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What can myosin VI do in cells?

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The recently solved structure of the myosin VI motor demonstrates that the unique insert at the end of the motor is responsible for the reversal of the normal myosin directionality. A second class-specific insert near the nucleotide-binding pocket contributes to myosin VI's unique kinetic tuning, allowing it to function either as an actin-based transporter or as an anchoring protein. Recent biochemical and biophysical studies have shown that the native molecule can form dimers upon clustering, and cell biological studies have demonstrated that it clearly does play both transport and anchoring roles in cells. These mechanistic insights allow us to speculate on how unusual aspects of myosin VI structure and function allow it to fill unique niches in cells.

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Introduction

Myosin VI is a myosin superfamily member with unique and intriguing features that allow it to fill a still-expanding number of cell biological roles. This actin-based motor produces force that acts towards the minus end of actin filaments, which is the opposite direction to all other characterized myosins [1]. Within the last two years, there have been structural, biophysical and biochemical studies that largely explain the mechanism by which myosin VI moves in the opposite direction to other myosin motors. Studies have also demonstrated that dimeric myosin VI can function *in vitro* either as a highly efficient transporter or as an actin-based anchor. There have also been new cell biological studies that definitively demonstrate that it plays both of these roles in cells. We review these findings, as well as other unique features of the myosin VI molecule, and postulate how these features enable and limit possible cellular functions.

Cell biological roles of myosin VI

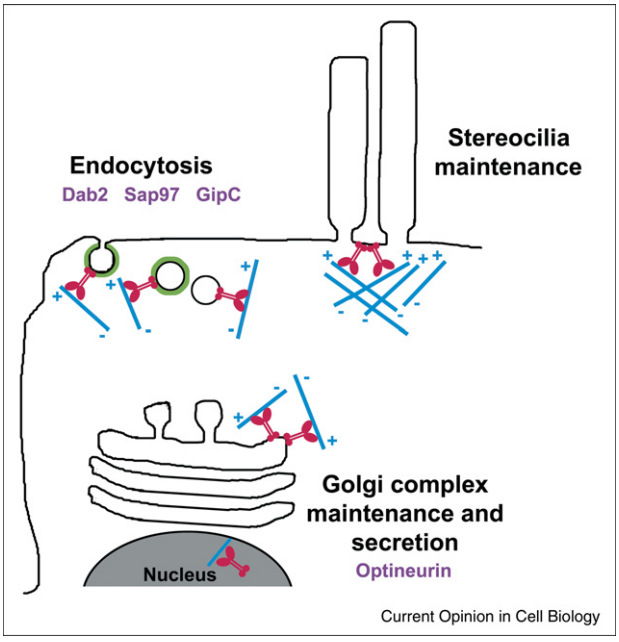
The myosin VI class was originally discovered in *Drosophila melanogaster* [2]. It was isolated as an F-actin binding protein that was released from actin by ATP, and was subsequently cloned from a cDNA expression library. In the initial characterization, it was noted that expression of myosin VI (referred to as *Drosophila* 95F myosin heavy chain) was widespread during development and in the adult, with differential expression of multiple splice forms [2]. Since that time, myosin VI has been proposed to play several roles in *D. melanogaster* during spermatogenesis [3,4••] and asymmetric cell division, including a role in spindle orientation [3]. Subsequent to its discovery in *Drosophila*, it has been found to be expressed in species ranging from *C. elegans* to human [5–7].

In mammalian cells, myosin VI is localized to endocytic vesicles, membrane ruffles, the cytosol and the Golgi complex [3,8] (see Figure 1). Its motor function is essential for several physiological functions of the cell, including normal rates of endocytosis [9,10,11••], maintenance of Golgi morphology and protein secretion [12]. The myosin-VI gene is defective in the deaf mouse, Snell's waltzer [6], where it was demonstrated that myosin-VI is required for the function of sensory hair cells; it appears to have a structural (anchoring) role in maintaining the stereocilia [3,13]. In humans, mutations in myosin VI cause hereditary hearing loss (DFNA22 and DFNB37 syndromes), which can be associated with hypertrophic cardiomyopathy [14,15]. Moreover, myosin VI regulates epithelial cell migration [16] and plays a role in the maintenance of adhesive cellular contacts within epithelial cell layers [17]. It is highly expressed in ovarian cancers [18] and its expression level, which is upregulated by DNA damage in a p53-dependent manner [19], correlates with the potential of the tumor to disseminate [18]. Myosin VI is involved in receptor clustering, and recently it has been shown that myosin VI is also present in the nucleus of mammalian cells, where it modulates the RNA polymerase II-dependent transcription of active genes [20•].

