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Minireview

Do unconventional myosins exert functions in dynamics of membrane compartments?

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Unconventional myosins have now been identified in amoeba as well as in higher eucaryotic cells. Their cellular localization, their ability to bind membrane vesicles and their ability to produce in vitro movement suggest that they can generate forces on the plasma membrane relative to actin filaments as well as on membrane compartments relative to actin. Genetic approaches and biochemical analysis of cells over-producing nonfunctional domains of unconventional myosins have provided direct evidence for a role of unconventional myosins in movement of intracellular vesicles and have allowed us to formulate hypotheses about the possible mechanisms by which unconventional myosins could participate in the intracellular transport of membrane proteins and secretory proteins.

Myosin I; Vesicular motion in eucaryotic cells; Intracellular transport

1. INTRODUCTION

Myosins are molecular motors for actin-based motility. Specific isoforms are expressed in muscle cells and nonmuscle cells. The persistence of normal cellular movements, including membrane ruffling, phagocytosis and cherniotaxis in amoeba cells in which the gene coding for the conventional myosin heavy chain was disrupted has confirmed the presence of a second type of myosin [1,2]. On the basis of the unusual monomeric structure of the first unconventional myosins identified, this class of myosins has been named myosins I and the conventional dimeric class of myosin, myosins II [3,4]. Myosins I, first identified in amoeba, have more recently been found in higher eucaryotic cells of both vertebrate and invertebrate organisms (for reviews see [5,6]). Given the structural diversity of the newly characterized members, we will refer to these new proteins as conventional and unconventional myosins [7]. Current theory is that a single cell type contains at least one form of conventional myosins and several unconventional myosins. In this review we will describe the structural diversity of the unconventional myosins and formulate a hypothesis about their role in membrane dynamics related to intracellular transport of proteins.

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2. STRUCTURAL COMPARISON OF CONVENTIONAL AND UNCONVENTIONAL MYOSINS

Conventional myosins form hexamers consisting of two heavy chains (200 kDa) and two pairs of light chains (between 14 and 20 kDa each). The carboxy-terminal tail domains of the heavy chains form an α-helical coiled-coil structure, and mediate the self-association of molecules into bipolar filaments. The amino-terminal ends form two globular heads which are often referred to as S1, a designation for the proteolytic fragments to which they correspond. The S1 domain contains both an actin-binding site and an ATP-binding site. Both unconventional myosins and conventional myosins contain an S1-like domain which exhibits mechanoenzymatic activity. In contrast, no homology is observed between tail domains of unconventional myosins and conventional myosins (Fig. 1). In addition, three different classes of tails have been observed thus far for unconventional myosins.

Myosins I isolated from *Acanthamoeba castellanii*, *Dictyostelium discoideum* and brush border myosin I have an amino-terminal S1-like domain linked to a short carboxy-terminal tail domain [8–13]. Their tail domain does not allow the molecules to form an α-helical coiled-coil structure but it exibits a lipid binding site in all cases. An actin-binding site for some of the amoeban myosins, and calmodulin-binding sites in the case of the brush border myosin I are also present in this domain.

The 215 kDa gene product of the mouse *dilute* coat

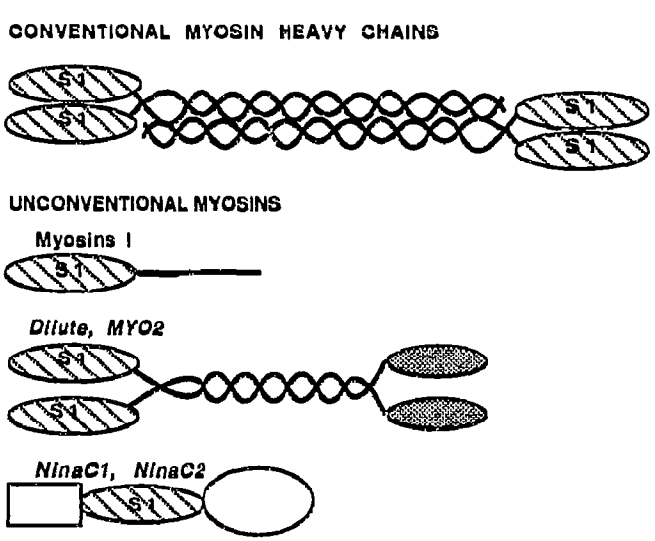

Fig. 1. Structural comparison of unconventional myosins (myosins I) with the heavy chain of the conventional myosins (myosins II).

