MYOSIN VI: Cellular Functions and Motor Properties

Folma Buss,[1] Giulietta Spudich,[2] and John Kendrick-Jones[2]

[1]Cambridge Institute for Medical Research, University of Cambridge, Cambridge CB2 2XY, United Kingdom; email: fb1@mole.bio.cam.ac.uk
[2]MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, United Kingdom; email: jkj@mrc-lmb.cam.ac.uk, gspudich@mrc-lmb.cam.ac.uk

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■ **Abstract** Myosin motor proteins use the energy derived from ATP hydrolysis to move cargo along actin tracks. Myosin VI, unlike almost all other myosins, moves toward the minus end of actin filaments and functions in a variety of intracellular processes such as vesicular membrane traffic, cell migration, and mitosis. These diverse roles of myosin VI are mediated by interaction with a number of different binding partners present in multi-protein complexes. Myosin VI can work in vitro as a processive dimeric motor and as a nonprocessive monomeric motor, each with a large working stroke. The possibility that both monomeric and dimeric forms of myosin VI operate in the cell may represent an important regulatory mechanism for controlling the multiple steps in transport pathways where nonprocessive and processive motors are required.

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INTRODUCTION

The Myosin Superfamily

Because movement is an essential part of life, eukaryotic cells have developed specialized methods of transport and motility in order to function as efficiently as possible. In membrane traffic pathways, for example, vesicles are rapidly moved between different locations within the cell using a variety of motor proteins moving along a network of actin filaments and microtubules. For the uptake and transport of molecules into the cell, cargo is first moved along actin filaments through the cortical actin network underneath the plasma membrane; long distance movement is then mediated along microtubules in order for the cargo to reach its correct intracellular location (Langford 1995, Brown 1999) (Figure 1). Short-range delivery around internal organelles such as the Golgi complex is also believed to involve transport along actin filaments. The two families of motor proteins that move along microtubules are known as kinesins and dyneins, whereas actin-based movement within the cell is the responsibility of the myosin superfamily. Thus for intracellular transport, two filamentous tracks (actin and microtubules) have evolved together with a multitude of motor proteins, each designed to carry specific cargoes under precise regulatory control.

Myosins are defined as actin-activated Mg^{2+} ATPases that use the energy derived from ATP hydrolysis to move along actin filaments. They all have a well-conserved, tripartite domain organization consisting of an N-terminal motor domain that binds actin and has ATPase activity, a neck region containing from one to six IQ motifs (with the consensus sequence IQXXXRGXXXR) that binds light chains or calmodulin, and a highly variable C-terminal tail domain essential for cargo binding. Myosins can be grouped into 18 distinct classes according to variations in the amino acid sequences of their motor domains (Hodge & Cope 2000, Berg et al. 2001). Comparing the sequences of their accompanying tail domains gives an identical classification. This indicates that the motor and tail domains have evolved together, so that the motor domain would have specific biochemical and kinetic properties when linked to different tail domains in order to perform distinct functions in the cell.

The completion of various genome-sequencing projects has allowed researchers to catalogue the myosins present in a number of different organisms. Humans, for example, possess 40 different myosin genes representing 12 different classes (Berg et al. 2001), whereas the nematode (*Caenorhabditis elegans*) has 17 myosin genes from 7 classes (Baker & Titus 1997), the fly (*Drosophila melanogaster*) appears to contain 13 myosin genes belonging to 8 classes (Tzolovsky et al. 2002, Yamashita et al. 2000), Dictyostelium (*Dictyostelium discoideum*) has 12 myosins grouped into 4 classes (Soldati et al. 1999), and yeast (*Saccharomyces cerevisiae*) contains
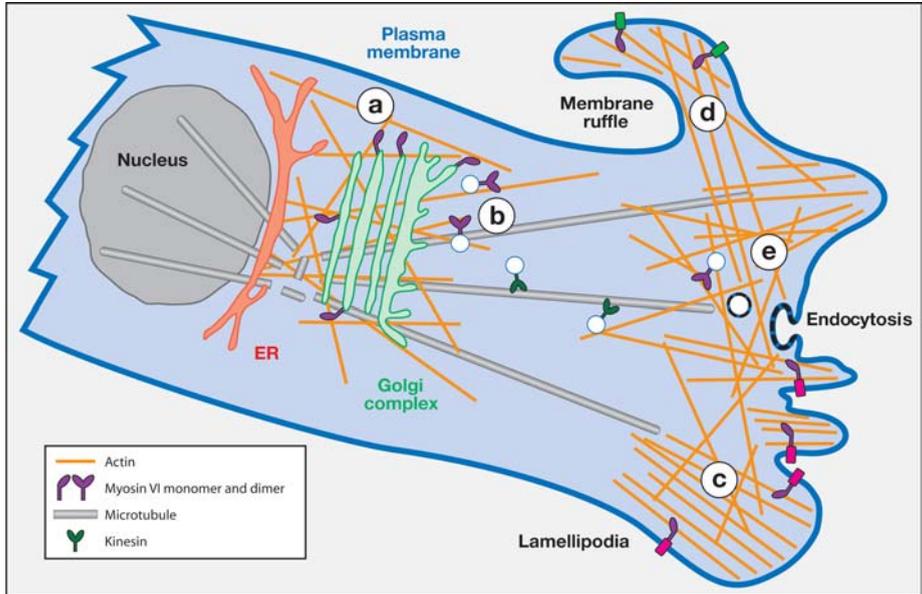
Figure 1   A cartoon illustrating some of the possible functions for myosin VI in a polarized fibroblast. (*a*) In the center of the cell, myosin VI may be involved in the steady-state organization and maintenance of the Golgi complex that links Golgi membranes to the actin cytoskeleton. (*b*) In post-Golgi membrane traffic at the *trans*-Golgi network (TGN), myosin VI might play a role in cargo sorting, vesicle formation, or the short-range transport of vesicles away from the Golgi complex (Warner et al. 2003). (*c*) At the plasma membrane, by analogy to border cell migration in *Drosophila* (Geisbrecht & Montell 2002), myosin VI could generate a protrusive force by pushing actin filaments outward, while binding to a cell adhesion complex and at the same time moving itself into the cell toward the minus end of actin filaments. (*d*) At the leading edge, myosin VI is also present in membrane ruffles; however, its binding partner(s) or function(s) has not yet been established (Buss et al. 1998). (*e*) During endocytosis in these cells, myosin VI associates with endocytic vesicles that have lost their clathrin coat and transports them through the cortical actin network (Aschenbrenner et al. 2003).

5 genes encoding myosins from 3 classes (Brown 1997). With just 3 classes of myosin (1 myosin II, 2 myosin Is, and 2 myosin Vs), yeast may contain the minimal number of myosin classes required for survival of eukaryotes. As more complete genome sequences are finished, will radically new unexpected myosins be identified?

The first crystal structure of a myosin II motor domain was solved 11 years ago by Rayment (Rayment et al. 1993), and since then other motor domain structures containing different nucleotide analogues mimicking specific steps in the ATPase cycle have been reported (Fisher et al. 1995, Smith & Rayment 1996, Houdusse et al. 1999). On the basis of these structures a model known as the lever arm

mechanism was proposed to explain how a myosin might generate movement (Holmes 1997). It was postulated that upon ATP hydrolysis small conformational changes generated in the motor domain are amplified by a converter region (found between the motor domain and neck domain), which causes this converter and the neck domain (the so-called lever arm) to swing, thereby resulting in movement. This basic mechanism is believed to operate in myosin II and V (Geeves & Holmes 1999), but in other classes of myosin, such as I and VI, additional features have been incorporated.

