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Usher syndrome: molecular links of pathogenesis, proteins and pathways

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Usher syndrome is the most common form of deaf-blindness. The syndrome is both clinically and genetically heterogeneous, and to date, eight causative genes have been identified. The proteins encoded by these genes are part of a dynamic protein complex that is present in hair cells of the inner ear and in photoreceptor cells of the retina. The localization of the Usher proteins and the phenotype in animal models indicate that the Usher protein complex is essential in the morphogenesis of the stereocilia bundle in hair cells and in the calycal processes of photoreceptor cells. In addition, the Usher proteins are important in the synaptic processes of both cell types. The association of other proteins with the complex indicates functional links to a number of basic cell-biological processes. Prominently present is the connection to the dynamics of the actin cytoskeleton, involved in cellular morphology, cell polarity and cell–cell interactions. The Usher protein complex can also be linked to the cadherins/catenins in the adherens junction-associated protein complexes, suggesting a role in cell polarity and tissue organization. A third link can be established to the integrin transmembrane signaling network. The Usher interactome, as outlined in this review, participates in pathways common in inner ear and retina that are disrupted in the Usher syndrome.

INTRODUCTION

Usher syndrome (MIM nos 276900-2, 276905 and 605472) is the most common form of deaf-blindness with a prevalence of ~1/20 000 and represents 50% of the cases with deaf-blindness (1–3). The hearing loss in the patients is sensorineural and most severe for high frequencies. Loss of vision is due to retinitis pigmentosa (RP), a progressive retinal degeneration leading to blindness (4–6). Usher syndrome can be associated with vestibular dysfunction, reduced odor identification and sperm motility and mental deficiency, cerebral atrophy and ataxia (reviewed in 4,7).

Three clinical subtypes of the syndrome are distinguished, mainly on the basis of the severity and progression of the hearing loss and the age of onset of RP (8). Usher syndrome type I (USH1) is the most severe with congenital severe to profound hearing loss and a prepubertal onset of RP. Usher syndrome type II (USH2) is the most common subtype and characterized by congenital moderate to severe hearing loss and onset of RP during or after puberty. Hearing loss is progressive in Usher type III (USH3), and onset of RP is variable. To date, five genes and two loci are known for USH1, two genes for USH2 and one gene and one locus for USH3 (Table 1; reviewed in 7). Mutations in the genes associated with USH1B, C, D, and F are also associated with non-syndromic hearing loss, and mutations in the *USH2A* gene are the most frequent cause of autosomal recessive RP (9,10) (Table 1; reviewed in 4,7,11).

USHER PROTEINS: THEIR STRUCTURE AND ISOFORMS

The proteins encoded by the Usher genes (Fig. 1) are members of protein classes with very different functions. Myosin VIIa is a motor protein, harmonin and SANS (scaffold protein containing ankyrin repeats and SAM domain) are scaffold proteins, cadherin 23 and protocadherin 15 are cell adhesion molecules and USH2A/usherin (isoform B) and VLGR1b (very large G-coupled protein receptor isoform b) are

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Table 1. Usher syndrome subtypes and associated genes and proteins

USH subtype	Location	Gene	Protein	Additional phenotype
USH1B	11q13.5	*MYO7A*	Myosin VIIa	DFNA11, DFNB2
USH1C	11p15.1	*USH1C*	Harmonin	DFNB18
USH1D	10q22.1	*CDH23*	Cadherin 23	DFNB12
USH1E	21q21	Unknown		
USH1F	10q21.1	*PCDH15*	Protocadherin 15	DFNB23
USH1G	17q25.1	*USH1G*	SANS	
USH2A	1q41	*USH2A*	USH2A	RP15
USH2C	5q14.3	*VLGR1*	VLGR1	Febrile seizures
USH3A	3q25.1	*USH3A*	Clarin-1	
USH3B	20q	Unknown		

The formerly described USH1A and USH2B loci have recently been withdrawn.

transmembrane proteins that could be involved in outside-in signaling. The protein encoded by the *USH3A* gene, clarin-1, is a member of the vertebrate-specific clarin family of four-transmembrane-domain proteins (12).

