
Axonal myosins

PAUL C. BRIDGMAN* and LISA L. ELKIN†

Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110, USA
Email: bridgmap@pcg.wustl.edu

Summary

The myosin super family is an extended family of actin-based motor proteins that can be divided into 15–18 structurally distinct classes (Sellers, J. R. (2000) *Biochimica et Biophysica Acta*, **1496**, 3–22; Hodge, T. & Cope, M. J. T. V. (2000) *Journal of Cell Science*, **113**, 3353–3354; Berg, J. S., Powell, B. C. & Cheney, R. E. (2001) *Molecular Biology of the Cell*, **12**, 780–794). Many myosin classes contain multiple members, including different isoforms within the same species as well as homologous proteins from different species. A number of the myosin classes are expressed in multiple cell types in vertebrates, including neurons. Surprisingly little is known about the neuronal function of these different myosins. In this review we concentrate on the vertebrate myosins known to be present in neuronal axons. We take a simplistic view of this topic, addressing a number of specific questions. (1) Which myosins are present in neurons? (2) Do their levels change during development? (3) Are the neuronal forms unique in any way? (4) Which neuronal myosins are located in axons and how are they distributed? (5) What do these myosins do and are they essential for a specific neuronal function?

Introduction

Neuronal axons are unique structures because of their extreme length and specialized cytoskeletal organization. During development they must grow and navigate through complex environments for long distances. The neuronal growth cone, a highly dynamic actin-rich cellular structure that generates the force essential for normal outgrowth rates (Lamoureux *et al.*, 1989), regulates axonal growth and pathfinding. The actin-rich peripheral regions of the growth cone are primarily involved in force production. Filopodia have a particularly important role in axonal guidance (Bentley & Toroian-Raymond, 1986) because they are essential for growth cone turning (Zheng *et al.*, 1996). This has been attributed to a sensory-motor role (Kater & Rehder, 1995), which may require actin-based force-producing proteins (Heidemann *et al.*, 1990).

After axons have made initial contacts with target cells, a pruning process occurs to establish the appropriate relationships between cells (Lichtman & Colman, 2000). The withdrawal or retraction of nerve endings may also involve an active process requiring force (Dennerll *et al.*, 1989; Amano *et al.*, 1998). In the adult nervous system, neuronal plasticity, which involves the remodeling of synaptic contacts, could potentially require force production similar to that seen during development. Thus, neurons have po-

tentially high demands for actin-myosin-based force-producing proteins both during development and into adulthood.

Intracellular transport is another process that requires force production, both during development and into adulthood. Proper neuronal function requires that materials be transported along the entire length of the axons into a branching network of specialized nerve endings, such as growth cones and synapses. Furthermore, many of these specialized terminals undergo local membrane recycling that requires transport of materials between the plasma membrane and other specialized organelle compartments. The important roles that the microtubule-based motor proteins kinesin and dynein play in fast transport in axons is well documented (Martin *et al.*, 1999). However, several important neuronal compartments lack microtubules, but are rich in actin filaments. Thus, it is possible that myosins also contribute to local transport of organelles in these actin-rich regions. In addition, the molecular basis of slow axonal transport is unknown. It is possible that myosins contribute to slow transport.

In vertebrates, most nonmuscle cells express multiple classes of myosin both during development and following maturation. Aside from an essential role for non-muscle myosin II in cytokinesis (Robinson & Spudich,

* To whom correspondence should be addressed.

† Present address: Dr. Lisa L. Elkin, Cellular Genomics Inc., 25 Science Park, Suite 691, New Haven, CT 06511.

2000), and several of the unconventional myosins (VI, VII and XV) in ear hair cell function (Wu *et al.*, 2000), it has been difficult to assign specific essential functions to these myosins. This difficulty is especially true in neurons because of a paucity of studies that specifically address neuronal myosin function. However, together with localization studies, recent studies of mouse mutations and knockouts have begun to address neuronal myosin function in vertebrates.

Which myosins are present in neurons?

Currently the myosin superfamily contains up to 18 classes of myosin each with multiple members (Sellers, 2000; Hodge & Cope, 2000; Berg *et al.*, 2001). Only myosin classes I, II, V, VI, and IX are clearly present in vertebrate neurons on the basis of either mRNA expression and/or protein staining (Itoh & Adelstein, 1995; Mercer *et al.*, 1991; Lewis & Bridgman, 1996; Warner *et al.*, 1992; Sherr *et al.*, 1993; Bahler *et al.*, 1994; Evans *et al.*, 1997; Suter *et al.*, 2000; Chieregatti *et al.*, 1998; Grewal *et al.*, 1999). While it is highly likely that neurons contain other myosins, published data to substantiate their presence is lacking.