Myosin tail domains are poorly conserved between the different classes. Whereas class II myosins contain helical tails able to assemble and function as filaments, less is known about the tail domains of the other myosin classes. Most are believed to operate as single molecules rather than as filaments and to bind specific cargoes. The tail domains are thought to specify distinct functions by providing an anchor for motor activity and/or by targeting the myosin to different intracellular compartments and structural complexes by interacting with specific binding partners.

Therefore, myosins appear to have a variety of different roles in the cell and vary widely in their kinetic properties and regulatory mechanisms. Apart from myosin II and V (Vale 2003, Reck-Peterson et al. 2000), the precise properties and functions of the other myosins are poorly understood. In this review we focus on one of these classes of myosins, namely myosin VI.

Myosin VI

Myosin VI was originally discovered in *Drosophila* and identified as the first member of a new class of myosins, class VI (Kellerman & Miller 1992). Sequence analysis revealed that myosin VI follows the basic myosin organization with a N-terminal motor domain, a short neck region containing one IQ motif, and a C-terminal tail domain (Figure 2) (Hasson & Mooseker 1994, Kellerman & Miller 1992). In the motor domain (in the upper 50-kDa domain) there is a unique 22 amino acid insert (Figure 2*b*, insert a) whose function is not yet known, and between the motor domain and IQ motif there is another unique 53 amino acid insert (Figure 2*b*, insert b) predicted to be the reverse gear (Wells et al. 1999). Initial sequence analysis of the helical region in the tail (Figure 2) suggested that it forms a double-helical coiled coil, and therefore myosin VI was thought to be a dimer (Hasson & Mooseker 1994, Kellerman & Miller 1992). However, more detailed analysis (Lister et al. 2004) using the COILS prediction program indicates that although the first segment of the helical region (Figure 2*b*, segment 1) has a strong heptad repeat, it is interrupted by two breaks where the helical propensity breaks down (prolines are present in some myosin VI sequences) and thus loops may occur. The central segment (Figure 2*b*, segment 2, charged region) contains a repeating pattern of alternating four positive and four negative amino acids that would favor the formation of a single, intramolecular salt-bridge stabilized helix rather than a double-helical coiled coil. The third segment of the tail has a weak coiled coil prediction (Figure 2*b*, segment 3). Detailed structural analysis
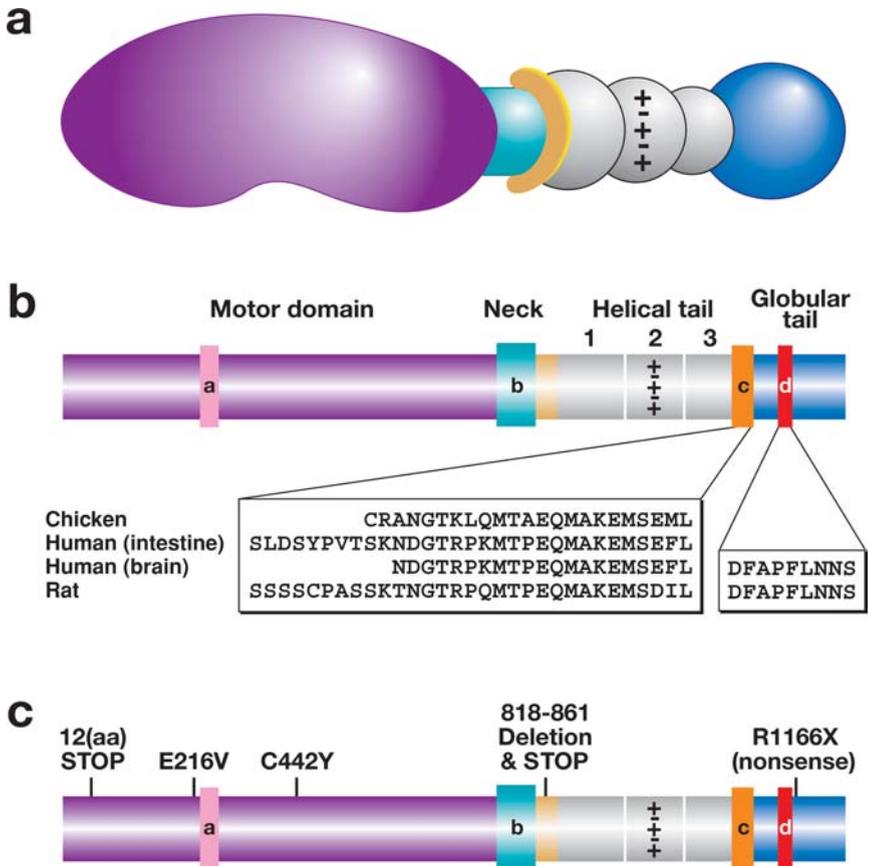
Figure 2 Cartoon illustrating (*a*) domain organization of myosin VI with the motor domain, neck region with single calmodulin, helical tail and globular tail. (*b*) Position of inserts (a–d) unique to myosin VI and the subdomains in the helical tail: (a) 22 amino acid in the motor domain (upper 50-kDa domain); (b) 53 aa insert in the neck region (reverse gear); (c) and (d) large and small splice inserts in the tail domain giving rise to four different isoforms of myosin VI. Comparing the sequence of these tail inserts shows high degree of homology between species. Subdomains of the helical tail are labeled as follows: (*1*) strong heptad repeats but with breaks, (*2*) charged region, (*3*) region with a weak heptad repeat prediction. (*c*) Position of mutations in the myosin VI gene that cause deafness in humans and mice.

[electron microscopy (EM) and X-ray crystallography] is thus urgently needed to establish the structure of this tail domain. The C-terminal globular domain has no obvious identifiable structural or functional motifs but does contain the sites with which myosin VI interacts with its binding partners (G. Spudich, unpublished observation).

Myosin VI can exist in a number of different isoforms, which represent splice variants with inserts in the tail. The number of isoforms differ between different organisms, and they are expressed in a tissue-specific manner. In *Drosophila*, three splice isoforms from a single gene have been identified (Kellerman & Miller 1992), whereas in fish, two isoforms possibly derived from two distinct genes have been described (Breckler et al. 2000). In mammalian cells, four myosin VI isoforms (Figure 2) from one gene are expressed (Buss et al. 2001a): one with a large (∼23/31 aa) insert in between the helical and the globular tail regions, one with a small (∼9 aa) insert within the globular domain, one with both inserts, and one with no insert. It is interesting to note that in humans two different variations of the large insert are expressed, a longer 31 aa insert in gut and a shorter 21 aa insert in brain (Figure 2*b*). The tissue distribution of these isoforms shows that the isoform with the 31 aa large insert is expressed mainly in tissues with polarized epithelial cells containing apical microvilli, such as liver, kidney, and small intestine, whereas the isoforms with the small or no insert are expressed in unpolarized tissue culture cell lines and in tissues such as testis and lung (Buss et al. 2001a). Myosin VI containing the 31 aa large insert appears to be involved in clathrin-mediated endocytosis in polarized epithelial cells (discussed below).

Myosin VI has another important fundamental property; unlike all the other myosins so far tested, it moves toward the minus end of actin filaments (Wells et al. 1999) and must therefore have unique cellular functions and properties. Although the polarity of actin filaments in cells is not well established, it is believed that actin filaments have their plus ends toward the plasma membrane (Cramer 1999) and toward the surface of internal organelles such as the phagosome (Defacque et al. 2000) or the Golgi complex. Therefore, as a minus-end-directed motor, myosin VI would move away from the plasma membrane into the cell and away from the surface of internal organelles such as the Golgi complex.

