In: LaVail M, Hollyfield JG, Andersson RE (eds) Retinal Degenerations: Mechanisms and Experimental Therapy. Kluwer Academic / Plenum Publishers, New York. 133-142, 2003

THE CELLULAR FUNCTION OF THE USHER GENE PRODUCT MYOSIN VIIA IS SPECIFIED BY ITS LIGANDS

Uwe Wolfrum[1]

1. Introduction

Defects in myosin VIIa are responsible for Usher Syndrome 1B (Weil et al., 1995). Human Usher syndrome (USH), named after the British ophthalmologist Charles Usher (Usher, 1914), is the most common hereditary form of combined blind- and deafness (≈ 50 % of cases in the developed countries). USH designates a group of clinically and genetically heterogeneous disorders with hearing loss and retinitis pigmentosa (RP). Three different USH types (USH1, 2 and 3; see Table 1) can be distinguished according to the degree of clinical symptomes. USH1 is the most severe subtype, characterized by severe to profound congenital sensorineuronal deafness, constant vestibular dysfunction (balance deficiency) and prepubertal onset of retinitis pigmentosa. USH1 is genetically heterogeneous. Out of at least seven distinct genetic loci (*USH1A-G*) four corresponding genes have been identified, namely *USH1B*, *C*, *D*, and *F* (Mustapha et al., 2002; Petit, 2001; Table 1). *USH1B* encodes for the molecular motor protein myosin VIIa (Weil et al., 1995), *USH1C* encodes harmonin (Bitner-Glindzicz et al., 2000; Verpy et al., 2000), a scaffold protein containing PDZ-domains, motifs which are known to organize supramolecular protein complexes (Sheng and Sala, 2001). Mutations in the cadherin-related proteins, cadherin 23 (Cdh23) and protocadherin 15 (PCdh15) underline USH1D and USH1F, respectively (Bolz et al., 2001, 2002; Bork et al., 2001; Ahmed et al., 2001; Alagramam et al., 2001a, b).

Type	Gene locus	Gene	Protein (cellular function)	Mouse model
1A	14q32			
1B	11q13.5	*MYO7A*	Myosin VIIa (molecular motor)	*shaker-1*
1C	11q15.1	*USH1C*	Harmonin (PDZ scaffold protein)	in prep.
1D	10q21-q22	*CDH23*	Cadherin 23 (cell-cell adhesion)	*waltzer*
1E	21q21			
1F	10q11.2-q21	*PCDH15*	Protocadherin 15 (cell-cell adhesion)	*ames waltzer*
1G	17q24-25			
2A	1q41	*USH2A*	Usherin (extracellular matrix)	in prep.
2B	3p23-24.2			
2C	5q14.3-21.3			
3A	3q21-25	*USH3A*	Clarin-1 (cell-cell adhesion)	in prep.

Table 1: *Usher syndrome (USH) subtypes (changed after Petit, 2001); fall 2002 update.*

As in other hereditary human diseases the knowledge of the cellular function of the USH gene products is a necessary prerequisite for the development of founded therapeutic strategies for the treatment of the disease. Essential insights into the cellular function of the *USH1B* gene product myosin VIIa were gathered by the identification and characterization of ligands to myosin VIIa. Recently obtained data further identify myosin VIIa as a component of a supramolecular USH1-protein complex composed of several USH gene products

* Johannes Gutenberg-Universität, Institut für Zoologie, 55099 Mainz, Germany

(Boëda et al., 2002; Reiners et al., 2003). The purpose of this chapter is to integrate the present knowledge on the function of myosin VIIa and its ligands in the cellular environment of the retina.

