Microtubule polymerases and depolymerases

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The variety of shapes and sizes of the microtubule cytoskeleton is as great as the number of different cell types. This large variety is a consequence of the dynamic properties of microtubules, which allow them to adopt distributions of arbitrary size and form. How is the distribution of microtubule lengths controlled? Recent work suggests that the length distribution is controlled, at least in part, by the activity of microtubule polymerases and depolymerases, which accelerate microtubule growth and shrinkage. Specifically, biochemical and single-molecule studies have shown how MCAK (kinesin-13) and Kip3p (kinesin-8) accelerate depolymerization and how XMAP215 may accelerate growth. Studies on the yeast Dam1 complex have shown how proteins can couple a cellular structure, the kinetochore, to the ends of polymerizing and depolymerizing microtubules.

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Current Opinion in Cell Biology 2007, **19**:31–35

This review comes from a themed issue on
Cell structure and dynamics
Edited by Daniel P Kiehart and Kerry Bloom

Available online 20th December 2006

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2006.12.009

Introduction

The structure of a microtubule and its dynamic properties have been extensively reviewed elsewhere [1,2]. In brief, microtubules typically comprise 13 protofilaments, which form the wall of a tube. Each of the protofilaments consists of a head-to-tail arrangement of α/β tubulin heterodimers. When bound to GDP, the tubulin dimer is in a bent conformation, which fits poorly into the straight wall of the microtubule. Exchange of GTP into its active site straightens the dimer, facilitating its incorporation into a sheet at the growing end of the microtubule ([3]; but see [4]). Closure of the sheet forms the tubular wall of the microtubule. Dimer addition at the end of a protofilament completes the hydrolysis pocket, triggering the hydrolysis of GTP to GDP and enabling the dimer to revert to a bent conformation; however, in the wall of the microtubule (the lattice), the dimer is constrained to remain straight and it is therefore trapped in a strained (bent) high-energy state. The release of the bending energy is the driving force for microtubule depolymerization of the mainly GDP-dimer-containing microtubule. Thus we can think of GTP-tubulin as the fuel for polymerization: the highly favorable binding energy of GTP-tubulin to the lattice drives polymerization, whereas the highly favorable dissociation of GDP-tubulin drives depolymerization [5].

The key question is how growth and shrinkage are balanced to produce the required cellular microtubule length distribution. A wide variety of proteins regulate the dynamics of microtubules [6,7]. Given this cornucopia of proteins and activities, are there any common principles that can explain the variety of microtubule structures? In this review we discuss one common principle, namely that the combined actions of microtubule depolymerases and polymerases could account, at least in part, for the regulation of microtubule length. Because the slower-growing minus end of a microtubule is usually anchored at a microtubule organizing site and not dynamic, this review focuses on the microtubule plus end.

Depolymerases

It is thought that there is a cap containing GTP-tubulin at the plus end of the microtubule that prevents a growing microtubule from depolymerizing [1,2]. This cap, whose structure is still uncertain, must be extremely robust to prevent the release of the large amount of bending energy stored in a microtubule (about half the free energy of GTP hydrolysis per GDP-tubulin dimer in the microtubule lattice). The discovery that kinesin-13 family proteins such as MCAK can depolymerize microtubules stabilized by the slowly hydrolyzible GTP analog GMP-CPP [8] led to the definition of a family of depolymerases that can remove the GTP-cap [9]. MCAK couples ATP hydrolysis to the removal of GTP-dimers from both ends of microtubules [10]. In its ATP or ADP-P_i state, MCAK binds to and stabilizes a bent protofilament conformation, which is the structural intermediate of depolymerization [11]. Following dissociation of a tubulin dimer from the end of the microtubule, the hydrolysis products (P_i and ADP) are released so that another round of ATP hydrolysis and tubulin dimer removal can proceed [10]. In this way, MCAK removes the GTP-cap, triggering what is called a 'catastrophe', whereby a growing microtubule is converted into shrinking one. Thus MCAK is an ATP-dependent depolymerase that acts as a catastrophe-inducing factor.

An open question was how MCAK can target rapidly to microtubule ends. MCAK, though it is a kinesin, does not move in a directed manner on the lattice [12•]. Rather, it targets by a 'diffusion and capture' mechanism in which it

associates weakly with the lattice, diffuses, and then binds to the end. One advantage of this strategy is that it allows targeting to both ends, which occurs *in vitro* and *in vivo*. The second advantage is that it allows very rapid end binding, because diffusion is a more rapid process than directed motility over short distances. Diffusion and capture is similar to the way that transcription factors and restriction enzymes find their binding sites on DNA (see [12•] for references).

