

Myosin VI: Roles for a Minus End-directed Actin Motor in Cells

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The recent discovery that the class VI myosin is minus end-directed (Schliwa, 1999; Wells et al., 1999) allows new mechanisms of actin-based motility to exist in cells. This will prompt reexamination of a broad range of cell movements previously difficult to explain by conventional force generating mechanisms. Myosins are a large family of molecular motor proteins divided into 15 or more classes, and are the only known type of actin-based motor. Intrinsically, an individual myosin converts energy derived from ATP hydrolysis into unidirectional movement towards only one specified end of an actin filament: either the plus (barbed-) or minus (pointed-) end. Minus end directionality in myosin VI is unique: all other myosins tested are plus end-directed actin motors. Myosin VI plays a key role in cell motility and shape change events in many animal species (Table I), and is essential for hearing in mice (Avraham et al., 1995) and fertility in flies (Hicks et al., 1999). This review discusses two plausible basic molecular functions for a minus end-directed actin motor in a cell: transportation of cargo, an expected role for an actin motor; and generation of cell expansion force, a new theory. I then speculate how cargo transport and cell expansion force participate in three distinct shape change events in which myosin VI activity is important.

Basic Functions for a Minus End-directed Myosin in Cells

For any myosin, the type of actin organization with which the myosin motor interacts is a key determinant of basic myosin function (for details see Cramer, 1999). Two distinct types of actin organization that exist in cells, uniform and opposite filament polarity actin networks (see below for descriptions), allow two extreme types of myosin function, respectively (Fig. 1): oriented transportation of cargo (Fig. 1 A) and sliding of actin filaments (Fig. 1 B). Myosin VI could have both of these basic functions as it associates with both of these types of actin organization (e.g., Table I).

To drive cargo transport, myosin (Fig. 1 A, single black ball and stick) moves (Fig. 1 A, arrows) attached cargo (Fig. 1 A, circles, curved line) over the surface of a uniform filament polarity actin network (Fig. 1 A, red lines). In this actin organization, all actin filament plus ends face

towards the cell surface (Fig. 1 A, as indicated). This allows oriented transportation of cargo into or out of the cell (e.g., Fig. 1 A, compare upward and downward arrows). Thus, a plus end-directed myosin will transport attached cargo outwards towards the cell surface, such as a vesicle on the exocytic pathway (Fig. 1 A, upward arrow). Conversely, a minus end-directed myosin will transport attached cargo inwards, away from the cell surface, such as a vesicle on the endocytic pathway or a region of the plasma membrane that is being retracted or tethered by the myosin (Fig. 1 A, downward arrows). Myosin-based cargo transport is, for the most part, spatially restricted to cell surface features (filopodia, lamellipodia, microvilli, stereocilia, growth cones, etc.), as this is where uniform filament polarity actin networks are principally located in cells. However, myosins may transport cargo on oriented actin networks that exist deeper in the cytoplasm of a few specialized cell types (Cramer, 1999). Plus end-directed transport on actin is known to occur in cells, e.g., outward movement of cell surface receptors in lamellipodia. Are there situations in cells for minus end-directed transport on actin? One long-standing mystery is dramatic actin-based inward flow (also termed retrograde flow or centripetal flow) of heterogeneous cellular particles and actin towards the minus end of actin filaments, also in lamellipodia and filopodia. Many theories explain this movement, but no one theory can account for all experimental data. A minus end-directed myosin has the correct directionality to actively transport at least a subpopulation of the particles inwards. The first direct evidence that myosin VI has transport motor capacity comes from studies in fly embryos, where the movement of myosin VI-containing particles on actin is blocked by injection of antimyosin VI antibodies (Mermall et al., 1994; Bohrmann, 1997). Paradoxically, it has been difficult to interpret this motility because particles move in random directions and for only short periods, questioning whether the motility reflects conventional transport or spatial retention of important molecules (see below).