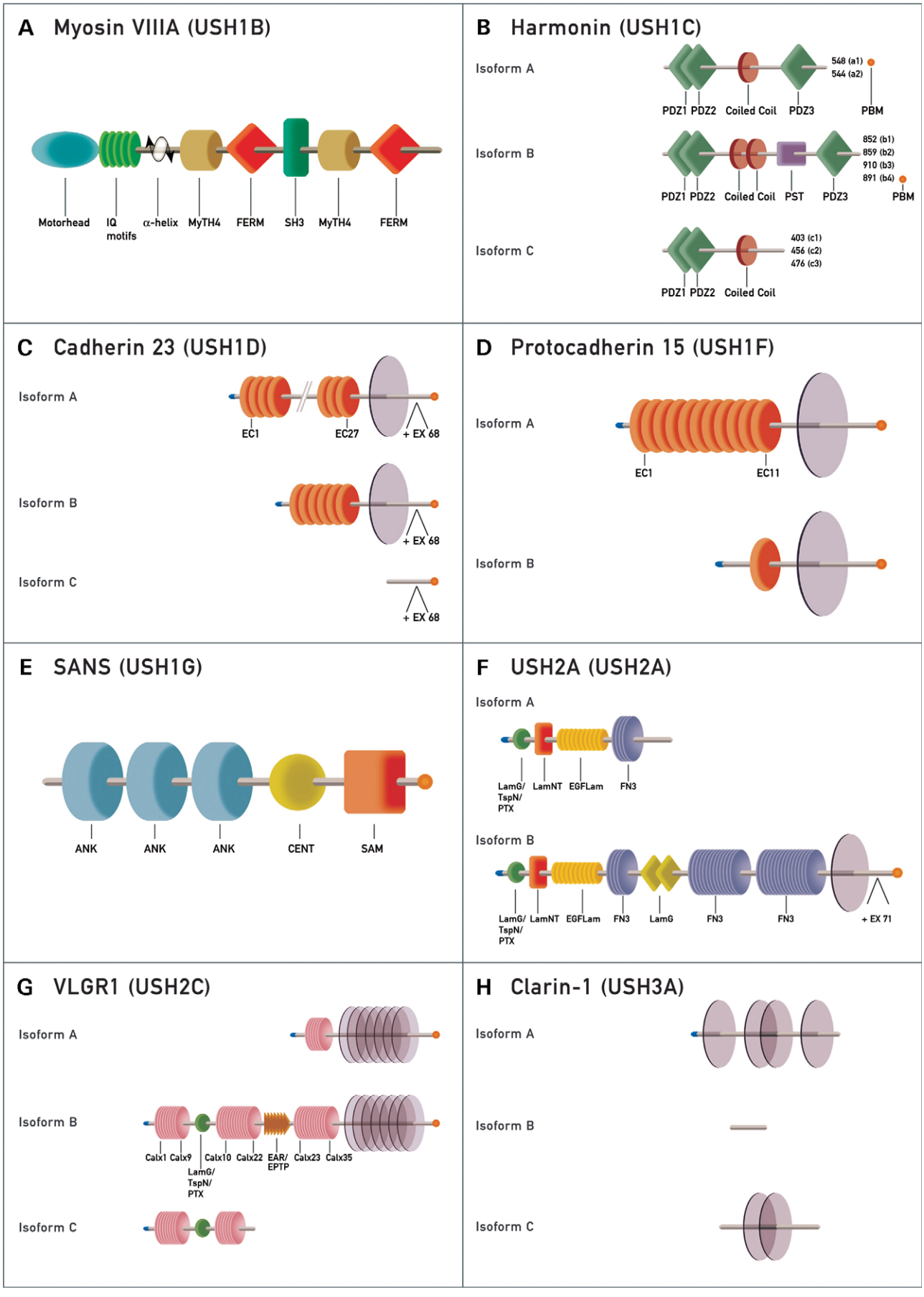
HARMONIN AND WHIRLIN MEDIATE THE FORMATION OF AN USHER PROTEIN MULTIASSEMBLY COMPLEX

