Cell

Myosin VI Rewrites the Rules for Myosin Motors

H. Lee Sweeney[1,*] and Anne Houdusse[2,*]
[1]Department of Physiology, University of Pennsylvania School of Medicine, B700 Richards Building, 3700 Hamilton Walk, Philadelphia, PA 19104-6085, USA
[2]Institut Curie CNRS, UMR144, 26 rue d'Ulm, 75248 Paris cedex 05, France
*Correspondence: lsweeney@mail.med.upenn.edu (H.L.S.), anne.houdusse@curie.fr (A.H.)
DOI 10.1016/j.cell.2010.04.028

Myosin VI is the only type of myosin motor known to move toward the minus ends of actin filaments. This reversal in the direction of its movement is in part a consequence of the repositioning of its lever arm. In addition, myosin VI has a number of other specialized structural and functional adaptations that optimize performance of its unique cellular roles. Given that other classes of myosins may share some of these features, understanding the design principles of myosin VI will help guide the study of the functions of myosins that adopt similar strategies.

Introduction

The myosin superfamily of molecular motor proteins power movements on actin filaments in eukaryotic cells. Although the thirty-five classes of myosins described to date (Odronitz and Kollmar, 2007) are highly divergent in their C-terminal cellular targeting domains, the N-terminal motor domain of the molecules, and thus the motor mechanism itself, is highly conserved in design and function (Figure 1A).

Directed movement powered by these myosin motor domains occurs via swinging of a myosin lever arm when a myosin head (the motor domain) is strongly bound to an actin filament. This is known as the lever arm hypothesis (see Holmes and Geeves, 1999 for review). This lever arm is attached to the converter subdomain of the motor and is formed by a C-terminal α-helical extension of the motor domain that contains a variable number of consensus binding sites (IQ motifs) for calmodulin (CaM) and calmodulin-related light chains (Figures 1A and 1B). In essence this extension functions as a mechanical lever arm, amplifying the movements of the converter subdomain of the myosin motor. The motor subdomain is in turn repositioned through a series of structural changes that are coupled to release of phosphate and magnesium-ADP (MgADP) when myosin is strongly bound to actin. This generates a force that swings the lever arm, a movement known as the myosin powerstroke (Figure 1B). It begins when myosin binds to actin in the prepowerstroke state with the products of ATP hydrolysis, inorganic phosphate and MgADP, trapped and the lever arm in a "primed" position. It ends upon dissociation of ADP and the formation of a high-affinity interface between myosin and actin, known as the rigor state. In general this movement is directed toward the plus end of an actin filament. Prior to 1999, it was thought that this was the case for all myosin motors.

Actin networks in cells do not maintain the high degree of uniform polarity as do microtubules. However, there are regions of cells where the filaments are largely of the same polarity, with the plus ends of actin filaments toward the cell membrane and minus ends directed into the cell body (Small et al., 1978, 1985; Cramer et al., 1997). The most dramatic examples of this are found in specialized actin-rich structures such as those found in the microvilli of intestinal epithelial cells and in the stereocilia of cochlear hair cells (Mooseker and Tilney, 1975; Tilney et al., 1980). Thus, analogous to the kinesin superfamily of microtubule motors, it is reasonable to imagine that a number of cellular functions would require a subset of myosin superfamily members to traffic in the reverse direction (toward the minus-end actin filaments).

Such logic led to the initial discovery that myosin VI moves in the opposite direction of other myosins (Wells et al., 1999). Somewhat surprisingly, myosin VI remains the only class of myosins that has been shown to traffic toward the minus ends of actin filaments. This may be due to the fact that in order to reverse its directionality this class had to evolve multiple structural and kinetic adaptations. However, this class also has a number of unusual features that appear to be present in a subset of plus-end-directed myosins. One of these features, cargo-induced dimerization, is involved in targeting within cells rather than in reversing directionality.

A Reverse Gear for Myosin

The mechanism for the reversal of directionality in myosin VI begins with repositioning the myosin lever arm. In fact the general postulate that any reverse-direction myosin must reposition the lever arm led to myosin VI being the initial candidate for reversal of directionality (Wells et al., 1999). Further validating this design principle, and the lever arm hypothesis itself, a later study elegantly demonstrated the engineering of a reverse myosin by introducing a domain at the end of the converter of a plus-end myosin (myosin I) to reposition the lever by ~180° (Tsiavaliaris et al., 2004). However, a complete 180° repositioning would only be possible for class I myosins, given that they alone lack an extended N terminus (generally a SH3 domain). This extended N terminus would block the swing of a 180° repositioned lever arm in any other myosin class. Furthermore, simply rotating the lever arm position by

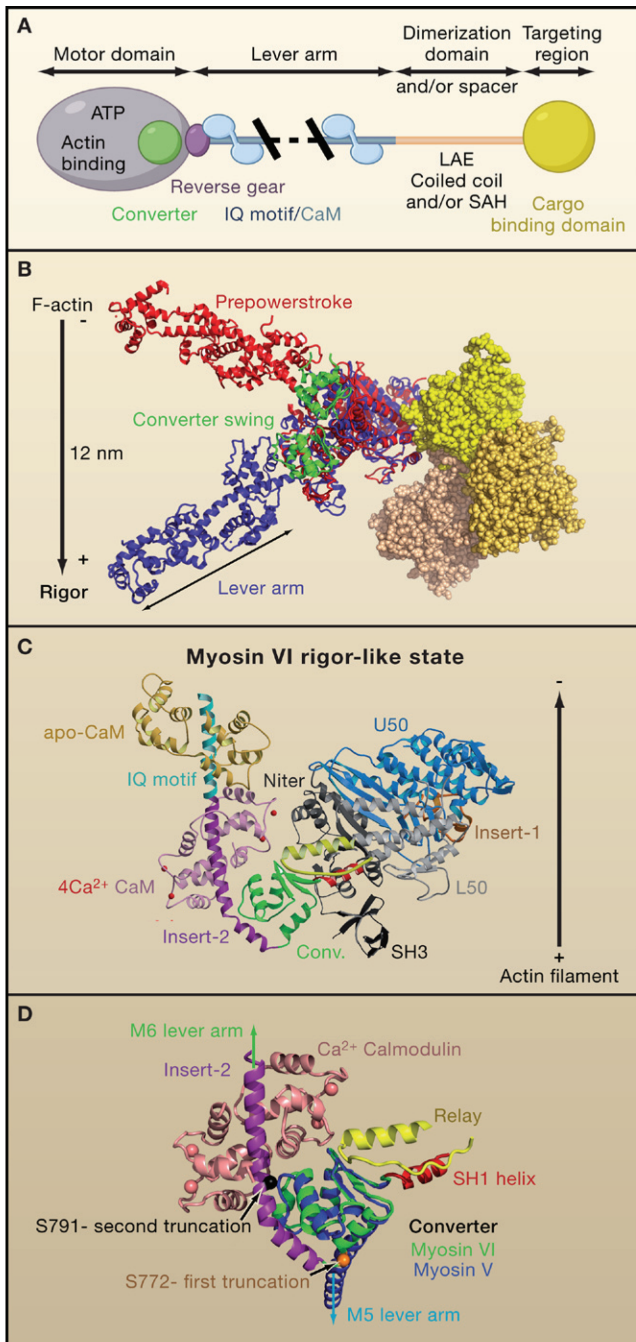

Figure 1. Mechanisms of Myosin Motor Movement

(A) Schematic of the organization of the myosin molecule. All myosins contain a motor domain with conserved elements able to bind ATP as well as more variable regions involved in actin binding. The C-terminal sequence of the motor domain forms a converter subdomain that plays a critical role in amplifying structural rearrangements of the motor domain and transmitting them to the adjacent region, the lever arm. The lever arm region is composed of a variable number of IQ motifs that form a helix that is the target for binding of calmodulin or specialized light chains of this superfamily. For myosin VI, there is an insertion between the converter and lever arm, denoted as the “reverse gear.” The rest of the sequence is variable, depending on the myosin class, but can contain coiled-coil sequences for dimerization and extended single alpha helix (SAH) domains. The regions nearest the C terminus play a role in targeting the myosin to specific cargos in the cell. The N-terminal motor domain also plays a role in targeting for a number of myosins.

