Human myosin-Vc is a novel class V myosin expressed in epithelial cells

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Summary

Class V myosins are one of the most ancient and widely distributed groups of the myosin superfamily and are hypothesized to function as motors for actin-dependent organelle transport. We report the discovery and initial characterization of a novel member of this family, human myosin-Vc (Myo5c). The Myo5c protein sequence shares ~50% overall identity with the two other class V myosins in vertebrates, myosin-Va (Myo5a) and myosin-Vb (Myo5b). Systematic analysis of the mRNA and protein distribution of these myosins indicates that Myo5a is most abundant in brain, whereas Myo5b and Myo5c are expressed chiefly in non-neuronal tissues. Myo5c is particularly abundant in epithelial and glandular tissues including pancreas, prostate, mammary, stomach, colon and lung. Immunolocalization in colon and exocrine pancreas indicates that Myo5c is expressed chiefly in epithelial cells. A dominant negative approach using a GFP-Myo5c tail construct in HeLa cells reveals that the Myo5c tail selectively colocalizes with and perturbs a membrane compartment containing the transferrin receptor and rab8. Transferrin also accumulates in this compartment, suggesting that Myo5c is involved in transferrin trafficking. As a class V myosin of epithelial cells, Myo5c is likely to power actin-based membrane trafficking in many physiologically crucial tissues of the human body.

Key words: Myosin-V, Organelle transport, Membrane trafficking, Transferrin receptor, rab8

Introduction

Although long distance organelle transport is generally attributed to microtubule-based motors, growing evidence indicates that actin-based motors, particularly the class V myosins, also play crucial roles in membrane trafficking (Brown, 1999; Reck-Peterson et al., 2000). The class V myosins have been widely hypothesized to provide a molecular basis for actin-dependent organelle transport (DePina and Langford, 1999; Tuxworth and Titus, 2000). Class V myosins are one of the most ancient groups of the myosin superfamily (Berg et al., 2001) and are essential for survival in both yeast (Johnston et al., 1991) and mouse (Mercer et al., 1991). Two of the five myosins in yeast are class V myosins, and one of these, Myo2p, is required for polarized secretion (Johnston et al., 1991; Schott et al., 1999). In vertebrates, most research on class V myosins has focused on myosin-Va (*dilute*, Myo5a), which is expressed at high levels chiefly in brain and melanocytes. Mice lacking Myo5a suffer neurological seizures and die within 2-3 weeks of birth (Mercer et al., 1991); these animals also exhibit serious defects in the distribution and trafficking of melanosomes (Provance et al., 1996; Wu et al., 1998). A second member of the myosin-V family in vertebrates, myosin-Vb (myr6, Myo5b), has been cloned from rat (Zhao et al., 1996), but has received relatively little attention. Given the fundamental importance of class V myosins in lower organisms and crucial role of Myo5a in brain and melanocytes, we sought to identify a novel member of the myosin-V family that might underlie actin-dependent organelle transport in the other cells and tissues of the human body.

Much of the evidence supporting the role of class V myosins in organelle or particle transport comes from studies in yeast. The yeast class V myosin Myo2p is required for polarized secretion (Schott et al., 1999), vacuolar inheritance (Catlett et al., 2000), Golgi inheritance (Rossanese et al., 2001) and spindle orientation (Yin et al., 2000). The other class V myosin in yeast, Myo4p, is required for the localization of an mRNA to the bud (Takizawa and Vale, 2000). In squid, myosin-V has been implicated in the movement of endoplasmic reticulum (ER)-derived vesicles in the axon (Tabb et al., 1998). In vertebrates, Myo5a is required for the transport/targeting of an ER-derived Ca^{2+} release compartment to dendritic spines (Takagishi et al., 1996) and the transport of melanosomes in the actin-rich periphery of melanocytes (Rogers et al., 1999; Wu et al., 1998). Myo5a also associates with SV2-labeled vesicles from brain (Evans et al., 1998).

Although the membrane trafficking pathways of eukaryotic cells are not yet fully understood, the rab family of small GTPases are essential components of these pathways and provide a useful set of molecular markers to define specific membrane compartments (Zerial and McBride, 2001). Each of the 60+ rab proteins in humans is believed to function at a specific step in membrane trafficking (Bock et al., 2001). Despite the essential roles of rab proteins in membrane trafficking, much remains to be learned about their mechanisms of action and the identity of their effectors. Intriguingly, several recent studies indicate that certain rabs may interact directly or indirectly with class V myosins in vesicle transport. In yeast, Myo2p and the rab protein Sec4p

exhibit genetic interactions such as synthetic lethality and have been hypothesized to be components of a motor-rab transport complex required for secretory vesicle transport (Finger and Novick, 1998; Schott et al., 1999). In melanocytes, Myo5a and rab27a are both required for normal melanosome trafficking and Myo5a appears to require rab27a to dock to melanosomes (Hume et al., 2001; Wu et al., 2001). Although biochemical evidence for a direct interaction between class V myosins and rab proteins has been difficult to obtain, recent two-hybrid data indicate that Myo5b binds specifically and directly to rab11a (Lapierre et al., 2001), a marker for a recycling compartment.

We report the discovery and cloning of a third member of the myosin-V family in vertebrates, human myosin-Vc (Myo5c). This myosin is relatively abundant in many secretory and glandular tissues, where it is expressed chiefly in epithelial cells. To investigate the function of Myo5c, we have pursued a dominant negative approach in a well-studied model of membrane trafficking, the HeLa cell. Dominant negative approaches using overexpression of the tail domain (the putative cargo interaction site) have proven very successful in investigating the functions of class V myosins in yeast, melanocytes and even in brain, where the *flailer* mutation leads to a brain-specific overexpression of the tail domain, resulting in neurological defects (Jones et al., 2000). Recently, a GFP-Myo5b tail construct was shown to selectively perturb the distribution of a recycling compartment in HeLa cells defined by the presence of the GFP-Myo5b tail, transferrin receptor and rab11a (Lapierre et al., 2001). We report that overexpression of a GFP-Myo5c tail selectively perturbs the distribution of an apparently different compartment in HeLa cells defined by the presence of GFP-Myo5c tail, transferrin receptor and rab8. Overexpressing the GFP-Myo5c tail also perturbs transferrin trafficking. We conclude that Myo5c is an actin-based motor protein involved in membrane trafficking and that Myo5c is a major class V myosin of epithelial cells.

Materials and Methods

Isolation and sequence analysis of MYO5c cDNA clones

Clones 2, 5 and 7 were amplified from Marathon Race Ready human pancreas cDNA (Clontech) and subcloned into pCR 2.1 TOPO (Invitrogen). Clone 7 was amplified using primers designed to a ~100 bp PCR product isolated from a previous screen for unconventional myosins (Bement et al., 1994) and an expressed sequence tag (EST) (T11378) that was similar but not identical to the tails of Myo5a and Myo5b. Repeated attempts to clone the 5′ end of the coding sequence succeeded only after a genomic clone (AC010674.7) containing the Myo5c sequence allowed us to PCR from putative 5′UTR into the known coding sequence (clone 5). The coding sequence of clone 7 was found to be interrupted at bp 3645 by a 137 bp insert containing an Alu-like sequence. Comparison with genomic sequence and PCR with primers designed to span this region (Clone 2) verified that this insert was in fact an intron that is normally spliced out of the mature mRNA. PCR from a different cDNA library to produce the GFP-Myo5c tail clone confirmed that this intron is normally spliced out. All clones and PCR constructs were validated by sequencing at the UNC-Chapel Hill Automated DNA Sequencing Facility.

mRNA expression analysis

To perform systematic analysis of the mRNA distribution of each class V myosin, ^{32}P-labeled cDNA probes for Myo5a, Myo5b or Myo5c were hybridized to a Human Multiple Tissue Northern Blot and a Human RNA Master Blot™ (Clontech). Each lane of the Northern Blot contained 2 μg of poly A^+ RNA, whereas the Master Blot contained normalized loadings of 89-514 ng of poly A^+ RNA per dot. Probes were labeled by random-priming and unbound counts were removed using spin columns. Blots were hybridized ($2\text{-}4\times10^6$ cpm/ml) and washed under high stringency conditions and exposed for 24 hours by autoradiography or phosphorimager. Blots were stripped and rehybridized under the same conditions with each probe. Human cDNA probes consisted of a portion of the carboxy-terminal tail and 3′ UTR and were amplified by PCR from the following ESTs: the 669 bp Myo5a probe, spanning the terminal 169 bp of the tail and the first 500 bp of the 3′ UTR, was amplified from EST N44526, corresponding to human Myo5A, GenBank accession #Y07759. The 646 bp human Myo5b probe, spanning the terminal 452 bp of the putative tail and the first 194 bp of the 3′ UTR, was amplified from EST W40522, of which the coding sequence shares ~88% nucleotide identity with rat Myo5b (myr6) and murine Myo5b. The 680 bp Myo5c probe, spanning the terminal 141 bp of the tail and the first 539 bp of the 3′ UTR, was amplified from EST 178419. To facilitate comparison of the dot blots, Adobe Photoshop was used to cut and paste the dot blots into the strips shown in Fig. 3.

Production of affinity-purified antibodies to Myo5b and Myo5c

Antibodies to mouse Myo5b and human Myo5c were raised against His-tagged fusion proteins. The Myo5b tail construct was amplified from a Marathon mouse kidney cDNA library (Clontech) and consists of part of the tail domain of a partial mouse Myo5b sequence (NM_008661; bp 341-1024), corresponding to amino acids (aa) 1209-1437 of rat myr6 (U60416). The Myo5c tail construct was amplified from a human bronchial epithelial cell cDNA library and consists of part of the tail domain (aa 1054-1350). The forward primers for each construct contained a *Bam*HI restriction site at their 5′ end, whereas the reverse primers contained a *Sal*I restriction site at their 5′ end. Each construct was cloned into pQE-30 or pQE-31 expression vectors (Qiagen). The bacterially expressed His-tagged fusion proteins were purified by elution from an SDS-polyacrylamide gel and injected into chickens (for Myo5b, Aves Labs, antibody 1962) or rabbits (for Myo5c, Covance, antibodies 199 and 200), and the antibodies were affinity-purified on CNBr activated Sepharose 4B columns coupled to the His-tagged fusion proteins used to generate them. Although immunoblots indicate that the Myo5b antibody reacts well with mouse Myo5b, it does not react well with human Myo5b. The rabbit anti-Myo5a antibody used in this study (32a) was generated against the tail and has been previously characterized (Espreafico et al., 1992).

