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New Cryo-EM Structures Reveal Molecular Insights into Microtubule Nucleation

Léa Mammri¹ and Paul Conduit^{1,2}

¹Université Paris Cité, CNRS, Institut Jacques Monod, F-75013, Paris, France.

²Corresponding author

Paul T. Conduit

Institut Jacques Monod

CNRS - Université Paris Cité

15 rue Hélène Brion

75013 Paris

France

0033 (1) 57 27 80 95

paul.conduit@ijm.fr

ORCID: 0000-0002-7822-1191

Microtubules within cells often have 13 protofilaments but are nucleated by γ -tubulin ring complexes (γ -TuRCs) that display 14 γ -tubulin molecules. Several new studies now use cryo-EM to provide high-resolution structures of γ -TuRCs post nucleation, revealing that these multi-protein complexes “close” during nucleation to display only 13 γ -tubulin molecules for protofilament assembly.

Microtubules are polarised polymers of α/β -tubulin dimers that are essential for intracellular organisation, cell function and development. They comprise a cylindrical arrangement of protofilaments, each consisting of α/β -tubulin dimers joined head-to-tail (Figure 1A). Protofilaments bind laterally with a slight longitudinal offset that creates a helical pitch and a microtubule “seam” between the first and last protofilaments (Figure 1A). Protofilament number can vary but only 13-protofilament microtubules have their protofilaments running straight along the microtubule shaft, allowing kinesin motors to “walk” in straight lines for cargo transport and microtubule sliding¹. This may be why microtubules typically have 13 protofilaments within cells, where microtubule nucleation is templated by γ -TuRCs. However, γ -TuRCs contain a helical array of 14 γ -tubulin molecules (Figure 1B). So how does a 14-fold template nucleate a 13-fold microtubule?

The γ -tubulin molecules of a γ -TuRC are held in a single-turn helical array by “ γ -tubulin ring complex proteins” (GCPs) (Figure 1B)². Each γ -tubulin-GCP unit can be considered as a “spoke”

that together form a cone-like structure with the γ -tubulin molecules at the open end providing binding sites for incoming α/β -tubulin dimers. All Eukaryotic γ -TuRCs contain multiple 2-spoke subcomplexes, known as γ -tubulin small complexes (γ -TuSCs), made from γ -tubulin, GCP2 and GCP3. Most Eukaryotes also contain a single 4-spoke subcomplex made from γ -tubulin, GCP4, GCP5 and GCP6 (hereafter the GCP4/5/4/6 complex). In these species, the GCP4/5/4/6 complex is essential for γ -TuRC assembly, as it seeds the oligomerisation of γ -TuSCs into the cone-like structure^{3,4}.

In recent years, ground-breaking high-resolution cryo-EM structures of purified native or recombinant γ -TuRCs have transformed our understanding of γ -TuRC assembly and regulation^{3,5-13}. In *S. cerevisiae*, which lack the GCP4/5/4/6 complex, structures of γ -TuSCs stimulated to oligomerise into helical assemblies by the binding of the spindle pole body (SPB) protein Spc110 revealed an overlap at spokes 1 and 14 such that only 13 γ -tubulin molecules are exposed for protofilament assembly⁶. In contrast, cryo-EM structures of native or recombinant human and *Xenopus* γ -TuRCs revealed that these γ -TuRCs assemble in a semi-open conformation, with no overlap between the γ -tubulins at spokes 1 and 14^{3,8-13}. This is due to the presence of the GCP4/5/4/6 complex at spokes 9 to 12, which causes this side of the γ -TuRC to splay outwards and down away from the geometry of a 13-protofilament microtubule, neatly linking cytosolic γ -TuRC assembly with a low activity conformation, presumably to avoid ectopic nucleation throughout the cytosol. It has therefore been proposed that these γ -TuRCs must make large-scale conformational changes (referred to as γ -TuRC closure) to become “active” once recruited to microtubule organising centres (MTOCs), such as centrosomes (the equivalent of the SPB in yeast)². How γ -TuRCs are “activated” has been major outstanding question in the field.

