



Published in final edited form as:

Trends Cell Biol. 2021 January ; 31(1): 50–61. doi:10.1016/j.tcb.2020.10.004.

Cutting, Amplifying, and Aligning Microtubules with Severing Enzymes

Yin-Wei Kuo^{1,2}, Jonathon Howard^{2,3,*}

¹Department of Chemistry, Yale University, New Haven, CT 06511, USA

²Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511, USA

³<https://howardlab.yale.edu>

Abstract

Microtubule-severing enzymes – katanin, spastin, fidgetin – are related AAA-ATPases that cut microtubules into shorter filaments. These proteins, also called severases, are involved in a wide range of cellular processes including cell division, neuronal development, and tissue morphogenesis. Paradoxically, severases can amplify the microtubule cytoskeleton and not just destroy it. Recent work on spastin and katanin has partially resolved this paradox by showing that these enzymes are strong promoters of microtubule growth. Here, we review recent structural and biophysical advances in understanding the molecular mechanisms of severing and growth promotion that provide insight into how severing enzymes shape microtubule networks.

Severing Enzymes Are Multifunctional Microtubule Regulators

The structure of the microtubule cytoskeleton is defined by the location, number, length, and orientation of the constituent microtubules. Microtubule organization differs between different cell types, different locations within one cell (e.g., mitotic spindle, membrane cortex, cilium), and different times (e.g., phase of the cell cycle, in response to external signals). The microtubule cytoskeleton is shaped by microtubule-associated proteins (MAPs), a diverse collection of proteins that regulate all aspects of microtubule growth and shrinkage [1-3]. They accelerate or decelerate microtubule polymerization and depolymerization, alter the rates of the transitions between growing and shrinking states known respectively as **catastrophe** (see Glossary) and **rescue** [4, 5], and nucleate new microtubules [6, 7].

Among this wide variety of microtubule regulators are the severing enzymes – **spastin**, **katanin**, and **fidgetin** – that cut microtubules into smaller fragments (Box 1). **Severases** play key roles in many cellular processes: mitosis and meiosis [8,9], ciliogenesis [10], neurodevelopment [11-13], cell migration [14], and cell wall biosynthesis [15] and **phototropism** [16] in plants [17, 18]. These diverse roles, which involve both assembly and

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

*Correspondence: joe.howard@yale.edu (J. Howard).

disassembly of the cytoskeleton, hint at severases having broader activities than just severing microtubules.

It has recently been discovered that severases are themselves powerful promoters of microtubule growth (Box 2) [19,20]. Purified spastin and katanin amplify the number and total mass of microtubules in buffers containing tubulin at cellular concentrations (see Figure IA in Box 2). This accounts for the decrease in microtubule numbers when severases are disrupted (see Figure IB in Box 2) [19,20]. Severases promote microtubule amplification by increasing the rate of conversion of shrinking microtubules to growing ones (called rescue) so that the microtubule fragments created by severing act as seeds for the growth of new microtubules. These new findings, together with earlier work showing that katanin and fidgetin have microtubule depolymerization and catastrophe activities [14,21], demonstrate that severases are multifunctional enzymes that both fragment microtubules and regulate their growth dynamics.

Concurrent with these discoveries, several recent cryoelectron microscopy structures have provided new insights into how severases cut microtubules. In this review, we discuss how these new results on the molecular mechanisms of severing and growth promotion can account for many of the diverse cellular phenotypes of severases.

Molecular Mechanisms of Severing and Growth Promotion

Katanin, spastin, and fidgetin belong to the meiotic clade of **AAA-ATPases** (Figure 1A) based on phylogenetic classification [22]. Purified katanin, spastin, and fidgetin all have microtubule-severing activity [18]. Vps4, the fourth member of the family, has no microtubule-severing activity but instead disassembles the ESCRT-III filaments during membrane fission [23,24]. The archaeal origin of the ESCRTIII–Vps4 system shows that the severases have evolved from an ancient family of cytoskeletal regulators [25,26]. All members of the meiotic clade have a conserved three-helix bundle that forms the microtubule interacting and trafficking domain, which is followed by a linker region that connects to the AAA domain (Figure 1B). The AAA-ATPases are the founding family of the **ATPases associated with diverse cellular activities (AAA+) protein** superfamily of ATP-powered molecular machines that include cytoskeletal motor proteins (dynein), DNA unwinders (helicases), protein complex disassemblers (e.g., NSF), and protein **unfoldases** (e.g., ClpX, Hsp104) [27]. Severases, like other AAA proteins, contain six AAA domains that form ring- or spiral-like structures (Figure 1C,D).

The prevailing model for how severases cut microtubules involves several steps shown in Figure 2.

Hexamer Assembly on the Microtubule Lattice

Katanin and spastin have low oligomerization tendencies in the absence of microtubules and the predominant forms in solution are most likely to be monomeric [28-30]. While spastin, katanin, and fidgetin hexamers have been observed in solution, the proteins are either ATPase defective or truncated [30-32] or bound to non-hydrolyzable ATP analogs at high-micromolar protein concentrations [33,34]. However, oligomers are readily seen by single-

molecule fluorescence and Forster resonance energy transfer on the microtubule lattice [28,35,36]. Thus, both microtubules and ATP greatly enhance the oligomerization. Mutations perturbing the subunit interaction inter-face reduce the severing activity of spastin [32,37], supporting oligomers as the active species.

C-Terminal Tail (CTT) Binding and Pulling

Recent cryoelectron microscopy structures show spastin [33,37] and katanin [31] hexamers bound to **CTT**-mimetic peptides in their central pores. While there is no direct evidence that severases generate force on the CTT to initiate tubulin unfolding, several lines of evidence show that CTTs are essential for severing. Removal of CTTs by the protease subtilisin inhibits severing by katanin [38,39] and spastin [30,40], as does the mutation of CTT-interacting residues in the severase central pore [30,37].

Tubulin Extraction by Unfolding

The current hypothesis proposes that severing enzymes function like AAA+ unfoldases such as Vps4, ClpX, and Hsp104 [27]: these hexameric rings are ATP-powered machines that thread the substrate polypeptide chains through their central pores to unfold or disaggregate the target proteins. Threading is thought to occur via a hand-over-hand mechanism [27]. Such a mechanism for severases is supported by recent structures of spastin and katanin bound with peptides in their pores. Katanin and spastin form open spirals when the subunits are mutated to trap them in an ATP-like conformation [31,37], while a spastin structure with **ADP-beryllium fluoride (ADP-BeF₃)** shows a 'broken spiral' in which the sixth AAA (magenta in Figure 1D) is in the **apo state** and has moved up to form a closed structure (Figure 1C,D) [33].

These structures fit a hand-over-hand mechanism in which ATP binding by the apo subunit (Figure 1D, magenta) moves it into a spiral-forming association with the adjacent ATP-bound subunit (green); this creates a steric clash with the microtubule surface leading to the extraction of an additional two amino acids from the tubulin peptide. Meanwhile, the yellow subunit is forced away from the microtubule surface, which triggers the hydrolysis of its ATP, while the cyan subunit unbinds ADP and moves upwards to break the spiral. This motion, where the AAA domains move up and down perpendicular to the surface of the microtubule, is analogous to the motion of the pistons in an axial engine (https://en.wikipedia.org/wiki/Axial_engine). For the spiral-forming subunits to lay flat on the microtubule surface, they must tilt by about 3° (the pitch of the spiral is 12 aa ≈ 2 nm and the diameter of the AAA ring is ≈ 12 nm); the ring is therefore predicted to wobble as the tubulin peptide chain is pulled through the pore during the ATPase cycle ([41], see movie for Vps4).

Creation of Defects

Fluorescence studies show that spastin and katanin catalyze the exchange of tubulin dimers from solution into the lattice [19] and negative-stain electron microscopy of stable microtubules *in vitro* [19] and EM tomography *in vivo* [42] have visualized severing-mediated defects in which subunits are missing from the lattice. The microtubule is thought

to break when enough tubulin subunits have been removed, although it remains unknown how many tubulin dimers need to be removed to induce this breakage.

