CHAPTER

6

The Cytoskeleton of Neurons and Glia

Gustavo Pigino, Yuyu Song, Laura L. Kirkpatrick, Scott T. Brady

OUTLINE

INTRODUCTION

Neurons and glia exhibit a remarkable diversity of shapes. These different morphologies are so characteristic and distinctive that they have been used since the time of Cajal to define neural functions. For example, Purkinje cells in the cerebellum have such distinctive morphologies that they are readily identifiable in any vertebrate. Neurons do not divide, so their distinctive morphologies are maintained throughout life. Biochemical and immunological markers are used to delineate neuronal populations. Their distributions are found to correlate well with populations previously defined on morphological grounds. This evidence indicates that the shape of cells in the nervous system is closely connected to their functions. Understanding the proteins and cellular structures that underlie cell morphology is thus essential for understanding the neural functions.

Proteins of the cytoskeleton play a central role in the creation and maintenance of cell shapes in all tissues. They serve multiple roles in eukaryotic cells. First, they provide structural organization for the cell interior, helping to establish metabolic compartments. Second, cytoskeletal structures serve as tracks for intracellular transport, which creates and maintains differentiated cellular functions. Finally, the cytoskeleton comprises the core framework of cellular morphologies.

Methods for visualizing individual neurons and glia *in vivo* have depended for more than 100 years on histochemical reactions with cytoskeletal elements, and even now these methods have not been surpassed. Because cytoskeletal structures play a particularly prominent role in the nervous

system, cytoskeletal proteins represent a large fraction of total brain protein, comprising perhaps a third or more of the total. In fact, much of our knowledge about cytoskeletal biochemistry is based on studies of proteins purified from brain. The aims of this chapter are twofold: first to provide an introduction to the cytoskeletal elements themselves and second to examine their role in neuronal and glial function. Throughout, the emphasis will be on the cytoskeleton as a vital, dynamic component of the nervous system.

MOLECULAR COMPONENTS OF THE NEURONAL CYTOSKELETON

Along with the nucleus and mitochondria, the cytoskeleton is one of several biological structures that define eukaryotic cells

The term 'cytoskeleton' is often used as if it described a single, unified structure, but the cytoskeleton of neurons and other eukaryotic cells comprises three distinct, interacting structural complexes that have very different properties: microtubules (MTs), neurofilaments (NFs) and microfilaments (MFs). Each has a characteristic composition, structure and organization that may be further specialized in a particular cell type or subcellular domain. The defining structural elements have long been identifiable in electron micrographs (Fig. 6-1), and a considerable amount is known about the detailed organization of these components in neurons and glia. Each set of cytoskeletal structures will be considered in turn.

Microtubules act as both dynamic structural elements and tracks for organelle traffic

Neuronal MTs are structurally similar to those found in other eukaryotic cells (reviewed in Amos & Schlieper, 2005; Hyams & Lloyd, 1994; Wade, 2007, 2009). The core structure is a polymer of 50 kDa tubulin subunits. Heterodimers of α-and β-tubulin align end to end to form protofilaments, 13 of which join laterally to form a hollow tube with an outer diameter

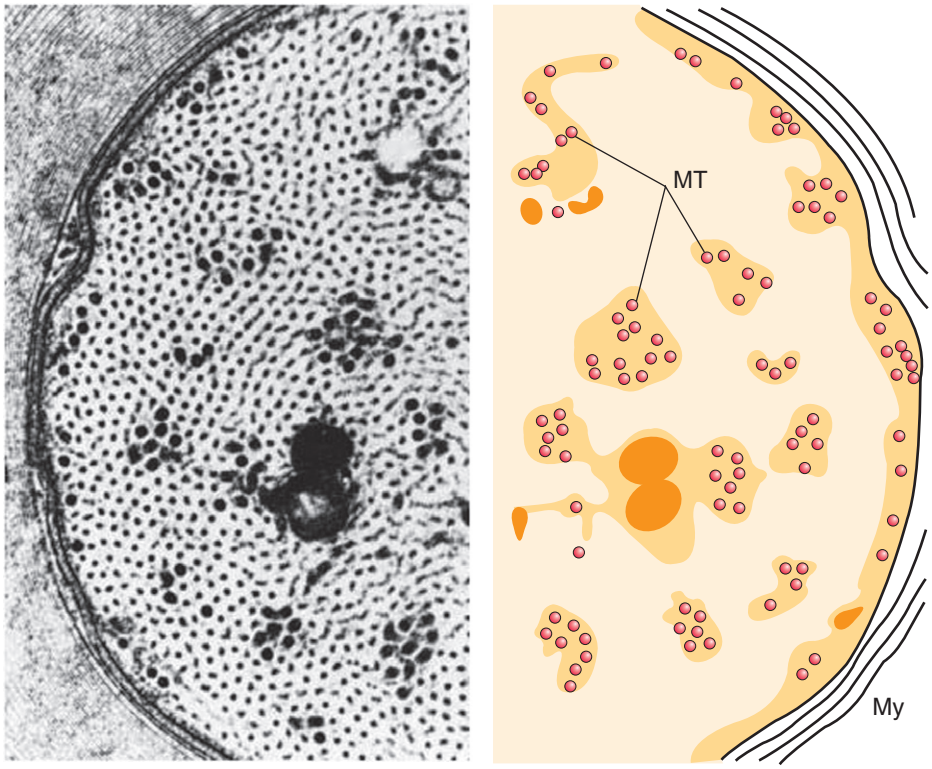

FIGURE 6-1 **The cytoskeleton and organization of the axon in cross-section. Left.** Electron micrograph of a myelinated toad axon in cross-section taken near a Schmidt–Lanterman cleft; axon diameter is slightly reduced and the different domains within the axoplasm are emphasized. **Right.** Diagram highlighting key features of the axoplasm. Portions of the myelin sheath surrounding the axon can be seen (*My*). Most of the axonal diameter is taken up by the neurofilaments (*clear area*). There is a minimum distance between neurofilaments and other cytoskeletal structures that is determined by the side arms of the neurofilaments. (These side arms are visible between some of the neurofilaments in the electron micrograph, **left.**) The microtubules (*MT*) tend to be found in bundles and are more irregularly spaced. They are surrounded by a fuzzy material that is also visible in the region just below the plasma membrane (*stippled areas*, **right**). These areas are thought to be enriched in actin microfilaments and presumably contain other slow component b (SCb) proteins as well. The *stippled regions* with embedded microtubules are also the location of membranous organelles in fast axonal transport (*larger, filled, irregular shapes*, **right**). Both microtubule and microfilament networks need to be intact for the efficient movement of organelles in fast transport. (Electron micrograph provided by Dr Alan Hodge. From Hodge, A. and Adelman, W. In: *Structure and Function in Excitable Cells*. New York: Plenum, 1983, pp. 75–111, with permission.)

of 25 nm (Fig. 6-2). Examples also exist of MTs with 12 and 14 protofilaments. The α- and β-tubulins are the best-known members of a unique protein family that have significant sequence similarity (Burns & Surridge, 1994). There is approximately 40% sequence identity between α- and β-tubulins, and even greater identity within α and β gene subfamilies (Luduena, 1998). Conservation of primary sequence for tubulins is also high across species, so tubulins from yeast can readily coassemble with tubulins from human brain. Tubulin dimers bind two GTPs and exhibit GTPase activity that is closely linked to assembly and disassembly of microtubules (Amos & Schlieper, 2005; Desai & Mitchison, 1997). While many questions remain about tubulin and its interactions, the structure of the αβ-tubulin dimer has recently been derived from electron diffraction studies (Nogales et al., 1998), providing a basis for dissection of MT functional architecture.

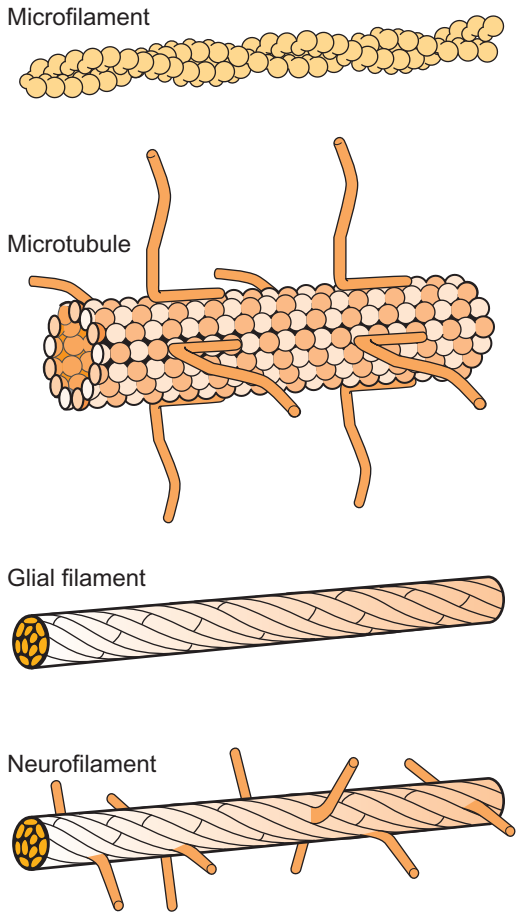

FIGURE 6-2 **Microfilaments, microtubules and intermediate filaments in the nervous system.** Each cytoskeletal structure has a distinctive ultrastructure. This schematic illustrates the major features of the core fibrils. The microfilament consists of two strands of actin subunits twisted around each other like strings of pearls. The individual subunits are asymmetrical, globular proteins that give the microfilament its polarity. The microtubule is also made from globular subunits, but in this case the basic building block is a heterodimer of α- and β-tubulins. These αβ dimers are organized into linear strands, or protofilaments, with β-tubulin subunits oriented toward the plus end of the microtubule. Protofilaments form sheets *in vitro* that roll up into a cylinder with 13 protofilaments forming the wall of the microtubule. Assembly of both microfilaments and microtubules is coupled to slow nucleotide hydrolysis, ATP for microfilaments and GTP for microtubules. The subunits of both glial filaments and neurofilaments are rod-shaped molecules that will self-assemble without nucleotides. The core filament structure is thought to be a ropelike arrangement of individual subunits. Glial filaments are typical type III intermediate filaments in that they form homopolymers without side arms. In contrast, neurofilaments are heteropolymers formed from three subunits, NFH, NFM and NFL for the high-, medium- and low-molecular-weight subunits. The NFH and NFM subunits have extended carboxy-terminal tails that project from the sides of the core filament and may be heavily phosphorylated.

Heterodimers in a MT are oriented in the same direction, so the resulting MT has asymmetric ends that differ in their assembly properties (Amos & Schlieper, 2005; Desai & Mitchison, 1997; Wade, 2007). The β-tubulin subunit is exposed at the 'plus' end, which is the preferred end for addition of tubulin dimers. The opposite 'minus' end grows more slowly at physiological concentrations of tubulin. In the case of free microtubules, the balance between assembly and disassembly at each end defines a critical concentration for net microtubule growth. MT assembly under *in vitro* conditions involves a slow nucleation step followed by a more rapid net growth phase; a kinetic pattern described as 'dynamic instability.' In glia and most other non-neuronal cells, however, the minus ends of MTs are usually bound at the site of nucleation, which is associated with the centrosome or pericentriolar complex of the cell (Amos & Schlieper, 2005; Desai & Mitchison, 1997), a site often called the microtubule organizing center (MTOC) (Luders & Stearns, 2007; Wiese & Zheng, 2006). Anchoring of MT minus ends helps establish and maintain the polarity of cellular MTs. Anchoring and nucleation of microtubules appears to require a third class of tubulin, γ-tubulin, which is detectable only as part of the pericentriolar complex (Joshi, 1994; Moritz & Agard, 2001; Raynaud-Messina & Merdes, 2007).

The organization of MTs in neurons differs several ways from that seen in non-neuronal cells (Fig. 6-3) (Conde & Caceres, 2009). Axonal and dendritic MTs are not continuous back to the cell body, nor are they associated with any visible MTOC. Axonal MTs can be more than 100 μm long, but they have uniform polarity, with all plus ends distal to the cell body. Dendritic MTs are typically shorter and often exhibit mixed polarity, with only about 50% oriented plus-end distal. Recent work suggests that MTs in both axons and dendrites are nucleated normally at the MTOC, but they are then released from the MTOC and delivered to neurites (Baas et al., 2006).

