Myosin-Driven Intracellular Transport

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SUMMARY

The delivery of intracellular material within cells is crucial for maintaining normal function. Myosins transport a wide variety of cargo, ranging from vesicles to ribonuclear protein particles (RNPs), in plants, fungi, and metazoa. The properties of a given myosin transporter are adapted to move on different actin filament tracks, either on the disordered actin networks at the cell cortex or along highly organized actin bundles to distribute their cargo in a localized manner or move it across long distances in the cell. Transport is controlled by selective recruitment of the myosin to its cargo that also plays a role in activation of the motor.

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1 INTRODUCTION

A remarkable feature of cells is the dynamic and often robust movement of their internal contents. Intracellular motility is used to position organelles where they are needed, such as targeting secretion to the apical regions of cells lining a lumen. The major cytoskeletal filament systems—actin and microtubules—serve as the tracks for movement of cellular cargo that is largely driven by their associated cytoskeletal motors, kinesins, dynein, and myosins. A variety of cellular content is transported, including intracellular vesicles such as endosomes and exosomes, organelles such as melanosomes or mitochondria, and particles such as ribonucleoproteins (RNPs). In metazoan cells, transport can be divided into two parts—long-distance movement along microtubules (reviewed by Barlan and Gelfand 2016) and relatively short-range transit along actin filaments. The polarized organization of the microtubule system, with many slow-growing ends embedded in the centrosome and the fast-growing ends extending all the way out to the periphery, provides tracks for movement to the cellular periphery and back into the center of the cell. Although these provide efficient highways for cargo, a local distribution system is needed, and that is provided by the actin cytoskeleton.

The cytoskeletal actin network is generally not polarized and, instead, filaments are randomly oriented in the cell, allowing for the movement of cargo all throughout the region in which filaments are found (for review, see Svitkina 2016). However, there are select regions of the cell in which actin is arranged in a more orderly manner. The actin filaments in cortical regions immediately beneath the plasma membrane are generally arranged with their barbed (or fast-growing) ends oriented toward the plasma membrane, providing a path for myosin-dependent movement of cargo either to or from the plasma membrane, based on the directionality of the associated motor. Specialized protrusive structures comprising parallel bundles of actin with their barbed ends at the membrane and pointed ends at the base, such as filopodia, stereocilia, and microvilli, also serve as cellular “superhighways” for cargo that is targeted for delivery within these structures, anchored at their tip or targeted for secretion.

The ability of cargo to move along either filament system means that intracellular transport is coordinated in a general sense. Intracellular vesicles or organelles have been shown to move along one filament system and then switch over to the other (for examples, see Rodionov et al. 1998; Rogers and Gelfand, 1998; Schuster et al. 2011; Schroeder et al. 2012). The mechanism by which an organelle can move along one filament type and then switch to another or change directionality when moving on a filament is not

yet well understood. It certainly requires that the appropriate motors are activated in the regions of the cell in which the microtubules and actin filaments overlap at the site of cargo transfer, and/or else already active motors might engage in a tug-of-war and the greater power or number of one motor type might win over the other. It should be noted that, in addition to translocation via motor-driven motility along filaments, actin polymerization can also power the intracellular movement of vesicles, typically endocytic vesicles, and even pathogenic bacteria, and that this occurs independently of microtubule motors. The focus of this review is on myosin-dependent movement of cargo along actin filaments. Selected examples will be presented to highlight the diversity and shared features of motor-driven transport along actin filaments in different cell types.

2 THE TRANSPORT MYOSINS—BASIC FEATURES

A variety of different myosins transport cargo within cells (reviewed by Sweeney and Holzbaur 2016). One key feature of these motors is their distinctive carboxy-terminal tail regions that bind to specific partner proteins to target the myosin to a particular cellular location or organelle. Another property of transporters is their ability to move continuously along actin filaments, typically on dimerization or multimerization. The myosin V family of myosins (reviewed in Hammer and Sellers 2011) are among the most widely used motors for actin-based transport and they are found in a diverse set of organisms ranging from amoebae to yeast to human, suggesting that they are among the most ancient of actin-dependent translocators. Myosin Vs have roles in the actin-based motility of organelles, vesicles, and RNPs, and a wide range of studies of the intracellular transport of these cargos have revealed several fundamental aspects of myosin-dependent movement along actin within a cell.

All known myosins with the exception of one, myosin VI, move in the same direction on the actin filament—toward the barbed end. Given that actin filaments within the cell are generally organized in a random fashion, the barbed end motors are all perfectly well-suited for distributing cargo throughout a network of filaments. However, in those regions of the cell in which actin filaments have a defined orientation, such as at the periphery where they are oriented with their barbed ends toward the membrane, barbed end-directed myosins, such as myosin V, will move cargo toward the plasma membrane, whereas the pointed end-directed myosin VI would work in opposition to transport vesicles away from the plasma membrane and into the interior of the cell. The same would be true for the movement of myosins along the parallel actin bundles found in filopodia, microvilli, and stereocilia.

The actin filaments themselves can also have an impact on translocation. For example, myosin Ic plays a role in the transport of vesicles containing GLUT4 in adipocytes, but it is unable to bind to actin filaments with the tropomyosin Tpm3.1 bound. Thus, this myosin tends to selectively drive cargo transport along filaments without tropomyosin (Kee et al. 2015). Regulating the levels of Tpm3.1 in the cortex might provide the cell with a mechanism to control the transit of GLUT4 vesicles in that region. The nucleotide state of an actin filament can also play a role in regulating myosin movement. Myosin Va takes more steps on a growing actin filament (the actin monomer has ADP·P_i bound) than on older filaments (ADP actin) (Zimmermann et al. 2015). In contrast, myosin VI prefers “older” ADP actin filaments. These differences in run length correlate with the directionality of each motor and could play a role in favoring the movement of one motor or another in regions of the cell with more stable, older filament networks versus regions in which the actin filaments turn over rapidly.

