

Myosin VIIa as a Common Component of Cilia and Microvilli

Uwe Wolfrum,¹ Xinran Liu,² Angelika Schmitt,¹ Igor P. Udovichenko,² and David S. Williams^{2*}

¹*Zoologisches Institut, Universität Karlsruhe, Germany*

²*Departments of Pharmacology and Neurosciences, University of California at San Diego School of Medicine, La Jolla, California*

The distribution of myosin VIIa, which is defective or absent in Usher syndrome 1B, was studied in a variety of tissues by immunomicroscopy. The primary aim was to determine whether this putative actin-based mechanoenzyme is a common component of cilia. Previously, it has been proposed that defective ciliary function might be the basis of some forms of Usher syndrome. Myosin VIIa was detected in cilia from cochlear hair cells, olfactory neurons, kidney distal tubules, and lung bronchi. It was also found to cofractionate with the axonemal fraction of retinal photoreceptor cells. Immunolabeling appeared most concentrated in the periphery of the transition zone of the cilia. This general presence of a myosin in cilia is surprising, given that cilia are dominated by microtubules, and not actin filaments. In addition to cilia, myosin VIIa was also found in actin-rich microvilli of different types of cell. We conclude that myosin VIIa is a common component of cilia and microvilli. *Cell Motil. Cytoskeleton* 40:261–271, 1998. © 1998 Wiley-Liss, Inc.

Key words: Usher syndrome; myosin; cilium; actin; microvillus

INTRODUCTION

Usher syndrome describes a group of inherited blindness-deafness disorders, resulting from retinal and cochlear degeneration. Usher syndrome 1B is caused by defects in the gene encoding myosin VIIa, a large unconventional myosin [Weil et al., 1995]. The myosin VIIa gene is also defective in shaker-1 (sh1) mice [Gibson et al., 1995; Mburu et al., 1997]. Myosin VIIa is present in the inner and outer hair cells of the cochlea and in the photoreceptor cells and pigmented epithelium of the retina. In the cochlear hair cell, it has been detected in the stereocilia, cuticular plate, and cell body [Hasson et al., 1995; El-Amraoui et al., 1996; Hasson et al., 1997a]. In the retina, it has been found in the apical microvilli of the pigmented epithelium [Hasson et al., 1995; El-Amraoui et al., 1996; Liu et al., 1997] and the connecting cilia of the photoreceptor cells [Liu et al., 1997]. Thus, in both the cochlea and retina, myosin VIIa is present in multiple cellular regions, with potentially different specific functions.

Usher syndrome patients have been reported to have some other abnormalities, besides blindness and

deafness. They include deficient olfaction [Zrada et al., 1996], decreased sperm motility [Hunter et al., 1986], abnormal nasal cilia [Arden and Fox, 1979], bronchitis [Bonneau et al., 1993], and asthma [Baris et al., 1994]. Although it is unclear from these reports whether the Usher patients tested included those with the type 1B syndrome (but note that type 1B patients make up 75% of all type 1 patients and 50% of all Usher syndrome patients), it is noteworthy that these other abnormalities could all result from defective cilia. Indeed, it has been proposed a number of times previously that dysfunctional

Contract grant sponsor: National Institutes of Health; Contract grant number: EY07042; Contract grant sponsor: Deutsche Forschungsgemeinschaft; Contract grant number: Wo548/3-1; Contract grant sponsor: FAUN-Stiftung (Nürnberg).

*Correspondence to David S. Williams, Department of Pharmacology, University of California at San Diego, School of Medicine, Mail code 0983, 9500 Gilman Drive, La Jolla, CA 92093-0983; E-mail: dswilliams@ucsd.edu

Received 16 January 1998; accepted 3 April 1998

cilia could be the basis of some types of Usher syndrome [e.g. Arden and Fox, 1979; Hunter et al., 1986; Barrong et al., 1992; Zrada et al., 1996]. As myosin VIIa has now been detected in the cilia of photoreceptor cells [Liu et al., 1997], this suggestion warrants further attention.

Accordingly, in the present study, we have addressed the question of whether myosin VIIa is a common component of cilia. We have investigated ciliated cells from different tissues, and demonstrate that myosin VIIa is indeed present in a variety of cilia, including the kinocilia of hair cells. Our results are therefore consistent with a common ciliary defect as the primary cause for disparate abnormalities in Usher syndrome 1B. However, we also found myosin VIIa in many types of actin-rich microvilli, often in association with the cilia, so that the protein appears to be a common component of both these subcellular structures.

MATERIALS AND METHODS

Antibodies

Myosin VIIa antibodies were generated and purified as described by Liu et al. [1997]. In the present paper, we used antibodies made against a recombinant protein, corresponding to amino acids 941-1071 of mouse myosin VIIa. The antibody used in all figures presented in this paper is the one referred to as pAb 2.2 in Liu et al. [1997]. A monoclonal antibody against acetylated α -tubulin was given to us by Dr D. Asai [Schultze et al., 1987], and is the same used to label photoreceptor cilia in Arikawa and Williams [1993]. A monoclonal antibody (MAb) against chicken gizzard actin [Lessard, 1988] was given to us by Dr. J. Lessard.

Western Blot Analysis

Tissues were prepared for Western blots of cochlea and olfactory epithelium from C57BL/6 mice. They were dissected, homogenized, and placed in boiling sodium dodecyl sulfate-polyacrylamide-gel electrophoresis (SDS-PAGE) sample buffer (62.5 mM Tris buffer, 10% glycerol, 2% SDS, pH 6.8). Kidneys from control and mutant shaker-1 mice (*sh1^{4626SB}*) mice were prepared in the same way by Dr. Karen Steel (Nottingham, UK), who then shipped the samples to us in the United States.

Proteins were separated by SDS-PAGE, transferred electrophoretically to Immobilon-P, blocked, and probed with primary and secondary antibodies. The latter was conjugated to alkaline phosphatase, so that labeling was detected by the formation of the insoluble product of 5-bromo-4-chloroindoyl phosphate hydrolysis.

Bovine eyes were obtained from a local slaughterhouse. Photoreceptor outer segments were purified from bovine retinas using sucrose density gradients as described by Azarian et al. [1995]. The purified outer

segments were collected by centrifugation, resuspended in buffer A (2% Triton X-100, 5 mM MgCl₂, 1 mM EGTA, 1 mM DTT, 24 μ M leupeptin, 0.2 mM PMSF, 20 mM Tris, pH 7.4, 4°C), and added to the top of a layer of 50% (w/w) sucrose in buffer A, which had been laid on top of a 50–60% (w/w) sucrose gradient in buffer A [cf. Fleischman et al., 1980; Horst et al., 1987]. Fractions (1 ml) of the gradient were collected and placed in boiling SDS-PAGE sample buffer. After transblotting, as above, immunoreactivity to acetylated α -tubulin and myosin VIIa antibodies was determined, using chemiluminescence of the Western blots and densitometric scanning of the resulting image. Thus, the relative amounts of acetylated α -tubulin and myosin VIIa in each fraction was measured. By also testing standard amounts of purified tubulin and recombinant 1-1075 amino acids of human myosin VIIa [Liu et al., 1997], we determined that the density of chemiluminescence labeling was proportional to the amount of protein in the band, over the range of protein amounts in our samples.

