Myosin-I in Retinal Pigment Epithelial Cells

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Purpose. Myosin-I is a nonfilamentous motor protein associated with the actin cytoskeleton and cellular membranes in several cell types. The occurrence and subcellular distribution of myosin-I in mammalian and fish RPE were investigated to examine the possible role of myosin-I in retinal pigment epithelium (RPE) motility processes.

Methods. Antibodies directed against myosin-I proteins from bovine adrenal medulla or chicken intestinal brush border were used to examine cultured fetal human RPE cells and freshly isolated bovine or green sunfish RPE by Western immunoblots and immunocytochemistry, using both conventional and confocal fluorescence microscopy.

Results. The monoclonal antibody directed against bovine adrenal myosin-I identified a single strong immunoreactive band at 116 kD in Western blots of homogenates of cultured human RPE cells and 114 kD in bovine RPE sheets. An immunoreactive band of similar molecular weight was also observed in bovine and rabbit retina. Cell fractionation studies of bovine RPE cells revealed that myosin-I was present in all fractions that included cell membranes. The polyclonal antibody directed against chicken brush border myosin-I identified doublet immunoreactive bands at 115/110 kD in Western blots of homogenates of fish retina but identified a strong predominant immunoreactive band at 140 kD in fish RPE and brain homogenates; minor bands at 115/110 kD were identified in fish RPE homogenates. Immunocytochemistry of cultured human RPE cells using the bovine adrenal myosin-I antibody revealed a broad distribution of myosin-I that appeared to be most concentrated along the length of the lateral membranes; no colocalization was seen with actin-rich stress fibers.

Conclusions. Proteins immunoreactive with myosin-I antibodies are present in both RPE and retina of mammals and green sunfish. In confluent cultures of human RPE cells, myosin-I is concentrated along the lateral cell membranes of the cuboidal cells. Invest Ophthalmol Vis Sci. 1994;35:2489-2499.

In nonmuscle cells, several classes of so-called “unconventional” myosins have now been described that do not form filaments. The major class known as myosin-I is associated with the actin cytoskeleton and membranes, and is thought to be a regulator of actin-based cytoskeletal movement in many eukaryotic cells. Although sharing the diagnostic traits of myosins (that is, actin-activated ATPase activity), myosin-I differs markedly in its structural properties and distribution from the conventional filament-forming class of myosins known as myosin-II. In vertebrates, myosin-I molecules possess only a single “head,” bind to phospholipids, and are usually associated with several tightly bound calmodulin light chains in vertebrates.

Myosin-I and other members of this growing multi-gene family of myosin motor proteins have been well described in such diverse cell types as amoeba, vertebrate cell lines, and chick intestinal epithelia. Myosin-I has recently been detected by Western blots and immunocytochemistry in a wide variety of mammalian tissues. Purified mammalian myosin-I has been biochemically characterized and several mammalian myosin-I-like genes have been cloned and sequenced. Due to the ability of myosin-I to bind to both cell membranes and actin, myosin-I is believed to play an important role in actin-based force-generating mechanisms active in such processes as vesicle movement, cell shape change and phagocytosis.
Retinal pigment epithelial (RPE) cells undergo several actin-dependent movements that are likely to entail myosin participation; examples include phagocytic engulfment of rod outer segments, pigmented granule dispersion and contraction of the circumferential microfilament bundle surrounding the RPE cell apex. We have therefore investigated the occurrence and distribution of the actin-based motor protein myosin-I in RPE and retinal cells. We have immunolocalized myosin-I using antibodies directed against other known vertebrate myosin-I molecules. In this report we describe the presence of myosin-I in RPE and retinal cells of mammals and fish.

