8 Breckenridge, D.G. *et al*. (2003) Caspase cleavage product of BAP31 induces mitochondrial fission through endoplasmic reticulum calcium signals, enhancing cytochrome c release to the cytosol. *J. Cell Biol.* 160, 1115–1127
9 Lee, Y.J. *et al.* Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. *Mol. Biol. Cell* (in press)
10 Sugioka, R. *et al*. (2004) Fzo1, a protein involved in mitochondrial fusion, inhibits apoptosis. *J. Biol. Chem.* 279, 52726–52734
11 Mukamel, Z. and Kimchi, A. (2004) Death-associated protein 3 localizes to the mitochondria and is involved in the process of mitochondrial fragmentation during cell death. *J. Biol. Chem.* 279, 36732–36738
12 Karbowski, M. *et al*. (2004) Endophilin B1 is required for the maintenance of mitochondrial morphology. *J. Cell Biol.* 166, 1027–1039
13 Karbowski, M. and Youle, R.J. (2003) Dynamics of mitochondrial morphology in healthy cells and during apoptosis. *Cell Death Differ.* 10, 870–880
14 Terrones, O. *et al*. (2004) Lipidic pore formation by the concerted action of proapoptotic BAX and tBID. *J. Biol. Chem.* 279, 30081–30091
15 Wissing, S. *et al*. (2004) An AIF orthologue regulates apoptosis in yeast. *J. Cell Biol.* 166, 969–974
16 Fannjiang, Y. *et al*. (2004) Mitochondrial fission proteins regulate programmed cell death in yeast. *Genes Dev.* 18, 2785–2797
17 James, D.I. *et al*. (2003) hFis1, a novel component of the mammalian mitochondrial fission machinery. *J. Biol. Chem.* 278, 36373–36379
18 Tondera, D. *et al*. (2004) Knockdown of MTP18, a novel phosphatidylinositol 3-kinase-dependent protein, affects mitochondrial morphology and induces apoptosis. *J. Biol. Chem.* 279, 31544–31555
19 Esseiva, A.C. *et al*. (2004) Temporal dissection of Bax-induced events leading to fission of the single mitochondrion in *Trypanosoma brucei*. *EMBO Rep.* 5, 268–273
20 McCormick, A.L. *et al*. (2003) Disruption of mitochondrial networks by the human cytomegalovirus UL37 gene product viral mitochondrion-localized inhibitor of apoptosis. *J. Virol.* 77, 631–641
21 Poncet, D. *et al*. (2004) An anti-apoptotic viral protein that recruits Bax to mitochondria. *J. Biol. Chem.* 279, 22605–22614
22 Arnoult, D. *et al*. (2004) Cytomegalovirus cell death suppressor vMIA blocks Bax- but not Bak-mediated apoptosis by binding and sequestering Bax at mitochondria. *Proc. Natl. Acad. Sci. U. S. A.* 101, 7988–7993
23 Pinton, P. *et al*. (2001) The Ca2+ concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the molecular mechanism of Bcl-2 action. *EMBO J.* 20, 2690–2701
24 Pacher, P. and Hajnoczky, G. (2001) Propagation of the apoptotic signal by mitochondrial waves. *EMBO J.* 20, 4107–4121
25 Brookes, P.S. *et al*. (2004) Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am. J. Physiol. Cell Physiol.* 287, C817–C833
26 Frieden, M. *et al*. (2004) Ca(2+) homeostasis during mitochondrial fragmentation and perinuclear clustering induced by hFis1. *J. Biol. Chem.* 279, 22704–22714
27 Karbowski, M. *et al*. (2002) Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis. *J. Cell Biol.* 159, 931–938
28 Karbowski, M. *et al*. (2004) Quantitation of mitochondrial dynamics by photolabeling of individual organelles shows that mitochondrial fusion is blocked during the Bax activation phase of apoptosis. *J. Cell Biol.* 164, 493–499
29 Olichon, A. *et al*. (2003) Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome *c* release and apoptosis. *J. Biol. Chem.* 278, 7743–7746
30 Griparic, L. *et al*. (2004) Loss of the intermembrane space protein Mgm1/OPA1 induces swelling and localized constrictions along the lengths of mitochondria. *J. Biol. Chem.* 279, 18792–18798