Myosin VI is found in higher, multicellular eukaryotic organisms and is ubiquitously expressed (Sellers 2000). It has been implicated in hearing (Avraham et al. 1995), in the development and maintenance of the stereocilia in the inner ear in mice (Self et al. 1999), in spermatogenesis in *C. elegans* (Kelleher et al. 2000), and in a wide variety of roles in *D. melanogaster*, including oogenesis, cell motility, and spermatogenesis. In mammalian cells, myosin VI is involved in endocytosis (Buss et al. 2001a, Aschenbrenner et al. 2003), in the maintenance of Golgi complex morphology and secretion (Warner et al. 2003), and in membrane ruffling (Buss et al. 1998). These functions of myosin VI are reflected by its intracellular localization in membrane ruffles, at the Golgi complex, and in clathrin-coated and -uncoated vesicles throughout the cell (Figure 3).

MYOSIN VI IN *DROSOPHILA*

Myosin VI was discovered in *Drosophila* and was first called 95F myosin owing to its localization on the polytene map (Kellerman & Miller 1992). At least three different splice variants of the C-terminal tail are expressed. Myosin VI is
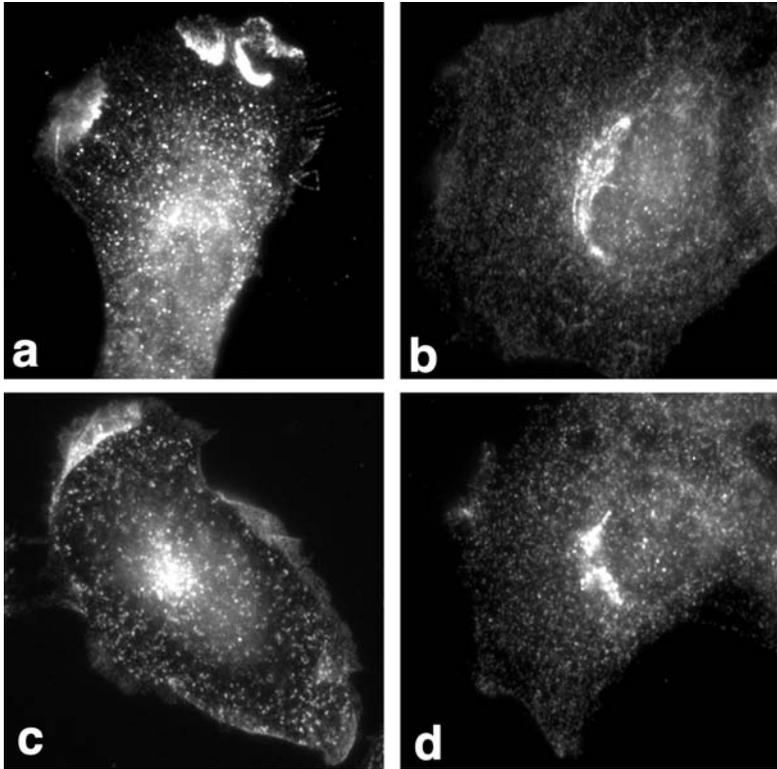
Figure 3   Intracellular localization of myosin VI. In normal rat kidney fibroblasts the
location of myosin VI was revealed by indirect immunofluorescence microscopy using
(*a*) polyclonal antibody to the whole tail of myosin VI, (*b*) polyclonal antibody to the
globular tail, (*c*) overexpression of green fluorescent protein myosin VI containing the
large insert, and (*d*) monoclonal antibody to the whole tail. Using these tools, myosin VI
can be localized at the leading edge in membrane ruffles, at/around the Golgi complex,
in clathrin-coated and -uncoated endocytic vesicles.

expressed throughout the life cycle of the fly, with the highest expression levels
in mid-embryogenesis (8–12 h) and in adults (Kellerman & Miller 1992). In the
early *Drosophila* embryo, myosin VI is required for the formation of pseudocleav-
age furrows in the syncytial blastoderm, where it is associated with cytoplasmic
particles that may represent either protein complexes or, more likely, membrane
vesicles. Microinjection of myosin VI antibodies into these embryos demonstrates
that myosin VI is an essential motor for the movement of particles along actin
filaments (Mermall et al. 1994). In the syncytial blastoderm, in a monolayer of nu-
clei underneath the plasma membrane, mitosis occurs without cytokinesis. These
nuclei divide parallel to the cell cortex, and adjacent spindles are separated by
actin-lined pseudocleavage furrows. When myosin VI activity is blocked, it leads

to disorganized cortical actin networks and reduced pseudocleavage furrow formation resulting in fused spindles and errors in chromosome segregation (Mermall & Miller 1995).

Recently, an additional role for myosin VI during asymmetric cell division in *Drosophila* embryonic neuroblasts has been described (Petritsch et al. 2003). In this process, cell fate determinants such as Miranda are targeted to the apical or basolateral domain of the cell, and the mitotic spindle is oriented parallel to the apical-basal axis. Asymmetric cell division in these neuroblasts produces a smaller ganglion mother cell and a large neuroblast (Gonczy 2002). Knocking out myosin VI in these embryos indicates that it is essential for both the delivery of the cell fate determinant Miranda to the basal crescent and the correct orientation of the mitotic spindle along the apical-basolateral axis (Petritsch et al. 2003). The localization of myosin VI in vesicles in these neuroblasts indicates that it may be involved in transporting Miranda-containing complexes to their basal destination. Positioning of the mitotic spindle is most likely to involve interactions between the plus ends of astral microtubules and the actin cytoskeletal network in the cortex. How myosin VI participates in the process of spindle orientation is not clear, but a protein complex containing myosin VI and microtubule plus-end-binding proteins such as D-CLIP190 or EB1 could be involved (Lantz & Miller 1998, Goodson et al. 2003, Petritsch et al. 2003). Myosin VI moving along cortical actin filaments and linked to astral microtubules via D-CLIP190 or EB1 could exert the pulling forces necessary for positioning the mitotic spindle.

In *Drosophila* testis, myosin VI is required for sperm individualization during spermatogenesis (Hicks et al. 1999, Rogat & Miller 2002). In this process, spermatids, joined together in a syncytium, are separated into individual sperm cells, each surrounded by its own membrane. Sperm individualization is characterized by extensive remodeling of the plasma membrane, which involves actin, the actin polymerization machinery, myosin VI, dynamin, and membranes concentrated in a structure called the investment cone. Myosin VI may have the following roles during sperm individualization: (*a*) At the leading edge of the investment cone, myosin VI could form a flexible link between sites of active actin filament growth and areas of the plasma membrane to be remodeled, (*b*) it could be involved locally in endocytosis at the plasma membrane or in tethering exocytic vesicles close to their site of fusion with the plasma membrane, and (*c*) it could be involved in shaping or keeping the newly formed plasma membrane close to the actin cone.

In *Drosophila* ovaries, myosin VI is essential for border cell migration (discussed below). In cells without myosin VI, both membrane protrusion in the leading edge and migration of these cells are abolished (Geisbrecht & Montell 2002, Deng et al. 1999).

MYOSIN VI AND DEAFNESS

Myosin VI is not the only myosin linked to deafness; mutations in myosin IIA, IIIA, VIIA, and XV also lead to auditory disorders (Friedman et al. 1999; Redowicz 1999, 2002).

