Unconventional myosins, actin dynamics and endocytosis: a ménage à trois?

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Abstract

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Review

Unconventional Myosins, Actin Dynamics and Endocytosis: A Ménage à Trois?

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Ever since the discovery of class I myosins, the first nonmuscle myosins, about 30 years ago, the history of unconventional myosins has been linked to the organization and working of actin filaments. It slowly emerged from studies of class I myosins in lower eukaryotes that they are involved in mechanisms of endocytosis. Most interestingly, a flurry of recent findings assign a more active role to class I myosins in regulating the spatial and temporal organization of actin filament nucleation and elongation. The results highlight the multiple links between class I myosins and the major actin nucleator, the Arp2/3 complex, and its newly described activators. Two additional types of unconventional myosins, myosinIX, and Dictyostelium discoideum MyoM, have recently been tied to the signaling pathways controlling actin cytoskeleton remodeling. The present review surveys the links between these three classes of molecular motors and the complex cellular processes of endocytosis and actin dynamics, and concentrates on a working model accounting for the function of class I myosins via recruitment of the machinery responsible for actin nucleation and elongation.

Key words: actin polymerization, Dictyostelium discoideum, endocytosis, myosins, Saccharomyces cerevisiae

Received 7 February 2003, revised and accepted for publication 26 February 2003

Actin Dynamics: A Brief Summary

In the cytosol, ATP-G-actin is kept in a readily polymerizable pool bound to profilin. Profilin-actin is thought to be recruited to the site of polymerization via the poly-Pro motifs of activator proteins like VASP or WASp. Because de novo nucleation of filaments is rate-limiting, the Arp2/3 complex, the major cellular F-actin nucleator, lies at the core of cytoskeleton dynamics. Recent advances propose that extracellular signals are relayed through effector proteins like Rho family GTPases to the WASp or SCAR family proteins, which in turn lead to the localized recruitment and activation of the Arp2/3 complex (1). Nucleation occurs mainly by branching off existing filaments, and polymerization of ATP-G-actin is thought to directly produce the force necessary to push membranes. Rapidly, capping of the newly generated filament is followed by stabilization through a variety of actin binding proteins. Binding of ADP/cofilin to ADP-F-actin initiates depolymerization. The pool of ATP-G-actin is reconstituted by the nucleotide exchange factor profilin (2). It has recently been demonstrated that although this Arp2/3-dependent mechanism may be the major one, it nevertheless does not account for all engagements of the actin polymerization machinery. For example, PAK kinase can recruit and phosphorylate filamin proteins and together generate orthogonally cross-linked actin meshworks (3). As another alternative to generate unbranched actin filaments, proteins of the formin family work downstream of Rho GTPases and appear able to nucleate filaments and stay associated with their growing barbed ends (4). The dendritic nucleation model offers a solid framework to further dissect the mechanisms of actin dynamics and their involvement in a vast array of cellular processes. Some of the pertinent questions discussed in this review are highlighted (A–E) in Figure 1.

The Myosin Superfamily

The myosin superfamily of mechanoenzymes comprises 18 classes (5). The human genome encodes about 40 myosin genes, among which about 25 are unconventional and come from at least 11 classes; Dictyostelium discoideum appears to have 13 myosins from about 6 classes (6,7) and Saccharomyces cerevisiae has 5 myosins from 3 classes (5). The relevance of myosins for mammalian physiology and pathology was recently emphasized by the finding that many human and murine genetic diseases are associated with mutations in myosins (5,8,9). While classical myosin II is almost exclusively involved in producing contraction power, unconventional myosins are active as cortical managers, mediate organelle and mRNA transporters, and function as regulators of signal transduction. In particular, class I myosins form the second largest group after conventional myosin II, and appear to be essential players in the establishment and maintenance of cortical tension and related functions such as motility, endocytosis, and exocytosis (9,10).
All members of the myosin family share a common structure; they are composed of three modules, the head, neck and tail domains. The N-terminal region harbors the motor unit, utilizing ATP to power movement along actin filaments. Almost all myosins follow the so-called TEDS rule by having a negatively charged amino acid residue [Asp (D), Glu (E) or phosphorylatable Ser (S) or Thr (T)] at a position of the head domain known as the cardiomyopathy loop, and crucial for activity. In class VI myosins and in class I myosins of lower eukaryotes, this site is phosphorylated by kinases of the PAK/Ste20 family, which are regulated by small GTPases of the Rho/Rac/Cdc42 family [reviewed in (12)]. The neck or middle domain acts as a lever arm stiffened by the binding of up to six light chains belonging to the superfamily of calmodulin-like EF hand proteins (14). Finally, via binding to specific proteins and cargoes, the tail is responsible for the specific function and location of myosins.

