

Myosin Function in Nervous and Sensory Systems

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ABSTRACT: Development of the nervous system requires remarkable changes in cell structure that are dependent upon the cytoskeleton. The importance of specific components of the neuronal cytoskeleton, such as microtubules and neurofilaments, to neuronal function and development has been well established. Recently, increasing focus has been put on understanding the functional role of the actin cytoskeleton in neurons. Important modulators of the actin cytoskeleton are the large family of myosins, many of which (classes I, II, III, V, VI, VII, IX, and XV; Fig. 1) are expressed in developing neurons or sensory cells. Myosins are force-pro-

ducing proteins that have been implicated in a wide variety of cellular functions in the developing nervous system, including neuronal migration, process outgrowth, and growth cone motility, as well as other aspects of morphogenesis, axonal transport, and synaptic and sensory functions. We review the roles that neuronal myosins play in these functions with particular focus on the first three events listed above, as well as sensory function. © 2003 Wiley Periodicals, Inc. *J Neurobiol* 58: 118–130, 2004

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NEURONAL MIGRATION

The migration of cells committed to a neuronal phenotype during development of the mammalian nervous system is well known (Sobeih and Corfas, 2002; Sun et al., 2002). Migration in the CNS involves several different forms of motility that differ in character but result in the final redistribution of developing neurons to specific layers or nuclei. In the periphery, neural crest cells undergo migration to various parts of the body prior to commitment to a neuronal phenotype (Hatten, 2002; Park et al., 2002). Little specific information is available about the role of the different myosin types in the migration of neurons or their precursors during these early developmental events. The onset of myosin expression in developing neurons has either not been studied or it is unknown if they contribute directly to migration. However, a

few recent studies suggest important roles for myosin II in migration. In addition, one can infer from studies of other cell types undergoing migration that multiple myosins are likely to play significant roles in this process. Myosin-dependent force production is a necessary part of most cell migration.

The most extensively studied neuronal myosin is myosin II. For this reason more is known about myosin II's role in early development and migration. There are three isoforms of nonmuscle myosin II expressed by mammalian neurons; myosin IIA, IIB, and IIC (Bridgman and Elkin, 2000; Berg et al., 2001). Because nonmuscle myosin II is essential for cytokinesis one must be careful in perturbation studies to differentiate between effects on cell division and migration during these early events.

The expression of myosin IIA is seen in mice as early as E6 and remains relatively constant throughout adulthood (Conti et al., 2000; Bridgman and Elkin, 2000). Furthermore, genetic ablation of myosin IIA results in early embryonic death (E7), making it impossible to study the role of myosin IIA in neuronal migration using the knockout approach. However,

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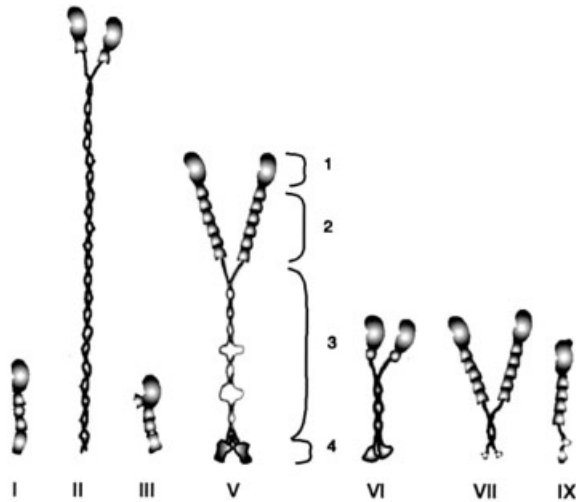


Figure 1 A diagram showing the basic structure of myosins known to be present in the nervous or sensory systems. The main domains associated with myosin structure are depicted: (1) head, (2) light chains, (3) coil-coil, (4) tail. (Adapted from Hodge and Cope, 2000.) Note that not all myosins have all domains. Myosins II, V, VI, and VII exist as dimers and therefore are two headed, while myosins I, III, and IX do not form dimers. The complete structure of myosin XV is not yet available.

because of its early expression it is likely that myosin IIA contributes to neuronal migration in a manner similar to the role it plays in other migrating vertebrate cells (Svitkina et al., 1997; Saitoh et al., 2001).

More is known about myosin IIB compared to myosin IIA. Myosin IIB expression levels depend upon two neuronal specific (mainly) myosin heavy chain (MHC) inserts (Itoh and Adelstein, 1995; Kawamoto, 1996). The expression of these MHC inserts also appears to be species specific and developmentally regulated. One of the inserts is a 10–16 amino acid sequence that is found near the ATP-binding region. The levels of mRNA encoding for this insert are seen to peak at E4, then gradually decrease until reaching adult levels. The other insert is a 21 amino acid sequence that becomes inserted near the actin-binding region of the motor domain in the MHC. MHCs containing this insert are expressed just prior to PO and then remain relatively high throughout adulthood. Some of the IIB MHCs found in the nervous system may lack both inserts, although this has not been established with certainty (Kawamoto, 1996).