Since the discovery that MCAK is a depolymerase, a number of other kinesins have been shown to modulate the dynamics of microtubules *in vivo* and *in vitro* [13–16]. Kip3p, a member of the kinesin-8 family, which is phylogenetically closely related to the kinesin-13 family, also depolymerizes GMP-CPP-stabilized microtubules, but only at the plus ends [17•,18•]. Interestingly, unlike MCAK, Kip3p uses ATP hydrolysis to move in a directed manner on the lattice to get to the plus end [17•,18•]. Kip3 is the most processive motor discovered so far: it moves on average 12 microns before falling off [17•]. Because budding yeast microtubules are shorter than 10 microns, almost every Kip3 that binds the lattice will get to the plus end. Thus in yeast the microtubule acts as an antenna to funnel Kip3 to just one end. When comparing Kip3 to MCAK, the high processivity allows the microtubule to act as larger antenna for Kip3 than is the case for MCAK, which remains on the lattice for only about 1 s, during which time it diffuses about 1 micron. Thus the target size for MCAK is about 1 micron from the end. On the other hand, the target size for Kip3 is the processivity length, 12 microns. Perhaps, by adjusting the processivity of different microtubule regulatory proteins, a cell could modulate the target size and as a consequence the depolymerase activity. An important corollary of processivity-based targeting is that the longer the microtubule the more protein will accumulate at the end and the higher the rate of depolymerization. This accounts for the fact that depolymerization by Kip3 is length-dependent and suggests a possible mechanism for cells to regulate microtubule length (see below).

Other kinesins have also been shown to destabilize microtubules. The kinesin-14 Kar3, which forms a heterodimer with a non-motor subunit Cik1, has been shown to be a minus-end-directed motor and to slowly depolymerize microtubules that have been partially stabilized by taxol: shortening occurs at the plus end [15] rather than the minus [19] end. Whether the translocase and possible depolymerase activities of Kar3 activity are coupled is not known.

Polymerases

Many proteins exert a positive effect on microtubule growth. How might a protein stimulate growth? Microtubules grow by the addition of tubulin dimers to their ends. At 10 μM tubulin a microtubule grows at about 1 micron/min, which corresponds to ~30 dimers per second or about two dimers per protofilament per second. The growth rate depends on the tubulin concentration, and the association rate of tubulin to the end is between 1 and 5 $\mu M^{-1}\,s^{-1}$. Because this association rate is comparable to the fastest protein–protein association rates [20], microtubule polymerization might be thought of as being a fast diffusion-limited association. However, one should not forget that there are 13 protofilaments in a microtubule, so in fact the association rate per protofilament end is ~10 times less. This leaves room for proteins that could increase the rate of addition of subunits to the end up to ten fold. Such proteins could act as catalytic enzymes that speed the association rate by stabilizing an intermediate in the association reaction. In other words they would act as polymerases. And in fact such a protein was identified twenty years ago: XMAP215 [21].

There are two ways in which XMAP215 might catalyze polymerization. XMAP215 could stimulate polymerization either by acting as an adaptor that brings multiple tubulins to the growing end or by changing the structure of the growing end so that soluble tubulin can more easily bind. Structurally, XMAP215 is a long thin molecule that could potentially bind multiple tubulin heterodimers and form small curved protofilaments [22]. Recent work shows that the XMAP215 homolog in yeast, STU2, binds tubulin through a 250 amino-acid heat-repeat-containing domain called the TOG domain, which is evolutionarily conserved from yeast through to humans. Like XMAP215, STU2 also localizes to the microtubule end [23•]. Thus STU2 could enhance the association of tubulin dimers to the growing end. In other work, mutation or inactivation of XMAP215 has been shown to lead to more punctuated growth of microtubules, suggesting that XMAP215 is an 'anti-pause' factor [24]. High resolution tracking of microtubule growth *in vitro* shows that in the presence of XMAP215 the microtubule growth is saltatory [25]; this has been interpreted as XMAP bringing several tubulin dimers to the end at once, thereby increasing the length in steps of up to 60 nm. Interestingly, the length steps of 60 nm correspond to the elongated form of the XMAP215 molecule [22]. An alternative possibility is that the jumps in length may not be true molecular steps, but may rather correspond to periods of rapid growth during which an XMAP215 molecule is bound at the end and recruits individual tubulin dimers to the end. Single-molecule studies will be needed to determine the mechanism of growth-rate acceleration by polymerases.