0962-8924/$ - see front matter
doi:10.1016/j.tcb.2005.02.005

Neuronal microtubules: when the MAP is the roadblock

Peter W. Baas and Liang Qiang

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

Recent studies shed new light on a potential cascade of events by which neurological diseases such as Alzheimer's lead to axonal degeneration. In this model, the pathology starts with an elevation in microtubule-associated proteins (MAPs) such as tau. This renders the microtubules less accessible to motor proteins, which impairs their capacity to sustain anterograde axonal transport of proteins and organelles. In response, the neuron hyperphosphorylates tau so that it dissociates from the microtubules. Unfortunately, the hyperphosphorylated tau forms abnormal filaments that are deleterious to the axon, and the tau-depleted microtubules become highly sensitive to microtubule-severing proteins such as katanin.

Corresponding author: Baas, P.W. (Peter.W.Baas@drexel.edu).
Available online 23 February 2005

Introduction

The days are gone when cell biologists could knock-down or overexpress a single cytoskeletal protein and then assume to interpret the results in a simple fashion. Microtubules and actin filaments are so functionally intertwined that it is virtually impossible to manipulate one without affecting the other [1]. Microtubule-based motor proteins such as cytoplasmic dynein require cofactors that interface with any number of other motor and non-motor proteins [2]. There is even an unusual myosin recently shown to interact directly with microtubules [3]. Despite the extra headaches for the investigator, the expanding knowledge of cytoskeletal interactions is now providing a view of the cytoskeleton in which cascades of interrelated factors regulate and fine-tune aspects of cellular motility and morphogenesis. Recent studies on traditional non-motor microtubule-associated proteins (MAPs) provide a new perspective on the manner by which various other microtubule-related

proteins might be regulated. These studies suggest that MAPs can restrict access to the microtubule for certain molecular motors. There appears to be specificity to these 'roadblocks' – for example in the types of motor proteins that are most sensitive. In addition, given that the association of MAPs with microtubules is regulated by phosphorylation events, there is the potential for tight regulation by signaling cues relevant to neuronal development and degeneration. These observations, buoyed by an exciting new paper by the Mandelkow laboratory [4], provide the basis for a provocative theory on how various proteins might be allowed to act on microtubules, and also provide the crux of a new model for how tau abnormalities contribute to axonal degeneration in diseases such as Alzheimer's.

Overexpression of MAPs suppresses organelle transport

The basis for these considerations is a series of studies in which overexpression of MAPs in fibroblasts and neurons causes inhibition of plus-end-directed microtubule transport [4–7]. For example, mitochondria accumulate within the cell body and fail to move into axons when excess tau protein is experimentally expressed. Interestingly, the levels of tau required to elicit this response need not be particularly high compared with endogenous neuronal levels. In principle, excess tau can inhibit the attachment of both kinesin and dynein motors, but, because kinesin is more severely affected, the net effect is a retrograde flow of organelles towards the cell center. Other MAPs such as MAP4 and MAP2c similarly inhibit motor-based transport, with the severity of the inhibition correlating with the strength of the binding affinity of the particular MAP. The new findings by Mandelkow and collaborators focus on MARK, which belongs to a family of kinases (which also includes Par-1) believed to be essential for the establishment of polarity in neurons and other cell types. Overexpression of MARK causes MAPs to be phosphorylated at their KXGS motifs, which in turn causes them to lose their association with microtubules. Inhibition of transport by overexpression of tau is relieved if MARK is also overexpressed, indicating that it is the association of tau with the microtubules that prohibits them from sustaining anterograde transport.

The conclusion evoked by these findings is that organelle transport in the axon can be regulated by MAPs (Figure 1). Available data suggest that MAPs do not slow the velocity of the transport once the motor is engaged, but they do diminish the frequency by which a motor can interact with the microtubule and thereby move along it [8,9]. If the density of the 'roadblocks' is high, there can be a significant impairment of anterograde transport, which can lead to axonal degeneration [4,8]. The authors suggest that the microtubule-based abnormalities in Alzheimer's disease might consist of multiple phases, the first of which is an increase in the levels of tau, which blocks anterograde transport. The second phase represents an effort on the part of the neuron to combat the excess tau by hyperphosphorylating it, thus causing it to dissociate from the microtubules. In the final phase, the hyperphosphorylated tau forms abnormal paired helical filaments. As it is stripped of its MAPs, the microtubule gradually becomes more accessible to motor proteins, but unfortunately also becomes highly susceptible to proteins that cause the microtubule to disintegrate (Figure 2). As a result, axonal degeneration ensues.