Immunoelectron Microscopy

Tissues, except cochleas, were removed from adult C57BL/6 mice and fixed in 0.2–0.5% glutaraldehyde and 2–4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at room temperature. Cochleas were obtained from mice at postnatal day 5 and fixed in periodate-lysine-paraformaldehyde fixative [McLean and Nakane 1974] for 3 h at 4°C. The tissue was dissected into small pieces during fixation. Fixed pieces were embedded in L.R. White, and polymerized at 60°C for 24 h or at 4°C under ultraviolet (UV) light for 50 h.

Ultrathin sections (60–70 nm) were collected on Formvar-coated nickel grids. Sections were first etched with saturated sodium periodate (Sigma Chemical Co., St. Louis, MO) then blocked with 4% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 30 min. The primary incubation was carried out with myosin VIIa antibodies in PBS containing 1% BSA, overnight at 4°C. The grids were rinsed with PBS, and then incubated with goat anti-rabbit IgG conjugated to 10-nm gold (Amersham) at a dilution of 1:30, in PBS with 1% BSA for 1 h. Alternatively, sections were preincubated with 0.1% Tween 20 in PBS, blocked with 50 mM NH₄Cl, in PBS and 0.5% fish gelatin (Sigma) plus 0.1% ovalbumin (Sigma) in PBS, and incubated with goat anti-rabbit IgG conjugated to nanogold (Nanoprobes, Stony Brook, NY), diluted in 0.1% ovalbumin, 0.5% fish gelatin, 0.01% Tween 20, 0.5 M NaCl in 10 mM phosphate buffer, pH 7.3. In this case, the nanogold labeling was silver-enhanced as described by Danscher [1981]. The sections were post-fixed in 2% glutaraldehyde for 20 min, washed in distilled water, and stained with 5% uranyl acetate for 5 min before observation in an electron microscope. Recombi-

nant protein (1 μ M) was added to the primary incubation as a negative control. The preimmune sera were also used as a negative control.

Immunofluorescence Microscopy

Cryosections were prepared from unfixed tissues of C57BL/6 mice, as described previously [Wolfrum, 1995]. Sections were incubated with 0.01% Tween 20 and 50 mM NH_4Cl_4 in PBS, and then washed in PBS. They were blocked with 0.5% fish gelatin (Sigma) plus 0.1% ovalbumin (Sigma) in PBS for 30 min, and then incubated with primary antibody in blocking buffer overnight at 4°C. Washed sections were subsequently incubated with secondary antibodies conjugated to fluorescein or rhodamine (Cappel Oreganon Teknika Corp., Durham, NC) in blocking buffer for 1 h at room temperature in the dark. After washing, sections were mounted in Mowiol 4.88 (Farbwerke Hoechst, Frankfurt, Germany), containing 2% n-propyl-gallate.

RESULTS

Myosin VIIa Antibodies

Our myosin VIIa antibodies have been analyzed by Western blots previously [Liu et al., 1997]. Here, we carried out further characterization. First, they were tested on western blots of whole-cell lysates from mouse cochlea and olfactory epithelium. They were found to be specific for a single polypeptide, corresponding to the apparent molecular mass of myosin VIIa (Fig. 1a). Second, the specificity for myosin VIIa was tested on western blots of kidney whole-cell lysate from an allele of shaker-1 mice (*sh1*^{4626B}). In the *sh1*^{4626B} allele, the mutation in the myosin VIIa gene results in a stop codon immediately after the codon for Gln720 [Mburu et al., 1997]. It is unknown whether a stable truncated product is made in these mice but, even if it were, it should not be recognized by the myosin VIIa antibodies used here. The antibodies recognized a polypeptide (of appropriate mobility for myosin VIIa) in mice expressing at least one wildtype myosin VIIa gene (+/?), but there was no immunoreactive polypeptide in mice homozygous for the mutant gene (-/-) (Fig. 1b). This result indicates that our antibodies do not detect a polypeptide that has the same mobility in SDS-PAGE as myosin VIIa but is different from myosin VIIa.

Hair Cells of the Cochlea

Previous studies have detected myosin VIIa in the stereocilia, cuticular plate, and cell body of rodent hair cells [Hasson et al., 1995; El-Amraoui et al., 1996]. By immunofluorescence of cochleas from 5-day-old mice, we observed that myosin VIIa is also located along the lateral cell membranes and in the apical extensions of

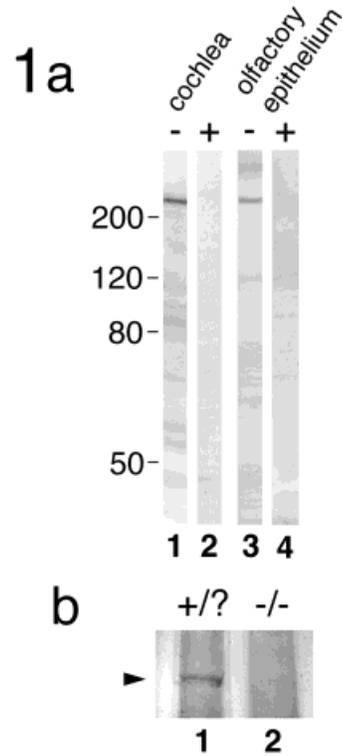
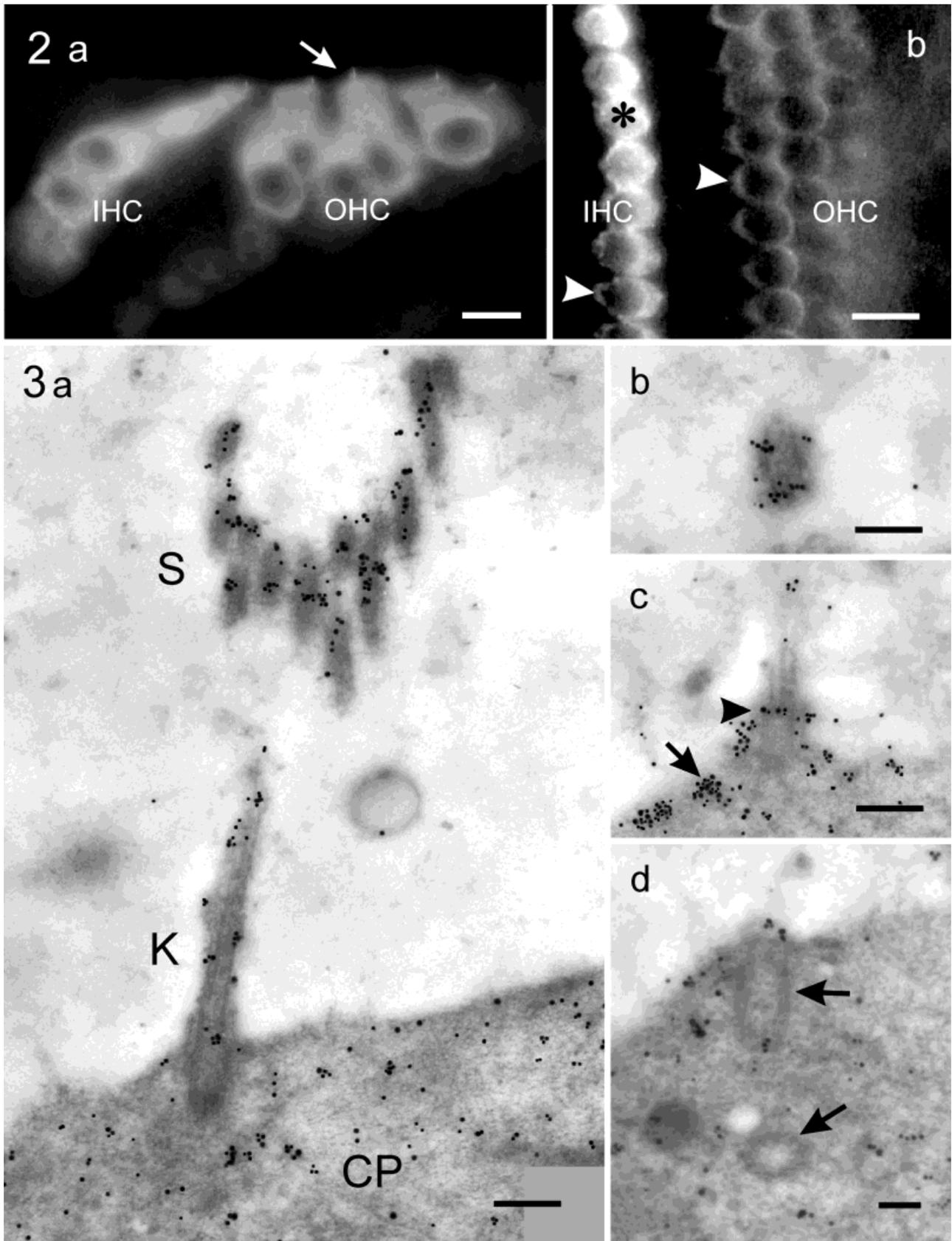


