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Expression in cochlea and retina of myosin VIIa, the gene product defective in Usher syndrome type 1B

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ABSTRACT **Myosin VIIa is a newly identified member of the myosin superfamily of actin-based motors. Recently, the myosin VIIa gene was identified as the gene defective in shaker-1, a recessive deafness in mice [Gibson, F., Walsh, J., Mburu, P., Varela, A., Brown, K. A., Antonio, M., Beisel, K. W., Steel, K. P. & Brown, S. D. M. (1995) *Nature (London)* 374, 62–64], and in human Usher syndrome type 1B, an inherited disease characterized by congenital deafness, vestibular dysfunction, and retinitis pigmentosa [Weil, D., Blanchard, S., Kaplan, J., Guilford, P., Gibson, F., Walsh, J., Mburu, P., Varela, A., Levilliers, J., Weston, M. D., Kelley, P. M., Kimberling, W. J., Wagenaar, M., Levi-Acobas, F., Larget-Piet, D., Munnich, A., Steel, K. P., Brown, S. D. M. & Petit, C. (1995) *Nature (London)* 374, 60–61]. To understand the normal function of myosin VIIa and how it could cause these disease phenotypes when defective, we generated antibodies specific to the tail portion of this unconventional myosin. We found that myosin VIIa was expressed in cochlea, retina, testis, lung, and kidney. In cochlea, myosin VIIa expression was restricted to the inner and outer hair cells, where it was found in the apical stereocilia as well as the cytoplasm. In the eye, myosin VIIa was expressed by the retinal pigmented epithelial cells, where it was enriched within the apical actin-rich domain of this cell type. The cell-specific localization of myosin VIIa suggests that the blindness and deafness associated with Usher syndrome is due to lack of proper myosin VIIa function within the cochlear hair cells and the retinal pigmented epithelial cells.**

Myosin VIIa is a newly identified member of the myosin superfamily of actin-based motors (1–3). The gene encoding myosin VIIa is responsible for human Usher syndrome type 1B (USH1B) (4), a disease characterized by sensorineural hearing loss, absence of vestibular function, and a progressive retinal degeneration termed retinitis pigmentosa. It affects ≈4.4 per 100,000 in the total United States population and 3–6% of deaf children (5, 6) and is the most frequent cause of deaf–blindness. Mutations in the myosin VIIa gene are also responsible for a recessive deafness in mice termed shaker-1 (*sh1*) (7). *sh1* mice exhibit hearing loss following early degeneration of the sensory epithelium in the cochlea, and head tossing behavior due to vestibular dysfunction (8). We have cloned a large portion of the human myosin VIIa heavy-chain cDNA** and have generated antibodies specific to a tail domain of myosin VIIa. Myosin VIIa heavy chain is a 240-kDa polypeptide and its expression is restricted to retinal pigmented epithelial (RPE) cells, cochlear hair cells, and certain cells of the testis, lung, and kidney. These results provide compelling evidence that the blindness and deafness phenotypes associated with Usher disease are due to the lack of functional myosin VIIa polypeptide within a specific subset of retinal and cochlear cells and suggest a role for myosin VIIa heavy chain in the development and maintenance of these sensory organs.

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MATERIALS AND METHODS

Isolation of Human and Porcine Myosin VIIa cDNAs. The 130-bp PCR products encoding a portion of myosin VIIa identified from human and porcine cell lines and tissues (ref. 1; EMBL accession nos. L29145 and L29133, respectively) were used to screen a number of cDNA libraries. Screening of a human liver λZAPII cDNA library (provided by James Anderson, Yale Medical School) and a human testis λgt10 cDNA library (Clontech) resulted in nine overlapping clones, one of which, clone 10C, was found to encode the entire N-terminal 1075 aa of human myosin VIIa (GenBank accession no. U34227). Screening of a λgt10 cDNA library prepared from LLC-PK_1 porcine kidney cells (provided by Robert Reilly, Yale Medical School) resulted in two clones, one of which, 14C, encompassed the N terminus of porcine myosin VIIa (aa 1–566; GenBank accession no. U34226).

