

## **Microtubule-severing in the axon: Implications for development, disease, and regeneration after injury**

**Wenqian Yu, Liang Qiang, and Peter W. Baas**

Department of Neurobiology and Anatomy  
Drexel University College of Medicine  
Philadelphia, PA

Please address correspondence to:

**Peter W. Baas, PhD**

Drexel University College of Medicine  
Department of Neurobiology and Anatomy  
2900 W. Queen Lane  
Philadelphia, PA 19129

Telephone: (215) 991-8298 Fax: (215) 843-9082

**Email: pbaas@drexelmed.edu**

### **ABSTRACT**

*Typical neurons extend a single elongated axon that can potentially grow over great distances within the nervous system. Axons from different neurons bundle together to form nerves. Within the axon are dense arrays of microtubules that provide structural support and also act as railways for organelle transport. Individual microtubules within the array assume a variety of lengths, from just a few micrometres to over a hundred micrometres. The distribution of microtubule lengths is strongly influenced by the presence of microtubule-severing proteins that are capable of cutting long microtubules into shorter pieces. The severing proteins, termed katanin and spastin, are enzymes that break the lattice of the microtubule using energy derived from ATP hydrolysis. The levels of the severing proteins are generally higher during development than in the adult, which is consistent with the fact that microtubules are generally longer in adult axons compared to developing axons. Short microtubules are highly mobile whereas longer microtubules are stationary. Greater mobility within the microtubule array correlates with more robust axonal growth. Here, we discuss the role of microtubule-severing proteins in the development of the axon. We then propose that abnormalities in microtubule-severing may be central to axonal degeneration in a variety of neurological diseases. Finally, we posit that clinical manipulation of the severing proteins may be useful in augmenting regeneration of damaged nerves.*

**Key words:** microtubule, axon, katanin, spastin, regeneration, degeneration

### **INTRODUCTION**

Microtubules form a continuous array within the axon. Each microtubule within the array is oriented with its assembly-favored “plus” end directed away from the cell body (Heidemann et al, 1981). Although the microtubule array is continuous, the individual microtubules that comprise the array are staggered along the length of the axon, and assume a variety of lengths (Bray and Bunge, 1981; Yu and Baas, 1994). Some microtubules are over a hundred micrometres long, while others are only a single micrometre in length or even shorter. Recent studies have shown that only the shortest microtubules are able to move rapidly within the axon, while the longer microtubules are effectively stationary (Wang and Brown, 2002). This important observation prompted us to propose the “cut and run” model, in which long microtubules need to be cut into short pieces if they are to be transported (Baas et al, 2005, 2006). According to this model, the mobility of microtubules within the axon is regulated not only

by the molecular motors that generate the movement, but also by microtubule-severing proteins that break the microtubules into pieces short enough to move.

Neurons express two known microtubule-severing proteins, both of which are enzymes that hydrolyze ATP in order to break the lattice of the microtubule. One of these proteins, termed katanin, was originally discovered on the basis of its microtubule-severing activity (McNally and Vale, 1993). The other, termed spastin, had been known for many years as a protein that goes awry during some forms of spastic paraplegia in humans (Hazan et al, 1999). Only recently was spastin discovered to be a microtubule-severing protein (Errico et al, 2002). The “cut and run” model posits that these two severing proteins share or divide up the duties of microtubule-severing required to mobilize the microtubule array to support axonal growth. Microtubules destined for the axon arise at the centrosome and must be severed from the centrosome in order to be transported into the axon

(Yu et al, 1993; Ahmad et al, 1994). After that, microtubules rapidly elongate by polymerization. Therefore a fraction of them must be severed within the axon to ensure that sufficient numbers remain short enough to undergo transport. Microtubule severing is accentuated at sites of morphological growth and plasticity, such as sites of impending interstitial branch formation (Yu et al, 1994) and growth cones (Dent et al, 1999). Once the axon has stopped growing, there is still a need for microtubule transport to maintain the microtubule array, but presumably fewer microtubules need to be in transit compared to axons that are still growing or undergoing major morphological changes. Consistent with this expectation, we have found that the levels of katanin are very high during axonal growth but plunge precipitously once the axon has reached its target tissues (Karabay et al, 2004).

