The contribution of αβ-tubulin curvature to microtubule dynamics

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Microtubules are dynamic polymers of αβ-tubulin that form diverse cellular structures, such as the mitotic spindle for cell division, the backbone of neurons, and axonemes. To control the architecture of microtubule networks, microtubule-associated proteins (MAPs) and motor proteins regulate microtubule growth, shrinkage, and the transitions between these states. Recent evidence shows that many MAPs exert their effects by selectively binding to distinct conformations of polymerized or unpolymerized αβ-tubulin. The ability of αβ-tubulin to adopt distinct conformations contributes to the intrinsic polymerization dynamics of microtubules. αβ-Tubulin conformation is a fundamental property that MAPs monitor and control to build proper microtubule networks.

Microtubules are polar polymers formed from αβ-tubulin heterodimers. These tubulin subunits associate head-to-tail to form protofilaments, and typically 13 protofilaments are associated side-by-side to form the hollow cylindrical microtubule. Most microtubules emanate from microtubule organizing centers, in which their minus ends are embedded. GTP-tubulin associates with the fast-growing plus ends as the microtubules radiate to explore the cell interior (see Box).

Unlike actin filaments, which grow steadily, microtubules frequently switch between phases of growth and shrinkage. This hallmark property of microtubules, known as "dynamic instability" (Mitchison and Kirschner, 1984), allows the microtubule cytoskeleton to be remodeled rapidly over the course of the cell cycle. "Catastrophes" are GTPase-dependent transitions from growing to shrinking, whereas "rescues" are transitions from shrinking to growing. Numerous microtubule-associated proteins (MAPs) regulate microtubule polymerization dynamics. Discovering how cells regulate and harness dynamic instability is a fundamental challenge in cell biology.

A recent accumulation of structural, biochemical, and in vitro reconstitution data has advanced the understanding of dynamic instability and the MAPs that control it. Fresh structural data have provided insight into the process of microtubule assembly and defined how some MAPs recognize αβ-tubulin in and out of the microtubule. In vitro reconstitution experiments are reshaping the understanding of catastrophe and also providing quantitative insight into the mechanism of MAPs. Here, we review this progress, paying special attention to the emerging theme of interactions that are selective for different conformations of αβ-tubulin, both inside and outside the microtubule lattice. We argue for the central importance of recognizing these distinct conformations in the control of microtubule dynamics by MAPs and hence in the construction of a functional microtubule cytoskeleton by cells.

Tubulin dimers and their curvatures

It was clear in early EM studies that αβ-tubulin could form a diversity of polymers (Kirschner et al., 1974). In particular, the first cryo-EM of dynamic microtubules (Mandelkow et al., 1991) revealed significant differences in the appearance of growing and shrinking microtubule ends. Growing microtubule ends had straight protofilaments and were tapered, with uneven protofilament lengths, whereas shrinking microtubule ends had curved protofilaments that peeled outward and lost their lateral contacts. These and other data established the canonical model that GTP-tubulin is "straight" but GDP-tubulin is "curved" (Melki et al., 1989). The idea that GTP binding straightened αβ-tubulin into a microtubule-compatible conformation before polymerization was appealing because it provided a structural rationale for why microtubule assembly required GTP and how GTP hydrolysis could lead to catastrophe. A subsequent cryo-EM study (Chrétien et al., 1995), however, revealed that growing microtubules often tapered and curved gently outward without losing their lateral contacts. These data suggested that GTP-tubulin might not be fully straight at the time of its incorporation into the microtubule lattice, an observation that set the stage for a

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Abbreviations used in this paper: DARPin, designed ankyrin repeat protein; DCX, doublecortin; GMPCPP, guanylyl 5′-α,β-methylenediphosphonate; MAP, microtubule-associated protein.

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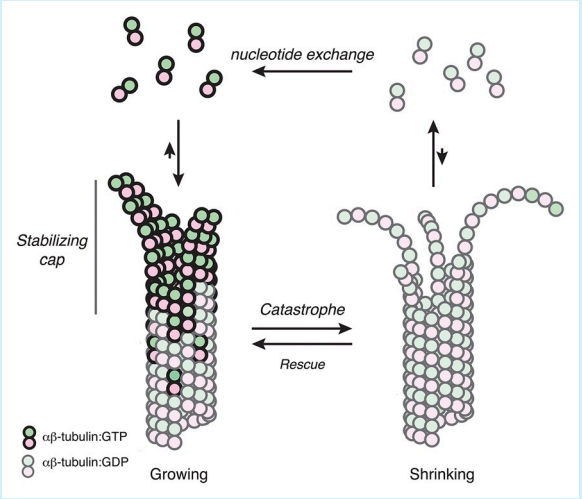
The cycle of microtubule polymerization.



Microtubules are hollow cylindrical polymers composed of αβ-tubulin subunits. Microtubule polymerization occurs through the addition of GTP-bound αβ-tubulin subunits onto microtubule ends. Growing microtubule ends show outwardly curved, tapered, and flattened end structures (left), presumably reflecting the conformational changes that occur during polymerization (see Fig. 1). The addition of a new subunit completes the active site for GTP hydrolysis, and consequently most of the body of the microtubule contains GDP-bound αβ-tubulin. The GDP lattice is unstable but protected from depolymerization by a stabilizing "GTP cap," an extended region of newly added GTP- or GDP.Pi-bound αβ-tubulin. The precise nature of the microtubule end structure and the size and composition of the cap are a matter of debate. Loss of the stabilizing cap leads to rapid depolymerization, which is characterized by an apparent peeling of protofilaments. "Catastrophe" denotes the switch from growth to shrinkage, and "rescue" denotes the switch from shrinkage to growth.

still-active debate on the structure of GTP-tubulin and of microtubule ends.

The atomic details of "straight" and "curved" became apparent when the first structures of αβ-tubulin were solved. The straight conformation of αβ-tubulin was determined from cryo-electron crystallographic studies of Zn-induced αβ-tubulin sheets (Nogales et al., 1998). The structure showed linear head-to-tail stacking of αβ-tubulin along the protofilament, both within and between αβ-tubulin heterodimers. The curved conformation of αβ-tubulin was determined from x-ray crystallographic studies of a complex between αβ-tubulin and Rb3 (Gigant et al., 2000; Ravelli et al., 2004), a microtubule-destabilizing factor in the Op18/stathmin family (Belmont and Mitchison, 1996). In this complex, the individual α- and β-tubulin chains adopted a characteristic conformation distinct from their straight one. Longitudinal interactions also differed from those in the straight conformation (Fig. 1): within and between the heterodimers, successive α- and β-tubulin chains were related by an ∼12° rotation. A chain of these curved αβ-tubulins generates an arc with a radius of curvature resembling that of the peeling protofilaments at shrinking microtubule ends (Gigant et al., 2000; Steinmetz et al., 2000).