Each of the myosin classes known to be present in neurons contains multiple members. The members of these different classes are products of different genes. Therefore, not all members may be present in neurons or may be expressed only at low levels. Complicating the interpretation of published immunolabeling data for some of the members of a particular class, is the lack of information about antibody cross-reaction between members or the use of antibodies made against a myosin in one species for labeling experiments in a different species. More details regarding specific members of myosin classes I, II, V, VI, and IX are given below.

MYOSIN I

The myosin I class is one of the largest, with several subclasses containing multiple members. This includes mammalian myosin 1 α (myr1), 1 β (myr2), IC (myr3), and 1 γ (myr4). Myosin 1 α , 1 β , and 1 γ are expressed in brain and are present in neurons (Wagner *et al.*, 1992; Sherr *et al.*, 1993; Bahler *et al.*, 1994; Ruppert *et al.*, 1993; 1995). Myosin 1 α in particular, is widely distributed in brain and spinal cord as assayed by immunoblotting and in situ hybridization (Ruppert *et al.*, 1993; Sherr *et al.*, 1993). Myosin IC, the vertebrate version of the "classical" or "amoeboid" myosin I, has not been reported to be present in neurons. Brush border myosin I, although closely related to myosin 1 α , has been recently separated into its own subclass (Sellers, 2000). Brush border myosin I is absent from neural tissue, but may be present in epithelial linings of the ventricles.

MYOSIN II

The nonmuscle form of this conventional myosin is present in all eukaryotic cells. Two isoforms of non-muscle myosin II heavy chain, myosin IIA and IIB, are expressed in most vertebrate cells including neurons (Kawamoto & Adelstein, 1991; Itoh & Adelstein, 1995). Myosin II protein is present at high levels in neurons of the cortex, cerebellum, and spinal cord as determined by immunocytochemistry (Miller *et al.*, 1992). Although it isn't known whether all neurons express both myosin IIA & B, in all cases where it has been investigated, both forms were present. Thus, myosin II appears to be one of the most widely distributed forms of myosin in vertebrate neurons.

A third member of the nonmuscle myosin II type has been identified from sequence information resulting from the cumulative efforts to sequence the genome of multiple species (Berg *et al.*, 2001). However, nothing is known about the tissue distribution of this new myosin II or if it is expressed in neurons.

MYOSIN V

In mammalian cells, three different myosin V heavy chains are expressed in a tissue-specific manner. Myosin Va mRNA is highly expressed in nervous tissue and the protein is broadly distributed throughout the brain and peripheral nerve (Mercer *et al.*, 1991; Espindola, *et al.*, 1992; Evans *et al.*, 1997; Drengk *et al.*, 2000; Suter *et al.*, 2000). In contrast to the distribution of myosin Va, myosin Vb has a much more limited distribution in the nervous system (Zhao *et al.*, 1996). In the CNS, myosin Vb mRNA expression appears to be high in hippocampus, dentate gyrus, and amygdala, with more limited expression in the cortex and mammillary nuclei. Myosin Vc appears to have a very limited amount of expression in the CNS. It is expressed in low levels only in the cerebellum (R. Cheney, personal communication).

MYOSIN VI

Only a single member of the vertebrate myosin VI class has been identified. Immunoblotting experiments indicate that myosin VI is present both in chicken brain and peripheral nerve (dorsal root ganglion cells) (Suter, *et al.*, 2000).

MYOSIN IX

Two isoforms of myosin IX, Myosin IXA (myr 7) and IXB (myr 5), have been identified and both are expressed in brain (Reinhard *et al.*, 1995; Wirth *et al.*, 1996; Chieregatti *et al.*, 1998; Grewal *et al.*, 1999). Although both myosin IXA and IXB appear to have a broad distribution of protein expression in different brain regions, myosin IXA levels appear more robust (Chieregatti

Table 1. A summary of the relative levels of the different types of myosin in nervous tissue according to developmental stage. The indicated levels are for comparison within an individual myosin type only. They should not be used for comparison between types.

Developmental stage	Myosin types								
	<i>I</i> α	<i>I</i> β	<i>I</i> γ	<i>IIA</i>	<i>IIB</i>	<i>Va</i>	<i>VI</i>	<i>IXA</i>	<i>IXB</i>
Early embryos (E6-E15)				+	+	+	+		
Late embryonic (E16-19)	+++	+	+++	+	++	++	+++	+	++
Early postnatal (P0-P10)	+++	+	+++	+	++	+++	++	++	+++
Late postnatal (P11-P20)	++	+	++	+	++	+++	++	++	++
Adult	++	+	+	+	+++	+++	++	++	+

et al., 1998). Myosin IXA is also highly expressed in the adrenal gland (Chieregatte *et al.*, 1998).

Do myosin levels change with development?

