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Microtubule-severing enzymes at the cutting edge

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Summary

ATP-dependent severing of microtubules was first reported in *Xenopus laevis* egg extracts in 1991. Two years later this observation led to the purification of the first known microtubule-severing enzyme, katanin. Katanin homologs have now been identified throughout the animal kingdom and in plants. Moreover, members of two closely related enzyme subfamilies, spastin and fidgetin, have been found to sever microtubules and might act alongside katanins in some contexts (Roll-Mecak and McNally, 2010; Yu et al., 2008; Zhang et al., 2007). Over the past few years, it has become clear that microtubule-severing enzymes contribute to a wide range of cellular activities including mitosis and meiosis, morphogenesis, cilia biogenesis and disassembly, and migration. Thus, this group of enzymes is revealing itself to be among the most important of the microtubule regulators. This Commentary focuses on our growing understanding of how microtubule-severing enzymes contribute to the organization and dynamics of diverse microtubule arrays, as well as the structural and biophysical characteristics that afford them the unique capacity to catalyze the removal of tubulin from the interior microtubule lattice. Our goal is to provide a broader perspective, focusing on a limited number of particularly informative, representative and/or timely findings.

Key words: AAA ATPase, Microtubule-severing enzyme, Microtubules

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Introduction

Microtubules are dynamic cytoskeletal polymers that perform integral roles in cell division, morphogenesis, motility and signaling. To function appropriately, microtubules must be assembled into a variety of higher-order arrays that undergo continual remodeling to meet the ever-changing needs of the cell. This plasticity is largely mediated by a host of regulatory proteins that bind to and modify the dynamic behaviors of individual microtubules. Whereas most of these microtubule regulators – including the microtubule destabilizing kinesin-13 family and the polymerization-promoting end binding protein 1 (EB1, also known as MAPRE1) (Gouveia and Akhmanova, 2010) – function at the ends of microtubules, a more mysterious, but no less important class of proteins, known as microtubule-severing enzymes, work by cutting microtubules into short fragments. On the surface it would seem that this 'severing' activity is particularly suited to catalyze the rapid destruction of portions of the microtubule network because the endpoint of the severing reaction in vitro is the complete loss of microtubules (McNally and Vale, 1993; Vale, 1991). However, the fates of severed microtubules within the cellular milieu are more complex and varied, as are the cellular functions of severing enzymes themselves. Indeed, work over the past two decades has strongly suggested that microtubule-severing enzymes are often employed to achieve highly constructive processes, such as seeding new microtubule growth, releasing microtubules from nucleation sites and allowing their transport from one domain of the cell to another (Baas et al., 2005; Roll-Mecak and McNally, 2010) (Fig. 1). In other cases, they have more nuanced roles in regulating the behaviors and composition of microtubule ends (Díaz-Valencia et al., 2011; Zhang et al., 2011). This Commentary focuses on our emerging understanding of both the mechanisms of action and cellular functions of this unique group of proteins.

The discovery of microtubule severing enzymes

To date, three classes of microtubule-severing enzymes have been identified, katanin, spastin and fidgetin, which all group together in the 'meiotic' subfamily of the ATPases associated with diverse cellular activities, or AAA, protein superfamily (Frickey and Lupas, 2004). A fourth member of this subfamily, vacuolar protein sorting 4 (VPS4), is not believed to sever microtubules (Babst et al., 1998; Bishop and Woodman, 2000; Yoshimori et al., 2000). AAA proteins are recognizable by the presence of a highly conserved ~230-amino-acid 'AAA domain', which contains the ATPase activity, and they generally function as hexameric rings or dodecameric stacked rings (Lupas and Martin, 2002; Neuwald et al., 1999; Vale, 2000).

The discovery and characterization of each of the three severing enzymes took very different routes. Katanin, the first microtubule-severing enzyme to be discovered, was initially identified as a biochemical microtubule-severing activity (Vale, 1991) and then purified from sea urchin eggs, where it exists as a heterodimer of a 60-kDa AAA-domain-containing catalytic subunit (p60, KATNA1) and an 80 kDa targeting and regulatory subunit (p80, KATNB1) (McNally and Vale, 1993). Katanin p60 and p80 were later found to be highly conserved in animals, higher plants, and protozoa. Many organisms, including *Drosophila melanogaster* and humans, also contain additional katanin-p60-like (katanin-like) proteins (Roll-Mecak and McNally, 2010).

In contrast with katanin, spastin was studied long before it was known to sever microtubules because of its link to the neurodegenerative disorder hereditary spastic paraplegia (HSP), which is caused by degeneration of specific subsets of axons in
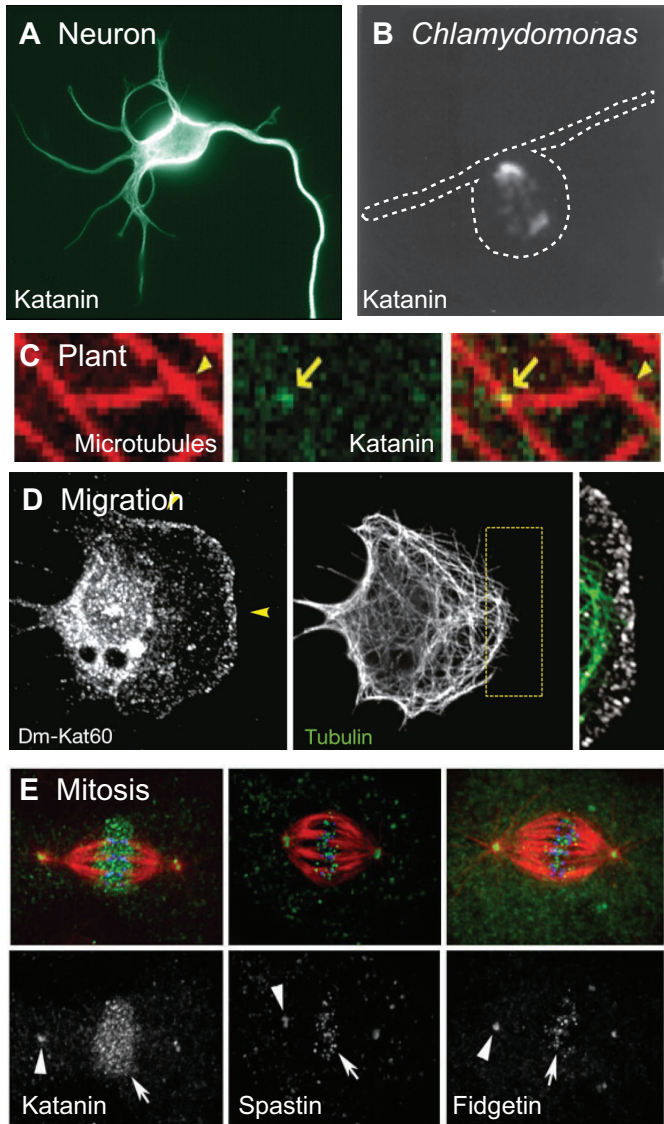
Fig. 1. Cell types and severing protein localization. Severing enzymes contribute to a variety of distinct microtubule organizations in an array of cell types. This figure shows several examples of the localization of severing enzymes as reported in the literature. (**A**) In neurons, katanin is highly expressed and is found at especially high levels in the axons. Reproduced with permission from Yu, et al., 2008. (**B**) In *Chlamydomonas*, katanin localizes to the basal bodies at the base of the cilia. Reproduced with permission from Lohret, et al. 1998. (**C**) Cortical microtubules of higher plants are nucleated as branches and released from the sides of older microtubules by katanin, which appears as a small punctum (arrow) just before severing of the branch (arrowhead marks the position of the other end of the microtubule). Reproduced with permission from Nakamura et al., 2010. (**D**) *Drosophila* S2 cell migration is mediated by severing enzymes, and katanin has been demonstrated to localize to the leading edge (arrowhead). Reproduced with permission from Zhang, et al. 2011. (**E**) Severing enzymes are found at spindle poles (arrowhead indicates centrosome) and on the chromosomes (arrow) during S2 cell mitosis. Reproduced with permission from Zhang, et al. 2007.

the peripheral nervous system and hallmarked by a progressive weakening of the lower extremities. Mutations in the spastin gene are the most common cause of autosomal dominant HSP (Hazan et al., 1999). Spastin has also been found to interact with a

number of distinct binding partners, which probably mediate its activities in different cellular contexts (Errico et al., 2004; Sanderson et al., 2006).

