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SPOTLIGHT

Microtubule nucleation without a ring?

Andreas Merdes

The native γ-tubulin ring complex is an asymmetric, imperfect template for microtubule nucleation. Wieczorek et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202009146) and Zimmermann et al. (2020. *Sci. Adv.* https://doi.org/10.1126/sciadv.abe0894) have reconstituted a recombinant complex that allows study of structure–function relationships and regulatory mechanisms.

γ-Tubulin has a conserved role in microtubule nucleation, in particular during spindle formation. γ-Tubulin assembles into multiprotein complexes with γ-tubulin complex proteins (GCPs): "γ-tubulin small complexes" (γ-TuSCs) are formed from two molecules of γ-tubulin and one copy each of GCP2 and GCP3. γ-TuSCs can form helical oligomers by lateral interaction, but these are of heterogeneous size and unstable (1). In fungi such as fission yeast, additional proteins such as Mto1/2 form a structural basis for the anchorage and lateral assembly of γ-TuSCs to enable microtubule nucleation (2). In most eukaryotes, additional proteins coassemble with γ-TuSCs into soluble "γ-tubulin ring complexes" (γ-TuRCs) of a fixed size of 2.2 MD.

Recent studies by three research groups have determined the composition and structure of native γ-TuRCs by cryo-electron microscopy combined with cross-linking and mass spectrometry (3, 4, 5, 6): 14 GCPs are aligned laterally into a short helix, whereby the fourteenth GCP overlaps with the first after a full helical turn. In a lateral view, the γ-TuRC resembles a cone, with the γ-tubulin molecules placed on top of the cone, at the C-terminal region of each GCP. Positions 1–8 in the complex are filled by four γ-TuSCs, whereas positions 9–14 are occupied by GCP4, GCP5, GCP4, GCP6, and a terminal γ-TuSC, respectively. The first four γ-TuSCs adopt a "closed conformation," resembling the geometry of a 13-protofilament microtubule wall, whereas the remaining GCPs are less closely aligned, leading to an asymmetry of the γ-TuRC, with the γ-tubulin molecules in positions 9–14 being spaced too far apart to act as a perfect template for microtubule nucleation ("open conformation"; Fig. 1 A). Additional proteins were assigned to the γ-TuRC, such as MOZART1, MOZART2, and actin, or an actin-related protein. Actin and two copies of MOZART1 are part of a "luminal bridge," together with N-terminal extensions of GCP6 and GCP3, spanning the inside basis of the cone.

Zimmermann et al. (7) and, in this issue, Wieczorek et al. (8), reconstitute the human γ-TuRC, enabling both groups to reveal the γ-TuRC structure using cryo-EM. Wieczorek et al. (8) reconstituted the γ-TuRC from 10 recombinant proteins produced in baculovirus-infected insect cells. The structure of the reconstituted γ-TuRC resembles closely that of native γ-TuRC. In a next step, Wieczorek et al. reconstituted the complex without actin and MOZART1 to eliminate the luminal bridge (γ-TuRC$^{\Delta LB}$), providing the first insights into the role of the luminal bridge. This γ-TuRC$^{\Delta LB}$ still assembles into a partial helix, whereas the remaining part of the complex is disorganized, without any region of overlap. The partial helix is formed by eight GCPs whose identity cannot be resolved, but it is tempting to speculate whether they comprise the first four γ-TuSCs as seen in a regular γ-TuRC. Their helical assembly may reflect their inherent potential to oligomerize (1) but nevertheless requires the presence of the N-terminal extension of GCP6, since deletion of this domain or complete loss of GCP6 prevents the assembly of GCPs into any higher order complexes (9).

Most interestingly, the loss of the luminal bridge does not interfere with microtubule nucleation, as γ-TuRC$^{\Delta LB}$ display nucleation kinetics similar to the full recombinant complex (8). This suggests that eight GCPs provide sufficient surface for the anchorage of tubulin dimers to build a small nucleus for microtubule assembly. In fact, the idea of a minimal nucleus is supported by recent findings demonstrating that the association of as few as four tubulin dimers may be sufficient for γ-TuRC–mediated nucleation (6, 10). Altogether, these data also illustrate how an asymmetric native γ-TuRC may function as a microtubule nucleator: starting with a minimal nucleus assembled from the surface of the "closed" part of the γ-TuRC, the microtubule cylinder can be completed by lateral addition of tubulin dimers. The shape of the γ-TuRC may then be rectified by allosteric effects of α/β-tubulin on the γ-TuRC conformation, or by stochastic switches of positions 9–14 into a "closed" state (Fig. 1 B). Such a mechanism is, of course, inefficient compared with microtubule elongation at growing plus-ends, since a considerable kinetic barrier has to be overcome, but it is

Centre de Biologie Intégrative, Université Paul Sabatier/Centre National de la Recherche Scientifique, Toulouse, France.