2. Myosin VIIa domain structure

The myosin VIIa gene (*USH1B*) was the first USH gene identified (Weil et al., 1995). It encodes myosin VIIa, an unconventional myosin composed of several functional domains (Figure in Table 2): a motor domain is linked by a neck region with the long tail. The motor or head domain bears an ATP-binding site and an actin filament-binding site common for myosins (Sellers, 2000). Recent studies demonstrated that myosin VIIa functions indeed as an actin filament-based molecular motor protein (Udovichenko et al., 2002). The neck region of myosin VIIa contains 5 IQ (isoleucine/glutamine) motifs expected to bind myosin light-chains regulating the motile function in other myosins. The Ca^{2+}-binding EF-hand protein calmodulin binds to one or more IQ-motifs and may serve as a myosin light-chain (Todorov et al., 2001; Udovichenko et al., 2002). Among the increasing number of myosin family members, the tail region is the most variable protein region indicating that the tail may be responsible for the specific cellular function of a particular myosin. The long myosin VIIa tail contains several domains. It begins with a short coiled-coil domain that has been shown to form homodimers (Weil et al., 1997). The coiled-coil domain is followed by two tandem repeats, each containing a MyTH4 (myosin tail homology 4) and a FERM (4.1 (four point one) protein, ezrin, radixin, moeosin)-like domain which are separated by a poor conserved SH3 (src homology type 3) domain (Chen et al., 1996; Mermall et al., 1998; Oliver et al., 1999). Since this tandem of MyTH4 and FERM domains also is present in other myosins, a functional significance has been suggested (Mermall et al., 1998; Oliver et al., 1999). Nevertheless, isolated FERM-domains have been shown to be responsible for protein attachment to the plasma membrane either via direct binding to phospholipids or through the interaction with specific transmembrane adaptor proteins (Chishti et al., 1998). This tail diversity in unconventional myosins is thought to specify their cellular function and it is commonly accepted that proteins interacting with the tail domain can determine the target (e.g. a vesicle or an organelle) to which the force generated by the motor domain is applied (Oliver et al., 1999). Therefore, it does not surprise that recent identification of proteins interacting with the tail of myosin VIIa provided essential insights into the function of myosin VIIa in retinal cells.

3. Myosin VIIa expression and subcellular localization in the mammalian retina

Myosin VII is expressed as a 230 kDa protein in a variety of tissues as an alternatively spliced truncated variant lacking portions of the c-terminal tail (Weil et al., 1995; Hasson et al., 1995; Self et al., 1998; Wolfrum et al., 1998). A comparative analysis of different tissues demonstrated myosin VIIa as a common component of the transition zone of motile and in-motile cilia and microvilli (Wolfrum et al., 1998).

In the retina, myosin VIIa contains its tail and is expressed in the cells of the retinal pigment epithelium and in both cone and rod photoreceptor cells (Hasson et al.,1995; El-Amraoui et al., 1996; Liu et al., 1997). In the cells of the retinal pigment epithelium, myosin VIIa is concentrated in the apical microvilli-like processes (Hasson et al.,1995; Liu et al., 1997). Since these extensions are involved in the phagocytosis of the distal ends of photoreceptor outer segments, it has been suggested that the retinitis pigmentosa in USH1B patients may result from defects in the phagocytotic ability of the retinal pigment epithelium (Hasson et al., 1995).

In retinal photoreceptor cells, myosin VIIa is predominantly localized at the membrane of the connecting cilium at the joint between the inner and the outer segment. In the ciliary membrane, the actin-based and membrane associated molecular motor myosin VIIa is associated with actin filaments and co-localizes with opsin (Wolfrum and Schmitt, 1999, 2000). Further, evidence for participation of myosin VIIa in the ciliary transport of opsin is coming from studies on myosin VIIa deficient *shaker-1* mice which revealed an abnormal opsin accumulation at the membrane of the connecting cilium (Liu et al., 1998; Wolfrum and Schmitt, 1999, 2000). Beside the ciliary localization, myosin VIIa was also detected in the synaptic terminals of photoreceptor cells (El-Amraoui et al., 1996; Reiners et al., 2003).

4. Myosin VIIa cellular function is determined by interacting proteins

Unconventional myosins play critical roles in the morphogenesis and maintenance of sensory cells (Hasson and Mooseker, 1997). A variety of publications shows that myosin VIIa is expressed in different subcellular compartments of cells (e.g. Wolfrum et al., 1998). This indicates that the motor activity of myosin VIIa serves in several distinct motile processes in the various cell types studied. As described above, in the retinal pigment epithelium, myosin VIIa contributes to the migration of melanosomes (Liu et al., 1998; El-Amraoui et al., 2002), whereas in the mechanosensory hair cells and photoreceptor cells it is suggested to participate in the transport of receptors and opsin (hair cells: Richardson et al., 1997; 1999; opsin transport in photoreceptors: Liu et al., 1999; Wolfrum and Schmitt, 1999; 2000). In *Dictyostelium,* myosin VII has been suggested to act as an important mediator of cell adhesion (Tuxworth et al., 2001) which is consistent with association of myosin VIIa with cell-

cell adhesions in sensory hair cells (Küssel-Andermann et al., 2000b). The tail domains of unconventional myosins are believed to be largely responsible for their specific function (Oliver et al., 1999). Recent identifications of proteins interacting with the domains encompassed in the tail of myosin VIIa indicate that these proteins specify the cellular function of myosin VIIa.

