Multiple unconventional myosin domains of the intestinal brush border cytoskeleton

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SUMMARY

Representatives of class V and class VI unconventional myosins are identified as components of the intestinal brush border cytoskeleton. With brush border myosin-I and myosin-II, this brings to four the number of myosin classes associated with this one subcellular domain and represents the first characterization of four classes of myosins expressed in a single metazoan cell type. The distribution and cytoskeletal association of each myosin is distinct as assessed by both biochemical fractionation and immunofluorescence localization. Myosin-VI exists in both the microvillus and terminal web although the terminal web is the predominant site of concentration. Myosin-V is present in the terminal web and, most notably, at the distal ends of the microvilli, thus becoming the first actin-binding protein to be localized to this domain as assessed by both immunohistochemical and biochemical methods. In the undifferentiated enterocytes of the intestinal crypts, myosin-VI is expressed but not yet localized to the brush border, in contrast to myosin-V, which does demonstrate an apical distribution in these cells. An assessment of myosin abundance indicates that while myosin-II is the most abundant in the cell and in the brush border, brush border myosin-I is only slightly less abundant in contrast to myosins-V and -VI, both of which are two orders of magnitude less abundant than the others. Extraction studies indicate that of these four myosins, myosin-V is the most tightly associated with the brush border membrane, as detergent, in addition to ATP, is required for efficient solubilization.

Key words: myosin (unconventional), brush border, microvillus

INTRODUCTION

The continuing identification of multiple classes of myosins expressed in a variety of cell types and subcellular domains across a wide variety of species begs the question of the unique and overlapping functions of so many actin-based motors. Recent reviews have already described seven major classes of myosins (Cheney and Mooseker, 1992; Cheney et al., 1993a), and it is apparent that additional classes of myosins do exist (Bement and Mooseker, 1993; Bement et al., 1994). With the demonstration that at least six different myosin classes may be expressed in the intestinal epithelial cell (Bement et al., 1994), we have used available probes to explore the organization of myosin domains in this cell type and in the intestinal brush border (BB) in particular.

Two myosin classes are currently known to be constituents of the BB cytoskeleton (Mooseker, 1985). BB myosin-I is a component predominately of the microvillus (MV) domain that forms the links between the MV core of actin filaments and the overlying MV membrane (for references see Coluccio and Bretscher, 1989). It is found to a lesser extent both in the subjacent terminal web (TW) domain where it may be associated with some vesicular elements (Drenckhahn and Dermietzel, 1988) and also on the basolateral cell membrane (Heintzelman and Mooseker, 1990). Myosin-II in the BB is associated with both the actin rootlets in the TW domain where it is seen to crosslink the lower part of the rootlets (Mooseker, 1985), and also with the junctional complex, specifically with the circumferential bundle of actin filaments of the adherens junction. This particular myosin-II has been cloned and sequenced and is more accurately referred to as chicken nonmuscle myosin heavy chain-IIA, just one isoform of nonmuscle myosin-II (Shohet et al., 1989; Kawamoto and Adelstein, 1991). In this report, we document the existence of a myosin-V and a myosin-VI in the intestinal epithelial cell BB.

To date, the characterized myosin-Vs include the products of the yeast MYO2 and MYO4 genes (Johnston et al., 1991; Haarer et al., 1994) and the mouse dilute gene (Mecer et al., 1991) the latter of which appears to be the mouse homologue of another class-V myosin identified in chick brain (Espreafico et al., 1992; Cheney et al., 1993b). Information derived from myosin-V mutations in mouse and in yeast, together with immunolocalization studies of chick brain myosin-V, suggest that this myosin type has a potential role in vesicular transport and membrane dynamics (Johnston et al., 1991; Mercer et al., 1991; Espreafico et al., 1992; Govinden and Novick, 1993). Similar functional implications have been made regarding the existing class VI myosins, which include the Drosophila 95F myosin heavy chain (95F MHC; Kellermeier and Miller, 1992),
and pig kidney myosin-VI from the proximal tubule cell line, LLC-PK1 (Hasson and Mooseker, 1994).

Here we show that each of the four distinct myosin classes, I, II, V and VI, has a unique but overlapping distribution within the BB domain. The demonstration that three of these unconventional myosins exist in the MV domain raises intriguing questions about the potential role of such myosins in a subcellular domain in which conventional motility phenomena have not been documented. In this regard, the most striking observation is the association of a subset of myosin-V with the distal ends of the MV, a potentially important domain that has, thus far, eluded characterization.

