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 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 (Fig. 1b). In yeast, the analogous structure is the spindle pole body. 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. 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 (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 and in cellular extracts 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 or soluble cues, 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 ends of growing microtubules. 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. 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.

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, the growth and shrinkage of these kinetochore-attached microtubules move the chromosome away from or towards the pole to which it is bound. Recent experiments have shown that microtubules grow out and if they made productive interactions with cellular structures or soluble cues, 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 ends of growing microtubules. 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).

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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 centrosome centres the nucleus. In the yeast \textit{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 \(^30\). 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 \(^30–32\), 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 \(^34\), 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 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 \(\beta\)-subunit resides at the plus end \(^35\). The structure...
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 are released? 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 subunits, 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

Proteins that modulate microtubule dynamics have been known traditionally as microtubule-associated proteins or MAPs. 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.

Two distinct classes of end-binding proteins have been described: the MCAKs (for mitotic centromere-associated kinesins),...
which bind to microtubule ends and destabilize them (Fig. 5a), and
the plus-end-binding proteins (or +TIPs\textsuperscript{45}), 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\textsuperscript{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\textsuperscript{53,54}.

Removal of the Xenopus MCAK (XKCM1) from egg extracts dramatically
increases the size of the microtubule arrays\textsuperscript{55} by suppressing
catastrophes\textsuperscript{56}. Overexpressing MCAK in tissue culture cells leads to
an almost complete loss of microtubules\textsuperscript{57}, perhaps by increasing cat-
strophes\textsuperscript{58}. The localization of MCAK at kinetochores suggests that
they could trigger depolymerization during mitosis\textsuperscript{59}. It has recently
been shown that the combination of XKCM1 and a MAP
(XMAP215) can reconstitute the physiological properties of
dynamic instability in vitro\textsuperscript{60}. Thus it seems that, by increasing the
catastrophe rate, MCAKs are central to the generation of dynamic
microtubules in decays.

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 curved protofilaments — the rams’
horns\textsuperscript{41,62}. 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\textsuperscript{63}, 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\textsuperscript{64}. 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\textsuperscript{65} (Fig. 5c). Both the S. pombe\textsuperscript{66} and the
S. cerevisiae\textsuperscript{67} 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\textsuperscript{68}. 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 sta-
bility 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\textsuperscript{69}. 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 pre-
venting the formation of stable populations of microtubules\textsuperscript{70}.

In S. pombe, removal of CLIP-170 leads to an increase in catastro-
phre rates so that few microtubules reach the end of the cell\textsuperscript{64}. 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 asso-
ciation 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 signalling\textsuperscript{71}.

Since the discovery of CLIP-170, many more plus-end-binding
proteins have been identified\textsuperscript{66,69,71}. CLASP proteins target micro-
tubule ends by binding to CLIP-170 (ref. 71). EB1 has been shown to
bind to the tips of growing microtubules\textsuperscript{69}, where it stabilizes the
polymer in mitosis by preventing catastrophes\textsuperscript{72} and may recruit
adenomatous polyposis coli (APC) to the microtubule end\textsuperscript{73}. Stu2,
the XMAP215 homologue in S. cerevisiae, also targets the ends of
growing microtubules\textsuperscript{74}.

The discovery of these different end-binding proteins is beginning
to shed light on how microtubule ends can couple to the cortex and
to the cell’s internal machinery. In S. cerevisiae, the Kar9 protein, which
may be the yeast analogue of APC, links microtubules to the cortex.
The binding of Kar9 to microtubule ends is dependent on the
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. The recent reconstitution of microtubule dynamics using a three-component system of tubulin, MCAK and XMAP215 is a step in this direction.

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