The activity of transport myosins is tightly regulated to prevent the cargo-attached motor from interacting with actin filaments until it is at the correct location and to avoid unproductive interactions with actin filaments when the myosin is not bound to cargo. A major mode of regulation is through autoinhibition. In the case of myosin V, as well as myosin VIIA and myosin X, the myosin is typically maintained in an “OFF” state when the globular tail domain (GTD) is folded over and interacts with the motor to inhibit activity. Conserved residues on both the motor domain and tail of myosin V mediate this interaction (Liu et al. 2006; Thirumurugan et al. 2006; Li et al. 2008; Nascimento et al. 2013). Mutation of these tail residues in yeast myosin V, Myo2p, results in a constitutively active motor (Donovan and Bretscher 2015).

Cargo binding to the myosin tail unfolds the myosin and relieves inhibition of the motor. Coupling cargo binding to activation ensures that motors are only turned on when cargo is available and ready for transport. Several studies show that myosin V is activated *in vivo* on binding of a cargo adaptor to the GTD. For example, the adaptor melanophilin increases the physiological actin-activated Mg-ATPase and *in vitro* motility of myosin Va (Li et al. 2005; Skolnick et al. 2013). Melanophilin binding switches myosin V from the closed inhibited conformation to a fully extended active conformation (Yao et al. 2015). This mechanism of activation for cargo transport is likely used by other myosins. Autoinhibition can also be overcome by myosin binding to membranes by direct interaction of the tail with lipids (typically phosphatidylinositol(4,5)-bisphosphate, PIP₂, or phosphatidylinositol(3,4,5)-trisphosphate, PIP₃). In the case of myosin X, lipid binding unfolds the autoinhibited myosin and turns on its activity (Umeki

et al. 2011). Another mechanism for activating myosins could be through Ca²⁺ binding to calmodulin light chains bound to the lever arm region that unfolds the myosin (see below).

Transport by myosins can also be activated, or significantly enhanced, by converting a monomer to a dimer by its binding partner(s), enabling the motor to move processively along actin. Cytosolic myosin VI is a monomer; however, binding of its partners optineurin or Dab2 to the tail dimerizes the motor, which is then able to move long distances along actin (Phichith et al. 2009; Yu et al. 2009). Similarly, myosin VIIA is a monomer and, when expressed in COS7 cells, it is largely cytosolic. Coexpression with its binding partner MyRIP dimerizes the motor, which can then translocate along actin filaments of a filopodium to the tip (Sakai et al. 2011).

Another mode of modulating transport is through the ability of myosins to sense and respond to forces. High load can significantly slow the mechanochemical cycles of some myosins, converting them to an anchor. For example, just 1 piconewton (pN) of force significantly slows ADP release from myosin Ib, increasing the time it remains bound to actin (Laakso et al. 2008), and thus it might not be suitable as a transporter. In contrast, 1–4 pN of force increases the processivity of myosin Ic (Greenberg et al. 2012).

Although the different modes of regulating myosins that transport cargo are now well understood at the molecular level, much remains to be learned about how these mechanisms operate *in vivo*. Many questions remain concerning how myosins are recruited to the appropriate cargo or activated at the right time and the right place, especially during switching from one track to another, and how these motors are able to propel this cargo along the dense actin network.

3 INTRACELLULAR TRANSPORT OF SECRETORY AND ENDOCYTIC VESICLES

Vesicles moving through the secretory and endocytic pathways must typically transit through the actin-dense cortex during internalization, recycling, and secretion. One motor implicated in this movement is myosin V, and the key first step for transport through the cortex is recruiting the myosin to the vesicle when and where it is needed. The Rab family of small GTPases and their effectors play a major role in motor recruitment to specific types of vesicles, because each is marked by a specific Rab (Hutagalung and Novick 2011; Jean and Kiger 2012). Myosin V isoforms are recruited to a Rab-containing vesicle either by direct binding of their GTD to the active Rab (GTP form) or to its Rab effector, or to both (Hammer and Sellers 2011). Alternative splicing in the tail can increase the targeting specificity of

myosin V. Structural studies of Rab and Rab effectors bound to mammalian myosin V tails have revealed the basis for Rab selectivity and how a Rab and its effector might bind simultaneously (Pylypenko et al. 2013; Wei et al. 2013).

Increasing Ca^{2+} levels locally (to $\geq 10 \mu\text{M}$) changes the conformation of myosin V (Krementsov et al. 2004; Li et al. 2004; Wang et al. 2004), but these concentrations of Ca^{2+} also reduce the motile activity because of dissociation of calmodulin light chains from the neck region (Cheney et al. 1993). This suggests that Ca^{2+} activation of myosin V would not be an effective mode of activating this motor in vivo. Instead, increasing Ca^{2+} levels might slow or inactivate the cargo-bound motor.