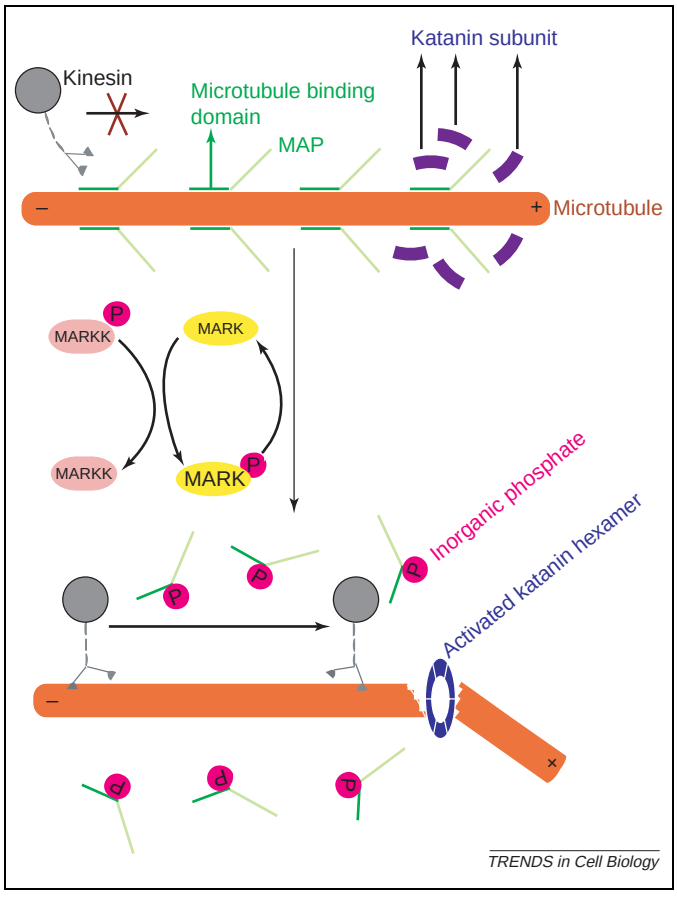

Figure 1. Model for microtubule-associated protein (MAP) suppression of organelle transport and microtubule severing. The microtubule is decorated by MAPs such as tau. The MAPs reduce the frequency by which motor molecules can interact with the microtubule lattice, and the frequency by which katanin subunits can hexamerize around the microtubule to break it. Phosphorylation of MARK by MARK kinase causes MARK to phosphorylate the MAP molecule, which dissociates from the microtubule, thereby enhancing the frequency by which motor and katanin molecules can interact with the microtubule lattice.

Microtubule disintegration by severing proteins

The observations reported by Mandelkow and collaborators prompted us to consider exactly how axonal microtubule levels are diminished after the MAPs dissociate from the microtubules. We use the word 'disintegrate' as a deliberately vague term because it is unknown exactly what causes the diminution of microtubule mass. There are proteins such as stathmin in neurons and other cell types that enhance the tendency of a microtubule to depolymerize by loss of subunits from the ends of the polymer. However, axonal microtubules can be hundreds of microns in length, and hence it probably makes more sense that the microtubules are broken at various positions along their length. After having been fragmented into shorter pieces, depolymerization of the microtubules would likely ensue owing to the creation of more microtubule ends from which subunits can be lost. Microtubule-severing proteins such as katanin and spastin have been identified in neurons (and other cell types) as enzymes that hydrolyze ATP in breaking the lattice of the

