OPINION

# Growth, fluctuation and switching at microtubule plus ends

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Abstract | Recent experiments suggest that microtubules do not grow steadily but instead elongate at a rate that varies in time. We argue that this variation might arise from fluctuations in the length of a dynamic GTP–tubulin cap at the microtubule end. We propose that these fluctuations can lead to a switch in the dynamics of a microtubule end between growth and shrinkage, and provide insight into how the sensitivity of this switch can be changed by microtubule polymerases, such as XMAP215, and tensile forces, through the stabilization of initial contacts in the cap.

Microtubules are polymers of the protein tubulin that form the rigid cores of organelles, such as axonemes and mitotic spindles. They also serve as tracks for motor proteins and give shape to cells. Microtubules grow by the addition and loss of α- and β-tubulin heterodimers at their ends[1,2]. When growing in buffers that contain only tubulin, microtubules exhibit two behaviours. Above a certain tubulin concentration, microtubules polymerize until most of the tubulin is in the polymer, whereas below this tubulin concentration, they exhibit a fascinating switch-like behaviour known as dynamic instability: they abruptly switch between periods of growth and shrinkage[3]. This dynamic instability allows the microtubule cytoskeleton to explore the interior of the cell so that microtubule ends can find and bind to specific structures, such as the kinetochores of chromosomes[4,5]. The dynamic conversion from growth to shrinkage at the cell cortex also allows an aster of microtubules to centre itself in the cell[6–9]. Despite the importance of this switch-like behaviour in controlling microtubule distribution, we still do not understand how it works.

The switch-like behaviour of microtubules depends on GTP hydrolysis. Most tubulin dimers in solution have GTP bound to their β-subunit, and the hydrolysis of this GTP to GDP is triggered by polymerization[10]. GTP–tubulin forms stable filaments, as shown by the high stability of microtubules grown in the presence of the slowly hydrolysed GTP analogue GMPCPP[11]. Following hydrolysis, GDP–tubulin-containing microtubules are highly unstable and rapidly depolymerize. A cap of GTP–tubulin (or GDP-$P_i$–tubulin, in which the hydrolysis products of GDP and phosphate are still bound to the protein) at the end of an otherwise GDP–tubulin-containing microtubule is thought to provide enough stability to sustain growth[12]. However, if this GTP–tubulin cap is lost, the microtubule switches to a shrinking phase by a process termed catastrophe[3] (FIG. 1).

> “...microtubules ... exhibit a fascinating switch-like behaviour known as dynamic instability: they abruptly switch between periods of growth and shrinkage.”

In physiological conditions, microtubules also switch between periods of growth and shrinkage, but these transitions, as well as the rates of growth and shrinkage themselves, are regulated by proteins called microtubule-associated proteins (MAPs)[13]. MAPs fall into several classes: polymerases, depolymerases and microtubule plus-end tracking proteins (+TIPs). The polymerase XMAP215 (also known as CKAP5 and chTOG in humans, Stu2 in budding yeast and Dis1 in fission yeast), for example, was originally isolated from frog eggs, in which it accelerates the growth of microtubules by tenfold, thereby allowing for rapid regrowth of the microtubule cytoskeleton after fertilization[14]. Depolymerases, such as kinesin 8 and kinesin 13, are thought to depolymerize the GTP–tubulin cap and thereby stimulate the transition rate of microtubules from growth to shrinkage[15]. Both polymerases and depolymerases preferentially bind to microtubule ends. The +TIPs end-binding protein 1 (EB1; also known as MAPRE1), cytoplasmic linker protein 170 (CLIP170; also known as CLIP1) and CLIP-associated proteins (CLASPs) also bind transiently near the plus ends of growing microtubules[16]. +TIPs require EB1 for their localization[17,18] and have various effects on microtubule dynamics[19–21]. A further set of MAPs, which includes TAU (also known as MAPT), bind along the length of microtubules and stabilize them.