(B) The myosin powerstroke illustrating the swinging lever arm hypothesis. Scallop striated muscle myosin II (S1) before the powerstroke (prepowerstroke) (1QVI; red) and afterward (rigor state) (1SR6; blue) are superimposed and docked on filamentous actin (F-actin, yellow). To visualize the swing, the converter, which controls the position of the lever arm, is indicated in green. The distance measured at the distal end of the lever arm is 12 nm for this fragment that contains two IQ motifs.

(C) Reversal of lever arm position in myosin VI. Shown is the structure of the motor domain and lever arm of myosin VI at the end of its powerstroke (rigor). The proximal portion of insert-2 (purple) introduces a bend in the final helix of the converter (green). In a plus-end-directed myosin, this helix continues unbroken and gives rise to the lever arm, as in (B) and (D).

(D) Details of the lever arm repositioning. The converter of myosin VI (green) is followed by a unique region called insert-2 (purple) that forms a kinked helix in order to redirect the lever arm position. The proximal region of insert-2 interacts with the converter in the structural states of myosin VI determined to date. The distal region of insert-2 binds a calmodulin (CaM, pink) with Ca^{2+} bound that interacts with the converter in the rigor state of the motor represented in this figure. This insert-2 sequence repositions the myosin VI lever arm (green arrow) by 120°, as compared to the myosin V lever arm direction (cyan arrow). A truncation at S772 (orange ball) causes reversal of the motor directionality, whereas truncation at S791 (black ball) maintains reverse directionality only if followed by a lever arm of at least one IQ motif in length (Ménétrey et al., 2007; Bryant et al., 2007; Park et al., 2007; Liao et al., 2009). Thus the proximal part of insert-2 is the minimum region necessary to reverse directionality.

180° places the end of the lever very close to the actin filament (Ménétrey et al., 2005), which could be problematic for cellular functions.

Lever arm repositioning is accomplished in myosin VI by a class-specific structural element, referred to as insert-2, which is in between the converter subdomain of the motor and the sole IQ motif (apo-CaM-binding site). This insert contains two parts. Its proximal part creates a bend of ~120° in the alpha helix that generally comes straight out of the converter to create the myosin lever arm (Figures 1C and 1D). This region makes specific interactions with the myosin VI converter. As predicted from the two myosin VI structures (Ménétrey et al., 2005, 2007) and confirmed in studies involving lever arm truncation and substitution (Bryant et al., 2007; Park et al., 2007; Liao et al., 2009), this region is sufficient to reverse directionality provided that it is followed by a lever arm of a minimal length. It is thus denoted as the “reverse gear” (Figure 1A).

The minimum length of the lever arm that is necessary for reversal of directionality is supplied by the second portion of insert-2, which is an unusual CaM-binding site (Bahloul et al., 2004). The CaM makes a number of interactions with the converter in the rigor state but may lose those interactions when the converter rearranges to create the prepowerstroke conformation. Although it has been shown (Liao et al., 2009) that this CaM is not necessary for movements of myosin VI in the absence of cargo it is unclear if the interactions are important once load is applied to the motor.

The 120° repositioning of the myosin VI lever arm avoids the steric clash with the N terminus of myosin VI that would have occurred with 180° repositioning, as described above. However, if the converter movements on actin were the same as in other myosins, then the powerstroke would be very small (~2.5 nm) and the end of the lever arm would be close to the actin filament at the beginning of the powerstroke (Ménétrey et al., 2005). Based on these considerations, we originally pro-

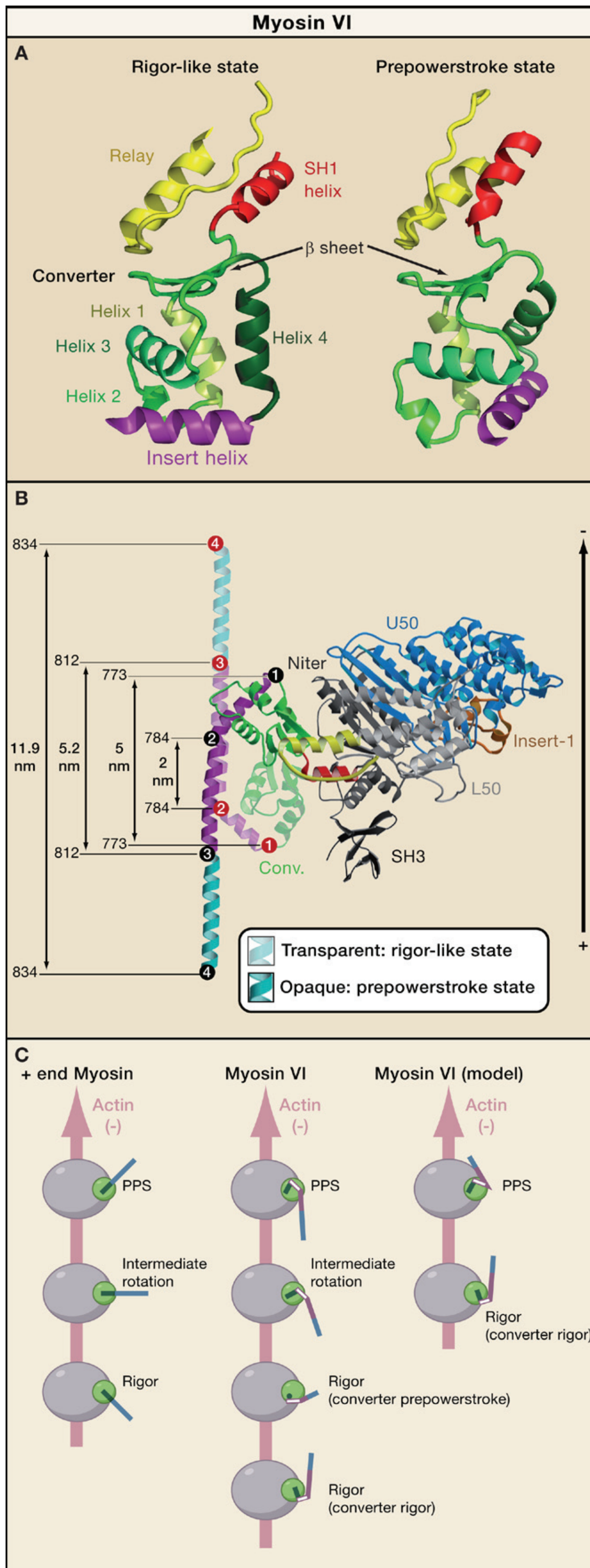

Figure 2. The Large Myosin VI Powerstroke

(A) Converter rearrangement in myosin VI. The adaptation that allows myosin VI truncated after the IQ motif to have a large powerstroke of 12 nm is a unique conformation of its converter in the prepowerstroke state. The rigor conformation of the myosin VI converter (left) is compared to the prepowerstroke conformation (right), as found in the myosin VI structure with ADP.P_i analogs bound. The lever arm is repositioned by a combination of the movement of the helices of the converter (indicated in shades of green) and of the proximal part of insert-2 (purple).

(B) Predicted stroke as a function of lever arm length. The structure of myosin VI in the prepowerstroke state (solid colors) is depicted as well as the position the converter/lever arm adopts at the end of the powerstroke (rigor-like state; transparent colors). Note that this converter rearrangement results in a predicted powerstroke of ~12 nm at the end of the IQ motifs, in agreement with measured values (Rock et al., 2005). Furthermore, the predicted stroke size and directionality from further truncations (as indicated) are in agreement with experimentally determined values (Bryant et al., 2007).

(C) Model of the myosin VI lever arm swing. Illustrated in the middle panel is a model showing the myosin VI powerstroke that begins (top) with the converter (in green with its last helix dark green) in a prepowerstroke conformation. The first helix of insert-2 is shown in open purple, the CaM-binding portion of insert-2 is closed purple, and the IQ motif is shown in cyan. The subsequent depictions show the lever arm after a half rotation of the converter and the lever arm after full converter rotation (rigor). The bottom illustration is of the complete lever arm swing after the converter rearranges to adopt its rigor conformation. Note that a component of the swing is into the plane of this figure, unlike that for a plus-end-directed myosin (left panel). In this model we assume that the converter does not rearrange to form its rigor conformation until it must do so due to steric hindrance (Ménétrey et al., 2007) near the end of its powerstroke. The swing keeps the myosin VI lever arm away from the actin filament, which would not be the case if the converter were to maintain a rigor conformation throughout the entire powerstroke (right panel).

posed that there would be a different rotation of the converter, as compared to plus-end myosins (Wells et al., 1999). We later proposed that mechanical uncoupling of the converter at the beginning of the powerstroke might be a simpler way to solve the problem (Ménétrey et al., 2005).