Tullio and colleagues (2001) noted multiple defects in cellular organization within the neuroepithelium of early embryos (E12.5) of myosin IIB knock-out mice, suggesting that myosin IIB plays a role in neuronal migration. The defects were similar to those

seen resulting from mutations in another cytoskeletal protein, filamin, which causes migration defects in the CNS (Fox et al., 1998). However, extensive studies of migration in these mice have been hindered by two factors. First, ablation of myosin IIB results in profound hydrocephalus thought to be caused by occlusion of the third ventricle and cerebral aqueduct. This blockage may occur because of faulty migration of neuronal precursor cells from the neuroepithelium into these cavities. Second, the mice often die between E13.5 and E17 for unknown reasons, or die at PO due to heart defects (Tullio et al., 2001). Partial ablation of myosin IIB caused similar but delayed defects and the mice survived for much longer times (Uren et al., 2000). It is unclear if the delayed appearance of hydrocephalus in these mice resulted from a defect in migration. Although the cerebral aqueduct showed stenosis, it was not completely occluded. The ependymal lining appeared to be disrupted, suggesting that other factors such as abnormal cell adhesion and leakage through the ependyma may contribute to the hydrocephalus. Thus, the exact nature of the myosin-IIB-dependent defect on cellular organization remains to be determined. However, studies on other cell types may put the potential role of myosin II in migration into perspective.

Most migrating cells appear to express at least two of the three types of myosin II (Kelly et al., 1996). Although the distributions sometimes overlap they often have distinct locations. In particular, this has been observed in growth cones and fibroblasts (Rochlin et al., 1995; Kelly et al., 1996). Because the different myosin II isoforms have different activity levels and turnover rates and may have different means of regulation, they are likely to have segregated and specific functions (Kelley et al., 1996). Thus, a model for some forms of neuronal or neuronal precursor migration may be rapidly migrating cells such as the fish keratocyte (Svitkina et al., 1997). The dynamic contraction model resulting from observations on the keratocyte fits well with the known location of myosin II and migratory behavior of rapidly moving mammalian cells, including migrating neurons and nerve growth cones (Brown and Bridgman, 2003a). However, because neuronal cells undergo different modes of migration, it will be important to more closely define the role that myosin II plays in migration of cells of the nervous system.

Little is known about the developmental regulation of myosin IIC or if it is present in migrating neurons. However, we have observed the subcellular localization of myosin IIC by immunostaining in embryonic peripheral nerves cultured from E13.5 embryos (P.C. Bridgman, unpublished observations). Thus, it seems

possible that it may participate in earlier events such as neuronal migration.

The only other myosin present in neurons that might have a role in neuronal migration for which data are available is myosin I. Myosin I is a single-headed, nonfilamentous member of the myosin superfamily that has been shown to be associated with the plasma membrane within many cell types. Since Pollard and Korn's original discovery of myosin I in *Acanthamoeba castellanii* in 1973 (reviewed by Barylko et al., 2000), there have been eight different isoforms of myosin I identified in mammalian cells (Berg et al., 2001). Of these eight, three are expressed within nervous tissue (reviewed by Bridgman and Elkin, 2000). These isoforms via HUGO nomenclature are MYO1B (myr 1, myosin-I α), MYO1C (myr 2, myosin-I β), and MYO1D (myr 4, myosin-I γ), respectively (Gillespie et al., 2001).

MYO1B has been shown to be expressed throughout the brain and spinal cord in both rats and mice via Western blot and *in situ* hybridization techniques (Ruppert et al., 1993; Sherr et al., 1993). MYO1B appears to be developmentally regulated in mouse brain and spinal cord, being present in relatively high levels only in late embryonic (E16–19) until early postnatal (P0–10) ages, and declining thereafter (Sherr et al., 1993). This suggests that this isoform of myosin I has little role in early neuronal cell migration, but may play a role in later development such as during axon outgrowth (see below).

Less is known about the other two myosin I isoforms that have been found within the nervous system. MYO1C has been shown to stain at least modestly in virtually all cultured rat brain cells (Wagner et al., 1992). The expression of MYO1C in brain has been shown to be relatively low and constant throughout all time frames examined even into the adult (Ruppert et al., 1995). It seems unlikely that MYO1C plays a major role in early neuronal migration, but similar to its function in other migrating cell types it may play a supportive role. MYO1C function in axon outgrowth is better established, but also controversial (see below). MYO1D expression is developmentally regulated, rising postnatally and reaching maximal levels in adult brain (Bahler et al., 1994).