How do these various MAPs modulate the dynamics of microtubules? To solve this problem, we need to understand how they regulate the structure and dynamics of the growing and shrinking ends. Here, we discuss recent high-resolution measurements of microtubule growth, as well as recent suggestions that the observed large fluctuations in growth rates can be accounted for by variations in the length of the GTP–tubulin cap. This dynamic cap model provides an elegant explanation for the acceleration of microtubule growth by the polymerase XMAP215, and offers new insights into both the switching of the microtubule end between growing and shrinking states and how this might be regulated by MAPs and tensile forces.

## Fluctuations in growth rates

The structure and biochemistry of the microtubule end determines the behaviour of the microtubule. However, progress in microtubule research has been hampered by the technical difficulties of tracking the microtubule end at high resolution and correlating this with the biochemistry of tubulin. For motor proteins, high-resolution tracking has been facilitated by attaching fluorophores or beads to the protein of interest. However, high-resolution measurements of microtubule growth have been confounded by the complexity of the microtubule end: each of the 13 protofilaments of a microtubule can potentially grow and shrink independently, whereas switching from growth to shrinkage must involve a collective transition of all the protofilaments. This structural complexity makes even the definition of the microtubule end unclear. For example, can the microtubule end be defined as the position of the last tubulin dimer on the longest protofilament? If so, it might not be possible to resolve the end in microscopy assays owing to limited sensitivity. In general, the definition of the length of a microtubule will depend on the assay used to measure it, and the length will vary depending on whether it is measured mechanically, optically or by electron microscopy.

Recent experiments to track microtubule growth have used optical tweezers, which have been applied successfully to the study

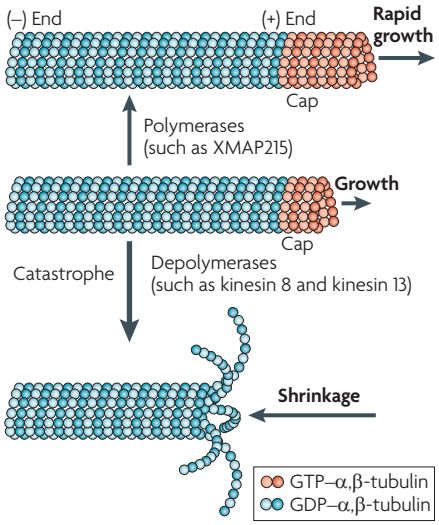


Figure 1 | **Regulation of growth and shrinkage of microtubules.** A cap of GTP–tubulin at the plus (+) end of GDP–tubulin-containing microtubules is thought to stabilize the growth phase (middle panel). Microtubule polymerases, such as XMAP215 (also known as CKAP5 and chTOG in humans, Stu2 in budding yeast and Dis1 in fission yeast), accelerate growth, perhaps by reducing the dissociation of incoming tubulin dimers and stabilizing the GTP cap (upper panel). Loss of the cap (for example, through the action of depolymerases such as kinesin 8 and kinesin 13), results in rapid shrinkage (termed catastrophe), with the protofilaments fraying or bending away from the microtubule axis (lower panel).

of motor proteins[22–25]. An important finding of these studies is that the length does not increase steadily but rather fluctuates on subsecond timescales, leading to apparent steps of 10–40 nm amplitude[23,24]. How do these fluctuations arise? Some variation will come from the random addition of tubulin dimers (BOX 1). Additional variation will arise because not all additions of tubulin will lead to the same length increase (BOX 1); for example, if we define the end of a microtubule to be the end of the longest protofilament, then addition to the longest protofilament will lead to a length increase of 8 nm (the length of a tubulin dimer), but addition to a lagging protofilament might lead to no length increase at all. The average length increase is 0.6 nm, which is calculated by dividing the dimer length by the number of protofilaments.