When a high-resolution structure of myosin VI was obtained at the beginning of the powerstroke (the prepowerstroke state) (Ménétrey et al., 2007), it was clear that we had not anticipated the manner in which myosin VI both increases the size of its powerstroke and moves the lever arm swing away from the actin filament. The myosin VI converter itself has been adapted to increase the stroke size via a rearrangement of the helices of the converter relative to each other in the prepowerstroke versus rigor-like states of the motor (Ménétrey et al., 2007; Figures 2A and 2B). This appears to create a lever arm swing that has an orthogonal component compared to the myosin powerstroke of other motors (Figure 2C), which keeps the end of the lever arm away from the actin filament. As previously noted (Ménétrey et al., 2007), this type of rearrangement would increase the stroke size of a plus-end-directed myosin as well. However, it remains to be demonstrated if this mechanism is ever exploited within other myosin classes.

Are there other myosins within the superfamily that move toward the minus ends of actin filaments? Using the search criteria of an insertion that could reposition the lever arm, there would appear to be at least one other strong candidate for reverse directionality, myosin XXX, among the known classes of myosins reported by Odronitz and Kollmar (2007). Perhaps some future study will evaluate this prediction.

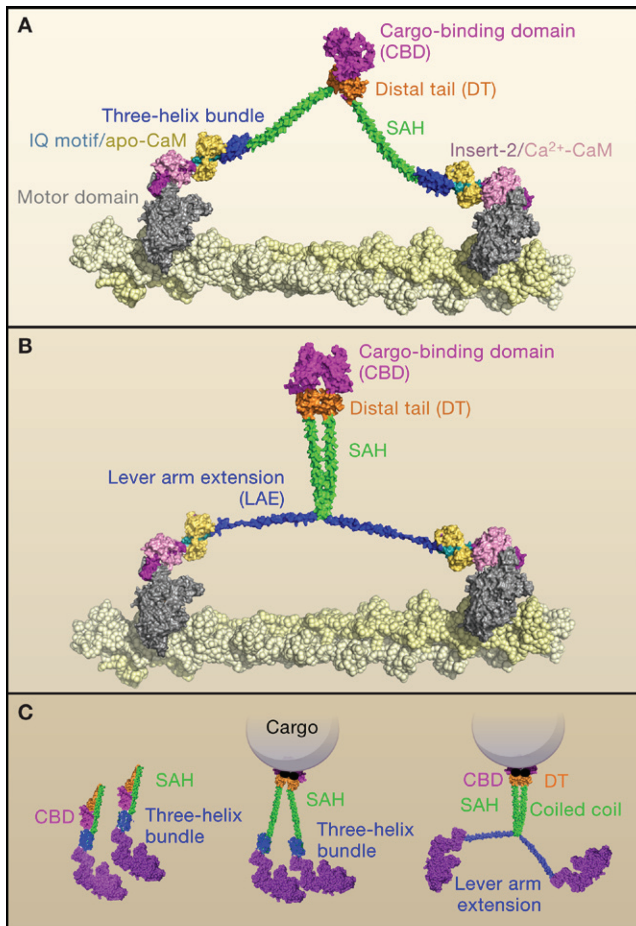

Figure 3. The Myosin VI Dimer

(A) Model of myosin VI dimer using stable single alpha helices (SAH) as lever arm extensions as proposed by Spink et al. (2008). To account for the 36 nm steps that the myosin VI dimer takes, Spink et al. (2008) propose that the SAH domain would be sufficiently stiff to provide a functional lever arm extension, in combination with the folded three-helix bundle domain (blue) that follows the myosin VI IQ domain. In this model, dimerization would only occur via interactions between the cargo-binding domains.

(B) Model of the dimerized molecule with unfolded three-helix bundle as lever arm extensions. This depicts a model of the myosin VI dimer consistent with the findings of Mukherjea et al. (2009). It illustrates that the lever arm extension (LAE, blue) is formed by unfolding of a three-helix bundle upon dimerization. The region immediately following the LAE participates in dimerization, as do segments of the distal tail (orange) and the cargo-binding domain (magenta). Most of the SAH acts as a spacer between motor and cargo. Thus the SAH is not contributing to the lever arm extension or to dimerization. In this model, the myosin VI heads through the first helix of the three-helix bundle are based on crystal structures (2BKH; 2V26; 3GN4) and the distal two-thirds of the cargo-binding domain is from the Dab2-tethered structure (3H8D; Yu et al., 2009).

(C) Model of cargo-induced dimerization. In this model the full-length myosin VI molecule primarily exists as a monomer folded in such a manner as to form intramolecular interactions involving the cargo-binding domain (magenta) that block potential dimerization sites, as shown in the first panel. This is consistent with small-angle X-ray scattering data obtained by Spink et al. (2008). The second panel illustrates that the unfolded monomers can be held in close proximity via tethering by an adaptor protein (black ovals) bound to a myosin VI cargo. The third panel depicts that this distal tethering of two cargo-binding domains allows internal dimerization (likely coiled coil) to occur immediately distal to the three-helix bundle, which drives its unfolding, forming an extension of the myosin VI lever arm (Mukherjea et al., 2009).

Stealth Dimerization

Reversible folding of dimeric myosins can provide a means to regulate their motor activity. This was first described for smooth muscle myosin II (Onishi and Wakabayashi, 1982; Sellers, 1991) and more recently has been demonstrated to occur for myosin V. Interactions between the myosin heads and cargo-binding domains have been revealed by high-resolution structures from electron microscopy and from models of the folded myosin V structure (Liu et al., 2006; Thirumurugan et al., 2006). The regulatory switch for unfolding and myosin V motor activity is cargo binding (Li et al., 2005), which is itself regulated by myosin phosphorylation (Karcher et al., 2001).

Cargo binding may be a common mechanism for regulating the activity of a number of myosin classes. What is emerging for some classes is a type of regulation and targeting involving dimerization that is induced by cargo binding. Perhaps not unexpectedly, there has been much debate as to whether these classes of myosins are in fact ever dimers in cells.

The surprising finding that full-length myosin VI isolated from cells is a monomer rather than a dimer (Lister et al., 2004) raised doubts as to the relevance of many of the initial results with myosin VI dimers (Lister et al., 2004). The earlier studies were performed on truncated myosin VI molecules forced to dimerize by the addition of either a GCN4 leucine zipper (Rock et al., 2001) or a myosin II coiled coil (Nishikawa et al., 2002) at the C terminus of a construct containing the motor domain, lever arm, and the predicted coiled coil. Lister et al. (2004) note that the region of the protein that was identified as coiled coil by prediction programs is in fact largely devoid of the necessary hydrophobic residues to form a coiled coil. This has subsequently been shown to also be the case for myosin VIIa and myosin X (Knight et al., 2005).

The predicted coiled-coil regions of these myosins contain alternating clusters of acidic and basic amino acids, consistent with a stable single alpha helix (SAH). This was first pointed out by Lister et al. (2004) but recently analyzed in more detail by Sivaramakrishnan et al. (2008). Further, it has been proposed that these SAH domains are sufficiently stiff to function as mechanical extensions of the myosin lever arms (Figure 3A; Knight et al., 2005; Sivaramakrishnan et al., 2008; Spink et al., 2008).

Inherent in the debate over whether or not the SAH domains do indeed function as lever arm extensions is the debate over whether or not myosins VI, VIIa, and X form dimers or not. This is a critical point to resolve given that much of the work with myosin VI (Rock et al., 2001; Nishikawa et al., 2002; Okten et al., 2004; Yildiz et al., 2004; Sweeney et al., 2007) and myosin VIIa (Yang et al., 2006) and recent work with myosin X (Nagy et al., 2008) have been performed on molecules that have been forced to dimerize by the addition of coiled coils at the truncated C termini of the constructs.