Preparation of Myosin VIIa-Specific Antibodies. A *Hin*dIII–*Eco*RI fragment encoding aa 877–1075 of human myosin VIIa was cloned into pGEX2-T (Amrad, Melbourne, Australia) to produce a 49-kDa glutathione *S*-transferase/myosin VIIa tail fusion protein. This insoluble fusion protein was extracted from the cell pellet with 0.5% (wt/vol) sodium *N*-lauroylsarcosine, diluted 1:10, brought to 1% (vol/vol) Triton X-100, and purified over a glutathione-Sepharose column. Rabbits were immunized with two 300-μg injections of purified fusion protein spaced 3 weeks apart and were bled 10 days after the second injection. To allow for affinity purification of serum, the same *Hin*dIII–*Eco*RI fragment was cloned into pQE-30 (Qiagen, Chatsworth, CA) and the resultant His_6-tagged myosin VIIa tail peptide was purified over a nickel-agarose column (Qiagen). The His_6-tagged myosin VIIa peptide was dialyzed into carbonate buffer containing 0.1% SDS and coupled to cyanogen bromide-activated Sepharose (Pharmacia). Rabbit serum was applied to the myosin-VIIa-tail affinity column and bound antibodies were eluted with low pH and dialyzed into phosphate-buffered saline (PBS: 10 mM sodium phosphate/137 mM NaCl, pH 7.4).

Immunoblot Analysis. Protein samples were homogenized in 5% (wt/vol) trichloroacetic acid and standardized for protein concentration by quantitation in the bicinchoninic acid (BCA) assay (Pierce). The trichloroacetic acid precipitation pellet was washed once with water before reconstitution in boiling 5× SDS/PAGE sample buffer to a final protein concentration of 0.9 mg/ml. After SDS/PAGE separation, proteins were transferred to poly(vinylidene difluoride) membrane and incubated with affinity-purified myosin-VIIa anti-

Abbreviations: DIC, differential interference contrast; RPE, retinal pigmented epithelial.

**The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U34226 and U34227).

bodies, and the bound antibodies were visualized with a chemiluminescence kit (Boehringer Mannheim).

Immunolocalization Studies. Guinea pig temporal bones were rapidly removed into PBS containing 50 mM EGTA (PBS/EGTA). The round window membrane and stapes footplate were removed, and a small fenestra was made in the apical cochlear bony capsule in order to rapidly and directly perfuse the cochlear scalae with 4% paraformaldehyde in PBS/EGTA. After 5 min of initial perfusion, the remaining bony capsule was chipped away, the spiral ligament and stria vascularis were peeled away, and the cochlea were immersed in fixative for an additional 15 min. Half-turns of the cochlea spiral were cut free, and nonspecific binding sites were blocked in PBS/EGTA/5% bovine serum albumin for 20 min. Affinity-purified anti-myosin-VIIa or control nonimmune IgG was added at a concentration of 10 μg/ml in PBS/EGTA/0.1% bovine serum albumin/1% normal goat serum for 1 hr at room temperature. Samples were washed with PBS/EGTA for 20 min before incubation for 1 hr at room temperature with a 1:150 dilution of rhodamine-conjugated goat anti-rabbit antibodies (Cappel) and 40 nM fluorescein-labeled phalloidin (Molecular Probes) in PBS/EGTA/0.1% bovine serum albumin/5% normal goat serum. Samples were washed with PBS/EGTA and placed in PBS/glycerol mounting medium (Citifluor, Kent, England). Samples were observed with a Bio-Rad MRC600 laser scanning confocal microscope.

Rat retina was excised and fixed for 5 min on ice in 4% paraformaldehyde in PBS/EGTA. Samples were quenched, cryoprotected, embedded for frozen sectioning, and prepared for immunostaining as described (9). Antibody incubations were as described above but were done for 20 min. Mounted slides were observed with a Nikon Diaphot 300 light microscope equipped with epifluorescence and differential interference contrast (DIC) imaging.