**MATERIALS AND METHODS**

**Antibodies**

Each of the antibodies used for immunoblotting and for immunolocalization has been previously characterized or is commercially available as follows: for BB myosin-I, a monoclonal antibody directed against the tail domain of the myosin (CX-7; Carboni et al., 1988) was used at a 1:200 dilution of an ascites stock for both blotting and staining. A commercial anti-human platelet myosin-II antibody (Biomedical Technologies, Inc., Stoughton, MA) was used at 1:250 for western blots and at 1:10 for immunolocalization. For myosin-V studies, an affinity-purified polyclonal antibody generated against a chick brain myosin-VI tail fusion protein (Espreafico et al., 1992) was used at 1 µg/ml for western blotting and 10 µg/ml for localization. An affinity-purified myosin-VI antibody was likewise generated against a pig kidney myosin-VI tail fusion protein (Hasson and Mooseker, 1994) and was used at 1 µg/ml for blotting and 20 µg/ml for tissue staining.

**Cell fractionation**

Brush borders were isolated as described by Keller and Mooseker (1982). In the initial stages of this procedure, a whole cell homogenate is generated, and this was used as starting material for standard subcellular fractionation. The homogenate was spun for 7 minutes at 1,500 g and samples were taken of this low-speed pellet (LSP) and supernatant (LSS). The LSS was further spun for 15 minutes at 27,000 g to generate the high-speed pellet (HSP) and supernatant (HSS), and the HSS was again spun for 1 hour at 100,000 g to yield the ultra-speed pellet and supernatant. Samples of the relevant fractions were collected, adjusted to equal volumes and processed for SDS-PAGE, and transferred to nitrocellulose using standard protocols. Immunoblots were first blocked in 5% non-fat dry milk for 1 to 2 hours at room temperature, incubated in primary antibody overnight at room temperature, and then processed with a secondary antibody conjugated to alkaline phosphatase.

**Micsrvilli (MV)** were purified from BBs as described by Mooseker et al. (1989), and samples of both BBs and MV were taken for quantification of total protein using the BCA method (Pierce Chemical Co., Rockford, IL) and for use in SDS-PAGE and immunoblotting.

To examine the association states of the four myosins within the BB-membrane cytoskeleton, samples of purified BBs were extracted under a variety of conditions. This involved resuspending BB pellets in buffer A alone (75 mM KCl, 10 mM imidazole, pH 7.2, 1 mM K-EGTA, 1.25 mM MgCl2), or with 10 mM ATP or 0.6 M NaCl, or ATP and salt together, for 15 minutes on ice. BB were pelleted, and samples of the pellets and supernatants were taken for SDS-PAGE and immunoblotting. The same protocol was used to extract BBs that had first been treated with 1% Triton X-100 for 15 minutes on ice and then pelleted (Mooseker and Tilney, 1975). This initial Triton supernatant (TXS) was also processed for SDS-PAGE and immunoblotting in order to assess the loss of BB components with detergent alone.

To examine the interaction of the myosin-V and -VI immunogens with actin, ATP extracts prepared from BBs using buffer A plus 10 mM ATP (see above) were dialyzed overnight against buffer A with MgCl2 added to 2 mM in order to remove ATP. F actin (6 µM) was added to samples of the dialysate in either the absence or the presence of 10 mM ATP and spun at 100,000 g for 1 hour. Controls included extract alone and F-actin in Buffer A. Pellets and supernatants were collected and processed for SDS-PAGE and immunoblotting.