In the *Snell's waltzer* (*sv*) mouse, an intragenic deletion leads to a truncation of the *myo6* gene in the neck region of the protein (Avraham et al. 1995) (see Figure 2*c*: deletion of aa 818–861 resulting in a stop). No myosin VI mRNA could be detected by Northern blotting; thus the *sv* mouse is referred to as a myosin VI null mutant. These mice exhibit circling, head-tossing, and hyperactivity (Deol & Green 1966), and direct cochlear stimulation confirmed their deafness (Avraham et al. 1995). A close examination of the inner ear hair cells in the cochlea of *sv* mice showed that at birth they appeared to be normal, but 3 days after birth the maturing stereocilia started to fuse so that by 20 days only giant disorganized stereocilia were observed on these cells (Self et al. 1999). In hair cells of normal mice, myosin VI is found in the pericuticular necklace and at the base of the stereocilia (Hasson et al. 1997), where it may be involved in membrane recycling and trafficking, assembly and/or anchoring the stereocilia.

Recent studies on an Italian family with autosomal-dominant nonsyndromic hearing loss revealed a *myo6* mutation (C442Y) in the motor domain at a highly conserved position (Melchionda et al. 2001) (Figure 2*c*). In this family, the onset of deafness occurs during childhood (at about 8–10 years) and by 50 years of age, adults have profound deafness. In a second detailed genetic study on three Pakistani families, recessive deafness was linked to several mutations identified in myosin VI (Ahmed et al. 2003). A single base mutation was predicted to generate a stop codon after the first 12 amino acids, resulting in a severe truncation of the protein. This VI null mutant is intriguing because, as in the *Snell's waltzer* mouse, it shows that a functionally null myosin VI mutant can survive. It is surprising that the *Snell's waltzer* mice and humans lacking myosin VI are viable because myosin VI appears to be involved in a whole host of essential cellular functions and is apparently unique in moving toward the minus end of actin. Although a second gene encoding myosin VI is present in *C. elegans* (Baker & Titus 1997) and fish (Breckler et al. 2000), no additional gene has been identified in mouse. Thus there must be a significant degree of redundancy during mouse development and/or other motor proteins must be able to compensate for the absent myosin VI. Myosin IX is a potential candidate, since initial experiments on expressed head fragments suggested that myosin IX, similar to myosin VI, might be a minus-end-directed motor (Inoue et al. 2002). However, more recent data with native myosin IX purified from tissue have demonstrated that it moves like the majority of the myosins toward the plus end of actin (O'Connell & Mooseker 2003). So far no other minus-end-directed myosin has been clearly identified. Can microtubule motor proteins at least partially compensate for the missing myosin VI? The major phenotype so far established in the *Snell's waltzer* mouse is deafness; however, further studies on the phenotypes of a wide variety of cells from this knock-out mouse may provide us with additional information on the role of myosin VI in vivo. Recent studies in humans have linked mutations in myosin VI to hyper-trophic cardiomyopathy (Mohiddin et al. 2004), and in ovarian cancer cells myosin VI expression is upregulated, indicating a potential role for myosin VI in cancer progression (Yoshida et al. 2004). These results may provide useful clues for our studies on the other possibly more subtle phenotypes present in the *Snell's waltzer*

mouse. Vestibular dysfunction and mild facial dysmorphology were also found to concur with profound hearing loss in homozygotes for the myosin VI mutations (Ahmed et al. 2003). It would be interesting to simulate these point mutations in mouse models and carry out cell biological and biochemical studies, to probe if these mutations lead to the same phenotype as the myosin VI null mutant.

INTRACELLULAR FUNCTIONS OF MYOSIN VI IN MAMMALIAN CELLS
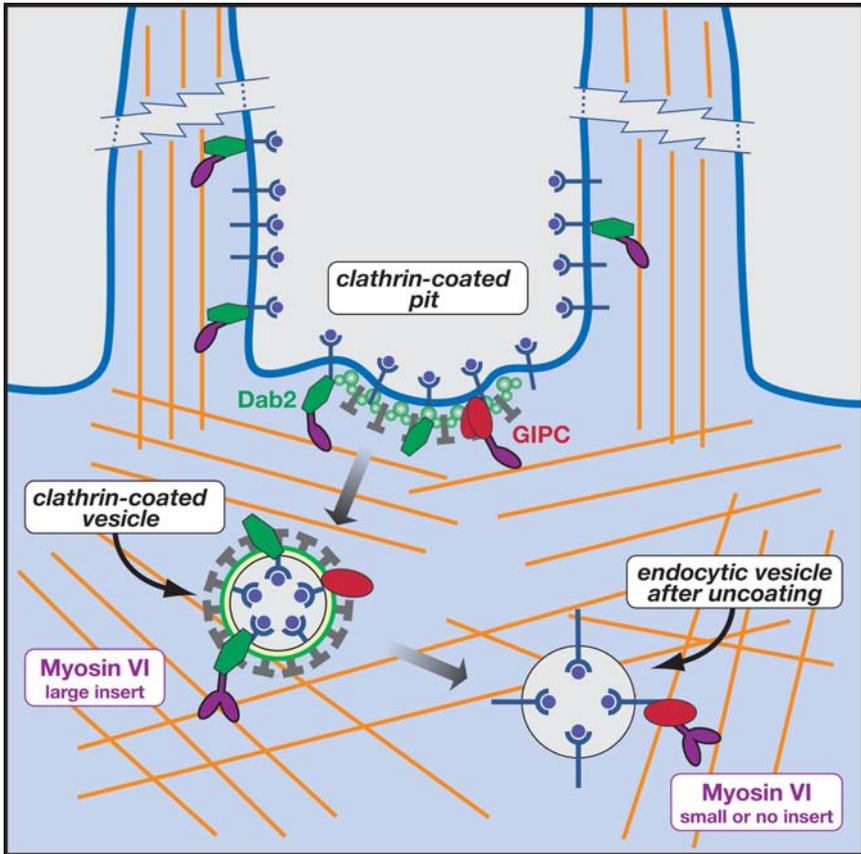
Myosin VI in Endocytosis

At the plasma membrane in mammalian cells, endocytosis is the fundamental process involved in the regulated uptake of macromolecules such as proteins, nutrients, and cell surface receptors, and in the retrieval of integral membrane proteins after vesicle fusion. Clathrin-mediated endocytosis is the best characterized uptake route for internalization of cell surface receptors in these cells (Brodsky et al. 2001). This process occurs in the following sequence of steps: (*a*) cargo selection and clustering, (*b*) coat assembly and formation of a coated pit, (*c*) invagination of the plasma membrane, (*d*) membrane fission and vesicle formation, and (*e*) movement of vesicles away from the plasma membrane. These steps require a machinery of structural coat components, as well as a large number of accessory proteins that now includes a growing list of actin-binding proteins (Engqvist-Goldstein & Drubin 2003, Schafer 2002, Qualmann & Kessels 2002). These latter proteins provide circumstantial evidence that in mammalian cells the cortical actin network underlying the plasma membrane is important for endocytosis. Although the link between the actin cytoskeleton and endocytosis is well documented by genetic studies in yeast (Engqvist-Goldstein & Drubin 2003, Geli & Riezman 1998), results in mammalian cells are more controversial and depend on the assay and the cell type tested (Fujimoto et al. 2000). However, in polarized as well as in unpolarized cells evidence indicates that actin filaments are involved in the early stages of endocytosis, specifically in receptor clustering and spatial organization of the endocytic machinery and also in vesicle scission and the transport of vesicles away from the plasma membrane. Furthermore, in unpolarized cells, clathrin-coated pits are aligned along actin filament bundles, and this association is disrupted by the actin depolymerizing drug, cytochalasin D (Bennett et al. 2001). Similarly, in live cells, the endocytic machinery is positioned in stationary hot spots in the plasma membrane by binding to the cortical actin network (Gaidarov et al. 1999). When the actin filaments were depolymerized, the mobility of the clathrin-coated pits in the plane of the cell surface was increased, implying that the clathrin-coated pits or vesicles were anchored to the actin filament network.

