong in the meristematic cells where it is targeted to the nucleus by a nuclear localization signal in the C-terminal tail [11].

### 4. Roles of EB1 in Regulating Microtubule Behavior

The dynamic properties of microtubules are regulated by multiple proteins. Two of them, EB1 and XMAP215 (chTOG in humans), are special in that they accumulate autonomously at microtubule ends [37]. EB1 is selective for growing ends (not distinguishing between plus and minus ends). And recruits several other proteins to growing microtubule ends and has seemingly antagonistic effects on microtubule dynamics [38] [39]. The first report regarding the possible role of EB proteins in MT dynamics came from the observation that, when overexpressed, these proteins induced the formation of acetylated MT bundles that were resistant to nocodazole treatment [40]. In addition, their ability to tip-track MTs led to the possibility that they might be involved in MT dy-

namics regulation, particularly in promoting MT growth [41]. This was confirmed in many independent studies using not only different model organisms such as budding and fission yeast, *Drosophila*, and human cells, but also *in vitro* systems [23] [42] [43]. Growing microtubule ends serve as transient binding platforms for essential proteins that regulate microtubule dynamics and their interactions with cellular substructures. End-binding proteins (EBs) autonomously recognize an extended region at growing microtubule ends with unknown structural characteristics and then recruit other factors to the dynamic end structure. The calponin homology (CH) domain of the fission yeast EB Mal3 bridges protofilaments except at the microtubule seam. By binding close to the exchangeable GTP-binding site, the CH domain is ideally positioned to sense the microtubule's nucleotide state. The same microtubule-end region is also a stabilizing structural cap protecting the microtubule from depolymerization. This supports that there is a common structural link between microtubule dynamic instability and end tracking [27]. EB1 senses conformational changes within the microtubule lattice induced by reactions taking place as part of the GTP hydrolysis cycle [27] [44]. This leads to the well-known comet-like accumulation of EBs at the end region of growing microtubules where high-affinity binding sites are gradually lost with time [45]. The impact of EB proteins on interphase MT dynamics may also involve their interaction with other +TIPs. In fact, differences in the expression and regulation of several +TIPs in different cell types may be responsible for the observed differences in specific MT populations [46]. In addition, data derived from *in vitro* assays demonstrated that EB1 can act cooperatively with other +TIPs such as CLIP170 in the regulation of MT dynamics. Accordingly, it was demonstrated that both EB1 and CLIP170 can synergize to modulate MT dynamics, possibly by modifying the MT-stabilizing cap [47]. Moreover, EB association with CLASPs was also reported to affect MT dynamics at the cell cortex by increasing MT rescue events [48]. Interestingly, EBs can also associate with and load MT depolymerizers such as MCAK to the MT plus ends [49]. This interaction is important for the localization of MCAK to the plus ends but also to enhance its catastrophe-inducing activity. Thus, by allowing the accumulation of polymerizers and depolymerizers at the MT plus end, EB proteins facilitate the rapid switching between MT growth and shortening. The overall picture that has emerged confirms the role of EB proteins in the regulation of MT dynamics, but their precise effect is still not fully understood. Recent work showed that modulation of MT dynamics by EB proteins can also be regulated by phosphorylation. In budding yeast, the single EB-like protein was described to be phosphorylated by Ipl1p/Aurora-B and this is important to regulate the association of EB to spindle MTs [50]. Moreover, a mutation in the fission yeast EB-like protein was sufficient to increase MT binding, leading to their stabilization [51]. In humans, less is known about the phosphorylation of EB proteins. Recent work demonstrated that EB3 is phosphorylated by Aurora kinases on S176 during mitosis [52]. This Aurora-mediated EB3 phosphorylation leads to a significant increase in MT growth, allowing stabilization of the midbody [53]. Dephosphorylation of EB3 restricts cortical MT growth, allowing proper daughter cell adhesion to the substrate. Inversely, phosphorylation of EB3 on S162 by the Src-PLCg2 signaling pathway was shown to block MT growth, leading to adherens junction stabilization in interphase cells [54]. Taken together, these data demonstrate that EB protein association to the MT plus ends can be regulated by phosphorylation, although it is still unclear how different phosphorylation events integrate to control EB function, thus regulating MT dynamics in different tissues.