METHODS

Human RPE Cell Cultures

Human fetal RPE cell cultures were obtained from two sources: (1) long-term cultures of fetal RPE for Western blots generously donated as whole cell lysates by L. Bost of the University of California, Davis, and prepared according to Bost et al.17 and (2) short-term secondary cultures of fetal RPE cells for immunofluorescence provided by Ann Raeber and S. Miller at University of California, Berkeley; fetal donor human eyes had been obtained according to the tenets of the Declaration of Helsinki (1992) and with the approval of the university Protection of Human Subjects Committee. The short-term cultures were prepared by a modification of Song and Lui18 and Ishigooka et al.19 as follows. Eyes from 18-22-weeks-gestation fetuses were obtained in a solution of Dexol human Ringer's solution containing 120 mM NaCl, 23 mM NaHCO₃, 10 mM glucose, 5 mM KCl, 1 mM MgCl₂, and 1.8 mM CaCl₂. Retinas and RPE tissues were dissected from the eye within 4 hours of removal. The neural retina was first separated from the RPE/choroid layer. To make RPE sheets, the RPE cells were gently scraped off the choroid with a rounded scalpel from either the pigmented or nonpigmented areas, according to Burke et al.21 The abundance of RPE sheets obtained by this method was verified by fluorescence microscopy using rhodamine-conjugated phalloidin, which revealed large polygonal networks of actin-rich circumferential microfilament bundles characteristic of RPE cells. This RPE sheet preparation was greatly enhanced in RPE cells, although small amounts of choroid remained attached to some of the RPE sheets, as visualized in phase-contrast microscopy.

Rabbit retinas were dissected in the light from 1.5 to 2 kg juvenile New Zealand white rabbits injected with 1 ml/kg of ketamine:xylazine:acepromazin (3:2:1) 30 minutes before euthanasia with Nembutal (1 ml/kg). Retinas were rinsed briefly in phosphate-buffered saline containing 136 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄ and 1.7 mM KH₂PO₄, pH 7.2, and retinas were then prepared for sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE).

Adult green sunfish Lepomis cyanellus were purchased from Fender Fish Hatchery (Baltik, OH), and maintained in indoor aquaria. Fish retinas and RPE sheets were obtained from fish previously dark-adapted for 1 hour. Additional green sunfish RPE sheet samples were generously provided by Christina King at the University of California, Berkeley. Fish brains were obtained from L. cyanellus or the cichlid Sarotherodon mossambicus (gifts of Howard Bern, the University of California, Berkeley) after pithing and spinal transection. All tissues were briefly rinsed in phosphate-buffered saline and prepared for SDS-PAGE.

Tissues were obtained and used with adherence to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

SDS-PAGE and Immunoblotting

Tissues were prepared for gels by first homogenizing them at 4°C with a plastic pestle and microfuge tube in a homogenization buffer that consisted of 50 mM Tris-HCl pH 7.4, 10 mM EDTA, and the protease in-
hbitors 1.3 mM benzamidine, 20 μM leupeptin, 1.5 
μM aprotinin, 26 μM TAME, and 0.5 mM PMSF. RPE 
samples were then spun at 800g in an IEC tabletop 
centrifuge at 4°C for 10 minutes to remove insoluble 
material and pigment, and the supernatant was used 
for gels. Protein determinations were performed on 
 aliquots using the BioRad Protein Assay reagent 
(BioRad Labs, Inc., Richmond, CA). Samples for elec-
trophoresis were boiled for 3 minutes in SDS sample 
buffer and 10% SDS-PAGE mini-gels were prepared 
as described by Pagh-Roehl et al; some samples were 
later spun in a microfuge (7800g for 5 minutes) before 
loading onto the gel. SDS-PAGE high molecular 
weight standards (Sigma) used were 200-kD rabbit skel-
etal muscle myosin, 116-kD β-galactosidase, 97-kD 
phosphorylase B, 66-kD bovine serum albumin, and 
45-kD ovalbumin. Proteins on gels were transferred 
onto nitrocellulose paper for Western blots, using a 
homemade semidy apparatus (similar to LKB), and 
the proteins were probed with antibodies.

Monoclonal antibodies directed against the tail 
portion of bovine adrenal medullary myosin-I (clone 
M2), hereafter referred to as bovine adrenal anti-myos-
in-I, were the generous gift of J. Albanesi, University 
of Texas Southwestern Medical Center, and provided 
in the form of mouse ascites fluid.8 Rabbit polyclonal 
abs antibodies directed against chick intestinal brush 
border myosin-I (anti-brush border myosin-I) were 
generously donated by R. Ezzell of the Massachusetts 
General Hospital Cancer Center at Harvard Medical 
School,5|2H and column-purified mouse intestinal 
gift of H. Sagara of the University of Tokyo. 24 For 
were either horseradish peroxidase (BioRad) or alka-