However, sources of variation involving the random addition of tubulin dimers are not sufficient to account for the measured size of the fluctuations. One possible explanation for the large fluctuations in microtubule growth rates might be that growth occurs by the addition of tubulin oligomers from solution, increasing the average step length increase (and its variance)[23]. This goes against the conventional wisdom that growth occurs by the addition of single dimers (for example, see REFS 26,27), but gains some support from recent electron microscopy and fluorescence correlation spectroscopy studies that suggest that the propensity for tubulin to form oligomers in solution might be higher than previously thought[28] (however, see online comments associated with REF.28).

## Dynamic cap model

An alternative explanation to account for the large fluctuations in microtubule growth rates is that the GTP–tubulin cap at the microtubule plus end varies in length[24]. The idea is that the GTP–tubulin cap is dynamic in that it grows and shrinks, with perhaps 90% of the GTP–tubulin dimers that bind to the cap dissociating before they are incorporated stably into the microtubule lattice (FIG. 2a,b). If both growth and shrinkage are random events, length variation will be much larger than variation that is due to random growth alone (BOX 1). The variation in cap size then adds to the overall variation in growth rate and even allows negative speeds if the cap is in a period of persistent shrinkage (FIG. 2a,b). This explanation fits with earlier measurements of fluctuations in growth rate using differential interference contrast video microscopy[29]. This dynamic cap model also resolves a potential thermodynamic inconsistency that is posed by the large apparent steps measured using optical tweezers: a length increase greater than 25 nm against a force of 4 pN (REF. 23) corresponds to mechanical work (force × distance) of greater than $100 \times 10^{-21}$ J, which exceeds the free energy that is available from GTP hydrolysis under cellular conditions[30]. Oligomer addition would therefore have to be associated with the concerted GTPase activity of the individual tubulin dimers in the oligomer.

One potential problem with the dynamic cap model is that it assumes that tubulin dissociation from the cap can be very fast. At a net growth rate of 1 μm per minute (which corresponds to a net addition of ~30 tubulin dimers per second), the rate of dissociation of GTP–tubulin from the cap would exceed 100 dimers per second. However, GMPCPP–tubulin (which is a model for GTP–tubulin) in stable microtubules dissociates from the plus end at only ~0.03 μm per minute, which corresponds to only 1 dimer per second[11]. These rates can be reconciled if it is assumed that GTP–tubulin dissociates quickly from 'unfavourable' sites, in which the tubulin dimers have few neighbours, but only slowly from 'favourable' sites, in which each dimer makes stabilizing contacts with many neighbours, such as when it is almost completely buried in the lattice. In a growing cap, many of the incoming GTP–tubulin dimers initially bind at unfavourable sites, such as at the end of the longest protofilament, and then dissociate before they are stabilized by subsequent

### Box 1 | **Cap fluctuations and Poisson steppers**

To explain the fluctuations in microtubule growth rates, we consider a Poisson stepper. This is a growth process in which subunits (of length $d$) bind at random times (at an average time interval of $\tau$). The average growth rate is $d/\tau$. Because the binding events occur at random, growth is not smooth. The average number of subunits added (that is, the average number of steps) in a time interval has a standard deviation that is equal to the square root of the number of steps — for example, if there are nine subunits added in an interval, the standard deviation is three (33% of the mean). The additional variation owing to the raggedness of the microtubule end (that is, some subunit additions lead to greater lengthening than others) increases the variance: if the length increase per subunit addition has a standard deviation equal to the mean length increase, the total variance will double.

