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SPOTLIGHT

GTP-tubulin loves microtubule plus ends but marries the minus ends

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Microtubule minus ends are inherently more stable than plus ends despite the fact that free tubulin associates more avidly to the plus end. In this issue, Strothman et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201905019) measure, for the first time, the off-rate for GTP-tubulin and find that it is different for the two ends, suggesting that this parameter may control the transition to disassembly at microtubule ends.

Dynamic instability of microtubule (MT) growth (1) is a behavior that is characterized by periods of prolonged assembly of αβ-tubulin dimers interrupted by sudden, rather unexpected, transitions to disassembly. Both ends of the MT exhibit this behavior, but with differing kinetics. Tubulin (αβ) dimers with GTP bound to the β subunit (called GTP-tubulin for simplicity) are the units of assembly of the polymerizing MT. However, the switch from assembly to disassembly requires GTP hydrolysis by the β-tubulin subunit once incorporated into the MT lattice. This inspired the hypothesis that rapidly polymerizing GTP-tubulin subunits provide a stabilizing structural "GTP cap" at the end of the MT that inhibits the transition to disassembly until GTP is hydrolyzed. Two technical problems have limited the detection and characterization of the cap. First, the signal from GTP hydrolysis by subunits at the MT end proved to be below the limit of detection of most phosphate release assays (2, 3), making it difficult to directly measure the cap size and correlate it with MT behavior. However, recent work suggests that end-binding (EB) proteins might associate with the stabilizing GTP cap (4). The cap seen by EB proteins is longer than previous biochemical assays suggested and may consist of both GTP-tubulin and GDP-Pi-tubulin hydrolysis intermediates (5). While this proposed cap region is recognizable by fluorescent EB proteins, and therefore measurable by light microscopy, there is still quite a bit of mystery surrounding the kinetic parameters that define the cap. For example, a second technical issue concerns the difficulty inherent in quantifying the contribution that protofilament neighbors make to both the structure and GTP hydrolysis rate of tubulin dimers in the MT lattice. It is likely that the kinetics driving MT end dynamics are driven, in part, by the mechanical coupling within the lattice (6), which is, most assuredly, a challenging structural problem to investigate.

Given the technical difficulties surrounding the measurement of GTP hydrolysis at the MT end, progress in understanding MT end kinetics at a molecular level has benefited from in silico modeling. Modeling assists researchers in determining what kinetic assumptions most closely match the experimental behavior of MTs. A recent consensus model for MT plus-end behavior is illustrated in Fig. 1 A. In this model, described as the "coupled-random" model for GTP hydrolysis (7), a newly incorporated GTP-tubulin subunit at the MT end will not hydrolyze GTP until another subunit incorporates distally. Once the terminal GTP-tubulin becomes the penultimate GTP-tubulin, it becomes competent to hydrolyze GTP but does so with random frequency. This behavior has the potential to produce a region of preferred EB1 binding, possibly consisting of a combination of GTP and GDP-Pi intermediates (5), at the end of an assembling MT. The model predicts that for an MT end to transition to disassembly, two events must take place: (1) the terminal GTP-tubulin subunit must detach and (2) the penultimate subunit must have already hydrolyzed its β-tubulin–bound GTP. Thus far, this model matches pretty well with the dynamic behavior of MT plus ends in a range of tubulin concentrations (8).

Most models, including this one, are derived from observing the plus end of the MT. They do not explain the behavior of the MT minus end. Previous work has shown that the stability of the plus end of the MT is correlated with the size of the GTP cap (4). Because the size of the cap is positively correlated with the MT growth rate (9) and because the MT growth rate at minus ends is slower, it follows that the minus-end cap should be shorter (Fig. 1 B, left). Thus, the minus end should be more prone to disassembly. In this issue, Strothman et al. demonstrate that, like the plus end, the minus-end cap size is determined by the GTP-tubulin assembly rate (10). Thus, for any given tubulin concentration, the cap marked by EB1 binding is shorter at the minus end than the plus end because GTP-tubulin subunits associate preferentially with the plus end. Yet the minus end has a longer lifetime. It is more stable to disassembly, not less stable as a shorter cap size would predict. This leads the group to hypothesize that another parameter likely controls the difference in stability between the minus end and the plus end.