While MTs in neurons are composed of the same basic constituents as those in non-neuronal cells, they are strikingly more diverse (Table 6-1). Brain MTs contain tubulins of many different isotypes, with many different post-translational modifications, and also have a variety of associated proteins (Amos & Schlieper, 2005; Halpain & Dehmelt, 2006). MT composition varies according to location, such as in axons or dendrites, suggesting that brain MTs exist in specialized forms to perform designated tasks in the unique environments of the neuron. For example, axonal MTs contain stable segments that are unusually resistant to treatments that depolymerize MTs in other cells (Brady, 1993). Such stable domains are preserved as short MT segments and may serve to nucleate or organize MTs in axons (Dent et al., 1999), particularly during

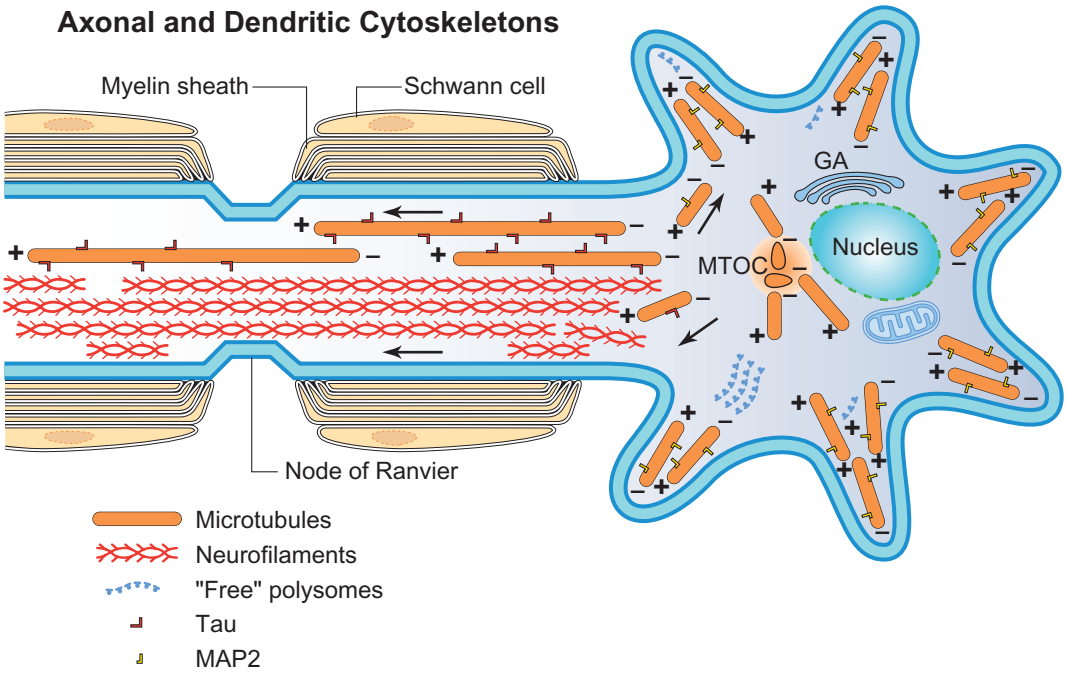

FIGURE 6-3 **The axonal and dendritic cytoskeletons differ in both composition and organization.** The major differences are illustrated diagrammatically in this diagram. With one exception, all cytoskeletal proteins are synthesized on free polysomes in the cell body, then transported to their different cellular compartments. The exception is MAP2, which is the major microtubule-associated protein of dendrites. While some MAP2 is synthesized in the cell body, MAP2 mRNA is specifically enriched in the dendritic compartment and a significant fraction is thought to be synthesized there. The microtubules of cell bodies, dendrites and axons are thought to be nucleated at the microtubule-organizing center (*MTOC*), then released and delivered to either the dendrites or axon. In the dendrite, microtubules often have mixed polarities with both plus and minus ends distal to the cell body. The functional consequence of this organization is uncertain but may help explain why dendrites taper with distance from the cell body. In contrast, all axonal microtubules are oriented with the plus end distal to the cell body and exhibit uniform distribution across the axon. Although some tau protein can be detected in cell bodies and dendrites, axonal microtubules are enriched in tau and axonal tau is differentially phosphorylated. MAP2 appears to be absent from the axon. Neurofilaments are largely excluded from the dendritic compartments but are abundant in large axons. The spacing of neurofilaments is sensitive to the level of phosphorylation. Microtubules and neurofilaments both stop and start in the axon rather than being continuous back to the cell body. The microfilaments are more dispersed in their organization and may be difficult to visualize in the mature neuron. They are most abundant near the plasma membrane but are also enriched in presynaptic terminals and dendritic spines. *GA*, Golgi apparatus.

regeneration. This and other specializations of axonal microtubules (see below) may reflect the unusual requirements of the neuronal cytoskeleton, where remarkably long microtubules are maintained at considerable distances from sites of new protein synthesis in the cell body.

Multiple genes exist for both α- and β-tubulins. Tubulin isotypes differ primarily at the carboxy terminus, the region where most post-translational modifications and MAP interactions occur. While most α- and β-tubulin isotypes are expressed in all tissues, some are expressed preferentially in different tissues. For example, class III and IVa β-tubulins are neuron specific (McKean et al., 2001). It is not known if such examples of tissue-specific expression imply that different isotypes are structurally suited to function in different tissues, or merely that different tubulin genes are part of different tissue-specific developmental programs. Where it can be evaluated, all isotypes available in a given cell appear capable of coassembly and typically can be detected in all cellular MTs.

Although precise cellular functions of each specific tubulin isotype remain virtually unknown, genetic evidence has accumulated that different tubulins may have different roles in the development and function of neurons (Tischfield & Engle, 2010). For example, mutations in the neuron-specific β-tubulin class III result in a spectrum of human neurological disorders known as TUBB3 syndromes. While some mutations in TUBB3 result in the ocular motility disorder CFEOM3, some others lead to behavioral and cognitive disorders, facial paralysis, and/or late-onset axonal sensorimotor polyneuropathy (Tischfield et al., 2010). Similarly a missense mutation in the tubulin-specific chaperone E (TBCE) gene leads to progressive motor neuropathy in mice. TBCE is a tubulin-binding chaperone that helps in the assembly of the tubulin α-β heterodimer (Bommel et al., 2002). These molecular observations suggest that perturbation of the normal MT dynamic properties is sufficient to cause neurodegeneration. Curiously, diseases associated with tubulins tend to affect the nervous system preferentially and different mutations may affect different

TABLE 6-1 Major Microtubule Cytoskeletal Proteins of the Nervous System

Protein	Expression pattern and distribution	Modifications
α-tubulin (multigene family)	In all cells, but some isoforms are preferentially expressed in brain	Acetylation and others
β-tubulin (multigene family)	In all cells, but some isoforms are preferentially expressed in brain	Phosphorylation and others
γ-tubulin	In all cells, pericentriolar region/MTOC	
MAP1a	Appears late, preferentially in dendrites	Phosphorylation
MAP1b (multigene family)	Appears early, then declines; enriched in axons	Phosphorylation
MAP2a	High molecular weight, dendritic in mature neurons	Phosphorylation
MAP2b	High molecular weight, dendritic expressed throughout lifetime	Phosphorylation
MAP2c (single gene)	Low molecular weight, dendritic in developing neurons	Phosphorylation
Tau – high MW	Peripheral axons with distinctive phosphorylation pattern	Phosphorylation
– low MW (alternative splicing of a single gene)	Enriched in CNS axons with distinctive phosphorylation pattern	Phosphorylation
MAP4	Primarily nonneuronal, multiple forms, widespread distribution	Phosphorylation at mitosis
Katanin	Widespread distribution, enriched at MTOC, severs MT	Phosphorylation
Stathmin	Widespread distribution, destabilizes MT	Phosphorylation
Spastin	Widespread distribution, enriched at branch points, severs MT	
Fidgetin	Widespread distribution, destabilizes MT	
APC	Localizes to the tip of early neurites and axons. Enrichment in axonal growth cones precedes axonal outgrowth.	Phosphorylation
EB1	Widespread distribution, binds to growing microtubule plus ends	
EB2	Widespread distribution, binds to growing microtubule plus ends	

populations of neurons (Tischfield & Engle, 2010) (see Box). Indeed, recent studies showed that use of drugs that alter MT dynamics *in vivo* might be neuroprotective (Trojanowski et al., 2005) suggesting that alterations in MT dynamics may be involved in some late-onset neurological disorders.

Brain MTs also contain a variety of post-translational modifications. When purified mammalian tubulin is analyzed by isoelectric focusing, over 20 different isoforms can be seen (Luduena, 1998). These are explained by a combination of multiple genes and various post-translational modifications. The two best-studied post-translational modifications of tubulin are α-tubulin detyrosination and acetylation (reviewed in Fukushima et al., 2009). Most α-tubulins are expressed with a carboxy-terminal tyrosine residue. This tyrosine can be removed by tubulin carboxypeptidase and then replaced by tubulin tyrosine ligase, in a rapid cycle that occurs on the majority of available tubulin. Since the carboxypeptidase acts only on assembled tubulin and the ligase acts on unassembled tubulin, this tyrosination/detyrosination cycle is linked to MT dynamics. These changes do not alter MT stability, but rather serve to report on MT turnover. Typically, newly assembled MTs will contain Tyr-tubulin. The longer a MT remains polymerized, the higher its content of detyrosinated tubulin, or Glu-tubulin. A second prominent post-translational modification of neuronal α-tubulin is lysine-ε-acetylation. The enzyme responsible for acetylation, tubulin acetyltransferase, like tubulin carboxypeptidase, acts only on assembled tubulin.

Neuronal β-tubulin isotypes appear to be subject to fewer post-translational modifications (Fukushima et al., 2009). For example, the β_{III} isotype of brain is phosphorylated. While the function of this modification is not clear, correlations with neurite outgrowth in neuroblastoma cells have been made. Another modification of β-tubulin is polyglutamylation, the addition of 1–5 glutamyl units to the γ-carboxyl group of a glutamate residue near the C-terminus (Kann et al., 2003). This summary of tubulin posttranslational modifications should not be considered exhaustive, as novel modifications continue to be reported. Despite the plethora of neuronal tubulin post-translational modifications, none are known to have a direct effect on MT properties, although they may correlate with specific states (i.e., assembly, microtubule age, etc.).

MTs *in vivo* invariably include members of a heterogeneous set of proteins known as microtubule-associated proteins (MAPs) (Amos & Schlieper, 2005; Cassimeris & Spittle, 2001; Dehmelt & Halpain, 2005; Halpain & Dehmelt, 2006). MAPs interact with MTs rather than with free tubulin and maintain constant stoichiometry with the tubulin in MTs through cycles of assembly and disassembly. Several categories of brain MAPs can be grouped in two families: MAP1 (1A and 1B) (Halpain & Dehmelt, 2006), as well as MAP2/tau (2A and 2B) and tau protein. MAP1 and MAP2 families are high molecular weight

(>270 kD), while tau is a single gene with extensive alternative splicing. In addition, there are other MAPs of intermediate molecular weight, such as MAP3 and MAP4. These groups of MAPs are collectively known as 'fibrous' or 'structural' MAPs because they have been observed to form lateral extensions between adjacent MTs and between MTs and other cytoskeletal elements (Schoenfeld & Obar, 1994). Some classify the MT-based molecular motor kinesin and dynein as MAPs, and these proteins drive the intracellular transport of membrane-bounded organelles along MT tracts as well as the movement of cytoskeletal elements (see Ch. 8, Axonal Transport), but these proteins do not meet the criteria of cycling with tubulin through assembly and disassembly.

