Microtubule Dynamics: an interplay of biochemistry and mechanics

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Abstract

Microtubules are dynamic polymers of αβ-tubulin that are essential for intracellular organization and chromosome segregation. Microtubule growth and shrinkage occur via addition and loss of αβ-tubulin subunits — biochemical processes. Dynamic microtubules can also exert forces by pushing or pulling against a load – mechanical processes. Recent advances at the intersection of biochemistry and mechanics have revealed the existence of multiple conformations of αβ-tubulin and their central role in dictating the mechanisms of microtubule dynamics and how microtubules do work. Microtubule associated proteins selectively target specific tubulin conformations to regulate microtubule dynamics, and mechanical forces can also influence microtubule dynamics by altering the balance of tubulin conformations. Importantly, the conformational states of tubulin dimers appear to be coupled throughout the lattice, in that the conformation of one dimer affects the conformation of its nearest neighbors and beyond. This coupling provides a long-range mechanism by which MAPs and forces can modulate microtubule growth and shrinkage. These findings provide evidence that the interplay between biochemistry and mechanics is essential for the cellular functions of microtubules.

Introduction

Microtubules are long, stiff polymers of αβ-tubulin that are structurally and functionally important components of the eukaryotic cell cytoskeleton, forming the mitotic spindle, the axonemes of cilia and flagella and serving as tracks for intracellular trafficking [1]. Microtubules are highly dynamic. They grow by the addition of GTP-tubulin dimers to the microtubule end, where a "stabilizing cap" is formed because GTP hydrolysis does not happen instantaneously[2]. This cap, which is enriched in GTP-tubulin, recruits "end-binding" (EB) proteins and a host of other microtubule-associated proteins (MAPs), including microtubule polymerases, depolymerases, and kinesins, which cooperate and compete to determine the microtubule's fate[3–5]. When the stabilizing cap is lost, the microtubule shrinks rapidly in an event known as a "catastrophe". Occasionally, a rapidly shrinking microtubule is "rescued", switching from shrinkage back to growth. This switching behavior, known as dynamic instability [6], can be harnessed by cells to exert forces, for example during chromosome segregation in mitosis [7].

Biochemical and mechanical phenomena are intimately intertwined in microtubules: rapid turnover and dynamic instability coexist with the rigidity of a stiff polymer [8]. This interplay between biochemistry and mechanics allows microtubules to be viewed from multiple perspectives. A biochemical perspective views microtubules as a collection of discrete subunits [9] that form a lattice with two main modes of interaction (Fig. 1). Recent developments in single-molecule microscopy [10] allow microtubule growth to be tracked with a resolution of ~40 nm [11]; this has enabled characterization of the behaviour of the stabilizing cap immediately prior to a catastrophe [12] and of the stochastic fluctuations in length that occur during elongation [13,14]. Recent measurements like these provide new insights into the biochemistry that underlies microtubule dynamics. A mechanical perspective, on the other hand, views microtubules as a continuous material [9]. Indeed, seminal measurements showed that microtubule stiffness is comparable to that of plexiglass [15].

The distinction between biochemical and mechanical perspectives is artificial[9], of course, because mechanical phenomena operate at the level of discrete subunits (Fig. 1C,D). The biochemical cycle of tubulin involves GTP-tubulin binding to the microtubule end, hydrolyzing the GTP and releasing phosphate while in the lattice, and then dissociating from the microtubule as GDP-tubulin. This review will advance the argument that changes in tubulin conformation that accompany this biochemical cycle are mechanical in nature. Conformational changes associated with nucleotide hydrolysis are harnessed to do mechanical work by a wide range of oligomeric proteins (e.g. [16,17] for some recent examples). We will argue that microtubules are unique because of the structural complexity of their growing and shrinking ends. First, we discuss the dynamics of the microtubule end and describe evidence that tubulin dimers in the growing end transition through multiple conformations in response to the biochemical cycle of GTP hydrolysis. We also describe new data that emphasize the importance of the tubulin conformational cycle for understanding how microtubule dynamics generates forces and how forces can affect microtubule dynamics. We then discuss data that makes it increasingly apparent that the conformational cycle of tubulin dimers is targeted by regulatory factors that control microtubule dynamics, providing additional evidence for the intimate links between mechanical and biochemical processes in microtubule dynamics and regulation[18]. Finally, we propose that long-range coupling between tubulin subunits may be the key for understanding microtubule dynamics, because it provides a framework that integrates mechanical and biochemical properties in the lattice.

Tubulin cycle at the microtubule end

The transition of a GTP-tubulin dimer in solution into a GDP-tubulin dimer entombed in the microtubule lattice is a complex process that involves multiple conformational changes. Below, we describe the structure that catalyzes this transition, the microtubule end, and the mechanical phenomena that underlie this process.

Structure of the microtubule end

For growing microtubules, "the end" is often synonymous with the "stabilizing cap", which describes the extended region near the growing end that is enriched in GTP-tubulin subunits. Although the exact distribution of nucleotide states is currently not known, the stabilizing cap contains several hundred tubulin dimers [19]. In cryo-EM, growing microtubule ends have been observed in a broad range of structures, from short, blunt ends [20] to long, outwardly-curved, and flattened structures interpreted as "sheets" of tubulin [21]. As a result, "the microtubule end" remains poorly defined. Our lack of certainty can be observed in the diverse schematics of microtubule ends that appear in reviews, research articles, and textbooks. These schematics range from large, elaborate sheets to simple, tapered structures that neglect the curvature of microtubule ends entirely. The most common schematics depict the growing microtubule end as a funnel. These funnels appear in undergraduate textbooks [22] and on conference posters. While scientists are not necessarily artists, and we differ in our tolerance for flourish, it's fair to say that the field has no consensus about what a microtubule end actually looks like.

The major features of microtubule ends are nevertheless consistent across the literature: some protofilaments are longer than others, the distal protofilaments curve outward to some degree, and there is a 'seam' where lattice contacts are perhaps weaker because the helical symmetry is interrupted there[23,24]. Box 1 describes our best faith effort to illustrate average values for taper length and curvature that are consistent with findings from cryo-EM[25,26] computational modelling [27,28] and fluorescence microscopy [11,13]. The data indicate that the microtubule end is tapered, ragged, and variably-curved (Box 1), even if the specifics of these features remain poorly determined.

