

Chapter 2

Microtubules in the Nervous System

Nobuyuki Fukushima

Abstract Neurons undergo various morphologic changes during development, including neuritogenesis, neurite outgrowth, neurite branching, and neurite retraction. Many studies have examined how microtubules (MTs) are reorganized or transported within the neurites of developing neurons and have revealed that MT dynamics are regulated by MT-interacting proteins and motor proteins, in concert with actin microfilaments. Here, I will describe recent progress in research on the behavior of MTs in the nervous system.

Keywords Microtubule · Tubulin · Actin · Neurite · Growth cone · Polarity · Transport · Microtubule-associated protein

Contents

2.1	Introduction	55
2.2	Tubulin in the Brain	56
2.3	Microtubules in Neuronal Development	57
2.3.1	Neuritogenesis	58
2.3.2	Neurite Outgrowth	59
2.3.3	Neurite Branching	61
2.3.4	Neurite Retraction	63
2.4	Microtubules in Neuronal Injuries and Diseases	65
2.5	Concluding Remarks	66
	References	67

2.1 Introduction

Microtubules (MTs) are one component of the cytoskeleton and are composed of heterodimers of α - and β -tubulin. These α/β heterodimers are polymerized in a

N. Fukushima (✉)
Department of Life Science, Kinki University, Higashiosaka, Japan
e-mail: nfukushima@life.kindai.ac.jp

head-to-tail fashion, resulting in a polarity to the MT, with one end designated the plus end and the other the minus end. The plus ends of the MTs exhibit cycles of growth and shrinkage, a behavior that is termed *dynamic instability*. In contrast, the minus ends are relatively unstable and shrink without stabilization. MT functions are highly regulated by the intrinsic GTPase activity of tubulins as well as MT-interacting proteins, including MT-associated proteins (MAPs), MT-severing proteins, MT plus-end tracking proteins (+TIPs), and motor proteins (Dent and Gertler, 2003; Rodriguez et al., 2003; Akhmanova and Steinmetz, 2008; Jaworski et al., 2008). The interactions between MTs and MT-interacting proteins are influenced by diverse posttranslational modifications (PTMs), which include acetylation, tyrosination, detyrosination, and polyglutamylation, and play a role in neuronal development (Westermann and Weber, 2003; Hammond et al., 2008). Although I will discuss some topics related to tubulin PTMs in this chapter, the current progress in PTM research will be reviewed in detail elsewhere.

For many years, MTs and actin microfilaments (MFs) (Fukushima et al., 2009) were viewed as functionally separate. In neurons, MTs serve as a scaffold for organelle transport and structural components of the neurite that play an important role in neurite elongation. In contrast, MFs regulate growth cone morphology and motility and play a role in proper axon guidance. Many studies over the past 20 years have provided clear evidence that MTs structurally and functionally interact with MFs in developing neurons. For example, MTs themselves are transported in an axon or a growth cone in an actin-dependent manner. Furthermore, a growing number of actin–MT cross-linking proteins, which include some of the known MAPs and +TIPs, have been identified. In this chapter, I will address how MTs are involved in the development of neurites and also review recent reports showing how interactions between actin and MTs regulate the shape and motility of neurites.

2.2 Tubulin in the Brain

In mice, there are seven α -tubulin genes and eight β -tubulin genes. Each α -tubulin isoform shows more than 90% amino acid identity to other α -tubulins, and β -tubulins, with the exception of β 1-tubulin, show more than 90% amino acid identity to other β -tubulins, but only approximately 78% to β 1-tubulin. The expression of the tubulin isoforms in brain is developmentally regulated. The α 1-, α 2-, α 4-, α 6-, and α 8-tubulin and the β 2-, β 3-, β 4-, and β 5-tubulin isoforms are expressed in the brain (Lewis et al., 1985; Villasante et al., 1986; Stanchi et al., 2000). Levels of α 1- and α 2-tubulin expression remain constant during postnatal brain development. In contrast, expression of α 4-, β 3-, and β 4-tubulin increases during postnatal brain development, whereas that of β 2- and β 5-tubulin decreases (Lewis et al., 1985; Villasante et al., 1986). The developmental expression profile of α 8-tubulin remains to be determined. Because any combination of α - and β -tubulin isoforms can copolymerize, the combination of α/β -tubulin heterodimers is thought to vary during brain development. However, the actual isoforms constituting individual MTs in developing neurons have not been directly demonstrated.

2.3 Microtubules in Neuronal Development

Neurons display various morphologic changes during development in culture (da Silva and Dotti, 2002). After neuronal commitment, spherical neurons make membrane sprouts, which are transformed into neurites (neuritogenesis) and are extended as the neurons differentiate (neurite outgrowth) (Fig. 2.1). Extending neurites may generate branches (neurite branching), corresponding with axon collaterals or dendritic arbors, or exhibit transient retraction (neurite retraction) (Fig. 2.1). Each event overlaps functionally and structurally with other events, and there are currently no clear boundaries that have been used to classify each step. However, for the purposes