Molecular Mechanism of Microtubule Rescue

The molecular mechanism of severase-dependent regrowth promotion is controversial. Three models have been proposed (see Figure IC in Box 2). In one model, spastin and katanin are thought to promote rescue through the exchange of GTP-tubulin from solution into sites on the microtubule in which GDP-tubulin has been extracted but where severing has not completed [19]. It is then hypothesized that the newly incorporated tubulin remains non-hydrolyzed and induces rescue according to the **GTP-island** hypothesis for rescue [43,44]. While it has been shown using fluorescent tubulin that spastin and katanin facilitate such lattice exchange of tubulin [19], whether the tubulin is hydrolyzed is unknown. Using either microtubule end-binding proteins (EBs) or a recombinant antibody raised against GTP-analog-loaded tubulin as markers of GTP-tubulin, it has been inferred that these lattice exchange sites contain GTP-tubulin [19,43,45]. However, recent evidence suggests that both methods preferentially recognize lattice defects in addition to GTP-tubulin [46,47]. Thus, the GTP-island hypothesis is not well established since the exact nucleotide states of tubulin subunits at these lattice exchange sites remain unclear.

An alternative model is that severases accumulate on the tips of shrinking microtubules where they slow shrinkage and promote rescue [20]. A key observation is that ATP hydrolysis, which is necessary for tubulin exchange into the lattice, is not necessary for rescue promotion. Therefore, the GTP-island mechanism is not necessary, although it could augment the ATP-independent pathway [20]. A third model posits that rescue is due to the binding of MAPs to the shrinking ends [12]. Additional mechanistic studies are needed to test between these (or other) models and to ascertain their relative contributions in cells.

Molecular Understanding of Cellular Phenotypes of Severases

The structural, biochemical, and biophysical studies of severing enzymes provide new insight into the outcomes of microtubule severing at the ensemble level. These outcomes can be classified into four groups: (i) disassembly of the microtubules (Box 1); (ii) amplification of microtubules (Box 2); (iii) control of microtubule length (Figure 3); and (iv) the spatial organization of microtubule arrays (Figure 4). Recent experimental and theoretical work indicates that these seemingly distinctive phenotypes can be largely accounted for by the combination of the rate and spatial distribution of severing, the dynamic properties of the microtubules (which depend on the tubulin concentration and MAPs), and the fate of the newly severed microtubule tips [20,48-51].

To Assemble or to Disassemble?

In vitro experiments and computer simulations have shown that there is a threshold of tubulin concentration that determines whether severing leads to the assembly or disassembly of dynamic microtubules [20,50,51]. If tubulin is above the **critical concentration** for net assembly [52] (termed the **unbounded microtubule growth** regime in the Dogterom and Leibler model of **dynamic instability** [53]), the shrinking plus ends of microtubules

generated by severing have a high chance of regrowth, leading to an increase in the total microtubule number and mass (see Figure IC in Box 2) [20,50,51]. In this way, spastin and katanin, which are strong rescue factors, lead to microtubule assembly at physiological tubulin concentrations [19,20]. Assembly will also be promoted by other rescue factors such as CLASPs [49] or microtubule stabilizers such as tau [54], as proposed in [12, 13]. This cut-and-regrowth amplification mechanism can function even in the absence of stabilized microtubule seeds or templates, which is suitable for the expansion of acentrosomal microtubule networks in neurons where the microtubule organizing centers are distant or absent [12, 13].

Conversely, if tubulin is below the critical concentration (the **bounded microtubule growth** regime), severing will rapidly disassemble the microtubules from the plus ends [50] (see Figure IC in Box 1). Therefore, at low tubulin concentrations or in the presence of destabilizing MAPs, severing is expected to cause disassembly [50,51]. Examples of destabilizing MAPs are depolymerizing kinesin 8 and 13, which increase the rate of microtubule catastrophe [55] and thereby raise the critical concentration. Fidgetin and katanin (but not spastin) have also been reported to have depolymerizing activities [14,21] and so are expected to promote disassembly as well. Thus, depending on the dynamic state of the tubulin, severing will lead to plus-end growth or shrinkage and the corresponding assembly or disassembly of microtubule networks [20,50,51].

The properties of newly created minus ends are also expected to influence microtubule amplification. Unlike plus ends, new minus ends are usually stable and regrow in the presence of tubulin [20], but can shrink in the presence of depolymerases [56]. Minus-end stabilizers such as CAMSAPs and SPR2 can protect the newly severed minus ends from depolymerization and therefore further increase microtubule assembly [56,57].

Targeting of severases to microtubule ends can also promote disassembly. Katanin can target minus ends specifically through interaction with the minus-end-binding proteins ASPM and CAMSAPs [57,58] and plus ends by localizing at the kinetochore [9]. Preferential end severing is expected to decrease the **GTP-cap** size and lead to disassembly, like the depolymerizing kinesin 13 [55]. Thus, whether severases disassemble or assemble the cytoskeleton depends on the dynamic state of the microtubules, the presence of other MAPs, and the location of severing.

Tubulin Degradation and Homeostasis

Early experiments showed that the majority of the tubulin subunits released on severing can repolymerize into microtubules [39], suggesting either that severases only partially unfold tubulin [39] or that the unfolding of only a small fraction of the tubulins in a microtubule is sufficient to disassemble it. If unfolding leads to degradation, severing may contribute to tubulin protein turn-over [59]. It is also possible that the lattice exchange activity of spastin and katanin serves as part of a tubulin quality control system to remove dysfunctional tubulin from the lattice [19]. Thus, severases may play a role in the homeostasis of tubulin and microtubules.

Microtubule Length Control by Severing Enzymes

Severases regulate microtubule lengths in cells (e.g., mitotic spindles; Figure 3A). If severing occurs uniformly over the lattice (as found for purified spastin [50]), the longer the microtubule, the more likely that it will be cut. Therefore, severing constitutes a form of negative feedback on microtubule length. Experimental and theoretical studies show that severases tighten the distribution of microtubule lengths, in addition to decreasing their mean length (Figure 3B) [50,60]. This is reminiscent of length control by the length-dependent depolymerase kinesin 8 [55,61,62]. The mean length is predicted to decrease with higher severing rates and increase with faster microtubule growth rates [50]. By contrast, microtubule number is controlled by the rescue frequency. This suggests that microtubule length and number can be independently regulated. The models predict that the phenotypes of severing enzymes are sensitive to protein expression levels, as observed in neurons [63,64] and meiotic spindles [65-67].

If severing enzymes cut at microtubule ends, they are likely to increase catastrophe, similar to the depolymerizing kinesin 13 [55], and decrease mean length. Thus, severases provide multiple mechanisms to regulate microtubule length.

Spatial Organization of Microtubule Arrays

Severases play key roles in aligning and reorienting the microtubule cytoskeleton. This is most extensively studied in plants where the cortical microtubules are frequently aligned circumferentially, perpendicular to the cellular axis (Figure 4A). In this orientation, the microtubule array promotes cell wall synthesis that elongates the cell [15]. The alignment of microtubules is expected to be self-organized because microtubule organizing centers are absent. The key principle for maintaining an aligned microtubule array is that the discordant (crossing) microtubules have shorter lifetimes and are less likely to survive than the aligned ones (Figure 4C). In *Arabidopsis* epidermal cells, this is achieved by selective severing of the crossing microtubules [68] by katanin [69]. Computational modeling shows that this mechanism can indeed align microtubules [48]. By contrast, the microtubules in the irregularly shaped *Arabidopsis* pavement cells are more disordered and isotropic: this is because the katanin-dependent selective severing is inhibited by augmin and SPR2 so that the crossovers are stabilized [70,71]. The different microtubule orientations in these two cell types highlight the importance of katanin severing in array alignment. The pruning effect of katanin has also been observed in *Caenorhabditis elegans* oocytes, where katanin is proposed to eliminate discordant microtubules to maintain the parallel/antiparallel spindle structure [67]. Thus, selective severing may be a general mechanism to align microtubules.