Targeting of myosin VI

Some of the adaptor proteins that recruit myosin VI to cellular targets have been identified (see [8] for a comprehensive review). It was recently demonstrated that optineurin is essential for myosin VI localization at the Golgi complex [21••], and binds to a site within the globular tail of myosin VI (Figure 2a). Sap97 [22] and Dab2 [23,24] mediate the recruitment of myosin VI to clathrin-coated pits and vesicles while GIPC recruits it to uncoated vesicles, allowing myosin

Figure 1



Proposed roles for myosin VI in mammalian cells. Both transporting (endocytosis) and anchoring (Golgi and stereocilia maintenance) roles are schematized. Myosin VI (red) moves toward the minus end of actin filament (blue). It powers several steps of endocytosis including the formation and movement of coated and uncoated vesicles via several adapters such as Dab2, Sap97 (coated vesicles, green) and GipC (uncoated vesicles). It is also important for the formation and maintenance of stereocilia in the inner ear hair cells. It localizes to the actin-dense cuticular plate region at the base of the stereocilia, where it appears to anchor the plasma membrane to the actin filaments. On the Golgi complex, myosin VI, via its adaptor optineurin, is likely to be involved in a tug-of-war with microtubule-based motors. This function is essential for the maintenance of Golgi size and for secretion. The mechanisms by which myosin VI fulfils these functions are largely unknown.

VI-mediated transport of these vesicles through the actin dense meshwork [10,11••].

Targeting Myosin VI to its different cargos may be modulated by tissue-specific differential splicing of regions within the tail [8,10,25•], resulting in variably expressed insertions at two sites (Figure 2a). A large insertion (LI) can be found next to the cargo binding globular tail, and has been postulated to alter folding back of the tail onto the rest of the molecule [8]. This splice-form is expressed in polarized epithelial cells and preferentially targets to Dab2 on clathrin-coated vesicles. The targeting is more likely to be mediated by affecting the overall conformation of the full-length molecule than by a binding site within the large insertion, as it can only target the full-length molecule [25•]. Additionally, the LI splice-form will bind to GIPC on uncoated vesicles in cells that do not express Dab2 [25•]. Overexpression of Dab2 in those same cells redirects the LI splice-form to Dab2-containing coated vesicles and away from GIPC on uncoated vesicles. The small insertion (SI) is found within the globular tail, and may be involved in targeting [8].