Box 1

Model for a growing microtubule end, and how it was constructed

The structure of the microtubule end is currently elusive. Here, we show our best-faith effort to construct an atomic model of a growing microtubule end (see figure). We considered two features that distinguish growing microtubule ends from the rest of the lattice, for which atomic models exist [127]. First, microtubule ends are tapered, with a "taper-length" defined as the distance between the longest protofilament and the fully-closed lattice. We chose a taper-length of 124 nm, which is consistent with the average value measured directly by cryo-EM (Fig. 3B of [25]) and with estimates based on sub-pixel fitting of fluorescence images (Fig 5D of [13] and Fig. S2 of [11]). Second, microtubule ends are variably curved due to the fact that GTP-tubulin adopts a curved conformation. Our model was informed by a theory wherein microtubule ends are shaped by two opposing forces: (1) the tendency to curve outward due to the intrinsic curvature of GTP-tubulin [30,33–35] and (2) the tendency to straighten due to lateral interactions between subunits that close up the lattice [125]. The precise relationship between curvature of the tip and its width is not understood. We assumed: (i) that a section of the microtubule end becomes fully-straight [24] when it is at least 6 αβ-tubulins wide (Fig. 4a″ of [25]); (ii) sections of the microtubule end that are 2 αβ-tubulins wide are approximately half-straightened [26]; (iii) sections of the microtubule end that are 3–5 subunits wide become

progressively straighter [125]. Conformations with curvature in between half-straight [26] and straight [24] were approximated by interpolation ('morphing'). Finally, we added some "raggedness" to the end, a feature that occurs frequently in computer simulations of microtubule dynamics [27,28].

Our final model used an average value for taper-length. The stochastic nature of subunit addition and loss will cause the taper length and overall curvature of growing microtubule ends to fluctuate considerably around their average values. These and other uncertainties highlight the challenge of understanding how the biochemical and mechanical properties of individual αβ-tubulin subunits dictate the structure and dynamics of the growing microtubule end.
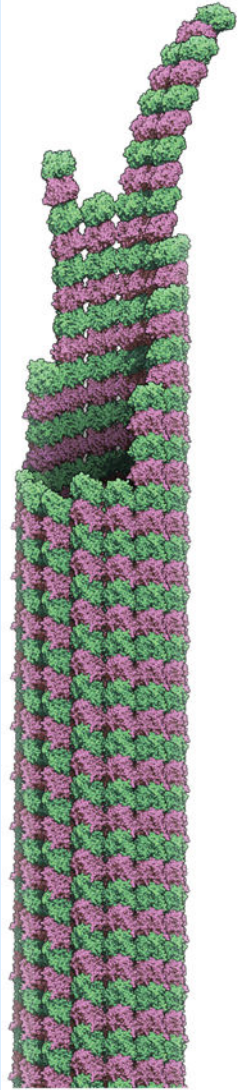
Tubulin dimers in the cap undergo a mechano-chemical cycle

Individual dimers pass through at least three conformations during microtubule growth and shrinkage (see Figure 1C, D). These conformations are described as (1) curved (Fig. 1C), (2) expanded (Fig. 1C), and (3) compacted (Fig. 1C) based on recent cryo-EM reconstructions [24,29] and crystal structures [30]. We argue that these conformations can be considered "mechanical states" because they are part of a cycle that stores and releases strain energy (Fig. 1D) [31,32]. Unpolymerized GTP-tubulin dimers start out in the curved conformation [33–35] (reviewed in [18]), which is characterized by a ~12° kink at the intradimer interface. After binding to the microtubule end, the dimers straighten into an expanded conformation, a process that is thought to introduce strain energy into the microtubule lattice. Following GTP hydrolysis and phosphate release, the dimers compact in the lattice, shortening in length by 3 Å through a movement of a subdomain of α-tubulin (Fig. 1C) [29]. Whether microtubules from all species undergo compaction has not yet been established. Indeed, recent structures of yeast microtubules did not show compaction in the GDP lattice [36,37]. Regardless, after catastrophe, GDP-tubulin dimers relax back to the curved state, peeling outward into the "ram's horns" observed by cryo-EM [20]. This relaxation releases the strain energy stored in the dimer, which can be harnessed to perform work [32], and completes the mechanical cycle (Fig. 1D).

Mechanics in the tubulin cycle

Direct evidence that the conformational cycle of tubulin is mechanical can be found in studies of how microtubules generate and respond to force. A growing microtubule generates a pushing force when it encounters a barrier [38]; this pushing force can center microtubule asters [39], mitotic spindles [40], and nuclei [41]. Conversely, a shrinking microtubule generates a pulling force on objects that are "coupled" to the microtubule end. The signature "coupler" is the kinetochore [32], a large molecular machine that attaches chromosomes to the growing and shrinking ends of a microtubule. Pulling forces arise from the outward peeling of protofilaments, which either push directly on the coupler [42] or bias its diffusion [43] on the microtubule lattice. Importantly, compressive stress and tensile stress generated by these forces can affect the dynamic instability of microtubules themselves.

The response to tensile stresses generated by pulling forces provides a clear example of forces altering the mechanical state of tubulin. Tensile stresses occur when a shrinking microtubule pulls on a "coupler" such as a kinetochore or, conversely, when the coupler pulls on a growing microtubule end. Both cases of tensile stress can be reconstituted *in vitro* and studied using optical tweezers. The couplers in these *in vitro* assays have included direct linkages [42], purified kinetochore proteins [43–46], as well as intact kinetochore particles from budding yeast [47]. Remarkably, applying tension to a growing microtubule via an intact kinetochore particle causes the microtubule to grow faster [47]. Similarly, applying tension via a microtubule polymerase in the XMAP215 family also caused faster growth[48] although other coupling proteins did not[45]. Perhaps tension increases the enzymatic cycle of XMAP215 family polymerases? Kinetochores contain a subunit with microtubule polymerase activity (e.g., in budding yeast the polymerase is Stu2 [49], an XMAP215 homolog), but, surprisingly, kinetochore particles completely depleted of Stu2 retain the

ability to make microtubules grow faster [50]. So why does tension applied through intact kinetochore particles lead to faster microtubule growth? The explanation may be that tension applied to the microtubule end may alter the distribution of tubulin conformations at the microtubule end and/or its taper and curvature. According to this view, pulling on a microtubule might promote the straightening of curved dimers at the end or antagonize the compaction of expanded dimers in the stabilizing cap. It remains unclear how such changes would lead to faster growth, and why intact kinetochores particles and XMAP215 do this while other couplers do not.

The key to understanding the relationship between microtubule dynamics and forces will be continued measurements of the relationship between the two. As with the experiments above, optical trapping currently provides the best way to do this. In a recent study, the amount of work performed by a shrinking microtubule was measured using optical tweezers [51], building upon a related assay pioneered by others [42]. In this assay, the depolymerizing microtubules "push" on a bead, and the magnitude of the bead deflection reflects the shape of the curling protofilaments. Interestingly, increasing tension on the bead led to reduced deflections, indicating that tension was reducing the curvature of the outwardly peeling protofilaments. The work output per tubulin dimer was estimated at ~4.7 k_BT, which is approximately 25% of the free energy available from GTP hydrolysis. This efficiency of this conversion of free energy to work is similar to that demonstrated for molecular motors like kinesin and dynein [52,53]. It is commonly assumed that the strain energy released as work is what makes microtubules shrink rapidly after a catastrophe. However, microtubules harbouring a slow-shrinking β-tubulin mutant (T238V, see Box 2) were shown to perform the same work as wild-type [51]. This counter-intuitive result could be explained by a physical model in which the T238V mutant makes stronger lateral bonds and the shrinking rate is determined by the breaking of lateral bonds rather than by the dissociation of dimers [51]. But the T238V mutation is buried and far from tubulin:tubulin interfaces, so its stronger lateral bonds cannot be explained by direct perturbation of that interface. Instead, the stronger lateral bonds must arise from an allosteric effect. Indeed, mutations at T238 alter the conformational cycle of αβ-tubulin dimers in the lattice [51,54]. Therefore, one hypothesis is that T238 mutants adopt a more GTP-like conformation in the GDP lattice, and this shift in conformational preference gives rise to stronger lateral bonds. Testing hypotheses like these will require structures of mutant tubulins. Whatever the precise structural mechanism, these optical trapping studies demonstrate that the balance of αβ-tubulin conformations in the lattice influences microtubule dynamics by tuning the strength of lateral bonds.

