Dynamics and mechanics of the microtubule plus end

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An important function of microtubules is to move cellular structures such as chromosomes, mitotic spindles and other organelles around inside cells. This is achieved by attaching the ends of microtubules to cellular structures; as the microtubules grow and shrink, the structures are pushed or pulled around the cell. How do the ends of microtubules couple to cellular structures, and how does this coupling regulate the stability and distribution of the microtubules? It is now clear that there are at least three properties of a microtubule end: it has alternate structures; it has a biochemical transition defined by GTP hydrolysis; and it forms a distinct target for the binding of specific proteins. These different properties can be unified by thinking of the microtubule as a molecular machine, which switches between growing and shrinking modes. Each mode is associated with a specific end structure on which end-binding proteins can assemble to modulate dynamics and couple the dynamic properties of microtubules to the movement of cellular structures.

The textbook functions of microtubules are to act as beams that provide mechanical support for the shape of cells, and as tracks along which molecular motors move organelles from one part of the cell to another (Fig. 1a). To perform these functions, a cell must control the assembly and orientation of its microtubule cytoskeleton. Microtubules assemble by polymerization of α-β dimers of tubulin. Polymerization is a polar process that reflects the polarity of the tubulin dimer, which in turn dictates the polarity of the microtubule (Fig. 2a). *In vitro*, purified tubulin polymerizes more quickly from the plus end, which is terminated by the β-subunit. The other, slow-growing end is known as the minus end, and is terminated by the α-subunit. In animal cells, minus ends are generally anchored at centrosomes, which consist of specialized microtubule-based structures called centrioles, surrounded by pericentriolar proteins[1] (Fig. 1b). In yeast, the analogous structure is the spindle pole body[2]. An important component of the centrosome is an unusual form of tubulin, γ-tubulin, which is thought to initiate nucleation by forming rings that act as templates for new microtubule growth[3,4]. After nucleation, microtubules grow out with their plus ends leading into the cytoplasm. Thus to a first approximation, the distribution of the microtubule cytoskeleton is determined by the location of the centrosome.

The first clue as to how cells rearrange the distribution of microtubules came from the discovery that during the polymerization of pure tubulin, plus ends switch between phases of slow growth and rapid shrinkage[5] (Fig. 2b). The conversion from growing to shrinking is called catastrophe, whereas the conversion from shrinking to growing is called rescue (Fig. 2b). Analysis in tissue culture cells[6,7] and in cellular extracts[8] soon confirmed that this behaviour, termed dynamic instability, is a feature of microtubules growing under physiological conditions (for a review, see ref. 9).

The importance of the discovery of dynamic instability was that it provided for the first time a mechanism by which microtubules could reassemble into different structures during the cell cycle or during development. It was hypothesized that microtubules could grow out and if they made productive interactions with cellular structures[10] or soluble cues[11,12], they would be stabilized. An early confirmation of this idea was the finding that kinetochores, specialized structures that connect microtubules to chromosomes, can 'capture' and stabilize the end of a growing microtubule[13]. Recently, soluble cues have also been shown to modulate microtubule dynamics during spindle assembly in *Xenopus* egg extracts. Here a Ran-dependent signal changes the local environment of cytoplasm around the chromosomes, stabilizing the plus ends and initiating the assembly of the mitotic spindle (for a recent review, see ref. 14).

Microtubules as molecular machines

Once assembled, polarized arrays of microtubules provide tracks for the transport of organelles and chromosomes[15]. This transport is driven by motor proteins such as kinesin and dynein that interact with and move along the lateral surface of the microtubule. Motor proteins are molecular machines — they transduce chemical energy derived from ATP hydrolysis into mechanical work used for cellular motility — and there has been considerable interest recently in understanding the biophysical mechanisms by which these protein machines work[16,17].