Table 2: *Scheme of myosin VIIa domain structure and binding sites of myosin VIIa binding proteins.*

Interacting protein	Myosin VIIa target domain	Reference
Calmodulin (Ca^{2+}-binding protein)	IQ-motifs	Todorov et al., 2001 Udovichenko et al., 2002
MAP2B (microtubule association)	SH3, MyTH4 (2), FERM (2)	Todorov et al., 2001
RIα of PKA (regulation)	MyTH4 (2), FERM (2)	Küssel-A. et al., 2000a
Keap1 (actin-associated protein)	SH3	Velichkova et al., 2002
Vezatin (transmembrane protein)	FERM (2)	Küssel-A. et al., 2000b
MyRIP (Rap-interacting protein)	MyTH4 (2), FERM (2)	El-Amraoui et al., 2002
Harmonin (scaffold protein)	FERM (2)	Boëda et al., 2002

The proteins identified as ligands to myosin VIIa are summarized in Table 2. As mentioned above, calmodulin binds to one or more IQ-motifs in the neck region of myosin VIIa and probably regulates the motor function of myosin VIIa (Todorov et al., 2001; Udovichenko et al., 2002). In biochemical approaches, Todorov et al. (2001) also found the microtubuli associated protein 2 (MAP2) to interact with the C-terminus portion of the tail domain of myosin VIIa. In neurons, MAP2 plays an important role in the integrative signaling between the actin and the microtubule cytoskeleton anchoring protein kinase A (PKA) in subcellular compartments (Harada et al., 2002). Interestingly, the regulatory subunit of the PKA, RIα, was identified as a ligand to the myosin VIIa-tail in yeast two hybrid screens (Küssel-Andermann et al. 2000a). It is worth to speculate that myosin VIIa brings the MAP2-PKA complex and the RIα in spatial vicinity at the myosin VIIa tail for the regulation of kinase activity.

In yeast two hybrid screens, further molecules were identified as myosin VIIa binding proteins: ***i)*** the actin-associated protein Keap1 specifically interacts with the SH3-domain in the myosin VIIa tail which was used as the bait in the screen (Velichkova et al., 2002). Keap1 is the human ortholog to *Drosophila* kelch-protein which is associated with filamentous actin at ring channels of fruit fly larvae. Mammalian Keap1 has been localized at the endoplasmic junctions which are complex cell-cell adhesion specializations at the contacts between Sertoli cells and the differentiating sperm heads in testes. Keap1 is also expressed in the retina. However, neither the subcellular distribution nor the cellular function of Keap1 in the retina has yet been studied. ***ii.)*** Vezatin (derived from Slowenian *vezati* = bind, connect) was identified as a myosin VIIa ligand by yeast two hybrid screens using the C-terminal region of myosin VIIa, corresponding to the second tandem of MyTH4 and FERM domains as bait (Küssel–Andermann et al., 2000b). Vezatin is an ubiquitous transmembrane protein of adherens cell-cell junctions, where it interacts with myosin VIIa and the cadherin/catenin complex. ***iii.)*** Applying the same bait for screening a human retinal cDNA library, an additional myosin VIIa-interacting protein MyRIP, named after its ability to interact with myosin VIIa and small Rab GTPases, was identified (El-Amraoui et al., 2002). ***iv.)*** More recently, we demonstrated that myosin VIIa specifically interacts via its second FERM domain with harmonin (Boëda et al., 2002). Harmonin is a scaffold protein previously identified as the *USH1C* gene product (Verpy et al., 2000). It contains three PDZ-domains (derived from the first described proteins containing this domain: post synaptic density protein PSD-95, *Drosophila* septated junction protein Disc large, and tight junction protein ZO-1) known to interact with other proteins (Fanning and Anderson, 1996; Sheng and Sala, 2001). Our studies revealed that harmonin PDZ1 binds to myosin VIIa (USH1B) whereas the PDZ2 specifically interacts with the cell-cell adhesion protein cadherin 23 (USH1D) (Boëda et al., 2002). In photoreceptors, the USH1 protein complex may contribute to specialized cortical cytoskeletal matrices of these synapses which are thought to play a fundamental role in the organization, structure and function of the synaptic junction (Garner et al., 2000).