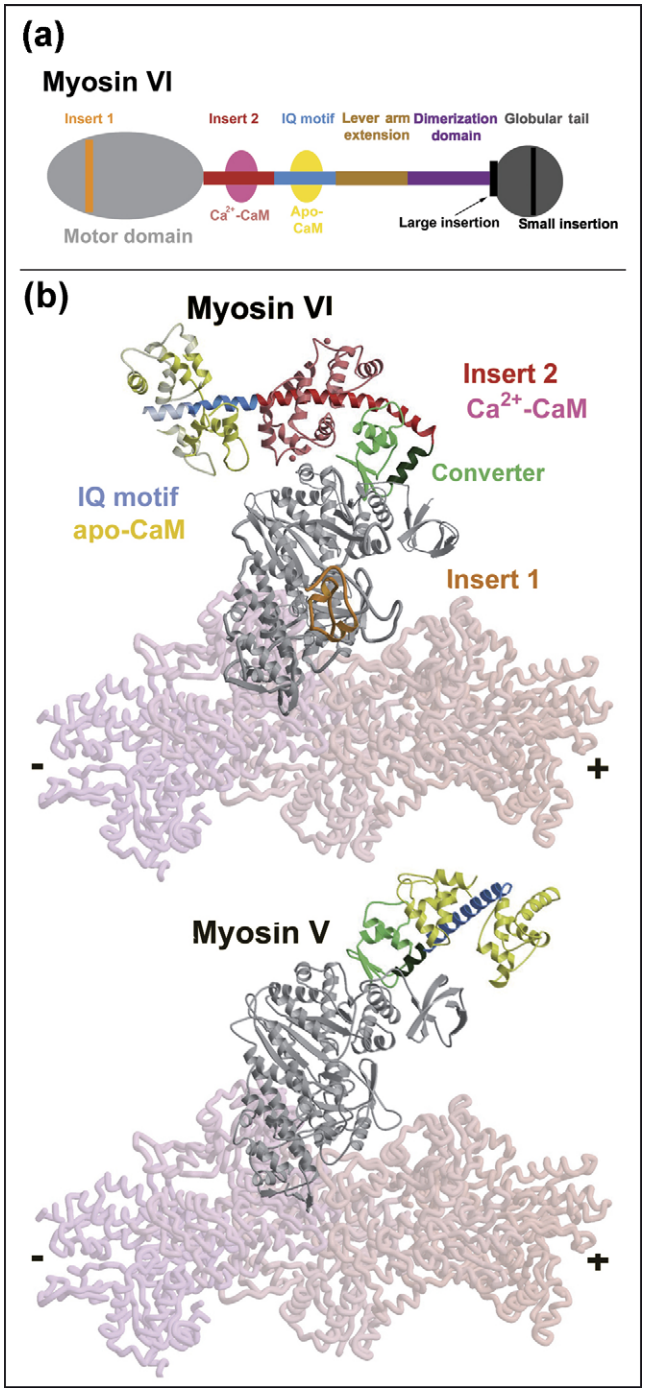
Structure of the myosin VI head

Myosin VI has two constitutively expressed, class-specific inserts within its motor domain (Figure 2). As revealed in the recently published high-resolution structure of the motor and lever arm of myosin VI [26••], and shown in Figure 2b, the first (insert 1) is in the upper 50 kDa subdomain near the nucleotide binding element, switch I. This insert slows the rate of ATP binding [26••], which is likely to be necessary for the mechanical coordination ('gating') of the two heads of a native myosin VI dimer during processive movement, and for ensuring that ATP does not rapidly dissociate myosin VI from actin during its role as an anchor [27••]. The second unique insert (insert 2) is part of a rather divergent converter domain and contains a unique CaM binding site [26••,28]. Based on speculation that myosin VI may possess an altered converter domain with repositioned lever arm, we initially hypothesized and then demonstrated that myosin VI had achieved reversal of directionality [1]. Indeed, additional *in vitro* assays confirmed the reverse directionality, but studies with chimeric myosins called into question whether or not the altered converter was in fact responsible for reversing direction [29]. Experiments with redesigned chimeras, based on the structure of myosin VI [30••], and experiments with truncated versions of monomeric myosin VI [31••] have recently revealed that insert 2 is in fact the sole determinant of directionality reversal, and that without insert 2, myosin VI is a plus-end directed motor.

Myosin VI is processive and takes surprisingly large steps

Myosin VI, like myosin V, does not form filaments and, for many of its cell biological roles, is likely to function as one of a few motors on a given cargo. Like myosin V, it is a processive motor [32,33] (i.e. the dimer undergoes multiple steps along an actin filament without detaching) that requires both a high duty ratio (i.e. the motor must spend the majority of its catalytic cycle strongly bound to actin) and mechanical coordination between the heads [34]. Not only is the myosin VI dimer processive, but it can take extremely large steps (an average of 30–36 nm) [32,33,35•,36•,37]. This was surprising, considering that, as depicted in Figure 2, it has a short lever arm. It was also demonstrated in all studies [32,33,35•,36•,37] that the distribution of step sizes is quite large compared to myosin V. This has been taken to indicate decoupling of insert 2 from the converter and/or the presence of a flexible element that extends the reach of the conventional lever arm. Indeed, it was demonstrated that a region following the IQ-CaM does extend the reach (increase the step size) of the myosin VI motor [38••]. We now refer to this as the lever arm extension (it was

Figure 2



The structure of myosin VI. **(a)** Schematic of the organization of the full-length myosin VI molecule. The motor domain contains two conserved, class-specific inserts. Insert 1 (orange in the diagram) is located near the nucleotide-binding pocket, and insert 2 (red) at the end of the motor. Insert 2 contains a calmodulin (CaM)-binding motif, and is followed by a second CaM-binding motif (IQ motif). This short lever arm is followed by a lever arm extension of unknown length and structure, which increases the myosin VI step size. Next is a region containing a putative coiled coil (dimerization), which in turn is followed by the globular tail. Several tail splice-forms exist, which involve a large insertion immediately preceding the globular tail, or a second, smaller insertion within the globular tail. **(b)** High-resolution structure of the nucleotide-free myosin VI (top) and myosin V (below) motor domains docked onto actin (pale purple). The converter (green) with its last helix (black) is found at a similar position in the two motors. Directionality is reversed in myosin VI by insert 2 (red) and its associated Ca^{2+}-CaM (pink).