Construction and expression of the GFP-Myo5c tail

The Myo5c tail construct was amplified from a human bronchial epithelial cell cDNA library (forward primer: 5′ GAACAAAGA-AAACCATGGGCTGGT-3′; reverse primer: 5′-CTGCTATAACCT-ATTCAGAAAGCCTA-3′) and was directionally cloned into pEGFP-C2 (Clontech) at the *Xho*I restriction site (5′ end) and the *Bam*HI restriction site (3′ end). The resulting fusion protein consists of GFP at the N-terminus followed by the entire tail of Myo5c (aa 902-1742). Cultured cells were transfected using Polyfect (Qiagen) with a DNA concentration of 0.625 μg/ml and fixed for immunofluorescence 12-24 hours later.

Immunoblots

Cultured cells (isolated by scraping) or mouse tissues were homogenized in 10 volumes of ice-cold TBS containing 2 mM EDTA, 2 mM DTT and 1 mM Pefabloc-SC. Homogenates were then immediately mixed with SDS sample buffer and boiled. Between ~20 and 50 μg protein was loaded and samples were separated on 4-20%

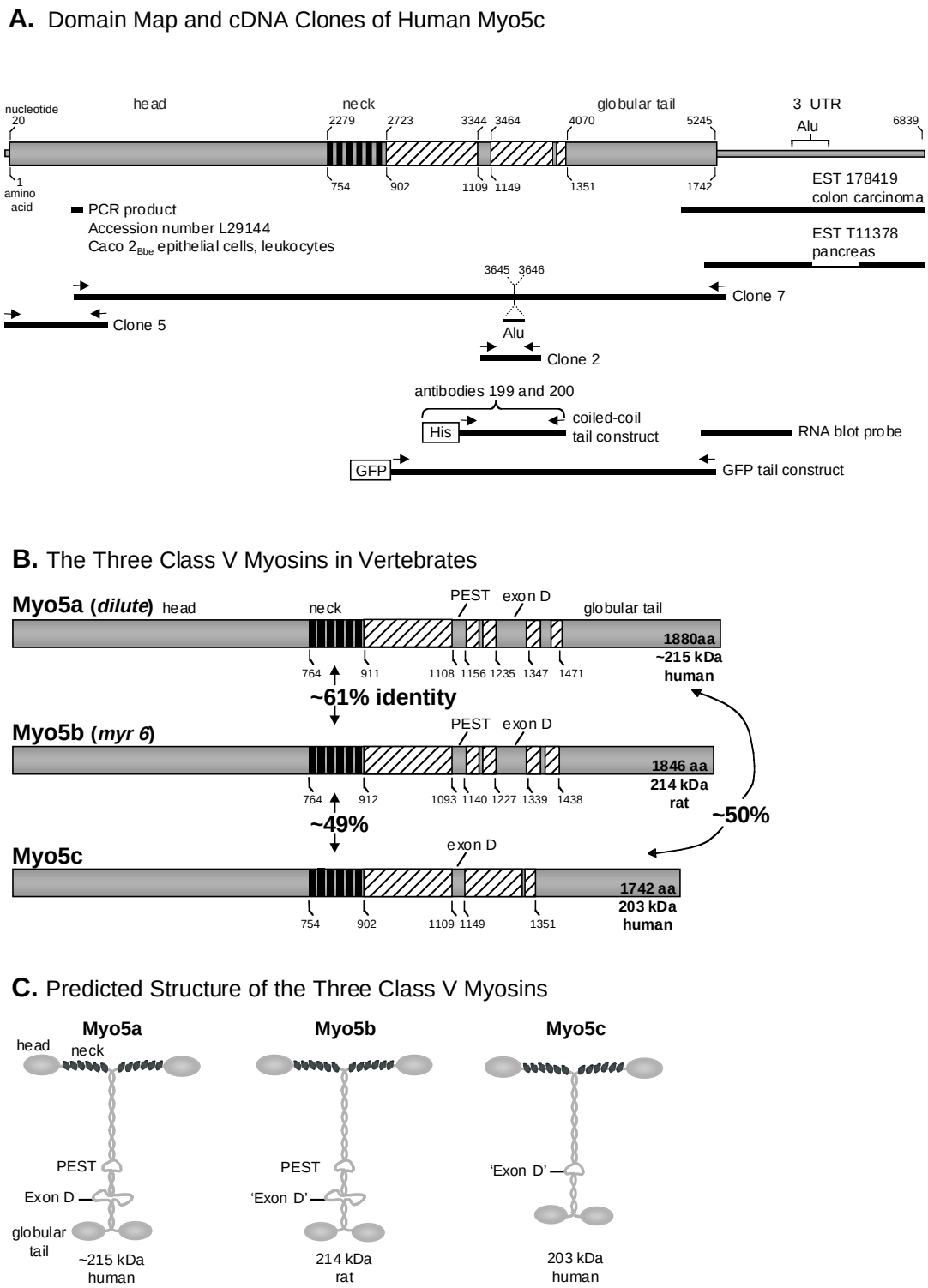
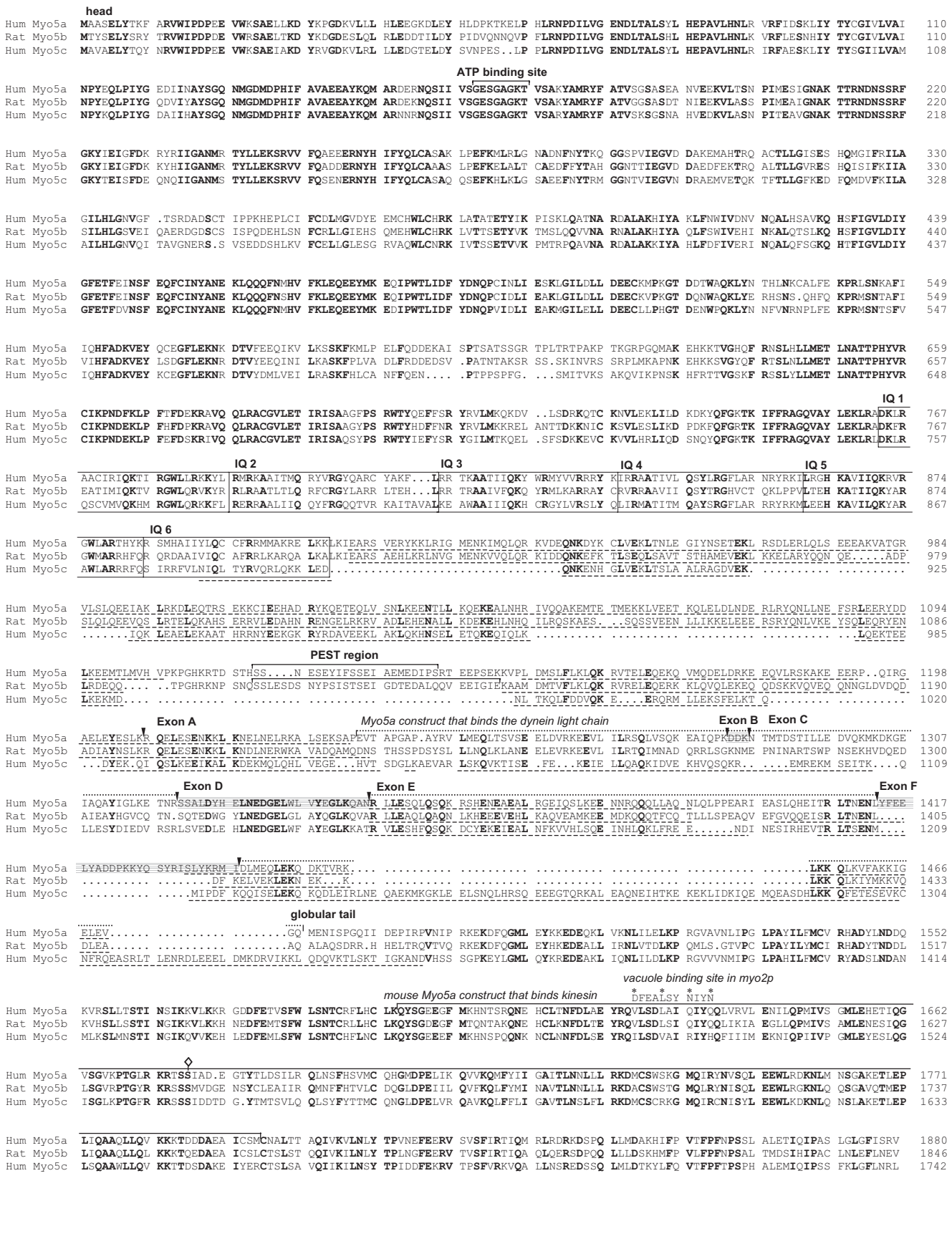


Fig. 1. Discovery and cloning of a novel class V myosin. (A) cDNA clones comprising the full coding sequence of human *MYO5C*. Combining the sequence of clones 2, 5 and 7 and EST 178419 results in 6839 bp of cDNA including 19 bp of 5′UTR, 5229 bp of coding sequence and 1591 bp of 3′UTR containing an Alu repeat and ending in a poly-A tail. Black shading indicates the sequenced portion of the clones. The locations of the mRNA probe, the His-tagged tail construct used to generate antibodies 199 and 200, and the GFP-tail construct are also depicted. Arrows indicate PCR primers used for amplification. (B) The three class V myosins in vertebrates. Note that the Myo5c tail lacks a PEST region but contains a small region similar to the alternatively spliced exon D mapped in Myo5a (Seperack et al., 1995). The human Myo5a sequence used here consists of a GenBank sequence (Y07759) into which we have inserted the 25 aa sequence of exon F. This Myo5a sequence thus includes all three alternatively spliced exons and will differ somewhat from actual splice forms in size and numbering. The diagrams of Myo5b and Myo5c are based on GenBank sequences U60416 and AF272390, respectively. (C) Predicted structure of the three class V myosins. Interestingly, the length of the initial segment of coiled-coil is predicted to be ~30 nm for all three myosins.