colour locus [14], its homologue in chicken brain known as p190 [15], and the 180 kDa product of the *MYO2* gene in *Sacharomyces cerevisiae* [16] display a second type of structure in their carboxy-terminal tail domain (Fig. 1). Their large tail domain is composed of several distinctive regions. The first region contains copies of a 24-residue repeat analogous to calmodulin-binding sites found in other unrelated proteins. This is followed by a region that contains the heptad repeat of hydrophobic residues characteristic of all sequences forming helical coiled-coils. Electron microscopic observations of p190 have revealed that this molecule is a dimer with two rather large heads, a central rod-like domain and two globular tail domains [7].

The two *ninaC* gene products (*ninaC1*:174 kDa, *ninaC2*:132 kDa) expressed in the photoreceptor cells of *Drosophila* also exhibit a S1-like domain but display a tail domain with a third type of structure [17]. The S1-like domain is in the center of the molecule. A 270-residue region binds to the amino-terminal end of the S1-like domain and displays a high similarity with the sequence of catalytic protein kinase domains. A 448-residue region (*ninaC1*) or a 82-residue domain (*ninaC2*) form the tail domains and bind to the carboxy-terminal end of S1-like domain. These tails have no homology with the tail domain of myosin II or any other unconventional myosins characterized thus far. Moreover, *ninaC1* and *ninaC2* have not yet been shown to be able to dimerize by a self-assembly process.

Although the sequences of the unconventional myosin tails are dissimilar, myosins I from amoeba, brush border myosin I, and the 174 kDa *ninaC* product have a region of 180–250 amino acids with a pronounced net positive charge. As discussed below, this domain may be involved in membrane binding.

3. ASSOCIATION OF UNCONVENTIONAL MYOSINS WITH SPECIFIC MEMBRANE DOMAINS

3.1. *Biochemical analysis*

Cell fractionation studies of *Acanthamoeba castellanii* have revealed that a substantial amout of myosin I is associated with membranes [18–20]. Brush border myosin I is recovered in membrane disks isolated from brush border enterocytes [21] and *ninaC1* interacts strongly with rhabdomere membrane fractions [22]. Membrane fractions from *Acanthamoeba castellanii*, stripped of peripheral proteins, are able to bind *Acanthamoeba castellanii* myosins IA and IB with binding capacities respectively of 0.65 and 0.85 nmol/mg proteins [20]. A protein containing the entire basic domain of *Acanthamoeba castellanii* myosin IC tail fused with *E. coli* β-galactosidase also binds to purified membranes with a K_d of 300 nM and competes for this binding with an intact myosin IC [23]. Altogether, these experiments suggest that myosins I are able to bind to isolated membrane fractions, and that binding sites are located in their carboxy-terminal tails. Moreover, membrane-associated proteins are not required for this binding since myosins I from *Acanthamoeba castellanii* and from chicken intestinal brush border bind directly to liposomes made of anionic lipids [18,24,25].

These observations suggest that electrostatic interactions between the basic domains of myosin I tails and the acidic phospholipids head groups would be sufficient for an high affinity association. However, these conclusions resulting from in vitro experiments cannot account for the specific in vivo distribution observed for unconventional myosins (see below). One can speculate that such specific localizations might involve a combination of interactions that comprise contacts with phospholipids (electrostatic associations) and contacts with membrane protein(s). The binding of the brush border myosin I to a microvillus membrane glycoprotein as demonstrated by an in vitro nitrocellulose binding assay, is in favour of this proposal [26].