The high molar ratio of structural MAPs to tubulin in brain suggests that MAPs may play an important role in determining MT properties. Some MAPs are differentially distributed in neurons (Cassimeris & Spittle, 2001). For example, MAP 2 is found primarily in cell bodies and dendrites, and tau is enriched in axons. Additionally, changes in MAP expression and MAP phosphorylation during development suggest they may play a role in modulating MT function in the developing brain (Schoenfeld & Obar, 1994). For example, MAPs 1A and 1B occur in both axons and somatodendritic domains, but MAP 1B is preferentially phosphorylated in axons and especially in developing axons. In humans, as many as six different tau proteins may be derived by alternative splicing from a single tau gene (Goedert, 2004; Deshpande et al., 2008). Both the expression and phosphorylation of the different tau isoforms are regulated throughout development. While MAPs affect nucleation and stability of MTs *in vitro*, these may not be their primary functions *in vivo*. Other likely functions include roles in MT spacing and organization, compartmentation, protection from microtubule severing proteins (Qiang et al., 2006), scaffolds for signaling molecules and interaction with other cellular structures.

Recently, another types of microtubule associated proteins have been defined that affect MT polymerization (stathmin, fidgetin) or lead to severing of MT (spastin and katanin). Stathmin destabilizes MT either by reducing available tubulin dimer or by altering the frequency of catastrophe at MT plus ends (Steinmetz, 2007). Fidgetin, katanin and spastin are all AAA-family ATPases and katanin/spastin sever MTs (Baas et al., 2005; Roll-Mecak & McNally, 2010). Fidgetin causes MT disassembly when overexpressed, and is important for chromosome movement in mitosis (Zhang et al., 2007), but has not been shown to play a role in mature neurons. Unlike katanin and spastin, purified fidgetin has not been shown to sever MT *in vitro*.

In neurons, katanin and spastin actions lead to release of MTs from the MTOC and allow transport of the assembled MT in axons and dendrites (Baas et al., 2005). MT reconfiguration and organization may require the association of molecular motors to short and mobile microtubule polymers. The "cut-and-run" model of MT reorganization proposes that long MTs are stationary, compared to short MTs that are motile, so neurons reorganize their MT arrays by severing MT polymers through the regulated activity of MT-severing enzymes (i.e., katanin and spastin). Although katanin is widely distributed within neurons it is highly expressed in developing axons; conversely its expression level decreases when axons reach their targets, suggesting a direct role of katanin in axonal remodeling and elongation. Recent experimental evidences showed that overexpression of katanin results in an increase in axonal processes development. On the contrary, inhibiting katanin induces profound deleterious effects on axonal outgrowth (Baas et al., 2005). These experimental findings suggest that MT severing and reconfiguration may play a role in axonal formation and development. Katanin has multiple regulatory mechanisms including tau binding to MTs, acetylation and phosphorylation (Baas et al., 2005; Sudo & Baas, 2010).

Spastin is a MT-severing enzyme that is more restricted to the nervous system than katanin (Baas et al., 2005; Roll-Mecak & McNally, 2010). One important property of spastin is its capacity to induce branch formation. Indeed, depletion of spastin in neurons result in a reduction of axonal length associated with a substantial reduction of axonal branching (Yu et al., 2008). Remarkably, mutations in spastin lead to a particular dying-back neuropathy collectively known as human hereditary spastic paraplegia (HSP), which appears to be unrelated to its role in severing MTs. Mutations in spastin are associated with dysfunction and degeneration of long axons of pyramidal tracts, apparently via a molecular mechanism involving deregulation of axonal transport (Solowska et al., 2008).

Finally, another group of MT binding proteins are known as microtubule plus-end tracking proteins (+TIPs) (Conde & Caceres, 2009). Members of the + TIP family include adenomatous polyposis coli (APC), a protein enriched at the tips of minor processes committed to become an axon (Votin et al., 2005). APC is also frequently mutated in colon cancer, but this may not be related to its action on MTs. End-binding proteins 1 and 3 (EB1 and EB3) are widely distributed within neurons in association to MT plus ends. EB1 is upregulated during axonal formation and may be required for axon elongation (Morrison et al., 2002). Interestingly, EB3 binds drebrin, an actin-binding protein involved in dendritic spine formation, and is enriched in growth cone filopodia. When the EB3–drebrin association is impaired, growth cone formation and process extension is impaired (Geraldo et al., 2008). Due to their ability to bind to MT plus ends, EB proteins fused to fluorescent proteins have been used to study the dynamic properties of MTs (Matov et al., 2010).

MTs serve multiple roles in neurons. Besides acting as the substrate for the transport of membrane-bounded organelles, MTs are necessary for the extension of neurites during development; they provide the structural basis for maintaining neurites after extension and they also help maintain the definition and integrity of intracellular compartments. The diversity of these functions is reflected in differences in the biochemistry and metabolic stability of different MTs.

Neuronal and glial intermediate filaments provide support for neuronal and glial morphologies

As a class of cytoskeletal structures, intermediate filaments (IFs) display an unusual degree of cell specificity and are often used as markers of cellular differentiation. They comprise a family of related genes that have been classified in five types (Herrmann et al., 2007). All five share homology in a core rod domain, which contains multiple α-helical domains that can

form coiled/coils. The sequence homology in this conserved domain is sufficient that some antibodies recognize all known IFs from mammals through a wide range of invertebrates. IFs are also ultrastructurally similar regardless of type, forming 8–10 nm ropelike filaments that may be several micrometers long. However, NFs differ from the other IFs because they have sidearms that project from their surface. The result is that IFs in non-neuronal cells are often seen in densely packed bundles, while NFs are widely spaced (Fig. 6-4).

Types I and II are the keratins that are found in various combinations in epithelial cells throughout the body. Keratin IFs must include representative subunits of both type I and type II IF subunits, with each tissue having a characteristic combination. In contrast, type III IF subunits typically form homopolymers. They include IFs that are characteristic of less-differentiated cells like glial or neuronal precursors, as well as those seen in more specialized cell types like smooth muscle cells and mature astrocytes. These three types of IF not only share sequence homology within their rod domains; in addition, their genes exhibit similar exon and intron structure. Type IV IFs are all neuron specific and have a common pattern of exons and introns that differs from that seen in types I–III. Finally, type V polypeptides are the nuclear lamins, which form the walls of the nuclear envelope structure rather than typical IF structures. All eukaryotes express type V lamins, but plants, many unicellular organisms and arthropods do not express other IF types. Cytoplasmic IFs are nearly ubiquitous in vertebrate cells, and some cells contain more than one type of IF in their cytoplasm. Curiously, oligodendrocyte precursors contain vimentin IFs, but these are lost during differentiation, making mature oligodendrocytes one of the few vertebrate cell types that lack cytoplasmic IFs.

The nervous system contains an unusually diverse set of intermediate filaments (Table 6-2) with distinctive cellular distributions and developmental expression (Herrmann et al., 2007; Lariviere & Julien, 2004). Despite their molecular heterogeneity, all intermediate filaments appear as solid, ropelike fibers 8–12 nm in diameter. Neuronal intermediate filaments

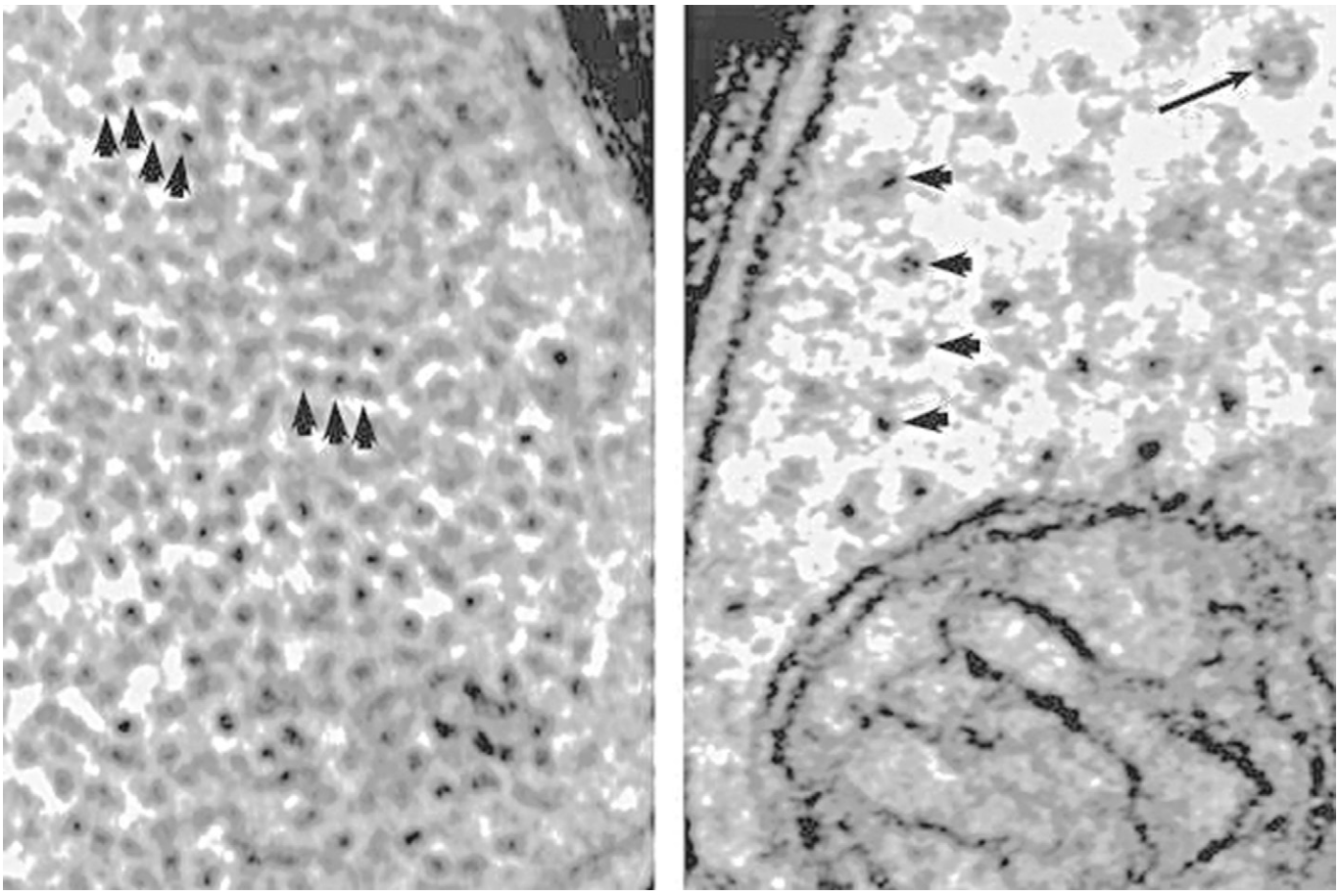

FIGURE 6-4 **Glial filaments and neurofilaments are easily recognized in electron micrographs.** The glial filaments lack side arms and often appear to be densely packed, with neighboring subunits almost touching (large arrows in **left** panel). In contrast, the spacing between neurofilaments is typically much greater (large arrows in **right** panel). This spacing is due to the side arms of neurofilaments formed by the tails of the high- and medium-molecular-weight neurofilaments (NFH and NFM, respectively). These tails are heavily phosphorylated in large axons such that NFH tails may have 50 or more phosphates added to the multiple repeats of a consensus phosphorylation site. NFM tails have fewer sites and typically only 10 to 12 phosphates. Charges on the surface of phosphorylated neurofilaments are thought to repel neighboring filaments, creating the large spacing. For comparison, a 25 nm microtubule is indicated in the **right** panel (*thin arrow*). (Micrograph provided by H. Ross Payne and Scott Brady.)