Because of the high degree of sequence conservation of the motor domain, all the myosins are expected to utilize F-actin as a track to power their movement, but it has recently been realized that the relationship between these motors and their tracks is likely more complex. Indeed, as this review surveys, class I myosins appear to be able to recruit directly or indirectly the Arp2/3 complex, and two other types of myosins possess enzymatic activities that modulate the function of Rho-family GTPases and their impact on cytoskeleton dynamics.

**Domain Analysis of Class I, and IX Myosins and of Dictyostelium discoideum MyoM**

In addition to their motor domains, class I myosins, have a variable tail domain structure that can directly or indirectly affect actin dynamics. Class I myosins can be divided into long-tailed and short-tailed. Most lower eukaryotes have only long-tailed myosins. In contrast, class I myosins in *D. discoideum* have either long tails (MyoB, MyoC, and MyoD), short tails (MyoA, MyoE and MyoF) or almost no tail at all (MyoK) (12,15). At the moment, only two homologues of long-tailed ameboid class I myosins, myosin IE (16) and IF (17), have been described in mammals, but many examples of short-tailed myosins have been extensively investigated (18,19). The long tails comprise three tail homology (TH) domains: TH1 rich in basic residues, TH2 with a high content of glycine and proline, and TH3, more commonly referred to as a Src homology 3 (SH3) domain. TH1 binds anionic phospholipids, while TH1 and TH2 bind F-actin in an ATP-independent manner (Figure 2) [reviewed in (12,15)]. The short tails encode only a TH1

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**Figure 1: A model for actin nucleation and elongation, and some important questions.** The popular dendritic nucleation model implies that upon reception of a stimulus (A) that leads to cell polarity through a signaling cascade that includes GTPases and lipid kinases (B), specific proteins can be relieved from an autoinhibitory conformation, recruit and activate the Arp2/3 complex (C) in close proximity to the membrane (D). During elongation, ATP-G actin is added at the growing barbed end of nascent actin filaments (E). These major steps are under intense investigation to solve the following questions. What is the range of stimuli that can lead to cell polarity and engagement of the actin dynamics machinery? (A?). Which GTPases, trimeric Gs and small GTPases of the Rho/Rac/Cdc42 family are involved, and by which GEF are they activated? (B?). Beside the best characterized Arp2/3 activators of the WASp family, what other proteins can act in this pathway? (C?). How is the seed F-actin positioned close to the plasma membrane? (D?). Is the ‘fuel’ for elongation, ATP-G actin, preconcentrated at the growing end, and is it bound by WH2 domain proteins and/or in the form of profilin-actin complex? (E?). Other factors, including capping proteins and cofilin, are crucial for motility but are not discussed here.
domain. *D. discoideum* MyoK is a highly divergent type I myosin with a very short neck region and a tail only 53 residues in length. However, MyoK has an insert of ~150 amino acids within the motor domain that, like TH2 domains, is extremely rich in Gly, Pro and Arg (GPR) (15,20). This TH2-like insert also binds F-actin in an ATP-independent manner (15). MyoK ends with a -CLIQ sequence, corresponding to a so-called -CAAX motif, a known protein farnesylation signal, and we have shown that it is sufficient to target a GFP fusion protein to the plasma membrane (E. Schwarz and T. Soldati, unpublished results).

Class IX myosins and *D. discoideum* MyoM [a myosin that does not officially belong to any class yet (5)] have enzymatic activities that are directly able to down-regulate or activate, respectively, signal transduction pathways that induce rearrangements of the actin cytoskeleton. Myosin IX, which is present in mammals and *Caenorhabditis elegans*, has an N-terminal extension of unknown function and an insertion in the loop2 of the motor domain that might modulate contact to actin (21). It has between 4 and 6 light chain binding sites in the neck and no recognizable dimerization domain. MyoM has two insertions in its motor domain, one at the same place as myosin VI and the second positioned similarly as in myosin IX, but the sequence of these insertions is not homologous (22,23). The tail of class IX myosins contain a zinc finger and a GTPase activating domain (GAP) for Rho family GTPases (21) and references therein), whereas the tail of MyoM carries a guanine nucleotide exchange factor (GEF) for Rac GTPases (22). In addition, the tail of MyoM contains a domain rich in Pro, Ser and Thr (together 50%) with one large poly Pro stretch that resembles profilin and SH3 binding motifs (22).