3.2. *Subcellular distribution*

Immunocytochemical studies with a variety of antibodies support the idea that different unconventional myosins are associated with a specific membrane compartment. Two myosins I from *Acanthamoeba castellanii* (myosins IA and IB) and one myosin I from *Dictyostelium discoideum* are localized in the leading edges of migrating amoeba [20,27,28]. These plasma membrane specializations interact with an important actin filaments network that excludes conventional myosin. In higher eukaryotic cells, two known examples of cell surface specializations are enriched in a specific myosin I the brush borders of the intestinal cells and the rhabdomeres of the *Drosophila* photo-receptor cells [22,29,30].

Several reports concerning the immunolocalization of unconventional myosins at the light and electron microscopic level also indicate that cytoplasmic vesicles are associated with these myosins. For instance, the myosin IC from *Acanthamoeba castellanii* is associated with the membrane of the contractile vacuole, the brush border myosin I is associated with the membrane of small vesicles present in the terminal web, and one unconventional myosin is associated with particles that are present in the cortical cytoplasm during the syncytial stages of *Drosophila* development [28,30,31].

The specific subcellular distribution observed for the unconventional myosins raises the question of how such homologous proteins can interact with a range of specialized membrane structures. The answer may be that their specific tail domain is able to interact with specific membrane binding sites.

4. FUNCTION OF UNCONVENTIONAL MYOSINS

4.1. *In vitro assays for motility*

As was observed with conventional myosins, beads coated with unconventional myosins move unindirectionally on actin cables of *Nitella* in an ATP-dependent manner with an average velocity of 0.03 μ m/s for the myosin I of *Acanthamoeba castellanii*, and 0.008 μm/s for the brush border myosin I [21,32,33]. A second assay in which fluorescent actin filaments move relative to myosin immobilized on a substrate has also been used. In this assay the same myosins I moved actin filaments 10-fold faster. *Acanthamoeba castellanii* myosin I bound to phosphatidylserine planar membranes was able to move actin filaments with a velocity of 0.2 μm/s and brush border myosin I bound to nirocellulose was able to move actin filaments with a velocity of 0.08 μm/s [25,34]. The velocity of brush border myosin I in this second assay is equal to the velocity of cytoplasmic conventional myosin motility. It is important to point out that under the lastest reported conditions, the velocity of actin filaments moved by *Acanthamoeba castellanii* is similar to the velocity of movement of crude organelles in the *Nitella* assay, and that this movement is inhibited by specific antibodies directed against *Acanthamoeba castellanii* myosin I [35].

4.2. *Function of unconventional myosins in cells*

Cellular localization of myosins I, their ability to bind lipids or membrane vesicles, and their ability to produce movement in vitro suggest that myosins I can generate forces on membranes relative to actin filaments. Therefore myosins I could be involved in dynamic processes of the plasma membrane and/or of the intracellular membranes.

4.2.1. *Role in dynamics of plasma membrane*

The role of myosin 1 in cell locomotion was directly established when several investigators demonstrated that *Dictyostelium discoideum* mutants defective for the gene encoding conventional myosin, were still able to move [1,2]. Two major phenomena have been observed during cellular locomotion. The first is the ability of actin to polymerize and depolymerize in a dynamic fashion, and the second is the rearwards transport of membrane particles at the cell surface as visualized after binding of concanavalin A coated beads to the cell surface. Movement of these particles has been shown to be actin-dependent [36–38]. The high concentration of myosin I in the leading edges and the fact that the *Dictyostelium discoideum* nul mutant for the gene encoding conventional myosin are still capable of active rearwards transport suggest that unconventional myosins could be responsible for this transport [27,39]. The concentration of unconventional myosin in phagosomes and the phenotype of *Dictyostelium discoideum* mutants, in which gene encoding conventional myosin or one of the myosins I have been disrupted, are also in favour of a role for unconventional myosins in the dynamics of these cellular structures [1,2,27,40]. Mutants in which gene coding for conventional myosin have been disrupted cannot cap surface particles but are still capable of phagocytosis. In contrast, mutants in which the gene encoding one myosin I has been disrupted exhibit a reduced rate of phagocytosis as well as delayed chemotactic streaming.