Growth and shrinkage of the GTP cap contribute additional noise to that of a stand-alone Poisson stepper. Suppose that microtubule growth is the sum of a cap-lengthening process with a mean speed of $v_+$ and a cap-shortening process with a mean speed of $v_-$. If these processes are independent, the mean speed is proportional to the net number of subunit additions (that is, $v_+ - v_-$). However, the standard deviation is not proportional to the square root of the net number of subunits added; instead, it is proportional to the square root of the total number added to and removed from the microtubule (that is, $v_+ + v_-$). For example, suppose there is a net addition of 9 subunits in a time interval, and that this is due to 45 additions and 36 subtractions. In this case, the standard deviation is not equal to $\sqrt{9} = 3$ (as it would be for a Poisson stepper) but instead is $\sqrt{(45 + 36)} = \sqrt{81} = 9$. The length of the cap fluctuates wildly (FIG. 2b) and this causes large fluctuations in growth rate. Indeed, sometimes the cap (and microtubule) will grow much faster than average, whereas at other times it might actually shorten. This dynamic cap model recapitulates the length traces shown in REF. 24 (FIG. 2a).

GTP–tubulin additions. By contrast, a depolymerizing microtubule that contains GMPCPP–tubulin will quickly lose these unfavourably bound GMPCPP–tubulin subunits (an initially ragged cap will thereby smoothen), and the shortening rate will be limited by dissociation of GMPCPP–tubulin from the favourable sites. Thus, the dynamic cap model is consistent with the stability of a GMPCPP-containing microtubule.

### Structural changes in tubulin

What determines whether a tubulin dimer is stably incorporated into the lattice or not, and why are some sites favourable and others not? For example, does a site need to be surrounded by enough other dimers to be favourable? Does stable dimer incorporation involve nucleotide changes (for example, hydrolysis or phosphate release), or does it involve conformational changes in the incoming tubulin itself?

The textbook model for the incorporation of tubulin into the microtubule end is as follows: the GTP–tubulin dimer is 'straight' and so fits well into the straight wall of the microtubule[31–33] (FIG. 3). By contrast, the GDP–tubulin dimer is 'kinked' at the interface between the α- and β-tubulin monomers within the dimer, and so prefers to form curled protofilaments that are energetically unfavourable when constrained in the straight wall of the microtubule[34,35].

Recent observations challenge this idea by suggesting that a conformational change in the tubulin takes place after polymerization: the full straightening of the GTP–tubulin only occurs after making contact with favourable sites in the lattice[36]. This view is based on the finding that γ-tubulin has a similar structure in both its GDP- and GTP-bound states. γ-Tubulin is a distant relative of α- and β-tubulin and nucleates microtubules by forming a ring-shaped complex at their minus ends. Although γ-tubulin does not form dimers, its structure is similar to the structure of the α- and β-tubulin monomers in the heterodimer in solution. Rice *et al.*[36] argue that if the GDP–tubulin and GTP–tubulin structures are similar in solution, then the 'unkinking' of the tubulin dimer must take place only after incorporation into the lattice. A difficulty with this argument is that small, unresolved changes at the interface between monomers that are not detected might be sufficient to influence the kink between monomers. Nevertheless, the hypothesis that straightening requires interactions with the lattice is appealing. Although the GTP–tubulin protofilament is straighter than a GDP–tubulin protofilament[31,37], it is not as straight as the dimer in the microtubule wall[10,38]. In other words, the full change in conformation from kinked to straight requires both changes in the nucleotide state and the formation of contacts in the lattice. Furthermore, straightening could have a role in GTP hydrolysis. Structural studies show that completion of the GTP hydrolysis pocket in the terminal tubulin subunit requires residues from the incoming dimer[10]. Strain developed during straightening in the lattice might further accelerate hydrolysis, coupling the GTPase cycle to polymerization[39].

