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Tipping microtubule dynamics, one protofilament at a time

Amol Aher and Anna Akhmanova

Microtubules are polymeric tubes that switch between phases of growth and shortening, and this property is essential to drive key cellular processes. Microtubules are composed of protofilaments formed by longitudinally arranged tubulin dimers. Microtubule dynamics can be affected by structural perturbations at the plus end, such as end tapering, and targeting only a small subset of protofilaments can alter the dynamics of the whole microtubule. Microtubule lattice plasticity, including compaction along the longitudinal axis upon GTP hydrolysis and tubulin dimer loss and reinsertion along microtubule shafts can also affect microtubule dynamics or mechanics. Microtubule behaviour can be fine-tuned by post-translational modifications and tubulin isotypes, which together support the diversity of microtubule functions within and across various cell types or cell cycle and developmental stages.

Address
Cell Biology, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Corresponding author: Akhmanova, Anna (a.akhmanova@uu.nl)

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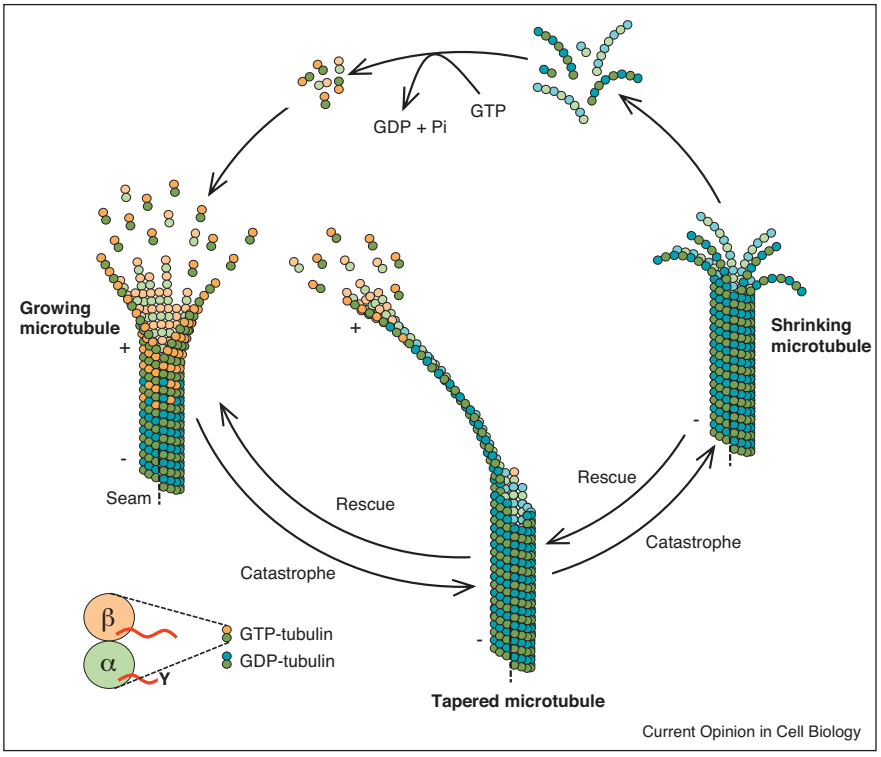
Introduction