Tris-glycine SDS-PAGE gels. Gels were transferred to nitrocellulose at 150 V-hours in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol, 0.02% SDS). Affinity purified antibodies were used at 1-2 μg/ml with 1:100,000 HRP-conjugated donkey anti-rabbit (Jackson Immunoresearch Laboratories, Inc.) or 1:1500 HRP-conjugated donkey anti-chicken (Jackson) secondary antibodies. Immunoblots were developed using SuperSignal West Pico ECL Substrate (Pierce) and films were scanned and imaged using Adobe Photoshop.



Cell culture and immunofluorescence

Human HeLa cells were obtained as a generous gift of Carol Otey (University of North Carolina, Chapel Hill, NC) and maintained in modified Eagle's medium (MEM**)** supplemented with 10% fetal bovine serum and 100 U penicillin-streptomycin. Frozen sections or cultured cells on coverslips were fixed with 3.7% paraformaldehyde in PBS for 20 minutes and permeabilized with 0.5% triton X-100 for 10 minutes. Sections or cells were then blocked in 5% goat serum for ~1 hour. All rabbit primary antibodies were used at 5 μg/ml: nonimmune serum IgG, anti-Myo5a antibody 32a, anti-Myo5c antibodies 199 and 200 and anti-rab11a (Zymed). Mouse monoclonal antibodies were used at the following concentrations: anti-rab8 (BD Transduction Laboratories) at 8 μg/ml; anti-transferrin receptor (Sigma C-2063) at 1:200; anti-LAMP1 (H4A3, Developmental Studies Hybridoma Bank) at 10 μg/ml; anti-golgin97 (Molecular Probes) at 8 μg/ml; anti-mannose-6-phosphate receptor (Affinity Bioreagents, Inc.) at 8 μg/ml. After incubation in primary antibodies for ~1 hour, sections or cells were washed 4× in PBS (5 minutes per wash), incubated with either Alexa 488-conjugated or Alexa 568-conjugated goat anti-rabbit at 1 μg/ml (Molecular Probes) or TRITC-conjugated goat anti-mouse at 1:200 (Jackson) secondary antibodies for ~1 hour, and washed 4× in PBS. Samples to be stained for F-actin or nuclei were then incubated with rhodamine-phalloidin at 13.2 nM or 4′,6-diamidine-2′-phenylindole, dihydrochloride (DAPI) at 200 nM for 15 minutes and washed 3× in PBS. Samples were mounted in Prolong Antifade media (Molecular Probes) and examined using a Zeiss fluorescence microscope with a 100× 1.4 NA lens, an Orca-II cooled CCD camera and Metamorph software. Control images were obtained and processed under identical conditions.

Transferrin internalization assay

Twenty-four hours after transfection with the GFP-Myo5c tail, HeLa cells were pre-incubated in serum-free MEM with 0.2% BSA ('uptake medium') for 30 minutes at 37°C, then incubated in uptake medium containing 25 μg/ml TRITC-transferrin (Molecular Probes) for 10 minutes at 37°C ('pulse'). After the pulse, cells were fixed immediately or chased by incubating in unlabeled transferrin for 5, 20, 60 or 120 minutes. To chase, cells were first rinsed three times in warm uptake medium, then incubated in uptake medium containing 25 μg/ml unlabeled holo-transferrin (Sigma) for the indicated time. Cells were then rinsed once in cold uptake medium, three times in cold PBS containing Ca^{2+} and Mg^{2+} and then fixed and immunostained as above.

Fig. 2. Alignment of the three class V myosins in vertebrates. Bolded letters denote identical residues in all three sequences. The head, ATP binding site and globular tail are marked. Solid boxes mark each of six IQ motifs in the neck domain. Regions predicted (Lupas et al., 1991) to form a coiled-coil with greater than 90% probability are denoted by dashed underlines. Carets indicate the boundaries of Myo5a exons A through F that have been mapped by PCR (Lambert et al., 1998; Seperack et al., 1995), and shading indicates the three exons known to undergo tissue-specific alternative splicing. Note that the human Myo5a sequence reported to bind the dynein light chain (dotted bracket) does not contain exons D and F (Naisbitt et al., 2000). The sequence corresponding to the mouse Myo5a construct reported to bind kinesin (Huang et al., 1999) is also indicated (solid bracket). The position of a conserved serine in the globular tail ('serine 1650' for mouse Myo5a), whose phosphorylation regulates binding of Myo5a to melanosomes, is marked by a diamond (Karcher et al., 2001). Rab11a-binding by rabbit Myo5b is reported to require the sequence corresponding to aa 1397-1418 and 1797-1811 of rat Myo5b (Lapierre et al., 2001). The sequence and location of a putative vacuole-binding site in the globular tail of Myo2p is also shown; asterisks denote functionally crucial residues (Catlett et al., 2000). The GenBank accession number for Myo5c is AF272390.

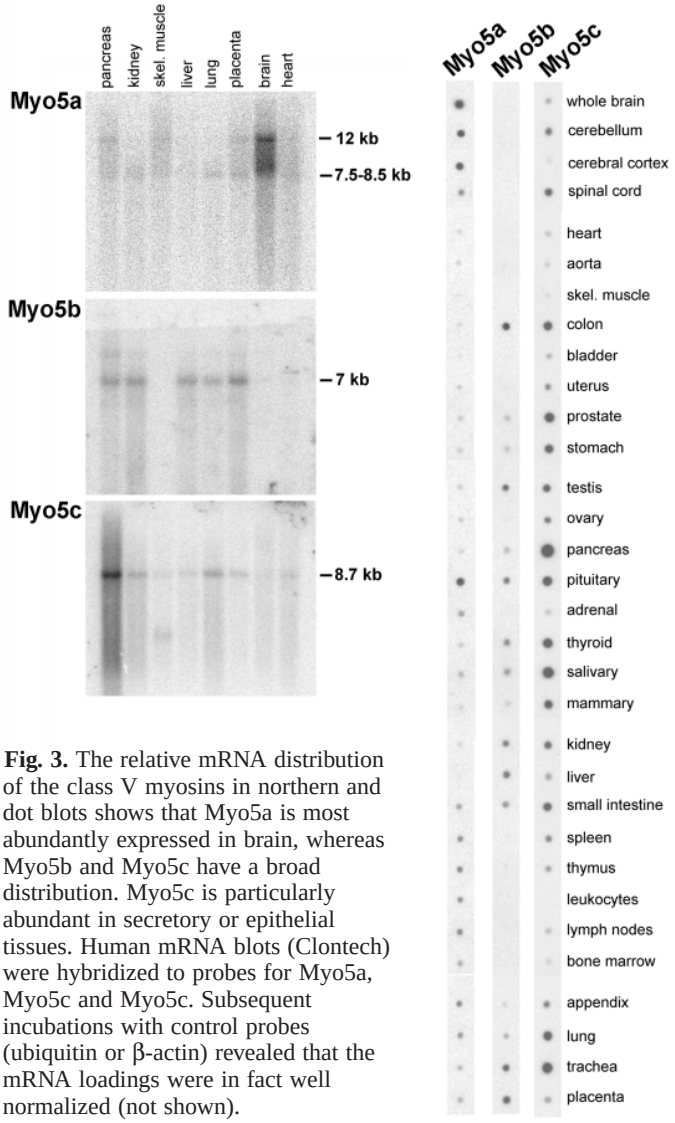


Fig. 3. The relative mRNA distribution of the class V myosins in northern and dot blots shows that Myo5a is most abundantly expressed in brain, whereas Myo5b and Myo5c have a broad distribution. Myo5c is particularly abundant in secretory or epithelial tissues. Human mRNA blots (Clontech) were hybridized to probes for Myo5a, Myo5c and Myo5c. Subsequent incubations with control probes (ubiquitin or β-actin) revealed that the mRNA loadings were in fact well normalized (not shown).

Online supplemental material

The nucleotide sequence and deduced amino acid translation for human MYO5C are provided as online supplemental material (http://jcs.biologists.org/supplemental/).

Results

Discovery of a third member of the myosin-V family in vertebrates, human Myo5c

Given that class V myosins appear to play crucial roles in cell function and that Myo5a is abundantly expressed chiefly in brain and melanocytes, we suspected that non-neuronal cells might express a novel class V myosin. When the sequence of rat Myo5b (*myr6*) was published (Zhao et al., 1996), it became clear that a small fragment of myosin head domain sequence (L29144) obtained in our initial PCR screen for unconventional myosins (Bement et al., 1994) probably represented a third member of the myosin-V family in human. We thus performed an extensive analysis of the human EST database and identified two ESTs that were similar but not identical to the tail domains of both Myo5a and Myo5b (Fig. 1). Perfect match PCR primers