**Unconventional Myosins, Cell Motility and Endocytosis**

In keeping with their potential role as cortical actin managers, immunolocalization studies of class I myosins in ameba [reviewed in (12,24)] and higher eukaryotes [reviewed in (5,25)] reveal their association with dynamic regions of the cell cortex, such as the leading pseudopod of migrating cells, endocytic structures, microvilli, cell–cell junctions (26) and sites of particle ingestion (27). In contrast to the extreme plasticity of the periphery in these eukaryotes, in the more rigid fungal cells the major site of myosin I localization is within the actin cortical patches [reviewed in (28)].

Cell movement, macropinocytosis and phagocytosis all use the actin cytoskeleton to extend membrane protrusions, and it has been shown that myosin motors are involved in these processes. In *D. discoideum*, cells lacking either MyoA, MyoB or MyoK move with reduced velocity, form more pseudopods, and turn more frequently than wild-type cells (15,29–31). MyoKnull cells and double mutants (A–/B–, B–/C–) have a lower cortical tension, while over-expression of MyoB, MyoC or MyoK augments cortical tension and restricts extension of actin-filled protrusions (15,32). MyoB and MyoK have been located to the phagocytic cup (15,33), and membrane ruffles (34). Cells lacking MyoB or MyoK have a reduced rate of phagocytosis (15,35,36), whereas overexpression of MyoB resulted in decreased macropinocytosis (32). In addition, MyoB null cells are deficient in membrane recycling from an endosomal compartment back to the surface (37). Single MyoC mutants have a decreased initial rate of fluid-phase uptake (36), and the slow growth of various double and triple mutants (A–/B–, B–/C–, B–/D–, B–/C–/D–) was interpreted as additive impairments of myosin I function in fluid-phase uptake (34,36). These results led to the proposition that class I myosins share partially overlapping but mainly nonredundant functions in endocytosis. Findings in *Acanthamoeba* (38) and *Entamoeba histolytica* (39) are in excellent agreement with these observations. Similarly, *Saccharomyces cerevisiae* mutants lacking both the myosin I proteins, Myo3p and Myo5p, exhibit a strong growth defect, accumulate intracellular vesicles and are severely impaired in endocytosis (40,41). MYOA is required for the viability of *Aspergillus* and is involved in the generation...
of cell polarity and focal secretion at the tip of growing hyphae (42,43).

These studies establish that class I myosins have a diverse array of functions related to endocytic trafficking. However, several studies suggest that these functions may not reflect their activity as molecular motors. For example, Aspergillus MYOA constructs with only 1% of wild-type ATPase activity retain their essential in vivo function (44). Instead, several more recent studies highlight the importance of the SH3 domain in myosin I function. The defects in growth, endocytosis and actin organization exhibited by D. discoideum cells lacking MyoA and MyoB can be fully rescued by wild-type MyoB but not by MyoB amputated of its SH3 domain (MyoBΔSH3) (45), whereas overexpression of MyoBΔSH3 does not generate the defects associated with overexpression of wild-type MyoB (32). In S. cerevisiae, the severe defects resulting from Myo3p/Myo6p double knockout can be complemented by either Myo3p or Myo6p lacking TH2 but not lacking their SH3 domain (46,47). In contrast, Schizosaccharomyces pombe Myo1p lacking TH2 and SH3 complements deletion of myo1 (48) and Aspergillus MYOA appears to function even when deleted from its SH3 domain (43). Interpretation of these data is complicated by the demonstrated in vivo redundancy between class I myosins and WASp and potentially other activators of the Arp2/3 complex (48,49).