## 5. Roles of Microtubule and EB1 in Cell Cycle

Proper regulation of MT (microtubule) dynamics is essential for various vital processes, including the segregation of chromosomes, directional cell migration and differentiation. Spindle dynamics through depolymerization or polymerization of microtubules (MT) generates the driving force required for chromosome movements in mitosis [55]. MT assembly and disassembly is modulated by a complex network of intracellular factors that cooperate or antagonize each other, are highly regulated in space and time and are thus attuned to the cell cycle and differentiation processes [56]. Cell-cycle progression is accompanied by changes in MT dynamics at very specific stages. This is accompanied by an increase in MT dynamics and an abrupt decrease in MT polymer level which tightly correlates with NEB [57]. So studies of roles of microtubule and EB1 are important to understand the life of plants.

Interactions between microtubules plus-end and the cell cortex are important for accurate positioning of the spindle [58]. During normal mitosis, the mitotic spindle positions itself at the geometric center of the cell. In S2 cells lacking Dm EB1, however, the spindle was frequently mis-positioned. This shows that mitotic spindle positioning requires EB1 activity. At the same time, the Loss of Dm EB1 function causes defects in mitotic spindle

structure in metaphase cells that could be classified into four general categories. The most common defect was a complete loss of astral microtubules. The second class of defects lacked astral microtubules and exhibited an overall compaction of the spindle into a basket-like meshwork of microtubules surrounding the chromosomes. The third type of defect was a detachment of a spindle pole from the bundles of microtubules that were connected to the kinetochores. The fourth category of defect was “barrel-shaped” spindles that maintained their symmetry, but failed to focus the microtubules at the poles and also lacked astral microtubules [12]. So, will these prevent normal chromosome segregation during mitosis? Whether chromosome segregation will be abnormal in these cases remains unclear. Dm EB1 plays a crucial role in mitotic spindle formation and elongation and is needed for the proper segregation of mitotic chromosomes during anaphase, but how it to induce chromosome segregation by regulating spindle movement is still not fully clear. The first reports indicated that depletion of EB1 in *Drosophila* leads to the formation of short spindles and short astral MTs [12]. In *Xenopus* egg extracts, EB1 was involved in spindle organization and chromosome segregation by interacting with XMAP215 [59]. In addition, together with APC, EB1 regulates chromosomal stability during mitosis [13].

Plants are prolific organisms covering the earth and are also irreplaceable partners for human beings. To enact morphogenesis, plants have evolved plant-specific MT arrays: cortical MTs, preprophase band, mitotic spindle, and phragmoplast. Plant microtubules (MTs) and MT-associated proteins (MAPs) are essential for fundamental morphogenesis, including controlling the direction of cell division and expansion, chromosome segregation, and cytokinesis. However, little is known about roles of EB1 in chromosome segregation in plants. Han *et al.* found that “partition-bundle” division would be happened in root-tip cells of wheat seedlings under the enhanced UV-B radiation [60]. Also, the distribution of microtubules in wheat cells was disordered under the enhanced UV-B radiation [61]. But whether there is a correlation between “partition-bundle” division and disordered microtubules and whether EB1 plays a role in this process require to further be tested. But in *Arabidopsis*, EB1c is thought to function primarily during mitosis; it localizes to mitotic microtubule arrays in dividing cells and is sequestered in the nucleus during interphase. EB1c mutants exhibit lagging chromosomes during anaphase, suggesting that this protein subtype plays a role in early mitotic phases, regulating spindle positioning and chromosome segregation [11]. Moreover, in plant, the mutants of EB1 have twisting macroscopic phenotypes that caused by the change of MT array orientation from transverse to oblique in young elongating cells, indicating that these MAPs are involved in cortical MT organization to keep specific cell wide orientation, such as transverse to the long axis of the organ [62]. And the phenotype of the triple null mutant of the three EB1 genes includes defective spindle or phragmoplast organization [11]. In addition, EB1 mutants have mild defects in gravity and touch sensing in roots [62] [63].

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