Two studies in this issue of NSMB have used cryo-EM to obtain high-resolution structures of *S. cerevisiae* and human γ -TuRCs post microtubule nucleation. Dendooven et al. purified spindles from *S. cerevisiae* cells and analysed the spindle-bound native γ -TuRCs (no small feat), while Aher et al. optimised conditions to analyse purified recombinant human γ -TuRCs that had nucleated microtubules *in vitro*. Strikingly, both studies showed that the γ -TuRCs invariably nucleated 13-protofilament microtubules and had fully closed to perfectly match the geometry of the microtubule (Figure 1C). Closure involved bending and twisting of the spokes and unexpected interactions between spoke 14 and the α/β -tubulin molecules above spoke 2, marking the position of the microtubule seam. These observations closely match those of another recombinant human γ -TuRC structure¹⁴, but not those of a native *Xenopus* γ -TuRC structure that revealed local mismatches between some γ -tubulins and protofilaments¹⁵. Whether these structural differences result from the γ -TuRC source or from sample handling will need to be resolved. Another interesting observation from *S. cerevisiae* was the presence of a luminal density that may functionally mimic the “luminal bridge”

previously identified within vertebrate γ -TuRCs, which supports γ -TuRC assembly. Interestingly, this vertebrate luminal bridge appears to be rearranged during microtubule nucleation, possibly by being squeezed during γ -TuRC closure.

Collectively, these studies show that γ -TuRCs can fully close during microtubule nucleation. This is not so surprising in *S. cerevisiae*, where assembled γ -TuRCs at the SPB are not so far from the closed state – they even dynamically sample this closed state prior to nucleation⁷. Other factors may, however, further promote the activity of these γ -TuRCs at the SPB. With this in mind, perhaps one of the most important findings from Dendooven et al. is the observation of a coiled-coil that binds across the γ -TuRC and the first row of α/β -tubulin dimers, potentially augmenting nucleation. The identity of the coiled-coil could not be determined, but cross-linking mass-spectrometry data were consistent with it belonging to Stu2, a protein of the TOG domain microtubule polymerase family that bind α/β -tubulin and are known to promote microtubule nucleation from γ -TuRCs¹⁶.

In contrast to *S. cerevisiae* γ -TuRCs, vertebrate γ -TuRCs have to make a large conformational change during microtubule nucleation to end up in the closed state. This suggests that the lateral interactions between protofilaments can force a flexible complex to make large-scale structural rearrangements, something that was previously suggested¹⁷. Intriguingly, the γ -TuRCs concomitantly impart a 13-fold symmetry on the microtubules, highlighting an interplay between the two structures. It must be pointed out, however, that a mutant form of α -tubulin was required to drastically increase the nucleation efficiency of the purified human γ -TuRCs, that were otherwise only $\sim 0.1\%$ efficient. Thus, in cells, additional factors must be required to promote microtubule nucleation from γ -TuRCs at MTOCs, which makes perfect sense given that cytosolic γ -TuRCs should remain at low activity to avoid promiscuous cytosolic microtubule nucleation. Good candidates that either change γ -TuRC structure or promote α/β -tubulin addition have already been identified. Many studies have shown that binding of a well conserved Centrosomin 1 motif (CM1) domain strongly promotes microtubule nucleation from cytosolic or purified γ -TuRCs and a recent pre-print reported that this binding imparts partial γ -TuRC closure¹⁸. Importantly, CM1 binding is normally regulated so that it occurs only at MTOCs. In terms of promoting α/β -tubulin addition onto γ -TuRCs (or preventing their loss), concentrating TOG domain proteins and γ -TuRCs together at MTOCs would be a convenient way to increase γ -TuRC activity. Other factors, such as TPX2 family members that stabilise newly forming microtubule seeds, could also be involved.

From these new studies, we have learnt that γ -TuRCs *can* close during microtubule nucleation, but questions still remain. In *S. cerevisiae*, it will be important to identify the coiled-coil protein and then test whether it supports α/β -tubulin addition onto the γ -TuRC to promote microtubule nucleation. For vertebrate γ -TuRCs, we need to better understand the regulation

of γ -TuRC activity at MTOCs in cells. Most likely, a combination of factors collectively and synergistically drive microtubule nucleation from γ -TuRCs, by either directly closing γ -TuRCs or by promoting tubulin addition. The presence and concentration of these factors may vary at different MTOCs, making certain MTOCs more or less proficient at nucleating microtubules. Whether this is an important control point during development will have to be determined.