Vezatin recruits myosin VIIa to specific sites of the plasma membrane - Vezatin is a transmembrane protein ubiquitously localized at cell-cell adhesion junctions associated with the cadherin/catenin complex (Küssel-Andermann et al., 2000b). Vezatin may anchor myosin VIIa to the adhesion junction and is a component of fibrillar ankle links that interconnect stereovilli (= stereocilia) of mechanosensitive hair cells (Küssel-

Andermann et al., 2000b). In the retina, vezatin is expressed in the cells of the retinal pigment epithelium and the photoreceptor cells at sites where it co-localizes with myosin VIIa (Schäfer et al., in prep.). Its localization indicates that vezatin recruits myosin VIIa to the microvilli-like extensions of the retinal pigment cells and to the complex membrane contacts at the ribbon synapses of the photoreceptor cells. In addition, vezatin may anchor myosin VIIa at the membrane of the connecting cilium of photoreceptor cells supporting the transport of opsin through the cilium (Wolfrum et al., 2001).

MyRIP bridges myosin VIIa to its cargo, namely RPE melanosomes and synaptic vesicles - Myosin VIIa also specifically binds MyRIP (MyosinVIIa- and Rab-Interacting Protein), a novel Rab effector protein that directly interacts with the small GTPase Rab27 in a GTP dependent manner (El-Amraoui et al., 2002). In cells of the retinal pigment epithelium, Rab27A, MyRIP, and myosin VIIa are found to be associated with melanosomes. This molecular motor complex bridges the melanosomes to actin filaments and thereby mediates the trafficking of these organelles. Defects in this complex are likely to account for the melanosome mislocalization in the retinal pigment epithelium of myosin VIIa defective *shaker-1* mice previously described by Liu et al. (1998). However, we also identified the molecular complex of MyRIP-myosin VIIa at the synapses of photoreceptor cells. It is well known that the targeting of synaptic vesicles is controlled by Rab proteins (e.g. Rab3) in crosstalk with Rab effectors (Rabphilin or RIM) (e.g. Wang et al., 1997). Although MyRIP does not interact with Rab3 nor with Rab5, our data suggested that the MyRIP–myosin VIIa complex is associated with the membrane of vesicles at the synapse of photoreceptor cells.

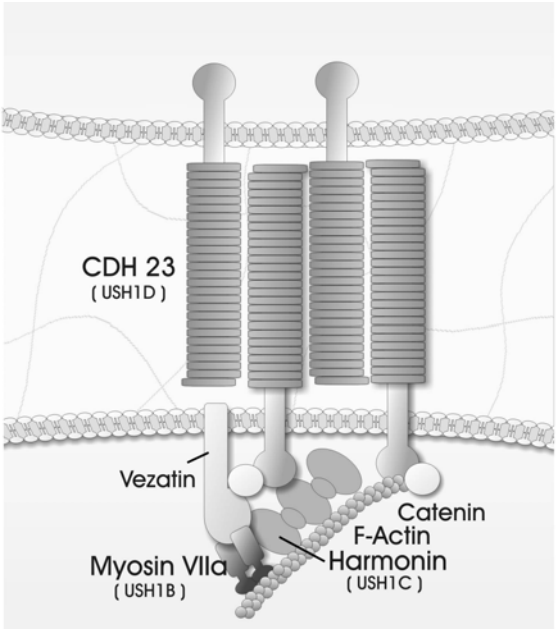

Figure 1: *Scheme of the supramolecular USH1 protein complex at cell-cell adhesions. Description see text.*