During the phototropic response to blue-light illumination, microtubules in *Arabidopsis* epidermal cells reorient from circumferential to axial (Figure 4A, WT 30 min). This halts axial growth on the illuminated side of the stem but not on the unilluminated side, leading to differential growth that bends the stem towards the light [16]. The reorientation is associated with katanin-dependent amplification of the discordant microtubules (Figure 4B). The key differences between array maintenance and reorientation are most likely to originate from the different microtubule dynamic regimes, with stabilizing MAPs such as CLASP biasing growth into the unbounded regime [49]. Stabilization of the newly severed plus and minus

ends can also promote the creation of unaligned microtubules through severing, thereby facilitating cortical reorientation and new array formation [49,56]. Furthermore, minus-end stabilization by SPR2 can prolong the lifetime of microtubule crossovers by preventing minus-end depolymerization, which consequently increases the probability of severing at the microtubule crossovers and promotes the reorientation of microtubule arrays in hypocotyl epidermal cells [56,72]. Thus, the interplay between severing and dynamics regulation can be tuned by MAPs to produce different morphologies.

It is tempting to speculate that similar array reorientation and organization strategies apply to acentrosomal microtubules in neuronal branching morphogenesis, where severases are involved in both the formation [54,73,74] and the destabilization [75,76] of branches. Thus, it is of interest to examine whether severases also sever preferentially at microtubule crossovers in neurons and compare the dynamics of microtubules when neurons branch or debranch.

How does katanin target the crossover sites? Katanin has been proposed to preferentially sever at the irregularities of the lattice and can directly target microtubule crossings by sensing lattice defects or damage caused by local mechanical stress [35,77]. Lattice defects can also locally enrich tubulin post-translational modifications like acetylation [78], which may enhance katanin localization and severing activity [79]. Recently, live-cell imaging and genetic analysis in plants have shown that the p80 regulatory subunit of katanin is the main determining factor for the crossover localization of katanin [80]. *C. elegans* katanin (the p60/p80 complex) also preferentially severs at microtubule intersections *in vitro* [67]. How does the p80 subunit localize katanin to crossovers? One possibility is that p80 facilitates the formation of hexamers, which sense microtubule crossovers by direct interaction with the two adjacent microtubules through multiple microtubule-binding domains. Alternatively, the p80 subunit may detect lattice irregularities directly or through association with other defect-sensing MAPs such as Clip170 and EB1 [46,47] inside the cells. Crossover- or lattice-defect-sensing activity has not been reported for spastin and fidgetin thus far. Further experiments are needed to understand how the severing sites of each severing enzyme are controlled spatially in the physiological environment.

Concluding Remarks and Future Perspectives

Extensive studies of microtubule-severing enzymes over the past decades have revealed versatile cellular functions. In this review, we have argued that severing under different microtubule dynamics regimes can result in drastically different outcomes based on a thresholding mechanism. The interplay between severing and microtubule dynamics constitutes an adaptable microtubule remodeling strategy, controlling the microtubule number, length, and array organization to serve the needs of development and homeostasis. This molecular framework stresses the importance of microtubule dynamics in interpreting the effects of severing enzymes. Elucidating the interplay between severing and dynamics *in vivo* will require improvement of the current imaging techniques to resolve single microtubule tips in the crowded cellular environment and the development of quantitative modeling to understand the combinatorial effects of severing and other microtubule regulators. Understanding the etiology of neuronal disorders caused by mutations in severing

enzymes, including **hereditary spastic paraplegia** and microcephaly [81-83], will require new molecular techniques to selectively interfere with the two activities of severases: microtubule cutting and the regulation of dynamics. Many questions remain on the molecular mechanisms underlying these activities and their interplay in cells (see Outstanding Questions).

Acknowledgments

We thank Dr Anna Luchniak, Dr Ivy Huang, Dr Maijia Liao, and Rafael C.A. Tavares for discussions and comments on the manuscript. This work was supported by NIH grants R01 GM110386, DP1 MH110065, and R01 NS118884 (to J.H.) and a fellowship from the Ministry of Education in Taiwan (to Y-W.K.).

Glossary

AAA-ATPase

a subfamily of the AAA+ proteins.

ADP-beryllium fluoride (ADP-BeF_x)

typically used as an ATP analog.

Apo state

the state where the cofactor of an enzyme is absent from the corresponding binding site. For the severase subunit, it refers to the conformational state where no adenosine nucleotide is bound.

ATPases associated with diverse cellular activities (AAA+) proteins

a large superfamily of molecular machines fueled by the hydrolysis of ATP. AAA+ proteins are found in bacteria, archaea, and eukaryotes.

Bounded/unbounded microtubule growth

the two dynamic regimes that originate from the Dogterom and Leibler model of dynamic instability (without severing). The average tubulin flux onto the polymer serves as the threshold between these two regimes. In the bounded growth regime, the average tubulin flux is negative and the microtubule population has an exponential length distribution with a finite mean length. In the unbounded growth regime, the average tubulin flux is positive and the microtubules continuously elongate, leading to infinite length in the absence of breakage or severing. The regime depends on the tubulin concentration and the activities of MAPs.

Catastrophe

the conversion of a growing microtubule into a shrinking one.

Critical concentration

the concentration of tubulin below which microtubules are in the bounded-growth regime and above which microtubules are in the unbounded-growth regime.

C-terminal tails (CTTs)

the highly negatively charged C termini of α - and β -tubulin are also known as 'E-hooks' due to the high density of glutamate residues. CTTs are hotspots for tubulin post-translational modifications.

Dynamic instability

the spontaneous, stochastic transitions of microtubules between growing and shrinking phases.

Fidgetin

the severing enzyme first discovered in mutant mice that display the fidget phenotype (frequent head shaking and circling).

GTP cap

layers of GTP or GDP-Pi tubulin at microtubule tips that are thought to prevent catastrophic disassembly of the microtubule from their ends.

GTP island

tubulin subunits in the microtubule lattice that contain GTP (or GDP-Pi), rather than GDP. GTP islands are thought to cause the regrowth of a depolymerizing microtubule (rescue), although this is not firmly established.

Hereditary spastic paraplegia

a neurodegenerative disease that is characterized by primary motor neuron degeneration and lower limb disorders such as weakness and spasticity.

Katanin

the severing enzyme named after the *katana* Japanese Samurai sword. Katanin comprises an AAA-ATPase p60 subunit with severing activity and a regulatory p80 subunit.

Phototropism

the growth of organisms such as plants towards a light stimulus.

Rescue

the conversion of a shrinking microtubule into a growing one.

Severase

an enzyme that has microtubule-severing activity.

Spastin

the severing enzyme named after hereditary spastic paraplegia. Mutations in the gene encoding spastin (SPAST) are the most common cause of HSP.

Unfoldases

enzymes that use the energy derived from the hydrolysis of ATP to unfold proteins.