RESULTS

A 130-bp PCR fragment encoding a portion of the motor domain of human myosin VIIa near the ATP binding site (1) was successfully used to screen a human testis λgt10 cDNA library. The isolated 3.5-kb cDNA encodes a myosin-like molecule (GenBank accession no. U34227) that has a 730-aa N-terminal motor domain, a 130-aa neck domain containing five light-chain binding repeats ("IQ" motifs; ref. 10), and a 210-aa tail. This tail is made up of two domains, a region of ≈70 aa predicted to form an α-helical coiled coil (11) and a unique C-terminal globular domain. The human myosin VIIa testis cDNA is 99% identical at the nucleotide level to the 1547-nt fragment of human myosin VIIa cDNA (ref. 4; GenBank accession no. U17180). The testis cDNA sequence differs from the U17180 sequence at 11 sites (Fig. 1), reflecting a frameshift (nt 1433–1440; U17180) and three single-base-pair discrepancies. These differences in the human myosin VIIa cDNA result in changes in the predicted amino acid sequence that match the murine ortholog at those sites (7). Human myosin VIIa is 96% identical to cloned portions of both mouse (7) and porcine (GenBank accession no. U34226) myosin VIIa at the amino acid level and is 83% identical to the frog homolog (2) (Fig. 1). By comparison, human myosin VIIa is only 25–40% identical to the motor domain of members of other unconventional myosin classes. In particular, the N-terminal 100 aa and the C-terminal tail of myosin VIIa are unique to this motor.

Repeated attempts to isolate the entire tail domain of myosin VIIa have been unsuccessful. Since the known portion of the myosin VIIa tail domain is novel, we generated antibodies directed against a bacterially expressed fusion protein containing the available myosin VIIa tail. These affinity-purified antibodies were used in immunoblot studies to analyze the expression profile of this protein. A 240-kDa polypeptide was detected in rat retina, kidney, temporal bone, and testis, while a 200-kDa polypeptide was detected in lung (Fig. 2). No expression was observed in rat liver, muscle, intestine, stomach, or brain. This finding is consistent with the mRNA expression data of Gibson *et al.* (7), which showed a 9.5-kb transcript in mouse kidney and testis and a slightly smaller species in lung. The antibodies did not recognize myosins II, I, or VI, all of which are known to be expressed within these tissues and which exhibit molecular weights different from that seen with the myosin VIIa-specific antibodies.

PCR evidence suggests that another member of the myosin VIIa family is expressed in humans, myosin VIIb (1). Myosin VIIb has not been cloned, so we cannot specifically compare it with myosin VIIa. Several lines of evidence suggest that myosin VIIa-specific probes do not recognize myosin VIIb. cDNA probes directed against either the conserved motor domain or the novel tail domains of myosin VIIa recognize a single mRNA of ≈9.5 kb in human testis by Northern blot analysis (data not shown). Screening of additional human and porcine cDNA libraries derived from liver, lung, small intestine, testis, and kidney tissues did not identify myosin VIIb clones when either motor- or tail-specific probes for myosin VIIa were used. When the 3.5-kb myosin VIIa cDNA clone was used as a probe for fluorescence *in situ* hybridization, it hybridized specifically to human chromosome 11q13–15, the position of *USH1B*, but not to additional loci (N. Mokady, T.H., and D. Ward, unpublished data). These examples of lack of cross-hybridization suggest that myosin VIIa and myosin VIIb are not highly homologous at the nucleotide level. The antibodies to myosin VIIa recognize appropriately sized polypeptides only in tissues known to express this motor as judged by Northern blot analysis (7) and by reverse transcription–PCR analysis (4) in human and mouse tissues. Taken together, these results suggest that myosin VIIa-directed probes are specific for this motor and do not recognize myosin VIIb.

An indirect-immunofluorescence study was undertaken to determine the cell-specific localization of myosin VIIa within the cochlea. Half-turns of guinea pig cochleal tissue were double-labeled with antibodies to myosin VIIa and with fluorescein-conjugated phalloidin and visualized as a whole mount by laser scanning confocal microscopy. Optical sections through the organ of Corti showed that myosin VIIa was concentrated within the outer and inner hair cells of the sensory epithelium (Fig. 3). It was detected within the stereocilia at the apex of the hair cell (Fig. 3 *a* and *b*) and within the cuticular plate which anchors the bases of the stereocilia (Fig. 3 *c* and *d*). Myosin VIIa was also present within the cytoplasm of the hair cells (Fig. 3 *e* and *f*), although this region is not enriched in F-actin. No myosin VIIa was detected within Hensen's cells or the pillar cells, two cell types within the organ of Corti that are enriched in F-actin. Nonimmune control experiments indicated that the staining of the stereocilia was not simply due to bleedthrough of the phalloidin signal. Also, no bleedthrough was seen from the bright phalloidin staining of the actin ring at the zona adherens (Fig. 3 *a* and *b*) or staining of the Hensen's cells and pillar cells (Fig. 3 *c–f*). Similar results were obtained with frozen sections of isolated cochlea and whole temporal bone (data not shown), indicating that this staining pattern was not dependent on the method of staining. No myosin VIIa-specific labeling was observed within any supporting cells or nerve cells within the cochlea. Similarly, no myosin VIIa-specific staining of the stria vascularis or other vascular tissue was seen. The image shown in Fig. 3 reflects the pattern of myosin VIIa expression along the entire length of the cochlea, with the staining intensity higher in inner hair cells than in outer hair cells. Labeling was not brighter at the tips