Myosin VIIa is a component of a supramolecular USH1 complex integrated via harmonin – Recently, two independent studies showed that USH1-proteins can interact with each other. Siemens et al. (2002) demonstrated that the USH1C protein harmonin binds cadherin 23 (USH1D). Moreover, our investigations provided striking evidence that harmonin interacts also with myosin VIIa (USH1B) (Boëda et al., 2002). In the USH1 complex, the scaffold protein harmonin binds the myosin VIIa tail via PDZ-domain 1 and cadherin 23 via PDZ-domain 2. In the mechanosensitive hair cells, this supramolecular USH1 complex is necessary for the proper differentiation of their stereovilli (Boëda et al., 2002). In our studies on the retina, all three components of the USH1 complex were found to be co-localized at the ribbon synapse of mature photoreceptor cells (Reiners et al., 2003). However, single components of the supramolecular USH1 protein complex are also localized in compartments of the retina separate from the other components (Reiners et al., 2003). Biochemical experiments with fractionated photoreceptor compartments in combination with cytochemical analysis strongly indicate that harmonin isoforms serve as scaffold proteins in the light sensitive outer segment. Nevertheless, at the photoreceptor synapse the USH1 protein complex may contribute to specialized cortical cytoskeletal matrices of the pre- and postsynaptic regions which are thought to play a fundamental role in the organization, structure and function of the synaptic junction (Garner et al., 2000). As other PDZ-domain proteins, based on its modular character and its ability to form multivalent interactions, harmonin is ideally suited to act as a potent organizer for a specialized protein complex at ribbon synapses of sensory cells. It physically and functionally bridges the motor activity of myosin VIIa probably involved in endo- or exocytotic processes with the cell-cell adhesion complex. The identified adhesion complex The transmembrane proteins of this complex, cadherin 23 and vezatin, may contribute to

holding the synaptic cleft in close register. The theoretical characteristics of the cadherin-like protocadherin 15 and the 4-tansmembrane protein clarin-1, the gene products of two additional USH genes, namely *USH1F* and *USH3A* (Alagramam et al., 2001a, b; Ahmed et al., 2001; Adato et al., 2002), respectively, indicate that both proteins may also be present in a membrane associated protein complex.

Although the defects in USH1 complex components should lead to abnormalities in retina function in USH1 animal models, all available USH1-mouse models (see Table 1) do not show progressive retinal degeneration. However, the minor reduction of a and b waves in electroretinograms (ERG) of mice with mutations in *Myo7a* (*shaker-1 mice*) may reflect defects at photoreceptor synapses introduced by the absence or dysfunction of the USH1 complex component, myosin VIIa (Libby and Steel, 2001). In patients, dysfunction of one USH1 complex partner protein may cause retinal dysfunction and the clinical Usher syndrome type 1 phenotype found in patients with USH1.

5. Acknowledgements

This work was supported by the FAUN-Stiftung, Nürnberg, Germany, Forschung contra Blindheit – Initative Usher Syndrom e.V., and the Deutsche Forschungsgemeinschaft Wo548/3 and Wo548/4. I would like to thank B. Reidel, J. Reiners, and R. Schäfer providing me with figures for this chapter and L. Clermont-Wocker for her help preparing the manuscript.

6. References

Adato, A., Vreugde, S., Joensuu, T., Avidan, N., Hamalainen, R., Belenskiy, O., Olender, T., Bonne-Tamir, B., Ben-Asher, E., Espinos, C., Millan, J. M., Lehesjoki, A. E., Flannery, J. G., Avraham, K. B., Pietrokovski, S., Sankila, E. M., Beckmann, J. S., Lancet, D., 2002, USH3A transcripts encode clarin-1, a four-transmembrane-domain protein with a possible role in sensory synapses, *Eur. J. Hum. Genet.* **10**: 339-350.

Ahmed, Z., Khan, S. N., Der Kaloustian, V. M., Li, X. C., Lalwani, A., Bitner-Glindzicz, M., Nance, W. E., Liu, X.-Z., Wistow, G., Smith, R. J. H., Griffith, A. J., Wilcox, E. R., Friedman, T. B., Morell, R. J., 2001, Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene *CDH23*, *Am. J. Hum. Genet*. **68**:26-37.

Alagramam, K. N., Murcia, C. L., Kwon, H. Y., Pawlowski, K. S., Wright, C. G., Woychik, R. P., 2001a, The mouse Ames waltzer hearing-loss mutant is caused by mutation of *Pcdh15*, a novel protocadherin gene, *Nat. Genet*. **27**:99-102.

