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Mechanics of microtubules

Taviare Hawkins [a], Matthew Mirigian [b], M. Selcuk Yasar [b], Jennifer L. Ross [b,*]

[a] Department of Physics, Mt. Holyoke College
[b] Department of Physics, 302 Hasbrouck Laboratory 666 N. Pleasant St. University of Massachusetts Amherst, MA 01003, United States

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ABSTRACT

Microtubules are rigid cytoskeletal filaments, and their mechanics affect cell morphology and cellular processes. For instance, microtubules for the support structures for extended morphologies, such as axons and cilia. Further, microtubules act as tension rods to pull apart chromosomes during cellular division. Unlike other cytoskeletal filaments (e.g., actin) that work as large networks, microtubules work individually or in small groups, so their individual mechanical properties are quite important to their cellular function. In this review, we explore the past work on the mechanics of individual microtubules, which have been studied for over a quarter of a century. We also present some prospective on future endeavors to determine the molecular mechanisms that control microtubule rigidity.

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1. Introduction

Microtubules are essential structural elements of cells. They outline the overall shape of cells by serving as the supports for extended morphologies such as axons, dendrites, and cilia (Fig. 1). The microtubules within the cell body are highly dynamic. They can rapidly polymerize and depolymerize at their free ends (Fig. 2). This stochastic switching between polymer growth and shrinkage is termed as "dynamic instability," and it occurs in live cells as well as *in vitro* with purified protein (Mitchison and Kirschner, 1984). In cells, associated proteins that can accelerate growth, shrinkage, catastrophe, and rescue rates manipulate the microtubule network (Cassimeris, 1993; LeBoeuf et al., 2008; Levy et al., 2005; Vandecandelaere et al., 1996) to afford active remodeling into new structures such as the mitotic spindle or to move the nucleus as a cell changes direction.

In keeping with their structural role, microtubules are the most rigid of the intracellular cytoskeletal filaments. The rigidity of microtubules is very important to all of their biological functions. They need to be stiff to create cell shape, especially for extended morphologies (Fig. 1). They need to remain fairly straight to enable efficient, long-range transport, since the microtubule network makes up the tracks for cargo-carrying motor proteins in the cell. Moreover, microtubules form a rigid structural network to which actin and myosin attach to create tensile forces during cell motility and membrane rearrangements. Microtubules must be mechanically steady under load in order to be able to withstand the forces of moving large cargo, such as the nucleus or chromosomes, or acting against the tugging of actin–myosin stress fibers during cell migration.

Physically, microtubules are hollow tubes composed of a lattice of α–β tubulin heterodimers (Fig. 2A). They are 25 nm in outer diameter with a 17 nm interior space diameter. Tubulin heterodimers stack end-to-end to form protofilaments. These protofilaments bind laterally to form sheets that are rolled into a tube (Amos and Hirose, 2007; Meurer-Grob et al., 2001). After the tube nucleates, heterodimers can associate to or dissociate from either end of the microtubule. The more dynamic, faster growing and faster shrinking "plus-end" is capped with beta subunits. In cells, there are typically 13 protofilaments nucleated by a special gammatubulin complex, but the lattice is adaptable. In cilia, there are doublets where a 13-protofilament microtubule with an A-lattice type has a 10-protofilament sub-tubule with a B-lattice type attached to the side (Amos and Klug, 1974). The A-lattice is a staggered lattice of heterodimers with α–β connections. The B-lattice has α–α and β–β lateral interactions except at the seam, where an A-lattice exists (Fig. 2C). Depending on the polymerization conditions *in vitro*, the microtubule forms a B-lattice, the protofilament number can vary from 8–17, the helical pitch can change from a 2 to 5 subunit rise, and the seam can exist or not (Fig. 2C).

From a bio-or nano-engineering view, microtubules are an ideal element for building nano-scale structures. They are long, stiff structures that readily polymerize at room temperature. They are aqueous, but can be fixed and dried. Additionally, they are easily visualized by fluorescence microscopy, electron microscopy, or scanning probe microscopy. Microtubules have an intrinsic polarity, and can bind a multitude of associated proteins. These associated proteins can manipulate their rigidity and stability as well as crosslink and bundle microtubules. Further, flow, molecular motors, or optical trapping can be harnessed to manipulate

* Corresponding author.
E-mail address: rossj@physics.umass.edu (J.L. Ross).

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their position. Thus, microtubules, alone or in networks, are intrinsically interesting structures to study for nano-and bio-engineering applications.

