

# Critical Roles for Microtubules in Axonal Development and Disease

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**Abstract** Axons are occupied by dense arrays of cytoskeletal elements called microtubules, which are critical for generating and maintaining the architecture of the axon, and for acting as railways for the transport of organelles in both directions within the axon. Microtubules are organized and regulated by molecules that affect their assembly and disassembly, their stabilization, their association with other cytoskeletal elements, and their alignment and bundling with one another. Recent studies have accentuated the role of molecular motor proteins and microtubule-severing proteins in the establishment and maintenance of the axonal microtubule array. The growing body of knowledge on the proteins and mechanisms that regulate axonal microtubules has fostered a better understanding of how many debilitating diseases cause axons to degenerate. The purpose of this chapter is to provide an update on current knowledge of axonal microtubules and the proteins that regulate them, and to reflect on cutting-edge findings linking these proteins and mechanisms to diseases that afflict the human population.

## 1 Microtubules in the Axon

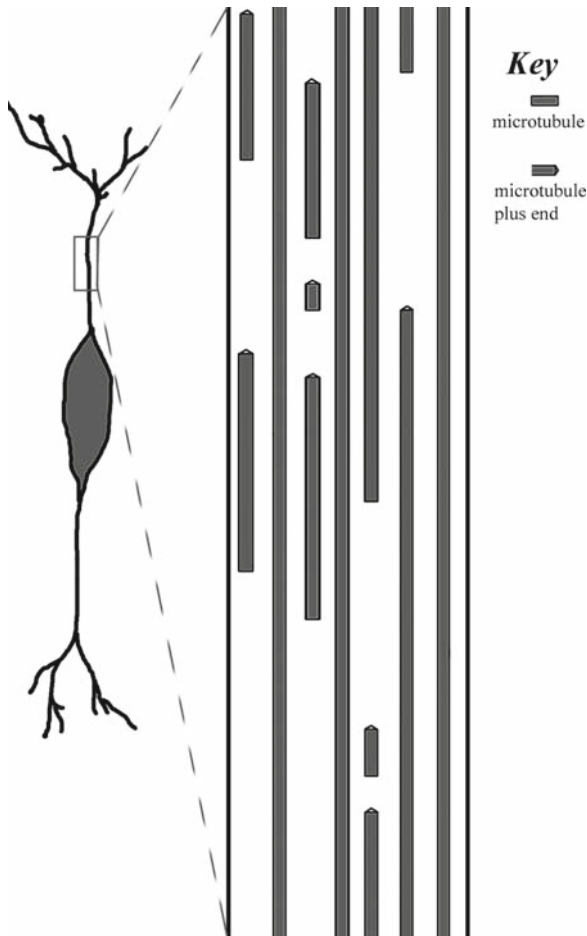
Each typical neuron of the body generates a single elongated axon that has the potential to traverse complex and often long journeys to reach its target tissue. The axon is effectively unlimited in its growth potential, as evidenced by the fact that it continues to grow unabated when neurons are transplanted into a culture dish (He and Baas 2003). This is particularly surprising because axons contain relatively little protein synthetic machinery, and hence need axonal transport from the cell body for their growth and maintenance (Baas and Buster 2004). In addition, the axon is an engineering marvel that clearly requires sophisticated architectural struts to generate and maintain its structure. The transport and architectural needs of the

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axon are fulfilled by cytoskeletal elements, and in particular, by microtubules. Microtubules are polymers of tubulin subunits that provide cells with their shape by resisting compression, while simultaneously acting as the substrate for the transport of organelles and proteins in both directions (Baas and Buster 2004). Microtubules are certainly not unique to axons, but rather fulfill architectural and motile needs of cellular apparatuses as diverse as mitotic spindles and cilia.

Microtubules form a continuous array within the axon, extending from the cell body into the growth cone at its distal tip (see Fig. 1). Almost every microtubule within the array is oriented with its assembly-favored “plus” end directed away from the cell body (Heidemann et al. 1981; Baas et al. 1987). 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



**Fig. 1** A continuous array of microtubules provides structural support to the axonal shaft. Individual microtubules within the array vary in length. Almost all of the microtubules exhibit uniform polarity with plus ends oriented distally, away from the cell body

Bunge 1981; Yu and Baas 1994). Some microtubules are over a hundred microns long, while others are only a single micron in length, or even shorter. Early studies on the kinetics of tubulin transport suggested that radiolabeled tubulin moves slowly down the axon, and in a relatively coherent manner compared to diffusion (Black and Lasek 1980). It was posited that tubulin is transported in the form of the microtubules, and that this transport consists of a slow and synchronous “march” of the polymers. Live-cell imaging analyses over the past several years have refined this model substantially by demonstrating that the transport of microtubules down the axon is actually not slow and coherent at all, but it is fast, intermittent, asynchronous, and bidirectional (Wang and Brown 2002; Hasaka et al. 2004; He et al. 2005; Myers and Baas 2007). Moreover, it is only the very shortest microtubules, those less than about 7  $\mu\text{m}$  in length, that are in transit.