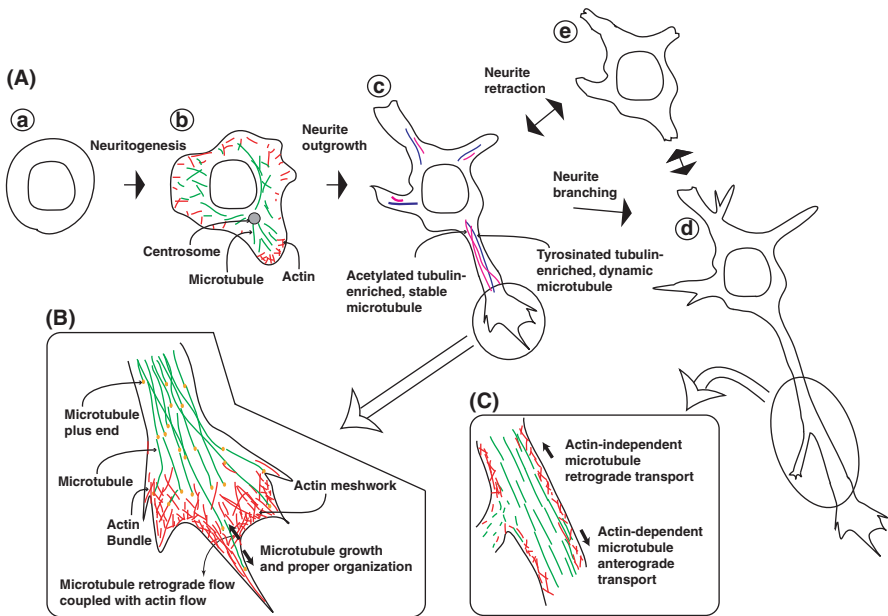


Fig. 2.1 Microtubule organization and dynamics in the developing neuron in culture. **a** and **b**; Shortly after plating, the spherical neurons produce protrusive membrane structures containing actin microfilaments (red) at the leading edges and microtubules (green) behind actin meshwork (neuritogenesis). **c**; These protrusions develop into several immature neurites and are guided by the advancement of motile growth cones as the neurons differentiate (neurite outgrowth). **d** and **e**; Extending neurites generate branches (neurite branching) and display transient retraction intrinsically or in response to extracellular repulsive signals (neurite retraction). Centrosome (green circle in **b**) localization or the presence of higher levels of stable microtubules (magenta in **c**) than dynamic microtubules (blue in **c**) is involved in the establishment of neuronal polarity. **b** The growth cone contains bundled microtubules in the center, an actin meshwork (red thin lines) in the lamellipodia, and actin bundles in filopodia. Dynamic microtubules extending from the C domain, which are oriented with the plus-ends (yellow) pointing to the periphery, grow along filopodial actin bundles (red thick lines) and move rearward coupled with actin retrograde flow. **c** Microtubules are bidirectionally transported within the axon. The anterograde microtubule transport is actin dependent, whereas the retrograde transport is actin independent. Short microtubules accumulate at the branching sites

of the current discussion, I will describe MT organization separately for these four events: neuritogenesis, neurite outgrowth, neurite branching, and neurite retraction.

2.3.1 Neuritogenesis

The roles of the cytoskeleton in neuritogenesis have been investigated in cultured neurons. Neurons initially form membrane sprouts consisting of MF-rich lamellipodia (Fig. 2.1) (Dotti et al., 1988; da Silva and Dotti, 2002). The actin cytoskeleton and its upstream modulators, including Rho, Rho-associated, coiled-coil containing protein kinase (ROCK), and profilin, have been well documented to be negative regulators of membrane sprouting (da Silva and Dotti, 2002). The membrane sprouts subsequently extend further to become short neurites containing MTs (Fig. 2.1). These processes clearly involve rearrangements of the cytoskeletal elements, which are thought to be mechanically and functionally integrated. Recent evidence using the neuronal cell line Neuro-2a showed that MAP2c, a juvenile MAP expressed in immature brains, is involved in neuritogenesis through accumulation and bundling of stable MTs (Dehmelt et al., 2003). This effect is reproduced by cotreatment of cells with the MT-polymerizing (stabilizing) drug paclitaxel and the actin-depolymerizing drug cytochalasin D, but not by treatment with paclitaxel alone. Thus, MAP2c seems to regulate the interactions between MTs and MFs, and not simply to cause MT stabilization. Indeed, it was reported that MAP2c binds not only MTs but also MFs via its MT-binding domain (Roger et al., 2004).

As multiple neurites extend during neuritogenesis, neurons choose one neurite as an axon and others as dendrites, leading to the establishment of neuronal polarity (Dotti et al., 1988). Such axon fate determination depends on intrinsic activities of both MTs and MFs in neurites. Local instability of the MF network within a single growth cone is known to determine neuronal polarization in cultured hippocampal neurons (Bradke and Dotti, 1999). Recently, MT stability has also been demonstrated to be a signal specifying neuronal polarization (Witte et al., 2008). Before the establishment of neuronal polarity or during neuritogenesis, the future axon contains more stable (acetylated) MTs versus dynamic (tyrosinated) MTs than do other neurites (Fig. 2.1). After establishment of polarity, the single axon still maintains higher levels of stable MTs. When neurons are treated with a glycogen synthase kinase-3 β (GSK-3 β) inhibitor that is a known inducer of multiple axons, many axon-like neurites contain stable MTs. In contrast, synapses of the amphid defective (SAD) kinase-deficient neurons that exhibit a loss of neuronal polarity have decreased levels of stable MTs in their neurites. Conversely, a low dose of paclitaxel induces the formation of multiple axons. In contrast, a low dose of the MT destabilizer nocodazole reduces dendrite formation without affecting axon formation, presumably due to the presence of more nocodazole-resistant, stable MTs in the axon and less in the dendrites. These observations suggest that changes in MT dynamics in neurites play a crucial role in the initial polarization of neuronal cells. Thus, the behavior of MTs and MFs must be coordinated to determine neuronal polarity. Although there is an increasing number of signaling molecules that

regulate the dynamics of MTs and MFs to affect neuronal polarity (Arimura and Kaibuchi, 2007), the molecular mechanisms are not fully understood.

Another study has demonstrated that the location of the centrosome, rather than cytoskeletal dynamics, may play an important role in establishment of neuronal polarity in the very early phase of neuritogenesis (de Anda et al., 2005). Shortly after plating hippocampal neurons on a substrate in culture, centrosomes, together with the Golgi apparatus and clusters of endosomes, accumulate beneath the first neurite that later develops into an axon (Fig. 2.1). Furthermore, suppression of centrosome function inhibits polarization and, conversely, neurons having multiple centrosomes develop multiple axons. Thus, localization of a functional centrosome is a key determinant in polarization, and MTs may be delivered only to the first neurite from the centrosome. However, the location of the centrosome is regulated by phosphoinositide 3-kinase (PI3K), Cdc42, and dynein (Palazzo et al., 2001; Etienne-Manneville and Hall, 2003; Arimura and Kaibuchi, 2007). Whether the location of the centrosome is cause or consequence is still under debate.

2.3.2 Neurite Outgrowth

2.3.2.1 Axon Outgrowth

Because extending axons are guided by the advancement of motile growth cones, it is important to understand the morphologic changes and their regulation through cytoskeletal rearrangements. In a growth cone, there are three distinct domains: the peripheral (P) domain composed of MF-rich filopodia and lamellipodia; the transitional (T) domain, an interface zone between the P domain and the central (C) domain; and the C domain composed of a thicker, MT-rich region containing organelles and vesicles and connected to the axon shaft (Fig. 2.1). Early studies have demonstrated that MFs are the primary elements that alter the growth cone shape and are essential for proper axon guidance, whereas MTs are essential to stabilize the axon structure and play an important role in axon elongation (Yamada et al., 1970, 1971).

