Published in final edited form as:

Curr Opin Struct Biol. 2016 April ; 37: 90–96. doi:10.1016/j.sbi.2015.12.009.

Visualizing Microtubule Structural Transitions and Interactions with Associated Proteins

Eva Nogales^{1,2,3} and Rui Zhang^{2,3}

¹Molecular and Cell Biology Department and QB3 Institute, UC Berkeley, CA 94720

²Howard Hughes Medical Institute, UC Berkeley, CA 94720

³Molecular Biophysics and Integrative Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Abstract

Microtubules (MTs) have been the subject of cryo-electron microscopy (cryo-EM) studies since the birth of this technique. Although MTs pose some unique challenges, having to do with the presence of a MT seam, lattice variability and disorder, MT cryo-EM reconstructions are steadily improving in resolution and providing exciting new insights into MT structure and function. Recent work has led to the atomic-detail visualization of lateral contacts between tubulin subunits and the conformational changes that give rise to strain in the MT lattice accompanying GTP hydrolysis. Cryo-EM has also been invaluable in describing the interactions between MTs and MT associated proteins (MAPs), which function to regulate MT dynamic instability, move cargoes, or contribute to other MT cellular processes.

Introduction

Microtubules (MTs) are essential cytoskeletal polymers involved in numerous cellular functions and essential for cell division. They are built of $\alpha\beta$ -tubulin heterodimers that associate longitudinally into protofilaments (PFs), which interact laterally, in parallel, to form the MT wall (Figure 1A). Tubulin binds GTP at a conserved site, which is non-exchangeable in α -tubulin (N-site, buried at the intra-dimer interface) and exchangeable in β -tubulin (E-site, exposed on the surface of the dimer) (Figure 1A) [1]. The nucleotide state in β -tubulin regulates MT dynamics. While GTP-tubulin incorporates efficiently at growing MT ends, polymerization is coupled to GTP hydrolysis, as catalytic residues in α -tubulin come in close proximity to the E-site nucleotide of β -tubulin at a longitudinal interface [2]. Hydrolysis gives rise to the phenomenon of dynamic instability by which MTs switch between growing phases (when MT ends are capped by GTP-bound tubulin) and shrinking phases (when MT ends lose their “GTP cap”) [3]. MTs can be stabilized against depolymerization by non-hydrolyzable GTP analogs [4], and by a variety of antimicrotubule agents [5]. For their many cellular functions, MTs interact with a myriad of MT associated

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

proteins (MAPs), including motor proteins, regulators of dynamic instability or kinetochore complexes.

The atomic structure of $\alpha\beta$ -tubulin was first obtained using electron crystallography of zinc-induced tubulin sheets [1], in which PFs associate in an antiparallel fashion [6]. A model of the MT was then generated by “docking” the electron crystallographic structure of the PF into a 20 Å cryo-EM reconstruction of the MT [7], thus identifying regions in tubulin facing the outside surface, the lumen or the lateral interfaces. This hybrid methodology has also been used for over fifteen years to visualize the interactions of MTs with a large number of MAPs.

A number of X-ray crystal structures of unpolymerized tubulin, bound to assembly inhibitors, have also been reported. They show $\alpha\beta$ -tubulin to be in a “curved” state, in contrast to the “straight” conformation within the MT (or the zinc-induced tubulin sheets [1]). This difference in curvature can be sensed by different MAPs, which in turn regulate MT dynamics. X-ray crystal structures describing the interactions between tubulin and MAPs that prefer the curved tubulin conformation (Op18/stathmin [8], tubulin tyrosine ligase (TTL) [9] and TOG-domain containing MAPs [10]) have been recently reviewed elsewhere [11]. Here we focus on recent cryo-EM studies that have: (1) pushed the resolution of MT structures to atomic resolution (Figure 1B); (2) characterized the link between tubulin nucleotide state and MT dynamic instability (Figure 2); and (3) described the interactions between MAPs and MTs (assembled, straight tubulin conformation) (Figure 3A).