In polarized epithelial cells with apical microvilli, the formation of a clathrin-coated pit is highly restricted to specialized plasma membrane domains in between

microvilli (Christensen et al. 1998). Depolymerization of actin filaments in these cells causes reduced movement of cell surface receptors down the microvilli into the microvillar cleft and leads to an increase in the number of clathrin-coated pits at the base of the microvillus (Gottlieb et al. 1993, Jackman et al. 1994, Shurety & Luzio 1995). These studies demonstrated that in the apical domain of polarized cells intact actin filaments are required for two essential steps in endocytosis: movement and clustering of receptors and vesicle scission.

So what is the function of myosin VI in clathrin-mediated endocytosis and at which stage of this process is it involved? The answer to these questions differ for different cell types depending on which myosin VI isoform is expressed (Aschenbrenner et al. 2003; Buss et al. 2001b, 2002). The splice variant of myosin VI containing the large insert in the tail domain (see Figure 2*b*) (expressed in polarized cells with apical microvilli) is predominantly found in clathrin-coated pits and vesicles concentrated in the terminal web underlying the apical plasma membrane, although a small amount of myosin VI is found in microvilli (Heintzelman et al. 1994, Biemesderfer et al. 2002). This localization of myosin VI and the identification of Dab2 (disabled 2) as a myosin VI–binding partner (Morris et al. 2002a, Inoue et al. 2002) suggest that myosin VI is involved in a very early step in endocytosis. Binding to Dab2 links myosin VI to cell surface receptors of the low-density lipoprotein receptor (LDLR) family (Morris & Cooper 2001) (see below), and this link might enable myosin VI to move receptor complexes from the tip of a microvillus to the base for clustering in a clathrin-coated pit (Figure 4). Such movement would specifically require a minus-end-directed myosin because actin filaments in the microvillus core are highly organized with the plus ends at the tips and the minus ends at the base (Figure 4).

Myosin VI may also provide a force for vesicle formation at later stages of endocytosis either by pulling the membrane associated with a clathrin-coated pit into the cell, thereby increasing the invagination, or by pushing actin filaments into the vesicle neck, supporting vesicle scission in conjunction with the host of other accessory endocytic proteins (Buss et al. 2001b). Finally, myosin VI may transport clathrin-coated vesicles through the actin-dense terminal web away from the plasma membrane before the vesicle uncoats. In unpolarized cells without apical microvilli, the splice variant of myosin VI with the small or no insert is expressed. This isoform localizes to uncoated endocytic vesicles before they fuse with the early endocytic compartment (Aschenbrenner et al. 2003). Targeting of myosin VI to these uncoated endocytic vesicles might involve binding to GIPC, which is found together with myosin VI on these peripheral vesicles (Figures 1 and 4). Overexpressing the myosin VI tail without the large insert in unpolarized epithelial cells does not affect clathrin-mediated uptake of the transferrin receptor; however, intracellular trafficking of endocytic vesicles through the actin-rich cell periphery in these cells to the perinuclear sorting endosome is delayed (Aschenbrenner et al. 2003). These results indicate that the different myosin VI isoforms have various roles in endocytosis in cells with different specialized plasma membrane domains.

Figure 4   A model depicting the possible roles for myosin VI during endocytosis. In polarized epithelial cells with apical microvilli, the myosin VI isoform containing the large insert is expressed. Two binding partners of myosin VI, Dab2 and GIPC (GAIP-interacting protein C terminus), are found associated with clathrin-coated vesicles and, in the case of GIPC, also with endocytic vesicles after uncoating. During the sequential steps of endocytosis, myosin VI may be involved initially in moving receptors down the microvillus for sequestering in a clathrin-coated pit and for restricting clathrin-coated pit formation to the base of a microvillus. Later, myosin VI could promote vesicle formation by increasing the plasma membrane invagination by pulling it into the cell and then transporting the newly formed clathrin-coated vesicles away from the plasma membrane into the cells. In unpolarized epithelial cells, myosin VI, without the large insert, and GIPC are also associated with endocytic vesicles after uncoating, and myosin VI is thought to be involved in transporting these vesicles through the dense cortical actin network toward the later endocytic compartment (Aschenbrenner et al. 2003).

Myosin VI in the Golgi Complex

The Golgi complex plays a central role in the secretory pathway. Every protein synthesized in the endoplasmic reticulum (ER) passes through the Golgi complex, where it undergoes post-translational modification and is sorted for its final destination. In higher eukaryotic cells, the Golgi complex appears as a single organelle, centered in the perinuclear position around the microtubule-organizing center (MTOC). It exists as a stack of flattened membrane cisternae, which can be classified using resident marker proteins as the *cis*, medial, and *trans* Golgi compartments (Ladinsky et al. 1999). Each side of the Golgi complex is flanked by an elaborate network of membrane tubules and vesicles: the *cis*-Golgi network (CGN) and the *trans*-Golgi network (TGN). The TGN is the exit site of the Golgi complex and therefore is central for the sorting of cargo into separate vesicles for dispatch to different locations in the cell (Griffiths & Simons 1986). This integrated network of membranous structures is thought to depend on interactions with the cytoskeleton and on associated motor proteins (Lippincott-Schwartz 1998). Perturbation of either actin filaments or microtubules leads to an altered Golgi complex morphology; for example, disruption of actin filaments with drugs such as latrunculin or cytochalasin D causes a collapse of the Golgi complex (Valderrama et al. 1998), whereas depolymerization of microtubules causes fragmentation of the Golgi complex and dispersion of mini Golgi stacks throughout the cell (Rogalski & Singer 1984, Thyberg & Moskalewski 1985). In addition to their structural role, actin filaments are also involved in post-Golgi membrane traffic to the cell surface (Hirschberg et al. 1998) and in retrograde protein transport from the Golgi complex to the ER (Valderrama et al. 2001).

The presence of myosin VI at the Golgi complex has been shown at the light microscope and at the EM level using a number of different polyclonal and monoclonal antibodies (Warner et al. 2003, Buss et al. 1998) (Figure 3). Within the Golgi complex myosin VI is associated with the TGN, as shown using the drug Brefeldin A, a fungal metabolite that inhibits ADP-ribosylation factor (ARF) function. This drug causes the TGN to fragment into vesicles containing myosin VI, which concentrate around the MTOC. At the ultrastructural level, myosin VI is present on vesicles in close proximity, about 200–400 nm on the *trans* side of the Golgi complex. Overexpression studies using deletion mutants of myosin VI indicate that the globular tail domain is essential for targeting to the Golgi complex.

Further insight into the function of myosin VI at the Golgi complex comes from studies using fibroblasts derived from the *sv* knockout mouse. In primary fibroblasts and also in immortalized fibroblastic cell lines derived from this *sv* mouse, the size of the Golgi complex is significantly reduced by about 40% compared with fibroblasts from wild-type mice. In addition, the absence of myosin VI also reduces the level of secretion from the TGN to the plasma membrane by about 40%. Both phenotypes can be rescued by overexpression of wild-type myosin VI in these fibroblasts. Deletion mutants of the tail domain are not able to substitute for the loss of myosin VI (Warner et al. 2003).