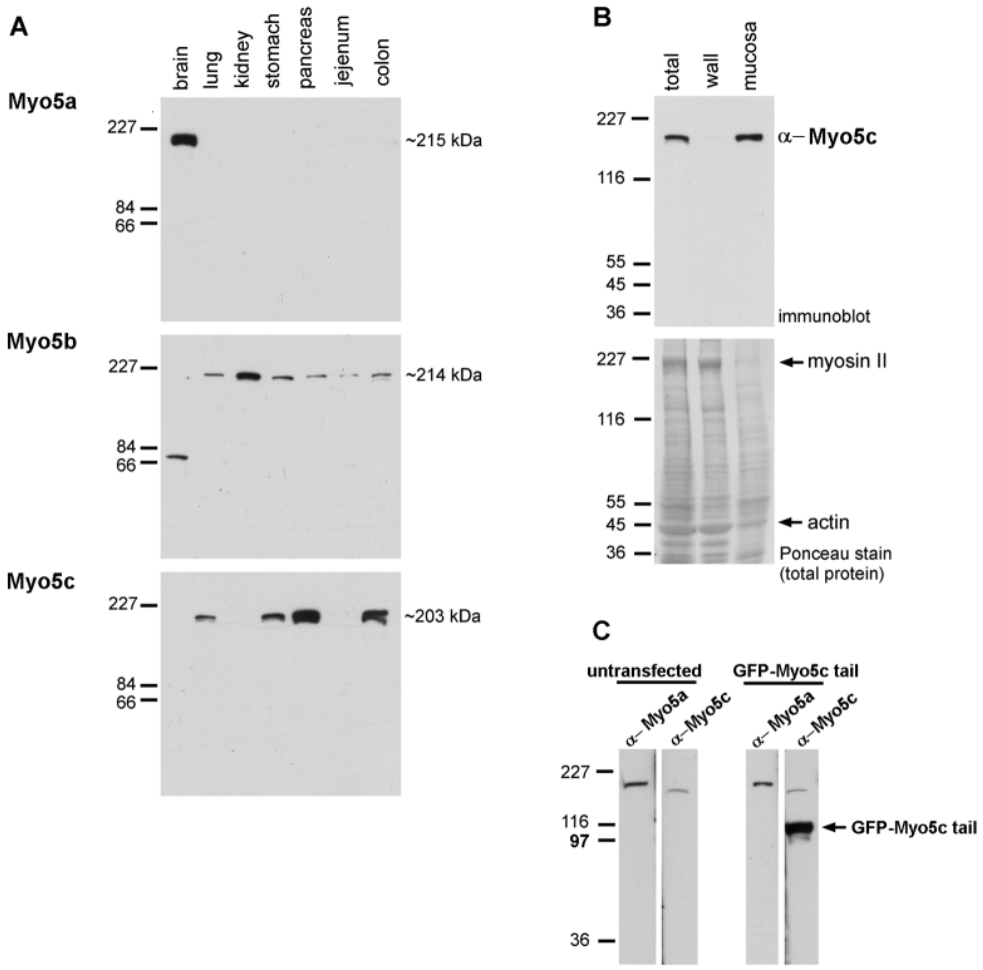


Fig. 4. Myo5c protein is abundant in epithelial and secretory tissue. (A) Immunoblot showing the protein distribution of class V myosins in mouse tissues. Myo5a is abundant in brain, whereas Myo5b and Myo5c are more widely distributed in non-neuronal tissues. Gels were run in triplicate, samples were loaded equally by wet tissue weight and blots were probed with antibodies to Myo5a, Myo5b or Myo5c. It is unclear if the lower band detected by the Myo5b antibodies in brain represents a breakdown product. (B) Immunoblot showing that Myo5c is enriched in the mucosa of mouse colon, but not the underlying muscle wall. The Ponceau stain of this blot shows that myosin-II and actin are found primarily with the muscle wall. The mucosa was dissected from 0.4 g of colon by scraping. The mucosal layer and the muscle wall were then separately homogenized in 4 ml of buffer as for the other mouse tissue homogenates, and equal volumes were loaded. (C) Immunblots of untransfected and transfected Hela cells. Blotting of HeLa cell lysates reveals that these cells endogenously express both Myo5a and Myo5c (left panels, 50 μg load). HeLa cells transfected with the GFP-Myo5c tail express a protein of the expected size, ~120 kDa (right panels, 20 μg load).

to these head and tail fragments were then used to clone a ~4.8 kb PCR product (Clone 7) from pancreas cDNA; this clone, together with a PCR clone containing 5′ sequence (Clone 5), includes the full-length coding sequence of a novel member of the myosin-V family we have named myosin-Vc (Figs 1, 2) (see also http://jcs.biologists.org/supplemental/).

Analysis of the now completed human genome indicates that Myo5c is the third and final member of its class (Berg et al., 2001). Analysis of genomic sequence also indicates that the human *MYO5C* gene has ~39 exons and extends over ~100 kb. Interestingly, the human *MYO5A* gene is located at 15q21 immediately adjacent (within 18 kb) to the 3′ end of the *MYO5A* gene. The *Myo5a*/*dilute* locus has been well characterized in mouse, and several other lethal mutations are known to lie near this locus (Rinchik et al., 1986). If mouse and human maintain synteny in this area, then the mouse *Myo5c* gene is likely to reside on chromosome 9 in the ~300 kb region between the *dilute* and *short ear* mutations. There are two known lethal mutations in this region and, on the basis of its position, *neonatal-lethal 2* is a good candidate to correspond to mouse *Myo5c*.

Myo5c shares only ~50% identity with Myo5a and Myo5b but has a generally similar structure

The deduced sequence of human MYO5C consists of 1742 amino acids (aa) encoding a 203 kDa heavy chain sharing ~50% overall identity with human or mouse Myo5a and rat Myo5b (Fig. 1B,C). Residues 1-753 encode a class V myosin motor domain sharing 68% identity with those of Myo5a and Myo5b. As expected for a class V myosin, Myo5c contains six IQ motifs and would thus be predicted to have as many as six calmodulin or calmodulin-like light chains (Espreafico et al., 1992). Following the sixth IQ motif are 411 aa predicted to form several segments of coiled-coil (Figs 1, 2). The coiled-coil regions share only 20-30% identity with the corresponding regions of Myo5a and Myo5b; in this same region, Myo5a and Myo5b share 42% identity. Myo5c entirely lacks the PEST region that interrupts the coiled-coil in Myo5a and Myo5b; in Myo5a this is a site of in vitro cleavage by the calcium-dependent protease calpain (Nascimento et al., 1996). Due in part to its lack of a PEST site, Myo5c is ~12 kDa smaller than Myo5a or Myo5b. The coiled-coil in Myo5c is interrupted by a ~40 aa region that shares similarity with portions of the ~112 aa non-coiled-coil region found in Myo5a and Myo5b. This similarity extends to exon D of Myo5a, which is a 26 aa exon expressed in melanocyte but not brain Myo5a (Lambert et al., 1998; Seperack et al., 1995). The C-terminal 392 aa of Myo5c share ~60% identity with the globular tails of Myo5a and Myo5b. The conserved globular tail of the class V myosins has been implicated in binding to cargos such as melanosomes (Wu et al., 1998), secretory vesicles (Schott et al., 1999) and

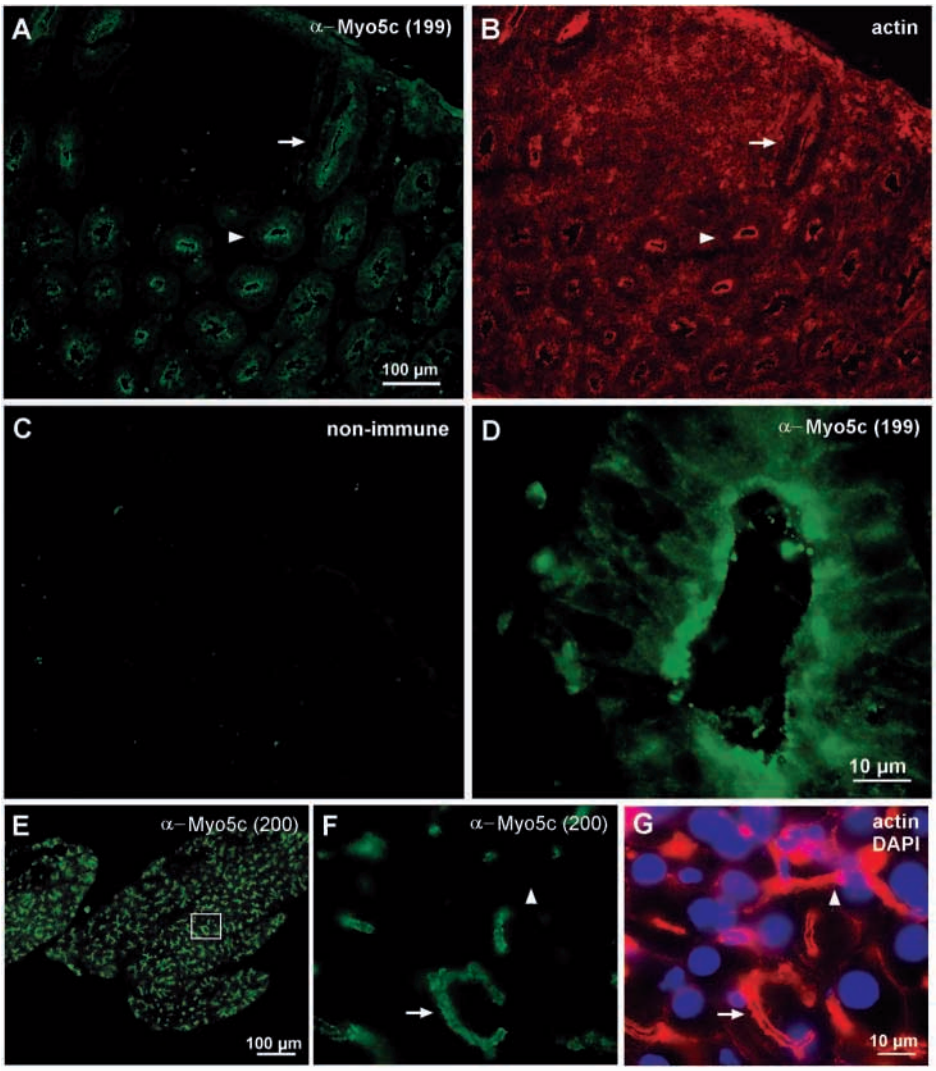


Fig. 5. Myo5c is expressed in epithelial cells of the colon and pancreas. In human colon sections double-labeled for Myo5c (A) and F-actin (B), Myo5c localizes to the single layer of epithelial cells constituting the intestinal crypts, as seen in longitudinal section (arrow) and cross section (arrowhead). Myo5c is particularly enriched in the apical portion of the epithelial cells, where it colocalizes with F-actin. The basolateral portions of these cells, which extend ~30 μm, exhibit some Myo5c staining but relatively little F-actin staining. The surrounding interstitial cells are faintly stained by F-actin but exhibit little or no Myo5c staining and appear black. (C) Non-immune serum IgG exhibits little or no staining of the adjacent section. (D) Higher magnification view of a single crypt showing Myo5c staining of individual intestinal epithelial cells. These cells, which surround the intestinal lumen, exhibit bright apical staining and fainter staining of cytoplasm and lateral cell margins. (E) In mouse pancreas sections, Myo5c localizes to the exocrine pancreas. (F,G) Higher magnification of the same section, triple-labeled for Myo5c (green), F-actin (red) and DAPI to label nuclei (blue), shows that Myo5c colocalizes with F-actin in the apical portions (arrows, F,G) of epithelial cells. Note that Myo5c does not colocalize with all F-actin-containing structures (arrowheads, F,G).

vacuoles (Catlett et al., 2000), and to proteins such as Smy1p (Lillie and Brown, 1998), kinesin (Huang et al., 1999) and rab11a (Lapierre et al., 2001). Interestingly, all three of the class V myosins in vertebrates contain a conserved serine whose phosphorylation in Myo5a regulates its binding to melanosomes (‘serine 1650’ of mouse Myo5a) (Karcher et al., 2001).