Growing evidence suggests that MF–MT interactions in the growth cone play a role in the shape changes of the growth cone and in axon outgrowth (Dent et al., 1999; Rodriguez et al., 2003; Kalil and Dent, 2005). In the P domain, dynamic MTs are present and sometimes penetrate into and retract from the filopodia (Fig. 2.1). This restricted MT organization in the P domain is likely to be regulated by actin assemblies. Forscher and Smith showed that, in an *Aplysia* bag cell neuron, MTs rapidly advance into the P domain when actin networks are completely abolished by treatment with cytochalasin B, suggesting an inhibitory role for MF structures in MT advance (Forscher and Smith, 1988). Further studies demonstrated that MTs advance along actin bundles within the filopodia, and retrograde actin flow is coupled with MT retrograde movements observed in the P domain (Schaefer et al., 2002). Closer analyses have revealed that selective loss of filopodia, but not the actin network in the lamellipodia, by treatment with low doses of cytochalasin B

induces increased lateral MT movements and a more randomized MT distribution in the P domain (Burnette et al., 2007). These results indicate that actin bundles in the filopodia are essential for proper MT organization, but not MT advancement, in the P domain. Interestingly, when the actin bundles are locally disrupted in the P domain, growth cones respond with repulsive turning (Zhou et al., 2002). This turning is accompanied by reorientation of dynamic MTs within the P domain from areas where actin bundles are lacking into the regions where actin bundles remain.

MTs exploring the P domain exhibit properties of dynamic instability (Schaefer et al., 2002). Although the precise mechanisms of MT invasion into the P domain are not well understood, MF–MT interactions are presumably involved in MT growth toward the leading edge in the direction of the growth cone extension and axon outgrowth. Dynamic MTs invading the P domain are oriented with their plus-ends pointing toward the leading edge of the growth cone and preferentially interact with plus-end tracking proteins (+TIPs) (Akhmanova and Steinmetz, 2008; Jaworski et al., 2008) (Fig. 2.1). Indeed, growth cones have been shown to contain several +TIPs, including CLIP-associated protein 1 (CLASP1), CLASP2, adenomatous polyposis coli (APC), dynein, and Miller-Dieker lissencephaly-1 (LIS1) (Lee et al., 2004; Zhou et al., 2004; Grabham et al., 2007). CLASPs stabilize MTs and interact with other +TIPs, such as end-binding protein-1 (EB1) and cytoplasmic linker protein-170 (CLIP-170), and MFs (Mimori-Kiyosue et al., 2005). APC also has MT-stabilizing properties and the ability to interact with actin or signaling molecules that modulate actin polymerization (Zhou et al., 2004). Direct evidence implicating +TIPs in MT advance in the P domain comes from a study showing that dynein and LIS1 inhibition reduced penetration of MTs into the P domain during laminin-induced axon outgrowth (Grabham et al., 2007). This suggests that dynein and LIS1 cooperatively play a prominent role in MT advance within the growth cones during axon outgrowth. Whether these +TIPs bind MF remains unknown. Rather, dynein interacts with the actin cytoskeleton, presumably via dynactin, and LIS1 regulates MF organization by affecting signaling molecules, such as IQ motif-containing GTPase-activating protein (IQGAP) and Cdc42 (Ahmad et al., 2000; Kholmanskikh et al., 2006). Thus, +TIPs may regulate direct and indirect MF–MT interactions crucial for growth cone extension or turning.

In addition to growth cone advancement, generation of a new segment of the axon is essential for axon outgrowth and is achieved by bundling of MTs at the growth cone neck (Dent and Gertler, 2003). Very recently, myosin II–mediated lateral movements of actin arcs, contractile structures present in the T domain, have been shown to promote MT bundling and to transport the MTs into the C domain, resulting in the formation of a new segment of the axon shaft contiguous with the growth cone neck (Burnette et al., 2008). Although these experiments employed pharmacologic manipulations of *Aplysia* bag cell neurons, similar observations have been obtained using local application of the Ig superfamily cell surface molecule *aplysia* cell adhesion molecule (apCAM), a physiologic guidance cue, to the growth cone of *Aplysia* neurons (Schaefer et al., 2008). Stimulation of growth cones with apCAM induced activation of Rho/Rho kinase/myosin II signaling pathways and

resulted in MT movements in the growth cone neck and subsequent axonal outgrowth. This response is in contrast with the well-known observations that the Rho pathway is required for repulsive cue-induced neurite retraction (see later). Thus, this pathway must be locally and precisely controlled within the growth cones.

Recent studies have also revealed a new aspect of MT behavior in the shaft of the growing axon. A live-cell imaging study using rat sympathetic neurons showed that short MTs (a few micrometers in length) are transported down the axon and that the transport is bidirectional, rapid, infrequent, and highly asynchronous (Wang and Brown, 2002). Baas and colleagues demonstrated that in chick sensory neurons, the anterograde transport of short MTs is mediated by cytoplasmic dynein in concert with actin, whereas the retrograde transport is presumably dependent on the kinesin-5 family, but not actin (Fig. 2.1) (Hasaka et al., 2004; He et al., 2005; Baas et al., 2006). Interestingly, the motor activities of dynein and kinesin are in part regulated by tau, a MAP present in axons (Dixit et al., 2008). Retarded axonal extension in tau-deficient hippocampal neurons may be due to reduced MT transport by lack of tau-mediated regulation of motor protein activities (Dawson et al., 2001).

2.3.2.2 Dendrite Outgrowth

In contrast with our understanding of the mechanisms regulating development of the axon, our knowledge of the roles of MTs in dendrite development is limited (Georges et al., 2008). As discussed above, axon/dendrite fate determination is partially mediated through selective MT stabilization in the future axon and localization of the centrosome beneath the cell surface where the future axon will extend (de Anda et al., 2005; Witte et al., 2008). However, whether dendrite fate is determined by a default mechanism, as a result of axon fate determination, or an active mechanism, which selectively decreases MT stability in the future dendrites, remains unclear.

Unlike axonal MTs, which exhibit a unidirectional polarity with the plus-ends directed away from the cell body, dendrite MTs are oriented bidirectionally, although more distal dendrites contain unipolar MTs oriented similarly to those in axons (Baas et al., 1988). Thus, the cytoskeletal mechanisms underlying dendrite outgrowth may differ from those in axons. Because dendrite outgrowth and development is associated with dendrite branching, I will discuss this issue in the next section.

2.3.3 Neurite Branching

The formation of neurite branches is essential for the establishment of neuronal networks and requires remodeling of the cytoskeleton. Branch formation can occur by different modes: splitting, delayed, and interstitial (Acebes and Ferrus, 2000; Dent and Gertler, 2003). The splitting mode involves branch formation via concomitant growth cone bifurcation and is likely to be related to dendritic branch formation. The delayed mode is mechanistically linked to behaviors of the growth cone. In this

mode, the primary growth cones of axons enlarge during prolonged pausing behaviors to leave remnants behind on the axon shaft after the axon resumes forward extension, and these remnants subsequently give rise to branches. The interstitial mode consists of development of sprouts from a stable axon shaft.

