Cell. Mol. Life Sci. 65 (2008) 2790 – 2800
1420-682X/08/182790-11
DOI 10.1007/s00018-008-8350-5

Cellular and Molecular Life Sciences

Myosins in the secretory pathway: tethers or transporters?

S. Loubéry[a, b] and E. Coudrier[a, b, *]

[a] Institut Curie, Centre de recherche 26 rue d'Ulm, Paris 75248 (France)
[b] CNRS, UMR144, Paris 75248 (France), Fax: 331 42346377, e-mail: coudrier@curie.fr

Online First 26 August 2008

Abstract. It is generally thought that microtubule-associated motors insure long-range movements of the secretory vesicles from the center of the cell to its periphery, while myosins insure short-range movements at the cell periphery. However, several of the myosins that have been reported during the last decade to be involved in the exocytic pathway are not processive, meaning that they do not have the ability to move cargos along actin polymers. We will review here the possible mechanisms by which these myosins could contribute to the traffic of secretory proteins from the Golgi complex to the plasma membrane. (Part of a Multi-author Review)

Keywords. Actin, myosin, exocytosis, exocyst, SNARE, microtubule.

Introduction

Proteins destined to be secreted move from the endoplasmic reticulum to the Golgi complex and then to the *trans*-Golgi network (TGN). From there, secretory proteins are transported into one of two types of vesicles depending on the cell type. In all cell types, some of the proteins are secreted continuously. These proteins are sorted in the TGN into transport vesicles that immediately move to and fuse with the plasma membrane, releasing their contents by exocytosis (constitutive exocytosis). In neuroendocrine cells, the secretion of neuropeptides and hormones is not continuous; these proteins are sorted in the TGN into secretory vesicles that are stored inside the cell awaiting a stimulus for Ca^{++}-dependent exocytosis (stimulated exocytosis). In addition to these two pathways secretion of some proteins, including the glucose transporter GLUT4 under insulin stimulation or newly synthesized basolateral membrane proteins in epithelial cells, is mediated by a subpopulation of endocytic vesicles [1, 2].

It is generally believed that vesicular transport between two compartments is achieved by microtubule- and actin-associated molecular motors along the microtubule and cortical actin meshwork. Microtubules form a radial polarized network with minus ends often localized to the juxtanuclear centrosomes and plus ends at the cell periphery in a large number of cell types. The minus-end-directed motors from the dynein and kinesin families are implicated in long-range movements towards the cell interior, while the plus-end-directed motors from the kinesin family mediate long-range movements towards the cell surface. A significant amount of work has studied the role of microtubule-associated motors in exocytosis-related processes, including Golgi positioning [3–9], intermediates between the endoplasmic reticulum and the Golgi complex [10, 11], and transport of secretory vesicles downstream of the Golgi complex towards the plasma membrane [12, 13]. The possible molecular mechanisms by which these motors contribute to the secretory pathway have been reviewed recently [14, 15].

Unlike microtubules, actin filaments form up to 15 distinct structures in metazoan cells, including stress fibers, cortical networks under the plasma membrane and peri-organellar structures [16]. It is generally accepted that the cortical actin filament networks provide tracks for short-range movements driven by motor proteins of the myosin family. The majority of the actin filament plus ends are thought to be facing the plasma membrane. Thus plus-end-directed myo-

* Corresponding author.

sins are expected to contribute to exocytosis. However, among the myosins that have been reported to be involved in the exocytic pathway, one of them moves towards the minus ends of the actin filaments and some of them are not processive, meaning that they do not have the ability to move cargo along actin polymers. We will discuss here how these different myosins could contribute to the traffic of secretory proteins from the Golgi complex to the plasma membrane.

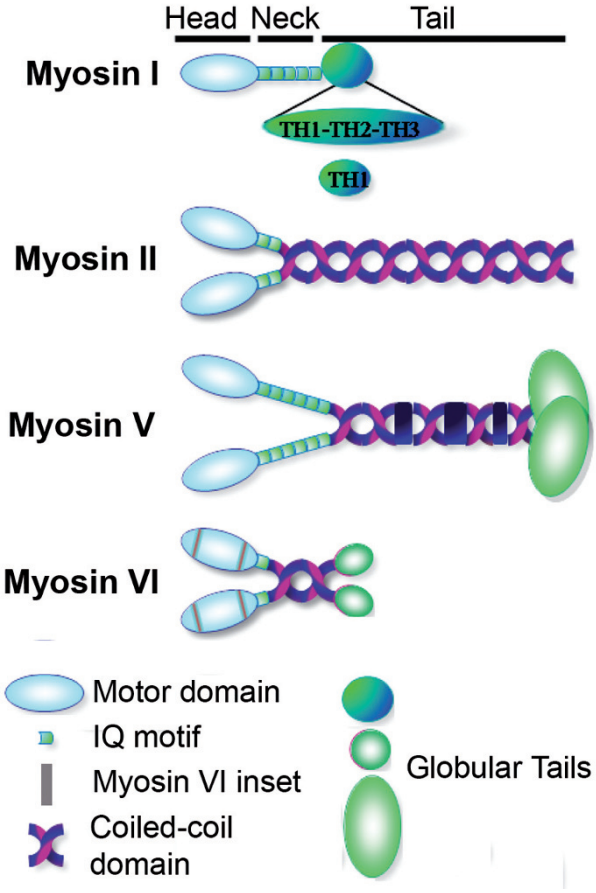

Figure 1. Organisation of the myosins from the classes I, II, V and VI in domains. Mammalian representatives of the myosins considered are depicted. Please note that Myo6 is represented as dimeric, although its dimerisation state in cells remains to be clarified.

Mechanochemical properties and regulation of the myosins involved in the secretory pathways

Myosins are mechanoenzymes that use ATP hydrolysis as a source of energy to exert a force along a cytoskeletal track – the actin filament. Among the 18 classes of myosins that have been categorized based on phylogenetic analysis of the conserved motor domain, members of four classes (I, II, V and VI) have been involved so far in exocytosis ([17, 18], see Table 1). Despite very different biochemical properties and functions, they all follow the same model of organization, which comprises three major domains (Fig. 1) [18, 19]. 1) The N-terminal motor domain, or 'head' which is a conserved ~80-kD globular region and that encompasses an ATP binding site and an ATP-dependant F-actin binding site. 2) The 'neck' region, also called 'lever arm', which can amplify the conformational change driven by ATP hydrolysis in the head to trigger the movement of the whole protein and its cargo. This domain encompasses one or more consensus sequence(s) (IVL)QXXXRGXXX(RK)-XX(FILVWY) named IQ motif(s) that can bind regulatory light chains, including calmodulin. 3) The C-terminal 'tail' domain, which provides the specificity for cargo binding and function. Depending on the myosin, this region encompasses coiled-coil domains enabling dimerization of the motor, and/or functional protein- or lipid-binding motifs such as SH3 (Src Homology type 3), PH (Pleckstrin Homology) or FERM (band 4.1/Ezrin/Radixin/Moesin) domains [19].

The ability of a given motor to transport cargos along a polymer depends on its processivity, an intrinsic property that relies on the kinetics of its enzymatic cycle and its structure [20]. Most highly processive motors function as dimers, and they usually have a high duty ratio, which means that they spend most of their ATPase cycle strongly bound to the cytoskeletal track: these properties enable them to take many steps along a polymer without falling off it [21, 22]. Non-processive motors that are monomeric motors or dimeric motors with low duty ratio could act as anchors in maintaining tensions between the compartment and the cytoskeleton polymer. Alternatively, the cooperative action of many motors of this type could induce a high effective processivity and thus trigger sustained movement.

Myosins II (Myo2) and myosin V (Myo5) contain coiled-coil domains and are dimeric *in vivo* (Fig. 1). Yet the isoform a of Myo5 (Myo5a) is highly processive, taking 36-nm steps which correspond to the helical periodicity of actin filaments, while Myo2 is non-processive [22–24]. Contrary to Myo2 and Myo5a, myosins from class 1 are monomeric (Fig. 1) and likely non-processive *in vivo*. The dimerization state and the processivity of Myo6 in cells remain a matter of debate. Myo6 was shown to be monomeric when purified from tissues or cell culture [25], but recent studies suggest that cargo binding may induce its dimerization *in vivo* (Fig. 1) and thus its processivity [26–29].