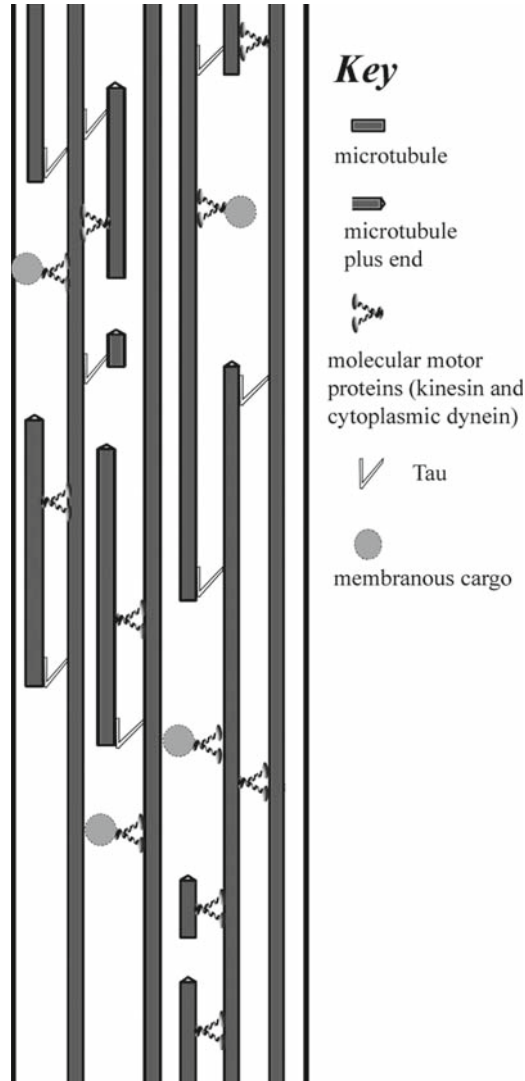
Recent observations have focused a great deal of attention on the molecular motor proteins that transport the short microtubules, as well as a category of proteins called microtubule-severing proteins that break long microtubules into ones that are short enough to be transported (Baas et al. 2006). In addition, strong evidence suggests that the same molecular motor proteins that transport the short microtubules also impinge upon the long microtubules, and thereby play important functional roles in such matters as determining whether the axon grows or retracts, as well as the navigation of the tip of the axon, called the growth cone (Myers et al. 2006; Nadar et al. 2008). The relevant microtubule-severing proteins are enzymes called katanin and spastin. The relevant molecular motor proteins appear to be cytoplasmic dynein, as well as a small number of specialized kinesins, typically thought of as “mitotic” motors because they were originally identified as crucial for generating forces on microtubules in the mitotic spindle (Baas 1999; Baas et al. 2006).

In addition to being subjected to motor-driven forces and the potential for severing, microtubules are classically known to be dynamic polymers, which means that they have the capacity for rapid assembly and disassembly. In the absence of accessory and regulatory proteins and signals, microtubules display extremely rapid bouts of intermittent assembly and disassembly known as “dynamic instability.” Such behavior is quite apparent in living cells as well; particularly, at the leading edge of motile cells (Wittmann et al. 2003), or in the case of the axon, within the growth cone (Suter et al. 2004). However, neurons are exceptionally rich in proteins that shift the dynamics toward assembly, and also stabilize the microtubules against disassembly. These proteins include classic fibrous microtubule-associated proteins (MAPs) such as tau, MAP1b, MAP1a, and MAP2, as well as other proteins such as STOP (stable tubule only protein), doublecortin, and crosslinking proteins such as plakins/plectins (Chapin and Bulinski 1992; Matus 1994; Bosc et al. 1996; Horesh et al. 1999; Leung et al. 2002). These proteins generally function by binding along the lattice of the microtubule, and thereby suppress the tendency of microtubules to disassemble. Other proteins, such as CRMP-2, may actually interact with the tubulin subunits to promote their assembly onto pre-existing microtubule polymers (Fukata et al. 2002). Notably, axons also contain proteins that promote microtubule disassembly, and these include stathmin and SCG10 (Curmi et al. 1999).

