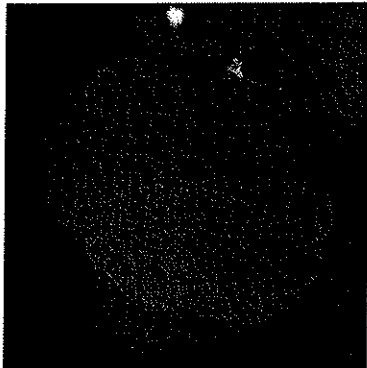


Looking under the hood of a
microtubule polymerase

Luke Rice
Department of Biophysics
UT Southwestern

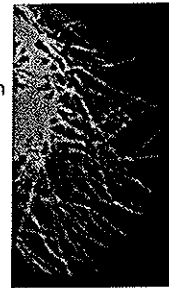
Dynamic microtubules organize the cytoplasm and
segregate chromosomes



C. elegans embryo *Hannak et al.,*
Elapsed time: 28min *JCB 157, 2002*

GFP-Microtubules
Drosophila S2 cell
Elapsed time: 5 min

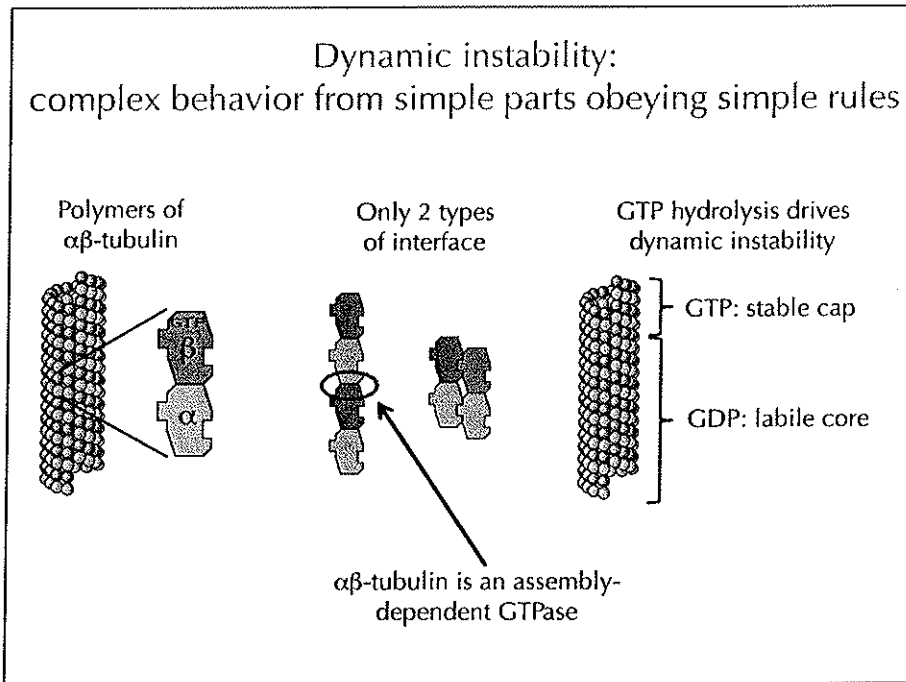
Rogers et al.,
JCB 158, 2002



Collections of molecules doing
'mesoscale' things

Ultimate driving force: biochemical
interactions between proteins

Molecular mechanisms, esp. of regulatory factors, not well understood
Understanding collective behavior is challenging for bottom-up approaches



Not so simple parts? Multiple conformations of $\alpha\beta$ -tubulin

'Straight'

'In between?'

'Curved'

Challenges:

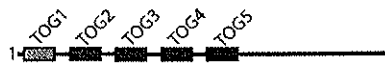
1. Little structure, biochemistry: polymerization gets in the way
2. No recombinant $\alpha\beta$ -tubulin (chaperones?): no mutagenesis

Micrographs: Mandelkow, Karsenti, Chretien, Milligan
 Straight structure: Nogales, Downing; Curved structure: Knossow
 'In between' EM reconstruction: Wang and Nogales

Today's focus: microtubule "polymerases" that use multiple TOG domains to promote MT growth

XMAP215: single polypeptide

Stu2p: homodimer

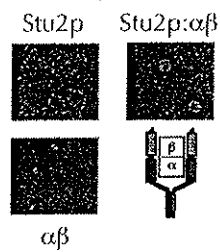


'TOGs on a string'