References

1. Akhmanova A and Steinmetz MO (2015) Control of microtubule organization and dynamics: two ends in the limelight. *Nat. Rev. Mol. Cell Biol* 16, 711–726 [PubMed: 26562752]
2. Goodson HV and Jonasson EM (2018) Microtubules and microtubule-associated proteins. *Cold Spring Harb. Perspect. Biol* 10, a022608 [PubMed: 29858272]
3. Brouhard GJ and Rice LM (2018) Microtubule dynamics: an interplay of biochemistry and mechanics. *Nat. Rev. Mol. Cell Biol* 19, 451–463 [PubMed: 29674711]

4. Howard J and Hyman AA (2007) Microtubule polymerases and depolymerases. *Curr. Opin. Cell Biol* 19, 31–35 [PubMed: 17184986]
5. Bowne-Anderson H et al. (2015) Regulation of microtubule growth and catastrophe: unifying theory and experiment. *Trends Cell Biol.* 25, 769–779 [PubMed: 26616192]
6. Petry S and Vale RD (2015) Microtubule nucleation at the centrosome and beyond. *Nat. Cell Biol* 17, 1089–1093 [PubMed: 26316453]
7. Roostalu J and Surrey T (2017) Microtubule nucleation: beyond the template. *Nat. Rev. Mol. Cell Biol* 18, 702–710 [PubMed: 28831203]
8. Mains PE et al. (1990) Mutations affecting the meiotic and mitotic divisions of the early *Caenorhabditis elegans* embryo. *Genetics* 126, 593–605 [PubMed: 2249759]
9. Zhang D et al. (2007) Three microtubule severing enzymes contribute to the “Pacman-flux” machinery that moves chromosomes. *J. Cell Biol* 177, 231–242 [PubMed: 17452528]
10. Lohret TA et al. (1998) A role for katanin-mediated axonemal severing during *Chlamydomonas* deflagellation. *Mol. Biol. Cell* 9, 1195–1207 [PubMed: 9571249]
11. Ahmad FJ et al. (1999) An essential role for katanin in severing microtubules in the neuron. *J. Cell Biol* 145, 305–315 [PubMed: 10209026]
12. Kapitein LC and Hoogenraad CC (2015) Building the neuronal microtubule cytoskeleton. *Neuron* 87, 492–506 [PubMed: 26247859]
13. Baas PW et al. (2016) Stability properties of neuronal microtubules. *Cytoskeleton* 73, 442–460 [PubMed: 26887570]
14. Zhang D et al. (2011) *Drosophila* katanin is a microtubule depolymerase that regulates cortical–microtubule plus-end interactions and cell migration. *Nat. Cell Biol* 13, 361–369 [PubMed: 21378981]
15. Burk DH et al. (2001) A katanin-like protein regulates normal cell wall biosynthesis and cell elongation. *Plant Cell* 13, 807 [PubMed: 11283338]
16. Lindeboom JJ et al. (2013) A mechanism for reorientation of cortical microtubule arrays driven by microtubule severing. *Science* 342, 1245533 [PubMed: 24200811]
17. Sharp DJ and Ross JL (2012) Microtubule-severing enzymes at the cutting edge. *J. Cell Sci* 125, 2561–2569 [PubMed: 22595526]
18. McNally FJ and Roll-Mecak A (2018) Microtubule-severing enzymes: from cellular functions to molecular mechanism. *J. Cell Biol* 217, 4057–4069 [PubMed: 30373906]
19. Vemu A et al. (2018) Severing enzymes amplify microtubule arrays through lattice GTP-tubulin incorporation. *Science* 361, eaau1504 [PubMed: 30139843]
20. Kuo Y-W et al. (2019) Spastin is a dual-function enzyme that severs microtubules and promotes their regrowth to increase the number and mass of microtubules. *Proc. Natl. Acad. Sci. U. S. A* 116, 5533–5541 [PubMed: 30837315]
21. Mukherjee S et al. (2012) Human fidgetin is a microtubule severing the enzyme and minus-end depolymerase that regulates mitosis. *Cell Cycle* 11, 2359–2366 [PubMed: 22672901]
22. Frickey T and Lupas AN (2004) Phylogenetic analysis of AAA proteins. *J. Struct. Biol* 146, 2–10 [PubMed: 15037233]
23. Davies BA et al. (2010) Coordination of substrate binding and ATP hydrolysis in Vps4-mediated ESCRT-III disassembly. *Mol. Biol. Cell* 21, 3396–3408 [PubMed: 20702581]
24. Schoneberg J et al. (2018) ATP-dependent force generation and membrane scission by ESCRT-III and Vps4. *Science* 362, 1423–1428 [PubMed: 30573630]
25. Samson RY et al. (2008) A role for the ESCRT system in cell division in archaea. *Science* 322, 1710–1713 [PubMed: 19008417]
26. Lindås A-C et al. (2008) A unique cell division machinery in the Archaea. *Proc. Natl. Acad. Sci. U. S. A* 105, 18942–18946 [PubMed: 18987308]
27. Puchades C et al. (2019) The molecular principles governing the activity and functional diversity of AAA+ proteins. *Nat. Rev. Mol. Cell Biol* 21, 43–58 [PubMed: 31754261]
28. Hartman JJ and Vale RD (1999) Microtubule disassembly by ATP-dependent oligomerization of the AAA enzyme katanin. *Science* 286, 782–785 [PubMed: 10531065]

29. Eckert T et al. (2012) Subunit interactions and cooperativity in the microtubule-severing AAA ATPase spastin. *J. Biol. Chem* 287, 26278–26290 [PubMed: 22637577]
30. White SR et al. (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 [PubMed: 17389232]
31. Zehr EA et al. (2020) Katanin grips the β -tubulin tail through an electropositive double spiral to sever microtubules. *Dev. Cell* 52, 118–131.e6 [PubMed: 31735665]
32. Roll-Mecak A and Vale RD (2008) Structural basis of microtubule severing by the hereditary spastic paraplegia protein spastin. *Nature* 451, 363–367 [PubMed: 18202664]
33. Han H et al. (2019) Structure of spastin bound to a glutamaterich peptide implies a hand-over-hand mechanism of substrate translocation. *J. Biol. Chem* 295, 435–443 [PubMed: 31767681]
34. Nithianantham S et al. (2018) Structural basis for disassembly of katanin heterododecamers. *J. Biol. Chem* 293, 10590–10605 [PubMed: 29752405]
35. Diaz-Valencia JD et al. (2011) *Drosophila* katanin-60 depolymerizes and severs at microtubule defects. *Biophys. J* 100, 2440–2449 [PubMed: 21575578]
36. Eckert T et al. (2012) Spastin's microtubule-binding properties and comparison to katanin. *PLoS One* 7, e50161 [PubMed: 23272056]
37. Sandate CR et al. (2019) An allosteric network in spastin couples multiple activities required for microtubule severing. *Nat. Struct. Mol. Biol* 26, 671–678 [PubMed: 31285604]
38. Johjima A et al. (2015) Microtubule severing by katanin p60 AAA+ ATPase requires the C-terminal acidic tails of both α - and β -tubulins and basic amino acid residues in the AAA+ ring pore. *J. Biol. Chem* 290, 11762–11770 [PubMed: 25805498]
39. McNally FJ and Vale RD (1993) Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell* 75, 419–429 [PubMed: 8221885]
40. Roll-Mecak A and Vale RD (2005) The *Drosophila* homologue of the hereditary spastic paraplegia protein, spastin, severs and disassembles microtubules. *Curr. Biol* 15, 650–655 [PubMed: 15823537]
41. Monroe N et al. (2017) Structural basis of protein translocation by the Vps4-Vta1 AAA ATPase. *eLife* 6, e24487 [PubMed: 28379137]
42. Srayko M et al. (2006) Katanin disrupts the microtubule lattice and increases polymer number in *C. elegans* meiosis. *Curr. Biol* 16, 1944–1949 [PubMed: 17027492]
43. Dimitrov A et al. (2008) Detection of GTP-tubulin conformation *in vivo* reveals a role for GTP remnants in microtubule rescues. *Science* 322, 1353–1356 [PubMed: 18927356]
44. Tropini C et al. (2012) Islands containing slowly hydrolyzable GTP analogs promote microtubule rescues. *PLoS One* 7, e30103 [PubMed: 22272281]
45. Aumeier C et al. (2016) Self-repair promotes microtubule rescue. *Nat. Cell Biol* 18, 1054–1064 [PubMed: 27617929]
46. de Forges H et al. (2016) Localized mechanical stress promotes microtubule rescue. *Curr. Biol* 26, 3399–3406 [PubMed: 27916523]
47. Reid TA et al. (2019) Structural state recognition facilitates tip tracking of EB1 at growing microtubule ends. *eLife* 8, 6710
48. Deinum EE et al. (2017) How selective severing by katanin promotes order in the plant cortical microtubule array. *Proc. Natl. Acad. Sci. U. S. A* 114, 6942–6947 [PubMed: 28630321]
49. Lindeboom JJ et al. (2018) CLASP stabilization of plus ends created by severing promotes microtubule creation and reorientation. *J. Cell Biol* 218, 190–205 [PubMed: 30377221]
50. Kuo Y et al. (2019) Predicted effects of severing enzymes on the length distribution and total mass of microtubules. *Biophys. J* 117, 2066–2078 [PubMed: 31708162]
51. Saltini M and Mulder BM (2020) Critical threshold for microtubule amplification through templated severing. *Phys. Rev. E* 101, 052405 [PubMed: 32575333]
52. Jonasson EM et al. (2020) Behaviors of individual microtubules and microtubule populations relative to critical concentrations: dynamic instability occurs when critical concentrations are driven apart by nucleotide hydrolysis. *Mol. Biol. Cell* 31, 589–618 [PubMed: 31577530]
53. Dogterom M and Leibler S (1993) Physical aspects of the growth and regulation of microtubule structures. *Phys. Rev. Lett* 70, 1347–1350 [PubMed: 10054353]

