Control of microtubule organization and dynamics: two ends in the limelight

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Abstract | Microtubules have fundamental roles in many essential biological processes, including cell division and intracellular transport. They assemble and disassemble from their two ends, denoted the plus end and the minus end. Significant advances have been made in our understanding of microtubule plus-end-tracking proteins (+TIPs) such as end-binding protein 1 (EB1), XMAP215, selected kinesins and dynein. By contrast, information on microtubule minus-end-targeting proteins (–TIPs), such as the calmodulin-regulated spectrin-associated proteins (CAMSAPs) and Patronin, has only recently started to emerge. Here, we review our current knowledge of factors, including microtubule-targeting agents, that associate with microtubule ends to control the dynamics and function of microtubules during the cell cycle and development.

Neurons
Cells that process and transmit information through electrical and chemical signals.

Protofilaments
Straight rows of longitudinally aligned tubulin dimers in microtubules.

Microtubule plus end
The dynamic end of a microtubule, which alternates *in vivo* between periods of growth and shrinkage and is often directed towards the cell surface. Microtubule plus ends grow quickly *in vitro*.

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Microtubules are cytoskeletal filaments implicated in a wide range of cellular processes: they serve as 'rails' for intracellular transport, drive chromosome separation during cell division, regulate cell polarity and morphogenesis and form the bases of cilia and flagella. Furthermore, microtubule-based structures, such as the mitotic spindle or parallel microtubule arrays in neurons, are major targets for the treatment of human diseases such as cancer and neurological disorders.

Microtubules form through the polymerization of αβ-tubulin heterodimers, which is regulated by the hydrolysis of β-tubulin-bound GTP, occurring with a delay after a tubulin dimer has been incorporated into the microtubule end (FIG. 1a). It is generally accepted that the newly formed microtubule tip contains a cap of GTP-tubulin (denoted the GTP cap), which has stabilizing properties, whereas the microtubule shaft is composed of GDP-tubulin and is intrinsically unstable. The GTP-cap model explains the dynamic instability of microtubules: in the presence of the cap, a microtubule continues growing, and loss of the cap leads to rapid microtubule shrinkage (reviewed in REF. 1). At the structural level, GTP hydrolysis triggers conformational changes in α-tubulin, leading to global lattice rearrangements and generation of lattice strain[2–4]; however, it has also been reported that GTP hydrolysis allosterically affects lateral contacts between protofilaments[5]. Together, these observations explain why GDP-microtubules are less stable.

Both the microtubule plus end (at which β-tubulin is exposed) and the microtubule minus end (at which α-tubulin is exposed) can grow in solutions of purified tubulin and thus can bear a GTP cap. However, the dynamic properties of the two ends are markedly different: the minus end grows more slowly and undergoes catastrophe less frequently than does the plus end. The two microtubule ends also behave differently when the stabilizing cap is severed *in vitro*: the plus end depolymerizes, whereas the minus end remains relatively stable and can resume growth[6,7].

In this Review, we discuss recent data that have deepened our understanding of both microtubule ends and of the proteins and ligands that interact with them. We first briefly focus on the intrinsic properties of the tubulin assembly–disassembly cycle. We then discuss how diverse factors specifically recognize dynamic microtubule plus ends and how cells use these factors to perform different functions. As important advances have been made recently in our understanding of proteins that regulate microtubule minus ends, we dedicate a section to this poorly studied topic. Finally, we discuss novel insights into microtubule-end regulation by pharmacological agents and expected future directions in the microtubule cytoskeleton field.

Microtubule end structure and dynamics

Cryo-electron microscopy studies show that microtubule plus ends growing *in vitro* often exhibit slightly curved, flattened and tapered sheet-like structures

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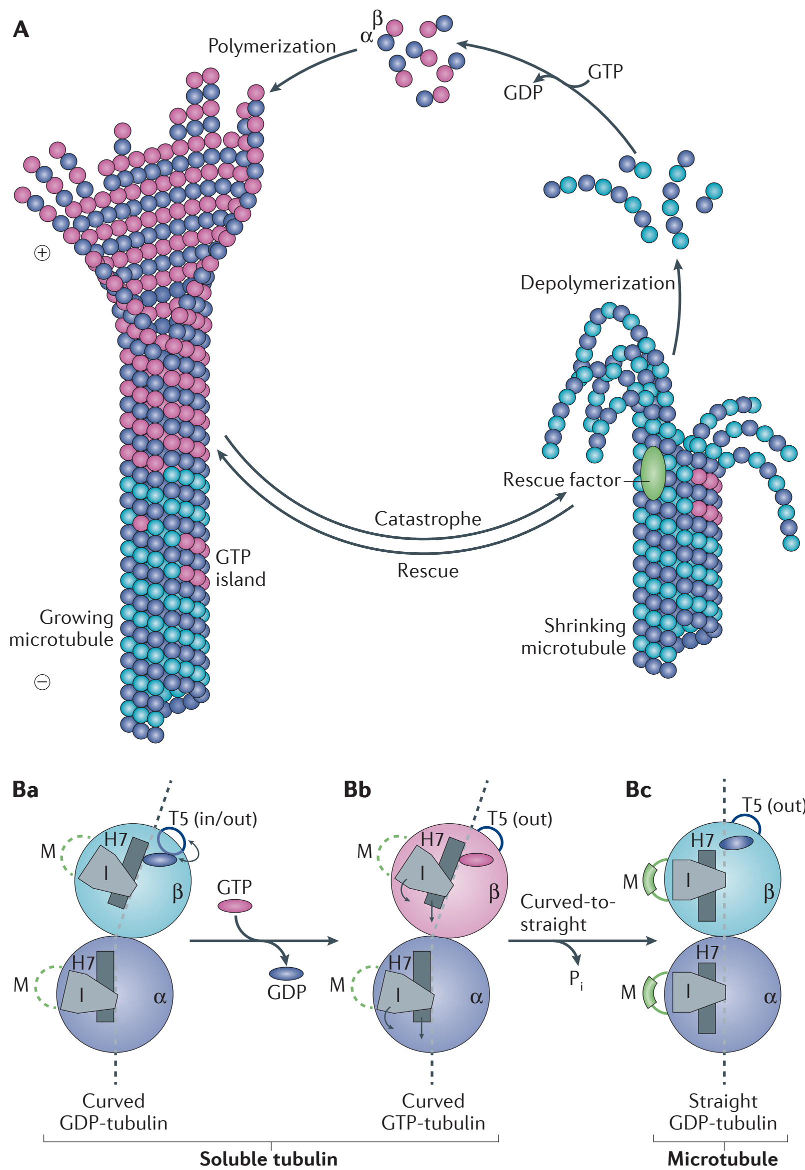

Figure 1 | **The tubulin assembly–disassembly cycle. A** | The cycle of tubulin assembly (that is, polymerization) and disassembly (that is, depolymerization) is powered by hydrolysis of the GTP bound to β-tubulin, which enables microtubules to switch between catastrophes and rescues. GTP-bound tubulin dimers are incorporated into growing (polymerizing) microtubules. GTP hydrolysis occurs, with a delay, after a GTP-tubulin dimer incorporates into the sheet-like structure of growing microtubule tips. Growing microtubule ends thus maintain a stabilizing GTP cap, the loss of which leads to a catastrophe and rapid depolymerization, resulting in shrinkage of the microtubule. Possible 'GTP islands' in the microtubule lattice or 'rescue factors' may induce rescues. **B** | In solution, both GTP-bound and GDP-bound tubulin assume a curved conformation. The M loop, which is the major structural element for mediating lateral tubulin contacts in microtubules, is disordered (represented by curved dashed lines)[168]. In the GDP-tubulin state (**Ba**), the T5 loop of β-tubulin switches between an 'in' and an 'out' conformation; in GTP-tubulin (**Bb**), this loop is stabilized in the 'out' conformation, thus promoting longitudinal tubulin contacts in microtubules[174]. Upon microtubule assembly (**Bc**), GTP-tubulin dimers undergo a gradual curved-to-straight conformational change in the microtubule end structure, whereby the intermediate domain (I) and helix H7 of both α- and β-tubulin undergo a rotational and a piston-like movement, respectively[170,175]. The lateral and longitudinal microtubule lattice contacts induce the structuring of both α-tubulin and β-tubulin M loops into short helices (represented by a solid line with green tube)[3] and promote GTP hydrolysis (thus converting GTP to GDP through the loss of inorganic phosphate (P_i))[176]. Figure adapted from REF. 170, Nature Publishing Group and with permission from REF. 174, Elsevier.

(denoted 'tubulin sheets'), whereas depolymerizing plus ends display strongly curved, 'peeling' protofilaments[8]. Growing and shrinking microtubule minus ends are probably characterized by similar structural features; however, to the best of our knowledge, a systematic study on this topic has not yet been reported. The curved protofilament structure of depolymerizing microtubule ends is an intrinsic feature of unpolymerized tubulin, in both the GDP- and the GTP-bound form (reviewed in REF. 9) (FIG. 1b). Transitions between the curved and the straight conformation of tubulin are thus generally acknowledged to be important for controlling microtubule dynamics.

The mechanisms underlying the transitions between growth and shrinkage (that is, microtubule rescues and catastrophes, respectively; FIG. 1a) are complex and poorly understood. *In vitro* work shows that microtubules that have been growing for a longer time ('older' microtubules) have a higher chance of undergoing a catastrophe. This 'ageing' behaviour suggests that the induction of catastrophe requires several molecular events to occur before a microtubule switches to depolymerization[10,11]. The nature of these catastrophe-promoting events is unknown, but they might involve the accumulation of microtubule-lattice defects or an increased tapering of the growing microtubule end[11,12]. In cells, catastrophes can be triggered by the exertion of pushing forces on growing microtubule tips by different obstacles, such as the cell cortex, which slow down microtubule growth and lead to the loss of the GTP cap and an eventual catastrophe[13].

Microtubule rescues are understood even less well than catastrophes. Their occurrence *in vitro* is not sensitive to tubulin concentration[14] and thus might not depend on the stochastic addition of GTP-tubulin dimers to the shrinking microtubule plus end. Instead, rescues might be induced by local lattice features that can halt microtubule disassembly, such as 'GTP islands' of GTP-tubulin that mimic the stabilizing GTP cap[15] (FIG. 1a).

Regulators of microtubule dynamics

The dynamic instability of microtubules and the connections between microtubules and cellular structures are spatially and temporally controlled by numerous factors, which can be broadly grouped into microtubule-associated proteins (MAPs) and molecular motors (reviewed in REF. 1). Among these, microtubule plus-end-tracking proteins (+TIPs), which include structurally and functionally diverse microtubule regulators, are distinguished by their ability to concentrate at growing microtubule ends[16,17].