Straight and curved are not the only two conformations, however. A cryo-EM study of αβ-tubulin helical ribbons trapped using guanylyl 5′-α,β-methylenediphosphonate (GMPCPP), a slowly hydrolyzable analogue of GTP, provided a molecular view of a possible microtubule assembly intermediate (Wang and Nogales, 2005). In these ribbons, GMPCPP-bound αβ-tubulin adopted a conformation roughly halfway (∼5° rotation) between the straight and curved conformations. These partially curved αβ-tubulin heterodimers formed two types of lateral bonds, only one of which resembled those in the microtubule. This structure suggested that at least some αβ-tubulin straightening occurs during polymerization.

Until recently, structural information about the conformation of unpolymerized GTP-bound αβ-tubulin was notably lacking. Three recent crystal structures (Nawrotek et al., 2011; Ayaz et al., 2012; Pecqueur et al., 2012) have now provided remarkably similar views of this previously elusive species. In all three structures, GTP-bound αβ-tubulin adopts a fully curved conformation, with its α- and β-tubulin subunits related by ∼12° of rotation (Fig. 1). This curvature is not consistent with models in which GTP binding straightens unpolymerized αβ-tubulin. In each of the structures, αβ-tubulin is bound to another protein, stathmin/Rb3 (Ozon et al., 1997), a designed ankyrin repeat protein (DARPin; Pecqueur et al., 2012), as well as a TOG domain from the Stu2/XMAP215 family of microtubule polymerases (Gard and Kirschner, 1987; Wang and Huffaker, 1997). Biochemical experiments have failed to detect GTP-induced straightening of αβ-tubulin, arguing against the possibility that these unrelated binding partners forced GTP-tubulin to adopt the curved conformation. For example, the affinity of stathmin–tubulin interactions is the same for GTP-tubulin and GDP-tubulin (Honnappa et al., 2003). Similarly, five small molecule ligands that target the colchicine binding site and are predicted to bind only curved αβ-tubulin have equivalent affinity for GTP-tubulin, GDP-tubulin, and αβ-tubulin in the stathmin complex (Barbier et al., 2010). Likewise, a TOG domain from Stu2p binds to GTP- and GDP-tubulin with comparable affinity (Ayaz et al., 2012). Finally, DARPin binds equally well to GTP- and GDP-tubulin even though it contacts a structural element that is positioned differently in the straight and curved conformations (Pecqueur et al., 2012). Taken together with early biochemical experiments (Manuel Andreu et al., 1989; Shearwin et al., 1994), these new data strongly support a model in which unpolymerized αβ-tubulin is curved whether it is bound to GTP or to GDP (Buey et al., 2006; Rice et al., 2008; Nawrotek et al., 2011). According to this model, the curved-to-straight transition occurs during the polymerization process, not before. We discuss some implications of this new view at the end of the following section.

Conformation and dynamic instability

How does GTP hydrolysis destabilize the microtubule lattice and trigger catastrophe? A recent structural study has compared high-resolution cryo-EM reconstructions of GMPCPP microtubules and GDP microtubules to provide some answers to this question (Alushin et al., 2014). The structures show that GTP hydrolysis induces a compaction at the longitudinal interface between dimers, immediately above the exchangeable nucleotide-binding site. This compaction is accompanied by conformational changes in α-tubulin. In contrast, lateral contacts between