Membrane receptors and channels are recycled back to the plasma membrane after internalization to maintain the normal physiological activity of a cell. After internalization, a receptor or channel is sorted into a recycling endosome for return to the plasma membrane. Myosin Vb moves the recycling endosome along actin filaments into position to dock for fusion with the plasma membrane (Fig. 1) (Hammer and Sellers 2011). The GTD of myosin Vb has binding sites for both the small GTPase Rab11a and its effector FIP2, which recruits myosin Vb to recycling endosomes in both polarized and nonpolarized mammalian cells (Schafer et al. 2014). Interaction of myosin Vb with both Rab11a and FIP2 stabilizes the complex on the vesicle. Myosin Vb is subsequently released from the recycling endosome when Rab11a is deactivated, presumably by a GTPase-activating protein (GAP) that stimulates hydroly-

sis of the bound GTP. The absence of myosin Vb function causes major defects in receptor recycling and results in decreased endocytosis (Roland et al. 2011). These interactions of myosin Vb with endosomes are similar to those of myosin Va with its adaptor complex of Rab27a and melanophilin (Hammer and Sellers 2011).

Elegant inducible recruitment strategies to manipulate motor-driven transport provide strong support for the role of myosin V in the transfer of cargo from microtubule-based transport to transport in the cortical actin cytoskeleton (Kapitein et al. 2013; van Bergeijk et al. 2015). Peroxisomes tagged with red fluorescent protein (RFP) normally move little in COS7 cells, but move robustly to the cell periphery if a kinesin motor is recruited to the organelle by fusing a peroxisome-targeting sequence (PEX) to its tail. Myosin Vb was inducibly recruited to the peroxisomes with bound kinesin using an adapter system consisting of FK506-binding protein (FKBP) on the peroxisome and FKBP12-rapamycin binding domain labeled with green fluorescent protein (GFP-FRB) replacing the cargo-binding GTD of myosin Vb. Addition of rapalog (an analog of rapamycin) induced the reversible association of FRB with FKBP and recruited myosin Vb to peroxisomes. Peroxisomes with both kinesin and myosin Vb moved rapidly toward the periphery of the cell, but movement slowed in the cortical regions in which individual peroxisomes moved irregularly with short pauses. These observations show the ability of myosin V to dominate over kinesin to move organelles into the cell periphery and then drive local delivery of vesicles to the plasma membrane in spite of the

Figure 1. Myosin-based transport of endocytic vesicles. Following the formation of a clathrin-coated vesicle (CCV), it is uncoated (forming an uncoated vesicle, UCV) and myosin VI is recruited through binding to the adaptor GIPC. Recycling endosomes (RE) return membrane receptors and channels (not shown) to the plasma membrane by myosin Vb-based transport. Note that the actin filaments (shown in yellow) are oriented with their barbed ends toward the membrane.

rather haphazard organization of actin filaments in the cortex.

A variation of this system using a light-sensitive light-oxygen voltage-sensing (LOV) domain targeted to peroxisomes (LOV-PEX) was developed to recruit myosin Vb fused to a peptide ligand (PDZb1) to this organelle in local regions of cells with illumination with blue light (i.e., an optogenetic approach) (van Bergeijk et al. 2015). This method was applied to dendritic spines of rat hippocampal neurons in primary culture. Spines are actin-rich protrusions that play a role in synaptic plasticity. Stimulation of *N*-methyl-D-aspartate (NMDA) receptors with glycine results in myosin V-dependent internalization of these receptors or targeting of the endoplasmic reticulum (ER) to the spines (Wang et al. 2008; Wagner et al. 2011). Illumination of spines in neurons that express LOV-PEX and myosin Vb–PDZb1 resulted in translocation of the peroxisomes into the spine, demonstrating that myosin Vb can move vesicular cargo along the actin filaments to deliver cargo into these spines.

The availability of systems in which motor activation/recruitment can be readily manipulated should allow for more detailed studies of myosin-driven movement in other actin-rich regions of the cell. However, it should be noted that this approach does not currently allow for any control over the numbers of each type of motor associated with the organelle, and so it will be necessary to further develop methods to study organelle movements powered by endogenous levels of bound motor.

Myosin VI has several roles in endocytosis, both in transport as well as in tethering organelles to the actin cytoskeleton for fusion or to aid in vesicle release (Bond et al. 2011; Tumbarello et al. 2013). Accordingly, the carboxy-terminal tail region of myosin VI has binding sites for several adapter proteins that are found on endosomes. Alternative splicing determines the presence or absence of long or short inserts (LI and SI, respectively) in the tail of myosin VI and contributes to specifying its binding partners. The myosin VI + LI isoform participates in receptor internalization by promoting the formation of clathrin-coated vesicles at the plasma membrane through association with the clathrin adapter Dab2. Myosin VI lacking inserts also participates in receptor uptake through interaction with the adapter GIPC that recruits myosin VI to endocytic vesicles following their uncoating. Myosin VI-associated uncoated vesicles (UCVs) containing the transferrin receptor (TfR) move through the actin-rich peripheral region of ARPE cells at ~35 nm/sec (Fig. 1), a velocity significantly lower than that of unloaded myosin VI in vitro (Rock et al. 2001). This could be caused by resistance from structures in the cytoplasm (including the actin network) or inhibition of motor activity. Myosin VI is lost from these

vesicles after they fuse with early endosomes marked with EEA1 (Aschenbrenner et al. 2003; Aschenbrenner et al. 2004).