The fact that mutations to spastin can give rise to spastic paraplegia provides one key example of how a flaw in a severing protein can lead to axonal degeneration. However, beyond this, very little work has been done to elucidate the potential role of the microtubule-severing proteins in human disease. For example, the mechanism by which the spastin mutations give rise to axonal degeneration remains unclear. It is instructive to note that the combined levels of katanin and spastin in the axon, even in the adult, are almost certainly high enough to continuously sever microtubules completely into subunits. Recent studies from our laboratory suggest that microtubules may be “protected” from too much severing by the protein known as tau (Qiang et al, 2006). We have speculated that abnormalities to this protective mechanism might contribute to the degradation of the microtubule array that occurs in neurodegenerative diseases such as Alzheimer’s (Baas and Qiang, 2005).

In this report, we summarize our findings and theories on microtubule-severing proteins during development and disease. In addition, we propose that damaged adult axons, for example after traumatic injury to the spinal cord, might be made to regenerate with more vitality if the levels of the severing proteins can be restored to juvenile levels.

### **Microtubule severing during development**

The “cut and run” model posits that longer microtubules are unable to move in a rapid concerted fashion until they are made shorter. In order to generate short microtubules and to do so at strategic locations, it seems reasonable that neurons would either have to utilize factors that promote disassembly of long polymers into short remnants, or factors that break long microtubules into short pieces. Certainly there are factors in the axon that destabilize and promote microtubule disassembly

(see for example, Grenningloh et al, 2004). However, shortening microtubules by disassembly would not increase the number of microtubules, but only decrease the length of individual microtubules. Thus the severing of long microtubules into multiple short microtubules is an attractive means by which neurons could generate high numbers of short microtubules in the axon.

Our first evidence for microtubule severing came from observations on the centrosome within the cell body of the neuron. Using a pharmacologic assay, we documented that microtubules are rapidly severed from the centrosome, and then transit through the cytoplasm in order to invade nascent axons (Yu et al, 1993; Ahmad and Baas, 1995). As for the axon itself, we performed serial reconstructions from electron micrographs of cultured neurons, and demonstrated high concentrations of short microtubules in the proximal region of the axon contiguous with the cell body and the most distal region of the axon contiguous with the growth cone (Yu and Baas, 2004). We also documented the presence of large numbers of short microtubules and the absence of long microtubules within the axon at sites where new interstitial branches were starting to emerge (Yu et al, 1994). More recently, using live-cell imaging, we directly observed the severing of short microtubules from looped bundles of microtubules within paused growth cones and their subsequent movement into filopodia (Dent et al, 1999). These observations strongly support the theory that long microtubules in the axon are strategically severed in order to generate high numbers of short microtubules when and where needed.

### **Katanin and Spastin**

Most of the attention in our laboratory on microtubule-severing proteins thus far has been focused on katanin. Katanin was originally purified from sea urchin eggs, where it was shown to sever microtubules by disrupting contacts within the polymer lattice using energy derived from ATP hydrolysis (McNally and Vale, 1993). Katanin has two subunit proteins of molecular weights 60kD and 80kD. The smaller subunit has the microtubule-severing activity (McNally and Thomas, 1989; Hartman and Vale, 1999). The larger subunit targets some of the katanin to the centrosome, and indeed many cells display a centrosomal enrichment of katanin (McNally et al, 1996). We have shown that katanin is present at the centrosome of cultured vertebrate neurons, but is also widely distributed within the axon and throughout all neuronal compartments (Ahmad et al, 1999). Experimental inhibition of katanin function prohibits microtubule release from the centrosome, and profoundly

increases microtubule length throughout the neuronal cell body (Ahmad et al, 1999; Karabay et al, 2004). As a result, axonal outgrowth is severely compromised. As noted above, the levels of katanin (in particular, the 60kD subunit) are very high in axons that are actively growing toward their targets, but then plunge when the axon reaches its target and stops growing (Karabay et al, 2004). Based on our studies so far, we have posited that microtubule-severing proteins play critical roles in various aspects of neuronal morphology such as the length, number, and branching patterns of neurites.