MYOSIN I

Myosin *I* α , *I* β , and *I* γ mRNAs have been detected in early neonatal mouse brain (Sherr *et al.*, 1993). Myosin *I* α protein has also been detected in embryonic brain, partially purified growth cone particles and peripheral nerve (Ruppert *et al.*, 1993; Lewis & Bridgman, 1996). Immunoblot data indicated that myosin *I* α was detectable in E17 brain and E19 superior cervical ganglia (SCG). In forebrain, protein levels were high in embryos and remained high through early postnatal days. Levels then dropped somewhat and remained steady through the adult. Myosin *I* β is detectable at modest levels in brain cells cultured from late embryonic rats (Wagner *et al.*, 1991). Myosin *I* γ (myr 4) protein was detectable at low levels on rat brain immunoblots as early as E17, but increased substantially after P14 (Bahler *et al.*, 1994). Developmentally dependent changes in neuronal myosin levels are summarized in Table 1.

MYOSIN II

Myosin II expression begins at the earliest stages of neural development, but the exact isoform expressed depends on several factors, including the presence of two different amino acid inserts in the myosin IIB heavy chain (see the next section).

MYOSIN V

Myosin *Va* protein was detectable by immunoblotting in embryonic rats by about E13 and continued to increase until reaching a maximum by about the time of birth (Espindola, *et al.*, 1992). Adult levels remained comparable to those at PO. In embryonic chick brain, myosin *Va* could be detected on immunoblots as early as E6 (Suter *et al.*, 2000).

MYOSIN VI

Myosin VI was detected in immunoblots of embryonic chick brain and peripheral nerve (Suter *et al.*, 2000). It was detectable as early as E6 in brain and in dorsal root ganglia (DRG) by E10. In DRGs it increased until about E16 and then decreased somewhat at later stages.

MYOSIN IX

Both myosin IXA and IXB show protein expression as early as E16 in brain (Chieregatti *et al.*, 1998). Myosin IXA protein expression appears to remain high in the adult, but the adult form migrates faster than the embryonic form on acrylamide gels indicating a developmentally regulated isoform switch (Chieregatti *et al.*, 1998). In contrast, myosin IXB is expressed at higher levels in embryonic and early postnatal stages than in adult brain (Chieregatti *et al.*, 1998).

Are the neuronal forms of the different myosins unique?

MYOSIN I

Although many of the members of the myosin I class were cloned from brain libraries, there is currently little information available about brain-specific characteristics within this class. Alternatively spliced forms of myosin *I* α (myr 1) are detected in brain and spinal cord on immunoblots, but their significance is unknown (Ruppert *et al.*, 1993).

MYOSIN II

The myosin IIB heavy chain has two amino acid inserts that appear to be mainly neuronal specific (Itoh & Adelstein, 1995). One insert (10–16 amino acids) is near the ATP binding region and the second (21 amino acids) is near the actin binding region. The expression pattern of each insert within the CNS depends on species and appears to be developmentally regulated. In chicken, mRNA encoding the 10-amino acid insert gradually increases after embryonic day 4, peaks at embryonic days

10–14, and then declines. The 21-amino acid insert is expressed just before birth and then remains high in the adult. In rats the myosin IIB containing the 21 amino acid insert is expressed throughout the brain, but has decreased levels in the cerebrum and is absent from the olfactory bulb (Takahashi *et al.*, 1999). During development it is first detectable in the cerebellum at P10 and increases from P14 to adult. In cerebrum it is not detected until P28.

MYOSIN V

Myosin Va shows alternative splicing that differs between neuronal and non-neuronal forms (Seperack *et al.*, 1995). An exon encoding additional sequence in the C-terminal tail domain is expressed in skin melanocytes and other non-neuronal tissues, but is absent from the neuronal form (Seperack *et al.*, 1995; Labert *et al.*, 1998).

MYOSIN VI

It is unknown if the neuronal form of myosin VI identified in chicken differs from the form expressed in the hair cells of the mouse ear (Hasson *et al.*, 1997).

MYOSIN IX

Myosin IXA shows alternative splicing that leads to a different isoform in adult brain. This isoform appears to contain a 40 amino acid insertion in the head sequence and a 19 amino acid insertion similar to that observed in myosin VI (Chiergatti *et al.*, 1998).

Which neuronal myosins are located in axons and what is their subcellular distribution?

All classes of myosin that are known to be present in neurons are found in neuronal axons to varying degrees. Myosins from different classes have distinct subcellular distributions, although in some cases the distribution overlaps. In most cases, it is not clear if individual members of each class are differentially distributed in separate neuronal compartments (cell body, axon, dendrites, spines, and terminals).

