It is an exciting time to be studying myosins and their roles in the function of cells and organisms. Past efforts aimed at finding new members of this family have now given way to a focus on identifying individual functions for each motor protein. These actin-based motors are now known to be intimately involved in the following processes: neurosensory function; vesicle trafficking; determinant partitioning; and cortical function. The following article reviews the inroads made into the functions of myosins in these processes over the past several years.

Myosins play key roles in neurosensation

A number of interesting studies have revealed that myosins play critical roles in neurosensation (see Table 1). Cells involved in this process extend and maintain highly specialized structures: stereocilia (e.g. in the inner and outer hair cells of the ear); microvilli-like projections (e.g. in Drosophila melanogaster rhabdomeres); membrane stacks (e.g. in photoreceptor cells); and growth cones (in nerves). Many of these structures are rich in actin and localization of myosins to these sites, and analyses of myosin mutants, suggest that several different myosins are essential for the maintenance and/or function of these extensions.

<table>
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<tr>
<th>Class</th>
<th>Function</th>
<th>Reference</th>
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<tr>
<td>I</td>
<td>Control of membrane extensions</td>
<td>[35,36,37,40]</td>
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<tr>
<td></td>
<td>Adaption motor</td>
<td>[4**,7]</td>
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<td>II</td>
<td>Determinant localization</td>
<td>[34**]</td>
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<td>III</td>
<td>Maintenance of Drosophila rhabdomeres</td>
<td>[1]</td>
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<td>V</td>
<td>Vesicle trafficking</td>
<td>[19**,21**,22**,25,26]</td>
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<td></td>
<td>Vacuole inheritance</td>
<td>[27]</td>
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<td></td>
<td>Organelle localization</td>
<td>[24]</td>
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<td></td>
<td>Message localization</td>
<td>[31**,32**]</td>
</tr>
<tr>
<td>VI</td>
<td>Anchoring of stereocilia</td>
<td>[4**]</td>
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<tr>
<td></td>
<td>Particle movement</td>
<td>[1]</td>
</tr>
<tr>
<td>VII</td>
<td>Function or maintenance of specialized actin-rich extensions</td>
<td>[4**,51,52]</td>
</tr>
<tr>
<td>IX</td>
<td>Unknown - could link intracellular signaling to actin cytoskeleton via GAP domain</td>
<td>[43**,44**]</td>
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Two different myosins, VI and VII, play essential roles in hearing and balance [1]. The inner and outer hair cells of the cochlea extend highly organized bundles of actin, the stereocilia, which are anchored to an actin-rich cuticular plate. Myosin VI in the inner and outer hair cells is localized to both this cuticular plate and a newly discovered actin-filled structure, the pericuticular necklace [3,4**]. Mice lacking myosin VI (snell's waltzer) exhibit cochlear degeneration and vestibular dysfunction [3]. Myosin VI might anchor the stereocilia in the cuticular plate or contribute to maintaining its rigidity. Myosin VIIa expressed in the inner and outer hair cells is found in the cuticular plate, the pericuticular necklace, and the stereocilia themselves [4**]. Myosin VIIa is also strikingly concentrated near the basal tapers of frog stereocilia where...
there are intracellular links between adjacent stereocilia [4°°]. Mutations in human VIIa result in hearing loss alone (as in the case of the human nonsyndromic deafness, DFNB2), or in combination with retinitis pigmentosa (Usher's syndrome) [5,6], while mutations in the mouse VIIa gene (shaker-1) only result in hearing loss [1].

Hearing adaptation is thought to occur via myosin-dependent closure of stretch-activated calcium channels located on stereocilia and evidence has accumulated that implicates a myosin as the tip-link motor responsible for adaptation [7-9]. Myosin Iβ is currently the best candidate for such an adaptation motor, based on its concentration at the tips of stereocilia [4°°,7]. Elegant biochemical studies using isolated hair bundles, in combination with electrophysiological measurements, support this hypothesis [7,10]. In addition, a comprehensive mapping study of human myosin genes has revealed that the gene encoding myosin Iβ, MYOIC, maps near a recessive nonsyndromic deafness disorder [11]. Myosin Iβ is also present in the pericuticular necklace of the inner and outer hair cells [4°°,7], suggesting that it might be responsible for functions other than hearing adaptation.