initially referred to as the proximal tail), and label it as such in Figure 2a. It was postulated that this was an extensible element, which would account for the variability of the myosin VI step size [38••]. Surprisingly, in the recent study in which insert 2 was removed and the lever arm replaced with the myosin V lever arm, resulting in reversal of myosin VI direction (toward the plus end), the step size distribution remained nearly identical to that of wild type myosin VI [30••]. Thus, the variability of the step size is inherent to the properties of the motor itself. This further implies that the lever arm extension is not a flexible linker, which would more easily explain the fact that the myosin VI step size is not load-dependent at loads less than the stall force [27••]. The mechanism underlying myosin VI's large step size remains unsolved and is a topic of ongoing research.

Is a unique form of gating needed for reverse processivity and anchoring?

Like myosin V, a myosin VI dimer contains two motor domains that alternate binding and release from actin in a hand-over-hand fashion [35•,36•] to generate processive movement of a single molecule. In both myosin V and myosin VI, there are kinetic specializations necessary to optimize this processive movement (i.e. increase the number of steps). First, the individual motor domains must possess high duty ratios: that is, the motor must spend the majority of its catalytic cycle strongly bound to actin so that the dimer does not dissociate and diffuse away. To further increase the degree of processivity, there must be a mechanism of communication between the two heads [39,40•]. One way of achieving communication (gating) is to utilize the intramolecular strain generated when both heads are strongly bound to actin. For myosin V, gating involves 'stalling' the lead head in a strongly bound state until the rear head can detach from actin. This is achieved by greatly slowing the release of ADP from the lead head until the rear head is released by ATP binding [40•].

Myosin VI dimers are processive [32,33] and display gating [34,41]. Owing to its reverse directionality, Myosin VI cannot achieve this by the same mechanism as myosin V or other processive classes of myosin. Based on the findings for myosin V [40•,42,43] and the reversal of the myosin VI lever arm position, one would predict that intramolecular strain might slow ADP release from the rear head of myosin VI, while either having no effect on the lead head or slightly accelerating its release. This arrangement would abolish processive movement, as the lead head would detach from actin before the rear head.

Clearly, myosin VI must have evolved a novel mechanism to circumvent this problem.

Clues to the mechanism of gating used by myosin VI come from a series of optical trap experiments with single molecules [27••]. They demonstrate that reverse (plus-end-directed) strain on a myosin VI dimer accelerates ADP binding, and further slows the already slow ATP binding [27••]. The net result is that ADP out-competes ATP for the active site of a myosin VI head, which greatly retards ATP from inducing the head's detachment from actin. Thus, as the amount of reverse strain, or load, on myosin VI is increased, the length of time it stays bound to an actin filament increases. This would allow the dimer to play a structural role (actin anchoring) under high loads. These unusual nucleotide-binding properties are due, at least in part, to the unique insert near the nucleotide-binding pocket, insert 1 [26••].

The same properties that allow myosin VI to be an efficient anchor could also be used to gate (stall) the lead head of a processive myosin VI dimer until the rear head detaches. In this model, both heads can release their ATP hydrolysis products and attach strongly to actin during processive movement, as is seen for myosin V [40•]. The lead head is stalled because it experiences reverse strain, and therefore, as shown by Altman *et al.* [27••], it will readily rebind ADP and not bind ATP until the strain is relieved (i.e. the rear head detaches). Thus there may be a common mechanism for gating the lead head during processive movement and anchoring under high loads.

An alternative mechanism has been proposed for myosin VI processive movement in which intramolecular strain is not generated. In this model, the lead head is prevented from releasing its ATP hydrolysis products and binding strongly to actin until the rear head detaches [34,39]. This model of gating, with the lead head weakly attached to actin, would predict a lower degree of processivity (i.e. a shorter run-length on an actin filament) than our model in which the lead head binds strongly to actin and is gated by prevention of ATP binding. Since we have recently observed that the average run length at physiological ATP concentrations for full-length dimeric myosin VI is over a micron [44••] (~30 steps), which is greater than the run-lengths seen with myosin V [45], an efficient gating mechanism must exist for myosin VI. Furthermore, a model in which the lead head is not attached strongly to actin would predict that the step size of the dimer would decrease as a function of load (below the stall force), which it does not [27••]. However, further kinetic experiments are needed to delineate the detailed mechanism of myosin VI gating.