To drive actin filament sliding, myosin oligomers (Fig. 1 B, two black balls and stick) sit between repeating units of actin filament subbundles (e.g., two subbundles are represented by red and blue lines in Fig. 1 B) in an opposite filament polarity actin network, and move subbundles relative to one another (e.g., Fig. 1 B top, compare thick arrows). In this actin organization, actin filament plus ends from adjacent subbundles face opposite directions (Fig. 1 B, compare red and blue lines) and myosin sits between overlapping/interdigitating filament minus ends from adja-

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Table I. Myosin VI Function and Localization in Cells

Organism/cell type	Myosin VI function	Localization in organism/cell	Actin filament polarity*	Reference
Human, mouse/hair cells	Stereocilia organization	Actin-rootlets of stereocilia cuticular plate microtubules?	Uniform and opposite (see text), random	Hasson et al., 1997; Self et al., 1999
Mouse, pig/absorptive epithelial cells	Microvilli organization	Actin-rootlets of microvilli, terminal web	Uniform, random	Hasson and Mooseker, 1994; Heintzelman et al., 1994; Self et al., 1999
Mammal/cultured cells	?	Golgi, Lamellipodium	Uniform	Buss et al., 1998
Chick/dorsal root ganglion neurons	?	Actin in growth cone and neurites, microtubules?	Uniform	Suter et al., 2000
Fish/photoreceptor cells	?	Mitochondria, cytoplasmic actin bundles	Uniform	Breckler et al., 2000
<i>Drosophila</i> ³ /blastoderm	Pseudocleavage	Pseudocleavage furrow particles	Likely opposite	Kellerman and Miller, 1992; Mermall et al., 1994; Mermall and Miller, 1995
<i>Drosophila</i> ³ /sperm	Individualization	investment cone (actin)	?	Hicks et al., 1999
<i>Drosophila</i> ³ /nurse cells in egg chamber	?	Particles	?	Bohrmann, 1997
<i>Drosophila</i> ³ /epithelium	Tissue organization	?	?	Deng et al., 1999
<i>Drosophila</i> ³ /migrating cells	Cell migration?	?	?	
<i>Drosophila</i> ³ /newly hatched flies	Expansion of wings and legs	?	?	
<i>Caenorhabditis elegans</i>	Sperm development	?	?	Kelleher ⁸

*Organization and filament polarity of the actin structure with which myosin VI colocalizes.

³In *Drosophila*, myosin VI is also known as 95F-myosin.

⁸Kelleher, J.F., G.L. Moulder, R.J. Barstead, and M.A. Titus. 1999. American Society for Cell Biology 39th Annual Meeting. 164a (Abstr.)

cent subbundles (Fig. 1 B, as indicated). Also, net filament plus ends are anchored to the plasma membrane (e.g., Fig. 1 B, junction of vertical and horizontal lines) via adhesion complexes or other connections. These structural criteria conceivably allow pulling or pushing forces to be exerted on the cell by a myosin. Thus, filament sliding driven by a plus end-directed myosin in an opposite filament polarity actin network exerts net pulling force or cell contraction force (Fig. 1 B, upper panel, arrows move together). Conversely a minus end-directed myosin in the same actin network exerts net pushing force or cell expansion force (Fig. 1 B, bottom, arrows move apart). Contraction force gener-

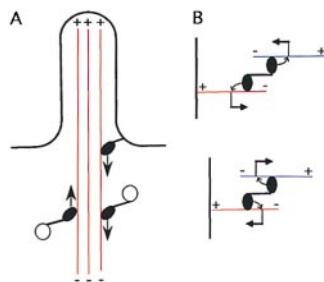


Figure 1. Basic functions for plus and minus end-directed myosins in cells. Myosin drives oriented transportation of cargo (A) and sliding of actin filaments (B). Myosin head (black balls) and tail (black stick) domains, actin filaments (red, blue lines), vesicle (circle), plasma membrane (curved and straight