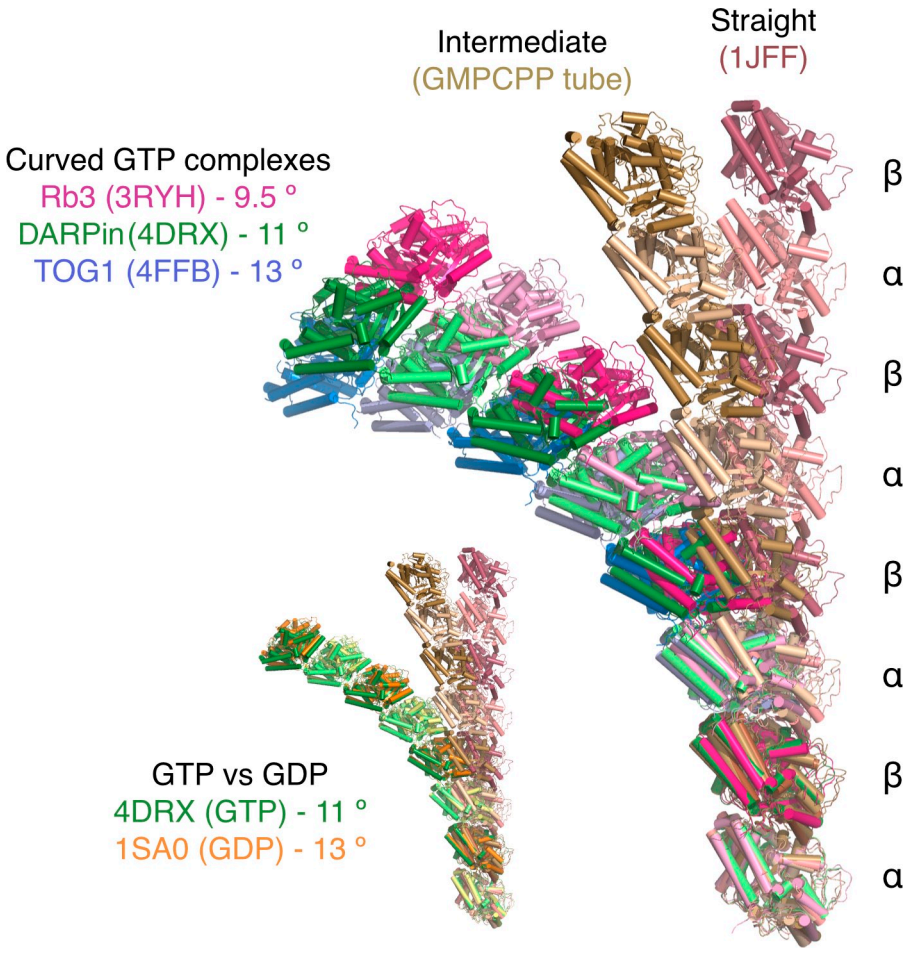


Figure 1. **Three structures of GTP-bound αβ-tubulin adopt similar curved conformations.** Different αβ-tubulin structures were superimposed using α-tubulin as a reference, and oligomers were generated by assuming that the spatial relationship between α- and β-tubulin within a heterodimer is identical to the relationship between heterodimers. Curvature is calculated from the rotational component of the transformation required to superimpose the α-tubulin chain onto the β-tubulin chain of the same heterodimer. All of the GTP-bound structures (Rb3 complex, Protein Data Bank [PDB] accession no. 3RYH [magenta]; DARPin complex, PDB accession no. 4DRX [green]; TOG1 complex, PDB accession no. 4FFB [blue]) show between 10° and 13° of curvature, which is very similar to the curvature observed in GDP-bound structures (see inset, where the αβ-tubulins from a GDP-bound stathmin complex [PDB accession no. 1SA0] are shown in yellow and orange). A straight protofilament (putty and dark red color, PDB accession no. 1JFF) and a partially straightened assembly (tan) from GMPCPP ribbons are shown for reference.

tubulins were essentially unchanged in the different nucleotide states. These observations suggest that GTP hydrolysis introduces strain into the lattice, but how this strain affects the strength of longitudinal and lateral bonds to destabilize the microtubule remains unknown. The GMPCPP and GDP microtubules also show distinct arrangements of elements that bind to MAPs, which suggests a structural mechanism some MAPs could use to distinguish GTP lattices from GDP lattices (discussed later).

In parallel with these structural advances, in vitro reconstitutions (Gardner et al., 2011b) have undermined the textbook view about the kinetics of catastrophe. The seminal measurements of catastrophe frequency (Walker et al., 1988, 1991) assumed that catastrophe occurred with the same probability on newly formed and old microtubules. In other words, the analysis implied that catastrophe was a first-order, single-step process. Although subsequent experiments (e.g., Odde et al., 1995; Janson et al., 2003) indicated that catastrophe involved multiple steps, the first-order view of catastrophe was widely adopted (Howard, 2001; Phillips et al., 2008). Recent experiments using a single-molecule assay for microtubule growth (Gell et al., 2010) have now shown definitively that catastrophe is not a single-step process; rather, newly formed microtubules undergo catastrophe less frequently than older ones (Gardner et al., 2011b). "Age-dependent" catastrophe implies that the stabilizing structure at the end of growing microtubules is evolving to become less effective. The timescale of this evolution is long compared with the kinetics of αβ-tubulin association (Gardner et al., 2011a). Thus, the ageing process probably reports on one or more structural properties of the microtubule end, such as the presence of "defects" in the lattice (Gardner et al., 2011b) or possibly increased tapering of microtubule ends (Coombes et al., 2013).

It now seems clear that changes in the curvature of αβ-tubulin during microtubule polymerization are fundamental to microtubule dynamics and the regulatory activities of MAPs. Having straight conformations of αβ-tubulin only occur appreciably in the microtubule lattice provides a simple structural mechanism by which MAPs can discriminate unpolymerized from polymerized αβ-tubulin. Biochemical properties that define microtubule dynamics, like the strength of lateral and longitudinal contacts and the rate of GTP hydrolysis, may differ for curved, straight, and intermediate conformations of αβ-tubulin; e.g., curved forms probably bind microtubule ends less tightly than straight forms. By regulating when and where these different conformations occur, MAPs can tune microtubule dynamics. More speculatively, the complex biochemistry associated with different conformations of αβ-tubulin may contribute to the aging of microtubule ends, which leads to catastrophe. Understanding the connections between αβ-tubulin conformation, biochemistry, and polymerization dynamics is a major challenge for the future. Expanding the current mathematical models (Bowne-Anderson et al., 2013) and computational models (VanBuren et al., 2005; Margolin et al., 2012) of microtubule

dynamics to incorporate these new findings about αβ-tubulin structure and age-dependent catastrophe may yield significant insights. In the following sections, we will examine recent studies that demonstrate how MAPs use selective interactions with distinct conformations of αβ-tubulin to control microtubule dynamics and thereby the physiology of the microtubule cytoskeleton.

Microtubule depolymerases stabilize curved conformations of tubulin

Perhaps the first direct evidence that MAPs might control the conformation of αβ-tubulin came from studies of microtubule depolymerases, which are proteins that promote, accelerate, or induce the depolymerization of microtubules (Howard and Hyman, 2007). Cells use microtubule depolymerases to maintain local control of microtubule catastrophe. Early electron microscopy studies of two unrelated depolymerases, Op18/stathmin and the kinesin-13 Xkcm1, showed that these proteins were able to induce/stabilize the curved conformation of αβ-tubulin and/or curved protofilaments (Desai et al., 1999; Gigant et al., 2000; Steinmetz et al., 2000). Depolymerases are also referred to as "catastrophe factors" because they trigger catastrophes in dynamic microtubules. The localized control of catastrophe is the essential function of depolymerases in cell physiology.

The microtubule depolymerase stathmin is inactivated around chromosomes and at the leading edge of migrating cells (Niethammer et al., 2004), creating a gradient of depolymerase activity in these zones. Proteins in the Op18/stathmin family form a tight complex with two curved tubulin dimers (Fig. 2 A). Op18/stathmin proteins have been critical for the crystallization of tubulin (Ravelli et al., 2004; Gigant et al., 2005; Prota et al., 2013) and for biochemical studies of tubulin conformation. Although stathmins are frequently described as tubulin-sequestering proteins, the effect they have on microtubule catastrophe frequencies in vitro is much stronger than would be predicted from the simple sequestration of tubulin (Belmont and Mitchison, 1996). The potency of stathmins suggests that they induce catastrophes through direct interactions with microtubule ends, presumably weakening the bonds of terminal subunits by inducing or stabilizing their curvature (Gupta et al., 2013).