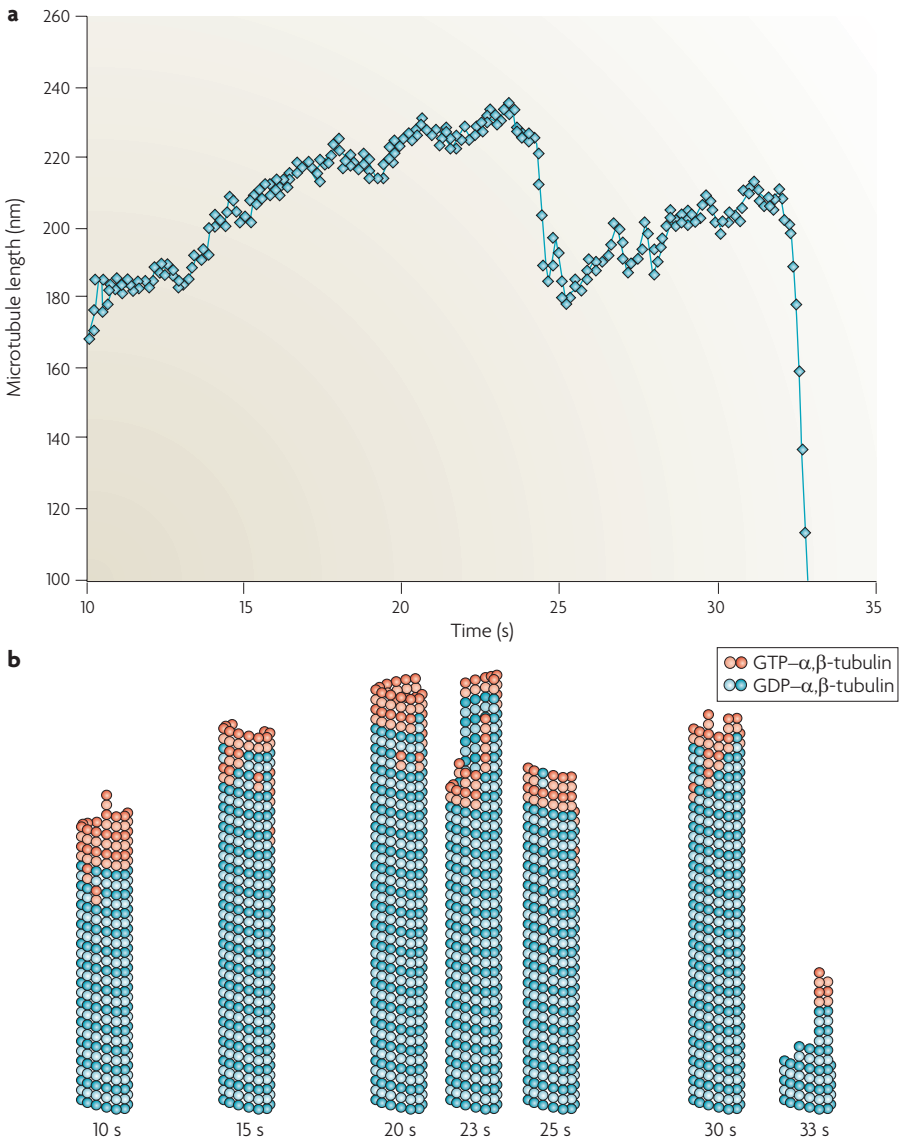


Figure 2 | **Dynamic cap model. a** | Simulated microtubule length over ~30 s. **b** | Microtubule simulations at selected time points. The GTP cap is shown at the end of a GDP–tubulin microtubule lattice. Changes in microtubule length result both from changes in cap length, as well as from elongation and shortening of the GDP–tubulin lattice (see REFS 24,27 for details). Figure is courtesy of M. Gardner, University of Minnesota, USA.