lines). Both indicated types of actin organization (A and B) exist in nonmuscle motile cells. A, Myosin pulls cargo on a uniform filament polarity actin network. Plus end- (upward arrow) and minus end- (downward arrows) directed myosins transport cargo in opposite directions. Myosins I and V are known plus end-directed transport motors. Myosin VI, the minus end-directed actin motor, also has transport motor activity in cells, but oriented movement of cargo remains to be demonstrated (see text). B, Myosin slides actin filaments in an opposite filament polarity (antiparallel) actin network. A plus end-directed myosin (top) pulls actin filaments together (thick arrows move together), generating pulling or contraction force in the cell. A minus end-directed myosin (bottom) pushes filaments apart (thick arrows move apart), generating pushing or expansion force in the cell. Contraction force generated by myosin II has been documented in many systems. However, expansion force resulting from minus end-directed myosins is, at this point, hypothetical.

ated by myosin II exists in all cell types tested. Generation of cell expansion or pushing force by a myosin is less discussed. Where might it occur? One broad situation is to balance contraction force in cells, necessary for cell homeostasis. In general, in the bulk of the cell, the microtubule system provides balancing force, but myosin-based cell expansion may account for balancing force at the periphery of cells where microtubules are scarce or absent. Another situation for myosin-based pushing force might include expansion of ring canals during *Drosophila* oocyte development. Also, when myosin VI activity is reduced by antisense depletion during *Drosophila* imaginal disc morphogenesis, defects in wing and leg expansion occur (Deng et al., 1999). Thus, myosin VI may play a role in powering tissue expansion in this situation. Actin networks may need strengthening to resist buckling and breaking before useful pushing force is generated by myosin. However filament-breaking in itself may be desirable in certain situations. For example, the actin network under the plasma membrane (cortical actin) or coating membrane organelles may need to locally expand and/or break to allow vesicle budding (Lorra and Huttner, 1999).

The mechanism of myosin VI in cargo transport or actin filament sliding is not yet clear. However, the predicted protein structure may give us some hints. There are no predicted membrane binding domains in the myosin VI tail. Instead, myosin VI-based transport may occur by protein-protein interactions mediated by a region of alternating positive and negative charge in the tail (Kellerman and Miller, 1992; Hasson and Mooseker, 1994; Buss et al., 1998). Actin filament sliding driven by a myosin requires formation of myosin oligomers or filaments. Myosin II is the only known filamentous myosin and forms homopolymers through a coil-coil domain in its tail. Whereas myosin VI also has a coil-coil domain and is predicted to form at least a dimer, a large globular region, also in the tail, may prevent conventional filament formation. Instead, myosin VI oligomerization may also be mediated by the region of alternating positive and negative charge in the tail, perhaps through a protein linker scaffold.

Role of Myosin VI in Diverse Biological Processes

Three actin-based shape change events known to require myosin VI activity (Fig. 2) are: pseudocleavage in fly embryo blastoderm (Fig. 2, A–C); sperm individualization in flies (Fig. 2 D–F); and development of stereocilia in mouse hair cells (Fig. 2, G–I). The precise roles of myosin VI revealed in these diverse processes will likely be relevant for other cell or tissue types (Table I), where myosin VI function is comparatively less clear. Whereas these shape change events differ in scale and gross morphology, a common feature is invagination of the plasma membrane (Fig. 2, compare B, E, and H). When myosin VI activity is blocked in these processes, related defects in membrane invagination and other events occur (Fig. 2, compare C, F, and I) and argues that myosin VI function in these tissue types is similar in at least some respects. In all cases, it is not thought that myosin VI produces the main force for morphogenesis (Mermall and Miller, 1995; Self et al., 1999) or actin motility (Hicks et al., 1999). Instead, observed spatial and temporal defects when myosin VI is defective are more consistent with a role for myosin VI in maintaining or stabilizing cellular structure.