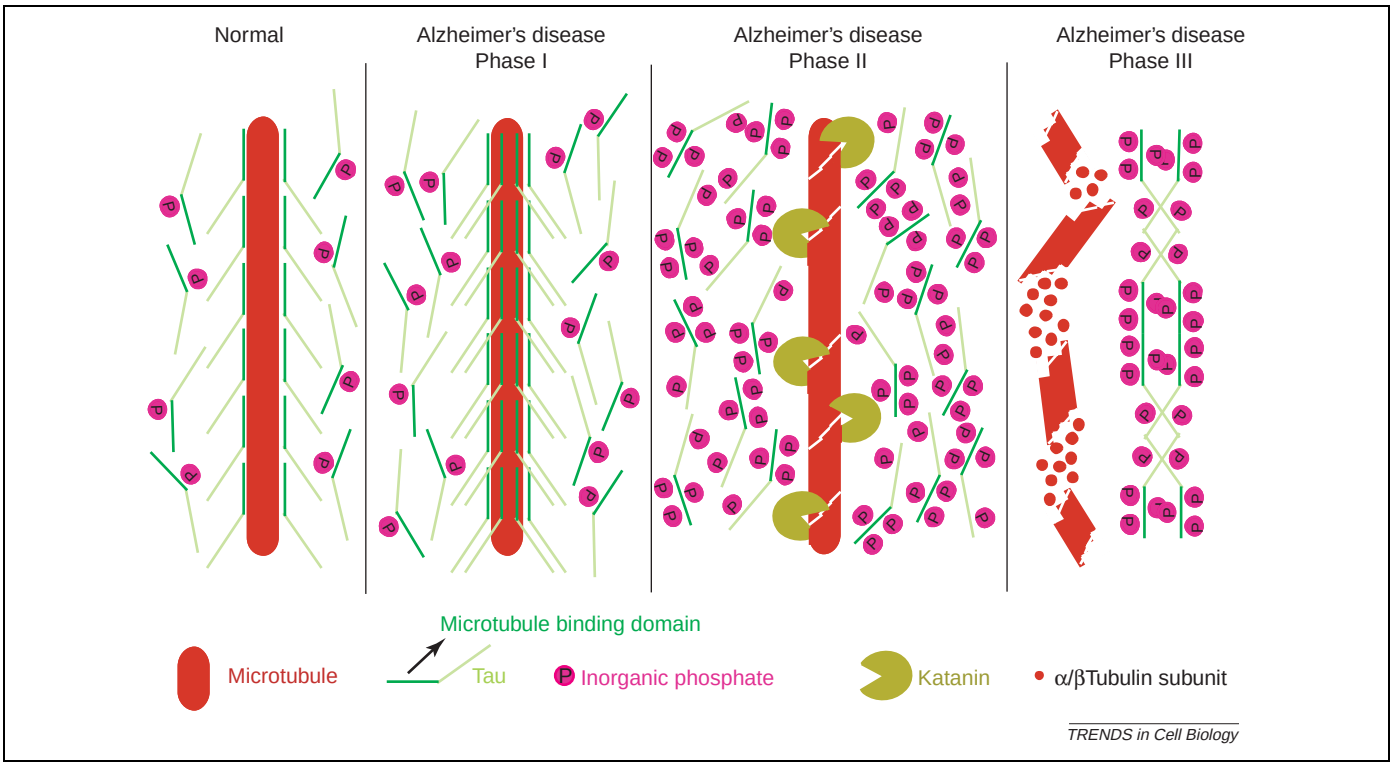

Figure 2. Model for microtubule-based axonal degeneration in Alzheimer's disease. The initial neuropathy results from overexpression of tau, which impedes motor-based transport on the microtubules (Phase I). The neuron responds by hyperphosphorylating tau, causing it to dissociate from the microtubule (Phase II). The microtubules become more susceptible to katanin access, thus resulting in the breakdown of the microtubule array. The hyperphosphorylated tau forms abnormal paired helical filaments (Phase III).

polymer [10,11]. Recent studies have shown that katanin, named for the Japanese samurai sword, is more highly expressed in neurons than it is in many other cell types and is present throughout all compartments of the neuron [10,12].