### Acceleration of growth by XMAP215

MAPs might provide insights into the mechanism of polymerization. As mentioned above, XMAP215 is a microtubule polymerase that accelerates growth by tenfold[40,41]. Electron microscopy images sometimes show XMAP215 to be highly elongated[42], which led to the hypothesis that XMAP215 accelerates growth by carrying several tubulin dimers *en masse* to the microtubule end. XMAP215 contains five tubulin-binding TOG domains (the structure of which was recently solved[43,44]) that could coordinate the formation of a large tubulin oligomer in solution. This 'shuttle' mechanism, also

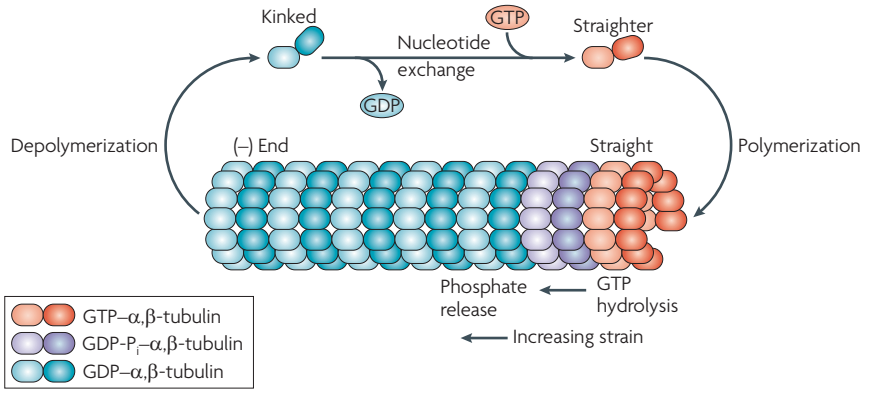


Figure 3 | **Tubulin GTPase cycle.** Exchange of GDP for GTP from the tubulin heterodimer in the cytoplasm partially straightens the kinked GDP–tubulin subunit. The GTP–tubulin subunit straightens fully when constrained in the wall of the microtubule after polymerization. Following hydrolysis, the GDP–tubulin dimer is even more strained, and this strain is the driving force for depolymerization, thus completing the cycle.

termed chaperoned growth, was supported by optical tweezer experiments[23] (see above), that showed larger growth fluctuations in the presence of XMAP215 (steps as large as 80 nm were reported). Because the tubulin dimer is only 8 nm long, the authors interpret these observations as the addition of tubulin oligomers that contain up to ten dimers.

However, a recent study[45] has tested the shuttle model and found three arguments against it. First, several experiments — analytic ultracentrifugation, gel filtration chromatography and single-molecule colocalization — indicate that the binding stoichiometry of tubulin to XMAP215 is 1:1. Second, for the shuttle mechanism to operate, the rate of targeting of XMAP215 to the microtubule end has to be high to sustain the high growth rates. Direct measurement of the end-association rate using single-molecule fluorescence, however, showed that it was too low to account for the high growth rate, even if each XMAP215 delivered as many as ten tubulin dimers. Third, in a shuttle mechanism, XMAP215 must leave the end quickly to allow another shuttle to land. However, the single-molecule fluorescence studies show that XMAP215 'surfs' the growing microtubule end, staying there for a time during which more than 25 tubulin dimers per protofilament (more than 300 per microtubule end) are added. A second microtubule-binding site is probably required for XMAP215 to remain associated with the microtubule end while promoting polymerization; this might involve an electrostatic interaction with the basic carboxy-terminal domain of tubulin[45].

How can the disparate conclusions from the optical tweezer and single-molecule fluorescence studies be reconciled? One possibility is that the fluctuations in microtubule growth rates observed by optical tweezer experiments are due to variations in the amount of XMAP215 at the microtubule end. Two arguments support this interpretation. First, the average growth rate in the optical tweezer experiments in the presence of XMAP215 is low (1–2 μm per minute), only one-tenth of the rate expected at saturating XMAP215 concentrations. If XMAP215 molecules bind to and unbind from the protofilament ends at random, considerable variation in the number of XMAP215 molecules at the end is to be expected under subsaturating conditions. When fewer XMAP215 molecules are at the end we would expect a lower growth rate, compared with a higher growth rate when more are at the end.

