Short-circuiting microtubule plus and minus end proteins in spindle positioning

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Proteins residing at the plus and minus ends of microtubules have been thought not to communicate with each other, but recent findings on *bona fide* nucleation factors also regulating microtubule dynamics have challenged this notion. New work by Bouissou *et al* (2014) in *The EMBO Journal* now reveals that interplay between the nucleation factor γ -TuRC and the plus-end tracking protein EB1 controls mitotic spindle positioning by affecting the stability and dynamics of astral microtubules.

See also: A Bouissou *et al* (January 2014)

One of the most fascinating aspects of microtubule biology is their hallmark behavior of assembly and disassembly, termed dynamic instability. At any given time within a cell, individual microtubules exhibit cycles of growth or shrinkage and switch between these two states without altering the total cellular mass of microtubule polymer. Dynamic instability allows the cell to rapidly reorganize the microtubule network in response to extracellular cues triggering cellular polarization, or internal cell cycle progression signals that initiate assembly of the mitotic spindle. Dynamic instability also underlies a 'search and capture' mechanism in which microtubules grow out from a centrosome or spindle pole in all directions and are captured at a site that stabilizes their interaction. These capture sites may be organelles to be transported by motor proteins, kinetochores during prophase, or the cell cortex. Microtubule-cortex interactions are important for polarization of the microtubule network

during directional migration, for example, or during oriented cell divisions. Dynamic instability is regulated both by the intrinsic properties of microtubules, as well as by a set of accessory molecules that control their polymerization dynamics. Growing microtubules are crowned at their plus end by a 'GTP cap'; when the rate of incorporation of new tubulin subunits at the end is outpaced by the inherent GTPase activity of polymerized tubulin, microtubules switch from growth to shrinkage in a process termed catastrophe (Howard & Hyman, 2009). Their behavior is also dictated by microtubule plus end interacting proteins (+TIPs) that govern dynamic instability by regulating the conformation of tubulin at the plus ends (Jiang & Akhmanova, 2011). EB1, for example, is a well-studied +TIP that functions to promote microtubule dynamic instability. +TIPs can also contribute to microtubule search and capture as binding partners for cortical protein complexes. Thus, elucidating the functional interplay between +TIPs and the endogenous GTPase activity of microtubules is an important goal towards understanding how cells regulate dynamic instability.

In addition to the activity that occurs at the plus ends, patterns of microtubule growth are also determined by their nucleation at microtubule organizing centers such as interphase centrosomes and mitotic spindle poles. Microtubule nucleation at these sites is mediated by γ -tubulin that is itself a component of a larger protein complex termed the γ -tubulin ring complex (γ -TuRC). Structural studies of the γ -TuRC revealed that it has a conical shape and a 13-fold symmetry and a widely-accepted model for γ -TuRC function proposes that it acts as a

nucleation complex for microtubule growth by positioning γ -tubulin as a template for each protofilament at the microtubule minus end (Moritz *et al*, 2000; Moritz & Agard, 2001). As microtubules often grow to lengths of many microns from the centrosome, the molecules that reside at the plus and minus ends have not, historically, been thought to communicate with each other to regulate microtubule dynamics. Over the last few years, however, a growing body of evidence suggests that there is cross-talk between proteins involved in microtubule nucleation and factors that regulate microtubules at the plus end. The first hints came from genetic studies in fungi that showed γ -tubulin and its interacting partners can regulate microtubule dynamics independently of nucleation (Paluh *et al*, 2000; Fujita *et al*, 2002; Cuschieri *et al*, 2006; Masuda *et al*, 2006; Anders & Sawin, 2011). More recently, the Raynaud-Messina group demonstrated that γ -TuRC proteins directly regulate dynamic instability in cultured *Drosophila* cells by limiting catastrophe events at the plus ends (Bouissou *et al*, 2009). From these studies, it is becoming clear that microtubules are regulated in unprecedented ways by factors that were previously thought to act as nucleation factors.

In their current study, Bouissou *et al* have extended these observations and now provide important mechanistic insight into how molecules of the γ -TuRC regulate microtubule dynamics independently of their role in nucleation. The authors began their study by examining the contributions of Dgrip75, a γ -TuRC subunit, to orientation of the mitotic spindle in cultured *Drosophila* S2 cells. During mitosis, each spindle pole

nucleates a halo of astral microtubules that interact with the cell cortex; in unpolarized cells, astral microtubules suppress rotation of the spindle while in polarized cells (such as neuroblasts) astral microtubules orient the axis of cell division in response to polarity cues. Bouissou *et al* found that depletion of Dgrip75 caused spindle mispositioning in unpolarized cells and misalignment in experimentally polarized cells, as well as in larval brain neuroblasts. This function was conserved as loss of the Dgrip75 homologue, GCP4, in HeLa cells also caused defects in spindle orientation. Consistent with a role for γ -TuRC components in spindle orientation, both γ -tubulin and γ -TuRC components localized to astral microtubules and loss of Dgrip75 led to an increase in both their rates of growth and shrinkage and a decrease in the amount of time they paused. The authors demonstrated that these effects are likely caused by direct modulation of dynamic instability as suppression of microtubule dynamics – either using drugs or by