Resolution Improvement towards a Better Understanding of MT Instability

Cryo-EM reconstructions of MTs, at the highest possible resolution and in different nucleotide states, should be invaluable towards a detailed molecular understanding of how GTP hydrolysis within β -tubulin controls MT dynamics. A major breakthrough towards such goal came with the MT structure at better than 10 Å resolution determined by Downing and Li in 2002 [12], which allowed a better definition of regions involved in lateral contacts between PFs. For years, cryo-EM studies of MTs, alone or decorated with MAPs, provided invaluable biological insights, but none reached better than 8 Å resolution. Limitations may have come from quality and quantity of the images, shortcomings in image processing, the similarity between α - and β -tubulin, and the presence of a MT “seam”. As shown in Figure 1A, lateral interactions between PFs typically involve α - α and β - β contacts, except for one unique site, the seam, where lateral contacts are heterotypic (α - β and β - α) [13], therefore breaking the helical symmetry.

The 8 Å resolution limit was recently broken with the implementation of two major strategies. The first involved the use of a real space image processing approach developed by Edward Egelman that uses single particle principles to overcome long range disorders of the helical assemblies (Iterative Helical Real Space Reconstruction (IHRSR) [14]). Long MTs are cut into small, overlapping segments that are aligned to each other to build up signal and produce a 3D reconstruction. Our modified IHRSR approach [15,16], inspired by previous work of Sindelar and Downing [17], takes advantage of the pseudo-helical symmetry of the

MT while still accounting for the presence of the seam. The second strategy was to use kinesin to “mark” the tubulin $\alpha\beta$ -dimer. The mass of the kinesin motor domain, which binds MT one copy per tubulin dimer, allowed us to effectively identify the α - and β -tubulin register and seam location for each MT. Using these two tactics, and a large number of cryo-EM images, we reported what were record resolution structures at the time for MTs bound to GMPCPP (4.7 Å), GDP (4.9 Å) and GDP+Taxol (5.5 Å) [15]. While building an atomic model directly into the EM density map was still not possible, the use of each map in conjunction with Rosetta modeling [18] (with an additional energy term to the standard Rosetta energy function to reflect the model-map agreement) allowed us to define an ensemble of 20 low-energy structural conformers, and used their average to describe each MT state. Comparison of the MT structures showed that GTP hydrolysis leads to a lattice compaction (Figure 2) around the E-site, at the inter-dimer interface, as well as a conformational change within α -tubulin, where the C-terminal region moves with respect to the N-terminal nucleotide binding domain. Interestingly, this study also showed that binding of Taxol partially counteracts the effects of GTP hydrolysis.

Most recently, we used new direct detector technology and improved seam-determination algorithms [16] to reach resolutions of 3.5 Å or better for MT structures, which allowed direct refinement of atomic models against the EM density maps [19]. These models revealed, for the first time, the atomic details of the native lateral contacts between PFs (Figure 1B–1D). The study included MTs bound to GMPCPP and GDP, as well as MTs bound to GTP γ S obtained by copolymerization with the MT plus end tracking protein EB3 (see below). The advance in resolution now allows a much better definition of the conformational changes in α -tubulin that accompany GTP hydrolysis. This study also provided reconstructions without any symmetry applied that reach 4.4 Å or better resolution, and therefore allowed the detailed visualization of the seam naturally present in the MT lattice [19]. Interestingly, the relative position of the two PFs at the seam makes this region of the MT deviate from the cylindrical geometry observed for the rest of the tube. This deviation is more significant for less stable MTs (i.e. GDP versus GMPCPP), suggesting that the seam may be a weak point in the MT lattice and play a role in MT disassembly.

Reading and Writing the Nucleotide State of the Microtubule End: EB Proteins

The plus end of the MT, crowned by β -tubulin subunits, is much more dynamic than the minus end. End-Binding proteins (EBs) constitute the central hub for a network of plus-end tracking proteins (+TIPs) that accumulate at growing MT ends [20,21]. EBs also play a role in MT dynamics by promoting MT growth and increasing catastrophe frequency (catastrophe is the switch from a growing to a shrinking phase) [22]. EBs bind MTs via an N-terminal calponin-homology domain (CH domain) [23,24]. It has been proposed that EBs recognize a nucleotide-dependent structural state at the growing ends, best mimicked by GTP γ S-bound MTs [25]. A cryo-EM structure by Surrey, Moores and coworkers of Mal3-bound GTP γ S-MT at 8.6 Å resolution [26] showed how this fission yeast EB binds to four neighboring tubulin subunits, at the junction between two PFs and two longitudinal interfaces, explaining how it promotes MT assembly.