Dent et al. examined MT reorganization during axonal branch formation, which resembled the delayed mode of neurite branching, in hamster cortical neurons (Dent and Kalil, 2001). Time-lapse imaging of neurons co-injected with fluorescently labeled tubulin and phalloidin revealed that dynamic MTs colocalize with MFs in transition regions of growth cones and at axon branch points. When neurons were treated with low doses of paclitaxel or nocodazole to inhibit MT dynamic instability, branch formation was prevented, whereas axonal elongation was not substantially affected. Similarly, treatment with cytochalasin D and latrunculin A, which attenuate MF dynamics, inhibited neurite branching. These data suggest that the coordinated dynamics of MTs and MFs regulate the delayed mode of branch formation.

In contrast, when chick sensory neurons are treated with low doses of paclitaxel or nocodazole, the growth of the axonal shaft is markedly suppressed, whereas interstitial branch formation is only minimally affected (Gallo and Letourneau, 1999). In addition, vinblastine treatment to inhibit MT repolymerization after depletion of MTs by a high dose of nocodazole results in the reappearance of branches. Therefore, branch formation is unlikely to be dependent on MT dynamics. Rather, transport of MT polymers appears to be involved in branch formation. Thus, the mechanism of MT reorganization in the interstitial mode of branch formation may be different from that of the delayed mode. However, the possibility that the mode of MT reorganization in axon branch formation varies depending on the neuronal cell type cannot be excluded.

Detailed analyses using electron micrographs and time-lapse imaging revealed that large numbers of short MTs are present, and that long MTs are absent, at the sites of axonal branches (Yu et al., 1994), suggesting that short MTs are transported into axon branches (Fig. 2.1). The questions to be resolved were the source of short MTs and the mechanism of their production. Baas and colleagues examined the behavior of spastin, an MT-severing protein concentrated at the sites of branch formation (Yu et al., 2008). They showed that exogenous expression of spastin results not only in production of short MT polymers but also in increases of the number of axonal branches. Generation of short MTs gives rise to an abundance of new MT ends, which could interact with various MT-interacting proteins, +TIPs, and motor proteins (Akhmanova and Steinmetz, 2008; Jaworski et al., 2008). These proteins may mediate anterograde transport of short MTs into branches, in concert with actin, which has been observed in the axon or growth cone (Hasaka et al., 2004; Grabham et al., 2007).

How guidance cues influence the cytoskeleton in axonal branch formation is still unknown. However, one report shows that some guidance cues change the organization and dynamics of the cytoskeleton at the growth cone and the axon shaft, resulting in promotion or suppression of branch formation without stimulation of axon elongation (Dent et al., 2004). FGF and netrin-1, which stimulate

branch formation, increase actin polymerization and formation of MT loops and splaying in growth cones. In contrast, semaphorin 3A, which inhibits branch formation, stimulated actin depolymerization and attenuated MT dynamics in growth cones. However, the signaling mechanisms by which these guidance cues regulate cytoskeletal dynamics and produce axon branching remain unclear.

Dendrites of most types of neurons are highly elaborated, and their outgrowth during development involves branch formation. A recent study has shown that stathmin, an MT destabilizer, is involved in dendrite branch formation (Ohkawa et al., 2007). Overexpression of stathmin in Purkinje cells leads to suppression of dendritic arborization. In contrast, inhibition of stathmin by neural activity and/or phosphorylation by calcium/calmodulin-dependent protein kinase increases dendritic arborization. However, knockdown of stathmin reduces dendrite growth. Thus, proper regulation of stathmin expression and activity is likely to be important in the development of dendritic arbors. Another study from the same laboratory has demonstrated that acetylated tubulin, present in stable MTs, plays a crucial role in dendrite branch formation (Ohkawa et al., 2008). These researchers identified an enzyme responsible for tubulin acetylation, an *N*-acetyltransferase complex consisting of ARD1 and NAD1, and found that inhibition of the complex limited dendrite branch formation in cortical neurons. Both studies implicate the importance of MT stability in dendrite branch formation. However, these results are in contrast with findings that MT stability determines the fate of the axon (Witte et al., 2008). Further investigation will be needed to understand how MTs are organized during dendrite growth.

2.3.4 Neurite Retraction

Transient neurite retraction is a fundamental process in neuronal network formation. This morphologic change occurs not only intrinsically but also in response to extracellular repulsive signals. Many studies have been conducted to further our understanding of the regulation of cytoskeletal rearrangements during repulsive signal-induced neurite retraction. Baas and colleagues have proposed an interesting model in which two motor proteins, dynein and myosin, are counterbalanced in their activities, and alterations in this balance determine whether the axon elongates or retracts (Baas and Ahmad, 2001; Baas et al., 2006). According to this model, dynein-dependent anterograde MT transport is predominant in growing neurites, and when neurites encounter repulsive signals, transient activation of actomyosin systems and the relative inhibition of dynein may result in enhancement of retrograde MT transport and neurite retraction. Indeed, nitric oxide (NO), a physiologic candidate for repulsive signals, induces neurite retraction that is reminiscent of that induced by dynein inhibition (Ahmad et al., 2000; He et al., 2002). Treatment of sensory neurons with an NO donor induces a rapid retraction of axons, characterized by an enlarged distal region, a thin trailing remnant, and unique sinusoidal MT structures. The NO-induced retraction is also accompanied by a lack of detectable changes in MT amounts and MT retrograde transport, and these effects

are not blocked by the MT stabilizer paclitaxel. These responses are similar to those observed in dynein-inhibited neurons (Ahmad et al., 2000). However, whether NO inhibits dynein or stimulates myosin remained to be determined.

A similar neurite retraction response is observed in cortical neurons treated with lysophosphatidic acid (LPA), a major lysophospholipid that exerts diverse cellular responses in many cell types (Fukushima et al., 2002; Fukushima and Morita, 2006). LPA causes neurite retraction through the Rho-actomyosin pathway in various neuronal cells (Fukushima, 2004). This effect is observed within 1~60 min after LPA exposure, depending on the cell types. Exposure of young cortical neurons that are initiating neurite extension to LPA results in neurite retraction accompanied by the rearrangement of MTs in neurites and the accumulation of MTs in cell bodies, without significant changes in the total amount of MTs in the cytoskeletal fraction of cultured neurons (Fukushima and Morita, 2006). These effects of LPA on MTs are blocked by pretreatment with inhibitors of the actomyosin and Rho pathways (cytochalasin D, blebbistatin and Y27632), but not by paclitaxel, although paclitaxel inhibits neurite retraction and MT depolymerization induced by nocodazole (Fig. 2.2). These observations suggest that LPA-induced MT rearrangement is not due to depolymerization of MTs and is dependent on actin polymerization during neurite remodeling.