These studies give us some ideas about the possible roles of myosin VI at the Golgi complex (Figure 1). Because the loss of myosin VI results in altered Golgi morphology, myosin VI appears to have a role in maintaining the elaborate reticulate structure of Golgi membranes. Perhaps it could form a flexible link between Golgi membranes and the surrounding actin filaments and, by pulling the membranes outward, keep them taut (Figure 1). Myosin VI may also transport cargo vesicles over short distances along actin filaments from the TGN to the microtubules, which are used for their long-distance transport to the plasma membrane. In analogy to a possible role of myosin VI at the plasma membrane during endocytosis, myosin VI may also be important for vesicle formation at the TGN, for example, either by pulling membrane along actin filaments, physically supporting the budding process, or by sorting different cargo molecules into budding vesicles and aiding their transport to different destinations inside the cell.

Myosin VI in Cell Migration

A migrating cell is polarized and typically has a leading edge and trailing edge. The protrusive force for forward movement is generated at the leading edge by the formation of pseudopodia, long, finger-like filopodia, or flat lamellipodia (Mitchison & Cramer 1996). If an extended lamellipodium fails to adhere to a substrate, it retracts and forms a membrane ruffle. This ruffling lamellipodium can lift off the substrate, undulate, and then move backward along the dorsal surface of the cell. Therefore, to generate a forward-driving movement, protrusion at the leading edge must be coupled to adhesion. The protrusive force to push the plasma membrane outward is generated by actin polymerization, regulated by Rho GTPases and the Arp2/3 complex (Hall 1998, Borisy & Svitkina 2000). This latter complex has now been shown to form a physical link with cell adhesion complexes, thereby coupling membrane protrusion and cell adhesion (DeMali et al. 2002, DeMali & Burridge 2003). Cell migration is thus a complex process characterized by (*a*) dynamic remodeling of the actin cytoskeleton, (*b*) cell adhesion to a substrate, and (*c*) reorganization of the plasma membrane and membrane trafficking (Nabi 1999).

Myosin VI forms a link between membrane protrusion and cell adhesion. For example, in *Drosophila*, myosin VI is essential for border cell migration in the ovary, especially for the formation of membrane protrusions at the leading edge of the cells (Geisbrecht & Montell 2002). In these migratory cells, myosin VI exists in a complex with the cell adhesion molecules β-catenin and E-cadherin. Thus by binding to stationary β-catenin/E-cadherin-containing adhesion complexes in the membrane, myosin VI could generate a protrusive force by pushing actin filaments outward, while moving itself into the cell toward the minus end of actin filaments (Buss et al. 2002). A role for myosin VI at the leading edge in migratory cells is also supported by our own work in mammalian cells where, after growth factor stimulation, myosin VI is actively recruited into membrane ruffles and phosphorylated in the motor domain (Buss et al. 1998) (Figure 3). The kinase possibly

involved could be the PAK (p21 rac-activated kinase), which has been shown to be important for cell motility in mammalian fibroblasts (Sells et al. 1999).

MYOSIN VI BINDING PARTNERS

Like myosin VI, its various binding partners are found throughout the cell: at the Golgi complex and in the secretory and endocytic pathway. This variety argues for the multi-functionality of myosin VI in the cell. Many of the proteins identified as binding to myosin VI have been shown to interact with a variety of other proteins in various cellular pathways (Table 1). Studying these binding proteins in detail may shed more light on the cellular functions of myosin VI.

Disabled-2

Disabled-2 (Dab2, DOC-2) was initially identified as a tumor suppressor involved in cell growth control, which was down-regulated in a number of different carcinomas (Fulop et al. 1998, Schwahn & Medina 1998). It has now been shown to participate in the regulation of several signaling cascades such as the Ras/MAPK (mitogen-activated protein kinase) (Xu et al. 1998), the TGF-β signaling pathway (Hocevar et al. 2001), and the Wnt signaling pathway (Hocevar et al. 2003). Overexpression of Dab2 in macrophages causes enhanced cell spreading and adhesion, together with pronounced lamella and filopodia formation (Rosenbauer et al. 2002). At the molecular level, Dab2 is thought to be a key player in the endocytosis of LDLRs. It recognizes the NPXY internalization signal in the cytoplasmic tail

TABLE 1 Myosin VI binding partners: functions and localizations

Binding partner	Localization	Proposed function
Dab2/DOC-2	Clathrin-coated pits and vesicles, perinuclear Golgi region	Tumor suppressor, in signaling pathways, cell spreading and adhesion, endocytosis of LDLR
GLUT1CBP/ GIPC/ SEMCAP-1 Synectin	Clathrin-coated pits and vesicles, uncoated endocytic vesicles, perinuclear Golgi region	Endocytosis, receptor tafficking at the Golgi complex, G protein–coupled signaling cell migration
SAP97/HDlg	Pre- and postsynaptic sites, cell-cell adhesion sites, Golgi region	Trafficking and localization of neurotransmitter receptors, intercellular adhesion
D-CLIP-190	Posterior pole of *Drosophila* embryos, vesicles in neurons	Microtuble-binding protein

of the LDL receptor family members via the N-terminal phosphotyrosine binding (PTB) domain (Morris & Cooper 2001). This PTB domain is also able to simultanously bind to phosphoinositides, thereby attaching Dab2 to the plasma membrane (Mishra et al. 2002). Outside the PTB domain, Dab2 binds to AP2, clathrin, and myosin VI, as well as to EH-domain-containing accessory endocytic proteins. Dab2 can therefore be classified as a cargo-specific adaptor protein (Mishra et al. 2002), linking cargo selection and coat formation (Traub 2003). Although Dab2 knockout mice are not viable, a conditional knockout yields live animals that secrete high levels of protein into the urine owing to a defect in the endocytosis of megalin (a scavenger receptor of the LDLR family required for readsorption of proteins from the urine) in renal proximal tubule cells. These cells also have fewer clathrin-coated pits (Morris et al. 2002b), which strongly supports a role for Dab2 in clathrin-mediated endocytosis.

Dab2 was found to be a myosin VI–binding partner by yeast two-hybrid studies (Inoue et al. 2002, Morris et al. 2002a). This interaction has been confirmed by mammalian two-hybrid assays, coimmunoprecipitation, and pull-down assays (Morris et al. 2002a). Green fluorescent protein-labeled myosin VI transfected into fibroblasts or unpolarized epithelial cells colocalizes with Dab2 in clathrin-coated vesicles only when the myosin VI isoform with the large tail insert is used (Aschenbrenner et al. 2003, Buss et al. 2001b). Myosin VI (the large insert isoform) binding to Dab2 may be a link between the initial steps of clathrin-mediated endocytosis such as receptor clustering and the actin cytoskeleton. Currently, the only established functional connection between myosin VI and Dab2 is in clathrin-mediated endocytosis in polarized cells with apical microvilli. Further experiments are needed to show what role myosin VI plays in the number of signaling pathways involving Dab2. Of particular interest would be a possible function of myosin VI as a Dab2-binding partner during cell adhesion and cell spreading.