Finally, the discovery of fidgetin began with the report in the 1940s of a spontaneous mutation in a stock of lab mice that results in a 'head-shaking-and-circling' phenotype, termed the 'fidget' phenotype (Gruneberg, 1943). 'Fidget' mice also display severe defects in auditory, ocular and skeletal development with an increased frequency of cleft palates (Truslove, 1956; Yang et al., 2006). Almost 60 years later it was revealed that these pleiotropic effects were caused by a single retrotransposon insertion into the second intron of the mouse fidget locus, which was then renamed fidgetin (Cox et al., 2000). Overexpression of *Drosophila* fidgetin in tissue culture cells results in the destruction of cellular microtubules (Zhang et al., 2007), suggesting that it, too, is a severing enzyme. Additionally, although it has not yet been reported in the literature, we have found that human fidgetin efficiently severs microtubules in an ATP-dependent fashion in vitro (D.J.S. and J.L.R., unpublished data). Vertebrates additionally contain two fidgetin-like proteins, fidgetin-like 1 and 2 (FIGNL1 and FIGNL2), whose functions remain relatively unexplored (Cox et al., 2000).

The mechanism of microtubule severing

Although katanin was identified 20 years ago, it has proven to be difficult to purify and work with in vitro, so our understanding of its biophysical and biochemical properties has progressed relatively slowly. However, it is clear that both katanin and spastin require ATP for severing activity, and that the Walker A and Walker B motifs of the AAA domain control ATP binding and hydrolysis, respectively (Hartman and Vale, 1999; Roll-Mecak and Vale, 2008). ATP binding induces hexamerization of the AAA domain and hydrolysis causes disassembly (Hartman et al., 1998; Hartman and Vale, 1999).

In addition, several lines of study point to a severing mechanism whereby the AAA domain of the severing enzyme binds to the C-terminal tail of tubulin and uses ATP hydrolysis to translocate the tubulin polypeptide through the central pore of the AAA hexamer (Fig. 2). As the polypeptide substrate moves through the pore, the tubulin is locally unfolded and/or interdimer bonds are weakened to allow the dimer to be removed (Fig. 2). The model is supported by the following data: (1) microtubules treated with subtilisin, a protease that cleaves the unstructured C-terminal tails of tubulin, cannot be severed (McNally and Vale, 1993; Roll-Mecak and Vale, 2005; White et al., 2007); (2) interestingly, the C-terminal tail is where most of the post-translational modifications of tubulin occur (Verhey and Gaertig, 2007), and certain modifications, such as polyglutamylation, enhance the activity of severing (Lacroix et al., 2010); (3) mutations in the pore-loop region restrict and inhibit severing (Roll-Mecak and Vale, 2008; White et al., 2007) and these same pore mutants are unable to sever microtubules in cells, but are still able to bind and bundle microtubules (White et al., 2007); and (4) antibodies against the C-terminal tails of microtubules block severing activity (Roll-Mecak and Vale, 2008).

In early 2008, the crystal structure of spastin was published (Roll-Mecak and Vale, 2008). Although the AAA domain is similar to that found in other members of the family, the microtubule-interacting and endosomal trafficking (MIT)-linker domains create interesting spokes that are all angled towards the smaller face, face A, of the AAA ring. These results enabled the
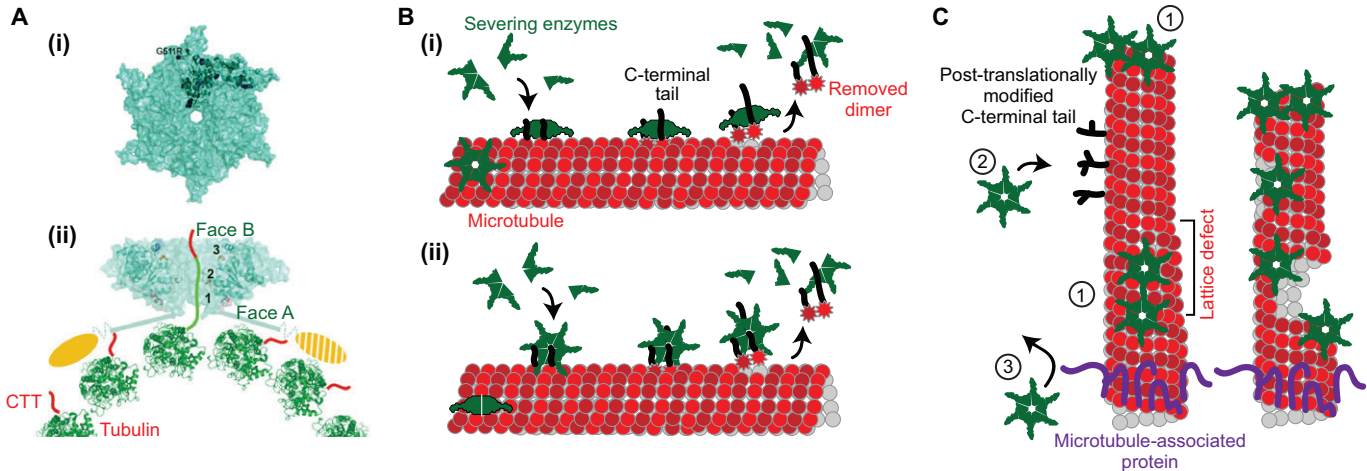
Fig. 2. Mechanism of severing. (**A**) The structure of the microtubule-severing enzyme spastin was solved in 2008. (i) Representative space-filling structure of the spastin hexamer with an inset of a monomer as a ribbon representation. (ii) Side view of the hexamer as it is envisioned to dock to the microtubule lattice. Face A docks on the side of the lattice, the C-terminal tail of the tubulin dimer is threaded through the 2-nm pore in the hexamer, and face B is facing away from the microtubule surface. Images are reproduced with permission from Roll-Mecak and Vale, 2008. (**B**) Microtubule severing is thought to occur through a molecular process wherein the severing protein AAA hexamer (green) assembles around the C-terminal tail (black line) of the tubulin dimer (red circles). The AAA domain uses ATP hydrolysis to deform the tubulin dimer and loosen the interdimer interactions to free it from the microtubule. The orientation of the microtubule-severing enzyme hexamer on the microtubule remains unknown. The hexamer face might sit flat, like a dinner plate, on the microtubule (i), or, the hexamer might stand up on the filament like a wheel (ii). (**C**) The cellular mechanisms that regulate severing location are likely to include: (1) localization to internal defects or filament ends, (2) localization to specific post-translational modifications, (3) steric inhibition of microtubule-severing enzyme binding and/or access to the microtubule lattice.

authors to propose a model for tubulin removal whereby the spastin face A faces the microtubule lattice with the radial spokes pointing towards the lattice to provide stabilization. Comparison of the relative positions of specific pore loops within the spastin structure with those of other AAA proteins known to directionally translocate peptides through their central pores provided further support for the hypothesis that the spastin face A is oriented towards the microtubule lattice.

Of course, there are still numerous open questions about the process of microtubule severing. For instance, we do not know exactly how severing enzymes bind to microtubules. They could bind flat onto the filament surface, like a plate, or they could stand up on it, like a wheel (Fig. 2). We do not know how the ring changes orientation and, perhaps, dissociates to cause the translocation of the C-terminal tail through the central pore. In addition, we do not know whether or not, or through which process, the severing enzymes identify specific regions of the microtubule on which to act, which is an aspect that is perhaps most relevant to the cellular activity of these enzymes. Interestingly, two studies have established that katanin preferentially severs at lattice defects, such as protofilament shifts and filament ends (Davis et al., 2002; Díaz-Valencia et al., 2011) (Fig. 2). It is less clear, however, how often such defects occur in cells, although some whole-cell electron microscopy studies have noted microtubules with large 'bites' taken from the filament walls (Srayko et al., 2006). Whether these defects are targets of or the result of severing enzymes remains unknown.