PROCESS OUTGROWTH AND GROWTH CONE MOTILITY

During development the growing axon must navigate through the surrounding tissue and find its way to its proper target. Axonal outgrowth depends upon and is directed by the growth cone (Mueller, 1999; Tessier-

Lavigne and Goodman, 2001). As is the case with other motile cells, movement of the growth cone involves multiple steps. Myosin II appears to be directly involved in most of the movement steps, indicating that it is important for growth cone motility and axon outgrowth (Brown and Bridgman, 2003a). Growth cone advance has been linked to retrograde flow of F-actin and is myosin dependent (Lin and Forscher, 1995; Lin et al., 1996; Diefenbach et al., 2002; Suter and Forscher, 2000). Recent data indicate that myosin IIB plays a role in retrograde flow (Brown and Bridgman, 2003b). Retrograde flow rate was observed to increase about twofold in growth cones from myosin IIB knockout mice. This suggests that myosin IIB may either directly contribute to generation of retrograde flow or is an important regulator of the flow rate. Because it is clear that myosin II does have an essential role in growth cone locomotion, and this directly influences the rate of axon outgrowth (Wylie et al., 1998; Bridgman et al., 2001), it is important to determine how myosin II is regulated in neurons. The other myosins that may contribute to growth cone motility and process outgrowth are the class I, V, and VI myosins. We will first discuss the regulation of myosin II and then discuss each of the other neuronal myosins below.

Myosin II

The role of myosin II in growth cone motility has been well documented and recently reviewed (Brown and Bridgman, 2003a). The regulation of myosin II in neurons is a much less well-developed area of research. The molecular mechanisms that regulate myosin II function in neurons are important but still unclear. However, it is likely that activity is regulated by at least two levels that are similar to nonmuscle myosin II in other cell types. The major source of regulation is through phosphorylation of the regulatory myosin light chain (MLC) (Adelstein and Klee, 1981; Fujita et al., 2001; Schmidt et al., 2002). The regulation is complex, involving a number of different pathways and enzymes that may affect other aspects of cytoskeletal dynamics. MLC phosphorylation activates actin-binding activity and permits bipolar filament formation by inducing a change in MHC conformation (Suzuki et al., 1978). Dephosphorylation has the opposite effects. Actin binding greatly potentiates the MHC ATPase activity necessary for generating force similar to that observed in skeletal muscle cells. There appears to be two primary pathways for regulating the phosphorylation of MLC, one that is calcium dependent, the other calcium independent (Fig. 2). Because myosin IIA and IIB share the same

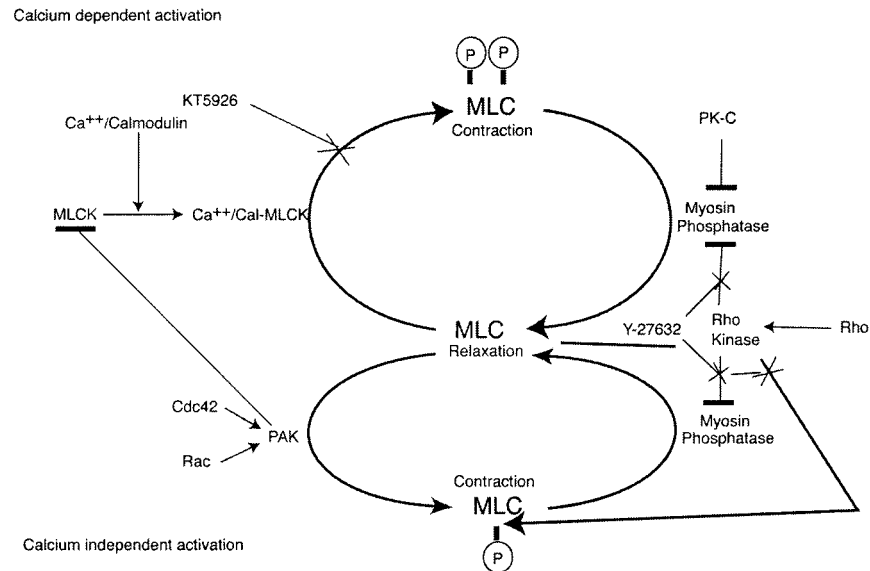


Figure 2 A diagram showing the regulation of myosin II gathered from information on smooth and nonmuscle cells. Two major pathways regulate myosin light chain (MLC) phosphorylation, one calcium dependent involving MLCK and the other calcium independent involving PAK. PAK can also inhibit MLCK if it interacts prior to activation by Ca^{++} /calmodulin. Dephosphorylation of the MLC by the myosin light chain phosphatase is regulated by at least two pathways, as indicated. Some of the drugs that inhibit these pathways are shown. Other more minor pathways are not depicted.

