

Chapter 8

Molecular Motors in Cargo Trafficking and Synapse Assembly

Robert van den Berg and Casper C. Hoogenraad

Abstract Every production process, be it cellular or industrial, depends on a constant supply of energy and resources. Synapses, specialized junctions in the central nervous system through which neurons signal to each other, are no exception to this rule. In order to form new synapses and alter the strength of synaptic transmission, neurons need a regulatory mechanism to deliver and remove synaptic proteins at synaptic sites. Neurons make use of active transport driven by molecular motor proteins to move synaptic cargo over either microtubules (kinesin, dynein) or actin filaments (myosin) to their specific site of action. These mechanisms are crucial for the initial establishment of synaptic specializations during synaptogenesis and for activity-dependent changes in synaptic strength during plasticity. In this chapter, we address the organization of the neuronal cytoskeleton, focus on synaptic cargo transport activities that operate in axons and dendrites, and discuss the spatial and temporal regulation of motor protein-based transport.

Keywords Actin • Axon • Cytoskeleton • Dendrite • Disease • Dynein • Kinesin • Microtubule • Myosin • Neuron • Synapse • Synaptic plasticity

8.1 Introduction

One apparent feature of neurons is that once the axon and dendrites have grown out, they establish synaptic contacts forming neuronal networks that propagate information in a unidirectional fashion. Excitatory synaptic signaling in the brain occurs by releasing glutamate from “sending” neurons and activating glutamate receptors

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at “receiving” neurons (Sheng and Hoogenraad 2007; Südhof 2004). Structurally, synapses are divided into two specialized domains: the presynaptic bouton on the axon side of the “sending neuron” and the postsynaptic compartment on the dendrite of the “receiving” neuron. The directional nature of signal relay requires that synaptic contacts are morphologically asymmetric with distinct protein components. Recent studies have identified the molecular components of synapses, particularly by using genetic and proteomic strategies, and have revealed that the specification of synaptic function, for example, excitatory or inhibitory, at both pre- and postsynapses is achieved via the recruitment and assembly of very distinct synaptic complexes (Jin and Garner 2008; Kim and Sheng 2004; Margeta et al. 2008; Sheng and Hoogenraad 2007). Proper arrangement of pre- and postsynaptic membranes and organization of pre- and postsynaptic compartments is essential for accurate synaptic signaling, neural network activity, and cognitive processes such as learning and memory formation (Kasai et al. 2010; Lisman et al. 2007; Yuste and Bonhoeffer 2001).

Most of the synaptic cargos, such as neurotransmitter receptors, ion channels, integral membrane proteins, signaling complexes, mRNAs, synaptic vesicle precursors, or mitochondria, are made and preassembled in the cell soma and need to be transported to the proper synaptic destinations. Studies on intracellular trafficking have demonstrated various mechanisms for compartment-specific localization (Goldstein and Yang 2000; Hirokawa and Takemura 2005; Hoogenraad and Bradke 2009; Winckler and Mellman 2010). For example, several synaptic cargos are nonspecifically transported to both axons and dendrites and are then selectively retained at the required compartments (Bel et al. 2009; Garrido et al. 2001; Letierri et al. 2006; Sampo et al. 2003; Wisco et al. 2003). Alternatively, many presynaptic cargos are correctly sorted into axons (Kaether et al. 2000; Pennuto et al. 2003), whereas postsynaptic components move specifically into dendritic branches and spines, which are specialized dendritic protrusions that mediate most of the excitatory synaptic transmission (Craig et al. 1993; Ruberti and Dotti 2000; Stowell and Craig 1999; Wang et al. 2008). In consequence, synaptic precursor vesicles need to be steered into axons in order to reach the presynaptic terminals, and glutamate receptors need to be transported into dendrites to be correctly inserted in the postsynaptic membrane. Importantly, several neurological diseases are linked to abnormalities in the machinery that controls synaptic cargo trafficking (Chevalier-Larsen and Holzbaur 2006; Gunawardena and Goldstein 2004; Lau and Zukin 2007; Shepherd and Huganir 2007; van Spronsen and Hoogenraad 2010).

Most intracellular cargo transport is driven by molecular motor proteins that move along two types of cytoskeletal elements: actin filaments and microtubules (Schliwa and Woehlke 2003; Vale 2003). Actin facilitates motility of motor proteins of the myosin superfamily, whereas microtubules serve as tracks for two families of motor proteins, kinesin and dynein. While many different motor proteins have been found to participate in neuronal cargo trafficking (Goldstein and Yang 2000; Hirokawa and Takemura 2005), for many of these their precise contribution to synaptic cargo transport has remained unclear. Most current models for neuronal trafficking rely heavily on microtubule plus-end-directed kinesin

family members (Hirokawa and Takemura 2005); however, recent work reported important roles for dynein and myosin in synaptic cargo transport (Kapitein et al. 2010; Lewis et al. 2009; Zheng et al. 2008). Further evidence suggests that the docking of molecular motors to synaptic cargo vesicles via adaptor molecules is an important mechanism to achieve transport specificity (Akhmanova and Hammer 2010; Schlager and Hoogenraad 2009).

It has intrigued scientists for a long time how synaptic cargo can be sorted in neurons along the cytoskeleton network to ensure precise cargo delivery. How are motor-cargo complexes able to choose between transport routes to the axon or dendrites? Here, it is important to consider that the actin and microtubule filaments themselves are intrinsically polarized structures with two functionally distinct ends, a “plus” and “minus” end. The polarity of the cytoskeleton filaments exists not only at the two ends but also all along the length of its lattice, which is critical for the directional movement of molecular motor proteins. For example, dynein transports cargo toward the minus end of microtubules, while kinesins motor proteins move toward the plus end of microtubules. In this way, local polarity patterns of microtubules and actin filaments in axon and dendrites can direct motor-driven cargo trafficking within neurons. Recent evidence suggests that a well-organized cytoskeleton network exists in neurons that can facilitate directional motor-driven cargo trafficking and establish asymmetric distributions of specific synaptic proteins (Kapitein and Hoogenraad 2010). Moreover, variations in cytoskeleton density, binding proteins, and posttranslational modifications could also drive synaptic cargo transport in specific directions. Thus, knowing the polarity and modification pattern of microtubules and actin in axon and dendrites is an instrumental piece of information for understanding how molecular motors direct synaptic cargo traffic.

In this chapter, we aim to give an overview of the molecular trafficking mechanisms important for the delivery of synaptic proteins. We will review current knowledge about the organization of the neuronal cytoskeleton, focus on synaptic cargo sorting and trafficking into axons and dendrites, and discuss the spatial and temporal regulation of motor protein-based transport. Studying the basic cellular machinery for synaptic cargo trafficking will help us to understand fundamental principles of synapse formation, function, and plasticity.

8.2 Microtubule and Actin Cytoskeleton in Neurons

The cytoskeletal organization in neurons is specialized in several ways, involving intracellular variations in density, orientations, binding proteins, and posttranslational modifications. Recently, it has become increasingly clear that these specific cytoskeletal properties directly modulate the activity of specific molecular motor proteins. In this section, we will review current knowledge about the structure, organization, and modifications of the microtubule and actin cytoskeleton (Fig. 8.1).

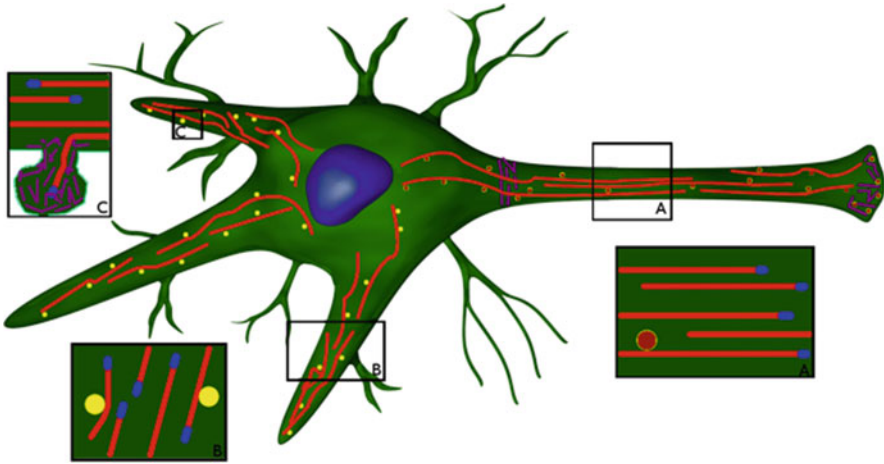


Fig. 8.1 Cytoskeleton organization in neurons. Microtubules (*red*) are present in both axons and dendritic shaft, while actin (*purple*) is enriched in the axon initial segment and dendritic spines. From the cell soma, synaptic cargo vesicles are sorted into the dendrites (*yellow vesicles*) or the axon (*red vesicles*). (a) In the axon, all microtubules are oriented with their plus ends (*blue comets*) pointing outward. (b) In dendrites, microtubules have a mixed orientation. (c) Dynamic microtubules occasionally enter spines

8.2.1 Actin and Microtubule Structure and Dynamics

The main transport infrastructure in eukaryotic cells is formed by the microtubules and actin cytoskeleton. To serve this function, the cytoskeleton must be organized into a wide variety of configurations, ranging from the higher-order actin-based networks in dendritic spines to the dense antiparallel microtubule array in dendritic shaft. Without the cytoskeleton, neurons would not be able to maintain their complex axonal and dendritic architectures and synaptic organization. Microtubules are noncovalent polymers of α - and β -tubulin dimers that form a hollow cylinder with a diameter of 25 nm and actin filaments consist of monomers of the protein actin that polymerizes to form 8-nm fibers (Howard and Hyman 2009; Pollard and Cooper 2009). There are multiple actin and α - and β -tubulin genes that are highly conserved among and between species and might be utilized for distinct neuronal functions. With the recent discovery of several congenital neurological disorders that result from mutations in genes that encode different α - and β -tubulin isotypes, novel paradigms have emerged to assess how selective perturbations in microtubule subunits affect neuronal functioning (Baran et al. 2010; Keays et al. 2007). For example, a series of heterozygous missense mutations in TUBB3, encoding the neuron-specific β -tubulin isotype III, have been described that result in a spectrum of human nervous system disorders (Tischfield et al. 2010). A knock-in disease mouse model reveals axon guidance defects without evidence of cortical cell migration abnormalities. Importantly, it was demonstrated that the

disease-associated mutations impair tubulin heterodimer formation and disrupt the interaction with kinesin motors.