By copolymerization with excess human EB3, we recently determined the structures of EB3 bound to its preferred substrate, GTP γ S-MT at 3.3 Å, allowing us a very detailed description of the interactions between the CH domain and the four tubulin molecules it contacts [19] (Figure 3B). Interestingly, the EB3-GTP γ S-MT structure displayed a compacted lattice, making it more similar to the GDP state than to the GMPCPP state. Compared to the EB-free GDP-MT structure, the EB3-GTP γ S MTs showed a small displacement between adjacent tubulin dimers along a PF that leads to a global “lattice twist” (Figure 2). By binding four tubulin subunits over two longitudinal and two lateral contacts, EB proteins are highly sensitive to the changes in MT lattice derived from nucleotide state, either lattice compaction or twist, thus selectively binding to a region near the growing end of the MT. We proposed that such region, mimicked by the GTP γ S bound MT, may correspond to a GDP-Pi state.

We also determined the structures of GMPCPP- and dynamic, GDP-MTs copolymerized with excess EB3. The lattice parameters of both the EB3-GMPCPP- and EB3-GDP-MTs shift towards those we observed for the EB3-GTP γ S state. When we analyzed the density at the E-site for all reconstructions, only the EB-free GMPCPP-MT structure, the only one with a non-compacted lattice, showed the presence of both the γ -phosphate and Mg²⁺. These two elements are missing in GDP-MTs, with and without EB3, as expected from hydrolysis of GTP during assembly. The density corresponding to the γ -phosphate is clearly present in the EB3-GTP γ S-MT structure, but the Mg²⁺ ion is not visible. The most interesting result is that for the EB3-coassembled GMPCPP-MT structure, both the Mg²⁺ and the γ -phosphate are missing from the E-site. This result indicates that EB3 stimulated hydrolysis of the otherwise slowly-hydrolyzable GMPCPP, as well as the consequent lattice compaction, within the two minutes of MT assembly that preceded sample vitrification. Thus, the interactions of EB3 across four tubulin subunits make it both sensitive to the lattice rearrangements that accompany GTP hydrolysis (essential for its tracking of the MT ends), and also capable of promoting a compacted structural intermediate state that increases the GTP hydrolysis rate, thus explaining its effects of MT dynamics.

Doublecortin (DCX), a MAP that functions to stabilize MTs in developing neurons, binds at a similar site to that of EB3/Mal3 [27] (Figure 3C), across four tubulin dimers and between two adjacent PFs. Like EBs, DCX also has a strong preference for 13-PF MTs (*in vitro* assembled MTs can have different PF numbers, most commonly 12–15, but by binding between PFs these proteins selectively stabilize 13-PF over any other PF number).

Stepping along the Microtubules: Kinesin and Dynein

All kinesin motor domains (Figure 3D) use a common ATP hydrolysis cycle to tune their affinity for the MT and allow them to “walk” on the MT lattice: kinesin in the apo (no nucleotide) or ATP-bound state binds strongly to MT, whereas kinesin in the ADP or ADP-Pi state binds weakly [28]. Recent cryo-EM studies have shed new light on the roles MTs play in the nucleotide-dependent mechanochemical cycle of kinesin [29–32]. Sindelar and coworkers [30] determined the cryo-EM structures (at 5–6 Å resolution) of MTs decorated with kinesin-1 motor domain in the apo and ADP-Al-Fx (transition analog) states. The authors proposed a model in which MT attachment allosterically triggers a clamshell

opening of the nucleotide cleft that facilitates the ADP release, which is the rate-limiting step [33]. In a parallel study, Moores and coworkers [31] reported a series of cryo-EM structures (at ~ 7 Å resolution) of MTs bound to kinesin-1 or kinesin-3 motor domain in apo, ADP, AMPPNP and ADP-Al-Fx states, and proposed a different allosteric pathway for the MT mediated ADP release. Higher resolution studies will undoubtedly help to refine these models toward a more complete and unified understanding of kinesin mechanochemistry.

The MT-binding domain (MTBD) of dynein (Figure 3E) also cycles through strong and weak MT binding states [28,34]. Cryo-EM structures have provided unique insight into the long-standing question that how structural information is transmitted bi-directionally over a 25 nm long coiled-coil stalk that physically connect the AAA+ ATPase motor domain and the MTBD of dynein on the MT surface. Coupling between MT binding and ATPase activation has been shown to occur through changes in the registry of the stalk coiled-coil [35]. Leschziner and coworkers [36] obtained a cryo-EM structure (9.7 Å resolution) of MTBD-MT complex corresponding to the strong binding state, revealing a MT-binding induced inward displacement of one of the helices in the MTBD in dynein, which is directly attached to the stalk coiled-coil. The authors further used molecular dynamics simulations to model the conformational changes of the dynein MTBD during the weak-to-strong state transition.

