

CryoEM Reveals Multi-step Activation of γ Tubulin and Assemblies to Optimize Microtubule Nucleation

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The microtubule cytoskeleton is essential in mediating a number of critical cellular processes, affecting cell shape, transport, organelle organization, and chromosomal segregation during mitosis [1]. Microtubule network dynamics are controlled by many factors including the efficiency and localization of the nucleation machinery. Microtubule nucleation is dependent on the universally conserved γ -tubulin small complex (γ TuSC), a 300 kDa heterotetramer composed of two copies of γ -tubulin and one each of accessory proteins GCP2 and GCP3 [2]. In yeast, nucleation is mediated by a heptameric ring of γ TuSC upon, which presents 13 γ -tubulins to form a template for microtubule nucleation [3]. Spc110p (a kendrin/pericentrin homologue) mediates the recruitment, assembly and activation of γ TuSC to the yeast spindle pole body [4].

Previous moderate resolution cryoEM structural studies (8Å) had shown that γ TuSCs complexed with the N-terminal domain of Spc110p self-assemble into filaments having 6.5 γ TuSCs/turn thereby presenting 13 γ -tubulins to template 13-protofilament MTs [3, 5, 6]. Although close to MT symmetry, the γ -tubulins within each γ TuSC were too far apart to correctly match the MT lattice. The relevant in vivo conformation was determined by cryo-tomography and sub-volume averaging, clearly showing an MT- matching geometry at the yeast spindle pole body, suggesting that γ TuSC closure might be an important regulatory step [3]. To validate this hypothesis, γ -tubulin was engineered with disulfides to stabilize a closed MT-like conformation, resulting in significantly enhanced MT nucleation [3].

Using single particle cryoEM, we present here maps of monomeric and dimeric γ TuSCs at resolutions of 3.6-4.6Å. In addition, we have obtained a \sim 3Å reconstruction of a filamentous closed Spc110p: γ TuSC complex using helical methods followed by symmetry expansion and asymmetric reconstruction. These have allowed de-novo model building of unknown regions and reinterpretation of significant regions of γ TuSC structure.

By comparison with a crystal structure of isolated γ -tubulin, it is clear that γ -tubulin is activated upon assembly into the γ TuSC, in a manner analogous to the bent to straight transition in $\alpha\beta$ -tubulin upon assembly into the microtubule lattice. Our structures allow us to map phosphorylation sites, revealing several at key interfaces, highly suggestive of their role in regulating spindle pole body attachment and assembly into rings. Our high-resolution structures also reveal conformational changes that occur within γ -tubulin when it assembles into a γ TuSC and some key origins for the remarkable 100-fold species preference of yeast γ TuSCs for yeast tubulin [7].

References:

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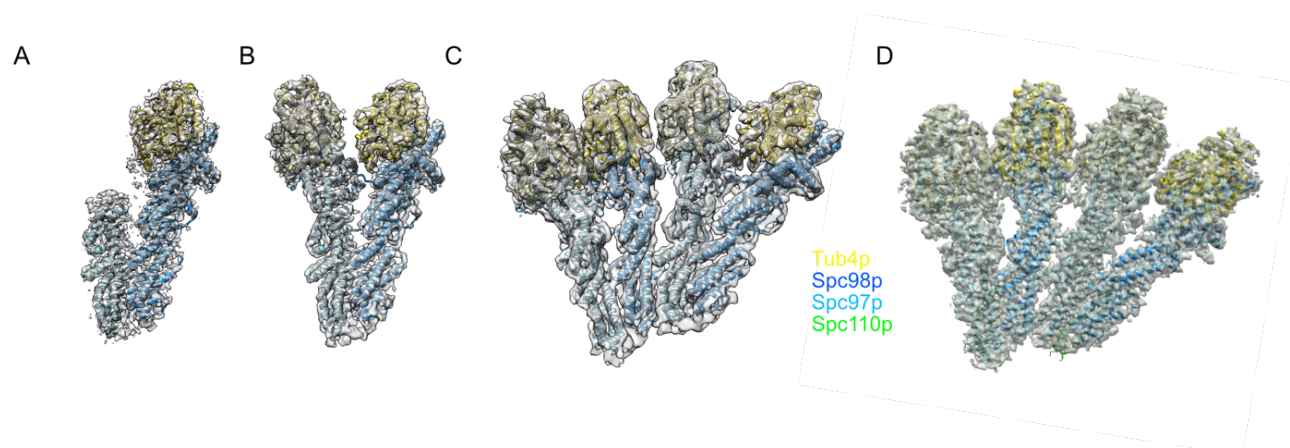


Figure 1. A) $\sim 3.6\text{\AA}$ reconstruction obtained by masking the mobile C-terminus of Spc97p showing the N-terminus of Spc97p (sky blue), Spc98p (dodger blue) and γ -tubulin bound to Spc98p (gold). B) $\sim 3.9\text{\AA}$ reconstruction of gamma-TuSC colored as in panel A, with gamma-tubulin bound to Spc97p colored in khaki. C) $\sim 4.6\text{\AA}$ reconstruction of a dimer of γ TuSC colored as in panel B. D) Segmented region from an asymmetric reconstruction of a $\sim 3\text{\AA}$ reconstruction of an Spc110p: γ TuSC filament. colored as in panel B with Spc110p in green.

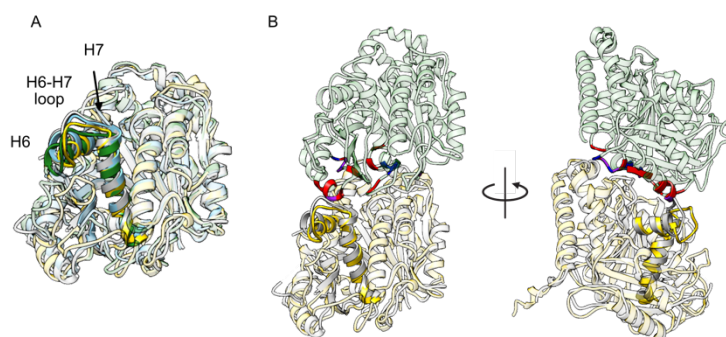


Figure 2. A) An overlay of yeast straight β -tubulin (forest green, PDB ID 5W3F) γ -tubulin bound to Spc98p (gold, this work), yeast bent β -tubulin (sky blue, PDB ID 4FFB) and the human free γ -tubulin (gray, PDB ID 3CB2) structures shows that the γ -tubulin bound to Spc98p H6-H7 loop adopts a conformation similar to that observed in straight microtubules. B) Clashes between γ -tubulin bound to Spc98p (blue) or human γ -tubulin (red, PDB ID 3CB2) or both (purple) with a straight α -tubulin are illustrated on a forest green α -tubulin. The yeast microtubule structure (PDB ID 5W3F) was used to model the $\beta\alpha$ -tubulin interface, and other proteins were aligned by the N-terminal domains. Clashes with a greater than 1.2\AA overlap are shown.