Alagramam, K. N., Yuan, H. J., Kuehn, M. H., Murcia, C. L., Wayne, S., Srisailpathy, C. R. S., Lowry, R. B., Knaus, R., Van Laer, L., Bernier, F. P., Schwartz, S., Lee, C., Morton, C. C., Mullins, R. F., Ramesh, A., Van Camp, G., Hagemen, G. S., Woychik, R. P., Smith, R. J. H., 2001b, Mutations in the novel protocadherin PCDH15 cause Usher syndrome type 1F, *Hum. Mol. Genet*. **10**:1709-1718.

Bitner-Glindzicz, M., Lindley, K. J., Rutland, P., Blaydon, D., Smith, V. V., Milla, P. J., Hussain, K., Furth-Lavi, J., Cosgrove, K. E., Shepherd, R. M., Barnes, P. D., O'Brien, R. A., Farndon, P. A., Sowden, J., Liu, X.-Z., Scanlan, M. J., Malcolm, S., Dunne, M. J., Aynsley-Green, A., Glaser, B., 2000, A recessive contiguous gene deletion causing infantile hyperinsulinism, enteropathy and deafness identifies the Usher type 1C gene, *Nat. Genet*. **26:**56-60.

Boëda, B., El-Amraoui, A., Bahloul, A., Goodyear, R., Daviet, L., Blanchard, S., Perfettini, I., Fath, K. R., Shorte, S., Reiners, J., Houdusse, A., Legrain, P., Wolfrum, U., Richardson, G., Petit, C., 2002, Myosin VIIa, harmonin and cadherin 23, three Usher 1 gene products that cooperate to shape the sensory hair cell bundle, *EMBO J.***21**:6689-6699.

Bolz, H., von Brederlow, B., Ramirez, A., Bryda, E. C., Kutsche, K., Nothwang, H. G., Seeliger, M., Salcedo Cabrera, M. D., Caballero Vila, M., Pelaez Molina, O., Gal, A., Kubisch, C, 2001, Mutation of *CDH23*, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D, *Nat. Genet*. **27**:108-112.

Bolz, H., Reiners, J., Wolfrum, U., Gal, A., 2002 The role of cadherins in Ca^{2+}-mediated cell adhesion and inherited photoreceptor degeneration, in: *Photoreceptors and Calcium*, W. Baehr, K. Palczewski, eds, Ronald G. Landes (Landes Bioscience), Georgetown, in press

Bork, J. M., Peters, L. M., Riazuddin, S., Bernstein, S. L., Ahmed, Z. M., Ness, S. L., Polomeno, R., Ramesh, A., Schloss, M., Srikumari, C. R. S., Wayne, S., Bellman, S., Desmukh, D., Ahmed, Z., Khan, S. N., Der Kaloustian, V. M., Li, X. C., Lalwani, A., Bitner-Glindzicz, M., Nance, W. E., Liu, X.-Z., Wistow, G., Smith, R. J. H., Griffith, A. J., Wilcox, E. R., Friedman, T. B., Morell, R. J., 2001, Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene *CDH23*, *Am. J. Hum. Genet*. **68**:26-37.

Chen, Z. Y., Hasson, T., Kelley, P. M., Schwender, B. J., Schwartz, M. F., Ramakrishnan, M., Kimberling, W. J., Mooseker, M. S., Corey, D. P., 1996, Molecular cloning and domain structure of human myosin- VIIa. The gene product defective in usher syndrome 1B, *Genomics* **36**:440-448.

Chishti, A. H., Kim, A. C., Marfatia, S. M., Lutchman, M., Hanspal, M., Jindal, H., Liu, S. C., Low, P. S., Rouleau, G. A., Mohandas, N., Chasis, J. A., Conboy, J. G., Gascard, P., Takakuwa, Y., Huang, S. C., Benz, E. J., Bretscher, A., Fehon, R. G., Gusella, A. F., Ramesh, V., Solomon, F., Marchesi, V. T., Tsukita, S., Arpin, M., Louvard, D., Tonks, N. K., Anderson, J. M., Fanning, A. S., Bryant, P. J., Woods, D. F., Hoover, K. B., 1998, The FERM domain: a unique module involved in the linkage of cytoplasmic proteins to the membrane, *Trends Biochem. Sci*. **23**:281-282.