Microtubule dynamics change dramatically during the cell cycle and during cell differentiation, owing to factors that promote microtubule growth or disassembly. Microtubule polymerases, such as the members of the XMAP215 family of +TIPs, bind to microtubule plus ends, recruit tubulin dimers and increase the rate of tubulin addition to growing tips[18,19]. The activity of these proteins helps to explain why microtubules assemble in cells much faster than they do *in vitro* from pure

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tubulin at the same concentration. *In vivo* studies support the importance of microtubule polymerases for promoting rapid and processive microtubule growth[20–22]. Other +TIPs, such as the end-binding proteins (EBs), can mildly increase microtubule polymerization rates *in vitro*[23,24], possibly by modulating the structure of microtubule ends[25].

Microtubule disassembly is regulated by microtubule depolymerases that belong to the kinesin families kinesin-13, kinesin-8 or kinesin-14. Kinesin-13 family members, such as mitotic centromere-associated kinesin (MCAK), do not step on microtubules; instead, they use the energy of ATP hydrolysis to remove terminal subunits from microtubule ends[26–30]. Kinesin-8 family members can walk to plus ends, where they seem to destabilize the GTP cap by cooperatively removing tubulin subunits as they reach the microtubule tip[11,31]. Owing to their motor processivity, they accumulate at higher levels at the tips of longer microtubules and depolymerize them more efficiently than shorter microtubules (reviewed in REF. 32). The minus-end-directed yeast kinesin-14 family member karyogamy 3 (Kar3) also has microtubule-depolymerizing activity[33]. In addition to these factors that can disassemble stabilized microtubules, the EBs promote microtubule catastrophe *in vitro*. They may do this by reducing the size of the stabilizing cap, to which they bind, and by accelerating the hydrolysis of tubulin-bound GTP or phosphate release[34]. In cells, EBs can make microtubules more dynamic, but they mostly promote rather than suppress microtubule elongation, possibly by counteracting the activity of more-potent depolymerases[23,35,36].

Members of the kinesin-4 family inhibit microtubule growth and suppress catastrophes by decreasing the overall tubulin turnover at the microtubule tip; this leads to the stabilization of microtubules of a particular length. This process is important for microtubule organization within the spindle and at the cell cortex[37,38]. Kinesin-4 motors typically show plus-end-directed motility, with the exception of the non-motile ciliary regulator kinesin-like 7 (KIF7), which mildly promotes, rather than suppresses, catastrophes and is involved in limiting the length of axonemal microtubules[39]. Certain members of the kinesin-8 family of motors can also stabilize microtubules and 'dampen' microtubule dynamics, which is important for regulating the movements of kinetochores attached to the kinetochore–microtubule fibres[40–42].

Of the factors that can suppress catastrophes and promote rescues, the best-understood are the cytoplasmic linker protein (CLIP)-associated proteins (CLASPs). Similar to XMAP215, members of this class of +TIPs can bind to microtubule lattices and recruit soluble tubulin dimers[43]. There are also indications that CLASPs might affect microtubule lattices, possibly by facilitating GTP hydrolysis[44]. CLASPs increase microtubule density and stability in different cellular settings in animals, plants and fungi (reviewed in REF. 45). Cytoskeleton-associated protein Gly-rich domain (CAP-Gly domain) family proteins such as CLIP-170 (also known as CLIP1) and CLIP-115 (also known as CLIP2) promote rescues, whereas the large subunit of dynactin, p150[Glued] (encoded by *DCTN1*), which is also a CAP-Gly family member, can suppress catastrophes[46,47].

An unusual type of microtubule regulator is the minus-end-directed motor cytoplasmic dynein, a multiprotein complex that, when attached to a barrier or a bead, can tether microtubule plus ends to it[48,49]. In an 'end-on' configuration, dynein can exert pulling forces and reduce microtubule depolymerization by holding on to a shrinking microtubule plus end[49]. Such end-on pulling is important, for example, for asymmetric spindle positioning during the first embryonic division in *Caenorhabditis elegans*[50,51] (reviewed in REF. 52). It should be noted that 'side-on' dynein–microtubule interactions that result in microtubules gliding along the cortex can also be important for spindle positioning, for example, in cultured mammalian cells[53].

Finally, a broad set of proteins can connect microtubule plus ends to different cellular organelles and cytoskeletal elements, the plasma membrane or mitotic kinetochores, typically through distinct protein domains with affinities for both microtubule tips and particular cellular structures. Such 'linker proteins' often function as parts of larger protein networks (see below).

Recognition of growing microtubule ends

+TIPs can be divided into 'autonomous tip trackers', which can recognize microtubule ends independently of other factors, and 'hitchhikers', which are proteins that often have some affinity for microtubules but concentrate at microtubule ends, primarily by binding to an autonomous tip tracker. In this section, we discuss the molecular mechanisms of autonomous tip trackers that have been identified and characterized by *in vitro* reconstitution experiments with purified components (BOX 1). In the subsequent section, we describe how autonomous tip trackers recruit hitchhikers to establish different types of complex +TIP networks.

End-binding proteins (EBs). EBs are considered to be master regulators of +TIP networks, as they autonomously recognize growing microtubule plus and minus ends, and they can recruit a range of different factors to these strategically important locations. Mammalian cells express up to three different EBs (EB1, EB2 and EB3), whereas yeasts contain only one EB (binding to microtubules 1 (Bim1) in *Saccharomyces cerevisiae* and Mal3 in *Schizosaccharomyces pombe*). EBs contain an amino-terminal calponin homology domain (CH domain)[54], which is followed by a variable linker region and a coiled-coil domain. The coiled-coil domain mediates the parallel homo- or heterodimerization of EB monomers[55,56], a process that was suggested to be controlled by the direct binding of GTP to EBs[57]. The coiled-coil domain extends into a four-helix bundle and a disordered tail that terminates with a carboxy-terminal EEY/F motif; the four-helix bundle and the first part of the tail region are termed the EB homology domain (EBH domain) (FIG. 2a).

The C-terminal domain of the EBs contains binding sites for numerous +TIP partners (see below). Together, the CH domain and the linker region are sufficient to

Microtubule minus end
The less-dynamic end of a microtubule. Microtubule minus ends do grow, albeit slowly. In cells, they are often stabilized (for example, by attachment to the centrosome) or function as sites of microtubule depolymerization. Microtubule minus ends grow slowly *in vitro*.

Catastrophe
The transition of a microtubule from growth to shortening.

Rescues
Transitions of microtubules from shortening to growth.

GTP islands
GTP-bound tubulin dimer patches in the microtubule shaft.

Kinesin
A microtubule-based molecular motor, often directed towards the plus ends of microtubules.

Motor processivity
The ability of motors to move long distances along a cytoskeletal filament without dissociation.

Axonemal microtubules
The central components of axonemes. Numerous eukaryotic cells carry whip-like appendages (cilia or eukaryotic flagella), the inner cores of which consist of a microtubule-based structure called the axoneme. The axoneme contains axonemal microtubules, which function as the 'skeleton' of these cell protrusions, giving them support, enabling transport and, in some cases, causing bending motion.

Kinetochores
Specialized regions on chromosomes that are connected to microtubules and motor proteins during cell division in eukaryotes. Kinetochores function in the separation of chromosome pairs.

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specifically recognize and track growing microtubule ends[23,58,59]; however, the interplay between the positively charged CH-linker region and the overall negatively charged C-terminal domain contributes to the fine-tuning of the specificity of EBs towards microtubule tips[60]. Notably, the linker region contains Ser, Thr and Tyr residues, the phosphorylation of which can regulate the function of EBs during the cell cycle[61–63], for example, by changing the overall conformation of the EB molecule and/or by causing electrostatic repulsion with the negatively charged surface of a microtubule. Other post-translational mechanisms of EB regulation include the acetylation of the C-terminal domain of EB1, which participates in the control of kinetochore–microtubule interactions by affecting the interaction of EB1 with +TIP binding partners[64], and ubiquitylation, which promotes the proteolysis of EB1 (REF. 65).

Fluorescence microscopy revealed that up to a few hundred EB molecules bind to a region of growing microtubule ends, where they form comet-like accumulations that are 0.5–2.0 μm long[66–68]. In these comets, EBs show rapid, diffusion-based turnover: that is, they undergo several cycles of binding and unbinding before the growing end matures into the microtubule lattice[67,69]. EBs show the highest accumulation several tens of nanometres away from the outermost microtubule plus end[25] (FIG. 2b). This result indicates that the structure, protofilament number and arrangement, and/or the tapering of the distal microtubule end reduce the number of high-affinity EB-binding sites.

What exactly do EBs recognize at growing microtubule ends? Recent studies revealed that EBs preferentially bind to stabilized microtubules assembled in the presence of the GTP or GDP–P_i analogues GMPCPP, GTPγS and GDP–BeF_3^- (REFS 34,70). These results suggest that EBs recognize the GTP-cap structure, which is consistent with the fact that EBs bind to both plus and minus ends of growing microtubules. Cryo-electron microscopy-based analyses of EB–microtubule interactions assembled from GTPγS-tubulin showed that the CH domain of EBs bridges protofilaments at the corners of four tubulin dimers[4,71] (FIG. 2c). The CH domain is thus ideally positioned to sense changes in the microtubule lattice, such as conformational alterations in the tubulin dimers resulting in global lattice rearrangements and the generation of lattice strain, which are induced on GTP hydrolysis[3,4,71]. An interesting implication of these data is that the length of the EB comet reflects the extent of the GTP cap[68]. However, this idea needs further validation, as the preference for microtubules assembled in the presence of non-hydrolysable GTP analogues is not an uncommon property among MAPs: for example, similar to EB1, doublecortin, tau and kinesin-1 show a preference for GTPγS- over GDP-microtubules[72].