But examples of cellular motility exist that do not rely exclusively on motor proteins. One is the movement of chromosomes during metaphase and anaphase of mitosis (Fig. 3a). After the plus ends of microtubules have attached to the chromosome via the kinetochore[18], the growth and shrinkage of these kinetochore-attached microtubules move the chromosome away from or towards the pole to which the minus end of the microtubule is attached[19]. Other examples are provided by the movement of the nucleus or the mitotic spindle through interactions between microtubules and the cell cortex, where the cortex is loosely defined as the plasma membrane and its associated protein components. Such cortical interactions, inferred from experiments in embryonic systems such as *Caenorhabditis elegans* (Fig. 3b) or *Drosophila*[20,21], have now been viewed directly in yeast. In the fission yeast *Schizosaccharomyces pombe*, microtubules grow out from the spindle pole bodies

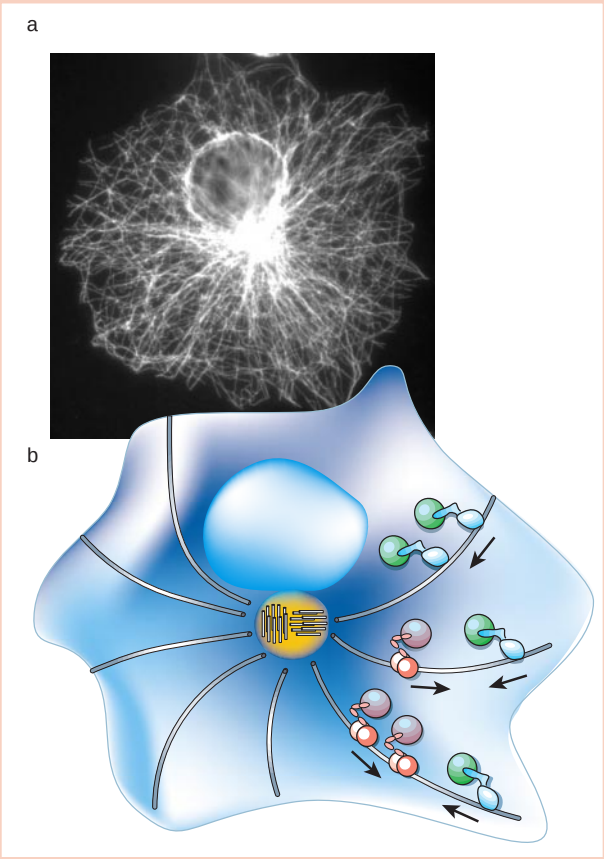

Figure 1 Microtubules are dynamic polymers. **a**, An interphase cell stained with an antibody to tubulin. Microtubules extend from the centrosome throughout the cell. (Image courtesy of A. Akhmanova.) **b**, A schematic diagram of the cell. Centrioles are shown in the centrosome (yellow). Red circles denote vesicles moving to the outside of the cell. Green circles denote vesicles moving to the centrosome.

and push back on the nucleus when their plus ends reach the ends of the cell[22]. The pushing from the two ends of the cell centres the nucleus. In the yeast *Saccharomyces cerevisiae*, cells divide by budding, resulting in a mother and a daughter cell. Prior to division, microtubules growing from one of the spindle pole bodies enter the bud where they attach to the cortex. The depolymerization of these cortex-attached microtubules is thought to reel in the spindle so that one of the poles is now located in the bud and will be inherited by the daughter following division[23–26] (Fig. 3c).

These examples suggest that microtubules themselves, in the absence of motors, can move cellular structures around inside cells by maintaining attachments as they grow or shrink[19]. *In vitro* studies with purified tubulin have confirmed that the end of a microtubule can act as a molecular machine that converts chemical energy into mechanical work, just like a motor protein. Polymerizing microtubules can deform membranes[27] or induce microtubule buckling[28], while depolymerizing microtubules can move beads attached to their ends[29]. Furthermore, the forces generated are high — up to 4 pN — which indicates that microtubule dynamics can generate as much force as motor proteins[16]. These forces can be used to form structures *in vitro*. Indeed, if an aster of outward-growing microtubules is placed in a microfabricated chamber, the pushing forces are capable of centring the aster[30,31], analogous to the centring of the nucleus in yeast[22]. Thus the microtubule end can be thought of as a molecular machine. Because microtubules grow and shrink by addition and loss of subunits from their ends, coupling of microtubule pulling and pushing to mechanical work can be distilled to the problem of the nature and control of the plus end of the microtubule.