TOG domain = $\alpha\beta$ -tubulin binding module
Minimal functional unit seems to be 2 TOG domains

TOG domains are used to discriminate between different states of $\alpha\beta$ -tubulin

1:1 complex with $\alpha\beta$



Bind MT plus end



Question:

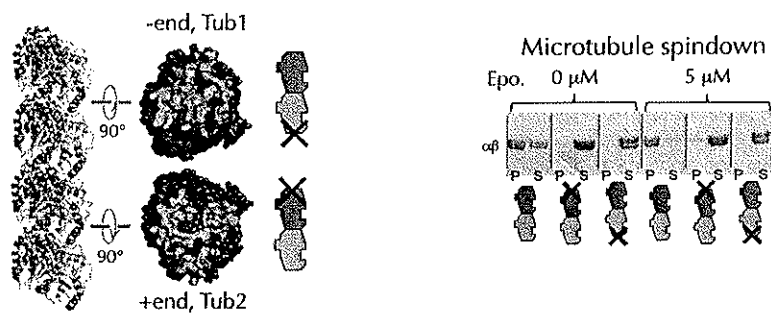
How do TOG domains do this?

Missing link:

How do TOG domains interact with $\alpha\beta$ -tubulin? A structure might help ...

Harrison & Howard & Hyman labs: Al-Bassam et al., JCB 2006; Brouhard et al., Cell 2008

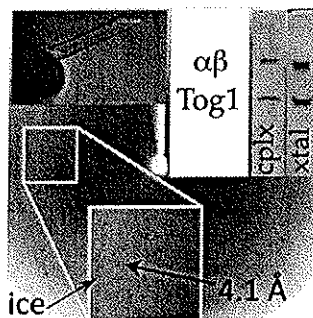
Making new, enabling reagents:
Polymerization-blocked $\alpha\beta$ -tubulin mutants



Mutants pass tests for 'otherwise normal':
purify as heterodimers, migrate normally on gel filtration, bind GTP

To make these mutants required overcoming a long-standing technical obstacle

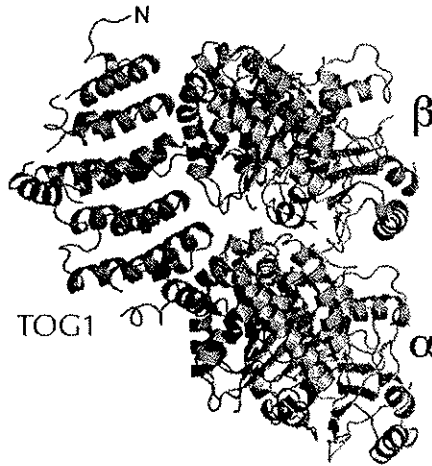
Crystallization of a TOG1: $\alpha\beta$ -tubulin complex



$\sim 3 \text{ \AA}$ diffraction (anisotropic), crystal growth variable
Phased by molecular replacement, refined with care
Refinement statistics: ($R_{\text{free}} = 26\%$, $R = 21\%$)

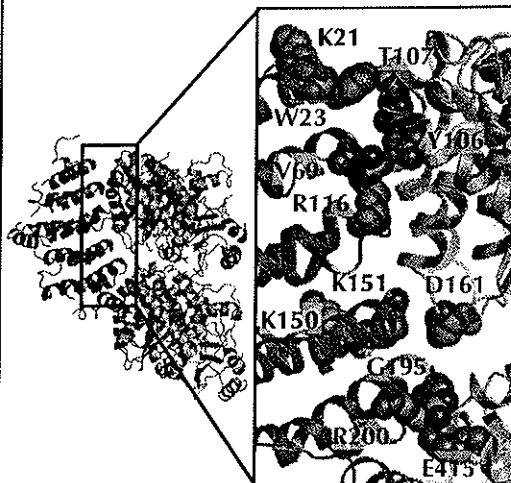
Pelin Ayaz

TOG1 makes substantial contacts with α - and β -tubulin



'Exclusive' binding is unexpected:
TOG1 leaves no room for TOG2 to bind equivalently to the same $\alpha\beta$ -tubulin

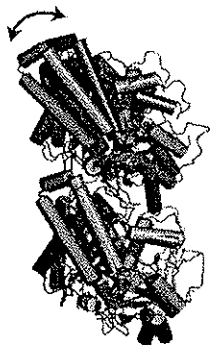
TOG1: $\alpha\beta$ -tubulin interactions require simultaneous contact with α - and β -tubulin