In the case of myosin VI the issue would now seem nearly resolved, with motor activity in cells being regulated by cargo-induced dimerization. It appears that myosin VI is normally a folded monomer (Lister et al., 2004; Spink et al., 2008) in cells unless it interacts with cargo. The ability of a dimeric adaptor

protein to initiate internal dimerization of myosin VI was anticipated based on a study that demonstrates the dimerization of full-length molecules when clustered at high density (Park et al., 2006). This is also the case for the dimeric adaptor protein optineurin (Phichith et al., 2009), which is involved in targeting myosin VI to the Golgi, where it is important for stabilization of Golgi structure and exocytosis (Sahlender et al., 2005).

Somewhat surprisingly, two studies find that fragments of the monomeric adaptor protein Dab2, which is involved in targeting of myosin VI for its role in clathrin-mediated endocytosis (Dance et al., 2004), can dimerize myosin VI (Yu et al., 2009; Phichith et al., 2009). Close proximity of myosin VI molecules, consistent with dimerization, had previously been detected on endocytic vesicles (Altman et al., 2007). The high-resolution structure of Dab2 bound to the cargo-binding domain of myosin VI reveals that the dimerization does not result from interactions between the cargo-binding domains themselves (Yu et al., 2009). Instead, the Dab2 peptide tethers the two cargo-binding domains in close proximity without any direct interactions between them. Presumably, this tethering will allow internal dimerization of more proximal myosin VI sequences (see below), analogous to the clustering experiments with full-length myosin VI (Park et al., 2006).

It remains to be clarified whether some cargos do not dimerize myosin VI and thus if it operates as a monomer for some cellular functions. Myosin VI has been shown to be capable of generating a large (~18 nm) powerstroke as a folded monomer (Lister et al., 2004) and an even larger powerstroke (~30 nm) for an unfolded monomer (Sivaramakrishnan et al., 2008). Intriguingly, a myosin VI monomer is capable of processively moving a 200 nm polystyrene bead with ~40 nm steps (Iwaki et al., 2006). However, this would likely not occur in the presence of load on the bead or vesicle in a cell. Recent work has demonstrated that at least four myosin VI monomers are required for long processive runs on cellular actin tracks (Sivaramakrishnan and Spudich, 2009). Ultimately, for myosin VI to function as a monomer in cells, the existence of monomeric cargos that do not tether two myosin monomers in close proximity is required. Alternatively, if the concentration of myosin VI were too low to saturate its cargo-binding sites, then it would function as a monomer, as seen in in vitro experiments in which only one of the two binding sites of optineurin is occupied by a myosin VI monomer (Phichith et al., 2009).

The dimerization of myosin VI induced by the binding of cargo parallels the phenomenon previously demonstrated for a kinesin family member, Kif1A (Tomishige et al., 2002). In fact, cargo-induced dimerization may represent a regulatory paradigm exploited by a subset of kinesins and myosins. Among the myosins, the three obvious candidates are those that contain SAH domains: VI, VIIa, and X.

Extending the Reach of Myosin VI

Another unusual feature of myosin VI is its lever arm. The conventional portion of the lever arm of myosin VI contains only the insert-2 CaM and a second CaM bound to a conventional IQ motif. However, myosin VI dimers can move processively (that is, as a single molecule) along actin filaments, with large (30–36 nm on average) but highly variable steps (Rock et al., 2001, 2005; Nishikawa et al., 2002). Therefore it has been a mystery how a myosin VI dimer could take steps as large as, or even larger than, myosin V, which has a lever arm composed of six CaM-binding IQ motifs. Such data appeared to represent a challenge to the lever arm hypothesis, ironic given that it was an extrapolation of the lever arm hypothesis that led to the discovery of the myosin VI directionality.

As noted above, one possible explanation for the large myosin VI step size is that the SAH domain of myosin VI, the longest found in any myosin, acts as a lever arm extension. Spink et al. (2008) propose such a model, which suggests that the sole point of internal dimerization is between the cargo-binding domains (Figure 3A). Even if there is not direct dimerization between cargo-binding domains, tethering via cargo adaptor proteins could potentially allow this structure to form (Yu et al., 2009).

The extension of the myosin VI lever arm has recently been shown to be comprised of a three-helix bundle that unfolds when myosin VI dimerizes (Mukherjea et al., 2009). It is dimerization that drives unfolding of the three-helix bundle to form the lever arm extension (Figure 3B). The unfolded bundle appears to maintain its helicity. The study of Mukherjea et al. (2009) further demonstrates that the dimerization of myosin VI occurs in a region preceding the SAH and immediately following the three-helix bundle (Figure 3B). Thus the SAH of myosin VI is not in position to extend the lever arm in the dimer and would simply act as a spacer between the motor domains and the cargo. Indeed removal of most of the SAH does not impact the average step size or step size distribution (Mukherjea et al., 2009). If under some conditions myosin VI functions as an extended monomer in cells, then the SAH could provide the major extension of the lever arm given that the three-helix bundle would remain folded (as depicted in Figure 3A), giving rise to a large powerstroke (Sivaramakrishnan et al., 2008). However, this would require an extension of the myosin VI monomer upon cargo binding given that the isolated compact myosin VI monomer has a powerstroke of 18 nm (Lister et al., 2004).

Talking Heads

For a dimeric myosin to be processive, each head must spend a high percentage of its actin-activated ATPase cycle strongly bound to actin in order to prevent simultaneous detachment of both heads. This property, known as a high duty ratio, is displayed by myosin VI (De La Cruz et al., 2001). A high duty ratio is sufficient for processive movement of a dimeric motor, or even monomeric motors coupled to the same cargo (Sivaramakrishnan and Spudich, 2009). However, the degree of processivity (as assessed by the average run length) can be further enhanced by communication between the heads, which is known as gating. The term gating refers to a phenomenon whereby intramolecular strain develops between the two heads of a dimer that is moving on an actin filament due to the relatively stiff linkage between the lever arms (Figure 4). This strain prevents completion of the lever arm movement (powerstroke) of the lead head, stalling it in a state that cannot release from actin. For plus-end-directed myosins, this is generally thought to be a state with MgADP trapped at the active site (Figure 4).

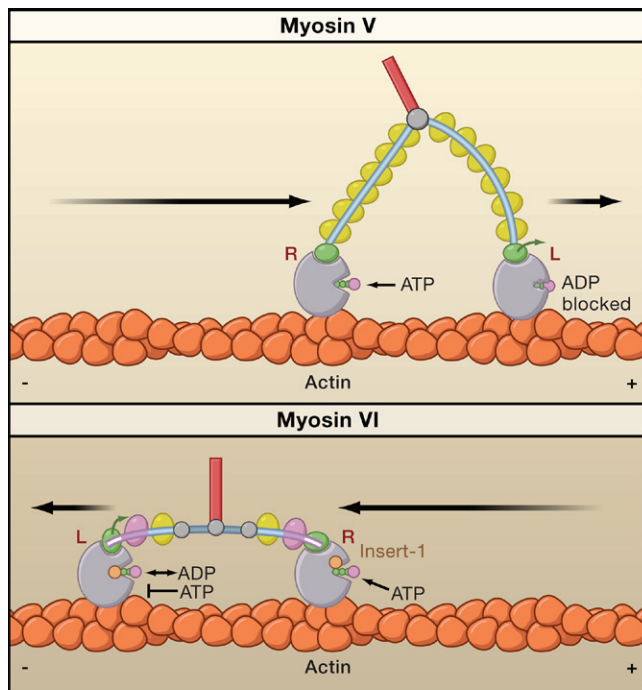

Figure 4. Gating between Two Myosin Heads of a Processive Dimer
Given that myosin V and myosin VI move in opposite directions, the gating mechanism allowing communication between the two heads of these processive motors must differ. Myosin motor domains are indicated in gray with the converter subdomain in green. The lever arm of myosin V is composed of six IQ motifs (cyan) to which six apo-calmodulins (yellow) are bound. The lever arm of myosin VI is composed of a unique insert-2 sequence (purple) that binds calmodulin with Ca^{2+} bound, a single IQ motif (cyan) with apo-calmodulin bound (yellow), followed by a lever arm extension (blue). A dimerization region is also represented (red). In myosin V, the lead head (L) cannot release ADP until the rear head releases ADP, binds ATP, and detaches from F-actin (brown line). In myosin VI, the lead head can release ADP and rebind ADP, but ATP binding is prevented until the rear head detaches. Another unique class-specific sequence of myosin VI, insert-1, is found near the nucleotide pocket (see also Figure 1C) and provides strain-dependent slowing of ATP binding.