GTP hydrolysis cycle

The energy to drive the microtubule machine comes from GTP hydrolysis. Tubulin is a GTPase whose activity is stimulated by polymerization[32]. A crucial observation is that tubulin polymerizes in the presence of non-hydrolysable GTP to form stable microtubules[33]. Thus, polymerization is driven by the high affinity of the tubulin–GTP dimer for the end of the microtubule. The high affinity means that polymerization will take place even against compressive forces, theoretically as high as several piconewtons[16], accounting for the ability of a growing microtubule to do work. But the high stability of the GTP microtubule poses a problem for disassembly, because GTP microtubules depolymerize at a negligible rate and evidently cannot do work while shortening. This problem is solved by GTP hydrolysis. The resulting GDP microtubule is very unstable and, if allowed to, will depolymerize even in the presence of tensile forces that oppose the depolymerization. Thus, binding of the GTP subunit can do work during the growth phase while unbinding of the GDP subunit can do work during the shrinkage phase.

There are two key regulatory events in the GTP cycle. The first is the coupling of hydrolysis to polymerization (for a detailed discussion, see ref. 34). An elegant coupling mechanism has been provided by the determination of the atomic structure of tubulin (Fig. 4a). In a microtubule, the β-subunit resides at the plus end[35]. The structure

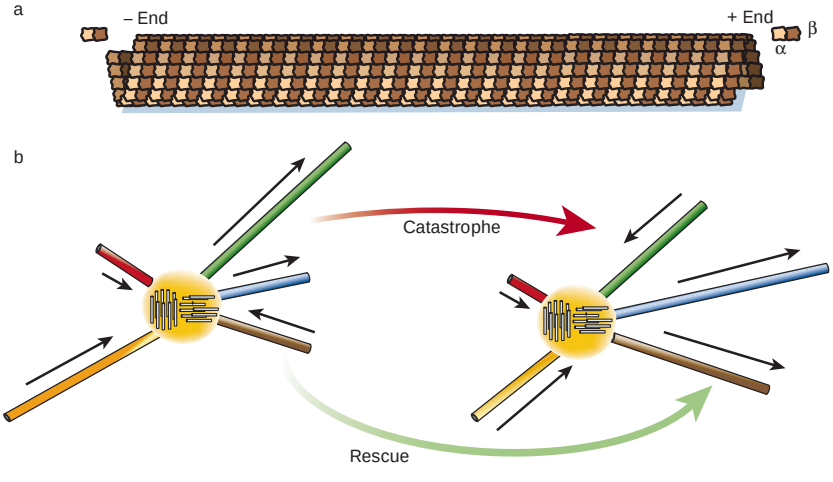

Figure 2 Microtubule structure and dynamics. **a**, A microtubule lattice. The β-subunit of tubulin is on the plus end. **b**, Dynamic instability of microtubules. Microtubules growing out from a centrosome switch between phases of growing and shrinking. The figure shows a hypothetical aster at two different times. The different colours represent different microtubules. The red and yellow microtubules are shrinking at both times. The blue microtubule is growing at both times. The green microtubule, growing at the first time, has undergone a catastrophe by the second time. The brown microtubule, shrinking at the first time, has undergone a rescue by the second time.