There are no human diseases known to be associated with mutations of katanin, probably because katanin is critically important across cell types for events as fundamental as mitosis. Thus mutations to katanin that compromise its function may be lethal. Spastin, on the other hand, appears to be more predominantly neuronal. Given that neurons express very high levels of katanin during development, a partial loss of spastin function would not be expected to be lethal. Spastin was known for many years as a gene whose mutations can give rise to spastic paraplegia (Hazan et al, 1999). Recently, it was noted that spastin has homology with P60-katanin in the region of the molecule that severs microtubules (Errico et al, 2002), and indeed, spastin was demonstrated to be a potent microtubule-severing protein (Errico et al, 2002; Evans et al, 2005). Studies on zebrafish suggest that spastin plays an important role in axonal development (Wood et al, 2006). Studies on spastin in *Drosophila* suggest complexity of function. One study suggests that depletion of spastin causes diminution of microtubules at the synapse (Sherwood et al, 2004) while the other study suggests the opposite (Trotta et al, 2004). While the reasons for this discrepancy are uncertain, one possibility is that diminished severing of microtubules can certainly lead to longer microtubules and an elevation in microtubule mass, but it can also lead to an impaired transport of microtubules, and hence a diminution in the delivery of microtubules to the distal axon. Thus, both results may be quite explicable by the “cut and run” model.

### **Regulation of microtubule severing**

The mechanisms by which the severing proteins are regulated remain elusive. There are no clear indications, at least as of yet, that the katanin or spastin molecules are directly regulated by modifications such as phosphorylation. However, the levels of the severing proteins appear to roughly correlate with the developmental stage of the axon. For example, as already discussed here, the levels of P60-katanin are high when axons are growing but drop precipitously when the axons reach their targets (Karabay et al, 2004). We suspect that this is

because signaling mechanisms target the protein for rapid degradation, which would be reminiscent of various proteins whose levels rapidly change during the cell cycle. Rapid degradation of the severing proteins could explain how axons slow their growth in response to various environmental cues. However, this phenomenon certainly could not explain the rapid and highly localized increases in microtubule severing that are required, for example, during the formation of axonal branches.

A clue to the local regulation of microtubule-severing may come from observations on simple fibroblasts and the behavior of microtubules assembled *in vitro* from purified tubulin. In typical fibroblasts, microtubules have been observed to break upon bending, even when the bending is fairly modest (Odde et al, 1999). However, microtubules do not break when bent in purified microtubule preparations (Jansen et al, 2004). A potential explanation for this discrepancy is that living cells contain katanin and spastin, which are absent from the *in vitro* preparations. When the microtubule bends, its lattice becomes more accessible to the severing proteins, thus leading to breakage. Interestingly, microtubules become highly contorted in neuronal axons that undergo retraction, but show no indication of breakage (He et al, 2002). We know that these developing axons are rich in severing proteins, so it may be that the microtubules within the axon are somehow “protected” from being accessed by katanin and/or spastin, even upon bending. Indeed, we have shown that axonal microtubules are more resistant to severing by overexpression of katanin than microtubules in any other neuronal compartment (Yu et al, 2005).

We have proposed a mechanism by which microtubule severing might be regulated. We call this the “MAP Protection Model.” In this model, fibrous microtubule-associated proteins (MAPs) regulate the access of the severing proteins to the microtubule lattice (Baas and Qiang, 2005). This model was inspired by the observation that katanin-induced microtubule severing becomes much more active in interphase extracts that are depleted of the frog homologue of MAP4 (McNally et al, 2002). In addition, severing is more active in mitotic extracts compared to interphase extracts, and this difference is based on phosphorylation of proteins, but apparently not of katanin itself. Interestingly, phosphorylation of MAP4 causes it to lose its association with the microtubules. In our model, it is the phosphorylation-induced detachment of the MAP molecules from the microtubule that enables katanin and spastin to gain access. In the axon, tau, rather than MAP4, would be the likely candidate to fulfill this role. Indeed, we have recently demonstrated that depletion of tau from cultured neurons renders

the microtubules in the axon dramatically more sensitive to severing by overexpression of katanin (Qiang *et al*, 2006).