light chains (Grant et al., 1990), they may both be equally regulated through these pathways. Ca^{++} /calmodulin regulates the activity of myosin light chain kinase (MLCK), which phosphorylates MLC at Ser-19 and sometimes Thr-18 (Goeckeler and Wysolmerski, 1995). Cdc42 and Rac regulate p21-activated kinase (PAK), which catalyzes monophosphorylation of MLC at Ser-19 (Chew et al., 1998). Mono versus diphosphorylation may correlate with the amount of myosin activation and allow a means of regulating the degree of force. PAK also inhibits the activity of MLCK (but only prior to its activation by Ca^{++} /calmodulin) indicating cross talk between the two pathways (Goeckeler et al., 2000). Various drug studies have implicated MLCK in growth cone activity (Bixby and Jhabvala, 1992; Jian et al., 1994; Oberstar et al., 1997). Our recent data indicate that MLCK and PAK are both abundant in growth cones (P.C. Bridgman, unpublished observations). It is therefore likely that both contribute to the regulation of myosin II in growth cones. It is still unclear if one of these pathways predominates or how they may interact in growth cones. The importance of the MLCK pathway in axon guidance has been demonstrated by recent studies on *Drosophila* (Kim et al., 2002). Rho kinase has also been shown to phosphorylate MLC and inhibit the activity of the myosin

phosphatase in smooth muscle and nonmuscle cells, including neuroblastoma cells and primary neurons, adding to the complexity (Amano et al., 1998; Hirose et al., 1998; Fritz and VanBerkum, 2002).

Dephosphorylation of MLC in smooth muscle occurs through the MLC phosphatase (Somylo and Somylo, 2000). Two primary pathways regulate the activity of the phosphatase. One is through phosphorylation by Rho kinase (Amano et al., 1998; Feng et al., 1999) and the other is less direct, through phosphorylation of an inhibitor protein (CPI-17) by protein kinase C (PK-C) (Murakami et al., 1998; Kitazawa et al., 2000). Both inhibit the activity of the phosphatase leading to sustained levels of MLC phosphorylation and myosin II activity. Originally, CPI-17 appeared to be smooth-muscle specific, but recent evidence indicates that it is present in neurons and thus may play an important role in developing neurons (Eto et al., 2002). In neuroblastoma cells over-expression of Rho kinase leads to retraction and cell rounding, while inhibition by a dominant negative or the drug Y27632 prevents LPA-induced retraction (Hirose et al., 1998). In cultures of primary neurons (granule cells) expression of constitutively active Rho kinase caused a decrease in the formation of new neurites (Bito et al., 2000). In contrast, application of the inhibitor Y27632 caused enhanced and precocious process outgrowth. It

also increased the size of growth cones and their motility. Therefore, it appears likely from the data available in the literature and our unpublished data that both light chain phosphorylation and dephosphorylation are major pathways for regulation of myosin II activity in neurons. However, more work needs to be done to substantiate this conclusion, separate out drug effects on different components of the cytoskeleton, and to determine the enzymes that are most important for controlling the state of growth cone MLC phosphorylation.

The second mechanism for regulation of non-muscle myosin II activity involves bipolar filament assembly through regulating the phosphorylation states of the MHCs (Hodge et al., 1992). Tight regulation of bipolar filament assembly is especially important for migrating cells because this allows quick and efficient responses to changes in the local environment. Bipolar filaments in moving nonmuscle cells are dynamic, undergoing rapid formation and disassembly (Kolega, 1998). Bipolar filaments are necessary for myosin-II-dependent contraction and are found in growth cones (Bridgman, 2002) so control of assembly may be important for outgrowth. Myosin IIB bipolar filament assembly appears to be inhibited by MHC phosphorylation at multiple sites by PK-C and casein kinase II (CK II), both of which are powerful activators of second messenger cascades (Murakami et al., 2000). Their ability to phosphorylate the IIB MHC was shown to be isoform-specific and occurs within the carboxyl-terminal domain. In contrast, phosphorylation of the IIA MHC does not inhibit filament assembly. However, the mts 1 protein (an S100 family protein; S100A4) does specifically inhibit myosin IIA assembly and also causes disassembly through the inhibition of IIA MHC phosphorylation by PK-C at a single 29 amino acid sequence located within the carboxyl terminus of the MHC protein. S100A4 protein has been useful to study this isoform-specific phosphorylation of the MHC. Binding of S100A4 to the MHC results in nonfunctional myosin IIA protein (Kriajevska et al., 1998), probably because function depends heavily on filament formation, although S100A4 has also been shown to partially inhibit the actin-activated MgATPase activity *in vitro* (Ford et al., 1997). Interestingly, the oligomeric form of S100A4 has been shown to induce differentiation of neuronal cells in rat hippocampal cultures, while the dimeric form did not (Novitskaya et al., 2000). These data seem to suggest that S100A4 protein may serve opposing roles depending on its expressed form.

Whether or not S100A4 plays a role in regulating myosin II function *in vivo* is unknown. S100A4 has

been shown to be expressed in both the developing and adult central nervous system (Aberg and Kozlova, 2000). Although it has been shown to be up-regulated in white matter astrocytes in the central nervous system following nerve injury (Kozlova and Lukanidin, 2002), no neuronal expression was detected.

In addition to the upstream mechanism of regulation there appears to be additional downstream mechanisms that regulate specific myosin-II-driven mechanisms. A general downstream mechanism is the regulation of myosin II binding to actin filaments by proteins associated with actin filaments. Caldesmon appears to regulate binding of myosin II to actin filaments in smooth muscle and nonmuscle cells, similar to the regulation of myosin II binding to actin by the troponin/tropomyosin complex in skeletal muscle (Yamashiro et al., 2001). However, it is unclear to what degree this mechanism contributes to regulations of growth cone myosin II activity.