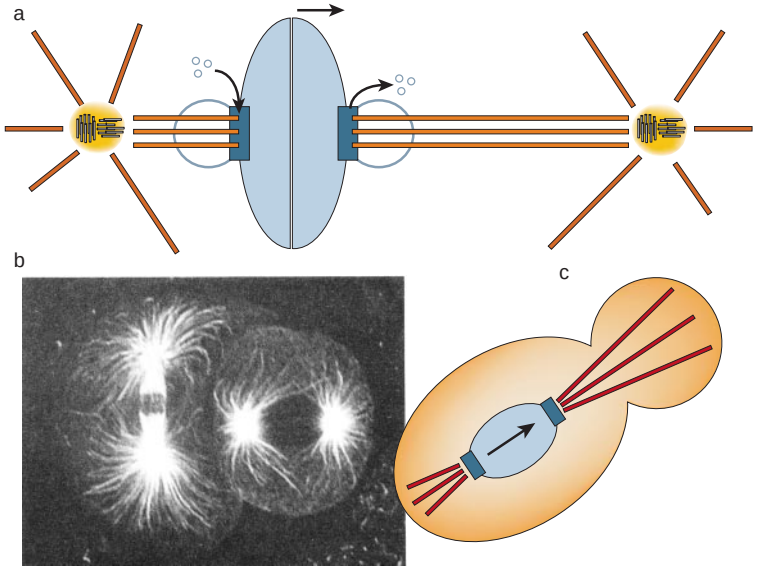

Figure 3 Interaction of microtubule ends with cellular structures. **a**, During metaphase of mitosis, movement of the chromosome (to the right) is associated with polymerization of microtubules on one side (left) and depolymerization on the other (right). **b**, Two-cell stage *Caenorhabditis elegans* embryo. One spindle (on the right) is rotated with respect to the other, perhaps through interactions between microtubules and a cortical site located between the two cells. **c**, Movement of the *Saccharomyces cerevisiae* spindle pole into the bud (at the right). Microtubules from one of the spindle pole bodies attach to the bud cortex. Depolymerization of these microtubules at the cortex may reel in the spindle into the bud.

shows that, although the β-subunit pocket can bind GTP, it lacks crucial residues necessary for hydrolysis. These residues are donated by the α-subunit when it docks to the end, and in this way hydrolysis is triggered[36] (Fig. 4b). If hydrolysis is faster than polymerization then the structural findings support a simple model in which a single ring of GTP subunits stabilizes the microtubule plus end by preventing internal GDP subunits from dissociating[37,38]. On the other hand, if hydrolysis lags behind polymerization, then a large cap of GTP subunits may form at the end and this could further stabilize the microtubule. Removal of this cap and the triggering of microtubule depolymerization constitutes the second key regulatory event. But we know a lot less about this event than the coupling of hydrolysis to polymerization. Recent work on the structure of the microtubule end, and proteins that bind to the end, is beginning to shed light on this issue.

Structure of the microtubule end

If a microtubule end is to act as a molecular machine, then it must undergo conformational changes in response to GTP hydrolysis. For example, motor proteins undergo a structural transition, known as the powerstroke, that is driven by the ATP hydrolysis cycle and that leads to the generation of force and the production of mechanical work[16,17,39]. Analogous changes do indeed take place at the ends of the microtubule. Viewing growing and shrinking microtubules in vitreous ice has shown that, both for pure tubulin and for microtubules growing under physiological conditions, the ends of growing microtubules (Fig. 4c) consist of two-dimensional sheets of protofilaments (head-to-tail arrangements of tubulin dimers)[40,41], whereas the ends of shrinking microtubules (Fig. 4d) are frayed, often resembling rams' horns[41,42]. Therefore it seems clear that there is a structural transition associated with the switch between growing and shrinking.

How does GTP hydrolysis control this structural transition? The early discovery of protofilament rings as depolymerization products of microtubules led to the hypothesis that GTP hydrolysis destabilizes the lattice by increasing the curvature of the protofilament[43,44]. Thus in the GTP state the subunits form straight protofilaments that fit nicely into the wall of the microtubules, whereas in the GDP state they form bent protofilaments that want to splay out from the lattice (Fig. 4d). Recent work has provided strong additional evidence for this model. First, protofilaments made from GTP–tubulin are straighter than those made from GDP–tubulin[45]. Second, the structure of the tubulin-sequestering protein Op18/stathmin complexed with two tubulin–GDP dimers shows the dimers are bent[46]. Although we do not know whether the bend is introduced by Op18 or not, it is suggestive that the bend within the dimer, together with rotation between the dimers, generates a protofilament with the same curvature as a GDP protofilament measured by other means.

We can now summarize with some confidence the relationship between GTP hydrolysis and the structural changes at the end of the microtubule. First, GTP–tubulin polymerizes onto the end of the microtubule (Fig. 2a). Second, docking of the α-subunit with the β-subunit of the lattice-attached dimer completes the hydrolysis pocket, triggering GTP hydrolysis (Fig. 4b). Third, GTP hydrolysis induces a bend within the subunit (or between subunits), inducing curvature in the lattice and destabilizing the microtubule (Fig. 4c). Thus the bending of the subunit induced by GTP hydrolysis is analogous to the powerstroke of a motor — the fuel driving the polymerization engine is GTP–tubulin binding to the end of the microtubule, whereas the fuel driving the depolymerization engine is release of mechanical strain from the lattice.