Microtubules and actin filaments are intrinsically polar structures and contain two distinct ends, a “plus end” favored for assembly/disassembly and a “minus end” which is less favored for these dynamics. Minus ends of microtubules are often, but not always, attached to the centrosome from which the microtubule is nucleated. It was recently found that the centrosome loses its function as a microtubule-organizing center during development of hippocampal neurons (Stiess et al. 2010). It is well possible that acentrosomal microtubule nucleation arranges the neuronal microtubule cytoskeleton in mature neurons and is responsible for the extended and complex morphology of neurons. In living cells, microtubules and actin filaments are highly dynamic, and their dominant kinetic behavior is known as dynamic instability, where the individual ends alternate between bouts of growth (“polymerization”) and shrinkage (“depolymerization”) (Mitchison and Kirschner 1984). Microtubules and actin filaments may also undergo treadmilling, a phenomenon in which filament length remains approximately constant, while monomers add at the plus end and dissociate from the minus end (Kueh and Mitchison 2009).

Regulation of microtubule and actin dynamics and turnover plays an important role in neuronal development and synaptic plasticity (Conde and Caceres 2009; Dent and Gertler 2003; Frost et al. 2010a; Hotulainen and Hoogenraad 2010). It is not surprising that recently, a lot of attention has been given to the plus ends of the microtubule, the site where most dynamics takes place. The microtubule plus end can grow, then undergo a shrinking event (“catastrophe”), pause, and grow again (“rescue”), all in a matter of seconds. The fate of the microtubule is determined by a large number of plus-end tracking proteins, most of them only found on the microtubule tip, that can control microtubule dynamics (Akhmanova and Steinmetz 2008; Gouveia and Akhmanova 2010). Plus-end tracking proteins regulate different aspects of neuronal architecture by mediating the cross talk between microtubule ends, the actin cytoskeleton, and the cell cortex, and participate in transport and positioning of signaling factors and membrane organelles (Hoogenraad and Bradke 2009; Jaworski et al. 2008). For example, it was recently found that the microtubule plus-end binding protein EB3 regulates actin dynamics in dendritic spines and is involved in spine morphology and synaptic plasticity (Jaworski et al. 2009).

8.2.2 Actin Organization in Neurons

Microtubules and actin filaments are present throughout the cell body and axonal and dendritic compartments (Cingolani and Goda 2008; Conde and Caceres 2009; Hotulainen and Hoogenraad 2010). In mature neurons, actin is the most prominent cytoskeletal protein at synapses, being present at both the pre- and the postsynaptic terminals but highly enriched at dendritic spines (Landis et al. 1988; Landis and

Reese 1983). Neurons treated with latrunculin, an inhibitor of actin polymerization, showed that the actin cytoskeleton is necessary for synapse formation, stability, and normal synaptic activity (Krucker et al. 2000; Okamoto et al. 2004). At the presynaptic terminal, actin filaments are important in organizing and recycling of synaptic vesicle (Cingolani and Goda 2008), while at the postsynaptic site, actin is involved in receptor trafficking and spine plasticity (Frost et al. 2010a; Hotulainen and Hoogenraad 2010). Spines exhibit a continuous network of both branched and long, linear actin filaments (Korobova and Svitkina 2010). In the spine heads, actin filaments are oriented with their plus ends to the postsynaptic density and synaptic membrane and their minus ends toward the dendritic shaft. The most likely role of actin in mature spines is to stabilize postsynaptic proteins and modulate spine head structure in response to postsynaptic signaling (Frost et al. 2010a; Hotulainen and Hoogenraad 2010). Several recent studies using advanced microscopy techniques present new insights in the organization and molecular composition of actin cytoskeleton in dendritic spines (Frost et al. 2010b; Honkura et al. 2008; Korobova and Svitkina 2010). For example, electron microscopy revealed that actin filaments in the neck of dendritic spines were shown to exhibit mixed polarity, although the plus ends are predominantly oriented away from the dendritic shaft (Hotulainen et al. 2009; Korobova and Svitkina 2010). Mutations in genes that code for actin regulatory proteins, like Rho GTPases, are commonly associated with mental retardation (Govek et al. 2004). Subtle variations in spine size and shape, mediated by the actin cytoskeleton, are associated with a variety of neurological and psychiatric disorders like schizophrenia and drug addiction (Blanpied and Ehlers 2004; van Spronsen and Hoogenraad 2010).

Apart from its organization in synapses and spines, little is known about the arrangement of the actin cytoskeleton in the axonal and dendritic shafts in mature neurons (Kapitein and Hoogenraad 2010). Evidence suggests that actin is present as short, branched filaments in the axon oriented perpendicular to the plasma membrane and is sometimes aligned with microtubules (Bearer and Reese 1999). Several papers showed that actin is enriched in the axon initial segment (Nakada et al. 2003; Winckler et al. 1999). Here, actin is an important regulator of Na^+ -channel stability at the initial segment membrane and is actively involved in the maintenance of neuronal polarity (Rasband 2010). The actin network in the initial segment could also function as a selective barrier for cargo transport to enter the axon. In fact, a recent paper presented evidence for an actin-based molecular sieve that prevents the diffusional entry of large macromolecules at the initial segment and isolates the axon from the cell body (Song et al. 2009). In contrast, other work proposed that the actin organization in axons promotes retrograde cargo transport by myosin Va motors (Lewis et al. 2009), which would require actin filaments of uniform polarity oriented parallel to the axon long axis. To better understand actin-based transport activity in neurons, detailed studies are required to reveal the actin cytoskeleton organization in axons, dendrites, and at the initial segment.

8.2.3 *Microtubule Organization in Neurons*

In mature neurons, most neuronal microtubules are not attached to the centrosome (Stiess et al. 2010) and form dense bundles running along the length of axons and dendrites (Fig. 8.1). Individual microtubules do not extend along the entire length of neuronal processes; instead, microtubule fragments stabilized at their minus ends form regularly spaced longitudinal arrays cross-linked by microtubule-associated proteins (MAPs) (Chen et al. 1992). Two abundant neuronal MAPs whose distributions are polarized between axons and dendrites are tau and MAP2 (Dehmelt and Halpain 2005). Since tau and MAP2 induce the formation of longitudinal microtubule bundles with a distinct spacing, it is plausible that MAPs are directly involved in the organizing of polarized cargo trafficking in axons and dendrites. So far, there is no evidence that this different spacing directly influences the transport machinery. In contrast, the presence of MAPs on the microtubule lattice seems to affect motor protein motility *in vitro* (Dixit et al. 2008). MAPs can cause decreased attachment and/or increased detachment of kinesin-1 motors and/or vesicles (Verhey and Hammond 2009). However, a direct role of MAPs in guiding polarized transport seems unlikely, as tau and MAP2 knockout mice show relatively mild phenotypes (Dehmelt and Halpain 2005).

Microtubule arrays within axon and dendrites are highly organized with respect to their intrinsic polarity (Hoogenraad and Bradke 2009; Kapitein and Hoogenraad 2010). In the late 1980s, an elegant hook-decoration technique was used to determine the orientation of microtubules in axons and dendrites by electron microscopy (Baas et al. 1988; Burton 1988). Surprisingly, microtubules in the axon are generally long and uniformly oriented, with their plus ends facing outward, while the microtubules in the dendrites are oriented in both directions (Fig. 8.1). Thinner, more distal dendrites, however, contain unipolar microtubules oriented the same way as axonal ones (Baas et al. 1988, 1989). This specialized microtubule organization has also been captured in action by visualizing EB3-GFP in living neuronal cells (Jaworski et al. 2009; Morrison et al. 2002; Stepanova et al. 2003). Stepanova et al. showed that in axons and distal dendrites, all dynamic microtubule plus ends point toward growth cones, while in proximal dendrites, significant EB3-GFP displacement was directed toward the cell body (Stepanova et al. 2003). Remarkably, the use of EB1-GFP tracking in *Drosophila* neurons provided strong evidence that axons and dendrites both have microtubule arrays of uniform polarity orientation. However, while axonal microtubules were uniformly plus-end distal, dendritic microtubules were of the opposite orientation, nearly all minus-end out (Rolls et al. 2007; Stone et al. 2008). These results show a striking difference in microtubule orientations in axons and dendrites and further prompt the idea that these distinct patterns might underlie polarized sorting of synaptic cargos (Fig. 8.2). Distinct patterns of microtubule orientation can directly generate asymmetries in the composition of each neuronal compartment by directing specific motor protein transport into axons or dendrites. Indeed, recent work in vertebrates, worms, and flies reported a specific role for plus- and minus-end-directed motor proteins in steering

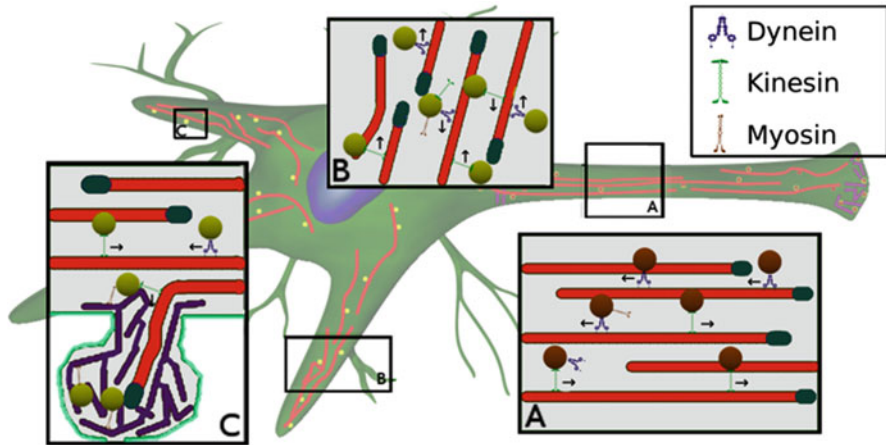


Fig. 8.2 Essential components of the synaptic cargo transport machinery. Kinesin, dynein, and myosin are the three classes of motor proteins that form the workforce of the transport system. (a) In the axon, dynein typically moves its cargo retrograde, toward the cell body, while kinesins move outward toward the synapse. Multiple active and inactive motors can be simultaneously attached to one synaptic cargo vesicle. (b) The situation in the dendrite is more complex, as both kinesins and dynein can move in antero- and retrograde direction, depending on the orientation of the underlying microtubule. (c) Upon arrival at the base of the spine, cargo vesicles are transported along the actin network by myosin motors

synaptic cargo transport into either axons or dendrites (Kapitein et al. 2010; Zheng et al. 2008) and found that the cyclin-dependent kinase pathway regulates polarized trafficking of presynaptic components (Ou et al. 2010).