The activities of these myosins are tightly regulated in cells. Changes in calcium concentration can

activate or inhibit the motor activity, most likely by regulating binding of the calmodulin light chains. Myosins from class I and class V in particular contain several IQ motifs that do not have identical sequences and that modulate the motor activity of these myosins by triggering partial or complete calmodulin dissociation upon variation of calcium concentration [30–32]. Phosphorylation is another mean of regulation commonly encountered for myosins. Both Myo2 heavy and light chains possess multiple phosphorylation sites enabling complex sets of kinases and phosphatases to regulate the Myo2 function [33,34]. These phosphorylations that are not exclusive can regulate both the motor activity and the dimerisation state of the myosin, allowing fine control of the grade of activation of the motor. Myo5 can be both positively and negatively regulated by phosphorylation. Akt-mediated phosphorylation of Myo5a activates the protein and facilitates insulin-stimulated GLUT4 vesicle translocation to the cell surface [35]; conversely, phosphorylation of Myo5 by calcium/calmodulin-dependant protein kinase II (CaMKII) results in the release of the motor from the surface of melanosomes [36]. The phosphorylation of Myo6 is a recently raised and not well understood issue, as it was suggested to regulate the ability of the motor to control actin organization by a yet unknown mechanism [37]. Combinations of all these different mechanisms of regulation enable the same myosin to function on different organelles in distinct cellular locations in order to fulfill specific tasks.

Myosin V (Myo5) transports secretory vesicles in yeast

Saccharomyces cerevisiae expresses five myosins among which Myo2p, which belongs to the myosin V family and has been involved in polarized secretion during cell growth [38–41]. Myo2p transports secretory vesicles to the growing bud of yeast [42]. Genetics evidence suggests that the Rab protein Sec4p interacts with the tail domain of Myo2p on the secretory vesicles, although direct interaction has not been demonstrated. In addition, mutation in Sec2p, a guanine nucleotide exchange factor (GEF) that inhibits Sec4p activation, uncouples Myo2p from the secretory vesicles. These observations support the model whereby a Rab protein could regulate the recruitment of a myosin to the surface of an organelle [43]. Sec4p is also one subunit of the exocyst complex that tethers incoming secretory vesicles to the plasma membrane prior their fusion [44]. This Rab protein may therefore coordinate the transport and tethering of the secretory vesicles to their site of exocytosis by interacting on the one hand with Myo2p and on the other with additional subunits of the exocyst complex.

Myosin Va (Myo5a) delivers secretory vesicles at the plasma membrane: lesson from melanosomes

1-Myo5a moves melanosomes on actin filaments

The first evidence for the participation of an actin-based motor in the transport of one organelle to the plasma membrane was provided by Mercer and colleagues [45], who reported that the gene mutated in the dilute mouse with a coat colour defect encodes the heavy chain of Myo5a. Equivalent mutation is observed in humans who suffer from Griscelli syndrome type 1 [46]. The distribution of melanosomes in epidermal melanocytes of dilute mice and patients who suffer from Griscelli syndrome type 1 is abnormal. Most of them are clustered in the perinuclear region. Based on the extensive colocalization of Myo5a with melanosomes in actin-rich regions at the periphery of mouse melanocytes, Wu and colleagues have proposed 'the capture model'. In this model Myo5a-dependent interaction of melanosomes with actin at the cell periphery prevents these organelles from returning to the cell center via microtubules and dynein [47]. In contrast to this static view for the role of Myo5a in the movement of melanosomes, Gross and colleagues showed that Myo5a in melanophores contributes to a certain amount of motility, and Wu and colleagues reported Myo5a-dependent movements of melanosomes *in vivo* (0.14 μm/s movements) in mammalian skin melanocytes [47]. Chabrillat and colleagues have also observed directional movements of melanosomes in the absence of microtubules, and they have reconstituted *in vitro* the actin-based movement of isolated melanosomes [48]. More recently Levi et al. have reported that trajectories of melanosomes in *Xenopus* melanophores present steps with a size compatible with the step size of Myo5 determined *in vitro* [49]. Together with the processive properties of Myo5a, these observations support the hypothesis in which Myo5a could move melanosomes at the cell periphery on the cortical actin meshwork.

The function of Myo5a on melanosomes is linked to its recruitment, which is mediated by a small Ras-like GTPase, Rab27a, and one of the effectors of Rab27, melanophilin. GTP-bound Rab27 interacts with melanophilin, which in turn recruits Myo5a to form a tripartite complex [47, 50–55]. Such tripartite complexes, comprising dmyosin V, Rab11 and dRip11, have been reported to contribute to rhodopsin transport in photoreceptor cells of *Drosophila* suggesting

that it may be a general way to recruit Myo5 on an organelle [56].

2-Myo5a moves and/or docks secretory granules to the plasma membrane

Myo5a has also been depicted on the secretory granules from neuroendocrine cells, pancreatic cells and on synaptic vesicles from neurons [57–60]. Release of neuropeptides and hormones at the plasma membrane is achieved by Ca^{++}-triggered stimulation of the secretory granules. Secretory vesicles are formed at the TGN and stored inside the cells. They are transported to the cell periphery along microtubules using a kinesin-1 [61], where they accumulate in the cortical actin meshwork [59]. Then the vesicles cross the actin meshwork and are docked to the plasma membrane (meaning that they are attached to the plasma membrane [62]) before fusion.

Myo5a is associated to the secretory vesicles depending on Ca^{++} [63–65]. Knockdown expression of Myo5a decreases the magnitude of the secretion in adrenal chromaffin cells and in pancreatic β cells [63–65]. The expression of a dominant negative domain of Myo5a induces extensive intracellular clustering of secretory granules in PC12 cells or insulin-secreting cells [65]. The first hypothesis suggesting that Myo5a contributes to the peripheral retention of the secretory vesicles at the cell periphery relied on the proposed mechanism for its function in the transport of melanosomes at the cell periphery (i.e. the capture model). In concordance with this hypothesis, Huet and colleagues have observed, using total internal reflection fluorescence microscopy (TIRFM), that secretory vesicles cannot attach to the plasma membrane directly from microtubules but must travel through the actin cortex before reaching the plasma membrane [66]. However, recent data suggest that Myo5a contributes to the docking step. Watanabe and colleagues have shown that syntaxin-1, a tSNARE involved in exocytosis, interacts with the neck region of Myo5a depending on the Ca^{++} concentration and that inhibition of this interaction affects exocytic release [67]. Desnos and colleagues have shown that Myo5a silencing in neuroendocrine cells reduces the duration of the immobilization period of the vesicles and thus reduces the number of docked vesicles [62, 68]. Additional studies will be needed to determine whether Myo5a contributes only to the docking step or whether it contributes also to short-distance transport along actin filaments of the cortical meshwork. Similarly to melanosomes, recruitment of Myo5a on the secretory vesicles could be mediated by Rab27 and one of its effectors, MyRip. Knockdown expression of Rab27 or MyRip reduces the magnitude of the secretory response [63, 69]. However, whether they form a tripartite complex with Myo5a to recruit this myosin on the secretory granules remains to be clarified.