A perplexing issue pertains to the mechanism by which katanin-based microtubule severing is regulated, given that constitutively active katanin would break microtubules completely into subunits [13]. One potential clue to the regulation of katanin is that its severing activity is stronger in mitotic extracts than in interphase extracts [14]. The enhanced activity in mitotic extracts has been shown to relate to phosphorylation events, but probably not to phosphorylation of katanin itself [14,15]. It is appealing to contemplate that katanin-based microtubule severing might be regulated by MAPs that block access of katanin to the microtubule (Figure 1). Indeed, the MAP4 homolog in *Xenopus* has been shown by *in vitro* studies to suppress microtubule severing by katanin [15].

Relevant to this discussion, microtubules appear to break more or less easily in different cell types. Microtubules have been shown to 'bend and break' in fibroblasts in which the bending of the microtubules need only be very slight to cause breakage [16]. By contrast, microtubules do not appear to break in retracting axons, despite being highly bent, even doubling back on themselves and forming loops [17]. It is unlikely that microtubules are broken by strain, given that microtubules *in vitro* can be dramatically contorted and bent without breaking if there are no MAPs and no katanin present [18]. The fact that neurons are far richer in MAPs than is the case for fibroblasts supports the view that the MAPs might protect the microtubules from being accessed by katanin. It is also appealing to speculate that signals relevant to axonal growth, growth cone motility and collateral branch formation might involve local phosphorylation of MAPs in key regions of the neuron, thus permitting katanin to access the microtubules by dissociation of the MAPs (Figure 3). This would result in localized microtubule severing at sites where microtubules need to be particularly short and mobile such as growth cones and sites of collateral branch formation [19,20]. Indeed, overexpression of MARK ultimately leads to the disintegration of the microtubule array in a response that is quite reminiscent of that observed when katanin is overexpressed [12,14,21].

The question arises as to how the presence of MAPs on the microtubules might modulate katanin-induced severing. Is it simply a physical blockade or is the mechanism more complex? Katanin is thought to preferentially attack 'lattice flaws' [22]; thus the MAPs would not need to coat the entire surface of the microtubule to potentially prohibit katanin from accessing the most sensitive sites on the polymer. In addition, it is relevant to note that katanin is thought to form a hexamer that wraps around the microtubule so that the torque produced by ATP hydrolysis can break the lattice [23]. Contact with the microtubule greatly enhances the process of hexamerization, suggesting that the hexamer is actually built around the microtubule. Therefore, the density of MAPs on the microtubule might well prevent sufficient contact from being made by enough subunits for them to form a hexamer at a particular site along the microtubule (Figure 1).