Microtubules are built from dimers of α-tubulin and β-tubulin, which interact with each other in a head-to-tail fashion to form protofilaments, and 11–15 protofilaments, depending on the species and cell type [1–3], interact with each other laterally to form a hollow tube. The microtubule end where β-tubulin is exposed, termed the plus end, grows fast *in vitro* and serves as the major site of microtubule elongation in cells [4]. Microtubules can switch spontaneously from growth to shortening (Figure 1); furthermore, in cells they can also exhibit a paused state. Microtubules rarely pause in solutions of purified tubulin alone but can do so, for example, when exposed to a combination of microtubule growth-promoting and inhibiting factors [5]. The alternation between phases of growth and shortening, the phenomenon termed dynamic instability, is fundamental for most microtubule functions, from chromosome separation during cell division to cell reorganization during migration and differentiation. In this review, we will focus on the recent insights into how nucleotide hydrolysis and small perturbations in microtubule plus end structure bring about the transitions between different phases of dynamic instability at the microtubule plus end. We will also discuss the dynamics of microtubule shafts, which have always been seen as stable highways, but have now emerged as important sites of tubulin exchange that can affect microtubule stability and rescue from a depolymerizing state. Microtubule minus ends, which grow slowly *in vitro*, were traditionally regarded exclusively as sites of microtubule stabilization or disassembly. Recent work showed that minus ends can also display interesting and functionally important dynamics, but since this topic was reviewed recently [6], it will not be covered here. At the end, we will briefly touch upon the regulation of microtubule dynamics by tubulin isoforms and modifications, which are a major source of microtubule heterogeneity within and between different cell types and tissues.

Coupling GTP hydrolysis to structural changes in microtubules and catastrophe induction

Tubulin subunit addition during microtubule polymerization is coupled to GTP hydrolysis: the residues in both α and β-tubulin subunits complete the catalytic core for GTP hydrolysis and undergo structural changes upon hydrolysis (see [7] and references therein). Polymerizing microtubules have a stabilizing cap of GTP-bound tubulin subunits (GTP cap) at their ends, whereas the loss of this cap results in a switch from growth to depolymerisation (catastrophe). It has been proposed that GTP hydrolysis leads to a conformational strain at the E-site (GTP-binding site on β-tubulin), and that this strain is relieved upon depolymerisation, resulting in the formation of peeling protofilaments [7,8]. Recent high resolution cryo-electron microscopy (cryo-EM) studies of mammalian microtubules bound to GDP or GTP analogue GMPCPP indicated that GTP hydrolysis leads to structural rearrangements in both α-tubulin and β-tubulin and to microtubule lattice compaction along the longitudinal inter-dimer interface [7,9]. However, GTP-hydrolysis-dependent lattice compaction was not observed in microtubules grown from tubulin derived from budding or fission yeast [10•,11•]. This highlights species-specific

Figure 1



Microtubule polymerization–depolymerization cycle. Microtubules polymerize by the addition of GTP-bound tubulin dimers. Loss of the GTP-cap leads to microtubule destabilization and the switch to a shrinking state (a catastrophe) with peeling protofilaments. Microtubules have been proposed to go through a tapered intermediate state [8,12,15], although we note that alternative models with a blunt end constituting an intermediate between different phases of microtubule dynamics also exist [17]. When the stabilizing GTP cap at such a tapered end is lost beyond a certain threshold, a microtubule can switch to catastrophe; alternatively, it can regain a complete plus end structure and keep growing. Depolymerising microtubules can transit back to the polymerization phase (a rescue). A scheme of tubulin dimer at the bottom shows the flexible acidic tails of α-tubulin and β-tubulin (red lines); Y, the C-terminal tyrosine of α-tubulin.

differences in the structural plasticity of microtubule lattices. It should be noted here that we are still missing a complete picture of the structural transitions in microtubule lattice associated with GTP hydrolysis and that the data obtained with GTP analogues should be interpreted with some caution.