El-Amraoui, A., Sahly, I., Picaud, S., Sahel, J., Abitbol, M., 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.

El-Amraoui, A., Schonn, J.-S., Küssel-Andermann, P., Blanchard, S., Desnos, C., Henry J.-P., Wolfrum, U., Darchen, F., Petit, C., 2002, MyRIP, a novel Rab effector, enables myosin VIIa recruitement to melanosomes and synaptic vesicles, *EMBO Rep*. **3**:463-470.

Fanning, A. S., Anderson, J. M., 1996, Protein-protein interactions: PDZ domain networks, *Curr. Biol*. **6**:1385-1388.

Garner, C. C., Nash, J., Huganir, R. L., 2000, PDZ domains in synapse assembly and signalling, *Trends Cell Biol*. **10**:274-280.

Harada, A., Teng, J., Takei, Y., Oguchi, H., Hirokawa, N., 2002, MAP2 is required for dendrite elongation, PKA anchoring in dendrites, and proper PKA signal transduction, *J. Cell Biol*. **158**:541-549.

Hasson, T., Heintzelman, M. B., Santos-Sacchi, J., Corey, D. P., 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. USA* **92**:9815-9819.

Hasson, T., Mooseker, M. S., 1997, The growing family of myosin motors and their role in neurons and sensory cells, *Cur.r Opin. Neurobiol.* **7**:615-623.

Küssel-Andermann, P., El-Amraoui, A., Safieddine, S., Nouaille, S., Perfettini, I., Lecuit, M., Cossart, P., Wolfrum, U., Petit, C., 2000a Vezatin, a novel transmembrane protein, bridges myosin VIIa to the cadherin/catenin complex, *EMBO J*. **19**:6020-6029.

Küssel-Andermann, P., El-Amraoui, A., Safieddine, S., Hardelin, J. P., Nouaille, S., Camonis, J., Petit, C., 2000b, Unconventional myosin VIIA is a novel A-kinase anchoring protein, *J. Biol. Chem*. **275**:29654-29659.

Libby, R. T., Steel, K. P., 2001, Electroretinographic anomalies in mice with mutations in Myo7a, the gene involved in human Usher syndrome type 1B, *Invest. Ophthalmol. Visual Sci*. **42**:770-778.

Liu, X. R., Vansant, G., Udovichenko, I. P., Wolfrum, U., 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.

Liu, X. R., Ondek, B., Williams, D. S., 1998, Mutant myosin VIIa causes defective melanosome distribution in the RPE of shaker-1 mice, *Nat. Genet*. **19**:117-118.

Liu, X., Udovichenko, I. P., Brown, S. D., Steel, K. P., Williams, D. S., 1999, Myosin VIIa participates in opsin transport through the photoreceptor cilium, *J. Neurosci*. **19**:6267-6274.

Mermall, V., Post, P. L., Mooseker, M. S., 1998, Unconventional myosins in cell movement, membrane traffic, and signal transduction, *Science* **279**:527-533.

Mustapha, M., Chouery, E., Torchard-Pagnez, D., Nouaille, S., Khrais, A., Sayegh, F. N., Mégarbané, A., Loiselet, J., Lathrop, M., Petit, C., Weil, D. 2002, A novel locus for Usher syndrome type I, USH1G, maps to chromosome 17q24-25, *Hum. Genet*. **110**:348-350.

Oliver, T. N., Berg, J. S., Cheney, R. E., 1999, Tails of unconventional myosins, *Cell Mol. Life Sci*. **56**:243-257.

Richardson, G. P., Forge, A., Kros, C. J., Fleming, J., Brown, S. D. M., Steel, K. P., 1997, Myosin VIIA is required for aminoglycoside accumulation in cochlear hair cells. *J. Neurosci*. **17**:9506-9519.

Richardson, G. P., Forge, A., Kros, C. J., Marcotti, W., Becker, D., Williams, D. S., Thorpe, J. R., Fleming, J., Brown, S. D., Steel, K. P., 1999, A missense mutation in myosin VIIA prevents aminoglycoside accumulation in early postnatal cochlear hair cells, *Ann. N. Y. Acad. Sci*. **884**:110-124.

Petit, C., 2001, Usher syndrome: from genetics to pathogenesis, Annu. Rev. Genomics Hum. Genet. **2**:271-297.