2. Background

2.1. Single microtubule mechanics

Since microtubules are structural elements of cells, much effort has gone into studying their mechanical properties. Moreover, microtubules are structures that typically work individually or in small groups instead of as a network, so it is very reasonable to study the mechanical properties of individual microtubules. In this section, we will review some of the experiments to study microtubule flexural rigidity using single microtubules. There is a large body of theoretical literature that describes the mechanics of microtubules from dimer to polymer using a variety of techniques. Unfortunately, we do not have the space to delve into that literature here.

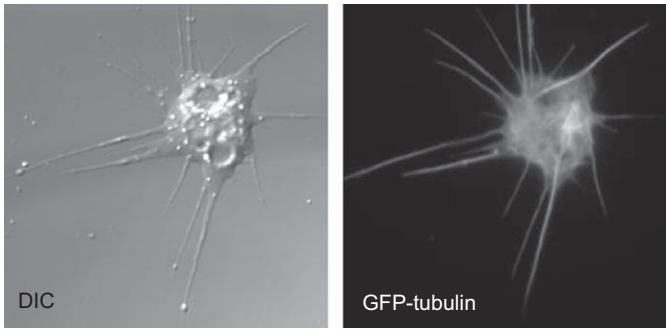

Fig. 1. Microtubule as cellular supports. Microtubules are support structures for the cell. When S2 cells are depleted of actin filaments, long, microtubule-filled processes remain. The outline of the cell is clearly seen in differential interference contrast microscopy (DIC) on the left. Fluorescence imaging of GFP-tubulin reveals that long extensions are supported by microtubules. Before actin is depleted, the cells are almost perfectly round. Cell images kindly provided by Shabeen Ally and Vladmir Gelfand.

Many groups with varied experimental approaches have studied microtubule flexural rigidity *in vitro* (reviewed in (Kasas and Dietler, 2007)). In general, in order to observe the elasticity of a material, one must probe it with a direct force. For microtubules, this has been accomplished by passive and active means. Passive forces refer to thermal fluctuations that cause long filaments to bend (Gittes, 1993). Active forces are more direct ways of implementing a force, such as using flow (Dye et al., 1993; Venier et al., 1994), optical tweezers (Felgner et al., 1996, 1997; Kikumoto et al., 2006; Kurachi et al., 1995; van Mameren et al., 2009), atomic force microscope probes (de Pablo et al., 2003; Kis et al., 2002, 2008; Schaap et al., 2004, 2006, 2007), rigid barriers (Dogterom and Yurke, 1997), membranes (Elbaum et al., 1996; Kaneko et al., 1998), or even motor proteins (Gittes et al., 1996; Kawaguchi et al., 2008; Roos et al., 2005; Vale et al., 1994). Several recent reviews illustrate and elaborate many of the techniques used for measuring microtubule mechanics in detail (Bicek et al., 2007; Gardel et al., 2008; Kasas and Dietler, 2007).

There have been over 20 published measurements of microtubule elasticity performed by various groups by diverse means. Table 1 has a list of several studies and the measured flexural rigidity (*EI*) for single microtubules, where *E* is the elasticity, often