Cryo-EM has recently also extended our molecular understanding of full-length dynein and its interaction with the dynactin cofactor. Lander and coworkers used negative stain and sophisticated 2D classification procedures to define the flexible architecture of the full dynein dimer, and to visualize a functional supracomplex of dynein and dynactin, itself a 23-subunit complex, on the MT surface [37]. A component of dynactin, p150^{glued}, contains a microtubule-binding domain that has itself been recently visualized bound to MTs by Mizuno and coworkers [38]. Finally, Carter and coworkers recently reported the groundbreaking cryo-EM structure of dynactin at 4 Å resolution [39] and a lower resolution reconstruction (8.2 Å) of a dynein-dynactin supracomplex with the cargo adaptor Bicaudal-D2.

Changing Tubulin Flavor: Tubulin Tyrosine Ligase-like (TTLL7) Enzyme

Most MT-MAPs interactions, including motor activities [40], are regulated by post-translational modifications on tubulin [41], which include tyrosination/detyrosination, polyglutamylation and polyglycylation within the intrinsically disordered C-terminal tails of tubulin extending on the surface of the MT (Figure 1), as well as acetylation of α -Lys40 on the MT lumen [42].

Glutamylation is carried out by tubulin tyrosine ligase-like (TTLL) enzymes. Recently, the interaction of human TTLL7 with MTs (Figure 3F) has been visualized at 8 Å resolution by Roll-Mecak and coworkers using cryo-EM, revealing a mechanism for MT recognition that involves disorder-to-order transitions by both tubulin and the enzyme [43]. Fitting of the TTLL7 X-ray structure into the cryo-EM reconstruction showed the presence of three rod-like densities near the MT surface that were interpreted as corresponding to regions in TTLL7 that are disordered in the isolated enzyme. Based on secondary structure predictions,

helices were modeled into these densities as part of the cationic MT binding domain (c-MTBD) of TTLL7. The reconstruction also showed clear densities corresponding to the C-terminal tails of α - and β -tubulin as they become structured through their interactions with TTLL7. Binding of the c-MTBD to helix H12 in α -tubulin appears to guide the flexible C-terminal tail of β -tubulin towards the active site, where it can contribute to enzyme activation.

The only other MT cryo-EM reconstruction where the tubulin tails had been visualized was a study of MT interaction with the human Ndc80 kinetochore complex, which bind to MTs with a monomer repeat [44] (Figure 3G). In that case disorder-to-order transitions in both Ndc80 and tubulin also contributed to the interactions, with the low-complexity N-terminal region of the NDC80 protein contributing to contacts along and across PFs that are regulated by Aurora B phosphorylation [45]. Interestingly, the branched character of the tubulin tails in the Ndc80 study was interpreted as polyglutamylation (the TTLL7 study used unmodified tubulin).

Closing Remarks

Progress in cryo-EM is making possible MT studies at higher and higher resolution that have recently allowed to model atomic structures directly into the EM density map. Such type of studies, when applied to MTs in different nucleotide states, have revealed the mechanism of microtubule destabilization by GTP hydrolysis as due to strain accumulated in α -tubulin at the longitudinal interface between dimers, where the nucleotide state is sensed, giving rise to different states of compaction and twist in the lattice (Figure 2). When applied to the study of MT-MAPs interactions, cryo-EM studies to date collectively show the presence of two major binding sites on the MT surface (Figure 1 and 3): (1) at the intra-dimer interface, as seen for kinesin, dynein and TTLL7; and (2) at the inter-dimer interface, often between adjacent PFs, as seen for EB3/Mal3 and DCX. The Ndc80 kinetochore complex is a hybrid that binds both intra and inter dimer interfaces and bridges PFs via an extended, regulatory tail. While group 1 MAPs are less sensitive to microtubule nucleotide state, group 2 MAPs can be exquisitely sensitive to lattice changes brought about by GTP hydrolysis, and in doing so, both affect and utilize MT dynamic instability in the regulation of MT cellular functions.

Acknowledgments

This work was funded by a grant from NIGMS (GM051487 to E.N.). E.N. is a Howard Hughes Medical Institute investigator.