The exact structure of a microtubule plus end is a matter of debate. Importantly, there are indications that such ends are often not blunt: cryo-EM analysis of microtubules assembled *in vitro* has shown that a ~30–40% of the growing microtubule ends can be tapered and display curved sheet-like extensions that range from 50 to 2000 nm in length [8,12,13]. The length of these sheet-like structures at the growing ends was shown to increase with increasing growth rates in a tubulin concentration-dependent manner [8]. Furthermore, it has been proposed that catastrophe induction involves the accumulation of defects like lagging protofilaments when a microtubule keeps growing for a longer period of time and undergoes ageing [14]. Such defects could lead to destabilization of a growing microtubule, possibly resulting in a tapered tip structure with a reduced stabilizing GTP-cap that predisposes it to a catastrophe [14,15,16•]. Experimentally observed sheet-like extensions might thus correspond to ageing, catastrophe-prone tip structures (Figure 1). It should be noted, however, that correlative observations of the structure and dynamics of microtubule ends in frog egg extracts led to the suggestion that metastable intermediates between different phases of microtubule dynamics correspond to blunt ends [17]. Furthermore, modelling suggested that the dynamic evolution of microtubule tip structure could explain age-dependent microtubule catastrophes in the absence of visible changes in microtubule tip configuration [18]. It is clear that more work is needed to understand what is happening at the microtubule plus end undergoing a catastrophe.

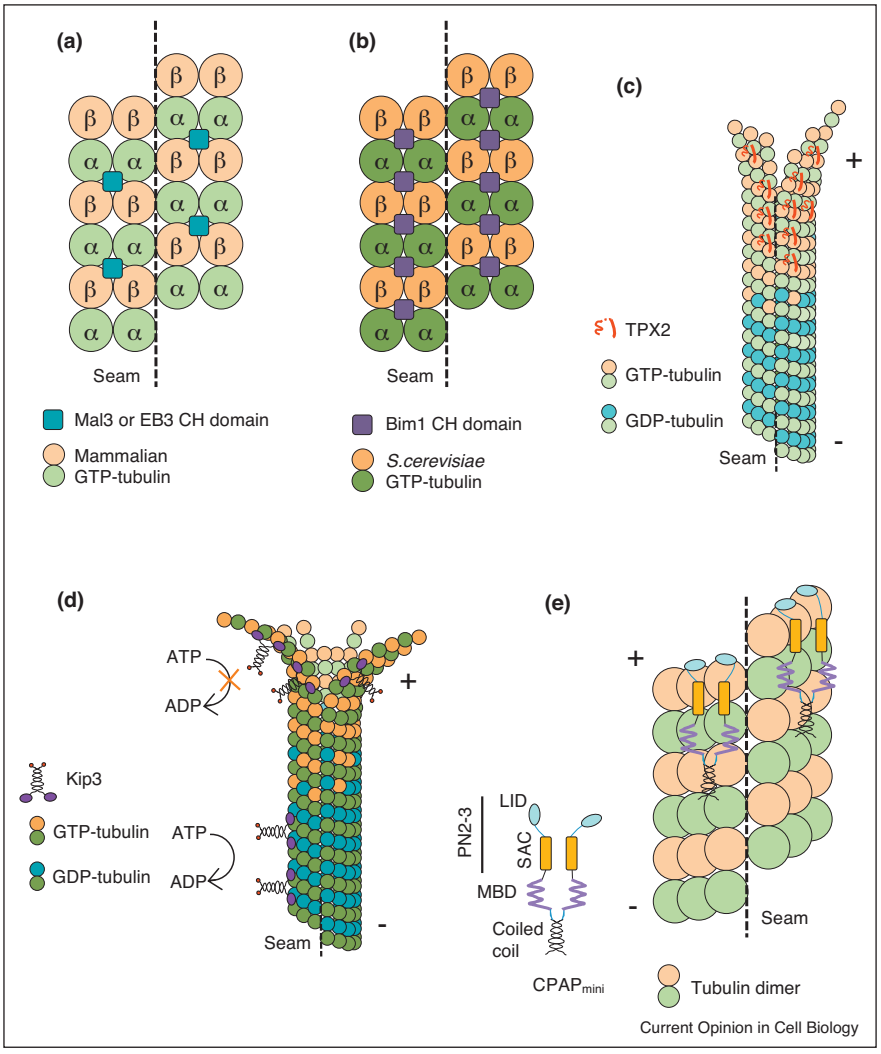
Visualization of stabilizing cap at microtubule ends was greatly facilitated by the discovery that the binding of the proteins of the End Binding (EB) family to microtubules is very sensitive to the nucleotide state of tubulin. EBs recognize a pocket on the microtubule in close proximity