Preparation of Cell Membranes From RPE 
Sheets

RPE cell membranes were prepared by differential 
ultracentrifugation using a modified protocol of 
Thomas and McNamee.25 All procedures were carried 
out at 4°C. Freshly isolated RPE sheets were collected 
as described above from pigmented areas of adult bo-
vine eyes and lysed in a microtube with plastic 
pestle in homogenization buffer consisting of 0.25 M 
sucrose, 10 mM EGTA, and the protease inhibitors 
described above. The homogenate was spun in an IEC 
table top centrifuge for 10 minutes at 800g to remove 

the initial pellet containing membrane-bound pigment 
granules, large pieces of membrane, and other insoluble 
material (P₁). The resultant supernatant (S₁) was 
then spun at 10,000g for 15 minutes using a Ti50 ro-
tor in a Beckman Model H ultracentrifuge. The result-

ing pellet and supernatant were obtained (P₂ and S₂, 
respectively). The supernatant S₂ was subjected to a 
final high-speed spin at 105,000g for 1 hour to pellet 
the fragmented membranes (P₃), consisting mainly of 
vesicles and fragmented microvilli;12,26 and the final 

supernatant was recovered (S₃). Protein contents were 
assayed using the Bradford reagent (BioRad), except 
P₃, which was directly resuspended in SDS sample 
buffer. All other samples were prepared for SDS-
PAGE and Western blotting by boiling aliquots in SDS 

sample buffer.

Immunofluorescence

Cultured cells grown on glass coverslips were fixed 
in 3.7% paraformaldehyde in phosphate-buffered saline 
for 10 minutes, followed by a 5-minute permeabiliza-
tion in 1% Triton X-100 in phosphate-buffered saline. 
Similar results were also obtained for cells fixed and 
permeabilized for 5 minutes at —20°C in 100% metha-
nol. Antibodies were applied to coverslips using the 
bovine adrenal anti-myosin-I antibodies described 
above diluted in Tris-buffered saline + 1% bovine 
serum albumin, followed by anti-mouse secondary an-
tibodies conjugated to Texas Red (Amersham, Inc.) or 
fluorescein (FITC; Cappell) for 1 hour at 37°C. Double-
labeled coverslips were additionally treated with 
0.165 μM rhodamine-conjugated phalloidin (Molecu-
lar Probes; Eugene, OR, USA) to detect actin fila-
ments, either before or after myosin labeling. Cover-
slips were mounted with Vectashield (Burlingame, 
CA).

Bovine retinal frozen sections were prepared by 
fixing freshly dissected bovine eyecups in: 4% para-
formaldehyde, 0.1% Triton X-100 and 5% sucrose in a 
buffered solution containing 50 mM K₂PIPEs pH 7.0, 
25 mM HEPES, 8 mM EGTA, and 2 mM MgCl₂. Tis-

tue was transferred to 15% and 30% buffered sucrose 
solutions, and then coated with OCT compound. Tis-
tue was frozen in liquid nitrogen and stored at —70°C. 
Frozen cross-sections of 5-μm thickness were cut at 
—20°C in a cryostat, placed on polylysine-coated glass 
coverslips, air dried for 1 to 2 minutes, and processed 
for immunofluorescence as described above.

Slides were viewed with a 63X oil immersion ob-
jective on a Zeiss photomicroscope (Carl Zeiss, Inc., 
Thornwood, NY) and photographed for 40 to 60 sec-
onds using Plus X film. Slides were also viewed with a 

laser dual-channel confocal microscope (Molecular Dy-
namics, Sunnyvale, CA) and images were transferred 
to Ektachrome 100 color slide film.
RESULTS

Western Immunoblots

Western blots using bovine adrenal anti-myosin-I revealed a 114 kD myosin-I band in freshly isolated bovine RPE sheets from either pigmented or hypopigmented areas of the eye (Fig. 1, lane 4, pigmented area shown). This molecular weight closely corresponds to the reported molecular weight of the myosin-I (116 kD) identified in several other bovine tissues and in cultured cell lines using the adrenal anti-myosin-I antibody. That our preparation was enriched in RPE sheets was verified by rhodamine-phalloidin fluorescent visualization of polygonal actin arrays corresponding to the apical microfilament bundles characteristic of the RPE (result not shown). In addition, the RPE-specific antibody S5D8 labeled a strong immunoreactive band of appropriate molecular weight (63 kD) in our preparation (Fig. 1, lane 5), previously reported for bovine RPE cells.