In summary, myosin II regulation is likely to depend upon multiple pathways, many of which have other effects on neurons and growth cones. Therefore it will be a challenge to sort out the important regulators of myosin II function in growth cones and developing neurons. It will be important to accomplish this because it is clear that myosin II has multiple force-dependent roles in growth cone motility and axon outgrowth that are likely to influence the navigational ability of an axon as it grows towards its target (Zhou et al., 2002).

Myosin I

As previously indicated, myosin II is not the only myosin present in growth cones. Myosin I may be the next most important class of myosin that contributes to growth cone motility. At least two of the three forms of myosin I identified in neurons are present in axons and growth cones: MYO1B and MYO1C.

As already stated, MYO1B is expressed throughout the brain and spinal cord in rodents. It is present in relatively high levels in late embryonic (E16–19) and early postnatal (P0–10) animals, declining slightly thereafter to low levels in adults (Sherr et al., 1993). Lechler et al. (2000) have reported direct involvement of MYO1B in actin polymerization within yeast cells, although this has not been established in higher species. MYO1B has been shown to be localized adjacent to the plasma membrane within the growth cones of superior cervical ganglion (SCG) neurons and was sometimes increased in association with leading edge ruffles (Lewis and Bridgman, 1996). The location of MYO1B adjacent to the plasma membrane in growth cones is at least consis-

tent with the possibility that it contributes to the regulation of actin polymerization. This is because many actin barbed or fast growing ends are located at the interface between the cytoskeleton and the inner membrane surface.

The expression of MYO1C in the mammalian nervous system is relatively low and constant throughout development (Ruppert et al., 1995). It is localized to cortical regions of cells in many mammalian cell types, including cells from brain (Wagner et al., 1992). However, in peripheral mammalian neurons growing in culture most of the staining was confined to neurites with little present in the growth cone (Brown and Bridgman, 2003b).

A recent study using a micro-chromophore-assisted laser inactivation (micro-CALI) technique to knock MYO1C function in chick dorsal root ganglion (DRG) neurons has shown that MYO1C may contribute to retrograde flow of F-actin within neuronal growth cones (Diefenbach et al., 2002). According to the Diefenbach study (2002), knocking out myosin II via the micro-CALI technique had little or no effect on retrograde flow. In contrast, when knocking out MYO1C function using the micro-CALI technique, the retrograde flow rate decreased 76% compared to unirradiated controls, while lamellipodial expansion increased. The latter result appears to be consistent with the clutch hypothesis proposed by Mitchison and Kirschner (1988); however, it does not fit with our recent data on the effects of myosin IIB on retrograde flow or the distribution of MYO1C in mouse peripheral nerve growth cones (Brown and Bridgman, 2003b). This is clearly an area that requires further study.

Even less is known about the function of MYO1D found within the nervous system. MYO1D has been shown to be expressed in low levels in the rat brain by E17 (Bahler et al., 1994). Thereafter it is seen to increase dramatically until maximal expression is reached in adult. MYO1D has a punctate distribution within subpopulations of neurons. The staining is mainly observed in the cell bodies and apical dendrites, suggesting that its function is associated with these compartments.

Myosin V

Myosin V is a dimeric, nonfilamentous myosin that occurs in three different isoforms (Wu et al., 2000; Rodriguez and Cheney, 2002). Based on their heavy chain sequences the myosin V's are myosin Va, myosin Vb, and myosin Vc. Of the three myosin V isoforms, myosin Va expression in the nervous system is seen to be the most broadly distributed (Mercer

et al., 1991). Developmentally, myosin Va protein is detectable at low levels in early embryonic development (Espindola et al., 1992). Myosin Va levels are seen to gradually increase until they are maximal around P0. Thereafter myosin Va protein levels remain relatively constant throughout adulthood.

Interest in myosin Va as a possible mediator of growth motility was stimulated by the report that CALI of myosin V in growth cones of chick neurons caused increased rates of filopodia extension (Wang et al., 1996). Subsequently, however, it was shown that neurons cultured from *Dilute lethal* (myosin Va null) mice have normal axon outgrowth rates and growth cones are normal in morphology (Evans et al., 1997). In cultured SCG growth cones the subcellular localization of myosin Va was mainly associated with organelle-rich areas (Evans et al., 1997). Myosin Va was specifically associated with a population of small 50–100 nm organelles, and was found in close association with both microtubules and actin bundles. Thus, a more likely function for myosin Va in growing neurons is in organelle transport (DePina and Langford, 1999). This possibility has been further strengthened and defined in recent studies (Bridgman, 1999; Rogers and Gelfand, 2000; Schott et al., 2002).

Myosin Vb expression has been shown to be more limited within the nervous system than myosin Va. *In situ* hybridization showed myosin Vb mRNA to be mainly localized to structures in the hippocampus, dentate gyrus, and amygdala (Zhao et al., 1996). Little is known about the developmental regulation of myosin Vb. However, myosin Vb appears to have a synaptic role in some cases (see below). Even less is known about the expression of myosin Vc in the nervous system, although expression has been detected in the cerebellum (Rodriguez and Cheney, 2002).