Mutations in the different Usher genes can lead to a broad spectrum of phenotypes in the ear and eye, but recent reports provide evidence for the existence of an integrated Usher protein network in both the inner ear and the retina (13–15) (Fig. 2). In this network, the USH1 and USH2 proteins are thought to be assembled in a multiprotein scaffold, with a central role for the PDZ domain containing protein homologs, harmonin and whirlin. Therefore, the gene encoding whirlin, *DFNB31*, was recently proposed as a functional candidate gene for Usher syndrome (15). In most of the cases, these two protein homologs bind with one or more of their PDZ domains to either a C-terminal class I PDZ binding motif (PBM; -X[ST]X[VIL]$^{-COOH}$ (16) or to internal PDZ binding domains of their interacting partners. To date, all proteins in this network that are associated with USH1 (myosin VIIa, cadherin 23, SANS and protocadherin 15) or type 2 (USH2A and VLGR1b) have been described to interact with both harmonin and whirlin (Fig. 2). Binding of VLGR1b (14,15), usherin/USH2A isoform B (14,17) and protocadherin 15 (18) to the PDZ domains of whirlin and/or harmonin was found to be strictly dependent on their C-terminal class I PBM, whereas binding of cadherin 23 (19) involves both a class I C-terminal PBM and an internal PBM resembling the PBM of RIL (20). As myosin VIIa does not contain a C-terminal PBM, its binding relies on one or more putative internal PBMs (21). Myosin XVa (22) and SANS (13) do contain a conserved C-terminal class I PBM, but their binding to harmonin and/or whirlin is not visibly affected by deletion of this motif, indicating that also one or more (putative) internal PBMs are involved in the binding. SANS (13) and protocadherin 15 (23) were also found to interact with myosin VIIa.

Although harmonin and whirlin share many protein partners, different partners have been identified for either harmonin [NBC3 (14,15), MCC2 (24), harp (25), DOCK4 (26), actin (21) and β-catenin (7,25)] or whirlin [NGL1 (22), CASK (27) and myosin XVa (22,28)], indicating that their binding repertoire is not fully overlapping (Fig. 2). It is likely that at least a subset of these will also bind to the other protein homolog, as in many of these proteins a conserved class I PBM can be identified. However, although the PDZ domains of whirlin and harmonin are quite homologous (15), differences in protein–protein interactions have been found. Both PDZ1 and 2 domains of whirlin were found to bind to USH2A isoform b (15), whereas this protein only binds to PDZ1 of harmonin (14). Furthermore, the harmonin interactor NBC3, binding to PDZ1 of harmonin, was found not to interact with whirlin (15).

Besides these protein–protein interactions, many of the proteins in this network, such as harmonin (13,19), whirlin (22), SANS (13), NGL-1 (22), RIα of PKA (29) and PHR1 (30), have been shown to form homodimers. The dimerization properties of myosin VIIa are under debate (31–33). In addition, different isoforms derived from splicing variation have been identified for many of the proteins, including the central organizers whirlin and harmonin, thus increasing the complexity of this interactome. This network very likely will expand in the future. For example, the Usher type III protein clarin-1, in line with the type I and type II proteins, is expected to belong to the network (12).

Figure 1. Outline of the Usher proteins and their different isoforms. (**A**) The Usher 1B protein, myosin 7a, consists of a motor head domain, five calmodulin-binding IQ motifs, two FERM domains, two MyTH4 domains and an Src homology 3 (SH3) domain. **(B)** The USH1C protein, harmonin, of which three different classes of isoforms are identified. All three isoforms consist of two PDZ (PSD95, discs large, ZO-1) domains (PDZ1 and 2) and one coiled-coil domain. In addition, class A isoforms contain an additional PDZ domain (PDZ3). The class B isoforms contain also this third PDZ domain, a second coiled-coil domain and a proline, serine, threonine-rich region (PST). Isoforms A1 and B4 contain a C-terminal class I PDZ binding motif (PBM). (**C**) Cadherin 23 (USH1D) is represented by three different isoforms. Isoform A is composed of 27 Ca^{2+}-binding extracellular cadherin domains (EC1-27), a transmembrane domain (grey disks) and a short intracellular domain with a C-terminal class I PBM. Isoform B is similar to isoform A, but only contains the last six EC domains. Isoform C only consists of the intracellular domain and C-terminal PBM. (**D**) Like cadherin 23, the non-classical cadherin protocadherin 15 (USH1F) consists of either 11 (isoform A) or one (isoform B) EC domain, a transmembrane domain and a C-terminal class I PBM. (**E**) The scaffold protein SANS (USH1G) consists of three ankyrin domains (ANK), a central region (CENT), a sterile alpha motif (SAM) and a C-terminal class I PBM. **(F)** Isoform A of the Usher 2A protein (USH2A) contains an N-terminal thrombospondin/pentaxin/laminin G-like domain, a laminin N-terminal (LamNT) domain, ten laminin-type EGF-like (EGF Lam) and four fibronection type III (FN3) domains. In addition to this region, isoform B contains two laminin G (LamG), 28 FN3, a transmembrane domain and an intracellular domain with a C-terminal class I PBM. (**G**) Isoform B of the very large G-coupled protein receptor, VLGR1 (USH1C), contains a thrombospondin/pentaxin/laminin G-like domain, 35 Ca^{2+}-binding calcium exchanger β (Calx) domains, seven EAR/EPTP repeats, a seven-transmembrane region and an intracellular domain containing a C-terminal class I PBM. (**H**) Clarin-1, the USH3A protein, only contains four (isoform A) or one transmembrane (isoform C) domain.