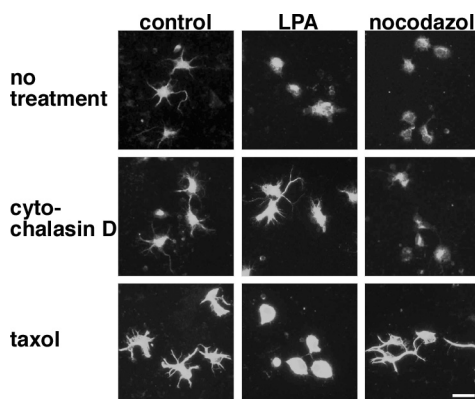


Fig. 2.2 Lysophosphatidic acid (LPA)-induced microtubule rearrangements in cultured cortical neurons. Cortical neurons were pretreated with 2 μ M cytochalasin D or 10 μ M paclitaxel for 5 min and exposed to vehicle, 1 μ M LPA, or 10 μ M nocodazole for 15 min. Then, cells were stained for α -tubulin. LPA-induced neurite retraction and concomitant microtubule rearrangements depend on actin polymerization. Bar = 20 μ m

Interestingly, two recent reports using MAP1B-deficient neurons have shown that MAP1B, which can cross-link MTs and MFs, is required for MT transport during NO- and LPA-induced neurite retraction (Bouquet et al., 2007; Stroissnigg et al., 2007). Exposure of neurons to an NO donor induces S-nitrosylation of MAP1B, leading to enhanced interaction with MTs. This interaction interferes with dynein motor function, resulting in neurite retraction. Whether LPA induces S-nitrosylation

is unknown. Rather, phosphorylation of MAP1B may be an important signal in LPA-induced neurite retraction, because LPA can activate many protein kinases including glycogen synthase kinase 3 β , which phosphorylates MAP1B, and phosphorylated MAP1B then alters the interaction between LIS1 and dynein (Jimenez-Mateos et al., 2005; Trivedi et al., 2005; Shano et al., 2008). Further investigation is needed to unveil the underlying mechanisms for LPA-induced neurite retraction.

2.4 Microtubules in Neuronal Injuries and Diseases

When injured, neurons respond with neurite regeneration or degeneration, depending on the property or intensity of the injury or the type of neuron. Generally, after injury, axons in the central nervous system (CNS) do not regenerate well, whereas those in the peripheral nervous system easily regenerate. A recent report has demonstrated that the state of MTs in the neurite tip after injury determines the ability of the neurite to regenerate (Erturk et al., 2007). When CNS axons are injured, the tips form swelled structures designated *retraction bulbs*, which contain disorganized MTs. MT stabilization inhibits the formation of retraction bulbs in CNS axons, resulting in axonal growth, whereas MT destabilization causes growth cones to transform into retraction bulb-like structures, resulting in restriction of axonal growth. The molecular mechanisms by which the MT state is controlled during nerve injury and regeneration remain unknown.

Another study has demonstrated that axotomy of *Aplysia* neurons induces the formation of swelling about 100 μ m proximal to the cut end (Erez et al., 2007). With time, the cut axonal end retracts and produces a flat lamellipodium, and a swelling develops at the center of the newly formed growth cone. During this process, MTs are reconstructed and play a role in vesicle transport into the swelling.

Many investigations have revealed that MTs and MAPs are involved in the onset and/or progression of neuronal diseases. For example, hyperphosphorylated tau is a well-known component of neurofibrillary tangles detected in Alzheimer's disease (Higuchi et al., 2002; Stoothoff and Johnson, 2005). Excellent reviews have discussed many other examples that have differential aspects of MTs in neuronal diseases, such as MT-based transport, MAP-related functions, and MT-targeted drugs (Benitez-King et al., 2004; Gerdes and Katsanis, 2005; Michaelis et al., 2005; Chevalier-Larsen and Holzbaur, 2006; Feng, 2006; Stokin and Goldstein, 2006; El-Kadi et al., 2007; Kerjan and Gleeson, 2007; Riederer, 2007; Sonnenberg and Liem, 2007). Here, I will discuss tubulin acetylation and mutations related to neuronal diseases.

Several lines of evidence have indicated that tubulin acetylation may be involved in neurodegenerative diseases, such as Huntington's disease (HD) and Parkinson's disease (PD) (Dompierre et al., 2007; Outeiro et al., 2007; Suzuki and Koike, 2007). HD is a neurodegenerative disorder characterized by cognitive and motor deficits. In HD, the MT-dependent transport of vesicles containing brain-derived neurotrophic factor (BDNF) is reduced, resulting in a reduction of trophic support and subsequent neuronal cell death. Pharmacologic inhibition of histone deacetylase

6 (HDAC6), a tubulin deacetylase, increases the level of acetylated MTs and concomitantly enhances BDNF transport in neuronal cell lines (Dompierre et al., 2007). Because this increased MT acetylation led to enhanced recruitment of kinesin-1 and dynein, these molecular motors are likely to be involved in BDNF transport. Interestingly, acetylated MT levels are reduced in HD patients, and the HDAC inhibitor trichostatin A (TSA) rescues the transport defect in neuronal cells carrying a polyglutamine expansion model of HD. These observations imply that HDAC inhibition and regulation of MT acetylation may be a therapeutic target in HD.

PD is characterized by motor deficits and loss of dopaminergic neurons, which may be induced by accumulation of α -synuclein in the midbrain. Inhibition of sirtuin 2/a mammalian homolog of yeast silent information regulator 2 (SIRT2), another tubulin deacetylase, results in protection of neurons from α -synuclein-induced cell death (Outeiro et al., 2007). SIRT2 inhibition also causes resistance to axonal degeneration in cerebellar granule neurons of mutant mice displaying slow Wallerian degeneration (Suzuki and Koike, 2007). However, in both cases, the mechanisms by which SIRT2 inhibition protects neurons from cell death remain unknown.

A recent mutagenesis study using mice has revealed that mutation in α 1-tubulin causes developmental brain abnormalities and that α 1-tubulin (*TUBA1A*) is a gene responsible for lissencephaly in human (Keays et al., 2007). Investigators screened mice treated with *N*-ethyl-*N*-nitrosourea, a strong mutagen, and identified a mutant mouse line that showed disorganized neuronal architecture of the hippocampus and cortex, impaired neuronal migration, and abnormal behavior, which resembled features observed in the lissencephaly mouse models (e.g., *Lis1*, *Dcx* mutants) (Paylor et al., 1999; Corbo et al., 2002). A mutation in the GTP-binding domain in α 1-tubulin was identified. Interestingly, several mutations in the human tubulin homolog were also found in patients with lissencephaly (Keays et al., 2007; Bahi-Buisson et al., 2008; Fallet-Bianco et al., 2008; Morris-Rosendahl et al., 2008), characterized by abnormal migration of neural progenitor cells in the cerebral cortex, although the location of these mutations differs from that in the mutant mice. The mutations in human may affect interactions of tubulin with MAPs.