Two heads are better than one

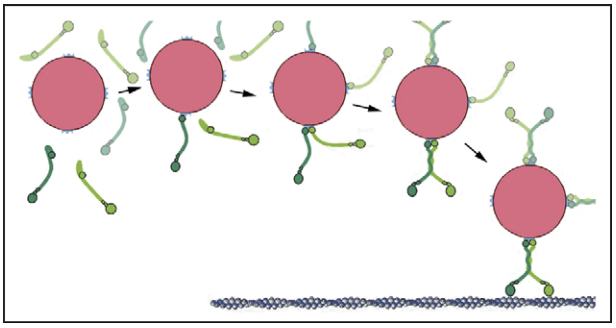
To further add to the intrigue of myosin VI, it was recently shown that native myosin VI does not form a dimer, is therefore not processive, and displays an unusually large stroke size indicative of a long lever arm [46••]. (Stroke size is the term used to describe the distance that the lever arm of an individual myosin head [measured at the end of the lever arm] can swing, while step size refers to the distance between the actin binding sites occupied by the two heads of a dimer as it 'walks' along an actin filament.) However, myosin VI can only be a processive motor and optimally function as an anchor when forced to dimerize. More recently, it was demonstrated that full-length native myosin VI can dimerize, and function as a dimer that has properties nearly identical to those of a forced dimer, if two molecules are held in close proximity [44••] (see Figure 3). The obvious implication is that binding to cargo (especially a dimeric cargo) would lead to dimerization of myosin VI *in vivo*. It was also proposed [44••] that other members of the myosin superfamily (myosin VII and myosin X) might undergo this cargo-regulated dimerization, on the basis of sequence similarities in the putative dimerization domains [47]. Evidence for this has now been obtained for myosin VIIa [48•].

Perhaps myosin VI, which is normally a monomer, can function both as a monomer and as a dimer in cells. However, on the basis of the functional properties of myosin VI that we have characterized, it is difficult to imagine that any of the proposed roles would be better served by a monomer. The dimer would more efficiently anchor and is a faster and more efficient transporter. That is not to say that myosin VI could not function as a monomer. It could do so, but at a much higher density of motors. However, if the local monomer concentration is high, then dimerization may occur.

Regulation of myosin VI

To date, there has been no demonstration of on/off regulation of the motor activity of myosin VI, as there has been for myosin V [49••,50••]. Increasing calcium concentration to >10 μM slows the motor activity and destroys coordination between the heads of a dimer, which would terminate a processive run. It is conceivable (but untested) that a sudden increase in calcium concentration could also disrupt anchoring. But the assumption in the literature has been that some yet-to-be-discovered on/off switch must exist for myosin VI. There are several putative phosphorylation sites in myosin VI, but none of these (apart from threonine 406, which has no measurable impact on steady state ATPase activity [41]) are in position to regulate motor activity directly. Recently, overexpression of a GFP-fusion of myosin VI with threonine 406 mutated to glutamate (406E) to mimic phosphorylation, or to alanine (406A) to mimic dephosphorylated myosin, revealed clustering of actin filaments by vesicle-associated 406E, but not 406A [51•]. This was cited as evidence that a phosphorylation event can promote anchoring by myosin VI. It has been shown that the kinetics of the 406E mutant differ from those of the 406A mutant [41]. Whether or not this truly

Figure 3



Dimerization of myosin VI: cargo-mediated dimerization hypothesis. Binding of myosin VI monomers (green) to cargo (indicated as binding to a dimeric adaptor protein in cyan) could alter the conformation of the molecule, possibly exposing the high probability coiled-coil region (dimerization domain). At the same time, close proximity of two monomers bound to cargo initiates dimerization. Once dimerized, the myosin VI can move a vesicle processively toward the minus-end of an actin filament, using a hand-over-hand motion. If the myosin VI cargo is anchored in a membrane, as is thought to be the case at the base of stereocilia [6,8,9,53•], then the myosin VI would exert force on the actin filament and hold it in close apposition to the membrane. Actin-induced dimerization might also be possible in some instances, as has been proposed to occur in the *Drosophila* investment cone [4••]. If monomeric myosin VI molecules bind in close proximity on actin filaments, they may dimerize and move processively towards the minus end of the filaments. Upon reaching the end, they would either dissociate, or possibly cross-link the branched filaments found at the minus end of the investment cone.

mimics phosphorylation is unclear. While the 406E mutant displays accelerated phosphate release compared to the 406A mutant, this difference was not seen when the wild type (406T) myosin VI was phosphorylated [41]. However, the more important functional difference between the 406E and 406A mutants, which has not been examined in the phosphorylated wild type protein, is the approximately three-fold increase in ADP affinity (from 30 μM to 9 μM) for the 406E mutant; this change, which is attributable to an increase in the ADP association rate, would have physiological impact in a cell. This would slow dissociation of a bound myosin VI monomer, perhaps promoting dimerization of two monomers. Could this play a role in the EGF-stimulated recruitment of myosin VI to the leading edge of cells, which is correlated with phosphorylation of Thr406 [52]? Potentially, phosphorylation could also increase the processive run-length of a dimer, and/or lower the stall force, which would promote anchoring.