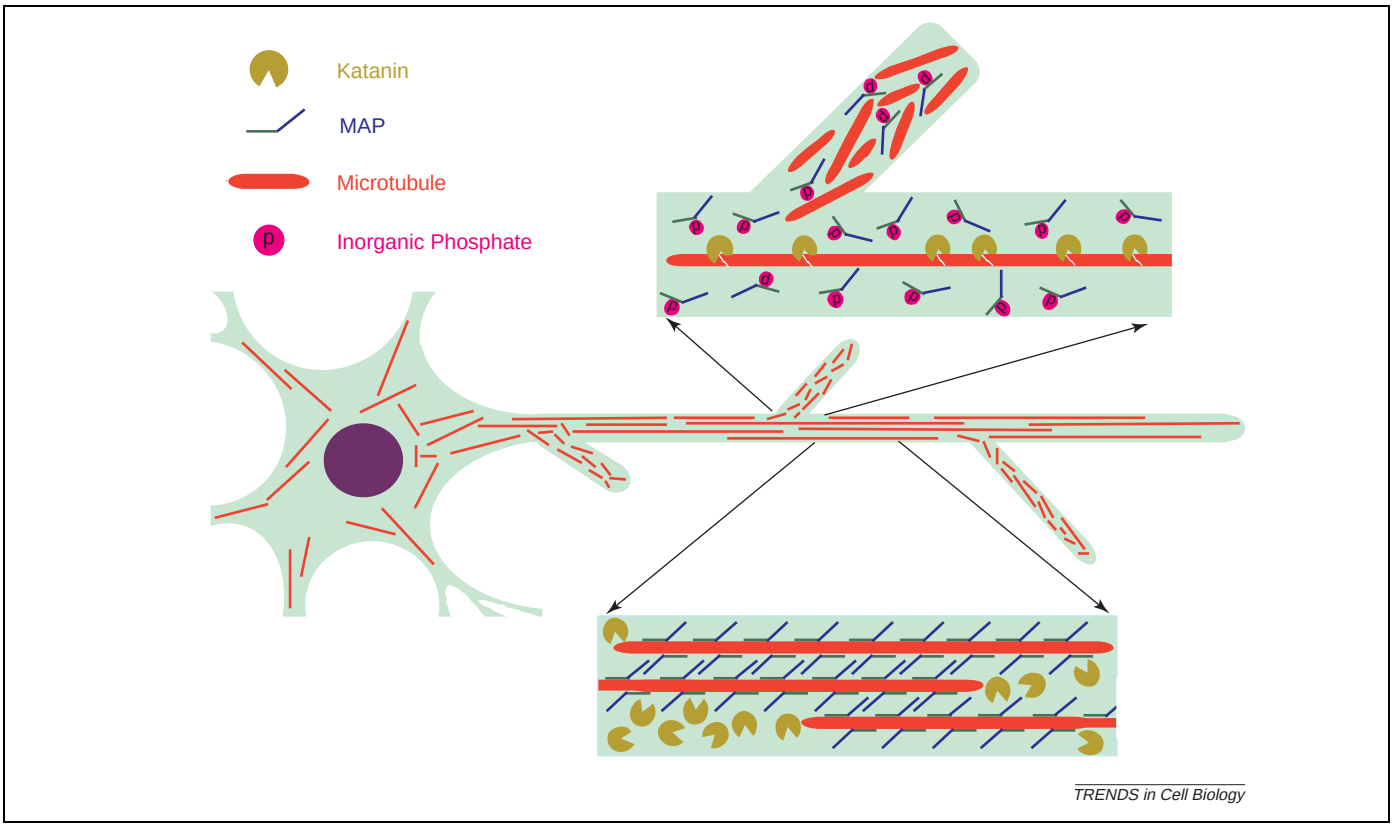

Figure 3. Model for local regulation of katanin access to the microtubule at sites of collateral branch formation along the axon. Microtubules within the axon are generally long but need to be locally severed at sites of branch formation so that short microtubules can move into the newly forming branch. Signals relevant to branch formation cause local phosphorylation of microtubule-associated proteins (MAPs) at the site of branch formation, causing the MAPs to dissociate from the microtubules in that discrete locale. As a result, the katanin can access the microtubules more readily, causing them to break.

Concluding remarks

Undoubtedly, a model in which MAPs regulate motor-based transport will raise some eyebrows. MAPs have been studied for decades, and there is an abundance of data that needs to be reconciled with the proposed model. For example, there are rather notable differences in MAP levels in various types of central and peripheral neurons, and sizable elevations at key phases of neuronal differentiation [24–28], and yet these fluctuations certainly do not cause organelle movements to cease. Perhaps the observations of Mandelkow and collaborators do not point to a normal means by which organelle transport is regulated but are only relevant to pathological conditions related to axonal degeneration. Alternatively, it is intriguing to contemplate that the levels of various cytoskeletal proteins and relevant kinases might be intimately co-regulated so that MAPs can, in fact, act as roadblocks that determine the frequency by which motor-based transport events (and katanin-based severing events) occur within the axon. Such a model would put MAP phosphorylation squarely at the heart of the various cellular events relevant to the generation, maintenance and pathological deterioration of neuronal polarity. Future studies will be required to further test these hypotheses in light of both the normal physiology of the neuron and axonal degeneration.