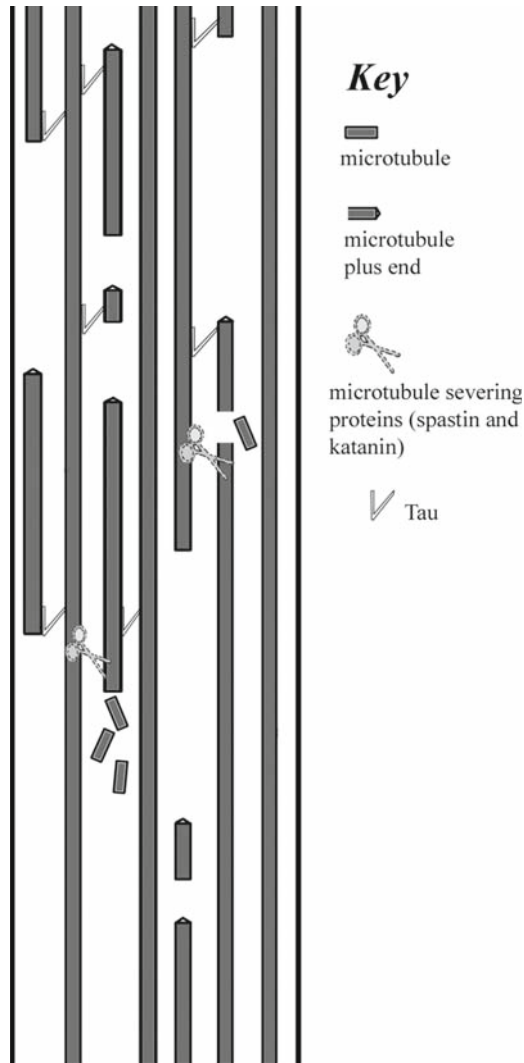
The relevant molecular motor proteins exert various complementary and antagonistic actions, so that desired effects can be achieved by regulating the balance of forces

either globally or locally in the axon. The same is true of the relevant proteins that impact the assembly, disassembly, and stabilization of the microtubules. By having a number of different participants that impact the properties of the microtubules, the axon can judiciously regulate the microtubule array to participate appropriately in events such as axonal growth, retraction, branch formation, and navigation.

Figure 2 schematically shows molecular motor proteins transporting membranous cargo and short microtubules in the axon, while figure 3 schematically illustrates



**Fig. 2** Molecular motor proteins power microtubule-mediated transport of membranous cargo, as well as that of short microtubules in anterograde and retrograde directions within the axon



**Fig. 3** Microtubule-severing proteins break long microtubules into shorter ones, thus regulating lengths, as well as numbers of microtubules within the axon. Tau limits the access of microtubule severing proteins, such as katanin to the microtubule lattice

the activity of microtubule-severing proteins. In both schematics, tau is also included, as it has been implicated in the potential regulation of molecular motors (Baas and Qiang 2005) and severing proteins (see Sect. 3.1).

Two other categories of proteins are worth mentioning with regard to the regulation of microtubules in the axon. The first is a relatively newly discovered family of proteins called +tips, which preferentially bind the plus ends of microtubules

during bouts of assembly. Shortly after the relevant tubulin subunits become part of the polymer, the +tips tend to lose their association, resulting in high concentrations of these molecules at the growing plus-end of the microtubule relative to elsewhere along the microtubule's length. These +tips, which include "end binding" proteins such as EB1 and EB3, as well as CLIPs and CLASPs, impact the dynamic properties of the microtubules, and also their interactions with cortical structures, molecular motors, and other proteins (Vaughan 2005; Akhmanova and Hoogenraad 2005). These proteins may be important for several functions, including, for example, the interaction of the plus-ends of the microtubules with structures in the cell cortex during branch formation and growth cone navigation (Stepanova et al. 2003; Jimenez-Mateos et al. 2005; Kornack and Giger 2005).

The other category of proteins worth mentioning is actin, and any protein that impacts the organization, distribution, or properties of the actin cytoskeleton, given that microtubules and actin filaments are well known for interacting with one another. We have recently published a chapter elsewhere on this topic (Myers and Baas 2009), and will refrain from including a discussion of it here.