Pseudocleavage

In flies and other insects, early embryonic development occurs by nuclear division without cellular division. This creates a multinucleate tissue, termed syncytial blastoderm. Pseudocleavage describes invagination of plasma membrane to keep mitotic spindles in the syncytium separate during mitosis (compare Fig. 2, A and B). When myosin VI activity is blocked with antibodies, membrane invagination (the pseudocleavage furrow) is less deep and mitotic spindles fuse (Fig. 2 C; Mermall and Miller, 1995). Myosin VI generally transports particles in the actin cortex of syncytial blastoderm (Fig. 2 A, arrows; Mermall et al., 1994), but it is unclear how this relates to substeps in the pseudocleavage process. Before mitosis, myosin VI-containing particles are relatively dispersed (Fig. 2 A, green circles). At mitosis, these particles relocate and concentrate in pseudocleavage furrows (Fig. 2 B, green circles). It is unlikely that myosin VI functions to directly transport particles that might contain important molecules to pseudocleavage furrows at mitosis: there are no oriented actin tracks leading to furrows and, moreover, observed myosin-VI driven particle motility is nonoriented (Fig. 2 A, compare arrows). Thus, myosin VI and its cargo must relocate to pseudocleavage furrows by other mechanisms. Before this relocation, any transport role for myosin VI must find an explanation for the randomness of particle motility. One idea is that this reflects intrinsic motor activity of myosin VI, perhaps functioning to spatially retain particles within the actin cortex, conveniently positioning them for subsequent relocation to pseudocleavage furrows at mitosis. Later during mitosis, when myosin VI is located at the furrow, it may function to slide actin filaments or transport cargo. In filament sliding, myosin VI may push actin filaments apart or mechanically break filaments in the actin cortex underneath the plasma membrane to allow invaginating membrane pass through the

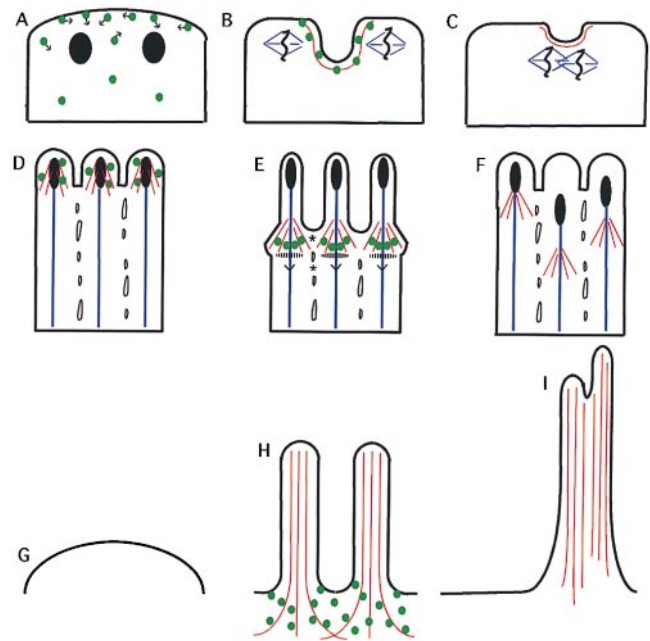


Figure 2. Biological processes requiring myosin VI activity. Simplified views of pseudocleavage (A–C, only two nuclei are shown), sperm individualization (D–F, only 3 out of 64 sperm are shown) in direction of arrowheads (E), and stereocilia development (G–I) representing early (A, D, and G) and late (B, E, and H) stages, and defects (C, F, and I) in these processes when myosin VI activity is blocked. Black lines (plasma membrane); black ovals and wavy lines (nuclei); red lines (actin filaments); blue lines (microtubules); green circles (localization of myosin VI); particle motility (A, arrows) before pseudocleavage, cytoplasmic bridge (e.g., asterisks in E), and accumulation of waste (E, hash) in sperm. Note when myosin VI activity is blocked, the position of plasma membrane in sperm (F) and organization of actin in stereocilia (I) has not been described and is theoretical as shown.

cortex. Alternatively, myosin VI may directly transport invaginating membrane over a short distance on cortical actin to stably tether new membrane position. Analysis of actin organization and membrane position in furrows and myosin VI conditional alleles may help distinguish these models and determine temporal importance of myosin VI function.