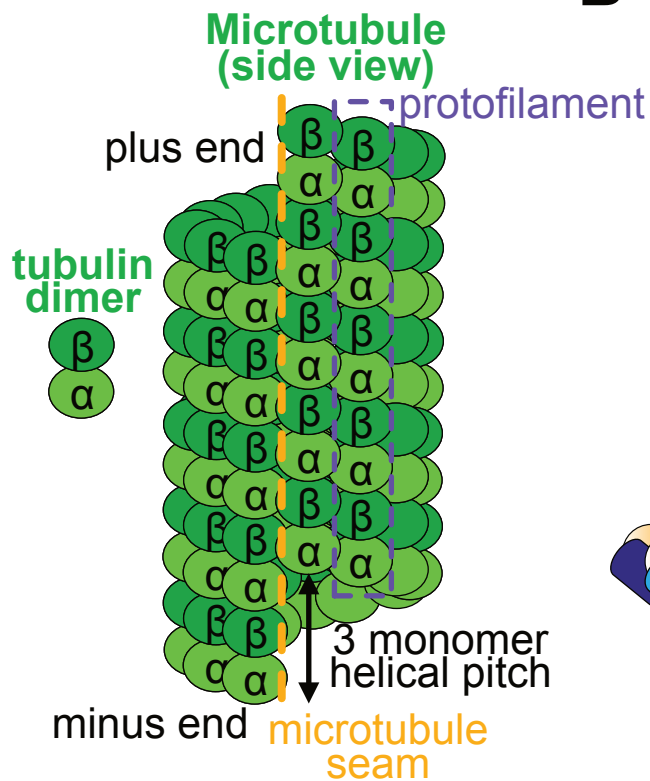
Perhaps the most important general finding from the new structures is that 14-spoke γ -TuRCs invariably nucleate 13-protofilament microtubules, even when the complex is in a very open conformation prior to nucleation. Thus, how γ -TuRCs become active at MTOCs becomes a separate question from how 13-protofilament microtubules are made – these microtubules will be made without any prior structural changes to γ -TuRCs. Nevertheless, many cellular microtubules do not have 13 protofilaments¹. So, are these microtubules nucleated independently of γ -TuRCs or by γ -TuRCs with different fold symmetries? And why do some cells benefit from these atypical microtubules? Clearly, we still have a lot to learn.

Figure Legend

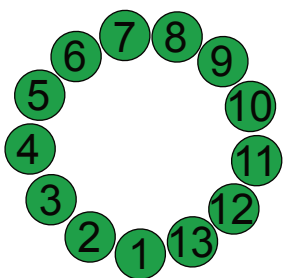
(A) Microtubules are built from α/β -tubulin dimers that join head-to-tail to form protofilaments. The microtubule depicted has 13 protofilaments, which allows the protofilaments to run straight along the longitudinal axis. There is a 3-monomer helical pitch that creates a “seam” where α and β -tubulin molecules associate laterally. (B) *S. cerevisiae* γ -TuRCs form when Spc110 binds and stimulates the oligomerisation of γ -TuSCs, made from γ -tubulin, GCP2 and GCP3 (Spc97 and Spc98 in *S. cerevisiae*). The highly conserved CM1 helix within the CM1 domain of Spc110 binds at the inter- γ -TuSC interface to promote the oligomerisation. These γ -TuRCs are built close to the optimal “closed” conformation for templating a 13 protofilament microtubule and they are known to dynamically sample this conformation. In contrast, vertebrate γ -TuRCs are built using a GCP4/5/4/6 complex, on which γ -TuSCs are added laterally. The side of the γ -TuRC containing only γ -TuSCs is close to the optimal confirmation but the side containing the GCP4/5/4/6 complex spays outwards away from the geometry of a 13 protofilament microtubule. (C) The new papers published in this edition of NSMB show that both *S. cerevisiae* and human γ -TuRCs are forced into the closed confirmation during microtubule nucleation under native conditions and *in vitro*, respectively. They also show that the γ -TuRCs dictate that the microtubules always have 13 protofilaments. In addition, Dendooven et al. identify a luminal density apparently consisting of 3 Spc98 N-terminal extension domains that may be functionally similar to the luminal bridge found in vertebrate γ -TuRCs. They also reveal a coiled coil of unknown identity that appears to help mediate the addition of the first row of α/β -tubulin dimers.