2.5 Concluding Remarks

During the past two to three decades, growing evidence has changed our understanding of the roles and functions of MTs. MTs are more dynamic cytoskeletal components than previously understood and produce a variety of changes in neuronal morphology; they are not simple, stable, static structures to merely support neuronal shapes. As I described in this chapter, MTs function in concert with MFs and are transported in an MF-dependent manner. Similar interactions with intermediate filaments (IFs) also play a crucial role in MT functions. This observation is supported by the finding of MT-IF cross-linkers, such as the plakin family proteins (Leung et al., 2002; Sonnenberg and Liem, 2007). Moreover, the identification and analyses of various types of molecules mediating the interactions of MTs with

MFs or other signaling molecules, such as +TIPs, suggest that MTs are the final acceptors of extracellular signals (Gundersen and Cook, 1999; Hollenbeck, 2001; Galjart, 2005; Akhmanova and Steinmetz, 2008). A good example has recently been published from two independent laboratories, which showed that MTs are also present in MF-rich dendritic spines that are correlated with neural development and activity-dependent plasticity, and the localization of MTs is mediated via the +TIP EB3 (Gu et al., 2008; Hu et al., 2008). A more systematic and integrated analysis will help us to understand MT functions and dynamics in the nervous system in development and disease.

References

- Acebes A, Ferrus A (2000) Cellular and molecular features of axon collaterals and dendrites. *Trends Neurosci* 23:557–565
- Ahmad FJ, Hughey J, Wittmann T, Hyman A, Greaser M, Baas PW (2000) Motor proteins regulate force interactions between microtubules and microfilaments in the axon. *Nat Cell Biol* 2: 276–280
- Akhmanova A, Steinmetz MO (2008) Tracking the ends: a dynamic protein network controls the fate of microtubule tips. *Nat Rev Mol Cell Biol* 9:309–322
- Arimura N, Kaibuchi K (2007) Neuronal polarity: from extracellular signals to intracellular mechanisms. *Nat Rev Neurosci* 8:194–205
- Baas PW, Ahmad FJ (2001) Force generation by cytoskeletal motor proteins as a regulator of axonal elongation and retraction. *Trends Cell Biol* 11:244–249
- Baas PW, Deitch JS, Black MM, Banker GA (1988) Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. *Proc Natl Acad Sci USA* 85:8335–8339
- Baas PW, Vidya Nadar C, Myers KA (2006) Axonal transport of microtubules: the long and short of it. *Traffic* 7:490–498
- Bahi-Buisson N, Poirier K, Boddaert N, Saillour Y, Castelnau L, Philip N, Buyse G, Villard L, Joriot S, Marret S, Bourgeois M, Van Esch H, Lagae L, Amiel J, Hertz-Pannier L, Roubertie A, Rivier F, Pinard JM, Beldjord C, Chelly J (2008) Refinement of cortical dysgeneses spectrum associated with TUBA1A mutations. *J Med Genet* 45:647–653
- Benitez-King G, Ramirez-Rodriguez G, Ortiz L, Meza I (2004) The neuronal cytoskeleton as a potential therapeutical target in neurodegenerative diseases and schizophrenia. *Curr Drug Targets CNS Neurol Disord* 3:515–533
- Bouquet C, Ravaille-Veron M, Propst F, Nothias F (2007) MAP1B coordinates microtubule and actin filament remodeling in adult mouse Schwann cell tips and DRG neuron growth cones. *Mol Cell Neurosci* 36:235–247
- Bradke F, Dotti CG (1999) The role of local actin instability in axon formation. *Science* 283: 1931–1934
- Burnette DT, Ji L, Schaefer AW, Medeiros NA, Danuser G, Forscher P (2008) Myosin II activity facilitates microtubule bundling in the neuronal growth cone neck. *Dev Cell* 15:163–169
- Burnette DT, Schaefer AW, Ji L, Danuser G, Forscher P (2007) Filopodial actin bundles are not necessary for microtubule advance into the peripheral domain of *Aplysia* neuronal growth cones. *Nat Cell Biol* 9:1360–1369
- Chevalier-Larsen E, Holzbaur EL (2006) Axonal transport and neurodegenerative disease. *Biochim Biophys Acta* 1762:1094–1108
- Corbo JC, Deuel TA, Long JM, LaPorte P, Tsai E, Wynshaw-Boris A, Walsh CA (2002) Doublecortin is required in mice for lamination of the hippocampus but not the neocortex. *J Neurosci* 22:7548–7557

- da Silva JS, Dotti CG (2002) Breaking the neuronal sphere: regulation of the actin cytoskeleton in neurogenesis. *Nat Rev Neurosci* 3:694–704
- Dawson HN, Ferreira A, Eyster MV, Ghoshal N, Binder LI, Vitek MP (2001) Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. *J Cell Sci* 114:1179–1187
- de Anda FC, Pollarolo G, Da Silva JS, Camoletto PG, Feiguin F, Dotti CG (2005) Centrosome localization determines neuronal polarity. *Nature* 436:704–708
- Dehmelt L, Smart FM, Ozer RS, Halpain S (2003) The role of microtubule-associated protein 2c in the reorganization of microtubules and lamellipodia during neurite initiation. *J Neurosci* 23:9479–9490
- Dent EW, Barnes AM, Tang F, Kalil K (2004) Netrin-1 and semaphorin 3A promote or inhibit cortical axon branching, respectively, by reorganization of the cytoskeleton. *J Neurosci* 24:3002–3012
- Dent EW, Callaway JL, Szebenyi G, Baas PW, Kalil K (1999) Reorganization and movement of microtubules in axonal growth cones and developing interstitial branches. *J Neurosci* 19:8894–8908
- Dent EW, Gertler FB (2003) Cytoskeletal dynamics and transport in growth cone motility and axon guidance. *Neuron* 40:209–227
- Dent EW, Kalil K (2001) Axon branching requires interactions between dynamic microtubules and actin filaments. *J Neurosci* 21:9757–9769
- Dixit R, Ross JL, Goldman YE, Holzbaur EL (2008) Differential regulation of dynein and kinesin motor proteins by tau. *Science* 319:1086–1089
- Domper JP, Godin JD, Charrin BC, Cordelieres FP, King SJ, Humbert S, Saudou F (2007) Histone deacetylase 6 inhibition compensates for the transport deficit in Huntington's disease by increasing tubulin acetylation. *J Neurosci* 27:3571–3583
- Dotti CG, Sullivan CA, Banker GA (1988) The establishment of polarity by hippocampal neurons in culture. *J Neurosci* 8:1454–1468
- El-Kadi AM, Soura V, Hafezparast M (2007) Defective axonal transport in motor neuron disease. *J Neurosci Res* 85:2557–2566
- Erez H, Malkinson G, Prager-Khoutorsky M, De Zeeuw CI, Hoogenraad CC, Spira ME (2007) Formation of microtubule-based traps controls the sorting and concentration of vesicles to restricted sites of regenerating neurons after axotomy. *J Cell Biol* 176:497–507
- Erturk A, Hellal F, Enes J, Bradke F (2007) Disorganized microtubules underlie the formation of retraction bulbs and the failure of axonal regeneration. *J Neurosci* 27:9169–9180
- Etienne-Manneville S, Hall A (2003) Cell polarity: Par6, aPKC and cytoskeletal crosstalk. *Curr Opin Cell Biol* 15:67–72
- Fallet-Bianco C, Loeuillet L, Poirier K, Loget P, Chapon F, Pasquier L, Saillour Y, Beldjord C, Chelly J, Francis F (2008) Neuropathological phenotype of a distinct form of lissencephaly associated with mutations in TUBA1A. *Brain* 131:2304–2320
- Feng J (2006) Microtubule: a common target for parkin and Parkinson's disease toxins. *Neuroscientist* 12:469–476
- Forscher P, Smith SJ (1988) Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. *J Cell Biol* 107:1505–1516
- Fukushima N (2004) LPA in neural cell development. *J Cell Biochem* 92:993–1003
- Fukushima N, Furuta D, Hidaka Y, Moriyama R, Tsujiuchi T (2009) Posttranslational modifications of tubulin in the nervous system. *J Neurochem* 109:683–693
- Fukushima N, Morita Y (2006) Actomyosin-dependent microtubule rearrangement in lysophosphatidic acid-induced neurite remodeling of young cortical neurons. *Brain Res* 1094:65–75
- Fukushima N, Weiner JA, Kaushal D, Contos JJA, Rehen SK, Kingsbury MA, Kim K-Y, Chun J (2002) Lysophosphatidic acid influences the morphology and motility of young, postmitotic cortical neurons. *Mol Cell Neurosci* 20:271–282
- Galjart N (2005) CLIPs and CLASPs and cellular dynamics. *Nat Rev Mol Cell Biol* 6:487–498
- Gallo G, Letourneau PC (1999) Different contributions of microtubule dynamics and transport to the growth of axons and collateral sprouts. *J Neurosci* 19:3860–3873