Surprisingly, studies in *Xenopus* egg extracts [26] have suggested that XMAP215 can destabilize microtubules, in addition to, or instead of, accelerating microtubule growth, as it was found to do in earlier experiments. Destabilizing activity has also been observed *in vitro* for STU2 [27]. The apparent contradiction between these papers and the earlier results may be reconciled when one realizes that a traditional catalytic enzyme accelerates a

reaction in both directions. For example, suppose that XMAP215 stabilizes an intermediate structural state in the pathway in which a tubulin dimer joins onto the end of a protofilament. If the conditions are such that growth is favorable, for instance when the tubulin concentration is high, then XMAP215 will accelerate the growth rate. But under conditions when growth is unfavorable, for instance at low tubulin concentrations, then XMAP215 may accelerate the removal of a tubulin dimer from the end [23•]. In this case it would be acting like a depolymerase, but with a very important difference to MCAK and Kip3p. Whereas XMAP215 is a passive enzyme (it has no ATPase activity), MCAK and Kip3 depolymerize GTP-microtubules not via a single transition state, but through an active ATP-dependent mechanism. In other words, the depolymerase activity of MCAK or Kip3 is not simply the reverse of the polymerase activity of XMAP215. Thus we must distinguish between depolymerases that are active (ATP-dependent) and those that are passive (catalysts).

Other MAPs, such as tau [28] and doublecortin [29•], have smaller effects on growth rates than XMAP215 does. These other MAPs exert their effect on microtubule dynamics more through the suppression of catastrophe, leading to more stably growing microtubules. This is nicely illustrated in experiments which show that the addition of up to 5 μM doublecortin has very little effect on the rate of growth of microtubules off axonemes, yet also completely abolishes catastrophes [29•]. Perhaps tau and doublecortin form a stabilized microtubule that is similar in structure and function to the GTP-cap of 'naked' microtubules. Interestingly, kinesin-13 can also depolymerize microtubules stabilized by doublecortin [29•]: thus, ATP-dependent depolymerases may be more than just catastrophe factors, as they may be important for depolymerizing MAP-stabilized microtubules. By contrast, XMAP215 has a profound effect on the growth rate without affecting the intrinsic catastrophe rate. Therefore there seems to be a crucial difference in the activities of XMAP215 and tau/doublecortin [28,29•]. XMAP215 uncouples growth and catastrophe and thus conforms to our definition of a polymerase. Further evidence for uncoupling of growth from catastrophe comes from reconstitution experiments with XMAP215 and XKCM1, which show that together these proteins create a microtubule that both grows quickly and is highly dynamic [30]. This circumvents the limitation of unregulated microtubule growth in which high growth is associated with high stability. The existence of proteins that can uncouple growth from catastrophe allows the cell a lot more flexibility to explore different regimes of microtubule dynamics.

Maintaining contact to a polymerizing or depolymerizing microtubule end

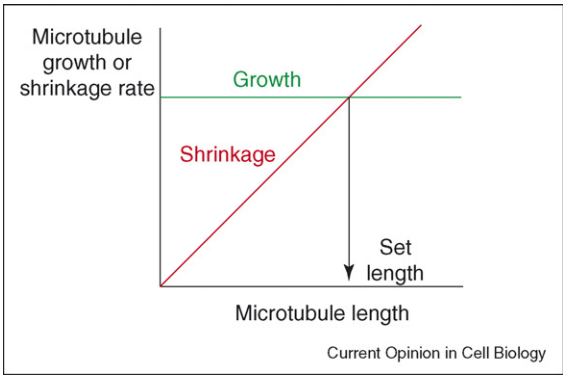
Some proteins, known as tip trackers or +TIPs, bind to the ends of growing microtubules [31–36]. These +TIPS include depolymerizing kinesins in the kinesin-13 and kinesin-8 families [17•,18•,37,38], as well as other proteins, such as EB1, CLIP-170 and APC. To date, however, there is little evidence *in vitro* that these latter proteins directly affect dynamics. Instead, they may be hitchhikers: if they remain bound they would be transported with the growing end.

