




SPOTLIGHT

Microtubule nucleation: How the NEDD1:MZT1:GCP3 trio captures the γ -TuRC

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In the cell, microtubules are nucleated by the γ -tubulin ring complex (γ -TuRC). In this issue, Muñoz-Hernández, Xu, and colleagues (<https://doi.org/10.1083/jcb.202410206>) combine cryo-EM and AlphaFold modeling to detail how the NEDD1 protein recruits the γ -TuRC to microtubule-organizing centers.

The reorganization of the microtubule cytoskeleton during the cell cycle is arguably one of the most fascinating processes in cell biology. Indeed, at the onset of mitosis, the cell disassembles its radial microtubule array present in the interphase to assemble the mitotic spindle. More generally, the cell constantly adapts its microtubule network in response to cellular cues through a process called microtubule dynamics. It requires the generation of new microtubules, or nucleation, which is a limiting factor in this process. This is where the γ -tubulin ring complex (γ -TuRC) comes in.

The γ -TuRC is a large and asymmetric cone-shaped assembly of about 2.3 MDa, which templates the $\alpha\beta$ -tubulin heterodimer into pseudo-helical microtubules of 13 protofilaments, the main type of microtubules found in the cell. The γ -TuRC is composed of 14 γ -tubulin molecules, each associated with a γ -tubulin complex protein (or GCP; five different GCPs, from GCP2 to GCP6, are found in humans), of Mozart proteins (MZT1 and MZT2), and of accessory/regulatory proteins. The study of the γ -TuRC has fully benefited from recent advances in cryo-EM, the so-called “resolution revolution,” with ~ 4 Å resolution structures determined concurrently by several laboratories a few years ago (for a review, see Ref. [1]). These breakthroughs have, in particular, established the order of the GCPs forming the γ -TuRC core from position 1 to position 14 to be (GCP2–GCP3)₄–GCP4–GCP5–GCP4–GCP6–GCP2–GCP3, whereas it

was previously thought that GCP4, GCP5, and GCP6 capped the γ -TuRC. Another unexpected finding was the identification of an actin molecule located in the γ -TuRC lumen, whose function is still debated. In these structures, the γ -TuRC was in an inactive, open conformation, whereas this complex has to be activated “in the right place at the right time” to nucleate microtubules. To address the underlying related questions, another series of structural results recently emerged.

Among them, the structure of the γ -TuRC in a fully closed shape anchored at the minus end of microtubules has been reported, nicely explaining how an assembly with a stoichiometry of 14 γ -tubulin: GCP nucleates 13-protofilament microtubules (2, 3, 4). The transition of the γ -TuRC into a partially closed conformation induced by the CDK5RAP2 activator has also been investigated (5, 6). In this issue, Muñoz-Hernández, Xu and colleagues (7) address yet another issue, namely how the γ -TuRC is recruited to where microtubule nucleation is to take place.

Neural precursor cell expressed, developmentally downregulated 1 (NEDD1) has been identified as a factor important for γ -TuRC localization in the cell (8, 9). Its N-terminal WD40 repeat domain targets NEDD1 to microtubule-organizing centers. It is connected by a flexible tether to the C-terminal domain, which binds to the γ -TuRC. Muñoz-Hernández, Xu and colleagues implemented cutting-edge integrative

structural biology approaches to characterize the NEDD1: γ -TuRC interaction (7). First, with the prior knowledge that NEDD1 acts as a tetramer and the assumption that it interacts with the GCP3 N-terminal domain and with MZT1, they generated an AlphaFold model of NEDD1: MZT1:GCP3. The model can be subdivided into a “pinwheel,” whose “axle” would be formed by the NEDD1 tetramer while the four MZT1:GCP3 subcomplexes would be the “blades,” and a “fishtail” of two pairs of helices protruding from the pinwheel, contributed by the C-terminal extensions of NEDD1 (Fig. 1A). Then the authors fitted this model into unassigned density of an in vitro reconstituted NEDD1-containing γ -TuRC cryo-EM map, obtained through a new processing of a previously collected dataset. The structural model was validated by a mutational approach targeting residues predicted to be important either for the stability of the NEDD1 tetramer or for the NEDD1:GCP3 interaction. The fishtail was also deleted, leading to a reduced interaction with the γ -TuRC in cells, therefore highlighting its contribution to NEDD1 function.