to the β-tubulin-bound GTP between two adjacent protofilaments and next to the longitudinal inter-dimer interface [19,20] (Figure 2a). In the context of growing microtubule ends, EBs bind to the outer surface of curved and straight sheets as well as the complete tubes [13]. Microfluidics-assisted microtubule dynamics assays revealed that a growing microtubule switches to a depolymerizing one when the number of EB binding sites, which is indicative of the protective GTP-cap is lost beyond a certain threshold [16•,21]. Modelling suggested that tapering of a microtubule end can explain the age-dependent broadening of the protective cap at the microtubule end, which would reduce the density and stability of the cap [21].

Factors affecting microtubule end tapering and catastrophes

Since microtubule protofilaments peel off during depolymerization, factors directly enhancing protofilament curvature can promote catastrophes. This view is supported by studies of microtubule depolymerases of the kinesin-13 family (see [22] and references therein). Recently, this concept was also extended to kinesin-8 family: it was shown that Kip3 tightly binds to curved

Figure 2



Different plus end targeting modes employed by microtubule-regulating factors. Through their calponin homology (CH) domains, mammalian and fission yeast End Binding (EB) protein family members bind autonomously to the growing plus ends at the inter-dimer repeat at the contact site of four tubulin dimers **(a)**. Budding yeast EB homologue Bim1 can bind to both inter-dimer and intra-dimer sites **(b)**. TPX2 targets plus ends by binding longitudinal inter-dimer and lateral interfaces with two short flexibly linked sequence elements; it shows preference for GTP-bound microtubule lattices **(c)**. Kinesin-8 family member Kip3 uses ATP hydrolysis to step along the microtubule; at the plus end it acts as a depolymerase by tightly interacting with curved tubulin dimers while its ATPase activity is suppressed **(d)**. CPAP targets the terminal tubulin dimer at the plus end with its tandemly arranged LID, SAC and MBD (Microtubule Binding Domain). The LID domain recognizes the β-tubulin surface exposed at the plus end and the SAC domain contacts the sides of both α and β tubulin in the dimer **(e)**.

tubulin and promotes microtubule depolymerisation by stabilizing curved protofilaments (Figure 2d) [23].

Another way to induce catastrophe is by accelerating GTP hydrolysis. EB proteins increase catastrophe frequency in solutions of purified tubulin by several fold in a concentration-dependent manner [24,25], and EB binding to the microtubule plus end was shown to accelerate the conformational maturation of the microtubule lattice [26]. High resolution cryo-EM of mammalian microtubules showed that EB promotes compaction of the microtubule lattice upon binding, thereby leading to a more GDP-like lattice, and can stimulate hydrolysis of GMPCPP, a slowly hydrolysable analogue of GTP [20]. These properties possibly enable EBs to sensitize microtubules to other perturbations of microtubule tip structures, such as those induced by microtubule-targeting drugs [27•,28]. It should be noted, however, that in cells, EBs suppress rather than promote catastrophes [29,30], likely due to numerous and complex interactions of EBs with other microtubule regulators. Furthermore, although all EB family members accumulate at microtubule plus ends in cells, structural studies have revealed species-specific differences in EB binding. For example, the budding yeast EB, Bim1, binds at both intra-tubulin and inter-tubulin dimer contacts between protofilaments in the context of budding yeast tubulin (Figure 2b) and induces lattice compaction [11•]. Fission yeast EB homologue Mal3, on the other hand, binds only at the contact site of four dimers, which is similar to the mammalian EB (Figure 2a), but unlike its mammalian homologue EB3, it does not induce longitudinal compaction of the microtubule lattice [10•]. How these differences relate to the functional properties of EBs in different species is currently unknown.