The “MAP Protection” model is appealing because it offers a means by which signaling cascades could regulate microtubule severing quite focally, for example at sites of impending axonal branch formation. The signaling cascades would cause tau (or other MAPs) to dissociate from the microtubules at the site where a branch is starting to form, thereby permitting katanin to break the microtubules into shorter highly mobile pieces precisely where needed (for more information and schematic illustrations, see Baas *et al*, 2006). Thus it will be of interest to determine whether growth factors that give rise to axonal branching utilize pathways that enhance tau phosphorylation.

### **Role of microtubule-severing proteins in neurodegenerative diseases**

Several neurodegenerative diseases involve the loss of tau molecules from the microtubules in the axon (Garcia and Cleveland, 2001). The classic example is Alzheimer’s disease, in which tau is gradually hyperphosphorylated, resulting in its disassociation with microtubules and subsequent assembly into abnormal filaments (Mandelkow *et al*, 2003). It has been known for many years that the microtubule array gradually deteriorates during axonal degeneration, but this has generally been attributed to enhanced microtubule depolymerization. However, there is no experimental evidence to support the view that loss of tau from axonal microtubules renders them less stable (see for example, Tint *et al*, 1998). Given our results suggesting that tau may be the “Chief Protector” of axonal microtubules from the severing proteins, it makes sense that microtubules in the axons of Alzheimer’s patients may fall victim to abnormal severing. As the tau gradually dissociates from the microtubules, the available katanin and spastin would presumably gain better access to the microtubules. Thus, we posit that the severing proteins may be central to the degenerative response during neuropathies such as Alzheimer’s disease.

Interestingly, the greater challenge may be to understand exactly how mutations to spastin result in the degeneration of axons during spastic paraplegia. Presumably a mutation to one of the two spastin genes results in a combination of some normal and some mutant spastin. The levels of katanin are generally higher than the levels of spastin, even in the adult axon (our unpublished data). Therefore, the question arises as to whether the observed axonal degeneration results from a fairly minor diminution in the total levels of the severing proteins. Although this might be the case, another possibility is that the

mutant spastin itself is deleterious to the axon for other reasons. For example, the mutant spastin may interact with other proteins in such a way as to inhibit important physiological processes in the axon that are not directly related to microtubule severing *per se*. In other words, is the neuropathy a “loss of function” or “gain of function” phenotype? More work needs to be done to resolve this issue.