Myosin VI

Myosin VI has been shown to be located in growth cones, suggesting a role in motility (Suter et al., 2000). Suter et al. (2000) noted that the distribution of immuno-positive staining was similar for both myosin V and myosin VI in DRG growth cones within the central (C) domain. However, within the peripheral (P) domain there was a less than 20% overlap in myosin V and VI staining. Interest in the myosin VI activity in growth cones is high because it is one of the few classes of myosin that has been shown to move to the pointed end of the actin filament through *in vitro* motility assays (Wells et al., 1999). It is currently uncertain if myosin VI demonstrates the same pointed end directed motility *in vivo*. However, it is clear that myosin VI has some potentially unique

properties and could contribute to specific aspects of growth cone motility.

In summary, the motility of the growth cone involves multiple classes of myosins. Currently the most important class of myosin for growth cone motility is myosin II. This is simply based upon the timing of developmental expression, relative abundance in growth cones, and the effects of genetic ablation. For this reason it will be important to explore the complexities of myosin II regulation in growth cones. However, as other neuronal myosins are studied in more detail, it may become clear that other myosins have equally or more important roles in outgrowth and growth cone motility.

NEURONAL MORPHOGENESIS

The actin cytoskeleton has been shown to play an important role in all aspects of neuronal morphogenesis (Luo, 2002). Other than the role in axon outgrowth, little is known about the role that myosins play in this process. Dendritic growth, guidance, branching, and spine formation all depend to some degree on the actin cytoskeleton, but for the most part, the roles of myosins in these processes have not been directly studied. The localization of the different myosins in the dendritic compartment may give some insight into the possible functions of myosins in dendrites.

Myosin I (MYO1D), myosin IIA and IIB, and myosin V (a and b) have all been found in the dendritic compartment via immunostaining (Espindola et al., 1992; Bahler et al., 1994; Hayashi et al., 1996; Bridgman, 1999; Vopicelli et al., 2002; P.C. Bridgman, unpublished observations). Only MYO1D appears to have a differential localization in dendrites as opposed to axons (Bahler et al., 1994). Both myosin IIB and myosin Va have been shown to be associated with dendritic spines and postsynaptic densities (Takagishi et al., 1996; Naisbitt et al., 2000; Vopicelli et al., 2002; Walikonis et al., 2000).

Only myosin V has been shown to be functionally important for dendritic spines. In *Dilute lethal* (myosin Va null) mice, Purkinje cell dendritic spines lack smooth endoplasmic reticulum and have abnormal morphologies (Takagishi et al., 1996; Bridgman, 1999). This results in abnormal calcium regulation within the spine and alters long-term synaptic depression (Miyata et al., 2000). It is unclear if the altered spine morphologies observed in *Dilute lethal* mice are a direct consequence of myosin V's absence or a result of the missing smooth endoplasmic reticulum. Although spines of other neuronal types do not show

the same absence of smooth endoplasmic reticulum and altered spine morphology, there is reason to believe that myosin V may have important general functions in dendritic spines. This is because myosin Va is bound by GKAP and CAM-KII (directly or indirectly), both of which are concentrated in postsynaptic membranes (Costa et al., 1999; Naisbitt et al., 2000). Myosin Va may also play a role presynaptically because *Dilute lethal* mice show enlarged presynaptic terminals at synapses between parallel fibers and Purkinje cells (Bridgman, 1999). The exact presynaptic role has remained elusive because synaptic transmission in hippocampal neurons is not altered (Schnell and Nicoll, 2001) and abnormal release characteristics were not noticed in a study of synapses on Purkinje cells (Miyata et al., 2000).

Recently, myosin Vb has been shown to be involved in the recycling of M₄-type muscarinic acetylcholine receptors (Volpicelli et al., 2002). This indicates that this isoform of myosin V may also be located in the dendritic compartment and have a functional role in spines.

Myosin IX, a proposed RhoGAP protein, has been shown to be expressed in brain, heart, and muscle, and is also associated with the hair cells of the inner ear (Chieriegatti et al., 1998; Grewal et al., 1999). Rho GTPases are thought to be regulators of neuronal morphology. Expression of *myr 7* and *myr 5*, the rat myosin IX isoforms, was shown to differ in developmental regulation (Chieriegatti et al., 1998). *Myr 5* expression was seen only moderately in embryonic neurons and decreased thereafter. *Myr 7* was elevated in embryonic neurons, and remained elevated through maturity. Immunostaining has revealed that both forms of myosin IX can be found in developing neurons, including Purkinje neurons.

Although myosin II has been localized to the cytoskeleton of dendritic spines (Cheng et al., 2000) and myosin IIB may associate with the postsynaptic density (Husi et al., 2000), it remains unknown if myosin II plays an essential role in either spine development or function. Myosin IIB knockout mice die prior to development of dendritic spines, making it difficult to study in this system.