Another protein which displays sensitivity for the GTP state of tubulin is the microtubule-stabilizing and nucleation-promoting factor TPX2 [31]. This protein uses short sequence motifs to bind across longitudinal and lateral tubulin interfaces, with the binding being optimal for the extended GTP-bound microtubule lattice (Figure 2c). TPX2 binding stabilizes microtubule lattice and may slow down lattice compaction and GTP hydrolysis, and thus suppress catastrophes and promote microtubule nucleation [31,32]. Engaging at lateral contacts between tubulin subunits might be a general property of protein domains promoting microtubule growth and nucleation, as it was also observed for one of the microtubule-binding TOG domains of the fly microtubule polymerase of the XMAP215 family, Msps [33]. Strengthening of lateral contacts, particularly those at the microtubule seam (the site where α-tubulin and β-tubulin subunits contact each other laterally within the microtubule lattice, see Figure 1) also underlies the effect of the microtubule-stabilizing drug peloruside [34].

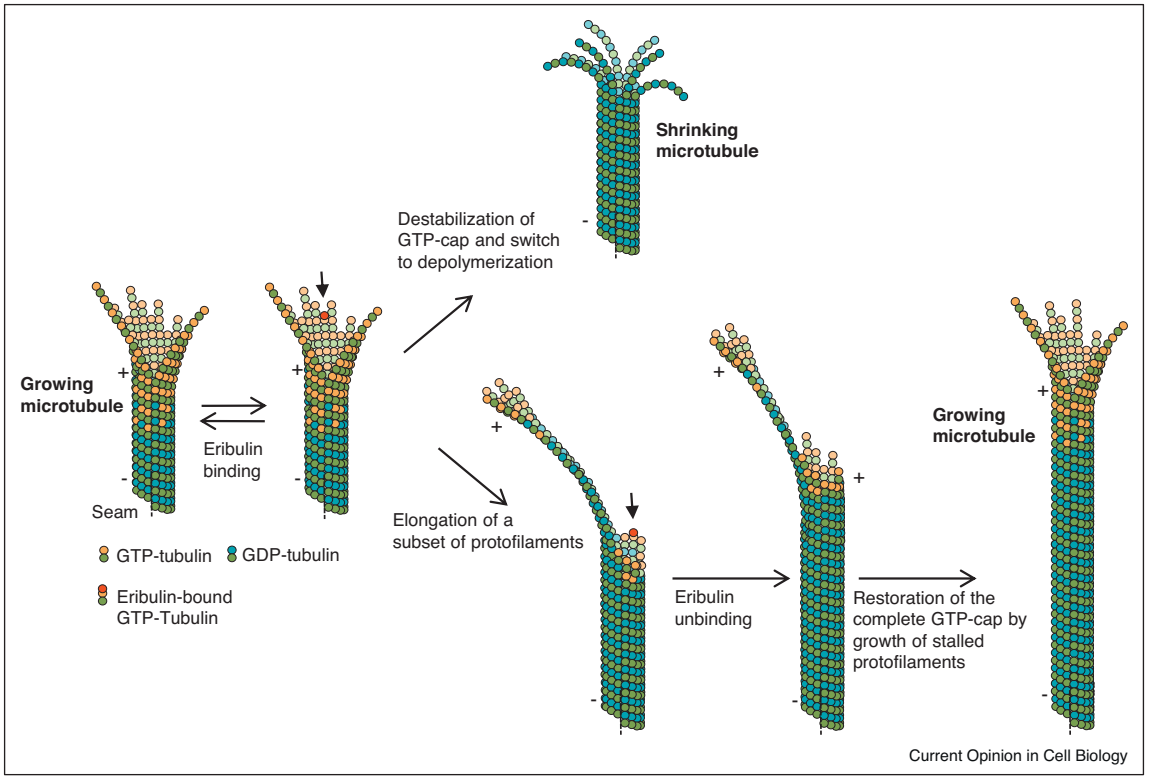
As indicated above, microtubule end tapering is a factor that might potentially lead to catastrophe. Microtubule protofilaments show asynchronous growth at the nanoscale but maintain the overall configuration as growth proceeds [35]. Interestingly, experiments with microtubule-destabilizing drug eribulin, which occludes the exposed surface of β-tubulin at the plus end and thus prevents the addition of the next tubulin dimer showed that blocking elongation of just a single protofilament could lead to catastrophe (Figure 3) [27•]. If microtubule growth was not immediately arrested by eribulin binding, it was typically perturbed, and such a microtubule often underwent a catastrophe at a later time point [27•]. Furthermore, low concentrations of eribulin that perturbed but did not block microtubule growth, caused an ~3-fold increase in the incidence of 'split' EB comets, which are indicative of the presence within the same microtubule of two longitudinally separated GTP caps (Figure 3). The two comets could merge together, resulting in restoration of normal microtubule growth, demonstrating that a microtubule with asynchronously growing protofilaments can recover its normal tip structure.