The retinitis pigmentosa observed in Usher's patients, who lack functional myosin VIIa, is due to degeneration of photoreceptor cells which is most probably caused by myosin VIIa deficiency [1]. Myosin VIIa is found in the outer and inner segments of photoreceptor cells [4°°,12] and in the connecting cilia joining these segments [6]. It is also present in the synaptic endings of photoreceptor cells [12] and in the adjacent retinal pigmented epithelia (RPE), where it is localized to the microvillar projections [6,13]. It has been suggested that myosin VIIA functions in the RPE to phagocytose shed membrane from the photoreceptor stacks [13] or in the photoreceptor cells to deliver membrane to the outer segments [6]. The degeneration of photoreceptor cells in Usher's patients also suggests a role for myosin VIIa in the maintenance of these specialized structures.

Analysis of a series of myosin VIIa alleles reveals that most mutations occur in the motor domain. A small number of mutations, however, have been found in either the light chain binding domain or the carboxy-terminal tail region [14]. The three identified tail mutations are predicted to result in full or partial truncation of the second talin homology domain [14], a region of the myosin tail that may play a role in anchoring it to membranes. It is possible that this truncated form of myosin VIIa is unstable or fails to localize properly. Continued cataloging of the myosin VIIa mutations in the context of what is known about myosin tertiary and quaternary structure should aid in mapping residues essential for force generation and myosin function. A combined analysis of mRNA, protein levels and morphological characterization of affected tissue has been initiated in shaker-1 mutants [15] and should help to further define the role of myosin VIIa in the maintenance and development of hair cells in the inner ear.

Like myosin VIIa, myosin V is also expressed in both ears and the eye. The distribution of myosin V, however, is quite distinct from that of myosins Iβ, VI and VIIa [4°°]. Myosin V in the ear is expressed in supporting cells, synaptic terminals at the base of inner hair cells in the ear, and af f e rent nerve fibers [4°°]. Myosin V in the eye is localized to the inner nuclear layer and to the synapses of rod photoreceptor cells [16], where it is most highly concentrated in the postsynaptic cell. The role of myosin V in these cells is currently unknown, but it is likely to be involved in synaptic vesicle transport in these cells (see below).

Myosins as vesicle/particle motors
Localization and biochemical studies have suggested that myosins are involved in the movement of vesicles and other particles through cell cytoplasm. Currently, myosin V is the strongest candidate for a particle transport motor.

Pigmentation defects in dilute mice — which possess a mutation in the myosin V gene— suggest that myosin V participates in the intracellular distribution of melanosomes [17]. Although earlier studies suggested the defect in dilute melanocytes is due to altered cell morphology, these cells have recently been shown to exhibit normal dendritic morphology both in vitro and in situ; however, the melanosome distribution in the melanocytes is abnormal [18°°,19°°,20°]. The intracellular distribution of myosin V is largely coincident with that of melanosomes, which are associated with both actin and microtubules, and myosin V is enriched in partially purified melanosome fractions [19°°,21°°,22°°]. The current working hypothesis is that melanosomes are normally transported from the center of the cell body via microtubule-dependent motors, and are then distributed (and possibly anchored to the cortical actin cytoskeleton) by myosin V (Figure 1). It should be noted that myosin V is not only associated with melanosomes, but is also associated with other intracellular organelles and enriched at the actin-rich periphery of cells [19°°,22°°].

The distribution of myosin V in neurons is also consistent with it participating in the intracellular transport of vesicles. It is colocalized with both microtubules and actin, and enriched in growth cones. Myosin V is associated with synaptic vesicles, possibly in a complex with synaptobrevin and synaptophysin [23°°], and as such may be involved in the transport of synaptic vesicles. Myosin V may also play a role in the intracellular distribution of membranous organelles in neurons. Examination of brains from dilute mice reveals that the smooth endoplasmic reticulum in the dendritic spines of Purkinje cells is missing [24°]. It is possible that mispositioning of the smooth ER in the dendritic spines, due to myosin V deficiency, could contribute to the observed neurological defects in these mice.
Speculative illustration of melanosome transport in mouse melanocytes. Melanosomes start as undifferentiated membrane-bound organelles near the nucleus (clear circles). They gain pigment as they mature to stage IV melanosomes (dark circles). The movement of melanosomes from the cell interior to the periphery appears to be dependent on both microtubules and actin. Myosin V is carried along with melanosomes as they begin their travel on microtubules in the cell interior. As the mature melanosomes reach the transition zone near the dendritic spines of melanocytes, they switch to actin, and are then translocated to the periphery by Myosin V. Myosin V is also localized to tubulovesicular organelles and concentrated at the cell membrane.