It is not yet clear whether the only homologues of long-tailed myosins identified in mammalian cells, myosins IE and IF, function in endocytosis (26), but evidence has been presented implicating two short-tailed mammalian class I myosins that lack TH2 and SH3 domains. Myosin IB is found on the surface of endosomes and lysosomes and is involved in the delivery of internalized molecules to lysosomes (50). Expression of a myosin IB mutant truncated in its motor domain was shown to act as a dominant negative inhibitor, that impaired the intracellular distribution of lysosomes and the directionality of their long-range movements. These results indicated, for the first time, that both actin filaments and myosin IB contribute to the trafficking of lysosomes in cooperation with microtubules and their associated molecular motors (51). Using an in vitro assay that follows transfer of an endocytosed marker from apical or basolateral early endosomes to recycling endosomes labeled with transferrin, Huber and colleagues showed that trafficking at these early steps of the endocytic pathway in MDCK epithelial cells depends on myosin IC, its light chain calmodulin, and polymerized actin (52).

As mentioned above, myosin IX and D. discoideum MyoM have antagonistic effects on Rho family GTPases. In mammalian cells, myosin IX has been shown to be partially membrane associated and to be recruited specifically to plasma membrane entry spots during Shigella flexneri invasion (53). The potential role of myosin IX in regulating bacteria entry and in more physiological endocytic processes has not been explored yet. In D. discoideum, GFP–MyoM fusions have been shown to be enriched in surface projections responsible for macropinocytosis, and on endosomal organelles (23). Exogenous stimulation of MyoM Rac-GEF activity by osmotic stress results in severe alterations of plasma membrane morphology and to compromised survival (22). The study of these two myosins is still in progress, but one might speculate that, as regulators of Rho-family GTPases, they may be acting upstream of the class I myosins discussed above, and play important roles in the control of actin dynamics leading to phagocytosis and macropinocytosis. The potential importance of their actin-activated ATPase motor domain to position them at their place of action in the actin cortex is not known yet.

Class I Myosins Recruit the Machineries for Actin Dynamics and Endocytosis

As indicated above, the SH3 domains are critical for the function of myosin I. SH3 domains are found in an impressive number of factors involved in signal transduction, actin dynamics and endocytic membrane trafficking (54–59), and have been shown to interact specifically with either one or a few proline-rich motifs of either class I (RxxPxPxxP) or class II (PxxPxxR) configuration (60). The discovery of complex networks of molecules associating through a cascade of interactions between SH3 and proline-rich domains (PRD) (61,62) sheds new light on the functional significance of TH2 and SH3 domains of class I myosins. For example, ligands of S. cerevisiae and D. discoideum myosin I SH3 domains have been identified, and shown to link these molecular motors with the endocytic machinery, as well as with the Arp2/3 complex and the machinery responsible for actin dynamics. Moreover, TH2 domains bear strong resemblance to the PRD of WASp, SCAR, and formins, all substrates for SH3–domain interactions, and involved in regulation of actin nucleation (4,63,64). It is also important to note that, besides being able to recruit and stimulate Arp2/3 nucleating activity via a C-terminal acidic domain, the proteins of the WASp/SCAR family have a WASp homology 2 (WH2) domain that can recruit G-actin (63). In yeast and mammalian cells Las17p/WASp also interacts with verprolin (Vrp1p)/WIP (WASp-interacting protein) (65,66). Many of the proteins shown to interact with WASp have both PRD and SH3 domains, emphasizing the potentially high complexity of the resulting networks (62).