To determine whether myosin-I was present in human RPE cells, we performed Western blots using cultured human fetal RPE cells (Fig. 1, lane 3), and a 116-kD myosin-I band was obtained.

To examine the subcellular distribution of myosin-I in mammalian RPE cells, we compared myosin-I content in the cytosol of several membrane-enriched subcellular fractions prepared from bovine RPE sheets by differential ultracentrifugation. Western blots showed myosin-I to be present in all supernatant and pellet fractions tested (Fig. 2). For example, myosin-I was detected in the initial low-speed pellet (P1), enriched in large membrane-bound melanin granules (result not shown), an intermediate-speed pellet (P2), as well as the final 105,000g high-speed membrane pellet (P3) shown by others to consist primarily of disrupted plasma membranes, Golgi vesicles and microvilli. Thus, myosin-I was found in all soluble fractions of RPE cells, and in several different types of pelletable membrane material.

To compare myosin-I in mammalian RPE cells with that in retina, we also examined retinal homogenates using Western immunoblots. The antibody directed against bovine adrenal myosin-I revealed a strong specific cross-reactive band at 116 kD in bovine and rabbit retinas (Fig. 1, lanes 1 and 2), similar to the immunoreactive band observed in mammalian RPE. Thus, both RPE and retina of all mammalian species we tested with the bovine adrenal anti-myosin-I antibody showed bands of similar molecular weight (114 to 116 kD).

In fish RPE, no cross-reactivity with the adrenal anti-myosin-I was observed; however, a polyclonal antibody directed against chicken brush border myosin-I (brush border anti-myosin-I) produced immunoreactive bands with fish tissues and was used to probe fish...
RPE, brain, and retinal samples for the presence of myosin-I. This antibody reacted strongly with a heavily loaded sample of purified mouse brush border myosin-I (Fig. 3a, lane 1), showed the major immunoreactive band reported previously at 116 kD, and also cross-reacted with bovine retina at a similar molecular weight as that observed with the adrenal anti-myosin-I antibody (Fig. 3a, lane 2). In isolated fish RPE cells, brush border anti-myosin-I strongly labeled a 140-kD band (Fig. 3b, lane 2). The RPE-enriched preparation was verified by phase-microscopy and by the presence of the 63-kD RPE-specific protein using the monoclonal antibody S5D8 (Fig. 3c, lane 1). Using the brush border anti-myosin-I, we compared the myosin-I in fish RPE with the fish brain, and detected a similar 140-kD band (see Fig. 3b, lanes 3 and 4); this fish brain myosin-I is larger than the size of mammalian brain myosin-I estimated from sequence information. In comparison to RPE and brain, the brush border myosin antibody labeled a 115/110-kD doublet in fish retina (Fig. 3b, lane 1). This 115/110-kD doublet was also seen in homogenates of fish rod inner-outer segment fragments known as RIS-ROS prepared according to Pagh-Roehl et al (data not shown). Thus, the molecular weights of the fish retinal antigens are close to the reported 116- and 110-kD values of mammalian and chick brush border myosin-I, respectively, as detected using the same myosin-I antibody. In fish RPE cells, the strong cross-reactive band at 140 kD is accompanied by several other weak bands including the doublet at 115/110 kD as seen in retina (Fig. 3b, lane 2). The additional bands may represent breakdown products of myosin-I or the presence of several different myosin-I isotypes in RPE. Multiple myosin isotypes have recently been reported in other highly specialized epithelial cells. Relative molecular weights observed in our Western blots surveys are summarized in Table 1.

### Immunolocalization

The human RPE cell cultures we employed for immunolocalization exhibited typical characteristics of cultured mammalian RPE cells described by others. Most of the cells had variable pigmentation, and after confluency assumed polyhedral packing of cells with linear cell borders, generally described as a "cobblestone" appearance. Some cells contained mature large oval melanin pigment granules observed as translucent bodies in phase and as dark spots in the fluorescence micrographs (Figs. 4a, 4b).