3-Myo5a contributes to the fusion of GLUT4 vesicles with the plasma membrane

Myo5a also contributes to glucose transporter 4 (GLUT4) vesicles translocation. In response to insulin stimulation, GLUT4-containing vesicles move to the cell cortex and fuse with the plasma membrane. Insulin stimulation induces phosphorylation of Myo5a in its motor domain depending on Akt2 (protein kinase Bβ), and the two proteins coimmunoprecipitate [35]. Knockdown expression of Akt2 by siRNA or expression of dominant negative Akt2 inhibits insulin-dependent phosphorylation of Myo5a and translocation of GLUT4-containing vesicles to the plasma membrane. Similarly, knockdown expression of Myo5a or expression of a dominant negative mutant attenuates GLUT4-containing vesicle translocation to the plasma membrane [35]. Whether Myo5a contributes to GLUT4 vesicle transport to the membrane or to the docking step remains to be clarified. Analysis of the dynamic behaviour of GLUT4 vesicles using TIRFM suggests that insulin stimulates recruitment of GLUT4 mobile vesicles to the plasma membrane. Thus these vesicles could be delivered to the plasma membrane directly from microtubules [70]. This hypothesis implies a mechanism distinct from that postulated for melanosome and secretory vesicle movements. Myo5a may contribute to the docking step, as proposed for the secretory vesicles. It may alternatively regulate microtubule-dependent movements. Indeed, impairment of Myo5a reduces the microtubule-dependent movements of melanosomes, suggesting that myosin Va may directly or indirectly coordinate actin and microtubule-dependent movements [47].

Myosin 1 (Myo1) and stimulated secretion

Several myosins from class I have been involved in regulated exocytosis. Two types of class 1 myosins can be distinguished depending on the size of their tail domain. All class 1 myosins present a basic tail homology domain (MyTH1) thought to be involved in membrane interaction. In addition, some of them, named long-tail class 1 myosins, present a proline-rich domain (MyTH2) that binds to actin filaments in protozoa and a domain containing a single Src homology domain 3 (SH3), a protein-protein interacting motif (MyTH3).

Long-tail myosins 1 and regulated secretion

Long-tail class 1 myosins in dictyostelium and yeast have been shown to contribute to actin remodelling [71–74]. One of these myosins (MYOA) is required for secretion in *Aspergillus nidulans* [75]. However, Liu and colleagues have demonstrated that the motor activity of MYOA does not contribute to its function. They have proposed that MYOA plays a structural role [76].

Another long-tail myosin 1, Myosin 1e (Myo1e), has been shown to control the regulated secretion of cortical granules in response to elevated calcium upon fertilization in *Xenopus* oocytes [77]. Its recruitment on cortical granules prior to exocytosis is mediated by its MyTH1 and MyTH2 domains, and its MyTH2 domain associates with assembling actin filaments *in vivo* [78]. Therefore, Myo1e may contribute to exocytosis in remodelling actin filaments at the surface of the cortical granules.

In a very elegant study Kim and collaborators have demonstrated that Myosin 1f (Myo1f), also a long-tail myosin 1 directs immune cell motility by controlling the regulated secretion at the cell surface of the β2 integrin [79]. In the absence of Myo1f the secretion of granules containing β2 integrin was highly increased. Similarly to Myo1e, Myo1f could modulate the cortical actin network via its MyTH2 domain and thereby inhibit the transfer to and/or the fusion of the secretion granule with the plasma membrane.

Myosin 1c (Myo1c) and the translocation of GLUT4 vesicles upon insulin stimulation

One short-tail myosin (Myo1c) has been also involved in the secretory pathway. Myosin 1c (Myo1c) contributes to the delivery of GLUT4 vesicles at the plasma membrane [35, 80, 81]. Myo1c is a ubiquitous monomeric myosin that is concentrated at the cell periphery and at the plasma membrane [82]. GLUT4 vesicles colocalize with Myo1c in membrane ruffles under insulin stimulation, and knockdown expression of Myo1c or overexpression of a dominant negative mutant blocks insulin-stimulated glucose transport [80, 81, 83]. It has been proposed that this non-processive myosin served as an anchor for the GLUT4 vesicles at the plasma membrane. In concordance with this hypothesis, the small GTPase Ral A that is linked with the adipocyte exocyst complex interacts with Myo1c, both being required for the corrected delivery of GLUT4 [84]. In the Xenopus oocyte, Myo1c controls actin assembly to membranes and so mediates force production during compensatory endocytosis [85]. Similarly, mammalian Myo1c could control actin polymerization using an unknown mechanism.

Myosin II (Myo2) in constitutive and stimulated secretion

It is generally acknowledged that Myo2 contributes to cell locomotion. However, Myo2 is also associated with the Golgi complex, vesicles emanating from the TGN and secretory granules, suggesting that it participates in constitutive and stimulated secretion [60, 64, 86].

Myo2 in constitutive secretion

A myosin toxin, butanedione monoxime (BDM), which is now believed to inhibit only the activity of Myo2 [87], induces compaction of the Golgi complex [88]. Myo2 has been involved in two different pathways associated with the Golgi complex: it is associated with vesicles from the TGN and contributes to basolateral transport in MDCK cells [89, 90] and to retrograde transport from the Golgi complex to the endoplasmic reticulum. Two isoforms of Myo2, Myo2a and Myo2b, are ubiquitously expressed in all non-muscle cells. Loss of Myo2b induces dispersion of the Golgi complex, while loss of Myo2a does not significantly alter it, indicating that Myo2b contributes to the organization of the Golgi complex; but the isoform that contributes to retrograde transport and transport of the secretory vesicles is still unknown [91].

Myo2 contributes to Ca^{++}-triggered release of the secretory granules

Overexpression of the unphosphorylated form of the regulatory light chains of Myo2 inhibits movement of secretory granules at the periphery of chromaffin cells, as well as the stimulated secretion, and decreases the fusion kinetics of individual granules [92]. Thus, Myo2 could participate in several steps of secretory granule secretion. Myo2 could contribute indirectly to the dynamics of the secretory vesicles in organizing the cortical actin meshwork or directly in controlling the actin dynamics at the surface of the vesicles, as has been suggested for cortical granules of the *Xenopus* oocyte [78]. In retaining transiently the secretory granules on actin filaments nearby the plasma membrane, it may also tether them to the membrane and favour their fusion. What is the Myo2 isoform that contributes to this process? It is presently unclear. Myo2b is required for exocytosis-dependent cell repair processes while Myo2a is required for cytolytic granule exocytosis in human natural killer cells [93, 94]. Whether one or both participate in the different steps of stimulated secretion remains to be elucidated.

Myosin VI (Myo6) contributes to constitutive exocytosis

One peculiarity of myosin VI (Myo6) is that it is the only one so far able to move towards the pointed ends (minus ends) of actin filaments [95]. Myo6 has been involved both in the endocytic and exocytic pathways. It is present on the Golgi complex and in vesicles at the plasma membrane. Loss of Myo6 reduces the size of the Golgi complex by approximately 40% [96]. Its downregulation also impairs exocytosis of vesicular stomatitis virus in NRK cells and alkaline phosphatase in cells derived from knockout mice (Snell's Waltzer) [96, 97]. Its impact on secretion might be related to its role in the organization of the Golgi complex. However, Myo6 is also present on vesicular structures suggesting that it may have a direct role on the secretory vesicles [97]. Similarly to Myo5a on melanosomes, a Rab protein mediates the recruitment of Myo6 on membrane domains. GTP-bound Rab8 links Myo6 to optineurin on the Golgi complex and on vesicles at the plasma membrane [97]. When optineurin is depleted from cells using RNA interference, Myo6 is lost from the Golgi complex and the Golgi is fragmented [97]. The way by which Myo6 contributes to constitutive exocytosis is unclear. Similarly to its proposed function during *Drosophila* spermatid individualization, Myo6 may act as a non-processive myosin (see above) and stabilize the actin filaments nearby the Golgi complex and the secretory vesicles [98]. Alternatively, as a processive motor, it could transport vesicles emanating from the Golgi complex and secretory vesicles along a short distance on actin filaments [27].

How do myosins contribute to the traffic events along the exocytic pathway if they do not move organelles and vesicles?