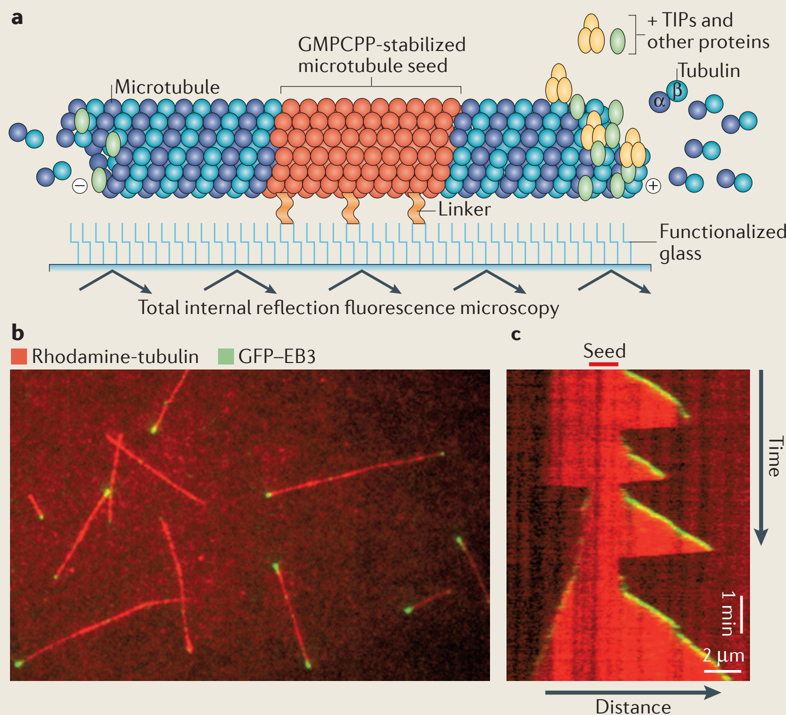
XMAP215 family of microtubule polymerases. Another major group of autonomous tip trackers are the members of the XMAP215 family of microtubule polymerases, which contain multiple tubulin-binding TOG domains (named after their discovery in the human protein ch-TOG (colonic and hepatic tumour-overexpressed gene; also known as CKAP5), which is the homologue of the *Xenopus laevis* protein XMAP215). Human ch-TOG and its frog orthologue XMAP215

Box 1 | *In vitro* reconstitution of microtubule end-tracking

Microtubules can spontaneously nucleate and grow in solutions of purified tubulin. For these experiments, tubulin is typically prepared from bovine or porcine brains, or from cultured mammalian cells. Such preparations represent a mixture of tubulin isotypes and post-translationally modified forms. Tubulin preparations that are well defined in terms of isotypes and post-translational modifications can be obtained from yeast[163–165]. Furthermore, specific recombinant human tubulin isotypes can be produced in insect cells[166].

To visualize microtubule growth *in vitro*, microtubules are typically nucleated from microtubule fragments ('seeds') that are stabilized using the slowly hydrolysable GTP analogue GMPCPP. The seeds are attached to a chemically functionalized glass surface using biotin–streptavidin links (if biotinylated tubulin is to be incorporated into the seeds)[67] or antibodies (see the figure, part **a**)[87]. Mixtures of purified proteins or cell extracts can be added to the assay, and microtubule growth and the behaviour of different fluorescent proteins can be observed using total internal reflection fluorescence microscopy or confocal microscopy. For example, when purified rhodamine-labelled brain tubulin and GFP-bound end-binding protein 3 (EB3) are added to such an assay, microtubules grow from both the plus and the minus end of the seed, as indicated by the presence of GFP (shown in green in the figure, part **b**). GFP–EB3 accumulates at the growing plus ends and to a lesser extent at the slower-growing minus ends; the behaviour of microtubule ends over time can be visualized using kymographs, which are time–space plots in which the fluorescence intensity along a single microtubule is shown for all frames of a movie (see the figure, part **c**).

Complex reconstitutions that involve at least five different purified proteins have been described[67,102,103]. Furthermore, using chemical micropatterning, this assay to reconstitute microtubule end-tracking *in vitro* was used to study, for example, the interactions between antiparallel aligned microtubules[167]. The assay has also been adapted to combine dynamic microtubules with actin filaments to study the coordination and co-alignment of the two types of cytoskeletal filaments[111]. Finally, in combination with microfabricated chambers, the assay has been used to study microtubule tip interactions with inert or functionalized barriers[49]. +TIPs, microtubule plus-end-tracking proteins. Images in parts **b** and **c** courtesy of R. Mohan, Sri Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, India.



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are monomeric members of this family of +TIPs, which contain five TOG domains; the yeast orthologue suppressor of tubulin 2 (Stu2) is a dimer with two TOG domains per polypeptide (reviewed in REF. 73). Several crystal structures of different TOG domains, either alone[58,74,75] or in complex with tubulin[76,77], have been solved. These structures revealed that TOG domains bind to the curved conformational state of the

Cytoskeleton-associated protein Gly-rich domain
(CAP-Gly domain). An ~70-residue protein domain that is characterized by several Gly residues, which are involved in shaping the loop regions of the globular fold. CAP-Gly domains contain a unique hydrophobic cavity that encompasses the highly conserved GKNDG sequence motif responsible for targeting CAP-Gly domains to the carboxy-terminal EEY/F motifs of γ-tubulin, end-binding protein, cytoplasmic linker protein 170 and SLAIN.

Dynein
A large, minus-end-directed, multisubunit microtubule motor protein that is involved in several cellular processes, including cell division, migration and intracellular transport.

Calponin homology domain
(CH domain). An ~100-residue actin- or microtubule-binding domain that is common to many actin-binding proteins, including cytoskeletal and signalling proteins, and microtubule-associated proteins such as end-binding proteins, calponin-homology and microtubule-associated protein (CLAMP) and highly expressed in cancer protein 1 (HEC1).

Coiled-coil domain
A protein structural motif that mediates subunit oligomerization. Coiled-coils contain between two and five α-helices that twist around each other to form a supercoil.

Carboxy-terminal EEY/F motif
A highly specific and conserved sequence motif found at the C termini of α-tubulin, end-binding proteins, cytoplasmic linker protein 170 and SLAIN. The EEY/F motif is the target of cytoskeleton-associated protein Gly-rich domains.

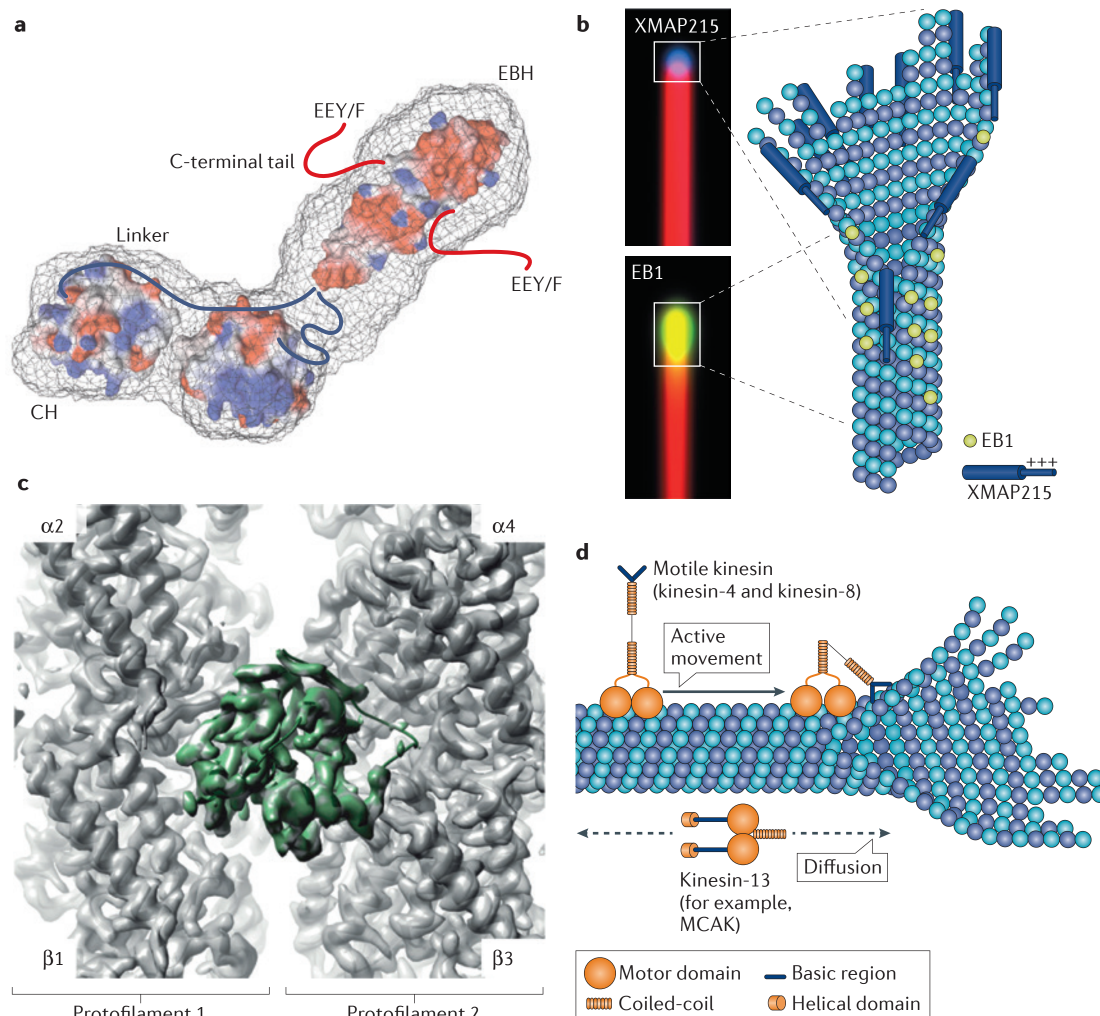

Figure 2 | **Recognition of growing microtubule ends by 'autonomous tip trackers'.** **a** | The structure of an end-binding protein dimer. The crystal structures of the predominantly positively charged calponin homology (CH) domain and the predominantly negatively charged carboxy-terminal domain of end-binding protein 1 (EB1)[54,177] are coloured according to their electrostatic potential (red, negatively charged; blue, positively charged). They were fitted into an envelope (grey mesh) obtained by small-angle X-ray scattering[60]. The predominantly positively charged linker regions and the predominantly negatively charged C-terminal tails containing C-terminal EEY/F motifs are indicated by lines. **b** | Illustration of the distribution of EB1 and XMAP215 at growing microtubule plus ends[25]. The particular arrangement of the XMAP215 tumour over-expressed gene (TOG) domains (blue signal within rectangle; top image) and the basic region (indicated by +++ in the schematic) drives the specificity of the polymerase for the growing microtubule plus end. Whereas XMAP215 binds to the distal microtubule plus end, EB1 displays the highest accumulation tens of nanometres away from the outermost tip (yellow signal within rectangle; bottom image). **c** | Cryo-electron microscopy reconstruction of a microtubule (grey) in complex with the CH domain of EB3 (REF. 4) (green). The CH domain binds at the corner of four αβ-tubulin dimers (numbered 1 to 4) that stem from two adjacent protofilaments. This figure was made based on coordinates and electron densities provided in the Electron Microscopy Data Bank (EMDB), EMD-6347, and the RCSB Protein Data Bank (PDB), PDB IDs 3C0I and 3JAK. **d** | Illustration of the microtubule plus-end-tracking mechanism of kinesin-4 and kinesin-8 family members (reviewed in REF. 32), as well as kinesin-13 family members[87]. Motile members of the kinesin-4 and kinesin-8 families processively 'walk' to the microtubule plus end; this requires ATP-driven active movement. Kinesin-13 family members, such as mitotic centromere-associated kinesin (MCAK), can reach microtubule ends by one-dimensional diffusion. The retention of kinesins at microtubule tips is promoted by the presence of basic regions within the kinesin molecules. EBH, EB homology domain. Figure part **a** republished with permission of The American Society for Cell Biology, from Buey, R. M. *et al.* Insights into EB structure and the role of its C-terminal domain in discriminating microtubule tips from lattice. *Mol. Biol. Cell*, **22** (16), 2011. Permission conveyed through Copyright Clearance Center, Inc. Figure part **b** adapted with permission from REF. 25, Elsevier.