![Western immunoblot illustrating cross-reactivity of an antibody directed against chicken brush border myosin-I with column-purified mouse brush border myosin-I.](image)

**FIGURE 3.** Western immunoblot illustrating cross-reactivity of an antibody directed against chicken brush border myosin-I with column-purified mouse brush border myosin-I (a, lane 1), bovine retina (a, lane 2), green sunfish retina (b, lane 1), green sunfish RPE cells (b, lane 2), green sunfish brain (b, lane 3), and cichlid brain (b, lane 4). (c) A Western immunoblot showing cross-reactivity of the RPE-specific antibody S5D8 with green sunfish RPE cells. Molecular weight standards correspond to 200, 116, 97, 66, and 45 kD (arrows, top to bottom respectively).

**TABLE 1. Summary of Western Immunoblots Using Myosin-I Antibodies**

<table>
<thead>
<tr>
<th>Tissue, Animal</th>
<th>Estimated Molecular Weight (kD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retina, bovine</td>
<td>116*†</td>
</tr>
<tr>
<td>Retina, rabbit</td>
<td>116*†</td>
</tr>
<tr>
<td>Retina, green sunfish</td>
<td>115/110†</td>
</tr>
<tr>
<td>RPE, bovine</td>
<td>114*†</td>
</tr>
<tr>
<td>RPE, human</td>
<td>116*†</td>
</tr>
<tr>
<td>RPE, green sunfish</td>
<td>140†</td>
</tr>
<tr>
<td>Brain, green sunfish</td>
<td>140†</td>
</tr>
<tr>
<td>Brain, cichlid</td>
<td>140†</td>
</tr>
</tbody>
</table>

* Bovine adrenal anti-myosin-I.
† Brush border anti-myosin-I.

Immunocytochemistry of post-confluent human RPE cell cultures with adrenal anti-myosin-I revealed preferential localization of myosin-I close to the lateral plasma membranes with some granular and diffuse intracellular staining above background levels (Fig. 4b). Identical staining patterns were obtained using anti-mouse Texas Red or anti-mouse FITC secondary antibodies. In very dense confluent cultures (usually after at least 6 to 7 days in culture), where cells were cuboidal/columnar and cross-sectional area of the cells was generally much smaller (about 8 to 12 μm), myosin-I staining appeared to be highest along the peripheral cell borders with granulations in the cytoplasm.

To determine whether myosin-I staining was coincident with actin in cultured fetal human RPE cells, we used rhodamine-conjugated phalloidin to localize F-actin. Abundant stress fibers throughout the cell were seen in subconfluent cultures, as described by others. In confluent cultures, many stress fibers were often present in cells closer to the edges of the colony, and structures resembling circumferential microfilament bundles were seen (Fig. 5a). In the dense con-
fluent regions, there were relatively few stress fibers and strong staining was mainly observed along the lateral edges of the cells (Fig. 5b).

In contrast to the staining of stress fibers by rhodamine-phalloidin, bovine adrenal anti-myosin-I did not stain stress fibers in the fetal human RPE cultures. The lack of myosin-I staining of stress fibers was easily observed in the subconfluent or early confluent areas of the RPE cultures, in which stress fibers were abundant. Myosin-I showed a strong lateral membrane distribution with some additional punctate staining beneath the membrane in some isolated cells (Figs. 6a, 6b) and in early confluent or subconfluent regions (Figs. 6c, 6d). This lack of bovine adrenal anti-myosin-I staining of stress fibers was also observed by others using the same antibody on vertebrate cell lines.7

For higher resolution of myosin-I distribution in RPE cultures, we employed confocal microscopy (Fig. 7). In Figure 7a, ten optical horizontal sections (XY scans) of a dense confluent area on the coverslip showed myosin-I distribution from the base of the RPE cells, that is, near the coverslip, through sequential planes of section toward the apex. This series revealed that myosin-I is primarily associated with the lateral cell membranes of the RPE cells, from base to apex. It also revealed the presence of a specialized distribution of myosin-I at the base of the RPE cells. Though still primarily associated with the lateral membranes of the cells, the myosin-I in the base of the cell appeared more ruffly and to extend out further from the regions of cell-cell contact observed more apically in the cells. This ruffly appearance may represent increased infoldings of the plasma membrane near the cell base. Continuous myosin-I staining was associated with the lateral walls of the RPE cells from the base to the dome-shaped apical surfaces. The cell apex appeared to be less rich in myosin-I than the lateral cell membranes. From these serial confocal images, a lateral view (Z scan) was digitally reconstructed (Fig. 7b). This image reemphasizes the general uniformity of myosin-I staining across the RPE monolayer, and the concentration of staining along lateral cell membranes.