Although blocking of a small number of protofilaments can induce catastrophe, stabilization of a few protofilaments can be sufficient to prevent depolymerization. An interesting example is provided by the centriolar protein CPAP/SAS-4, which has both microtubule stabilizing and autonomous plus-end capping properties. The presence of 2–3 dimers of CPAP at the growing microtubule plus end, which could maximally engage and cap 4–6 protofilaments, was sufficient not only to slow down microtubule growth rate by 5–8 fold but also suppress catastrophe frequency by an order of magnitude [36]. Microtubule stabilization occurred through the combinatorial action of CPAP domains responsible for the binding at plus-end-exposed surface of β-tubulin and at the side of the same tubulin dimer, and microtubule lattice stabilization (Figure 2e) [36,37]. Similarly, a single dimer of kinesin-4 family member KIF21B was shown to be able to pause microtubule growth in a manner dependent on the motor domain and microtubule binding elements in the tail region [38]. Similar to eribulin, a single KIF21B molecule sometimes arrested a subset of protofilaments, while the remaining ones continued growth for some time before eventually switching to depolymerization. These findings suggest that targeting a few protofilaments is sufficient to destabilize the plus end, pause its growth or promote microtubule plus end stability.

Microtubule lattice repair and rescues

Microtubule lattices were traditionally regarded as intrinsically static structures that exchange dimers only from the ends. This view has been recently revised, and it is now clear that microtubule dynamics, longevity (the time between nucleation of a microtubule and its complete depolymerization) and mechanical properties (e.g., the

Figure 3



Perturbations at a growing plus end due to capping of a single protofilament. Capping of an exposed site on β-tubulin at the outmost growing plus end by eribulin leads to perturbed growth leading to a catastrophe or stalling of a subset of protofilaments. After eribulin unbinding, the stalled protofilaments can reinitiate growth, and a complete growing end can be restored.