Analysis of myosin V function in *Saccharomyces cerevisiae* is consistent with this myosin acting as a transport motor. Previous work had established that *myo2* mutants (which lack Myo2p, a yeast class V myosin) accumulated vesicles within the cytoplasm and failed to bud [25,26]. *myo2* mutants are synthetically lethal with a group of late-acting secretory mutants (responsible for post-Golgi vesicular transport to the plasma membrane) indicating that Myo2p may play a role in the later steps of secretion [26]. Myo2p is also required for transporting the yeast vacuole into the daughter cell prior to division [27*] and for delivering chitin synthase 3 (Chs3p) to the growing bud [28*]. Significantly, the yeast myosin V mutant exhibits a disorganized actin cytoskeleton which could be due to a general effect of the mutation on actin organization.

**Myosins play roles in determinant localization**

Directed localization or positioning of messages or determinants is of vital importance to any cell. In multicellular organisms, development cannot occur without the specification of polarity. Recently, several groups have presented evidence to suggest that an important function of certain myosins may be determinant localisation/positioning.

Mating type switching in budding yeast takes place in the mother cell immediately after budding, in order to avoid the mother cell mating with its daughter. Switching in the daughter cell is prevented by the specific suppression of HO endonuclease (an enzyme necessary for mating type switching) by Ash1p. The *myo4* mutant—deficient in another yeast type V myosin—is
unable to suppress switching due to the failure of Ashlp
to become specifically restricted to the daughter cell
[29°,30]. Interestingly, since this spatial restriction occurs
by controlling the transport of the Ashlp mRNA into the
growing bud [31°,32°], these results raise the intriguing
possibility that myosin V plays a role in either the directed
transport or anchoring of mRNAs required for cell fate
determination.

The development of the Caenorhabditis elegans embryo
consists of a series of asymmetric divisions which specifi-
cally segregate developmental determinants such as the
p-granules that mark the germline progenitor cells. The
par (partitioning defect) genes were identified in a
genic screen to identify genes involved in the early rounds of asymmetric division [33]. One of these, par-1,
was used as a probe in interaction cloning experiments
using a Caenorhabditis elegans cDNA expression library.
The major interacting protein identified was NMY-2,
a non-muscle myosin II [34°°]. Studies using antisense
nmy-2 mRNA revealed that depletion of this myosin
reproduced the par phenotype. Cytokinesis proceeded
normally, but the orientation of the spindle, and thus the
cleavage plane, were altered such that the cells divided
symmetrically—which led to early death of the embryo
[34°°]. While myosin II has traditionally been thought
to play roles in whole cell contractile activities (such as
in driving the contraction of the cleavage furrow),
this study suggests a novel role for myosin II. NMY-2
might play a role in transportation of cleavage plane
determinants to one end of the embryo, or be responsible
for local organization of the actin cortex in a manner
that allows determinants to remain anchored in the appropriate
location.

**Myosin contributes to cortical function**

Myosins play important roles in driving activities carried
out by the actin-rich cell cortex. The localization of
the class I myosins to the actin-rich cortex of the cell
suggests that these myosins participate in the extension
or retraction of actin-rich protrusions. Deletion of a single
myosin I gene from Dicyostelium discoideum results in
defects in cell migration and pseudopod formation [1].
Because they produce excess pseudopodia, these mutants
turn too frequently and this results in a concomitant
decrease in instantaneous velocity [35•]. Support for a fun-
damental role for class I myosins is found in neuron mutant
experiments. Deletion of a single myosin gene from
neuronal growth cones results in increased lamellipodial expansion
and an increased tendency of that region of the growth
cone to turn [36•]. Thus, the class I myosins are likely to
control the placement and activity of extensions required
for efficient translocation.

Deletion of multiple class I myosins from Dicyostelium
does not result in any additional effects on motility, but
does have an unexpected effect on pinocytosis [37,38].

Pinocytosis in Dicyostelium occurs through the extension
and retraction of actin-filled 'crowns' on the cell surface
[39]. Single mutants display normal rates of pinocytosis,
but myosin I double and triple mutant cells exhibit a
significant decrease in fluid uptake [37,38]. The pinocytic
defect is not due to a failure to form the pinocytic crowns
[37], so it is likely to be a consequence of a failure to
retract them. Cells in which myosin I is overexpressed fail
to make crowns and have a more severe pinocytic defect
[40]. The morphology of the overexpressing cells suggests
that the cortex is hypercontracted, consistent with a role
for myosin I in the retraction of pinocytic crowns.

