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Structural Biology 2019: An Atomistic View of Microtubule Stabilization by GTP - Liliane Mouawad- Institute Curie

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Abstract
A microtubule is a powerful framework shaped of αβ-tubulins. The nearness of nonhydrolyzable guanosine-5'- triphosphate (GTP)/guanosine diphosphate (GDP) on the β-tubulins incites microtubule polymerization/depolymerization. In spite of the huge number of trial investigations of this dynamical procedure, its system is as yet muddled. To give experiences into this instrument we examined the main depolymerization steps of GDP/GTP-bound microtubules by ordinary mode examination with the all-molecule model. We likewise built a depolymerizing microtubule and contrasted it with cryo-electron microscopy tomograms (cyro-ET). The outcomes show that during depolymerization, the protofilaments bend as well as turn to debilitate their horizontal cooperations. These connections are balanced out by GTP, yet not equally. Not the entirety of the interface deposits is of equivalent significance: five of them, having a place with the H2-S3 circle, assume an extraordinary job; going about as a lock whose key is the γ-phosphate of GTP. Arrangement arrangements of a few tubulins affirm the significance of these deposits.

Microtubules (MTs) are dynamic constituents of the cytoskeleton involved in many important functions of the cell, such as cell division, intracellular transport of vesicles and organelles, compartmentalization of the cytoplasm, and flagellar motility. An MT is a hollow flexible tube of variable length, which may reach several micrometers in length. The most widespread MT is formed of 13 protofilaments (PFs), and its diameter is 25 nm. Each PF is composed of ab-tubulin dimers. The a-tubulin always contains a nonhydrolyzable guanosine-50-triphosphate (GTP), whereas the b-tubulin contains a GTP molecule that can be hydrolyzed to guanosine diphosphate (GDP) during MT polymerization. GTP hydrolysis in the b-tubulin plays an essential role in the modulation of the MT dynamic polymerization/depolymerization process (Howard and Hyman, 2003), which is the basis of its functional power. Indeed, the presenceof GTP in the b-tubulin plus-end layer favors polymerization and growth of the MT, whereas the presence of GDP provokes its depolymerization and shrinkage (Desai and Mitchison,1997). It has been shown that the presence of only a one-layer of GTP-bound b-tubulins at the plus-end tip is sufficient to prevent MT depolymerization despite the fact that all the other b-tubulins are GDP-bound (Caplow and Shanks, 1996; Drechsel and Kirschner, 1994). The mechanism of this dynamic process is not yet well understood. The allosteric model postulates that GDP favors curved dimers and PFs, whereas GTP favors straighter conformations (Nogales and Wang, 2006b; Wang and Nogales, 2005). However, this model is contested by the results of several experimental (Barbier et al., 2010; Buey et al., 2006; Nawrotek et al., 2011; Rice et al., 2008) and in silico (Gebremichael et al., 2008; Grafmu¨ ller and Voth, 2011) studies. Those studies seem to support the lattice model, which posits that dimer conformational straightening occurs only upon recruitment into the growing MT lattice (Rice et al., 2008). Recently, cryo-electron microscopy structures were resolved for MTs bound to either GDP-taxol or guanylyl 5'-a, b-methylenediphosphonate (GMPCPP), a slowly hydrolyzable GTP analog (Yajima et al., 2012). However, even the direct structural comparison in that study does not reveal the mechanism by which GTP stabilizes the most widespread MT. Indeed, that study concerns the 15-PF MT, which is different from the 13-PF MT considered here, and because GMPCPP is longer than GTP, its way of action may be different. Therefore, the question remains open. Here, we approach the problem from a different angle. Our aim is to understand the mechanism according to which the 65 additional atoms of the g-phosphates of the 13 GTPs in the b-tubulin plus-end layer modify the dynamics not only of this layer, which is composed of more than 105 atoms, but of the entire MT. For this purpose we constructed a 13-protofilament MT of three layers (Figure 1), i.e., three ab-tubulin dimers for each PF, with GTP in the nonexchangeable site of all a-tubulins and GDP in the exchangeable site of the b-tubulins of the first two layers (starting from the minus end). For the plus-end layer, two states were considered: either the 13 b-tubulins were GDP-bound, in which case the MT was called MT-GDP, or they were GTP-bound, and the MT was called MT-GTP. More precisely, MT-GDP and MT-GTP refer to the energy-minimized structures.

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