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tubulin dimer and thus are likely to recognize outwardly curved tubulin sheets and/or particular curved protofilament configurations of growing microtubule ends. XMAP215 family members also contain a disordered basic region that has a weak affinity for the negatively charged outer surface of the microtubule lattice; this is known as the C-terminal microtubule lattice-binding domain. This region allows XMAP215 to diffuse along microtubules to reach their ends[18,78]. The current data on XMAP215 and Stu2 suggest a model in which these autonomous +TIPs specifically and processively track microtubule plus ends, catalysing the addition of multiple tubulin dimers while remaining bound to the microtubule end. As predicted by this model, fluorescence microscopy data showed that the XMAP215 binding sites are indeed located at the outermost microtubule ends; XMAP215 accumulation thus precedes the peak of the EB1 comet[25,79] (FIG. 2b). How do XMAP215 proteins discriminate between microtubule ends? As TOG domains bind to tubulin in a specific orientation, the current view is that the simultaneous binding of XMAP215 proteins to both curved tubulin (through their N-terminal TOG domains) and the microtubule shaft (through their C-terminal microtubule lattice-binding domain) is only possible at the plus end[76] (FIG. 2b). The linked TOG domains within a protein would then promote microtubule growth using a tethering mechanism that increases the local concentration of unassembled tubulin near the distal plus end[77].

Motor +TIPs. Motile motor proteins, such as kinesin-4 and kinesin-8 family members, accumulate at microtubule ends on the basis of their ATP hydrolysis-driven movement (FIG. 2d). These kinesins are dimeric molecules containing N-terminal motor domains. Basic microtubule-binding regions present in the kinesin tails can enhance motor processivity and the ability of motors to remain attached to microtubule ends, as has been shown for the kinesin-8 family members kinesin-related protein 3 (KIP3) and KIF18A and the kinetochore kinesin-7 member centromere protein E (CENP-E)[80–83].

Non-motile kinesin-13 proteins, such as MCAK, can also accumulate at microtubule ends. They disassemble microtubules in an ATP hydrolysis-dependent manner by promoting the curved conformational state of tubulin dimers at microtubule ends (FIG. 1b), and possibly also by disrupting lateral tubulin interactions in microtubules[26–30]. On the basis of cryo-electron microscopy data, it has been proposed that, in addition to promoting curvature, MCAK induces or stabilizes a displaced arrangement between two tubulin monomers within a dimer on isolated protofilaments[29]. Whether tubulin dimers can inherently adopt such 'sheared' configurations, and whether this conformational transition is inducible only at microtubule tips, remains to be resolved. The association of MCAK with microtubules is enhanced by its positively charged neck[84] and is negatively regulated by an autoinhibitory interaction between its C-terminal-tail peptide and its motor domain[85]. The accumulation and activity of this depolymerase at microtubule ends depends on complex conformational changes within the molecule[30,85,86]. *In vitro*, MCAK effectively accumulates at microtubule ends by diffusing along the microtubule lattice[87] (FIG. 2d); however, the contribution of this mechanism to the localization of MCAK in cells remains to be determined.

Other autonomous tip trackers. In this subsection, we briefly discuss autonomous tip trackers that are typically not referred to as canonical +TIPs but that have had their interactions with microtubule ends investigated in detail. One such example is the brain MAP doublecortin. Like the CH domain of the EBs, the ubiquitin-like doublecortin domains (DC domains) of doublecortin bind to microtubule lattices between protofilaments at the corners formed by four tubulin dimers[88]. However, in contrast to EBs, which recognize the GTP cap (see above), *in vitro* studies suggest that doublecortin primarily senses the outwardly curved structure of tubulin sheets, which enables the protein to track growing microtubule ends[72].

Two additional, non-canonical autonomous tip trackers are the highly conserved nuclear division cycle 80 complex (NDC80 complex) and the fungi-specific DUO1 and MPS1-interacting 1 complex (DAM1 complex), which organize kinetochore–microtubule attachments in the mitotic spindle. The NDC80 complex tracks only depolymerizing ends[89], whereas the DAM1 complex tracks both growing and shrinking ends[90]. In contrast to EBs and doublecortin, which bind between protofilaments, the CH domains of the NDC80 complex interact along protofilaments[91]. It is thought that the binding properties and oligomerization of the NDC80 and DAM1 complexes may allow them to stay stably attached to the end of a depolymerizing microtubule and thus to transmit force to kinetochores during chromosome segregation (reviewed in REF. 92).

+TIP networks

Considering the large number of +TIPs in cells, the growing microtubule plus end must be a crowded place, and +TIPs can either cooperate or compete with each other to access this strategically important location. The rules that determine which of the many +TIPs are recruited to the limited number of available binding sites at microtubule ends, and at which point during the cell cycle and in which spatial location this occurs, is an important issue that has only recently started to be addressed.

Protein domain- and motif-mediated +TIP interactions. As mentioned above, the autonomous tip trackers of the EB family can target a large range of structurally and functionally diverse +TIPs to growing microtubule ends. Two major +TIPs, CLIP-170 and p150^{Glued} (the large subunit of the dynein accessory complex dynactin) bind to the C termini of EBs through their CAP-Gly domains. CAP-Gly domains are globular modules that specifically recognize C-terminal EEY/F motifs that are found in EBs, α-tubulin, CLIP-170 and the ch-TOG- and CLIP-170-binding protein SLAIN[21,93–95].

The EB-dependent recruitment mechanism of other +TIPs was enigmatic, as they seemed to lack any

EB homology domain
(EBH domain). An ~50-amino-acid domain found at the carboxyl termini of end-binding proteins. It constitutes of a pair of helix–loop–helix segments forming an antiparallel four-helix bundle. The highly conserved residues of the EBH domain form a surface patch that contains a deep hydrophobic cavity, which serves as an interaction site for binding partners.

TOG domains
HEAT (Huntington, elongation factor 3, protein phosphatase 2A and target of rapamycin1) repeat-containing tubulin-binding domains named after their discovery in the human microtubule-associated protein ch-TOG (colonic and hepatic tumour-overexpressed gene).

Doublecortin domains
(DC domains). Regions found in the amino terminus of the protein doublecortin that consist of tandem repeated copies of an approximately 80-amino-acid sequence region.

Nuclear division cycle 80 complex
(NDC80 complex). A multisubunit protein complex that mediates the attachment of microtubule plus ends to kinetochores.

DUO1 and MPS1-interacting 1 complex
(DAM1 complex). A multisubunit protein complex (also known as the DASH complex) that is involved in kinetochore–microtubule attachments in yeast and can form rings around microtubules. It may provide a dynamic linkage at depolymerizing or polymerizing microtubule ends for force generation.

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recognizable, common EB-binding domain. However, this issue was solved by the discovery that the small amino acid motif SxIP (where x is any amino acid) is embedded in a disordered and basic region of many +TIPs, and that it is specifically recognized by the EBH domain of EBs[96] (reviewed in REF. 97). The SxIP motif is necessary and sufficient to target +TIPs to growing microtubule ends, and it is generally acknowledged to represent a major 'microtubule tip-localization signal' (MtLS)[96]. Recent proteome-wide screens for mammalian +TIPs that contain an SxIP motif revealed that they form a complex group that comprises several tens of different proteins[98,99].

It is well known that many microtubule-binding proteins contain basic regions that are predicted to be disordered (see above); in +TIPs, such regions contribute to the overall affinity of the +TIP for both the negatively charged microtubules and C-terminal domains of EBs (reviewed in REF. 17). This requirement offers a possibility for control: spatially or temporally regulated kinases that target Ser residues in the vicinity of SxIP restrict the tip-tracking activity of +TIPs by causing electrostatic repulsion[63,96,100] (reviewed in REF. 97).

Competition, hierarchy and synergism in +TIP networks. As outlined above, +TIP networks are formed by a limited set of interactions that are mediated by small domains, linear motifs and/or basic regions. Notably, many of these individual interactions display dissociation constants in the micromolar range and are thus likely to be transient (reviewed in REF. 17). As +TIPs are often large, multidomain and thus multivalent proteins, it is reasonable to assume that +TIP networks are typically built from numerous moderate- to low-affinity modular binding sites in different combinations. These structural and biophysical properties allow +TIP networks to dynamically remodel while the microtubule end elongates in a particular cellular location.

As XMAP215 family members are autonomous tip trackers, they do not directly interact with EBs. However, *in vitro* reconstitution experiments showed that EB1 and the frog protein XMAP215 increase microtubule growth rates in a synergistic manner to a level that is comparable to that observed in cells[24]. This suggests the presence of an allosteric interaction between EBs and XMAP215 that is mediated by conformational changes in the microtubule end region. In mammalian cells, ch-TOG binds SLAIN2, a +TIP that contains multiple SxIP motifs and that enables ch-TOG to access growing microtubule tips in the crowded cellular environment[21] (FIG. 3a). Consistent with this observation, experiments with fly proteins revealed that EB1 recruits the +TIP Sentin, which — similar to SLAIN2 in mammalian cells — can interact with the fly homologue of XMAP215 (Mini spindles (Msps)) to cooperatively promote microtubule dynamics[101]. SLAIN2 also contains a C-terminal EEY/F motif that is recognized by the CAP-Gly domain of CLIP-170 (REF. 21) (FIG. 3a).

Owing to the large number of +TIPs, the organization of +TIP networks depends on both hierarchical and non-hierarchical interactions, as well as on competition. For example, the SxIP motif- and CAP-Gly-domain-containing +TIPs can compete for EB binding and thus displace each other from the microtubule tips[102]. *In vitro* reconstitution experiments demonstrated that a hierarchical +TIP network involving the preferential sequence of interactions between the microtubule plus end, EB1, CLIP-170 and p150^{Glued} allowed the formation of a platform for the docking of the mammalian dynein motor complex even in the presence of a competing SxIP motif-containing peptide[102] (FIG. 3b). Another *in vitro* study reported that the yeast +TIPs Pac1 (perish in the absence of Cin8 1; the yeast homologue of human LIS1) and Bik1 (bilateral karyogamy defect 1; the yeast homologue of CLIP-170) work together to load dynein onto the plus-end-directed kinesin Kip2, which, with the help of Bim1 and Bik1, processively transports the minus-end-directed dynein motor towards growing microtubule plus ends[103] (FIG. 3c). Similarly, the complex formed between the kinesin-8 family member KIF18B and the kinesin-13 family member MCAK in mammalian cells is targeted to microtubule plus ends by SxIP-dependent interactions of both kinesins with EB1 and the motor activity of KIF18B[104]. The EB1–KIF18B–MCAK complex, which is negatively regulated by Aurora kinase phosphorylation, is an important regulator of microtubule plus-end dynamics in mitotic cells[104].