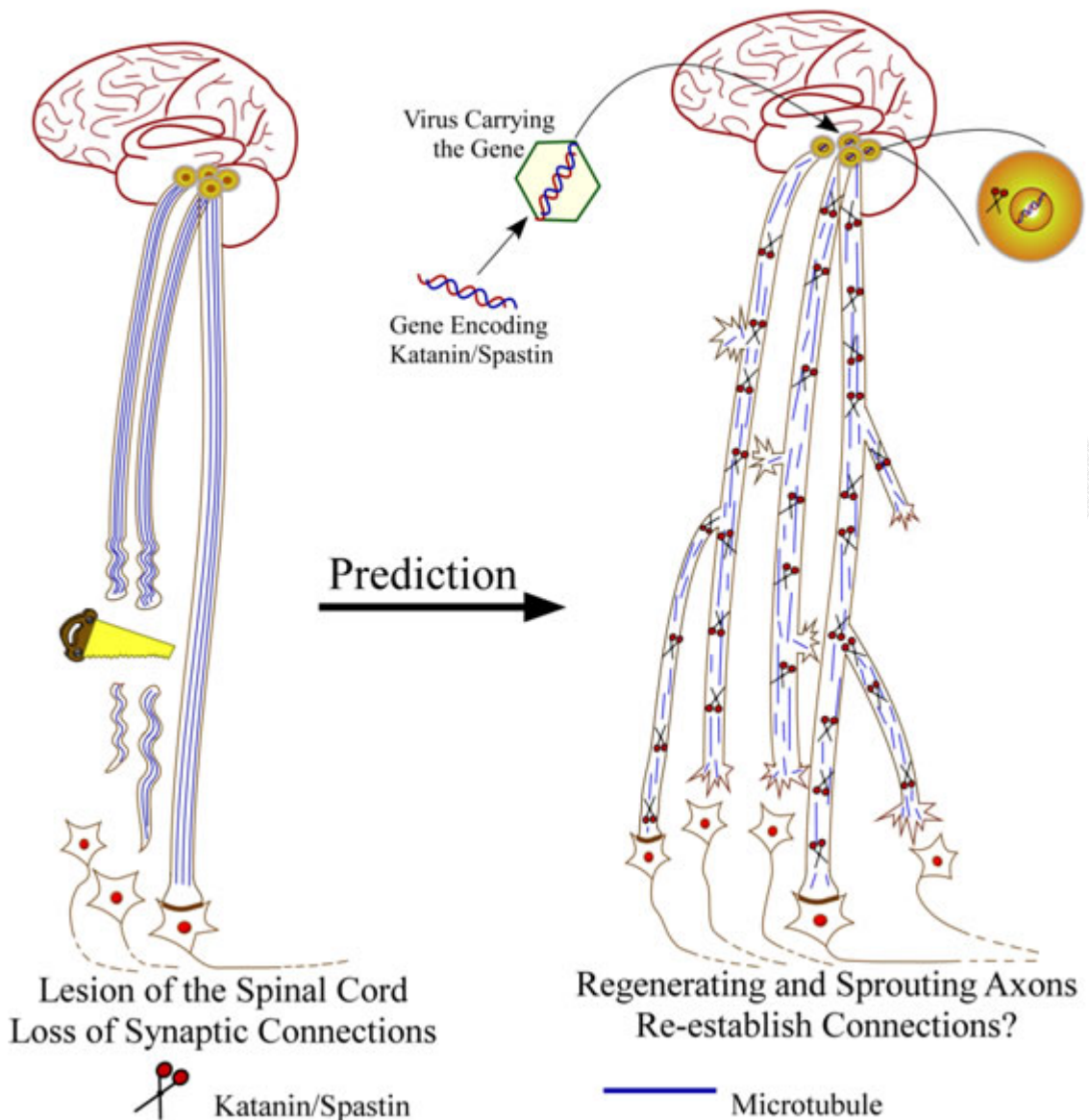
### **Microtubule-severing during axonal regeneration after injury**

Approximately 12,000 to 15,000 people per year in the USA (and thousands more around the world) are afflicted by injuries to the spinal cord. The majority of these patients, if they survive, are permanently paralyzed. Most spinal cord injuries afflict relatively young individuals who are otherwise healthy, and result in a dramatically diminished quality of life as well as huge costs to the individual and the health care system. The ongoing challenge for the research community is to develop strategies that will restore injured adult axons to the vitality of growth they enjoyed during development so that they can re-innervate their appropriate targets. Decades ago, a great deal of effort was focused on the axonal transport systems of damaged and regenerating axons (for example, see Ingoglia *et al*, 1982; for review, see Roy *et al*, 2005) because it was surmised that the capacity of the axon to grow is directly related to the advance of the cytoskeletal elements within the axon. An underlying premise of these early studies was that adult axons could be made to grow at rates comparable to juvenile axons if only the transport of cytoskeletal elements could be restored to the levels that occur during development. Unfortunately, until recently, cytoskeletal transport had never been well enough understood at the molecular level for strategies to be developed for achieving this goal.

We believe the “cut and run” model may represent the mechanistic breakthrough needed to open the door to the development of effective methods for augmenting microtubule transport in injured adult axons. Adult axons contain mainly very long microtubules, sometimes exceeding many hundreds of micrometres in length (Bray and Bunge, 1981; Tsukita and Ishikawa, 1981). The fact that the total levels of the severing proteins are lower in adult axons compared to growing axons is consistent with the presence of far fewer short microtubules, and less robust microtubule transport. Therefore, it makes good sense that injured axons in the spinal cord cannot rally their microtubules to undergo the vitality of transport characteristic of developing axons. Restoring the levels of the microtubule-severing proteins to their juvenile levels via gene

therapy may be a fruitful avenue for augmenting regeneration of injured adult axons (see figure 1).