Much of what is known about the various microtubule-related molecules in the axon has been elucidated within the context of development. Adult axons are relatively "hard-wired," and do not undergo the degree of morphological plasticity as they do during development. Even so, it would appear that the vast majority of the microtubule-related molecules important for development are still present throughout the lifetime of the axon. Microtubules still need to be transported down the axon; albeit, somewhat less robustly compared to development. That means the molecular motor proteins and microtubule-severing proteins still have important roles to play. In addition, it is very important that a substantial portion of the microtubule mass is relatively stable to ensure that the axon has architectural support and railways for the ongoing transport and trafficking of organelles, RNA, and proteins needed for the vitality of the axon. Of course, damaged axons have some regenerative capacity, especially in the peripheral nervous system, and so preserving the necessary elements for axonal growth makes sense.

Over the past few decades, scientists interested in axonal microtubules have made the argument that microtubules are so important for the axon that undoubtedly studying how they are regulated would someday yield a huge payoff in terms of understanding the neurological disorders that afflict the human population. Recent years have proven especially fruitful in this regard. For example, we now know that mutations to microtubule-related proteins such as doublecortin and Lis1 can cause congenital flaws in brain lamination, resulting in mental retardation (Gleeson et al. 1998; Reiner et al. 1993), and it is clear that misregulation of tau is a critical component of Alzheimer's disease (see Sects. 3 and 3.1). It also appears that microtubule-based axonal transport is particularly sensitive to toxins and misfolded proteins, which can lead to nerve degeneration as a result of compromised axonal transport (De Vos et al. 2008). Therefore, in developing a thorough understanding of axonal microtubules, the microtubule research community is making rapid progress toward characterizing some of the most debilitating neurological diseases, as well as effective strategies for their treatment and prevention. The purpose of this

chapter is not to be exhaustive, but rather to provide a few examples of how careful, in-depth study of axonal microtubules has led to breakthroughs in the understanding of diseases that impact the nervous system.

## 2 Microtubule-Severing and Hereditary Spastic Paraplegia

Hereditary spastic paraplegia (HSP) is a heterogeneous group of genetic diseases that mainly affect the corticospinal tracks, resulting in spasticity in the lower limbs (Bruyn 1992). Although there are variable forms of the disease, the age of onset is usually in early adulthood, with no developmental deficiencies. Mutations to several different genes can give rise to HSP, but roughly 40–60% of the cases result from autosomal dominant mutations to a gene called *SPG4*, which codes for a protein called spastin (Hazan et al. 1999). The discovery in 2002 that spastin is a microtubule-severing protein (Errico et al. 2002) prompted enormous interest, because it suggested that the axons of patients afflicted with this form of HSP may degenerate due to insufficient severing of microtubules. With a mechanism in hand, the potential existed for rapid progress on therapies.

Our interest in microtubule-severing activity first arose from our early work on the length-distribution of microtubules in the axon, which was conducted before katanin or spastin had been identified as severing proteins. We had determined that new microtubules do not spontaneously nucleate in the axon (Baas and Ahmad 1992), and hence, we speculated that microtubule number could only be increased in the axon by either release and transport of new microtubules from the centrosome (located in the cell body of the neuron) or by severing of pre-existing microtubules in the axon such that a single microtubule would be transformed into many short ones (Joshi and Baas 1993). The short microtubules could then either elongate into longer ones or disassemble to provide subunits used by their neighbors to elongate. We performed serial reconstructions from electron micrographs of cultured neurons and demonstrated the appearance of large numbers of short microtubules and the absence of long microtubules within the axon at sites where new branches were starting to form (Yu et al. 1994). These observations provided support for a model in which long microtubules are severed in a very localized and tightly regulated fashion during the formation of collateral branches. Using live-cell imaging, we directly observed the severing of short microtubules from looped bundles of microtubules within paused growth cones, and the subsequent movement of the short microtubules into filopodia (Dent et al. 1999). Producing more free ends of microtubules, via severing, may also be important because free ends of microtubules are known to interact with a variety of proteins and structures, such as those within the cell cortex (see discussion on +tips in Sect. 1). Microtubule-severing would certainly appear to be particularly important for the plasticity of the axon during development; however, as noted earlier, having a sufficient balance of long and short microtubules is crucial for proper axonal transport throughout the lifetime of the neuron (Yu et al. 2007).

Katanin was the first microtubule-severing enzyme to be well characterized. It was originally purified from sea urchin eggs, in which it was shown to sever microtubules by disrupting contacts within the polymer lattice using energy derived from ATP hydrolysis (McNally and Vale 1993). In studies on neurons, we showed that katanin is present at the centrosome, consistent with observations in other cell types, and that it is also widely distributed within the axon, and throughout all neuronal compartments (Ahmad et al. 1999; Yu et al. 2005). Inhibition of katanin by various experimental approaches 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. In addition, we found that the levels of katanin are very high in axons that are actively growing toward their targets, but then decrease precipitously when the axon reaches its target and stops growing (Karabay et al. 2004).