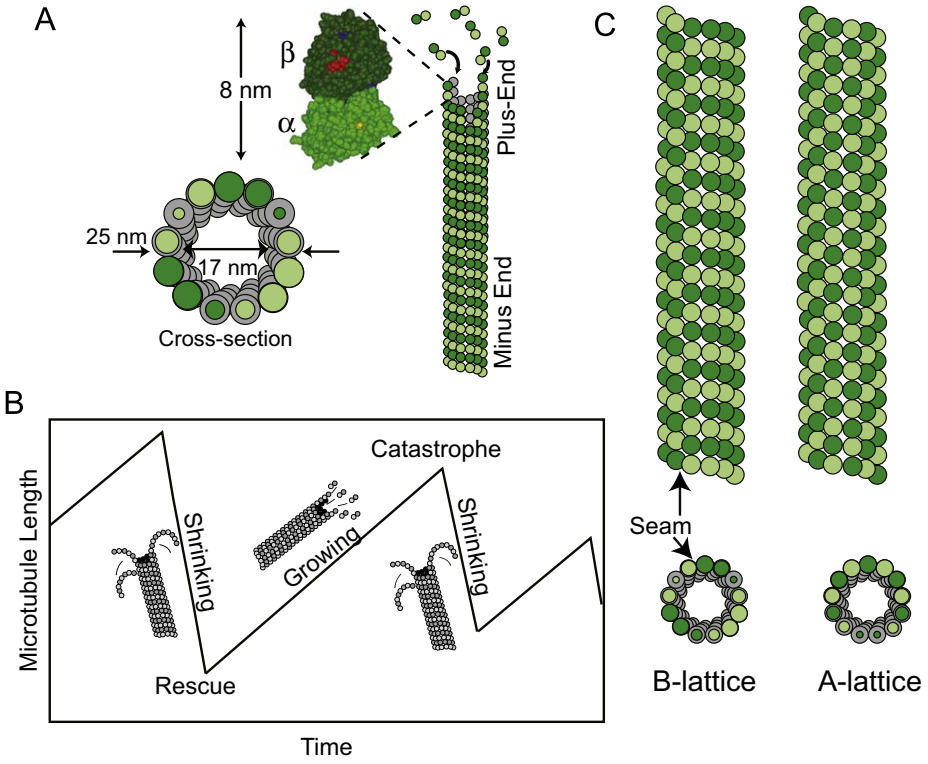

Fig. 2. Microtubule structure and dynamics. (A) Microtubules are polymer filaments made from tubulin dimers. The tubulin heterodimer is made of a beta (dark green) and alpha subunit (light green). A few hundred dimers bind together to nucleate the polymer, and individual dimers add on to the ends to grow the microtubule. The plus-end is the more dynamic and rapidly growing and shrinking end. The minus-end is less dynamic. The microtubule is a tube with an outer diameter of 25 nm and an inner diameter of 17 nm. (B) Microtubules grow by the addition of dimers and shrink by the loss of dimers. Stochastic transitions from shrinking to growing are called "rescues." Stochastic transitions from growing to shrinking are called "catastrophes." Microtubules are more stable if they have reduced catastrophes and increased rescue frequencies. (C) Microtubule B-lattice and A-lattice. In B-lattice, the alpha (light green) touches a neighboring alpha except at the seam. In A-lattice, the alpha (light green) touches the beta (dark green) throughout the lattice. There is no seam in A-lattice.

Table 1
Flexural Rigidity of Single Microtubules.

Citation	Tubulin source	Temp (°C)	Variation	EI (× 1024 Nm^2)	Lp (mm)	Measurement technique
Mizushima-Sugano et al. (1983)	Porcine	25	GDP tubulin	0.45	0.74	Affixed to cover slip
Gittes (1993)		37	With Taxol Rhodamine tubulin with Taxol	22 ± 1.5 21 ± 1	5.1 5.6	Thermal fluctuations
Dye et al. (1993)		37	GDP tubulin with Taxol with MAP mix	1.36 0.13 0.30	0.33 ± 0.09 0.031 ± 0.008 0.074 ± 0.009	Calibrated flow
Venier et al. (1994)	Porcine	37	GDP tubulin with Taxol with Taxotere GDP–BeF3 tubulin GDP tubulin GDP–BeF3 tubulin GDP–AlF4 tubulin	9.2 ± 0.9 4.7 ± 0.4 4.8 ± 0.4 26 ± 2.7 8.5 ± 2 29 ± 5 25 ± 5	2.2 1.1 1.1 6.1 2.0 6.8 5.8	Thermal fluctuations one end calibrated flow
Mickey and Howard (1995)	Bovine	37 25	GMPCPP tubulin with Tau with Taxol GDP capped with GMPCPP tubulin with Taxol	62 ± 9 34 ± 3 32 ± 2 26 ± 2 21 ± 1	14.5 7.9 7.5 6.1 5.1	Thermal fluctuations
Kurz and Williams (1995)	Bovine	37	GDP tubulin with MAP mix GDP tubulin with MAP mix	35.8 ± 0.95 39.5 ± 1.25 26.4 27.3	8.4 ± 2.2 9.4 ± 2.7 6.2 ± 0.8 6.5 ± 0.8	Calibrated flow thermal fluctuations