4.2.2. *Role in vesicle motion*

The binding in vitro and the subcellular localization of some of the unconventional myosins with intracellular vesicles allow one to postulate a role for these proteins in vesicle movement. Such movements on actin cables have been observed in plants and in *Algae* [41,42]. The inhibition of budding and the accumulation of vesicles at the restrictive temperature in the *MYO2* temperature-sensitive yeast mutant, provided direct evidence for a role of unconventional myosins in this movement [16]. Such a role is consistent with the fact that actin is involved in the movement of secretory vesicles to the cell surface in *Sacharomyces cerevisiae* [43]. Defective transport of melanosomes in the keratinocytes of the *dilute* mouse mutant also suggests a role of actin filaments in vesicle motion in higher eucaryotic cells.

In order to investigate the involvement of unconventional myosins in vesicles movement in higher eucaryotic cells, we have over-produced a nonfunctional domain of an unconventional myosin that could potentially compete with endogenous unconventional myosin(s) and could inhibit its(their) function(s). As shown in Fig. 2, we have observed that the over-production of the brush border myosin I carboxy-terminal tail domain in a hepatoma cell line (BWTG3) results in an atypical distribution of albumin, as characterized by its defective accumulation in the Golgi region. A similar

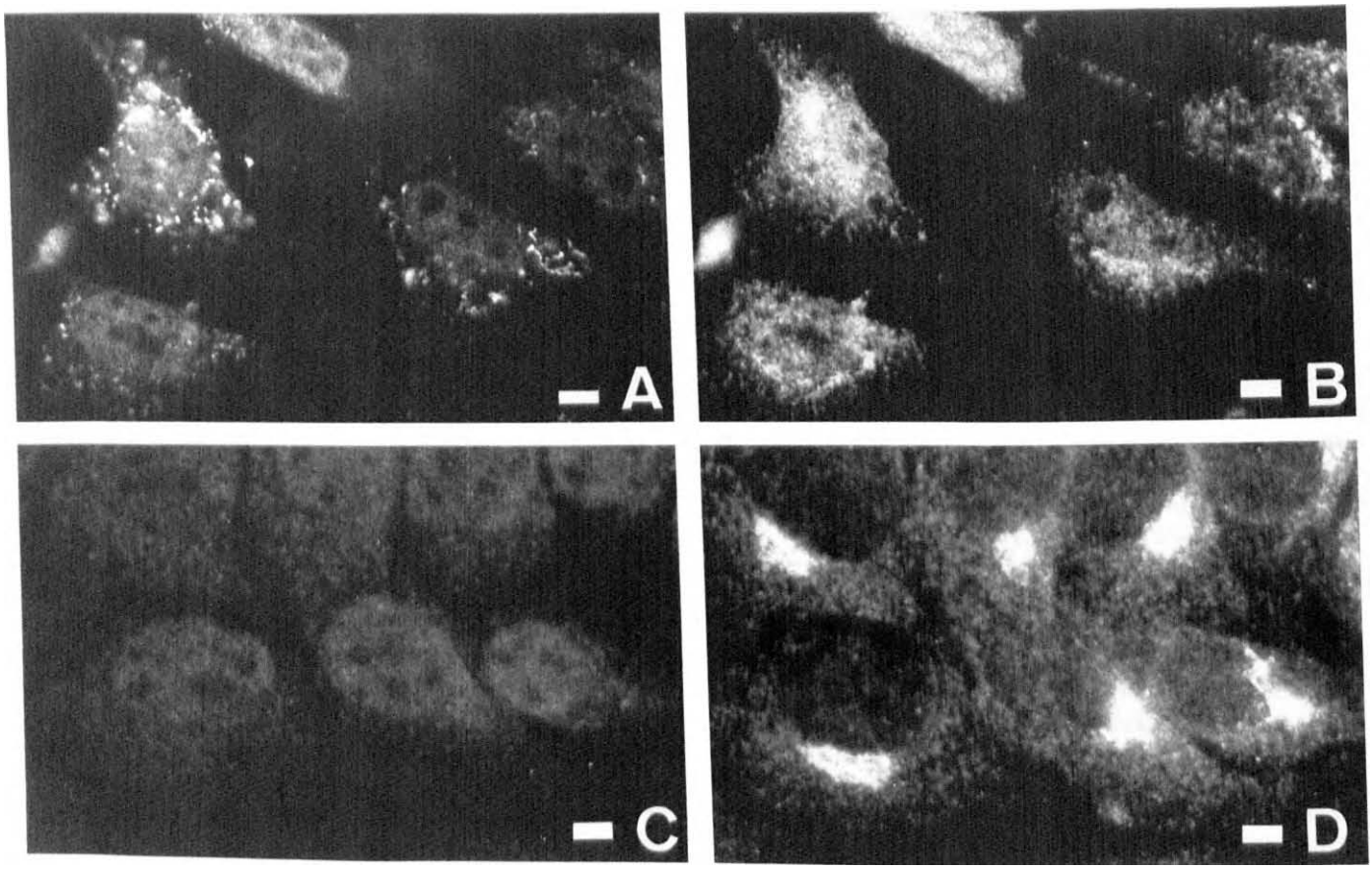

Fig. 2. Over-production of a nonfunctional domain of the chicken brush border myosin I in a hepatoma cell line (BWTG3) [50]. BWTG3 cell line (C,D) and permanent clone derived from BWTG3 cell line (A,B) selected after transfection with the cDNA encoding the brush border myosin I tail domain have been analyzed by double immunofluorescence staining. Micrograph A shows over-production of the tail domain detected with specific monoclonal antibodies. The same antibodies have been incubated with nontransfected cells in micrograph C. Micrographs B and D show the localization of albumin in the same cells. Note