References and recommended reading

Papers of particular interest, published within the last three years, have been highlighted as:

- of special interest
- of outstanding interest

1. Nogales E, Wolf SG, Downing KH. Structure of the alpha beta tubulin dimer by electron crystallography. *Nature*. 1998; 391:199–203. [PubMed: 9428769]

2. Nogales E, Downing KH, Amos LA, Lowe J. Tubulin and FtsZ form a distinct family of GTPases. *Nat Struct Biol.* 1998; 5:451–458. [PubMed: 9628483]
3. Mitchison T, Kirschner M. Dynamic instability of microtubule growth. *Nature.* 1984; 312:237–242. [PubMed: 6504138]
4. Hyman AA, Salser S, Drechsel DN, Unwin N, Mitchison TJ. Role of GTP hydrolysis in microtubule dynamics: information from a slowly hydrolyzable analogue, GMPCPP. *Mol. Biol. Cell.* 1992; 3:1155–1167. [PubMed: 1421572]
5. Dumontet C, Jordan MA. Microtubule-binding agents: a dynamic field of cancer therapeutics. *Nat Rev Drug Discov.* 2010; 9:790–803. [PubMed: 20885410]
6. Lowe J, Li H, Downing KH, Nogales E. Refined structure of alpha beta-tubulin at 3.5 Å resolution. *J Mol Biol.* 2001; 313:1045–1057. [PubMed: 11700061]
7. Nogales E, Whittaker M, Milligan RA, Downing KH. High-resolution model of the microtubule. *Cell.* 1999; 96:79–88. [PubMed: 9989499]
8. Gigant B, Curmi PA, Martin-Barbey C, Charbaut E, Lachkar S, Lebeau L, Siavoshian S, Sobel A, Knossow M. The 4 angstrom X-ray structure of a tubulin : stathmin-like domain complex. *Cell.* 2000; 102:809–816. [PubMed: 11030624]
9. Prota AE, Bargsten K, Zurwerra D, Field JJ, Diaz JF, Altmann KH, Steinmetz MO. Molecular mechanism of action of microtubule-stabilizing anticancer agents. *Science.* 2013; 339:587–590. [PubMed: 23287720]
10. Ayaz P, Ye X, Huddleston P, Brautigam CA, Rice LM. A TOG:alphabeta-tubulin complex structure reveals conformation-based mechanisms for a microtubule polymerase. *Science.* 2012; 337:857–860. [PubMed: 22904013]
11. Brouhard GJ, Rice LM. The contribution of alphabeta-tubulin curvature to microtubule dynamics. *J Cell Biol.* 2014; 207:323–334. [PubMed: 25385183]
12. Li H, DeRosier D, Nogales E, Downing KH. Structure of the microtubule at 8 Å resolution. *Structure.* 2002; 10:1317–1328. [PubMed: 12377118]
13. Mandelkow EM, Schultheiss R, Rapp R, Muller M, Mandelkow E. On the surface lattice of microtubules: helix starts, protofilament number, seam, and handedness. *J Cell Biol.* 1986; 102:1067–1073. [PubMed: 3949873]
14. Egelman EH. The iterative helical real space reconstruction method: surmounting the problems posed by real polymers. *J Struct Biol.* 2007; 157:83–94. [PubMed: 16919474]
15. Alushin GM, Lander GC, Kellogg EH, Zhang R, Baker D, Nogales E. High-resolution microtubule structures reveal the structural transitions in alphabeta-tubulin upon GTP hydrolysis. *Cell.* 2014; 157:1117–1129. [PubMed: 24855948] This study, which broke the previous resolution ceiling of ~8 Å, produced ~5 Å cryo-EM reconstructions of MTs in different functional states. Atomic modeling was carried out using Rosetta with the density map as an additional term in the energy function. Comparison of the GMPCPP- and GDP-MT states showed that GTP hydrolysis causes a lattice compaction at the intradimer interface and a conformational change in α -tubulin. Taxol partially reversed those effects.
16. Zhang R, Nogales E. A new protocol to accurately determine microtubule lattice seam location. *J Struct Biol.* 2015; 192:245–254. [PubMed: 26424086]
17. Sindelar CV, Downing KH. The beginning of kinesin's force-generating cycle visualized at 9-Å resolution. *J Cell Biol.* 2007; 177:377–385. [PubMed: 17470637]
18. Song Y, DiMaio F, Wang RY, Kim D, Miles C, Brunette T, Thompson J, Baker D. High-resolution comparative modeling with RosettaCM. *Structure.* 2013; 21:1735–1742. [PubMed: 24035711]
19. Zhang R, Alushin GM, Brown A, Nogales E. Mechanistic Origin of Microtubule Dynamic Instability and Its Modulation by EB Proteins. *Cell.* 2015; 162:849–859. [PubMed: 26234155] In this highest resolution cryo-EM study of MTs to date, atomic models were refined directly against the 3.5 Å resolution maps of MTs in different states. The GTP γ S-bound MT, obtained by copolymerization