To compare myosin-I localization to actin localization in individual cells, double-staining studies were performed. In Figure 5a, rhodamine-conjugated phalloidin was used to stain actin filaments in the culture. In Figure 5b, the rhodamine-phalloidin staining was observed in cells near the edges of confluent cultures, with abundant stress fibers (small arrows) and structures resembling circumferential microfilament bundles near the cell periphery (large arrow). In all cultures, stress fibers were observed with rhodamine-phalloidin. (Bar, 10 μm.)
performed using anti-myosin-I immunofluorescence with rhodamine-conjugated phalloidin to visualize F-actin in the cells (Figs. 7c, 7d). In a confluent area, F-actin is preferentially localized along lateral cell walls and in a relatively small number of stress fibers (Fig. 7c). Occasional actin-rich stress fibers were usually present in low abundance in these dense confluent areas as shown in Figure 5b. Double-labeling of myosin-I and actin filaments revealed similar lateral distribution of these two proteins in confluent human RPE cell cultures (Figs. 7c, 7d). A digital comparison of the confocal images (Fig. 7c) revealed that the staining patterns were largely coincident but unique localizations for both myosin-I and actin were observed. Both myosin-I and actin were concentrated near the border of the cell; however, unlike actin, myosin-I was not localized in stress fibers nor any other visible fibers in the cultures.

To determine whether phalloidin treatment in double-labeled cultures might interfere with the binding of anti-myosin-I and therefore explain why stress fibers did not show myosin-I staining, we performed double-labeling experiments as follows: myosin-I and secondary antibody labeling, followed by rhodamine-phalloidin. A second set of slides was treated in the reverse order, that is, treated first with rhodamine-phalloidin. Staining distribution was identical in both sets of slides whether the cells were treated with phalloidin before or after myosin-I detection. This suggests that the fluorescent staining of F-actin and myosin-I were independent.

In contrast to the distinct localization of myosin-I in human RPE cultured cells, immunostaining of bovine RPE/retina frozen cross-sections revealed only a diffuse staining slightly above background throughout the retina and RPE (data not shown). The actin-rich circumferential microfilament bundles, which stained strongly with rhodamine-phalloidin, did not stain at all with anti-myosin-I. We attributed the lack of definitive localization for myosin-I in retina with possible changes in antigenicity upon freezing the tissues for sectioning, because a variety of blocking, fixation, and permeabilization procedures had no influence on the myosin-I staining we observed. Because we were unable to see distinct localization of myosin-I in cryosections, we therefore cannot yet address the issue of in vivo distribution of this protein.

**DISCUSSION**

This is the first report of the presence of an unconventional nonfilamentous myosin known as myosin-I in RPE cells. We have shown that antibodies directed against bovine adrenal myosin-I cross-react with an immunoreactive band at 114 kD in Western blots of bo-
FIGURE 7. Confocal microscopy of confluent cultured human RPE cells (19 days after plating) labeled with antibody directed against bovine adrenal medullary myosin-I. (a) Myosin-I distribution is illustrated by a sequence of ten optical sections (XY scans) taken at approximately 1-μm intervals from the base of the cells near the coverslip, up to the dome-shaped apex of the cells. Depth of focus in each optical section is 0.17 μm. (b) Digital reconstruction (Z scan) of a lateral view of the cultured cells in (a). Myosin-I is primarily associated with lateral cell membranes in confluent cells. Double labeling of confluent RPE cells using (c) rhodamine-phalloidin and (d) bovine adrenal anti-myosin-I to stain myosin-I. A partial scan performed midway through the cells reveals that F-actin distribution is concentrated along the lateral cell membranes and in a few stress fibers (arrows, c). A complete scan of the cells shows that myosin-I is concentrated along lateral cell boundaries but is not found in stress fibers. (e) Computer reconstruction of the double-labeled RPE cell images in (c, d), includes regions of colocalization. Both actin and myosin-I are associated with lateral cell borders, but only actin is associated with stress fibers (arrows, same stress fibers as in c). (Numbers, distance in micrometers.) See cover also for e.