Disruption of myosin VI function significantly reduced movement of UCVs through the actin network, resulting in their accumulation in the cell periphery (Aschenbrenner et al. 2004) and a block in several endocytic processes. For example, slowed transit of UCVs through the cortex in cells deleted of myosin VI causes a decrease in the uptake of the TfR. Delays in the uptake and downstream delivery of receptors to early endosomes via GIPC-positive UCVs can also impact receptor-mediated signaling. Development of the zebrafish arterial system depends on vascular endothelial growth factor (VEGF) signaling in endothelial cells, and so reducing the expression of the fish GIPC (syntectin) or myosin VI results in abnormal arterial morphogenesis (Lanahan et al. 2010). VEGF binding to endothelial cells results in phosphorylation of the VEGF receptor (VEGF-R) on tyrosine 1175 (P-Y¹¹⁷⁵) and promotes internalization through clathrin-mediated endocytosis. Activated VEGF-R is delivered to EEA1-positive early endosomes through myosin VI-dependent transit through the cortex, where it then signals to both the phospholipase C γ (PLC γ)–mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/Akt pathways to promote endothelial development. In the absence of myosin VI, slowed delivery and fusion of UCVs with the early endosome correlates with notably reduced levels of VEGF-R P-Y¹¹⁷⁵, resulting in diminished signaling through these pathways. In the case of both the motility of endocytic UCVs and signaling from the VEGF-R following internalization, the minus end-directed myosin VI takes advantage of the orientation of actin filaments at the plasma membrane to move vesicles into the cell interior.

4 DISEASES CAUSED BY DEFECTS IN ACTIN-BASED ORGANELLE TRANSPORT

Loss of organelle transport by myosin V isoforms is the underlying cause of two rare diseases. Mutations of myosin Va cause Griscelli syndrome, and mutations of myosin Vb cause microvillus inclusion disease (Van Gele et al. 2009; van der Velde et al. 2013) (see OMIM #214450 for Griscelli GS1 syndrome and 251850 for MVID [microvillus inclusion disease]).

Griscelli syndrome patients have hypopigmentation (pigment dilution) of the skin and hair and neurological disorders. Patients with Elejalde disease (OMIM #256710) have symptoms similar to those of Griscelli GS1 patients, and so mutations in myosin Va that await identification might cause the disease (Van Gele et al. 2009). The Griscelli phenotypes are strikingly similar to those of the mouse

myosin Va *dilute* mutant (Mercer et al. 1991). The pigmentation defect is due to loss of melanosome transport and transfer of pigment to the keratinocytes of the skin and hair cell shafts. Kinesin transports melanosomes to the periphery of melanocytes, where they are transferred to myosin Va for distribution through the cortex and anchoring on actin just below the plasma membrane (Wu and Hammer 2014). A Rab27–melanophilin complex links myosin Va to melanosomes, and loss-of-function mutations in each protein lead to essentially identical pigmentation defects in human and mouse (referred to as Griscelli syndrome GS2 and GS3; *ashen* and *leaden*) (Van Gele et al. 2009). The neurological disorder associated with mutations in myosin Va is consistent with the finding that ER is not transported into the dendritic spines of hippocampal neurons in the *dilute* mouse (Takagishi et al. 1996; Wagner et al. 2011).

The mutations in *MYO5B* that cause microvillus inclusion disease disrupt the microvilli on the apical surface of intestinal epithelial cells and result in a devastating loss of intestinal absorption (Cutz et al. 1989; Ruemmele et al. 2006; van der Velde et al. 2013). The diminished absorptive surface area acutely impacts nutrient absorption, resulting in a high incidence of death of newborns. Intestinal epithelial cells in patients have large inclusions filled with microvillar structures and accumulate enzymes normally found in the brush border in the cytoplasm. The loss of myosin Vb function causes the disease by disrupting the transport of recycling endosomes through the actin cortex to the surface of cells, where they fuse with the plasma membrane. These cells depend on recycling internalized receptors and channels back to the plasma membrane for their absorptive and uptake activities. The phenotypes of mice with mutations of their *Myo5b* gene resemble those of patients with microvillus inclusion disease (Cartón-García et al. 2015; Schneeberger et al. 2015). These powerful models can be used to provide important insights into how loss of myosin Vb causes such serious dysfunction of the intestinal epithelium and will potentially aid in devising therapeutic strategies for restoring function.

5 RNA TRAFFICKING

A subset of messenger RNAs (mRNAs) is packaged into RNPs for transport and localization to specific regions of a cell, such as the leading edge of a migrating cell, that require timely local control over protein production. The majority of RNP transport in metazoan cells occurs by means of microtubules; however, once these RNPs reach their destination, they can be moved locally along actin filaments by myosin V family members. In contrast, RNPs move exclusively along actin filaments in yeast, and so studies of RNA

trafficking in yeast have provided key insights into the basis of their myosin-dependent transport.

A select set of mRNAs is targeted to the bud of a growing daughter yeast cell. These RNAs can either be packaged into an RNP or associated with cortical endoplasmic reticulum (cER), and the yeast myosin V—Myo4p—is essential for their transport into the bud (Estrada et al. 2003; Shepard et al. 2003; Aronov et al. 2007). In the nucleus, the adaptor protein She2p binds to the mRNA zip code that contains sequences for targeting the mRNA. On export from the nucleus, this RNP complex binds to a complex of the She3p adaptor protein and Myo4p. Depending on the zip code sequence in the RNA, the RNP–motor complex can either bind to a receptor on the cER or remain in the cytoplasm (Singer-Krüger and Jansen 2014). Myo4p is a monomeric, nonprocessive myosin that is unable to move continuously on actin filaments by itself (Reck-Peterson et al. 2001). However, binding to the She2p–She3p complex assembles it into a dimeric transport complex that is able to move processively. Reconstitution of this complex in vitro showed that the dimeric adaptor protein She3p binds two Myo4p motors and a She2p tetramer that, in turn, binds to an *ASH1* mRNA (Heym et al. 2013; Sladewski et al. 2013). The She2p binds directly to the zip code sequence, allowing for more motors to be recruited to the complex with increasing numbers of zip code sequences. The complex has in vitro run lengths of 1–2 μm and moves at velocities comparable to those in vivo, or faster (0.6–2 $\mu\text{m}/\text{sec}$) (Bertrand et al. 1998). RNA binding might be crucial for the optimal movement of this complex, especially at physiological ionic strengths, but this remains to be firmly established (Heym et al. 2013; Sladewski et al. 2013). Thus, as is true for many of the transport myosins, such as myosin VI and myosin VIIA, cargo binding is the key event that activates the motor for transport.