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          1                                                                                                  100
HumM7     MVILQQGDHV WMDLRSGQEF DVPIGAVVKL CDSGQVQVVD DEDNEHWISP QNATHIKPMH PTSVHGV.ED MI.RLGDLNE AGILRNLLIR YRDHLIYTYT
PigM7     MVILQQGDyV WMDLRSGQEF DVPIGAVVKL CDSGQiQVVD DEgNEHWISP QNATHIKPMH PTSVTGmmED MIqhLGDLNE AGILRNLLIR YRDHLIYTYT
MusM7     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......TYT
FrogM7    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          101                                                                                                200
HumM7     GSILVAVNPY QLLSIYSPEH IRQYTNKKIG EMPPHIFAIA DNCYFNMKRN SRDQCCIISG ESGAGKTEST KLILQFLAAI SGQHSWIEQQ VLEATPILEA
PigM7     GSILVAVNPY QLLSIYSPEH IRQYTNKKIG EMPPHIFAIA DNCYFNMKRN SRDQCCIISG ESGAGKTEST KLILQFLAAI SGQHSWIEQQ VLEATPILEA
MusM7     GSILVAVNPY QLLSIYSPEH IRQYTNKKIG EMPPHIFAIA DNCYFNMKRN nRDQCCIIrG ESGAGKTEST KLILQFLAAI SGQHSWIEQQ VLEATPILEA
FrogM7    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......LEA

          201                                                                                                300
HumM7     FGNAKTIRND NSSRFGKYID IHFNKRGAIE GAKIEQYLLE KSRVCRQAlD ERNYHVFYCM LEGM.SEDQK KKLGLGQAsD YNYLAMGNCI TCEGRVDSQE
PigM7     FGNAKTIRND NSSRFGKYID IHFNKRGAIE GArIEQYLLE KSRVCRQAPD ERNYHVFYCM LEGM.SEeQK KKLGLGQATD YNYLAMGNCI TCEGReDSQE
MusM7     FGNAKTIRND NSSRFGKYID IHFNKRGAIE GAKIEQYLLE KSRVCRQAPD ERNYHVFYCM LEGM.nEeeK KKLGLGQAaD YNYLAMGNCI TCEGRVDSQE
FrogM7    FGNAKTIRND NSSRFGKYID IHFNKkGAIE GAKIEQILLE KSRVCRQAsD ERNYHiFYCM LkGMlppDhK KKLGLGkATD fNYLcMGkCt TCdGRdDSkE

          301 *                                  *                                    *                  ***   400
HumM7     YANIhSAMKV LMFTDTENWE ISKLLAAILH LGNLQYEART FENLDACEVL FSPSLATAAS LLEVNPPDLM SCLTSRTLIT RGETVSTPLS REQALDVRDA
PigM7     YANIRSAMKV LMFTDTENWE ISKLLAAILH LGNLQYkdRT FENLDACEVL FStaLATAAS LLEVNPPDLM nCLTSRTLIT RGETVSTPLS REQALDVRDA
MusM7     YANIRSAMKV LMFTDTENWE ISKLLAAILH mGNLQYEART FENLDACEVL FSPSLATAAS LLEVNPPDLM SCLTSRTLIT RGETVSTPLS REQALDVRDA
FrogM7    YANIRSAMKV LMFTDTENWE ISrLLAAILH mGNLrYEARm ydNLDACEVv yftSLtTAAt LLEVfPqDLM nCLTSRTiIT RGETVSTPLS kEQALDVkDA