We next expanded our studies to include spastin, starting with detailed quantitative and functional comparisons between katanin and spastin in the nervous system. We found that katanin has widespread expression in the various cells and tissues of the body; however, spastin is more enriched in the nervous system, with only low levels of expression elsewhere (Solowska et al. 2008). Nonetheless, during the development of the nervous system, the levels of katanin are many times higher than the levels of spastin. After development is complete, at which time katanin levels plunge, spastin levels are only slightly diminished. Despite this, the levels of katanin remain notably higher than the levels of spastin throughout the adult brain and spinal cord. These observations provided some support for a “loss of function” scenario for HSP, because a diminished level of functional spastin would presumably be more consequential in the adult, due to the lower levels of katanin. However, nothing in these studies suggested an obvious explanation of why the corticospinal tracks should be particularly sensitive to a diminution in functional spastin levels.

In terms of the functions of spastin, we found that over-expressing spastin in cultured neurons profoundly increased the frequency of axonal branch formation (Yu et al. 2008). This phenomenon did not occur when katanin was over-expressed (Yu et al. 2005), suggesting that spastin has some specialized properties to promote branch formation that are not shared by katanin. Depletion of spastin diminished, but did not eliminate branch formation, suggesting that katanin can provide sufficient severing of microtubules for branches to form, but that spastin is more optimal to perform in this capacity (Yu et al. 2008). In part, this may be due to the fact that spastin tends to accumulate at sites of branch formation far more than katanin. Patients with spastin mutations as well as spastin knockout mice do not show deficiencies in axonal branching during development (Fink and Rainier 2004), supporting the conclusion that katanin is sufficient to carry out this function in the absence of spastin.

Additional observations caused us to further doubt whether a “loss of function” scenario makes sense to explain the degeneration of the corticospinal tracks in HSP patients. The *spastin* gene is interesting in that it contains a second start-codon, not far downstream from the first one (Claudiani et al. 2005; Mancuso and Rugarli 2008). In rodents, this results in two spastin isoforms, which we refer to as M1 and

M85, with respective molecular weights of 68 and 60 kDa (note: they are named according to the relevant methionine; in humans, the shorter isoform would be M87 rather than M85; nonetheless, the term M85 will be used for simplicity). Notably, we found that M85 is expressed in all regions of the central nervous system at all times during development and in the adult (Solowska et al. 2008). By contrast, there is little or no detectable M1 at any time during development, or in the adult, with one exception. About 20% of the total spastin in the adult spinal cord is the M1 isoform. Given that this corresponds to the location and time where degeneration occurs in spastin-based HSP, we wondered if mutant M1 may pose a problem for the axon, while mutant M85 does not.

In this view, spastin-based HSP would be a “gain-of-function” disease in which axonal degeneration is not caused by insufficient microtubule-severing activity, but rather by a cytotoxic mutant protein. To test this hypothesis, we compared the effects of truncated versions of M1 and M85 spastin, lacking the AAA ATPase domain critical for severing function, on cultures of embryonic cortical neurons (Solowska et al. 2008). These studies demonstrated just how detrimental M1 mutants would be if they were robustly expressed during development. Neurons induced to express the truncated M1 are slower to develop, have shorter axons, and have generally less robust morphologies. Interestingly, the truncated M85-spastin, which would correspond to the mutant spastin expressed during development in HSP patients, did not cause any developmental problems in neuronal cell cultures.

To further investigate the mechanism of HSP, we tested the effects of truncated spastin M1 and M85 on fast axonal transport. In studies on squid axoplasm, perfusion of full-length M1 plus M85 spastins showed no effect on fast axonal transport, and the same was true for the truncated M85, which lacks the ability to sever microtubules (Solowska et al. 2008). However, the truncated M1 polypeptide strongly inhibited fast axonal transport, indicating that it is the 8 kDa amino terminal region of M1 that elicits these deleterious effects. One possibility is that pathogenic spastin mutations induce a conformational change that results in abnormal exposure of the 8 kDa amino terminal unique to M1. Consistent with this view, intragenic polymorphisms of spastin have been found within the 8 kDa amino terminus, which dramatically modify the HSP phenotype. In addition, spastin is known to interact with another HSP-related protein called atlastin via the 8 kDa amino-terminal region of M1 (Sanderson et al. 2006). Interestingly, recessive mutations in *atlastin* also lead to HSP (Zhao et al. 2001), suggesting that the binding of atlastin or other polypeptides to the amino terminal of M1 could help prevent M1-induced pathology (Zhao et al. 2001).