The exact role of myosins in the transport of RNAs in multicellular organisms is less clear. The *Drosophila* oocyte provides an example of where the final targeting or localization of an RNP depends on actin and myosin V. In this system, myosin V (*didum*) is required for germline specification. The fly myosin V is found in a complex with the RNA-binding protein Staufien and is required for the localization of *oskar* RNA to the posterior pole (Krauss et al. 2009).

Similarly, myosin V has a role in targeting RNA to the dendritic spines of mouse hippocampal neurons that have a role in memory and learning. Specific localization of mRNAs plays a role in neuronal plasticity by allowing rapid synthesis of proteins following the stimulation of glutamate receptors with the agonist dihydroxyphenylglycine (DHPG). Mouse myosin Va forms a complex with TLS, an RNA-binding protein in RNPs, and the associated

mRNA for the kelch family protein Nd1-L, which stabilizes actin filaments (Yoshimura et al. 2006). Myosin Va and TLS colocalize at dendritic spines. Loss of myosin Va function in *dilute-lethal* mutant mice results in displacement of TLS from spines and loss of actin-dependent transport of the RNP into the spine following DHPG stimulation (Yoshimura et al. 2006).

Loss of myosin Va from human HeLa cells or fibroblasts results in perinuclear accumulation of RNAs or RNPs (Salerno et al. 2008; Lindsay and McCaffrey 2014). This is consistent with myosin Va playing a role in the distribution of RNPs throughout the cell by movement along local actin networks. Together, these findings suggest that myosin V transports RNPs in both yeast and in multicellular organisms, consistent with this being a fundamentally conserved role for this ancient family of myosins.

6 MOVEMENT ALONG ACTIN BUNDLES

A variety of cell types makes specialized membrane projections supported by parallel bundles of actin filaments (see Svitkina 2016). These include filopodia, stereocilia, and microvilli. The uniform orientation of the actin filaments, with the barbed end toward the plasma membrane, creates a superhighway for myosins to deliver cargo either to the tips or the base of these structures. Several different myosins have been implicated in the generation and/or organization of these structures and are proposed to transport components, such as adhesion receptors or regulators of actin polymerization, along the length of the actin core to the tip.

Formation of filopodia requires the function of a myosin—myosin X in mammalian cells and the functionally related amoeboid myosin VII in *Dictyostelium* (Tuxworth et al. 2001; Bohil et al. 2006). Growth of filopodia depends on two actin-binding proteins, VASP and formins (reviewed by Pollard 2016). VASP facilitates the addition of actin monomers to growing filament ends, prevents capping of the filaments, and bundles actin filaments. Formins nucleate and stimulate elongation while associating processively with actin filament barbed ends (Faix et al. 2009). Myosin X and VASP move together along the length of filopodia toward the tip at a rate of ~ 600 nm/sec, similar to the rate that myosin X dimers move on actin filaments in vitro (Tokuo and Ikebe 2004; Kerber et al. 2009). Myosin X transport has been proposed to contribute to the extension of filopodia by delivering VASP to the tip to promote growth of actin filaments (Kerber and Cheney 2011).

Filopodia are important for mediating the interaction between cells and their environment. The carboxy-terminal FERM domain of myosin X interacts with the cytoplasmic tails of several different types of receptors, including the

cytoplasmic tail of the $\beta 1$ -integrin adhesion receptor. As the two proteins colocalize in punctae along the length of filopodia, myosin X might transport the adhesion receptor to the filopodial tip (Zhang et al. 2004). Observations of cells expressing mutant myosin X, which does not bind the $\beta 1$ -integrin tail, show that this myosin is not required for the adhesion receptor to enter filopodia but has a role in concentrating and anchoring the receptors at the tip, where they can serve as the first point of contact between the cell and the extracellular matrix (Arjonen et al. 2014). Furthermore, the tips in the cells with reduced Myo10 levels appear to fail to be stabilized by substrate contact during growth, resulting in the formation of short filopodia (Zhang et al. 2004; Watanabe et al. 2010).

Myosin X has also been implicated in mediating receptor signaling. The netrin receptor DCC (“deleted in colorectal cancer”) has crucial roles in axon guidance, and it has been shown that these receptors require Myo10 for cell signaling (Zhu et al. 2007). Myosin X and DCC are colocalized at filopodial tips, and netrin stimulates the formation of filopodia by activation of VASP (Menon et al. 2015). Similar to $\beta 1$ -integrin, myosin X interacts with the cytoplasmic tail of DCC, and loss of filopodia due to a reduction in myosin X expression results in reduced neurite outgrowth in cortical explants and embryos. These findings are consistent with filopodial-mediated signaling through netrin having an important role in neuronal pathfinding. Other work shows that myosin X participates in cellular responses to the growth factor bone morphogenetic protein (BMP). Endothelial cells exposed to BMP increase myosin X expression and filopodia number. The BMP receptor ALK6 moves within filopodia, is present at the tip, and it coprecipitates with ectopically expressed GFP–myosin X. Reducing myosin X expression by RNA silencing abrogates BMP signaling and inhibits endothelial tube formation (Pi et al. 2007), suggesting that myosin X trafficking of ALK6 into filopodia is required for BMP signaling. The details of the myosin X–ALK6 interaction, whether it is direct or indirect and how it is regulated, are not yet known.