Fig. 1. Western blot analysis of myosin VIIa antibodies. **a:** Western blots of whole-cell lysates from cochlea (lanes 1 and 2) and olfactory epithelium (lanes 3 and 4). The myosin VIIa antibody was incubated with the Immobilon in either the absence (-) or presence (+) of 1 μ M recombinant protein (indicated at the top of each lane), corresponding to amino acids 941-1071 of mouse myosin VIIa. In the absence of competing recombinant protein, only a single band is evident. This band represents a polypeptide of \sim 230 kD. **b:** Western blot of kidney proteins from *sh1*^{4626B} mice. Lane 1, from mouse with at least one wild-type gene (+/?). Lane 2, from a homozygous mutant (-/-) littermate. No polypeptide is labeled in lane 2, demonstrating that the labeled band in lane 1 (arrowhead, \sim 230 kD) represents myosin VIIa.

undifferentiated inner and outer hair cells (Fig. 2). Stereocilia are not actually cilia, but actin-rich microvilli [Tilney et al., 1980]. During development of the cochlea, a bone fide cilium, the kinocilium is present no longer than postnatal day 10 in mice [Kikuchi and Hilding, 1965; Kimura, 1966]. Immunoelectron microscopy revealed that not only stereocilia, but also the kinocilia were labeled in 5-day-old mice with myosin VIIa antibodies (Fig. 3). Labeling was evident along the length of each kinocilium, although the basal bodies were devoid of label (Fig. 3c,d). Labeling appeared most dense where sections grazed the periphery of the proximal cilium (Fig. 3a-c), indicating a predominance of myosin VIIa in this region.

Olfactory Epithelium

Myosin VIIa mRNA has been detected in the olfactory epithelium [Weil et al., 1996]. In this tissue, the



Figures 2 and 3. Localization of myosin VIIa in cochlear hair cells.

sensory cells are associated with basal cells and supporting cells [Menco, 1984]. At the apical surface of the epithelium, microvilli of supporting cells surround the olfactory vesicle or bulb of each olfactory neuron. Numerous cilia extend from the bulb of each olfactory cell and project into the nasal mucosa. By immunofluorescence of cryosections, myosin VIIa antibodies were observed to label the apical surface of the epithelium (Fig. 4). Immunoelectron microscopy demonstrated that the cilia of each olfactory bulb contained myosin VIIa. In addition, the actin-rich microvilli of the supporting cells were labeled (Fig. 5).

Kidney

The kidney has been demonstrated previously to contain myosin VIIa by Western blot analysis [Hasson et al., 1995, 1997b]. By immunofluorescence microscopy, intense labeling was evident around the inner surface of each proximal tubule, but the distal tubules showed only faint labeling (Fig. 6). Immunoelectron microscopy detected myosin VIIa predominantly in the actin-rich microvilli that project into the lumen of the proximal tubule (Fig. 7). It also showed that, in the distal tubules, the bases of the widely scattered single cilia were immunoreactive (Fig. 8).

Lung

The lung was also previously shown to contain myosin VIIa by Western blot analysis, although in this tissue the protein has a slightly smaller apparent molecular mass [Hasson et al., 1995, 1997b]. Electron immunomicroscopy showed that the cilia lining the inner walls of the bronchi were labeled by myosin VIIa antibodies. Label was most concentrated in the proximal region of each cilium and was especially evident in cilia that had

been sectioned obliquely in this region (arrowheads, Fig. 9), indicating that the myosin VIIa is present mainly in the periphery of cilium. Between the cilia, numerous microvilli are present; they were also labeled by myosin VIIa antibodies (Fig. 9).

Testis

By immunofluorescence microscopy, Hasson et al. [1997b] found myosin VIIa in the Sertoli cells of the testis, concentrated in a region of amplified membrane that surrounds each developing spermatozoon head. It is rich in actin and is known as ectoplasmic specialization [Russell and Peterson, 1985; Vogl et al., 1991]. Myosin VIIa was not detected in the spermatozoa themselves [Hasson et al., 1997]. Our observations by immunoelectron microscopy confirm this distribution pattern (Fig. 10a). Comparison of actin and myosin VIIa labeling demonstrates that myosin VIIa colocalizes with actin in Sertoli cells (Fig. 10b). The axonemes of spermatozoa were heavily labeled by tubulin antibodies (Fig. 10d), but anti-myosin VIIa labeling was not evident (Fig. 10c).