MYOSIN I

The localization of myosin I α has been studied in detail in primary cultured rat SCG neurons using both light- and EM-based immunolabeling techniques (Lewis & Bridgman, 1996). Immunofluorescence microscopy showed that myosin I α has a punctate distribution throughout the neuronal cell body, neurites, and growth cones. In growth cones, the staining was elevated in thin peripheral regions of high actin filament content. Immunoelectron microscopy using colloidal gold conjugated antibodies showed that in growth cones myosin I α was concentrated primarily in actin-

rich regions of the cell cortex adjacent to the cell membrane. By contrast, in neuronal cell bodies and proximal portions of neurites, myosin I α was associated mainly with tubulovesicular structures.

Myosin I β has been reported to be present in neurites and growth cone-like structures of primary-cultured rat brain neurons and PC12 cells using immunofluorescence staining (Wagner *et al.*, 1992). The most intense staining was at the actin rich periphery of the growth cone-like structures, similar to the myosin I α staining observed in rat SCG growth cones.

Myosin I γ (myr4) was detected by immunofluorescence staining of brain sections in cell bodies and apical dendrites of a subpopulation of CNS neurons (Bahler *et al.*, 1994). It is unclear if myosin I γ is absent from axons or simply expressed at lower levels and is therefore difficult to detect in brain sections.

MYOSIN II

In cultured SCG neurons both myosin IIA & B are present throughout the neuron, including the axon (Rochlin *et al.* 1995). Myosin IIB has a cortical location in the cell body and axon. Myosin IIA is also cortically located in cell bodies, but has a more even distribution through the depth of the neurite than myosin IIB. In growing neurons both myosin IIA & B are found in growth cones. They have overlapping but slightly different localization. Myosin IIB was most concentrated in the margin bordering the thickened, organelle-rich central region and the thin, actin-rich peripheral region. Immunofluorescence staining colocalized with actin bundles proximal and distal to the marginal zone. The trailing edge of growth cones and the distal portion of the neurite often had a rimmed appearance. A monoclonal antibody raised to myosin II from a neural blastoma cell line gave a similar staining pattern to myosin IIB in cultured DRG neurons (Miller *et al.*, 1992). In SCG neurons, myosin IIA showed an overlapping but slightly different distribution than IIB (Rochlin *et al.*, 1995). It lacked the elevation in the marginal zone that was characteristic of IIB staining. Myosin IIA staining was most intense in the organelle rich central region of the growth cone.

The distribution of myosin IIB in cultured hippocampal neurons is essentially the same as that observed in SCG neurons. In addition, dendritic growth cones also show staining for myosin IIB (Bridgman, unpublished).

MYOSIN V

Myosin Va is detectable in all brain regions tested by immunoblotting (Espindola *et al.*, 1992). Immunocytochemical techniques have been used to investigate the distribution of myosin Va in a number of different neuronal cell types, but has been most frequently studied in the cerebellum and peripheral nervous system. A systematic study of neuronal staining in

additional brain regions has not been reported. In the cerebellum, myosin Va protein is highest in Purkinje cells (Espindola *et al.*, 1992; Bridgman unpublished). The Purkinje cell dendritic tree stains very intensely. Additional components (granule cell axons) of the surrounding molecular layer also stain, but less intensely.

Myosin Va protein is highly expressed in peripheral nerves of the rat autonomic nervous system, including neurons from the SCG (Evans *et al.*, 1997) and the enteric ganglia (Drengk *et al.*, 2000). In cultured SCG neurons myosin Va immunofluorescence staining was concentrated in neurites and the organelle-rich regions of the growth cone (Evans *et al.*, 1997). A very similar distribution of stain has been observed in cultured chick DRG neurons (Suter *et al.*, 2000). Immunoelectron microscopy of rat SCG neurons revealed that the concentration in areas normally associated with an abundance of microtubules resulted mainly from the association of label with a distinct population of organelles (50–100 nm diameter) (Evans *et al.*, 1997). Subsequent fractionation studies on chick brain revealed that a similar population of organelles enriched in myosin Va could be isolated (Evans *et al.*, 1998). Consistent with other reports (Prekaris *et al.*, 1997), these vesicles were enriched in synaptic vesicle markers SV2 and synaptophysin. The vesicles could also support the movement of F-actin in an *in vitro* motility assay, indicating that myosin Va may contribute to their transport or docking in actin-rich regions of the cell.

Related to the above observations is the finding that granule cell presynaptic terminals in *dilute-lethal* mice are greatly enlarged and contain increased numbers of 90–100 nm vesicles. Immunoelectron microscopy of myosin Va at these synapses revealed that it was associated with both 90–100 nm vesicles and a subset of synaptic vesicles (Bridgman, 1999; Bridgman, unpublished).

In addition, information is available on the distribution of myosin V in cultured hippocampal neurons from mice. Myosin Va stains in a punctate pattern throughout the neuron, but is most intense at synaptic contacts (Bridgman, unpublished). Double staining of hippocampal neurons using antibodies to myosin Va and Vb revealed that most hippocampal neurons show equivalent levels of staining intensity for both myosin V isoforms. However, subsets of neurons show staining primarily for one or the other. Myosin Vb staining is less punctate than Va and is distributed throughout cell bodies, dendrites, and axons.