- Georges PC, Hadzimichalis NM, Sweet ES, Firestein BL (2008) The yin-yang of dendrite morphology: unity of actin and microtubules. *Mol Neurobiol* 38:270–284
- Gerdes JM, Katsanis N (2005) Small molecule intervention in microtubule-associated human disease. *Hum Mol Genet* 14(Spec No 2):R291–R300
- Grabham PW, Seale GE, Bennecib M, Goldberg DJ, Vallee RB (2007) Cytoplasmic dynein and LIS1 are required for microtubule advance during growth cone remodeling and fast axonal outgrowth. *J Neurosci* 27:5823–5834
- Gu J, Firestein BL, Zheng JQ (2008) Microtubules in dendritic spine development. *J Neurosci* 28:12120–12124
- Gundersen GG, Cook TA (1999) Microtubules and signal transduction. *Curr Opin Cell Biol* 11:81–94
- Hammond JW, Cai D, Verhey KJ (2008) Tubulin modifications and their cellular functions. *Curr Opin Cell Biol* 20:71–76
- Hasaka TP, Myers KA, Baas PW (2004) Role of actin filaments in the axonal transport of microtubules. *J Neurosci* 24:11291–11301
- He Y, Francis F, Myers KA, Yu W, Black MM, Baas PW (2005) Role of cytoplasmic dynein in the axonal transport of microtubules and neurofilaments. *J Cell Biol* 168:697–703
- He Y, Yu W, Baas PW (2002) Microtubule reconfiguration during axonal retraction induced by nitric oxide. *J Neurosci* 22:5982–5991
- Higuchi M, Lee VM, Trojanowski JQ (2002) Tau and axonopathy in neurodegenerative disorders. *Neuromolecular Med* 2:131–150
- Hollenbeck P (2001) Cytoskeleton: microtubules get the signal. *Curr Biol* 11:R820–R823
- Hu X, Viesselmann C, Nam S, Merriam E, Dent EW (2008) Activity-dependent dynamic microtubule invasion of dendritic spines. *J Neurosci* 28:13094–13105
- Jaworski J, Hoogenraad CC, Akhmanova A (2008) Microtubule plus-end tracking proteins in differentiated mammalian cells. *Int J Biochem Cell Biol* 40:619–637
- Jimenez-Mateos EM, Wandosell F, Reiner O, Avila J, Gonzalez-Billault C (2005) Binding of microtubule-associated protein 1B to LIS1 affects the interaction between dynein and LIS1. *Biochem J* 389:333–341
- Kalil K, Dent EW (2005) Touch and go: guidance cues signal to the growth cone cytoskeleton. *Curr Opin Neurobiol* 15:521–526
- Keays DA, Tian G, Poirier K, Huang GJ, Siebold C, Cleak J, Oliver PL, Fray M, Harvey RJ, Molnar Z, Pinon MC, Dear N, Valdar W, Brown SD, Davies KE, Rawlins JN, Cowan NJ, Nolan P, Chelly J, Flint J (2007) Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. *Cell* 128:45–57
- Kerjan G, Gleeson JG (2007) Genetic mechanisms underlying abnormal neuronal migration in classical lissencephaly. *Trends Genet* 23:623–630
- Kholmanskikh SS, Koeller HB, Wynshaw-Boris A, Gomez T, Letourneau PC, Ross ME (2006) Calcium-dependent interaction of Lis1 with IQGAP1 and Cdc42 promotes neuronal motility. *Nat Neurosci* 9:50–57
- Lee H, Engel U, Rusch J, Scherrer S, Sheard K, Van Vactor D (2004) The microtubule plus end tracking protein Orbit/MAST/CLASP acts downstream of the tyrosine kinase Abl in mediating axon guidance. *Neuron* 42:913–926
- Leung CL, Green KJ, Liem RK (2002) Plakins: a family of versatile cytolinker proteins. *Trends Cell Biol* 12:37–45
- Lewis SA, Lee MG, Cowan NJ (1985) Five mouse tubulin isotypes and their regulated expression during development. *J Cell Biol* 101:852–861
- Michaelis ML, Seyb KI, Ansar S (2005) Cytoskeletal integrity as a drug target. *Curr Alzheimer Res* 2:227–229
- Mimori-Kiyosue Y, Grigoriev I, Lansbergen G, Sasaki H, Matsui C, Severin F, Galjart N, Grosveld F, Vorobjev I, Tsukita S, Akhmanova A (2005) CLASP1 and CLASP2 bind to EB1 and regulate microtubule plus-end dynamics at the cell cortex. *J Cell Biol* 168:141–153