Intestine

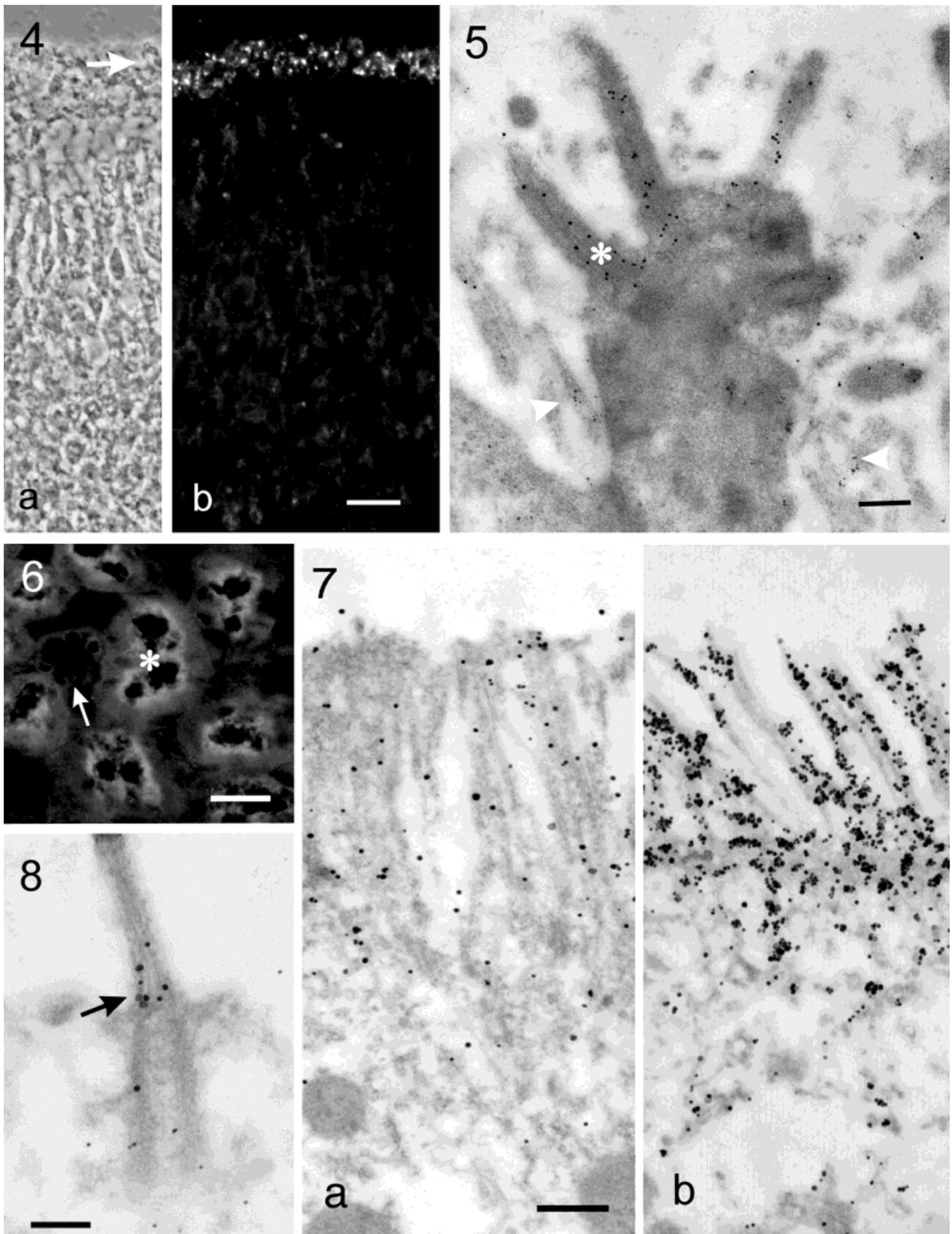
Because myosin VIIa appeared to be present in microvilli of various types, we tested whether it was present in the brush-border microvilli of the small intestine. The brush border microvilli have provided a model system for cell biological studies of an actin filament cytoskeleton [Mooseker, 1985]. As illustrated in Figure 11, myosin VIIa was detected in brush-border microvilli, but, interestingly, primarily at the distal ends of the microvilli, where a "cap-like" structure was described previously [Mooseker and Tilney, 1975; Tilney 1983].

Photoreceptor Cell Axonemes

The above and previous immunoelectron microscopic observations [Liu et al., 1997] indicate that myosin VIIa is a component of cilia. For further evidence, we tested biochemically whether myosin VIIa associated with the axonemal fraction of photoreceptor outer segments. Purified bovine rod outer segments were suspended in buffer containing Triton X-100 detergent and were loaded on top of a sucrose gradient to purify the axonemal fraction [cf. Fleischman et al., 1980; Horst et al., 1987]. Western blots of the fractions are shown in Figure 12. The fractions enriched in axonemal proteins were identified by their acetylated tubulin content; microtubules in other domains of the photoreceptor cell do not contain acetylated α -tubulin [Sale et al., 1988; Arikawa and Williams, 1993]. These fractions were also found to contain the most myosin VIIa, indicating that myosin VIIa is associated with the axonemal fraction of photoreceptor outer segments.

Fig. 2. Immunofluorescence labeling of myosin VIIa in cochlear hair cells of 5-day old mice. **a:** In longitudinal section, labeling of the cell bodies of both the inner hair cells (IHC) and outer hair cells (OHC) is evident. *Arrow*, apical extensions of the hair cells; i.e., the stereocilia and a kinocilium. **b:** Transverse sections through the cochlear hair cells reveal that myosin VIIa is present in the cuticular plate (*asterisk*) and along the lateral cell membrane of the hair cells (*arrowhead*). Scale bars = 8 μ m.

Fig. 3. Electron micrographs of sections through an outer hair cell of the cochlea from a 5-day-old mouse, illustrating silver-enhanced immunogold labeling of myosin VIIa. An oblique section of part of a kinocilium is shown in each panel. **a:** Near-longitudinal section through the proximal part of a kinocilium (K), which is labeled, as are the stereocilia (S) and the cuticular plate (CP) of the hair cell. **b:** Near-transverse section through the proximal part of a kinocilium. Label is predominantly in the periphery. **c:** The base of a kinocilium. Label is present in the transition zone (*arrowhead*), but not in the basal body region below it. Parts of the cuticular plate are densely labeled (*arrow*). **d:** The basal bodies of the kinocilium (*arrows*) lack labeling. Scale bars = 400 nm.



Figures 4 and 5. Localization of myosin VIIa in the olfactory epithelium.
Figures 6-8. Localization of myosin VIIa in the kidney.

DISCUSSION

Myosin VIIa was detected in a variety of different cells. In the cochlear hair cells, it was found throughout most of the cell, but in other cells it was localized mainly in cilia or microvilli. The main aim of the present study was to determine whether myosin VIIa is a common component of cilia. Our results indicate that it is present in motile and sensory cilia. Although above-background levels of label were found in the distal cilium, at least in the kidney and lung cilia, a higher concentration was present in the proximal region, or transition zone. This zone is immediately distal to the basal bodies [Gibbons, 1961]. Oblique sections through this region demonstrated that myosin VIIa was mainly in periphery of the transition zone, outside of the ring of microtubules. In photoreceptor cells, the transition zone corresponds to the connecting cilium, (i.e., the region between the inner and outer segments) [Röhlich, 1975; Besharse and Horst, 1990]. In the present experiments, myosin VIIa was found to cofractionate with the photoreceptor axoneme. Previously, we found that myosin VIIa labeling was concentrated in the connecting cilium of the photoreceptor cell [Liu et al., 1997]. Indeed, labeling of the photoreceptor connecting cilium, by the same antibodies as in the present study, was typically more striking than that found here for other cilia. Part of the reason for this impression may be because the photoreceptor connecting cilium is longer than the transition zone of other cilia, so that a larger domain contains label. However, overall, the results obtained with the photoreceptor cilium, and the less specialized cilia from other tissues, indicate that

myosin VIIa is a common component in the periphery of the transition zone of cilia. Interestingly, we and others [Hasson et al., 1997b] did not detect myosin VIIa in the axoneme of spermatozoa. Note, however, that the flagellum of a mammalian spermatozoon differs from most flagella and cilia in that the microtubule ring is surrounded by outer dense fibers, which are composed mainly of keratin [Bellvé and O'Brien, 1983]. Except for spermatozoa, the localization of myosin VIIa in cilia elsewhere is consistent with previous suggestions that a variety of abnormalities in different tissues of Usher syndrome patients might stem from a general ciliary defect.