Kinesin-13s, first identified by their central motor domain (Aizawa et al., 1992; Wordeman and Mitchison, 1995), depolymerize microtubules catalytically using the energy of ATP hydrolysis (Hunter et al., 2003). Kinesin-13s depolymerize microtubules at spindle poles to generate poleward flux (Ganem et al., 2005), at kinetochores to drive anaphase chromosome segregation (Maney et al., 1998; Rogers et al., 2004), and in neuronal processes (Homma et al., 2003). Evidence that kinesin-13s depolymerized microtubules came from the discovery of the *Xenopus laevis* homologue, Xkcm1, in a screen for kinesin-related proteins involved in spindle assembly (Walczak et al., 1996). Incubation of Xkcm1, also known as MCAK, with GMPCPP microtubules caused peeled protofilaments and significant "ram's horns" structures to appear at microtubule ends (Desai et al., 1999), which indicates that MCAK binds more tightly to curved structures than to straight ones. As with all kinesins, tight binding of the motor domain is coupled to its ATP hydrolysis cycle. Kinesin-13s first bind the microtubule lattice with an on-rate constant that strongly influences its depolymerase activity (Cooper et al., 2010). Kinesin-13s then target the end of the microtubule via "lattice diffusion," a random walk mediated by electrostatic interactions that occurs in the ADP state (Helenius et al., 2006). Exchange of ADP to ATP occurs at microtubule ends; in the ATP state, MCAK binds tightly to tubulin dimers and either induces or stabilizes their outward curvature and detachment from the microtubule lattice (Friel and Howard, 2011). The subsequent hydrolysis of ATP causes kinesin-13 to release its tubulin subunit, now detached from the lattice, and begin another cycle of depolymerization (Moores et al., 2002).

A distinguishing feature of the kinesin-13 motor domain is an extension of loop L2, known as the KVD finger (Ogawa et al., 2004; Shipley et al., 2004), which protrudes from the motor domain toward the minus end of the microtubule (Fig. 2 B). Alanine substitution of the KVD motif inhibits depolymerase activity in cell-based assays (Ogawa et al., 2004) and in vitro (Shipley et al., 2004). A recent cryo-EM study showed that the kinesin-13 motor domain contacts curved tubulin on three distinct surfaces (Asenjo et al., 2013) that differ from the contact surfaces of kinesin-1 (Sindelar and Downing, 2010; Gigant et al., 2013). The location of the kinesin-13 contact surfaces could allow kinesin-13 to stabilize spontaneous curvature of tubulin dimers at either microtubule end. Alternatively, tight binding of the kinesin-13 motor domain could directly induce curvature in the tubulin dimer. In either case, by promoting curvature at the growing microtubule end, kinesin-13s weaken the association of terminal subunits and induce catastrophes.

Kinesin-8s are motile depolymerases (Gupta et al., 2006; Varga et al., 2006) that establish the length of microtubules in the mitotic spindle (Goshima et al., 2005; Rizk et al., 2014), position the spindle (Gupta et al., 2006), and modulate the dynamics of kinetochore microtubules (Stumpff et al., 2008; Du et al., 2010). Unlike the nonmotile kinesin-13s, whose motor domain is fully specialized for depolymerization, kinesin-8 proteins walk to the microtubule end and remove tubulin upon arrival (Gupta et al., 2006; Varga et al., 2006). Although it is unclear if depolymerase activity is fully conserved (Du et al., 2010; Mayr et al., 2011), all kinesin-8s combine motility with a negative effect on microtubule growth. For *Saccharomyces cerevisiae* Kip3p, the combination of motility and depolymerase activity has a significant functional consequence: Kip3p depolymerizes longer microtubules faster than shorter ones (Varga et al., 2006). This length-dependent depolymerization can be explained by an "antenna model." In this model, longer microtubules will accumulate more kinesin-8s, which then walk toward the microtubule end, forming length-dependent traffic jams in some cases (Leduc et al., 2012). Because the rate of depolymerization depends on the number of kinesin-8s that arrive at the microtubule end, longer microtubules will be depolymerized more quickly. The "antenna model" depends critically on the high processivity of kinesin-8, which is thought to result from an additional C-terminal microtubule-binding element (Mayr et al., 2011; Stumpff et al., 2011; Su et al., 2011; Weaver et al., 2011); the C terminus may also contribute to a recently described microtubule sliding activity in Kip3p (Su et al., 2013). Intriguingly, a single Kip3p appears to be insufficient to