Is cargo-mediated dimerization a novel form of regulation?

An indirect type of regulation that could depend on phosphorylation of sites in the tail of myosin VI would involve folding, as is seen with the constitutively dimeric myosins, smooth and non-muscle myosin II and myosin V. While this is a formal possibility, and could be more prevalent in some splice forms than others, the observation remains that native myosin VI purified from cells is an unregulated monomer [46••].

However, if myosin VI is normally a monomer unless bound to a cargo or target, is it necessary to regulate its motor activity, or is regulation of dimerization sufficient? While there would be an energetic cost associated with having an active monomer free to diffuse in cells, the benefit would be that the reversible ATP-driven interaction with actin would provide facilitated diffusion of myosin VI into areas of the highest F-actin concentration. Thus myosin VI would be most available to interact with cargo in the areas where F-actin is abundant.

If myosin VI were a constitutive dimer, then its diffusion in cells would be quite limited without regulation of motor activity, since every time it encountered an actin filament, it would walk a considerable distance along that filament before falling off. If the actin filaments are polarized with the minus ends away from the membrane, then myosin VI would never diffuse to its target sites at the cell membrane. In fact for any dimeric, processive myosin, efficient diffusion in the cytoplasm would require that the motor activity be regulated, or that diffusion take place as a monomer. Myosin V can diffuse as a dimer in the cytoplasm when not bound to cargo because the dimer forms a folded complex that inhibits motor activity [49••,50••]. Both of the heads of myosin V are still free to interact with actin, but this interaction does not initiate walking along the filament. This could enable facilitated diffusion of the myosin V folded-dimer into the regions of highest F-actin concentration, similar to our proposal for the full-length myosin VI monomer, if the myosin V is blocked from hydrolyzing ATP [50••].

Regulated dimerization of myosin VI might represent a new paradigm within the myosin superfamily that could extend to myosins VIIa and X. It has been speculated [8,44••] that this may allow myosin VI to work either as monomers or dimers in cells. While this is still a possibility, we now propose an alternative hypothesis: that all of these myosin classes function primarily as dimers in cells, but that the regulation of dimerization via cargo interaction replaces the need for regulating motor activity.

Did myosin VI evolve to be an anchor?

As discussed above, myosin VI possesses kinetic properties that are well suited to an anchoring role in cells [27••]. While there are other myosins with kinetics that would facilitate anchoring, in a plus-end directed motor this involves greatly slowing ADP release, which also would slow movement. Myosin VI is unique in that its structural and kinetic properties would optimize both a transport and an anchoring role.

However, initial *in vitro* studies with myosin VI seemed to indicate that it was not highly processive [32,33], and took

only six to eight steps before detaching from actin. Partly on the basis of this fact, it was speculated that all of the cell biological functions of myosin VI could be best accomplished by its role as an anchor [3]. Our more recent work has demonstrated that in single molecule *in vitro* assays, the full-length myosin VI dimer is capable of considerably longer run lengths (greater than a micron; >30 steps) than the zippered dimer [44••]. (The zippered dimer was formed by removal of the cargo-binding domain and addition of a leucine zipper to insure constitutive dimerization.) This does not seem due to altered kinetics, and may be related to the fact that, in rotary shadowing EM, the full-length molecule appears to be more compact than the zippered dimer [44••]. This might allow it to move under an anchored actin filament and avoid contacting the underlying substrate. This physical limitation of the *in vitro* assays may lead to an underestimate of processive run lengths for all processive myosins. Recently we have observed (PR Selvin and HL Sweeney, unpublished) that increasing the ADP concentration in a range likely to be found in cells (up to 100 μM) further increases the average run length of myosin VI and individual runs of over 3 μm (>100 steps) are observed. Thus myosin VI is well suited to perform a transport role in situations where it moves against loads that are less than its stall force.