References

1 Rodriguez, O.C. *et al*. (2003) Conserved microtubule–actin interactions in cell movement and morphogenesis. *Nat. Cell Biol.* 5, 599–609
2 Schroer, T. (2004) Dynactin. *Annu. Rev. Cell Dev. Biol.* 20, 759–779
3 Weber, K.L. *et al*. (2004) A microtubule-binding myosin required for nuclear anchoring and spindle assembly. *Nature* 431, 325–329
4 Mandelkow, E.M. *et al*. (2004) MARK/PAR1 kinase is a regulator of microtubule-dependent transport in axons. *J. Cell Biol.* 167, 99–110
5 Ebneth, A. *et al*. (1998) Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. *J. Cell Biol.* 143, 777–794
6 Trinczek, B. *et al*. (1999) Tau regulates the attachment/detachment but not the speed of motors in microtubule-dependent transport of single vesicles and organelles. *J. Cell Sci.* 112, 2355–2367
7 Stamer, K. *et al*. (2002) Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. *J. Cell Biol.* 156, 1051–1063
8 Seitz, A. *et al*. (2002) Single-molecule investigation of the interference between kinesin, tau and MAP2c. *EMBO J.* 21, 4896–4905
9 von Massow, A. *et al*. (1989) Interaction between kinesin, microtubules, and microtubule-associated protein 2. *Cell Motil. Cytoskeleton* 14, 562–571
10 Ahmad, F.J. *et al*. (1999) An essential role for katanin in severing microtubules in the neuron. *J. Cell Biol.* 145, 305–315
11 Errico, A. *et al*. (2002) Spastin, the protein mutated in autosomal dominant hereditary spastic paraplegia, is involved in microtubule dynamics. *Hum. Mol. Genet.* 11, 153–163
12 Karabay, A. *et al*. (2004) Axonal growth is sensitive to the levels of katanin, a protein that severs microtubules. *J. Neurosci.* 24, 5778–5788
13 McNally, F.J. and Vale, R.D. (1993) Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell* 75, 419–429
14 Vale, R.D. (1991) Severing of stable microtubules by a mitotically activated protein in *Xenopus* egg extracts. *Cell* 64, 827–839
15 McNally, K.P. *et al*. (2002) Katanin-mediated microtubule severing can be regulated by multiple mechanisms. *Cell Motil. Cytoskeleton* 53, 337–349

16 Waterman-Storer, C.M. and Salmon, E.D. (1997) Actomyosin-based retrograde flow of microtubules in the lamella of migrating epithelial cells influences microtubule dynamic instability and turnover and is associated with microtubule breakage and treadmilling. *J. Cell Biol.* 139, 417–434
17 He, Y. *et al*. (2002) Microtubule reconfiguration during axonal retraction induced by nitric oxide. *J. Neurosci.* 22, 5982–5991
18 Janson, M.E. *et al*. (2003) Dynamic instability of microtubules is regulated by force. *J. Cell Biol.* 161, 1029–1034
19 Yu, W. *et al*. (1994) Microtubule fragmentation and partitioning in the axon during collateral branch formation. *J. Neurosci.* 14, 5872–5884
20 Dent, E. *et al*. (1999) Reorganization and movement of microtubules in growth cones and developing interstitial branches. *J. Neurosci.* 19, 8894–8908
21 Ebneth, A. *et al*. (1999) Phosphorylation of MAP2c and MAP4 by MARK kinases leads to the destabilization of microtubules in cells. *Cell Motil. Cytoskeleton* 44, 209–224
22 Davis, L.J. *et al*. (2002) The importance of lattice defects in katanin-mediated microtubule severing *in vitro*. *Biophys. J.* 82, 2916–2927
23 Hartman, J.J. and Vale, R.D. (1999) Microtubule disassembly by ATP-dependent oligomerization of the AAA enzyme katanin. *Science* 286, 782–785
24 Meixner, A. *et al*. (2000) MAP1B is required for axon guidance and is involved in the development of the central and peripheral nervous system. *J. Cell Biol.* 151, 1169–1178
25 Przyborski, S.A. and Cambray-Deakin, M.A. (1995) Developmental regulation of MAP2 variants during neuronal differentiation *in vitro*. *Brain Res. Dev. Brain Res.* 89, 187–201
26 Charriere-Bertrand, C. and Nunez, J. (1992) Regulation of tubulin, Tau and microtubule associated protein 2 expression during mouse brain development. *Neurochem. Int.* 21, 535–541
27 Mangin, G. *et al*. (1989) Timing of expression of tau and its encoding mRNAs in the developing cerebral neocortex and cerebellum of the mouse. *J. Neurochem.* 53, 45–50
28 Couchie, D. *et al*. (1988) Expression of the mRNA for tau proteins during brain development and in cultured neurons and astroglial cells. *J. Neurochem.* 50, 1894–1899

0962-8924/$ - see front matter
doi:10.1016/j.tcb.2005.02.001