Myo5c is widely distributed in secretory and epithelial tissue

To systematically determine the expression patterns of the three class V myosins in vertebrate tissues, we first analyzed the distributions of their mRNAs. A northern blot and a dot blot (Fig. 3) containing mRNA samples from many human tissues were hybridized under high stringency conditions with probes for human Myo5a, Myo5b and Myo5c. As expected from previous analyses (Espreafico et al., 1992; Mercer et al., 1991), Myo5a mRNA (~8 and 12 kb) is most abundant in brain and is expressed at lower levels in other tissues. The Myo5b mRNA (7 kb) has a broader distribution and is relatively abundant in kidney, liver and placenta, with little or no signal detected in brain. Most importantly, we found that the Myo5c mRNA (8.7 kb) has a broad distribution and is expressed in epithelial and glandular tissues such as salivary, stomach, colon, pancreas, lung, thyroid, prostate and mammary.

To determine the protein distribution of class V myosins, we first generated specific antibodies to Myo5b and Myo5c. We then probed immunoblots of mouse tissues and a human cell line with these antibodies and our existing Myo5a antibodies (Fig. 4A-C). As expected, Myo5a protein is most abundant in brain, with little or no protein detected in the other tissues tested (Fig. 4A). By contrast, Myo5b and Myo5c are easily detected in many non-neuronal tissues. Myo5b is most abundant in kidney, whereas Myo5c is especially abundant in pancreas, colon and stomach (Fig. 4A). These different patterns of distribution also provide strong evidence that our antibodies are specific. The slightly smaller size of the band recognized by the Myo5c antibodies (most obvious in samples blotted side by side, as in Fig. 4C) also indicates that the Myo5c antibodies do not react with Myo5a or Myo5b. On the basis of the use of immunoprecipitated Myo5c as a standard in western blots, we estimate that Myo5c constitutes ~0.02% of the total protein in mouse stomach. To determine whether Myo5c associates with the mucosa or the underlying muscle wall in tissues such as mouse colon, we isolated the mucosa from the wall by scraping

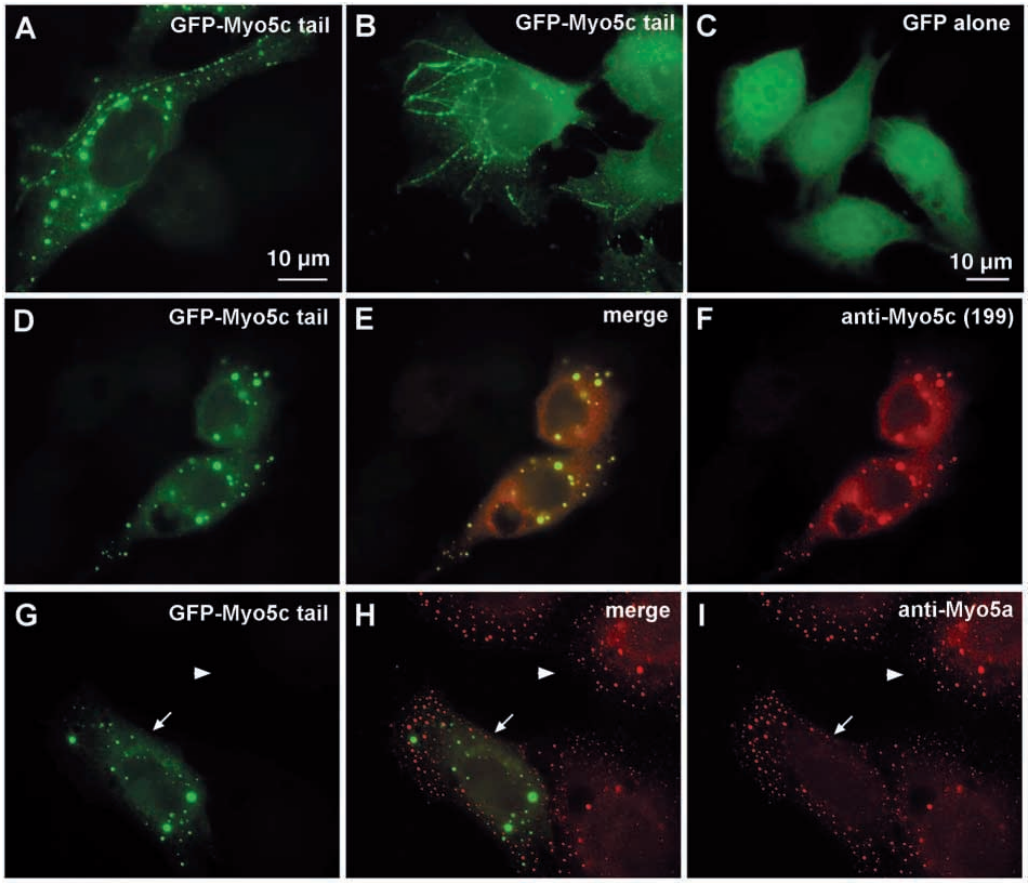


Fig. 6. Overexpressing the GFP-Myo5c tail leads to the formation of puncta and tubulovesicular structures. In transfected cells, the GFP-Myo5c tail forms puncta and tubules (A,B,D,G), suggestive of a membranous compartment, whereas GFP alone has a diffuse distribution (C). (D-F) Antibodies to Myo5c stain the puncta. (G-I) The puncta formed by overexpression of the GFP-Myo5c tail do not colocalize with endogenous Myo5a, which shows a striking localization to small puncta in the periphery of the cell.

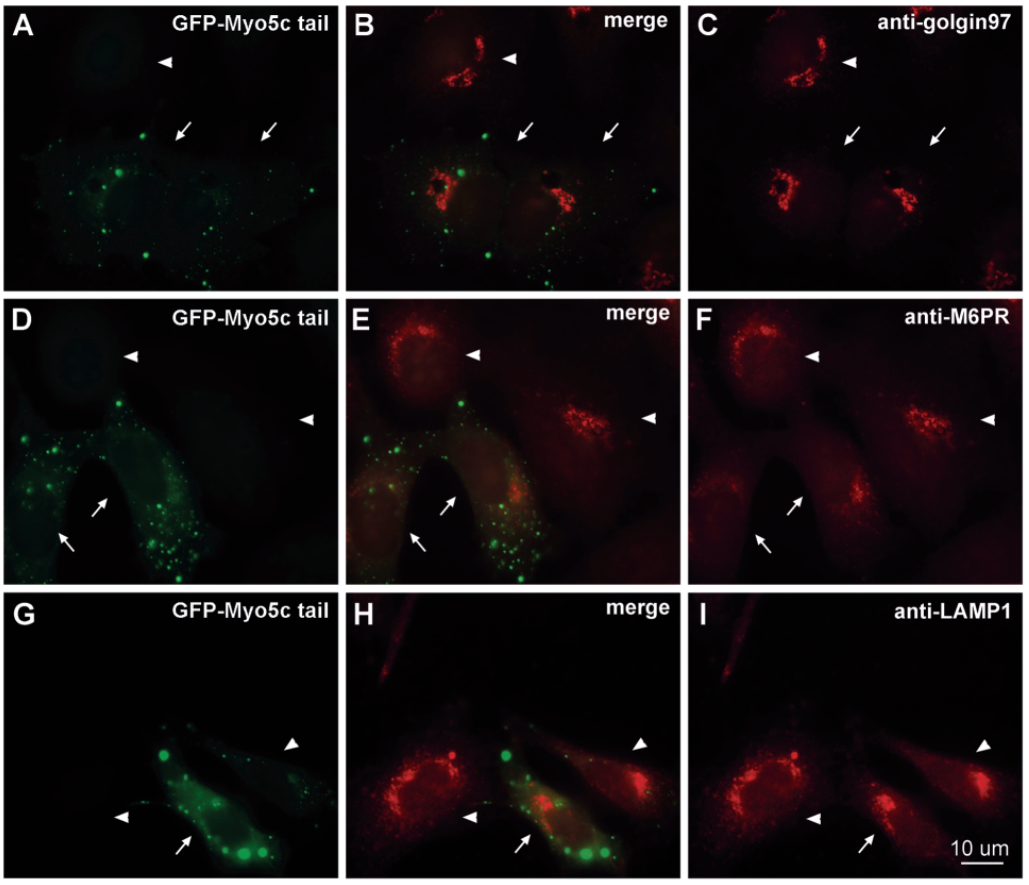


Fig. 7. The GFP-Myo5c tail does not colocalize with or perturb the Golgi compartment, late endosomes or lysosomes. Arrows denote cells expressing the GFP-Myo5c tail, whereas arrowheads mark untransfected cells. (A-C) The GFP-Myo5c tail does not colocalize with golgin97, a membrane marker for the Golgi compartment. (D-F) Although the mannose-6-phosphate receptor cycles between the TGN, late endosomes, and lysosomes, at steady state the majority of the receptor is found on late endosomes, where it does not colocalize with the GFP-Myo5c tail. (G-I) The GFP-Myo5c tail also fails to colocalize with LAMP1, a membrane marker for lysosomes.