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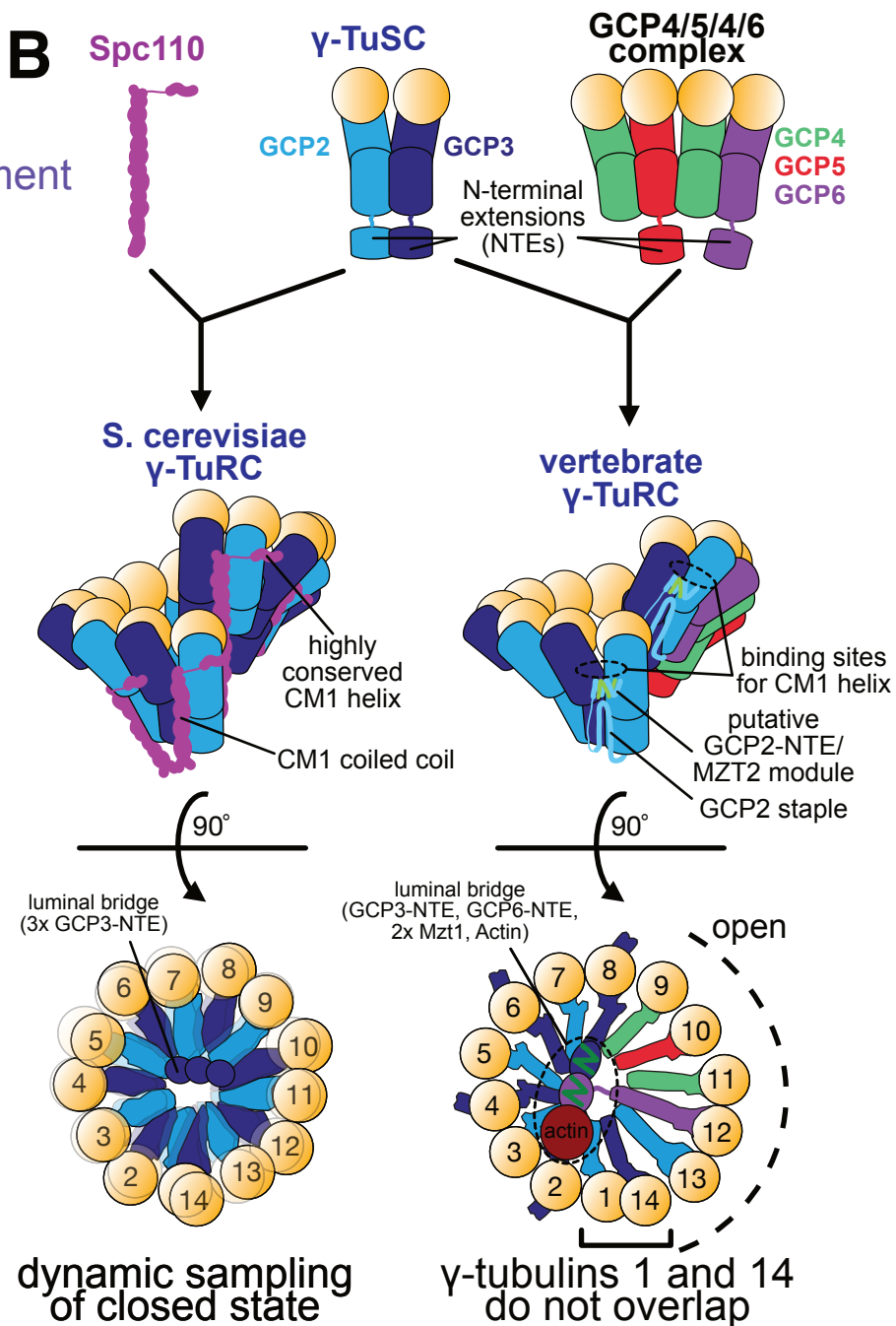
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A

Microtubule (cross section)



13 protofilament microtubule

B**C**

Microtubule forced by γ -TuRC to have 13 protofilaments

interplay \updownarrow

γ -TuRCs forced into closed state when nucleating a microtubule