          401                                                                                                500
HumM7     FVKGIYGRLF VWIVDKINAA IYKPPSQdVK NSRRSIGLLD IFGFENFAVN SFEQLCINFA NEHLQQFFVR HVFKLEQEEY DLESIDWLHI EFTDNQDALD
PigM7     FVKGIYGRLF VWIVDKINAA IYKPPSQEVK NpRRSIGLLD IFGFENFAVN SFEQLCINFA NEHLQQFFVR HVFKLEQEEY DLESIDWLHI EFTDNQDALD
MusM7     FVKGIYGRLF VWIVeKINAA IYKPPplEVK NSRRSIGLLD IFGFENFtVN SFEQLCINFA NEHLQQFFVR HVFKLEQEEY DLESIDWLHI EFTDNQeALD
FrogM7    FVKGIYGRLF VWIVeKINAA IYrPvSiEpK mmRRSIGLLD IFGFENFtVN SFEQLCmNFA NEkLQQ.... .......... .......... ..........

          501                                                                                                600
HumM7     MIANKPMNII SLIDEESKFP KGTDTTMLHK LNSQHKLNAN YIPPKNNHET QFGINHFAGi VYYEtQGFLE KNRDTLHGDI IQLVHSSRNK FIKQIFQADV
PigM7     MIANKPMNII SLIDEESKFP KGTDTTMLHK LNSQHrLNsN YIPPKyNHET QFGINHFAGV VYYESQG... .......... .......... ..........
MusM7     MIANrPMNvI SLIDEESKFP KGTDaTMLHK LNSQHKLNAN YvPPKNsHET QFGINHFAGV VYYESQGFLE KNRDTLHGDI IQLVHSSRNK FvKQIFQADV
FrogM7    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          601                                                                                                700
HumM7     AMGAETRKRS PTLSSQFKRS LELLMRTLGA CQPFFVRCIK PNEFKKPMLF DRHLCVRQLR YSGMMETIRI RRAGYPIRYS FVEFVERYRV LLPGVKPAYK
PigM7     .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
MusM7     AMGAETRKRS PTLSSQFKRS LELLMRTLGA CQPFFVRCIK PNEFKKPMLF DRHLCVRQLR YSGMMETIRI RhAGYPIRYS FVEFVERYRV LLPGVKPAYK
FrogM7    .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          701                       729
HumM7     QGDLRGTCQR MAEAVLGTHD DWQIGKTKI
PigM7     .......... .......... .........
MusM7     QGDLRGTCQR MAEAVLGTHD DWQIGKTKI
FrogM7    .......... .......... .........
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FIG. 1. Alignment of the myosin VII-class motors. The motor sequence of myosin VIIa (HumM7) is compared with the available partial head sequences of porcine myosin VIIa (PigM7), mouse myosin VIIa (MusM7; ref. 7), and bullfrog myosin VIIa (FrogM7; ref. 2). Nonidentical amino acids are shown in lowercase type. The amino acid sequence identified previously by Weil *et al.* (4) in their analysis of Usher patients is underlined, and amino acid differences between the two sequences are marked with an asterisk.

of the stereocilia, suggesting that myosin VIIa is not the adaptation motor which is hypothesized to regulate tension on the mechanically sensitive transduction channels (12).

To determine the distribution of myosin VIIa in the eye, immunostaining of frozen sections of rat retina was undertaken. DIC imaging (Fig. 4 *c* and *f*) revealed eight layers within the retina. Fluorescein-conjugated phalloidin labeled four of these layers within the neural retina as well as the apical domain of the RPE cells (Fig. 4 *b* and *e*) and the vascular choroid. Antibodies to myosin VIIa, however, specifically bound only to the RPE cell layer (Fig. 4 *a* and *d*). Myosin VIIa was not found within the neural retina, although these cell layers contain F-actin. The staining of the vascular tissue (Fig. 4*a*) was not specific to the myosin VIIa antibody, as it was seen in nonimmune controls. Myosin VIIa was enriched at the apical domain of the RPE cells and also was found throughout the cytoplasm of these cells. The apical domain contains actin-rich junctional complexes and numerous microvilli. Analysis at higher magnifications (Fig. 4 *d–f*) indicated that myosin VIIa was found specifically within these microvilli and colocalized with F-actin within these structures.