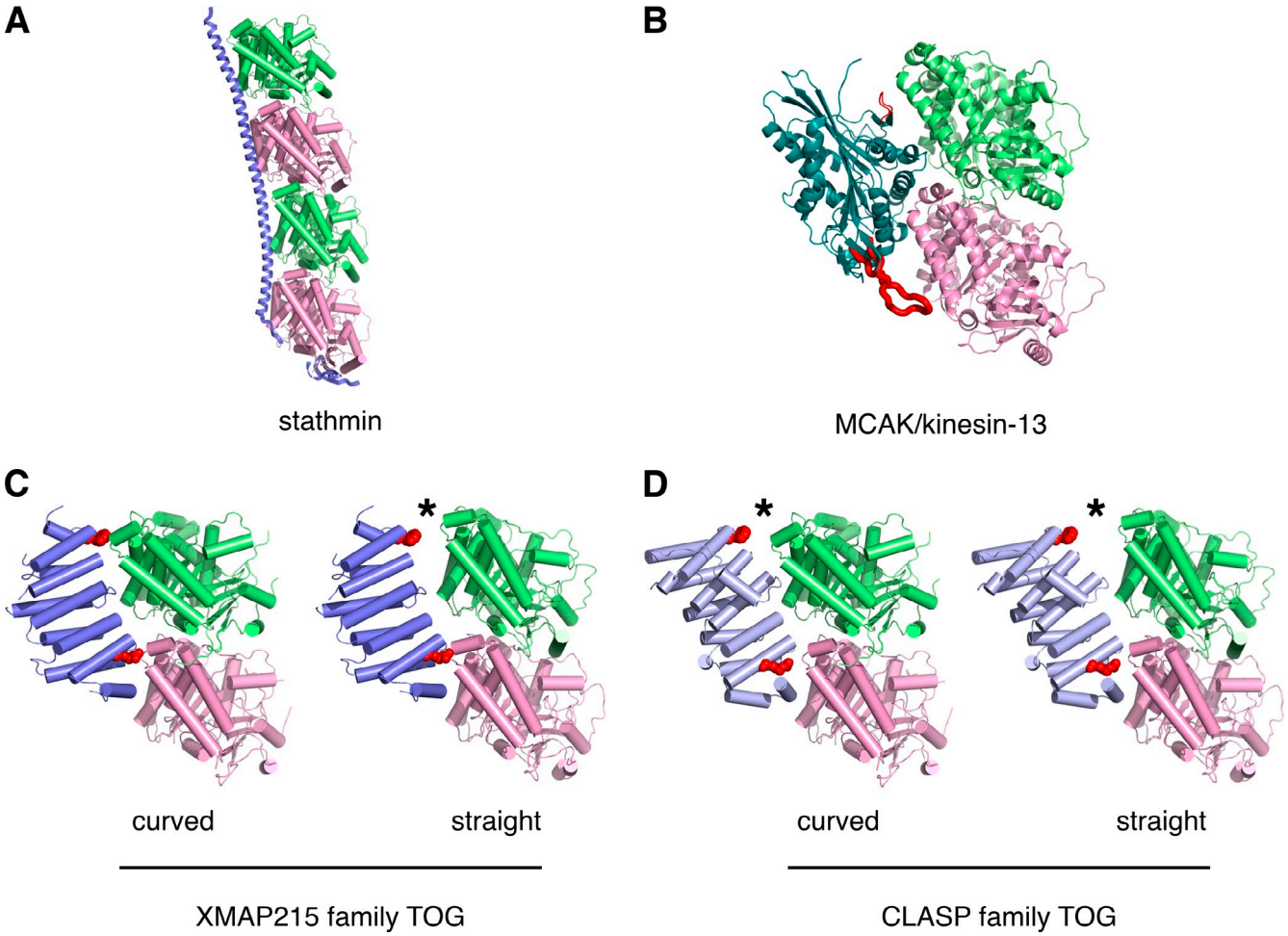


Figure 2. **Proteins that recognize curved αβ-tubulin tend to make long interfaces that span both α- and β-tubulin.** (A) A stathmin family protein (blue) forms a long helix that binds two αβ-tubulin heterodimers (pink and green; PDB accession no. 3RYH). (B) The structure of a complex between kinesin-1 and αβ-tubulin (PDB accession no. 4HNA) is shown with the motor in dark green and αβ-tubulin in pink and lime. Depolymerizing kinesins have insertions (red segments modeled based on a crystal structure of MCAK; PDB accession no. 1V8K), such as the KVD finger, that expand the contact region compared with purely motile kinesins. (C) The TOG1 domain (blue) from Stu2, an XMAP215 family polymerase, contacts regions of α- and β-tubulin (pink and green) that move relative to each other in the curved (left, PDB accession no. 4FFB) and straight (right, model substituting straight αβ-tubulin; PDB accession no. 1JFF) conformations of αβ-tubulin. The asterisks show where this relative movement would disrupt the TOG–tubulin interface. Red side chains indicate conserved tubulin-binding residues at the top and bottom of the TOG domain. (D) The TOG2 domain from human CLASP1 (light blue, PDB accession no. 4K92) shows an "arched" interface that in docked models like the ones shown here is not complementary to curved (left) or straight (right) conformations of αβ-tubulin. Curved and straight structures are PDB 4FFB and 1JFF, respectively. Red side chains indicate binding residues similar to those in the polymerase family TOG domains, and asterisks highlight where the arched nature of this TOG prevents a conserved binding residue from contacting its interaction partner on β-tubulin.

remove a tubulin dimer. Rather, a second Kip3p must arrive at the microtubule end to bump off the first one (Varga et al., 2009).

There are less structural and mutagenesis data available to explain the unique ability of kinesin-8s to walk and depolymerize. It is also not clear that all kinesin-8s use the same cooperative mechanism described for Kip3p. Like kinesin-13, the motor domain of kinesin-8 has an extended loop L2. This loop is disordered in the available crystal structure, but has been observed to contact α-tubulin in a cryo-EM reconstruction (Peters et al., 2010). The kinesin-8 loop L2 lacks a KVD sequence, however, and systematic mutations of L2 have not yet determined its role in depolymerase activity. The extent to which kinesin-8s recognize/induce curvature at microtubule ends remains unresolved. Truncated kinesin-8 motor domains can create small peels at the ends of GMPCPP microtubules (Peters et al., 2010), which suggests that kinesin-8 can induce or stabilize curvature. The fact that two kinesin-8s are required to dissociate a tubulin subunit, however, indicates that single motors alone do not substantially weaken the bonds holding the terminal tubulin subunit. Perhaps kinesin-8s do not stabilize curved forms of αβ-tubulin as strongly as kinesin-13s do.

Reconstitution of microtubule dynamics in vitro showed that the depolymerizing kinesins affect catastrophe in different ways (Gardner et al., 2011b): kinesin-13s eliminate the aging process described earlier, whereas kinesin-8s accelerate it. Importantly, the local control of catastrophes by depolymerases is accomplished primarily through the local modulation of curvature at microtubule ends.

Growth-promoting MAPs also use conformation-selective interactions with αβ-tubulin

MAPs that accelerate growth or stabilize the microtubule lattice counteract microtubule depolymerases (Tournebize et al., 2000; Kinoshita et al., 2001). XMAP215 was discovered as the major protein in *Xenopus* extracts that promotes microtubule growth (Gard and Kirschner, 1987). Later, functional homologues were discovered in *S. cerevisiae* (Stu2p) (Wang and Huffaker, 1997) and other organisms (e.g., Charrasse et al., 1998; Cullen et al., 1999). XMAP215 family proteins localize to kinetochores and microtubule organizing centers, where they contribute to chromosome movements and to spindle assembly and flux (Wang and Huffaker, 1997; Cullen et al., 1999). Loss of XMAP215 family polymerase function leads to shorter, slower-growing microtubules and often gives rise to smaller and/or aberrant spindles (Wang and Huffaker, 1997; Cullen et al., 1999). All family members contain multiple TOG domains that bind αβ-tubulin (Al-Bassam et al., 2006; Slep and Vale, 2007). The molecular mechanisms underlying the activity of these proteins, and the collective action of their arrayed TOG domains, have until recently remained obscure. Recent progress is defining the structure and biochemistry of TOG domains and their interactions with αβ-tubulin. The emerging view is that XMAP215 family polymerases, like the depolymerases, bind to curved αβ-tubulin dimers as an important part of their biochemical cycle. In this section, we will focus on the most recent developments that are shaping the molecular understanding of growth-promoting MAPs, emphasizing the somewhat better studied XMAP215 family.

Affinity chromatography using immobilized TOG domains from Stu2p revealed that the TOG1 domain binds directly to unpolymerized αβ-tubulin (Al-Bassam et al., 2006). TOG domains can also bind specifically to one end of the microtubule (Al-Bassam et al., 2006). Crystal structures of TOG domains, sequence conservation, and site-directed mutagenesis defined the αβ-tubulin–interacting surface, which forms a narrow "spine" of the book-shaped domain (Al-Bassam et al., 2007; Slep and Vale, 2007).