Sperm Individualization

In *Drosophila* testis, the process of individualization simultaneously converts a network of interconnected sperm in a syncytium into individual sperm cells (Fig. 2, compare D and E). Individualization is driven by an actin structure, termed the investment cone (Fig. 2 E, red lines), which travels from the head to the tail of each sperm (Fig. 2 E, in direction of arrowheads). As the cone progresses, plasma membrane invaginates at the sperm connection sites (cytoplasmic bridges, two are denoted with asterisks in Fig. 2 E) so that each sperm is encased in its own membrane (Fig. 2 E, compare above and below the investment cone). The mechanism of these events is unknown, but must take into account the supply of plasma membrane required for invagination. Plasma membrane in the syncytium is sufficient

for this, even excess to requirements. (Total surface area is $\sim 1.2\text{--}2.4 \times 10^5 \mu\text{m}^2$ around 64 sperm in the syncytium, and $1.4 \times 10^5 \mu\text{m}^2$ for 64 individualized sperm; calculated from data presented in Tokuyasu et al., 1972.) In myosin VI mutants, investment cones stall partway down sperm tails and the cones, and nuclei are misaligned (Hicks et al., 1999; Fig. 2 F, compare red lines and black ovals). Myosin VI may function to transport membrane in one of two distinct pathways (Fig. 3, compare A and B) that use syncytial plasma membrane as a supply for required membrane invagination at cytoplasmic bridges. In the plasma membrane transport model (Fig. 3 A), myosin VI (Fig. 3 A, green balls) binds syncytial plasma membrane (Fig. 3 A, black lines) and directly participates in membrane invagination by repositioning (tethering) this membrane towards the bottom of actin investment cones (Fig. 3 A, in direction of short arrows). In the membrane vesicle transport model (Fig. 3 B), myosin VI (Fig. 3 B, green balls) instead binds endocytic vesicles (Fig. 3 B, circles) derived from syncytial plasma membrane and moves them (Fig. 3 B, short arrows) across the investment cone (Fig. 3 B, red lines). This provides required membrane at cytoplasmic bridges for subsequent exocytosis and membrane invagination (Fig. 3 B, dashed line). In either mechanism, stalled motility of investment cones in mutants is explained by their physical blockage in cytoplasmic bridges, which in normal sperm would be removed. Since position and size of bridges varies down sperm (Tokuyasu et al., 1972), blockage will occur out of synch, accounting for observed misalignment of investment cones in the mutants. Also, continued generation of motile force exerted on stalled investment cones may instead transfer to nuclei and cause their misalignment (Hicks et al., 1999). The models predict that actin filament minus ends face towards the bottom of the investment cone, in the direction of investment cone motility. Nothing is known about structural organization of actin in investment cones, nor of the mechanism of investment cone motility in sperm. However, during hair cell development, actin filaments grow from the bottom of stereocilia actin bundles with minus ends leading (Tilney and Tilney, 1992), and may be similar to the situation in sperm. Distinguishing these models and other potential roles for myosin VI during sperm individualization requires information on membrane organization and vesicle trafficking in myosin VI mutants, and actin organization and filament polarity of investment cones.