The structural basis for gating in myosin VI is an unresolved question. Due to the repositioning of its lever arms, the strain experienced by each head within the processive myosin VI dimer will be reversed, as compared to plus-end-directed myosins such as myosin V (Figure 4). Thus rearward strain would not be communicated to the nucleotide-binding pocket in such a manner as to slow the release of MgADP. The kinetics of the initial encounter of a myosin VI dimer with actin reveals that the product release steps from both heads are as fast as for the unloaded monomer (Sweeney et al., 2007). Instead of MgADP release being gated by intramolecular strain (as for a plus-end-directed myosin), ATP binding to the lead head is blocked by intramolecular strain, whereas MgADP is free to rebind (Sweeney et al., 2007). This gating requires a second structural element specific to myosin VI, insert-1 (Figure 4), which is in position to obstruct entry of the gamma phosphate of ATP into the nucleotide-binding pocket (Ménétrey et al., 2005; Sweeney et al., 2007).

How the strain is transmitted to insert-1 of the lead head of myosin VI is not at all understood. Two studies have examined the motions of the myosin VI lever arms during processive movement on actin (Sun et al., 2007; Reifenberger et al., 2009) at low ATP concentrations and conclude that both lever arm positions are at ~180° (Figure 3B), essentially parallel to the actin filament. However, whether this is the only angle seen, as described by Reifenberger et al. (2009), or whether the angles can be variable, as reported by Sun et al. (2007), is a source of controversy. In either case, this is a very different result as compared to myosin V (Toprak et al., 2006), where the lever arm of the lead head moves part of the way through its powerstroke before being stalled by intramolecular strain (Figure 4). The intramolecular strain that stalls the lever arm of the lead head of myosin V is transmitted to the nucleotide-binding elements, preventing them from rearranging and releasing MgADP. With MgADP trapped in the lead head, ATP cannot bind and dissociate the head.

In contrast, the results of Reifenberger et al. (2009) suggest that the lever arm of the lead head of myosin VI does not move from its initial prepowerstroke position (Figure 3B), despite the fact that the lead head is attached to actin and generates force. Whatever the mechanism that constrains the lever arm of the lead head it must also position insert-1 so that ATP binding, but not ADP binding, is blocked. The lead head is known to release ADP at a rate that is similar to the unstrained rate (Sweeney et al., 2007). This does not imply that the lead head is not experiencing intramolecular strain. However, as Oguchi et al. (2008) demonstrate, load has a minimal effect on ADP dissociation from a myosin VI monomer, whereas it accelerates ADP binding. Thus strain has the opposite effect on ADP affinity of the myosin VI motor when compared to that of myosin V (Oguchi et al., 2008). At this point, it is unclear how strain is transmitted to the nucleotide-binding elements of any myosin, and thus there are no mechanistic insights into the differential effects seen for myosin V and VI.

A recent paper (Iwaki et al., 2009) demonstrates that reverse strain on a myosin VI head accelerates binding to actin, which was interpreted as accelerating phosphate release from the head. Further, it would imply that in a processive dimer, phosphate release from the lead head should be accelerated by intramolecular strain. This is not contradictory to the reported kinetics of the myosin VI dimer (Sweeney et al., 2007), given that faster release from the lead head could not have been measured. If reverse strain greatly accelerates the release of phosphate from the lead head of a processive dimer, this could be important for myosin VI function. As previously shown (Rock et al., 2001; Nishikawa et al., 2002; Park et al., 2007), the myosin VI step size is highly variable. The acceleration of strong actin binding by rearward strain would increase the probability of a lead head binding strongly to actin when the distance between the heads is sufficient to create intramolecular strain, which would allow gating. This also will increase the probability of large steps with the desired directionality.

It is unknown if this "search and capture" mechanism (Iwaki et al., 2009) for the myosin VI head is a general feature of myosins. The myosin VI lever arm is positioned such that reverse strain on a myosin VI head would amount to forward strain on the head of a plus-end-directed myosin (Figure 4). Thus the significance, or even existence, of the mechanism beyond myosin VI is unclear. As this "search and capture" mechanism

would be advantageous for all myosin motors, it is important for future studies to examine a plus-end-directed myosin to ascertain if this is a general feature of myosin motors.

What Is the Purpose of Myosin SAH Domains?

For myosin VI, experimental data rule out a role of the SAH domain as an extension of the lever arm in the dimer, as discussed above. Presumably, the dimerization of myosin VI proximal to the SAH that unfolds the three-helix bundle involves a short segment of coiled coil, but this has yet to be established. There is also the possibility of a short segment of coiled coil that precedes the SAH domain found in myosin VIIa. Furthermore, the lever arm of myosin VIIa, with its five IQ motifs, is sufficient to explain its observed step size (Yang et al., 2006) without any need for a lever arm extension.

In the case of myosin X, its three IQ motifs are immediately followed by what appears to be an SAH with no possibility of intervening dimerization. Thus in this myosin the SAH likely does function as a lever arm extension, as proposed from studies with myosin X monomers (Knight et al., 2005). Assuming that myosin X is more likely to function as a dimer rather than as a monomer in cells, then what is the purpose of the SAH as a lever arm extension? Why does myosin X simply not add a fourth IQ motif? The twenty residues of SAH of myosin X would be ~3 nm in length, as opposed to the 3.5 nm for an IQ motif, so the lengths are similar. Perhaps the SAH has some role in allowing myosin X dimers to preferentially traffic on bundles of actin, rather than on single actin filaments (Nagy et al., 2008).

If the SAH domains of myosins VI and VIIa do not function as lever arm extensions, then what purpose do they serve? Modeling suggests that the SAH of myosin VI could act as a scaffold for folding the monomer (Spink et al., 2008). In the case of *Drosophila* myosin VIIa, recent findings show that the full-length monomer folds tightly and has little activity (Yang et al., 2009). However, the protein unfolds and is activated at high actin concentrations by the weak (~30 μM) interaction of the C-terminal FERM domain with actin. This provides a possible mechanism for myosin VIIa to diffuse in an inactive form to regions where actin filaments are abundant and then unfold and crosslink the actin filaments due to binding via the motor and FERM domains. The authors go on to conjecture that myosin VIIa has no region capable of internal dimerization and therefore would be acting as a monomer in cells unless some as yet unknown cargo-binding protein can dimerize it. This is surprising, as an earlier study by this group (Yang et al., 2006) points out that a myosin VIIa construct truncated at the end of the putative SAH/coiled coil will indeed form a processive dimer when the monomers are clustered.

Regulation via Cargo-Induced Dimerization

All of these observations beg the question as to whether or not myosins VI, VIIa, or X ever function as monomers in cells. Functional dimerization (that is, generation of processive dimers) has been shown for both VI and VIIa, using high-density clustering of truncated constructs without cargo-binding domains (Park et al., 2006; Yang et al., 2006). We propose a general model of regulation via cargo-induced dimerization for these three myosin classes. This model is mostly conjecture for myosins VIIa and X but is supported by data for myosin VI (Mukherjea et al., 2009; Phichith et al., 2009; Yu et al., 2009). The first aspect of the model is that all three of these myosin classes exist as nonfunctional, folded monomers in cells unless bound to a cargo. Once two monomers are held closely together by binding to cargo, these myosin classes function as dimers (likely most of the time, if not solely). As they all are highly processive as dimers, there must be internal dimerization that is proximal to the cargo-binding region and that immediately follows their effective lever arms in order to allow gating between the heads to occur. But in order to exist in cells as monomers, the coiled-coil regions of myosins VI, VIIa, and X are short and relatively weak, preventing constitutive dimerization. Internal dimerization only occurs when the monomers are held in close apposition by cargo binding, creating a high effective concentration. Furthermore, cargo binding likely promotes unfolding of the monomer, exposing the internal dimerization region (Park et al., 2006; Mukherjea et al., 2009; Phichith et al., 2009). This model is depicted in Figure 3C.

