Structural basis of EB1 effects on microtubule dynamics

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Abstract

+TIPS (plus-end tracking proteins) are an increasing group of molecules that localize preferentially to the end of growing microtubules. +TIPS regulate microtubule dynamics and contribute to the organization of the microtubular network within the cell. Thus they participate in a wide range of cellular processes including cell division, motility and morphogenesis. EB1 (end-binding 1) is a highly conserved key member of the +TIP group that has been shown to modulate microtubule dynamics both in vitro and in cells. EB1 is involved in accurate chromosome segregation during mitosis and in the polarization of the microtubule cytoskeleton in migrating cells. Here, we review recent in vitro studies that have started to reveal a regulating activity of EB1, and its yeast orthologue Mal3p, on microtubule structure. In particular, we examine how EB1-mediated changes in the microtubule architecture may explain its effects on microtubule dynamics.

The microtubule, a dynamic structure

Microtubules are polar and dynamic polymers involved in the functional organization of most eukaryotic cells. They are hollow cylinders approx. 25 nm in diameter resulting from the assembly of αβ-tubulin heterodimers [1]. Tubulin dimers are arranged head-to-tail to form protofilaments that interact laterally, making the cylindrical microtubule wall (Figure 1A). This particular arrangement confers on the microtubule a structural polarity with the β-subunit exposed at the fastest-growing plus end [2]. Protofilaments are longitudinally shifted from one to the other, so that tubulin monomers describe left-handed lateral helices within the microtubule lattice. The architecture of a classical cytoplasmic microtubule consists of 13 protofilaments aligned parallel to the tube axis and three lateral helices (hereafter denoted by 13_3 microtubule; Figure 1A) [3]. All other protofilament and/or lateral helix number configurations exhibit a small twist of the protofilaments, so that the same helical geometry found in the 13_3 microtubule is maintained (Figure 1B) [4,5]. As a result of this protofilament skewing, electron micrographs of vitreous-ice embedded microtubules display characteristic moiré patterns that allow us to accurately determine the surface-lattice organization and microtubule polarity [5–8]. Protofilament skewing and changes in the microtubule radial curvature have been proposed to induce the accumulation of an excess of energy in the polymer, capable of destabilizing interactions between tubulin dimers [9,10]. As a consequence, unskewed 13_3 microtubules, which represent the most common in vivo architecture, would also be the most energetically favourable ones.

Other remarkable structural features have been characterized at the end of microtubules, which are clearly distinguishable from the tube. Outwardly curled, separated protofilaments appear at the depolymerizing microtubule extremity [11–14], whereas a two-dimensional sheet of tubulin has been observed at the end of growing microtubules in vitro [11,12,15–18] (Figure 1D) and in cells [19–23]. These observations suggested that microtubule assembly is a two-dimensional process occurring through the extension of an outwardly curved tubulin sheet that gradually closes into a tube [24]. According to the proposed model, sheets display two opposite curvatures resulting from (i) the longitudinal intrinsic curvature of protofilaments and (ii) the lateral interactions between protofilaments (green and red arrows respectively in Figure 1C; reviewed in detail in [29]). The latter increases with the number of protofilaments, which leads to the gradual straightening and closure of the sheet into a cylinder. Hence, the closure of the sheet is intimately linked to the balance between its lateral and longitudinal growth. This two-dimensional assembly model also implies that the lattice organization is determined by the tubulin-sheet closure, unlike the template model based on microtubule helical growth [25].

Microtubules undergo dynamic instability, an intrinsic property characterized by stochastic transitions between growing and shrinking states [1]. This striking behaviour, which is essential for diverse cellular processes, is characterized by the following four parameters: the growth rate, the shrinkage rate, the catastrophe frequency (transition from growth to shrinkage) and the rescue frequency (reverse transition). Despite extensive research in the field, the molecular mechanisms sustaining these random transitions are still widely unknown, but models have been developed. Dynamic
instability is fuelled by GTP hydrolysis on β-tubulins, which led primarily to the model of the so-called GTP-cap [26]. This cap of GTP-bound β-tubulin was proposed to protect microtubules from catastrophes by reinforcing lateral and/or longitudinal tubulin interactions at microtubule ends [27,28]. Later on, the presence of outwardly curved tubulin sheets at the end of growing microtubules led to the conformational cap model [29]. Tubulin-sheet closure compels protofilaments to straighten up, accumulating elastic energy into the lattice. As a consequence, the tubulin sheet appears more stable than the cylindrical wall, and would therefore constitute a protective cap at microtubule ends. Recent structural studies and computer modelling support the relevance of such a conformational cap [24,30–32]. Today, the relationship between the conformational cap and GTP hydrolysis is not clearly understood, even though a conformational antibody raised against GTP-bound tubulin has revealed the end of growing microtubules in immunostaining experiments [33]. Whether this antibody recognizes the curved conformation of tubulin present in two-dimensional sheets remains to be determined. One possibility would be that GTP hydrolysis is triggered by the tubulin sheet straightening, making a rough overlap between the sheet and the GTP-cap, as suggested elsewhere [29].