MYOSIN VI

The distribution of myosin VI has been examined by immunofluorescence staining of cultured chicken dorsal root ganglion neurons (Suter *et al.*, 2000). It is present in cell bodies, axons, and growth cones. Myosin VI shows a punctate distribution similar to myosin V. In growth

cones the labeling is most concentrated in the growth cone central domain. In double-labeling experiments for myosin V and VI, about 20% of the puncta colocalized.

MYOSIN IX

Myosin IXA immunofluorescence staining of brain sections revealed immunoreactivity primarily in cell bodies and dendrites of multiple neuronal cell types (Chieriegatti *et al.*, 1998). This included Purkinje cells and granule cells in the cerebellum, pyramidal cells in the cortex and neuronal cell bodies in the CA3 and dentate gyrus in the hippocampus. Myosin IXB showed a similar distribution, but was markedly decreased in the molecular layer of the cerebellum.

Immunofluorescence staining of cultured hippocampal neurons with an antibody to myosin IXA revealed that, in addition to cell bodies and dendrites, axons were also labeled (Chieriegatti *et al.*, 1998). Although the staining was cytoplasmic, it was slightly increased near the plasma membrane.

What do axonal myosins do?

Direct information on the function of the different classes of myosin in neurons is scarce. The available data, however, does suggest some reasonable possibilities.

MYOSIN I

Little data is available to address the function of myosin I in neurons. However, the location of myosin I α in growing axons may give insight into its function during development. In growth cones myosin I α is associated with the cortical actin meshwork adjacent to the cell membrane (Lewis & Bridgman, 1996). This means that it is likely to have at least a partial overlap in distribution with myosin IIB. Unlike myosin IIB, myosin I α does not form bipolar filaments and is therefore less likely to participate directly in cortical tension. However, it could provide a linkage between the membrane and the actin cytoskeleton, since its tail domain may bind phospholipids (Pollard *et al.*, 1991). In yeast, myosin I has recently been shown to play a role in actin polymerization (Lechler *et al.*, 2000). Although it is unknown if vertebrate myosins I play similar roles in locomoting cells, the location of myosin I α in the cortical actin of the growth cone peripheral domain would be consistent with such a role.

Myosin I α was also found to associate with tubulovesicular organelles in proximal portions of neurites (Lewis & Bridgman, 1996). More recently, microinjection of a fluorescently labeled antibody to myosin I α was found to produce bright spots in cultured SCG neurons (Bridgman, 1999). These spots moved bidirectionally in neurites at rates considerably slower than that

observed for myosin Va-labeled spots. Thus, myosin I α may associate with organelles in axons and could play a similar role to myosin Va in local transport of a different set of organelles. Alternatively myosin I α may be a passenger on these organelles, which may deliver this myosin to the plasma membrane in highly dynamic regions of the periphery. It remains unclear whether myosin I α is essential for neuronal function.

MYOSIN II

Like all myosins in the type II class, myosin II A & B are capable of forming bipolar filaments through protein-protein interactions within the rod domain (Matsumura *et al.*, 1985). Bipolar filaments are necessary for conventional contractile schemes in which the bipolar filaments pull against oppositely oriented actin filaments, similar to the arrangement in muscle cells. The myosin II puncta seen in immunofluorescence micrographs may represent small bipolar filaments similar to those seen in fibroblasts (Verkhovskiy *et al.*, 1995; Bridgman, unpublished). Thus, one possibility is that myosin II forms small bipolar filaments in certain cortical regions that act to stabilize the cell cortex through a contractile mechanism (perhaps by increasing cortical tension). Protrusion would be hindered in cortical regions containing bipolar filaments and would be free to occur in regions lacking the filaments. Consistent with this possibility is the normal localization of punctate myosin IIB in submembrane regions of the rear 1/3 of the cone and the lack of myosin IIB at the leading edge (Rochlin *et al.*, 1995). In growth cones, myosin IIB is more abundant and has a slightly more peripheral location than myosin IIA. In addition, myosin IIB is up-regulated during outgrowth of cultured cells whereas myosin IIA is not (Itoh & Adelstein, 1995; Wylie *et al.*, 1998). Therefore, it is assumed that myosin IIB plays the major role in generating tension in growth cones.