It became clear, in the present study, that myosin VIIa is also a common component of actin-rich regions of amplified membrane, in particular, microvilli. The presence of myosin VIIa in the stereocilia of inner and outer hair cells from the cochlea has already been well described [Hasson et al., 1995; El-Amraoui et al., 1996]. Moreover, this point has been made by Sahly et al. [1997], in a paper published during the preparation of the present one. These investigators describe the expression of the myosin VIIa gene during mouse embryogenesis and note that it is expressed by a number of epithelial cells which all possess microvilli. Here, we demonstrate that myosin VIIa is also in the microvilli of the kidney proximal tubule, supporting cells of the olfactory epithelium, lung bronchi, and brush border of the intestine in the adult mouse. In addition, we detected it in two other actin-rich regions associated with amplified membrane: the ectoplasmic specializations of testicular Sertoli cells [cf. Hasson et al., 1997b] and the lateral margins of undifferentiated inner and outer cochlear hair cells.

The presence of myosin VIIa in these two different types of structure—one based on a microtubule cytoskeleton, the other on an actin filament cytoskeleton—suggests two different general functions. In microvilli, myosin VIIa is often found in conjunction with other unconventional myosins. For example, in the cochlear hair cells, myosins I and VI are also present in the stereocilia [Hasson et al., 1997a], and in brush border microvilli, myosins I, II, V, and VI have been detected [Heintzelman et al., 1994]. Presumably then, in microvilli, myosin VIIa carries out a very specific function that the other unconventional myosins do not.

By contrast, the presence of myosin VIIa in cilia represents a first for unconventional myosins. Its localization in cilia is somewhat surprising, given that cilia are primarily the domain of microtubules rather than actin filaments. Indeed, it remains to be shown whether all the cilia found to contain myosin VIIa also contain actin filaments. So far, actin filaments have been demonstrated only in the photoreceptor cilium [Vaughan and Fisher, 1987; Arikawa and Williams, 1989; Chaitin and Burnside, 1989], although actin has also been detected in

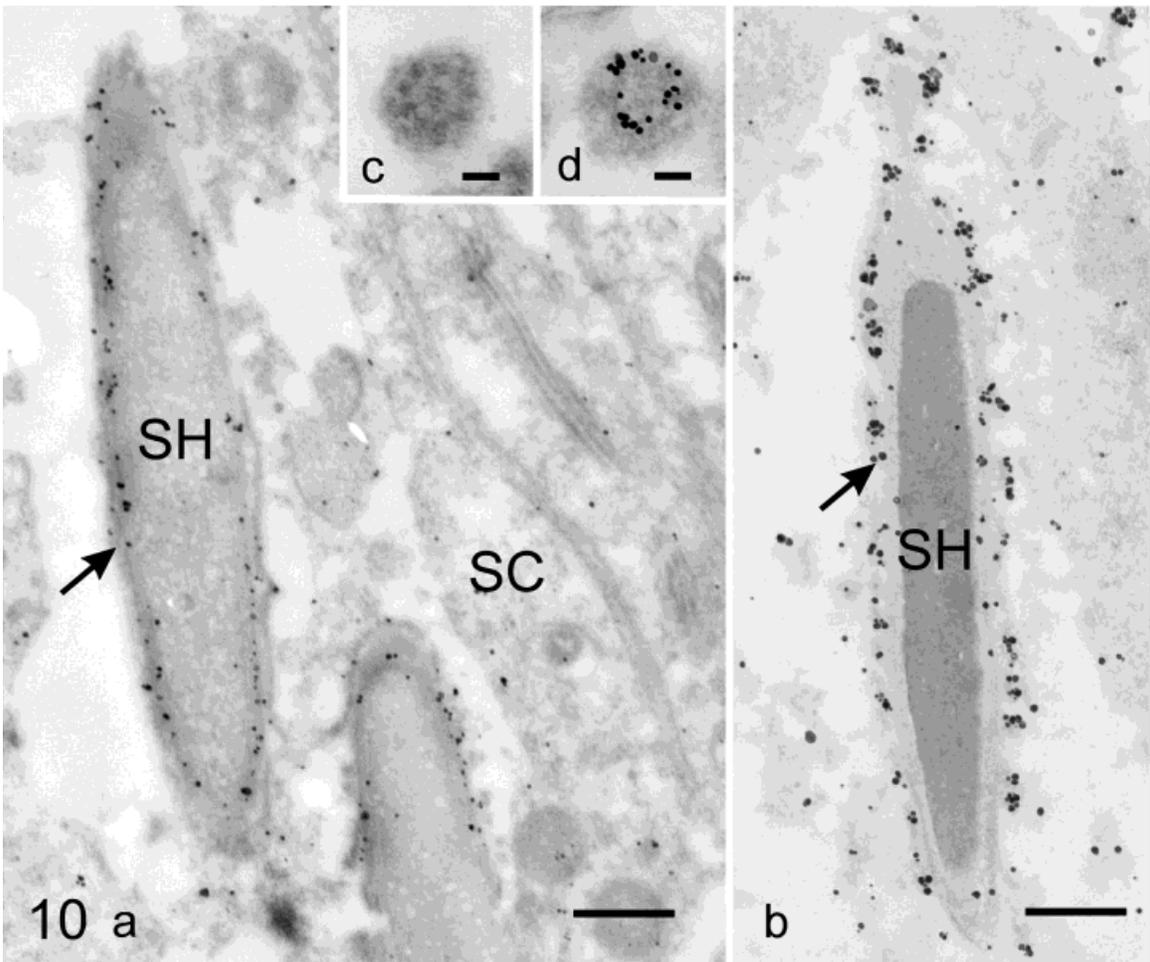
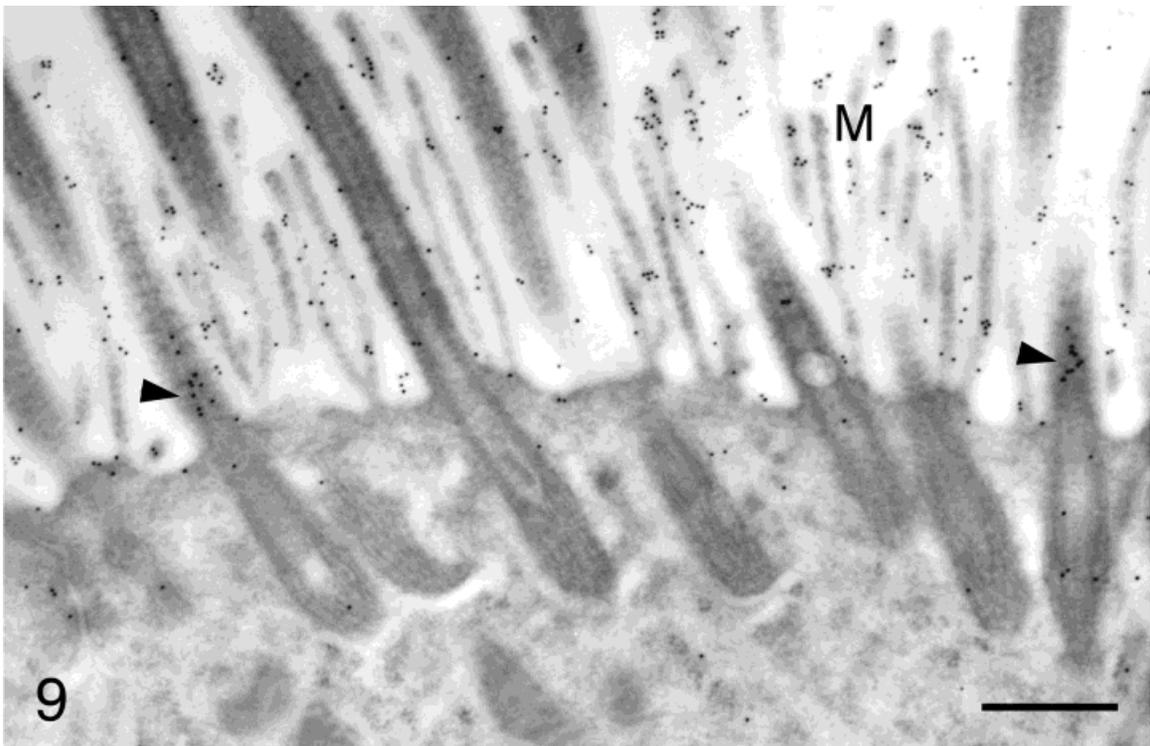
Fig. 4. Longitudinal section through a mouse olfactory epithelium. **a**: Phase-contrast image. **b**: Indirect immunofluorescence labeling of myosin VIIa. *Arrow* (**a**) the apical region of the olfactory cells, where the olfactory vesicles reside. Punctate labeling is evident in this region (**b**). Scale bar = 10 μ m.