TABLE 6-2 IF Proteins of the Nervous System

IF Type	Subunit	Cell type
Type III	Vimentin	Neural and glial precursors
	GFAP	Astrocytes, some Schwann cells
	Peripherin	Subset of neurons, particularly in PNS, may coassemble with NFH/NFM/NFL
	Desmin	Smooth muscle cells in vasculature
Type IV	NFH	
	NFM	
	NFL	Most neurons, most abundant in large neurons
	α-Internexin	Subset of neurons, particularly parallel fibers in cerebellum, may also coassemble with NFH/NFM/NFL
Type V	Nuclear lamins	Nuclear envelope
Type VI	Nestin	Neuroectodermal precursors in developing brain

(NFs) can be hundreds of micrometers long and have characteristic sidearm projections, while filaments in glia or other nonneuronal cells are shorter and lack sidearms (Fig. 6-2). The existence of NFs was established long before much was known about their biochemistry or properties. As stable cytoskeletal structures, NFs were noted in early electron micrographs, and many traditional histological procedures that visualize neurons are based on a specific interaction of metal stains with NFs.

The primary type of IF in large myelinated axons is formed from three subunit proteins known as the neurofilament triplet: NF high-molecular-weight subunit (NFH, 180–200 kDa); NF middle-molecular-weight subunit (NFM, 130–170 kDa); and NF low-molecular-weight subunit (NFL, 60–70 kDa) (Herrmann & Aebi, 2000; Larivere & Julien, 2000; Lee & Cleveland, 1996). A separate gene encodes each of the subunit proteins. The NF triplet proteins are type IV IF proteins that are expressed only in neurons and have a characteristic domain structure. The amino-terminal regions of all three subunits interact via α-helical-coiled coils to form the core of the filament. NFM and NFH also have long carboxy-terminal regions, which project from the core filaments as sidearms. NFH, and to a lesser extent NFM, have a large number of consensus phosphorylation sites for proline-directed kinases in this carboxy-terminal extension (>50 on NFH and >10 on NFM in many species). In large myelinated axons, most, if not all, of these sites are phosphorylated (Herrmann et al., 2007; Lariviere & Julien, 2004). This phosphorylation of NFH and NFM sidearms alters the charge density on the NF surface, repelling adjacent NFs with similar charge. Such mutual repulsion by the sidearms of NFs is thought to be a major determinant of axonal caliber (de Waegh et al., 1992).

Other IFs are also found in the nervous system (Herrmann et al., 2007; Lariviere & Julien, 2004). Vimentin is the most widely expressed type III IF and is found in a variety of cell types like fibroblasts, microglia and smooth muscle cells as well as in embryonic cell types including neuronal and glia precursors. Astrocytes and some Schwann cells contain the type III IF glial fibrillary acidic protein (GFAP). This distribution has led to the widespread use of GFAP immunoreactivity to identify astrocytes in culture and in tissue. In contrast to NFs, type III IFs like GFAP lack sidearms and often appear to be tightly bundled.

At least three other IFs occur in selected neurons or neuronal precursors: α-internexin, peripherin and nestin (Herrmann & Aebi, 2000; Lariviere & Julien, 2004). All of these are most prominently expressed during development, then downregulated. Based on sequence and gene structure, peripherin is a type III IF, while α-internexin is a type IV IF. Nestin is the most divergent of the IFs that form filaments, and the nestin sequence has characteristics of both type III and type IV IFs. As a result, nestin is sometimes considered to be a distinct IF type (type VI). While the NF triplet proteins and α-internexin are components of both CNS and PNS neurons, peripherin appears to be expressed preferentially in PNS neurons. Both can coassemble with NF triplet proteins *in vitro* and may do so *in vivo*, but can also form homopolymeric filaments. Both are expressed at higher levels in a variety of developing neurons, and expression becomes more restricted as neurons mature. Many neurons cease to express α-internexin during maturation. However, a few neurons retain the expression of IFs containing α-internexin. Notably, IFs of parallel fibers in the cerebellar cortex contain only α-internexin subunits. Peripherin continues to be expressed in some peripheral neurons in maturity. Although nestin is specific to the nervous system, it is expressed in multipotent neuroectodermal precursors and suppressed during subsequent development (Wiese et al., 2004). For this reason it can be used as an early marker for differentiation of precursor cells.

Not all neurons have NFs. Indeed, one entire phylum in the animal kingdom, arthropods, expresses only type V nuclear lamins, so arthropod cells have no IF cytoskeletal structures at all. In addition, mature oligodendrocytes lack IFs although their embryonic precursors contain vimentin. Clearly, the IFs are not essential for cell survival. Yet, in large myelinated fibers, NFs make up the bulk of axonal volume and represent a substantial fraction of the total protein in brain. In most organisms, IFs in both glia and neurons contribute to the distinctive morphologies of these cells. They are thought to provide mechanical strength and a stable cytoskeletal framework. In neurons, NFs play an important role in regulating cellular and axonal volumes and are a primary determinant of axonal caliber in large fibers. Finally, NFs exhibit an unusual degree of metabolic stability, which makes them well suited for a role in stabilizing and maintaining neuronal morphology (Lariviere & Julien, 2004; Lasek, 1988).

Actin microfilaments and the membrane cytoskeleton play critical roles in neuronal growth and secretion

This major class of cytoskeletal elements is perhaps the oldest family. Certainly, the actin cytoskeleton has the most diverse composition and organization. MFs are formed from 43 kDa actin monomers that are arranged like two strings of pearls intertwined into fibrils 4–6 nm diameter (Fig. 6-2) [26].

Actins are a gene family with six distinct genes expressed in mammals, four of which are expressed in brain (Perrin & Ervasti, 2010), as are several actin-related proteins (Steinmetz et al., 1997). A remarkable variety of proteins have been found to interact with actin MFs, ranging from myosin motors to cross-linkers, bundling proteins to anchoring proteins, and sequestration proteins to small GTPase regulatory proteins (dos Remedios et al., 2003; Letourneau, 2009).

Actin MFs are found throughout neurons and glia, but they are enriched in cortical regions of the cell near the plasmalemma and are particularly concentrated in presynaptic terminals, dendritic spines and growth cones. Under most circumstances in the nervous system, MFs are short oligomers organized into a meshwork most apparent near the plasma membrane and in the vicinity of axonal MTs. MFs are the main components of the membrane cytoskeleton, and this may be their primary role in mature neurons. The actin cytoskeleton also plays an important role in the Golgi complex (GC) morphology. Actin and actin-associated proteins, like spectrin and myosin, are associated with the GC, although their exact functions are not fully understood. However, the cytoplasmic arrangement of actin filaments is directly involved in the subcellular localization and the shape of GC (Valderrama et al., 1998). Spectrin is thought to provide a cross-linked matrix surrounding GC membranes, similar to its role at the plasma membrane, providing structural integrity, as well as defining putative GC microdomains and possibly mediating interactions with dynactin and dynein (Holleran et al., 2001). The prominent actin bundles (stress fibers) seen in fibroblasts and many other non-neuronal cells in culture are not characteristic of neurons *in vivo* or *in vitro*. Most neuronal MFs are less than a micron in length. However growth cones contain many longer MFs, with bundles of MFs in the filopodia and lamellipodia in addition to the typical more dispersed actin network (Fig. 6-4) (Lowery & Van Vactor, 2009; Pak et al., 2008). The role of actin MFs in the growth cone will be considered in greater detail below.

Many MF-associated proteins (dos Remedios et al., 2003; Letourneau, 2009) have been described in the nervous system (Table 6-3). In general, a good deal is known about their distribution and function in primary cultures of neurons and glia, but less is known about their role in the mature nervous system. Two that have been characterized more extensively are the major non-actin structural elements of the membrane cytoskeleton: spectrin and ankyrin. Spectrin is a flexible rod-shaped molecule composed of homologous a- and b-subunits, originally characterized as a component of the erythrocyte membrane cytoskeleton. Neurons were the first cell type, other than erythrocytes, shown to contain spectrins, and the brain form was initially called fodrin. Spectrin heterodimers align end to end to form tetramers, which are cross-linked by short-actin MFs. This spectrin–actin meshwork is tightly coupled to the plasma membrane through direct binding to membrane proteins (Beck & Nelson, 1996). Some of these interactions occur via the protein ankyrin, which has separate binding sites for specific membrane proteins and b-spectrin (Fig. 6-5). In neurons, specific isoforms of spectrin and ankyrin are localized to axons, dendrites and paranodal regions. The spectrin and ankyrin isoforms in perikarya and dendrites tend to be highly homologous to the erythrocyte forms and distinct from the spectrin and ankyrin isoforms that occur in axons.

A variety of additional MF bundling or linking proteins have been described in the nervous system (dos Remedios et al., 2003). For example, fimbrin may play a role in the formation of microfilament bundles in growth cone filopodia. Still other actin-binding proteins may regulate MF assembly. Gelsolin fragments MFs in a Ca^{2+}-sensitive manner, but also caps and nucleates MFs. In contrast, profilin and β-thymosins

TABLE 6-3 Selected Microfilament-Associated Proteins Expressed in the Nervous System

Protein	Activity	Cellular location
Actin	Core subunit of MFs	Throughout neurons and glia, enriched in growth cones and in membrane cytoskeleton
Tropomyosin	Stabilize MFs	Co-distributed with most MFs
Spectrin/fodrin	Cross-link MFs in membrane cytoskeleton	Enriched in membrane cytoskeleton
Ankyrin	Links MF/spectrin to membrane proteins	Membrane cytoskeleton, distinct forms in axon, dendrite and nodes of Ranvier
Fimbrin	MF bundling and cross-linking	Growing neurites
Gelsolin	Fragments MFs and nucleates assembly, regulated by Ca^{2+}	Growing neurites, glia, mature neurons
β-thymosin	Binds actin monomers and regulates MF assembly	Growing neurites
Profilin	Binds actin monomers, inhibits MF formation, regulated by selected signal transduction pathways	Growing neurites, glia, mature neurons
Arp2/3	Nucleation of actin MF assembly in cortex and initiation of MF branches	Enriched in cell cortex where MF assembly is active
N-WASP	Interacts with Arp2/3 complex to nucleate branched actin MF assembly	Enriched in cell cortex where MF assembly is active, binds to Cdc42/Rac small GTPases and Arp2/3 complexes
Formin	Nucleates straight actin filaments	Enriched in cell cortex where MF assembly is active, binds to Rho small GTPase

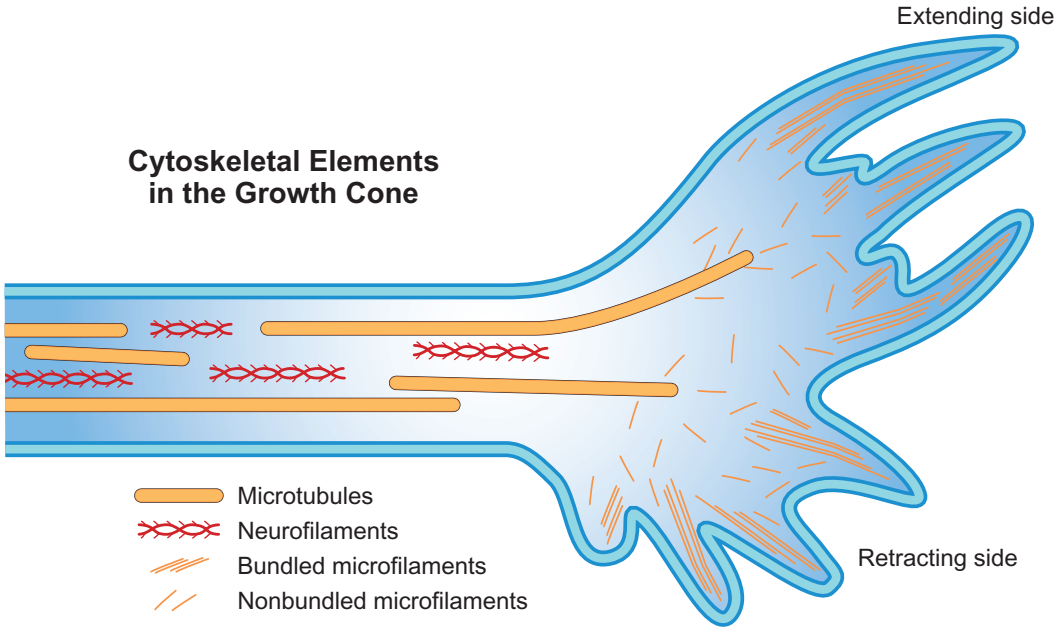

FIGURE 6-5 **The cytoskeletal elements of a growth cone are organized for motility.** In this diagram of a growth cone, a typical distribution of major cytoskeletal structures is shown. The microfilaments are longer and more prominent in the growth cone than in other regions of a neuron. They are bundled in the lamellipodia and particularly in the filopodia. A combination of actin assembly, microfilament cross-linking and myosin motors (see Ch. 28) is thought to mediate this movement. In the central core of the growth cone, the microfilaments may interact with axonal microtubules, which do not extend to the periphery. These microtubules may be pulled toward the preferred direction of growth and appear to be necessary for net advance. In the absence of microtubules, filopodia extend and retract but the growth cone does not advance. Microtubule movements are thought to be a combination of assembly and contractility. Finally, the neurofilaments appear to stabilize the neurite and consolidate advances but appear to be excluded from the growth cone proper.

bind to actin monomers and may act in part by sequestering actin subunits, although this oversimplifies the effects of these proteins. The list of actin MF-associated proteins has become quite long, and this diversity reflects the many forms of MF-based cytoskeletal structures.