In early models for XMAP215, the arrayed TOG domains were thought to bind multiple αβ-tubulins (Gard and Kirschner, 1987). Subsequent fluorescence-based reconstitution of XMAP215 activity, however, gave results that were not consistent with this "shuttle" model (Brouhard et al., 2008). The reconstitution assays showed that XMAP215 acted processively, residing at the microtubule end long enough to perform multiple rounds of αβ-tubulin addition. Intriguingly, XMAP215 increased the rate of, but not the apparent equilibrium constant for, microtubule elongation. XMAP215 also stimulated the rate of shrinkage in the absence of unpolymerized αβ-tubulin. Similar observations were made using Alp14 (Al-Bassam et al., 2012), a *Schizosaccharomyces pombe* XMAP215 homologue. These studies showed that XMAP215 catalyzes polymerization: it promotes microtubule growth by using its TOG domains to repeatedly bind and stabilize an intermediate state that otherwise limits the rate of polymerization.

How do TOG domains recognize the microtubule end and promote elongation? Recent structural studies (Ayaz et al., 2012, 2014) suggest that interactions with curved αβ-tubulin play a central role. The crystal structures of complexes between αβ-tubulin and the TOG1 or TOG2 domains from Stu2p revealed that both TOG domains bind to curved αβ-tubulin (Ayaz et al., 2012, 2014; Fig. 2 C). The TOG domains do not interact strongly with microtubules even though the TOG-contacting epitopes are accessible on the microtubule surface (Ayaz et al., 2012). Preferential binding to curved αβ-tubulin (Ayaz et al., 2014) occurs because the arrangement of the TOG-contacting regions of α- and β-tubulin differs between curved and straight conformations (Fig. 2 C). Conformation-selective TOG–αβ-tubulin interactions explain how XMAP215 family proteins discriminate unpolymerized αβ-tubulin from αβ-tubulin in the body of the microtubule. XMAP215 family proteins require a basic region in addition to TOG domains for microtubule plus end association and polymerase activity (Widlund et al., 2011). The polarity of TOG–αβ-tubulin interactions and the ordering of domains in the protein together explain the plus end specificity of these polymerases: only at the plus end can TOGs engage curved αβ-tubulin while the C-terminal basic region contacts surfaces deeper in the microtubule (Ayaz et al., 2012). A recent study proposed that the linked TOG domains catalyze elongation using a tethering mechanism that effectively concentrates unpolymerized αβ-tubulin near curved subunits already bound at the microtubule end (Ayaz et al., 2014). The mechanisms by which these proteins catalyze depolymerization are less understood, although depolymerization can be explained by the catalytic stabilization of an intermediate state (Brouhard et al., 2008). By analogy with the depolymerases described earlier, the stabilization of such a state by arrayed TOG domains seems likely to also depend on the preferential interactions with curved αβ-tubulin.

CLASP family proteins (Pasqualone and Huffaker, 1994; Akhmanova et al., 2001) also contain TOG domains, but they are used to different effect: CLASPs do not make microtubules grow faster but instead appear to regulate the frequencies of catastrophe and rescue. For example, in vitro reconstitutions using Cls1p, a CLASP protein from *S. pombe*, showed that Cls1p promoted rescue (Al-Bassam et al., 2010). CLASP family proteins also localize to kinetochores and contribute to spindle flux (Maiato et al., 2005). Loss of CLASP function affects microtubule stability and causes spindle defects (Akhmanova et al., 2001; Maiato et al., 2005), but does so without significantly affecting microtubule growth rates (Mimori-Kiyosue et al., 2006). CLASPs can also stabilize microtubule bundles/overlaps (Bratman and Chang, 2007). The recently published structure of a CLASP family TOG domain (Leano et al., 2013) provided an unexpected hint about a possible origin of the different activities. Indeed, the structure revealed significant differences with XMAP215 family TOG domains even though the CLASP TOG maintains evolutionarily conserved αβ-tubulin–interacting residues (Fig. 2 D). Whereas the αβ-tubulin binding surface of XMAP215 family TOGs is relatively flat, the equivalent surface of the CLASP TOG is arched in a way that appears to break the geometric match with curved αβ-tubulin (Leano et al., 2013; Fig. 2 D). This suggests that CLASP TOG domains might bind to an even more curved conformation of αβ-tubulin that has not yet been observed, that they do not simultaneously engage

α- and β-tubulin, or that they do something else. It is not yet clear how these different possibilities might contribute to the rescue-promoting activity of CLASPs. However, even though the biochemical and structural understanding of how CLASP TOGs interact with αβ-tubulin is less advanced than for XMAP215 family TOGs, the conservation of critical αβ-tubulin–interacting residues makes it seem likely that conformation-selective interactions with αβ-tubulin will play a prominent role.

The modulation of microtubule dynamics by XMAP215/CLASP family proteins ensures proper microtubule function in both interphase and dividing cells. As for the depolymerases, specific interactions with curved αβ-tubulin likely underlie the different regulatory activities of XMAP215/CLASP family proteins.

Sensing conformation at lattice contacts

Thus far, we have described how microtubule polymerases and depolymerases bind selectively to curved conformations of the αβ-tubulin dimer. These interactions play a significant role in the movement of tubulin dimers into and out of the microtubule polymer. Once in the polymer, αβ-tubulin dimers make contacts with neighboring tubulins. Recently, three MAPs were shown to bind microtubules at lattice contacts: (1) the Ndc80 complex, a core kinetochore protein; (2) doublecortin (DCX), a neuronal MAP; and (3) EB1, the canonical end-binding protein. Here we will summarize recent progress demonstrating how these proteins recognize distinctive features of lattice contacts.