Fig. 5. Electron micrograph of the olfactory vesicle of a single olfactory cell, illustrating silver-enhanced immunogold labeling of myosin VIIa. The *asterisk* indicates the base of an olfactory cilium. *Arrowheads*, microvilli, which extend from supporting cells of the olfactory epithelium, and are also labeled. Scale bar = 300 nm.

Fig. 6. Immunofluorescence labeling of myosin VIIa in a cross section of mouse renal tubules stained. *Asterisk*, a proximal tubule, labeled around its inner surface. *Arrow*, the distal part of a tubule, which is not labeled. Scale bar = 30 μ m.

Fig. 7. Silver-enhanced immunogold labeling of the microvilli that project into the interior of a proximal tubule of a mouse kidney. **a**: Labeled with myosin VIIa antibodies. **b**: Labeled with an actin antibody. Scale bar = 500 nm.

Fig. 8. Silver-enhanced immunogold image of the base of a single cilium projecting from the epithelium of a mouse renal distal tubule. *Arrow*, transition zone of the cilium, where label is most evident. Scale bar = 800 nm.



Figures 9 and 10.

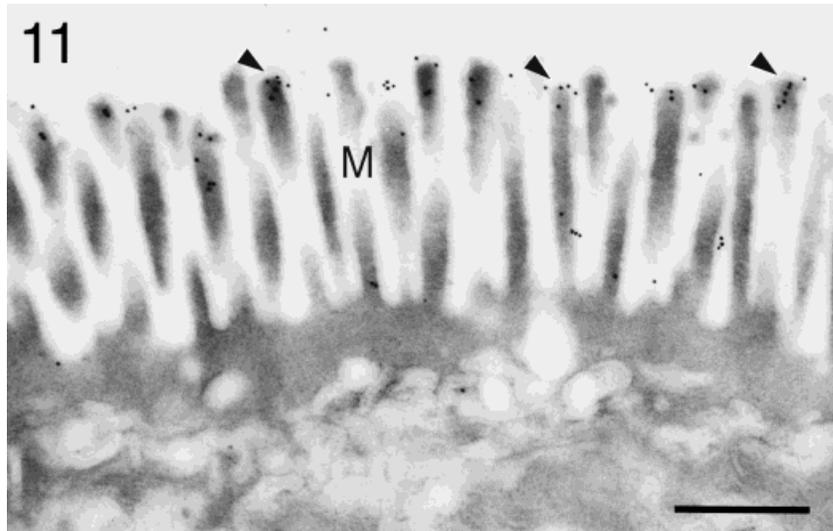


Fig. 11. Localization of myosin VIIa in the brush border of the small intestine. The microvilli (M) are immunogold-labeled, primarily at their distal ends. Scale bar = 500 nm.

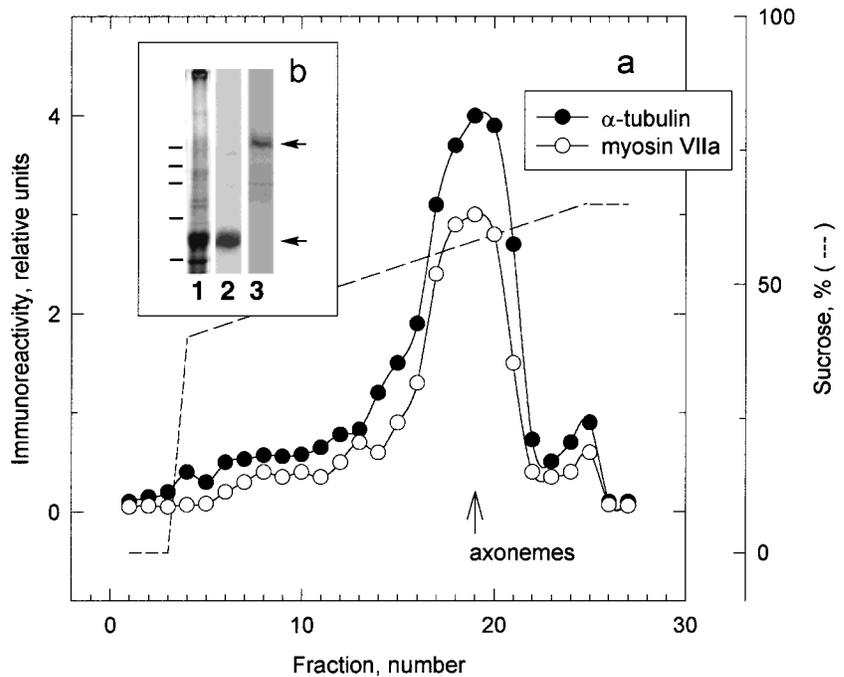


Fig. 12. Co-sedimentation of myosin VIIa with acetylated α -tubulin after fractionation of detergent-lysed bovine photoreceptor outer segments in a sucrose gradient (40–65% w/w). Fractions were analyzed by Western blots, using antibodies against myosin VIIa and acetylated α -tubulin. Acetylated α -tubulin is an indicator of the photoreceptor axoneme [cf. Arikawa and Williams, 1993]. **a:** Graph indicating relative amount of protein from densitometric scan of immunolabeled Western blots. **b:** Illustration of fraction 19. **Lane 1**, Coomassie blue-stained gel; **lane 2**, Western blot labeled with antibody against acetylated α -tubulin; **lane 3**, Western blot labeled with antibody against myosin VIIa. *Upper arrow*, position of myosin VIIa; *lower arrow*, position of α -tubulin. The positions of molecular mass standards are indicated by marks to the left (205, 116, 97, 66, 45 kD, from top to bottom).