The two S. cerevisiae type I myosins, Myo3p and Myo5p, are linked to the Arp2/3 complex by multiple interactions (Figure 3A). First, these myosins bind directly to Pro-rich motifs in both Vrp1p and Las17p by means of their SH3 domain (47,49,67). The interaction with Vrp1p is required for their proper localization to cortical patches that mark sites of polarized cell growth (46). In addition, the Vrp1p-Las17p complex recruits the Arp2/3 complex in a pathway
of actin nucleation parallel to the one dependent on class I myosins (68). Second, both myosins directly interact with the Arp2/3 complex via a C-terminal acidic motif (47–49). Related acidic sequences are similarly located at the C-terminus of other fungal class I myosins (Candida albicans, S. pombe and Aspergillus), and of Las17p and human WASp. In S. cerevisiae, Myo3p, Myo5p and Las17p function in a redundant manner to activate the Arp2/3 complex, as removal of the acidic sequence from class I myosins or Las17p has little effect, but deletion of all acidic domains virtually eliminates actin filament assembly in cortical patches (48). In an assay based on permeabilized urea-treated cells, Myo3p or Myo5p were shown to promote actin polymerization only when a specific site important for regulation of their motor activity was intact. Nucleation activity was impaired by dephosphorylation or mutation of the TEDS site (49). This finding contrasts with the observation in Aspergillus that a barely functional motor domain does not (completely) impair the in vivo function of MYOA (44). Thus, the exact role of the motor domain in actin nucleation in vivo requires further clarification, but in vitro reconstitution data appear to indicate that it is dispensable for the core nucleation activity. Indeed, the tail of Myo5p was shown to induce cytosol-dependent actin polymerization around Sepharose beads. This assay appears to mimic the formation of dynamic cortical dependent actin patches (67,69), structures that have been implicated in endocytosis. Importantly, the agreement between the in vitro biochemical requirements for actin nucleation and the in vivo genetic requirements for uptake are striking and indicate an intricate link between actin dynamics and endocytosis, in yeast. Both processes depend on the Arp2/3 complex, Vrp1p and cofilin (67,69). Interestingly, profilin seems to be dispensable, perhaps highlighting that the WH2 domain (present in Las17p and also Vrp1p), which can bind G-actin directly, may bypass the need for the recruitment of monomeric ATP-actin by profilin. These findings are similar but not identical to the requirements for the in vitro reconstitution of the motility of Listeria from purified components, which is commonly considered to be a model for lamellipodium extension (70). These data might reflect the fact that lamellipodium extension clearly relates to phagocytosis and macropinosis, but the morphological correlate of endocytosis in yeast is still unclear.

In ameba, class I myosins are also able to recruit the Arp2/3 complex, but do so with a twist, involving an adapter protein (Figure 3B). CARMIL and Acan125 were isolated from extracts using recombinant GST fusions with the SH3 domains of D. discoideum MyoB and MyoC (71), and Acanthamoeba MyoA (72) and MyoC (73) as affinity baits, respectively. These homologous adapters consist of multiple leucine-rich repeat sequences and bear two C-terminal PXPF motifs that are ligands of the myosin I SH3 domains (74). CARMIL was shown to bind the Arp2/3 complex through an acidic motif similar to the ones found in WASp family proteins and class I myosins (71). As mentioned above, MyoK has a TH2-like insertion in its motor domain that has about 40% identity (over 60% homology) with the Pro-rich domain of some WASp proteins. Closer analysis revealed that it contains a variety of Pro-rich motifs that have been shown to work as profilin-binding sites (for example, ZPPF, where Z generally is Pro, Ala, Gly, or occasionally a charged residue, P is Pro, and F is...
preferentially a hydrophobic residue) (75). Finally, it contains a canonical class I SH3 binding motif (RxxPxxP). This so-called GPR loop might therefore serve as a multifunctional protein–protein interaction domain (15). Preliminary data indicate that the GPR loop of MyoK is able to bind the profilin–actin complex as well as a protein identified as the D. discoideum homologue of the yeast and mammal Abp1p/SH3P7r2 protein (Figure 3C, C. Kistler and T. Soldati, unpublished results). Abp1p is conserved from yeast to humans and has been shown in yeast to recruit and activate the Arp2/3 complex through two acidic motifs (76), and in humans to recruit dynamin through its SH3 domain to plasma membrane sites marked by other endocytic markers (77). Abp1p is thus a perfect paradigm for the link between actin dynamics and endocytosis (78).

Interestingly, in addition to the seven components of the Arp2/3 complex, the native CARMIL complex also contains the α and β subunits of capping protein (71), whose function is to block the barbed end of actin filaments. Determining the significance of the presence of both an actin nucleator and a polymerization terminator in the same complex will require more investigation, but it invites further speculation about their functions in shaping the very architecture of the cortical actin meshwork. Also noteworthy is that, in addition to their concentration in actin-driven cellular protrusions of the cell cortex, especially crown-shaped macropinocytic cups and the leading edge of migrating cells, MyoB, CARMIL and the Arp2/3 complex are found on a variety of endosomal compartments (37,71). These findings highlight the importance of class I myosin function not only at the plasma membrane, as has been mainly discussed here, but also on the surface of endocytic (and possibly other) organelles.