## Possible Katanin/Spastin Gene Therapy For Spinal Cord Repair



**Figure** Schematic illustration showing a possible strategy for clinically augmenting nerve regeneration after injury to the spinal cord.

### Concluding remarks

Microtubule-severing proteins were initially studied in the context of the centrosome and cell division, but today we know that two major severing proteins are present in the axons of postmitotic neurons. The levels of the severing proteins are particularly high during phases of active axonal growth and morphological plasticity. In the adult axon, the levels of the severing proteins are diminished. Even so, the severing proteins are theoretically high enough to have profound implications for the degeneration of certain axons during neuropathies in

which the microtubules lose their tau-based protection. In axons with comparatively lower total levels of the severing proteins, mutations to spastin may diminish the capacity of the axon to sever microtubules sufficiently well to maintain the necessary degree of mobility within the microtubule array. Thus axonal degeneration could result from either too much or too little severing. These considerations suggest that a better understanding of the severing proteins may prove quite beneficial for developing new approaches for diminishing the extent of axonal degeneration in a variety of neurological diseases. It is also exciting to speculate

that clinical manipulation of the severing proteins may be a fruitful means for augmenting the mobility of microtubules in injured adult axons, thereby enhancing their capacity to regenerate.

The underlying hypothesis is based on the observations that adult neurons express lower levels of microtubule-severing proteins than juvenile axons and that short microtubules are motile whereas long microtubules are not. Thus, we propose that gene therapy to augment katanin and/or spastin expression in the damaged neurons may be successful in altering the length-distribution of microtubules. This would theoretically generate more mobility within the microtubule array, reminiscent of that displayed by developing axons. This, in turn, may cause the damaged axons to regenerate with more vitality. In addition, heightened microtubule mobility within neighboring non-damaged axons may cause them to sprout with more vitality, thus further augmenting the possibility for functional recovery.

#### REFERENCES

- Ahmad FJ, Joshi HC, Centonze VE and Baas PW. Inhibition of microtubule nucleation at the neuronal centrosome compromises axon growth. *Neuron* 1994; 12:271-280.
- Ahmad FJ and Baas PW. Microtubules released from the neuronal centrosome are transported into the axon. *J Cell Sci* 1995; 108:2761-2769.
- Ahmad FJ, Yu W, McNally FJ and Baas PW. An essential role for katanin in severing microtubules in the neuron. *J Cell Biol* 1999; 145:305-315.
- Baas PW and Qiang L. Neuronal microtubules: when the MAP is the roadblock. *Trends Cell Biol* 2005; 15:183-187.
- Baas PW, Karabay A and Qiang L. Microtubules cut and run. *Trends Cell Biol* 2005; 15:518-524.
- Baas PW, Vidya Nadar C and Myers KA. Axonal transport of microtubules: the long and short of it. *Traffic*. 2006; 7:490-498.
- Bray D and Bunge MB. Serial analysis of microtubules in cultured rat sensory axons. *J Neurocytol.* 1981; 10:589-605.
- Dent EW, Callaway JL, Szebenyi G, Baas PW and Kalil K. Reorganization and movement of microtubules in growth cones and developing interstitial branches. *J Neurosci* 1999;9:8894-8904.
- Errico A, Ballabio A and Rugarli EI. Spastin, the protein mutated in autosomal dominant hereditary spastic paraplegia, is involved in microtubule dynamics. *Hum. Mol Genet* 2002; 11:153-163.
- Evans KJ, Gomes ER, Reisenweber SM, Gundersen GG and Lauring BP. Linking axonal degeneration to microtubule remodeling by spastin-mediated microtubule severing. *J Cell Biol* 2005;168:599-606.
- Garcia ML and Cleveland DW. Going new places using an old MAP: tau, microtubules and human neurodegenerative disease. *Curr Opin Cell Biol.* 2001;13:41-48.
- Grenningloh G, Soehrman S, Bondallaz P, Ruchti E and Cadas H. Role of microtubule destabilizing proteins SCG10 and stathmin in neuronal growth. *J Neurobiol* 2004;58:60-69.
- Hartman JJ and Vale RD. Microtubule disassembly by ATP-dependent oligomerization of the AAA enzyme katanin. *Science* 1999;286:782-785.
- Hazan J, Fonknechten N, Mavel D, Paternotte C, Samson D, Artiguenave F, Davoine CS, Cruaud C, Durr A, Wincker P, Brottier P, Cattolico L, Barbe V, Burgunder JM, Prud'homme JF, Brice A, Fontaine B, Heilig B and Weissenbach J. Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. *Nat. Genet.* 1999; 3:296-303.
- He Y, Yu W and Baas PW. Microtubule reconfiguration during axonal retraction induced by nitric oxide. *J Neurosci* 2002; 22:5982-91.
- Heidemann SR, Landers JM and Hamborg MA. Polarity orientation of axonal microtubules. *J Cell Biol* 1981; 91:661-665.
- Ingoglia NA, Sharma SC, Pilchman J, Baranowski K and Sturman JA. Axonal transport and transcellular transfer of nucleosides and polyamines in intact and regenerating optic nerves of goldfish: speculation on the axonal regulation of periaxonal cell metabolism. *J Neurosci.* 1982;2:1412-1423.