These studies reveal the importance of filopodia in cellular signaling and are consistent with the proposal that trafficking of receptors to the filopodia tip is crucial for these responses. However, it has not yet been established whether the primary role of myosin X is in filopodia formation, receptor trafficking to the tip to mediate signaling responses, or both processes.

The hair cells of the cochlea and utricle are the primary sensory cells essential for hearing and balance. These specialized cells extend long projections, called stereocilia, from their apical regions that mediate the response to a sound wave or change in the body’s orientation. Stereocilia

are organized in rows of increasing height and contain a core of parallel bundles of actin. Their formation and function depend on several different myosins, including myosin VI, myosin VIIA, and myosin XVA. Mutations of genes encoding each of these myosins results in deafness: Myosin VI mutations cause deafness types DFNA22 and DFNB37; myosin VIIA mutations cause Usher syndrome Type 1B, DFNB2, and DFNA11; and myosin XVA mutations cause DFNB3 (Dror and Avraham 2009). It has been technically challenging to study the functions of stereocilia myosins given the difficulties inherent in working with cochlear tissue explants embedded in bone. The general similarity between filopodia, which are also made of parallel bundles of actin, and stereocilia has greatly facilitated studies of these myosins and provided crucial molecular insights into their roles in stereocilia.

Mice lacking myosin XVA initiate the growth of stereocilia but their growth is significantly stunted (Probst et al. 1998). Myosin XVA is localized to the tips of stereocilia along with the scaffolding protein whirlin and an actin regulator, Eps8 (Belyantseva et al. 2005; Manor et al. 2011). Myosin XVA is a barbed end-directed motor (Bird et al. 2014) with a PDZ-binding domain at the carboxyl terminus of the tail that interacts directly with the PDZ protein whirlin that, in turn, binds Eps8. Myosin XVA also interacts with Eps8 directly. The phenotypes of whirlin, Eps8, and myosin XVA mutants are almost identical—lack of any one of these proteins results in short stereocilia. Myosin XVA is required for the localization of both whirlin and Eps8 to the stereocilia tip, whereas its own localization is independent of whirlin and Eps8. Based on these findings, it has been proposed that myosin XVA transports Eps8 to the stereocilia tips to support their elongation (Fig. 2). Indeed, myosin XVA and whirlin expressed in COS7 cells move along filopodia together and colocalize at the tip (Belyantseva et al. 2005). Interestingly, the amino-terminal extension of myosin XVA is crucial for its targeting to distinct rows of stereocilia (Fang et al. 2015). Analysis of myosin XVA function suggests that it selectively transports a crucial actin regulator to the tips of stereocilia to control their differential elongation.

Myosin IIIA is also a stereocilia myosin implicated in trafficking of components to the stereocilia tip (Fig. 2). This myosin is of particular interest as it is a monomeric kinase–myosin hybrid possessing motor activity autoregulated by phosphorylation (Quintero et al. 2010). Myosin IIIA concentrates at the tips of stereocilia in cochlear hair cells and filopodia when expressed in COS7 cells. Targeting of this myosin in filopodia requires the presence of a second actin-binding site at its carboxyl terminus (THDII) (Erickson et al. 2003; Schneider et al. 2006). Similar to myosin XVA, myosin IIIA interacts with an actin regulator—Espin 1 in

this case. Overexpression of both proteins results in longer hair cell stereocilia in cochlear explants, and their coexpression promotes filopodia elongation in COS7 cells. The proteins move together along the length of filopodia at a rate of 40 nm/sec and accumulate at the tip (Salles et al. 2009; Merritt et al. 2012). The rate of motility in vivo is similar to that reported for in vitro motility, ~100 nm/sec (Koma-ba et al. 2003). Myosin IIIA is a monomer, and so it cannot move processively in the same hand-over-hand mode used by the dimeric transport myosins such as myosin V or myosin VI. However, the motor has a high affinity for actin in the presence of ATP, whereas the THDII actin binding site has a high off-rate. Thus, the combined properties of the two sites would enable an inchworm-type mechanism allowing myosin IIIA to remain bound to actin filaments but also be released from actin and progress toward the tips of stereocilia or filopodia as the motor undergoes a step (Dosé et al. 2007; Merritt et al. 2012). Surprisingly, the auditory hair cells from mice lacking both myosin IIIA and the closely related myosin IIIB have elongated stereocilia with espin-1 still present at the tips (Lelli et al. 2016). These findings indicate that the two myosin IIIs work in concert to control stereocilia length, potentially by delivering a cohort of actin regulators to the tip.

An interesting example of potential myosin-based transport along specialized actin structures is found in intestinal epithelial cells. Microvilli are thin projections of the plasma membrane supported by a bundle of actin filaments on the apical surface of these cells. They increase the absorptive surface area of the intestine and are also an effective barrier against the bacteria in the gut. Microvilli are of uniform height and are linked together all along their lengths. Myosin Ia is the major myosin of the intestinal epithelium and it was proposed to provide a stable link between the actin-rich core of the microvillus and the overlying membrane, acting mainly as tether rather than a motor (Tyska et al. 2005). A mouse mutant lacking myosin Ia does not have any overt defects in brush border formation or intestinal function, except accumulation of membrane and bulges at the tips of some microvilli (Tyska et al. 2005). Beautiful in vitro assays revealed that myosin Ia is not a passive membrane tether, but rather it can provide forces at the tip of microvilli required for shedding of membrane vesicles into the lumen of the intestine (Fig. 3) (McConnell et al. 2009). These luminal vesicles are rich in alkaline phosphatase and are proposed to be essential for dephosphorylating bacterial lipopolysaccharides, thus, limiting bacterial growth and preventing the adhesion of enteropathogenic *Escherichia coli* (EPEC) to the epithelial cells (Shifrin et al. 2012). The mechanism by which myosin Ia moves promotes the movement of membranes or membrane domains near the tip or possibly along the length