Many +TIPs are also found at kinetochores, where they could form a higher order complex to regulate the coupling of the kinetochore to a growing or shrinking microtubule. Recent studies on the 10-protein yeast Dam1 complex suggest that coupling of the microtubule end to the kinetochore could be topological [39–41•]. The Dam1 complex forms a ring that can encircle the microtubule, and *in vitro* studies show that it can remain on the end of a shrinking microtubule. This is consistent with the hypothesis that the peeling protofilaments at the shrinking end could exert a force on the complex. Recent experiments using optical tweezers show that contact can be maintained in the presence of forces of up to a few piconewtons [42•]. Thus, the Dam1 complex forms a strong link between the kinetochore and the shrinking microtubule that can potentially couple the force associated with microtubule depolymerization to the chromosome. The complex can also associate with a growing microtubule [42•], consistent with the preferential binding of the Dam1 complex to GTP-tubulin [43] in the cap at the growing end. Interestingly, some truncated MCAK constructs can also form rings and spirals around microtubules with remarkable structural similarity to the Dam1 rings [44,45], though their functional relevance is not clear. Genetic evidence in fission yeast suggests that the Dam1 complex and the Kip3p homologs Klp5/Klp6 (kinesin-8) coordinate to control bipolar kinetochore attachment [46]. In yeast, Kip3p synchronizes poleward kinetochore movement during anaphase [47]. Thus motor proteins may work in concert with the Dam1 complex to regulate kinetochore attachment to, and movement at, the ends of microtubules.

Microtubule length regulation *in vivo*

Now let us consider how these polymerases and depolymerases could regulate microtubule length and distribution *in vivo*. Pioneering work by Hoyt showed that the opposing activities of motors are able to regulate the length of the mitotic spindle (reviewed in [48]). In *Xenopus* egg extracts, the steady-state length of microtubules is determined by the opposing activities of XMAP215 and MCAK [30]. Similarly, in *Drosophila* S2 cells, interfering with the various depolymerizing kinesins and polymerases, such as the XMAP215 homolog and other +TIPS, alters the lengths of the metaphase mitotic spindles [49]. Another example of length regulation is cilial growth, where length regulation may in part be explained by the balance of polymerization and depolymerization [50]. These studies suggest that cells may use

Figure 1



Length control is achieved by a balance between polymerization and length-dependent depolymerization.

different families of polymerases and depolymerases or other microtubule regulatory proteins to control microtubule length, according to cellular circumstance.

The stochastic nature of microtubule dynamics makes it an unsuitable mechanism for the precise specification of microtubule length. Though the combination of random catastrophes and a fixed growth rate will generate a distribution of microtubules of defined mean length, the distribution is very broad. In contrast, many cellular structures, such as the metaphase mitotic spindle, have a very narrow distribution of microtubule lengths. A tight distribution could be achieved by balancing microtubule growth and shrinkage. However, there is a difficulty: if the growth rate exceeds the shrinkage rate, then microtubules will grow indefinitely (an unbounded condition) and conversely, if the shrinkage rate exceeds the growth rate, then the microtubules will disappear altogether. Therefore, there must be some kind of feedback between length and growth to set a tight distribution of lengths. One possibility is the existence of length-dependent forces between antiparallel microtubules in an array such as the mitotic spindle [49]. An alternative is length-dependent depolymerization. Consider the end of a microtubule with several different proteins bound to it — some of them destabilizing the cap, others stabilizing the cap, and perhaps others accelerating growth. If the depolymerization is length-dependent, as shown for Kip3 [17•], this would define a set length of microtubules at which the growth is exactly balanced by shrinkage (Figure 1) and lead to a tight distribution of lengths. The set length would in turn depend on the activities of the polymerases and depolymerases.

Conclusions

The intrinsic ability of the microtubule lattice to transition between extended periods of growth and catastrophic disassembly has fascinated biologists since its first discovery. The cell has acquired mechanisms to modulate the basic reactions of dynamic instability by evolving depolymerases and polymerases that combine to set the steady state length of microtubules. Kinesin-family depolymerases disassemble stabilized microtubules; polymerases enhance the binding of tubulin subunits to microtubule ends. It will be crucial to discover how the cell regulates the relative activity of these two classes of proteins in order to set the length of microtubules according to cellular circumstances.

Acknowledgements

These ideas have benefited from discussion with members of our laboratories, especially Gary Brouhard, Kazuhisa Kinoshita, Tim Noetzel, Martin Srayko, Jeff Stear and Vladimir Varga, and our collaborators Jawdat Al-Bassam David Drechsel and Steve Harrison. We thank Claire Walczak and Gohta Goshima for comments on an earlier version of this manuscript.

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