with the +Tip protein EB3, shows a compacted lattice, which is yet distinct from the GDP state by a different lattice twist. By binding between four dimers, EB3 is ideally placed to discriminate lattice states. The study also supports an effect of EB proteins in the promotion of GTP hydrolysis that explains their effects on MT dynamics.

20. Akhmanova A, Steinmetz MO. Tracking the ends: a dynamic protein network controls the fate of microtubule tips. *Nat Rev Mol Cell Biol.* 2008; 9:309–322. [PubMed: 18322465]
21. Galjart N. Plus-end-tracking proteins and their interactions at microtubule ends. *Curr Biol.* 2010; 20:R528–R537. [PubMed: 20620909]
22. Maurer SP, Cade NI, Böhner G, Gustafsson N, Boutant E, Surrey T. EB1 accelerates two conformational transitions important for microtubule maturation and dynamics. *Curr Biol.* 2014; 24:372–384. [PubMed: 24508171]
23. Hayashi I, Ikura M. Crystal structure of the amino-terminal microtubule-binding domain of end-binding protein 1 (EB1). *J Biol Chem.* 2003; 278:36430–36434. [PubMed: 12857735]
24. Slep KC, Vale RD. Structural basis of microtubule plus end tracking by XMAP215, CLIP-170, and EB1. *Mol Cell.* 2007; 27:976–991. [PubMed: 17889670]
25. Maurer SP, Bieling P, Cope J, Hoenger A, Surrey T. GTPgammaS microtubules mimic the growing microtubule end structure recognized by end-binding proteins (EBs). *Proc Natl Acad Sci U S A.* 2011; 108:3988–3993. [PubMed: 21368119]
26. Maurer SP, Fourniol FJ, Böhner G, Moores CA, Surrey T. EBs recognize a nucleotide-dependent structural cap at growing microtubule ends. *Cell.* 2012; 149:371–382. [PubMed: 22500803] This work described the interaction of the fission yeast Mal3 with MTs. It showed how this EB protein binds between two protofilaments and across two inter-dimer interfaces, physically stapling four tubulin subunits together. It also convincingly showed that EB proteins do not bind to the MT seam. Structure-based mutations in the MT-binding regions of EB were shown to have effects on MT binding and end recognition.
27. Fourniol FJ, Sindelar CV, Amigues B, Clare DK, Thomas G, Perderiset M, Francis F, Houdusse A, Moores CA. Template-free 13-protofilament microtubule-MAP assembly visualized at 8 Å resolution. *J Cell Biol.* 2010; 191:463–470. [PubMed: 20974813]
28. Gennerich A, Vale RD. Walking the walk: how kinesin and dynein coordinate their steps. *Curr Opin Cell Biol.* 2009; 21:59–67. [PubMed: 19179063]
29. Morikawa M, Yajima H, Nitta R, Inoue S, Ogura T, Sato C, Hirokawa N. X-ray and Cryo-EM structures reveal mutual conformational changes of Kinesin and GTP-state microtubules upon binding. *EMBO J.* 2015; 34:1270–1286. [PubMed: 25777528]
30. Shang Z, Zhou K, Xu C, Csencsits R, Cochran JC, Sindelar CV. High-resolution structures of kinesin on microtubules provide a basis for nucleotide-gated force-generation. *Elife.* 2014; 3:e04686. [PubMed: 25415053] In this study modeling of kinesin-1 motor (in different nucleotide states) into cryo-EM reconstruction (~5 Å resolution), together with existing X-ray structures, led to the proposal of a detailed mechanistic model for the ATP-driven cycle of kinesin motility. The authors identified a conserved residue in kinesin that, when the motor is bound to a MT, triggers the opening of the nucleotide cleft and therefore the release of ADP. Their model is in line with that proposed for myosin movement on actin.
31. Atherton J, Farabella I, Yu IM, Rosenfeld SS, Houdusse A, Topf M, Moores CA. Conserved mechanisms of microtubule-stimulated ADP release, ATP binding, and force generation in transport kinesins. *Elife.* 2014; 3:e03680. [PubMed: 25209998] Cryo-EM reconstructions of MTs bound by either kinesin-1 or kinesin-3, in different nucleotide states, were compared to show

similar conformational changes through the mechanochemical cycle of kinesin. The authors proposed a conserved mechanism of ATP-driven motility across the kinesin family members.