These observations not only provide insight into the underlying causes of the disease, but are also critically important for the development of effective treatment strategies. We do not believe the “loss/gain of function” issue is completely resolved; however, if our view on this problem is correct, then there is no utility in developing strategies to compensate for a hypothetical insufficiency in microtubule-severing activity in afflicted axons. Instead, a more productive approach would be to try to rid the afflicted neurons of the mutant M1 spastin. According to our results thus far, it would be preferable for the neurons to risk losing the viable

spastin than to tolerate the pathogenic spastin molecules. Thus, a therapeutic RNAi approach may prove highly effective in depleting the problematic molecules from the neurons of patients with spastin-based HSP.

### 3 Tauopathies

Tauopathies are a class of neuro-degenerative disorders caused by abnormalities of the tau protein. As mentioned earlier, tau is one of the many microtubule-associated proteins that can influence microtubule dynamics in the axon. In addition, tau is thought to regulate the spacing between microtubules (Black 1987; Chen et al. 1992), as well as interactions between microtubules and other proteins and structures in the axon (Ebner et al. 1998). The binding of tau to microtubules is regulated by its phosphorylation at specific sites (Fath et al. 2002). Tauopathies are generally caused by either mutations to tau that affect its capacity to bind to microtubules or by abnormalities in the pathways that phosphorylate tau. There are two proposed ways in which abnormal tau behavior is believed to lead to axonal degeneration. The first is that the microtubules are impaired because of the loss of normal levels of tau binding to their lattice. The second is that the abnormal tau may itself be toxic, as evidenced by its tendency to self-assemble into various types of aggregates, including straight filaments and paired helical filaments (Bunker et al. 2006; Arrasate et al. 1999), and by recent studies of the effects of the amino terminus of tau on axonal transport (Lapointe et al. 2009). At least 34 different pathology-associated mutations in various regions of the *tau* gene have been identified so far. Such pathologies include frontotemporal dementia with Parkinsonism, linked to chromosome 17 (FTDP-17). Other examples of tauopathies linked to specific tau mutations include progressive supranuclear palsy, corticobasal degeneration, and Pick's disease (Wang and Liu 2008). Interestingly, in Alzheimer's disease, there are no known mutations of tau, but only alterations in tau phosphorylation.

The *tau* gene, located on human chromosome 17, gives rise to six well-recognized isoforms of tau protein through the process of alternative splicing of mRNA. Missense mutations mentioned above act by causing misregulation of alternative splicing. Exon 2, 3, and 10 of *tau* RNA transcript are known to undergo alternative splicing. While the exons 2 and 3 encode amino terminal inserts, the exon 10 codes for a part of the microtubule-binding region of the tau protein. Exon 10 is also the exon in which the maximum numbers of disease-causing mutations have been identified. Alternative splicing is responsible for generating tau isoforms, containing either four or three microtubule binding repeats (4R tau, or 3R tau). Of the six known tau isoforms, three belong to each category. The number of microtubule binding repeats present dictates the microtubule binding ability of a given tau isoform. 4R tau isoforms have a stronger affinity for microtubules (Wang and Liu 2008). Interestingly, under normal physiological conditions, alternative splicing is regulated such that the ratio of 4R tau-to-3R tau is maintained at 1:1 (Donahue et al. 2007; D'Souza and Schellenberg 2005). It has been suggested that perturbation

of this ratio lies at the root of tau-related neurodegeneration (Stanford et al. 2003). A mouse model of tauopathies generated by over-expression of the smallest tau isoform mimics characteristic features of human tauopathies, such as progressive accumulation of tau inclusions and neurodegeneration (Lee and Trojanowski 2001). However, it still remains controversial as to whether 3R or 4R tau isoforms are more toxic to the cell.

### ***3.1 Tauopathies and Microtubule-Severing***

One of the most common claims about tauopathies is that the loss of tau from the microtubules in the axon destabilizes the microtubules and leads to their depolymerization. This interpretation is hard to defend, however, in light of the fact that experimental depletion of tau from axons does not render the microtubules less stable, nor are microtubules less stable in tau knockout mice (Tint et al. 1998; Harada et al. 1994). These observations do not entirely invalidate the idea, however, because the mix of other microtubule-stabilizing molecules may largely be able to compensate for the loss of tau, with modest effects accumulating over time to give rise to microtubule loss.