8.2.4 Microtubule Modifications in Neurons

One other way to directly influence synaptic cargo transport is to generate functional diversity by modifying the cytoskeleton; motor proteins recognize the various spatial cues and establish specific synaptic cargo trafficking routes. It has also recently been demonstrated that posttranslational modification of microtubules can alter their stability and motor protein-binding characteristics (Verhey and Hammond 2009). Stable microtubules in neurons typically accumulate a variety of posttranslational modifications, like acetylation, deetyrosination, and (poly) glutamylation. Although both dendrites and axons have high levels of acetylated microtubules, acetylated microtubules are abundantly present in axons (Witte et al. 2008). The selective enrichment of acetylated microtubules in axons can be abolished by inhibition of a known α -tubulin deacetylase histone deacetylase 6 (HDAC6), suggesting that in normal situations, the activity of tubulin-modifying enzymes differs between axons and dendrites rather than that the acetylation reaction is restricted to the axon (Janke and Kneussel 2010). Biochemical evidence

revealed that kinesin-1 has an increased affinity for acetylated microtubules, consistent with the observation that in fibroblast cells, kinesin-1 motility occurs predominantly over modified microtubules (Cai et al. 2009). It was proposed that acetylation of microtubules was the major determinant for the selective motility of kinesin-1 motors into specific neurites. Kinesin-1 also preferentially binds detyrosinated microtubules (Liao and Gundersen 1998), which are also enriched in the axon. Recent work identified a specific region in kinesin-1, termed β 5-L8, to be responsible for this preference (Konishi and Setou 2009). Interestingly, upon knockdown of tubulin tyrosine ligase (TTL), the fraction of dendrites that contained kinesin-1 increased. Recently, synaptic activity has been shown to modify microtubules posttranslational modifications (Maas et al. 2009). Treatment of neurons with strychnine, an inhibitor of the glycine receptor, increases neuronal activity, leads to increased polyglutamylation, and influences synaptic cargo transport. In this way, microtubule modifications are intimately related to synaptic changes and synaptic cargo transport.

8.3 Microtubule- and Actin-Based Motor Proteins

In general, cargos are transported over long distances along microtubules before transferring to the actin cytoskeleton for the final part of their journey. A common feature of both actin- and microtubule-based transport is that the force required for cargo transport is generated by molecular motor proteins through ATP hydrolysis (Kardon and Vale 2009; Schliwa and Woehlke 2003; Vale 2003; Woolner and Bement 2009). The motor proteins that use microtubules as tracks are the minus-end-directed dynein and plus-end-directed kinesins, whereas myosins move along actin filaments. Neuronal cargo trafficking is achieved by the concerted efforts of both microtubule-based and actin-based motors (Hirokawa and Takemura 2005; Schlager and Hoogenraad 2009). Several classes of myosin motors participate in synaptic cargo transport in axon and dendrites—most commonly used are myosin V and myosin VI. Although the basic machinery for microtubule- and actin-dependent transport in neurons is well established, how synaptic cargos achieve specificity and directionality to their site of action is an emerging field of investigation. In this section, we will introduce the actin and microtubule transport system and its main components. We will also explain the role the actin network plays in selecting the destination of cargo and its role in synaptic function.

8.3.1 *Myosin Motor Proteins in Neurons*

Myosins were the first molecular motors to be discovered and comprise a large (~25 classes) evolutionarily conserved family of actin-based motor proteins (Conti and Adelstein 2008). Early studies focused on the role of myosins as force generators in

muscle tissue. The head region in the myosin heavy chain contains the motor domain; this is the site of ATP hydrolysis, which leads to a force generating conformational change (Conti and Adelstein 2008). It was long thought that the sole function of myosin was to generate force in muscles; however, with the discovery of nonmuscle (unconventional) myosin, new roles for myosin motors were uncovered. Several myosin motors can move directionally along actin filaments, such as myosin V toward the plus end and myosin VI toward the minus end (Sweeney and Houdusse 2010; Woolner and Bement 2009). Myosin motors have been implicated in short-range transport of synaptic cargos, especially in the areas of the neuron where there is hardly any microtubule network, like dendritic spines and presynaptic terminals. For example, myosin V and VI regulate the mobility of synaptic vesicles at the presynapse (Cingolani and Goda 2008) and AMPA receptor-containing recycling endosomes in dendritic spines (Nash et al. 2010; Osterweil et al. 2005; Rudolf et al. 2010; Wang et al. 2008). Moreover, myosin motors can also be involved in regulating microtubule-based cargo transport, either by making direct physical contact with kinesin motors and enhancing each other's processivity (Ali et al. 2008; Huang et al. 1999) or by cooperative actions of actin- and microtubule-based motors on a single cargo (Gross et al. 2007). These motor-motor interactions may represent a mechanism by which the transition of vesicles from microtubules to actin filaments or vice versa is regulated. In contrast, recent data suggest that myosin V and VI can facilitate organelle docking by opposing, rather than complementing, microtubule-based movements (Pathak et al. 2010). Emerging data show that myosin motors are not only important for transport of cargo, they also regulate the secretion of exocytotic vesicles by docking them in actin-rich areas (Desnos et al. 2007). Moreover, myosin II can also regulate actin dynamics in spines in response to synaptic stimulation (Rex et al. 2010; Ryu et al. 2006). All these different actions of myosin motors, such as regulating synapse shape and stability, transporting and docking synaptic vesicles, and influencing actin dynamics, are important for synaptic function and plasticity (Hotulainen and Hoogenraad 2010).

8.3.2 *Kinesin Motor Proteins in Neurons*

Kinesin-1 was the neuronal transport motor to be identified based on assays of vesicular motility along microtubules in extruded axoplasm (Vale et al. 1985). Similar to the myosin superfamily nowadays, many different kinesin motors have been found. It is thought that approximately 45 different mammalian kinesin genes exist which, by virtue of alternative splicing, code for as many as 90 different kinesin proteins (Hirokawa et al. 2009). The vast majority of kinesin proteins share a number of structural characteristics: a highly conserved kinesin motor domain, responsible for microtubule binding and force generation by ATP hydrolysis; one or more coiled-coil domains for protein dimerization; and a cargo-binding domain (Hirokawa and Noda 2008; Lawrence et al. 2004; Schliwa and Woehlke 2003;

Vale 2003). The motor domain is ATP bound and upon attachment to the microtubule, ATP is hydrolyzed to ADP, resulting in a conformational change of the kinesin protein. This conformational change effectively results in a step of 8 nm along the microtubule (Schnitzer and Block 1997). Since most kinesin form a homodimer, the two heads of the kinesin molecule move in a hand-over-hand mechanism along the microtubule (Yildiz et al. 2004). Kinesin movement is highly processive, meaning that once bound to the microtubule, the motor will move prior to detaching, allowing it to transport cargo over long distances in axons and dendrites. The position of the kinesin motor domain determines the direction in which it moves: kinesin proteins with an N-terminal motor domain (the most common layout) move to the microtubule plus end, whereas kinesins with a C-terminal motor domain move toward the minus end. Kinesins with a motor domain in the middle are involved in regulating microtubule dynamics (Hirokawa and Noda 2008; Verhey and Hammond 2009). The nonmotor regions of kinesin motor proteins are poorly conserved and have been shown to regulate both motor function (by intramolecular folding and inhibition of ATPase activity) (Verhey and Hammond 2009) and cargo binding (by interacting with adaptor proteins) (Schlager and Hoogenraad 2009).

Most cargos bound to kinesin-1 motor proteins, such as amyloid precursor protein (APP) and Reelin receptor AporER2, interact indirectly via kinesin-1 light chains (Hirokawa et al. 2010). However, some cargos bind directly to the kinesin heavy chain, such as the AMPA receptor, which binds via adaptor protein GRIP1 (Setou et al. 2002), and mitochondria, which bind via adaptor protein Milton (Glater et al. 2006). A recent study showed that kinesin-1 binds synaptic precursor vesicles via syntabulin and syntaxin-1 (Cai et al. 2007). Knockdown of syntabulin impairs the anterograde transport of synaptic vesicle precursors. Members of the kinesin-3 family, including KIF1A and KIF1B β , also transport synaptic vesicle precursors in the axon. Both KIF1A and KIF1B β knockout mice exhibit defects in sensory and motor neurons, including a loss of synaptic vesicles (Hirokawa et al. 2010). The delivery of GABAA receptors to synapses is mediated by the kinesin-HAP-1 complex and is disrupted by mutant huntingtin (Twelvetrees et al. 2010). Several kinesin motors are now critically involved in neuronal disease pathogenesis (Goldstein 2001; Hirokawa and Noda 2008; Mandelkow and Mandelkow 2002; Morfini et al. 2009). For example, mutations in kinesin-1/KIF5A are associated with spastic paraplegia (Ebbing et al. 2008), truncating mutations in KIF17 are associated with schizophrenia (Tarabeux et al. 2010), and heterozygous missense mutations in KIF21A have been found to cause congenital fibrosis of the extraocular muscles (CFEOM), a rare congenital eye movement disorder that results from the dysfunction of the oculomotor nerve (Yamada et al. 2003).