Transfer of secretory proteins from one donor compartment to the acceptor requires four essential steps, including vesicle budding and fission from the donor compartment, transport, tethering and fusion with the acceptor compartment [99]. It is generally thought that molecular motors insure transportation of the vesicles. It is very likely that myosin V fulfills this function in yeast, in melanocytes for the movement of melanosomes as well as its homologue myosin XI in plants for polar auxin transport. But it is rather unclear for Myo5a associated with secretory vesicles (Fig. 2A) [100]. Most of the other myosins involved in the secretory pathway do not have the ability to move vesicles on actin tracks. They may interfere with the other steps required to transfer the secretory proteins

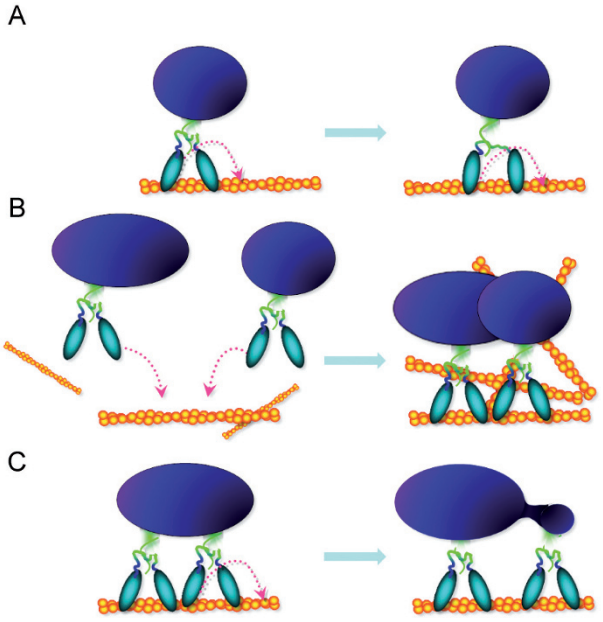

Figure 2. Possible roles for myosins in membrane trafficking and dynamics. (*A*) Processive myosins can move cargos along actin filaments. (*B*) Myosins can regulate tethering, docking and/or fusion by reorganizing the actin network and transiently immobilizing compartments on actin filaments. (*C*) Myosins can also regulate budding and/or fission by exerting tensions on membranes.

from one donor compartment to one acceptor. Some of these myosins, such as Myo2, Myo6, Myo1e and Myo1f, can control the organization of the actin network. It has been proposed that muscle Myo2 induces contraction by forcing two sets of actin filaments of opposite polarity to slide towards each other. Based on this model it is generally thought that non-muscle Myo2 causes the sliding of actin filaments. Recent experiments demonstrating that Myo6 stabilizes the actin network during spermatid individualization and in cultured cells depending on its phophorylation argue for Myo6 acting as an actin cross-linker [37, 98, 101]. Myo1e and Myo1f may also contribute to actin nucleation as has been shown for the long-tail myosins 1 in yeast and amoeba in addition to presenting a MyTH2 domain that has the ability to bind actin filaments [71–73]. In controlling the organization of the actin filaments in the vicinity of the secretory vesicles, Myo2, Myo6, Myo1e and Myo1f may contribute indirectly to the dynamics of secretory vesicles. They may also trap secretory vesicles in the actin meshwork and favour their fusion with the plasma membrane (Fig. 2B) [102].

Several myosins participate in the fusion step by interacting with proteins from complexes required for fusion. Myo1c and yeast myosin V interact with the exocyst, while Myo5a interacts with syntaxin-1. Additionally, recent observations in MDCK cells favour

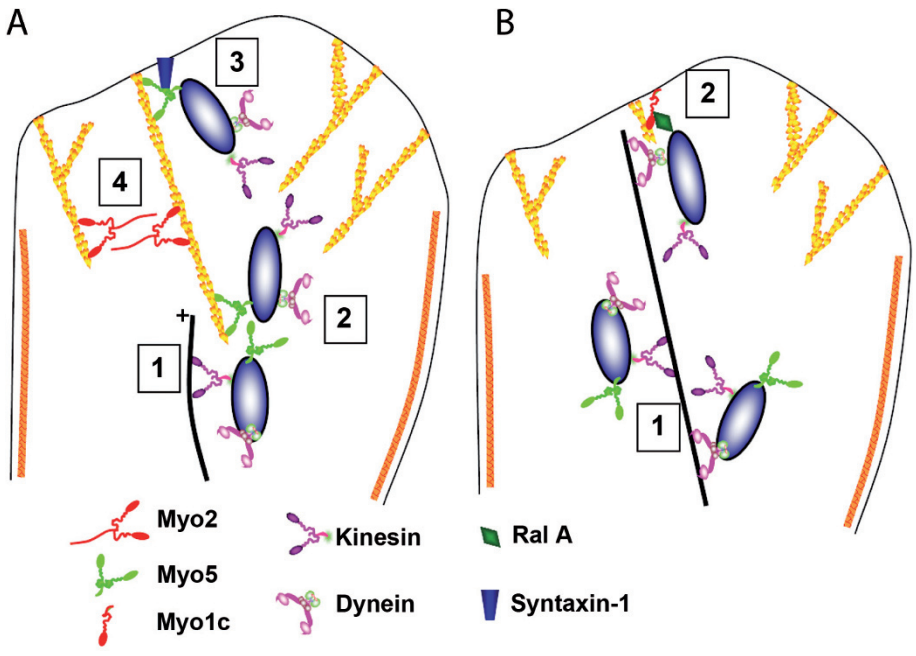

Figure 3. Motors involved in secretory granules and GLUT4 secretions. A and B1- Secretory granules and GLUT4 vesicles move bidirectionally on microtubules depending on kinesins and dynein. A2- Secretory granules are transferred on the cortical actin meshwork depending on Myo5a. A3- Secretory granules interact with the plasma membrane depending on Myo5a and syntaxin-1. A4- Myo2 controls the dynamics of the cortical actin meshwork to facilitate delivery of the secretory granules at the plasma membrane. B2- GLUT4 vesicles are transported to the plasma membrane via the microtubules and bind to the plasma membrane depending on Myo1c and Ral A. The role of Myo5a remains to be clarified.

such interaction between Myo6 and the exocyst for the sorting of newly synthezised proteins to the basolateral membrane domain of these cells [103, 104]. However, the way they contribute to fusion might vary from one myosin to another. In yeast, myosin V transports part of the exocyst complex to the plasma membrane, and activation of Rab Sec4p releases it from the vesicles before fusion, while in mammalian cells Myo5a may dock the secretory vesicles to the plasma membrane by interacting with one protein of the SNARE complex located at the plasma membrane; and Myo1c, which is located at the plasma membrane, may recruit the exocyst complex by interacting with Ral A.

Finally, by exerting a force between the actin filaments and the membrane of the donor compartment, myosins may control morphological changes and participate in budding and/or fission of the cargo vesicles as suggested for myosins involved in the endocytic pathway (Fig. 2C) [71, 105–107]. As a whole, the experimental evidence available suggests that multiple mechanisms could contribute to the function of myosins along the constitutive and stimulated exocytic pathways.

Coordination of microtubule- and actin-based movements

One important issue of myosin-actin-based translocation of secretory granules is that they have to work together with microtubule-based motors to mediate the delivery of the secretory granules to the plasma membrane. It has been suggested that Myo5 could coordinate the movements occurring on the two cytoskeleton networks. In yeast, the myosin V Myo2p interacts with a kinesin-related protein Smy1p, which participates with it in the delivery of vesicles at the bud tip [108]. In melanocytes, impairment of Myo5a reduces the microtubule-dependent movements of melanosomes [47]. Similarly to yeast, Myo5a may regulate microtubule-based transport in mammalian cells by interacting with a kinesin such as kinesin-1 [109].

Rab proteins could also act as coordinators of microtubule-associated motors and myosins. In melanocytes, the carboxy-terminal domain of the Rab27 effector melanophilin comprises a binding site for actin and EB1, a protein associated with the plus ends of the microtubules [110]. This effector has therefore been proposed to coordinate actin- and microtubule-based movements. Indeed, GFP-melanophilin is associated with microtubules, depending on its EB1 interaction. However, the distribution of melanosomes at the cell periphery requires neither the EB1 binding site nor the actin binding site [110, 111]. Recent observations suggest an alternative to this hypothesis. The orthologue of melanophilin in zebrafish regulates dynein independent of its interaction with Myo5 [112]. The authors have proposed that activation of melanophilin suppresses dynein activity and thereby favours movement of the melanosomes towards microtubule plus ends. Whether Rab27 and its effector on the secretory granules, MyRip, can play a similar role is an unsolved question. MyRip also presents an actin-binding site, but neither a micro-

Table 1. Myosins involved in secretion-related processes. The myosins known so far to be involved in secretion-related processes are reported here. When known, their partners and the small GTPases involved in the regulation of their activity and their recruitment on membranes are specified.

