The Atomic Structure of the Microtubule Nucleating γ-tubulin Small Complex and its Implications for Regulation

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Microtubule nucleation by $\gamma$-tubulin Complexes

$\gamma$TuSC/$\gamma$TuRC structure, assembly, activation
Attachment factor Spc110 stabilizes $\gamma$TuSC assembly

$\gamma$TuSCs

Individual $\gamma$TuSCs

$\gamma$TuRCs/filaments

$\text{Spc110p}$

$\text{spindle pole body}$

$\text{6.5 } \gamma\text{TuSCs/turn} = 13 \gamma\text{-tubulins} = \text{in vivo MT protofilament } \#$

J. Kollman
Open - closed transition enhances $\gamma$-TuRC MT nucleation

- closed state better MT nucleator
- suggests closure as a regulatory mechanism
γTuSC pseudo-atomic model built using 6.5Å oxidized map

GCP4 crystal structure

C-terminal domain directly binds γ-tubulin

Merdes, Mourney Guillet, et al. 2011

less than 20% similarity
γTuSC pseudo-atomic model built using 6.5Å oxidized map

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less than 20% similarity

Missing 234 aa from gcp2, 275 aa from gcp3
Built into a ~6.5 Å map
The Image Data

Polara Data
~80 e-/A²
Dose filtered &
aligned with MotionCorr2
Thon rings 5Å
or better
γTuSC monomer/dimer by single particle cryoEM (3.8Å)

- first true atomic description of γTuSC, numerous inserts, etc
- differences in the interfaces between the γTuSCs vs internal interface
- conformational changes in γ-tubulin upon assembly into γTuSC
- interpretation of phosphorylation sites, mutations
Workflow

Drift correct & pick
  ↓
Determine CTF
  ↓
extract particles
  ↓
2D Classification
  ↓
3D Classification
  ↓
Extract classes
  ↓
Align into one class
  ↓
3D Classification
Improving the Map

Increase Dataset size (+0.5M particles)

Various Programs (Relion, cryosparc)
  Full workflow, as well as feeding them classification results from Frealign

Focused Classification in Frealign

Various Masks
  Half-Tusc, Base only, Base plus one tubulin arm
Improving the Map - Frealign, Shaped Masks and Weighting
Assembly driven global conformation changes

both assembly & allosteric conformational changes required
Assembly driven global conformation changes

both assembly & allosteric conformational changes required
Assembly driven global conformation changes

Monomer
Closed
Open

GCP3 N-terminus

Twist of the conserved GCP domains is the major re-arrangement
What is the conformation of $\gamma$-tubulin on the $\gamma$-TuSC

98-bound $\gamma$

Human $\gamma$ xtal (3CB2)

straight $\beta$

yeast MT from Nogales & Rice
What is the conformation of \( \gamma \)-tubulin on the \( \gamma \)-TuSC

straight \( \alpha \)

98-bound

Human \( \gamma \) xtal (3CB2)

straight \( \beta \)
What is the conformation of $\gamma$-tubulin on the $\gamma$-TuSC

clashes with human and yeast $\gamma$-tubulin

straight $\alpha$

98-bound

Human $\gamma$ xtal (3CB2)

straight $\beta$

clashes w/human $\gamma$-tubulin
What is the conformation of $\gamma$-tubulin on the $\gamma$-TuSC?
GCP2 phospho sites suggests functional roles

GCP2
GCP3
Spc110
γ-tubulin
new phos
GCP2 phospho sites suggests functional roles

Spc110 binding

GCP2
GCP3
Spc110
γ-tubulin
new phos

γTuSC recruitment

Spc110 binding
GCP2 phospho sites suggests functional roles
Models for $\gamma$-complex mediated attachment and nucleation

$\gamma$-tubulin “poised” for $\alpha$-tubulin binding

attachment $\Rightarrow$ assembly

partially active for nucleation

$\square$ closure & activation

fully active for nucleation

MT nucleation
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