Kurachi et al. (1995)	Bovine	37	With MAPs (10 um length) with MAPs (30 um length) with Taxol (5 um length) with Taxol (20 um length)	34 ± 17 200 ± 60 1 ± 0.65 20 ± 6	7.9 46.8 0.2 4.7	Optical trap buckling
Elbaum et al. (1996)	Bovine	27	GDP tubulin	26 ± 10	6.3 ± 2.4	Vesicle deformation
Felgner et al. (1996)	Porcine	22–25	GDP tubulin with Taxol with MAPs GDP tubulin with Taxol with MAPs	3.7 ± 0.8 1 ± 0.3 16 ± 3 4.7 ± 0.4 1.9 ± 0.1 18 ± 3	0.9 0.2 39.2 1.2 0.5 4.4	Optical trap RELAX method optical trap WIGGLE method
Felgner et al. (1997)	Porcine	24–27	GDP tubulin with Taxol with 2% full-length Tau with 18% full-length Tau with 48% full-length Tau with 85% full-length Tau with tau binding repeat constructs with tau projection domain constructs with double tau binding domains with MAP 2c2 with MAP 2d2 with MAP 2c3 with MAP mix	3.8 ± 0.9 1.0 ± 0.3 4.5 ± 1.5 8.9 ± 1.3 9.4 ± 2.6 10.4 ± 3.1 5.8 ± 1.5 to 8.5 ± 1.6 6.1 ± 2 to 9.2 ± 1.4 29.6 ± 9.3 15.1 ± 3.3 16.1 ± 2.7 14.5 ± 3.8 16.0 ± 3.0	0.9 0.2 1.1 2.2 2.3 2.5 1.4 2.2 7.2 3.7 3.9 3.5 3.9	Optical trap RELAX method
Dogterom and Yurke (1997)	Bovine	22	GDP tubulin	34 ± 7	8.4	Thermal fluctuations
Cassimeris et al. (2001)	Porcine		GDP tubulin with XMAP215	18.5 ± 2.0 17.5 ± 2.2	7.1 4.4	Thermal fluctuations one end
Janson and Dogterom (2004)	Bovine	23	Fast polymerization slow polymerzation	18 28	4.2 ± 0.3 6.6 ± 0.9	Thermal fluctations of shape
Pampaloni et al. (2006)	Porcine		With Taxol (2.6 um length) with Taxol (47.5 um length)	0.45 21	0.11 ± 0.05 5.035 ± 0.8	Thermal fluctuations one end
Kikumoto et al. (2006)	Bovine	33	GDP tubulin with Taxol	7.9 ± 0.7 2.0 ± 0.8	1.9 0.5	Optical trap buckling
Brangwynne et al. (2007)	Bovine		With Taxol (25–66 um length) with Taxol (18–25 um length)	12 6.2	2.8 ± 1 1.5 ± 0.7	Thermal fluctuations
van den Heuvel et al. (2007)	Bovine	37	With Taxol	1 ± 0.1	0.24 ± 0.03	Microtubule trajectories
Kawaguchi et al. (2008)	Porcine	20–35	With Taxol with Taxol	2.5 ± 0.5 2.7 ± 0.4	0.6 0.6	Thermal fluctuations one end kinesin forced buckling
van den Heuvel et al. (2008)	Bovine	37	With Taxol (short length) with Taxol (long length)	0.34 ± 0.086 15 ± 1.28	0.08 ± 0.02 3.6 ± 0.3	Microtubule trajectories
Van Mameren et al. (2009)	Porcine		With Taxol	6.1 ± 1.3	1.4	Optical trap buckling