Box 2

Recombinant αβ-tubulin: a new tool to study microtubule dynamics

The current understanding of microtubule biochemistry has relied almost exclusively on αβ-tubulin derived from mammalian (mostly bovine) brain preparations. While 'brain preps' have large yields, the resulting αβ-tubulin is a mixture of multiple α- and β-tubulin isotypes and varies in its degree of post-translational modifications[128]. The path to recombinant αβ-tubulin began with its purification from genetically tractable model organisms[129,130]. Subsequent work showed that fusing an affinity tag to β-tubulin could

improve purification without compromising function[131], showed isotype-specific polymerization dynamics[132], and probed the binding sites for taxol[133] and kinesin[134,135]. The potential value of recombinant tubulin was clear.

These early methods had poor yields and were restricted to viable mutants. Inducible overexpression in yeast[136] improved yields and expanded the scope of purifiable mutants to include non-viable mutants. Methods for purifying recombinant human αβ-tubulin from baculovirus-infected insect cells soon followed[137–139].

Illuminating mechanisms of MAPs and motors

A polymerization-blocked mutant of yeast tubulin[136] led to structures of complexes between αβ-tubulin and the TOG1 or TOG2 domains from Stu2 (see main text)[81,82]. Recombinant point mutants of tubulin created microtubules that separated the motility and depolymerase activities of the kinesin-8 family Kip3[73]. Motor proteins generally interact with the unstructured C-termini of αβ-tubulin, which previously could only be removed by proteolytic cleavage. Recombinant, chimeric microtubules in which human C-terminal tails were fused to a yeast tubulin core, demonstrated that specific tubulin tails or post-translational modifications affected different motor proteins in distinct ways[140].

Revealing properties of individual tubulin isotypes

Humans have 7 α-tubulin genes and 8 β-tubulin genes, some only expressed in specific cell types. Studies with recombinant, single-isotype mammalian αβ-tubulin discovered different polymerization dynamics for the isotypes relative to bovine brain tubulin[138,139,141], echoing observations for different yeast isotypes[132]. Although αβ-tubulin isotypes differ mainly in the sequence of their C-terminal tails, in at least one case the differences in dynamics could be attributed to sequence variation in the more conserved structured core of the protein[141]. Thus, all positions, not just those in the tails, can contribute to the functional divergence of tubulin genes.

Insights into allosteric properties of the lattice

Insight into the connection between the αβ-tubulin conformational cycle and polymerization dynamics came from studies of the buried T238A mutation in the yeast β-tubulin[54]. β:T238 mutations suppress catastrophe, slow microtubule shrinking, and increase spontaneous nucleation, with little effect on microtubule growth rates[54]. β:T238 αβ-tubulin retains a GTP-like conformation in the GDP lattice[51,54], providing a structural correlate to the observed changes in dynamics. Point mutations in β-tubulin associated with a human genetic disorder altered microtubule growth rates and/or catastrophe frequencies, even though the mutations are not found at polymerization interfaces[138], but the underlying mechanisms remain to be clarified.

Together, these studies emphasize the mechanical nature of the αβ-tubulin conformational cycle. Microtubules push and pull, and the energetics of individual dimers are altered by the resulting compressive and tensile stresses.

Conformational cycle and the role of MAPs

The conformational cycle of tubulin establishes the size and composition of the microtubule's stabilizing cap, loss of which is the cause of dynamic instability [6]. In recent years, enabled by improved fluorescence microscopy assays [10], studies of the EB proteins [4,11,12,19,55–60] have provided a wealth of new insights into the size, composition, and dynamics of the stabilizing cap. Studies of EBs and other MAPs have revealed that MAPs take advantage of the tubulin conformational cycle in order to regulate microtubule dynamics. Yet knowledge about the distributions of tubulin conformations and nucleotide states in the stabilizing cap, and how they relate to each other, remains an important gap in understanding. In this section we will discuss how MAPs interact with the mechanical cycle of tubulin, providing evidence that biochemical regulation of microtubule dynamics through MAPs is strongly tied to the conformational changes of tubulin dimers.

Interplay between EB proteins and tubulin conformations

Autonomous plus-end tracking by an EB protein was first reconstituted in vitro using Mal3, the S. pombe EB ortholog [19]. Mal3 marked a "comet" region hundreds of nm in length (Fig. 2A) and also increased the frequency of microtubule catastrophe [19]. Similar observations followed for vertebrate EBs [57]. Single-molecule and kinetic analyses revealed the high-affinity EB-binding site on the microtubule to be transient, with a lifetime of about 10s [19]. Simultaneous imaging of the comet and the microtubule demonstrated that the comet region does not extend all the way to the very end of the growing microtubule [11]. Instead, there is an "EB-free" zone at the very end (Fig. 2A) [11] that might overlap with the partially curved, tapered region [25]. The presence of this zone indicates that the high-affinity EB binding site takes time to develop.

Interestingly, increasing the concentration of EB decreases the size of the EB comet and causes it to shift closer to the microtubule end (Fig. 2A) [11], thereby reducing the size of the "EB-free" zone. Thus, it seems clear that EBs both recognize particular sites on the lattice and influence the creation and destruction of these sites. These EB concentration-dependent effects have led to the hypothesis that EBs are "maturation factors" that increase the rate of GTP hydrolysis in the lattice [11,24]. This hypothesis explains how increasing the concentration of EB can increase the catastrophe frequency, because faster GTP hydrolysis should lead to smaller stabilizing caps. By revealing and quantifying a transient and distinctive region at the growing end of MTs that could be marked and altered by EBs, these studies provided provocative new insight into the molecular events occurring near the growing microtubule end.

Although the dynamics of EB comets have been measured with increasing accuracy and precision [11], the nature of the high-affinity EB site remains poorly defined. Important clues have come from experiments using GTP analogs. EBs bind with comparable affinity to comets and to static microtubules containing GTPγS, a hydrolysis-resistant GTP analog, so GTPγS microtubules are thought to mimic the comet region of growing microtubules [58]. In contrast, EBs bind less tightly to microtubules containing GMPCPP, a different hydrolysis-resistant GTP analog that yields an 'expanded' conformation of tubulin in the lattice [29]. GMPCPP is thought to provide the best model for the GTP state of the lattice because it

supports microtubule polymerization better than GTPγS or other analogs [61]. These experiments have been taken to suggest that EB proteins bind preferentially to the GDP-Pi state.