Of the many cellular roles proposed to date for myosin VI, only a role in endocytosis has been definitively shown to involve a transport function, although it could be working as a transporter in several other situations [53•,54]. The work of Aschenbrenner *et al.* [11••] quantified endocytic vesicle movements, and demonstrated that they travel only short distances before apparently detaching, reattaching and resuming movement. While the calculated viscous drag on the vesicles is small compared to the myosin VI stall force [27••], vesicles may become physically entrapped in a dense actin network or actin-binding sites may not be readily accessible to the detached head of a processive dimer. This could lead to dissociation of myosin VI from its actin track, perhaps preceded by a prolonged period of stall. Aschenbrenner *et al.* [11••] also observed what they termed 'vesicle stretching', which may be due to myosin VI pulling on a trapped vesicle, or could be due to myosin VI motors that are moving away from each other on two different actin filaments.

From the first time we assayed the ATPase activity of a myosin VI dimer [34], it was evident that unlike myosin V dimers, which readily cross-link actin at low actin concentrations [40•], myosin VI dimers do not cross-link. (In these assays, myosin V rapidly begins to bundle actin filaments, which is easily seen in EM, and is manifested as decreasing ATPase activity.) This would seem problematic for the proposed cell biological functions of myosin VI that require it to function as an actin cross-linker (see below). However, lack of cross-linking would facilitate transport in a dense actin network, as it would tend to keep the myosin VI dimer processing along a single actin filament. Crossing over to another filament at an oblique angle to the first would risk cross-linking and stalling movement. This has been proposed to occur in the case of myosin V transport of melanosomes, and may be exploited to 'capture' the melanosomes in the cortical actin network [55].

Having both heads bound to the same actin filament would also be ideal for a true anchoring role, and recapitulates the *in vitro* geometry in the optical trap that demonstrated anchoring [27••]. The case of stereocilia may be the best example of this; see Figure 1. The proposed role, based on myosin VI localization and the impact of myosin VI mutations in both mice and zebrafish [3,13,53•], is to anchor the apical membrane between individual stereocilia to the cuticular plate, as indicated in Figure 1. The optimal geometry would be to have individual dimers attached by their cargo-binding tails to a target in the membrane, and for them then to attempt to pull that target along actin filaments with their minus ends pointing away from the membrane. As the myosin VI moves away from the membrane, it will eventually stop and anchor when the load exceeds the stall force. This anchoring can be maintained for long periods of time that could be increased by further elevations in ADP concentration and possibly by phosphorylation at Thr406 [51•]. Binding of the two heads of a dimer to two different actin filaments could also lead to stall if the load on the rear head exceeds the stall force. However, unless the filaments are rigidly anchored relative to each other, motions of the filaments might relieve the strain and promote dissociation of the myosin from actin. Thus, a much more efficient anchor would entail both heads remaining on the same filament.

While it has been proposed that monomers of myosin VI may also play an anchoring role [8], it would not be possible for a single monomer to do so. Anchoring of a target would require the binding of multiple monomers, and each individual monomer would have to be externally loaded within the time of an unloaded duty cycle (~200 msec), as in optical trap experiments, or it would simply dissociate. In the cell, this could only be accomplished by the actions of another motor protein(s), which could be additional myosin VI monomers. However, given the unique kinetics of gating within the dimer, a single dimer, or multiple dimers, would be more likely to provide efficient anchoring than multiple monomers.

Why does myosin VI not use a conventional lever arm?

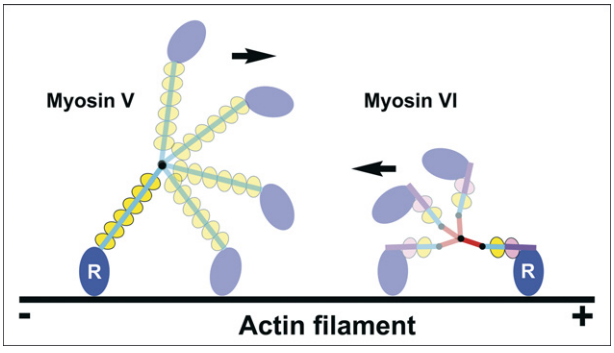
Why is it that myosin VI does not cross-link filaments under conditions where myosin V does? While the answer to this question is not known, we speculate that the

answer lies in answering another question. Why does myosin VI have a lever arm that is unlike that of any other myosin? All myosin classes are thought to have lever arms that amplify the motions of the motor core. The myosin V motor has a lever arm that is long enough (six calmodulin binding sites) to allow it to walk with 36 nm steps along an actin filament. Myosin VI takes approximately the same size steps, but, as diagrammed in Figure 4, has a short lever arm comprised of two calmodulins [26••,28], followed by a short extension.