In neuronal growth cones, both lamellipodia and filopodia can exert tension on the axon (Heidemann *et al.*, 1990). Contraction of filopodia (or bundles within lamellipodia) may contribute to traction force by pulling the growth cone forward (Bray, 1982; Heidemann *et al.*, 1990). Alternatively, myosin driven retrograde flow of actin may produce traction force through an "escalator" mechanism and pull the growth cone forward (Heidemann *et al.*, 1990; Lin *et al.*, 1996). Actin bundles that course from the leading edge of growth cones to the marginal region primarily contain f-actin with barbed ends oriented toward the leading edge (Lewis & Bridgman, 1992). Myosin IIB or IIA organized as bipolar filaments in the central domain (Rochlin *et al.*, 1995) could interact with oppositely oriented filaments and pull the peripheral f-actin centripetally. If these bundles were linked to the substratum, then tension exerted on them would generate traction force. This would effectively pull the growth cone

forward and exert tension on the axon. Furthermore, myosin II bipolar filaments in the marginal zone of growth cones would pull against both the peripheral cytoskeleton and the proximal cytoskeleton, exerting tension on each, thereby pulling up weakly adherent connections and pulling the growth cone towards strongly adherent connections. A similar model has been proposed for fish keratocytes (Svitkina *et al.*, 1997).

Consistent with the possibility that myosin IIB plays a role in nerve growth cone motility is the observation that antisense suppression of myosin IIB in neuroblastoma cells causes decreased rates of outgrowth (Wylie *et al.*, 1998). Targeted gene disruption of the myosin heavy chain B in mice causes a number of developmental defects that include hydrocephalus (Tullio *et al.*, 1997). Characterization of neural specific defects in this knockout is underway. However, it is clear that the rate of nerve outgrowth and growth cone traction force in cells cultured from these mice is also reduced, consistent with the proposed role of myosin IIB in growth cone function (Tullio *et al.*, 2001).

MYOSIN V

A number of recent studies on pigment cells have provided support for myosin Va dependent movement of melanosomes (Wu *et al.*, 2000). Similar support for an organelle motor role for myosin V has also come from work on squid axoplasm (Tabb *et al.*, 1998). A more recent study addressed the role of myosin Va in axonal transport of organelles in vertebrate neurons (Bridgman, 1999). Live cultured SCG neurons microinjected with a fluorescent antibody to myosin Va or expressing a GFP-myosin Va tail construct showed bright fluorescent puncta in neurites. The bright spots, which were identified as myosin Va associated vesicles by photoconversion and electron microscopy, were observed to move bidirectionally in neurites. A substantial portion of these vesicles could be labeled with an antibody to the synaptic vesicle associate protein SV2. The rapid movements of myosin Va-associated vesicles were shown to be microtubule dependent. In contrast, slower movements that could be detected in absence of microtubules were shown to be myosin Va dependent. This was shown by comparing microtubule-independent movements in normal and myosin Va null cells. These results indicate that myosin Va appears to be mainly a passenger on organelles during long range axonal transport, but can potentially contribute to local transport in actin rich regions. Direct observation of particle movements along actin filaments in growth cones supports this possibility (Evans & Bridgman, 1995). Particles were observed to move along actin bundles in the actin rich periphery at rates consistent with myosin V activity.

The possibility that myosin V contributes to slow axonal transport has not been tested. While it is clear that a

substantial fraction of the myosin Va present in axons is not associated with organelles (Evans *et al.*, 1998), most neurons in mice that lack myosin Va expression grow and differentiate without detectable deficits in slowly transported materials.

The only myosin that clearly has an essential non-sensory nervous system specific function is myosin Va. *Dilute-lethal* (myosin Va-null) mice die at about P20 probably as a result of the inability to feed. Their abnormal behavior probably reflects a direct effect of myosin Va on neuronal function. Several pieces of evidence point towards defects in synaptic transmission and localization of endoplasmic reticulum in dendritic spines (Langford & Molyneaux, 1998). In *dilute-lethal* mice, Purkinje cell dendritic spines are devoid of endoplasmic reticulum (Takagishi *et al.*, 1996), which is necessary for the storage and release of calcium in response to local depolarization. Interestingly, mice deficient in the $\alpha 1(A)$ subunit of the P/Q-type voltage-dependent calcium channel exhibit neurological abnormalities similar to those seen in *dilute-lethal* mice (Mori *et al.*, 2000). This type of channel is found in both Purkinje and granule cells. Furthermore, mice with related mutations showed enlarged parallel fiber varicosities that contacted multiple Purkinje cell spines (Rhyu *et al.*, 1999). This is similar to defects observed in *dilute-lethal* mice where enlarged granule cell varicosities also contact multiple Purkinje cell spines (Bridgman, 1999). Thus, abnormal regulation of intracellular calcium levels may lead to multiple morphological changes. However, in the latter case the enlarged varicosities also contained increased numbers of 90–100 nm vesicles. This suggests that, in the absence of myosin Va, local transport may also be defective in these presynaptic terminals. It has also been proposed that myosin Va plays an essential role in growth cone filopodial extension (Wang *et al.*, 1996). It is difficult to confirm this possibility since *dilute-lethal* neurons show normal neuronal outgrowth, growth cone shapes, and filopodia numbers (Evans *et al.*, 1997).