Recent studies on mechanisms for regulation of MF assembly have identified a number of components critical for creating and maintaining the actin membrane cytoskeleton including Arp2/3 and the WASp/Scar family (Pollard, 2007). Arp2 and Arp3 are actin-related proteins that form a complex with five other unique polypeptides [28, 36]. The first member of the WASP/Scar family was isolated from hematopoietic cells and is mutated in a rare disease known as Wiskott–Aldrich syndrome. Other family members were expressed at high levels in brain and other tissues. Briefly, members of the Cdc/Rac family of small GTPases activate WASp/Scar proteins. Activated WASp/Scars bind to the Arp2/3 complex to initiate a new MF or a branch of an existing MF. Often actin monomer is sequestered in association with profilin to limit assembly. A second signal (PIP or PIP_2) stimulates dissociation of actin and profilin and rapid MF assembly occurs. This pathway is particularly important in neuronal activities like growth cone motility. A second pathway for initiating and regulating actin filament assembly is mediated by the formins (Goode & Eck, 2007; Pollard, 2007), and is also mediated by a small GTPase, but in this case Rho is responsible (Goode & Eck, 2007; Pollard, 2007). Formins create straight filaments, whereas the WASP/Arp2/3 pathway produces branched filament networks.

MFs in the nervous system appear to have a variety of functions. The neuronal membrane cytoskeleton plays a role in maintaining the distribution of plasma membrane proteins, establishing cell morphologies and segregating axonal and dendritic proteins into their respective compartments. MFs and the membrane cytoskeleton also mediate the interactions between neurons and the external world, including cell adhesion molecules, extracellular matrix components and neighboring cells (see Ch. 9). In neurons and glia, cell adhesion sites such as tight junctions and focal adhesion plaques interact with the MF cytoskeleton either directly or indirectly. The cortical MF meshwork also restricts access of organelles to the plasma membrane and is involved in both regulated and constitutive secretion. Finally, MFs are the basis of filopodia and lamellipodia, which are essential for cell migration, growth cone motility and myelination.

ULTRASTRUCTURE AND MOLECULAR ORGANIZATION OF NEURONS AND GLIA

A dynamic neuronal cytoskeleton provides for specialized functions in different regions of the neuron

The cytoskeleton in different regions of a neuron differs with regard to both composition and function. While both

neurons and glia exhibit distinctive specializations in their cytoskeletal elements, distinct domains are most easily visualized within the neuron. In order to maintain the polarized morphology of a neuron, cytoskeletal, cytoplasmic and membranous elements must be assembled and targeted in a characteristic and consistent manner. The biochemical diversity of each of these elements is striking, as the composition of proteins in the axon differs significantly from that in cell bodies and dendrites. There is diversity even along the length of a single axon. In this section, several examples are given that illustrate how characteristic features of the somatodendritic and axonal cytoskeletons are generated and maintained. A common theme underlying each example is the dynamic nature of the cytoskeleton.

Both the composition and organization of cytoskeletal elements in axons and dendrites become specialized early in differentiation

A brief description of how the cytoskeletal elements within axons and dendrites are established illustrates the dynamic nature of the cytoskeleton. The sequence of events has been described for neurons in primary culture (Conde & Caceres, 2009; Mandell & Banker, 1995). Postmitotic neurons initially extend and retract multiple neuritic processes. These early short neurites are of comparable length and growth rate. All contain MTs oriented with plus ends distal to the cell body and all have both MAP2 and tau microtubule-associated proteins. Eventually, in neurons that elaborate an axon, one neurite outgrows the others. This first long neurite continues to grow without tapering and becomes the single axon. Under culture conditions that lack directional information, the choice of neurite that becomes the axon appears to be stochastic. Cues in the local environment are likely to specify a particular direction for neurite outgrowth *in vivo*. As the axon grows, it loses MAP2, while tau is enriched and become differentially phosphorylated (Dehmelt & Halpain, 2005). Subsequent to axonal outgrowth, some of the other neurites are stabilized and begin to extend. The number of dendrites varies with type of neuron, ranging from the single dendritic process that partially fuses with the axon to produce the single branched process of dorsal root ganglion pseudo-unipolar neuron, to the elaborate branching dendrite of the Purkinje cell and to multipolar motor neurons with both apical and basal dendritic arbors. Most neurites that develop into dendrites become tapered as they grow. When dendrites reach this stage, they begin to contain MTs in both orientations. More or less concurrently, MAP2 becomes enriched in the dendritic processes. Initially, the smallest form of MAP2 is abundant, but a shift to predominantly high molecular weight isoforms occurs with maturation (Dehmelt & Halpain, 2005).

As axons and dendrites mature, their differences become more apparent (Conde & Caceres, 2009; Mandell & Banker, 1995). In axons, MTs have a uniform orientation with plus ends distal to the cell body, but dendrites contain MTs in both orientations. Dendrites and to a lesser extent perikarya contain MAP2, which is excluded from axons at an early stage. Curiously, MAP2 mRNA is one of several mRNAs that are specifically transported into the dendrite and translated locally. No other cytoskeletal protein has been found to display a similar expression pattern, and protein synthetic machinery for the other proteins is excluded from the axon. Expression of dendritic mRNAs is affected by synaptic activity, but its physiological function is uncertain. In contrast, tau is enriched in axons and NFs similarly occur primarily in axons. The phosphorylation of MAP 1B, tau and NFs is maintained at a high level in axons (Lee & Cleveland, 1996; Schoenfeld & Obar, 1994). Neuron-specific isoforms of spectrin and ankyrin exist only in axons (Beck & Nelson, 1996).

While tau is not restricted to the growing axonal process, tau expression appears to be critical for the initial elongation event that defines an axon-to-be. If tau expression is blocked before the commitment to axonal outgrowth through use of antisense oligonucleotides, no axon is formed (Caceres & Kosik, 1990). If tau expression is blocked after commitment, the axon is retracted, suggesting that tau–MT interactions are important for axonal differentiation, but the phenotype of the tau knockout mouse is very mild (Harada et al., 1994), suggesting that other MAPs may substitute for tau in development. Similarly, reduction in MAP2 expression inhibits dendrite differentiation. That MAP2 and tau seem to bestow upon MTs the ability to form 'dendrite-like' or 'axon-like' processes respectively is suggested by experiments in which these MAPs have been expressed in non-neuronal cells. MAP2 and tau differentially affect the packing density of MTs, so, while these MAPs may not trigger the initial polarization of neurons, they no doubt contribute to maintenance of the polarized phenotype (Conde & Caceres, 2009; Mandell & Banker, 1995). For both axons and dendrites, cytoskeletal composition and organization are carefully orchestrated during differentiation and maturation.

Although axonal and dendritic microtubules differ in their associated proteins and organization, both are thought to originate in the cell body. Microtubules are likely to continue to grow after entry in the axon or dendrite, but there is little evidence for *de novo* formation of microtubules in either region. The evidence suggests that both are nucleated at the MTOC as in other cell types. Instead of remaining associated with the MTOC, neuritic MTs are released from the MTOC and transported into the neurite. The sites where most elongation occurs and where specific microtubule-associated proteins are added remains a matter of dispute. Similarly, the molecular mechanisms by which different cytoskeletal compositions are maintained in different neuronal compartments are unknown.

As the size and shape of neurons change during development, the composition of the IF cytoskeleton varies coordinately. Different types of neurons and neurons in different stages of development vary in the number and composition of their IFs (Lee & Cleveland, 1996). For example, in many neurons peripherin and α-internexin are expressed very early in neuronal differentiation, then downregulated. NFL and NFM are detectable during initial neurite outgrowth, while NFH is not expressed at significant levels until much later. Phosphorylation of NFH and NFM occurs even later in development, and only reaches its full extent in large myelinated axons. Additionally, NFs in different neuronal populations

may not contain the same subunit stoichiometry for NFL, NFM and NFH.

CYTOSKELETAL STRUCTURES IN THE NEURON HAVE COMPLEMENTARY DISTRIBUTIONS AND FUNCTIONS

While each set of cytoskeletal elements has a distinctive spectrum of composition, stability and distribution, all three interact with each other. They have complementary functions and may be coordinately regulated. Such interactions can be seen during development of the nervous system, in mature neuron/glia interactions and in neuropathologies.

Microfilament and microtubule dynamics underlie growth cone motility and function

The mechanisms by which neurons make appropriate synaptic connections are a subject of great interest. The first stages of this process are neurite elongation and pathfinding. As the growing tip of a growth cone advances across a substrate, the growth cone must interpret extracellular cues to steer the growing neurite in the right direction (Lowery & Van Vactor, 2009; Pak et al., 2008). Growth cones receive both attractive and repulsive cues and respond by selectively stabilizing, destabilizing or rearranging actin and MT cytoskeletons to allow for directed growth (see also Ch. 32). This section briefly describes how extracellular signals mediate rearrangements of the cytoskeleton.

The periphery of growth cones can be roughly divided into two domains. *Filopodia* are long, thin, spike-like projections that grow and retreat rapidly from the growth cone surface; *lamellipodia* are web-like veils of cytoplasm that also spread and retract, often between filopodia. The body of the growth cone similarly includes two domains: the area of cytoplasm beyond the cylindrical neurite that adheres to the substratum, which is actin rich, and the central microtubules that enter from the shaft of the axon (Lowery & Van Vactor, 2009). Filopodia and lamellipodia sample the environment for favorable conditions; then the body of the growth cone moves forward as the axon elongates. The distribution of the three cytoskeletal elements in the growth cone is well established (Fig. 6-4) (Lowery & Van Vactor, 2009; Pak et al., 2008). In neurites MTs occur as bundles, which then splay out into single filaments in the body of the growth cone, but MTs do not extend beyond the base of the filopodia. NFs are limited to the shaft of the neurite and do not extend beyond the proximal edge of the growth cone. Actin MFs make up the majority of the growth cone cytoskeleton. They form a complex meshwork that includes a number of actin-associated proteins beneath the entire plasma membrane of lamellipodia and the growth cone body. Bundles of actin MFs form the cores of filopodia (Lowery & Van Vactor, 2009; Pak et al., 2008). Forces generated by rearrangement of the actin MFs help realign the MTs, and eventually the NFs, to the preferred direction for growth (Lowery & Van Vactor, 2009).

The diameter of a growth cone is often much greater than that of the neurite, allowing it to sample a large volume of the environment. Typically, the shape of a growth cone is constantly changing, with filopodia and lamellipodia extending and retracting, receiving signals from the surface of other cells, the extracellular matrix or the surrounding media. A number of factors may elicit a growth response, including soluble neurotrophins and membrane or matrix-bound ligands. In addition, repulsive signals have been identified that lead to collapse of filopodia or lamellipodia and to retraction of growth cones. The extension of filopodia and lamellipodia are regulated in part by members of the Rho and Rac family of low-molecular-weight GTPases (Burridge & Wennerberg, 2004).