referred to as the Young's modulus for bending, and I is the moment of inertia that describes the shape of the object, in this case a cylinder. The measurements agree that microtubules are stiff polymers with a flexural rigidity similar to carbon nanotubes ($EI \sim 10^{-24}$ Nm^2), although the actual numbers found by different groups vary by an order of magnitude. We list the measurements in terms of flexural rigidity, but different groups measured different parameters, which depended on the measurement type and the model assumed for the microtubule. Another quantity frequently measured is the persistence length (L_p), *which is related to flexural rigidity by the thermal energy* of the system: $L_p = EI/k_BT$ (Gittes, 1993).

The first measurement of microtubule flexural rigidity was performed in 1983 by Mizushima-Sugano et al., 1983. They measured the end-to-end distance of microtubules stuck to a cover slip using dark-field microscopy. The average end-to-end distance is a function of the microtubule length and the persistence length. This method revealed a very small persistence length of ~75 μm for microtubules. Although this was an order of magnitude greater than the persistence length of actin, it is a far

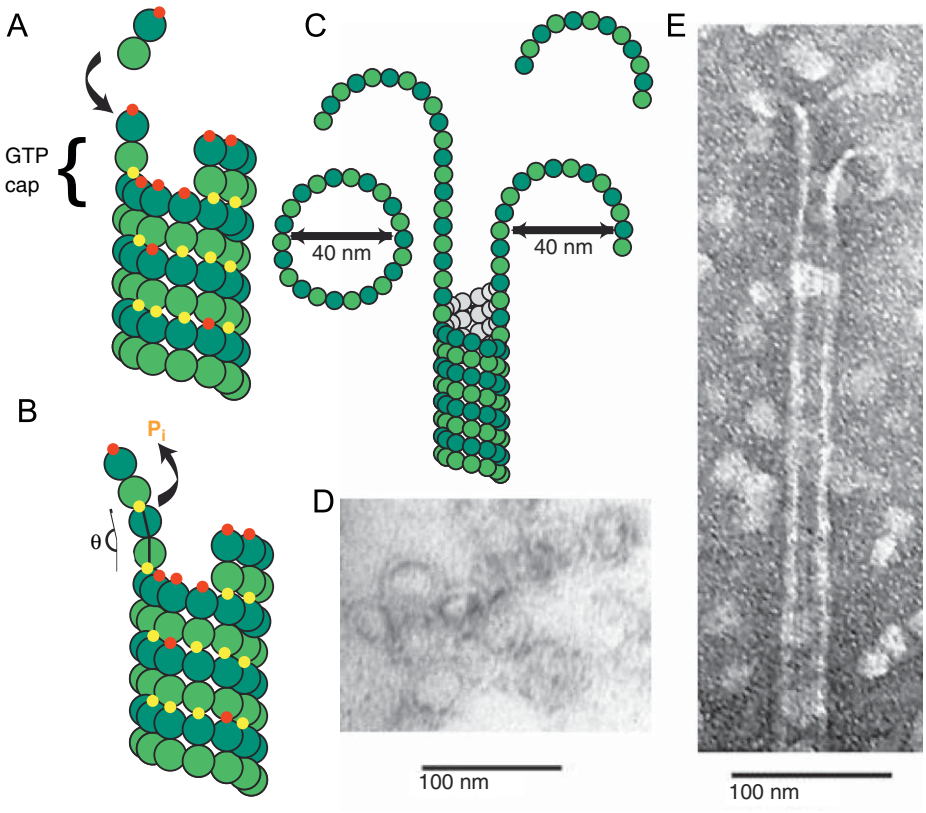

Fig. 3. Microtubule GTP cap and shapes. (A) Microtubules during polymerization have a cap of GTP-bound dimers at the top (denoted with a red-dot). The GTP-tubulin has a straight conformation. (B) Upon hydrolysis of the GTP into GDP, the tubulin dimer kinks back (GDP-tubulin denoted with a yellow dot). (C) Protofilaments bend back when depolymerizing to form GDP-tubulin rings. This shows that the longitudinal bonds are stronger than the lateral bonds, which break more easily. (D) Electron micrograph of GDP tubulin rings and (E) electron micrograph of depolymerizing microtubule.

cry from the accepted values of 1–10 mm in recent literature (Table 1). The discrepancy probably arises from the fact that the microtubules surveyed were affixed to the glass cover slip, possibly in atypical positions. Thus, the end-to-end distance was likely under-estimated.

2.1.1. *Effects of Taxol*

A particular discrepancy in the literature is about the effect of taxol. Taxol, or paclitaxel, is a chemotherapy drug used to stabilize microtubules. It is commonly used *in vitro* to stabilize microtubules against depolymerization after GTP-hydrolysis has occurred on the microtubule. Most groups agree that taxol decreases the stiffness of microtubules. On the other hand, two groups have seen either no effect (Vale et al., 1994) or an increase of stiffness (Mickey and Howard, 1995) in the presence of taxol. Theoretical modeling also implies that taxol-stabilized microtubules should be less stiff than un-stabilized microtubules (VanBuren et al., 2005), but personal anecdotes from cell biological researchers about stiffer or more flexible taxol-microtubules keeps this question open (Table 1).

2.1.2. *Effects of nucleotide*

More recent literature has found some very interesting results about how microtubule stiffness can be tuned. For instance, if microtubules polymerize with GMPCPP, a slowly hydrolyzable analog of GTP, the polymer is much stiffer (Table 1). GMPCPP is thought to enhance the longitudinal bonds along the microtubules, resulting in straighter protofilaments, more akin to the straight microtubule structure (Fig. 3). Since, GTP is thought to hydrolyze stochastically along the length of the microtubule, with GDP throughout the older, middle region and GTP at the end (called the "GTP cap"), the majority of the microtubule will have GDP-tubulin (Fig. 3A and B). GDP-tubulin's natural shape is a ring composed of a protofilament, peeled back and flipped inside-out (Fig. 3D). This result implies that the longitudinal bonds are still very stable, but they have a preferred kinked conformation. This kinked conformation does not conform to the straight structure of the microtubule (Fig. 3B). It is this activity that destabilizes the microtubule to cause depolymerization (Fig. 3E). It also affects the mechanics of the microtubule, making the filament less rigid (Table 1).

2.1.3. *Effects of MAPs*

In addition to GMPCPP, microtubule-associated proteins (MAPs) can alter the flexibility of microtubules. Most of the proteins studied have been neuronal MAPs that are shown to stiffen microtubules (Felgner et al., 1997; Mickey and Howard, 1995). Tau is found in the axon and is important to stabilize and stiffen microtubules against depolymerization while supporting the extended structure of the axon. MAP2 is found in the dendrites, and it also stiffens the structure, although there are discrepancies about the extent to which these proteins can enhance the stiffness (Table 1). Interestingly, a mitotic MAP, XMAP215, was shown to have no effect on microtubule flexural rigidity (Cassimeris et al., 2001). This may reflect the mechanism of action for XMAP215, which is known to act at microtubule plus-ends to enhance polymerization (Brouhard et al., 2008; Kerssemakers et al., 2006).