**Quantification of myosin content in whole cells and brush borders**

Samples of whole cell and BB fractions, the protein concentration of which had been determined using the BCA assay, were run on SDS-PAGE together with dilutions of a known concentration of purified BB myosin-I, BB myosin-II, chick brain myosin-V or purified pig kidney myosin-VI tail fusion protein. These gels were immunoblotted with their respective antibodies and the immunoreactive bands quantified by scanning densitometry on a Visage 2000 scanner (BioImage/Millipore, Bedford MA) in reflectance mode. Concentrations of the unknowns were determined by comparison to the standard curve for the purified myosin samples. An assessment of the relative affinity of the myosin-V and myosin-VI antibodies for their BB-associated antigens compared to their original antigens was accomplished as follows. Chicken BBs were purified and extracted for 15 minutes with 10 mM ATP and 1% Triton X-100. The residual BBs were pelleted and the supernatant was incubated for 1 hour with Pansorbin cells (Protein A-coated Staphylococcus aureus; Calbiochem, La Jolla, CA). These cells were spun out and myosin-V or -VI antibody was added to the supernatant and allowed to incubate for 2 hours at 4°C prior to the addition of Pansorbin cells for an additional hour. Cells were pelleted, washed 3 times with TBS, and the pellets prepared for SDS-PAGE. Approximate concentrations of BB myosin-V and -VI were assessed on Coomassie Blue-stained polyacrylamide gels by comparison with known concentration of chick brain myosin-V or myosin-VI fusion protein. Serial dilutions of the pure proteins and those immunoprecipitated from BBs were then immunoblotted and the immunoreactive bands compared. As additional controls, myosin-V was immunoprecipitated from chick brain, and myosin-VI from LLC-PK1 cells and immunoblotted at matching concentrations as above.

**Immunohistochemistry**

Pieces of chicken duodenum were dissected and fixed for 5 minutes on ice in 4% formaldehyde in PBS containing 50 mM K-EGTA. Tissue was rinsed in the PBS/EGTA buffer, quenched for 10 minutes in 0.05% sodium borohydride, rinsed and then cryoprotected in 1 M sucrose prior to embedding in OCT media (Miles Inc., Elkhart, IN) and freezing in liquid nitrogen-cooled isopentane. Frozen sections (4 µm) were cut and applied to Vectabond (Vector Labs, Burlingame CA)-coated slides, and stored at ~20°C until used. Staining was accomplished by first immersing slides in ~20°C acetone, then into water, and into the PBS/EGTA buffer containing 0.1% BSA. Primary antibodies, or non-immune IgGs used as controls, were applied to the sections for 20 minutes, the slides washed 3 times, and then incubated for 20 minutes with an FITC-conjugated secondary antibody (1:1000, Cappell, Durham, NC). Slides were again washed and then observed with a Zeiss light microscope equipped for epifluorescence. The use of blocking agents (fetal calf serum, 5% milk powder) was tested, but as no difference in staining patterns was observed, blocking was determined to be unnecessary and was routinely omitted.

**RESULTS**

**Analysis of myosin content in subcellular fractions**

The initial observation was made that antibodies generated...
against the tail domain of either chick brain myosin-V or porcine kidney myosin-VI each reacted with a single band of the expected molecular mass of 190 kDa and 140 kDa, respectively, on immunoblots of purified chicken BBs (Fig. 1). These immunogens were distinct in molecular mass from the two known myosins, and the antibodies showed no cross-reactivity with any other BB components. To assess more carefully the expression of these myosin immunogens in the intestinal epithelial cell, standard cell fractionation was performed and analysis for the expression of myosins in different subcellular compartments (Fig. 2). As predicted from the initial immunoblot of purified BBs, all four myosins were detected in whole-cell homogenates, in the low-speed pellet that contains the bulk BB fraction, and in the high-speed pellet that contains smaller fragments of BBs, MV and the larger organelles. A fraction of each myosin was pelletable under ultra-high speeds, suggesting an association with the microsomal fraction or with the few fragments of MV that may still be present. In contrast to myosins-I and -V, myosins-II and -VI both demonstrated a substantial soluble component. It should be noted that in addition to the predominant 140 kDa band detected with the myosin-VI antibody, a fainter, higher molecular mass immunogen was seen in the ultraspeed pellet and supernatant fractions (Fig. 2). This band was not, however, detected in any of the BB-containing fractions.

To define further the localization of the myosins in the BB domain itself, MV were purified from BBs, and immunoblotted in comparison to intact BBs, each sample being loaded so as to equalize total protein content (Fig. 2, lanes 6 and 7). As myosin-II is known not to be a component of the MV, the detection of only a trace amount of myosin-II compared to that seen in BB, attests to the purity of the MV fraction. In these samples, and in other preparations in which no myosin-II was detected in the MV sample, a fraction of both myosin-V and myosin-VI was demonstrated to reside in the MV fraction.