Take-home messages:

1. The entire length of the TOG domain contacts $\alpha\beta$ -tubulin
2. Simultaneous contact with α - and β -tubulin is required for binding
3. TOG: $\alpha\beta$ -tubulin interactions are exclusive

In the TOG1 complex, GTP-bound $\alpha\beta$ -tubulin is 'curved'



Yeast $\alpha\beta$ on straight animal $\alpha\beta$:
~12° difference in curvature

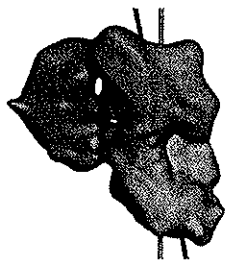


Yeast $\alpha\beta$ on curved animal $\alpha\beta$:
~1° difference in curvature

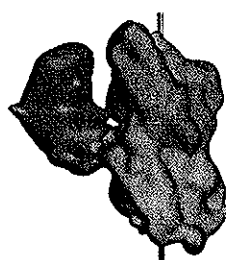
Straight $\alpha\beta$ -tubulin structure: Nogales; Curved $\alpha\beta$ -tubulin structure: Knossow

TOG1 contacts curved $\alpha\beta$ -tubulin across a hinge

TOG1 complex

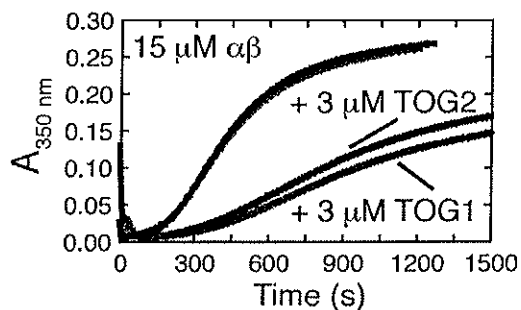


Fake model with straight $\alpha\beta$



Can TOG1 'read' the $\alpha\beta$ -tubulin conformation? Easy to test:
if TOG1 prefers curved $\alpha\beta$ -tubulin, it should antagonize microtubule assembly

The isolated TOG1 or TOG2 domains inhibit microtubule assembly

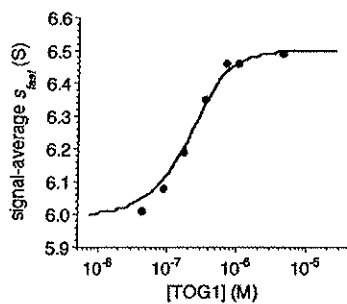


TOG: $\alpha\beta$ -tubulin interactions are indeed conformation selective!
TOG domains prefer non-straight conformations

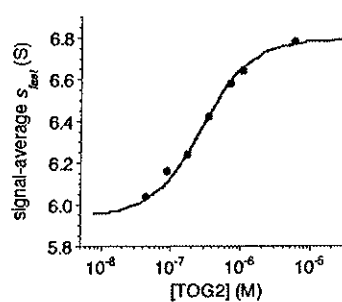
Different states, different conformations -> recipe for discrimination

TOG1 and TOG2 each bind $\alpha\beta$ -tubulin with comparable affinity

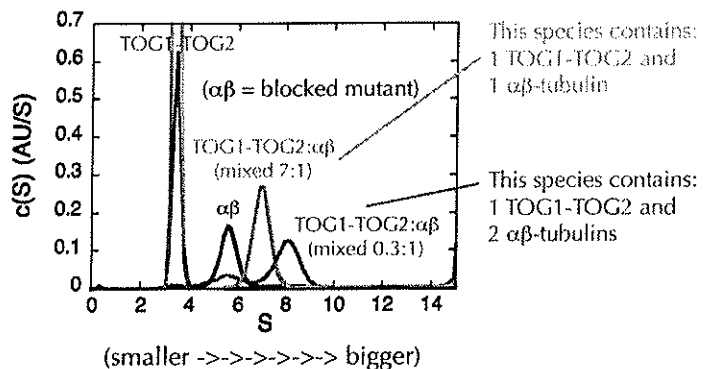
TOG1: $\alpha\beta$ -tubulin $K_D = 50 \text{ nM}$



TOG2: $\alpha\beta$ -tubulin $K_D = 150 \text{ nM}$



If TOG1 and TOG2 can each bind one $\alpha\beta$ -tubulin, can TOG1-TOG2 bind two $\alpha\beta$ -tubulins?