Finally, there is now a good deal of evidence indicating that severing appears to be regulated by post-translational modifications of microtubule-associated proteins and tubulin. Structural microtubule-associated proteins, such as MAP4 and tau, have been shown to inhibit katanin in cells (McNally et al., 2002; Qiang et al., 2006) (Fig. 2). Numerous cellular studies have

pointed to post-translational modification of the C-terminal tails of tubulin being the greatest indicators of severing activity (Lacroix et al., 2010; Sharma et al., 2007; Sudo and Baas, 2010) (Fig. 2). Unfortunately, very few studies have been performed in vitro with associated proteins or post-translationally modified tubulins. A recent publication examined the effects of polyglutamylation of various lengths on spastin-severing activity both in cells and using purified microtubules in vitro. They found that spastin preferentially severs microtubules with long polyglutamylated side chains on the C-terminal tails (Lacroix et al., 2010). Further studies of this kind need to be performed, so that our understanding of the molecular mechanisms of microtubule-severing enzymes catches up with the numerous observations regarding their functions inside cells.

Roles of microtubule-severing enzymes in mitosis and meiosis

Active microtubule severing was first observed as an M-phase specific activity (Vale, 1991), and thus a good deal of effort has gone towards understanding the roles of severing proteins in the formation and function of the spindle apparatus. During animal cell mitosis, most of the spindle microtubules are nucleated from duplicated centrosomes and are oriented with their plus-ends facing the spindle equator, although some microtubules also extend from chromosomes. As the spindle matures, microtubule minus-ends are thought to be released from their nucleating γ-tubulin ring complexes at centrosomes and incorporated into a focused pole where their persistent depolymerization by members of the kinesin-13 family constrains spindle length and promotes poleward chromosome movement (Rogers et al., 2005). Poleward chromosome motility also involves kinesin-13-catalyzed depolymerization of microtubule plus-ends embedded within kinetochores, the multiprotein complexes assembled on the

centromere (Rath and Sharp, 2011). Meiotic spindles in females are structurally similar but form in the absence of centrosomes and thus all of their microtubules must be nucleated from other sites. Current data indicate that microtubule-severing enzymes are utilized to promote the assembly of the spindle itself and the segregation of chromosomes on it (also see below). Intriguingly, they appear to employ a variety of mechanisms to do so, which include the seemingly diametrically opposed activities of microtubule amplification and microtubule end depolymerization.

Katanin in *Caenorhabditis elegans* meiosis

The function of katanin in M phase has been most thoroughly investigated during oocyte meiosis in *C. elegans*. Indeed, the genes encoding the *C. elegans* katanin p60 and p80 subunits, termed *mei-1* and *mei-2* respectively, were identified in a screen for embryonic lethal mutants before the discovery of microtubule severing (Mains et al., 1990). Null mutations in *mei-1* and *mei-2* strongly perturb meiotic spindle assembly and result in the microtubules forming a disorganized mass around chromosomes (Clandinin and Mains, 1993; Clark-Maguire and Mains, 1994b; Srayko et al., 2000). In hypomorphic katanin mutants (in which the protein is present but is less functional), bipolar spindles do form but are aberrantly elongated and display defects in their orientation and dynamics at the oocyte cortex (McNally et al., 2006). Tellingly, the loss of katanin activity in this system results in fewer, but longer, spindle microtubules, leading to a model in which katanin-mediated severing events increase the number and density of spindle microtubules by generating numerous short polymers that are stabilized to serve as seeds for new microtubule growth (Roll-Mecak and Vale, 2006; Srayko et al., 2006) (Fig. 3). *C. elegans* katanin concentrates on meiotic chromosomes and spindle poles, which further suggests that microtubule amplification occurs particularly at these sites (Clark-Maguire and Mains, 1994a; Srayko et al., 2000). There is also evidence that katanin works in parallel with γ-tubulin to generate spindle microtubules, as katanin, γ-tubulin double mutants display a synergistic loss in microtubule polymers (McNally et al., 2006) (Fig. 3). It should be noted, however, that microtubule amplification is not the sole meiotic function of *C. elegans* katanin. Recent work has shown that it can support aspects of bipolar spindle assembly independently of its severing activity, probably through a secondary role in microtubule bundling (McNally and McNally, 2011). *C. elegans* katanin is degraded at the end of meiosis, which means that it does not have role in mitosis (Lu and Mains, 2007). However, there is evidence that both katanin and a katanin-like protein amplify mitotic spindle microtubules in vertebrate cells, although this occurs to a much lesser extent than in *C. elegans* meiosis (Buster et al., 2002; Sonbuchner et al., 2010). The reason for this could be that the absence of centrosomes in oocytes results in an increased reliance on severing as a means of generating enough microtubules to support normal spindle morphogenesis.

Severing enzymes in *Drosophila* mitosis

More recent work in mitotic *Drosophila* S2 cells has identified completely different spindle functions for microtubule-severing enzymes (Zhang et al., 2007). In this system, katanin, spastin and fidgetin (CG3326) all work together to promote poleward chromatid motility during anaphase A by promoting the simultaneous depolymerization of the plus- and minus-ends of kinetochore microtubules (Fig. 1). On one hand, spastin and
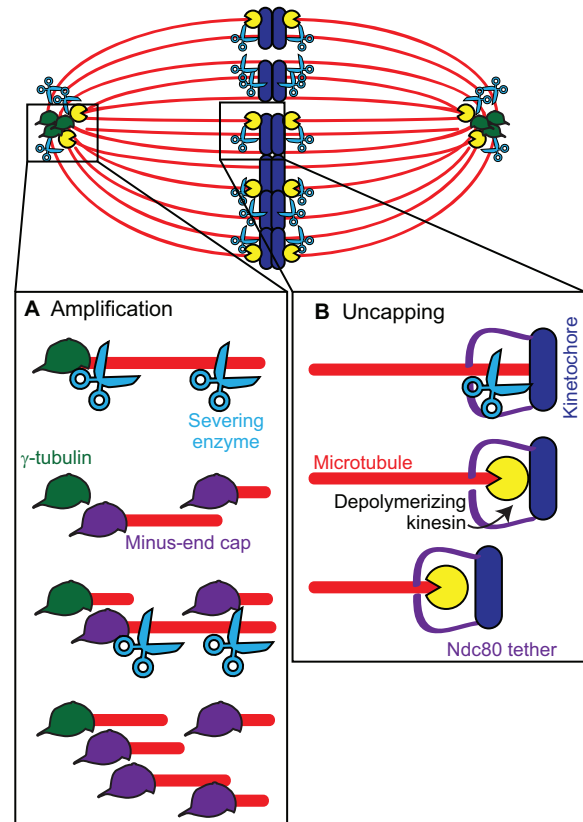
Fig. 3. Severing enzymes in mitosis and meiosis. Microtubule-severing enzymes (blue scissors) perform two functions in mitosis and meiosis: (**A**) to amplify the number of microtubules in the spindle, and (**B**) to uncap microtubule ends to enable depolymerizing kinesins to rapidly degrade microtubules, as, for example, in anaphase A. (A) Severing of microtubules nucleated by γ-tubulin (green caps) creates seeds to nucleate more growth. After severing, the seeds are protected by additional minus-end cappers, such as patronin (purple caps). Newly growing microtubules continue to be severed, to be recapped and to grow in order to enhance the density of microtubules. (B) Microtubules capped, for instance, at the plus-end and stabilized by the kinetochore (purple), are severed at the onset of anaphase A. Severing allows access to the end by depolymerizing kinesins (yellow) that shrink microtubules rapidly, thereby pulling the chromosomes apart.

fidgetin concentrate at the centrosome and contribute to anaphase A by promoting the depolymerization of pole-focused microtubule minus-ends. Why both enzymes are used for this same task remains unclear. On the other hand, katanin localizes primarily to chromosomes, kinetochores and centrosomes and drives anaphase A primarily by promoting the depolymerization of kinetochore-associated microtubule plus-ends.