2.1.4. *Effects of polymerization rate*

Even more interesting is the effect of polymerization on microtubule flexural rigidity. Janson and Dogterom found that

faster growth rates resulted in smaller persistence lengths (Janson and Dogterom, 2004). For growth rates from 1.2–2.7 μm/min, the persistence length can change by a factor of two. This difference may be due to an increased number of lattice defects. Fast-growing microtubules are more likely to have defects, or errors, in the lattice. Such defects affect dimer–dimer bonds and likely result in reduced bonding energy, resulting in more flexible filaments.

2.1.5. Length dependence

Perhaps the most intriguing result about microtubule flexural rigidity is that there is a length dependence. Shorter microtubules appear to be less rigid, or softer, than longer microtubules. Research groups using passive (Pampaloni et al., 2006) and active techniques (Kurachi et al., 1995) have observed a length dependence of the persistence length. Microtubules from 5–30 μm can have a difference in flexural rigidity of up to an order of magnitude (Kurachi et al., 1995). These differences are most likely due to the anisotropic nature of the microtubule. By anisotropic, we refer to the differential strength of the longitudinal and lateral bonds between dimers. The longitudinal bonds between dimers within the protofilament are stronger than the lateral interprotofilament bonds. This is evidenced by the fact that, upon depolymerization, the dimers often stay attached as protofilaments that bend back into GDP-rings.

2.1.6. Anisotropic stiffness

Atomic force microscope (AFM) studies can image the stiffness of the microtubule using "jump mode." The mechanical maps reveal that the space between protofilaments is weaker than the locations on top of the protofilaments (Schaap et al., 2006). A following study showed that the neuronal MAP, tau, did not affect the lateral rigidity of microtubules, implying that it only enhances the longitudinal bonds to enhance the flexural rigidity of the whole microtubule (Schaap et al., 2007). The anisotropy of microtubules proves they are not homogeneous plastic beams. This property is similar to other biological materials, such as wood and bamboo, which are also stiffer longitudinally.

2.1.7. Microtubules under tension

The protofilaments of microtubules are strong enough to perform work during depolymerization, thus giving a biological reason for the longitudinal bond strength of microtubules. As microtubules rapidly depolymerize, the protofilaments peel back (Fig. 3E). Any molecule or complex loosely attached to the microtubule will be forced back toward the minus-end as the microtubule plus-end depolymerizes. The yeast kinetochore complex, Dam1, is pulled in this way (Asbury et al., 2006; Franck et al., 2007; Grishchuk et al., 2008; Westermann et al., 2006). Dam1 is attached to the kinetochore of the chromosome, which is also pulled back by the depolymerizing protofilaments of the microtubule. For yeast, where only one microtubule is attached to each kinetochore, the energy of microtubule depolymerization is harnessed to pull apart the sister chromatids. In this case, the microtubule and the individual protofilaments are pulling against a tension created by the chromatid attachment and another kinetochore microtubule on the other side. Quantitative studies with optical traps on the Dam1 system estimate the tensile force of a single microtubule to be 0.5–3 pN (Asbury et al., 2006).

Microtubules are also likely to feel a tension at the cell membrane, where they are sometimes coupled to motor proteins, such as cytoplasmic dynein (Ligon et al., 2001). In particular in budding yeast, cortical cytoplasmic dynein pulls on the microtubule embedded in the spindle-pole body to reel the mitotic spindle into the bud neck during cell division (Markus et al., 2009). Thus, microtubules are not just for pushing and compressive forces, but they are also for pulling, especially during cell division processes in a variety of species.

One study used microtubules bundled by depletants to measure the radial flexibility of microtubules (Needleman et al., 2005). When 20k poly-ethylene-oxide (PEO) is used at low concentration, the microtubules are not bundled and form a nematic phase at relatively high concentration. As the level of PEO is increased, the polymer is excluded from the microtubule interior, thus increasing the osmotic pressure on the outside of the tube. The microtubules collapse radially, and the radial elastic modulus can be measured from the known osmotic pressure of the polymer solution. As more PEO is added, the microtubules reversibly decompress and return to a cylindrical shape as PEO enters the lumen.

In cells, microtubule networks often manifest as focused arrays emanating from organizing centers containing microtubule-nucleating complexes. Several *in vitro* studies have probed the mechanics of a microtubule-nucleating center to show that the intrinsic dynamic instability of microtubules can create forces to center the organizing complex inside a confined system (Cosentino Lagomarsino et al., 2007; Faivre-Moskalenko and Dogterom, 2002). They found that microtubule growth can push the organizing center, but that prolonged growth is unproductive and can lead to microtubule buckling. On the other hand, the addition of microtubule catastrophe agents leads to more dynamic searching for the center of the chamber. This line of research is leading this group to a new understanding of microtubule mechanics during tension, which is a yet-unexplored area.