that the transfected cells do not exhibit a strong staining for albumin in the Golgi zone. (Bar = 1 μm in each case.) Monoclonal antibodies directed against the brush border myosin I are generous gifts from Dr. Mooseker (Yale University, USA) and polyclonal antibodies directed against albumin were purchased from Nordic-Copenhagen.

effect has been observed for two other proteins secreted by the same cell line (α-fetoprotein and α_2-macroglobulin). These observations suggest that an unconventional myosin could contribute to the transort of secreted proteins in or out of the Golgi complex.

4.2.3. *Hypothesis about the role of unconventional myosins in the movement of vesicles from a donor compartment to an acceptor compartment*

The data illustrated in Fig. 2 support our proposal for a requisite acto-myosin mechanism involvement in the budding of vesicles and/or in the motion of newly formed vesicles from the rough endoplasmic reticulum to the Golgi. The fact that several *Acanthamoeba Castellani* (28) myosins I have been found in association with different intracellular vesicles allowed us to postulate, more generally, that such a mechanism, using different unconventional myosins, could be involved in several transport steps of exocytosis and/or endocytosis. In this respect, this proposal is reminiscent of recent evidence for the diverse motility functions (in mitosis, meiosis, vesicle transport and organelle transport) of the super family of kinesin-like microtubule motor proteins. The functional diversity of the members of this superfamily is related to the high structural diversity of their tail domains [44]. Similarly, the specific subcellular distribution of the small GTP-binding proteins depends of the sequence of their tail domain. It has been demonstrated that this tail sequence is necessary and sufficient to account for the association of the small GTP binding proteins with their target membrane (for review see [45]).

We propose at least three kinds of mechanisms for intracellular transport that could involve unconventional myosins (see Fig. 3).