Reiners, J., Reidel, B., El-Amraoui, A., Boëda, B., Huber, I., Petit, C., Wolfrum, U., 2003, Spatial colocalization of the PDZ-protein harmonin with the other Usher I syndrome gene products Cadherin 23, and myosin VIIa at ribbon synapses, *submitted*.

Self, T., Mahony, M., Fleming, J., Walsh, J., Brown, S. D. M., 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.

Sellers, J. R., 2000, Myosins: a diverse superfamily, *Biochim. Biophys. Acta* **1496**:3-22.

Sheng, M., Sala, C., 2001, PDZ domains and the organization of supramolecular complexes, *Ann. Rev. Neurosci*. **24**:1-29.

Siemens, J. Kazmierczak, P., Reynolds, A., Sticker, M., Littlewood Evans, A., Müller, U., 2002, The Usher syndrome proteins cadherin 23 and harmonin form a complex by means of PDZ-domain interactions, *Proc. Natl. Acad. Sci. USA* **99**: 14946-14951.

Todorov, P. T., Hardisty, R. E., Brown, S. D. M., 2001, Myosin VIIA is specifically associated with calmodulin and microtubule-associated protein-2B (MAP-2B), *Biochem. J.* **354**:267-274.

Tuxworth, R. I., Weber, I., Wessels, D., Addicks, G. C., Soll, D. R. , Gerisch, G., Titus, M. A. 2001, A role for myosin VII in dynamic cell adhesion, *Curr. Biol*. **11**:318-329.

Udovichenko, I. P., Gibbs, D., Williams, D. S., 2002, Actin-based motor properties of native myosin VIIa, *J. Cell Sci.* **115**:445-450.

Usher, C. H., 1914, On the inheritance of Retinitis pigmentosa, with notes of cases, *R. Lond. Ophthalmol. Hosp. Rep*. **19**:130-236.

Velichkova, M., Guttman, J., Warren, C., Eng, L., Kline, K., Vogl, A. W., Hasson, T., 2002, A human homologue of *Drosophila* kelch associates with myosin-VIIa in specialized adhesion junctions, *Cell Motil. Cytoskeleton* **51**:147-164.

Verpy, E., Leibovici, M., Zwaenepoel, I., Liu, X.-Z., Gal, A., Salem, N., Mansour, A., Blanchard, S., Kobayashi, I., Keats, B. J. B., Slim, R., Petit, C. 2000, A defect in harmonin, a PDZ domain-containing protein expressed in the inner ear sensory hair cells, underlies Usher syndrome type 1C, Nature Genet. **26**:51-55.

Wang, J., Powers, N. L., Hofstetter, P., Trautwein, P., Ding, D., Salvi, R. 1997, Effects of selective inner hair cell loss on auditory nerve fiber threshold, tuning and spontaneous and driven discharge rate, *Hearing Res*. **107**:67-82.

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, Defective myosin VIIA gene responsible for Usher syndrome type 1B, Nature **374**:60-61.

Weil, D., Küssel, P., Blanchard, S., Levy, G., Leviacobas, F., Drira, M., Ayadi, H., Petit, C., 1997, The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene, *Nat. Genet*. **16**:191-193.

Wolfrum, U., Liu, X. R., Schmitt, A., Udovichenko, I. P., Williams, D. S., 1998, Myosin VIIa as a common component of cilia and microvilli, *Cell Motil. Cytoskeleton* **40**:261-271.

Wolfrum, U., Schmitt, A., 1999, Evidence for myosin VIIa driven transport of rhodopsin in the plasma membrane of the photoreceptor-connecting cilium, in: *Retinal degeneration diseases and experimental therapy*, J. G. Hollyfield, R. E. Andersson, M. LaVail, eds, Kluwer Academic/Plenum Publishers, New York, pp. 3-14.

Wolfrum, U., Schmitt, A. 2000, Rhodopsin transport in the membrane of the connecting cilium of mammalian photoreceptor cells, *Cell Motil. Cytoskeleton*. **46**:95-107.

Wolfrum, U., El-Amraoui, A., Fath, K. R., Petit, C., Schmitt, A., 2001, Identification and characterization of the membrane-associated actin cytoskeleton in the photoreceptor cilium, *Invest. Ophthalmol. Vis. Sci*. **42**:296