- Morris-Rosendahl DJ, Najm J, Lachmeijer AM, Sztriha L, Martins M, Kuechler A, Haug V, Zeschneigk C, Martin P, Santos M, Vasconcelos C, Omran H, Kraus U, Van der Knaap MS, Schuierer G, Kutsche K, Uyanik G (2008) Refining the phenotype of α -*Ia Tubulin* (*TUBA1A*) mutation in patients with classical lissencephaly. *Clin Genet* 74: 425–433
- Ohkawa N, Fujitani K, Tokunaga E, Furuya S, Inokuchi K (2007) The microtubule destabilizer stathmin mediates the development of dendritic arbors in neuronal cells. *J Cell Sci* 120: 1447–1456
- Ohkawa N, Sugisaki S, Tokunaga E, Fujitani K, Hayasaka T, Setou M, Inokuchi K (2008) N-acetyltransferase ARD1-NAT1 regulates neuronal dendritic development. *Genes Cells* 13:1171–1183
- Outeiro TF, Kontopoulos E, Altmann SM, Kufareva I, Strathearn KE, Amore AM, Volk CB, Maxwell MM, Rochet JC, McLean PJ, Young AB, Abagyan R, Feany MB, Hyman BT, Kazantsev AG (2007) Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease. *Science* 317:516–519
- Palazzo AF, Joseph HL, Chen YJ, Dujardin DL, Alberts AS, Pfister KK, Vallee RB, Gundersen GG (2001) Cdc42, dynein, and dynactin regulate MTOC reorientation independent of Rho-regulated microtubule stabilization. *Curr Biol* 11:1536–1541
- Paylor R, Hirotsune S, Gambello MJ, Yuva-Paylor L, Crawley JN, Wynshaw-Boris A (1999) Impaired learning and motor behavior in heterozygous Pafah1b1 (Lis1) mutant mice. *Learn Mem* 6:521–537
- Riederer BM (2007) Microtubule-associated protein 1B, a growth-associated and phosphorylated scaffold protein. *Brain Res Bull* 71:541–558
- Rodriguez OC, Schaefer AW, Mandato CA, Forscher P, Bement WM, Waterman-Storer CM (2003) Conserved microtubule-actin interactions in cell movement and morphogenesis. *Nat Cell Biol* 5:599–609
- Roger B, Al-Bassam J, Dehmelt L, Milligan RA, Halpain S (2004) MAP2c, but not tau, binds and bundles F-actin via its microtubule binding domain. *Curr Biol* 14:363–371
- Schaefer AW, Kabir N, Forscher P (2002) Filopodia and actin arcs guide the assembly and transport of two populations of microtubules with unique dynamic parameters in neuronal growth cones. *J Cell Biol* 158:139–152
- Schaefer AW, Schoonderwoert VT, Ji L, Medeiros N, Danuser G, Forscher P (2008) Coordination of actin filament and microtubule dynamics during neurite outgrowth. *Dev Cell* 15:146–162
- Shano S, Hatanaka K, Ninose S, Moriyama R, Tsujiuchi T, Fukushima N (2008) A lysophosphatidic acid receptor lacking the PDZ-binding domain is constitutively active and stimulates cell proliferation. *Biochim Biophys Acta* 1783:748–759
- Sonnenberg A, Liem RK (2007) Plakins in development and disease. *Exp Cell Res* 313:2189–2203
- Stanchi F, Corso V, Scannapieco P, Ievolella C, Negrisola E, Tiso N, Lanfranchi G, Valle G (2000) TUBA8: a new tissue-specific isoform of α -tubulin that is highly conserved in human and mouse. *Biochem Biophys Res Commun* 270:1111–1118
- Stokin GB, Goldstein LS (2006) Axonal transport and Alzheimer's disease. *Annu Rev Biochem* 75:607–627
- Stoothoff WH, Johnson GV (2005) Tau phosphorylation: physiological and pathological consequences. *Biochim Biophys Acta* 1739:280–297
- Stroissnigg H, Trancikova A, Descovich L, Fuhrmann J, Kutschera W, Kostan J, Meixner A, Nothias F, Propst F (2007) S-nitrosylation of microtubule-associated protein 1B mediates nitric-oxide-induced axon retraction. *Nat Cell Biol* 9:1035–1045
- Suzuki K, Koike T (2007) Mammalian Sir2-related protein (SIRT) 2-mediated modulation of resistance to axonal degeneration in slow Wallerian degeneration mice: a crucial role of tubulin deacetylation. *Neuroscience* 147:599–612
- Trivedi N, Marsh P, Goold RG, Wood-Kaczmar A, Gordon-Weeks PR (2005) Glycogen synthase kinase-3 β phosphorylation of MAP1B at Ser1260 and Thr1265 is spatially restricted to growing axons. *J Cell Sci* 118:993–1005

- Villasante A, Wang D, Dobner P, Dolph P, Lewis SA, Cowan NJ (1986) Six mouse α -tubulin mRNAs encode five distinct isoforms: testis-specific expression of two sister genes. *Mol Cell Biol* 6:2409–2419
- Wang L, Brown A (2002) Rapid movement of microtubules in axons. *Curr Biol* 12:1496–1501
- Westermann S, Weber K (2003) Post-translational modifications regulate microtubule function. *Nat Rev Mol Cell Biol* 4:938–947
- Witte H, Neukirchen D, Bradke F (2008) Microtubule stabilization specifies initial neuronal polarization. *J Cell Biol* 180:619–632
- Yamada KM, Spooner BS, Wessells NK (1970) Axon growth: roles of microfilaments and microtubules. *Proc Natl Acad Sci USA* 66:1206–1212
- Yamada KM, Spooner BS, Wessells NK (1971) Ultrastructure and function of growth cones and axons of cultured nerve cells. *J Cell Biol* 49:614–635
- Yu W, Ahmad FJ, Baas PW (1994) Microtubule fragmentation and partitioning in the axon during collateral branch formation. *J Neurosci* 14:5872–5884
- Yu W, Qiang L, Solowska JM, Karabay A, Korulu S, Baas PW (2008) The microtubule-severing proteins spastin and katanin participate differently in the formation of axonal branches. *Mol Biol Cell* 19:1485–1498
- Zhou FQ, Waterman-Storer CM, Cohan CS (2002) Focal loss of actin bundles causes microtubule redistribution and growth cone turning. *J Cell Biol* 157:839–849
- Zhou FQ, Zhou J, Dedhar S, Wu YH, Snider WD (2004) NGF-induced axon growth is mediated by localized inactivation of GSK-3 β and functions of the microtubule plus end binding protein APC. *Neuron* 42:897–912

Cytoskeleton of the Nervous System

Nixon, R.A.; Yuan, A. (Eds.)

2011, XV, 774 p., Hardcover

ISBN: 978-1-4419-6786-2