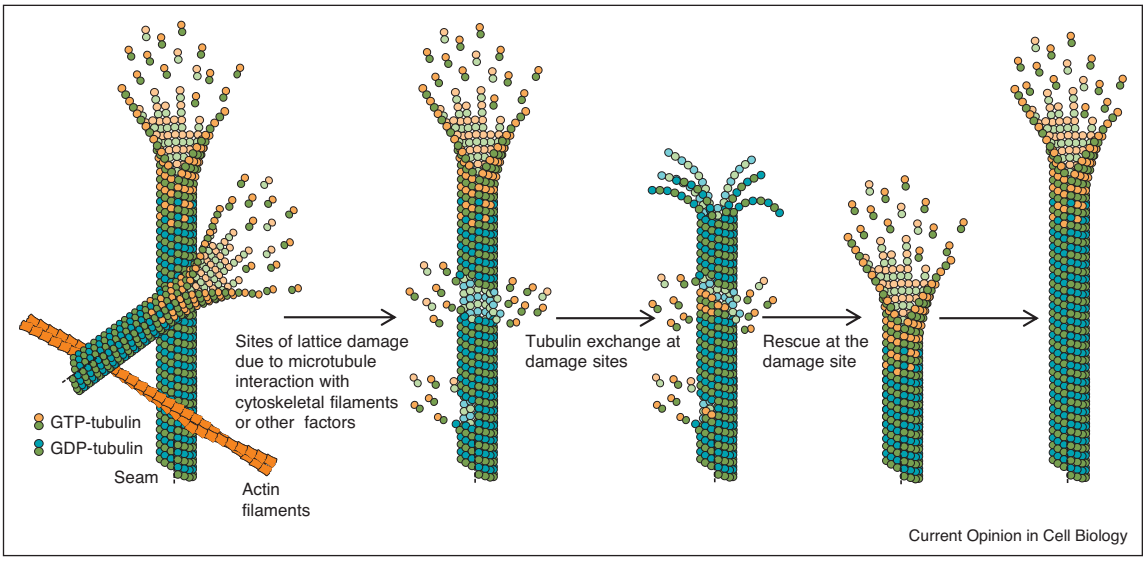
ability to deform or withstand mechanical stresses) are modulated by tubulin turnover within the lattice [39,40••]. *In vitro* assays using a microfluidic device to induce microtubule bending showed that microtubules soften when subjected to repeated cycles of mechanical stress, because they lose dimers at the sites experiencing the highest strain. These sites can incorporate new tubulin dimers enabling microtubule recovery into complete tubes as indicated by restoration of microtubule stiffness (Figure 4) [40••]. Self-repair at the sites of microtubule damage that might be caused by interactions with other cytoskeletal filaments, such as other microtubules can lead to incorporation of GTP-tubulin dimers from solution (Figure 4) [39]. The sites of microtubule damage and repair can subsequently induce rescue of depolymerizing microtubules either because they are intrinsically more stable than GDP-bound lattice or because they can recruit microtubule rescue factors such as CLIP-170 (Figure 4) [39,41]. It will be important to know whether the incorporation of GTP-tubulin dimers into microtubule lattice creates a rescue-prone region only until GTP is hydrolysed to GDP or whether it induces persistent changes.

Interestingly, rescue-prone regions can be also triggered by the microtubule-stabilizing drug taxol [28], which, similar to GTP-bound tubulin induces an extended microtubule lattice conformation [7,34]. Furthermore, microtubules grown *in vitro* have been shown to display spontaneous defects, such as transitions in protofilament number [42], and it will be important to know whether such defects also occur in cells and serve as a source of microtubule lattice turnover. Future studies comparing the kinetics of tubulin turnover in the lattice *in vitro* and in cells, where it is most likely modulated by regulatory proteins, will shed light on the impact of microtubule repair on modulating microtubule behaviour.

Microtubule heterogeneity: tubulin isotypes and modifications affecting microtubule dynamics

The structured core as well as the flexible tails of α-tubulin and β-tubulin (Fig. 1), which are hotspots for different post-translational modifications, can modulate microtubule stability directly or indirectly. Microtubules assembled from specific tubulin isoforms and lacking extensive post-translational modifications typical for brain microtubules, for example, α1B/βI + βIVb [43] or a human β-isotype, βIIB [44] displayed 2–3-fold reduced transitions to catastrophes compared to brain tubulin or βIII-tubulin, respectively. Such effects are similar to those

Figure 4



Dynamics within the microtubule lattice. Microtubule lattices subjected to mechanical stress, for example due to interactions with other microtubules, or, potentially, also actin filaments, can be damaged and lose tubulin dimers. Incorporation of GTP-bound tubulin dimers at the damage sites can restore microtubule integrity. A depolymerizing microtubule can undergo a rescue at the site of microtubule repair.

induced by some microtubule regulators (e.g., EBs) but smaller than those caused by highly potent microtubule-stabilizing and destabilizing drugs or proteins, which can completely arrest microtubule growth or depolymerization. Interestingly, microtubules grown from the recombinant α1B/βI + βIVb tubulin exhibited a shortened tapered region at the plus ends as seen by cryo-EM [43]. This property might explain their stability and reduced catastrophe frequency. The differential effect of tubulin isotypes on microtubule stability also underlies their role in sensitivity and resistance to microtubule-targeting chemotherapeutic agents, such as taxol or vinca alkaloids (reviewed in [45]).