Another aspect of morphogenesis that may depend upon myosin II is the specification of axons and dendrites. Myosin IIB is present in both axonal and dendritic growth cones of hippocampal neurons (Fig. 3). This is intriguing because it has recently been shown that mechanical tension can determine the fate of growing processes in hippocampal neurons (Lamoureux et al., 2002). Differential activation of myosin IIB could potentially play a role in determining the amount of traction force and thus degree of tension on

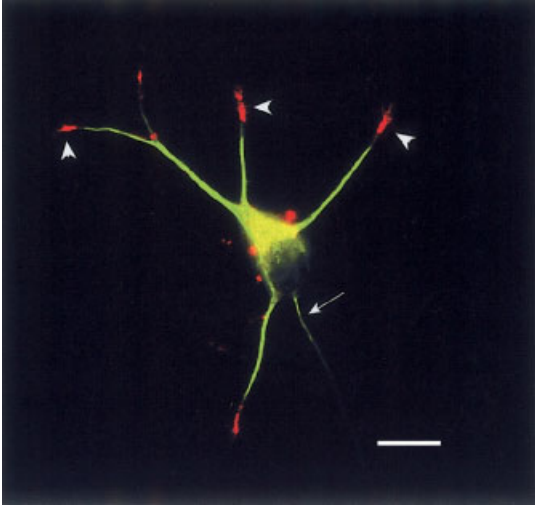


Figure 3 A rat hippocampal neuron grown in cell culture for 6 days and then fixed and stained with antibodies to MAP2 (green) to identify dendrites and myosin IIB (red). Myosin IIB is concentrated in dendritic growth cones (arrowheads). A portion of the proximal axon (arrow) can be seen. The axonal growth cone also stained but is not shown. Bar = 12 μm .

growing processes. This could determine the ultimate fate or phenotype of these processes.

AXONAL TRANSPORT

The role of microtubule-based motors such as kinesins and dynein in axonal transport is well known (reviewed by Cyr and Brady, 1992; Mercer et al., 1994). Whether myosins contribute directly to long range axonal transport in mature neurons is not known. However, there is some evidence that certain classes of myosins do contribute to transport during development and possibly to short range transport in specific regions of the neuron (Buss et al., 2002; Langford, 2002). The most evidence for myosin-based transport in neurons is for the class V myosins (Langford, 2002). Myosin V has been directly associated with organelle transport in yeast (Govindan et al., 1995; Catlett and Weisman, 1998; Catlett et al., 2000), as well as melanocytes and other pigmented cells, and so it seems likely that it plays a similar role in neurons (Mermall et al., 1998; DePina and Langford, 1999; Schott et al., 2002). Myosin-V-based axonal transport is the subject of another review in this volume.

SENSORY SYSTEMS

Possibly the clearest functional role for myosins in the nervous system is in sensory systems. Myosins have been shown to play a role in the morphology, function, and maintenance of sensory tissues. For instance, normal hearing and vestibular function in mammals has been shown to require myosin I, V, VI, VII, IX, and XV (for reviews see Wu et al., 2000; Maniak, 2001). Similarly, myosin III and VII have been shown to be required for normal vision in mammals (Wu et al., 2000; Dose and Burnside, 2002). Much of the work has been facilitated by naturally occurring mutations that lead to sensory defects. We will briefly discuss the myosins known to be involved in these sensory systems.

Myosin VIIa has been shown to play a role in auditory function in many vertebrate systems. Mutations in myosin VIIa have been shown to be associated with the sensory losses seen in Usher syndrome type Ib, *shaker-1* mice, and *mariner*, in zebra fish. These mutations result in hearing deficits and vestibular abnormalities (reviewed by Wu et al., 2000; Maniak et al., 2001). Myosin VIIa is mainly located along stereocilia in association with the lateral linkages that interconnect the actin cytoskeletons of adjacent stereocilia to one another (Hasson et al., 1997). Myosin VIIa may represent the intracellular anchor for these linkages and is essential for maintaining the integrity of the hair bundle.

Snell's Waltzer syndrome affects vestibular function in mice and has been shown to be associated with mutations in type VI myosin (Self et al., 1999; Wu et al., 2000). Myosin VI is found in the cuticular plate at the base of hair cells closely associated with the rootlets. It is thought to anchor the apical membrane between stereocilia to the actin of the cuticular plate. Mutations in myosin VI results in the progressive fusion of stereocilia membranes, eventually leading to degeneration of the hair cells by P0 (Self et al., 1999).

MYO1C staining has been shown to be localized at the tips of hair cell stereocilia (Hasson et al., 1997; Wu et al., 2000), and MYO1C is thought to function in slow transducer adaptation of the stereocilia in response to stimulation (Holt and Corey, 2000; Dumont et al., 2002). The exact mechanism of transducer adaptation is not clear, but MYO1C may act to couple the actin filaments of the stereocilia to the calcium-dependant transduction channels, providing the tension forces needed to control adaptation (Cyr et al., 2002). Holt et al. (2002) showed that mutation of MYO1C eliminated slow adaptation. It is also possible that MYO1C functions in the proper assembly of the transduction apparatus.