A General Convergence of Actin Dynamics and Endocytosis

In addition to the above discussion of the specific function of class I myosins in bridging processes of endocytosis and actin dynamics, a general convergence of cellular, genetic and biochemical studies supports the hypothesis that the actin cytoskeleton is coupled to endocytic processes. Recent studies have identified several proteins that may functionally link the endocytic machinery with actin filament dynamics. Three of these proteins, Abp1p, Pan1p and cortactin, are activators of actin assembly nucleated by the Arp2/3 complex (79). Two others, intersectin and syndapin, bind N-WASp, a potent activator of actin assembly via the Arp2/3 complex. All of these proteins are themselves part of the endocytic machinery or bind further components, and thus, could coordinate actin assembly and trafficking events. Such generalization is extremely exciting but not directly within the scope of this review. Therefore, interested readers are invited to survey recent articles that highlight the double life of proteins such as Srv2p/CAP, Prk1p/Ark1p, End3p, Lsb1–5p, Ysc84, Bzz1p, Mti1p/Bbc1p, Rvs161/167p in machineries at the interface of endocytosis and cytoskeleton polarity and remodeling (54–59,62,80).

Conclusions

Class I myosins are multidomain proteins capable not only of coupling their actin-based motor activities to membrane dynamics, but of directly and indirectly affecting actin assembly and disassembly. As a consequence of the properties of their basic TH1 domain, all class I myosins (including MyoK that has a C-terminal farnesylation in place of TH1) have the potential to associate to a membrane and to translocate F-actin along its surface. In addition, the Gly, Pro and Arg-rich TH2 (or MyoK TH2-like) domain probably confers on many class I myosins the capacity to bind two actin filaments and slide them relative to each other. These features might help myosin I accomplish the task predicted in Figure 1D.

The C-terminal acidic domain that mediates the direct association of yeast myosin I with the Arp2/3 complex is missing in the D. discoideum and Acanthamoeba myosins I, but the connection to the Arp2/3 complex has been maintained through the adaptor protein CARMIL. Homologues of CARMIL exist in Caenorhabditis elegans (71), Drosophila and other higher eukaryotes including mammals, providing an important clue that the long-tailed class I myosins present in animals may be similarly linked to the Arp2/3 complex via their SH3 domain. Importantly, the example set by D. discoideum MyoK of an alternative mode for the indirect recruitment of the Arp2/3 complex by an adaptor bound via its GPR loop offers the attractive possibility that a comparable mechanism is active in higher eukaryotes, where the PRD of class I myosins and other linker proteins might fulfill the job. In general, these properties fulfill the requirement proposed in Figure 1C.

Finally, in addition to the three ways of linking to the Arp2/3 complex illustrated in Figure 2, class I myosins make use of two different ways to concentrate G-actin, the fuel for F-actin elongation. Class I myosins either bind profilin-actin through a Pro-rich domain (MyoK GPR loop, Figure 3C) or bind G-actin indirectly via the WH2 domains of the adaptor proteins WASp, CARMIL and Vrp1p (81, Figure 3(A,B)). Concentrating monomeric actin is not essential for actin-based motility (70) but greatly enhances the efficiency and speed of the process (70,82–85). This capacity of class I myosins adequately fulfills the role suggested in Figure 1E.

Many candidates have been proposed for the role of regulators of the Rho-family GTPases, and recently a specific GEF, for example, has been linked to phagocytic uptake of particles (86). In this context, it is remarkable that class IX myosins and D. discoideum MyoM are demonstrated down-regulators and activators of such GTPases.
respectively (Figure 1B), and might thus play an important role in connecting actin dynamics and endocytic processes.

Overall, the evidence that class I myosins and actin dynamics play a crucial role in endocytosis in yeasts and other lower eukaryotes is overwhelming. However, their involvement in the endocytic machinery of mammalian cells is still acutely lagging. The major challenges for the future will be to determine the roles played by actin filaments at different steps in the internalization of proteins and fluid, and to determine how the interface of the endocytic machinery and the actin cytoskeleton is structured and regulated.

Acknowledgments

I thank all the lab members who along the years have contributed to a better understanding of endocytosis and myosin function. The work has been supported by the Max-Planck Society, the Deutsche Forschungsgemeinschaft, the BBSRC and The Wellcome Trust.

References

43. Liu X, Osherov N, Yamashita RA, Chung YS, May GS. Myosin I mutants with only 1% of wild-type actin-activated MgATPase activity retain essential in vivo function (s). Proc Natl Acad Sci USA 2001;98:9122–9127.