The first cryo-EM reconstruction of EB-bound GTPγS microtubules showed that EBs bind at the vertex of four αβ-tubulin dimers (Fig. 2B) [59]. At higher resolution, αβ-tubulin in the EB-bound GTPγS lattice adopted a "twisted and compacted" conformation that differs from the compacted, GDP-bound conformation with respect to the relative rotation between αβ-tubulins along a protofilament (Fig. 2C) [24]. Consistent with this structural difference, EBs bind to the GDP lattice about 10-fold less tightly than they bind to the comet region or GTPγS microtubules [58]. EB binding can also influence the conformation of tubulin in the lattice [36]. The EB-GTPγS structure may indicate that EBs bind preferentially to a "post-GTPase" nucleotide state of the lattice such as GDP-Pi [24]. It is not yet clear whether EBs from all species induce/prefer a "twisted and compacted" state; the structure of Mal3 bounds to *S. pombe* microtubules was distinct[37]. Another caveat is that EB proteins have some affinity for all lattices[58,60], a fact which may explain why EBs have been observed by cryo-EM within the "EB-free zone[25]." Finally, whether GTPγS faithfully mimics a GDP. Pi state is not known. Nevertheless, preferential binding to a post-GTPase state of the lattice would also explain the "EB-free zone" at the very end of a microtubule, because in this zone tubulin would be in its expanded conformation.

This "post-GTPase" hypothesis is at odds with the observation that *increasing* the concentration of EB *decreases* the size of the EB comet [11]; this effect on comet size has been proposed to occur because EB binding increases the rate of GTP hydrolysis in the microtubule [11,24]. However, to increase the rate of GTP hydrolysis, EBs should bind to the lattice *before* hydrolysis (pre-GTPase), not after. Thus, there is an apparent conflict between EB's preference for a "twisted and compacted" GTPγS lattice (supposedly a post-GTPase phenomenon) and the concentration dependence of EB comet size (rationalized as a pre-GTPase phenomenon). One way to resolve this conflict is to move away from the viewpoint that the conformation of tubulin is only determined by its nucleotide state. As will be discussed in the final section of the article, we argue that long-range mechanical coupling between subunits in the lattice could explain how EBs can stimulate GTP hydrolysis while binding preferentially to what appears to be a post-GTPase state of the lattice.

The conformation cycle underlies regulation by microtubule polymerases and depolymerases

EB proteins currently provide one of the best-understood regulatory mechanisms for microtubule dynamics, but recent studies of other MAPs indicate that, like for EBs, they bind selectively to specific conformations of tubulin and thereby modulate the tubulin conformation cycle.

Whereas EB proteins modulate the transitions between expanded and compacted conformations within the microtubule lattice, a growing number of proteins regulate transitions between straight and curved conformations of αβ-tubulin at the growing microtubule end. The first protein shown to bind preferentially to the curved conformation of αβ-tubulin was Op18/stathmin [62,63], which uses this activity to sequester unpolymerized

αβ-tubulin. It is now clear that microtubule depolymerases of the kinesin-13 family also bind preferentially to, and can induce, curved αβ-tubulin [64] on the microtubule end [65,66]. Kinesin-13's use this activity to trigger catastrophe [67–69]. Binding preferentially to curved tubulin may be a universal feature of microtubule depolymerases. Indeed, a recent study of the cooperative microtubule depolymerase Kip3 [70–72] from the kinesin-8 family indicates that it also interacts preferentially with curved αβ-tubulin (see also Box 2) [73], and that this property underlies its depolymerase activity [73]. Despite sharing the same overall kinesin fold, Kip3 and MCAK evolved distinct ways to bind preferentially to curved αβ-tubulin [64,73–75]. These studies demonstrate that microtubule dynamics depends on the balance between straight and curved conformations on the microtubule end and that depolymerases act by favouring curved conformations.

Surprisingly, preferential binding to curved αβ-tubulin can also be used to promote microtubule growth. Indeed, microtubule polymerases in the Stu2/XMAP215 family make microtubules grow faster [76–80] using TOG domains that bind preferentially to curved αβ-tubulin [81,82]. Recent advances in the mechanistic understanding of these proteins further illuminate the central role of mechanical coupling and conformational transitions in microtubule dynamics and regulation.

In the absence of unpolymerized αβ-tubulin, isolated polymerase TOG domains will depolymerize stabilized microtubules, presumably by stabilizing a curved, more dissociation-prone conformation of αβ-tubulin on the microtubule end [54]. Yet when linked in the intact protein, in the presence of unpolymerized αβ-tubulin multiple TOGs catalyze polymerization. How can this be? While unpolymerized αβ-tubulin is curved and binds tightly to TOGs, lattice interactions induce straighter conformations that bind weakly to TOGs. As long as the straightening-inducing interactions with the lattice are stronger than the TOG:αβ-tubulin interactions they compete with, the TOG domains will 'hand-off' their bound tubulins to the lattice, allowing the polymerase to promote growth by accelerating the delivery of new subunits.

These examples drew mainly on studies of how selected MAPs control microtubule dynamics by influencing and being influenced by the conformation of tubulin subunits. It will be fascinating to discover if related logic underlies the activity of less understood regulatory proteins like Kinesin-family polymerases [83–86], TOG-based rescue factors (e.g. CLASPs [87,88]), anti-catastrophe factors like TPX2 (see below), and others [89,90]. Combining these MAPs together with αβ-tubulin mutants that have altered conformational cycles ([54], see also Box 2) should yield more direct insight into the mechanical underpinnings of microtubule regulation.

Implications for nucleation and rescue

In the previous sections, we focused on how the conformational cycle of tubulin dimers is linked to microtubule growth and catastrophe. Notably, similar processes also operate during microtubule nucleation [91,92] and rescue [93].

Microtubules can nucleate spontaneously (form 'de novo') when unpolymerized αβ-tubulin oligomerizes in solution (Fig. 3A). Oligomers below a certain size are unstable but growth becomes favourable after a "critical nucleus" is formed [94,95]. Because tubulin oligomers grow from soluble GTP-tubulin, which is curved, these small oligomers must also have curvature and may resemble growing microtubule ends. The kinetics of microtubule nucleation show a high degree of cooperativity [96,97], suggesting that the critical nucleus requires multiple, kinetically unfavourable steps to form.

Cells are thought to bypass these unfavourable steps by using templates like the γ-tubulin ring complex (γ-TuRC) [98,99] or newly severed microtubule ends (e.g., [100]) to nucleate microtubules. In vitro, templates like the γ-TuRC or stabilized microtubule 'seeds' do accelerate microtubule formation and reduce the tubulin concentration required to observe elongation, but this 'templated nucleation' still shows a time lag [101]. The presence of this lag, which is unexpected [102], indicates that templates imperfectly mimic the microtubule end (Fig. 3B).