The work of Rock *et al.* [38••] demonstrated that the region C-terminal to the IQ-motif of myosin VI extends the reach of the short lever arm. While it could be that some aspect of directionality reversal requires an alternative type of lever arm, we suggest that the hybrid lever arm of myosin VI also restricts the volume explored by an unbound head of a processive dimer and minimizes the probability of cross-linking actin filaments. This hypothesis is illustrated in Figure 4.

The most compelling case for myosin VI playing a cross-linking role in cells comes from recent experiments demonstrating that myosin VI can stabilize the actin network (investment cone) during spermatid individualization [4••]. At the base of these actin cones, where myosin VI is located, the filaments are aligned at somewhat oblique angles to each other. With this geometry and sufficiently high concentrations of actin filaments, one could envision that the two heads of a dimer could load each other and stall, thus cross-linking the filaments. But how could this situation arise if an unbound myosin VI head has a high probability of remaining on the same filament? Perhaps if a dimer reaches the minus end of an actin filament, the detached head will explore a volume that may include another filament. If that filament is at a sufficiently large angle, and if the unbound head binds to it, then both heads will be loaded as if they are lead heads and unable to detach for an extended period of time. Thus cross-linking would only occur near the minus ends of filaments. This could explain why myosin VI is seen only in a narrow band at the edge of the investment cones. It is possible that the Arp2/3 complexes that create the filament branches at the base of the actin cones provide a barrier that the lead head of a dimer cannot easily cross, which promotes binding to an adjacent actin filament [56]. This mechanism would allow cross-linking only where Arp2/3 branches are abundant and explain the restricted myosin VI distribution seen in the cones.

Figure 4



Schematic comparison of myosin V and VI. The myosin V and VI motor domains are in blue, with 'R' indicating the rear head of a processive dimer. The hypothetical volume explored by the lead head during hand-over-hand movement is illustrated. The long lever arm (containing six calmodulins, shown as yellow ovals) of myosin V allows it to take large (36nm) steps [42,43]. Without an extension (shown in red) of its short lever arm (two calmodulins), large steps cannot be achieved by myosin VI [38••]. Both EM reconstructions and the crystal structure (Figure 2b) reveal that the rigor (end of the powerstroke) position of the myosin VI lever arm is more parallel to the actin filament than that of myosin V [1,26••]. Furthermore, single molecule measurements on myosin VI monomers indicate that the angular swing of the lever arm may be larger for myosin VI than for myosin V [30••,31••,38••,46••]. This would allow a step equivalent or larger than that of myosin V to be accomplished by a shorter lever arm. Also shown is a hypothetical hinge between the IQ-CaM and lever arm extension of myosin VI. The combination of a shorter lever arm and this hinge could allow the lead head of myosin VI to explore less volume than that of myosin V, which could reduce the probability of binding to another actin filament (i.e. cross-linking). Whether myosin VI will cross-link actin filaments at higher concentrations than is the case with myosin V is unknown.

One last question raised by Miller *et al.* [4••] is whether or not the cargo-binding tail is needed for the putative myosin VI cross-linking in the actin cones. A GFP-tail fragment failed to localize to the cones, demonstrating that motor activity was required for proper localization, as well as for filament anchoring. If there is no soluble binding partner for the myosin VI tail present, then myosin VI dimerization could occur simply as a result of a high concentration of myosin VI and actin being present, which could then promote cross-linking, as speculated above. However, the experiments did not rule out a role for a soluble tail-binding protein, as it was not shown that myosin VI could provide its cross-linking role with its cargo-binding globular tail removed. A soluble target protein complex could also potentially promote coupling of dimers, allowing pairs of dimers to cross-link, or could simply initiate monomer dimerization.

Conclusions

To achieve reverse directionality, myosin VI has evolved several unique structural and functional adaptations. Interestingly, these adaptations allow it to function as a highly processive minus-end directed transporter on actin, and convert it to an actin-anchoring protein under high loads. While both of these roles are best performed by a dimer, myosin VI exists primarily as a monomer in cells. Future work is needed to delineate which of its functions are used in the variety of cellular processes it serves, whether it functions as a monomer or dimer in each of its roles, and how it is targeted and regulated in the cell.

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