GIPC

GIPC—homologous to GLUT1 C-terminal binding protein, SEMCAP-1, and synectin—(Bunn et al. 1999, De Vries et al. 1998, Gao et al. 2000, Wang et al. 1999) is a second myosin VI–binding partner that can bind to LDL receptors such as megalin (Gotthardt et al. 2000, Lou et al. 2002). In addition, GIPC, by binding to a variety of other transmembrane proteins such as human lutropin receptor (LHR) (Hirakawa et al. 2003) and the β1-adrenergic receptor (Hu et al. 2003), may link myosin VI to a number of different cell surface molecules for receptor clustering and endocytosis. Furthermore, because GIPC is also associated with the melanosomal membrane protein, gp75 (Liu et al. 2001), the myosin VI present in the TGN (Buss et al. 1998, Warner et al. 2003) could be involved in the local trafficking of proteins such as gp75 from the Golgi complex to melanosomes. GIPC has now been shown to localize to both clathrin-coated vesicles (Lou et al. 2002) and -uncoated vesicles containing transferring receptor (Aschenbrenner et al. 2003). Whereas the myosin isoform with the large insert seems to be involved in the initial steps

of clathrin-mediated endocytosis (possibly through its association with Dab2 or GIPC), the other myosin VI isoforms (with the small or no insert) colocalize with GIPC in uncoated vesicles and appear to be involved in movement of uncoated endocytic vesicles (Aschenbrenner et al. 2003).

SAP97

SAP97 is a member of the postsynaptic density-95 (PSD-95)-like MAGUK (membrane-associated guanylate kinases) family of proteins that contains three PDZ domains, a src homology 3 (SH3) domain, and a guanylate-kinase-type sequence (Fujita & Kurachi 2000). Most family members are localized to the synapse; however, SAP97 is also expressed in epithelial cells (Wu et al. 1998). SAP97 binds to the Glu1 subunit of AMPA-type glutamate receptors (Leonard et al. 1998), and it may have a role in trafficking newly synthesized AMPA receptors through the Golgi complex to the plasma membrane (Sans et al. 2001). Myosin VI was identified as a SAP97-binding partner in a yeast two-hybrid assay (Wu et al. 2002), and GluR1, myosin VI, and SAP97 can be precipitated in a complex from rat brain, suggesting a role for myosin VI in AMPA receptor trafficking around the Golgi complex or underneath the plasma membrane (Buss et al. 1998, Warner et al. 2003). Interestingly, myosin VI is not the only motor protein that binds to SAP97 because in axons and dendrites of cultured hippocampal neurons, SAP97 associates with KIF1 kinesins, which mediate transport along microtubules (Mok et al. 2002).

In *Drosophila*, mutations of SAP97 (*Dlg*) cause a loss in intercellular adhesion of epithelial cells within imaginal discs (Woods & Bryant 1991) and a loss of apical-basolateral epithelial polarity. Recruitment of SAP97 to these cell-cell contact sites on the plasma membrane requires the presence of E-cadherin and the cortical actin cytoskeleton (Reuver & Garner 1998, Wu et al. 1998). SAP97 coimmunoprecipitates with E-cadherin, α-catenin, and β-catenin in fibroblasts. In *Drosophila* border cells, myosin VI is present in a protein complex with E-cadherin and β-catenin (Geisbrecht & Montell 2002), which suggests that myosin VI might be the linker molecule between SAP97 and the actin network at cell-cell adhesion sites.

D-CLIP-190

D-CLIP-190 was identified as a myosin VI–binding partner in coimmunoprecipitation experiments using *Drosophila* embryo extracts (Lantz & Miller 1998). D-CLIP-190 binds to microtubules (Lantz & Miller 1998) as does the mammalian homologue CLIP-170 (Pierre et al. 1992). Myosin VI and D-CLIP-190 colocalize in particulate possibly vesicular structures in neurons and are found in the posterior pole of early embryos, where both microtubule and actin-based processes occur. Depolymerizing the actin filaments with cytochalasin D disrupts the localization of both myosin VI and D-CLIP-190 in this region, whereas disrupting the microtubules does not appear to affect localization (Lantz & Miller 1998). CLIP-170

possibly has a role in the loading of endocytic vesicles onto the plus ends of microtubules (Pierre et al. 1992); perhaps D-CLIP-190 has a similar role, accepting vesicles from myosin VI and acting as a bridge between the actin cytoskeleton and the microtubule network.

WHAT TYPE OF MOTOR IS MYOSIN VI?

Clues to the role(s) that myosin VI might play in the wide range of cellular events (discussed above) may be provided by examining its in vitro properties. Ever since myosin VI was discovered in *Drosophila* and sequence analysis revealed that the tail domain contained a significant region of heptad repeats, it was assumed to exist as a stable dimer molecule similar to myosin II and V (Hasson & Mooseker 1994, Kellerman & Miller 1992). For this reason, almost all the in vitro motility studies have been carried out on myosin VI dimer constructs where dimerization has been ensured by the inclusion of either a C-terminal leucine zipper (Rock et al. 2001) or a portion of the myosin II rod domain (Nishikawa et al. 2002). Kinetic and motility studies revealed that myosin VI has a high duty ratio (it remains strongly bound to the actin filament for >90% of its actomyosin ATPase cycle) (De La Cruz et al. 2001), and the dimer constructs move processively along actin filaments with a large step size of 30–36 nm (the two heads walk straight along the filament spanning the actin helical repeat of 36 nm) (Rock et al. 2001). Recently, it was shown by hydrodynamic and EM single molecule imaging that whole myosin VI can exist as a stable monomer with motile properties similar to those previously measured. These monomers, however, generate single nonprocessive movements on actin with a step size of 18 nm (Lister et al. 2004).

For myosin II and V there is now general, if not universal, agreement that the size of the working stroke (step size) of a myosin per single ATPase cycle depends in a linear fashion on the length of the light chain–binding domain (classical lever arm mechanism) (Purcell et al. 2002, Tanaka et al. 2002, Uyeda et al. 1996, Veigel et al. 1999, Warshaw et al. 2000). The long step sizes produced by both monomer and dimer myosin VIs were therefore unexpected because the neck region of myosin VI is short (it appears to contain only a single light chain–binding domain) and should be able to contribute a lever arm movement of only 4 nm. Thus the step size in myosin VI cannot be generated solely by the classical lever arm mechanism that operates in myosin II and V unless other parts of the molecule contribute to the effective lever. A number of models have been proposed to explain how myosin VI might achieve its long step size. Rock et al. (2001) postulated that in their myosin VI dimer construct, the large step size was generated by a small working stroke coupled to a large conformational change caused by the unfolding of the 53 aa insert (between the motor domain and light chain–binding domain), which would allow the free head to extend and bind to the next available binding site (36 nm away) on the actin filament. In contrast, Nishikawa et al. (2002) proposed, based on EM studies of their myosin VI dimer construct, that binding of the first head to

the actin filament caused conformational changes in the actin filament such that the second head could slide down the filament until it reached the next high affinity site 36 nm away. In this model the length of the lever arm in myosin VI would not be crucial for its large step size. We have argued in favor of a model where the size of the working stroke in monomeric myosin VI is dependent not only on the length of the lever arm but also on the changes in the orientation and degree of rotation of the lever arm as it goes through the acto-myosin ATPase cycle (Lister et al. 2004). Perhaps the unique 53 aa insert, the light chain–binding domain, and possibly the first segment of the tail (Figure 2) may all contribute to form the lever arm. Solving the structure of these regions of myosin VI may help to decide which of the models operates.

Is Myosin VI Able to Function as a Monomer or Dimer or Both?