Another scenario is suggested by our observations on microtubule-severing function. Quantitatively, neurons contain total levels of microtubule-severing proteins far higher than would be required to completely break down microtubules in the test tube (Solowska et al. 2008). Therefore, neurons must have regulatory mechanisms that either attenuate the activity of the severing proteins or protect the lattice of the microtubules from being fully accessed by the severing proteins. Whatever the case, it is not hard to understand how misregulation of these mechanisms could result in abnormal levels of microtubule-severing activity that could be profoundly deleterious to the axon.

The question arises as to how microtubule-severing function is regulated, such that severing of microtubules occurs when and where needed? We have proposed a model 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, a fibrous MAP similar to tau (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 (Vale 1991; McNally et al. 2002). Interestingly, phosphorylation of MAP4 causes it to lose its association with the microtubules (McNally et al. 2002), consistent with a model that we call the “MAP protection model.” In this model, fibrous MAPs protect the lattice of the microtubule from being accessed by katanin. Phosphorylation of the MAPs results in their detachment from the microtubule, thereby enabling katanin to gain access (Baas and Qiang 2005). In the axon, tau, rather than MAP4, would be the likely candidate to fulfill this role (see Fig. 3). The MAP protection model offers a potential means by which signaling cascades can regulate microtubule-severing activity in a spatially discrete manner, for example,

at impending sites of 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.

To test the premise of this idea, we conducted studies in which we over-expressed katanin or spastin in fibroblasts in which we had first expressed a neuronal MAP, the katanin or spastin causes dramatic severing of microtubules and loss of microtubule mass. We found that robust levels of tau and MAP2 are able to protect the microtubules from being severed by katanin, but MAP1b was not able to do so (Qiang et al. 2006). Tau and MAP2 have a very similar microtubule-binding domain to MAP4, and so these results make sense. Tau, being the MAP enriched in axons, therefore, appeared to be the prime suspect for being the principal protector of microtubules in the axon. Indeed, when we depleted various MAPs from neurons with siRNA, it was only tau whose loss caused the microtubules in the axon to become notably more sensitive to the over-expression of katanin (Qiang et al. 2006). In subsequent studies, we found that tau is less effective at protecting microtubules from spastin (Yu et al. 2008), which is probably one reason why spastin is always expressed at much lower levels than katanin.

On the basis of these observations, we posited that one of the contributing factors to axonal degeneration in tauopathies (such as Alzheimer's disease) may be a gradually heightened sensitivity of the microtubules to abnormal microtubule-severing activity, mainly of katanin (Baas and Qiang 2005; Qiang et al. 2006). This hypothesis has not yet been tested, but if it is valid, it may suggest new avenues for therapies based on downregulating microtubule-severing activity in the afflicted axons.

## 4 Other Neurodegenerative Diseases

A number of review papers have been written on neurodegenerative disorders over the past few years, and these include substantive sections on how flaws in microtubules and microtubule-related events such as axonal transport give rise to degeneration of axons (see, e.g., Roy et al. 2005; Chevalier-Larsen and Holzbaur 2006; Duncan and Goldstein 2006; Morfini et al. 2002). Although it is not possible to provide an in-depth discussion of the various relevant diseases within the limited space available here, three additional diseases will be discussed briefly below.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that is believed to arise from accumulation of misfolded protein aggregates (Boillee et al. 2006). Motor neurons of the central nervous system are preferentially degenerated in the pathology of ALS. Degeneration of motor neurons leads to atrophy of muscles supplied by these neurons, which gradually leads to complete loss of voluntary muscle movement as the disease progresses. Although the exact cause of the disease is not known, mutations in the gene that encodes copper-zinc superoxide dismutase (SOD1) have been linked to cases of familial ALS (Rosen et al. 1993).

Under normal physiological conditions, SOD1 protects the cell from toxic superoxide radicals. A mouse model of ALS was created by over-expressing an ALS-linked SOD1 mutant; motor neurons in these mice exhibit defective axonal transport (Ligon et al. 2005). Similar to the situation discussed earlier with regard to spastin and HSP, loss of function of SOD1 seems unlikely to be the cause of the observed motor neuron defects in ALS (Boillee et al. 2006).