co-depletion of EB1 – was able to partially rescue spindle orientation defects caused by the loss of GCP4 in HeLa cells, or Dgrip75 in fly cells, respectively. Loss of Dgrip75 or GCP4 also altered the localization of EB1, causing it to distribute from its typical distal association with the microtubule plus end to a more proximal localization along the microtubule lattice. Since EB1 exhibits a preference to bind to microtubules composed of GTP-tubulin over GDP-tubulin (Maurer *et al*, 2012), this unexpected effect led the authors to ask whether loss of γ -TuRC components could change the composition of the microtubule lattice *in vivo*. To address this experimentally, the authors made use of a monoclonal antibody that specifically recognizes tubulin in the GTP-bound conformation (Dimitrov *et al*, 2008) and used this reagent to examine microtubules in interphase HeLa cells depleted of GCP4. The surprising result was that the number of GTP-tubulin ‘islands’ in the microtubule lattice of these cells was significantly higher.

Collectively, this study demonstrates that γ -TuRC subunits may play a role as microtubule stabilizers independently of their role in microtubule nucleation and this function is particularly important to spindle orientation during mitosis (Fig 1).

Altogether, this paper represents a significant advance to our understanding about the regulation of microtubule dynamic instability, and importantly, introduces new questions to prime future studies. The evidence that γ -TuRC subunits regulate dynamic instability to promote microtubule stabilization is strong, but we lack insight into the mechanism at work. Dgrip75/CGP4 localize to the microtubule lattice throughout the cell cycle, is this the pool of protein that is regulating microtubule dynamics? Do γ -TuRC components regulate dynamic instability by direct interaction with the microtubule, as the GTP-tubulin monoclonal antibody might suggest? Or do these proteins act indirectly, perhaps by exerting regulation over EB1 or other +TIPs that govern microtubule behavior? Finding answers to these questions will have far-ranging impact on our understanding of asymmetric cell division and microtubule dynamics.

Conflict of interest

The author declares that he has no conflict of interest.

References

- Anders A, Sawin KE (2011) Microtubule stabilization *in vivo* by nucleation-incompetent gamma-tubulin complex. *J Cell Sci* 124: 1207–1213
- Bouissou A, Verollet C, de Forges H, Haren L, Bellaiche Y, Perez F, Merdes A, Raynaud-Messina B (2014) γ -tubulin ring complexes and EB1 play antagonistic roles in microtubule dynamics and spindle positioning. *EMBO J* 33: 114–128
- Bouissou A, Verollet C, Sousa A, Sampaio P, Wright M, Sunkel CE, Merdes A, Raynaud-Messina B (2009) γ -tubulin ring complexes regulate microtubule plus end dynamics. *J Cell Biol* 187: 327–334
- Cuschieri L, Miller R, Vogel J (2006) Gamma-tubulin is required for proper recruitment and assembly of Kar9-Bim1 complexes in budding yeast. *Mol Biol Cell* 17: 4420–4434
- Dimitrov A, Quesnoit M, Moutel S, Cantaloube I, Poüs C, Perez F. (2008) Detection of GTP-tubulin conformation *in vivo* reveals a role for GTP remnants in microtubule rescues. *Science* 322: 1353–1356

Figure 1. Schematic of a dividing metazoan cell.

γ -TuRCs at mitotic microtubule-organizing centers (MTOCs) nucleate both kinetochore and polar microtubules, as well as astral microtubules that provide MTOC anchoring at the cell cortex and thereby control mitotic spindle positioning. In addition, γ -TuRCs are also found along the length of astral microtubules, where their presence serves to limit redistribution of the plus-end tracking protein EB1 and keep astral microtubule dynamics in check.

- Fujita A, Vardy L, Garcia MA, Toda T (2002) A fourth component of the fission yeast γ -tubulin complex, Alp16, is required for cytoplasmic microtubule integrity and becomes indispensable when γ -tubulin function is compromised. *Mol Biol Cell* 13: 2360–2373
- Howard J, Hyman AA (2009) Growth, fluctuation and switching at microtubule plus ends. *Nat Rev Mol Cell Biol* 10: 569–574
- Jiang K, Akhmanova A (2011) Microtubule tip-interacting proteins: a view from both ends. *Curr Opin Cell Biol* 23: 94–101
- Masuda H, Miyamoto R, Haraguchi T, Hiraoka Y (2006) The carboxy-terminus of Alp4 alters microtubule dynamics to induce oscillatory nuclear movement led by the spindle pole body in *Schizosaccharomyces pombe*. *Genes Cells* 11: 337–352
- Maurer SP, Fourniol FJ, Bohner G, Moores CA, Surrey T (2012) EBs recognize a nucleotide-dependent structural cap at growing microtubule ends. *Cell* 149: 371–382
- Moritz M, Agard DA (2001) Gamma-tubulin complexes and microtubule nucleation. *Curr Opin Struct Biol* 11: 174–181
- Moritz M, Braunfeld MB, Guénebaut V, Heuser J, Agard DA (2000) Structure of the gamma-tubulin ring complex: a template for microtubule nucleation. *Nat Cell Biol* 2: 365–370
- Paluh JL, Nogales E, Oakley BR, McDonald K, Pidoux AL, Cande WZ (2000) A mutation in gamma-tubulin alters microtubule dynamics and organization and is synthetically lethal with the kinesin-like protein pkl1p. *Mol Biol Cell* 11: 1225–1239