A Myosin VIIIA (USH1B)
Motorhead
IQ motifs
α-helix
MyTH4
FERM
SH3
MyTH4
FERM
B Harmonin (USH1C)
Isoform A
548 (a1)
544 (a2)
PDZ1 PDZ2 Coiled Coil PDZ3 PBM
Isoform B
852 (b1)
859 (b2)
910 (b3)
891 (b4)
PDZ1 PDZ2 Coiled Coil PST PDZ3 PBM
Isoform C
403 (c1)
456 (c2)
476 (c3)
PDZ1 PDZ2 Coiled Coil
C Cadherin 23 (USH1D)
Isoform A
EC1
EC27
+ EX 68
Isoform B
+ EX 68
Isoform C
+ EX 68
D Protocadherin 15 (USH1F)
Isoform A
EC1
EC11
Isoform B
E SANS (USH1G)
ANK
ANK
ANK
CENT
SAM
F USH2A (USH2A)
Isoform A
LamG/ TspN/ PTX
LamNT
EGFLam
FN3
Isoform B
LamG/ TspN/ PTX
LamNT
EGFLam
FN3
LamG
FN3
FN3
+ EX 71
G VLGR1 (USH2C)
Isoform A
Isoform B
Calx1
Calx9
LamG/ TspN/ PTX
Calx10
Calx22
EAR/ EPTP
Calx23
Calx35
Isoform C
H Clarin-1 (USH3A)
Isoform A
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Isoform C

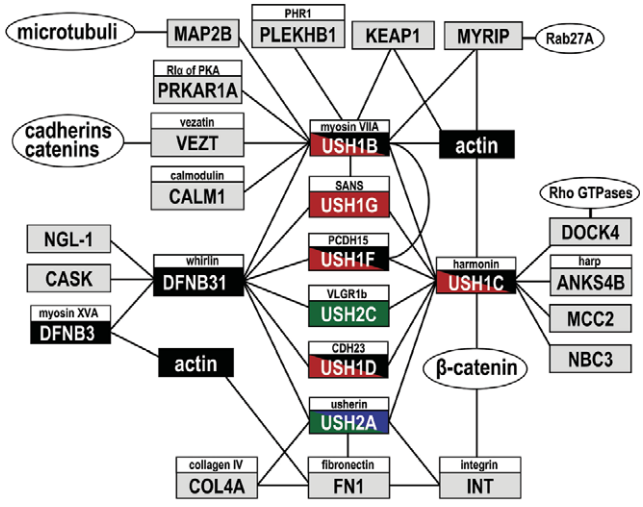

Figure 2. The Usher protein network. All identified protein–protein interactions are indicated. Red colored boxes indicate association with Usher syndrome, blue color indicates association with isolated RP and black indicates association with isolated deafness. The binding of cadherin 23 and protocadherin 15 to whirlin has been identified in a yeast two-hybrid assay (van Wijk *et al*., unpublished data).