To obtain an estimate of the relative abundance of the four myosins in the whole cell and in the BB domain itself, quantitative densitometry of immunoblots was done by comparing samples of known total protein concentration with standards of the purified myosins or a myosin-VI fusion protein as this myosin has not yet been purified from tissue. To validate the use of this method for assessing myosin abundance, it was first necessary to determine the relative affinity of the brain myosin-V and kidney myosin-VI antibodies for their myosin antigens

<table>
<thead>
<tr>
<th>Myosin</th>
<th>% of whole cell protein</th>
<th>% of brush border protein</th>
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<tbody>
<tr>
<td>I</td>
<td>1.4±0.6*</td>
<td>6.3±3.3</td>
</tr>
<tr>
<td>II</td>
<td>5.2±1.2</td>
<td>16.5±4.3</td>
</tr>
<tr>
<td>V</td>
<td>0.01±0.005</td>
<td>0.05±0.02</td>
</tr>
<tr>
<td>VI</td>
<td>0.03±0.01</td>
<td>0.09±0.04</td>
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*Values are ± standard deviation.
from membrane-intact BBs. All the myosins were solubilized in the presence of ATP, but while myosins-II and -VI were most completely released, only a small fraction of myosin-V was subject to ATP solubilization, a pattern shared, albeit to a lesser degree, by myosin-I. Salt alone had minor effects on the release of the myosins, but did potentiate the ATP release of myosin-I and possibly myosin-VI as well. Although detergent treatment itself did not release any of the myosins (Fig. 3b, TXS lane), the ATP-dependent solubilization of myosin-I and of myosin-V were greatly enhanced by having had the membrane removed (Fig. 3b). The pattern of myosin-II and myosin-VI extraction was similar in both intact and detergent-treated BBs.

To determine if the BB-associated immunogens that we are detecting with the antibodies against chick brain myosin-V and pig kidney myosin-VI may, in fact, be myosins, we examined the potential of these proteins to bind to actin in the presence and absence of ATP. As neither myosin has yet been purified from the BB, we assayed actin binding using whole ATP extracts of purified BBs and monitored this by immunoblotting. As illustrated in Fig. 4, both the BB-associated myosin-V and myosin-VI immunogens demonstrate the ability to bind to F-actin in the absence but not the presence of ATP, behavior characteristic of bona fide myosins.

**Immunolocalization of myosins in the intestinal epithelial cell**

We next sought to describe and compare the localization of the BB-associated myosins using indirect immunofluorescence. Staining for each of the four myosins in the chicken intestinal BB revealed a unique distribution for each. The BB staining patterns were most accurately assessed in high-magnification, underexposed photographs in which bleeding of the intense BB-associated immunofluorescent signals was minimized. The most striking staining pattern was that obtained with the myosin-V antibody, which detected distinct concentrations of this protein in both the TW zone and at the distal ends of the MV (Fig. 5). This pattern contrasts with that of myosin-I (Fig. 6a), which is predominantly MV, and with myosin-II (Fig. 6c), which is exclusively associated with the TW as documented previously (see Mooseker, 1985, for review). Myosin-VI is expressed predominantly in the TW domain but does show some faint staining of the MV domain (Fig. 6e), consistent with the biochemical fractionation seen in Fig. 2. In addition to the concentration of these myosins in the BB, each also demonstrated some non-BB-associated component that is most evident with routinely processed, lower-magnification images. Staining of the supranuclear cytoplasm was quite evident with antibodies against myosins-II, -V and -VI, in contrast to BB myosin-I in which the non-BB-associated staining was minor compared to the signal in the BB domain (Fig. 7).

The staining patterns of the myosins in the intestinal crypts was also documented to assess any changes in their localization that might occur during differentiation of the BB (Fig. 8). As previously documented, BB myosin-I is not yet concentrated in the apical domain, unlike myosin-II, which does localize to the TW in these cells. The pattern of myosin-V staining is akin to that of myosin-II in that this protein is already expressed in the BB domain of the crypt cells although...
the ‘railroad track’ staining pattern seen in mature enterocytes cannot be discerned, possibly due to the short size of the MV in these cells and our limits of resolution. Myosin-VI staining demonstrated a pattern similar to that of myosin-I. That is, myosin-VI, although expressed in the crypts, has not yet assumed a BB localization characteristic of that seen in the mature villus cell population.