A question of great relevance to cell biology is what is the purpose of designing motors that work optimally as dimers if they only dimerize when bound to cargo? The answer may be that diffusion through cortical actin networks in cells is more efficient as monomers for classes of myosin motors whose targets/cargos are found at the cell periphery (such as myosins VI, VIIa, and X). Once they bind to their target/cargo, they can internally dimerize and function as processive, gated dimers. In the myosin VI or VIIa dimer, the SAH plays the role of a spacer between the cargo and the two heads. However, for myosins VI and VIIa, the true role of the SAH may be to serve as a scaffold for folding the monomers, as previously suggested (Mukherjea et al., 2009; Phichith et al., 2009). This is consistent with a proposed model for the folded monomer of myosin VI (Spink et al., 2008). Monomer folding could be an additional role for the SAH of myosin X.

For myosin VIIa, monomer folding may allow it to preferentially reach areas with a high density of actin filaments (Yang et al., 2009), such as the stereocilia of hair cells (Hasson et al., 1997). A similar mechanism may allow myosin X to reach the actin bundles at the base of filopodia (Berg and Cheney, 2002) before forming a functional dimer that is required to traffic toward the filopodia tip (Tokuo et al., 2007). For many of the functions of myosin VI, its cargo, or binding target, is located at the plasma membrane. Myosin VI must diffuse through a network of cortical actin in order to reach these targets, and thus a compact structure will diffuse more readily. This may be one reason that a "normal" lever arm consisting of multiple calmodulin/light chain-binding sites is not used by myosin VI, even though single molecules of engineered myosin VI can function in vitro with either myosin V lever arms (Park et al., 2007) or extended lever arms that are entirely artificial (Liao et al., 2009). Diffusion through the actin network would likely be extremely difficult if the myosin VI had to diffuse as an active dimer with the lever arm extensions unfolded. Not only would the size pose a problem, but as soon as the myosin VI encountered an actin filament, it would walk processively away from the plasma membrane. The fact that the full-length monomer is not inactive but maintains actin-activated ATPase activity

may be advantageous, given that reversible actin interactions of the monomer could create facilitated diffusion of myosin VI, concentrating it in regions where filamentous actin (F-actin) is most abundant.

Are Gating and Dimerization Necessary for Cellular Functions?

A recent study addresses the importance of dimerization of myosin VI during spermatid individualization in *Drosophila melanogaster* (Noguchi et al., 2009). A forced dimer, in which a leucine zipper is introduced between the SAH and distal tail, provides limited rescue of function. Although interpreted as evidence that myosin VI does not function as a dimer during actin cone stabilization (Noguchi et al., 2009), the inability of a constitutive dimer of myosin VI to stabilize actin cones alternatively could be interpreted as evidence that myosin VI must traffic to its target as a monomer before undergoing dimerization. Thus the results of the study could be interpreted as evidence for the need for regulated dimerization, rather than dimerization not being necessary. Clearly further experimentation is needed.

Noguchi et al. also address the importance of gating. Although none of the mutant myosin VI constructs in the study rescue function to the same degree as wild-type, the construct that best rescues function is the one that lacks insert-1. This deletion kept overall myosin VI geometry intact but without any gating between the heads, based on earlier kinetic studies (Sweeney et al., 2007). This suggests that gating may not be essential but is necessary to optimize the function of myosin VI during actin cone stabilization.

Another recent study would argue that neither gating nor dimerization are essential for the processive movements of myosin VI on the actin cytoskeleton. Sivaramakrishnan et al. (2008) examined movements of quantum dots transported by either two myosin VI dimers or four myosin VI monomers on the surface of an actin cytoskeleton derived from fish keratinocytes. They observe identical straight, 10 μm movements in either case. Thus the high duty ratio of each myosin VI head is sufficient to enable processive movements of multiple myosin VI motors.

A cellular role in which gating (and thus dimerization) is essential has been demonstrated by Hertzano et al. (2008), who show that a missense mutation in the myosin VI motor that results in loss of gating in the dimer (based on in vitro measurements) causes deafness in mice. The cause of deafness is the loss of the stereocilia (bundles of polarized actin filaments designed to sense different frequencies of sound) and the subsequent death of the cochlear hair cells. This recapitulates what is seen in mice that are null for myosin VI (Avraham et al., 1995). Thus gating may be essential for myosin VI function in cellular processes that must be precisely tuned, such as maintenance of the stereocilia.

Although the discussion to this point has focused on gating as a means to increase the processive movement of myosin VI, myosin VI can also act as a load-dependent anchor in vitro (Altman et al., 2004). This would allow it in essence to crosslink its cargo to an actin filament when under high loads and undoubtedly would be critically dependent upon gating between the heads (Sweeney et al., 2007). It has been argued that the role of myosin VI in the maintenance of stereocilia is most likely as a load-dependent anchor (Frank et al., 2004; Sweeney and Houdusse, 2007). Thus gating of myosin VI heads may be more important for its anchoring function than for processive movement in a cell. Indeed, recent studies have revealed that multiple myosin VI monomers, which would not experience gating, can processively move a cargo in a manner similar to myosin VI dimers (Sivaramakrishnan and Spudich, 2009). On the other hand, as noted above, a myosin VI dimer that is defective in gating is incapable of stabilizing stereocilia in mouse hair cells, resulting in deafness (Hertzano et al., 2008).

Conclusions and Perspectives

In rewriting the rules for myosin motors, myosin VI has undergone a number of adaptations that allow it to function as a reverse-direction, processive motor as well as a load-dependent anchor. Among these are repositioning of the lever arm, gating of the lead head of a dimer that involves a mechanism to block ATP binding, a converter that rearranges, and an unprecedented lever arm design that encompasses a three-helix bundle that can be induced to unfold. Although there has been much progress in establishing these mechanisms, there are unresolved questions and controversies surrounding the details of the head gating, the nature of dimerization of the molecule, and under what conditions in cells the molecule functions as a dimer. It is also unclear how many of the new features uncovered during the examination of myosin VI may be used by a subset of other myosin superfamily members to optimize their cellular functions.

The cellular roles described for myosin VI encompass diverse processes, including endocytosis (Hasson, 2003; Aschenbrenner et al., 2004; Dance et al., 2004), Golgi function (Sahlender et al., 2005), maintenance of stereocilia (Avraham et al., 1995: Self et al., 1999), cytokinesis (Arden et al., 2007), cell adhesion (Millo et al., 2004), nuclear transcription (Vreugde et al., 2006), tumorigenesis (Yoshida et al., 2004; Jung et al., 2006), and localization to neuronal growth cones (Suter et al., 2000). The details of what precise type of function, either processive transporter or load-dependent anchor (or both), that myosin VI is performing in these cellular processes are not clear. To date, the only cellular function for which myosin VI has been proposed to serve primarily as a processive transporter is vesicle transport during endocytosis (Hasson, 2003; Aschenbrenner et al., 2004; Dance et al., 2004). For this transport role, it appears that either a myosin VI monomer or dimer is sufficient, whereas anchoring roles that require gating require dimerization of myosin VI. Ultimately, the binding partners and their proximity to each other will determine whether myosin VI functions as a monomer or dimer in cells. What is lacking are cellular studies designed to distinguish which role(s) myosin VI is performing, and whether it is acting as a monomer or dimer in the many cellular tasks it can perform due to its unique design and functionalities.

ACKNOWLEDGMENTS

H.L.S. is supported by grants from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) and the National Institute on Deafness and Other Communication Disorders (NIDCD). A.H. is supported by grants from the CNRS, the HFSPO, the ANR blanche BLAN07-3_193368, and the ACI BCMS.

REFERENCES

Altman, D., Sweeney, H.L., and Spudich, J.A. (2004). The mechanism of myosin VI translocation and its load-induced anchoring. Cell *116*, 737–749.

Altman, D., Goswami, D., Hasson, T., Spudich, J.A., and Mayor, S. (2007). Precise positioning of myosin VI on endocytic vesicles in vivo. PLoS Biol. *5*, e210.