8.3.3 *Dynein Motor Proteins in Neurons*

Similar to kinesin motors, the motor proteins of the dynein family move along microtubule structures. However, while most kinesin motors move toward the

microtubule plus end, dyneins move toward the minus end of the microtubule. In most species, more than 15 dynein encoding genes have been identified, where the majority of dynein motor proteins are involved in nontransport actions but drive flagellar beating (Kardon and Vale 2009). In stark contrast to kinesin proteins where many different motor types perform many different tasks, only one dynein motor, cytoplasmic dynein 1, is responsible for the bulk of the minus-end-directed cargo transport. In other respects, dynein is very different from kinesin and myosin motor proteins. One of the most obvious differences is the sheer size of the dynein motor complex: with a mass of 1–2 MDa, it is several times larger than a typical myosin or kinesin motor. At the core of the motor complex is the dynein heavy chain (DHC1), a polypeptide of over 500 kDa, which is essential for the motor activity consisting of three different domains: the stalk, motor, and tail domains. While in kinesin and myosin motors, the polymer-binding site and catalytic site are integrated within a single globular motor domain; in dynein, the microtubule-binding domain is separated from the motor domain by a ~15-nm stalk (Carter et al. 2008). The stalk is a coiled-coil structure that extends directly from the motor domain and is thought to function as a lever (Houdusse and Carter 2009; Kardon and Vale 2009). The circular motor domain consists of six ATPase domains, the tail domains mediate dimerization and form the interaction site for five additional dynein subunits. The intermediate chain (IC) and light intermediate chain (LIC) bind directly to the heavy chain tail, whereas light chain 8 (LC8), light chain 7 (LC7 or roadblock), and T-complex testis-specific protein 1 (Tctex1) bind to the intermediate chain (Kardon and Vale 2009). The dynein subunits are essential in determining the binding of dynein to specific cargos, the cellular localization, and even intrinsic properties of dynein, like its processivity. Interestingly, missense point mutations in the tail domain of cytoplasmic dynein heavy chain have been shown to result in progressive motor neuron degeneration in mice (Hafezparast et al. 2003). Recent data show that the mutations in the nonmotor part of the protein inhibit dynein motor run length and significantly alter motor domain coordination (Ori-McKenney et al. 2010). These results suggest a potential role for the dynein tail in motor function and provide direct evidence for a link between single-motor processivity and disease.

The efficient function of cytoplasmic dynein critically depends on the dynactin (dynein activator) accessory complex. Most dynein-dependent processes from yeast and filamentous fungi to invertebrates and mammals require dynactin (Schroer 2004). Dynactin has been shown to regulate dynein transport in several ways; it is involved in targeting of dynein, functions as adaptor protein, and regulates processive dynein movement (Kardon and Vale 2009; Schroer 2004). Dynactin is a large complex that contains 11 different subunits, and since some subunits are present in multiple copies, the complete assembly can be comprised of as many as 20 proteins. Detailed EM studies have revealed that dynactin basically consists of two parts, a rod domain and arm domain projecting from the rod (Schroer 2004). Mutations in the p150glued gene, coding for one of the large dynactin subunits, are found in a family with motor neuron disease (Puls et al. 2003). Affected patients develop adult-onset vocal fold paralysis, facial weakness, and distal limb muscle

weakness, mainly caused by the selective loss of motor neurons. Mutant mice with impaired dynein/dynactin function showed disrupted retrograde axonal transport and develop motor neuron disease similar to amyotrophic lateral sclerosis (ALS) (LaMonte et al. 2002; Teuling et al. 2008). This illustrates both the importance of subunits in the dynactin complex as well as the crucial role of dynein-based transport in the nervous system.

Cytoplasmic dynein transports neurotrophic tyrosine kinase receptor family (Trk) (Ha et al. 2008), Rab6-positive neuropeptide-containing secretory vesicles (Colin et al. 2008; Schlager et al. 2010), the piccolo/bassoon complex (Fejtova et al. 2009), and mitochondria (Hollenbeck and Saxton 2005) retrogradely in the axon, while in the dendrites, the cargos carried by cytoplasmic dynein include glycine receptor vesicles (Maas et al. 2006), messenger ribonucleoproteins (mRNPs) (Villace et al. 2004), Rab5 endosomes (Satoh et al. 2008), and AMPA receptor vesicles (Kapitein et al. 2010). In these cases, the cargos bind to the dynein complex either directly or through adaptor proteins such as gephyrin (glycine receptor) or bicaudal-D and related proteins (Rab6, BDNF, NPY) (Fig. 8.3). Regulation of the binding is controlled via phosphorylation, GTP hydrolysis of the small Rab-GTPases, or Ca^{2+} signaling (Schlager and Hoogenraad 2009).

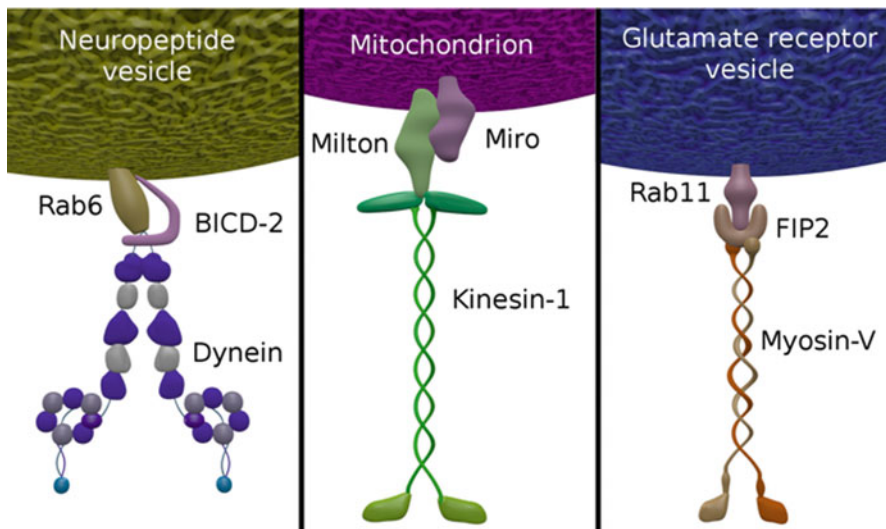


Fig. 8.3 Motor-adaptor-cargo transport complexes. Three typical examples of motor-adaptor-cargo transport machineries. Each motor protein connects to its cargo via adaptor molecules. Although the motor proteins, adaptors, and cargos vary, the essential building blocks are the same in each case. (a) Dynein motor complex connected to neuropeptide containing Rab6-positive vesicles via adaptor BICD-2. (b) Kinesin-1 is connected to mitochondria via Miro and Milton. This complex is regulated by calcium. (c) Myosin V transports glutamate receptor-containing vesicles by connecting to Rab11-FIP2

8.4 Regulating Motor Protein–Based Transport

Precise regulation of motor-based transport is essential to ensure precise cargo delivery to synapses. The list of molecules that are known to link specific transport motors to synaptic cargos is rapidly expanding (Hirokawa et al. 2010; Schlager and Hoogenraad 2009). Biochemical and proteomics approaches and high-throughput yeast two-hybrid screens have identified more than 100 proteins that bind to kinesin-1 in mammals, flies, and worms (Gindhart 2006). Most of these proteins act as cargo molecules themselves or function as motor-adaptor proteins (scaffolding proteins, Rab GTPases, signaling proteins); some are regulators of motor activity. Emerging data from several organisms and different experimental systems suggest that transport motors can be regulated at several points, including motor-cargo binding, motor activation, motor switching, microtubule track selection, cargo release at the destination, and the recycling of motors (Schlager and Hoogenraad 2009; Verhey and Hammond 2009). It is becoming increasingly clear that motor-adaptor-cargo interactions play a key role in identifying synaptic cargos and regulating synaptic cargo trafficking. In this section, we discuss recent work that has shed light on the regulation of the synaptic cargo-motor interactions. To illustrate the common layout and components of motor-adaptor-cargo complexes, we will focus here on two calcium-regulated motor adaptors, the endosomal myosin-V-FIP2 and mitochondrial kinesin-1-Milton-Miro complexes (Fig. 8.3).

8.4.1 Motor-Adaptor-Cargo Interactions

In the large majority of cases, motor proteins do not bind directly to vesicles or synaptic proteins, but they interact with cargo via so-called adaptor proteins. The main role of adaptor proteins is to provide an additional layer of regulation for transport specificity and selectivity. Adaptors can be single proteins, such as GRIP linking KIF5 to AMPA receptors (Hoogenraad et al. 2005; Setou et al. 2002), or protein complexes such as the Mint/CASK/MALS linking KIF17 to NR2B subunits of NMDA receptors (Setou et al. 2000). Interestingly, recent findings show that CaMKII activity is correlated with regulated cargo release near the postsynaptic membrane. Here, CaMKII has been shown to phosphorylate KIF17, which induces the dissociation of the Mint scaffolding protein complex and release of NMDA receptor-containing cargo near the postsynaptic membrane (Guillaud et al. 2008). In this way, regulated CaMKII activity provides an attractive mechanism for targeting NMDA receptor complexes to active synapses where an activity regulated kinase is switched “on.” The significance of KIF17 function to brain function is further illustrated by the observation that transgenic mice overexpressing KIF17 show enhanced spatial and working memory (Wong et al. 2002). Additional examples of regulated adaptor proteins are the DENN/MADD adaptor protein that binds KIF1A and KIF1B β and interacts with Rab3 vesicles (Niwa et al. 2008),

liprin family proteins as adaptors that link KIF1A to synaptic vesicle precursors (Miller et al. 2005; Wagner et al. 2009), and bicaudal-D family proteins connecting dynein motors to Rab6-positive neuropeptide secretory vesicles (Grigoriev et al. 2007; Matanis et al. 2002; Schlager et al. 2010). Interestingly, these Rab6-positive secretory vesicles also contain semaphorin 3A and BDNF and are anterogradely transported in axons by kinesin-3 motors (Barkus et al. 2008; de Wit et al. 2006; Schlager et al. 2010). Thus, regulation of cargo binding can be controlled via phosphorylation or GTP hydrolysis of the small Rab-GTPases (Schlager and Hoogenraad 2009).