Figure 2. Transport of actin elongation proteins in stereocilia. Two different myosins, myosin IIIA/B and myosin XVA, move up the polarized actin core of the stereocilium (shown in yellow), which is oriented with barbed ends pointed toward the membrane at the tip. Each myosin carries a cargo that includes an adaptor protein(s) (Espn-1 for myosin III, whirlin and Eps8 for myosin XVA) and an actin monomer that is added at the tip. Note that, as it is not known whether myosin XVA is a dimer, it might move as a processive dimer or by facilitated diffusion (gray arrow) if it is monomer.

of the microvillus is not yet clear. It is a monomeric non-processive motor, and so it seems likely that several motors must be organized in a cluster and linked to the phosphatase to promote vesicle shedding by an as yet unknown mechanism.

Myosin VI is found in the actin-rich apical regions of both cochlear hair cells and polarized epithelial cells that extend brush borders, such as intestinal epithelial cells and kidney proximal tubule cells. This myosin has a role in the internalization of clathrin-coated vesicles at the base of the apical stereocilia or microvilli but might also play a role in

transporting receptors down the length of the microvillus to the base for internalization (Fig. 3). Intestinal epithelial cells of the myosin VI-null mutant mouse have increased apical membrane levels of the cystic fibrosis transmembrane conductance regulator (CFTR) channel, consistent with a defect in endocytic uptake (Ameen and Apodaca 2007). The sodium and phosphate cotransporters, types IIa and IIc, on the brush border are internalized in kidney proximal tubule cells on stimulation with parathyroid hormone, but this process is significantly decreased in the absence of myosin VI (Blaine et al. 2009). Intestinal epithe-

Figure 3. Myosin-based transport in microvilli. The drawing illustrates the tip-to-base movement of the sodium-hydrogen exchanger (NHE3) powered by myosin VI (Myo6). Note that myosin VI is monomeric and that binding to its cargo promotes dimerization. In contrast, myosin Ia (Myo1a) binds directly to membrane lipids and may directly move components in the opposite direction, from base to tip, or effect changes in membrane tension to promote the shedding of vesicles (LV) enriched for alkaline phosphatase (AP) into the lumen of the gut.

lial cells stimulated with either carbachol or forskolin typically internalize the sodium-hydrogen exchanger NHE3. However, in the absence of myosin VI, the levels of the exchanger are significantly higher than normal in apical surface membranes (Chen et al. 2014) and isolated brush border membranes (Hegan et al. 2012). Examination of the

distribution of NHE3 along the length of the microvilli revealed a greater amount of the exchanger in the apical-most region of the protrusion in these cells (Chen et al. 2014), consistent with myosin VI having a role in the movement of NHE3 from the tip of the microvillus toward the base where internalization would occur.

The finding that myosin VI is required for efficient uptake of several different epithelial receptors/transporters following stimulation, together with the orientation of the actin bundle within microvilli (pointed ends toward the interior of the cell), supports a role for this motor in moving these proteins within the plane of the brush border membrane to the base, where abundant uptake machinery resides. An outstanding question is how the motor is linked to these integral membrane proteins and how various signals activate the myosin to engage the receptor or transporter for movement down the microvillus and either transfer the transporter to the endocytic machinery or switch myosin VI to having a role in recruiting components of the clathrin-coated vesicle.

7 LONG-DISTANCE TRANSPORT

Myosin-based transport typically occurs over relatively short distances in mammalian cells. However, an unusual example of long-range transport has been described in the mouse oocyte (Schuh 2011; Holubcová et al. 2013). During maturation, these cells move their spindle to the cortex, where, following meiotic division, they produce a large oocyte and a small daughter cell called a polar body. Spindle positioning depends on the actin cytoskeleton that is organized into a large network connected by nodes that are separated by $\sim 5 \mu\text{m}$ from each other. Each node includes a vesicle associated with Rab11a and myosin Vb. These vesicles move at $12 \mu\text{m}/\text{min}$ (the speed of myosin Vb in vitro; Watanabe et al. 2006) for distances of up to $30 \mu\text{m}$ from the cell center to periphery. The vesicles serve as nucleation centers for actin polymerization, as does the plasma membrane, recruiting the actin elongation proteins Spire and formin that promote the elongation of individual actin filaments. Actin filaments growing from the vesicles provide tracks for myosin Vb-dependent movement of the vesicles toward each other, resulting in vesicle fusion. But because the vesicles are connected, myosin Vb translocation results in movement of the entire network. Ultimately, the network moves toward the plasma membrane, and this is crucial for positioning the spindle that is moved along with it. Loss of myosin Vb activity inhibits vesicle fusion and network movement, and spindle position at the membrane no longer occurs. This is potentially a widely used mechanism for promoting intracellular vesicle transport as it has recently been shown that myosin Va and Spir are coordinately recruited to Rab 11 vesicle membranes in mammalian cultured cells (Pylypenko et al. 2016). Much remains to be learned about the molecular details of this unusual and intriguing form of myosin-based organelle transport, and it will be of interest to see

if this form of organelle motility that is driven by formation of its own network and tracks is indeed seen in other contexts.