Collectively, these data support and expand an earlier model whereby microtubule-severing enzymes function within the spindle by cutting away stabilizing caps from microtubule ends which in turn provides a suitable substrate for kinesin-13-catalyzed depolymerization (Buster et al., 2002; Zhang et al., 2007) (Fig. 3). We note that anaphase A in *Drosophila* S2 cells is stimulated by different kinesin-13 proteins that also localize to and depolymerize the opposite ends of kinetochore-associated microtubules (Buster et al., 2007; Rath et al., 2009). In particular, spastin and fidgetin could release microtubule minus-ends from centrosomes where they are capped and shielded from depolymerization by their

nucleating γ-tubulin ring complexes and/or the minus-end capping protein patronin (Goodwin and Vale, 2010). In fact, the latter has been shown to selectively associate with minus-ends and to prevent their depolymerization by kinesin-13 in vitro. We note, however, that double knockdowns of patronin with katanin or spastin did not appear to induce any synergistic phenotypes (Goodwin and Vale, 2010). At chromosomes and/or kinetochores, katanin might sever away the stabilizing caps formed by the plus-end tracking protein EB1 and proteins associated with it. This hypothesis is supported by the findings that EB1 associates with polymerizing microtubule plus-ends within the kinetochore and that a vertebrate EB protein family member inhibits kinesin-13-mediated end depolymerization in vitro (Montenegro Gouveia et al., 2010). Whether microtubule-severing enzymes are utilized in the same way in other organisms remains an open question. If so, katanin must be functionally replaced by a different severing enzyme, as its localization to mitotic chromosomes appears to be restricted to *Drosophila*.

Severing enzymes in cytokinesis

Both katanin and spastin are also important for cytokinesis, but their roles in this process are not well established. Cytokinesis starts with the formation and contraction of the actomyosin contractile ring, which drives the ingression of the plasma membrane between the newly forming daughter cells. The underlying central spindle microtubules are compacted into a tight midbody structure, which is then disassembled during abscission of the daughter cells through a process that appears to involve microtubule severing. Both katanin and spastin localize to the midbody through interactions with LAPSER1 (for 'rich in leucine, alanine, proline, serine, glutamate and arginine 1'; also known as LZTS2) and components of the endosomal sorting complex required for transport (ESCRT) complex, respectively, and their inhibition delays midbody disassembly and abscission in some cell types (Errico et al., 2004; Guizetti et al., 2011; Schiel and Prekeris, 2010; Sharma et al., 2007; Sudo and Maru, 2007). However, it is currently unclear whether these proteins actually directly sever and/or disassemble midbody microtubules

and two very recent studies on the role of spastin in cytokinesis have come to opposite conclusions as to whether this is so (Guizetti et al., 2011; Schiel and Prekeris, 2010). Thus, this is an area ripe for further investigation.

Microtubule severing in the regulation of neuronal morphogenesis and function

Multiple microtubule-severing enzymes have also been found to have crucial roles in neuronal morphogenesis, function and plasticity. Indeed, katanin, katanin-like proteins, spastin and fidgetins are highly expressed in the nervous systems of diverse organisms, and all of these enzymes, apart from the fidgetins, have been found to impact upon neuronal microtubule arrays, although their specific roles can vary depending on the neuronal cell type and organism (Ahmad et al., 1999; Butler et al., 2010; Lee et al., 2009; Sherwood et al., 2004; Solowska et al., 2008; Trotta et al., 2004; Wharton et al., 2003; Yang et al., 2005; Yu et al., 2005).

Katanin in the nervous system

A neuronal function for katanin was first demonstrated in cultured rat neurons, where the inhibition of katanin p60 strongly compromises axon outgrowth and causes an increase in the number of centrosome-associated microtubules and in microtubule length in the cell body and axon (Ahmad et al., 1999). On the basis of these data, it has been proposed that katanin stimulates axon growth by releasing microtubules from centrosomes and dissecting them into shorter segments that can be transported along longer microtubules down the axon to seed new microtubule growth (Fig. 4). These activities are analogous to those described for *C. elegans* katanin in the section on mitosis and meiosis above.

Given the potentially destructive impact of uncontrolled microtubule severing within the neuron, it is not surprising that the neuronal activities of katanin are maintained under tight regulatory control that is mediated at multiple levels. The best characterized of these is the negative regulation of katanin by the axon-specific microtubule-associated protein (MAP) tau, which
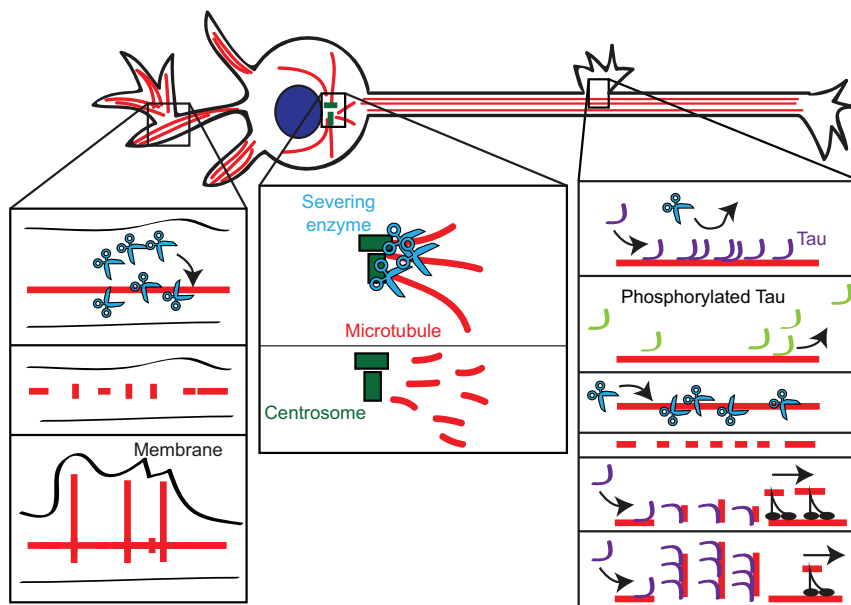
Fig. 4. Roles of severing enzymes in neuronal morphogenesis. Severing enzymes (blue scissors) are highly enriched in developing and adult neurons in all three major compartments of the neuron: the cell body, the dendrites and the axon. In the dendrites (left-hand panel), spastin and a katanin-like protein sever microtubules at branch sites to promote arborization. In the cell body (middle panel), katanin is required to sever microtubules from the centrosome (dark green) to allow microtubule transport down the axon. In axons (right-hand panel), katanin is used to create short microtubules for transport up the axon during elongation. The activity of katanin is regulated by tau, whose dephosphorylated form (purple) protects microtubules from katanin. When phosphorylated (light green), tau dissociates from the microtubules and exposes them to severing. Spastin is used to control branching in the axon, and short microtubules can rotate to grow into forming branches.

shields microtubules from katanin-mediated severing (Qiang et al., 2006) (Fig. 4). There is also evidence that katanin is locally activated on specific subsets of axonal microtubules by the phosphorylation of the microtubule-binding domains on tau, which result in the release of the MAP from the microtubule (Qiang et al., 2010). This observation has fueled speculation that the misregulation of katanin might contribute to tauopathies, such as Alzheimer's disease, which are hallmarked by tau hyperphosphorylation (Baas and Qiang, 2005). Similarly, a very recent study has shown that katanin is inhibited by the neuroprotective peptide (NAP), which has been found to ameliorate some Alzheimer's disease symptoms in animal models (Sudo and Baas, 2011). In addition to its regulation by tau, katanin has been found to selectively attack acetylated microtubules (Sudo and Baas, 2010), which are abundant in the axon, and its expression levels have been shown to vary dramatically during neuronal development, namely peaking during periods of rapid axon outgrowth and falling precipitously thereafter (Karabay et al., 2004). Fitting all of these pieces together into a coherent picture of how different katanin activities are controlled within the neuron will require a great deal of additional work.

Roles for spastin in the nervous system

Whereas spastin has long been known to affect axonal morphology and function, our understanding of its specific influences on the organization of axonal microtubules has proceeded more slowly. Studies in *Drosophila* have suggested that spastin is utilized to amplify microtubules within synaptic boutons (button-like projections of the axon that form at synapses) (Sherwood et al., 2004), whereas work in zebrafish supports the possibility of broader roles for spastin in the generation of axonal microtubules (Butler et al., 2010). Interestingly, in cultured rat neurons, spastin works alongside katanin to regulate axon morphology by selectively promoting the formation of collateral branches. Spastin also accumulates at nascent branch sites where it might generate a population of short mobile microtubules that are specifically delivered into the developing branch (Yu et al., 2008). The fragmentation of microtubules into short segments has been observed at axon branch sites, which is consistent with this hypothesis (Yu et al., 1994). Unlike katanin, spastin activity is not strongly affected by tau, but is enhanced on polyglutamylated microtubules, which are abundant in the axon (Lacroix et al., 2010; Qiang et al., 2006).