If the coupled-random model is representative of real MT behavior, it underscores the importance of GTP-tubulin off-rates from the end of the MT as the parameter most likely to control the transition of MT depolymerization (rather than GTP-hydrolysis rates). Unfortunately, at tubulin concentrations required for MT assembly, this off-rate is so small as to be almost nonexistent and is accordingly

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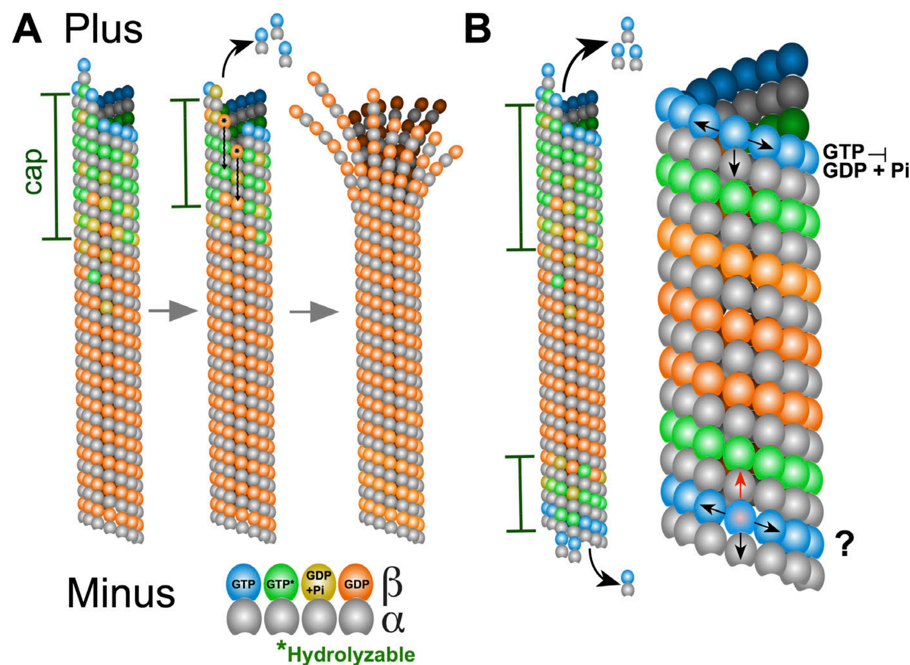

Figure 1. **Microtubule minus ends possess a smaller GTP cap, yet they exhibit greater stability against disassembly than the MT plus end. (A)** The coupled-random model for GTP hydrolysis in which terminal GTP-tubulin dimers (blue) do not become competent to randomly (stochastically) hydrolyze GTP until they are capped by another dimer and become penultimate subunits (green). The GTP cap, as delineated by EB1 binding, is thought to consist of GTP-tubulin and GDP-Pi-tubulin (yellow) subunits. GDP-tubulin subunits are shown as orange. **(B)** The off-rate for GTP-tubulin is greater at the plus end relative to the minus end. Left: For any tubulin concentration, the cap is smaller at the minus end. Right: The hydrolysis state of neighboring subunits has the potential to structurally drive GTP hydrolysis through lateral and longitudinal interactions (small arrows). It is unknown why, or if, the β-tubulin subunit at the minus end would not hydrolyze GTP, as it is nominally "capped" by longitudinal interactions (red arrow).

difficult to quantify (8). Strothman et al. (10) have heroically measured this parameter in highly stable GMPCPP MTs by imaging their slow disassembly over the course of 15 h. In doing so, they have made the important discovery that the loss of GTP-tubulin (as modeled by GMPCPP tubulin) is more than twice as rapid at the plus end versus the minus end of the MT (Fig. 1 B, left). This may explain the aforementioned long-standing mystery in the MT dynamics field: Why are the more slowly assembling MT minus ends less prone to disassembly? Once either end transitions to disassembly, both ends of the MT disassemble at the same rate, suggesting that the GDP-tubulin off-rate is similar for both ends. Thus, Strothman et al. (10) demonstrate that it is the off-rate for GTP-tubulin that is distinct for the two ends.

Like any study that adds a key piece to the puzzle of MT end kinetics, Strothman et al. (10) makes us eager for more puzzle pieces. For example, if the coupled-random model can explain both the plus and the minus end, are the GTP hydrolysis rules the same at each end? One would not think so because the β-tubulin subunit at each end sees a rather different structural and mechanical environment within the lattice (Fig. 1 B, right). Yet, the similar scaling of EB1 comet size with growth rate suggests that the rate of GTP hydrolysis at the minus end is similar to the plus end (10). Perhaps β-tubulin is also sensitive to the lattice state of its intra-dimer α-tubulin, which must also be capped by another dimer before the minus-end β-tubulin can become competent to hydrolyze GTP. Both the α- and β-tubulin subunits of a single dimer, then, would contribute to sensing penultimate dimer status. And what is the contribution of the GDP-Pi-tubulin intermediates to this process? The more deeply we understand this process, the better we will understand MT behavior and regulation in cells because cellular MT regulators have evolved to see and operate on these fundamental structural and biochemical features of MTs.

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