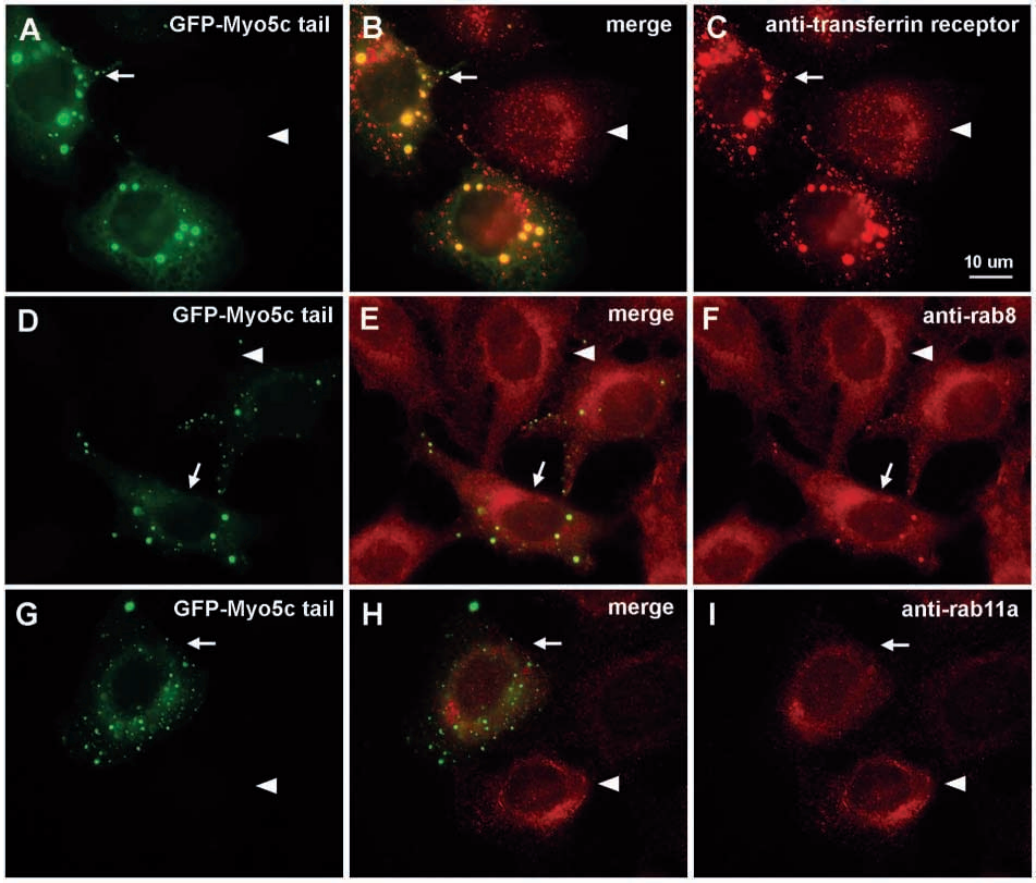


Fig. 8. The overexpressed GFP-Myo5c tail colocalizes with and perturbs a compartment containing the transferrin receptor and rab8, but not rab11a. In all panels, arrows denote transfected cells and arrowheads denote untransfected cells. (A-C) The overexpressed GFP-Myo5c tail colocalizes with the transferrin receptor, a marker for the endosomal pathway. Furthermore, the distribution of the transferrin receptor is perturbed in cells expressing the GFP-Myo5c tail (arrows) as compared to untransfected cells (arrowheads). (D-I) Transfected cells were also stained for rab proteins to define specific membrane compartments. The GFP-Myo5c tail puncta colocalize with rab8 (D-F), but not rab11a (G-I). Similar to the results with the transferrin receptor, at least some of the rab8 distribution appears perturbed in transfected cells (arrow, F), whereas the distribution of rab11a does not appear to be significantly altered in transfected cells (arrows, I) versus untransfected cells (arrowhead, I).

and blotted the resulting fractions with Myo5c antibodies (Fig. 4B). Although most of the actin and conventional myosin remained with the muscle wall, virtually all of the Myo5c was found in the mucosal fraction containing the intestinal epithelial cells. Immunoblotting also indicates that endogenous Myo5a and Myo5c are present in HeLa cells, a cell line derived from a human cervical carcinoma (Fig. 4C, left panels).

Myo5c localizes to epithelial cells of the colon and pancreas

To determine which types of cells express Myo5c, we stained human colon sections for Myo5c and found that it localizes to the single layer of epithelial cells lining the colonic crypts (Fig. 5A). Double-labeling with F-actin revealed that Myo5c is particularly prominent in the F-actin-rich apical regions of the intestinal epithelial cells (Fig. 5B). Non-immune antibodies showed little or no staining (Fig. 5C). Higher magnification showing individual epithelial cells reveals that Myo5c is present in both the apical and basolateral regions, but is not detected in the surrounding interstitial cells.

In mouse pancreas, Myo5c localizes to the exocrine pancreas rather than the islets of the endocrine pancreas (Fig. 5E). Higher magnification of triple-labeled sections shows that Myo5c (Fig. 5F, green) largely overlaps with apical F-actin (Fig. 5G, red) in epithelial cells of the exocrine pancreas. It is not yet clear whether Myo5c localizes to the acinar cells, centroacinar cells, or both. Taken together, the evidence from northern blotting, immunoblotting and immunolocalization clearly indicates that Myo5c is a broadly distributed class V myosin that is especially abundant in epithelial cells.

Localization and dominant negative effects of the GFP-Myo5c tail

If Myo5c functions in organelle transport, overexpressing its tail domain should saturate the binding sites for the endogenous motor and thus inhibit its function. This dominant negative strategy has been very successful with other class V myosins, including Myo2p (Catlett et al., 2000; Reck-Peterson et al., 1999; Schott et al., 1999), Myo5a (Rogers et al., 1999; Wu et al., 1998) and Myo5b (Lapierre et al., 2001), as well as for class I myosins (Durrbach et al., 2000; Raposo et al., 1999). In these studies, overexpression of a tail construct led to an accumulation or altered distribution of a specific set of organelles, presumably due to failure of the organelle to be transported or anchored to its normal location. We thus generated a dominant negative construct consisting of GFP fused to the entire tail of Myo5c (Fig. 1A). We used HeLa cells for our dominant negative studies because they are easily transfected and their membrane trafficking pathways are relatively well characterized. Immunoblots show that HeLa cells express low levels of endogenous Myo5a and Myo5c and that cells transfected with the GFP-Myo5c tail express a protein of the expected size (Fig. 4C). On the basis of a transfection efficiency of ~20-50%, we estimate that the GFP-Myo5c tail is overexpressed on the order of 100-fold relative to endogenous Myo5c.

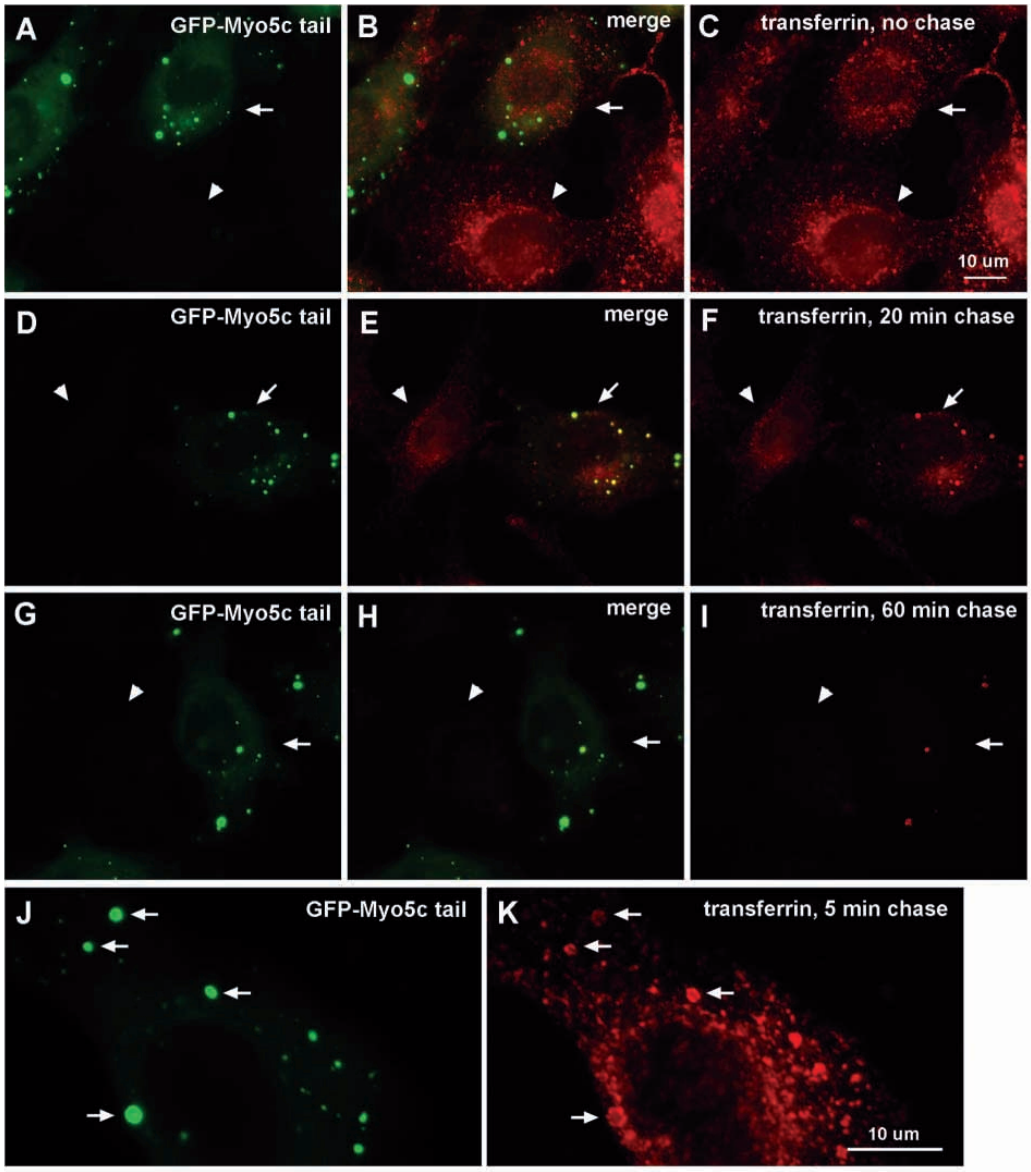


Fig. 9. In cells expressing the GFP-Myo5c tail, transferrin is taken up normally and colocalizes with the Myo5c tail after a short delay, but transferrin trafficking is inhibited. Cells were incubated in TRITC-transferrin for 10 minutes, followed by incubation in unlabeled transferrin for various 'chase' times of 0, 5, 20 or 60 minutes. (A-C) Cells expressing the GFP-Myo5c tail (arrows) appear to internalize transferrin similar to untransfected cells (arrowhead). After 10 minutes of internalization, transferrin does not significantly colocalize with the GFP-Myo5c tail puncta, but by 20-60 minutes (D-I), transferrin shows obvious colocalization with the GFP-Myo5c tail. Furthermore, transferrin remains in cells expressing the GFP-Myo5c tail (arrows) for much longer than in untransfected cells (arrowheads), indicating that the GFP-Myo5c tail impedes transferrin trafficking. (J-K) Close examination of cells after 5 minutes of chase, when transferrin first begins to colocalize with the GFP-Myo5c tail, reveals that transferrin appears to associate with the margins of the GFP-Myo5c tail puncta (arrows).