Together, these studies offer detailed molecular insights into the biophysical properties, architecture and modes of regulation of dynamic +TIP networks. They highlight how connectivity, hierarchy and the activity of motors within multivalent +TIP networks can be orchestrated by engaging a limited set of motifs with weak binding affinities for their targets and how phosphorylation can regulate individual interaction nodes within +TIP networks. They further suggest that microtubule-directed functions of +TIPs can be synergistically modulated through the conformation of the microtubule-end region itself. In this context, it has been proposed that +TIP networks stimulate microtubule growth by limiting fluctuations in the microtubule tip structure and could thus act as 'microtubule polymerization chaperones' (REF. 105).

Finally, a basic principle to enable +TIPs to access the crowded environment of microtubule ends is to integrate +TIPs that contain multiple SxIP motifs, such as SLAIN2, which act as 'adhesive' factors to enhance +TIP interactions and promote the localization of +TIPs to growing microtubule ends. Notably, the properties of +TIP networks are reminiscent of phase transitions, which are generally observed in assemblies of multivalent macromolecular systems[106]. Complex formation between proteins that contain multiple weak interaction sites can generate sharp transitions between small assemblies and macroscopic polymer gels (known as sol–gel transitions) as the total number of connections in the network increases[107]. These liquid–liquid demixing phase separations can produce micrometre-sized liquid droplets under physiological conditions[107]. Along these lines, +TIP networks could be viewed as liquid droplets that are specifically formed around growing microtubule ends.

SxIP motif
An amino acid sequence motif of Ser-any amino acid-Ile-Pro, embedded in an intrinsically disordered positively charged sequence region that is found in many microtubule plus-end-tracking proteins. The SxIP motif is specifically recognized by the EB homology domain of EBs.

Kinases
Enzymes that catalyse the transfer of phosphate groups from ATP to specific substrates, such as particular amino acid residues in proteins. This process is termed phosphorylation.

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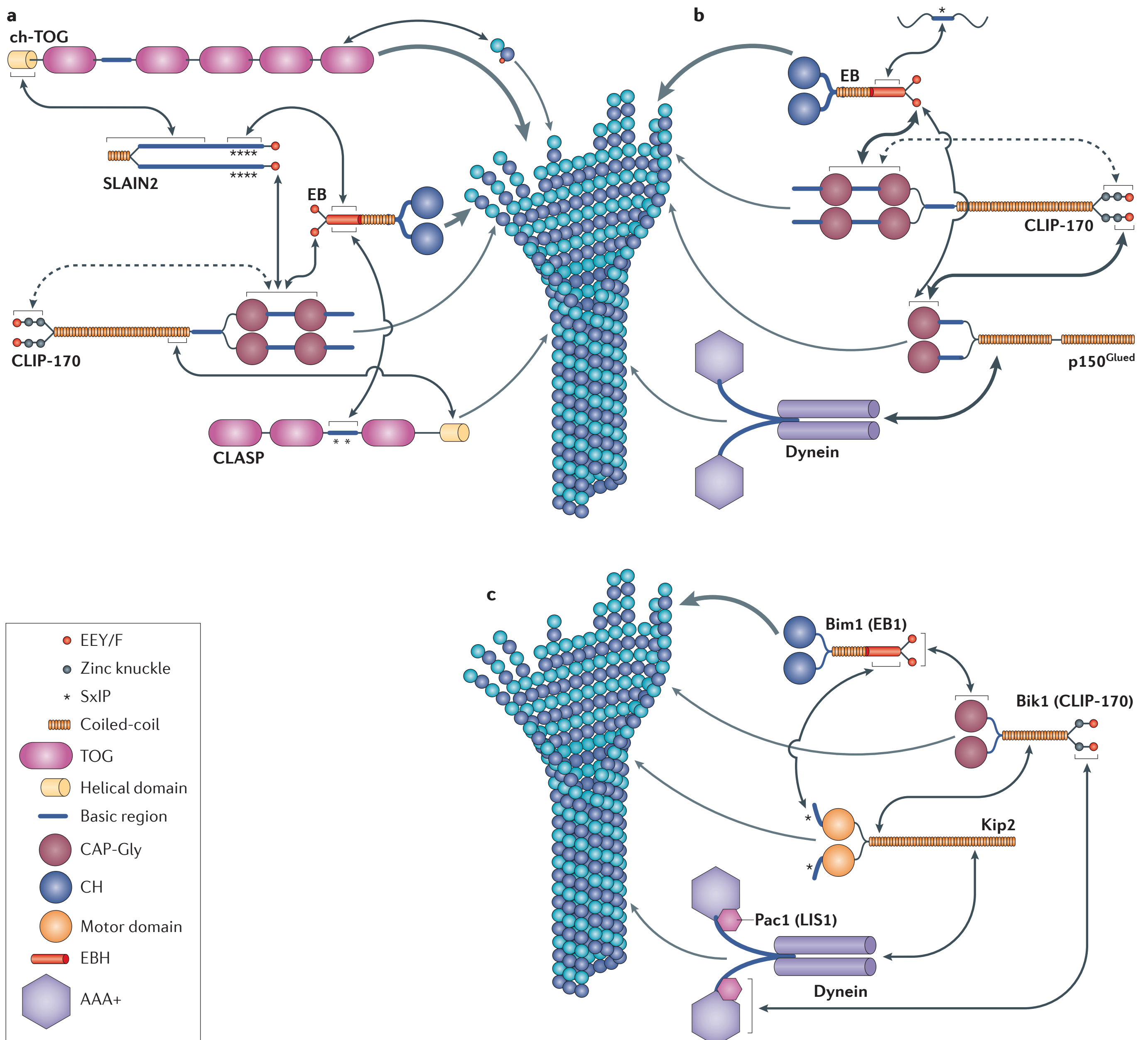

Figure 3 | **Microtubule plus-end-tracking protein (+TIP) networks.** **a** | The role of SLAIN2 as an 'adhesive' +TIP factor is illustrated. SLAIN2 combines multiple SxIP motifs with additional +TIP-binding sites, including two carboxy-terminal EEY/F motifs, to ensure continuous access to growing microtubule ends, despite a crowded environment, through end-binding proteins (EBs). This unique property of SLAIN2 is expected to enable it to associate simultaneously with several different +TIPs, including ch-TOG (colonic and hepatic tumour-overexpressed gene) and cytoplasmic linker protein 170 (CLIP-170), to promote the localization of these proteins to microtubule tips. CLIP-associated proteins (CLASPs) can bind to microtubules directly and accumulate at growing microtubule plus ends by binding to EBs (via SxIP motifs) or to CLIP-170. **b** | Hierarchical microtubule plus end–EB–CLIP-170–p150^{Glued}–dynein network. This network (in which EB binds to CLIP-170 and p150^{Glued}, CLIP-170 binds to p150^{Glued}, and p150^{Glued} binds to dynein) is established owing to the ability of the cytoskeleton-associated protein Gly-rich (CAP-Gly) domains of CLIP-170 to bind to the EEY/F motifs of EB, and of the CAP-Gly domains of p150^{Glued} to interact with a composite binding site formed by the zinc knuckles and EEY/F motifs of CLIP-170 (highlighted with thick double arrows) (reviewed in REF. 178). The importance of this hierarchical network for recruiting p150^{Glued} and dynein to microtubule plus ends becomes apparent in the presence of a peptide containing an SxIP motif that competes with p150^{Glued} for EB binding. **c** | In yeast, dynein is linked to the plus-end-directed and SxIP motif-containing kinesin Kip2 (kinesin-related protein 2) by Pac1 (perish in the absence of Cin8 1; LIS1 in humans) and Bik1 (bilateral karyogamy defect 1; CLIP-170 in humans)[103]. Binding of Kip2 to Bim1 (binding to microtubules 1; EB1 in humans) localized at microtubule tips enables the minus-end-directed dynein to 'hitchhike' to the plus end on the Bim1–Bik1–Pac1–Kip2 network. In all panels, grey arrows highlight the interaction between +TIPs and the microtubule; interactions playing the major part in protein recruitment to microtubule tips are depicted by thick arrows. Brackets highlight +TIP regions or domains that are involved in protein–protein interactions. Black double arrows indicate a direct interaction between regions or domains of +TIPs. In the CLIP-170 scheme, the dashed double arrow indicates an intramolecular interaction that leads to a folded-back, autoinhibited state of the +TIP[179]. CH domain, calponin homology domain; EBH, EB homology domain.

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Actin
A protein that forms microfilaments in most cells. Actin filaments represent one of the three cytoskeletal filament systems besides microtubules and intermediate filaments.

Myosin V
A subclass of the myosin family of actin-dependent motor proteins that is required for the transport of vesicles or mRNA cargo.

Phosphoinositide
A phosphorylated form of phosphatidylinositol, a member of a family of lipids that has important roles in lipid signalling, cell signalling and membrane trafficking.

Focal adhesions
Integrin-mediated cell–substrate adhesion structures that anchor the ends of actin filaments (also known as stress fibres) and mediate strong attachments to substrates. They also function as integrin signalling platforms.

Neuromuscular junctions
Structures that connect the nervous system to the muscular system through synapses between nerve fibres and muscle cells. In vertebrates, the small-molecule neurotransmitter acetylcholine is released by the neuron and binds acetylcholine receptors in the plasma membrane of the muscle cell.

Retrograde transport
The movement of molecules or organelles inwards, away from the plasma membrane and towards the cell body. Often mediated by the microtubule minus-end-directed motor dynein.

Guanine nucleotide exchange factors
(GEFs). Proteins that facilitate the exchange of GDP for GTP in the nucleotide-binding pocket of GTP-binding proteins.

Neurites
Projections extending from the cell body of a neuronal cell. In differentiated neurons, neurites are distinguished into axons (projections that transmit signals) and dendrites (projections that receive signals).

Cellular functions of +TIP networks

The coordinated recruitment of multiple proteins with distinct activities to microtubule ends allows cells to couple the control of microtubule dynamics to specific cellular sites or signalling events. In the subsequent sections, we discuss how +TIP networks can guide microtubule growth, control the attachment of microtubule tips to cellular structures and concentrate molecules for signalling and transport purposes.