54. Yu W et al. (2008) The microtubule-severing proteins spastin and katanin participate differently in the formation of axonal branches. *Mol. Biol. Cell* 19, 1485–1498 [PubMed: 18234839]
55. Gardner MK et al. (2011) Depolymerizing kinesins Kip3 and MCAK shape cellular microtubule architecture by differential control of catastrophe. *Cell* 147, 1092–1103 [PubMed: 22118464]
56. Nakamura M et al. (2018) SPR2 protects minus ends to promote severing and reorientation of plant cortical microtubule arrays. *J. Cell Biol* 217, 915–927 [PubMed: 29339437]
57. Jiang K et al. (2014) Microtubule minus-end stabilization by polymerization-driven CAMSAP deposition. *Dev. Cell* 28, 295–309 [PubMed: 24486153]
58. Jiang K et al. (2017) Microtubule minus-end regulation at spindle poles by an ASPM–katanin complex. *Nat. Cell Biol* 19, 480–492 [PubMed: 28436967]
59. Gasic I and Mitchison TJ (2018) Autoregulation and repair in microtubule homeostasis. *Curr. Opin. Cell Biol* 56, 80–87 [PubMed: 30415186]
60. Tindemans SH and Mulder BM (2010) Microtubule length distributions in the presence of protein-induced severing. *Phys. Rev. E* 81, 2821–2828
61. Hough LE et al. (2009) Microtubule depolymerization by the kinesin-8 motor Kip3p: a mathematical model. *Biophys. J* 96, 3050–3064 [PubMed: 19383451]
62. Varga V et al. (2006) Yeast kinesin-8 depolymerizes microtubules in a length-dependent manner. *Nat. Cell Biol* 8, 957–962 [PubMed: 16906145]
63. Karabay A et al. (2004) Axonal growth is sensitive to the levels of katanin, a protein that severs microtubules. *J. Neurosci* 24, 5778–5788 [PubMed: 15215300]
64. Stone MC et al. (2012) Normal spastin gene dosage is specifically required for axon regeneration. *Cell Rep.* 2, 1340–1350 [PubMed: 23122959]
65. Loughlin R et al. (2011) Katanin contributes to interspecies spindle length scaling in *Xenopus*. *Cell* 147, 1397–1407 [PubMed: 22153081]
66. McNally K et al. (2006) Katanin controls mitotic and meiotic spindle length. *J. Cell Biol* 175, 881–891 [PubMed: 17178907]
67. McNally K et al. (2014) Katanin maintains meiotic metaphase chromosome alignment and spindle structure *in vivo* and has multiple effects on microtubules *in vitro*. *Mol. Biol. Cell* 25, 1037–1049 [PubMed: 24501424]
68. Wightman R and Turner SR (2007) Severing at sites of microtubule crossover contributes to microtubule alignment in cortical arrays: microtubule dynamics. *Plant J.* 52, 742–751 [PubMed: 17877711]
69. Zhang Q et al. (2013) Microtubule severing at crossover sites by katanin generates ordered cortical microtubule arrays in *Arabidopsis*. *Curr. Biol* 23, 2191–2195 [PubMed: 24206847]
70. Wang G et al. (2018) Augmin antagonizes katanin at microtubule crossovers to control the dynamic organization of plant cortical arrays. *Curr. Biol* 28, 1311–1317.e3 [PubMed: 29657114]
71. Wightman R et al. (2013) SPIRAL2 determines plant microtubule organization by modulating microtubule severing. *Curr. Biol* 23, 1902–1907 [PubMed: 24055158]
72. Fan Y et al. (2018) The *Arabidopsis* SPIRAL2 protein targets and stabilizes microtubule minus ends. *Curr. Biol* 28, 987–994.e3 [PubMed: 29526586]
73. Jinushi-Nakao S et al. (2007) Knot/Collier and Cut control different aspects of dendrite cytoskeleton and synergize to define final arbor shape. *Neuron* 56, 963–978 [PubMed: 18093520]
74. Stewart A et al. (2012) Katanin p60-like1 promotes microtubule growth and terminal dendrite stability in the larval class IV sensory neurons of *Drosophila*. *J. Neurosci* 32, 11631–11642 [PubMed: 22915107]
75. Brill MS et al. (2016) Branch-specific microtubule destabilization mediates axon branch loss during neuromuscular synapse elimination. *Neuron* 92, 845–856 [PubMed: 27773584]
76. Lee H-H et al. (2009) *Drosophila* IKK-related kinase Ik2 and katanin p60-like 1 regulate dendrite pruning of sensory neuron during metamorphosis. *Proc. Natl. Acad. Sci. U. S. A* 106, 6363–6368 [PubMed: 19329489]
77. Davis LJ et al. (2002) The importance of lattice defects in katanin-mediated microtubule severing *in vitro*. *Biophys. J* 82, 2916–2927 [PubMed: 12023214]

78. Coombes C et al. (2016) Mechanism of microtubule lumen entry for the α -tubulin acetyltransferase enzyme α TAT1. *Proc. Natl. Acad. Sci. U. S. A* 113, E7176–E7184 [PubMed: 27803321]
79. Sudo H and Baas PW (2010) Acetylation of microtubules influences their sensitivity to severing by katanin in neurons and fibroblasts. *J. Neurosci* 30, 7215–7226 [PubMed: 20505088]
80. Wang C et al. (2017) KTN80 confers precision to microtubule severing by specific targeting of katanin complexes in plant cells. *EMBO J.* 36, 3435–3447 [PubMed: 28978669]
81. Hazan J 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 [PubMed: 10610178]
82. Hu WF et al. (2014) Katanin p80 regulates human cortical development by limiting centriole and cilia number. *Neuron* 84, 1240–1257 [PubMed: 25521379]
83. Mishra-Gorur K et al. (2014) Mutations in KATNB1 cause complex cerebral malformations by disrupting asymmetrically dividing neural progenitors. *Neuron* 84, 1226–1239 [PubMed: 25521378]
84. Vale RD (1991) Severing of stable microtubules by a mitotically activated protein in *Xenopus* egg extracts. *Cell* 64, 827–839 [PubMed: 1671762]
85. Walker RA et al. (1989) Asymmetric behavior of severed microtubule ends after ultraviolet-microbeam irradiation of individual microtubules *in vitro*. *J. Cell Biol* 108, 931–937 [PubMed: 2921286]
86. Sherwood NT et al. (2004) *Drosophila* spastin regulates synaptic microtubule networks and is required for normal motor function. *PLoS Biol.* 2, e429 [PubMed: 15562320]
87. Wood JD et al. (2006) The microtubule-severing protein spastin is essential for axon outgrowth in the zebrafish embryo. *Hum. Mol. Genet* 15, 2763–2771 [PubMed: 16893913]
88. Nakamura M et al. (2010) Microtubule and katanin-dependent dynamics of microtubule nucleation complexes in the acentrosomal *Arabidopsis* cortical array. *Nat. Cell Biol* 12, 1064–1070 [PubMed: 20935636]
89. Madeira F et al. (2019) The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* 47, W636–W641 [PubMed: 30976793]

Highlights

Disruption of microtubule-severing enzymes leads to multiple, seemingly contradictory phenotypes: destruction, production, and disorganization of cellular microtubules.