LOCALIZATION OF THE USHER PROTEIN COMPLEX IN INNER EAR AND RETINA

The Usher protein complex in inner ear and retina seems to have its major function in the neurosensory cells, respectively, in the hair cells and photoreceptor cells (Fig. 3). In the inner ear, hair bundles are located at the apical surface of both the auditory and vestibular hair cells. The displacement of the hair bundle by a sound wave opens the mechanotransduction channel at the tip of the stereocilia, which initiates the signaling cascade for sound perception (34,35). The hairs, or stereocilia, develop from microvilli and have a stiff core of parallel actin filaments anchored in the cuticular plate, a meshwork of horizontal actin filaments beneath the apical cell membrane. The kinocilium, a true cilium, is connected to the developing stereocilia bundle and essential for its orientation. The morphogenesis of hair cells is reviewed in (36,37). The major sites of colocalization of Usher proteins in the inner ear are the stereocilia and the synaptic regions of hair cells (13–15,19,21,23). In addition, the spiral ganglion neurons harbor several of the Usher proteins such as USH2A (15), protocadherin 15 (38) and USH3A (12). In the cuticular plate, harmonin and myosin VIIa are co-expressed (21,39–41). Cadherin 23 is also found in Reissner's membrane (42,43) and SANS, protocadherin 15 and USH2A in the supporting cells (13,17,38).

In the retina, the visual signaling cascade is associated with disk membranes within the outer segments of the photoreceptor cells. Its activation leads to a hyperpolarization of photoreceptor cells and the reduction of neurotransmitter release at their synapses, located in the outer plexiform layer (OPL) (44). The USH proteins colocalize in this synaptic layer (7,14,15,18,45), as well as in the ciliary region between the outer and inner segments, more particularly in the connecting cilium, and the calycal processes (Fig. 4) (14,15,41,46–48). Protocadherin 15, USH2A and VLGR1 have also been detected in the outer limiting membrane, the region of adherens junctions (14,15) between photoreceptor cells and Müller glia cells. Myosin VIIa is also located, although under debate, in the OPL (7) and in the retinal pigment epithelium (48). Harmonin and protocadherin 15 are also present in the outer segments (18,45), and harmonin and SANS were found to colocalize in the inner segments (7,45).

THE USHER PROTEIN COMPLEX IN STEREOCILIA DEVELOPMENT

The phenotype of *shaker, waltzer*, *deaf-circler*, *ames waltzer* and *Jackson shaker* mice, which harbor a mutation in one of the USH1 genes, indicate that several of the Usher proteins are essential for the development and cohesion of the stereociliar bundle of hair cells in both the cochlea and the vestibular organ (reviewed in 4,11). During growth and maturation and also in adulthood, stereocilia maintain their cohesion by fibrous interstereociliar links and by links with the kinocilium. In addition, the stereocilia are covered with the cell coat material during development, also described as shaft connectors, and at the tip with the tectorial membrane attachment crown. The type of links in the hair bundle rapidly changes during development in a species- and hair cell type-specific manner (reviewed in 49). From the earliest stages of stereocilia development in the outer hair cells of mouse cochlea, transient lateral links are present that diminish at early postnatal stages when ankle links appear at the base of the stereocilia. Subsequently, the latter diminish and horizontal top connectors appear that are maintained in adulthood. The tip link that connects the tip of a stereocilium to the shaft of the neighboring taller stereocilium is present from E17.5 onwards. The general figure that emerges for the localization of Usher proteins in stereociliar development is that the large extracellular regions of the transmembrane proteins cadherin 23, protocadherin 15, USH2A isoform b and VLGR1b are part of the links that are intracellularly attached to the scaffold proteins harmonin and/or whirlin. These scaffold proteins are directly or indirectly (via myosin VIIa, myosin XV and/or vezatin) connected to the actin core of the stereocilia [for discussion see also (11)]. The molecular composition of the different types of links is being elucidated on the basis of the spatiotemporal expression, immuno-histochemistry and immuno-electronmicroscopy. Cadherin 23 has been shown to be a component of the transient lateral and kinocilial links and, although still under debate, might be a component of the tip link (50–52). For USH2A and VLGR1, evidence is emerging that they are components of the ankle links (17,53). Protocadherin 15 might also be part of these links, although not essential (23). In addition, the myosin VIIa-binding protein vezatin has been suggested to be associated with the ankle links (54) and, interestingly, whirlin appears at the base of the stereocilia in the period during which ankle links are present (22). This suggests that whirlin might be involved in the anchoring of the ankle links through its association with USH2A and VLGR1 and with myosin VIIa (15,17). The actin bundling and stabilization activity of