Interestingly, Saccharomyces cerevisiae myosin I double
mutants exhibit an endocytic defect similar to that observed in the Dicyostelium mutants [41,42]; however,
the yeast mutants differ from those in Dicyostelium, in
that the former exhibit an apparent disorganization of
the actin cytoskeleton [42]. Other defects typical of actin
disorganization are exhibited by the yeast myosin I double
mutants. These include defects in bud site selection and
osmotic sensitivity and decreased receptor-mediated
endocytosis [41,42]. These results suggest that the class
I myosins play fundamental roles in organizing the actin
cytoskeleton in diverse organisms.

**Rising stars of the myosin superfamily**

Several novel members of the myosin family have
been identified recently, and analyses of these new
classes promise to uncover interesting functions for this
superfamily of motor proteins. Analyses of these new
classes promise to uncover interesting functions for this
superfamily of motor proteins. First and foremost is myosin
IX, which has a novel insertion near the position where
myosin is known to contact actin [1]. This could alter
the way the motor interacts with the actin cytoskeleton,
possibly by increasing its affinity for actin and allowing
it to act as a processive motor, i.e. it would not diffuse
away from actin during translocation. Recent motility
experiments have revealed that the myosin IX motor
is capable of driving ATP-dependent actin movement
in vitro, albeit at a slow rate (14 nm/s, in the presence
of calcium) [43•]. The tail of this myosin contains two
intriguing domains, a GTPase activating (GAP) domain
followed by a zinc-finger motif thought to provide a
protein interaction site. The GAP domain has been found
to function as a potent activator of the GTPase activities
of the RHO family of G-proteins [43•,44•].

Other novel myosins have been identified in a range
of interesting organisms. The Caenorhabditis elegans motor
protein HUM-4 is among the most highly divergent of
the myosins. It has a large amino-terminal extension as
well as several non-conserved insertions in the head. The
tail contains MyTH-4 and talin-like domains similar to
those found in class VII and X myosins [45]. The talin-like
domain may serve as a membrane binding- or protein
interaction site [46,47]. The function of the MyTH-4
domain is unknown but its conservation between highly divergent myosins suggests it has an important role in the function of these motors [46,47]. The Toxoplasma gondii and Plasmodium falciparum (accession number Y09693) myosins together define a new class of myosins, class XIV [48]. The Toxoplasma myosins have a conserved head followed by a small tail domain which is highly basic. Similar polybasic domains in class I myosins are known membrane binding domains [1].

A potentially interesting myosin, CsmA, has been identified in Emericella nidulans. The csmA gene was originally identified in a screen for chitin synthases. Analysis of the full sequence of this new synthase gene revealed that it encoded an active chitin synthase with a conserved myosin head domain at its amino terminus [49]. Phylogenetic analysis reveals that CsmA is one of the most highly divergent myosins identified to date, being more divergent than either myosin III or myosin XII from all the other classes (JP Baker, MA Titus, unpublished data). The chitin synthase region of the csmA gene is identical to the previously identified chsD gene, which is required for cell wall integrity [50]. It is likely that these two genes are the same and that the region encompassing the myosin head domain was not recognized in the original paper. The precise functions of these novel myosin motors are currently unknown; however, the advantage gained by using organisms in which genetic and molecular approaches can be readily combined is likely to mean that they will soon be added to the growing library of known myosin functions.

**Conclusions**

Clearly, myosins are required for many different functions within a cell or organism; however, two general trends do emerge from studies of this superfamily of actin-based motors. The first is that myosins play important roles in the movements of membranes or membrane-bound organelles. Only a few functions described above do not involve the cell membrane or internal vesicles/organelles at some level. The second, even more interesting trend, is the role of these motors in the activity of cellular extensions. Myosins have been found in abundance in almost every type of extension/projection examined, from pseudopods to microvilli and stereocilia. The production, maintenance or function of these extensions appears to require one or more myosins. In many cases the motors are so important that removal of their function leads to the progression of major disorders in neurosensory and neurological function. We are left with the burning question—what are they doing? At present the question remains unanswered but it is a safe bet this will not be the case for long!

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


A comprehensive review bringing together the structural data available for the myosin II motor domain with a comparative analysis of the motor domain sequences for all known classes of myosins. The authors highlight functionally important conserved sequences.


A beautiful study on the expression and localization of myosins I, V, VI, and VII in the ear. The authors correlate the distribution of each myosin with different actin-rich structures in the ear of several different vertebrates and draw conclusions regarding the specialized function of each type of myosin. A ‘must read’ for anyone interested in myosins!