Second, the rising phases of the steps are not instantaneous, as would be expected for a discrete event such as the addition of a large tubulin–XMAP215 complex. Instead, the steps have finite slopes, corresponding to growth rates that are still less than expected under saturating XMAP215 conditions[45,46]. Perhaps the rising phases of the steps are periods of high XMAP215 occupancy at the microtubule end and therefore high growth rate. Another possibility is that the rising phases of the steps seen by the optical tweezer experiments correspond to the zippering of adjacent soft protofilaments growing from the ends of the microtubules, thereby creating a stiffer end that can be resolved in the presence of the force exerted by the tweezers. The steps could therefore be concerted conformational changes in the dynamic cap. If force influences the end structure, then the length measured in the optical tweezer experiments might differ from those measured optically or by electron microscopy, in which there is no force.

## The dynamic cap model and XMAP215

The dynamic cap model provides an elegant explanation for how XMAP215 accelerates microtubule growth. Although 90% of the incoming GTP–tubulin dimers normally dissociate, XMAP215 slows down this rate and therefore at high XMAP215 concentrations, the growth rate could be accelerated by up to tenfold. In other words, XMAP215 inhibits dissociation of GTP–tubulin from the cap[45].

How might XMAP215 inhibit dimer dissociation from the dynamic cap? The recent structural model of the GTP–tubulin cap discussed above suggests some possible mechanisms of action of XMAP215. XMAP215 could provide additional contacts that stabilize the binding of GTP–tubulin. An alternative mechanism is suggested by the activity of taxol, which also stimulates microtubule growth. Taxol straightens the protofilament[33,47] (FIG. 4), suggesting that it might act by straightening the tubulin dimer and helping it to bind in the lattice. Perhaps XMAP215 also straightens the incoming tubulin and leads to more favourable inter-protofilament interactions. Thus, the straightening of the slightly bent GTP–tubulin into the lattice might provide a regulatory point for the activity of microtubule polymerases.

## Switching in a new light

The model of the cap, in which the dissociation rate, the nucleotide state and even the conformation of a tubulin dimer are influenced by its neighbours, leads naturally to the abrupt switching between growing and shrinking phases that is at the heart of the dynamic instability process[3]. The higher the growth rate, the more GTP subunits in the cap and the more stable the cap; the lower the growth rate, the less stable the cap. The net result is a positive-feedback loop between growth and stability. Higher growth rates will stimulate more favourable contacts, which will further stimulate growth, and lower growth rates will stimulate fewer favourable contacts, leading to catastrophe. An important consequence of positive feedback is that if the contacts are sufficiently cooperative (that is, if each dimer is influenced by a sufficient number of neighbours), then the cap can switch stably between a growing and a shrinking state. Such switching is analogous to that discussed in signalling and genetic networks[48,49].

Switching is an emergent property that requires feedback and is facilitated by cooperativity[50]. A tubulin dimer makes six contacts within the microtubule lattice. This large number lends itself naturally to a high degree of cooperativity. This is contrasted by

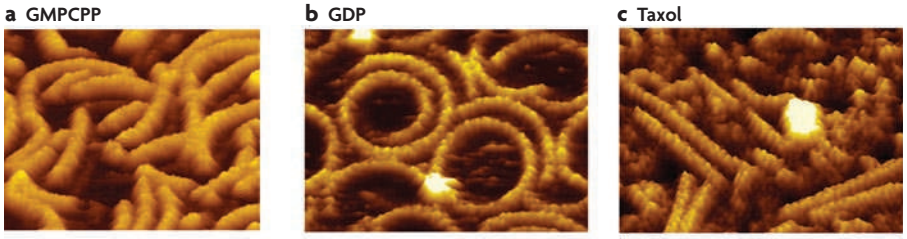


Figure 4 | **Atomic force microscopy images of microtubule protofilaments. a** | Slightly curved protofilaments formed from the depolymerization of GMPCPP–tubulin microtubules by the addition of $Ca^{2+}$ (GMPCPP is a slowly hydrolysed GTP analogue). **b** | Strongly curved protofilaments formed by the depolymerization of GDP–tubulin microtubules. **c** | Straight protofilaments formed by GTP–tubulin in the presence of taxol. The periodicity seen in the protofilaments corresponds to the length of the tubulin monomers (4.1 nm). The topographic contrast corresponds to a height scale of 10 nm[33]. Images (see also REF. 33) are courtesy of D. Mueller, Dresden University of Technology, Germany.