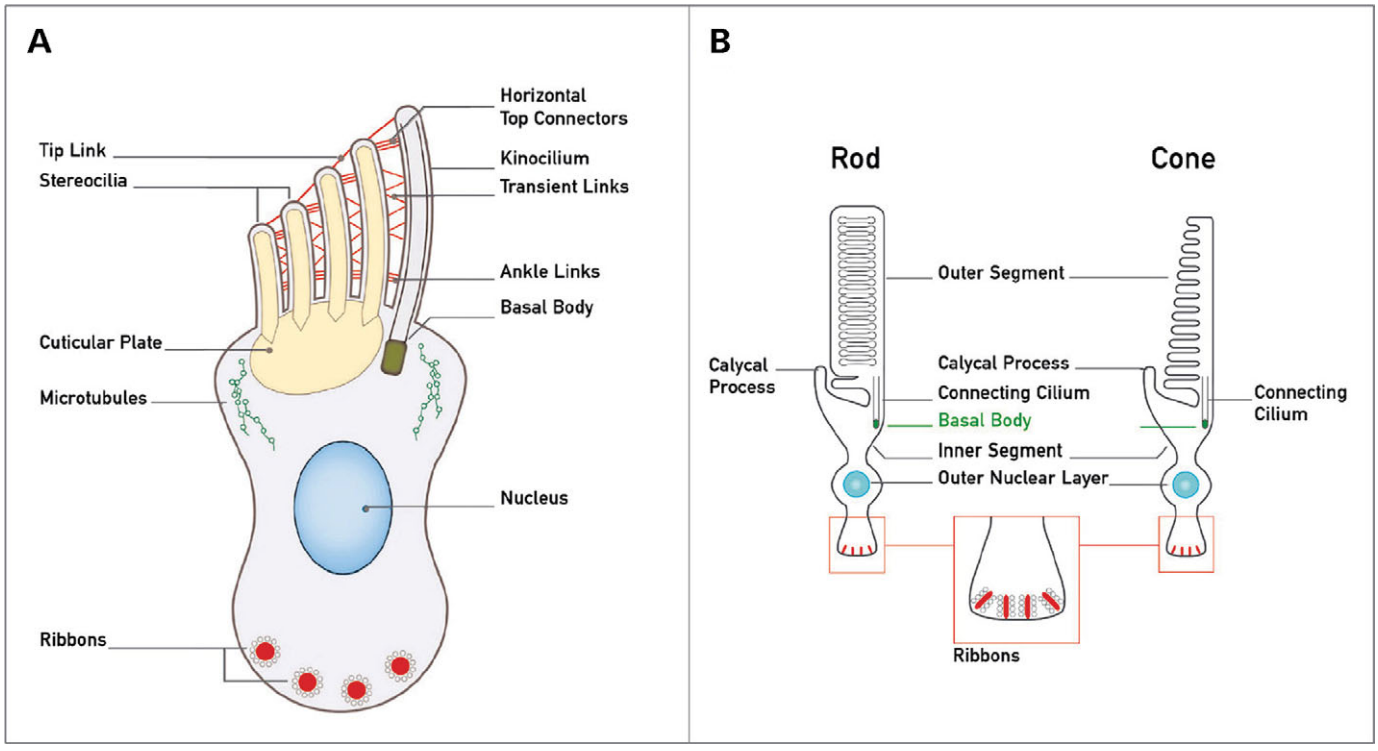

Figure 3. Diagram of the sensory cells in the inner ear and retina. (**A**) The apical side of the inner ear hair cell carries the highly organized, actin-filled stereocilia, in which the mechanotransduction takes place. The stereocilia are kept together by the tip links, horizontal links and ankle links. The stereocilia are anchored in the actin-rich cuticular plate. The kinocilium is located lateral to the largest stereocilium and is formed from the basal body. The synaptic junction between hair cell, efferent and afferent neurons at the basal side of the hair cell, contains the ribbons. (**B**) The rod and cone photoreceptors, which are the main morphological subtypes of photoreceptor cells, are highly polarized. The photoreceptor outer segment, a modified cilium containing the phototransduction proteins, is separated from the inner segment by the connecting cilium. The calycal processes are situated next to the proximal outer segment. The nuclei of the photoreceptor cells are situated in the outer nuclear layer. The synaptic terminals, containing the ribbons, connect the photoreceptors with horizontal cells, bipolar cells and ganglion cells.

harmonin b might contribute to the growth of the stereocilia (21). The scaffold protein SANS, not present in stereocilia but in the kinocilium of OHC of P3 mice (17), might have a role in anchoring kinociliar links via myosin VIIa.