Microtubule guidance. The interaction of growing microtubule ends with more stable cytoskeletal elements, such as actin bundles and other microtubules, can steer the direction of microtubule polymerization to determine the architecture of microtubule arrays. +TIPs that contain actin-binding domains, such as spectraplakins and growth arrest-specific 2 (GAS2)-like proteins, guide microtubule growth along actin bundles and induce microtubule–actin co-alignment[98,108] (FIG. 4a). Spectraplakins are huge cytoskeletal 'crosslinkers' with vital functions in various tissues in worms, flies and mammals[109]. They can coordinate microtubule and actin organization in an EB-dependent manner in certain biological settings, such as axonal growth[110]. The complex between an EB and an engineered truncated version of spectraplakin that contains an SxIP motif and actin-binding CH domains (termed TipAct) was sufficient to induce microtubule–actin co-organization in a system with purified components[111] (FIG. 4a). Microtubule plus ends can also be actively transported along actin cables by myosin motor proteins. This mechanism guides astral microtubules to the bud tip during early stages of spindle positioning in budding yeast; it depends on myosin V, which is connected to microtubule plus ends by the adaptor protein Kar9 and the EB1 homologue Bim1 (REFS 112,113) (FIG. 4b).

Passive MAP-based crosslinkers and motors can both guide microtubule growth along other microtubules. In the dendrites of fly neurons, this process, which is important for the uniform minus-end-out orientation of microtubules, relies on the complex of the plus-end-directed, heterotrimeric kinesin-2 with the fly adenomatous polyposis coli (APC) tumour suppressor homologues and EB1 (REF. 114) (FIG. 4c). *In vitro* reconstitution assays showed that the targeting of a kinesin motor domain to growing microtubule plus ends through an SxIP motif or a direct fusion with EB1 is sufficient to bend and steer dynamic microtubules along pre-existing microtubule tracks[115,116].

EB-mediated microtubule tip localization is also a conserved feature of the microtubule minus-end-directed kinesin-14 family[117–119]. Sliding of microtubule plus ends towards the minus ends of other microtubules promotes the formation of antiparallel microtubule bundles and regulates nuclear positioning in fission yeast, and contributes to the formation of the mitotic apparatus in animals, plants and fungi[117,119–122].

Attachment of plus ends to cellular structures. The concerted action of multiple microtubule regulators becomes particularly apparent in the case of microtubule interactions with cellular structures, such as mitotic kinetochores or the cell cortex. During chromosome alignment and segregation, both specialized complexes, such as NDC80 and DAM1, and microtubule-end stabilizers and destabilizers, such as CLASPs and MCAK, orchestrate the formation and maintenance of bi-oriented end-on attachments of kinetochore pairs to dynamic microtubule ends (reviewed in REF. 123).

Microtubule plus ends also interact with the cell cortex. Force-dependent induction of microtubule catastrophe[13] and the action of microtubule depolymerases (such as the kinesin-13 family member KIF2A[124]) or microtubule-growth inhibitors (such as the kinesin-4 family member KIF21A[38]) promote the termination of microtubule polymerization at the cell margin. At the same time, microtubule-stabilizing factors, such as CLASPs, APC, CLIPs, spectraplakins, dynein and dynactin, can capture microtubules at the cell cortex (reviewed in REF. 125). The binding to the cortex can be mediated by a direct interaction between +TIPs and lipids, as is the case for the phosphoinositide-binding SxIP-containing protein APC membrane recruitment 2 (AMER2)[98,126], but more frequently this depends on membrane-associated adaptors or the association of +TIPs with the cortical actin meshwork. Similar to the situation at kinetochores, cortical scaffolding proteins can bring together microtubule regulators that do not bind to each other directly, such as CLASPs and KIF21A[38] (FIG. 4d). Cortical microtubule-attachment complexes can induce the formation of stable routes for vesicle transport: for example, the attachment of microtubules to the cell cortex by CLASPs promotes the delivery of exocytotic cargo required for the turnover of focal adhesions[127] (FIG. 4d) and the delivery of acetylcholine receptors to neuromuscular junctions[128].

In the case of cytoplasmic dynein, its simultaneous interaction with microtubule plus ends and the plasma membrane can be accompanied by force generation and is important for microtubule network positioning (reviewed in REF. 52). For small membrane organelles, the interaction with microtubule tip-associated dynein motors leads to their loading and movement in the minus end direction, a process that is important for efficient retrograde transport in neurons[129].

Microtubule plus ends as concentration devices. The accumulation of a high concentration of molecules at microtubule plus ends can promote signalling. Microtubule tips can concentrate signalling molecules, such as RHO GTPase guanine nucleotide exchange factors (GEFs) or kinases[98,130]. An interesting recent example is provided by a microtubule tip-associated complex that contains EB1, the microtubule- and actin-binding +TIP neuron navigator 1 (NAV1) and the RHO-specific GEF triple functional domain protein (TRIO). This complex activates the small GTPase RAC1 to stimulate the extension of neurites in neuronal cells by triggering actin remodelling[131] (FIG. 4e). The concentration of proteins at microtubule tips can have another consequence: although each EB or its partner shows only transient immobilization at the microtubule plus end and thus does not

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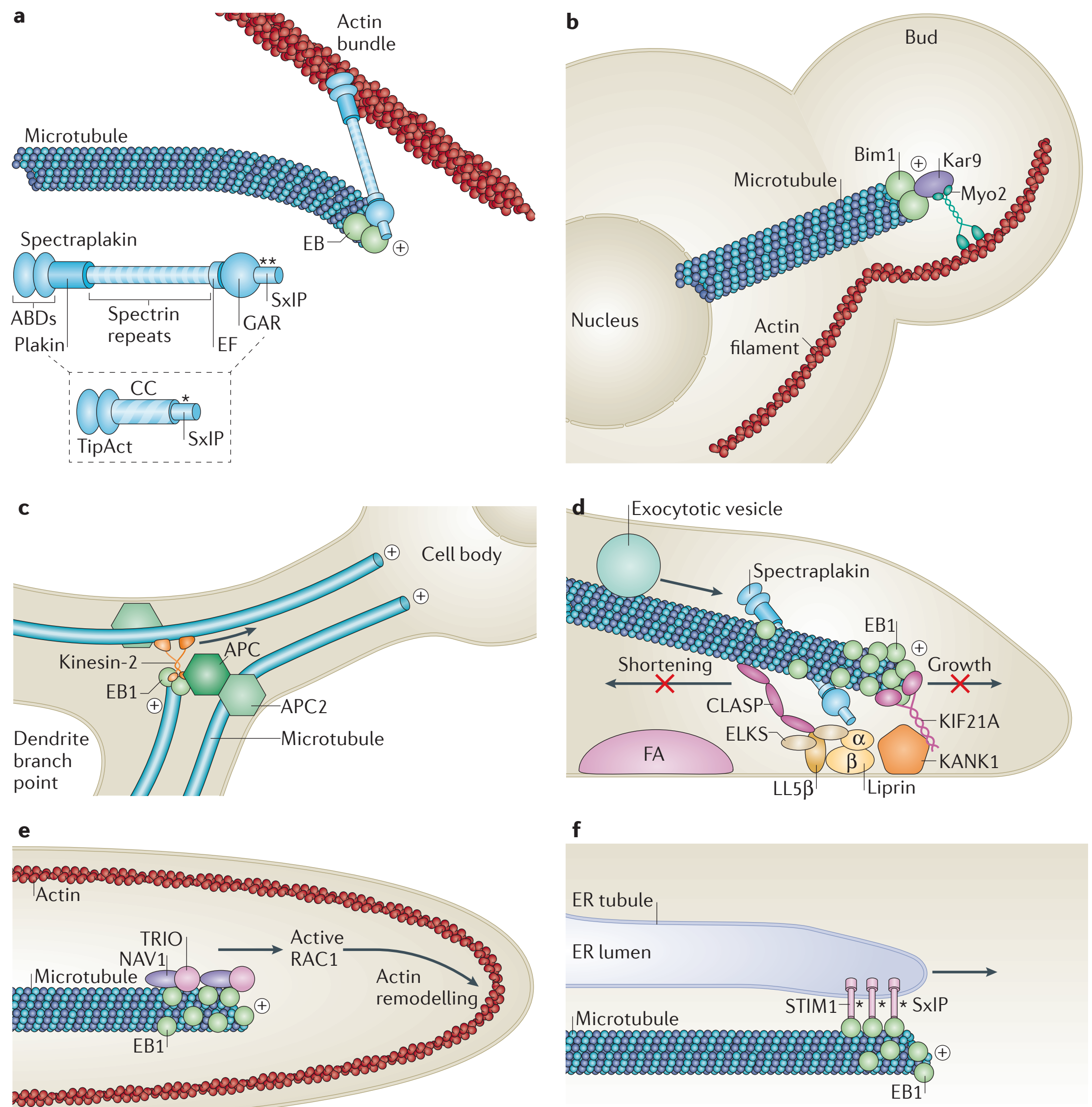

Figure 4 | **Cellular functions of microtubule plus-end-tracking protein (+TIP) networks. a** | Guidance of microtubule growth by actin bundles is mediated by the large cytoskeletal 'crosslinker' spectraplakin, which contains amino-terminal actin-binding calponin homology (CH) domains (ABDs), plakin and spectrin repeats, EF hands, a microtubule-binding Gly- and Arg-rich (GAR) domain and carboxy-terminal SxIP motifs (indicated by asterisks)[108]. A short version of this protein, named TipAct, which contains the ABDs and an SxIP motif connected by a coiled coil (CC), was sufficient to promote the guidance of growing microtubules along actin cables *in vitro*[111]. **b** | Growing microtubules can be targeted into the yeast bud by a complex composed of the end-binding protein 1 (EB1) orthologue Bim1 (binding to microtubules 1), the adaptor protein karyogamy 9 (Kar9) and the actin-based motor protein myosin 2 (Myo2; a class V myosin), which moves along actin filaments[112,113]. This microtubule–actin-dependent process is required for positioning the mitotic spindle. **c** | Microtubule guidance in neuronal branch points is carried out by kinesin-2, which promotes maintenance of the 'minus-end-out' localization of microtubules in fly dendrites. EB1, adenomatous polyposis coli (APC) and APC2 are required for this process, but the exact composition of the +TIP complex involved is currently unknown. **d** | The stabilization of microtubule plus ends and their attachment to the cell cortex promotes the localized delivery of vesicles to focal adhesions (FAs). Microtubules are stabilized by cytoplasmic linker-associated proteins (CLASPs) and spectraplakins, and their growth at the cell cortex is terminated by the kinesin-4 family member kinesin-like 21A (KIF21A). The cortical recruitment of these factors depends on multiple scaffold proteins, including KN motif and ankyrin repeat domain-containing 1 (KANK1), liprin, LL5β and ELKS[38,180]. **e** | The activation of RAC1 by +TIPs is required for neurite extension. EB1 at growing microtubule ends promotes the formation of a complex between the SxIP motif-containing +TIPs neuron navigator 1 (NAV1), which is dependent on EB1 for localization at microtubule plus ends, and the RHO-specific guanine nucleotide exchange factor (GEF) triple functional domain protein (TRIO), leading to TRIO-dependent activation of RAC1 and actin remodelling[131]. **f** | The protrusion of endoplasmic reticulum (ER) tubules by growing microtubules is driven by a complex between EB1 and the transmembrane ER calcium-entry regulator stromal interaction molecule 1 (STIM1), which contains an SxIP motif in its cytosolic domain [96,132]. Figure part **c** adapted with permission from REF. 114, Elsevier. Figure part **d** adapted with permission from REF. 38, Elsevier.