Why might blunt templates be imperfect substrates for elongation compared to a growing microtubule end? One source of imperfection may be that templates like the γ-TuRCs are blunt rings rather than the tapered, variably-curved arrangements of αβ-tubulin normally found at growing microtubule ends (Fig. 3B; also see Box 1). αβ-tubulin may associate onto a blunt template with lower affinity because blunt templates require more straightening of the incoming subunits, and/or because blunt templates lack the 'raggedness' that generates favourable binding sites. The maturation of a blunt template into a tapered, variably-curved microtubule end can be accelerated in vitro by MAPs that interact preferentially with partially curved tubulin conformations, that stabilize αβ-tubulin:αβ-tubulin interfaces, or that accelerate the association of αβ-tubulin [101].

Several MAPs have been shown to regulate nucleation (e.g. [103–105], and new data shows they use conformation-based mechanisms to do so. XMAP215 family proteins provide an example of how accelerating the binding of αβ-tubulin to microtubule ends can promote nucleation [101,105,106], which depends on XMAP215's conformation-specific interactions with tubulin dimers (Fig. 3C, left). TPX2 (targeting factor for Xklp2) is a spindle assembly factor in the chromatin-mediated microtubule nucleation pathway [107–109]. TPX2 suppresses catastrophe and slows post-catastrophe shrinking in vitro [101,106,110]. TPX2 also reduces the lag for templated nucleation [101], which may be critical to its function given that TPX2 also interacts directly with γ-TuRCs during branching nucleation [111,112]. TPX2 can also stabilize "stubs" (small oligomers) of tubulin that act as nuclei for microtubule formation in vitro [106]. Suppressing catastrophe and stabilizing small oligomers of αβ-tubulin indicates that TPX2 stabilizes an αβ-tubulin:αβ-tubulin interface, and a recent cryo-EM structure showed TPX2 binding across both longitudinal and lateral interfaces [113]. Interestingly, TPX2 also binds preferentially to partially curved tubulin structures at microtubule ends [106]. The unrelated protein doublecortin (DCX), which is expressed in developing neurons, provides another example of a MAP that regulates nucleation in vitro [114]. DCX also binds to partially curved tubulin structures [115] by binding at the vertex of 4 αβ-tubulins [116]. DCX nucleates microtubules spontaneously with extraordinary efficacy [117,118,119]. Thus, two MAPs that stabilize αβ-tubulin:αβ-tubulin interfaces in partially curved tubulin assemblies at

microtubule ends (Fig. 3C, right) show strong nucleation activity, indicating that microtubule ends and nucleation intermediates are structurally similar. Interacting with conformations found in these intermediates may be the key to regulating nucleation.

Depending on the conformation being stabilized, MAPs can also inhibit nucleation (Fig. 3C, right). For example, EB1 and MCAK each reduce the rate of templated nucleation and increase the concentration of tubulin required to observe elongation [101]. As discussed above, EB1 may accomplish this by promoting a twisted and compacted state of αβ-tubulin [24] in the GTP lattice that may have higher GTPase activity and/or be a less favourable substrate for elongation. MCAK antagonizes nucleation because it prefers fully curved conformations of tubulin that are not compatible with the variably-curved structures present at the growing microtubule end and in nucleation intermediates (Fig. 3C, right).

The lag associated with nucleation on a blunt template thus reflects an energetic barrier to develop a properly structured microtubule end. By modulating what must be a delicate balance between different conformations of tubulin in the lattice, MAPs can either promote or inhibit this development.

The transition from a blunt template to a "true", persistently-growing microtubule end is hard. It must be even harder to rescue a shrinking microtubule by converting the outwardly peeling protofilaments back into a growing microtubule end. This difficulty may explain why rescues are rarely observed *in vitro*. In contrast, rescues are readily observed in tissue culture cells likely owing to the presence of "rescue factors". Perhaps the best-characterized rescue factor is Cls1, the CLASP family protein in *S. pombe* [88]. CLASP-family proteins [87] contain TOG domains, but some of these TOGs appear to prefer a different conformation of tubulin [120] compared to the TOGs from microtubule polymerases. Thus, CLASPs may facilitate rescue by interacting with a specific conformation of tubulin that occurs during protofilament peeling. However, little is currently known about how the CLASP TOGs actually contribute to rescue.

An unexpected connection between rescue and microtubule mechanics comes from recent work examining how microtubules respond to bending stress [121], which exemplifies how mechanical forces can alter the biochemical properties of tubulin in the lattice. In this work, microtubules were bent repeatedly by controlled fluid flow in a microfluidics chamber; the microtubule became increasingly flexible with each bend. Normal stiffness could be restored over time in the presence of soluble tubulin, suggesting that the mechanical stress from repeated bending damaged the lattice, and that fresh tubulin could enter and repair these damaged sites. Sites of photodamage can also be repaired by fresh tubulin [121]. Interestingly, microtubule rescues cluster at sites of repair *in vitro* [122]. In cells, sites of damage also appear to undergo repair and facilitate rescue [122]. Indeed, cells may protect their microtubules from damage via acetylation [123,124], but whether and how acetylation is linked to mechanics remains unclear.. Although we do not understand what "repair" entails, rescues may occur near repaired sites because the fresh tubulin stabilizes the region and reduces or eliminates protofilament peeling. Under normal circumstances, regardless of nucleotide state, tubulin below the cap and in the body of the lattice is thought to be so tightly incorporated that it does not dissociate appreciably. By demonstrating that the mechanical deformations

accompanying bending can significantly alter how strongly tubulin is bound in the lattice, this line of work provides clear new evidence for the intimate connections between material properties of microtubules and the biochemical properties of the individual subunits.

Long range mechanical coupling in the lattice

The complex structure of the microtubule end and the presence of expanded, compacted, and variably-curved conformations of tubulin therein that differently interact with each other and that are modulated by MAPs raise a number of questions about how the mechanical states of tubulin affect the biochemistry of tubulin–tubulin interfaces and vice versa. For example, what drives the straightening of curved GTP-tubulin dimers when they assemble into the lattice at the growing end or in nucleation intermediates? It has been proposed that straightening would be driven by the alignment of lateral interfaces between neighboring dimers [34]. The presence of variably-curved conformations at the end implies that the straightening of one dimer both requires and facilitates the straightening of its neighbors [125] (Fig. 4B). These interactions describe a kind of allostery, wherein the effect of one tubulin binding to another is transmitted to other interfaces via changes in curvature.

Likewise, how does the compaction of a single tubulin dimer affect its neighbors? The expanded GTP-tubulin lattice and compacted GDP-tubulin lattice are incommensurate [29]. The mismatch between these conformations must introduce structural conflicts near the microtubule end where the lattice contains a mixture of nucleotide states. Resolution of these conflicts, wherein tubulin dimers change conformation to accommodate their mismatched neighbors, may give rise to long-range coupling in the lattice: the effects of a conformational transition in one tubulin may propagate beyond its immediate neighbors to alter the conformation or biochemistry of more distant subunits in the lattice. For example, when one αβ-tubulin compacts in an otherwise expanded GTP-lattice, the resulting structural mismatch likely creates a kind of stress that makes neighboring tubulins more likely to compact, which would in turn make their neighbours more likely to compact, and so on (Fig. 4A). This conformational coupling could in principle over-ride or modulate the effect of nucleotide state on tubulin conformation. Because of this mechanical coupling in the lattice, the conformation of an individual αβ-tubulin might not be uniquely determined by its nucleotide state but rather also by the conformation of its neighbors. Such long-range mechanical coupling would make the microtubule end a highly-cooperative structure capable of abrupt transitions.