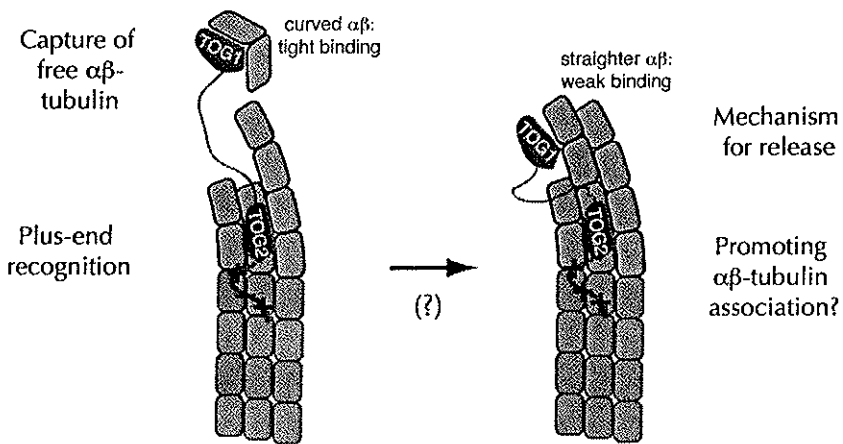


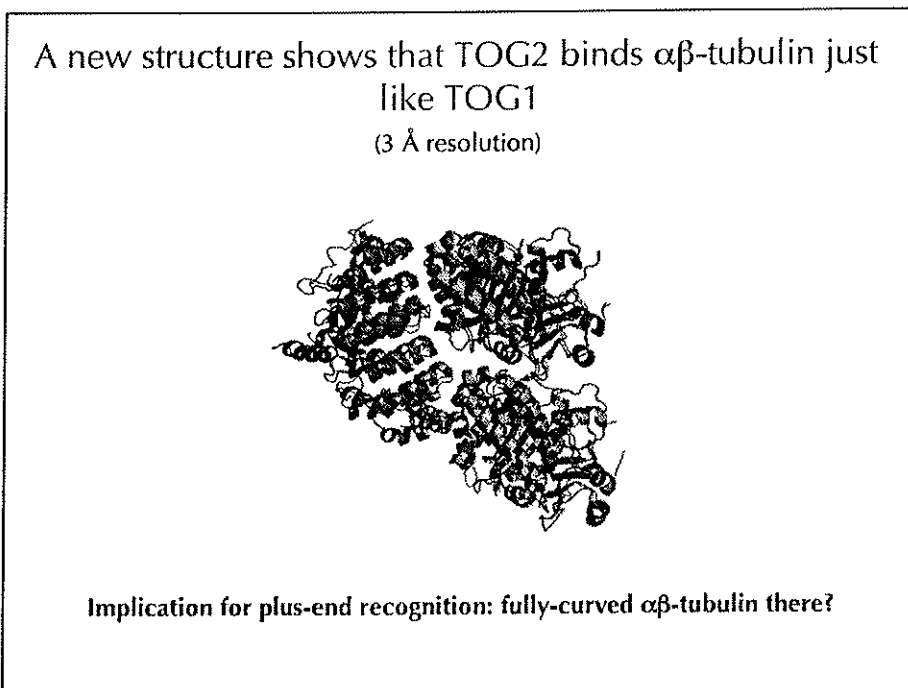
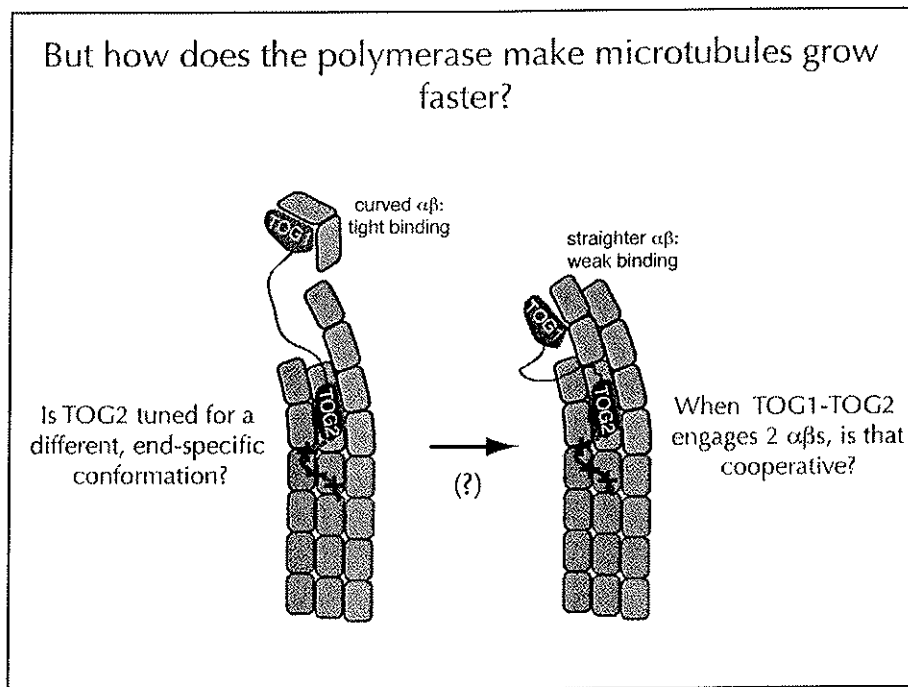
Surprise!