Proteins that bind to microtubule ends

Coupling of dynamic microtubule ends to cellular structures requires proteins with unusual properties. If a protein binds to the end of a shrinking microtubule, will it not detach as the tubulin dimers at the end detach? Conversely, if a protein binds to the end of a growing microtubule, will it not block the association of additional tubulin dimers?

Proteins that modulate microtubule dynamics have been known traditionally as microtubule-associated proteins or MAPs[47]. Such proteins, originally isolated from bovine brain, but since identified in all systems studied, increase the growth rate and prevent microtubule catastrophes. So far, studies of MAPs have told us little about the mechanisms by which proteins modulate the dynamics of the microtubule ends. The reason is that they bind all along the microtubule lattice, yet we expect that their effect on dynamics should take place only at the microtubule end. A significant step forward in understanding the dynamics of the plus end was taken with the introduction of green fluorescent protein (GFP) technology to describe proteins that specifically target microtubule ends and in many cases mediate their dynamics[48–50]. Two distinct classes of end-binding proteins have been described: the MCAKs (for mitotic centromere-associated kinesins),

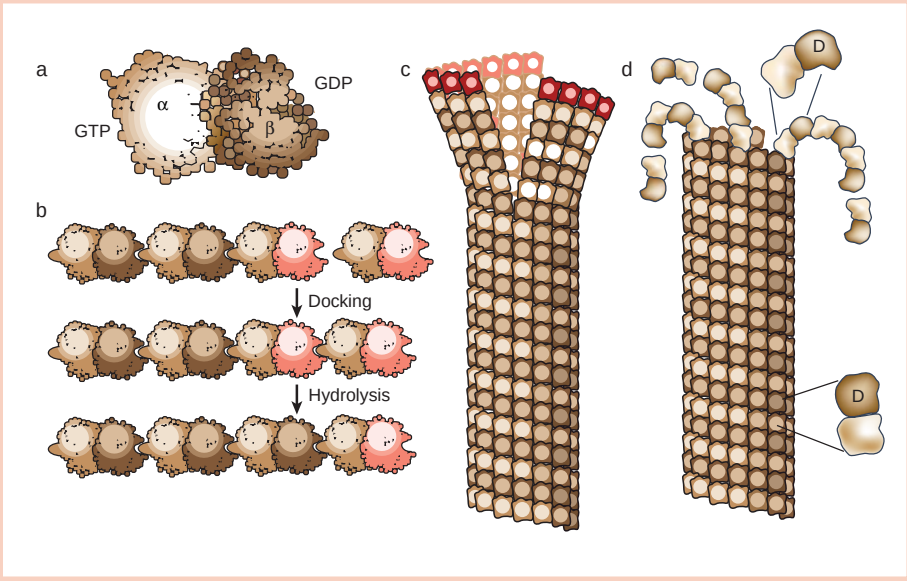

Figure 4 Model for how the GTP hydrolysis cycle is coupled to structural changes in the microtubule. **a**, Atomic structure of the tubulin dimer as seen in the wall of the protofilament. **b**, Docking of the α-β subunit to the microtubule end. Residues from the incoming α-subunit trigger hydrolysis of the GTP bound to the lattice-attached β-subunit. **c**, **d**, Microtubules at growing ends contain sheets of protofilaments while microtubules at shrinking ends curl. The straight–bent transition is also shown in panel **d**. The GTP dimer is thought to have a straight conformation that fits nicely into the straight wall of the microtubule. Hydrolysis of GTP induces a bend in the subunit, but this bend is constrained within the lattice. The constraint places stress on the lattice, which is released during depolymerization, allowing the protofilament to adopt a curled conformation.

which bind to microtubule ends and destabilize them (Fig. 5a), and the plus-end-binding proteins (or +TIPs[48]), which bind to the growing end of the microtubule and at least in some cases stabilize the microtubule during its growth phase (Fig. 5c).