New findings that spastin and katanin regulate microtubule dynamics, together with earlier observations on katanin and fidgetin, help to explain these phenotypes. Whether severing is destructive or productive depends on whether the microtubules are in their bounded-growth or unbounded-growth regimes.

A wide range of severing enzyme functions can be understood based on the dynamic state of the microtubule, the location of the severing events on the microtubule filament, and the stability of the new ends created by severing.

Outstanding Questions

Do severing enzymes remove tubulin from the microtubule lattice via a hand-over-hand mechanism? How processive are severases: do they unfold the entire tubulin protein or does unfolding terminate to release a partially folded protein that can reassemble into microtubules? What forces are required to remove a tubulin? How many tubulins need to be removed to induce microtubule breakage?

How do severases regulate microtubule dynamics? Do severases act at the depolymerizing end or do they create GTP islands in the lattice that induce rescue? Both oligomerization and the tubulin CTT (E-hook) are key for severing: what role do they play in the regulation of dynamics?

What are the relative contributions of the severing and dynamics activities to the phenotypes of spastin and other severases?

Do severing enzymes promote tissue morphogenesis in animals, as they do in plants, by aligning microtubule arrays? Does severing of microtubules in neurons initiate branching?

Box 1.**Microtubule Destruction by Severases**

Severing was first identified as a destroyer of the microtubule cytoskeleton through polymer fragmentation and disassembly (Figure IA,B). Following the identification of severing activity in frog egg extracts [84], the founding member of the severase family, katanin, was later purified from sea urchin eggs [39]. Spastin, katanin, and fidgetin disassemble microtubules when overexpressed in tissue culture cells and are necessary for microtubule disassembly in some organismal systems [17,18].

It is easy to see how severing could disassemble a network of dynamic microtubules (Figure IC). Microtubules are capped at their ends by stabilizing regions enriched in GTP tubulin (the **GTP cap**) that protects the central, GDP-containing region from depolymerization. Cutting a microtubule with spastin exposes the GDP region, and the newly created plus end shrinks [20]. Shrinkage is seen following laser cutting *in vitro* [85] and katanin cutting *in vivo* [16,49,69]. The disappearance of the fragments with uncapped plus ends and further severing of the still-capped fragment therefore leads to net disassembly of microtubules.

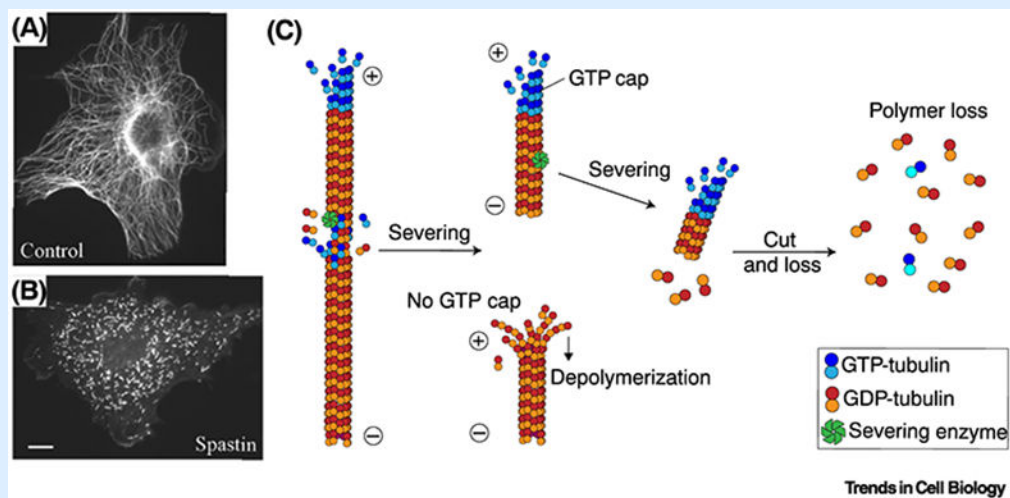


Figure I. Severing-Dependent Microtubule Disassembly.

(A,B) Overexpression of spastin in a rat fibroblast fragments the microtubules. Bar, 10 μm . Adapted from [54]. (C) Model scheme of microtubule loss due to severing. The newly created plus end without a GTP cap rapidly depolymerizes. Under low- or no-rescue conditions, severing eventually leads to a decrease of microtubule length, number, and mass.

Box 2.**Microtubule Amplification by Severases**

In contrast to the disassembly of microtubules, spastin and katanin facilitate the assembly of microtubules in reconstituted assays (Figure IA). Microtubule assembly activity is also found *in vivo*. Early examples include the reduction of microtubule bundles in the neuromuscular junctions of *Drosophila* loss-of-function spastin mutants [86] and the decrease of meiotic microtubule number and density in a *Caenorhabditis elegans* katanin-null mutant (Figure IB) [42]. Mutations of *Drosophila* kat-60L1 also show decreased numbers of polymerized microtubules in dendrites, along with reduced numbers and length of dendritic branches [74], similar to the spastin mutant phenotype [73]. Spastin knockdown in zebrafish embryos causes loss of axonal microtubules and impaired outgrowth [87] and overexpression of spastin in cultured neurons increases microtubule number [54]. Furthermore, katanin-mediated microtubule generation has been directly visualized in *Arabidopsis* hypocotyl cells [16,88]. Thus, severases are capable of increasing microtubule mass.

To explain these paradoxical findings – disassembly in some cases and assembly in others – it has been proposed that the microtubule fragments created by severases act as seeds to generate new microtubules [42,66]. However, this idea of nucleation-like activity is hard to reconcile with the GTP-cap model because severed microtubules expose a new GDP plus end and are expected to depolymerize (Figure IC). Also, why does regrowth occur in some situations but not in others? One possible resolution of the paradox is that severases act in conjunction with other MAPs [12,13], which is certainly true in plants where katanin-mediated microtubule reorganization involves microtubule nucleators like the γ -tubulin ring complex (γ -TuRC) and augmin [16,88].

There is, however, a simpler resolution of the paradox. Spastin and katanin are rescue factors that facilitate the conversion of shrinking microtubules to growing ones [19,20], leading to microtubule amplification (Figure IC). This occurs at physiological concentrations of tubulin, but not in earlier assays that used artificially stabilized microtubules as the *in vitro* substrates. Spastin also decreases the microtubule shrinkage rate, further promoting regrowth [20]. Together, these effects on dynamics shift microtubules from the bounded- to the unbounded-growth regime so that microtubules regrow before they disappear [50]. Thus, the newly created microtubule will act as a seed to increase the total microtubule number and mass (Figure IC).