Stereocilia Organization

Stereocilia are finger-like structures that transduce sensory information on the apical surface of hair cells (Fig. 2 H). Each stereocilium comprises a bundle of actin filaments (Fig. 2 H, red lines) that extend below the apical surface to form actin rootlet anchors (Fig. 2 H, splayed red lines). In mice effectively null for myosin VI, stereocilia are normal before birth (Fig. 2 H). Starting at birth in mutants (Fig. 2 I), the apical plasma membrane (black line) loses its position and rises up between adjacent stereocilia. Also in mutants, stereocilia themselves lose position, moving within the plane of the cell surface (position of stereocilia does not correlate before and after apical surface membrane has risen-up) towards each other (fat-

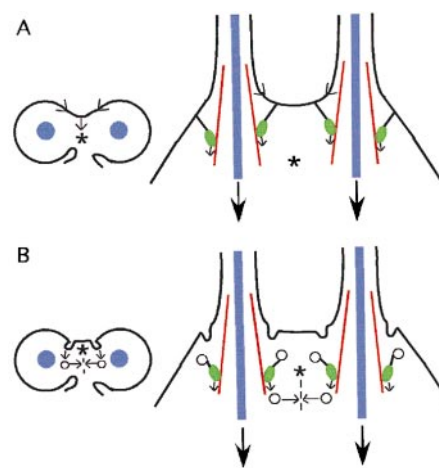


Figure 3. Transport motor functions for myosin VI during sperm individualization. Myosin VI transports plasma membrane (A) or recycling membrane vesicles (B) during individualization. Both models account for observed accumulation of myosin VI towards the bottom of actin investment cones, and use syncytial plasma membrane as a required supply of membrane (total surface area is $\sim 1.2\text{--}2.4 \times 10^5 \mu\text{m}^2$ around 64 sperm in the syncytium, and $1.4 \times 10^5 \mu\text{m}^2$ for 64 individualized sperm; calculated from data presented in Tokuyasu et al., 1972), which is consistent with observed shrinkage of sperm circumference after individualization. Left, Top view of sperm; right, side view of sperm; asterisk, fudiciary mark on cytoplasmic bridge; blue lines and circles, microtubule axoneme; long thick arrows, direction of investment cone motility and individualization. A, Myosin VI (green ball and stick) tethers syncytial plasma membrane (black lines) onto investment cones (red lines). As myosin VI (short arrows) and/or the investment cone move downwards, attached syncytial plasma membrane is consequently pulled downwards (arrowheads), thus resulting in membrane invagination. B, Endocytosis (black circles) removes plasma membrane from the syncytium. Myosin VI (green ball and stick) transports endocytic membrane vesicles across the investment cone (red lines). Exocytosis (long thin arrows) and membrane invagination (dashed line) occurs subsequently. In a related mechanism, myosin VI is instead placed on the exocytic pathway (Hicks et al., 1999).

ness of fused stereocilia is less than the combined fatness of individual stereocilia before fusion; measured from images presented in Self et al., 1999; Fig. 2, compare H and I). By about two weeks after birth, fused giant stereocilia structures have formed in mutant mice (Fig. 2, compare H and I; Self et al., 1999). These defects are consistent with two separate roles for myosin VI in newborn mice: one in stable positioning of apical plasma membrane, and the other in anchoring stereocilia. Considering positioning of plasma membrane, the surface membrane topology of a group of stereocilia on a single hair cell, though on a different scale, is similar to individualized sperm. Related endocytic vesicle and plasma membrane transport pathways described for sperm (see above) can explain the plasma membrane positioning defect observed in myosin VI-deficient hair cells. However, in hair cells, experimental data strongly favors a plasma membrane transport route (Self et al., 1999) because in myosin VI-deficient hair cells, fluid phase endocytosis is not blocked. In this membrane transport model (Fig. 4 A), myosin VI (Fig. 4, single green

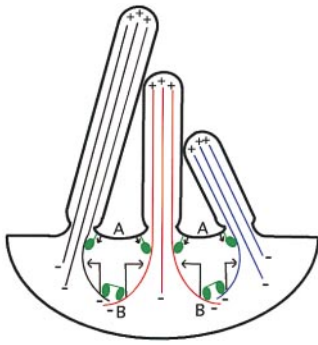


Figure 4. Two distinct functions for Myosin VI in stereocilia organization. Myosin VI functions as a motor to transport plasma membrane (A) and generate expansion force (B) in hair cells. Actin filament minus ends face towards rootlets (Tilney et al., 1980) and arguably interdigitate where rootlets splay (as indicated, thin black, red, and blue lines). A, Myosin VI (single green ball and stick)