2.1.8. In vivo studies

Unknown forces generated by other filament networks, associated proteins, and crowding effects complicate studying microtubule mechanics in cells. Moreover, microtubule networks are very dense, so assessing the mechanics of any one or subset of microtubules is quite difficult. It is perhaps easiest to study structures that are composed of microtubules, such as cilia and axons. Mechanical manipulation experiments of these structures with calibrated pipettes have been performed for decades (Baba, 1972). As well, mechanical experiments on mitotic spindles, which are mostly composed of microtubules and associated proteins, have been performed for almost as long to show that microtubules are under tension during cell division (Ault and Nicklas, 1989).

With higher resolution fluorescence imaging of live cells, researchers have been able to image individual microtubules at the cell periphery (Fig. 4A) (Brangwynne et al., 2006; Odde et al., 1999). There are several recent reviews that describe these techniques and results (Bicek et al., 2007; Gardel et al., 2008). The overall result is that microtubules in the cell periphery are experiencing large forces due to actin filaments and myosin motors that cause low-wavelength bending modes of the microtubule.

3. Future

3.1. Microtubule-associated proteins

Although several groups have studied a few types of microtubule-associated proteins extensively, there are over 600 known MAPs. Besides the MAPs discussed here, other MAPs of interest include the microtubule plus-tip tracking proteins (plus-tips), such as EB1 and CLIP-170 (Lansbergen and Akhmanova, 2006).

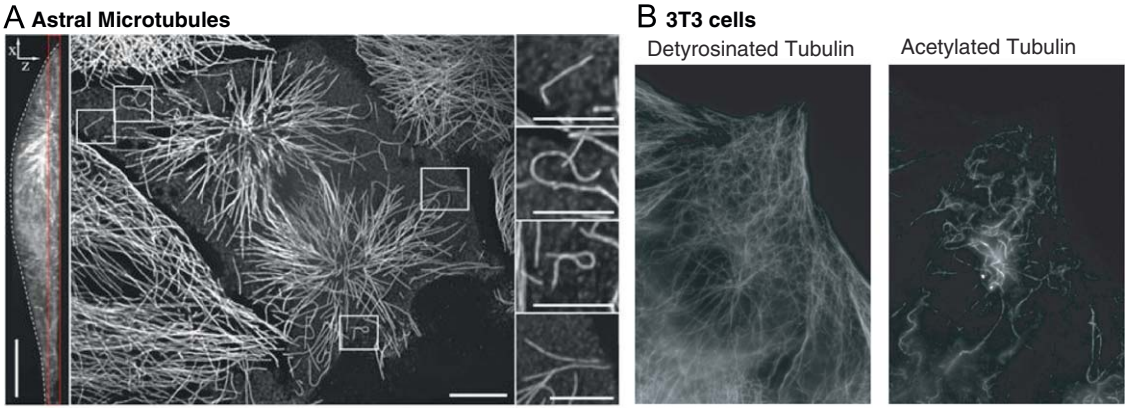

Fig. 4. Curvey microtubules. (A) Microtubules in cell periphery can take on a number of highly curved confirmations. These shapes are most likely caused by actin retrograde flow, actin–myosin contraction, or other motor proteins. Image adapted from Rusan and Wadsworth, JCB, 2005. (B) 3T3 cells stained for detyrosinated or acetylated tubulin show acetylated microtubules, which are cold and drug stable, are highly curved near the nucleus of the cell.

These proteins are known to track growing microtubule ends and may aid in reducing defects in the microtubule structure during rapid polymerization by slightly hindering the incorporation of dimers or rejecting poorly incorporated dimers. If they could correct for defects, microtubules polymerized in the presence of plus-tips should be more rigid than those without plus-tips. On the other hand, they may have no effect on the microtubule rigidity, if they are added after microtubule polymerization. Or, these proteins may have no effect on microtubule rigidity because they act at the plus-end of the microtubule, as was the case with XMAP215 (Cassimeris et al., 2001).