Overexpressing the GFP-Myo5c tail in HeLa cells leads to the formation of numerous bright 'puncta' distributed throughout the cell and less frequently to tubulovesicular structures (Fig. 6A,B,D,G). Transfection with GFP alone yields only diffuse cytoplasmic staining (Fig. 6C). The localization of the GFP-Myo5c tail to puncta and structures with a tubulovesicular appearance suggests that it is associated with a membranous compartment. Although our current Myo5c antibodies appear unsuitable for immunofluorescent localization of the low levels of endogenous Myo5c present in HeLa cells, these antibodies do stain the puncta constituted by the GFP-Myo5c tail (Fig. 6D-F). Non-immune antibodies do not detect the puncta (not shown). Interestingly, staining with our Myo5a antibodies reveals that the endogenous HeLa cell Myo5a exhibits a striking localization to peripheral puncta (Fig. 6G-I). It is important to note that the GFP-Myo5c tail does not colocalize with this endogenous Myo5a compartment and does not perturb its distribution. It is also important to note that the formation of the GFP-Myo5c puncta is not exclusive to HeLa cells; we observe similar puncta when we express the GFP-Myo5c tail in HEK 293 cells, 3T3 cell or polarized LLC-PK_1 cells (not shown).

The GFP-Myo5c tail colocalizes with and perturbs the distribution of a compartment containing the transferrin receptor

To identify the compartment associated with the GFP-Myo5c tail, we transiently transfected cells and then used immunofluorescence to determine if the GFP-Myo5c tail colocalizes with markers for well-characterized membrane compartments. The GFP-Myo5c tail does not colocalize with markers for the Golgi compartment (golgin97, Fig. 7A-C), late endosomes (mannose-6-phosphate receptor, Fig. 7D-F) or lysosomes (LAMP1, Fig. 7G-I). In addition, the GFP-Myo5c tail does not noticeably perturb the organization or distribution of these compartments. The GFP-Myo5c tail puncta do not appear to be aggresomes (Kopito, 2000) as they fail to

colocalize with aggresome markers such as vimentin baskets or hsc70 (not shown).

The GFP-Myo5c tail does exhibit striking colocalization with the transferrin receptor, an integral membrane protein frequently used as a marker for the endosomal pathway (Fig. 8A-C). Furthermore, the distribution of the transferrin receptor is dramatically perturbed in cells expressing the GFP-Myo5c tail. Although virtually all of the GFP-Myo5c tail puncta colocalize with the transferrin receptor, note that some of the transferrin receptor-containing membranes do not colocalize with the GFP-Myo5c tail and do not appear to be obviously perturbed by it. The GFP-Myo5c tail thus specifically colocalizes with a subset of membranous organelles that contain the transferrin receptor.

The GFP-Myo5c tail colocalizes with and perturbs the distribution of Rab8 but not Rab11a

Because the class V myosin Myo2p and the rab protein Sec4p exhibit genetic interactions and are essential for polarized secretion in yeast (Finger and Novick, 2000), we asked if the GFP-Myo5c tail colocalizes with rab8, one of the vertebrate rab proteins most similar to Sec4p (Chavrier et al., 1990). Rab8 is also a ubiquitously expressed rab originally implicated in regulating transport of basolaterally targeted proteins from the TGN to the plasma membrane (Huber et al., 1993a; Huber et al., 1993b). As shown in Fig. 8D-F, some rab8 clearly colocalizes with and is perturbed by the GFP-Myo5c tail. Given that a GFP-Myo5b tail construct has recently been shown to colocalize with and perturb a recycling compartment containing the transferrin receptor and rab11a in HeLa cells (Lapierre et al., 2001), we also asked if the GFP-Myo5c tail colocalizes with rab11a. As shown in Fig. 8G-I, the GFP-Myo5c tail does not colocalize with or perturb rab11a. This indicates that the compartment associated with the GFP-Myo5c tail and rab8 is distinct from the rab11a compartment associated with the GFP-Myo5b tail. The GFP-Myo5c tail also failed to colocalize with rab4, although it should be noted that both of the rab4 antibodies that we used yielded only faint staining (not shown). In summary, the GFP-Myo5c tail selectively perturbs a compartment containing transferrin receptors and rab8, but not rab11a.

Overexpressing the GFP-Myo5c tail perturbs transferrin trafficking

To investigate whether the Myo5c tail perturbs a secretory pathway (as might be expected from its association with rab8) or an endosomal pathway (as might be expected from its association with transferrin receptor), we next performed pulse-chase experiments with TRITC-labeled transferrin. In this experiment, HeLa cells were incubated (pulsed) with TRITC-labeled transferrin for 10 minutes and then chased through the endocytic and recycling pathways by incubating in unlabeled transferrin for 0, 5, 20, 60 or 120 minutes. In cells that were pulsed and fixed without chasing, TRITC-transferrin was internalized normally in both transfected and untransfected cells (Fig. 9A-C). Thus, expression of GFP-Myo5c tail does not grossly impair endocytosis of transferrin. At this early time point, when transferrin is known to be passing through the early endosomal pathway (Yamashiro et al., 1984), there is no colocalization with the GFP-Myo5c tail.

When the cells are chased with unlabeled transferrin for 5 minutes, TRITC-transferrin begins to colocalize with the GFP-Myo5c tail puncta. After 20 minutes of chase, much of the TRITC-transferrin obviously colocalizes with the GFP-Myo5c tail puncta (arrow, Fig. 9D-F). In cells expressing the GFP-Myo5c tail, some TRITC-transferrin remains colocalized with the GFP-Myo5c tail puncta at 60 (Fig. 9G-I) and 120 minutes (not shown), long after the TRITC-transferrin in untransfected cells has recycled back to the plasma membrane. Thus, overexpressing the GFP-Myo5c tail either blocks or greatly delays the recycling of transferrin back to the plasma membrane. These experiments reveal that the GFP-Myo5c tail labels a compartment accessible to transferrin, and that overexpressing the GFP-Myo5c tail perturbs the trafficking of at least some transferrin.

Interestingly, the internalized TRITC-transferrin initially appears to associate with the edges of the GFP-Myo5c tail puncta (Fig. 9J,K), as if newly arrived vesicles are adding to an existing clump of vesicles. To summarize, cells overexpressing the GFP-Myo5c tail appear to endocytose transferrin similarly to untransfected cells. After a short delay, transferrin exhibits clear colocalization with the GFP-Myo5c tail in a compartment that appears to be downstream of the early endosome. Lastly, the GFP-Myo5c tail appears to inhibit the recycling of at least some transferrin to the surface.

Discussion

We report here the discovery of a new member of the myosin-V family, human Myo5c. Our analysis reveals that Myo5c is a broadly expressed class V myosin that is especially abundant in tissues with prominent secretory or epithelial functions such as exocrine pancreas, prostate and mammary gland. Immunofluorescence localization shows that Myo5c is expressed at the highest levels in epithelial cells. A dominant negative strategy using overexpression of the GFP-Myo5c tail in HeLa cells indicates that it selectively associates with and perturbs a specific membrane compartment that contains transferrin receptor and is accessible to transferrin. This compartment colocalizes with rab8 but not rab11a, and thus appears to be distinct from the recycling compartment recently associated with Myo5b and rab11a (Lapierre et al., 2001).

What are the relative roles of the three class V myosins in vertebrates?

The presence of three class V myosins in vertebrates raises several obvious questions. Does a given cell express only one class V myosin or all three? Do class V myosins generally function in the actin-rich cortex to move organelles towards the plasma membrane? Are these myosins found on the same organelle or do they each associate with a specific organelle or set of organelles? Given that Myo5a is expressed at high levels chiefly in brain and melanocytes, do Myo5b and Myo5c underlie actin-based organelle transport in most other cells and tissues? Although fully answering these and related questions will require much additional research, the systematic analysis of the class V myosins presented here allows us to tentatively answer some of these questions. We find that HeLa cells

express low levels of both Myo5a and Myo5c and the work of Lapierre et al. (Lapierre et al., 2001) suggests that HeLa cells also express Myo5b. In preliminary immunoblotting experiments we find that Myo5a and Myo5c are both detected in several cell lines including HEK 293, Hs766T, LLC-PK$_1$, Caco-2 and MDCK cells. This shows that several cell types express multiple class V myosins. Although Myo5a is most abundant in neurons, neurosecretory cells and melanocytes, work from our lab and many others indicates that low levels of Myo5a are detected in most cell types. Given the relatively broad tissue distribution that we observe for Myo5b and Myo5c, it is likely that many cells express multiple class V myosins, with the relative levels of expression varying greatly among different cell types.