DISCUSSION

In addition to the well-characterized BB myosins-I and -II, we describe in this report the existence of two additional myosin classes that are associated with the intestinal epithelial cell BB domain. Antibodies generated against chick brain myosin-V (Espreafico et al., 1992) react with a 190 kDa protein that exists in both the TW and at the distal ends of the MV as assessed by both indirect immunofluorescence localization and cell fractionation studies. Anti-pig kidney myosin-VI antibodies react with a 140 kDa protein in the BB that is seen to exist in both the TW and the MV domain, although the TW localization is predominant. Several attempts have been made to localize these two proteins at the ultrastructural level using immunoelectron microscopy, but to date only our BB myosin-I antibody has proved useful for such studies. (Heintzelman and Mooseker, 1990).

The BB-associated myosins-V and -VI are not abundant in the BB, each comprising less than 0.1% of total BB protein, and we have not yet attempted to purify these proteins from the intestinal epithelial cell in order to fully characterize them biochemically. However, two lines of evidence would suggest that these two proteins may indeed be myosins and not simply immuno-crossreactive species. First, both proteins were detected and localized using antibodies generated against the tail domains of myosin-V or myosin-VI, domains that are thought to be unique to these myosin classes. The pig kidney myosin-VI tail domain shows homology only to the one other class-VI myosin yet characterized, the Drosophila 95F MHC (Kellerman and Miller, 1992; Hasson and Mooseker, 1994). The tail domain of chick brain myosin-V, apart from its homology to the other known myosins-V, shows homology to only one protein, a 80 kDa putative glutamic acid decarboxylase from mouse, but this homology is suspect for several reasons (see Espreafico et al., 1992). Notably, the immunogens we detect in the BB, both by immunoblot and by immunoprecipitation and visualization on Coomassie-stained gels, are of the expected sizes of 190 kDa and 140 kDa for the known myosins-V and -VI, respectively.
The second piece of evidence suggesting that these BB-associated proteins are myosins comes from actin cosedimentation experiments done using ATP extracts from isolated BBs. Both immunogens were observed to bind to actin in the absence but not in the presence of ATP, behavior diagnostic of genuine myosins. Further characterization of these BB proteins as myosins, including enzymatic activity and mechanochemical potential, must await their stringent purification away from contaminating BB myosins-I and II, both of which are much more abundant in this system.

Myosin-VI in the intestinal epithelial cell is localized predominately to the TW domain of the BB although some association with the MV was evident. In addition to the major 140 kDa immunogen we detected, with pig kidney myosin-VI antibody, a minor, higher molecular mass immunogen was also seen in the ultrasspeed pellet and supernatant cell fractions. This band was absent, however, from the BB and MV fractions. The significance of this additional immunogen is unknown, although the precedent for expression of multiple myosin-VI isoforms has been set by the work of Kellerman and Miller (1992) with Drosophila. It is possible that this additional immunogen may account for some percentage of the non-BB-associated myosin-VI that we detect by immunofluorescence.

The concentration of myosin-VI in the TW is intriguing as this is a region through which the incoming and outgoing traffic of the apical domain must pass. If myosin-VI is associated with such vesicular trafficking, as suggested for the 95F MHC in Drosophila (Kellerman and Miller, 1992), the question arises of whether it is a motor or simply a passenger. Recent work on Drosophila 95F MHC suggests that this myosin-VI may be a motor, as antibodies against this protein can inhibit some intracellular particle transport (Mermall et al., 1994). Such data are relevant to the population of myosin-VI, in the TW, though probably not for MV-associated myosin-VI as such motile activity has not been observed in this domain. The role of myosin-VI in the MV is not at all apparent at this time, but as myosin-VI is evident only in the BB of mature enterocytes and not in the undifferentiated cells of the crypts, we might assume...
that myosin-VI function is related to the physiology of the fully mature cell and not, for example, involved in the assembly of the BB architecture. A similar picture has been painted for BB myosin-I, which also demonstrates a redistribution during the differentiation of crypt cells into mature enterocytes (Heintzelman and Mooseker, 1990). The expression pattern of myosin-VI in the kidney proximal tubule may also change in concert with differentiation as discussed by Hasson and Mooseker (1994). In this system, myosin-VI is associated with the BB only in the fully differentiated proximal tubule cell, while in the differentiating cell the protein segregates, predominately, with the soluble cell fraction. It will be of interest to define further the subcellular associations of myosin-VI, as has been done by Fath and Burgess (1993) for BB myosin-I, when we examine in more detail the dynamics of these myosins during enterocyte differentiation.