Signals in response to extracellular guidance cues that cause growth cones to steer probably involve multiple pathways (Gallo & Letourneanu, 2004; Lowery & Van Vactor, 2009). When one or a few filopodia receive an attractive cue, the growth cone will turn in that direction. At least two things occur in the region of the growth cone chosen for further growth. First, extracellular signals activate cell surface receptors to recruit a multiprotein complex that links the receptor to the actin meshwork beneath the surface. This is likely to involve a variety of signals including local Ca^{2+} transients, release of PIP and PIP_2, activation of Cdc42/Rac small GTPases, and phosphorylation of cytoskeletal proteins in the vicinity. Rapid polymerization of actin MFs leads to a protrusion of the membrane at the site of growth. This burst of actin polymerization is probably due to the concerted actions of a number of different actin-binding proteins (Lowery & Van Vactor, 2009; Pak et al., 2008), and the membrane protrusion may also involve actin-based motors like myosins (Brown & Bridgman, 2004) (see Ch. 8). More or less concurrent with MF rearrangements, the splayed MTs in the growth cone body begin to invade the selected site (Gordon-Weeks, 2004; Zhou & Cohan, 2004). This invasion may involve both MT elongation and MT movements. Once MTs form an ordered bundle oriented in the new direction, the membrane of the growth cone collapses around the MT bundle to create an extension of the neurite cylinder and the NFs are advanced to consolidate new growth. Then the growth cone begins looking for the next signal. While this description is an oversimplification of growth cone steering events, it nevertheless illustrates that growth cones are highly motile and very dynamic entities.

The axonal cytoskeleton may be influenced by glia

In cross-sections of large myelinated axons, most of the volume is occupied by NFs separated from each other by sidearm spacers. Spaces between fields of NFs are occupied by one of two specialized regions: MTs with membrane-bounded organelles or electron-dense regions adjacent to MTs and to the plasma membrane cortex (Fig. 6-1). These electron-dense areas are enriched in short actin MFs. Such images suggest a static cross-linked cytoskeleton and do not reveal the underlying dynamics of the axonal cytoskeleton. In fact, fully mature neurons also have a dynamic cytoskeleton that is both engaged in axonal transport (Chapter 8) and responsive to the local environment.

The relationships between an axon and its myelinating glia are both intimate and extensive. Originally, these relationships

were thought to be specified by signals from the axon that elicited specific responses in glial cells after contact, including proliferation and myelination (Brady, 1993). Little thought was given to the possibility of glia influencing neurons. However, more recent studies indicate that the axonal cytoskeleton is also altered locally by glial contacts. Axonal cytoskeletal elements are subject to constant modulation via signals from the axonal environment, including both target cells and cells forming the myelin sheath. Such signals appear to influence axonal branching, synapse formation and axonal caliber.

The response of the axon to loss of myelin is instructive. In the Trembler mutant mouse, with a mutation in the PNS myelin protein PMP22 (see Chapter 38), axons in the PNS undergo a continuing cycle of partial myelination followed by demyelination. The result is a thin or absent peripheral myelin sheath and a reduction in axonal caliber (Fig. 6-6). Remarkably, this reduction in axonal caliber is highly localized to segments of axon with disrupted myelin. The local nature of these changes was proven by studies in which regions of Trembler sciatic nerve were grafted into normal nerves. Only those axon segments surrounded by Trembler Schwann cells have reduced diameters. More importantly, the reduction in axon caliber is due to an increase in NF density in Trembler nerves by a factor of two, even though the actual amount of NF protein is not changed. The increased NF packing density means that the same number of NFs now occupies a smaller volume producing a smaller axon. This change in density appears to be due to a reduction in the phosphorylation of NFH and NFM tail domains, which allows the individual NFs to be packed more tightly (de Waegh et al., 1992; Witt & Brady, 2000). Such changes are restricted to axons in contact with Trembler Schwann cells. Thus, it is the direct

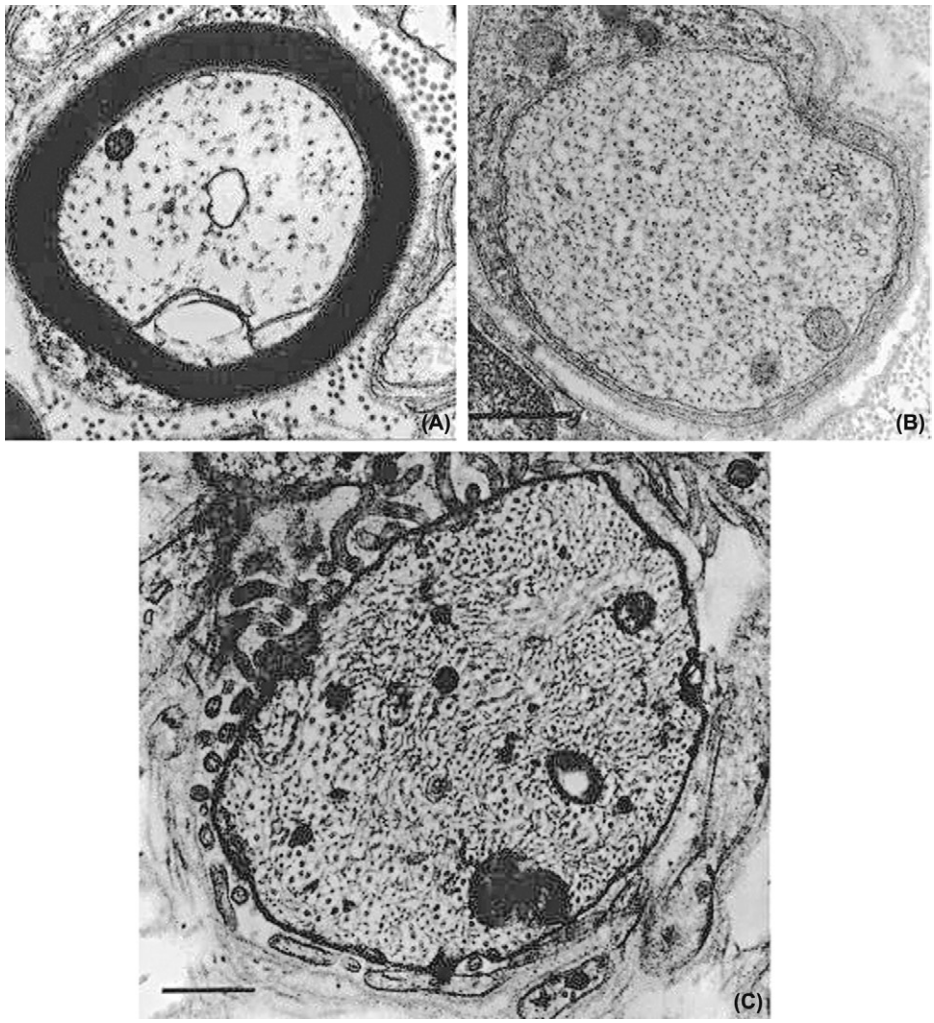

FIGURE 6-6 **The local environment can alter the organization of the axonal cytoskeleton. (A)** In a normal myelinated axon of the sciatic nerve, neurofilaments and microtubules are widely spaced, so they occupy considerable volume. **(B)** In contrast, a comparably sized axon from the sciatic nerve of the demyelinating Trembler mutant mouse has a denser cytoplasm, with neurofilaments densely packed. This has been shown to result from a shift in the net dephosphorylation of neurofilaments produced by demyelination. This effect on the axonal cytoskeleton is highly localized. **(C)** Similar changes in the organization and phosphorylation of the axonal cytoskeleton occur even over the short gap in the myelin sheath which occurs at the node of Ranvier. Such changes illustrate the dynamic nature of the axonal cytoskeleton. Bars represent 0.5 μm with **(A)** and **(B)** at the same magnification. (Micrographs supplied by Sylvie de Waegh and Scott Brady.)

interaction between the axons and the Schwann cells that modulates the axonal cytoskeleton. The influence of myelinating Schwann cells is not restricted to NFs, because the stability, organization and composition of the MT cytoskeleton are also altered in Trembler nerves.

The effects of myelin can be observed in intact normal nerve as well. A similar change in cytoskeletal organization occurs in normal myelinated nerves at nodes of Ranvier (Scherer et al., 2004). Beginning in the paranodal regions where compact myelin is lost, the diameter of the axon is reduced; the packing density of NFs in increased; and NF phosphorylation is reduced. Thus, the myelinating glial environment constantly influences the axonal cytoskeleton, providing a dramatic example of the dynamic nature of the neuronal cytoskeleton.

Levels of cytoskeletal protein expression change after injury and during regeneration

As described above there are substantial changes in the composition and organization of neuronal and glial cytoskeletal elements during development. Changes are equally dramatic following injury to the nervous system. Some changes reflect the switch from maintaining cellular structures to growth or repair modes. The response of PNS neurons during regeneration may fall into this category. In other cases, the response is incomplete or may be a reactive response to the injury. For example, after CNS injury, astrocytes become reactive, rapidly proliferate and frequently form a glial scar. The hallmark of this glial scar is a dramatic increase in GFAP IF bundles, which allows the astrocytes to fill the injured zone and provide mechanical support for surrounding uninjured tissues (Qiu et al., 2000). Unfortunately, the glial scar often represents a physical barrier to neuronal elongation and repair.

The neuronal response to injury has been studied in some detail to gain a better understanding of why mammalian CNS neurons regenerate poorly or not at all, while PNS neurons regenerate effectively (see also Chapter 32). Although regeneration is often described as paralleling developmental growth of neurons, the distinctive pattern of changes in the neuronal cytoskeleton during regeneration differs in some important ways from changes during development. First, there is a coordinate downregulation in NF subunit expression following injury. NFH, NFM and NFL protein and mRNA levels in the perikarya decline rapidly after injury and do not recover until after reconnection with an appropriate target cell has occurred. Unlike the case during development, where NFH expression lags behind the other two, all three NF subunits decline and recover coordinately during regeneration. Concurrent with the reduction in NF protein levels following injury, both tubulin and actin increase significantly. While NF proteins are low throughout the elongation phase of regeneration, tubulin and actin expression remains high during neurite growth and synaptogenesis. As might be expected, there are associated changes in the expression of MAPs and MF-associated proteins during regeneration as well.

The observation that selected tubulin genes are preferentially upregulated during regeneration has led to proposals that some tubulin isoforms are better suited for neurite growth (see Box 6-1). Nonetheless, the idea that changes in cytoskeletal protein are required for effective regeneration is consistent with observations that changes in cytoskeletal protein expression after injury are limited or altered in CNS neurons that fail to regenerate after injury. The precise regulation of cytoskeletal gene expression during both development and regeneration suggests the importance of these structures for neuronal growth.

Alterations in the cytoskeleton are frequent hallmarks of neuropathology

A definitive diagnosis for many neurological disorders depends on a histological examination. The identifying characteristic for a number of neuropathologies is a disrupted or aberrant cytoskeleton. This is a feature of many toxic neuropathies and a number of neurodegenerative conditions, including motor neuron disease, frontotemporal dementia (FTDP-17) and Alzheimer's disease. Although other neuronal functions may also be affected and the initial cause of the disease may not directly involve the cytoskeleton, the associated pathogenic disruption of cytoskeletal function may be a key element in the loss of neurons or neuronal function. In recent years, a new class of neurological disorders captured a lot of attention in the neuroscience community. Both sporadic and familial forms of these puzzling disorders exist, but all of them exhibit a characteristic accumulation of tau protein aggregates, which have come to be known as tauopathies (Hernandez & Avila, 2007) (see Ch. 47). In the broadest sense, any disease that involves pathological changes in tau may be considered a tauopathy, including Alzheimer's disease (Duyckaerts et al., 2009). Mutations in the tau gene have now been linked to familial forms of these tauopathies, with differences in the severity of symptoms and the cellular location of tau accumulations depending on the specific mutation. These mutations comprise a heterogeneous group of neurological disorders known as FTDP-17 (Hernandez & Avila, 2007). The pathological hallmark in both sporadic and familial tauopathies is the presence of intracellular tau filamentous inclusions that are typically abnormally phosphorylated.