MCAK/Kin I kinesins

The best understood end-binding proteins are the MCAKs, also called Kin I kinesins. These unusual kinesins[51,52], rather than moving along the surface of microtubules like other motor proteins, use energy from ATP hydrolysis to bind to the ends of microtubules, remove tubulin subunits and thus trigger depolymerization[53,54]. Removal of the *Xenopus* MCAK (XKCM1) from egg extracts dramatically increases the size of the microtubule arrays[55] by suppressing catastrophes[56]. Overexpressing MCAK in tissue culture cells leads to an almost complete loss of microtubules[57], perhaps by increasing catastrophes. The localization of MCAK at kinetochores suggests that they could trigger depolymerization during mitosis[58]. It has recently been shown that the combination of XKCM1 and a MAP (XMAP215) can reconstitute the physiological properties of dynamic instability *in vitro*[59]. Thus it seems that, by increasing the catastrophe rate, MCAKs are central to the generation of dynamic microtubules inside cells.

How might the interaction of MCAKs with the end of a growing microtubule convert it to a shrinking one? In the presence of non-hydrolysable ATP analogues, MCAK-family proteins bind to the ends of microtubules and form curled protofilaments — the rams' horns[53,60,61]. These observations suggest that MCAK proteins bind preferentially to the bent form of the tubulin dimer (Fig. 5b). Even growing microtubules are expected to have a small flair at their ends, owing to internal strain of the GTP subunits[62], and MCAK may discriminate between the ends of a microtubule and the lattice (that is, the lateral surface) by recognizing these slightly bent subunits in the flared region. A plausible hypothesis for how MCAK destabilizes a growing microtubule is that, after it binds to the end, it causes additional bending, inducing the formation of the curl, which weakens the association of the terminal GTP–tubulin dimer and catalyses its dissociation into solution. Thus by triggering release of GTP subunits from the end of the microtubule, MCAK gates the release of the strained GDP subunits that were trapped in the lattice.

Plus-end-binding proteins

The first bona fide plus-end-binding protein described was CLIP-170, a linker between membranes and microtubules[63]. As microtubules grow in the presence of GFP–CLIP-170, bright patches can be seen at the growing end; these patches then disappear when the microtubule stops growing[63,64] (Fig. 5c). Both the *S. pombe*[65] and the *S. cerevisiae*[66] homologues of CLIP-170 have also been shown to target microtubule ends. Work in tissue culture cells illustrates the interaction between CLIP-170 and dynamic microtubules. Here, microtubules growing from centrosomes initially exhibit similar dynamic instability properties as described *in vitro*[67]. That is, they have a low catastrophe rate and if a microtubule does catastrophe, it usually shrinks back to the nucleation centre because the rescue rate is also low. But when a microtubule reaches the cell periphery, the stability of its plus end changes markedly. Here, microtubules that undergo catastrophe rapidly rescue, and microtubules close to the membrane show frequent fluctuations between phases of growing and shrinking[67]. This is thought to allow the microtubules to adapt rapidly to changes in cell shape. Recent work has suggested that these rescue events near the cell periphery are determined by CLIP-170. Removal of CLIP-170 binding to microtubules by dominant negative constructs inhibits rescue of microtubules near the cortex, thus preventing the formation of stable populations of microtubules[64].

In *S. pombe*, removal of CLIP-170 leads to an increase in catastrophe rates so that few microtubules reach the end of the cell[65]. As a result, polarized growth that takes place at the end of the cell is impaired, leading to an aberrant cell morphology. The results in yeast suggest that microtubule dynamics play a role in cell signalling by providing a mechanism for the targeting of signals (perhaps by association with the CLIP-170 complex) that are necessary for polarized growth. Studies on the interaction between microtubules and focal contacts provide further evidence for a role of the microtubule end in cell signaling[68].

Since the discovery of CLIP-170, many more plus-end-binding proteins have been identified[48,69,70]. CLASP proteins target microtubule ends by binding to CLIP-170 (ref. 71). EB1 has been shown to bind to the tips of growing microtubules[49], where it stabilizes the polymer in mitosis by preventing catastrophes[72] and may recruit adenomatous polyposis coli (APC) to the microtubule end[49]. Stu2, the XMAP215 homologue in *S. cerevisiae*, also targets the ends of growing microtubules[73].