Additional issues are raised by our attempt to understand the biology of myosin-V because of the presence of this protein at the distal ends of MV. As immunoelectron microscopy has not yet been possible using this antibody, we are uncertain if BB myosin-V is located precisely in the MV tip complex or is just diffusely localized at the distal ends of the MV. If at the tip, this concentration of myosin-V would suggest an association of myosin-V with another protein or a complex of proteins that itself is anchored at the tips. Our biochemical data would suggest an association with the BB membrane, since myosin-V was efficiently extracted with ATP only after detergent solubilization of the BB membrane. The dense cap at the MV tip into which the actin core is inserted is still largely uncharacterized. Z-protein has been localized to this structure by immunofluorescence, but no biochemical data are available to confirm this localization (Ohashi and Maruyama, 1989). Cap Z has been identified in the MV fraction by immunoblotting, but has not yet been localized, although the MV tip would be the obvious place to find such a protein (Schafer et al., 1992). Myosin-V, then, may be the best handle we currently have to define further this interaction site between the MV membrane and the cytoskeleton. Characterization of myosin-V's membrane association in the MV becomes critical as the first step in expanding our understanding of this attachment site.
That myosin-V is expressed apically in the crypt cells also raises the possibility that this myosin may be important in early differentiation, perhaps in MV morphogenesis or other aspects of BB assembly.

We must also try to understand the relationship between the two distinct concentrations of myosin-V in the TW and at the distal ends of the MVs. If myosin-V is destined for the end of the MV, what keeps the TW population sequestered where it is? What events might be involved in moving myosin-V from the TW to the MV? Myosin-V may simply be playing two distinct roles in the BB, but if so, how are these functions segregated? These same questions arise for brain myosin-V as discussed by Espreafico et al. (1992). In neuronal cells, intense Golgi-like perinuclear staining of myosin-V was evident as was staining of cell processes, most notably at their distal tips where an array of F-actin is also present. This dichotomous staining pattern may be homologous to the bright TW staining and the staining at the distal ends of MV as seen in the intestinal epithelial cell.

These staining patterns also show some similarities with respect to the dynamics of BB myosin-I. In the fully differentiated enterocyte, the large majority of myosin-I is associated with the BB, with lesser amounts identified in association with the basolateral membrane and with vesicles in the TW (Heintzelman and Mooseker, 1990; Drenchkahn and Dermietzel, 1988). However, in the differentiating cells of the intestinal crypts, when only a fraction of myosin-I resides in the BB, Fath and Burgess (1993) have demonstrated the association of BB myosin-I with a Golgi-derived membrane fraction. As with myosin-VI, the question arises of whether the BB-associated myosins-I and -V are delivered as cargo or are the actual motors powering their own translocation to their final destination, be it the BB, the tips of neurite processes, or in the case of the yeast myosin-V, MYO2, the bud site (Johnston et al., 1991; Govinden and Novick, 1993). If it is simply cargo, then the actual work done by each myosin may be at the site of the plasma membrane and associated cytoskeletal infrastructure. Currently, both scenarios are feasible and need not be mutually exclusive.

It is apparent that our identification of four myosin classes in the BB domain, although quite interesting, is also a bit overwhelming, in that the complexity of this system is continuing to grow. If, indeed, multiple subclasses of these myosins also coexist in a given domain, then the situation will only become more complicated. This forces us to ask the question of how many myosins can be supported by a single actin-membrane cytoskeletal domain? That three unconventional myosins have been identified in the MV domain alone also pushes us to explore unconventional roles for these myosins, since typical motility phenomena such as contraction and vesicular translocation are not known to occur in the MV. Such unconventional roles including mechanochemical regulation of specific membrane channels (e.g. for discussion see Mooseker et al., 1991; Mooseker, 1993) are purely speculative at this time but do offer alternatives to the textbook roles of those myosins involved in contraction and vesicle movement. Recent work on the Drosophila NINAC unconventional myosin implicated in phototransduction and regulation of calmodulin localization already attest to the potential diversity of myosin function in the cell (Porter et al., 1993).

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REFERENCES


Myosins of the brush border cytoskeleton


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