The interaction with the γ -TuRC involves the two regions of the NEDD1:MZT1:GCP3 complex (Fig. 1, B and C). First, two MZT1:GCP3 blades of the pinwheel (the other two are likely disordered) interact with several GCP subunits to form an intricate interface. Second, whereas one pair of the fishtail helices is mainly disordered, the

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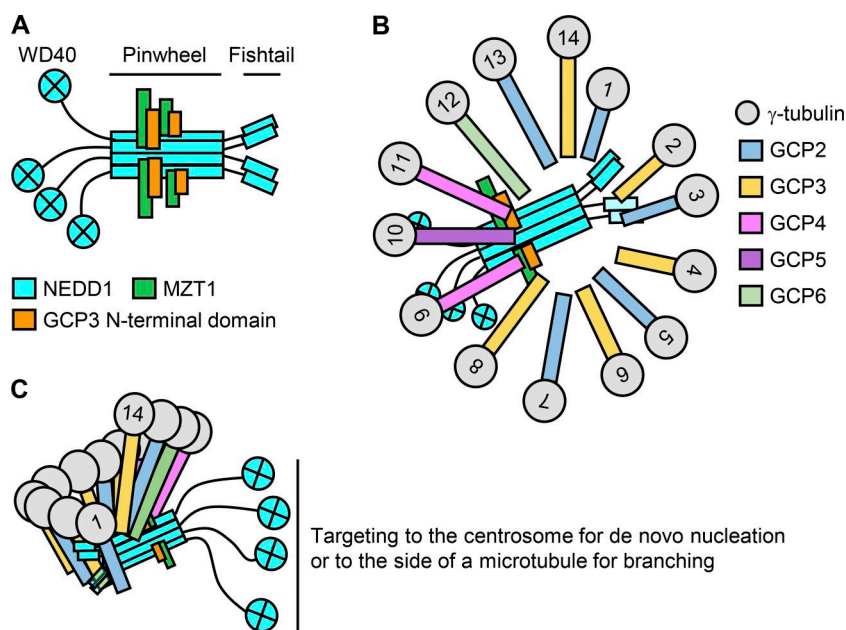


Figure 1. The dual binding mode of NEDD1 to the γ -TuRC. (A) Model of the NEDD1 tetramer bound to MZT1 and to the N-terminal domain of GCP3. (B and C) Top and side views of the γ -TuRC (only γ -tubulin and the GCPs are shown) with bound NEDD1:MZT1:GCP3. The NEDD1 N-terminal WD40 domains are available to anchor the complex to microtubule-organizing centers.

second one interacts with GCPs at positions 1 and 2, with a contribution of a loop from GCP6. Interestingly, despite the fourfold symmetry of the NEDD1:MZT1:GCP3 AlphaFold model, the binding to the (asymmetric) γ -TuRC breaks this symmetry. In addition, the authors established that the NEDD1-bound γ -TuRC is in an open state. The comparison with partially or fully closed conformations, however, predicts that the presence of NEDD1 does not interfere with the open-to-closed γ -TuRC transition. This feature suggests that the nascent microtubule remains attached to its microtubule-organizing center via the γ -TuRC:NEDD1 assembly. The authors also determined the structure of the γ -TuRC in presence of both NEDD1 and CDK5RAP2 and found that the γ -TuRC remained in an open conformation. This somewhat unexpected result raises the possibility that NEDD1 would prevent the CDK5RAP2-induced γ -TuRC conformational changes (5, 6). It was, however, not confirmed in a concurrent study (10) and could be due to a sample undersaturated in CDK5RAP2. This point will deserve further investigation. Finally, an unanticipated benefit of this study has been the attribution of an unassigned

density of the cryo-EM map to a GCP5 region. The authors propose that this motif contributes to the “latch,” a structure that stabilizes the partially closed conformation of the γ -TuRC but should be removed for complete closure (4).

As usual in the case of such complex systems, the work of Muñoz-Hernández, Xu and colleagues (7) and of other laboratories has provided many answers while leaving outstanding questions. First, although recent studies have served to resolve missing features of the γ -TuRC, as illustrated above for a GCP5 region, a better understanding of how this large assembly functions still requires a more complete structural model. A second question concerns the activation of the γ -TuRC. A fully closed state has been observed only at the minus end of a microtubule, leading to the proposal that a nascent microtubule is needed to stabilize this conformation. This model agrees with the power law dependence on $\alpha\beta$ -tubulin concentration observed for γ -TuRC-mediated nucleation, which suggests that templated nucleation requires $\alpha\beta$ -tubulin to assemble to a critical size (11), likely corresponding to the minimal size for complete γ -TuRC closure. However, it remains possible that the

γ -TuRC anchored to a microtubule-organizing center can switch to a (fully) active conformation before microtubule nucleation. Relatedly, how NEDD1 is targeted to the centrosome for initiating new microtubules or to preexisting microtubules for branching is still unclear. In this context, extending the studies to other regulators important for the localization of the γ -TuRC, for its activation, or for promoting elongation from the nucleus (12) is required. The combination of cryo-EM and cryo-electron tomography approaches seems particularly suited to address these questions. We can be confident that a new series of structural results on microtubule nucleation will soon be available.

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