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undergo directional transport, an interaction with a structure bearing multiple EB-binding sites can, through an as-yet-uncharacterized mechanism, 'push' this structure in the direction of microtubule growth[111]. This type of mechanism is likely to be responsible for the formation of microtubule growth-dependent protrusions from the endoplasmic reticulum (ER), which are driven by the transmembrane SxIP-containing ER protein stromal interaction molecule 1 (STIM1), which binds to EB1 at microtubule tips[132] (FIG. 4f).

Control of microtubule minus ends

Microtubule minus ends and their associated factors have received considerably less attention than have microtubule plus ends and their binding factors. The main factor known to specifically associate with microtubule minus ends is the γ-tubulin ring complex (γ-TURC)[133]. The γ-TURC localizes to microtubule minus ends as a consequence of its microtubule-nucleating activity, but it can also bind and cap the minus ends of pre-formed microtubules[134]. The localization and activity of the γ-TURC is controlled by different factors that can recruit it to the centrosomes, membrane organelles or, in the case of augmin, the microtubule lattice, resulting in the formation of 'branched' microtubule arrays[135]. Ninein, which is a large coiled-coil protein that binds to the γ-TURC, has been implicated in anchoring microtubule minus ends at centrosomes, the apical side of epithelial cells and desmosomes[136–139]. Interestingly, a recent study showed that microtubule polymerization from γ-TURCs is strongly affected by microtubule plus-end regulators: catastrophe-promoting factors, such as MCAK and EB1, inhibited microtubule outgrowth, whereas XMAP215 facilitated the onset of microtubule elongation[140].

Recently, members of a protein family that contains calmodulin-regulated spectrin-associated protein 1 (CAMSAP1), CAMSAP2 and CAMSAP3 (also known as Nezha) in mammals and Patronin in invertebrates have emerged as regulators of free microtubule minus ends that function independently of γ-tubulin (FIG. 5). These proteins can specifically associate with both stable and dynamic microtubule minus ends[141–144]. They bind to and stabilize the minus ends of non-centrosomal microtubules in different cell types, including neurons, in which the regulation of such microtubules is particularly important owing to the presence of very long membrane extensions[142–149].

In vitro reconstitution experiments with dynamic microtubules showed that CAMSAP1 dynamically tracks growing microtubule minus ends[142]. The other two mammalian family members, CAMSAP2 and CAMSAP3, show more peculiar behaviours: they are rapidly recruited to the free microtubule minus ends and, when these ends grow, CAMSAP2 and CAMSAP3 stably decorate stretches of microtubule lattices[141,142] (FIG. 5c). These stretches are stabilized against depolymerization from both ends and form sites of non-centrosomal microtubule outgrowth[142,145,146]. CAMSAPs can therefore be described as microtubule minus-end-targeting proteins (–TIPs) that control the stability of microtubule minus ends in a manner dependent on minus-end polymerization. CAMSAPs also dramatically slow down microtubule minus-end growth, which probably explains why the polymerization of microtubules from the minus end is very difficult to observe in cells[142,150]. The biochemical bases of microtubule minus-end recognition and tracking by CAMSAPs is currently unclear; it seems to involve their highly conserved C-terminal CKK domain (CAMSAP, KIAA1078 and KIAA1543 domain), as well as additional microtubule-lattice-binding domains[142] (FIG. 5a). However, the situation might be different for the fly protein Patronin, which requires the coiled-coil domain to bind to minus ends[141]. As the formation of CAMSAP-stabilized microtubule stretches depends on minus-end polymerization, it can occur only when the γ-TURC is detached from the minus end or when a new minus end is generated by microtubule breakage or severing. Consistent with this view, in neurons, γ-TURC and CAMSAPs act in separate, although possibly sequential, steps to generate non-centrosomal microtubule arrays[146].

Similarly to +TIPs that promote microtubule polymerization and stability, –TIPs such as Patronin can counteract the depolymerase activity of kinesin-13 (REF. 144). This function is particularly important in dividing fly cells, in which Patronin is required to maintain proper spindle length and promote spindle elongation in anaphase B by suppressing poleward microtubule flux[144,151]. In mammals, the CAMSAP-induced formation of stabilized microtubule stretches is antagonized by the microtubule-severing protein katanin[142]. It is unclear whether katanin cuts CAMSAP-stabilized microtubules or inhibits microtubule minus-end growth and the concomitant CAMSAP deposition; the latter mechanism is not unlikely, as katanin can depolymerize microtubules from their ends[152].

In addition to CAMSAPs and Patronin, other minus-end-stabilizing factors are likely to exist. The worm homologue of Patronin, for example, is not essential for neuronal function[147–149], suggesting that other factors must regulate non-centrosomal minus ends in worm neurons. In mammalian cells, CAMSAPs seem to be mainly involved in controlling interphase microtubules[142,153]. Therefore, other factors, such as the *X. laevis* RAN GTPase-regulated protein microspherule 1 (mcrs1)[154], might protect microtubule minus ends in the spindle during mitosis. –TIP-interaction networks are, so far, poorly studied, but they can be expected, for example, to play a part in bringing together the minus ends at mitotic and meiotic spindle poles. Finally, specific regulators of microtubule minus ends have yet to be discovered in plants, in which γ-tubulin alone is unlikely to account for all minus-end stabilization and katanin-based severing is important for the organization of microtubule arrays[155].

Ligands acting at microtubule ends

Microtubule-targeting agents (MTAs), such as taxol, are some of the most potent chemotherapeutic drugs. They inhibit cell proliferation and promote apoptotic cell death by suppressing microtubule dynamics (reviewed in REF. 156). Besides being of high medical relevance, MTAs are widely used to manipulate microtubule networks, the most recent example being a photoswitchable

Endoplasmic reticulum
(ER). A membrane organelle in eukaryotic cells that has important functions in protein synthesis, folding and processing, as well as in lipid and sugar metabolism and calcium storage. It forms an interconnected network of flattened, membrane-enclosed sacs or tubes.

γ-tubulin ring complex
(γ-TURC). A multisubunit protein complex that binds to microtubule minus ends and nucleates microtubules, for example, at the centrosome. The complex contains a special isoform of tubulin, γ-tubulin.

Centrosomes
Organelles that form the main microtubule-organizing centre (MTOC) of most animal cells. They consist of cylindrical, microtubule-based structures called centrioles and their surrounding material, which can nucleate and anchor microtubules.

CKK domain
(Calmodulin-regulated spectrin-associated protein (CAMSAP), KIAA1078 and KIAA1543 domain). A domain that occurs at the carboxyl termini of the CAMSAPs and Patronin. CKK domains are thought to bind microtubules.

Poleward microtubule flux
The process of translocation of spindle microtubules towards spindle poles, coupled to the depolymerization of their minus ends.

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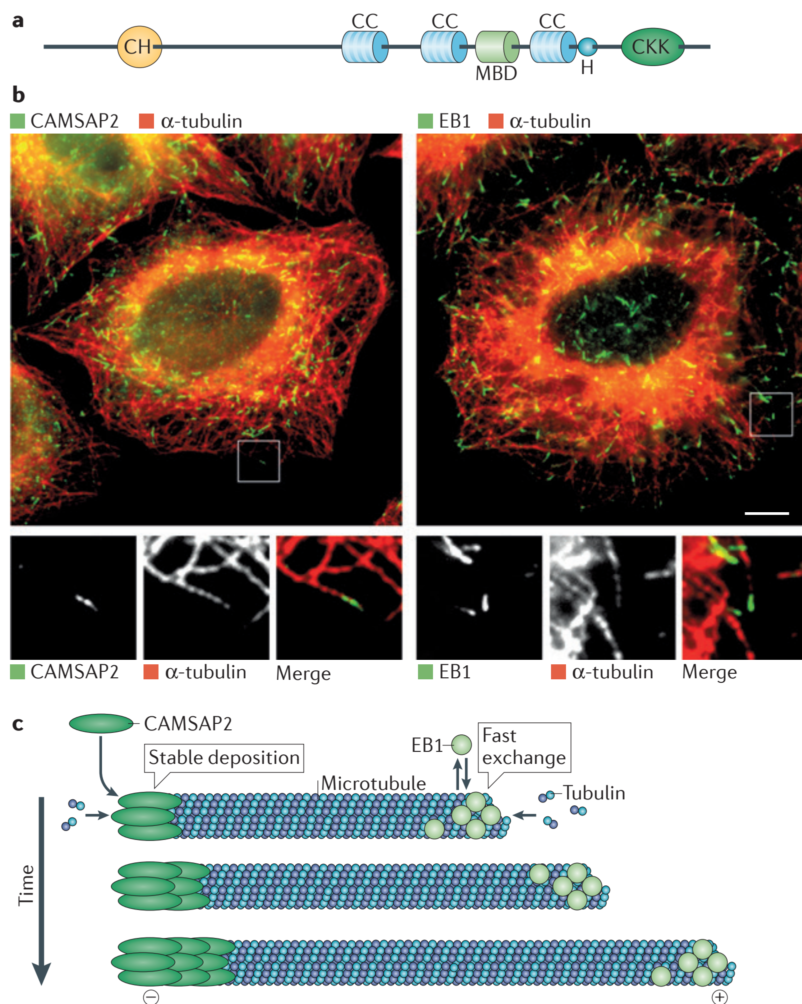

Figure 5 | **Comparison of the localization and turnover of the microtubule plus-end-tracking protein (+TIP) EB1 and the minus-end-targeting protein (–TIP) CAMSAP2 at growing microtubule ends.** **a** | The schematic shows the domain organization of calmodulin-regulated spectrin-associated proteins (CAMSAPs). The carboxy-terminal CKK (CAMSAP, KIAA1078 and KIAA1543 domain) is required for CAMSAPs to recognize microtubule minus ends. Depending on the CAMSAP, the microtubule-binding domain (MBD) and the helical domain (H) may also contribute to microtubule binding; the tightest microtubule association is observed for CAMSAP3, as it involves all three microtubule-binding regions. **b** | Staining for endogenous CAMSAP2 (left) or end-binding protein 1 (EB1; right) and α-tubulin in interphase HeLa cells. CAMSAP2 and EB1 decorate 1–2-μm-long stretches at the minus ends and plus ends of microtubules, respectively. Both the plus and the minus ends are distributed throughout the cytoplasm, with the minus ends being somewhat more abundant in the central part of the cell. Enlarged images (of the region in the white square) at the bottom of each image show single microtubule ends decorated with CAMSAP2 or EB1. **c** | Dynamics of CAMSAP2 and EB1 on growing microtubule ends. EBs form a comet-like accumulation and turn over rapidly at microtubule plus ends. CAMSAP2 (as well as CAMSAP3) is recruited to the growing microtubule minus ends and dissociates only slowly, if at all, resulting in the formation of CAMSAP-decorated microtubule stretches. EBs can also bind to growing microtubule minus ends (not shown); in the presence of CAMSAP2 or CAMSAP3, microtubule minus-end growth is slowed down and EB accumulation at the minus end is low. CH, calponin homology domain; CC, coiled-coil domain. Images in part **b** courtesy of S. Hua, Utrecht University, the Netherlands.