Fig. 9. Localization of myosin VIIa in lung. The cilia and microvilli (M) of the cells lining the wall of a bronchus are shown to contain myosin VIIa by immunogold-labeling. In the cilia, label is more concentrated in the proximal region, corresponding to the transition zone. Label is most evident where sections graze the periphery of the proximal cilium (*arrowheads*), indicating a predominance of myosin VIIa in this region. Scale bar = 500 nm.

Fig. 10. Localization of myosin VIIa in testis. Electron micrographs of sections through adult mouse testes, illustrating silver-enhanced immunogold labeling of myosin VIIa (**a**) and actin (**b**). Label for myosin VIIa and actin is most concentrated in the projections (*arrows*) of Sertoli cells (SC), surrounding the spermatozoa heads (SH). Transverse sections through the axonemes of the spermatozoa are not labeled by myosin VIIa antibodies (**c**), but are labeled by antibodies against acetylated α -tubulin (**d**). Scale bars: **a** = 900 nm; **b** = 500 nm; **c,d** = 150 nm.

axonemes of *Chlamydomonas* flagella [Piperno and Luck, 1979].

The localization of myosin VIIa in the kinocilium of cochlear hair cells indicates a ciliary function as well as a microvillar one in the ear. The presence of myosin VIIa in photoreceptor cilia, in addition to the apical microvilli of the RPE, indicated the same for the eye. Thus, in the two organs most obviously affected in Usher syndrome 1B patients (the ear and the eye), myosin VIIa functions both in actin-rich microvilli and in cilia. Its function in the kinocilium may well be a critical one. Although the kinocilium does not persist in differentiated cochlear hair cells, and therefore is not required for mechanosensation, it appears to be responsible for orga-

nizing the array of stereocilia [e.g. Sobkowicz et al., 1995; Kelley et al., 1992]. Hence, myosin VIIa in the kinocilium could be important in hair cell development. Indeed, analysis of different alleles of shaker-1 mice (*sh1^{6J}* and *sh1^{816SB}*) shows that myosin VIIa is required for normal hair cell development, as well as for the function of the mature hair cells [Self et al., 1997, 1998]. Moreover, in these mice, the kinocilium appears abnormally positioned relative to the stereocilia [Self et al., 1997, 1998], a finding consistent with the involvement of this structure in the perturbed hair cell development.

In conclusion, myosin VIIa is present in a variety of cilia as well as microvilli. In cilia, it is concentrated in the periphery of the transition zone. The presence of a putative actin-based mechanoenzyme, such as myosin VIIa, as a common component of cilia is surprising. Our results are consistent with previous notions that defects in ciliary function may be responsible for many of the different tissue abnormalities experienced by patients with Usher syndrome.

ACKNOWLEDGMENTS

We thank Dr Karen Steel for helpful comments and providing the *sh1^{4626SB}* mouse kidney lysates, Dr Larry Goldstein for helpful discussions, Joana Yamada for technical assistance, and Drs. David Asai and J. Lessard for gifts of tubulin and actin antibodies. The study was supported by National Institutes of Health grant EY07042 (to D.S.W.) and by Deutsch Forschungsgemeinschaft (Wo548/3-1) and FAUN-Stiftung (Nürnberg) grants (to U.W.).

REFERENCES

- Arden, G.B., and Fox, B. (1979): Increased incidence of abnormal nasal cilia in patients with retinitis pigmentosa. *Nature* 279:534–536.
- Arikawa, K., and Williams, D.S. (1989): Organization of actin filaments and immunocolocalization of alpha-actinin in the connecting cilium of rat photoreceptors. *J. Comp. Neurol.* 288:640–646.
- Arikawa, K., and Williams, D.S. (1993): Acetylated alpha-tubulin in the connecting cilium of developing rat photoreceptors. *Invest. Ophthalmol. Vis. Sci.* 34:2145–2149.
- Azarian, S.M., King, A.J., Hallett, M.A. and Williams, D.S. (1995): Selective proteolysis of arrestin by calpain: Molecular characterization and its effect on rhodopsin dephosphorylation. *J. Biol. Chem.* 270:24375–24384.
- Baris, B., Ataman, M., Sener, C., and Kalyoncu, F. (1994): Bronchial asthma in a patient with Usher syndrome: Case report. *J. Asthma* 31:487–490.
- Barrong, S.D., Chaitin, M., Fliesler, S., Possin, D., Jacobson, S., and Milam, A. (1992): Ultrastructure of connecting cilia in different forms of retinitis pigmentosa. *Arch. Ophthalmol.* 110:706–710.
- Bellvé, A.R., and O'Brien, D.A. (1983): The mammalian spermatozoon: Structure and temporal assembly. In Hartmann, J.F. (ed.): "Mechanism and Control of Animal Fertilization." San Diego: Academic Press, pp. 56–137.
- Besharse, J.C., and Horst, C.J. (1990): The photoreceptor connecting cilium: A model for the transition zone. In Bloodgood, R.A. (ed.): "Ciliary and Flagellar Membranes." New York: Plenum Press, pp. 409–431.
- Bonneau, D., Raymond, F., Kremer, C., Klossek, J.-M., Kaplan, J., and Patte, F. (1993): Usher syndrome type I associated with bronchiectasis and immotile nasal cilia in two brothers. *J. Med. Genet.* 30:253–254.
- Chaitin, M.H., and Burnside, B. (1989): Actin filament polarity at the site of rod outer segment disk morphogenesis. *Invest. Ophthalmol. Vis. Sci.* 30:2461–2469.
- Danscher, G. (1981): Histochemical demonstration of heavy metals. A revised version of the sulphide silver method suitable for both light and electron microscopy. *Histochemistry* 71:1–16.
- El-Amraoui, A., Sahly, I., Picaud, S., Sahel, J., Abitbol, M., and Petit, C. (1996): Human Usher 1B/mouse *shaker-1*: The retinal phenotype discrepancy explained by the presence/absence of myosin VIIA in the photoreceptor cells. *Hum. Mol. Genet.* 5:1171–1178.
- Fleischman, D., Denisevich, M., Raveed, D., and Pannbacker, R.G. (1980): Association of guanylate cyclase with the axoneme of retinal rods. *Biochim. Biophys. Acta* 630:176–186.
- Gibbons, I.R. (1961): The relationship between the fine structure and direction of the beat in gill cilia of a lamellibranch mollusc. *J. Biophys. Biochem. Cytol.* 11:179–205.
- Gibson, F., Walsh, J., Mburu, P., Varela, A., Brown, K.A., Antonio, M., Beisel, K.W., Steel, K.P., and Brown, S.D.M. (1995): A type VII myosin encoded by mouse deafness gene shaker-1. *Nature* 374:62–64.
- Hasson, T., Heintzelman, M.B., Santos-Sacchi, J., Corey, D.P., and Mooseker, M.S. (1995): Expression in cochlea and retina of myosin VIIa, the gene product defective in Usher syndrome type 1B. *Proc. Natl. Acad. Sci. U.S.A.* 92:9815–9819.
- Hasson, T., Gillespie, P.G., Garcia, J.A., MacDonald, R.B., Zhao, Y., Yee, A.G., Mooseker, M.S., and Corey, D.P. (1997a): Unconventional myosins in inner-ear sensory epithelia. *J. Cell Biol.* 137:1287–1307.
- Hasson, T., Walsh, J., Cable, J., Mooseker, M.S., Brown, S.D.M., and Steel, K.P. (1997b): Effects of shaker-1 mutations on myosin-VIIa protein and mRNA expression. *Cell Motil. Cytoskeleton* 37:127–138.
- Heintzelman, M.B., Hasson, T., and Mooseker, M.S. (1994): Multiple unconventional myosin domains of the intestinal brush border. *J. Cell Sci.* 107:3535–3543.
- Horst, C.J., Forestner, D.M., and Besharse, J.C. (1987): Cytoskeletal-membrane interactions: A stable interaction between cell surface glycoconjugates and doublet microtubules of the photoreceptor connecting cilium. *J. Cell Biol.* 105:2973–2987.
- Hunter, D.G., Fishman, G.A., Mehta, R.S., and Kretzer, F.L. (1986): Abnormal sperm and photoreceptor axonemes in Usher's syndrome. *Arch. Ophthalmol.* 104:385–389.
- Kelley, M.W., Ochiai, C.K., and Corwin, J.T. (1992): Maturation of kinocilia in amphibian hair cells: Growth and shortening related to kinociliary bulb formation. *Hear. Res.* 59:108–115.
- Kikuchi, K., and Hilding, D. (1965): The development of the organ of Corti in the mouse. *Acta Otolaryngol.* 60:207–222.
- Kimura, R.S. (1966): Hairs of the cochlear sensory cells and their attachment to the tectorial membrane. *Acta Oto-Laryngol.* 61:55–72.
- Lessard, J.L. (1988): Two monoclonal antibodies to actin: One muscle selective and one generally reactive. *Cell Motil. Cytoskeleton* 10:349–362.