Spastin and katanin have also been found to work together to control dendritic morphology in selected sensory neurons within *Drosophila* larvae. Similarly to the situation in rat axons, null mutations in *Drosophila* spastin reduce dendritic arborization (branching) (Jinushi-Nakao et al., 2007), raising the possibility that it seeds microtubule growth within newly forming regions of the dendrite. Furthermore, mutation of the gene encoding a katanin-like protein, *kat-60L1*, inhibits the destruction of these same dendrites during metamorphosis. This 'dendritic pruning' is a necessary step in the rewiring of the adult nervous system. In this case, Kat-60L1 probably works by severing and depolymerizing microtubules in the proximal dendrite to mark the site at which the process will ultimately disjoin from the cell body (Lee et al., 2009) (Fig. 4). Adding complexity to this picture, however, are the results of a very recent unpublished study showing that loss of Kat-60L1 activity also reduces dendritic arborization during earlier developmental stages (Nina

Sherwood, personal communication) (Fig. 4). A systematic comparative analysis of the neuronal functions of katanins, spastin and fidgetins in a single cell type, though a daunting task, has the potential to provide a wealth of information.

Katanin regulates cilia biogenesis and disassembly

The roles of microtubule-severing enzymes are not limited to mitosis, meiosis and neuronal morphogenesis, as katanin, in particular, has been shown to be involved in numerous additional activities. The assembly and disassembly of cilia and flagella is one of the best characterized of these functions. Cilia and flagella are complex and highly-conserved microtubule-based extensions that are generally motile and designed to move the cell itself or substances around the cell. Cilia and flagella are structurally similar, comprising a cylindrical arrangement of nine doublet microtubules with a central pair of single microtubules that is collectively known as the axoneme. Immotile cilia lack the central pair of axonemal microtubules. The axoneme is attached to the basal body, which has a secondary function as a centriole, with the interface between these two structures referred to as the transition zone (Fig. 1).

Mutation of the genes encoding the katanin subunits in *Chlamydomonas* and *Tetrahymena*, two evolutionarily distant organisms, results in a strikingly similar phenotype, namely the formation of immotile cilia that lack the central pair of axonemal microtubules (Dymek et al., 2004; Sharma et al., 2007). It has been proposed that katanin-mediated microtubule severing within the cilia and/or cell body generates a pool of precursor tubulin subunits or short microtubule fragments that are in some way specified for the assembly of the central pair of axonemal microtubules (Fig. 5). In addition, in *Chlamydomonas*, but not *Tetrahymena*, the depletion of katanin inhibits de-ciliation at the onset of mitosis. De-ciliation allows the basal bodies to be reutilized as the centrioles of mitotic centrosomes. There is evidence to suggest that katanin releases the basal body from the axoneme by selectively severing microtubules at the transition
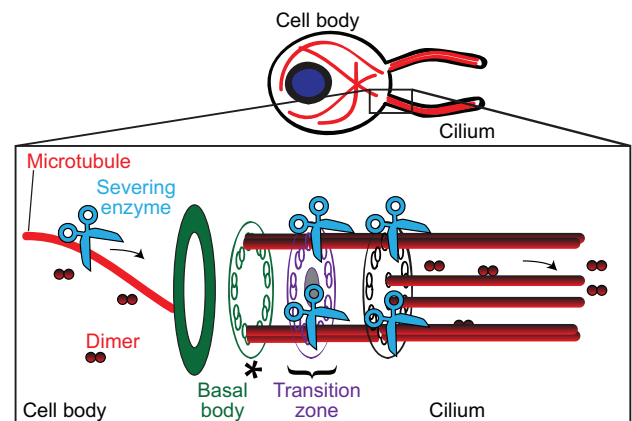
Fig. 5. Microtubule-severing enzymes in cilia biogenesis and disassembly. The severing of microtubules within cilia or the cell body by katanin (blue scissors) releases tubulin dimers that subsequently assemble in the central microtubule pair and enable the formation of motile cilia. Before going into mitosis or meiosis, ciliated cells retract their cilia. They disassemble cilia using severing enzymes that specifically cut in the transition zone (purple outline, denoted with a curly bracket) to release the basal body (green outline, denoted with an asterisk) to be used as centrioles during division.

zone (Rasi et al., 2009) (Fig. 5) – an activity that might mirror that of Kat-60L1 in the dendritic pruning described above.

Analysis of the effects of katanin overexpression in *Tetrahymena* also suggests that there is an extraordinary selectivity for certain ciliary microtubule subsets. For instance, overexpressed katanin does not impact upon the central axonemal microtubule pair but does attack the outer doublets, with the B-tubule being more prone to severing than the A-tubule. This selectivity can be controlled, at least in part, by tubulin post-translational modifications, as the B-tubule contains higher levels of polyglutamylated tubulins relative to the A-tubule or central microtubules. Consistent with this, *Tetrahymena* katanin-null mutants display an increase in polyglutamylated microtubules within cilia with a corresponding decrease in tubulin polyglycylation (Sharma et al., 2007). Understanding whether and how katanin influences the formation and function of both motile and immotile cilia in higher-order organisms should be an emphasis of future studies.

Microtubule severing in cell migration

A new set of functions for microtubule-severing enzymes has come from analyses of their roles in cell migration. The inhibition of katanin p60 has been shown to reduce neuronal migration in mice (Toyo-Oka et al., 2005) whereas the inhibition of katanin p80 slows the movement of cultured rat epithelial cells (Sudo and Maru, 2008). A more recent study also indicates that canonical katanin p60 is aberrantly expressed in bone metastases of prostate cancer, and that its overexpression in prostate cancer cells enhances their motility (Ye et al., 2011). It is possible that, at least in these situations, katanin promotes cell motility in the same way that it supports axon outgrowth, by releasing microtubules from centrosomes and promoting their transport towards the leading edge of cell movement. Indeed, the release of centrosomal microtubules has been observed in some migratory cell types (Abal et al., 2002), yet, how this would impact upon cell movement remains poorly defined.

In stark contrast with mammalian cells, the depletion of katanin p60 from *Drosophila* cells results in a substantial increase in their motility rates, indicating that katanin can also work as a negative regulator of cell migration. In this case, katanin localizes to the cell cortex and promotes the depolymerization of the microtubule plus-ends that contact cortical sites (Zhang, 2011) (Fig. 1). The human katanin-like protein KATNAL1 displays a similar localization pattern and has a similar impact on cell migration in breast cancer cells (Zhang et al., 2011), which raises the intriguing possibility that it works antagonistically with katanin p60 and that disrupting the balance of the activities of these two enzymes might contribute to metastasis. Cortical katanin has been proposed to promote microtubule plus-end depolymerization in a way that is similar to the role of kinetochore katanin in mitosis, by removing stabilizing removing EB1 caps from microtubule plus-ends to trigger kinesin-13-mediated microtubule depolymerization. The *Drosophila* kinesin-13, Klp10A, is loaded onto polymerizing microtubule plus-ends but promotes their depolymerization primarily at the cortex (Mennella et al., 2005).

How *Drosophila* katanin and human KATNAL1 suppress cell motility is unknown, but this process might involve the small GTPase Rac, which normally stimulates actin-based protrusions of the membrane. Rac is activated by microtubule polymerization, perhaps because it binds to and is sequestered by free tubulin, and katanin can locally inhibit Rac by suppressing microtubule polymerization at the leading edge (Best et al., 1996; Waterman-Storer et al., 1999). Consistent with this, RNA interference (RNAi)-mediated depletion of katanin causes an increase in both the amplitude and frequency of membrane protrusions in *Drosophila* S2 cells (Zhang et al., 2011). Alternatively, or in addition to this, the increased density of microtubules at the cortex of katanin-depleted cells could enhance the delivery of membrane to the protrusive zone and/or influence the turnover of focal adhesion complexes that link cells to their substrata to provide traction for cell movement (Watanabe et al., 2005). A more thorough understanding of the mechanisms and pathways through which katanin and katanin-like proteins influence cell motility will likely prove to be a very fruitful research topic with basic and clinical implications.