A third way to control intracellular trafficking is to regulate motor-cargo interactions by responding to changes in local ion concentrations. It is well known that activation of NMDA receptors causes a rapid influx of Ca^{2+} in dendritic spines. A recent study shows that myosin Vb is a “ Ca^{2+} sensor” for actin-based postsynaptic AMPA receptor trafficking (Wang et al. 2008). Increased Ca^{2+} levels lead to unfolding of myosin Vb motors and allows for binding to Rab11-FIP2 adaptors on recycling endosomes (Schlager and Hoogenraad 2009) (Fig. 8.3). The association of myosin Vb with Rab11-FIP2 transports AMPA receptor-containing recycling endosomes into the actin-rich spines. Thus, elevated Ca^{2+} levels in spines promote local postsynaptic trafficking. On the other hand, Ca^{2+} influx reduces mitochondrial motility (Boldogh and Pon 2007). The Milton-Miro complex was identified as an adaptor between kinesin-1 and mitochondria and a candidate for Ca^{2+} -dependent regulation of mitochondrial transport. Indeed, recent studies suggest that the mitochondrial Miro-Milton adaptor complex is important for the Ca^{2+} -dependent regulation of mitochondria trafficking (Wang and Schwarz 2009) (Fig. 8.3). Elevated Ca^{2+} levels permit Miro to interact directly with the motor domain of kinesin-1. The interesting aspect of this model is that kinesin-1 remains associated with mitochondria regardless of whether they are moving or stationary. In the “moving” state, kinesin-1 is bound to mitochondria by binding to Milton, which in turn interacts with Miro on the mitochondrial surface. In the “stationary” state, in the presence of high Ca^{2+} levels, the kinesin-1 motor domain interacts directly with Miro and prevents microtubule interactions. In contrast, another recent paper showed that the presence of Ca^{2+} inhibits the Miro1/kinesin-1 protein-protein interaction and that the motor is dissociated from mitochondria yielding arrested movement (Macaskill et al. 2009). Both findings imply the existence of “ Ca^{2+} sensors” that detect neuronal activity stimuli and convert Ca^{2+} influx into mechanisms regulating cargo trafficking.

8.5 Conclusion and Future Directions

A typical fully differentiated neuron within an active neuronal circuitry faces an enormous logistical challenge. Synaptic cargo needs to be sorted into dendrites and axons both during basal neuronal activity and changes in activity, such during firing of action potentials. Not all synapses are equally active, and their requirements can

vary greatly; some may require a constant flow of receptors and neurotransmitters, while others undergo depression and mainly need transport out of the synapse into a reserve pool or back to the cell body. Moreover, the distance between the cell soma and the most distant synapse can be huge, for example, up to 1 m for a motor neuron. And all these transported proteins, mitochondria, neurotransmitters, and synaptic vesicle precursors flow through an axon of only 5 micrometer in diameter. Therefore, neurons are equipped with a well-balanced and meticulously regulated transport system in order to facilitate synapse formation, function, and plasticity. First of all, the actin and microtubule cytoskeleton play a pivotal role in synaptic plasticity—together they determine synaptic architecture, organize subcellular compartments, and transport intracellular synaptic constituents. Second, the characteristic dynamics, polarity, and modifications patterns of cytoskeleton elements are instrumental for establishing and maintaining the structural and compositional polarity of synapses. Third, this highly specialized microtubule and actin cytoskeletal organization facilitates local, polarized transport by guiding specific motor proteins to specific directions. Fourth, synaptic activity may regulate the cytoskeleton organization and motor protein transport in neurons. All these mechanisms occur simultaneous and can influence each other, creating a highly dynamic infrastructure that is able to rebuild itself in order to adapt to changes in the cellular environment. Without this highly dynamic cytoskeleton system, synaptic plasticity and cognitive brain functions would be impossible.

The relationship between cytoskeleton, motor protein transport, and synaptic signaling is never more apparent than when the brain becomes dysfunctional. Molecular motor proteins, especially kinesin proteins, are prime candidates to be involved in several psychiatric and neurological disorders (Goldstein 2001; Mandelkow and Mandelkow 2002), ranging from schizophrenia (Tarabeux et al. 2010) to spastic paraplegia (Ebbing et al. 2008). Both KIF5 and dynein may be involved in the pathology of Huntington and other polyQ diseases (Colin et al. 2008; Twelvetrees et al. 2010). The role of dynein and dynactin in neurological diseases is best described in the motor neuron disease ALS (Chevalier-Larsen and Holzbaur 2006), and mutations in tubulin isotypes have been observed in patients with severe neurodevelopmental disorders (Keays et al. 2007; Tischfield et al. 2010). There is also a strong link between the activity of tubulin modifying enzymes and neuronal abnormalities. Mutant mice that lack the gene for tubulin tyrosine ligase (TTL), the enzyme that catalyzes the addition of a C-terminal tyrosine residue to α -tubulin in the tubulin tyrosination cycle, die shortly after birth because of neuronal disorganization, including premature axon specialization (Erck et al. 2005). On the other hand, mice that lack functional cytosolic carboxypeptidase (CCP1), the enzymes catalyzing deglutamylation, have increased microtubule hyperglutamylation and Purkinje cell degeneration (Rogowski et al. 2010). However, a number of key mechanistic questions remain to be answered. What are the downstream effects of transport deficits that lead to neurodegeneration? In some cases, it may be a failure to supply new material to the distal axons and dendrites, so that synapses degrade over time. Consistently, defects in both axon and dendritic transport in various organisms can lead to neuropathies (Hirokawa et al. 2010).

In other cases, neuronal degeneration may result from the accumulation of toxic substances in the processes. The molecular motor machinery itself could be critically involved in removing toxic waste in neurons, but these protein accumulations may also lead to traffic jams and disrupt normal synaptic trafficking routes.

In this chapter, we have shown how the cellular infrastructure is essential for neuronal development and plasticity. This highly adaptive network of filaments, motor, adaptor, and cargo proteins is able to answer the ever-changing demands of neuronal networks in action. As a large number of neurological diseases illustrate, there is little room for error. If this fascinating intracellular transport system is not working at peak efficiency, neurons are not able to function properly and will eventually degenerate and die. Future studies on neuronal cytoskeletal dynamics and the synaptic transport machinery may lead to new insights and, hopefully, new treatments for neurological and psychiatric disorders.

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References

- Akhmanova, A., & Hammer, J. A., 3rd. (2010). Linking molecular motors to membrane cargo. *Current Opinion in Cell Biology*, 22, 479–487.
- Akhmanova, A., & Steinmetz, M. O. (2008). Tracking the ends: A dynamic protein network controls the fate of microtubule tips. *Nature Reviews Molecular Cell Biology*, 9, 309–322.
- Ali, M. Y., Lu, H., Bookwalter, C. S., Warshaw, D. M., & Trybus, K. M. (2008). Myosin V and kinesin act as tethers to enhance each others' processivity. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 4691–4696.
- Baas, P. W., Black, M. M., & Banker, G. A. (1989). Changes in microtubule polarity orientation during the development of hippocampal neurons in culture. *The Journal of Cell Biology*, 109, 3085–3094.
- Baas, P. W., Deitch, J. S., Black, M. M., & Banker, G. A. (1988). Polarity orientation of microtubules in hippocampal neurons: Uniformity in the axon and nonuniformity in the dendrite. *Proceedings of the National Academy of Sciences of the United States of America*, 85, 8335–8339.
- Baran, R., Castelblanco, L., Tang, G., Shapiro, I., Goncharov, A., & Jin, Y. (2010). Motor neuron synapse and axon defects in a *C. elegans* alpha-tubulin mutant. *PLoS One*, 5, e9655.
- Barkus, R. V., Klyachko, O., Horiuchi, D., Dickson, B. J., & Saxton, W. M. (2008). Identification of an axonal kinesin-3 motor for fast anterograde vesicle transport that facilitates retrograde transport of neuropeptides. *Molecular Biology of the Cell*, 19, 274–283.
- Bearer, E. L., & Reese, T. S. (1999). Association of actin filaments with axonal microtubule tracts. *Journal of Neurocytology*, 28, 85–98.
- Bel, C., Oguievetskaia, K., Pitaval, C., Goutebroze, L., & Faivre-Sarrailh, C. (2009). Axonal targeting of Caspr2 in hippocampal neurons via selective somatodendritic endocytosis. *Journal of Cell Science*, 122, 3403–3413.