32. Goulet A, Major J, Jun Y, Gross SP, Rosenfeld SS, Moores CA. Comprehensive structural model of the mechanochemical cycle of a mitotic motor highlights molecular adaptations in the kinesin family. *Proc Natl Acad Sci U S A*. 2014; 111:1837–1842. [PubMed: 24449904]
33. Hackney DD. Kinesin ATPase: rate-limiting ADP release. *Proc Natl Acad Sci U S A*. 1988; 85:6314–6318. [PubMed: 2970638]
34. Roberts AJ, Kon T, Knight PJ, Sutoh K, Burgess SA. Functions and mechanics of dynein motor proteins. *Nat Rev Mol Cell Biol*. 2013; 14:713–726. [PubMed: 24064538]
35. Gibbons IR, Garbarino JE, Tan CE, Reck-Peterson SL, Vale RD, Carter AP. The affinity of the dynein microtubule-binding domain is modulated by the conformation of its coiled-coil stalk. *J Biol Chem*. 2005; 280:23960–23965. [PubMed: 15826937]
36. Redwine WB, Hernandez-Lopez R, Zou S, Huang J, Reck-Peterson SL, Leschziner AE. Structural basis for microtubule binding and release by dynein. *Science*. 2012; 337:1532–1536. [PubMed: 22997337] The subnanometer structure of MTs decorated with the dynein MTBD, together with the crystal structure of the isolated dynein MTBD, were used to visualize the transition to a high-affinity MT-bound state using molecular dynamics. The authors proposed a model of the conformational changes in the dynein MTBD that involves dynamic salt bridges. Mutations affecting these salt bridges gave rise to longer run lengths, indicating that dynein has evolved to have suboptimal processivity.
37. Chowdhury S, Ketcham SA, Schroer TA, Lander GC. Structural organization of the dynein-dynactin complex bound to microtubules. *Nat Struct Mol Biol*. 2015; 22:345–347. [PubMed: 25751425] This study used negative stain electron microscopy and a sophisticated 2D classification and averaging scheme to produce stunning images of full dynein dimers, dynactin complexes and the engagement of the dynein-dynactin complex with the microtubule.
38. Wang Q, Crevenna AH, Kunze I, Mizuno N. Structural basis for the extended CAP-Gly domains of p150^{glued} binding to microtubules and the implication for tubulin dynamics. *Proc Natl Acad Sci U S A*. 2014; 111:11347–11352. [PubMed: 25059720] This study reported the first cryo-EM reconstruction of a CAP-Gly domain bound to the MT surface. It showed how this domain in p150^{glued}, a component of dynactin, binds to the crest of the protofilament in a position that appears compatible with simultaneous binding of the MTBD of dynein, as well as the CH domain of EB proteins. The latter participate in the recruitment of p150^{glued} to the MT ends.
39. Urnavicius L, Zhang K, Diamant AG, Motz C, Schlager MA, Yu M, Patel NA, Robinson CV, Carter AP. The structure of the dynactin complex and its interaction with dynein. *Science*. 2015; 347:1441–1446. [PubMed: 25814576] This study reported a stunning 4 Å resolution cryo-EM reconstruction of a full dynactin complex, revealing the complex architecture of this remarkable dynein cofactor, built around a filament of 7 Arp1 subunits. This architecture sheds unique light on the possible biogenesis of the complex. The study also includes a 8.2 Å resolution cryo-EM reconstruction of a supra-complex of dynactin bound to dynein tails and the cargo binding Bicaudal-D2.
40. Sirajuddin M, Rice LM, Vale RD. Regulation of microtubule motors by tubulin isotypes and post-translational modifications. *Nat Cell Biol*. 2014; 16:335–344. [PubMed: 24633327]