Early investigators postulated that the mouse dilute phenotype was due to the improper outgrowth of dendritic melanocyte spines. The authors of this paper isolated dilute melanocytes and showed that they were still dendritic, but that the melanosomes failed to migrate into the arbor.


Immunofluorescent and immunoelectron microscopy were utilized to explore the in vivo association of myosin V with melanosomes. The results of this paper point to both actin- and microtubule-dependent motility of these organelles. The acto-myosin driven segment appears to be in the dendritic endings alone.


These authors also explore the morphology of mouse dilute melanocytes and demonstrate that these cells are still dendritic, but the melanosomes fail to migrate into the arbor both in vitro and in situ.


A beautiful study of myosin V localization in superior cervical ganglion neurons. Myosin V is enriched in organelle-rich zones of the extended growth cone and associated with a distinct subset of small organelles which are themselves associated with both actin and microtubules. The authors also explore the hypothesis that structural abnormalities in the mouse dilute neurons could account for neurological dysfunction. The morphology of the dilute-lethal neurons appears to be normal. These results are somewhat in conflict with earlier analyses that suggested that myosin V was required for neuronal extension; however, they might be reconciled with earlier studies if a different myosin V compensate for the loss of the dilute protein during neuronal growth and development.


A study examining the association of myosin V with organelles in the cultured melanocyte. Immunolocalization results demonstrate that myosin V colocalizes with melanosomes as well as other membrane-bound organelles. Subcellular fractionation experiments reveal that myosin V colocalizes with melanosomes. Treatment of cells with serotonin to stimulate melanogenesis results in increased levels of myosin V present in the cell.


The authors present biochemical data demonstrating a functional association of myosin V with synaptic vesicles. They also show that myosin V is associated with the syntaxobrevin/synaptophysin complex. They suggest that myosin V may play an important role in the transport of syntaxobrevin/synaptophysin-containing vesicles in nerve terminals.


The original description of the neurological disorders in dilute mice is unknown. The brains appear normal at the gross level. These authors carried out a morphological analysis of the brains of dilute-lethal mice and found that the smooth endoplasmic reticulum of dilute mice was incorrectly localized and did not extend into the dendritic spines of Purkinje cells. The mislocalization of the smooth ER may contribute to neurological dysfunction in these mice.


Myosin V is thought to act in trafficking of membrane-bound organelles. Further support for this hypothesis is provided by this analysis of the inheritance of the yeast vacuole. The yeast myo2 mutants were shown to fail to efficiently move the vacuole into their daughter cells. The authors find that Myo2 is localized to the vacuole and the tip of the growing bud (where the vacuole is anchored in wild type cells).


Yet another interesting function for myosin V in yeast. This time for the first myosin V identified, myo2. This paper presents data showing that myosin V mutants fail to properly localize the chitin synthase to the growing bud. This chitin synthase is crucial for determining the position of the daughter bud cell.


The authors devised a clever screen to identify genes required for the suppression of mating type switching in the yeast daughter cell. They identified several genes essential for this process, one of which encodes the second yeast myosin V, Myo4p.


There is a more interesting twist to the Myo4p story than originally thought. myo4 mutants fail to properly localize the Ash1 mRNA to the tip of the growing bud. A similar effect is observed upon irradiation of the actin cytoskeleton.


These authors also show that myo4 mutants fail to properly localize the Ash1 message to the daughter cell. This suggests that myosin V may not simply act as an organelle motor but may participate in the localization of mRNAs as well.


A surprising result from this lab showed a non-muscle myosin II to be involved in correct asymmetric partitioning in Caenorhabditis elegans. It was found that this protein is co-precipitated with another protein involved in this process, Par-1.


The original data on myosin I in Dictyostelium showed that deletion of these genes reduced the instantaneous velocity of these cells. This paper shows that this is probably due to excess turning which is the result of too many pseudopods being produced adjacent to the substratum. This points to a role for this class in suppression of membrane extensions.


The first demonstration that myosin IX is a functional actin-based motor. These authors enrich for myosin IX by immunoaffinity precipitation of the native protein from tissue culture cells. They show that the entire protein has the same GAP activity as the expressed version. Even more importantly, they show that this motor is capable of moving along actin filaments.


This paper presents the result of functional tests on the GAP domain in the myosin IX tail. They express the GAP domain alone and show that it specifically activates the GTPase activity of the RHO subfamily. Overexpression of this domain in either insect tissue or cultured rat kidney cells alters the actin distribution.