- Blanpied, T. A., & Ehlers, M. D. (2004). Microanatomy of dendritic spines: Emerging principles of synaptic pathology in psychiatric and neurological disease. *Biological Psychiatry*, *55*, 1121–1127.
- Boldogh, I. R., & Pon, L. A. (2007). Mitochondria on the move. *Trends in Cell Biology*, *17*, 502–510.
- Burton, P. R. (1988). Dendrites of mitral cell neurons contain microtubules of opposite polarity. *Brain Research*, *473*, 107–115.
- Cai, D., McEwen, D. P., Martens, J. R., Meyhofer, E., & Verhey, K. J. (2009). Single molecule imaging reveals differences in microtubule track selection between Kinesin motors. *PLoS Biology*, *7*, e1000216.
- Cai, Q., Pan, P. Y., & Sheng, Z. H. (2007). Syntabulin-kinesin-1 family member 5B-mediated axonal transport contributes to activity-dependent presynaptic assembly. *Journal of Neuroscience*, *27*, 7284–7296.
- Carter, A. P., Garbarino, J. E., Wilson-Kubalek, E. M., Shipley, W. E., Cho, C., Milligan, R. A., Vale, R. D., & Gibbons, I. R. (2008). Structure and functional role of dynein's microtubule-binding domain. *Science*, *322*, 1691–1695.
- Chen, J., Kanai, Y., Cowan, N. J., & Hirokawa, N. (1992). Projection domains of MAP2 and tau determine spacings between microtubules in dendrites and axons. *Nature*, *360*, 674–677.
- Chevalier-Larsen, E., & Holzbaur, E. L. (2006). Axonal transport and neurodegenerative disease. *Biochimica et Biophysica Acta*, *1762*, 1094–1108.
- Cingolani, L. A., & Goda, Y. (2008). Actin in action: The interplay between the actin cytoskeleton and synaptic efficacy. *Nature Reviews Neuroscience*, *9*, 344–356.
- Colin, E., Zala, D., Liot, G., Rangone, H., Borrell-Pages, M., Li, X. J., Saudou, F., & Humbert, S. (2008). Huntingtin phosphorylation acts as a molecular switch for anterograde/retrograde transport in neurons. *EMBO Journal*, *27*, 2124–2134.
- Conde, C., & Caceres, A. (2009). Microtubule assembly, organization and dynamics in axons and dendrites. *Nature Reviews Neuroscience*, *10*, 319–332.
- Conti, M. A., & Adelstein, R. S. (2008). Nonmuscle myosin II moves in new directions. *Journal of Cell Science*, *121*, 11–18.
- Craig, A. M., Blackstone, C. D., Huganir, R. L., & Banker, G. (1993). The distribution of glutamate receptors in cultured rat hippocampal neurons: Postsynaptic clustering of AMPA-selective subunits. *Neuron*, *10*, 1055–1068.
- de Wit, J., Toonen, R. F., Verhaagen, J., & Verhage, M. (2006). Vesicular trafficking of semaphorin 3A is activity-dependent and differs between axons and dendrites. *Traffic*, *7*, 1060–1077.
- Dehmelt, L., & Halpain, S. (2005). The MAP2/Tau family of microtubule-associated proteins. *Genome Biology*, *6*, 204.
- Dent, E. W., & Gertler, F. B. (2003). Cytoskeletal dynamics and transport in growth cone motility and axon guidance. *Neuron*, *40*, 209–227.
- Desnos, C., Huet, S., Fanget, I., Chapuis, C., Bottiger, C., Racine, V., Sibarita, J. B., Henry, J. P., & Darchen, F. (2007). Myosin va mediates docking of secretory granules at the plasma membrane. *Journal of Neuroscience*, *27*, 10636–10645.
- Dixit, R., Ross, J. L., Goldman, Y. E., & Holzbaur, E. L. (2008). Differential regulation of dynein and kinesin motor proteins by tau. *Science*, *319*, 1086–1089.
- Ebbing, B., Mann, K., Starosta, A., Jaud, J., Schols, L., Schule, R., & Woehlke, G. (2008). Effect of spastic paraplegia mutations in KIF5A kinesin on transport activity. *Human Molecular Genetics*, *17*, 1245–1252.
- Erck, C., Peris, L., Andrieux, A., Meissirel, C., Gruber, A. D., Vernet, M., Schweitzer, A., Saoudi, Y., Pointu, H., Bosc, C., et al. (2005). A vital role of tubulin-tyrosine-ligase for neuronal organization. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 7853–7858.
- Fejtova, A., Davydova, D., Bischof, F., Lazarevic, V., Altmann, W. D., Romorini, S., Schone, C., Zuschratter, W., Kreutz, M. R., Garner, C. C., et al. (2009). Dynein light chain regulates axonal trafficking and synaptic levels of Bassoon. *The Journal of Cell Biology*, *185*, 341–355.

- Frost, N. A., Kerr, J. M., Lu, H. E., & Blanpied, T. A. (2010a). A network of networks: Cytoskeletal control of compartmentalized function within dendritic spines. *Current Opinion in Neurobiology*, *20*, 578–587.
- Frost, N. A., Shroff, H., Kong, H., Betzig, E., & Blanpied, T. A. (2010b). Single-molecule discrimination of discrete perisynaptic and distributed sites of actin filament assembly within dendritic spines. *Neuron*, *67*, 86–99.
- Garrido, J. J., Fernandes, F., Giraud, P., Mouret, I., Pasqualini, E., Fache, M. P., Jullien, F., & Dargent, B. (2001). Identification of an axonal determinant in the C-terminus of the sodium channel Na(v)1.2. *EMBO Journal*, *20*, 5950–5961.
- Gindhart, J. G. (2006). Towards an understanding of kinesin-1 dependent transport pathways through the study of protein–protein interactions. *Briefings in Functional Genomics & Proteomics*, *5*, 74–86.
- Glater, E. E., Megeath, L. J., Stowers, R. S., & Schwarz, T. L. (2006). Axonal transport of mitochondria requires Milton to recruit kinesin heavy chain and is light chain independent. *The Journal of Cell Biology*, *173*, 545–557.
- Goldstein, L. S. (2001). Kinesin molecular motors: Transport pathways, receptors, and human disease. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 6999–7003.
- Goldstein, L. S., & Yang, Z. (2000). Microtubule-based transport systems in neurons: The roles of kinesins and dyneins. *Annual Review of Neuroscience*, *23*, 39–71.
- Gouveia, S. M., & Akhmanova, A. (2010). Cell and molecular biology of microtubule plus end tracking proteins: End binding proteins and their partners. *International Review of Cell and Molecular Biology*, *285*, 1–74.
- Govek, E. E., Newey, S. E., Akerman, C. J., Cross, J. R., Van der Veken, L., & Van Aelst, L. (2004). The X-linked mental retardation protein oligophrenin-1 is required for dendritic spine morphogenesis. *Nature Neuroscience*, *7*, 364–372.
- Grigoriev, I., Splinter, D., Keijzer, N., Wulf, P. S., Demmers, J., Ohtsuka, T., Modesti, M., Maly, I. V., Grosveld, F., Hoogenraad, C. C., et al. (2007). Rab6 regulates transport and targeting of exocytotic carriers. *Developmental Cell*, *13*, 305–314.
- Gross, S. P., Vershinin, M., & Shubeita, G. T. (2007). Cargo transport: Two motors are sometimes better than one. *Current Biology*, *17*, R478–R486.
- Guillaud, L., Wong, R., & Hirokawa, N. (2008). Disruption of KIF17-Mint1 interaction by CaMKII-dependent phosphorylation: A molecular model of kinesin-cargo release. *Nature Cell Biology*, *10*, 19–29.
- Gunawardena, S., & Goldstein, L. S. (2004). Cargo-carrying motor vehicles on the neuronal highway: Transport pathways and neurodegenerative disease. *Journal of Neurobiology*, *58*, 258–271.
- Ha, J., Lo, K. W., Myers, K. R., Carr, T. M., Hums, M. K., Rasoul, B. A., Segal, R. A., & Pfister, K. K. (2008). A neuron-specific cytoplasmic dynein isoform preferentially transports TrkB signaling endosomes. *The Journal of Cell Biology*, *181*, 1027–1039.
- Hafezparast, M., Klocke, R., Ruhrberg, C., Marquardt, A., Ahmad-Annuar, A., Bowen, S., Lalli, G., Witherden, A. S., Hummerich, H., Nicholson, S., et al. (2003). Mutations in dynein link motor neuron degeneration to defects in retrograde transport. *Science*, *300*, 808–812.
- Hirokawa, N., Niwa, S., & Tanaka, Y. (2010). Molecular motors in neurons: Transport mechanisms and roles in brain function, development, and disease. *Neuron*, *68*, 610–638.
- Hirokawa, N., & Noda, Y. (2008). Intracellular transport and kinesin superfamily proteins, KIFs: Structure, function, and dynamics. *Physiological Reviews*, *88*, 1089–1118.
- Hirokawa, N., Noda, Y., Tanaka, Y., & Niwa, S. (2009). Kinesin superfamily motor proteins and intracellular transport. *Nature Reviews Molecular Cell Biology*, *10*, 682–696.
- Hirokawa, N., & Takemura, R. (2005). Molecular motors and mechanisms of directional transport in neurons. *Nature Reviews Neuroscience*, *6*, 201–214.
- Hollenbeck, P. J., & Saxton, W. M. (2005). The axonal transport of mitochondria. *Journal of Cell Science*, *118*, 5411–5419.

- Honkura, N., Matsuzaki, M., Noguchi, J., Ellis-Davies, G. C., & Kasai, H. (2008). The subspline organization of actin fibers regulates the structure and plasticity of dendritic spines. *Neuron*, *57*, 719–729.
- Hoogenraad, C. C., & Bradke, F. (2009). Control of neuronal polarity and plasticity—A renaissance for microtubules? *Trends in Cell Biology*, *19*, 669–676.
- Hoogenraad, C. C., Milstein, A. D., Ethell, I. M., Henkemeyer, M., & Sheng, M. (2005). GRIP1 controls dendrite morphogenesis by regulating EphB receptor trafficking. *Nature Neuroscience*, *8*, 906–915.
- Hotulainen, P., & Hoogenraad, C. C. (2010). Actin in dendritic spines: Connecting dynamics to function. *The Journal of Cell Biology*, *189*, 619–629.
- Hotulainen, P., Llano, O., Smirnov, S., Tanhuanpaa, K., Faix, J., Rivera, C., & Lappalainen, P. (2009). Defining mechanisms of actin polymerization and depolymerization during dendritic spine morphogenesis. *The Journal of Cell Biology*, *185*, 323–339.
- Houdusse, A., & Carter, A. P. (2009). Dynein swings into action. *Cell*, *136*, 395–396.
- Howard, J., & Hyman, A. A. (2009). Growth, fluctuation and switching at microtubule plus ends. *Nature Reviews Molecular Cell Biology*, *10*, 569–574.
- Huang, J. D., Brady, S. T., Richards, B. W., Stenolen, D., Resau, J. H., Copeland, N. G., & Jenkins, N. A. (1999). Direct interaction of microtubule- and actin-based transport motors. *Nature*, *397*, 267–270.
- Janke, C., & Kneussel, M. (2010). Tubulin post-translational modifications: Encoding functions on the neuronal microtubule cytoskeleton. *Trends in Neurosciences*, *33*, 362–372.
- Jaworski, J., Hoogenraad, C. C., & Akhmanova, A. (2008). Microtubule plus-end tracking proteins in differentiated mammalian cells. *The International Journal of Biochemistry & Cell Biology*, *40*, 619–637.
- Jaworski, J., Kapitein, L. C., Gouveia, S. M., Dortland, B. R., Wulf, P. S., Grigoriev, I., Camera, P., Spangler, S. A., Di Stefano, P., Demmers, J., et al. (2009). Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity. *Neuron*, *61*, 85–100.
- Jin, Y., & Garner, C. C. (2008). Molecular mechanisms of presynaptic differentiation. *Annual Review of Cell and Developmental Biology*, *24*, 237–262.
- Kaether, C., Skehel, P., & Dotti, C. G. (2000). Axonal membrane proteins are transported in distinct carriers: A two-color video microscopy study in cultured hippocampal neurons. *Molecular Biology of the Cell*, *11*, 1213–1224.
- Kapitein, L. C., & Hoogenraad, C. C. (2010). Which way to go? Cytoskeletal organization and polarized transport in neurons. *Molecular Cell Neuroscience*, *46*(1), 9–20.
- Kapitein, L. C., Schlager, M. A., Kuijpers, M., Wulf, P. S., van Spronsen, M., MacKintosh, F. C., & Hoogenraad, C. C. (2010). Mixed microtubules steer dynein-driven cargo transport into dendrites. *Current Biology*, *20*, 290–299.
- Kapitein, L. C., & Hoogenraad, C. C. (2011). Which way to go? Cytoskeletal organization and polarized transport in neurons. *Mol Cell Neurosci* *46*, 9–20
- Kardon, J. R., & Vale, R. D. (2009). Regulators of the cytoplasmic dynein motor. *Nature Reviews Molecular Cell Biology*, *10*, 854–865.
- Kasai, H., Fukuda, M., Watanabe, S., Hayashi-Takagi, A., & Noguchi, J. (2010). Structural dynamics of dendritic spines in memory and cognition. *Trends in Neurosciences*, *33*, 121–129.
- Keays, D. A., Tian, G., Poirier, K., Huang, G. J., Siebold, C., Cleak, J., Oliver, P. L., Fray, M., Harvey, R. J., Molnar, Z., et al. (2007). Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. *Cell*, *128*, 45–57.
- Kim, E., & Sheng, M. (2004). PDZ domain proteins of synapses. *Nature Reviews Neuroscience*, *5*, 771–781.
- Konishi, Y., & Setou, M. (2009). Tubulin tyrosination navigates the kinesin-1 motor domain to axons. *Nature Neuroscience*, *12*, 559–567.
- Korobova, F., & Svitkina, T. (2010). Molecular architecture of synaptic actin cytoskeleton in hippocampal neurons reveals a mechanism of dendritic spine morphogenesis. *Molecular Biology of the Cell*, *21*, 165–176.