MYOSIN VI

Myosin VI is the only myosin known to move towards the pointed end of an actin filament (Wells *et al.*, 1999). For this reason it is particularly interesting to determine if myosin VI is involved in organelle transport in neurons. Although immunolocalization data is consistent with such a possibility, no direct data on myosin VI function in neurons is available. However, it is interesting to note that the mouse mutation Snell's Waltzer, which may be a myosin VI null, only shows obvious defects in hearing and balance (Self *et al.*, 1999). Thus, if myosin VI is involved in organelle transport in neurons, it is either not essential for this function, or any deficits that arise are subtle.

MYOSIN IX

This class of myosin is unique because the tail domain contains a region that stimulates the GTPase-activity of Rho (Chieriegatti *et al.*, 1998). Overexpression of myosin IXA (myr 7) in HeLa cells leads to inactivation of Rho, fragmentation of actin filaments, and morphological changes. Cells transfected with myosin IXA lost their well-spread morphology, rounded up, and formed narrow processes, whereas cells transfected with a mutated form of myosin IXA with an inactive RhoGAP domain remained well spread. This data suggests that myosin IXA regulates Rho activity in neurons and contributes to the regulation of neuronal morphology.

Conclusions

It is increasingly clear that myosins can play important roles in neuronal development and function. Many of these roles may not be neuronal cell specific; however, many appear to be required for normal development and function. Although it is clear that myosins play a role in multiple neuronal compartments, we have concentrated this review on the role of neuronal myosins in axons and their terminals. The different axonal myosins studied to date have overlapping but slightly different locations, which may give insight into their functions. We summarize the localization and potential functions of these myosins below (Fig. 1), concentrating on the most thoroughly studied members of the different classes. What is striking from this diagram is the realization that the cytoplasm of neurons is very rich in myosins. Although the different classes may show peak expression levels at different developmental stages, the levels remain sufficiently high to expect multiple classes to be present in a single neuron as shown.

Both myosins $I\alpha$ and $I\beta$ are located in axons and growth cones (Fig. 1). Myosin $I\alpha$ associates with organelles in axons and with the cortical actin adjacent to the plasma membrane in the leading edge of growth cones. Myosin $I\beta$ has a similar location, although it is not clear if both isoforms are present in the same cells. In contrast, myosin IIB has a cortical location in axons and the rear of growth cones. It also associates with actin bundles in the growth cone marginal region and to a lesser extent with actin bundles at the base of filopodia. Myosin IIA has an overlapping distribution, but is more concentrated centrally. Myosin Va is primarily associated with membranous organelles in axons and growth cones. It is also located within presynaptic terminals, but is usually not found close to active zones. Myosin VI shares a similar distribution to that of myosin Va in growth cones, although less than a third of myosin VI puncta colocalize with myosin Va by immunofluorescence microscopy. It remains unclear whether myosin