It is generally accepted that, in plants and yeasts, actin microfilaments are used as tracks for the movement of membrane vesicles. In contrast, in higher eukaryotic cells microtubules and their associated motors are the only cytoskeleton that have been proposed for organelle movements. However, Kuznetsov et al. have recently reported that organelles can move on actin fil-

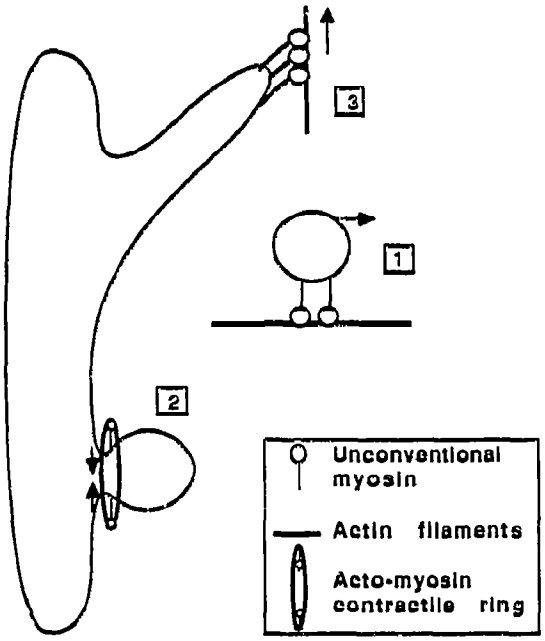

Fig. 3. Model for the role of unconventional myosins in intracellular transport. Three kinds of participation in intracellular vesicle transport are proposed for unconventional myosins: (1) undirectional movement of vesicles on actin filaments, (2) formation of a contractile ring inducing the fission of the vesicle, (3) stretching of the membrane of the donor compartment to form a tubular structure.

aments in squid axoplasm in an ATP-dependent manner [46]. It is reasonable to postulate that this transport requires the participation of unconventional myosins. This hypothesis raises questions about the functional redundancy between actin filaments and microtubules in secretory pathways. Surprisingly Lillie and Brown have recently reported that when a kinesin-like protein is over-expressed in the temperature-sensitive *MYO2* mutant encoding a defective myosin I, it can rescue the abnormal phenotype of this mutant [47]. Could the acto-myosin mechanism be substituted for microtubules and their motor-associated proteins in the secretory pathway? Or is the acto-myosin mechanism used only for some specific vesicle transports steps? These questions remain to be answered.

The accumulation of coated pits in the *shibire* neurogenic mutant of *Drosophila* suggests that clathrin alone is not sufficient to induce the fission of clathrin-coated vesicles [48]. The *shibire* mutated gene encodes for a microtubule-associated protein that is a dynamin-like protein [49]. This protein can induce the sliding of microtubules in an in vitro assay and thus could participate in the fission of vesicles. Such a mechanism would be analogous to the actin sliding process described for conventional myosin and actin when they form a contractile ring during cytokinesis. Similarly, unconventional myosins that display two actin-binding sites in a monomeric form, or a potential for dimerization, could also induce actin microfilaments to slide with respect to each other. We propose that unconventional myosins may form a contractile ring and thus contribute to the fission of budding membranes.

Unconventional myosins could exert tension between actin filaments and a membrane compartment causing a stretching of this membrane. Subsequently the tubular structure so formed may give rise to a vesicle by the fission mechanism discussed above. In this respect it is worth recalling that tubular structures are commonly observed in intracellular membrane compartments such as rough endoplasmic reticulum, Golgi stacks and endosomes, but that the establishment and maintenance of these particular shapes is still poorly understood.

Indeed, experiments must be carried out to demonstrate these hypotheses. A combination of molecular genetic approaches that could lead to the identification of new unconventional myosins in higher eukaryotic cells with the biochemical analysis of eukaryotic cells over-producing functional or nonfunctional unconventional myosins and in vitro assays to study intracellular transport will allow us to understand the function and the interrelation of the new members of the myosin family.

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