The relationship between variations in tubulin sequences and their effects on microtubule dynamics is complex and not yet well understood. Interestingly, point mutations in β-tubulin located far from the tubulin site responsible for GTP-hydrolysis could impart stability to both ends of dynamic microtubules without significantly altering the lattice structure [46]. Another β-tubulin mutation, which is buried in the tubulin core, reduced catastrophe frequency by two fold and the shrinkage rate by two orders of magnitude, by suppressing a conformational change in the lattice that normally occurs in response to GTP hydrolysis [47].

Microtubule properties can also be affected by post-translational modifications of tubulin. For example, the depletion of the acetylating enzyme TAT1 from cells and *in vitro* reconstitution experiments demonstrated that intra-luminal acetylation makes microtubules more resistant to breakage [48••]. It has been proposed to be most likely due to weakening of lateral contacts between protofilaments, which would increase lattice flexibility and resilience against mechanical stress [49]. These changes could explain the increased longevity of acetylated microtubules [48••]. Another potential example is provided by detyrosinated microtubules, which buckle under load in beating cardiomyocytes; perturbing detyrosination by overexpressing tubulin tyrosine ligase or by a pharmacological approach showed that this microtubule modification confers mechanical resistance to contraction in these cells [50]. Since detyrosination occurs at the outmost tail of α-tubulin, it is unlikely to affect the mechanical properties of microtubules directly, but it could do so indirectly, by preferentially recruiting or repelling specific factors. For example, members of kinesin-13 family of microtubule depolymerases prefer tyrosinated microtubules as a substrate compared to detyrosinated ones [51]. Another interesting example is tubulin glutamylation, which was shown through biochemical approaches to tune microtubule severing by spastin depending on the number of glutamates per tubulin dimer: when glutamylation was increased up to a certain threshold, it promoted spastin activity, whereas beyond this threshold it had an inhibitory effect [52].

Importantly, post-translational modifications tend to accumulate in microtubules stabilized by other factors, and through the mechanisms described above, these modifications can exert a positive or a negative feedback on microtubule stability. This can lead to emergence of microtubule subsets with distinct properties, which can

be recognized by microtubule motors responsible for differential sorting of specific cargo. An interesting example of such sorting is provided by mitotic cells, in which the spindle, but not astral microtubules are enriched in detyrosination and serve as tracks for preferential chromosome transport [53•]. Another example are dendrites of mammalian neurons, in which stable and acetylated microtubules are mostly oriented towards the cell body, while dynamic, tyrosinated microtubules are oriented in the opposite direction, and the two microtubule subsets are preferentially used by different kinesins transporting various cargos [54••]. These examples illustrate how differences in microtubule dynamics are ultimately translated into fundamental pathways controlling cell architecture and polarity.

Conclusions and future directions

Genetic modification, such as knockout technologies in mammalian cells, combined with *in vitro* reconstitution systems using recombinant proteins, including tubulin itself, provide a powerful approach to dissect the complexity of the factors responsible for different aspects of microtubule behaviour. Improved light and electron microscopy methods generate an increasingly precise picture of microtubule structure and dynamics. For example, such studies recently illuminated the importance of microtubule perturbations and defects in controlling different microtubule properties and identified some factors that can induce, modulate or repair these defects. The next outstanding challenge is to generate a quantitative framework in which the relative importance and contribution of each factor is put into the context of the organization and behaviour of the whole system.

Acknowledgements

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