The presence of the conventional filament-forming motor protein myosin-II, with a heavy chain molecular weight of approximately 200 kD, has previously been reported in chick, fish, and mammalian RPE cells. Myosin-II is concentrated in mature differentiated RPE sheets in the apical region associated with the circumferential microfilament bundle, a purse-string-like actin-rich structure that encircles the cell apex. In cultured RPE cells, myosin-II has also
been observed in striated stress fibers in the nonconfluent areas. We reported here that although myosin-I shows substantial colocalization with peripheral actin filaments in cultured human RPE cells, the myosin-I pattern is mainly localized on or near the lateral plasma membrane from apex to base. Myosin-I is not preferentially localized at the circumferential microfilament bundle, nor does it show striations nor colocalization with stress fibers. Therefore, the immunolocalization pattern we report here differs substantially from that reported by others for myosin-II.

Myosin-I is thought to play a role in actin-dependent motility processes associated with cell membranes. Molecular sequence information and biochemical studies show that all myosin-I proteins so far described probably bind to both phospholipids and F-actin. Immunocytochemical studies of other vertebrate tissues have shown myosin-I to be closely associated with intracellular membranous structures such as membrane-bound granules, and transport vesicles, with plasma membranes and with microvilli. An association of myosin-I with membranes in human RPE cells is suggested by the concentration of myosin-I at the RPE lateral membranes we observed and by the presence of myosin-I in immunoblots of pelleted membranes after cell fractionation.

Myosin-I is thought to play a role in phagocytosis of rod outer segments, because actin filaments are required at the site of membrane engulfment. Previous studies in macrophages and myosin-mutant Dictyostelium amoebae have shown that certain isotypes of myosin-I are localized to the sites of particle ingestion in the actin-rich phagocytic cup. In preliminary experiments immunolocalizing myosin-I in fetal human RPE cultures fed bovine ROS or latex beads we have found no obvious association of myosin-I with attached or ingested particles. These findings do not of course preclude the possibility that other nonfilamentous myosins not cross-reactive with our antibody may be involved in RPE phagocytosis, or perhaps RPE cells may differ from amoeba and macrophages by employing myosin-II rather than myosin-I in phagocytic engulfment.

A functional role for myosin-I in the region of the lateral membranes in human RPE cultured cells is suggested by the strong lateral membrane localization we observed. A similar lateral distribution of the neural cell-adhesion molecule (N-CAM), a molecule involved in cell-to-cell attachment, has been observed in cultured rat RPE cells. Although N-CAM and myosin-I have similar distributions in cultured RPE cells, their functional roles in this region may be different. The concentration of myosin-I in ruffled edges near the base of the RPE cells (ie, basal infoldings) that we observed suggests that myosin-I may be involved in cell attachment processes, because basal junctions are known to appear in cultured RPE cells in addition to the prominent apical attachments seen in vivo. We are currently investigating the ultrastructure of our human RPE cell cultures to help determine the morphologic structures involved in these regions.

Our Western blot screens using antibodies to bovine adrenal myosin-I and chicken brush border myosin-I detected cross-reactive bands in fish and mammalian retinas, as well as in RPE cells. The bovine adrenal myosin-I antibody identified a 116-kD band in Western blots of homogenates of mammalian retina. The polyclonal antibody directed against intestinal brush border myosin-I identified 115/110-kD bands in fish retina and also in rod inner-inner segments. In contrast, Hofer and Drenckhahn showed in the chicken retina that the same brush border anti-myosin-I cross-reacted mainly with components of Müller cells, rather than of photoreceptors. This difference in immunodetection may reflect differences in abundance of myosin-I in the two cell types or it may reflect species-specific retinal myosin-I differences. Conventional filament-forming myosin-II within the photoreceptor has been immunolocalized to the base of the outer segment and nonfilamentous myosin-V localized to the synaptic terminal. The photoreceptor has thus already been shown to possess multiple myosins, each of which may play a unique role in normal retinal function.

The existence of myosin-II and our report of myosin-I suggests that there may be multiple myosins playing separate roles in motile processes and/or organelle movement within the RPE. The coexistence of multiple nonfilamentous myosins in the same cell has already been reported for other specialized cell types such as rat hepatocytes, mouse neurons, and Acanthamoeba. It has been suggested that different motile functions may be mediated by different myosin homologues, and that various aspects of actin-dependent motility will turn out to be mediated by various members of a large superfamily of myosins, comparable to the numerous regulatory roles currently attributed to the G-protein superfamily.

Key Words
myosin, retinal pigment epithelium, retina, bovine, human

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References