Each TOG domain in TOG1-TOG2 can engage its own $\alpha\beta$ -tubulin.

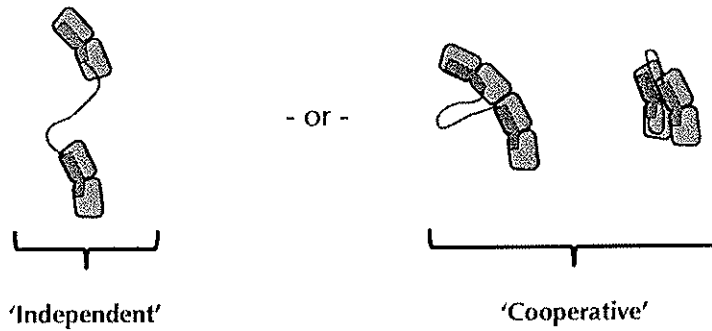
Analytical ultracentrifugation in collaboration with Chad Brautigam, UTSW

Insight: Conformation-selective recognition is how TOGs discriminate between different states of $\alpha\beta$ -tubulin



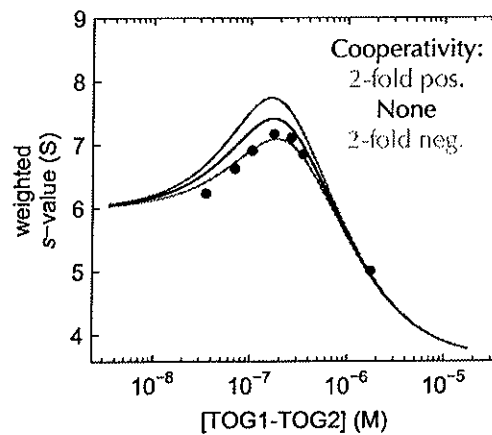


When TOG1-TOG2 binds two $\alpha\beta$ -tubulins, is it stabilizing an $\alpha\beta:\alpha\beta$ interface?



Cooperativity in the TOG1-TOG2:($\alpha\beta$)₂ complex?

Experiment: add different amounts of TOG1-TOG2 to a constant amount of $\alpha\beta$ -tubulin, then analyze the size distribution of oligomers.

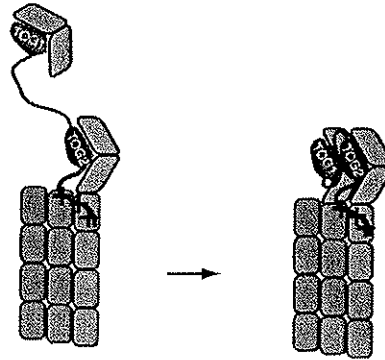


No evidence for positive cooperativity?! The two TOG domains act independently

Summary

- 1) TOG domains bind preferentially to a curved conformation of $\alpha\beta$ -tubulin that does not 'fit' in the microtubule lattice
- 2) Conformation-selective interactions explain how TOG domains discriminate different states of $\alpha\beta$ -tubulin and recognize the microtubule end
- 3) TOG domains do not stabilize $\alpha\beta$ -tubulin: $\alpha\beta$ -tubulin interactions

Simple 'tethering' mechanism
 Delivery of $\alpha\beta$ -tubulin to the MT end is accelerated because the TOG1-bound $\alpha\beta$ -tubulin is confined nearby



Acknowledgements

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UTSW Structure core

Diana Tomchick, Chad Brautigam

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Dominika Borek, Youxing Jiang

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