- Liu, X., Vansant, G., Udovichenko, I.P., Wolfrum, U. and Williams, D.S. (1997): Myosin VIIa, the product of the Usher 1B syndrome gene, is concentrated in the connecting cilia of photoreceptor cells. *Cell Motil. Cytoskeleton* 37:240–252.
- Mburu, P., Liu, X.Z., Walsh, J., Saw, D., Jamie, M., Cope, T.V., Gibson, F., Kendrick-Jones, J., Steel, K.P., and Brown, S.D.M. (1997): Mutation analysis of the mouse myosin VIIA deafness gene. *Genes Funct.* 1:191–203.
- McLean, I.W., and Nakane, P.K. (1974): Periodate-lysine-paraformaldehyde fixative. A new fixation for immunoelectron microscopy. *J. Histochem. Cytochem.* 22:1077–1083.
- Menco, B.P.M. (1984): Ciliated and microvillous structures of rat olfactory and nasal respiratory epithelia. *Cell Tissue Res.* 235:225–241.
- Mooseker, M. (1985): Organization, chemistry, and assembly of the cytoskeletal apparatus of the intestinal brush border. *Annu. Rev. Cell Biol.* 1:209–241.
- Mooseker, M.S., and Tilney, L.G. (1975): Organization of actin filament–membrane complex. *J. Cell Biol.* 67:725–743.
- Piperno, G., and Luck, D.J.L. (1979): An actin-like protein is a component of axonemes from *Chlamydomonas* flagella. *J. Biol. Chem.* 254:2187–2190.
- Röhlich, P. (1975): The sensory cilium of retinal rods is analogous to the transitional zone of motile cilia. *Cell Tissue Res.* 161:421–430.
- Russell, L.D., and Peterson, R.N. (1985): Sertoli cell junction: Morphological and functional correlates. *Int. Rev. Cytol.* 94:177–211.
- Sahly, I., El-Amraoui, A., Abitbol, M., Petit, C., and Dufier, J.L. (1997): Expression of myosin VIIA during mouse embryogenesis. *Anat. Embryol.* 196:159–170.
- Sale, W.S., Besharse, J.C., and Piperno, G. (1988): Distribution of acetylated α -tubulin in retina and in in vitro-assembled microtubules. *Cell Motil. Cytoskeleton* 9:243–253.
- Schulze, E., Asai, D.J., Bulinski, J.C., and Kirschner, M. (1987): Post-translational modification and microtubule stability. *J. Cell Biol.* 105:2167–2177.
- Self, T.J., Avraham, K. and Steel, K.P. (1997): A scanning electron microscope study of the development of the mouse mutant Snell's waltzer. *Br. J. Audiol.* 31:82–82.
- Self, T.J., Mahony, M., Fleming, J., Walsh, J., Brown, S.D.M., and Steel, K.P. (1998): Shaker-1 mutations reveal roles for myosin VIIA in both development and function of cochlear hair cells. *Development* 125:557–566.
- Sobkowitz, H.M., Slapnick, S.M., and August, B.K. (1995): The kinocilium of auditory hair cells and evidence for its morphogenetic role during the regeneration of stereocilia and cuticular plates. *J. Neurocytol.* 24:633–653.
- Tilney, L.G. (1983): Interactions between actin filaments and membranes give spatial organization to cells. *Mod. Cell Biol.* 2:163–199.
- Tilney, L.G., DeRosier, D.J., and Mulroy, M.J. (1980): The organization of actin filaments in the stereocilia of cochlear hair cells. *J. Cell Biol.* 86:244–259.
- Vaughan, D.K., and Fisher, S.K. (1987): The distribution of f-actin in cells isolated from vertebrate retinas. *Exp. Eye Res.* 44:393–406.
- Vogl, A.W., Pfeiffer, D.C., and Redenbach, D.M. (1991): Ectoplasmic (“junctional”) specializations in mammalian Sertoli cells: influence on spermatogenic cells. *Ann. N.Y. Acad. Sci.* 637:175–202.
- 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., and Petit, C. (1995): Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature* 374:60–61.
- Weil, D., Levy, G., Sahly, I., Levi-Acobas, F., Blanchard, S., El-Amraoui, A., Crozet, F., Philippe, H., Abitbol, M., and Petit, C. (1996): Human myosin VIIA responsible for the Usher 1B syndrome: A predicted membrane-associated motor protein expressed in developing sensory epithelia. *Proc. Natl. Acad. Sci. U.S.A.* 93:3232–3237.
- Wolfrum, U. (1995): Centrin in the photoreceptor cells of mammalian retinae. *Cell Motil. Cytoskeleton* 32:55–64.
- Zrada, S.E., Braat, K., Doty, R.L., and Latis, A.M. (1996): Olfactory loss in Usher syndrome—another sensory deficit. *Am. J. Med. Genet.* 64:602–603.