In addition to stabilizing MAPs, there are also microtubule destabilizing proteins. These come in two varieties: the depolymerizing kinesins, such as MCAK (Hunter et al., 2003), and the microtubule severing proteins, such as katanin (McNally and Vale, 1993). Either of these types of associated proteins could affect the microtubule lattice and alter the flexural rigidity. Depolymerizing kinesin act at the microtubule ends to actively remove dimers (Helenius et al., 2006), so these may not affect the mechanics of the microtubule lattice very much. Unlike depolymerizing kinesins, severing proteins can act anywhere along the length of the microtubule. They are likely to loosen interdimer interactions to remove one or several dimers from the lattice. If this is the case, severing proteins may cause a decrease in filament rigidity as they produce work to tear apart the microtubule lattice. A recent paper on cofilin, an actin severing protein without ATPase activity, showed that cofilin worked to cut actin filaments in this manner (McCullough et al., 2008). Mutated or defective microtubule-associated proteins have been linked to various diseases: Alzheimer's disease, Parkinson's disease, Hereditary Spastic Paraplegia and Huntington's disease. Although much *in vivo* work is occurring, it is not clear how the stability or flexibility of microtubules relates to the disease states. It is becoming clear that the flexibility and stability of microtubules are essential to their functions, and defective MAPs may alter those properties and directly result in disorder conditions.

3.2. Microtubule structure

Tubulin dimers can assemble into a variety of structures—from rings to sheets to tubes (Fig. 3). Even microtubules themselves can range in protofilaments number from 8–17, and can have altered lattice positions of dimers (Fig. 2). It was elaborated earlier that this plasticity in the lattice – particularly in the lateral connections – creates the anisotropic flexural rigidity of the microtubule. In this section, we will discuss some unexplored areas of microtubule structure that can lead to insights into how lattice structure can affect microtubule rigidity. Such explorations will likely have implications for microtubule regulation inside cells.

As explained above, the microtubule lattice could affect the flexural rigidity. In particular, large changes in the protofilament number could cause a change in the moment of inertia (I) and change the flexural rigidity. This idea is explored as a possible explanation for the two-fold change in *EI* as a function of polymerization rate, but was dismissed as not being a strong enough effect (Janson and Dogterom, 2004). When the microtubule lattice changes or a tubulin subunit is missing or altered, that creates a defect in the microtubule wall (Chrétien et al., 1992; Schaap et al., 2004). Lattice defects are likely to have altered interdimer interactions and thus affect the flexural rigidity of microtubules. Indeed, one study suggests that polymerization rates affect microtubule flexibility because fast-polymerizing microtubules are likely to have more defects along their lattice (Janson and Dogterom, 2004). The defect-laden nature of the microtubules was inferred in these experiments, but future experiments with known defect locations will illuminate the effect that imperfections can have on microtubule mechanics.

Another possible alteration of the microtubule lattice is the different lattice types: A-lattice and B-lattice. As explained in the introduction, the A-lattice is the lattice of the complete microtubule in the cilia outer doublet, and the B-lattice is the lattice of the B-tubule attached to the side of the A-tubule. The B-lattice is found most often when microtubules are spontaneously nucleated *in vitro*. The interdimer interactions of these two lattices should have different strengths, which may translate into different flexural rigidities for these two types of microtubule lattices. Interestingly, there is new evidence that microtubules in cells may be mostly A-lattice type. A recent paper showed that fission yeast microtubules polymerized in the presence of the fission yeast EB1 are A-lattice using cryo-electron microscopy and reconstruction (des Georges et al., 2008). In addition, A-tubules are nucleated from axonemes *in vivo*. Given that the nucleation site is likely to confer particular lattice structure on the nucleated microtubule, *in vitro* microtubules nucleated from axonemes will likely have A-lattices (Scheele et al., 1982). They may also have distinct mechanical properties from spontaneously nucleated B-lattice microtubules.

Further modifications to the microtubule, such as post-translational modifications (PTMs), may also affect the mechanical properties of microtubules (Verhey and Gaertig, 2007). Post-translational modifications are additional chemical modifications that proteins can acquire after translation. For microtubules, there are several known modifications. Most

modifications occur on the carboxy-terminal tail (CTT) of the dimer. The CTT is a highly negatively charged, unstructured polypeptide. Many MAPs use the CTT to bind or enhance binding to microtubules, and these modifications are likely used for signaling. One PTM, acetylation, is found on alpha tubulin, and is located inside the microtubule lumen. It is unknown if PTMs or even the CTT itself can modify the flexural rigidity of microtubules. Indirect evidence from cellular staining reveals that acetylated and tyrosinated microtubules compose a stable subset of microtubules, often found in cilia, axons, and near the microtubule-organizing center by the nucleus. Interestingly, the acetylated microtubules near the nucleus are highly curved, implying they may be more flexible than other microtubules (Fig. 4B). If they are not more flexible, then they are under tremendous pressure. Their shape does not change upon depolymerization of microtubules, suggesting some other load-bearing filament network may be compressing them into this very curved conformation.

After almost three decades of measurements, understanding microtubule mechanics is just as important today. There are plenty of avenues to study, only a few of which are elaborated in this review. These future directions will reveal new information to elucidate how microtubule rigidity is regulated in the cell.

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