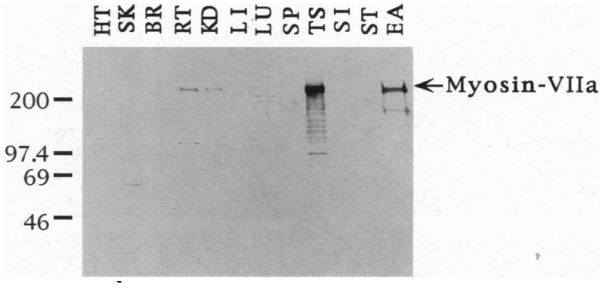

FIG. 2. Protein expression of myosin VIIa in rat tissues. Total cell extracts from rat tissues were electrophoresed in an SDS/5–16% polyacrylamide gel for immunoblot analysis with the affinity-purified rabbit anti-myosin-VIIa antibodies. Samples (4.5 μg of protein per lane) are indicated as follows: HT, heart; SK, skin; BR, brain; RT, retina; KD, kidney cortex; LI, liver; LU, lung; SP, spleen; TS, testis; SI, small intestine; ST, stomach; EA, temporal bone (ear). Three times the standard amount of protein was loaded in the ear lane. The antibody recognized a 200- to 240-kDa polypeptide. The lower bands seen in the testis and ear lanes were attributed to myosin VIIa degradation products, since they increased in intensity upon storage of samples. Molecular size (kDa) markers are shown at left.

DISCUSSION

Our data establish that myosin VIIa is present within the hair cells of the cochlea and the pigmented epithelium cells of the retina, two tissues known to be affected in Usher disease. Some patients with Usher disease exhibit abnormal sperm cells (13) and defects in bronchial function (14); in keeping with this, myosin VIIa was also found to be expressed in the testis and lung. Myosin VIIa is also expressed in the kidney, but kidney problems have not been reported in Usher patients. The kidney, and in particular the proximal-tubule epithelial cells contains a number of myosins, including two myosins II, at least four myosins I, and myosin VI (reviewed in ref. 9). In addition, at least six other unconventional myosins are expressed within this cell type (1). Perhaps these myosins serve overlapping functions in the kidney, masking the loss of myosin VIIa in Usher patients and *sh1* mice.

sh1 mice exhibit degeneration of the organ of Corti and the afferent spiral ganglion neurons in the first few weeks after birth, at a time when the organ of Corti is normally still developing (8). Hair cells are first detectably abnormal within 3 days after birth. Spiral ganglion neurons are also abnormal at 3 days. Little is known of the histopathology of type 1 Usher patients, as existing studies are apparently on usher types 2 or 3. Given the restricted localization of myosin VIIa to the hair cells, we suggest that the defect lies within this cell type and that the degeneration of the afferent neurons is secondary.

Usher syndrome type 1 is also characterized by absence of vestibular function. Although we have not located myosin VIIa in the vestibular system, amphibian myosin VIIa was identified in the sensory epithelium of the bullfrog sacculus (2), suggesting that myosin VIIa may be expressed in mammalian vestibular hair cells as well. Gillespie *et al.* (15) identified three myosin-like proteins in bullfrog saccullar stereocilia by photoaffinity labeling with uridine nucleotides; one of them had an apparent molecular mass of 230 kDa. In addition, Walker *et al.* (16) identified a 240-kDa calmodulin-binding protein in saccular hair bundles which may be the same protein and, perhaps, myosin VIIa. The neck domain sequence of myosin VIIa has five light-chain-binding IQ repeats. These repeats have been shown to bind calmodulin in other unconventional myosins (10).

The primary cause of the retinal degeneration observed in Usher disease is not known. The problem could lie in the photoreceptors themselves or in the RPE cells, which support the photoreceptors and function in photoreceptor renewal by phagocytosing disks shed by the rod outer segments (17). Myosin VIIa expression is restricted to the pigmented epithelium, suggesting that defects in these cells are the cause of the retinitis pigmentosa in Usher type 1B. One animal model of retinitis pigmentosa, the RCS rat, provides supportive evidence. In these animals, the RPE layer is defective in phagocytosis (18). Cellular debris accumulates in the intercellular space between the outer segments and the RPE layer, leading to photoreceptor cell death. The genetic defect in the RCS rat is not known, but studies in chimeric animals have determined that the cellular defect lies in the RPE layer itself (19).