Although we do not yet know the precise numbers of molecules of Myo5a, Myo5b and Myo5c expressed in various kinds of cells, we do know that Myo5a levels, like those of kinesin, correspond to ~0.2% of total protein in brain (Cheney et al., 1993). We find that Myo5c is expressed in many tissues and is most abundantly expressed in epithelial cells. Given that Myo5c constitutes ~0.02% of total protein in stomach (of which epithelial cells constitute only a fraction) and that tissues such as pancreas and prostate express several-fold higher levels of Myo5c, it is likely that the abundance of Myo5c in some epithelial cells approaches that of Myo5a in brain. Myo5c is thus a major class V myosin of at least some epithelial cells and would be expected to play an important role in the physiological functions of these cells. Current work is thus directed at determining whether the *Myo5c* gene is required for survival, as might be suggested by the location of the *neonatal lethal-2* deletion in mouse.

Although there is still a great deal of uncertainty about the precise functions of the class V myosin and the identity of their cargoes, Myo5a is clearly associated with the trafficking of melansomes in melanocytes (Rogers et al., 1999; Wu et al., 1998) and the trafficking of an inositol triphosphate receptor-containing compartment in neurons (Takagishi et al., 1996). Myo5b has recently been implicated in the function of a rab11a-associated recycling endosome in HeLa cells and in a rab11a-associated recycling compartment required for transcytosis in polarized MDCK cells (Lapierre et al., 2001). As discussed below, our data implicate Myo5c in the function of a transferrin-accessible compartment in HeLa cells that appears to be distinct from the Myo5b/rab11a recycling compartment as well as the endogenous Myo5a compartment. Taken together, these data strongly suggest that each of the class V myosins is associated with a specific set of membrane trafficking events. It will thus be important to determine whether the variation in the expression levels of the class V myosins observed between cells such as Myo5a-rich neurons and Myo5c-rich epithelial cells corresponds with the elaboration of specific membrane trafficking pathways. In this regard it is interesting to note that *Drosophila* (and *Caenorhabditis elegans*) expresses only a single class V myosin that is equally related to each of the three vertebrate class V myosins.

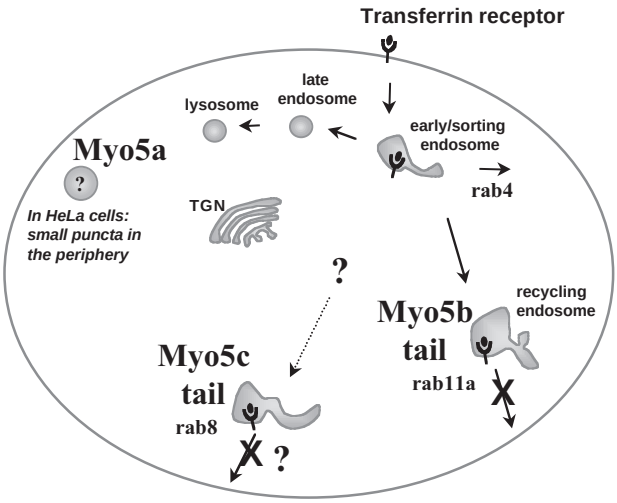


Fig. 10. A working model of class V myosins in membrane trafficking in HeLa cells. Myo5a exhibits a striking localization to small puncta of an unknown identity in the periphery. In HeLa cells Myo5b appears to associate with a major recycling compartment containing rab11a, as a dominant negative construct consisting of the Myo5b tail inhibits transferrin recycling and colocalizes with the transferrin receptor and rab11a (Lapierre et al., 2001). By contrast, the Myo5c tail associates with an apparently distinct transferrin-accessible compartment containing rab8 but not rab11a. Although the precise nature of the pathway associated with Myo5c in HeLa cells is unclear, this pathway is likely to be of particular importance in the epithelial cells where this myosin is most abundantly expressed.

What compartment is the Myo5c tail associated with?

We hypothesize that the GFP-Myo5c tail acts as a dominant negative by displacing endogenous Myo5c from its tail-associated cargos. Because the GFP-Myo5c tail includes the coiled-coil portion of the tail, this construct might also suppress the function of endogenous Myo5c by dimerizing with it, thus forming dimers with only one motor domain. In either case, our dominant negative approach appears to be highly specific as it does not obviously perturb the Golgi compartment, late endosomes, lysosomes, endogenous Myo5a or the rab11a-associated recycling compartment. The dominant negative studies with the GFP-Myo5c tail reported here also provide strong evidence that the Myo5c tail associates with a membranous compartment. This evidence includes: (1) localization to structures with a punctate or tubulovesicular appearance; (2) colocalization with transferrin receptor, an integral membrane protein; (3) colocalization with a specific rab protein; and (4) colocalization with transferrin, a lumenal marker for membranes involved in endosomal/recycling pathways.

Our results showing that the Myo5c tail colocalizes with and perturbs the distribution of the transferrin receptor and rab8 in HeLa cells share some striking similarities to recent work with Myo5b (Lapierre et al., 2001). Lapierre et al. (Lapierre et al., 2001) discovered that overexpression of the GFP-Myo5b tail in HeLa cells results in the elaboration of a compartment containing the transferrin receptor, the Myo5b tail, and rab11a. As a control for specificity, these investigators showed that an analogous GFP-Myo5a tail construct failed to colocalize with the transferrin receptor or alter its distribution. In support of these data showing that the tails of the class V myosins exhibit highly specific targeting, we find that the GFP-Myo5c tail does not colocalize with or perturb the distribution of endogenous

Myo5a. More importantly, given that our overexpression experiments with the GFP-Myo5c tail in HeLa cells show no evidence of colocalization with rab11a, Myo5c appears to be associated with a different membrane compartment than Myo5b. It also appears that there are morphological differences between these two compartments as the puncta formed by the GFP-Myo5b tail are relatively large and centrally located (Lapierre et al., 2001), whereas the puncta we observe with the GFP-Myo5c tail are smaller, more numerous and located throughout the cell.

Our current working model of the roles of the class V myosins in membrane trafficking in HeLa cells is illustrated in Fig. 10. Although Myo5a is expressed at only low levels in HeLa cells, it exhibits an intriguing punctate localization in the periphery of the cell. Although the precise identity of this compartment is unknown, it does not appear to be endosomal as it fails to colocalize with the transferrin receptor (not shown). On the basis of use of a GFP-Myo5b tail construct, Myo5b in HeLa cells is reported to associate with a recycling endosome containing the transferrin receptor and rab11a (Lapierre et al., 2001). Furthermore, overexpressing the Myo5b tail led to an accumulation of transferrin in this compartment and blocked the recycling of transferrin back to the plasma membrane. Our work in HeLa cells indicates that the Myo5c tail associates with a compartment containing the transferrin receptor and rab8. Overexpressing the GFP-Myo5c tail also leads to an accumulation of transferrin and perturbs transferrin trafficking. Because this Myo5c-associated compartment does not contain rab11a, it appears to be distinct from the recycling compartment associated with Myo5b. Although Myo5b appears to play a major role in transferrin recycling in HeLa cells, in polarized MDCK cells it seems to be involved in transcytosis rather than transferrin recycling (Lapierre et al., 2001). We thus suggest that Myo5c functions in a pathway different from that of Myo5b and that this pathway is likely to be of particular importance in the polarized epithelial cells where Myo5c is most abundantly expressed.

The colocalization of the GFP-Myo5c tail with rab8 is intriguing, given that rab8 may be the vertebrate homolog of Sec4p (Chavrier et al., 1990). This raises the exciting possibility that Myo5c and rab8 have a role in vertebrate epithelial cells analogous to the role of Myo2p and Sec4p in polarized secretion in yeast. Some caution is warranted here, however. First, we have only used rab8 as a compartment marker, and have no evidence that it directly interacts with Myo5c. Second, the accumulation of rab8 that we observe (as well as the accumulation of transferrin) could be due to blockade of a membrane trafficking event that occurs downstream of the usual sites of action of rab8. Third, rab8 was originally implicated in regulating the secretory pathway transporting proteins from the TGN to the basolateral plasma membrane (Huber et al., 1993a; Huber et al., 1993b), whereas our localization data indicate that Myo5c is most prominent in the apical portions of epithelial cells. Although there are many possible explanations for this apparent discrepancy, one possibility is that rab8 may have functions in addition to its role in regulating traffic to the basolateral plasma membrane. In fibroblasts rab8 appears to play only a minor role in regulating transport of proteins to the plasma membrane (Chen et al., 1998; Peranen et al., 1996) and one study suggests that rab8 is associated with the promotion of organelle transport via reorganization of the actin and microtubule cytoskeleton (Peranen et al., 1996). Testing Myo5c for the ability to interact with rab proteins and characterizing the compartments associated with endogenous Myo5c in polarized and unpolarized cells will obviously be important steps in resolving these issues.

Although our data implicate Myo5c in membrane trafficking, the precise identity of the compartment(s) involved remains unknown, as does the precise stage at which Myo5c functions. Although class V myosins might be expected to function in the transport of shuttle vesicles to targets such as the plasma membrane (with the transport complex defined by the presence of an active motor), they could be involved in other essential trafficking processes such as budding, tethering or fusion. In this regard it will be interesting to examine the EM appearance of the puncta induced by overexpressing the GFP-Myo5c tail to determine if they consist of an aggregate of small vesicles. In preliminary live-cell imaging experiments we find that a subset of the small puncta labeled by the GFP-Myo5c tail shortly after transfection undergo linear movements over distances of at least 5-10 μm. This suggests that at least some of the structures associated with the GFP-Myo5c tail at these early times undergo motor-driven movement.

In conclusion, we report the discovery of the third and final member of the myosin V family in vertebrates. Myo5c is present in many tissues and appears to be a major class V myosin of epithelial cells. Overexpressing the GFP-Myo5c tail in HeLa cells leads to colocalization with the transferrin receptor and rab8, and also perturbs transferrin trafficking. Because class V myosins appear to function as motors for organelle trafficking, the dominant negative strategy used here provides a powerful approach to selectively inhibit the function of a specific unconventional myosin and thus delineate the molecular basis of actin-dependent organelle transport. The discovery of Myo5c also represents an important opportunity to extend studies of the class V myosins beyond useful model systems such as yeast and melanocytes to include many of the physiologically crucial tissues and cells of the human body.

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