tethers (short arrows) the apical cell surface (thick black line) onto stereocilia rootlets to maintain surface membrane topology. B, Myosin VI (two green balls and stick) slides interdigitating filament–minus ends apart between adjacent stereocilia rootlets (long arrows move apart). Opposing pushing forces are exerted on each rootlet (e.g., long arrows acting on the middle rootlet), which keeps each stereocilium in place. Net outwards force is exerted on peripheral rootlets due to fewer nearest neighbors (e.g., long arrow acting on the left and right rootlet, respectively) causing stereocilia to tilt inwards above the cell surface.

balls) binds apical plasma membrane (Fig. 4, thick black line) and moves attached membrane downwards a short distance (Fig. 4, short arrows) towards actin filament minus ends in rootlets, thereby effectively tethering the plasma membrane at the base of stereocilia. Even if myosin VI is required for other types of endocytosis or membrane vesicle traffic, this does not contribute to maintaining position of plasma membrane over stereocilia as there is no change in total surface membrane area over stereocilia in hair cells in the absence of myosin VI function (measured from images in Self et al., 1999).

Turning to stereocilia anchoring, myosin VI may transport particles or generate expansion force to stabilize stereocilia position in the apical cell region. In the transport scenario, myosin VI indirectly stabilizes stereocilia by transporting a strengthening component to rootlets. Alternatively, myosin VI generates expansion (pushing) force between adjacent stereocilia rootlets (Fig. 4 B) to directly stabilize stereocilia. Because each stereocilium is surrounded by several neighbors, opposing pushing forces are exerted on each stereocilium rootlet, thus keeping each stereocilium in place (e.g., Fig. 4 B, opposing long arrows acting on the middle stereocilium). Of these alternative roles, a simple experiment is persuasive of the rootlet-pushing idea. Myosin II–based contractile (pulling) force anchors microvilli, but not stereocilia (Tilney et al., 1980). Thus, alternative anchoring force must exist for stereocilia. That this anchoring may come from rootlet-pushing, but not pulling, explains why mature stereocilia tilt towards each other above the cell surface (e.g., Fig. 4). It is difficult to assess tilting in myosin VI mutants due to complex morphology, but when myosin VI activity is naturally absent earlier in hair cell development, younger stereocilia tend to splay outwards as predicted by the model. Rootlet-pushing requires that filament minus ends from adjacent rootlets interdigitate. Where investigated in lizard, filament minus ends splay towards each other (Tilney et al.,

1980). I estimate interdigitation of splayed filaments is plausible, though further studies are required.

Future Directions and Conclusions

The existence of minus end directionality in myosin VI adds a new dimension to the actin cytoskeleton and cell motility. A role for myosin VI in other membrane remodeling events, such as cytokinesis, cellularization, endocytosis, and membrane budding may be revealed in the future. In this article, I have described several distinct possible functions for myosin VI. Determining their relative importance in any given cell event will require elucidation of precise actin organization with which myosin VI interacts, temporal analysis of membrane position, and different myosin VI alleles. Whether myosin VI functions to transport particles to a specific destination requires further examination. Minus end directionality allows existence of myosin-based cell expansion force, but this needs direct testing. It also opens the possibility of as yet undiscovered novel types of actin organization in cells. Also, distinct myosin VI isoforms may have different cellular functions, as has been seen with different isoforms of other myosin classes. In fact, two distinct myosin VI genes exist in fish (Breckler et al., 2000), and myosin VI is alternatively spliced in flies (Kellerman and Miller, 1992). Further diversity may come from regulation by phosphorylation (Buss et al., 1998) and interaction of myosin VI with microtubules (Lantz and Miller, 1998). As with most cytoskeleton motors, the directionality of myosin VI needs to be formally demonstrated in cells.

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