Correspondence to Andreas Merdes: andreas.merdes@univ-tlse3.fr.

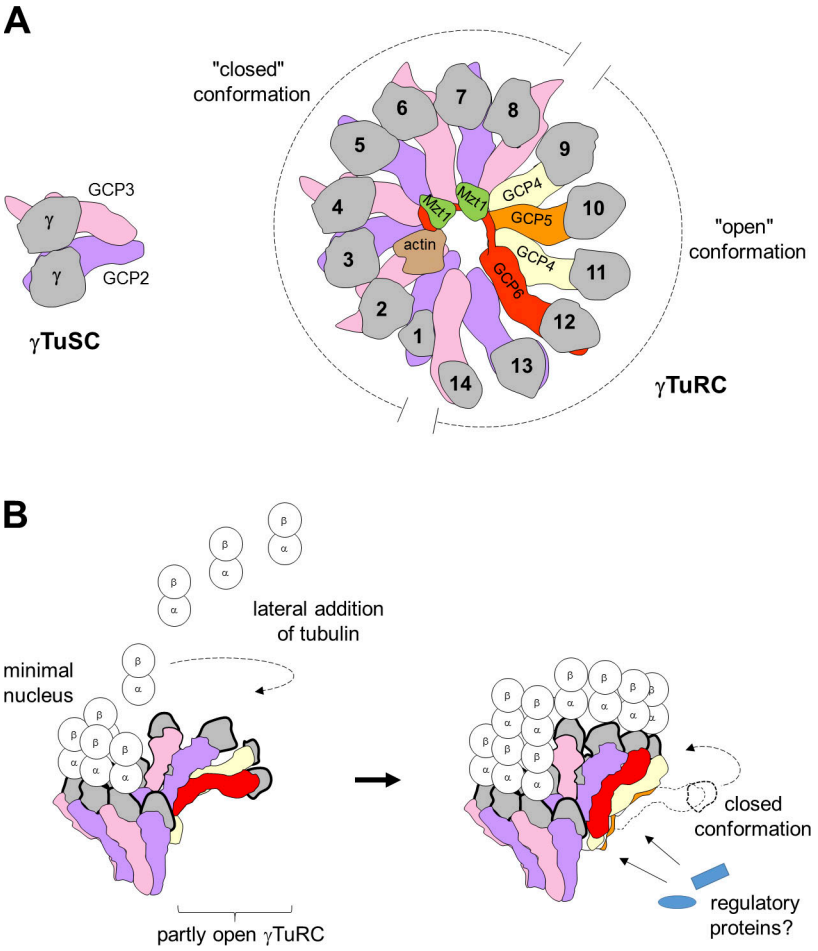

Figure 1. **Microtubule nucleation from an asymmetric γ-TuRC. (A)** γ-TuSC and γ-TuRC viewed from the top. A "closed" conformation at positions 1–8 enables contacts between neighboring γ-tubulin molecules. GCPs in positions 9–14 are spaced farther apart ("open"). Actin, MOZART1 (Mzt1), and an N-terminal extension of GCP6 are part of the luminal bridge. **(B)** Side views of γ-TuRCs in partly open and closed conformations. A minimal nucleus of tubulin dimers (α/β) can form at positions 1–8. Lateral association of additional dimers completes the cylinder, thus matching a perfect template for nucleation. GCPs in positions 9–14 undergo a conformational switch that might occur stochastically or driven by allosteric effects of α/β-tubulin or regulatory proteins.

still more efficient than spontaneous nucleation in solution (6, 10). γ-TuRC–dependent nucleation of microtubules might be boosted if regulatory proteins or post-translational modification of the γ-TuRC were able to alter its conformation directly into a "closed" state. An intact luminal bridge may contribute to this process, since it influences directly the conformation of positions 9–14 in the γ-TuRC (8). It can be speculated whether GCPs 4, 5, and 6 interact with regulatory proteins (5, 6). Moreover, the exact role of nucleation activators such as Cdk5rap2 or other proteins carrying CM1 sequence motifs remains to be determined, since their addition to native γ-TuRC is insufficient to induce a "closed" conformation (3, 5). Reconstituted γ-TuRCs, as developed by Wieczorek et al. (8) and by Zimmermann et al. (7), should pave the way for future analyses, since specific protein interactions can be manipulated by mutagenesis or by the reconstitution of partial complexes.

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