Motor neurons are large cells with long axons. Owing to their great lengths, these axons heavily depend on microtubule-driven transport of cargo for their maintenance and viability. For example, retrograde transport of trophic factors is thought to be essential for neuronal survival (Ye et al. 2003). The mechanism by which mutant SOD1 disrupts axonal transport is not entirely understood. Cytoplasmic dynein, the principal minus-end-directed microtubule-associated motor protein, is known to colocalize with aggregates of mutant SOD1 (Ligon et al. 2005). A recent report has shown interaction between mutant SOD1 and cytoplasmic dynein (Zhang et al. 2007). These observations suggest disruption of dynein-mediated transport of cargo along microtubules as an explanation for the degeneration and death of motor neurons caused by mutant SOD1. The hypothesis that perturbed axonal transport is the underlying cause of neuronal degeneration is further supported by a recent report linking mutations in the *spastin* gene to etiology of ALS (Munch et al. 2008). As mentioned earlier, mutations in the *spastin* gene can lead to impaired axonal transport. Finally, another gene whose mutations are linked with ALS, termed *ALS2*, generates a protein called alsin, which partially associates with the centrosome and appears to impact the microtubule system (Millecamps et al. 2005).

Potential links between impaired axonal transport, neuronal degeneration, and cell death are being actively explored in other neurological diseases as well. Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by uncontrolled, uncoordinated jerky muscle movements along with decline in mental abilities. Mutations in the protein huntingtin, resulting in an abnormally large number of polyglutamine repeats in its sequence, is believed to be the cause of this disease. Despite widespread expression of huntingtin protein in non-nervous as well as nervous tissue, mutations of *huntingtin* gene lead to degeneration selectively in brain tissue. The mechanism of this degeneration is thus far unclear. However, it is known that mutant huntingtin exhibits a propensity to form aggregates within neurons (DiFiglia et al. 1997) and also has altered interactions with other proteins (Harjes and Wanker 2003). The hypothesis that neuronal death is caused by toxic gain of function by misfolded mutant proteins in the case of huntingtin is questioned by reports suggesting that neuronal dysfunction precedes aggregate formation (Gunawardena and Goldstein 2005). Recently, it has been demonstrated that huntingtin is a positive regulator of axonal transport and that it directly interacts with dynein (Caviston et al. 2007; Colin et al. 2008). Observations on mouse models of HD and also human HD patients provide evidence for disrupted axonal transport in the form of swollen neuronal projections, accumulation of vesicles and organelles, and mutant huntingtin (Gunawardena and Goldstein 2005). These pieces of evidence present a strong case for the role of disrupted axonal transport in the pathology of Huntington's disease. It has been hypothesized that, owing to

the mutations, neuronal degeneration may be due to loss of function of huntingtin, thereby leading to the observed impairment in axonal transport (Her and Goldstein 2008; Szebenyi et al. 2003).

Perturbed axonal transport and also direct effects on neuronal microtubules are implicated in the pathology of Parkinson's disease (PD). PD is characterized by degeneration of dopaminergic neurons in the substantia nigra of the brain. Loss of these neurons leads to reduced dopaminergic input to the striatum, the part of the brain that controls voluntary muscle movement. The attendant functional disinhibition results in nonselective, excessive muscle tone, which produces muscle rigidity, and abnormalities in speech. Mutations in the *parkin* gene are associated with some of the familial cases of Parkinson's (Moore et al. 2005). *Parkin* codes for a protein-ubiquitin E3 ligase. Under normal physiological conditions, *parkin* independently binds to tubulin subunits and microtubules. Interaction of *parkin* with tubulin facilitates its degradation, whereas *parkin*'s interaction with microtubules stabilizes them. PD-linked *parkin* mutants retain their ability to bind to microtubules, but are unable to bind to tubulin. This leads to intracellular accumulation of misfolded tubulin, leading to neurodegeneration (reviewed in Feng 2006). Furthermore, toxins implicated in PD, such as rotenone, cause depolymerization of microtubules (Marshall and Himes 1978). This disrupts microtubule-mediated transport of dopamine vesicles, leading to intracellular accumulation of leaky vesicles, which leads to neurodegeneration (Ren et al. 2005).

## 5 Concluding Remarks

In conclusion, as research investigators of microtubules, we are gratified that progress on the regulation of microtubules in neurons has led to a greater understanding of the normal physiology of the axon, as well as new mechanistic breakthroughs on neurodegenerative diseases that plague the human population. We look forward to further progress, and in particular to a new focus on potential therapies for treating, preventing, and perhaps even curing these microtubule-related diseases.

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## Critical Roles for Microtubules in Axonal Development and Disease

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