In some cases, the primary pathogenic mechanism is an effect on one or more cytoskeletal structures. For example, some widely used chemotherapeutic agents for treatment of tumors act by disrupting microtubule function in the spindles of rapidly dividing cancer cells. Such drugs can affect MTs in both neuronal and non-neuronal cells. Perhaps the two best known examples are vincristine and paclitaxel (taxol). While these two compounds have opposite actions on microtubules, long-term or high-dosage treatment with either leads to a high frequency of associated neuropathies. Vincristine is a classic antimicrotubule drug that destabilizes existing MTs and blocks assembly of new MTs. In contrast, paclitaxel stabilizes MTs and leads to formation of numerous short MTs in inappropriate sites. Despite these differences, peripheral neuropathies are a troublesome side effect of both drugs. Neuronal viability is compromised because MT function is compromised. Moreover, other environmental toxins or chemicals may interfere with neurofilaments.

In fact, a number of neuropathologies with diverse pathogenic mechanisms have associated disruptions in axonal NF. Neurofilaments accumulate in the cell bodies of motor neurons in patients with amyotrophic lateral sclerosis and related motor neuron diseases (Pasinelli & Brown, 2006). The example of motor neuron disease is particularly interesting, because the symptoms of motor neuron disease can be produced in animal models in several very different ways. Some of these animal models have little obvious relevance to the cytoskeleton, such as those resulting from point mutations in

TUBULIN MUTATIONS AND NEUROLOGICAL DISEASES

Yuyu Song, Scott T. Brady

Heterozygous missense mutations in genes for different tubulin isotypes, such as TUBA1, TUBB2B and TUBB3, as well as a homozygous splice-site mutation in TUBA8, have been associated with a wide spectrum of neurological diseases. Based on the major phenotypes observed and likely underlying cellular mechanisms in patients and animal models, these mutations can be divided into two categories: the first shows lissencephaly and polymicrogyria (e.g., TUBA1, TUBA8, TUBB2B and a subset of TUBB3 mutations) due primarily to cortical cell migration problems; the second shows misinnervation / disinnervation in certain nerves (e.g., the other subset of the TUBB3 mutations) due mainly to aberrant guidance and maintenance of axons. Additional neurological symptoms can be found in both categories, including intellectual impairment and motor defects, ranging from mild to severe. Potential molecular mechanisms include disrupted formation of MT heterodimers; perturbed MT polymerization; altered interactions between MAPs or motor proteins (kinesin and dynein) and MTs; impaired axonal transport and changes in intrinsic dynamic properties of MTs. Some of these phenotypes are shared among different mutations at various sites on several isotypes, but others appear to segregate with specific tubulin isotypes and may be confined to structural domains or even certain amino acid residues. Why some amino acid substitutions lead to distinct phenotypes while others exhibit more similarities is still not clear. One possibility is that different tubulin isotypes may play different roles in neuronal structures and functions during development and maturation, as well as maintenance and modeling throughout life. Four distinct functional aspects may be seen with a given mutation: (1) altered interactions with other tubulin monomers in forming heterodimers or with other tubulin dimers in forming protofilaments; (2) differential binding to GTP/GDP and MAPs that regulate intrinsic properties of MT dynamics and stability; (3) reduced affinity for motor proteins needed to maintain axonal transport and axon guidance; (4) changed responses to environmental cues that determine MT polarity and direction of axon growth. Here, we use a subtype of TUBB3 mutations to illustrate genotype–phenotype correlations and to demonstrate some potential cellular/molecular mechanisms.

Neuron-specific β-III tubulin (TUBB3) mutations may produce congenital fibrosis of extraocular muscles 3 (CFEOM3)

Recently, a class of TUBB3 mutations were shown to produce CFEOM3 (Tischfield et al., 2010), which is a group of eye movement disorders caused by dysfunction of the oculomotor nerve and/or the extraocular muscles innervated by it, and can result from genetic errors in axon growth and guidance. Children suffering from CFEOM are usually affected at birth due to dominant negative mutations. The classical CFEOM symptoms (ptosis and restricted eye movements) are observed as well as additional nervous system disorders, such as peripheral axonal neuropathy, facial paralysis, or intellectual and behavioral impairments. Conventional neuroimaging reveals a spectrum of abnormalities including hypoplasia of oculomotor nerves, as well as dysgenesis of the corpus callosum, anterior commissure, and corticospinal tracts. The most common mutation, which causes relatively isolated CFEOM3, results from an R262C amino acid substitution. A $Tubb3^{R262C}$ knock-in mouse model reveals axon guidance defects of the oculomotor nerve and central axon tracts, without evidence of cortical cell migration abnormalities (Tischfield et al., 2010). These defects suggest that aberrant axon growth and guidance might provide the cellular basis for the disorder, but what is the underlying molecular mechanism? Structural analysis showed that the mutation site for R262C is located in the loop between helix H8 and strand 7 of β-tubulin, below helices H12 and H11, which normally forms a putative hydrogen bond with H12 through the carbonyl oxygen of residue D417. The R262C mutation would abolish this hydrogen bond, potentially affecting motor protein interactions with MTs and leading to isolated CREOM3. Several severe disease-associated TUBB3 substitutions (e.g., E410K and D417N/H) reside directly at putative kinesin interaction sites on β-tubulin. Further, kinesin-microtubule interactions were decreased both in the $Tubb3^{R262C}$ knock-in mouse model and in yeast models bearing the entire allelic series of mutations (Tischfield & Engle, 2010). Therefore, mutations in TUBB3 may cause aberrant axon growth and maintenance by directly or indirectly altering MT interactions with other proteins, including but not necessarily limited to molecular motors in the kinesin family.

Thus, the genetics showed that tubulin mutations could be associated with a clinical neurological disease, CFEOM3. This phenotype is a sensitive indicator of errors in axon growth and guidance, providing insights into which functions of tubulin and MTs affect axon growth and guidance. The genetic studies generate basic neurochemical studies that illuminate pathogenic mechanisms as well as elucidating molecular pathways essential for axon guidance and circuit formation throughout the mammalian nervous system. In turn, these studies may lead to new therapeutic strategies, advancing the care of patients.

a superoxide dismutase gene. Others directly involve neurofilaments, such as transgenic mice that express defective NF subunits or overexpress normal NF subunits. Regardless of how these animal models are produced, a characteristic feature of motor neuropathology is the accumulation of poorly phosphorylated NFs in cell bodies and initial segments (Lariviere & Julien, 2004).

Other diseases with disruptions in neurofilament organization include diabetic neuropathy and Charcot-Marie-Tooth disease (see Chapter 38). For these diseases, the disruption of neurofilaments may either be a secondary effect, as in the case of Trembler axons, or a direct effect. For example, some forms of Charcot-Marie-Tooth peripheral neuropathy result from mutations in a neurofilament subunit (Brownlees et al., 2002). In most cases, neuronal degeneration is an eventual consequence, but neuronal function may be impaired prior to substantial loss of neurons. Generally, disruptions of neurofilaments have the most severe consequences in large motor neurons, which is consistent with the fact that the largest neurons have the highest levels of neurofilament expression.

Phosphorylation of cytoskeletal proteins is involved both in normal function and in neuropathology

An entire book could be devoted to research on the phosphorylation of cytoskeletal proteins. Phosphorylation of cytoskeletal components may affect their assembly and organization as well as their associated function. As described above, levels of phosphorylation on NFs are a major determinant in regulation of axonal caliber, and a specific set of phosphorylations is a hallmark of tau protein in axons. The phosphorylation of other MAPs is carefully regulated during development and maturation of the nervous system. Similarly, many MF-associated proteins are regulated by phosphorylation, so that the local action of kinases and phosphatases may underlie many changes in the membrane cytoskeleton and growth cone motility.

Phosphorylation of cytoskeletal proteins has also been linked to various neuropathologies. For example, the NF accumulations seen in many diseases have aberrant phosphorylation patterns. A more significant change may be the hyperphosphorylation of brain MAP tau, a well-known regulator of neurite extension and MT stability (Conde & Caceres, 2009), which is associated with the formation of neurofibrillary tangles in Alzheimer's disease (AD) (Goedert, 2004). Abnormally phosphorylated and folded protein tau is missorted from axons to somatodendritic compartments in neurons from AD brain, as well as in animal models of AD. One potential functional consequence of abnormal tau phosphorylation and folding is the loss of MT binding capacity, with the concomitant reduction of MT assembly, stability and integrity (Conde & Caceres, 2009; Goedert, 2004). Neurofibrillary tangles contain distinctive paired helical filaments that are extremely resistant to solubilization and analysis. They were initially thought to be aberrant NFs based on their dimensions, but a careful immunochemical and biochemical dissection showed that the primary polypeptide present in tangles was tau, the axonally enriched MAP (Kosik et al., 1988). The tau in neurofibrillary tangles is differentially phosphorylated and this misphosphorylation is thought to play a role in the formation of tangles. While the precise etiology of neurofibrillary tangles and their relationship to the deposition of amyloid are not yet certain, the appearance of these tangles is closely correlated with loss of neurons and their number is a good indication of how far the disease has advanced. Clearly, alterations in the axonal cytoskeleton are an important component of the Alzheimer's neuropathology (Hernandez & Avila, 2007) (see Chs. 46–47).

SUMMARY

The architectures of neurons and glia are generated by the diverse specializations of MTs, NFs and MFs. Each of these components forms a set of structures that are constantly changing and subject to the influence of extracellular signals. While the importance of the cytoskeleton for development, maintenance and regeneration of nerve fibers is now well documented, many details about their activities remain to be delineated. Continued exploration of these phenomena will provide the basis for a deeper understanding of neuronal development, regeneration and neuropathology.

References

Amos, L. A., & Schlieper, D. (2005). Microtubules and maps. *Advances in Protein Chemistry*, *71*, 257–298.

Baas, P. W., Karabay, A., & Qiang, L. (2005). Microtubules cut and run. *Trends in Cell Biology*, *15*(10), 518–524.

Baas, P. W., Vidya Nadar, C., & Myers, K. A. (2006). Axonal transport of microtubules: The long and short of it. *Traffic*, *7*(5).

Beck, K. A., & Nelson, J. (1996). The Spectrin-based membrane skeleton as a membrane protein-sorting machine. *American Journal of Physiology*, *270*, C1263–C1270.

Bommel, H., Xie, G., Rossoll, W., Wiese, S., Jablonka, S., Boehm, T., et al. (2002). Missense mutation in the tubulin-specific chaperone E (Tbce) gene in the mouse mutant progressive motor neuronopathy, a model of human motoneuron disease. *Journal of Cell Biology*, *159*(4), 563–569.

Brady, S. T. (1993). Axonal dynamics and regeneration. In A. Gorio (Ed.), *Neuroregeneration* (pp. 7–36). New York, NY: Raven Press.

Brown, M. E., & Bridgman, P. C. (2004). Myosin function in nervous and sensory systems. *Journal of Neurobiology*, *58*(1), 118–130.

Brownlees, J., Ackerley, S., Grierson, A. J., Jacobsen, N. J., Shea, K., Anderton, B. H., et al. (2002). Charcot-Marie-Tooth disease neurofilament mutations disrupt neurofilament assembly and axonal transport. *Human Molecular Genetics*, *11*(23), 2837–2844.

Burns, R. G., & Surridge, C. D. (1994). Tubulin: Conservation and structure. In J. S. Hyams & C. W. Lloyd (Eds.), *Microtubules* (Vol. 13, pp. 3–31). New York, NY: Wiley.

Burridge, K., & Wennerberg, K. (2004). Rho and Rac take center stage. *Cell*, *116*(2), 167–179.

Caceres, A., & Kosik, K. S. (1990). Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. *Nature*, *343*, 461–462.

Cassimeris, L., & Spittle, C. (2001). Regulation of microtubule-associated proteins. *International Review of Cytology*, *210*, 163–226.