Katanin in higher-order plants

Whereas this Commentary has, so far, focused almost entirely on the utilization of microtubule-severing enzymes in animal cells, the most clearly demonstrated katanin-mediated cellular function has emerged from studies of the higher-order plant *Arabidopsis*. The gene encoding *Arabidopsis* katanin, termed *ATKN1*, was originally identified in a screen for ethyl-methanesulfonate-induced mutations that cause a reduction in the mechanical strength of the stem (Burk et al., 2001). The reduction in stem strength in *ATKN1* mutants is accompanied by global alteration in plant morphology and these pleiotropic effects were found to result from defects in cell morphogenesis and cell wall biosynthesis caused by a disruption in the arrangement of cortical microtubules. In wild-type *Arabidopsis* cells, microtubules form parallel arrays beneath the plasma membrane, which control, among other things, the direction of cellulose deposition (Fig. 6). A loss-of-function mutation in the *ATKN1* gene delays the formation of cortical microtubule arrays, whereas the overexpression of ATKN1 causes cortical microtubules to form dense bundles that ultimately depolymerize (Burk et al., 2001; Stoppin-Mellet et al., 2006).

Elegant live-cell analyses have provided detailed insights into how the cortical microtubule arrays of plant cells form and have highlighted a role for katanin in this process. In particular, these
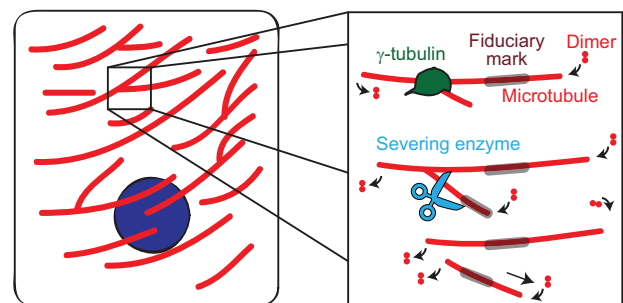
Fig. 6. Severing in higher plants. In higher plants, microtubule severing is used to create and enhance microtubule density, and to align the cortical microtubule array. γ-Tubulin ring complexes (green cap) bind to the sides of microtubules to nucleate new microtubules. These branched microtubules are cut by katanin (blue scissors) and the new free microtubules treadmill within the cortical array. Darkened areas represent photobleached 'fiduciary' markers to demonstrate that microtubules are treadmilling by adding dimers (red circles) to growing ends and dissociating dimers from the depolymerizing ends, and to show that microtubules are not gliding.

studies have revealed that the majority of cortical microtubules form as branches from the sides of pre-existing microtubules at an angle of roughly 40° (Chan et al., 2003; Murata et al., 2005; Shaw et al., 2003). These new microtubules, which are nucleated by γ-tubulin ring complexes that transiently associate with the wall of the parent microtubule, then detach from their sites of origin (namely the parent microtubule and the nucleating γ-tubulin ring complex) and move away from it by treadmilling, presumably to be incorporated into the larger parallel array of cortical microtubules (Fig. 6). A loss-of-function mutation in *ATKN1* nearly completely prevents these detachment events, which strongly supports the hypothesis that they are mediated by katanin. This hypothesis is also supported by the observation that GFP–ATKN1-containing puncta can often be observed at the presumptive severing site just before it occurs (Nakamura et al., 2010) (Fig. 1). Thus, in this system, katanin has the dual function of seeding new microtubule growth and uncapping the microtubule minus-end, which is probably necessary for treadmilling to occur. Understanding how ATKN1 is specifically directed to the branch sites should be the focus of future research efforts.

Concluding remarks

We hope that this Commentary has conveyed the impressive recent rate of growth in our understanding of both the cellular functions and mechanisms of action of the microtubule-severing enzymes. Nonetheless, our true understanding of these issues remains in a very nascent state. There are several general observations that can be garnered from recent studies. Microtubule-severing enzymes create and maintain non-centrosomal arrays of microtubules in a variety of cell types. Severing enzymes are often partnered with the microtubule nucleator γ-tubulin. They control microtubule behavior by creating new 'bare' ends, either by cutting the lattice or removing tubulins directly from the end, which are then highly responsive to the local environment of other microtubule regulators. These ends can become stabilized, grow or shrink depending on which other cellular components associate with them. But is this it or can severing enzymes also control the topology of the microtubule lattice by removing individual tubulins that have been marked by specific post-translational modifications or correct errors in the microtubule lattice? This is a question that remains completely unexplored. Other important questions that need to be addressed include: what is the specific contribution of hexamerization to the severing reaction? How are distinct severing enzymes directed to different tubulin post-translational modifications and what is the cellular impact of this aspect of their regulation? What are the roles of the katanin-like and fidgetin-like proteins? And finally, do katanin, spastin and/or fidgetin perform functions entirely unrelated to their microtubule severing activities? Our ability to answer these questions will determine our rate of progress in the coming decade of research on microtubule severing enzymes.

Funding

References

Abal, M., Piel, M., Bouckson-Castaing, V., Mogensen, M., Sibarita, J. B. and Bornens, M. (2002). Microtubule release from the centrosome in migrating cells. *J. Cell Biol.* **159**, 731-737.

Ahmad, F. J., Yu, W., McNally, F. J. and Baas, P. W. (1999). An essential role for katanin in severing microtubules in the neuron. *J. Cell Biol.* **145**, 305-315.

Baas, P. W. and Qiang, L. (2005). Neuronal microtubules: when the MAP is the roadblock. *Trends Cell Biol.* **15**, 183-187.

Baas, P. W., Karabay, A. and Qiang, L. (2005). Microtubules cut and run. *Trends Cell Biol.* **15**, 518-524.

Babst, M., Wendland, B., Estepa, E. J. and Emr, S. D. (1998). The Vps4p AAA ATPase regulates membrane association of a Vps protein complex required for normal endosome function. *EMBO J.* **17**, 2982-2993.

Best, A., Ahmed, S., Kozma, R. and Lim, L. (1996). The Ras-related GTPase Rac1 binds tubulin. *J. Biol. Chem.* **271**, 3756-3762.

Bishop, N. and Woodman, P. (2000). ATPase-defective mammalian VPS4 localizes to aberrant endosomes and impairs cholesterol trafficking. *Mol. Biol. Cell* **11**, 227-239.

Burk, D. H., Liu, B., Zhong, R., Morrison, W. H. and Ye, Z. H. (2001). A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. *Plant Cell* **13**, 807-827.

Buster, D., McNally, K. and McNally, F. J. (2002). Katanin inhibition prevents the redistribution of gamma-tubulin at mitosis. *J. Cell Sci.* **115**, 1083-1092.

Buster, D. W., Zhang, D. and Sharp, D. J. (2007). Poleward tubulin flux in spindles: regulation and function in mitotic cells. *Mol. Biol. Cell* **18**, 3094-3104.

Butler, R., Wood, J. D., Landers, J. A. and Cunliffe, V. T. (2010). Genetic and chemical modulation of spastin-dependent axon outgrowth in zebrafish embryos indicates a role for impaired microtubule dynamics in hereditary spastic paraplegia. *Dis. Model. Mech.* **3**, 743-751.

Chan, J., Calder, G. M., Doonan, J. H. and Lloyd, C. W. (2003). EB1 reveals mobile microtubule nucleation sites in Arabidopsis. *Nat. Cell Biol.* **5**, 967-971.

Clandinin, T. R. and Mains, P. E. (1993). Genetic studies of mei-1 gene activity during the transition from meiosis to mitosis in Caenorhabditis elegans. *Genetics* **134**, 199-210.

Clark-Maguire, S. and Mains, P. E. (1994a). Localization of the mei-1 gene product of Caenorhaditis elegans, a meiotic-specific spindle component. *J. Cell Biol.* **126**, 199-209.

Clark-Maguire, S. and Mains, P. E. (1994b). mei-1, a gene required for meiotic spindle formation in Caenorhabditis elegans, is a member of a family of ATPases. *Genetics* **136**, 533-546.

Cox, G. A., Mahaffey, C. L., Nystuen, A., Letts, V. A. and Frankel, W. N. (2000). The mouse fidgetin gene defines a new role for AAA family proteins in mammalian development. *Nat. Genet.* **26**, 198-202.

Davis, L. J., Odde, D. J., Block, S. M. and Gross, S. P. (2002). The importance of lattice defects in katanin-mediated microtubule severing in vitro. *Biophys. J.* **82**, 2916-2927.

Díaz-Valencia, J. D., Morelli, M. M., Bailey, M., Zhang, D., Sharp, D. J. and Ross, J. L. (2011). Drosophila katanin-60 depolymerizes and severs at microtubule defects. *Biophys. J.* **100**, 2440-2449.