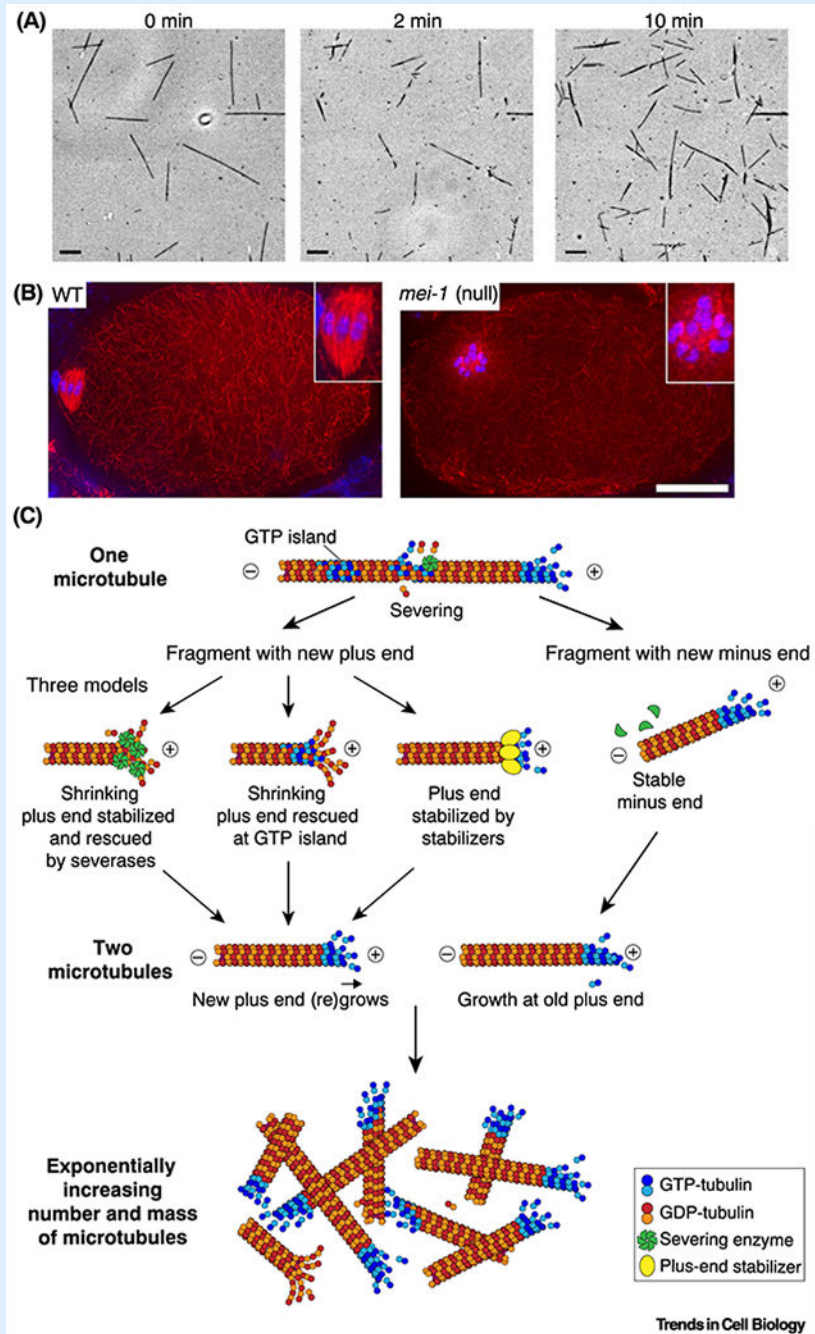


Figure I. Increase in Microtubule Number and Mass by Severases.

(A) Example of microtubule amplification *in vitro*. Adapted from [20]. Unlabeled microtubules are visualized by interference reflection microscopy (IRM); the times after addition of ATP and spastin are indicated. Severing of dynamic microtubules by spastin increases the total microtubule number and mass exponentially over time. Bar, 5 μm . (B) *Caenorhabditis elegans* katanin-null mutant (*mei-1*) shows a decrease in microtubule (red) number and mass in the embryo. Adapted from [42]. Insets show the magnified meiotic spindles. Bar, 10 μm . (C) Proposed models of severase-dependent microtubule

amplification. Severing generates a stable minus end and an unstable plus end. The new plus end can either shrink or be stabilized by plus-end stabilizers. The shrinking plus end can rescue due to its direct stabilization by severases or can rescue when it encounters a GTP island. Abbreviation: WT, wild type.

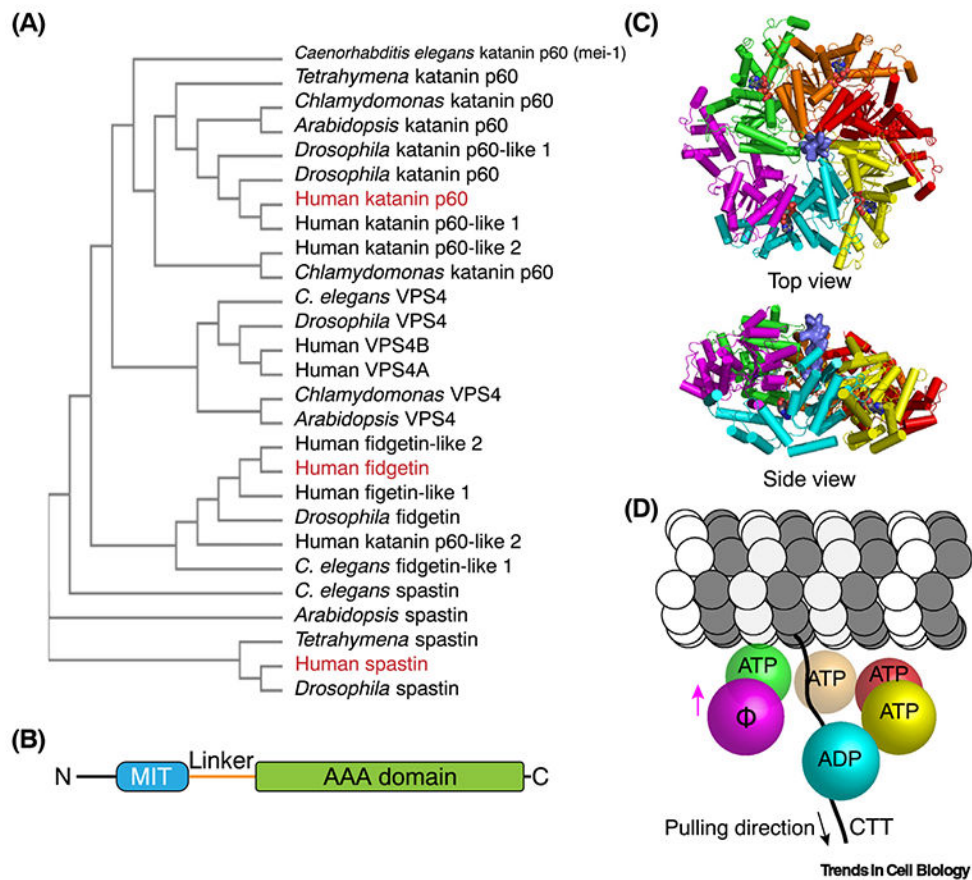


Figure 1. Phylogeny and Structure of Microtubule-Severing Enzymes.

(A) Phylogenetic tree of the meiotic clade AAA-ATPases from model organisms [89]. (B) General architecture of severing enzymes. The predominant microtubule-binding site is located in the microtubule interacting and trafficking (MIT) domain and/or the linker regions. (C) Cryoelectron microscopy structure of a human spastin hexamer bound to a tubulin C-terminal tail (CTT)-mimetic peptide in the presence of the ATP analog ADP-BeF_x based on PDB structure 6PEN [33]. The hexamer is not a flat ring. Five protomers of the hexamer are in a right-handed helix, while the sixth protomer (magenta) is in an intermediate position, which closes the ring. (D) Hand-over-hand model of tubulin polypeptide being unfolded by the hexamer (microtubule not to scale). The magenta (apo) subunit moves up to the top of the spiral when binding to ATP (magenta arrow), causing the extraction of an additional two amino acids from the CTT peptide. The yellow subunit is pushed down, leading to ATP hydrolysis, while the cyan subunit subsequently releases ADP and moves up to the position where the apo subunit breaks the spiral.