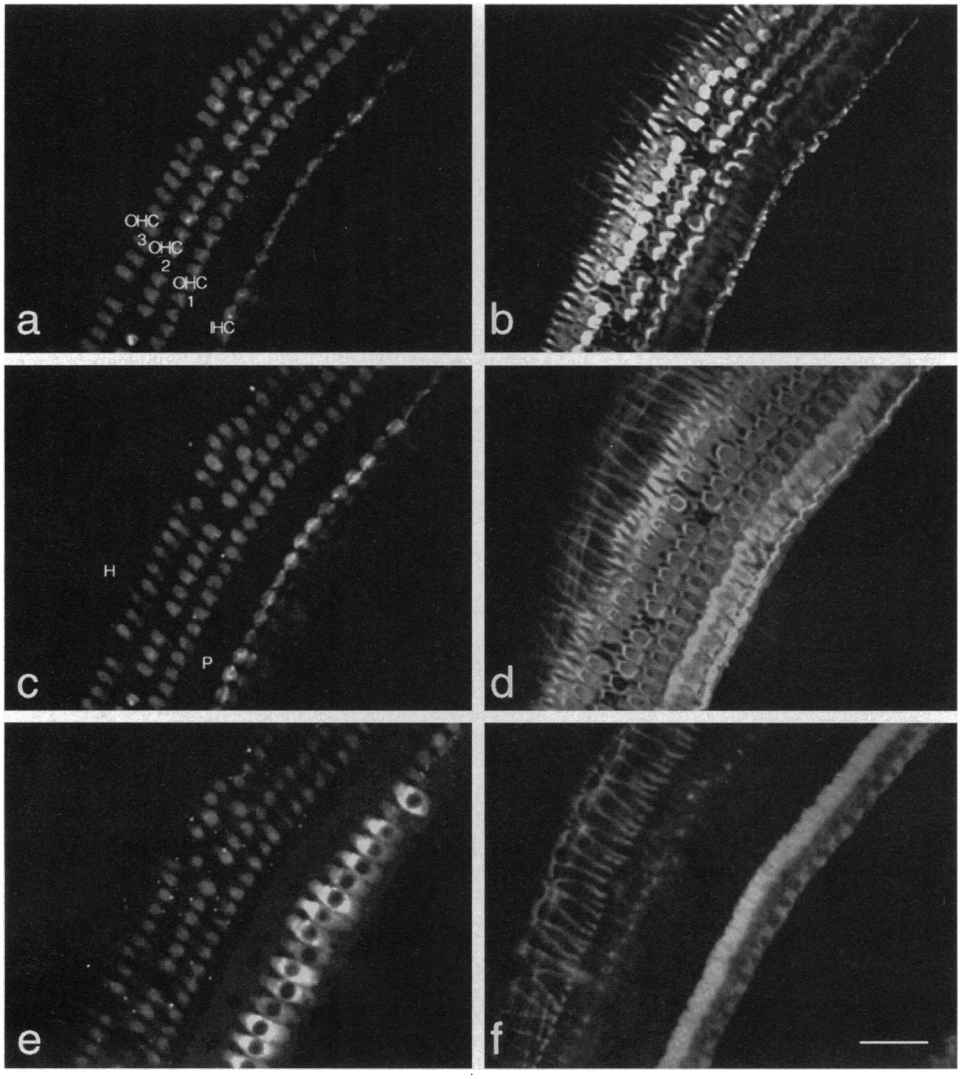

FIG. 3. Localization of myosin VIIa and F-actin in guinea pig cochlea. Immunofluorescence micrographs show optical sections through the organ of Corti perpendicular to the cochlear axis stained for myosin VIIa (*a*, *c*, and *e*) and F-actin (*b*, *d*, and *f*). (*a* and *b*) Apical domain of the organ of Corti. The locations of the inner hair cell (IHC) and three outer hair cell (OHC) rows are marked. The stereocilia are clearly visible as V-shaped, brightly stained bundles in the OHC and as a row of brightly stained material in the IHC. (*c* and *d*) Optical section 2.4 μm lower, at the level of the cuticular plate. The location of the actin-rich pillar cells (P) and Hensen's cells (H) are shown. (*e* and *f*) Optical section 7.8 μm lower, at the level of the IHC nuclei. (Bar = 50 μm.)