MTA allowing for optical control of microtubule dynamics[157]. At high concentrations (in the micromolar range), MTAs stabilize or destabilize microtubules by shifting the equilibrium towards the microtubule polymer, or tubulin dimers and small oligomers, respectively. They are thus often grouped into microtubule-stabilizing and microtubule-destabilizing agents (BOX 2). However, at low, therapeutically relevant concentrations (in the nanomolar range), both classes of compounds potently suppress microtubule dynamics in a similar manner, suggesting that under such conditions they primarily act on microtubule ends without significantly affecting the microtubule-polymer mass (reviewed in REF. 156).

The consequences of MTA interactions with microtubule ends are likely to be influenced by different +TIPs. Indeed, *in vitro* work showed that EBs synergize with different MTAs, irrespective of whether they are stabilizers or destabilizers, to promote catastrophes[158]. Along similar lines, the destabilizing MTAs vinflunine and vincristine are more effective in inhibiting cell migration and proliferation in cells with increased levels of EB1 (REF. 159). In another study, EB1 was shown to suppress the binding of the microtubule stabilizer taxol to microtubules, thus rendering the drug less potent[160]. These observations suggest that the choice of optimal chemotherapy for a particular patient might depend on the level of EB1 expression in the tumour.

Although research on MTAs is very advanced and several anti-tubulin drugs are widely used to treat several types of cancers, much less progress has been made in developing small-molecule ligands that target MAPs. Agents that interfere, for example, with +TIPs could open completely new routes for therapeutic intervention: it is conceivable that the exact protein composition, architecture and connectivity of +TIP networks may be different in diseased and in healthy cells, and may thus help to increase the specificity of drugs for cancer cells. Moreover, such ligands would represent valuable compounds for manipulating MAP networks. In this context, a recent study in *Drosophila melanogaster* showed that peptide aptamers of the SxIP motif perturbed the EB-dependent recruitment of +TIPs through competition and affected fly viability and microtubule dynamics in larval cells[161]. Another study reported that pregnenolone, which is a neurosteroid that improves memory and neurological recovery, targets CLIP-170 and enhances its interaction with microtubules, $p150^{Glued}$ and LIS1 (REF. 162). These two studies highlight the feasibility of identifying agents that target +TIP networks.

Future directions

We have come a long way in understanding the complexity of the regulators that control microtubule-end structures, dynamics and functions. Nevertheless, important questions remain. How are the dynamic end structures of microtubules (curved tubulin sheets versus straight lattice regions) and their conformational transitions correlated with the overall architecture of the GTP cap and +TIP-binding sites? What is the exact molecular basis of catastrophes and rescues in the absence and the presence of different microtubule regulators? How can we understand the collective activities of microtubule regulators within complex, non-stoichiometric networks? Can we obtain a complete systems-wide description of the entire microtubule cytoskeleton in any cellular system?

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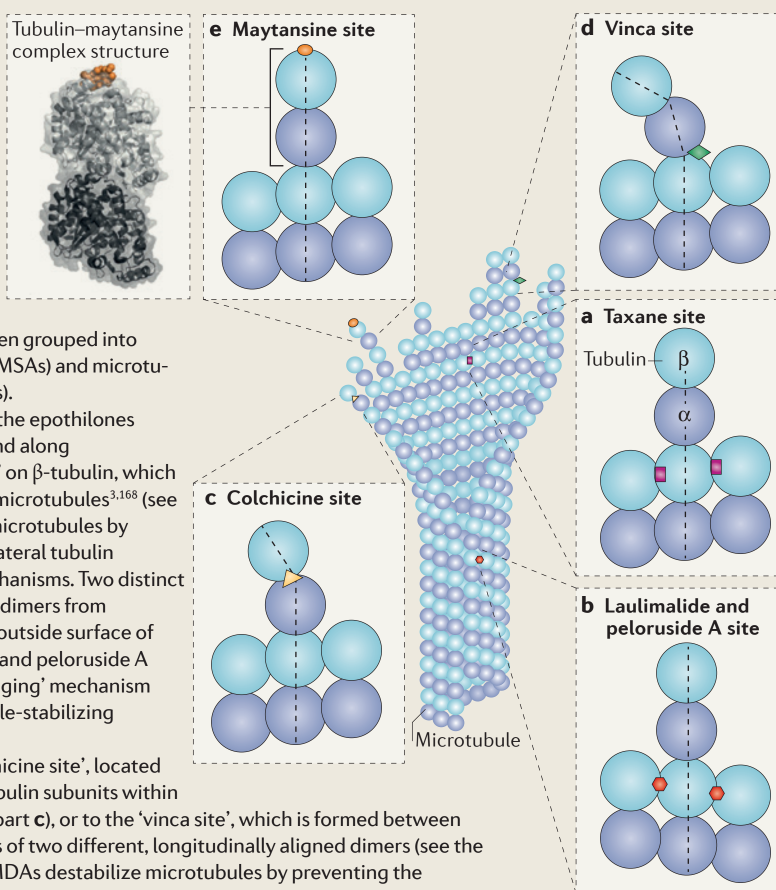
Box 2 | **Molecular mechanisms of microtubule-targeting agents (MTAs)**

MTAs are a chemically highly diverse class of cytotoxic agents that comprise several dozens of different chemical scaffolds. The vast majority of MTAs are natural products originating from various organisms, including bacteria, plants and marine sponges. On the basis of their effects on microtubules at high concentrations (in the micromolar range), MTAs are often grouped into microtubule-stabilizing agents (MSAs) and microtubule-destabilizing agents (MDAs).

Most MSAs, such as taxol and the epothilones (which are used in the clinic), bind along microtubules to the 'taxane site' on β-tubulin, which is located in the luminal side of microtubules[3,168] (see the figure, part **a**). They act on microtubules by stabilizing longitudinal and/or lateral tubulin contacts through allosteric mechanisms. Two distinct MSAs that bridge two β-tubulin dimers from adjacent protofilaments on the outside surface of the microtubule are laulimalide and peloruside A (see the figure, part **b**). This 'bridging' mechanism readily explains their microtubule-stabilizing effect[169].



MDAs bind to either the 'colchicine site', located between the α-tubulin and β-tubulin subunits within the same dimer (see the figure, part **c**), or to the 'vinca site', which is formed between α-tubulin and β-tubulin subunits of two different, longitudinally aligned dimers (see the figure, part **d**). Colchicine-site MDAs destabilize microtubules by preventing the curved-to-straight conformational transition of tubulin that accompanies microtubule assembly[170] (FIG. 1b). Tubulin–colchicine complexes can incorporate into growing microtubules[171] and may thus perturb microtubule ends. Vinca-site MDAs, such as vinblastine, destabilize microtubule ends by introducing a molecular wedge between two longitudinally aligned tubulin dimers. This mode of action induces the formation of curled protofilament assemblies that are not compatible with the straight protofilament configuration in microtubules[172]. An interesting class of MDAs, the representatives of which were only recently found to bind to a new site on β-tubulin, comprises the clinically relevant 'maytansine-site' compounds. Maytansine-site compounds bind to β-tubulin at the tip of the microtubule plus end and are predicted to suppress microtubule dynamics by inhibiting protofilament elongation[173] (see the figure, part **e**). The structure of maytansine in complex with tubulin has recently been solved to high resolution by X-ray crystallography (RCSB Protein Data Bank ID: 4TV8; see the figure, part **e**)[173].

It is well established that at low, therapeutically relevant concentrations (in the nanomolar range), both MSAs and MDAs kinetically stabilize microtubules without changing the microtubule-polymer mass (reviewed in REF. 156). Thus, the effects of drugs on the dynamics of microtubule during chemotherapy are often more relevant for their antitumour activities than their effects on polymer mass. Why do MTAs have different effects on microtubule dynamics at high and low concentrations? At high concentrations, MTAs shift the tubulin–microtubule equilibrium by acting on unassembled and/or assembled tubulin to either promote (MSAs) or inhibit (MDAs) microtubule polymerization. By contrast, at low concentrations, MTAs act primarily on microtubule ends. On the basis of the mechanisms described above, it is conceivable that the binding of a few MTAs to a microtubule tip locally perturbs its structure and in this way affects both microtubule assembly and disassembly.

Some of these questions would be resolved if we could, in line with the famous quote from Richard Feynman, "just look at the thing" with sufficient spatial and temporal resolution, for example, by improving correlative light–electron microscopy methodologies. Such studies could reveal whether propagating defects, such as loss of protofilaments, asynchronous protofilament growth or differences in curvature, and the effects of different regulators on these microtubule end structure, would account for the induction of catastrophe or the acceleration of microtubule polymerization by MAPs and ligands. Time-resolved super-resolution fluorescence microscopy can provide a way to obtain a more complete picture of the structure and dynamics of the cellular microtubule cytoskeleton, including the currently poorly accessible dense microtubule arrays in the vicinity of centrosomes or in neuronal processes. The engineering of differentially labelled or photoswitchable MTAs can potentially bring microtubule pharmacology to the single-molecule level and allow

Peptide aptamers
Small proteins containing a 10–25-amino-acid peptide that binds with high specificity and affinity to a molecular target.

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individual microtubules to be perturbed in cells with high temporal and spatial resolution. *In vitro* reconstitutions of increasing complexity, combined with microfabricated chambers or liposomes to mimic the cellular confinement performed in parallel with cell-biological experiments and computational modelling will allow the fundamental mechanisms controlling the geometry, size, mechanics and dynamics of cytoskeletal networks to be deciphered. Finally, we have reached a stage at which the comparison of +TIP networks in different eukaryotic cell types and from patient tissues will be important for understanding their underlying architectures and functions in healthy and diseased states, and might open up the exciting possibility of developing novel strategies to target the microtubule cytoskeleton in the framework of personalized medicine.

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Competing interests statement
The authors declare no competing interests.

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