- Krucker, T., Siggins, G. R., & Halpain, S. (2000). Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, *97*, 6856–6861.
- Kueh, H. Y., & Mitchison, T. J. (2009). Structural plasticity in actin and tubulin polymer dynamics. *Science*, *325*, 960–963.
- LaMonte, B. H., Wallace, K. E., Holloway, B. A., Shelly, S. S., Ascano, J., Tokito, M., Van Winkle, T., Howland, D. S., & Holzbaur, E. L. (2002). Disruption of dynein/dynactin inhibits axonal transport in motor neurons causing late-onset progressive degeneration. *Neuron*, *34*, 715–727.
- Landis, D. M., Hall, A. K., Weinstein, L. A., & Reese, T. S. (1988). The organization of cytoplasm at the presynaptic active zone of a central nervous system synapse. *Neuron*, *1*, 201–209.
- Landis, D. M., & Reese, T. S. (1983). Cytoplasmic organization in cerebellar dendritic spines. *The Journal of Cell Biology*, *97*, 1169–1178.
- Lau, C. G., & Zukin, R. S. (2007). NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. *Nature Reviews Neuroscience*, *8*, 413–426.
- Lawrence, C. J., Dawe, R. K., Christie, K. R., Cleveland, D. W., Dawson, S. C., Endow, S. A., Goldstein, L. S., Goodson, H. V., Hirokawa, N., Howard, J., et al. (2004). A standardized kinesin nomenclature. *The Journal of Cell Biology*, *167*, 19–22.
- Leterrier, C., Laine, J., Darmon, M., Boudin, H., Rossier, J., & Lenkei, Z. (2006). Constitutive activation drives compartment-selective endocytosis and axonal targeting of type 1 cannabinoid receptors. *Journal of Neuroscience*, *26*, 3141–3153.
- Lewis, T. L., Jr., Mao, T., Svoboda, K., & Arnold, D. B. (2009). Myosin-dependent targeting of transmembrane proteins to neuronal dendrites. *Nature Neuroscience*, *12*, 568–576.
- Liao, G., & Gundersen, G. G. (1998). Kinesin is a candidate for cross-bridging microtubules and intermediate filaments. Selective binding of kinesin to detyrosinated tubulin and vimentin. *Journal of Biological Chemistry*, *273*, 9797–9803.
- Lisman, J. E., Raghavachari, S., & Tsien, R. W. (2007). The sequence of events that underlie quantal transmission at central glutamatergic synapses. *Nature Reviews Neuroscience*, *8*, 597–609.
- Maas, C., Belgardt, D., Lee, H. K., Heisler, F. F., Lappe-Siefke, C., Magiera, M. M., van Dijk, J., Hausrat, T. J., Janke, C., & Kneussel, M. (2009). Synaptic activation modifies microtubules underlying transport of postsynaptic cargo. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 8731–8736.
- Maas, C., Tagnaouti, N., Loebrich, S., Behrend, B., Lappe-Siefke, C., & Kneussel, M. (2006). Neuronal cotransport of glycine receptor and the scaffold protein gephyrin. *The Journal of Cell Biology*, *172*, 441–451.
- Macaskill, A. F., Rinholm, J. E., Twelvetrees, A. E., Arancibia-Carcamo, I. L., Muir, J., Fransson, A., Aspenstrom, P., Attwell, D., & Kittler, J. T. (2009). Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. *Neuron*, *61*, 541–555.
- Mandelkow, E., & Mandelkow, E. M. (2002). Kinesin motors and disease. *Trends in Cell Biology*, *12*, 585–591.
- Margeta, M. A., Shen, K., & Grill, B. (2008). Building a synapse: Lessons on synaptic specificity and presynaptic assembly from the nematode *C. elegans*. *Current Opinion in Neurobiology*, *18*, 69–76.
- Matanis, T., Akhmanova, A., Wulf, P., Del Nery, E., Weide, T., Stepanova, T., Galjart, N., Grosveld, F., Goud, B., De Zeeuw, C. I., et al. (2002). Bicaudal-D regulates COPI-independent Golgi-ER transport by recruiting the dynein-dynactin motor complex. *Nature Cell Biology*, *4*, 986–992.
- Miller, K. E., DeProto, J., Kaufmann, N., Patel, B. N., Duckworth, A., & Van Vactor, D. (2005). Direct observation demonstrates that Liprin-alpha is required for trafficking of synaptic vesicles. *Current Biology*, *15*, 684–689.
- Mitchison, T., & Kirschner, M. (1984). Dynamic instability of microtubule growth. *Nature*, *312*, 237–242.

- Morfini, G. A., Burns, M., Binder, L. I., Kanaan, N. M., LaPointe, N., Bosco, D. A., Brown, R. H., Jr., Brown, H., Tiwari, A., Hayward, L., et al. (2009). Axonal transport defects in neurodegenerative diseases. *Journal of Neuroscience*, *29*, 12776–12786.
- 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*, 145–152.
- Nakada, C., Ritchie, K., Oba, Y., Nakamura, M., Hotta, Y., Iino, R., Kasai, R. S., Yamaguchi, K., Fujiwara, T., & Kusumi, A. (2003). Accumulation of anchored proteins forms membrane diffusion barriers during neuronal polarization. *Nature Cell Biology*, *5*, 626–632.
- Nash, J. E., Appleby, V. J., Correa, S. A., Wu, H., Fitzjohn, S. M., Garner, C. C., Collingridge, G. L., & Molnar, E. (2010). Disruption of the interaction between myosin VI and SAP97 is associated with a reduction in the number of AMPARs at hippocampal synapses. *Journal of Neurochemistry*, *112*, 677–690.
- Niwa, S., Tanaka, Y., & Hirokawa, N. (2008). KIF1Bbeta- and KIF1A-mediated axonal transport of presynaptic regulator Rab3 occurs in a GTP-dependent manner through DENN/MADD. *Nature Cell Biology*, *10*, 1269–1279.
- Okamoto, K., Nagai, T., Miyawaki, A., & Hayashi, Y. (2004). Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. *Nature Neuroscience*, *7*, 1104–1112.
- Ori-McKenney, K. M., Xu, J., Gross, S. P., & Vallee, R. B. (2010). A cytoplasmic dynein tail mutation impairs motor processivity. *Nature Cell Biology*, *12*, 1228–1234.
- Osterweil, E., Wells, D. G., & Mooseker, M. S. (2005). A role for myosin VI in postsynaptic structure and glutamate receptor endocytosis. *The Journal of Cell Biology*, *168*, 329–338.
- Ou, C. Y., Poon, V. Y., Maeder, C. I., Watanabe, S., Lehrman, E. K., Fu, A. K., Park, M., Fu, W. Y., Jorgensen, E. M., Ip, N. Y., et al. (2010). Two cyclin-dependent kinase pathways are essential for polarized trafficking of presynaptic components. *Cell*, *141*, 846–858.
- Pathak, D., Sepp, K. J., & Hollenbeck, P. J. (2010). Evidence that myosin activity opposes microtubule-based axonal transport of mitochondria. *Journal of Neuroscience*, *30*, 8984–8992.
- Pennuto, M., Bonanomi, D., Benfenati, F., & Valtorta, F. (2003). Synaptophysin I controls the targeting of VAMP2/synaptobrevin II to synaptic vesicles. *Molecular Biology of the Cell*, *14*, 4909–4919.
- Pollard, T. D., & Cooper, J. A. (2009). Actin, a central player in cell shape and movement. *Science*, *326*, 1208–1212.
- Puls, I., Jonnakuty, C., LaMonte, B. H., Holzbaur, E. L., Tokito, M., Mann, E., Floeter, M. K., Bidus, K., Drayna, D., Oh, S. J., et al. (2003). Mutant dynactin in motor neuron disease. *Nature Genetics*, *33*, 455–456.
- Rasband, M. N. (2010). The axon initial segment and the maintenance of neuronal polarity. *Nature Reviews Neuroscience*, *11*, 552–562.
- Rex, C. S., Gavin, C. F., Rubio, M. D., Kramar, E. A., Chen, L. Y., Jia, Y., Haganir, R. L., Muzyczka, N., Gall, C. M., Miller, C. A., et al. (2010). Myosin IIb regulates actin dynamics during synaptic plasticity and memory formation. *Neuron*, *67*, 603–617.
- Rogowski, K., van Dijk, J., Magiera, M. M., Bosc, C., Deloulme, J. C., Bosson, A., Peris, L., Gold, N. D., Lacroix, B., Grau, M. B., et al. (2010). A family of protein-deglutamylating enzymes associated with neurodegeneration. *Cell*, *143*, 564–578.
- Rolls, M. M., Satoh, D., Clyne, P. J., Henner, A. L., Uemura, T., & Doe, C. Q. (2007). Polarity and intracellular compartmentalization of *Drosophila* neurons. *Neural Development*, *2*, 7.
- Ruberti, F., & Dotti, C. G. (2000). Involvement of the proximal C terminus of the AMPA receptor subunit GluR1 in dendritic sorting. *Journal of Neuroscience*, *20*, RC78.
- Rudolf, R., Bittins, C. M., & Gerdes, H. H. (2010). The role of myosin V in exocytosis and synaptic plasticity. *Journal of Neurochemistry*, *116*(2), 177–191.
- Rudolf, R., Bittins, C. M., Gerdes, H. H. (2011) The role of myosin V in exocytosis and synaptic plasticity. *J Neurochem* *116*, 177–191