8 CYTOPLASMIC STREAMING IN PLANTS

One of the first-studied examples of actin-based transport is the cytoplasmic streaming of plant cells. In contrast to animal cells but similar to yeast, plants use actin filaments as their major cytoskeletal highway. Cytoplasmic streaming is observed in a wide range of plant species and cell types, such as leaf cells of *Arabidopsis*, which move intracellular contents at a rate of $4 \mu\text{m}/\text{sec}$ (Tominaga et al. 2013). The intermodal cells of Characean algae are quite large (up to 10-cm long for a single cell), and they show robust cytoplasmic streaming occurring at a remarkable rate of $60 \mu\text{m}/\text{sec}$. Early elegant experiments by Kamiya and colleagues established that a motive force was generated at regions of the cell close to the plasma membrane, in which cables of uniformly polarized actin filaments reside to support an actomyosin-based mechanism for streaming (Shimmen 2007; Tominaga and Ito 2015). The movement of endoplasmic vesicles along actin filaments generates the motive force for cytoplasmic streaming and appears to be essential for the efficient distribution of organelles throughout the cells. The myosin responsible for streaming is myosin XI (Fig. 4). This myosin is similar in overall domain organization to the more widely expressed myosin V, but it is a distinct class of plant-specific myosin, with myosin XI family members having biochemical and biophysical motor properties that differ from those of myosin V in several respects (Avisar et al. 2009; Tominaga and Ito 2015). Purified *Chara corallina* (Cc) myosin XI is indeed a remarkable motor protein—it moves actin filaments in vitro at a speed of $60 \mu\text{m}/\text{sec}$ (Kimura et al. 2003), a speed significantly faster than vertebrate fast skeletal muscle myosin. The tobacco (Nt) myosin XI is also a fast motor—it moves at $7 \mu\text{m}/\text{sec}$ (Tominaga et al. 2003). Although both motors can move actin filaments at high rates of speed, their motor properties differ—Nt myosin XI is processive, whereas Cc myosin XI is most likely nonprocessive owing to differences in their biochemical properties. Only a small number of processive myosins would be needed to move an organelle, whereas larger numbers of the nonprocessive motors would be necessary to propel continuous organelle movement.

Plants express multiple class XI myosins—for example, there are 14 in *Arabidopsis* (Madison and Nebenfuhr 2013), presenting a challenge for studying the role of a given motor owing to redundancy between the different motors. However, studies of mutants in multiple myosin genes or the effects of the overexpression of tails in *Arabidopsis* and

Figure 4. Cytoplasmic streaming and nuclear movement in plant cells. The drawing shows the movements of a variety of organelles, including the nucleus (N), endocytic vesicles (Endo), Golgi, endoplasmic reticulum, and mitochondria (Mito). Note that these are likely to be driven by several different myosin XI isoforms, and a given organelle can be associated with more than one type of myosin XI. The adapter(s) linking myosin XI to each organelle are not known, but the WIT1/2 proteins are known to recruit myosin XI-i to the nuclear membrane in *Arabidopsis* (Tamura et al. 2013).

RNA interference in the moss *Physcomitrella* have provided strong support for a role for myosin XI in organelle transport and streaming in these two plant systems (Madison and Nebenfuhr 2013). Several different myosins have been implicated in the transport of Golgi, peroxisomes, mitochondria, and the ER. It appears that disrupting one myosin can impact the motility of several different organelles, possibly owing to the presence of more than one type of myosin XI on a given organelle. Alternatively, the transport could be indirect, and the rapid movement of some cargo along actin tracks can drive the overall movement of many other organelles. Myosin XI family members have also been implicated in actin organization, and so loss of one could lead to defects in organization of actin tracks (Cai et al. 2014). In contrast, *Arabidopsis* myosin XI-I has a specific role in movement of the nucleus and plays a role in linking the nucleus to the actin cytoskeleton. Loss of this myosin results in alterations in shape, dark-induced movements, and positioning of the nucleus (Tamura et al. 2013). Working out the relationship between specific plant myosins and

organelle motility will be challenging, given the large number of myosin genes and the range of cellular contexts and developmental stages that they operate in.

Cytoplasmic streaming has long been suggested to play a crucial role in overall plant growth (Prokhnovsky et al. 2008; Ojangu et al. 2012), but only recently has support for this proposal been obtained. A clever experiment using chimeric myosins was used to investigate the link between streaming velocity and growth in *Arabidopsis*. The motor domain of At myosin XI-2 was replaced by the much faster Cc myosin XI motor or a significantly slower human myosin Vb motor and the chimeric myosins expressed in the *Arabidopsis* *myo11-2* null mutant (Tominaga et al. 2013). Amazingly, plants expressing the Cc myosin XI—At myosin XI-2 chimera showed a threefold faster rate of streaming, and the overall size of the plant and leaf tissue was increased significantly compared with controls. In contrast, the plants expressing the Hs myosin Vb—At myosin XI-2 chimera showed little or no streaming, and the plants were smaller. These findings are quite consistent with

a model whereby rapid myosin XI-based motility of organelles plays a crucial role in distributing these throughout the plant cell to favor optimal growth. There remain many questions about how this movement of organelles has such a dramatic effect on plant size, but it now seems clear that myosin XI-based motility plays a critical role in plant growth by driving the cytoplasmic streaming of organelles.

9 CONCLUSION

Myosins transport a remarkable variety of intracellular cargoes. The underlying biochemical and biophysical properties of transport motors are now well understood and much is also known about how the myosin is targeted to a specific cargo. The future challenge is to gain an understanding of how motor recruitment and activity are controlled at the local level and how motors are organized on the cargo itself.

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