Long-range coupling in the lattice provides a new way to think about how regulatory proteins like EBs could interact with microtubules and influence tubulin conformation in the lattice. Recall the pre-GTPase vs. post-GTPase paradox: EBs appear to accelerate the rate of GTP hydrolysis while binding preferentially to what has been thought to be a post-GTPase conformation in the lattice. These behaviours are hard to reconcile with a view in which an individual αβ-tubulin's conformation is uniquely determined by its nucleotide state. Long-range coupling in the lattice may resolve this paradox (Fig. 4C). An interesting recent study of XMAP215 and EB1 revealed synergistic action that may result from long-range coupling in the lattice [126]. Indeed, when EBs bind to and stabilize a "twisted and compacted" conformation at one site, long-range coupling means the EB-induced change in

conformation could propagate some distance through the lattice. If the corresponding changes in conformation also elevate the rate of GTP hydrolysis at remote sites in the stabilizing cap, then EB's preference for a "twisted and compacted" conformation at one site has the potential to accelerate GTP hydrolysis elsewhere. This hypothesis, which is speculative, should ultimately be testable using αβ-tubulin mutants with altered conformational cycles. The idea that long-range mechanical coupling might influence GTP hydrolysis throughout the stabilizing cap also has implications for the mechanism of microtubule catastrophe: mechanical coupling creates the possibility for positive feedback and threshold effects, wherein increasing compaction within the stabilizing cap drives GTP hydrolysis and cap loss. We speculate that whether acting through GTPase activity or not, lattice-propagated modulation of αβ-tubulin conformation and biochemistry, and the threshold-dependent effects that may accompany this long-range coupling, will be important for understanding fundamental mechanisms of microtubule dynamics in general.

Conclusion and perspective

The field has made remarkable progress advancing the biochemical and mechanical understanding of microtubule polymerization dynamics. New structural states of αβ-tubulin have been defined in atomic detail [24,29]. We are beginning to appreciate how the transitions between these states create the phenomenon of dynamic instability [54], and how MAPs can manipulate these transitions to modulate microtubule dynamics [73,74,81,82,90,113]. Our understanding of material properties of microtubules – how they generate and respond to force, and transmit information over nearest-neighbour and longer distances – has increased apace [42,51,126]. However, we still lack an understanding of how biochemistry determines mechanics and of how mechanics modulates biochemistry. Integrating these two perspectives remains a major challenge for the future.

An integrated understanding of microtubule dynamics and regulation that unifies mechanical and biochemical perspectives will require deeper insight into the conformations of αβ-tubulin and their relative energetics, new knowledge about how regulatory factor binding can modulate αβ-tubulin conformation and energetics, and the ability to detect and quantify the contribution of mechanical coupling effects. Although formidable challenges remain, the remarkable recent leaps forward leave us optimistic about the prospects for substantial progress in the near future.

Acknowledgments

Glossary

Microtubule plus end

The end of the microtubule that is crowned by β-tubulin subunits. This more dynamic end of the polymer typically grows from the middle of the cell toward the periphery.

Microtubule minus end

The end of the microtubule that is crowned by α-tubulin subunits. This less dynamic end of the polymer is typically capped or anchored in a microtubule organizing structure like the centrosome.

Catastrophe

The switch from microtubule growing to microtubule shrinking.

Rescue

The switch from microtubule shrinking to microtubule growing.

Nucleation

The process of forming a microtubule *de novo* (from unpolymerized αβ-tubulin subunits) or on a template such as the γ-tubulin ring complex or a severed microtubule end.

γ-Tubulin ring complex (γ-TuRC)

A conical oligomer of γ-tubulins and gamma-ring complex proteins (GCPs) that creates a template for microtubule nucleation.

TPX2

A spindle-assembly factor that functions in the Ran-GTP pathway. TPX2 is released from importins in the vicinity of chromosomes, where it stimulates microtubule nucleation and interacts with motor proteins.

Compaction

A conformational change posited to occur upon GTP hydrolysis in the microtubule lattice, based on distinct conformations of αβ-tubulin observed in structures of mammalian microtubules.

End-binding (EB) protein

a family of evolutionarily conserved proteins that autonomously track the growing microtubule end. EB proteins regulate microtubule dynamics and are also responsible for recruiting the majority of other plus-end tracking proteins to the growing microtubule end.

Microtubule polymerase

Regulatory factor that promotes microtubule elongation, typically by making microtubules grow faster.

XMAP215/Stu2

The best-studied family of microtubule polymerases. Polymerases in this family are the major factors that promote fast microtubule growth in cells, and their activity is important for proper formation of the mitotic spindle.

Microtubule depolymerase

Regulatory factor that promotes microtubule depolymerisation. Well-studied microtubule depolymerases include the MCAK/kinesin-13 and the Kip3/kinesin-8 families.

MCAK/kinesin-13

Kinesin-family proteins that act as microtubule depolymerases. Kinesin-13's use ATP hydrolysis to induce outward curvature in protofilaments, triggering catastrophes.

Kip3/kinesin-8

Microtubule depolymerases that combine the motility of conventional kinesins with depolymerase activity. Because of this unique combination, some kinesin-8's have been shown to depolymerize microtubules in a length-dependent manner.

Rescue factor

Regulatory factor that increases the likelihood of microtubule rescue. The best-studied rescue factors belong to the CLASP family.

Free energy

a thermodynamic quantity that describes the amount of energy that can do work in a given system. The change in free energy associated with a chemical reaction provides the amount of work that is released by that reaction, or that must be input to drive that reaction.

Conformational strain

Describes internal energy stored by a molecule when that molecule is held in a conformation that differs from the strain-free conformation.

Optical tweezers

Device in which radiation pressure from a laser beam focused by a microscope objective is used to trap a small bead or other object. Optical tweezers provide a sensitive way to measure both the position of the trapped object and the forces experienced by the trapped object.

'Morphing' (interpolation)

A computational procedure that creates a series of structural intermediates between two known conformations of a molecule. Intermediate conformations generated by morphing provide a useful way to visualize steps along a conformational change, but do not always capture the actual pathway for the conformational change.