Arden, S.D., Puri, C., Au, J.S., Kendrick-Jones, J., and Buss, F. (2007). Myosin VI is required for targeted membrane transport during cytokinesis. Mol. Biol. Cell *18*, 4750–4761.

Aschenbrenner, L., Naccache, S.N., and Hasson, T. (2004). Uncoated endocytic vesicles require the unconventional myosin, Myo6, for rapid transport through actin barriers. Mol. Biol. Cell *15*, 2253–2263.

Avraham, K.B., Hasson, T., Steel, K.P., Kingsley, D.M., Russell, L.B., Mooseker, M.S., Copeland, N.G., and Jenkins, N.A. (1995). The mouse Snell's waltzer deafness gene encodes an unconventional myosin required for structural integrity of inner ear hair cells. Nat. Genet. *11*, 369–375.

Bahloul, A., Chevreux, G., Wells, A.L., Martin, D., Nolt, J., Yang, Z., Chen, L.Q., Potier, N., Van Dorsselaer, A., Rosenfeld, S., et al. (2004). The unique insert in myosin VI is a structural calcium-calmodulin binding site. Proc. Natl. Acad. Sci. USA *101*, 4787–4792.

Berg, J.S., and Cheney, R.E. (2002). Myosin-X is an unconventional myosin that undergoes intrafilopodial motility. Nat. Cell Biol. *4*, 246–250.

Bryant, Z., Altman, D., and Spudich, J.A. (2007). The power stroke of myosin VI and the basis of reverse directionality. Proc. Natl. Acad. Sci. USA *104*, 772–777.

Cramer, L.P., Siebert, M., and Mitchison, T.J. (1997). Identification of novel graded polarity actin filament bundles in locomoting heart fibroblasts: implications for the generation of motile force. J. Cell Biol. *136*, 1287–1305.

Dance, A.L., Miller, M., Seragaki, S., Aryal, P., White, B., Aschenbrenner, L., and Hasson, T. (2004). Regulation of myosin-VI targeting to endocytic compartments. Traffic *5*, 798–813.

De La Cruz, E.M., Ostap, E.M., and Sweeney, H.L. (2001). Kinetic mechanism and regulation of myosin VI. J. Biol. Chem. *276*, 32373–32381.

Frank, D.J., Noguchi, T., and Miller, K.G. (2004). Myosin VI: a structural role in actin organization important for protein and organelle localization and trafficking. Curr. Opin. Cell Biol. *16*, 189–194.

Hasson, T. (2003). Myosin VI: two distinct roles in endocytosis. J. Cell Sci. *116*, 3453–3461.

Hasson, T., Gillespie, P.G., Garcia, J.A., MacDonald, R.B., Zhao, Y., Yee, A.G., Mooseker, M.S., and Corey, D.P. (1997). Unconventional myosins in inner-ear sensory epithelia. J. Cell Biol. *137*, 1287–1307.

Hertzano, R., Shalit, E., Rzadzinska, A.K., Dror, A.A., Song, L., Ron, U., Tan, J.T., Shitrit, A.S., Fuchs, H., Hasson, T., et al. (2008). A Myo6 mutation destroys coordination between the myosin heads, revealing new functions of myosin VI in the stereocilia of mammalian inner ear hair cells. PLoS Genet. *4*, e1000207.

Holmes, K.C., and Geeves, M.A. (1999). Structural mechanism of muscle contraction. Annu. Rev. Biochem. *68*, 687–728.

Iwaki, M., Tanaka, H., Iwane, A.H., Katayama, E., Ikebe, M., and Yanagida, T. (2006). Cargo-binding makes a wild-type single-headed myosin-VI move processively. Biophys. J. *90*, 3643–3652.

Iwaki, M., Iwane, A.H., Shimokawa, T., Cooke, R., and Yanagida, T. (2009). Brownian search-and-catch mechanism for myosin-VI steps. Nat. Chem. Biol. *5*, 403–405.

Jung, E.J., Liu, G., Zhou, W., and Chen, X. (2006). Myosin VI is a mediator of the p53-dependent cell survival pathway. Mol. Cell. Biol. *26*, 2175–2186.

Karcher, R.L., Roland, J.T., Zappacosta, F., Huddleston, M.J., Annan, R.S., Carr, S.A., and Gelfand, V.I. (2001). Cell cycle regulation of myosin-V by calcium/calmodulin-dependent protein kinase II. Science *293*, 1317–1320.

Knight, P.J., Thirumurugan, K., Xu, Y., Wang, F., Kalverda, A.P., Stafford, W.F., 3rd, Sellers, J.R., and Peckham, M. (2005). The predicted coiled-coil domain of myosin 10 forms a novel elongated domain that lengthens the head. J. Biol. Chem. *280*, 34702–34708.

Li, X.D., Ikebe, R., and Ikebe, M. (2005). Activation of myosin Va function by melanophilin, a specific docking partner of myosin Va. J. Biol. Chem. *280*, 17815–17822.

Liao, J.C., Elting, M.W., Delp, S.L., Spudich, J.A., and Bryant, Z. (2009). Engineered myosin VI motors reveal minimal structural determinants of directionality and processivity. J. Mol. Biol. *392*, 862–867.

Lister, I., Schmitz, S., Walker, M., Trinick, J., Buss, F., Veigel, C., and Kendrick-Jones, J. (2004). A monomeric myosin VI with a large working stroke. EMBO J. *23*, 1729–1738.

Liu, J., Taylor, D.W., Krementsova, E.B., Trybus, K.M., and Taylor, K.A. (2006). Three-dimensional structure of the myosin V inhibited state by cryoelectron tomography. Nature *442*, 208–211.

Ménétrey, J., Bahloul, A., Wells, A.L., Yengo, C.M., Morris, C.A., Sweeney, H.L., and Houdusse, A. (2005). The structure of the myosin VI motor reveals the mechanism of directionality reversal. Nature *435*, 779–785.

Ménétrey, J., Llinas, P., Mukherjea, M., Sweeney, H.L., and Houdusse, A. (2007). The structural basis for the large powerstroke of myosin VI. Cell *131*, 300–308.

Millo, H., Leaper, K., Lazou, V., and Bownes, M. (2004). Myosin VI plays a role in cell-cell adhesion during epithelial morphogenesis. Mech. Dev. *121*, 1335–1351.

Mooseker, M.S., and Tilney, L.G. (1975). Organization of an actin filament-membrane complex. Filament polarity and membrane attachment in the microvilli of intestinal epithelial cells. J. Cell Biol. *67*, 725–743.

Mukherjea, M., Llinas, P., Kim, H.J., Travaglia, M., Safer, D., Ménétrey, J., Franzini-Armstrong, C., Selvin, P.R., Houdusse, A., and Sweeney, H.L. (2009). Myosin VI dimerization triggers an unfolding of a three-helix bundle in order to extend its reach. Mol. Cell *35*, 305–315.

Nagy, S., Ricca, B.L., Norstrom, M.F., Courson, D.S., Brawley, C.M., Smithback, P.A., and Rock, R.S. (2008). A myosin motor that selects bundled actin for motility. Proc. Natl. Acad. Sci. USA *105*, 9616–9620.

Nishikawa, S., Homma, K., Komori, Y., Iwaki, M., Wazawa, T., Hikikoshi Iwane, A., Saito, J., Ikebe, R., Katayama, E., Yanagida, T., and Ikebe, M. (2002). Class VI myosin moves processively along actin filaments backward with large steps. Biochem. Biophys. Res. Commun. *290*, 311–317.

Noguchi, T., Frank, D.J., Isaji, M., and Miller, K.G. (2009). Coiled-coil-mediated dimerization is not required for myosin VI to stabilize actin during spermatid individualization in Drosophila melanogaster. Mol. Biol. Cell *20*, 358–367.

Odronitz, F., and Kollmar, M. (2007). Drawing the tree of eukaryotic life based on the analysis of 2,269 manually annotated myosins from 328 species. Genome Biol. *8*, R196.

Oguchi, Y., Mikhailenko, S.V., Ohki, T., Olivares, A.O., De La Cruz, E.M., and Ishiwata, S. (2008). Load-dependent ADP binding to myosins V and VI: implications for subunit coordination and function. Proc. Natl. Acad. Sci. USA *105*, 7714–7719.