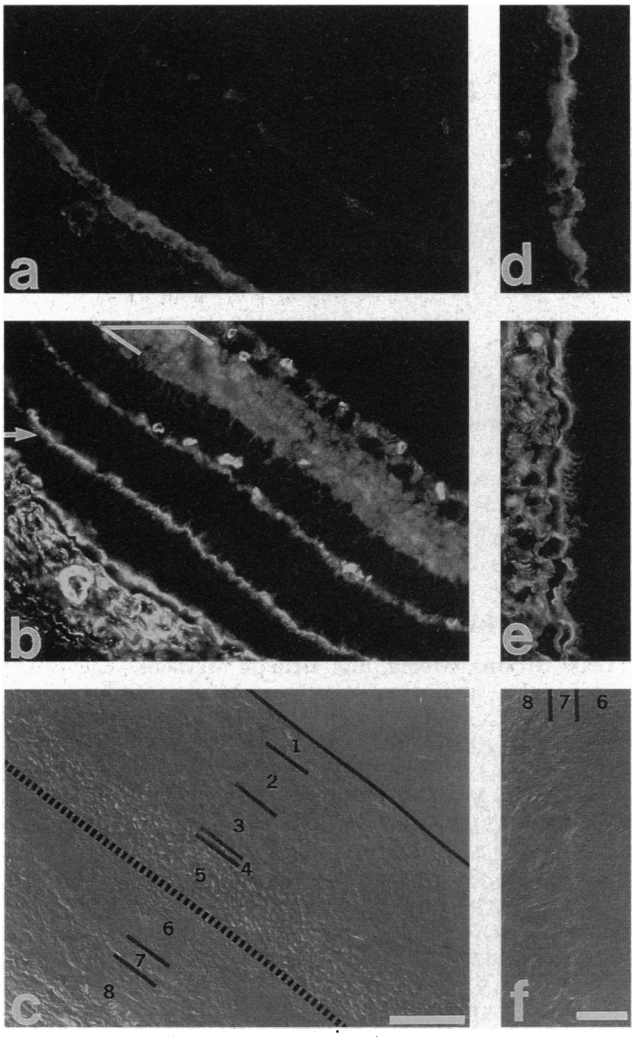

FIG. 4. Adult rat retina stained with myosin VIIa (*a* and *d*) and F-actin (*b* and *e*) and visualized by DIC optics (*c* and *f*). Retinal layers (demarcated in *c* and *f*) are as follows: solid line, inner limiting membrane; broken line, external limiting membrane; 1, ganglion cell layer; 2, inner plexiform layer; 3, inner nuclear layer; 4, outer plexiform layer; 5, outer nuclear layer; 6, rod and cone outer segments; 7, pigmented epithelium; 8, choroid layer. In *b*, the external limiting membrane is marked by an arrow, and the inner plexiform layer by a bracket. (Bar in *c* = 50 μm; bar in *f* = 25 μm.)

sh1 mice do not exhibit retinitis pigmentosa. As discussed by Weil *et al.* (4), this may reflect the type of myosin VIIa mutation observed within the *sh1* mice. The three *sh1* mutations identified by Gibson *et al.* (7) reflected two different single amino acid changes and a 10-aa deletion. All mutants expressed myosin VIIa at the mRNA level and therefore may express the mutant protein (7). A human recessive nonsyndromic deafness locus (*DFNB2*) also maps to 11q13 (20). Perhaps this human deafness is more closely related to *sh1*. Alternatively, another unconventional myosin could be compensating for the lack of myosin VIIa in the *sh1* retina.

Unconventional myosins have been shown to be associated with numerous actin-based motilities, such as phagocytosis, endocytosis, chemotactic movement, and vesicular transport (21, 22). Perhaps myosin VIIa serves a similar role in membrane movements in sensory hair cells and in the RPE cells. Both cell types undergo continuous and essential membrane movements—specifically, phagocytosis in the retina and release of transmitter-containing vesicles at the afferent hair-cell synapses (17, 23). Outer hair cells exhibit a much lower level of constitutive transmitter release than inner hair cells (24), consistent with the differential levels of expression of myosin VIIa between these two hair-cell types. Our results, showing a specific location of myosin VIIa within the cochlea and retina, should allow for an enhanced understanding of the molecular causes of blindness and deafness in Usher patients.

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