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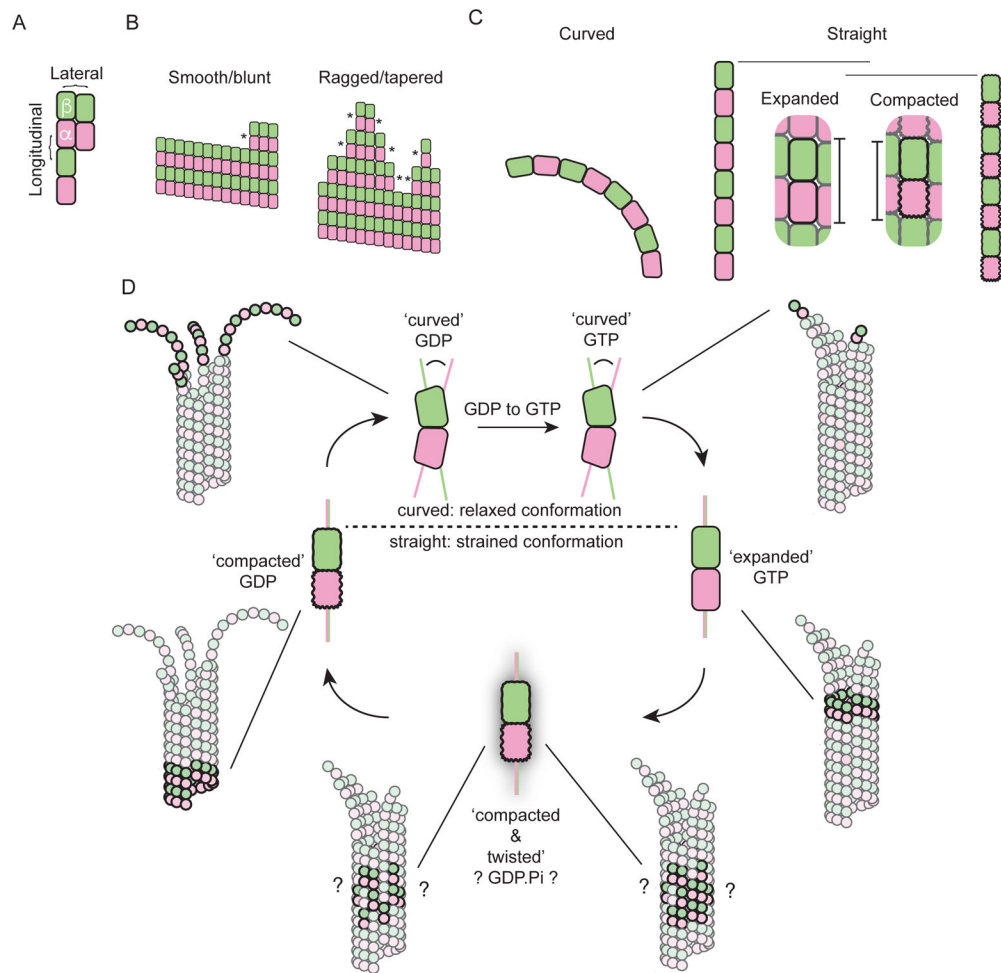
Figure 1.

Tubulin biochemistry, conformation, and mechanics. **A**. Cartoon illustrating the fundamental longitudinal (vertical) and lateral (horizontal) interactions between αβ-tubulins (pink and green) that make up the microtubule lattice. **B**. Two dimensional schematics of blunt (few 'corner' sites, *) and ragged/tapered (more corner sites, *) microtubule ends. Taper refers to the extension of some protofilaments beyond others; ragged describes an uneven or rough distribution of protofilament lengths. **C**. Cartoons of longitudinal assemblies of αβ-tubulin illustrating the three major conformations in structural detail. (left) Tubulin adopts a curved conformation in single protofilaments and when unpolymerized. (right) Tubulin adopts straight 'expanded' or 'compacted' conformations in the body of the microtubule. The horizontal bars and the inset indicate that the compacted conformation is somewhat 'shorter' than the expanded one. **D**. The tubulin conformation cycle is a mechanical cycle. Straight conformations of αβ-tubulin are strained but stabilized by interactions with the microtubule lattice (not illustrated here). Release of that strain during depolymerization can do mechanical work. The dark/bold subunits in the thumbnail cartoons of growing or shrinking microtubules indicate the region of the microtubules where the different conformations occur. The 'glow' indicates the compacted and twisted state, and the ?'s indicate uncertainty

about whether this conformation reflects a specific nucleotide state and about the distribution of these sites in the microtubule.
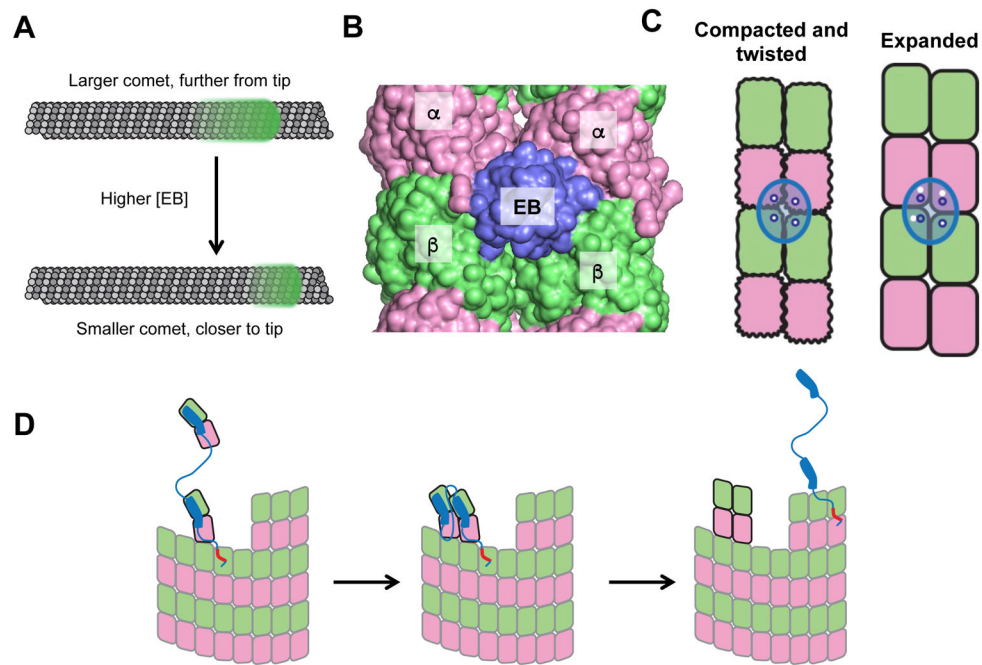
Figure 2.
Regulatory proteins and the tubulin conformation cycle. **A**. EBs form a 'comet' (green glow) near but somewhat behind the growing microtubule end (top). Increasing the concentration of EB (arrow) reduces the size of the comet and moves it closer to the growing end (bottom). **B**. EB (purple) binds at a vertex of 4 αβ-tubulin dimers (α: pink; β: green) in the lattice. **C**. EB (purple oval; the 4 empty blue circles illustrate the arrangement of EB's tubulin-binding epitopes) binds most tightly to a 'compacted and twisted' conformation of αβ-tubulin. Red circles indicate the EB-contacting surfaces on the lattice (white circles). EB binds poorly to the expanded lattice because the EB-contacting epitopes are improperly spaced (mismatch between white and blue circles). **D**. Conformation-based mechanism of a microtubule polymerase. Linked TOG domains like those in Stu2 bind selectively to curved tubulin and increase the rate of tubulin:microtubule associations by concentrating unpolymerized tubulin near the microtubule end via a tethering mechanism (left). The tethered TOG-bound tubulin associates faster with the microtubule end, and lateral interactions between TOG-bound tubulins on the microtubule end (middle) leads to straightening, which releases the TOGs (right) for another round of incorporation. The red segment indicates a basic region that mediates lattice binding.
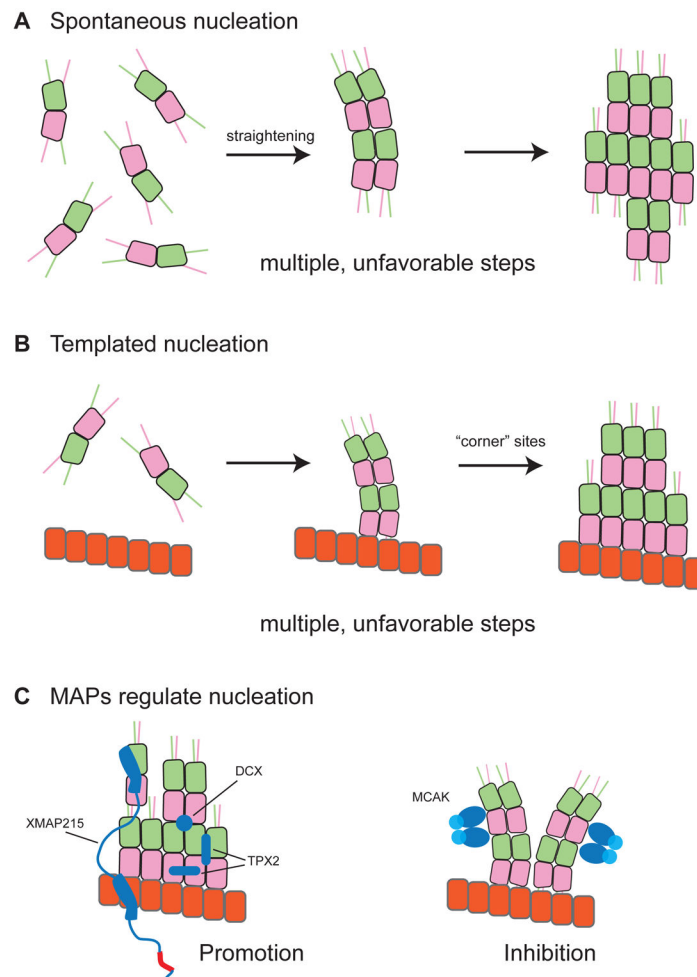
A Spontaneous nucleation