Myosin class	Protein	Intracellular localisation	Recruitment and regulation	References
Myosin I	Myo1c	GLUT4-positive vesicles	Under the control of Ral A	[80, 81, 84, 114]
	Myo1f	Granule-containing β2 integrin	Unknown	[79]
	Myo1e	Cortical granules	Cystein string protein	[77, 78]
Myosin II	Myo2a	Trans-Golgi network vesicles	Unknown	[93]
		Cytolytic granules	Unknown	[94]
	Myo2b	Golgi apparatus	Unknown	[91]
Myosin V	Myo2p	Secretory vesicles	Under the control of Sec4p	[38–42]
	Myo5a	Melanosomes	Recruited by melanophilin under the control of Rab27a	[47, 48, 50, 51, 53, 54]
		Secretory vesicles	Unknown	[59, 61, 63–65, 68]
		GLUT4-positive vesicles	Unknown	[35]
Myosin VI	Myo6	Golgi apparatus Secretory vesicles	Recruited by optineurin under the control of Rab8	[96, 97]

tubule-binding site nor interaction with microtubule-binding proteins have been reported up to now [113]. Another Rab protein, Rab4, is a potential candidate for coordinating microtubule- and actin-dependent movements of GLUT4 vesicles. According to Yoshizaki and colleagues, Rab4 may serve as an adaptor between the kinesin-2, KIF3 and GLUT4 vesicles for their microtubule-based movements on the one hand and Myo5a and GLUT4 vesicles for their actin-based movement on the other hand [35].

Concluding remarks

Recent progress in our understanding of the myosins involved in secretion has provided new insight on the link between these motors and the other protein complexes involved in trafficking (Table 1). The presence of Myo2 and Myo6 on vesicular structures in addition to their association with the Golgi complex suggests that both myosins play a role at least in two steps of the constitutive exocytic pathway. Whether they act sequentially, for the transport of the same cargo, or whether they transport distinct cargos is an interesting issue. Myo5 and Myo2 both contribute to the delivery of secretory vesicles at the plasma membrane. Myo5 could move and dock the secretory vesicles at the plasma membrane, while Myo2 could reorganize the actin filaments to facilitate the passage of the secretory vesicles through the cortical actin meshwork. Myo5a and Myo1c both participate in delivery of GLUT4 at the plasma membrane. Both may contribute to the docking steps: Myo5a on the GLUT4 vesicles could likely tether them at the plasma membrane via its interaction with syntaxin-1, while Myo1c at the plasma membrane can tether the vesicles via its interaction with Ral A (Fig. 3). The challenge now is to understand the molecular mechanisms by which these myosins contribute to these processes and whether they act in a coordinated way. The recent progress in live cell imaging and *in vitro* assays to reconstitute vesicle motility and fusion should enable the exploration of such mechanisms.

Acknowledgment. This work was supported by grants to E. C. from the Association pour la Recherche sur le Cancer (Arc 3508) and from the Institut National du Cancer [Réseau Mélanome Idf (RS#13)]. S. L. has been the recipient of a fellowship from the 'Association pour la Recherche sur le Cancer.'

1 Hou, J. C. and Pessin, J. E. (2007). Ins (endocytosis) and outs (exocytosis) of GLUT4 trafficking. Curr. Opin. Cell Biol. 19, 466 – 473.
2 Cresawn, K. O., Potter, B. A., Oztan, A., Guerriero, C. J., Ihrke, G., Goldenring, J. R., Apodaca, G. and Weisz, O. A. (2007). Differential involvement of endocytic compartments in the biosynthetic traffic of apical proteins. EMBO J. 26, 3737 – 3748.
3 Allan, V. J., Thompson, H. M. and McNiven, M. A. (2002). Motoring around the Golgi. Nat. Cell Biol. 4, E236 – 242.
4 Harada, A., Takei, Y., Kanai, Y., Tanaka, Y., Nonaka, S. and Hirokawa, N. (1998). Golgi vesiculation and lysosome dispersion in cells lacking cytoplasmic dynein. J. Cell Biol. 141, 51 – 59.
5 Hoogenraad, C. C. Akhmanova, A., Howell, S. A., Dortland, B. R., De Zeeuw, C. I., Willemsen, R., Visser, P., Grosveld, F. and Galjart, N. (2001). Mammalian Golgi-associated Bicaudal-D2 functions in the dynein-dynactin pathway by interacting with these complexes. EMBO J. 20, 4041 – 4054.
6 Lane, J. D. and Allan, V. J. (1999). Microtubule-based endoplasmic reticulum motility in Xenopus laevis: activation of membrane-associated kinesin during development. Mol. Biol. Cell 10, 1909 – 1922.
7 Matanis, T. Akhmanova, A. Wulf, P., Del Nery, E., Weide, T., Stepanova, T., Galjart, N., Grosveld, F. Goud, B., De Zeeuw, C. I., Barnekow, A., Hoogenraad, C. C. (2002). Bicaudal-D regulates COPI-independent Golgi-ER transport by recruiting the dynein-dynactin motor complex. Nat. Cell Biol. 4, 986 – 992.

8 Wozniak, M. J. and Allan, V. J. (2006). Cargo selection by specific kinesin light chain 1 isoforms. EMBO J. 25, 5457 – 5468.
9 Xu, Y., Takeda, S., Nakata, T., Noda, Y., Tanaka, Y. and Hirokawa, N. (2002). Role of KIFC3 motor protein in Golgi positioning and integration. J. Cell Biol. 158, 293 – 303.
10 Le Bot, N., Antony, C., White, J., Karsenti, E. and Vernos, I. (1998). Role of xklp3, a subunit of the Xenopus kinesin II heterotrimeric complex, in membrane transport between the endoplasmic reticulum and the Golgi apparatus. J. Cell Biol. 143, 1559 – 1573.
11 Stauber, T., Simpson, J. C., Pepperkok, R. and Vernos, I. (2006). A role for kinesin-2 in COPI-dependent recycling between the ER and the Golgi complex. Curr. Biol. 16, 2245 – 2251.
12 Bowman, A. B., Kamal, A., Ritchings, B. W., Philp, A. V., McGrail, M., Gindhart, J. G. and Goldstein, L. S. (2000). Kinesin-dependent axonal transport is mediated by the sunday driver (SYD) protein. Cell 103, 583 – 594.
13 Kreitzer, G., Marmorstein, A., Okamoto, P., Vallee, R. and Rodriguez-Boulan, E. (2000). Kinesin and dynamin are required for post-Golgi transport of a plasma-membrane protein. Nat. Cell Biol. 2, 125 – 127.
14 Caviston, J. P. and Holzbaur, E. L. (2006). Microtubule motors at the intersection of trafficking and transport. Trends Cell Biol. 16, 530 – 537.
15 Hehnly, H. and Stamnes, M. (2007). Regulating cytoskeleton-based vesicle motility. FEBS Lett. 581, 2112 – 2118.
16 Chhabra, E. S. and Higgs, H. N. (2007). The many faces of actin: matching assembly factors with cellular structures. Nat. Cell Biol. 9, 1110 – 1121.
17 Hodge, T. and Cope, M. J. (2000). A myosin family tree. J. Cell Sci. 113 Pt 19, 3353 – 3354.
18 Foth, B. J., Goedecke, M. C. and Soldati, D. (2006). New insights into myosin evolution and classification. Proc. Natl. Acad. Sci. USA 103, 3681 – 3686.
19 Krendel, M. and Mooseker, M. S. (2005). Myosins: tails (and heads) of functional diversity. Physiology (Bethesda) 20, 239 – 251.
20 De La Cruz, E. M. and Ostap, E. M. (2004). Relating biochemistry and function in the myosin superfamily. Curr. Opin. Cell Biol. 16, 61 – 67.
21 De La Cruz, E. M., Wells, A. L., Rosenfeld, S. S., Ostap, E. M. and Sweeney, H. L. (1999). The kinetic mechanism of myosin V. Proc. Natl. Acad. Sci. USA 96, 13726 – 13731.
22 Higuchi, H. and Endow, S. A. (2002). Directionality and processivity of molecular motors. Curr. Opin. Cell Biol. 14, 50 – 57.
23 Mehta, A. D., Rock, R. S., Rief, M., Spudich, J. A., Mooseker, M. S. and Cheney, R. E. (1999). Myosin-V is a processive actin-based motor. Nature 400, 590 – 593.
24 Baker, J. E., Krementsova, E. B., Kennedy, G. G., Armstrong, A., Trybus, K. M. and Warshaw, D. M. (2004). Myosin V processivity: multiple kinetic pathways for head-to-head coordination. Proc. Natl. Acad. Sci. USA 101, 5542 – 5546.
25 Lister, I., Schmitz, S., Walker, M., Trinick, J., Buss, F., Veigel, C. and Kendrick-Jones, J. (2004). A monomeric myosin VI with a large working stroke. EMBO J. 23, 1729 – 1738.
26 Altman, D., Goswami, D., Hasson, T., Spudich, J. A. and Mayor, S. (2007). Precise positioning of myosin VI on endocytic vesicles in vivo. PLoS Biol. 5, e210.
27 Park, H., Ramamurthy, B., Travaglia, M., Safer, D., Chen, L. Q., Franzini-Armstrong, C., Selvin, P. R. and Sweeney, H. L. (2006). Full-length myosin VI dimerizes and moves processively along actin filaments upon monomer clustering. Mol. Cell 21, 331 – 336.
28 Spudich, G., Chibalina, M. V., Au, J. S., Arden, S. D., Buss, F. and Kendrick-Jones, J. (2007). Myosin VI targeting to clathrin-coated structures and dimerization is mediated by binding to Disabled-2 and PtdIns(4,5)P(2). Nat. Cell Biol. 9, 176 – 183.
29 Sweeney, H. L., Park, H., Zong, A. B., Yang, Z., Selvin, P. R. and Rosenfeld, S. S. (2007). How myosin VI coordinates its heads during processive movement. EMBO J. 26, 2682 – 2692.
30 Barylko, B., Binns, D. and Albanesi, J. (2000). Regulation of the enzymatic and motor activities of myosin I. Biochim. Biophys. Acta. 1496, 23 – 35.
31 Lin, T., Tang, N. and Ostap, E. M. (2005). Biochemical and motile properties of Myo1b splice isoforms. J. Biol Chem. 280, 41562 – 41567.
32 Taylor, K. A. (2007). Regulation and recycling of myosin V. Curr. Opin. Cell Biol. 19, 67 – 74.
33 Bresnick, A. R. (1999). Molecular mechanisms of nonmuscle myosin-II regulation. Curr. Opin. Cell Biol. 11, 26 – 33.
34 Matsumura, F. (2005). Regulation of myosin II during cytokinesis in higher eukaryotes. Trends Cell Biol. 15, 371 – 377.
35 Yoshizaki, T., Imamura, T., Babendure, J. L., Lu, J. C., Sonoda, N. and Olefsky, J. M. (2007). Myosin 5a is an insulin-stimulated Akt2 (protein kinase Bbeta) substrate modulating GLUT4 vesicle translocation. Mol. Cell Biol. 27, 5172 – 5183.
36 Karcher, R. L., Roland, J. T., Zappacosta, F., Huddleston, M. J., Annan, R. S., Carr, S. A. and Gelfand, V. I. (2001). Cell cycle regulation of myosin-V by calcium/calmodulin-dependent protein kinase II. Science 293, 1317 – 1320.
37 Naccache, S. N. and Hasson, T. (2006). Myosin VI altered at threonine 406 stabilizes actin filaments in vivo. Cell Motil. Cytoskeleton 63, 633 – 645.
38 Govindan, B., Bowser, R. and Novick, P. (1995). The role of Myo2, a yeast class V myosin, in vesicular transport. J. Cell Biol. 128, 1055 – 1068.
39 Pruyne, D. W., Schott, D. H. and Bretscher, A. (1998). Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. J. Cell Biol. 143, 1931 – 1945.
40 Lillie, S. H. and Brown, S. S. (1992). Suppression of a myosin defect by a kinesin-related gene. Nature 356, 358 – 361.
41 Pruyne, D., Legesse-Miller, A., Gao, L., Dong, Y. and Bretscher, A. (2004). Mechanisms of polarized growth and organelle segregation in yeast. Annu. Rev. Cell Dev. Biol. 20, 559 – 591.
42 Schott, D. H., Collins, R. N. and Bretscher, A. (2002). Secretory vesicle transport velocity in living cells depends on the myosin-V lever arm length. J. Cell Biol. 156, 35 – 39.
43 Seabra, M. C. and Coudrier, E. (2004). Rab GTPases and myosin motors in organelle motility. Traffic 5, 393 – 399.
44 Boyd, C., Hughes, T., Pypaert, M. and Novick, P. (2004). Vesicles carry most exocyst subunits to exocytic sites marked by the remaining two subunits, Sec3p and Exo70p. J. Cell Biol. 167, 889 – 901.
45 Mercer, J. A., Seperack, P. K., Strobel, M. C., Copeland, N. G. and Jenkins, N. A. (1991). Novel myosin heavy chain encoded by murine dilute coat colour locus. Nature 349, 709 – 713.
46 Pastural, E. Barrat, F. J., Dufourcq-Lagelouse, R., Certain, S., Sanal, O., Jabado, N., Seger, R., Griscelli, C., Fischer, A., de Saint Basile, G. (1997). Griscelli disease maps to chromosome 15q21 and is associated with mutations in the myosin-Va gene. Nat. Genet. 16, 289 – 292.
47 Wu, X., Bowers, B., Rao, K., Wei, Q. and Hammer, J. A. (1998). Visualization of melanosome dynamics within wild-type and dilute melanocytes suggests a paradigm for myosin V function In vivo. J. Cell Biol. 143, 1899 – 1918.
48 Chabrillat, M. L., Wilhelm, C., Wasmeier, C., Sviderskaya, E. V., Louvard, D. and Coudrier, E. (2005). Rab8 regulates the actin-based movement of melanosomes. Mol. Biol. Cell 16, 1640 – 1650.
49 Levi, V., Gelfand, V. I., Serpinskaya, A. S. and Gratton, E. (2006). Melanosomes transported by myosin-V in Xenopus melanophores perform slow 35 nm steps. Biophys. J. 90, L07 – 9.
50 Barral, D. C. and Seabra, M. C. (2004). The melanosome as a model to study organelle motility in mammals. Pigment Cell Res. 17, 111 – 118.

51 Fukuda, M. and Itoh, T. (2004). Slac2-a/melanophilin contains multiple PEST-like sequences that are highly sensitive to proteolysis. J. Biol Chem. 279, 22314 – 22321.

52 Fukuda, M. and Kuroda, T. S. (2002). Slac2-c (synaptotagmin-like protein homologue lacking C2 domains-c), a novel linker protein that interacts with Rab27, myosin Va/VIIa, and actin. J. Biol. Chem. 277, 43096 – 43103.

53 Fukuda, M., Kuroda, T. S. and Mikoshiba, K. (2002). Slac2-a/melanophilin, the missing link between Rab27 and myosin Va: implications of a tripartite protein complex for melanosome transport. J. Biol. Chem. 277, 12432 – 12436.

54 Itoh, T., Watabe, A., Toh, E. A. and Matsui, Y. (2002). Complex formation with Ypt11p, a rab-type small GTPase, is essential to facilitate the function of Myo2p, a class V myosin, in mitochondrial distribution in Saccharomyces cerevisiae. Mol. Cell Biol. 22, 7744 – 7757.

55 Wu, X., Sakamoto, T., Zhang, F., Sellers, J. R. and Hammer, J. A., 3rd. (2006). In vitro reconstitution of a transport complex containing Rab27a, melanophilin and myosin Va. FEBS Lett. 580, 5863 – 5868.

56 Li, B. X., Satoh, A. K. and Ready, D. F. (2007). Myosin V, Rab11, and dRip11 direct apical secretion and cellular morphogenesis in developing Drosophila photoreceptors. J. Cell Biol. 177, 659 – 669.

57 Takagishi, Y., Futaki, S., Itoh, K., Espreafico, E. M., Murakami, N., Murata, Y. and Mochida, S. (2005). Localization of myosin II and V isoforms in cultured rat sympathetic neurones and their potential involvement in presynaptic function. J. Physiol. 569, 195 – 208.

58 Takagishi, Y. and Murata, Y. (2006). Myosin Va mutation in rats is an animal model for the human hereditary neurological disease, Griscelli syndrome type 1. Ann. N. Y. Acad. Sci. 1086, 66 – 80.

59 Rudolf, R., Kogel, T., Kuznetsov, S. A., Salm, T., Schlicker, O., Hellwig, A., Hammer, J. A., 3rd and Gerdes, H. H. (2003). Myosin Va facilitates the distribution of secretory granules in the F-actin rich cortex of PC12 cells. J. Cell Sci. 116, 1339 – 1348.

60 Rose, S. D., Lejen, T., Casaletti, L., Larson, R. E., Pene, T. D. and Trifaro, J. M. (2002). Molecular motors involved in chromaffin cell secretion. Ann. N. Y. Acad. Sci. 971, 222 – 231.

61 Varadi, A., Ainscow, E. K., Allan, V. J. and Rutter, G. A. (2002). Molecular mechanisms involved in secretory vesicle recruitment to the plasma membrane in beta-cells. Biochem. Soc. Trans. 30, 328 – 332.

62 Toonen, R. F., Kochubey, O., de Wit, H., Gulyas-Kovacs, A., Konijnenburg, B., Sorensen, J. B., Klingauf, J. and Verhage, M. (2006). Dissecting docking and tethering of secretory vesicles at the target membrane. EMBO J. 25, 3725 – 3737.

63 Ivarsson, R., Jing, X., Waselle, L., Regazzi, R. and Renstrom, E. (2005). Myosin 5a controls insulin granule recruitment during late-phase secretion. Traffic 6, 1027 – 1035.

64 Rose, S. D., Lejen, T., Casaletti, L., Larson, R. E., Pene, T. D. and Trifaro, J. M. (2003). Myosins II and V in chromaffin cells: myosin V is a chromaffin vesicle molecular motor involved in secretion. J. Neurochem. 85, 287 – 298.

65 Varadi, A., Tsuboi, T. and Rutter, G. A. (2005). Myosin Va Transports Dense Core Secretory Vesicles in Pancreatic MIN6 {beta}-Cells. Mol. Biol. Cell. 16, 2670 – 2680.

66 Huet, S., Karatekin, E., Tran, V. S., Fanget, I., Cribier, S. and Henry, J. P. (2006). Analysis of transient behavior in complex trajectories: application to secretory vesicle dynamics. Biophys. J. 91, 3542 – 3559.

67 Watanabe, M. Nomura, K., Ohyama, A., Ishikawa, R, Komiya, Y., Hosaka, K., Yamauchi, E., Taniguchi, H., Sasakawa, N., Kumakura, K. Ushiki, T., Sato, O., Ikebe, M., Igarashi, M. (2005). Myosin-Va regulates exocytosis through the submicromolar Ca2+-dependent binding of syntaxin-1A. Mol. Biol. Cell 16, 4519 – 4530.

68 Desnos, C. Huet, S., Fanget, I., Chapuis, C., Bottiger, C., Racine, V., Sibarita, J. B., Henry, J. P. and Darchen, F. (2007). Myosin va mediates docking of secretory granules at the plasma membrane. J. Neurosci. 27, 10636 – 10645.

69 Desnos, C. Schonn, J. S., Huet, S., Tran, V. S., El-Amraoui, A., Raposo, G., Fanget, I., Chapuis, C., Menasche, G., de Saint Basile, G., Petit, C., Cribier, S., Henry, J. P., Darchen, F. (2003). Rab27A and its effector MyRIP link secretory granules to F-actin and control their motion towards release sites. J. Cell Biol. 163, 559 – 570.

70 Lizunov, V. A., Matsumoto, H., Zimmerberg, J., Cushman, S. W. and Frolov, V. A. (2005). Insulin stimulates the halting, tethering, and fusion of mobile GLUT4 vesicles in rat adipose cells. J. Cell Biol. 169, 481 – 489.

71 Jonsdottir, G. A. and Li, R. (2004). Dynamics of yeast Myosin I: evidence for a possible role in scission of endocytic vesicles. Curr. Biol. 14, 1604 – 1609.

72 Jung, G., Remmert, K., Wu, X., Volosky, J. M. and Hammer, J. A., 3rd. (2001). The Dictyostelium CARMIL protein links capping protein and the Arp2/3 complex to type I myosins through their SH3 domains. J. Cell Biol. 153, 1479 – 1497.

73 Sirotkin, V., Beltzner, C. C., Marchand, J. B. and Pollard, T. D. (2005). Interactions of WASp, myosin-I, and verprolin with Arp2/3 complex during actin patch assembly in fission yeast. J. Cell Biol. 170, 637 – 648.

74 Sun, Y., Martin, A. C. and Drubin, D. G. (2006). Endocytic internalization in budding yeast requires coordinated actin nucleation and myosin motor activity. Dev. Cell 11, 33 – 46.

75 McGoldrick, C. A., Gruver, C. and May, G. (1995). *myoA* of *Aspergillus nidulans* encodes an essential myosin I required for secretion and polarized growth. J. Cell Biol. 128, 577 – 587.

76 Liu, X., Osherov, N., Yamashita, R., Brzeska, H., Korn, E. D. and May, G. S. (2001). Myosin I mutants with only 1 % of wild-type actin-activated MgATPase activity retain essential in vivo function(s). Proc. Natl. Acad. Sci. USA 98, 9122 – 9127.

77 Schietroma, C., Yu, H. Y., Wagner, M. C., Umbach, J. A., Bement, W. M. and Gundersen, C. B. (2007). A role for Myosin 1e in cortical granule exocytosis in Xenopus oocytes. J. Biol Chem. 282, 29504 – 29513.

78 Yu, H. Y. and Bement, W. M. (2007). Multiple myosins are required to coordinate actin assembly with coat compression during compensatory endocytosis. Mol. Biol. Cell 18, 4096 – 4105.

79 Kim, S. V. Mehal, W. Z., Dong, X., Heinrich, V., Pypaert, M., Mellman, I., Dembo, M., Mooseker, M. S., Wu, D. and Flavell, R. A. (2006). Modulation of cell adhesion and motility in the immune system by Myo1 f. Science 314, 136 – 139.

80 Bose, A., Robida, S., Furcinitti, P. S., Chawla, A., Fogarty, K., Corvera, S. and Czech, M. P. (2004). Unconventional myosin Myo1c promotes membrane fusion in a regulated exocytic pathway. Mol. Cell Biol. 24, 5447 – 5458.

81 Bose, A., Guilherme, A., Robida, S. I., Nicoloro, S. M., Zhou, Q. L., Jiang, Z. Y., Pomerleau, D. P. and Czech, M. P. (2002). Glucose transporter recycling in response to insulin is facilitated by myosin Myo1c. Nature 420, 821 – 824.

82 Barylko, B., Jung, G. and Albanesi, J. P. (2005). Structure, function, and regulation of myosin 1C. Acta Biochim. Pol. 52, 373 – 380.

83 Huang, J., Imamura, T., Babendure, J. L., Lu, J. C. and Olefsky, J. M. (2005). Disruption of microtubules ablates the specificity of insulin signaling to GLUT4 translocation in 3T3-L1 adipocytes. J. Biol Chem. 280, 42300 – 42306.

84 Chen, X. W., Leto, D., Chiang, S. H., Wang, Q. and Saltiel, A. R. (2007). Activation of RalA is required for insulin-stimulated Glut4 trafficking to the plasma membrane via the exocyst and the motor protein Myo1c. Dev. Cell 13, 391 – 404.

85 Sokac, A. M., Schietroma, C., Gundersen, C. B. and Bement, W. M. (2006). Myosin-1c couples assembling actin to membranes to drive compensatory endocytosis. Dev. Cell 11, 629 – 640.

86 Fath, K. R. (2005). Characterization of myosin-II binding to Golgi stacks in vitro. Cell Motil. Cytoskeleton 60, 222 – 235.

87 Ostap, E. M. (2002). 2,3-Butanedione monoxime (BDM) as a myosin inhibitor. J. Muscle Res. Cell Motil. 23, 305 – 308.
88 Duran, J. M. Valderrama, F., Castel, S., Magdalena, J., Tomas, M., Hosoya, H., Renau-Piqueras, J., Malhotra, V., Egea, G. (2003). Myosin motors and not actin comets are mediators of the actin-based Golgi-to-endoplasmic reticulum protein transport. Mol. Biol. Cell 14, 445 – 459.
89 Ikonen, E., Parton, R. G., Lafont, F. and Simons, K. (1996). Analysis of the role of p200-containing vesicles in post-Golgi traffic. Mol. Biol. Cell 7, 961 – 974.
90 Musch, A., Cohen, D. and Rodriguez-Boulan, E. (1997). Myosin II is involved in the production of constitutive transport vesicles from the TGN. J. Cell Biol. 138, 291 – 306.
91 Vicente-Manzanares, M., Zareno, J., Whitmore, L., Choi, C. K. and Horwitz, A. F. (2007). Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells. J. Cell Biol. 176, 573 – 580.
92 Neco, P., Giner, D., Viniegra, S., Borges, R., Villarroel, A. and Gutierrez, L. M. (2004). New roles of myosin II during vesicle transport and fusion in chromaffin cells. J. Biol Chem. 279, 27450 – 27457.
93 Togo, T. and Steinhardt, R. A. (2004). Nonmuscle myosin IIA and IIB have distinct functions in the exocytosis-dependent process of cell membrane repair. Mol. Biol. Cell 15, 688 – 695.
94 Andzelm, M. M., Chen, X., Krzewski, K., Orange, J. S. and Strominger, J. L. (2007). Myosin IIA is required for cytolytic granule exocytosis in human NK cells. J. Exp. Med. 204, 2285 – 2291.
95 Sweeney, H. L. and Houdusse, A. (2007). What can myosin VI do in cells? Curr. Opin. Cell Biol. 19, 57 – 66.
96 Warner, C. L., Stewart, A., Luzio, J. P., Steel, K. P., Libby, R. T., Kendrick-Jones, J. and Buss, F. (2003). Loss of myosin VI reduces secretion and the size of the Golgi in fibroblasts from Snell's waltzer mice. EMBO J. 22, 569 – 579.
97 Sahlender, D. A., Roberts, R. C., Arden, S. D., Spudich, G., Taylor, M. J., Luzio, J. P., Kendrick-Jones, J. and Buss, F. (2005). Optineurin links myosin VI to the Golgi complex and is involved in Golgi organization and exocytosis. J. Cell Biol. 169, 285 – 295.
98 Noguchi, T., Lenartowska, M. and Miller, K. G. (2006). Myosin VI stabilizes an actin network during Drosophila spermatid individualization. Mol. Biol. Cell 17, 2559 – 2571.
99 Cai, H., Reinisch, K. and Ferro-Novick, S. (2007). Coats, tethers, Rabs, and SNAREs work together to mediate the intracellular destination of a transport vesicle. Dev. Cell 12, 671 – 682.
100 Peremyslov, V. V., Prokhnevsky, A. I., Avisar, D. and Dolja, V. V. (2008). Two Class XI myosins function in organelle trafficking and root hair development in Arabidopsis thaliana. Plant Physiol. 82, 2836 – 2843.
101 Rogat, A. D. and Miller, K. G. (2002). A role for myosin VI in actin dynamics at sites of membrane remodeling during Drosophila spermatogenesis. J. Cell Sci. 115, 4855 – 4865.
102 Kjeken, R. Egeberg, M., Habermann, A., Kuehnel, M., Peyron, P., Floetenmeyer, M., Walther, P., Jahraus, A., Defacque, H., Kuznetsov, S. A., Griffiths, G (2004). Fusion between phagosomes, early and late endosomes: a role for actin in fusion between late, but not early endocytic organelles. Mol. Biol. Cell 15, 345 – 358.
103 Au, J. S., Puri, C., Ihrke, G., Kendrick-Jones, J. and Buss, F. (2007). Myosin VI is required for sorting of AP-1B-dependent cargo to the basolateral domain in polarized MDCK cells. J. Cell Biol. 177, 103 – 114.
104 Ang, A. L., Taguchi, T., Francis, S., Folsch, H., Murrells, L. J., Pypaert, M., Warren, G. and Mellman, I. (2004). Recycling endosomes can serve as intermediates during transport from the Golgi to the plasma membrane of MDCK cells. J. Cell Biol. 167, 531 – 543.
105 Chibalina, M. V., Seaman, M. N., Miller, C. C., Kendrick-Jones, J. and Buss, F. (2007). Myosin VI and its interacting protein LMTK2 regulate tubule formation and transport to the endocytic recycling compartment. J. Cell Sci. 120, 4278 – 4288.
106 Salas-Cortes, L., Ye, F., Tenza, D., Wilhelm, C., Theos, A., Louvard, D., Raposo, G. and Coudrier, E. (2005). Myosin Ib modulates the morphology and the protein transport within multi-vesicular sorting endosomes. J. Cell Sci. 118, 4823 – 4832.
107 Neuhaus, E. M. and Soldati, T. (2000). A myosin I is involved in membrane recycling from early endosomes. J. Cell Biol. 150, 1013 – 1026.
108 Beningo, K. A., Lillie, S. H. and Brown, S. S. (2000). The yeast kinesin-related protein Smy1p exerts its effects on the class V myosin Myo2p via a physical interaction. Mol. Biol. Cell 11, 691 – 702.
109 Huang, J. D. Huang, J. D., Cope, M. J., Mermall, V., Strobel, M. C., Kendrick-Jones, J. Russell, L. B., Copeland, N. G. and Jenkins, N. A. (1998). Molecular genetic dissection of mouse unconventional myosin-VA: head region mutations. Genetics 148, 1951 – 1961.
110 Hume, A. N., Tarafder, A. K., Ramalho, J. S., Sviderskaya, E. V. and Seabra, M. C. (2006). A coiled-coil domain of melanophilin is essential for Myosin va recruitment and melanosome transport in melanocytes. Mol. Biol. Cell 17, 4720 – 4735.
111 Wu, X. S., Tsan, G. L. and Hammer, J. A., 3rd. (2005). Melanophilin and myosin Va track the microtubule plus end on EB1. J. Cell Biol. 171, 201 – 207.
112 Sheets, L., Ransom, D. G., Mellgren, E. M., Johnson, S. L. and Schnapp, B. J. (2007). Zebrafish melanophilin facilitates melanosome dispersion by regulating dynein. Curr. Biol. 17, 1721 – 1734.
113 Kuroda, T. S. and Fukuda, M. (2005). Identification and biochemical analysis of Slac2-c/MyRIP as a Rab27A-, myosin Va/VIIa-, and actin-binding protein. Methods Enzymol. 403, 431 – 444.
114 Huang, S., Lifshitz, L., Patki-Kamath, V., Tuft, R., Fogarty, K. and Czech, M. P. (2004). Phosphatidylinositol-4,5-bisphosphate-rich plasma membrane patches organize active zones of endocytosis and ruffling in cultured adipocytes. Mol. Cell Biol. 24, 9102 – 9123.