the case of actin, in which the dimers make fewer contacts and dynamic instability has not been observed. The packing of the dimers in the lattice might therefore explain why dynamic instability in microtubules exhibits such a strong switch-like behaviour.

One way to think of polymerases and depolymerases is as proteins that bind to the cap and alter the sensitivity of the switch. The sensitivity of the switch might also be altered by tensile forces, which have been shown to inhibit catastrophe when applied to the growing microtubule end by the DAM1 complex[51]. Perhaps tension, like taxol, straightens protofilaments, thereby stabilizing lateral tubulin–tubulin contacts. Slender filaments, observed by electron microscopy to connect kinetochores to the ends of microtubules, might transfer tension to the protofilaments, stabilizing the microtubules in the mitotic spindle[52]. Thus, the straightening of the slightly bent GTP–tubulin by ligands (for example, taxol), proteins (for example, XMAP215) or tension, might regulate the set point of the catastrophe switch. By straightening bent protofilaments, ligand binding and tension will also change the length of the microtubule. This highlights our earlier point that the length of a microtubule will depend on the method used to measure it.

The growing ends of microtubules might also be modified by +TIPs, such as EB1. Rapidly growing microtubules have gently curved sheets at their ends[53]. The relationship of this end structure to the GTP–tubulin cap is not known; however, recent work has shown that EB1 can contribute to the formation and closure of the sheets[20] by binding to the seam formed when the microtubule sheet closes[54]. The two protofilaments on either side of the seam are staggered with respect to each other (A-type interface), whereas the rest of the protofilaments are almost in register (B-type interaction). Consistent with this finding, EB1 has been shown to promote the formation of A-lattice microtubules, in which most of the interfaces are A-type[19]. Thus, EB1 might modify microtubule dynamics by altering the strength and type of lateral interactions between tubulin dimers.

The ability to directly visualize the GTP state of tubulin is an important requirement. This would provide insight into the signalling-like processes in the cap, just as phospho-specific antibodies have proven crucial for understanding signalling by phosphorylation cascades, and GTP-sensitive antibodies have been useful in understanding signalling by G proteins. An exciting recent paper characterizes a new antibody directed against GMPCPP–tubulin, which binds to the plus ends of microtubules *in vivo* and *in vitro*[55]. Interestingly, the antibody marks internal sites in the microtubule, suggesting that GTP hydrolysis might not always be completed. Intriguingly, the positions of these internal sites correlate with the transition from shrinkage to growth *in vivo*, providing a molecular explanation for the occurrence of these so-called 'rescues'. This antibody should be useful in testing whether MAPs act by influencing the hydrolysis reaction.

## Concluding remarks

The growth of microtubules is accompanied by large fluctuations in rates and abrupt transitions between phases of growth and shrinkage. There are many potential sources of this variation but we argue that fluctuation in the length of the GTP–tubulin cap might be the main source. In this view, length fluctuations might reflect highly cooperative structural and chemical transitions in the cap that are essential for switching. Moreover, plus end-binding proteins might modulate microtubule growth and catastrophe by interfering with these transitions.

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## DATABASES

**UniProtKB:** http://www.uniprot.org
CLIP170 | EB1 | XMAP215

## FURTHER INFORMATION

**Jonathon Howard's homepage:** http://www.mpi-cbg.de/research/research-groups/joe-howard.html
**Anthony A. Hyman's homepage:** http://hymanlab.mpi-cbg.de/hyman_lab

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