Plus-end Tracking Proteins

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TIPS: Plus-end Tracking Proteins

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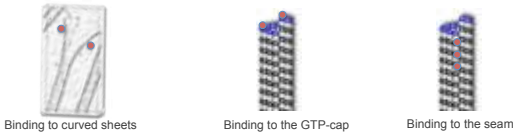
Context

Plus-ends tracking proteins (+TIPs) are a special class of MAPs (Microtubule Associated Proteins) that localize specifically to the growing plus-end of microtubules, where they regulate their dynamics and their interaction with various cellular targets (cell cortex, kinetochores, …). Yet, the precise molecular structure recognized by +TIPs remains unknown. Current hypotheses include the curved tubulin sheets at microtubule ends, the nucleotide state of tubulin (GTP versus GDP), or the « seam » of heterologous lateral interactions between α and β-subunits inside the microtubule lattice.



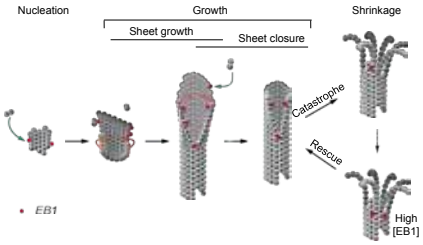
Models of microtubule end tracking mechanisms by +TIPs.

Objectives

Our aim is to understand how +TIPs remain attached to the growing ends of microtubules. For this purpose, we use video-light and cryo-electron microscope approaches to understand how they regulate microtubule structure and dynamics.

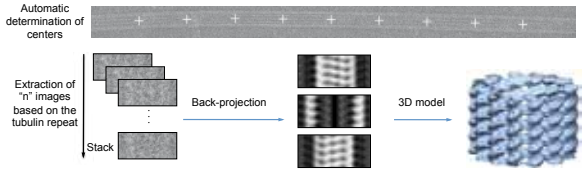
Results

➢ *EB1 regulates microtubule dynamics and tubulin sheet closure in vitro*



We have analyzed the interaction of mouse EB1 with microtubules *assembled in vitro* from pure tubulin. Our results indicate that EB1 stimulates microtubule dynamicity (increase in growth rate, catastrophes and rescues), while favoring the formation of curved sheets and their closure into tubes. We also found that EB1 favors the 13 protofilament structure of microtubules commonly found in cells. These results allowed us to propose a model where EB1 stimulates microtubule assembly while eliminating stressed lattice configurations (Vitre *et al.*, 2008).

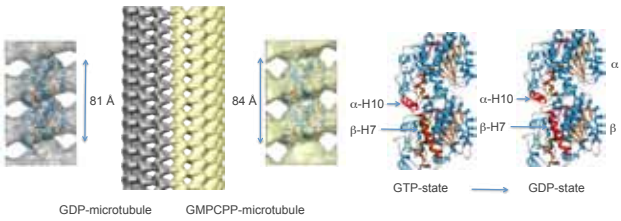
➢ *TubuleJ: a plugin to the ImageJ software to reconstruct MTs in 3D*



Microtubules with low protofilament skew angles cannot be reconstructed in 3D using classical helical algorithms. To overcome this limitation, we have designed TubuleJ, a plugin to the ImageJ software (*http://rsbweb.nih.gov/ij/*). TubuleJ is composed of two main modules. The first one can be applied to any type of fiber and allows automatic curvature determination, fiber straightening, and filtration of layer lines in Fourier space. The second module is specific to microtubules and allows 3D reconstructions of MTs composed of any protofilament and lateral helix-start number (Blestel *et al.*, 2009, APP patent under way).

http://www.umr6026.univ-rennes1.fr/english/home/research/tips/Software/

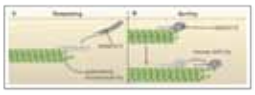
➢ *GTP-hydrolysis destabilizes microtubule longitudinal interactions: a potential recognition site for +TIPs*

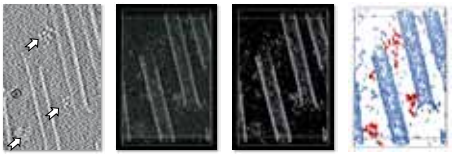


The conformational changes that the tubulin molecule undergoes during the hydrolysis of the GTP bound to its β-subunit during microtubule assembly remain unknown. To address this issue, we have compared the structure of microtubules assembled in the presence of GTP ("GDP-microtubules") and of its slowly hydrolyzable analogue GMPCPP. We found that, at a resolution of ~15 Å, the two structures are very similar, apart from a difference in size and a lack of density in GDP-microtubules at the inter-subunits longitudinal interactions. These results suggest that GTP-hydrolysis destabilizes the MT lattice by weakening the longitudinal bonds, instead of the lateral ones. In addition, this site is a potential candidate to be specifically recognized by +TIPs (work in progress).

➢ *XMAP215 binds along the microtubule lattice and at MT ends*

XMAP215 is a large and flexible protein involved in the formation of the mitotic spindle. Several models exist to explain its stimulating effect on microtubule polymerization.





We have used cryo-electron tomography of vitrified specimens to analyze its interaction with microtubules (Coquelle *et al.*, 2011). In collaboration with C. Kervrann (INRIA, Rennes), we have developed CryoSeg, an original software that allows semi-automatic extraction of densities inside tomograms based on a patch recognition approach. Results obtained on XMAP215 show that this protein binds along the microtubule lattice and at its ends, localizations which are consistent with its passive diffusion along microtubules and its targeting at microtubule ends.

Conclusions and perspectives

Our results suggest that +TIPs such as EB1 or XMAP215 modulate microtubule dynamics by recognizing tubulin sheets at microtubule ends. In addition, the conformational difference between the "GDP" and "GTP-like" state of tubulin might explain how +TIPs recognize its nucleotide state. Thus, the curved sheets at microtubule growing ends might be partly composed of non-hydrolyzed GTP-tubulin molecules, and their straightening might induce GTP-hydrolysis. We are currently labeling His-tagged +TIPs with Ni-NTA functionalized nanoparticles to visualize them at the video-light and cryo-electron microscope levels.

Impact

- Vitre *et al.*, 2008. Nat. Cell Biol., 10:415-421.
- Blestel *et al.*, 2009. Proceedings of the IEEE International Symposium on Biomedical Imaging. 298-301.
- Coquelle et al., 2009, Biochem. Soc. Trans., 37:997-1001.
- Coquelle *et al.*, 2011, Meth. Mol. Biol. *In press.*

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