The Ndc80 complex is a core component of the kinetochore–microtubule interface (Janke et al., 2001; Wigge and Kilmartin, 2001; McCleland et al., 2003), forming a "sleeve" that connects the outer kinetochore to microtubules of the mitotic spindle (Cheeseman et al., 2006; DeLuca et al., 2006). Loss of Ndc80 function leads to chromosome segregation errors in mitosis (McCleland et al., 2004; DeLuca et al., 2005). Ndc80 binds to microtubules at the longitudinal interface between α- and β-tubulin and extends outward toward the plus end at an ∼60° angle (Cheeseman et al., 2006; Wilson-Kubalek et al., 2008). Ndc80 binds to both the intradimer and interdimer interface and forms oligomeric arrays (Alushin et al., 2010). The binding of Ndc80 to this longitudinal lattice contact may confer a preference for straight rather than curved microtubule lattices, because the shape of the Ndc80 binding site is expected to change as a protofilament bends (Alushin et al., 2010; Fig. 3 A). Preferential binding to straight protofilaments might allow the Ndc80 complex to remain attached to the end of a shrinking microtubule. Indeed, reconstitutions of the Ndc80 complex interacting with dynamic microtubules show that the curved shrinking end acts as a "reflecting wall," giving rise to "biased diffusion" (Powers et al., 2009). Interestingly, the Ndc80 complex also promotes rescue (Umbreit et al., 2012), and selective binding to straight lattice contacts may contribute to this rescue activity.

DCX, a MAP expressed in developing neurons (Francis et al., 1999; Gleeson et al., 1999) and mutated in cases of subcortical band heterotopia (des Portes et al., 1998; Gleeson et al., 1998), is unique in its ability to bind specifically to 13-protofilament microtubules over other protofilament numbers (Moores et al., 2004; Fig. 3 B). DCX contains two nonidentical, microtubule-binding "DC" domains (Taylor et al., 2000) that share a ubiquitin-like fold (Kim et al., 2003). A cryo-EM reconstruction showed that a single DC domain binds to microtubules at the vertex of four tubulin dimers in the so-called "B" lattice configuration (Fourniol et al., 2010). The DCX binding site is ideally situated to detect the subtle changes at lattice contacts that result from different protofilament numbers, which range from 11 to 16 for mammalian microtubules (Sui and Downing, 2010). Despite their ideal location, protofilament preference is not a property of single DCX molecules. Rather, it is cooperative interactions between neighboring DCX molecules that are sensitive to the spacing between protofilaments (Bechstedt and Brouhard, 2012). In vitro, this selectivity enables DCX to nucleate homogeneous, 13-protofilament microtubules (Moores et al., 2004). The function of DCX in developing neurons remains unclear, with models ranging from microtubule stabilization (Gleeson et al., 1999) to regulation of kinesin traffic (Liu et al., 2012).

EB1, the canonical end-binding protein (Morrison et al., 1998), uses its calponin homology (CH) domain (Hayashi and Ikura, 2003) to bind the same lattice contact as DCX (Maurer et al., 2012). EB1 forms "comets" by binding rapidly and tightly to a distinct feature at the growing microtubule end but only weakly to the "mature" lattice (Bieling et al., 2007). Recent work has defined this distinctive feature as the nucleotide state. EB1 binds preferentially to microtubules built from GTP analogues (Zanic et al., 2009; Maurer et al., 2011). Combined with careful analysis of the size, shape, and dynamics of EB1 comets (Bieling et al., 2007), these results established that EB1 recognizes microtubule ends by binding specifically to the "GTP cap," which is an extended region of the microtubule end that is enriched with GTP- and GDP-Pi-tubulin dimers. A recent cryo-EM reconstruction of the CH domain of Mal3 (the *S. pombe* EB1) bound to GTPγS microtubules provided a possible structural mechanism for how EB1 might differentiate GTP from GDP lattices (Maurer et al., 2012; Fig. 3 C). Mal3 was observed to contact helix H3 of β-tubulin, which connects directly to the exchangeable nucleotide-binding site. EB1 also contacts the regions of α-tubulin that move during the compaction of the lattice that follows GTP hydrolysis (Alushin et al., 2014). Mutation of conserved EB1 residues that contact either helix H3 or the compacting region of α-tubulin disrupts the end-tracking behavior of EB1 (Slep and Vale, 2007; Maurer et al., 2012). Interactions with helix H3 and the compacting region of α-tubulin also enable EB1 to accelerate the transitions of tubulin from the GTP state to the GDP state; in other words, EB1 acts as a "maturation factor" for the microtubule end (Maurer et al., 2014). EB1 recruits a large network of plus-end-tracking proteins (Akhmanova and Steinmetz, 2008) through interactions with the EB1 C terminus (Hayashi et al., 2005; Honnappa et al., 2006) and EB1 homology domain (Honnappa et al., 2009). This diverse and complex protein network is essential for the regulation of microtubule dynamics, the capture of microtubule ends by the cell cortex (Kodama et al., 2003) and endoplasmic reticulum (Grigoriev et al., 2008), and the positioning of the mitotic spindle (Liakopoulos et al., 2003).

As mentioned earlier, microtubule ends also show unique structural configurations, namely tapered, outwardly flared, and flattened structures collectively described as "sheets" (Chrétien