Dymek, E. E., Lefebvre, P. A. and Smith, E. F. (2004). PF15p is the chlamydomonas homologue of the Katanin p80 subunit and is required for assembly of flagellar central microtubules. *Eukaryot. Cell* **3**, 870-879.

Errico, A., Claudiani, P., D'Addio, M. and Rugarli, E. I. (2004). Spastin interacts with the centrosomal protein NA14, and is enriched in the spindle pole, the midbody and the distal axon. *Hum. Mol. Genet.* **13**, 2121-2132.

Frickey, T. and Lupas, A. N. (2004). Phylogenetic analysis of AAA proteins. *J. Struct. Biol.* **146**, 2-10.

Goodwin, S. S. and Vale, R. D. (2010). Patronin regulates the microtubule network by protecting microtubule minus ends. *Cell* **143**, 263-274.

Gouveia, S. M. and Akhmanova, A. (2010). Cell and molecular biology of microtubule plus end tracking proteins: end binding proteins and their partners. *Int. Rev. Cell. Mol. Biol.* **285**, 1-74.

Grüneberg, H. (1943). Two new mutant genes in the house mouse. *J. Genet.* **45**, 22-28.

Guizetti, J., Schermelleh, L., Mäntler, J., Maar, S., Poser, I., Leonhardt, H., Müller-Reichert, T. and Gerlich, D. W. (2011). Cortical constriction during abscission involves helices of ESCRT-III-dependent filaments. *Science* **331**, 1616-1620.

Hartman, J. J. and Vale, R. D. (1999). Microtubule disassembly by ATP-dependent oligomerization of the AAA enzyme katanin. *Science* **286**, 782-785.

Hartman, J. J., Mahr, J., McNally, K., Okawa, K., Iwamatsu, A., Thomas, S., Cheesman, S., Heuser, J., Vale, R. D. and McNally, F. J. (1998). Katanin, a microtubule-severing protein, is a novel AAA ATPase that targets to the centrosome using a WD40-containing subunit. *Cell* **93**, 277-287.

Hazan, J., Fonknechten, N., Mavel, D., Paternotte, C., Samson, D., Artiguenave, F., Davoine, C. S., Cruaud, C., Dürr, A., Wincker, P. et al. (1999). Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. *Nat. Genet.* **23**, 296-303.

Jinushi-Nakao, S., Arvind, R., Amikura, R., Kinameri, E., Liu, A. W. and Moore, A. W. (2007). Knot/Collier and cut control different aspects of dendrite cytoskeleton and synergize to define final arbor shape. *Neuron* **56**, 963-978.

Karabay, A., Yu, W., Solowska, J. M., Baird, D. H. and Baas, P. W. (2004). Axonal growth is sensitive to the levels of katanin, a protein that severs microtubules. *J. Neurosci.* **24**, 5778-5788.

Lacroix, B., van Dijk, J., Gold, N. D., Guizetti, J., Aldrian-Herrada, G., Rogowski, K., Gerlich, D. W. and Janke, C. (2010). Tubulin polyglutamylation stimulates spastin-mediated microtubule severing. *J. Cell Biol.* **189**, 945-954.

Lee, H. H., Jan, L. Y. and Jan, Y. N. (2009). Drosophila IKK-related kinase Ik2 and Katanin p60-like 1 regulate dendrite pruning of sensory neuron during metamorphosis. *Proc. Natl. Acad. Sci. USA* **106**, 6363-6368.

Lohret, T. A., McNally, F. J. and Quarmby, L. M. (1998). A role for katanin-mediated axonemal severing during Chlamydomonas deflagellation. *Mol. Biol. Cell* **9**, 1195-1207.

Lu, C. and Mains, P. E. (2007). The C. elegans anaphase promoting complex and MBK-2/DYRK kinase act redundantly with CUL-3/MEL-26 ubiquitin ligase to degrade MEI-1 microtubule-severing activity after meiosis. *Dev. Biol.* **302**, 438-447.

Lupas, A. N. and Martin, J. (2002). AAA proteins. *Curr. Opin. Struct. Biol.* **12**, 746-753.

Mains, P. E., Kemphues, K. J., Sprunger, S. A., Sulston, I. A. and Wood, W. B. (1990). Mutations affecting the meiotic and mitotic divisions of the early Caenorhabditis elegans embryo. *Genetics* **126**, 593-605.

McNally, F. J. and Vale, R. D. (1993). Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell* **75**, 419-429.

McNally, K. P. and McNally, F. J. (2011). The spindle assembly function of Caenorhabditis elegans katanin does not require microtubule-severing activity. *Mol. Biol. Cell* **22**, 1550-1560.

McNally, K., Audhya, A., Oegema, K. and McNally, F. J. (2006). Katanin controls mitotic and meiotic spindle length. *J. Cell Biol.* **175**, 881-891.

McNally, K. P., Buster, D. and McNally, F. J. (2002). Katanin-mediated microtubule severing can be regulated by multiple mechanisms. *Cell Motil. Cytoskeleton* **53**, 337-349.

Mennella, V., Rogers, G. C., Rogers, S. L., Buster, D. W., Vale, R. D. and Sharp, D. J. (2005). Functionally distinct kinesin-13 family members cooperate to regulate microtubule dynamics during interphase. *Nat. Cell Biol.* **7**, 235-245.

Montenegro Gouveia, S., Leslie, K., Kapitein, L. C., Buey, R. M., Grigoriev, I., Wagenbach, M., Smal, I., Meijering, E., Hoogenraad, C. C., Wordeman, L. et al. (2010). In vitro reconstitution of the functional interplay between MCAK and EB3 at microtubule plus ends. *Curr. Biol.* **20**, 1717-1722.

Murata, T., Sonobe, S., Baskin, T. I., Hyodo, S., Hasezawa, S., Nagata, T., Horio, T. and Hasebe, M. (2005). Microtubule-dependent microtubule nucleation based on recruitment of gamma-tubulin in higher plants. *Nat. Cell Biol.* **7**, 961-968.

Nakamura, M., Ehrhardt, D. W. and Hashimoto, T. (2010). Microtubule and katanin-dependent dynamics of microtubule nucleation complexes in the acentrosomal Arabidopsis cortical array. *Nat. Cell Biol.* **12**, 1064-1070.

Neuwald, A. F., Aravind, L., Spouge, J. L. and Koonin, E. V. (1999). AAA+: A class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes. *Genome Res.* **9**, 27-43.

Qiang, L., Yu, W., Andreadis, A., Luo, M. and Baas, P. W. (2006). Tau protects microtubules in the axon from severing by katanin. *J. Neurosci.* **26**, 3120-3129.

Qiang, L., Yu, W., Liu, M., Solowska, J. M. and Baas, P. W. (2010). Basic fibroblast growth factor elicits formation of interstitial axonal branches via enhanced severing of microtubules. *Mol. Biol. Cell* **21**, 334-344.

Rasi, M. Q., Parker, J. D., Feldman, J. L., Marshall, W. F. and Quarmby, L. M. (2009). Katanin knockdown supports a role for microtubule severing in release of basal bodies before mitosis in Chlamydomonas. *Mol. Biol. Cell* **20**, 379-388.

Rath, U. and Sharp, D. J. (2011). The molecular basis of anaphase A in animal cells. *Chromosome Res.* **19**, 423-432.

Rath, U., Rogers, G. C., Tan, D., Gomez-Ferreria, M. A., Buster, D. W., Sosa, H. J. and Sharp, D. J. (2009). The Drosophila kinesin-13, KLP59D, impacts Pacman- and Flux-based chromosome movement. *Mol. Biol. Cell* **20**, 4696-4705.

Rogers, G. C., Rogers, S. L. and Sharp, D. J. (2005). Spindle microtubules in flux. *J. Cell Sci.* **118**, 1105-1116.

Roll-Mecak, A. and Vale, R. D. (2005). The Drosophila homologue of the hereditary spastic paraplegia protein, spastin, severs and disassembles microtubules. *Curr. Biol.* **15**, 650-655.

Roll-Mecak, A. and Vale, R. D. (2006). Making more microtubules by severing: a common theme of noncentrosomal microtubule arrays? *J. Cell Biol.* **175**, 849-851.

Roll-Mecak, A. and Vale, R. D. (2008). Structural basis of microtubule severing by the hereditary spastic paraplegia protein spastin. *Nature* **451**, 363-367.