The possibility that myosin VI might be able to function both as a nonprocessive monomeric motor and as a processive dimer in vivo is intriguing. At present there is no information regarding which forms of myosin VI are present in vivo. However, one could imagine that certain functions (illustrated in Figure 4 and outlined in Table 2), such as maintaining tension, clustering transmembrane receptors, or tethering membranes and vesicles to actin filaments, might require a nonprocessive monomer, whereas other processes, such as the retrograde transport of vesicles along actin filaments to different cellular compartments, might rely on a processive dimer. It is conceivable, for example, that during endocytosis at the plasma membrane, myosin VI monomers could be recruited in sufficient concentrations to coated pits and/or budding vesicles by binding to one of its binding partners such as Dab2, or it could be modified by phosphorylation/dephosphorylation or by binding to the lipid membrane so that dimerization could take place via part of the helical

TABLE 2   Possible myosin VI movements (toward the minus end of actin filaments) in different intracellular functions

Intracellular function	Type of movement	Processive/nonprocessive
Tethering of vesicles and membranes to actin filaments	Holding	Nonprocessive
Clustering of trans-membrane receptors	Short-range transport	Nonprocessive/Processive
Vesicle formation at the plasma membrane	Pulling	Nonprocessive/Processive
Pushing actin filaments at the leading edge	Protrusive	Nonprocessive/Processive
Retrograde transport of endocytic vesicles	Long-range transport	Processive

tail domain. Although there is no direct evidence thus far for such a proposal, it has been demonstrated in the Kif1/Unc104 kinesin family that dimerization of these motor proteins on the surface of lipid vesicles triggers their processivity (Tomishige et al. 2002, Vale 2003).

Regulation of Myosin VI Functions

Very little is known about how myosin VI is regulated within the cell. However, given the diversity of cellular localization and functions discussed above, one would assume that tight regulation is required to ensure that myosin VI is active only where and when required. In addition it is likely that there is cross talk between the different myosins and other motor proteins so as to maintain the high degree of dynamic organization present within the cell. For example, the activities of both plus-end- and minus-end-directed myosins must be regulated and coordinated in order to achieve the correct distribution of organelles and macromolecules in the cell. Although the regulatory mechanisms involved are not yet understood, myosin VI has at least four potential ways it might be regulated.

ALTERNATIVE SPLICING   At the primary level of regulation myosin VI exists in four alternatively spliced isoforms with large, small, both, or no inserts in the tail domain (see Figure 2) (Buss et al. 2001a). The myosin VI isoform with the large insert is required for targeting to the apical surface in polarized cells, whereas those isoforms with the small or no insert appear to be involved in vesicle transport in unpolarized cells.

PHOSPHORYLATION   When A431 cells are stimulated with epidermal growth factor (EGF), myosin VI is recruited into ruffles at the plasma membrane and there is a three- to fourfold increase in myosin VI phosphorylation (Buss et al. 1998). It was suggested that a PAK, a member of the small G protein rac- and cdc 42–activated kinase family, might be responsible for phosphorylation, and T406 might be the phosphorylation site (obeying the TEDS rule for myosin motor domain phosphorylation sites proposed by Bement & Mooseker (1995). T406 is equivalent to the serine/threonine residue identified in *Acanthamoeba* myosins of class I, which must be phosphorylated for full myosin I activity in vitro (Brzeska et al. 1997). In myosin VI, however, the most recent in vitro data indicate that phosphorylation at T406 has no observable effect on myosin VI activity, i.e., it does not alter either its rate of actin movement in the in vitro motility assay or its maximal actin-activated ATPase rate (Morris et al. 2003). The role of T406 phosphorylation/dephosphorylation in the motor domain may be more subtle, possibly to finely tune myosin VI/actin cytoskeletal interactions in the cell. Preliminary results have identified additional phosphorylation sites in the globular tail domain (R. Roberts, unpublished results), but their regulatory role has not yet been established. They may be involved in regulating the binding of myosin VI to its binding partners.

Although PAK can phosphorylate myosin VI in vitro, little is known about the actual kinases, phosphatases, and phosphorylation sites that are employed in cells. Thus identification of the kinases/phosphatases, their roles, and that of the cell signaling pathways involved are exciting areas for future exploration.

CALCIUM/CALMODULIN   Myosin VI has a single light chain–binding domain (IQ motif) that binds calmodulin and is believed to act as the movement generating lever arm (see above) and calcium-binding component. Recent kinetic studies have shown that at calcium concentrations $>10~\mu$M, both the rate of ADP release from actomyosin VI and the in vitro motility rate of myosin VI are slowed (Morris et al. 2003). Interestingly calcium also leads to a loss of coordination between the two heads in the dimer construct such that the degree of processive movement is reduced. Thus calcium binding and phosphorylation do not appear to operate as on/off switches like those in vertebrate nonmuscle myosin II but rather seem to act to modulate myosin VI functions.

CONFORMATIONAL CHANGES IN THE TAIL DOMAIN   Because myosin VI can exist as a stable monomer (Lister et al. 2004) and also appears to be able to form a dimer (Nishikawa et al. 2002), the tail domains must be able to undergo dramatic conformational changes. A large number of potential binding partners have been identified that bind to the globular tail domain of myosin VI, and a mechanism to regulate their binding is essential. Conformational changes in the tail could be used to regulate/block these protein-protein interactions. Secondary structure predictions (http://cubic.bioc.columbia. edu/predictprotein/) indicate that the first part of the tail contains helical regions with hinge regions, and a central domain with alternating repeats of negatively and positively charged amino acids, which suggest a tail domain with considerable flexibility. One could therefore imagine the following scenario: In the folded state a monomeric myosin VI tail could exist in a folded conformation such that the binding sites in the globular tail domain would be hidden/blocked from the interacting proteins. The dimer interfaces might also be blocked and the actin-binding site in the motor domain also inhibited. Indeed, immuofluorescence location studies (Buss et al. 1998) have shown that myosin VI does exist in a diffuse cytoplasmic pool in the cell that is not associated with any obvious cellular structures. Under appropriate signals the tail could unfold to allow interactions with binding partners, possibly dimer formation, and activation of the motor domain. Although at present there is no direct evidence to support this model, such a regulatory system does occur in nonmuscle myosin II (Craig et al. 1983). In the nonphosphorylation state the myosin II tail is folded into a compact inactive state (actin binding is also blocked), and upon phosphorylation of the light chain the tail unfolds and, together with other tails, assembles into a filament that can now interact with actin to generate force and movement. One of the signals that might control the folding of the myosin VI tail could be the phosphorylation sites recently identified in the tail domain. Interestingly, the EM single molecule imaging data on myosin VI monomers confirm that the tail domain is extremely

flexible and appears to exist in a large number of different conformations (Lister et al. 2004).

PERSPECTIVES

The *Drosophila* homologue of myosin VI was discovered over 12 years ago (Kellerman & Miller 1992) and subsequently myosin VI was identified in most higher eukaryotic organisms. Interesting results are now starting to accumulate on the properties and intracellular functions of this class of myosins. Progress in the myosin field is being driven by the development of exciting new techniques such as optical tweezers to measure force and step size at the molecular level, single-molecule fluorescence probe techniques, live-cell microscopy, atomic force microscopy, rapid single-molecule enzyme kinetics, protein-protein interaction screens, protein engineering, and knockout technology together with structural analysis. Thus far myosin VI appears to have a wide variety of roles in membrane trafficking pathways and cell motility in *Drosophila* and mammalian cells. One of the most intriguing observations was the demonstration that myosin VI could exist, at least in vitro, as a processive dimer and as a nonprocessive monomer molecule, both with unexpectedly large working strokes. How myosin VI can achieve such a large working stroke with a short lever arm and whether both monomer and dimer species exist in the cell programmed for different intracellular functions are just a few of the exciting questions that need to be answered. We are just beginning to understanding how, where, and when myosin VI functions in different cells, and with the wonderful tools that are being developed to study single molecules both inside and outside cells, answers to our questions will start to emerge. Myosin VI will become the focus of attention and take its place at center stage in the very near future.

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