straightening

multiple, unfavorable steps

B Templated nucleation

"corner" sites

multiple, unfavorable steps

C MAPs regulate nucleation

DCX

XMAP215

TPX2

MCAK

Promotion

Inhibition

Figure 3.
Barriers to spontaneous and templated nucleation. A. Spontaneous nucleation involves multiple, unfavorable steps. Unpolymerized tubulin is curved (right) and must straighten to form small oligomers (middle). The growth of these oligomers (left) requires increased straightening for newly added tubulins and of those already in the oligomer. B. Templated nucleation also involves multiple, unfavourable steps. A blunt template (right) presents fewer high-affinity "corner" sites than a tapered growing end; curved tubulin binding to the blunt template may also have to straighten more than at the partially curved microtubule end (middle). The transition of the blunt template into a growing microtubule end requires many tubulins to straighten (left). C. MAPs regulate microtubule nucleation by altering the conformation of tubulin. Nucleation-promoting MAPs like XMAP215, TPX2, and DCX help form a nascent plus end (left). Nucleation-inhibiting MAPs like MCAK prevent this formation (right).
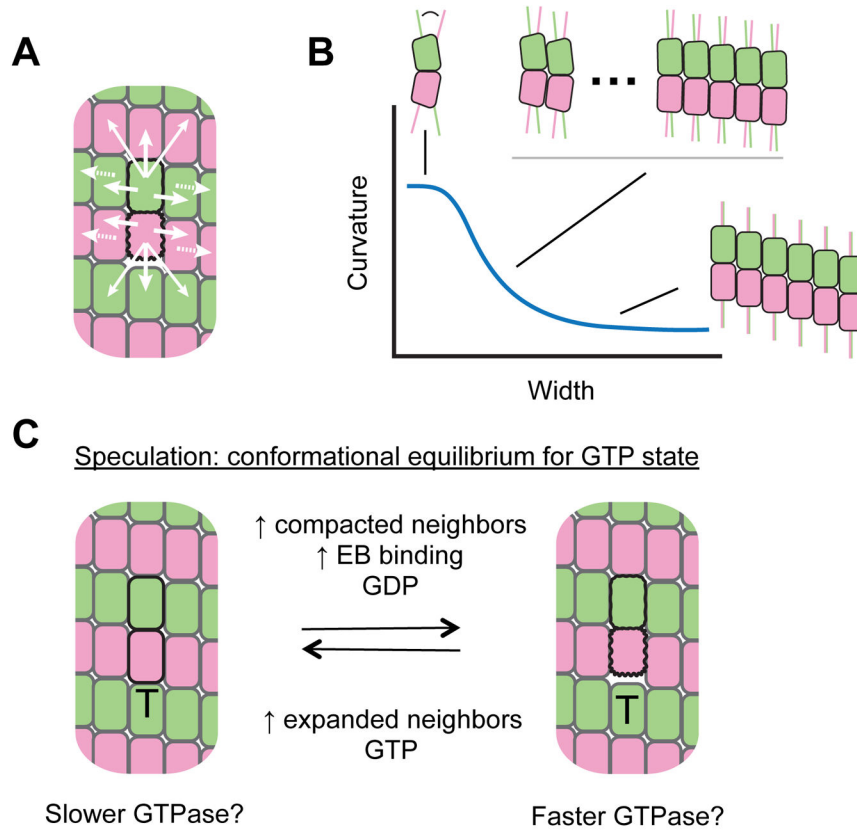
Figure 4.
Mechanical coupling in the microtubule lattice. **A**. Cartoon illustrating a rationale for mechanical coupling in the lattice. A compacted tubulin (dark outline) is shown in a majority expanded lattice (light outlines). The mismatch between expanded and compacted conformations is likely resolved through conformational changes near the mismatch (white arrows). The periodic nature of the lattice means that such accommodation could propagate beyond nearest-neighbors (dashed arrows). **B**. At the microtubule end or in nucleation intermediates, tubulin curvature is thought to vary with width of the taper, affecting the strength of tubulin:tubulin interactions. Colored lines on the αβ-tubulin cartoons provide a visual reference for the amount of curvature. This provides another example of long-range mechanical coupling. **C**. Model for conformational control of GTPase that was inspired by observations about EB proteins. The model speculates that expanded tubulins may have slower GTPase than compacted tubulins. 'T' on the cartoons indicates GTP nucleotide state. Combined with long-range coupling, such a model is expected to display threshold-type behaviours and could give rise to cooperative, positive-feedback enhancement of GTPase activity in the stabilizing cap.