Roll-Mecak, A. and McNally, F. J. (2010). Microtubule-severing enzymes. *Curr. Opin. Cell Biol.* **22**, 96-103.

Sanderson, C. M., Connell, J. W., Edwards, T. L., Bright, N. A., Duley, S., Thompson, A., Luzio, J. P. and Reid, E. (2006). Spastin and atlastin, two proteins mutated in autosomal-dominant hereditary spastic paraplegia, are binding partners. *Hum. Mol. Genet.* **15**, 307-318.

Schiel, J. A. and Prekeris, R. (2010). Making the final cut - mechanisms mediating the abscission step of cytokinesis. *Scientific World Journal* **10**, 1424-1434.

Sharma, N., Bryant, J., Wloga, D., Donaldson, R., Davis, R. C., Jerka-Dziadosz, M. and Gaertig, J. (2007). Katanin regulates dynamics of microtubules and biogenesis of motile cilia. *J. Cell Biol.* **178**, 1065-1079.

Shaw, S. L., Kamyar, R. and Ehrhardt, D. W. (2003). Sustained microtubule treadmilling in Arabidopsis cortical arrays. *Science* **300**, 1715-1718.

Sherwood, N. T., Sun, Q., Xue, M., Zhang, B. and Zinn, K. (2004). Drosophila spastin regulates synaptic microtubule networks and is required for normal motor function. *PLoS Biol.* **2**, e429.

Solowska, J. M., Morfini, G., Falnikar, A., Himes, B. T., Brady, S. T., Huang, D. and Baas, P. W. (2008). Quantitative and functional analyses of spastin in the nervous system: implications for hereditary spastic paraplegia. *J. Neurosci.* **28**, 2147-2157.

Sonbuchner, T. M., Rath, U. and Sharp, D. J. (2010). KL1 is a novel microtubule severing enzyme that regulates mitotic spindle architecture. *Cell Cycle* **9**, 2403-2411.

Srayko, M., Buster, D. W., Bazirgan, O. A., McNally, F. J. and Mains, P. E. (2000). MEI-1/MEI-2 katanin-like microtubule severing activity is required for Caenorhabditis elegans meiosis. *Genes Dev.* **14**, 1072-1084.

Srayko, M., O'Toole, E. T., Hyman, A. A. and Muller-Reichert, T. (2006). Katanin disrupts the microtubule lattice and increases polymer number in C. elegans meiosis. *Curr. Biol.* **16**, 1944-1949.

Stoppin-Mellet, V., Gaillard, J. and Vantard, M. (2006). Katanin's severing activity favors bundling of cortical microtubules in plants. *Plant J.* **46**, 1009-1017.

Sudo, H. and Maru, Y. (2007). LAPSER1 is a putative cytokinetic tumor suppressor that shows the same centrosome and midbody subcellular localization pattern as p80 katanin. *FASEB J.* **21**, 2086-2100.

Sudo, H. and Maru, Y. (2008). LAPSER1/LZTS2: a pluripotent tumor suppressor linked to the inhibition of katanin-mediated microtubule severing. *Hum. Mol. Genet.* **17**, 2524-2540.

Sudo, H. and Baas, P. W. (2010). Acetylation of microtubules influences their sensitivity to severing by katanin in neurons and fibroblasts. *J. Neurosci.* **30**, 7215-7226.

Sudo, H. and Baas, P. W. (2011). Strategies for diminishing katanin-based loss of microtubules in tauopathic neurodegenerative diseases. *Hum. Mol. Genet.* **20**, 763-778.

Toyo-Oka, K., Sasaki, S., Yano, Y., Mori, D., Kobayashi, T., Toyoshima, Y. Y., Tokuoka, S. M., Ishii, S., Shimizu, T., Muramatsu, M. et al. (2005). Recruitment of katanin p60 by phosphorylated NDEL1, an LIS1 interacting protein, is essential for mitotic cell division and neuronal migration. *Hum. Mol. Genet.* **14**, 3113-3128.

Trotta, N., Orso, G., Rossetto, M. G., Daga, A. and Broadie, K. (2004). The hereditary spastic paraplegia gene, spastin, regulates microtubule stability to modulate synaptic structure and function. *Curr. Biol.* **14**, 1135-1147.

Truslove, G. M. (1956). The anatomy and development of the fidget mouse. *J. Genet.* **54**, 64-86.

Vale, R. D. (1991). Severing of stable microtubules by a mitotically activated protein in Xenopus egg extracts. *Cell* **64**, 827-839.

Vale, R. D. (2000). AAA proteins. Lords of the ring. *J. Cell Biol.* **150**, F13-F19.

Verhey, K. J. and Gaertig, J. (2007). The tubulin code. *Cell Cycle* **6**, 2152-2160.

Watanabe, T., Noritake, J. and Kaibuchi, K. (2005). Regulation of microtubules in cell migration. *Trends Cell Biol.* **15**, 76-83.

Waterman-Storer, C. M., Worthylake, R. A., Liu, B. P., Burridge, K. and Salmon, E. D. (1999). Microtubule growth activates Rac1 to promote lamellipodial protrusion in fibroblasts. *Nat. Cell Biol.* **1**, 45-50.

Wharton, S. B., McDermott, C. J., Grierson, A. J., Wood, J. D., Gelsthorpe, C., Ince, P. G. and Shaw, P. J. (2003). The cellular and molecular pathology of the motor system in hereditary spastic paraparesis due to mutation of the spastin gene. *J. Neuropathol. Exp. Neurol.* **62**, 1166-1177.

White, S. R., Evans, K. J., Lary, J., Cole, J. L. and Lauring, B. (2007). Recognition of C-terminal amino acids in tubulin by pore loops in Spastin is important for microtubule severing. *J. Cell Biol.* **176**, 995-1005.

Yang, Y., Mahaffey, C. L., Bérubé, N., Nystuen, A. and Frankel, W. N. (2005). Functional characterization of fidgetin, an AAA-family protein mutated in fidget mice. *Exp. Cell Res.* **304**, 50-58.

Yang, Y., Mahaffey, C. L., Bérubé, N. and Frankel, W. N. (2006). Interaction between fidgetin and protein kinase A-anchoring protein AKAP95 is critical for palatogenesis in the mouse. *J. Biol. Chem.* **281**, 22352-22359.

Ye, X., Lee, Y. C., Choueiri, M., Chu, K., Huang, C. F., Tsai, W. W., Kobayashi, R., Logothetis, C. J., Yu-Lee, L. Y. and Lin, S. H. (2012). Aberrant expression of katanin p60 in prostate cancer bone metastasis. *Prostate* **72**, 291-300.

Yoshimori, T., Yamagata, F., Yamamoto, A., Mizushima, N., Kabeya, Y., Nara, A., Miwako, I., Ohashi, M., Ohsumi, M. and Ohsumi, Y. (2000). The mouse SKD1, a homologue of yeast Vps4p, is required for normal endosomal trafficking and morphology in mammalian cells. *Mol. Biol. Cell* **11**, 747-763.

Yu, W., Ahmad, F. J. and Baas, P. W. (1994). Microtubule fragmentation and partitioning in the axon during collateral branch formation. *J. Neurosci.* **14**, 5872-5884.

Yu, W., Solowska, J. M., Qiang, L., Karabay, A., Baird, D. and Baas, P. W. (2005). Regulation of microtubule severing by katanin subunits during neuronal development. *J. Neurosci.* **25**, 5573-5583.

Yu, W., Qiang, L., Solowska, J. M., Karabay, A., Korulu, S. and Baas, P. W. (2008). The microtubule-severing proteins spastin and katanin participate differently in the formation of axonal branches. *Mol. Biol. Cell* **19**, 1485-1498.

Zhang, D., Rogers, G. C., Buster, D. W. and Sharp, D. J. (2007). Three microtubule severing enzymes contribute to the "Pacman-flux" machinery that moves chromosomes. *J. Cell Biol.* **177**, 231-242.

Zhang, D., Grode, K. D., Stewman, S. F., Diaz-Valencia, J. D., Liebling, E., Rath, U., Riera, T., Currie, J. D., Buster, D. W., Asenjo, A. B. et al. (2011). Drosophila katanin is a microtubule depolymerase that regulates cortical-microtubule plus-end interactions and cell migration. *Nat. Cell Biol.* **13**, 361-370.