41. Janke C. The tubulin code: molecular components, readout mechanisms, and functions. *J Cell Biol.* 2014; 206:461–472. [PubMed: 25135932]
42. Szyk A, Deaconescu AM, Spector J, Goodman B, Valenstein ML, Ziolkowska NE, Kormendi V, Grigorieff N, Roll-Mecak A. Molecular basis for age-dependent microtubule acetylation by tubulin acetyltransferase. *Cell.* 2014; 157:1405–1415. [PubMed: 24906155]
43. Garnham CP, Vemu A, Wilson-Kubalek EM, Yu I, Szyk A, Lander GC, Milligan RA, Roll-Mecak A. Multivalent Microtubule Recognition by Tubulin Tyrosine Ligase-like Family Glutamylases. *Cell.* 2015; 161:1112–1123. [PubMed: 25959773] This study reported the X-ray crystal structure of TTLL7 and the cryo-EM structure of TTLL7-decorated MTs. Disorder-to-order transitions in both tubulin and TTLL7 occur upon binding to guide the acidic tail of β -tubulin into the active site of the enzyme.
44. Alushin GM, Ramey VH, Pasqualato S, Ball DA, Grigorieff N, Musacchio A, Nogales E. The Ndc80 kinetochore complex forms oligomeric arrays along microtubules. *Nature.* 2010; 467:805–810. [PubMed: 20944740]
45. Alushin GM, Musinipally V, Matson D, Tooley J, Stukenberg PT, Nogales E. Multimodal microtubule binding by the Ndc80 kinetochore complex. *Nat Struct Mol Biol.* 2012; 19:1161–1167. [PubMed: 23085714] In this study the C-terminal tails of tubulin are seen engaged with both the CH domain of the NUF2 subunit within the Ndc80 complex, and with a region of the N-terminal domain of the NDC80 protein that becomes ordered upon interaction with the MT (between Ndc80 complexes oligomerized on the MT surface). This interaction involves the C-terminal tail of tubulin in the adjacent protofilament.

Highlights

Progress in cryo-EM resolution now allows building atomic models into microtubule (MT) maps

GTP hydrolysis induces strain in α -tubulin at longitudinal interfaces between dimers

MAPS bind either at intra or interdimer contacts, the latter are sensitive to MT nucleotide state

Cryo-EM studies have led to models of kinesin/dynein mechanochemistry on MTs

Disorder-to-order transitions guide β -tubulin tail to the active site of its glutamylase

Figure 1. MT structure and interactions. (A) Schematic of a 13-PF, 3-start helix MT showing the seam (α -tubulin: green, β -tubulin: blue). The tubulin with black thick outlines depicts one turn of tubulin dimer along the 3-start helix. Major binding sites for different MAPs on the outside surface of MT are indicated schematically. (B) Cryo-EM density map at 3.3 Å resolution (EMD-6349) showing homotypic lateral interactions, viewed from the MT lumen (α -tubulin: green, β -tubulin: blue). (C and D) Zoom-in view of the density and model (EMD-6349, PDB: 3JAK) for the β - β lateral interactions (C) and α - α lateral interactions (D).

Figure 2.

Global lattice changes proposed to accompany GTP hydrolysis and EB binding. (A) Schematic of one PF from MTs in three different nucleotide states (GMPCPP, EB3-GTP γ S and GDP), viewed from the outside of the MT. Directions of the lattice twist and lattice compaction are indicated in blue. All the horizontal lines mark the center positions of β -tubulin. The three consecutive tubulin dimers are aligned on the $\beta 1$ subunits marked by red stars. (B and C) Comparison of atomic models of the top tubulin dimer ($\alpha 3$ and $\beta 3$) as indicated by red thick outlines in (A). Tubulin models for the GMPCPP (PDB: 3JAT), EB3-GTP γ S (PDB: 3JAK) and GDP (PDB: 3JAS) states are colored in gold, blue and light purple, respectively.

Figure 3.

Interaction of different MAPs with MTs. (A) End-on view of a helical turn of 13 tubulin dimers, viewed from the minus end, showing a gallery of MAPs that have been characterized by cryo-EM studies. (B–G) Interactions of tubulin with MAPs, shown from an outside view. In (B) and (C) MAPs binding sites are between two PFs and across two inter-dimer interfaces. From (D) to (H) MAPs binding sites are within one tubulin dimer. The crystal structures of the different MAPs were placed at their respective MT binding sites based on previous publications: $\alpha\beta$ -tubulin (PDB:3JAK); (B) EB3 (PDB:3JAK); (C) DCX (PDB:

2XRP); (D) kinesin (PDB:4HNA); (E) dynein (PDB:3J1T); (F) TTLL7 (4YLS) and (G) Ndc80 complex (PDB:3IZ0, NDC80: dark blue, NUF2: yellow).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript