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Review

Structure, function, and regulation of myosin 1C✪

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Myosin 1C, the first mammalian single-headed myosin to be purified, cloned, and sequenced, has been implicated in the translocation of plasma membrane channels and transporters. Like other forms of myosin I (of which eight exist in humans) myosin 1C consists of motor, neck, and tail domains. The neck domain binds calmodulins more tightly in the absence than in the presence of Ca^{2+}. Release of calmodulins exposes binding sites for anionic lipids, particularly phosphoinositides. The tail domain, which has an isoelectic point of 10.5, interacts with anionic lipid headgroups. When both neck and tail lipid binding sites are engaged, the myosin associates essentially irreversibly with membranes. Despite this tight membrane binding, it is widely believed that myosin 1C docking proteins are necessary for targeting the enzyme to specific subcellular location. The search for these putative myosin 1C receptors is an active area of research.

Keywords: myosin 1, domain structure, membrane protein translocation

Myosins comprise a large superfamily of actin-dependent molecular motors which have been grouped into 17 classes based on the sequences of their catalytic domains (Cope J. & Hodge T.) (The Myosin Home Page, http//www.mrc.lmb.com.ac.uk/myosin/myosin.html). Until the 1970s only one class of myosin was known, the so-called "conventional" or "Type II" myosins that promote actin filament sliding in muscle cells. The first unconventional myosins were discovered by Pollard and Korn (1973) in *Acanthamoeba castellanii*, and were termed myosins I because, unlike the two-headed conventional myosins, they were found to be single-headed and incapable of forming bipolar filaments. There are now known to be more than thirty forms of myosin I (Korn, 2000); eight isoforms are present in humans (Gillespie *et al.*, 2001). The cellular function of mammalian myosins I remain poorly understood. Although it is generally believed that their major roles are to transport organelles along actin filaments, the failure to identify specific binding partners or to generate knockout animals has hampered attempts to link a given myosin isoform to its unique cargo.

This review focuses on the structure, function, and regulation of the myosin I isoform which is now termed myosin 1C. This myosin I was purified from bovine adrenal medulla in our laboratory (Barylko *et al.*, 1992), and was molecularly cloned

and sequenced shortly thereafter (Reizes *et al.*, 1994; Zhu *et al.*, 1996). We originally called this enzyme "mammalian myosin I", as it was the first single-headed myosin to be isolated from a mammalian source. However, based on its similarity to a partial sequence previously determined from a mouse cDNA library (Sherr *et al.*, 1993), we renamed it "myosin Iβ" (Reizes *et al.*, 1994). The ortholog from rat was named "myr 2" (Bahler & Rhoads, 2002) Its current name, "myosin 1C" stems from an effort to unify the myosin I nomenclature (Gillespie *et al.*, 2001).

DOMAIN STRUCTURE OF MYOSIN 1C

With few exceptions (e.g., myosin III), myosins show a common arrangement of functional domains. ATP- and actin-binding catalytic domains are located in the N-terminal region of about 700 amino acid ; the adjacent "neck" regions are of various lengths and contain the binding sites for one or more light chains; and the C-terminal domains are capable of either self-associating into bipolar fila-

✪Paper dedicated to the memory of Professor Witold Drabikowski and Professor Gabriela Sarzała-Drabikowska.

Abbreviations: CALI, chromophore-assisted laser inactivation; Glut4, glucose transporter protein 4; PI, phosphatidylinositol; PI3K, phosphatidylinositol 3-kinase; PIP2 and PI 4,5-P2, phosphatidylinositol 4,5-bisphosphate; siRNA, small interfering RNA.

ments or of binding to cargo transported by the myosins.

The catalytic core of myosin 1C contains consensus actin- and nucleotide-binding sequences conserved among all myosin motor domains, and is expected to adopt the same general conformation as that of the chicken skeletal muscle myosin head domain, solved by Rayment's group using X-ray crystallography (Rayment *et al.*, 1993). The catalytic domains of myosin I family members can be divided into two classes (El Mezgueldi *et al.*, 2002; De La Cruz & Ostap, 2004); Class 1 catalytic domains are found in most protozoan isoforms and in mammalian myosin 1E, all of which also have extended tail domains; class 2 catalytic domains are found in the majority of mammalian myosins I, including myosin 1C. The class 2 myosins I typically have slower ATPase rates than do class 1 myosins, prompting speculation that they are more suited for structural roles or for the maintenance of tension (Coluccio & Geeves, 1999; El Mezgueldi *et al.*, 2002), whereas the class 1 isoforms may be better suited for rapid contractile events such as pseudopod extension (De La Cruz & Ostap, 2004).

Immediately C-terminal to the motor domain (i.e., beginning approx. at residue 685 of the bovine sequence) is a neck domain that contains three calmodulin binding IQ motifs. Although it is difficult to define the junction between the neck and tail domains, we designate the C-terminus of the neck as the end of the third IQ motif, approx. residue 775. Thus, the myosin 1C neck has a molecular mass of approximately 10 kDa.

IQ motifs, named for the presence of isoleucine (I) and glutamine (Q) residues near their N-termini, are α-helical segments of 20–25 residues found in nearly every myosin isoform, as well as in dozen of other cytoskeletal and signaling proteins (reviewed in Bahler & Rhoads, 2002). Often, these motifs interact with calmodulins or calmodulin-like proteins in a manner independent of, or inhibited by, Ca^{2+}. As shown in Fig. 1, the IQ motifs of myosin 1C are rich in hydrophobic and positively charged residues. Myosin 1C heavy chain binds 2–3 calmodulin in the absence of Ca^{2+} (1 mM EGTA) and at least two of three calmodulins are released in the presence of 0.1 mM free Ca^{2+} (Zhu *et al.*, 1996). Association of calmodulin with myosin 1C apparently inhibits its actin-activated ATPase activity, as the turnover number increases from 6 min^{-1} to 15 min^{-1} either by elevating the Ca^{2+} concentration (Barylko *et al.*, 1992) or by proteolytically cleaving the myosin heavy chain between the head and the neck (unpublished results). Surprisingly, although Ca^{2+} stimulates myosin ATPase activity (Barylko *et al.*, 1992; Zhu *et al.*, 1996), it inhibits force generation in *in vitro* motility assays (Zhu *et al.*, 1996; 1998). The inhibition of motility by Ca^{2+} results from calmodulin release, because Ca^{2+} did not inhibit myosin 1C in the presence of calmodulin point mutant impaired in Ca^{2+}-binding (Zhu *et al.*, 1998). This result suggests that bound calmodulins have the same role in myosin I as light chains have in conventional myosins, i.e., to stabilize the lever arm during force generation (Rayment *et al.*, 1993). However, as discussed below, IQ motifs of myosin 1C have also been implicated in Ca^{2+}-sensitive membrane binding.

The C-terminal tail regions of myosins are the sites of greatest sequence diversity. Hence, they are assumed to be responsible for tethering the myosins to specific cargo. This inference was confirmed when globular domains in the C-termini of myosins V and VI were found to confer subcellular targeting information to those isoforms (Hammer & Wu, 2002; Buss *et al.*, 2004; Seabra & Coudrier, 2004). Like other myosins 1, the myosin 1C tail is rich in basic amino-acid residues (isoelectric point of 10.5) (Reizes *et al.*, 1994). It does not contain any currently recognized interaction motifs, such as the SH3 domain found in myosins 1E and F.

ASSOCIATION OF MYOSIN 1C WITH MEMBRANES

As discussed below, there is evidence that myosin 1C facilitates the translocation of membrane proteins, such as mechanosensitive ion channels in the ear and glucose transporters in adipocytes. To carry out these functions it is necessary for the myosin to interact with membranes, either directly or indirectly. Despite extensive efforts from several laboratories (including our own), attempts at identifying specific myosin 1C "receptors" or membrane docking proteins have failed. However, there are ample data that indicate a direct electrostatic association between the noncatalytic portion of myosin 1C and negatively charged phospholipids.

Pollard's group was the first to demonstrate the binding of a myosin 1 (from *Acanthamoeba*) to membrane lipids (Adams & Pollard, 1989). They further showed that the interaction was mediated by the basic tail domain of the myosin (Doberstein & Pollard, 1992) and speculated that the tail recognizes membrane-bound cargo for translocation along actin filaments. Shortly thereafter, Mooseker's laboratory reported lipid binding of avian brush border myosin I (Hayden *et al.*, 1990) and we showed that both full-length myosin 1C and a bacterially expressed tail

Figure 1. The IQ motifs of bovine myosin 1C.

portion (residues 820–1025) bound to anionic phospholipid vesicles (Reizes *et al.*, 1994).

Whereas we analyzed the lipid binding properties of the myosin 1C tail alone, Tang and coworkers (Tang *et al.*, 2002) used stopped-flow kinetics to examine the binding to liposomes of a myosin 1C fragment containing both the tail and the calmodulin-binding neck domain (residues 690–1028). According to these authors, myosin 1C remains bound to liposomes for a sufficient length of time to accommodate multiple cycles of the ATPase force-generating reaction. Nevertheless binding was transient and off-rates were relatively high. Interestingly, more than 60% of the neck-tail constructs were irreversibly bound to liposomes if the measurements were carried out in the presence of 250 μM free Ca^{2+}. Because one or more calmodulins are released from the myosin 1C neck at this Ca^{2+} concentration, this result raised the possibility that the IQ motifs represent a second lipid binding site that is normally occluded by calmodulin. Because these motifs are relatively rich in basic and hydrophobic residues they may be able to interact both electrostatically with anionic phospholipid head groups and with the hydrophobic core of the bilayer. The electrostatic interactions may also be regulated by phosphorylation (Fig. 2).

The lipid binding ability of myosin 1C IQ motifs was recently confirmed by Hirono and others (Hirono *et al.*, 2004). Using a combination of lipid overlay and solid-state binding assays, they found that a construct consisting of three IQ motifs of frog myosin 1C binds preferentially to the phosphoinositide, PIP_2, and somewhat less well to other anionic lipids. This interaction was Ca^{2+}-dependent, and was reduced by addition of excess calmodulin. Thus, it is possible that the neck domain of myosin 1C functions as a molecular switch, binding alternatively to lipids or to calmodulins depending on the presence of Ca^{2+}. The interaction between myosin 1C and PIP_2 also raises the possibility of reversible membrane anchoring, responsive to signaling pathways that regulate phospholipase C, PI kinase, and PI phosphatase activities.

FORCE GENERATION BY MYOSIN 1C

Batters and others (Batters *et al.*, 2004) recently carried out a comprehensive analysis of myosin 1C force generation using optical-tweezer tranducers. Their data suggest that the myosin 1C powerstroke occurs in two phases, the first producing an average displacement of 3.1 nm and the second yielding a displacement of 1.1 nm. Interestingly, three-dimensional mapping based on cryo-electron microscopy data indicate that the neck domain of myosin 1C swivels 33° upon release of ADP, suggesting a maximal displacement of about 5 nm from this step alone. The authors speculate that these displacement values (4.3 nm *vs* > 5 nm) provide a range of positions available to the myosin during its translocation. Image analysis also indicates that upon ADP release the three IQ motifs swivel as a single rigid body that projects from the motor domain. The motor domain itself did not exhibit obvious conformational differences when bound to actin in the presence of ADP *vs*. in the nucleotide-free state.

Based on force analysis and rapid-reaction kinetic experiments, Batters and others (2004) propose a model in which myosin 1C can exist in two conformations, one allowing ATP to bind readily, the other containing trapped ADP and requiring a slow conformational change which promotes ADP release and allows ATP to bind. In the presence of Ca^{2+} (77 μM free) the fast phase is lost, suggesting that Ca^{2+} favors the ADP-trapped conformation. An external strain on the myosin (as apparently occurs during adaptation in the ear, see below) might accelerate ADP release from this trapped state and enhance cycling through the ATPase cycle.

SUBCELLULAR DISTRIBUTION OF MYOSIN 1C

Using a panel of monoclonal antibodies prepared against bovine myosin 1C, we found that this isoform is expressed in a wide variety of tissues and cultured cell lines (Wagner *et al.*, 1992). These antibodies were also used to localize myosin 1C in fibroblasts, kidney epithelial cells, and neuroendocrine PC12 cells (Wagner *et al.*, 1992). In general, our immunofluorescence microscopy studies showed a punctate distribution of the myosin concentrated in the cell periphery as well as plasma membrane staining, particularly at the leading edges of motile cells. The same antibodies were used by other groups to localize myosin 1C to the phagocytic cup in macrophages (Allen & Aderem, 1995), the stereociliary tip in outer hair cells of the frog ear (Garcia *et al.*, 1998), and mitotic junctions of retinal epithelial cells (Breckler & Burnside, 1994).

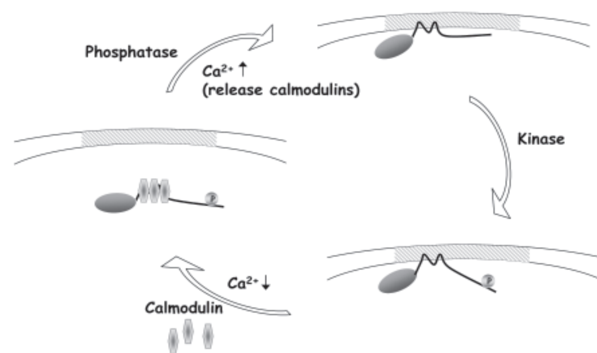

Figure 2. Hypothetical model showing dissociation of myosin 1C from lipid rafts by phosphorylation and Ca^{2+}/calmodulin.

A proteomics analysis of lipid rafts from human Raji B-lymphocyte cell line revealed the enrichment of myosin 1C in rafts (Saeki *et al.*, 2003). Lipid rafts are cholesterol- and sphingolipid-rich membrane domains that incorporate a subset of membrane proteins while excluding others (reviewed in Pike, 2004; Simons & Vaz, 2004). Rafts apparently originate in the trans-Golgi network where sphingolipids are synthesized, and are further concentrated in post-Golgi elements of the secretory/endocytic pathway, being particularly abundant in the plasma membrane. They have been defined operationally according to a number of criteria, most commonly by their insolubility in cold Triton X-100 and their low buoyancy in density gradient after centrifugation. Because certain established raft proteins can be solubilized by cold Triton X-100, procedures involving milder detergents (Claas *et al.*, 2001; Shogomori & Brown, 2003; Chamberlain, 2004) or detergent-free buffers (Smart *et al.*, 1995; Song *et al.*, 1996) have also been employed to isolate rafts. Our laboratory has recently demonstrated that myosin 1C preferentially distributes to lipid rafts prepared from rat fibroblasts and HeLa cells using two detergent-free isolation methods (unpublished data). Lipid rafts are enriched in PI(4,5)P_2 (Caroni, 2001; Golub *et al.*, 2004) which, as mentioned above, binds to the IQ motifs of myosin 1C. Thus, it is possible that the myosin is recruited to rafts in the presence of Ca^{2+}, which releases calmodulin from the IQ motifs and exposes PIP_2-binding sites.

Myosin 1C may participate in two distinct modes of raft-based membrane transport. First, it may generate force to translocate raft-containing vesicles through the cytoplasm along actin or microtubule tracks (Fig. 3). Second, it may participate in the translocation of raft domains within the plane of the bilayer, (Fig. 4). Results of fluorescence resonance energy transfer, laser trap, and chemical crosslinking experiments indicate that lipid rafts in resting cells are small entities, ranging in diameter from about 50 nm (Pralle *et al.*, 2000) to as little as 7 nm (Anderson & Jacobson, 2002). Given these small sizes, it is unlikely that a single raft contains more than a few protein molecules and, hence, is incapable of accommodating multiprotein signaling complexes. Presumably, complexes formed in response to extracellular stimuli (e.g., activation of B- or T-lymphocyte receptors) require the coalescence of small rafts into larger patches (Fig. 4). Although such a stimulus-triggered event may occur by simple diffusion of raft proteins in the bilayer, there is evidence for a role of actin and myosin in this process (Nebl *et al.*, 2002; Jordan & Rodgers, 2003; Tharaux *et al.*, 2003).

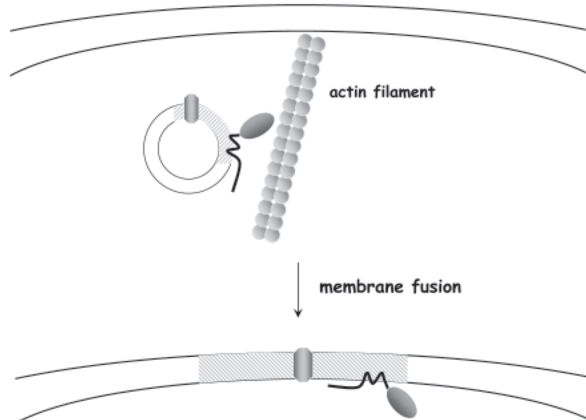

Figure 3. Translocation of raft-containing vesicle to the plasma membrane.

FUNCTIONS OF MYOSIN 1C

Role of myosin 1C in glucose transport

Insulin stimulates glucose transport in adipocytes by promoting exocytosis of a population of vesicles containing the glucose transporter protein, Glut4 (reviewed in Ducluzeau *et al.*, 2002; Watson *et al.*, 2004). Czech's group implicated myosin 1C in this process when they showed reduced glucose uptake in cells treated with siRNAs to deplete expression of the myosin, and in cells overexpressing the myosin 1C tail domain as a dominant inhibitor of the endogenous myosin.

Insulin-stimulated glucose uptake also requires phosphatidylinositol 3-kinase (PI3K) activity (Nakanishi *et al.*, 1995). Pretreatment of adipocytes with the PI3K inhibitor, LY294002, inhibits exocytosis of Glut4-containing vesicles and results in accumulation of these vesicles beneath the plasma membrane. Overexpression of myosin 1C reverses the effect of LY294002 (Bose *et al.*, 2004), whereas overexpression of the tail domain prevents the sub-plasmallemal accumulation of vesicles.

Bose and others (2004) observed that in addition to stimulating glucose uptake, overexpression of full-length myosin 1C also induced membrane ruffling in adipocytes, even in the absence of insulin.

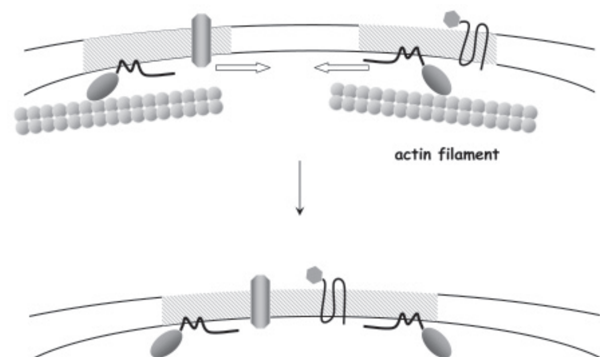

Figure 4. Coalescence of rafts within the plasma membrane.

Myosin tagged with yellow fluorescent protein was particularly enriched in regions of the membrane that were most active in ruffling, consistent with our earlier localization of endogenous myosin 1C to ruffles in a variety of cultured cells (Wagner *et al.*, 1992). Interestingly, Glut4 vesicles colocalized with myosin 1C in ruffles only if the cells were treated with insulin. This suggests that myosin 1C does not reside on vesicles as they translocates through the cytoplasm, but may facilitate their exocytosis only when they reach the cell periphery. Presumably, vesicle transport through the cytoplasm depends on the microtubule motor, kinesin, whereas myosin 1C serves to anchor and/or translocate vesicles on actin filaments associated with the plasma membrane. However, it is also possible that the myosin facilitates exocytosis indirectly, without binding to Glut4 vesicles.

Role of myosin 1C in hair cell adaptation

Myosin 1C has been implicated in translocating ion channels and their associated transduction complexes within the membranes of stereocilia in vestibular hair cells of the ear (recently reviewed in Gillespie & Cyr, 2004). These complexes are located near the tips of stereocilia, and are connected to adjacent stereocilia by flexible proteins known as "tip links". Under resting conditions the stereocilia are upright and the tip links are unstretched, favoring a closed state for the ion channels. However, excitatory stimuli cause the stereocilia to tilt, resulting in extension of the tip links and opening of the ion channels. The process of adaptation, which prevents prolongation of the response, is initiated while the stereocilia are still in the tilted position. It occurs by the sliding of the transduction complex down the stereocilium, resulting in relaxation of the tip link and closure of the ion channel. Finally, upon termination of the stimulus the stereocilia resume their upright positions and the transduction complex must again move upward within the membrane.

There is evidence that movement of the transduction complex during adaptation and recovery is mediated by myosin 1C, which connects the complex to a bundle of actin filaments within the stereocilium. Approximately 100–200 myosin 1C molecules are tethered to structure known as the "insertional plaque", which houses the ion channel and is also connected to the tip link. During adaptation, the myosins slip down toward the pointed ands of actin filaments (opposite direction of their normal active movement). Upon recovery, the myosin 1C ATPase cycle is used to generate force needed to climb up the actin cables, pulling the insertional plaque forward.

Role of myosin 1C in neuronal growth cone extension

Jay's laboratory has pioneered the use of chromophore-assisted laser inactivation (CALI) to investigate the functions of distinct myosin isoforms in cell motility. In this procedure, anti-myosin antibodies labeled with malachite green (MG) are introduced into cells, which are then irradiated by laser light to release hydroxyl free radicals from the dye. The radicals damage and inactivate MG-antibody-bound myosins (or other targeted proteins) without seriously affecting nearby proteins (Jay, 1988; Jay & Keshishian, 1990; Wang *et al.*, 1996). Jay's group has been particularly interested in examining the roles of different myosins in filopodial and lamellipodial extension in neuronal growth cones. They found that inactivation of myosin V causes retraction of filopodia from chick dorsal root ganglion growth cones (Wang *et al.*, 1996) whereas inactivation of myosin II causes reduction in the size of lamellipodia (Diefenbach *et al.*, 2002). In contrast, CALI of myosin 1C resulted in lamellipodial extension indicating that myosin 1C actually represses membrane protrusion (Wang *et al.*, 1996; Diefenbach *et al.*, 2002; Wang *et al.*, 2003). To explain this result Jay and his colleagues invoke a "clutch mechanism", in which myosin 1C acts as a motor to propel F-actin filaments rearward with respect to the substratum (Jay, 2000). When the myosin is inactivated by CALI, this rearward motion is inhibited and the lamellipodia move

Table 1. Selected functions of mammalian unconventional myosins.

Myosin	Function	Reference
Myosin Iα (myosin 1b)	Endosome-to-lysosome trafficking	Raposo *et al.*, 1999
Myr 4(myosin 1d)	Early stages of endosome	Huber *et al.*, 2000
Myosin V	Transport of melanosome	Mercer *et al.*, 1991; Wu *et al.*, 1997
Myosin VI	Clathrin-dependent endocytosis in polarized cells	Buss *et al.*, 2001
Myosin VI	AMPA receptor trafficking	Wu *et al.*, 2002
Myosin VI	Golgi trafficking and secretion	Warner *et al.*, 2003
Myosin X	Intracellular adhesion	Yonezawa *et al.*, 2003
Myosin X	Intergrin relocalization	Zhang *et al.*, 2004
Myosin X	Phagocytosis	Cox *et al.*, 2002
Myosin IX	Bacterial entry (based on localization data)	Graf *et al.*, 2000

forward due to net actin polymerization at the leading edge of the growth cone.

PERSPECTIVES

Fifteen years after the discovery of vertebrate myosin 1C, several questions still remain to be answered. First and most important, what are the unique functions carried out by this myosin isoform? At present, the preponderance of data point to the plasma membrane as the major site of myosin 1C action. However, it is still unclear whether it serves to translocate proteins or protein complexes within the plane of the bilayer, to deliver vesicles to the cell periphery, or to modify the cortical actin cytoskeleton as occurs during membrane ruffling. Of course, it is possible that myosin 1C promotes all of these modes of cell motility.

A second question, which pertains to all of the unconventional myosins, regards the function of the IQ motifs. Based on structural data and force measurements, the neck domains of myosins represent lever arms, and the light chains (calmodulins, in the case of myosin 1C) serve to stabilize them during the powerstroke. However, there is also evidence that IQ motifs interact with membranes when calmodulins are released upon Ca^{2+} ligation. Both possibilities may be correct. For example, only the C-terminal IQ motif might bind to membranes, leaving the remaining two available to serve in lever arm stabilization. Unfortunately, there are no reliable data regarding the *in vivo* stoichiometry of myosin 1C heavy chain to calmodulin light chains.

A third area that merits further investigation is the potential role of myosin 1C phosphorylation. In the case of most protozoan and yeast myosins 1 the importance of heavy chain phosphorylation is evident, as unphosphorylated myosins are essentially inactive. However, in myosin 1C and other mammalian myosin 1 isoforms, the corresponding phosphorylated serine or threonine of lower eukaryotic myosins is replaced by phosphomimetic residues, glutamate or aspartate. Thus, phosphorylation of myosin 1C appears to have a non-catalytic role, perhaps in modulating interactions of the myosin with calmodulin, lipids, or yet unidentified binding partners. Indeed, Williams and Coluccio showed a reciprocal relationship between myosin 1C phosphorylation and calmodulin binding (Williams & Coluccio, 1995).

The fourth unanswered question concerns the existence of putative myosin 1C binding partners. Aside from actin and calmodulin, no myosin 1C binding protein has been identified. This situation is in marked contrast to myosin V, which binds to organelles *via* direct interaction with Slac2/melanophilin (synaptotagmin-like protein homologue lacking C2 domain), which in turn binds the small GTPase, rab27 (Fukuda *et al.*, 2002). Although it is formally possible that myosin 1C localizes to the cell periphery solely by virtue of interactions with lipids and cortical actin filaments, we favor the view that myosin 1C docking proteins serve to target the myosin to specific subcellular locations. If so, discovery of these proteins will greatly increase our understanding of myosin 1C function.

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