Okten, Z., Churchman, L.S., Rock, R.S., and Spudich, J.A. (2004). Myosin VI walks hand-over-hand along actin. Nat. Struct. Mol. Biol. *11*, 884–887.

Onishi, H., and Wakabayashi, T. (1982). Electron microscopic studies of myosin molecules from chicken gizzard muscle I: The formation of the intramolecular loop in the myosin tail. J. Biochem. *92*, 871–879.

Park, H., Ramamurthy, B., Travaglia, M., Safer, D., Chen, L.Q., Franzini-Armstrong, C., Selvin, P.R., and Sweeney, H.L. (2006). Full-length myosin VI dimerizes and moves processively along actin filaments upon monomer clustering. Mol. Cell *21*, 331–336.

Park, H., Li, A., Chen, L.Q., Houdusse, A., Selvin, P.R., and Sweeney, H.L.

(2007). The unique insert at the end of the myosin VI motor is the sole determinant of directionality. Proc. Natl. Acad. Sci. USA *104*, 778–783.

Phichith, D., Travaglia, M., Yang, Z., Liu, X., Zong, A.B., Safer, D., and Sweeney, H.L. (2009). Cargo binding induces dimerization of myosin VI. Proc. Natl. Acad. Sci. USA *106*, 17320–17324.

Reifenberger, J.G., Toprak, E., Kim, H.J., Safer, D., Sweeney, H.L., and Selvin, P.R. (2009). Myosin VI undergoes a 180° power stroke implying an uncoupling of the front lever arm. Proc. Natl. Acad. Sci. USA, in press.

Rock, R.S., Rice, S.E., Wells, A.L., Purcell, T.J., Spudich, J.A., and Sweeney, H.L. (2001). Myosin VI is a processive motor with a large step size. Proc. Natl. Acad. Sci. USA *98*, 13655–13659.

Rock, R.S., Ramamurthy, B., Dunn, A.R., Beccafico, S., Rami, B.R., Morris, C., Spink, B.J., Franzini-Armstrong, C., Spudich, J.A., and Sweeney, H.L. (2005). A flexible domain is essential for the large step size and processivity of myosin VI. Mol. Cell *17*, 603–609.

Sahlender, D.A., Roberts, R.C., Arden, S.D., Spudich, G., Taylor, M.J., Luzio, J.P., Kendrick-Jones, J., and Buss, F. (2005). Optineurin links myosin VI to the Golgi complex and is involved in Golgi organization and exocytosis. J. Cell Biol. *169*, 285–295.

Self, T., Sobe, T., Copeland, N.G., Jenkins, N.A., Avraham, K.B., and Steel, K.P. (1999). Role of myosin VI in the differentiation of cochlear hair cells. Dev. Biol. *214*, 331–341.

Sellers, J.R. (1991). Regulation of cytoplasmic and smooth muscle myosin. Curr. Opin. Cell Biol. *3*, 98–104.

Sivaramakrishnan, S., and Spudich, J.A. (2009). Coupled myosin VI motors facilitate unidirectional movement on an F-actin network. J. Cell Biol. *187*, 53–60.

Sivaramakrishnan, S., Spink, B.J., Sim, A.Y., Doniach, S., and Spudich, J.A. (2008). Dynamic charge interactions create surprising rigidity in the ER/K {alpha}-helical protein motif. Proc. Natl. Acad. Sci. USA *105*, 13356–13361.

Small, J.V., Isenberg, G., and Celis, J.E. (1978). Polarity of actin at the leading edge of cultured cells. Nature *272*, 638–639.

Small, J.V., Herzog, M., and Anderson, K. (1985). Actin filament organization in the fish keratocyte lamellipodium. J. Cell Biol. *129*, 1275–1286.

Spink, B.J., Sivaramakrishnan, S., Lipfert, J., Doniach, S., and Spudich, J.A. (2008). Long single α-helical tail domains bridge the gap between structure and function of myosin VI. Nat. Struct. Mol. Biol. *15*, 591–597.

Sun, Y., Schroeder, H.W., 3rd, Beausang, J.F., Homma, K., Ikebe, M., and Goldman, Y.E. (2007). Myosin VI walks "wiggly" on actin with large and variable tilting. Mol. Cell *28*, 954–964.

Suter, D.M., Espindola, F.S., Lin, C.H., Forscher, P., and Mooseker, M.S. (2000). Localization of unconventional myosins V and VI in neuronal growth cones. J. Neurobiol. *42*, 370–382.

Sweeney, H.L., and Houdusse, A. (2007). What can myosin VI do in cells? Curr. Opin. Cell Biol. *19*, 57–66.

Sweeney, H.L., Park, H., Zong, A.B., Yang, Z., Selvin, P.R., and Rosenfeld, S.S. (2007). How myosin VI coordinates its heads during processive movement. EMBO J. *26*, 2682–2692.

Thirumurugan, K., Sakamoto, T., Hammer, J.A., 3rd, Sellers, J.R., and Knight, P.J. (2006). The cargo-binding domain regulates structure and activity of myosin 5. Nature *442*, 212–215.

Tilney, L.G., Derosier, D.J., and Mulroy, M.J. (1980). The organization of actin filaments in the stereocilia of cochlear hair cells. J. Cell Biol. *86*, 244–259.

Tokuo, H., Mabuchi, K., and Ikebe, M. (2007). The motor activity of myosin-X promotes actin fiber convergence at the cell periphery to initiate filopodia formation. J. Cell Biol. *179*, 229–238.

Tomishige, M., Klopfenstein, D.R., and Vale, R.D. (2002). Conversion of Unc104/KIF1A kinesin into a processive motor after dimerization. Science *297*, 2263–2267.

Toprak, E., Enderlein, J., Syed, S., McKinney, S.A., Petschek, R.G., Ha, T., Goldman, Y.E., and Selvin, P.R. (2006). Defocused orientation and position imaging (DOPI) of myosin V. Proc. Natl. Acad. Sci. USA *103*, 6495–6499.

Tsiavaliaris, G., Fujita-Becker, S., and Manstein, D.J. (2004). Molecular engineering of a backwards-moving myosin motor. Nature *427*, 558–561.

Vreugde, S., Ferrai, C., Miluzio, A., Hauben, E., Marchisio, P.C., Crippa, M.P., Bussi, M., and Biffo, S. (2006). Nuclear myosin VI enhances RNA polymerase II-dependent transcription. Mol. Cell *23*, 749–755.

Wells, A.L., Lin, A.W., Chen, L.Q., Safer, D., Cain, S.M., Hasson, T., Carragher, B.O., Milligan, R.A., and Sweeney, H.L. (1999). Myosin VI is an actin-based motor that moves backwards. Nature *401*, 505–508.

Yang, Y., Kovács, M., Sakamoto, T., Zhang, F., Kiehart, D.P., and Sellers, J.R. (2006). Dimerized Drosophila myosin VIIa: a processive motor. Proc. Natl. Acad. Sci. USA *103*, 5746–5751.

Yang, Y., Baboolal, T.G., Siththanandan, V., Chen, M., Walker, M.L., Knight, P.J., Peckham, M., and Sellers, J.R. (2009). A FERM domain autoregulates Drosophila myosin 7a activity. Proc. Natl. Acad. Sci. USA *106*, 4189–4194.

Yildiz, A., Park, H., Safer, D., Yang, Z., Chen, L.Q., Selvin, P.R., and Sweeney, H.L. (2004). Myosin VI steps via a hand-over-hand mechanism with its lever arm undergoing fluctuations when attached to actin. J. Biol. Chem. *279*, 37223–37226.

Yoshida, H., Cheng, W., Hung, J., Montell, D., Geisbrecht, E., Rosen, D., Liu, J., and Naora, H. (2004). Lessons from border cell migration in the Drosophila ovary: A role for myosin VI in dissemination of human ovarian cancer. Proc. Natl. Acad. Sci. USA *101*, 8144–8149.

Yu, C., Feng, W., Miyanoiri, Y., Wen, W., Zhao, Y., and Zhang, M. (2009). Myosin VI undergoes cargo-mediated dimerization. Cell *138*, 537–548.