- Ryu, J., Liu, L., Wong, T. P., Wu, D. C., Burette, A., Weinberg, R., Wang, Y. T., & Sheng, M. (2006). A critical role for myosin IIb in dendritic spine morphology and synaptic function. *Neuron*, *49*, 175–182.
- Sampo, B., Kaech, S., Kunz, S., & Banker, G. (2003). Two distinct mechanisms target membrane proteins to the axonal surface. *Neuron*, *37*, 611–624.
- Satoh, D., Sato, D., Tsuyama, T., Saito, M., Ohkura, H., Rolls, M. M., Ishikawa, F., & Uemura, T. (2008). Spatial control of branching within dendritic arbors by dynein-dependent transport of Rab5-endosomes. *Nature Cell Biology*, *10*, 1164–1171.
- Schlager, M. A., & Hoogenraad, C. C. (2009). Basic mechanisms for recognition and transport of synaptic cargos. *Molecular Brain*, *2*, 25.
- Schlager, M. A., Kapitein, L. C., Grigoriev, I., Burzynski, G. M., Wulf, P. S., Keijzer, N., de Graaff, E., Fukuda, M., Shepherd, I. T., Akhmanova, A., et al. (2010). Pericentrosomal targeting of Rab6 secretory vesicles by Bicaudal-D-related protein 1 (BICDR-1) regulates neuritogenesis. *EMBO Journal*, *29*, 1637–1651.
- Schliwa, M., & Woehlke, G. (2003). Molecular motors. *Nature*, *422*, 759–765.
- Schnitzer, M. J., & Block, S. M. (1997). Kinesin hydrolyses one ATP per 8-nm step. *Nature*, *388*, 386–390.
- Schroer, T. A. (2004). Dynactin. *Annual Review of Cell and Developmental Biology*, *20*, 759–779.
- Setou, M., Nakagawa, T., Seog, D. H., & Hirokawa, N. (2000). Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science*, *288*, 1796–1802.
- Setou, M., Seog, D. H., Tanaka, Y., Kanai, Y., Takei, Y., Kawagishi, M., & Hirokawa, N. (2002). Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. *Nature*, *417*, 83–87.
- Sheng, M., & Hoogenraad, C. C. (2007). The postsynaptic architecture of excitatory synapses: A more quantitative view. *Annual Review of Biochemistry*, *76*, 823–847.
- Shepherd, J. D., & Huganir, R. L. (2007). The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annual Review of Cell and Developmental Biology*, *23*, 613–643.
- Song, A. H., Wang, D., Chen, G., Li, Y., Luo, J., Duan, S., & Poo, M. M. (2009). A selective filter for cytoplasmic transport at the axon initial segment. *Cell*, *136*, 1148–1160.
- Stepanova, T., Slemmer, J., Hoogenraad, C. C., Lansbergen, G., Dortland, B., De Zeeuw, C. I., Grosveld, F., van Cappellen, G., Akhmanova, A., & Galjart, N. (2003). Visualization of microtubule growth in cultured neurons via the use of EB3-GFP (end-binding protein 3-green fluorescent protein). *Journal of Neuroscience*, *23*, 2655–2664.
- Stiess, M., Maghelli, N., Kapitein, L. C., Gomis-Ruth, S., Wilsch-Brauninger, M., Hoogenraad, C. C., Tolic-Norrelykke, I. M., & Bradke, F. (2010). Axon extension occurs independently of centrosomal microtubule nucleation. *Science*, *327*, 704–707.
- Stone, M. C., Roegiers, F., & Rolls, M. M. (2008). Microtubules have opposite orientation in axons and dendrites of *Drosophila* neurons. *Molecular Biology of the Cell*, *19*, 4122–4129.
- Stowell, J. N., & Craig, A. M. (1999). Axon/dendrite targeting of metabotropic glutamate receptors by their cytoplasmic carboxy-terminal domains. *Neuron*, *22*, 525–536.
- Südhof, T. C. (2004). The synaptic vesicle cycle. *Annual Review of Neuroscience*, *27*, 509–547.
- Sweeney, H. L., & Houdusse, A. (2010). Myosin VI rewrites the rules for myosin motors. *Cell*, *141*, 573–582.
- Tarabeux, J., Champagne, N., Brustein, E., Hamdan, F. F., Gauthier, J., Lapointe, M., Maios, C., Piton, A., Spiegelman, D., Henrion, E., et al. (2010). De novo truncating mutation in Kinesin 17 associated with schizophrenia. *Biological Psychiatry*, *68*, 649–656.
- Teuling, E., van Dis, V., Wulf, P. S., Haasdijk, E. D., Akhmanova, A., Hoogenraad, C. C., & Jaarsma, D. (2008). A novel mouse model with impaired dynein/dynactin function develops amyotrophic lateral sclerosis (ALS)-like features in motor neurons and improves lifespan in SOD1-ALS mice. *Human Molecular Genetics*, *17*, 2849–2862.
- Tischfield, M. A., Baris, H. N., Wu, C., Rudolph, G., Van Maldergem, L., He, W., Chan, W. M., Andrews, C., Demer, J. L., Robertson, R. L., et al. (2010). Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. *Cell*, *140*, 74–87.

- Twelvetrees, A. E., Yuen, E. Y., Arancibia-Carcamo, I. L., MacAskill, A. F., Rostaing, P., Lumb, M. J., Humbert, S., Triller, A., Saudou, F., Yan, Z., et al. (2010). Delivery of GABAARs to synapses is mediated by HAP1-KIF5 and disrupted by mutant huntingtin. *Neuron*, *65*, 53–65.
- Vale, R. D. (2003). The molecular motor toolbox for intracellular transport. *Cell*, *112*, 467–480.
- Vale, R. D., Schnapp, B. J., Reese, T. S., & Sheetz, M. P. (1985). Movement of organelles along filaments dissociated from the axoplasm of the squid giant axon. *Cell*, *40*, 449–454.
- van Spronsen, M., & Hoogenraad, C. C. (2010). Synapse pathology in psychiatric and neurologic disease. *Current Neurology and Neuroscience Reports*, *10*, 207–214.
- Verhey, K. J., & Hammond, J. W. (2009). Traffic control: Regulation of kinesin motors. *Nature Reviews Molecular Cell Biology*, *10*, 765–777.
- Villace, P., Marion, R. M., & Ortin, J. (2004). The composition of Staufen-containing RNA granules from human cells indicates their role in the regulated transport and translation of messenger RNAs. *Nucleic Acids Research*, *32*, 2411–2420.
- Wagner, O. I., Esposito, A., Kohler, B., Chen, C. W., Shen, C. P., Wu, G. H., Butkevich, E., Mandalapu, S., Wenzel, D., Wouters, F. S., et al. (2009). Synaptic scaffolding protein SYD-2 clusters and activates kinesin-3 UNC-104 in *C. elegans*. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 19605–19610.
- Wang, Z., Edwards, J. G., Riley, N., Provance, D. W., Jr., Karcher, R., Li, X. D., Davison, I. G., Ikebe, M., Mercer, J. A., Kauer, J. A., et al. (2008). Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity. *Cell*, *135*, 535–548.
- Wang, X., & Schwarz, T. L. (2009). The mechanism of Ca²⁺-dependent regulation of kinesin-mediated mitochondrial motility. *Cell*, *136*, 163–174.
- Winckler, B., Forscher, P., & Mellman, I. (1999). A diffusion barrier maintains distribution of membrane proteins in polarized neurons. *Nature*, *397*, 698–701.
- Winckler, B., & Mellman, I. (2010). Trafficking guidance receptors. *Cold Spring Harbor Perspectives in Biology*, *2*, a001826.
- Wisco, D., Anderson, E. D., Chang, M. C., Norden, C., Boiko, T., Folsch, H., & Winckler, B. (2003). Uncovering multiple axonal targeting pathways in hippocampal neurons. *The Journal of Cell Biology*, *162*, 1317–1328.
- Witte, H., Neukirchen, D., & Bradke, F. (2008). Microtubule stabilization specifies initial neuronal polarization. *The Journal of Cell Biology*, *180*, 619–632.
- Wong, R. W., Setou, M., Teng, J., Takei, Y., & Hirokawa, N. (2002). Overexpression of motor protein KIF17 enhances spatial and working memory in transgenic mice. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 14500–14505.
- Woolner, S., & Bement, W. M. (2009). Unconventional myosins acting unconventionally. *Trends in Cell Biology*, *19*, 245–252.
- Yamada, K., Andrews, C., Chan, W. M., McKeown, C. A., Magli, A., de Berardinis, T., Loewenstein, A., Lazar, M., O’Keefe, M., Letson, R., et al. (2003). Heterozygous mutations of the kinesin KIF21A in congenital fibrosis of the extraocular muscles type 1 (CFEOM1). *Nature Genetics*, *35*, 318–321.
- Yildiz, A., Tomishige, M., Vale, R. D., & Selvin, P. R. (2004). Kinesin walks hand-over-hand. *Science*, *303*, 676–678.
- Yuste, R., & Bonhoeffer, T. (2001). Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annual Review of Neuroscience*, *24*, 1071–1089.
- Zheng, Y., Wildonger, J., Ye, B., Zhang, Y., Kita, A., Younger, S. H., Zimmerman, S., Jan, L. Y., & Jan, Y. N. (2008). Dynein is required for polarized dendritic transport and uniform microtubule orientation in axons. *Nature Cell Biology*, *10*, 1172–1180.