Dynamic instability is modulated within the cell by a myriad of MAPs (microtubule-associated proteins) [34]. Their effects on microtubule dynamics and polarization have been well described in the past two decades, but their precise molecular mechanisms are still unknown. Nonetheless, recent concepts have started to arise concerning the effects of MAPs on microtubule structure, including lattice architecture and tubulin-sheet morphology. As an example, the neuronal MAP doublecortin seems to force specifically the assembly of 13_3 microtubules while modulating microtubule dynamics in vitro [35,36]. Thus the current challenge consists of deciphering the link between the control of microtubule structure and the regulation of microtubule dynamics.
EB1 (end-binding 1), a +TIP (plus-end tracking protein) that tunes microtubule dynamics

+TIPs are the most recently described group of MAPs that localize preferentially to the growing microtubule end, where they constitute an intricate molecular network [37]. +TIPs play fundamental roles in the control of microtubule dynamics and polarization during the cell cycle, so that they participate in diverse cellular processes, such as cell division, migration and differentiation [38].

EB1 is a highly conserved key member of the +TIP group. First described as an interactor of the tumour suppressor APC (adenomatous polyposis coli), it is now better known for being the ‘key stone’ of the +TIP network [37,39–41]. EB1 tracks microtubule ends by treadmilling at the end of microtubules. This process, which has recently been reconstituted in vitro with purified components [40–42], is characterized by the association of the protein with the newly growing end coupled with a rapid dissociation from the lattice older part. The recognition of a specific structure at microtubule plus end, such as the tubulin sheet, is the favourite targeting mechanism rather than a co-polymerization with tubulin heterodimers, since EB1 has a weak affinity for the latter [43–46]. Furthermore, the dwelling time of EB1 at microtubule ends is too brief to account for the whole comet decoration through a co-polymerization process [40,41]. Afterwards, EB1 could come unstuck from the microtubule lattice after the conformational change induced by tubulin-sheet closure. Phosphorylation and/or interaction with other factors could influence this behaviour in vivo. GTP hydrolysis might also be involved in EB1 tracking, even if it does not modify drastically the αβ-heterodimer conformation [30–32]. Further experiments, including precise EB1 localization by cryo-electron tomography, will be necessary to superimpose clearly EB1 comets observed in fluorescence microscopy with tubulin sheets. However, a recent in vitro study, based on TIRF (total internal reflection fluorescence) experiments, has revealed that the mean comet length increases with the microtubule growth rate [40] as shown previously for the tubulin sheet [12].

It is now clear that EB1 has strong effects on microtubule dynamics in vitro and in vivo, but its precise action on each parameter of dynamic instability is still contradictory and controversial in the literature. First, EB1 has been shown to stimulate both catastrophes and rescues in Saccharomyces cerevisiae and Drosophila melanogaster [47,48], while it inhibits catastrophes in Schizosaccharomyces pombe, Xenopus laevis egg extracts and cultured mammalian cells [43,49,50]. Secondly, based on in vitro turbidimetry assays, several studies have revealed that EB1 is able to promote, on its own, tubulin assembly [42,45,51–53], whereas others have suggested the need of an additional +TIP to get the same activity [46,54,55]. Finally, the use of VE-DIC (video-enhanced differential interference contrast) light microscopy, which allows us to record individual microtubule behaviour, did not clarify much our understanding regarding the precise effects of EB1 on microtubule dynamics. Indeed, in two studies, EB1 has been shown to strongly increase microtubule dynamics [42,53], whereas in another one it decreases catastrophes [52]. Furthermore, a very recent report has demonstrated that EB3, another member of the conserved EB1 protein family, promotes microtubule dynamics in vitro [50]. These discrepancies among all the above mentioned studies may be explained by differences in the cDNA employed (e.g. human EB1 versus mouse EB1) and the experimental conditions used, including variable EB1/tubulin ratios and diverse microtubule assembly buffers. As an example, assembly buffers may contain KCl or NaCl at various concentrations, while these salts are known to affect not only the ionic strength but also the microtubule assembly itself [56] and B. Vitre, F.M. Coquelle, C. Heichette, D. Chrétien and I. Arnal, unpublished work). In addition, VE-DIC studies have been performed under conditions in which the concentration of free tubulin heterodimers either remained roughly constant [42,53] or decreased progressively during the experiment [52].

EB1 shapes microtubule lattice during tubulin-sheet closure

Structural studies have recently been performed in order to decipher in detail the molecular mechanisms sustaining EB1/Mal3p activity on microtubule dynamics [45,53,57].