The discovery of these different end-binding proteins is beginning to shed light on how microtubule ends can couple to the cortex and thus mediate mechanical work. In *S. cerevisiae*, the Kar9 protein, which may be the yeast analogue of APC, links microtubule ends to the cortex. The binding of Kar9 to microtubule ends is dependent on the

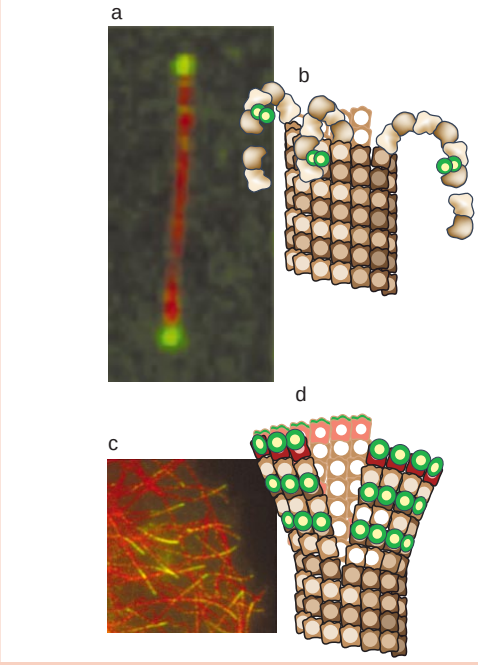

Figure 5 Proteins that recognize microtubule ends. **a**, GFP–MCAK bound to microtubule ends *in vitro*. **b**, Model for MCAK (green) binding to the lattice. **c**, GFP–CLIP-170 bound to the ends of growing microtubules in cells. The yellow segments represent GFP–CLIP-170 at microtubule ends, and the red is microtubules. (Image courtesy of A. Akhmanova.) **d**, Model for CLIP-170 (green) binding to microtubule ends.

end-binding protein EB1. Thus EB1 loads Kar9 onto microtubule ends. When these Kar9 ends reach the cell periphery, they apparently interact with the cortex via cytoplasmic myosin[23,25,74,75]. This interaction provides a secure coupling so that depolymerization at the plus end pulls the spindle pole body towards the bud. It has been suspected for some time that microtubules also interact with the dynein/dynactin complex at the cortex[76]. Recent work suggests that the dynein/dynactin complex associates with CLIP-170 and in this way targets microtubule ends[77]. Because the dynein/dynactin complex can bind to the actin cortex, this may provide the molecular linkage that allows the complex to mediate spindle positioning in various species[21,76].

Plus-end-binding proteins bind to microtubule ends in a different manner to MCAK. The original studies with CLIP-170 suggested a mechanism by which CLIP-170 loads on with the tubulin dimer, but the observation of sheets at the ends of growing microtubules (Fig. 4c) suggests another possible mechanism. Examination of the dynamics of CLIP-170 plus-end segments shows them to be about 1 μm long[63]. Sheets of over 1 μm in length have been measured in *Xenopus* egg extracts[41]. An attractive possibility is that CLIP-170-like proteins target the sheets of microtubules and dissociate as the sheet closes into a tube (Fig. 5d). Recent studies with EB1 provide additional support for this idea[72], as small sheet-like structures can be seen at the ends of microtubules in the presence of GFP–EB1. A unifying hypothesis could be that the end-binding proteins act by binding to and stabilizing the appropriate end structure — the curled protofilament in the case of MCAK and the sheet in the case of CLIP-170. The sheet stabilizes the end against depolymerization whereas the curl destabilizes the microtubule end.

Outlook

It is clear that studies on the relationship between the biochemistry of end-binding proteins and the physiology of the microtubule end are at an early stage. Do the proteins modulate the structure of the end? Do they change the rate of GTP hydrolysis? Do they catalyse nucleotide exchange? Do they induce structural transitions as suggested by the work with MCAKs? All these mechanisms are possible and it will be crucial to reconstitute the activities of these proteins with dynamic microtubules, as has been done for the proteins that regulate the dynamics of the actin cytoskeleton[78]. The recent reconstitution of microtubule dynamics using a three-component system of tubulin, MCAK and XMAP215 is a step in this direction[59]. □

doi:10.1038/nature01600

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Acknowledgements We thank Y. Barral and G. Borisy for sharing unpublished data. Research in the authors' laboratories is supported by the Human Frontier Science Program, the National Institutes of Health and the Max Planck Gesellschaft.