19. Janson ME and Dogterom M. A bending mode analysis for growing microtubules: evidence for a velocity-dependent rigidity. *Biophys J* 2004;87:2723-2736.
20. Karabay A, Yu W, Solowska JM, Baird DH and Baas PW. Axonal growth is sensitive to the levels of katanin, a protein that severs microtubules. *J Neurosci* 2004;24:5778-5788.
21. Mandelkow EM, Stamer K, Vogel R, Thies E and Mandelkow E. Clogging of axons by tau, inhibition of axonal traffic and starvation of synapses. *Neurobiol Aging*. 2003;24:1079-1085.
22. McNally FJ and Vale RD. Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell* 1993;75:419-429.
23. McNally FJ, Okawa K, Iwamatsu A and Vale RD. Katanin, the microtubule-severing ATPase, is concentrated at centrosomes. *J Cell Sci* 1996;109:561-567.
24. McNally FJ and Thomas S. Katanin is responsible for the M-phase microtubule-severing activity in *Xenopus* eggs. *Mol Biol Cell* 1998;9:1847-1861.
25. McNally KP, Buster D and McNally FJ. Katanin-mediated microtubule severing can be regulated by multiple mechanisms. *Cell Motil Cytoskeleton* 2002;53:337-49.
26. Odde DJ, Ma L, Briggs AH, DeMarco A and Kirschner MW. Microtubule bending and breaking in living fibroblasts. *J Cell Sci* 1999;112:3283-3288.
27. Qiang L, Yu W, Andreadis A, Luo M and Baas PW. Tau protects microtubules in the axon from severing by katanin. *J Neurosci*. 2006;26:3120-3129.
28. Roy S, Zhang B, Lee VM and Trojanowski JQ. Axonal transport defects: a common theme in neurodegenerative diseases. *Acta Neuropathol*. 2005;109:5-13.
29. Sherwood NT, Sun Q, Xue M, Zhang B and Zinn K. *Drosophila* spastin regulates synaptic microtubule networks and is required for normal motor function. *PLoS Biol* 2004;2:e429:2094-2111.
30. Tint I, Slaughter T, Fischer I and Black MM. Acute inactivation of tau has no effect on dynamics of microtubules in growing axons of cultured sympathetic neurons. *J Neurosci*. 1998;18:8660-8673.
31. Trotta N, Orso G, Rossetto MG, Daga A and Broadie K. The hereditary spastic paraplegia gene, spastin, regulates microtubule stability to modulate synaptic structure and function. *Curr Biol* 2004;14:1135-1147.
32. Tsukita S and Ishikawa H. The cytoskeleton in myelinated axons: serial section study. *Biomed. Res*. 1981;2:424-437.
33. Wang L and Brown A. Rapid movement of microtubules in axons. *Curr Biol* 2002;12:1496-1501.

JENB