Myosin IXB (myr 5) is expressed in the inner ear, suggesting that it may also contribute to the function of hair cells (Grewal et al., 1999). It is unknown if myosin IXB may also play a role in hair cell development similar to its potential role in brain (Chieriegatti et al., 1998).

The expression of myosin XV has also been verified within the inner ear of vertebrates. Mutations in myosin XV expression have been associated with nonsyndromic deafness (Weil et al., 1997), and profound hereditary deafness DFNB3 in humans (Liang et al., 1999), as well deafness and vestibular abnormalities in *shaker-2* mice. Through *in situ* hybridization techniques, myosin XV has been localized to the sensory epithelia of both the cochlear and vestibular systems in mice. Immuno-localization in adult mice has shown myosin XV to be associated with the cuticular plate and stereocilia of the hair cells (Liang et al., 1999). Abnormal expression of myosin XV in hair cells leads to aberrant F-actin distribution in the basal portion of the stereocilia. As a result, shortening of the hair cells occurs. Although not yet conclusive, these results seem to implicate myosin XV's participation in the normal organization of actin filaments within the basal section of the stereocilia. Although the domain structure of myosin XV has been studied (Anderson et al., 2000), a complete picture of its structure has not yet emerged.

The function of myosin V within sensory tissue seems to be related to its proposed function as an actin-based motor protein. As with the other myosin isoforms discussed previously, myosin V has also been shown to be localized within the inner ear (Coling et al., 1997; Hasson et al., 1997). However, myosin V localization was seen only in the afferent nerves that innervate the hair cells, not within the hair cells themselves. Consistent with this observation, myosin V isoforms have also localized extensively

within brain tissue, where they make up approximately 0.3% of the total protein (Cheney et al., 1993).

Myosin III expression has been shown to be associated with the photoreceptor cells in *Drosophila melanogaster* (Ng et al., 1996), spotted bass, and human (Dose and Burnside, 2002). Two variants of type III myosins, a full-length version, MYO3A, and a truncated version, MYO3B, have been discovered. Myosin III expression in vertebrates was shown to require two separate genes, while invertebrate expression was accomplished using spliced variants of the same gene (Dose and Burnside, 2002). Mutations in the genes controlling myosin III expression results in retinal degeneration (Ng et al., 1996; Dose and Burnside, 2002).

The exact function of myosin III in the photoreceptor cells remains unknown; however, Ng and colleagues (1996) reported that myosin III has serine/threonine kinase activity. They have also shown myosin III is capable of autophosphorylation, and phosphorylates the smooth muscle myosin regulatory light chain (LC₂₀), indicating that myosin III may have some yet undetermined motor function within the photoreceptor cells.

Recently, MYO3B expression has been linked to the gene responsible for Bardet-Biedl syndrome in humans. There is no direct evidence aberrant MYO3B expression is the causative agent behind Bardet-Biedl syndrome; however, MYO3B expression within the retina, kidneys, and testes, primary areas that are affected by this syndrome, has been verified (Dose and Burnside, 2002).

CONCLUSIONS AND PERSPECTIVE

Our knowledge of the roles that the myosins play in normal neuronal development or the mature nervous

Table 1 Associations between the Different Myosin Classes (and Their Isoforms) and the Different Developmental Functions or Sensory Systems Are Summarized

	Myosins												
	I			II			III	V		VI	VII	IX	XV
	B	C	D	A	B	C	A	B	A	B	A	B	
Neuronal migration	X			XX									
Process outgrowth and growth cone motility	XX			XX			X		X				
Neuronal morphogenesis	XXX			XX			XX		X			XX	
Axonal transport	X						X		X				
Sensory systems													
Hearing/vestibular	X			X			X		X	X		X	X
Vision							XX						

system is still relatively rudimentary. The emerging data indicate that most of the myosins discussed in this review are likely to play key roles in various stages of neuronal development (Table 1). During neuronal migration, force-producing myosin proteins such as myosin II are likely to be required for directed movement. Similarly, during neuronal morphogenesis myosin motors (I, II) are likely to be responsible for many of the forces required to form the elaborate processes and connections between neurons. In the mature nervous system it is clear the myosins are important for sensory functions in general, but are especially important for hair cell function. However, in both developing and mature systems it is still necessary to sort out the essential roles of the different myosin types from general indirect effects on the actin cytoskeleton. In particular, acute studies of neurons or sensory cells would greatly benefit from drug reagents that are more specific for myosin activity or their regulating enzymes. Long-term studies of both developing and the adult nervous system would benefit from conditional knockouts so that only the nervous or sensory systems are affected.

Nerve outgrowth, growth cone motility, and neuronal morphogenesis remain promising areas for future discoveries of important myosin-dependent functions in the developing nervous system. However, the least understood, but most exciting area for future research, may be related to transport function within the multiple compartments of the neuron.

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