Conde, C., & Caceres, A. (2009). Microtubule assembly, organization and dynamics in axons and dendrites. *Nature Reviews Neuroscience*, *10*(5), 319–332.

de Waegh, S. M., Lee, V. M.-Y., & Brady, S. T. (1992). Local modulation of neurofilament phosphorylation, axonal caliber, and slow axonal transport by myelinating schwann cells. *Cell*, *68*, 451–463.

Dehmelt, L., & Halpain, S. (2005). The MAP2/Tau family of microtubule-associated proteins. *Genome Biology*, *6*(1), 204.

Dent, E. W., Callaway, J. L., Szebenyi, G., Baas, P. W., & Kalil, K. (1999). Reorganization and movement of microtubules in axonal growth cones and developing interstitial branches. *Journal of Neuroscience*, *19*(20), 8894–8908.

Desai, A., & Mitchison, T. J. (1997). Microtubule polymerization dynamics. *Annual Review of Cell and Developmental Biology*, *13*, 83–117.

Deshpande, A., Win, K. M., & Busciglio, J. (2008). Tau isoform expression and regulation in human cortical neurons. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *The FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, 22(7), 2357–2367. doi: 10.1096/fj.07-096909.

dos Remedios, C. G., Chhabra, D., Kekic, M., Dedova, I. V., Tsubakihara, M., Berry, D. A., et al. (2003). Actin binding proteins: Regulation of cytoskeletal microfilaments. *Physiological Reviews*, *83*(2), 433–473.

Duyckaerts, C., Delatour, B., & Potier, M. C. (2009). Classification and basic pathology of Alzheimer disease. *Acta Neuropathologica*, *118*(1), 5–36.

Fukushima, N., Furuta, D., Hidaka, Y., Moriyama, R., & Tsujiuchi, T. (2009). Post-translational modifications of tubulin in the nervous system. *Journal of Neurochemistry*, *109*(3), 683–693.

Gallo, G., & Letourneau, P. C. (2004). Regulation of growth cone actin filaments by guidance cues. *Journal of Neurobiology*, *58*(1), 92–102.

Geraldo, S., Khanzada, U. K., Parsons, M., Chilton, J. K., & Gordon-Weeks, P. R. (2008). Targeting of the F-actin-binding protein drebrin by the microtubule plus-tip protein EB3 is required for neuritogenesis. *Nature Cell Biology*, *10*(10), 1181–1189.

Goedert, M. (2004). Tau protein and neurodegeneration. *Seminars in Cell and Developmental Biology*, *15*(1), 45–49.

Goode, B. L., & Eck, M. J. (2007). Mechanism and function of formins in the control of actin assembly. *Annual Review of Biochemistry*, *76*, 593–627.

Gordon-Weeks, P. R. (2004). Microtubules and growth cone function. *Journal of Neurobiology*, *58*(1), 70–83.

Halpain, S., & Dehmelt, L. (2006). The MAP1 family of microtubule-associated proteins. *Genome Biology*, *7*(6), 224.

Harada, A., Oguchi, K., Okabe, S., Kuno, J., Terada, S., Ohshima, T., et al. (1994). Altered microtubule organization in small-calibre axons of mice lacking tau protein. *Nature*, *369*(6480), 488–491.

Hernandez, F., & Avila, J. (2007). Tauopathies. *Cellular and Molecular Life Sciences*, *64*(17), 2219–2233.

Herrmann, H., & Aebi, U. (2000). Intermediate filaments and their associates: Multi-talented structural elements specifying cytoarchitecture and cytodynamics. *Current Opinion in Cell Biology*, *12*(1), 79–90.

Herrmann, H., Bar, H., Kreplak, L., Strelkov, S. V., & Aebi, U. (2007). Intermediate filaments: from cell architecture to nanomechanics. *Nature Reviews Molecular Cell Biology*, *8*(7), 562–573.

Holleran, E. A., Ligon, L. A., Tokito, M., Stankewich, M. C., Morrow, J. S., & Holzbaur, E. L. (2001). beta III spectrin binds to the Arp1 subunit of dynactin. *Journal of Biological Chemistry*, *276*(39), 36598–36605.

Hyams, J. S., & Lloyd, C. W. (Eds.), *Microtubules* (Vol. 13). New York, NY: Wiley.

Joshi, H. C. (1994). Microtubule organizing centers and gamma-tubulin. *Current Opinion in Cell Biology*, *6*, 54–62.

Kann, M. L., Soues, S., Levilliers, N., & Fouquet, J. P. (2003). Glutamylated tubulin: Diversity of expression and distribution of isoforms. *Cell Motility and the Cytoskeleton*, *55*(1), 14–25.

Kosik, K. S., Orecchio, L. D., Binder, L., Trojanowski, J. Q., Lee, V., & Lee, G. (1988). Epitopes that span the tau molecule are shared with paired helical filaments. *Neuron*, *1*, 817–825.

Lariviere, R. C., & Julien, J. P. (2004). Functions of intermediate filaments in neuronal development and disease. *Journal of Neurobiology*, *58*(1), 131–148.

Lasek, R. J. (1988). Studying the intrinsic determinants of neuronal form and function. In R. J. Lasek & M. M. Black (Eds.), *Intrinsic Determinants of Neuronal Form and Function* (pp. 1–60). New York, NY: Liss.

Lee, M. K., & Cleveland, D. W. (1996). Neuronal intermediate filaments. *Annual Review of Neuroscience*, *19*, 187–217.

Letourneau, P. C. (2009). Actin in axons: Stable scaffolds and dynamic filaments. *Results & Problems in Cell Differentiation*, *48*, 65–90.

Lowery, L. A., & Van Vactor, D. (2009). The trip of the tip: Understanding the growth cone machinery. *Nature Reviews Molecular Cell Biology*, *10*(5), 332–343.

Luders, J., & Stearns, T. (2007). Microtubule-organizing centres: A re-evaluation. *Nature Reviews Molecular Cell Biology*, *8*(2), 161–167.

Luduena, R. (1998). Multiple forms of tubulin: Different gene products and covalent modifications. *International Review of Cytology*, *178*, 207–275.

Mandell, J. W., & Banker, G. A. (1995). The microtubule cytoskeleton and the development of neuronal polarity. *Neurobiology of Aging*, *16*, 229–238.

Matov, A., Applegate, K., Kumar, P., Thoma, C., Krek, W., Danuser, G., et al. (2010). Analysis of microtubule dynamic instability using a plus-end growth marker. *Nature Methods*, *7*(9), 761–768.

McKean, P. G., Vaughan, S., & Gull, K. (2001). The extended tubulin superfamily. *Journal of Cell Science*, *114*(Pt 15), 2723–2733.

Moritz, M., & Agard, D. A. (2001). Gamma-tubulin complexes and microtubule nucleation. *Current Opinion in Structural Biology*, *11*(2), 174–181.

Morrison, E. E., Moncur, P. M., & Askham, J. M. (2002). EB1 identifies sites of microtubule polymerisation during neurite development. *Brain Research Molecular Brain Research*, *98*(1–2), 145–152.

Nogales, E., Wolf, S. G., & Downing, K. H. (1998). Structure of the $\alpha\beta$ tubulin dimer by electron crystallography. *Nature*, *391*, 199–203.

Pak, C. W., Flynn, K. C., & Bamburg, J. R. (2008). Actin-binding proteins take the reins in growth cones. *Nature Reviews Neuroscience*, *9*(2), 136–147.

Pasinelli, P., & Brown, R. H. (2006). Molecular biology of amyotrophic lateral sclerosis: Insights from genetics. *Nature Reviews Neuroscience*, *7*(9), 710–723.

Perrin, B. J., & Ervasti, J. M. (2010). The actin gene family: Function follows isoform. *Cytoskeleton (Hoboken)*, *67*(10), 630–634.

Pollard, T. D. (2007). Regulation of actin filament assembly by Arp2/3 complex and formins. *Annual Review of Biophysics and Biomolecular Structructure*, *36*, 451–477.

Qiu, J., Cai, D., & Filbin, M. T. (2000). Glial inhibition of nerve regeneration in the mature mammalian CNS. *Glia*, *29*(2), 166–174.

Raynaud-Messina, B., & Merdes, A. (2007). Gamma-tubulin complexes and microtubule organization. *Current Opinion in Cell Biology*, *19*(1), 24–30.

Roll-Mecak, A., & McNally, F. J. (2010). Microtubule-severing enzymes. *Current Opinion in Cell Biology*, *22*(1), 96–103.

Scherer, S. S., Arroyo, E. J., & Peles, E. (2004). Functional organization of the nodes of ranvier. In R. A. (2004). Lazzarini (Ed.), *Myelin Biology and Disorders* (Vol. 1, pp. 89–116). Amsterdam: Elsevier Academic.

Schoenfeld, T. A., & Obar, R. A. (1994). Diverse distribution and function of fibrous microtubule-associated proteins in the nervous system. *International Review of Cytology*, *151*, 67–137.

Solowska, J., Morfini, G., Falnikar, A., Himes, T., Brady, S., Huang, D., et al. (2008). Quantitative and functional analyses of spastin in

the nervous system: Implications for hereditary spastic paraplegia. *Journal of Neuroscience*, *28*, 2147–2157.

Steinmetz, M. O. (2007). Structure and thermodynamics of the tubulin-stathmin interaction. *Journal of Structural Biology*, *158*(2), 137–147.

Steinmetz, M. O., Stoffler, D., Hoenger, A., Bremer, A., & Aebi, U. (1997). Actin: From cell biology to atomic detail. *Journal of Structural Biology*, *119*(3), 295–320.

Sudo, H., & Baas, P. W. (2010). Acetylation of microtubules influences their sensitivity to severing by katanin in neurons and fibroblasts. *Journal of Neuroscience*, *30*(21), 7215–7226.

Tischfield, M. A., Baris, H. N., Wu, C., Rudolph, G., Van Maldergem, L., He, W., et al. (2010). Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. *Cell*, *140*(1), 74–87.

Tischfield, M. A., & Engle, E. C. (2010). Distinct alpha- and beta-tubulin isotypes are required for the positioning, differentiation and survival of neurons: New support for the "multi-tubulin" hypothesis. *Bioscience Reports*, *30*(5), 319–330.

Trojanowski, J. Q., Smith, A. B., Huryn, D., & Lee, V. M. (2005). Microtubule-stabilising drugs for therapy of Alzheimer's disease and other neurodegenerative disorders with axonal transport impairments. *Expert Opinion on Pharmacotherapy*, *6*(5), 683–686.

Valderrama, F., Babia, T., Ayala, I., Kok, J. W., Renau-Piqueras, J., & Egea, G. (1998). Actin microfilaments are essential for the cytological positioning and morphology of the Golgi complex. *European Journal of Cell Biology*, *76*(1), 9–17.

Votin, V., Nelson, W. J., & Barth, A. I. (2005). Neurite outgrowth involves adenomatous polyposis coli protein and beta-catenin. *Journal of Cell Science*, *118*(Pt 24), 5699–5708.

Wade, R. H. (2007). Microtubules: An overview. *Methods in Molecular Medicine*, *137*, 1–16.

Wade, R. H. (2009). On and around microtubules: An overview. *Molecular Biotechnology*, *43*(2), 177–191.

Wiese, C., Rolletschek, A., Kania, G., Blyszczuk, P., Tarasov, K. V., Tarasova, Y., et al. (2004). Nestin expression—A property of multi-lineage progenitor cells? *Cellular and Molecular Life Sciences*, *61*(19–20), 2510–2522.

Wiese, C., & Zheng, Y. (2006). Microtubule nucleation: Gamma-tubulin and beyond. *Journal of Cell Science*, *119*(Pt 20), 4143–4153.

Witt, A., & Brady, S. T. (2000). Unwrapping new layers of complexity in axon/glial relationships. *Glia*, *29*, 112–117.

Zhang, D., Rogers, G. C., Buster, D. W., & Sharp, D. J. (2007). Three microtubule severing enzymes contribute to the "Pacman-flux" machinery that moves chromosomes. *Journal of Cell Biology*, *177*(2), 231–242.

Zhou, F. Q., & Cohan, C. S. (2004). How actin filaments and microtubules steer growth cones to their targets. *Journal of Neurobiology*, *58*(1), 84–91.