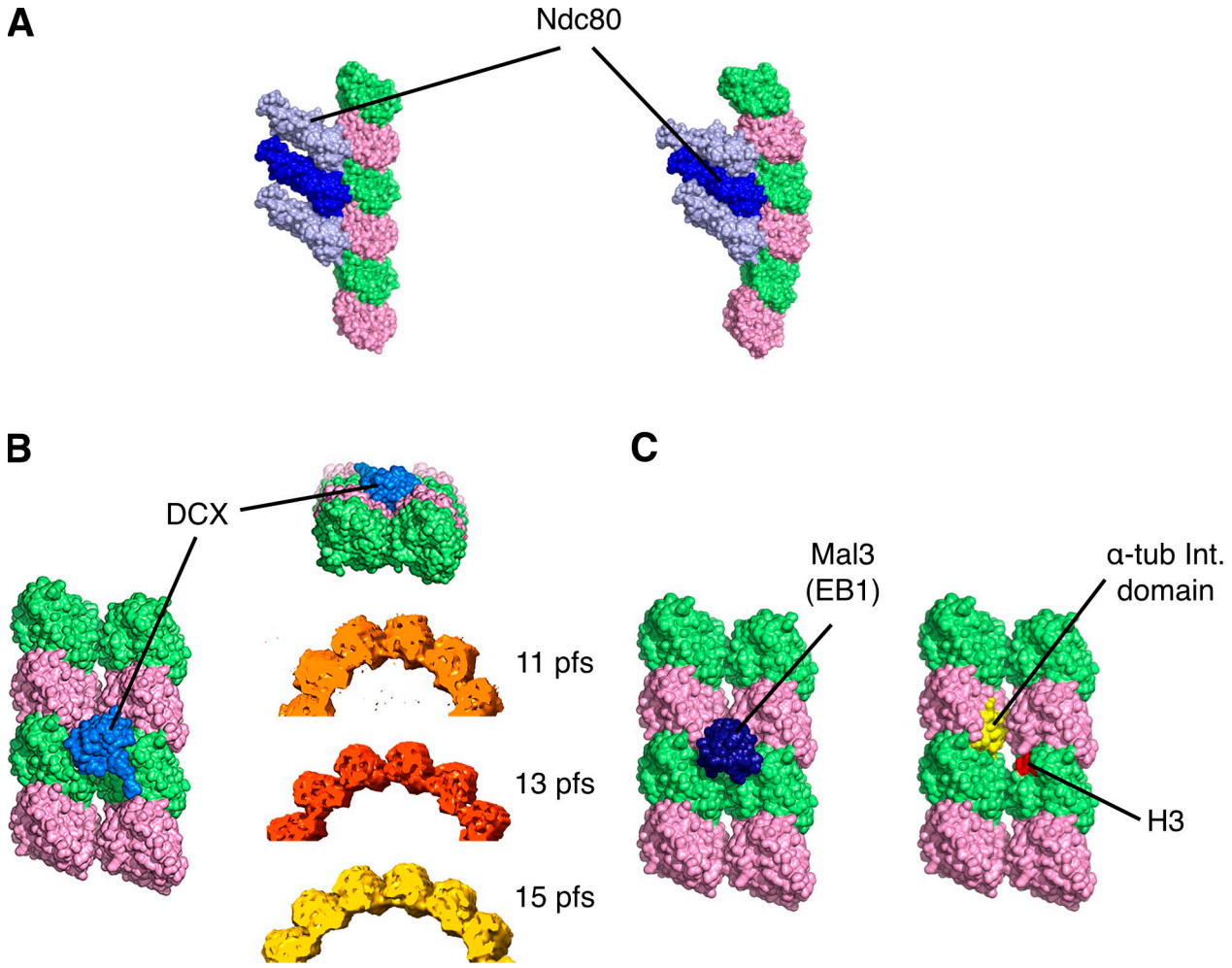


Figure 3. **Proteins that bind microtubules can distinguish unique configurations at lattice contacts.** (A) Ndc80 (light and dark blue) binds the contact within (dark blue) and between (light blue) αβ-tubulin heterodimers (pink and green). The left shows part of an Ndc80 array on straight protofilaments (PDB accession no. 3IZ0). The right shows that neighboring Ndc80 molecules clash when modeled onto a curved protofilament. Individual Ndc80s may read the conformation at a single joint, or the change in conformation may disrupt cooperative interactions between adjacent Ndc80s. (B) Two views of DCX (blue) binding a lattice contact at the vertex of four αβ-tubulins, PDB accession no. 4ATU. Cooperative interactions on the microtubule allow DCX to discriminate between the subtle changes that accompany different protofilament numbers (11: orange, EMDataBank [EMD] accession no. 5191; 13: red, EMD accession no. 5193; 15: yellow, EMD accession no. 5195). (C) EB1 (left, dark blue) binds at the same vertex as DCX (PDB accession no. 4AB0), but EB1 binds preferentially to GTP vertices over GDP vertices, and is not sensitive to protofilament number. The same section of microtubule with EB1 removed (right) shows the location of nucleotide-dependent changes at the four-way vertex: helix H3 of β-tubulin (red patch at the lower right of the four-way junction), and the intermediate (Int.) domain of α-tubulin (yellow patch at the top left of the four-way junction). pfs, protofilaments.

et al., 1995). The sheets contain distinctive lattice contacts, and recent work shows that the microtubule-binding activities of DCX and EB1 are sensitive to these structural features. DCX, for example, binds specifically to the outwardly flared sheets (Bechstedt et al., 2014), which enables DCX to track microtubule ends. Evidence for the ability of EB1 to recognize or control a distinct lattice configuration comes from the reconstitutions showing that EB1 promotes elongation synergistically with XMAP215 (Zanic et al., 2013): lack of a detectable direct EB1–XMAP215 interaction suggested that the observed synergy was mediated through alterations of the microtubule end structure itself. Further evidence that EB1 can affect the structure of the microtubule lattice comes from data showing that EB1 can nucleate "A" lattice microtubules in vitro (des Georges et al., 2008) and influence protofilament number distributions (Vitre et al., 2008; Maurer et al., 2012). The connection between the structure of microtubule ends, their nucleotide state, and microtubule dynamics is an important open question.

Conclusions and outlook

The αβ-tubulin dimer adopts a range of conformations as it moves in and out of the microtubule polymer, including changes to its intrinsic curvature and changes to its lattice contacts. These different conformations affect microtubule dynamics by altering the strength of lattice association and the rate of GTP hydrolysis. The work we discussed here has revealed an intimate linkage between these different conformations and the activities of key proteins that regulate microtubule dynamics. It is now clear that selective interactions with distinct conformations of unpolymerized and polymerized αβ-tubulin define the cell physiology of the microtubule cytoskeleton. Recently developed methods for purifying or overexpressing αβ-tubulin

(des Georges et al., 2008; Johnson et al., 2011; Widlund et al., 2012; Minoura et al., 2013) are facilitating structural studies and allowing the biochemistry of αβ-tubulin polymerization to be dissected in unprecedented detail. Microtubule structural biology is entering a golden age, where the pace of new structural information is accelerating. We anticipate that future crystallographic and high-resolution cryo-EM studies will define the strategies used by other MAPs to recognize and control the conformation of αβ-tubulin, and may reveal new conformations of αβ-tubulin inside and outside of the microtubule. Reconstitutions of microtubule dynamics are rapidly increasing in complexity and are beginning to reveal how the activities of multiple MAPs can reinforce or antagonize each other (Zanic et al., 2013). More complex reconstitutions are also defining the minimal requirements for creating cellular-scale structures like the mitotic spindle (Bieling et al., 2010; Subramanian et al., 2013). Reconstitutions will also greatly advance the understanding of the dynamics and regulation of microtubule minus ends. As the ever-advancing structural data are integrated with reconstitution data, incorporated into computational models, and correlated with cell biology experiments, a robust, multiscale understanding of microtubule biology will come within reach.

Due to our focus on structural biology, biochemistry, and in vitro reconstitutions, it was not possible to cite many of the outstanding cell biology papers germane to this review. L.M. Rice and G.J. Brouhard would like to thank S. Wolfson for editing, and M. Steinmetz for providing helpful comments. L.M. Rice would like to thank G.J. Brouhard for tolerating his taste in colors for tubulin dimers.

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