Using high-resolution surface shadowing and electron microscopy, Mal3p has first been localized along the microtubule seam, which is thought to be the resulting tube junction area. Consistent with the previous analysis, a three-dimensional reconstruction based on cryo-electron microscopy images revealed that the N-terminal part of Mal3p closely binds to tubulin monomer on the outer surface of microtubules and occupies the groove between protofilaments. Overall, these primary structural data strongly suggest that Mal3p would reinforce the lateral interactions between adjacent protofilaments, in particular at the microtubule closure junction. This lattice-based activity may stabilize the microtubule wall, and thereby account for the stimulating effects of EB1/Mal3p on rescue events [53]. It is worth noting that the stimulation of rescues by EB1, unlike its other effects on microtubule dynamics, has been observed only for the higher concentrations used in in vitro assembly experiments [53]. This is consistent with the very low affinity of EB1/Mal3p for the microtubule lattice [40,45,52], and suggests that the other modulating activities of this +TIP on microtubule dynamics are based on its preferential binding to microtubule ends. However, recent publications favour a solely lattice-based activity to explain the diverse effects of EB1 on dynamic instability [40,41]. Hence, further experiments will be needed to clearly discriminate between the lattice-based activities of EB1 and the ones that are confined to microtubule extremities.

Microtubules assembled in vitro display a reduced mean sheet length at their extremities and a lower overall proportion of tubulin sheet in the presence of either Mal3p or EB1 [45,53]. Given the localization of Mal3p on microtubule seams, it has been primarily proposed that this +TIP may
Alternative models to account for EB1/Mal3p effects on microtubule structure and dynamics

(A) EB1/Mal3p (in red) would bind to the sheet edges and accelerate tube closure either through a zippering activity or an increase in the sheet lateral growth (see the text for details). Both models result in the reduction of the conformational cap thought to stabilize microtubules, making the polymers more prone to undergo a catastrophe. Such a mechanism would indirectly favour the selection of the most stable 13_3 microtubules. (B) EB1 forces the tube to close into 13_3 configurations, probably by controlling lateral interactions between adjacent tubulin dimers in the area around the tube junction. Figure based on data taken from [45,53,57].

Figures 2A and 2B: EB1 stimulates sheet closure and constrains tube closure, respectively.

Process sheet closure through a zippering activity (Figure 2A) [45]. Alternatively, EB1 may bind preferentially to the edges of tubulin sheet where it could facilitate the incorporation of tubulin heterodimers and/or oligomers [53]. Through this mechanism, EB1 would increase the tubulin-sheet lateral curvature, resulting in their straightening up and closure into a tube (as described in Figure 1C). Both models may account for EB1 stimulating effects on the catastrophe frequency, since microtubules with a shorter conformational cap are thought to be more prompt to undergo a depolymerization event [12]. However, only the second model can explain the EB1-mediated stimulation of the microtubule growth rate by the increase in the incoming tubulin flux.

EB1 and Mal3p favour the unskewed 13_3 lattice architecture in co-assembly experiments [53,57]. Such an effect has already been observed with doublecortin and was attributed to a constraining activity [35,36]. Since Mal3p localizes, like doublecortin, in between protofilaments, it has been proposed that EB1/Mal3p forces the 13_3 configuration by controlling the microtubule radial curvature [57]. Nevertheless, unlike doublecortin, EB1/Mal3p preferentially localizes to microtubule ends, suggesting that its constraining activity would be confined around the microtubule closure junction (Figure 2B). Interestingly, this eventual modus operandi does not explain how EB1 stimulates the catastrophe frequency and the growth rate, which opens the possibility of two independent EB1-mediated mechanisms at microtubule ends.

In parallel, based on the EB1/Mal3p sheet closing activity, a more indirect mechanism has been put forward to explain the selective accumulation of 13_3 microtubules [53]. Indeed, EB1/Mal3p-mediated reduction of the conformational cap destabilizes microtubules, leading to the elimination, at first, of the more unstable lattice configurations through catastrophe events, as proposed previously [10]. Thus this phenomenon is thought to contribute indirectly to the selection of 13_3 microtubules by discarding the more stressed configurations over time (Figure 2A). Additionally, a more direct proofreading activity has been considered that remains to be demonstrated [53]. In all likelihood, this mechanism would be localized close to the tube junction area where EB1/Mal3p would be able to detect, as a sensor, any lattice defect and trigger a catastrophe event to eliminate it (Figure 2B). The localization of Mal3p in the groove between protofilaments [57] is compatible with a radial curvature sensor activity, but further investigations are required to shed light on the intimate molecular mechanisms involved. Three-dimensional models combining electron microscope and X-ray crystallography data represent promising future directions to identify, at the atomic level, the molecular interactions in play between EB1 and the microtubule.

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**References**


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