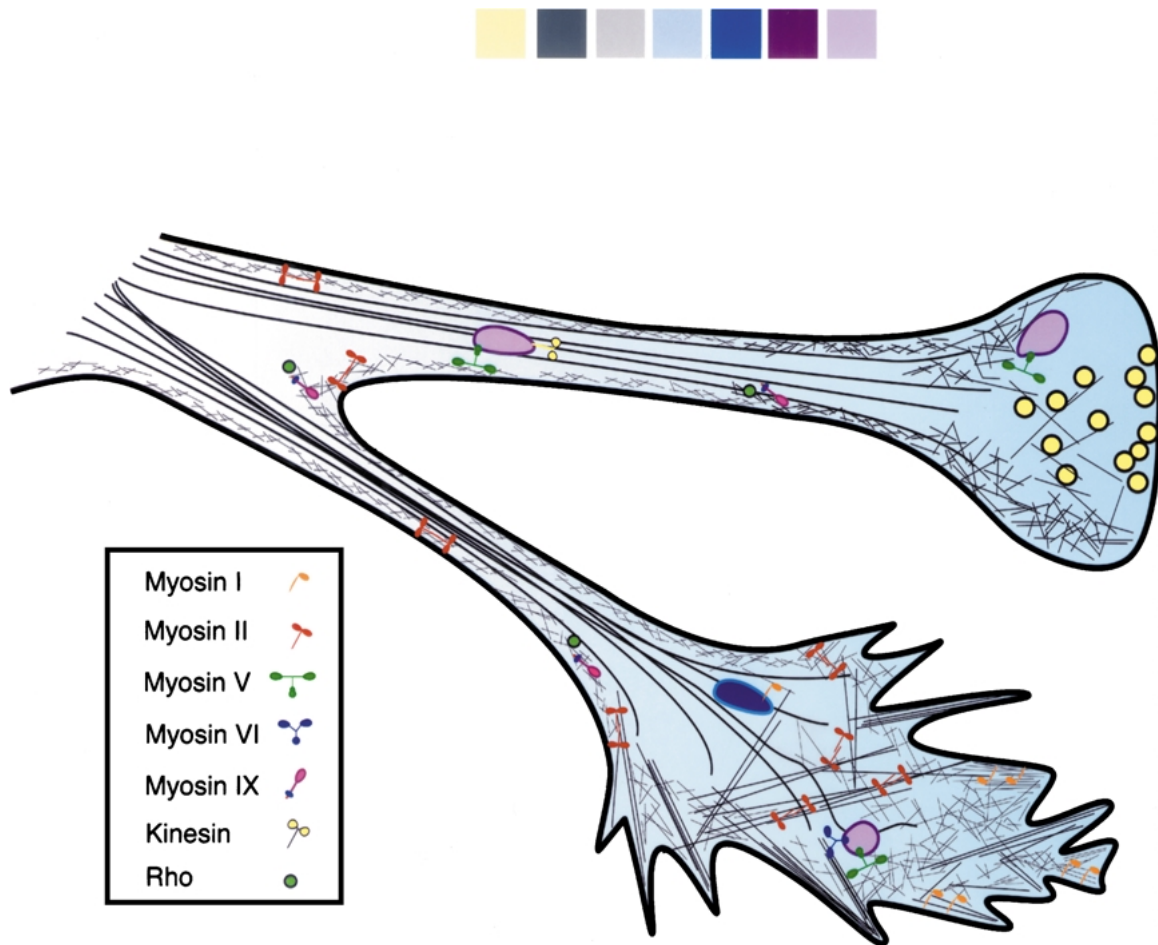


Fig. 1. A diagram summarizing the distribution of the different myosin classes in the axonal, growth cone and presynaptic compartments. Synaptic vesicles are depicted as yellow; other membranous vesicles are shown in magenta and blue. Actin filaments are depicted as fine short lines, or intermediate length lines, while microtubules are depicted as long curvilinear lines.

VI is associated with organelles because there are no published studies of myosin VI-immunoelectron microscopy in growth cones. Myosin IXA is distributed throughout the axon, but is increased cortically.

Although much remains to be learned about neuronal myosins, we can begin to draw a model of myosin function in these cells based on localization studies, analysis of mutations/knockouts, and studies of the dynamic properties of these myosins. Studies to date suggest that myosin function in neurons may be roughly divided into two categories: changes in cell shape and intracellular transport.

Contributions of myosins to neuronal cell shape may be apparent in the force producing activity of the growth cone during axonal outgrowth or in the more subtle changes associated with remodeling of synaptic contacts. The main player in shape changes is probably myosin II, although myosin I and IX may also contribute. Myosin II can form bipolar filaments that can produce a contraction when bound to actin filaments of opposite polarities. Contractile activity can contribute to cortical tension in cell bodies or neurites

(Fig. 1). This could have an important role in "focusing" the sites of new protrusion driven by actin polymerization. In addition, contraction could produce retrograde actin flow and traction force necessary for growth cone advance (Fig. 1). Myosin I α also has a cortical location in growth cones, but is more concentrated at the leading edge, suggesting that it may play a role in either the interaction between actin and the membrane or in actin polymerization (Fig. 1). Myosin IX may be able to regulate neuronal morphology through a combination of its motor domain activity and regulation of Rho. Rho has been shown to control actin organization (Ridley & Hall, 1992). Therefore, myosin IX could regulate branching or other protrusive-dependent changes.

Neuronal myosins also appear to play a role in intracellular transport. While myosins are unlikely to be responsible for fast axonal transport over long distances, they may play a role in modulating fast transport or could contribute to slow transport of certain constituents. Myosins may also contribute to local transport in actin-rich regions of the cell, such as in the periphery of growth cones or in presynaptic terminals.

Of the myosins known to be present in neurons, myosins I, V, and VI may contribute to local transport.

Clearly, a great deal of work has been done towards elucidating the function of myosins in neurons; however, many questions still remain. What are the actual functions of these proteins in neurons and how does this compare to their function in other cell types? What are the functional differences between different isoforms of each myosin class? What other myosin classes are present in neurons? How are these myosins distributed and what is their subcellular localization at the ultrastructural level? How can we adapt our current model of myosin function in neurons to more accurately reflect the distributions and functions of these proteins?

Although existing data has given hints as to the function of the myosins we have discussed here, additional studies must be performed to confirm our current hypotheses of the function of these myosins in neurons. More localization studies at the EM level, further analysis of myosin mutations/knockouts, and additional dynamic studies should help to provide a more complete model of myosin function in neurons.

Acknowledgments

This work was supported by NIH grants NS26150 and NS35162 (PCB). We thank Colleen Abel for her assistance with the illustration.

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