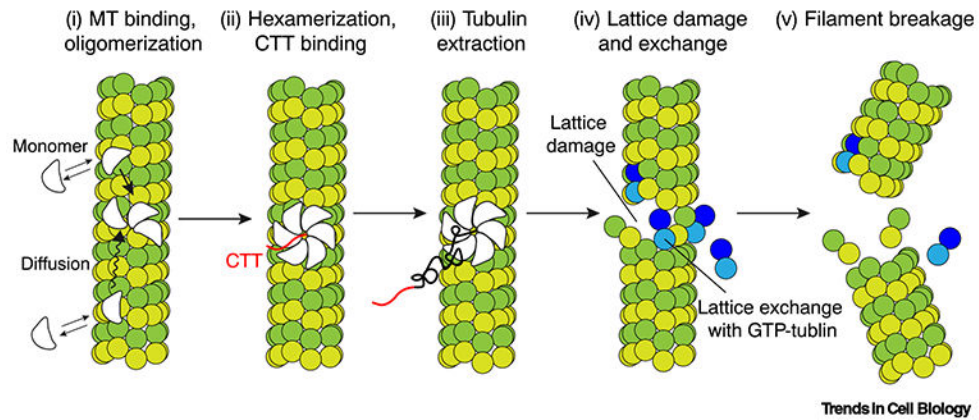


Figure 2. Molecular Mechanism of Microtubule Severing by Severases.

The prevailing model of microtubule severing requires several steps: assembly of hexamers, binding to the tubulin C-terminal tail (CTT), pulling the tubulin polypeptide through the pore, and the generation of lattice defects that eventually lead to microtubule breakage. GTP-tubulin can be added to the defects before a severing event occurs, leading to lattice exchange.

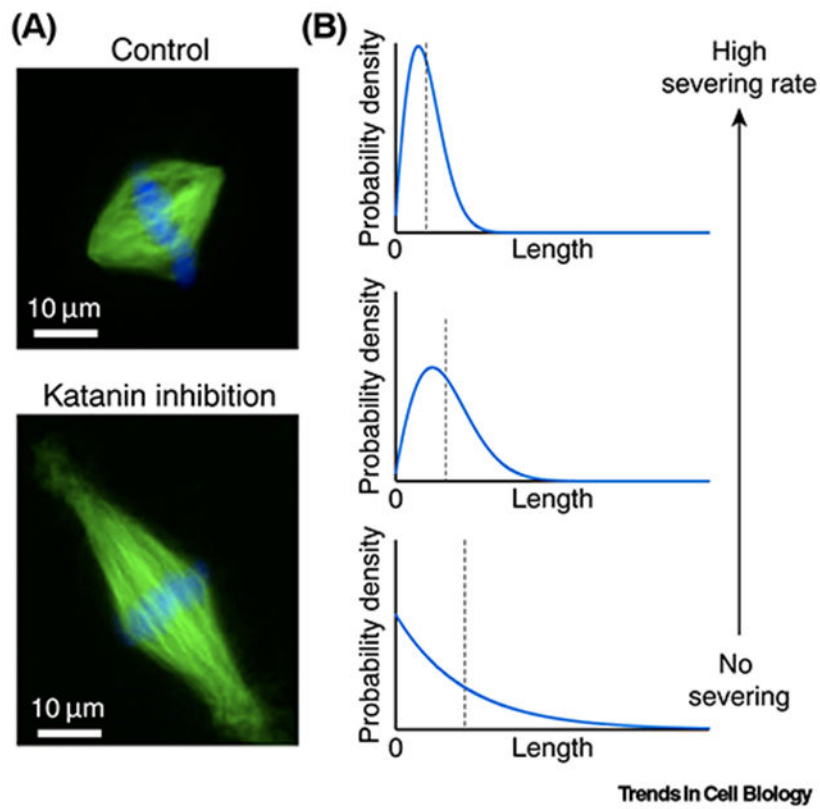


Figure 3. Microtubule Length Control by Severing Enzymes.

(A) Inhibition of katanin results in abnormally long spindle length in *Xenopus* extracts. Adapted from [65]. Top: control spindle; bottom: inhibition of katanin by addition of katanin antibodies. (B) Theoretical microtubule length distributions in the presence of severing [50]. Increased severing activity leads to shorter and more uniform microtubule lengths. Broken lines indicate the average length in each case.

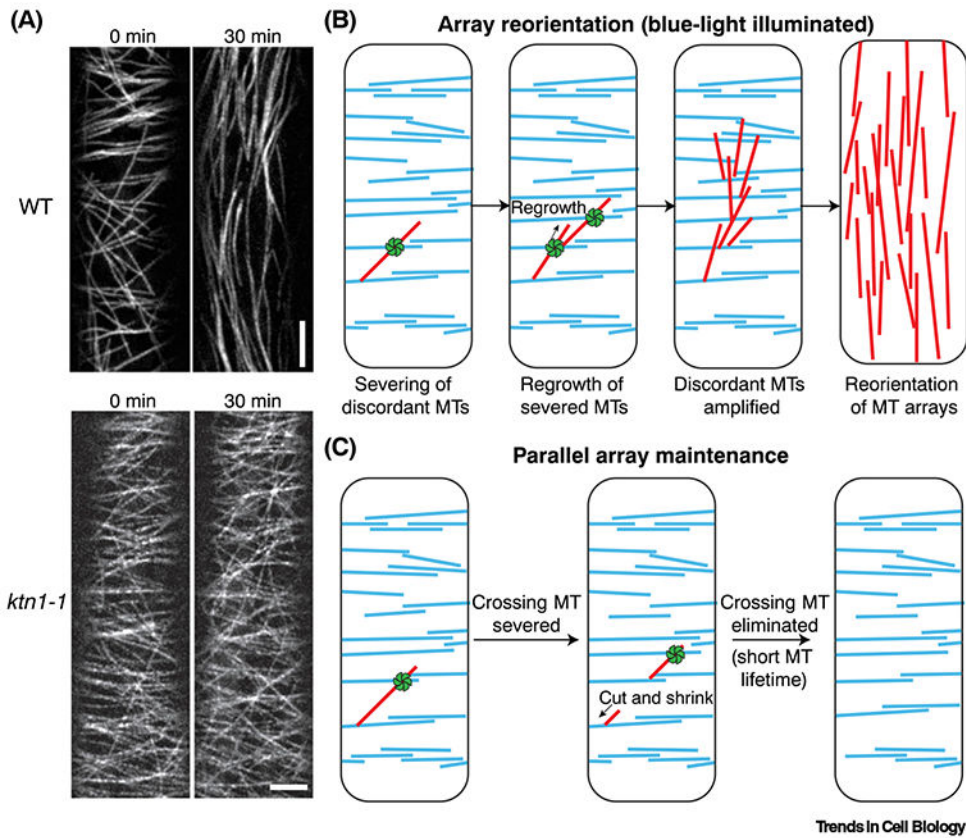


Figure 4. Maintenance and Reorientation of Cortical Microtubule Arrays by Selective Severing. (A) Stimulation by blue light induces cortical microtubule reorientation in *Arabidopsis* hypocotyl cells. Adapted from [16]. This phototropic response requires the severing activity of katanin. Wild-type (WT) (top) cortical microtubule arrays are highly parallel and reorient to $\sim 90^\circ$ after the illumination with blue light, while mutations in katanin (bottom) abolish the array reorientation. Bars, 5 μm . (B) Model for plant cortical microtubule array reorientation by phototropic signals. Microtubules crossing the original parallel arrays are severed by katanin. The rapid regrowth of severed fragments amplifies the discordant microtubules and gives rise to reorientation in response to blue-light illumination. (C) Model for the maintenance of parallel microtubule arrays by severing. The microtubule that crosses the original parallel microtubules at a large angle is severed, leading to rapid shrinkage and thus a shorter lifetime. The elimination of the discordant microtubules by severing and collision-induced catastrophe (not shown) together promotes order and maintains microtubule arrays.