SANS is mainly concentrated below the cuticular plate in IHCs and in the OHCs, especially below the kinociliar basal body where the cuticular plate is thinner (13). The localization of SANS overlaps with the concentration of vesicles and microtubules in the apical peri- and subcuticular regions including the subkinocilial region (55). Therefore, SANS has been suggested to function in the trafficking of the Usher proteins along microtubules and actin filaments toward the stereocilia and kinocilium (7,11,13). Indeed, in mice defective for SANS or myosin VIIa, harmonin b is absent from the stereocilia and accumulates in the apical region of the hair cells (11,21).

USHER PROTEINS AT SYNAPTIC REGIONS OF PHOTORECEPTOR AND HAIR CELLS

All Usher proteins (except for clarin-1) have been shown to be present in the OPL of the mouse and/or rat retina (7,14,18,45). For the inner ear, the localization of Usher proteins at the synaptic terminals of the neurosensory cells has been less extensively studied. The presence of harmonin, SANS, USH2A and VLGR1 at these sites has been reported for both IHCs and OHCs (13,14). Whirlin was demonstrated in the synaptic region of OHCs only (15). It has not been sorted out yet whether the USH proteins function at the pre- or postsynaptic sides of synapses or at both sides. Also, it remains to be determined whether Usher proteins function directly in the synaptic ribbon. For whirlin, this is unlikely because it was only detected in the OHC synaptic region which only contains ribbon synapses in the apical coil of the cochlea (15). Only for *Myo7a* mutants, the ultrastructure of synaptic regions of hair cells, more specifically the ribbon synapses in IHCs and OHCs, has been investigated and found to be normal (40). Thus, we can only speculate about the function of the Usher protein complex in synapses. Myosin VIIa has been suggested to have a role in either transporting synaptic molecules or in endocytosis (56). Cadherin 23, protocadherin 15, USH2A and VLGR1, anchored by harmonin, SANS or whirlin, might function in keeping the synaptic membranes closely apposed by homo- or heterotypic interaction of their large extracellular regions (7,15,57). In addition, VLGR1, being a G-coupled protein receptor, might be involved in signaling and in ion homeostasis because of its Ca^{2+}-binding calcium exchanger β-domains.

PDZ proteins are known to be a major component of excitatory synapses, and several members of the PSD-95

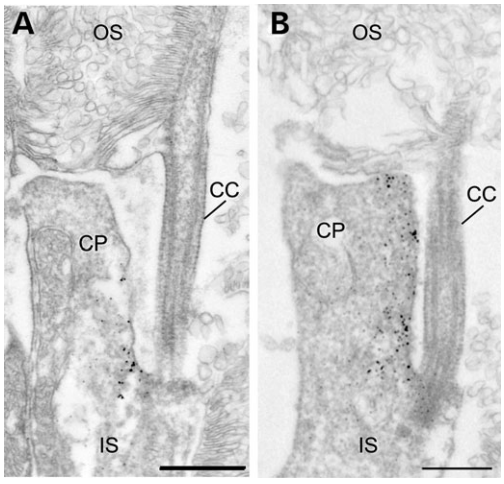

Figure 4. Subcellular localization of USH2A and whirlin in the ciliary region of photoreceptor cells. Electron micrographs of mouse photoreceptor cells illustrating silver-enhanced immunogold labeling of (**A**) USH2A and (**B**) whirlin in longitudinal sections of the outer segment (OS) and the apical inner segment (IS) of a rod photoreceptor cell. Labeling is restricted to the periciliary region in calycal processes (CP) of IS facing the connecting cilium (CC). Scale bars: 0.5 μm.

(postsynaptic density protein 95) family of PDZ proteins are differentially expressed in the synaptic regions of inner ear hair cells and photoreceptors (58,59). Whether harmonin, whirlin and SANS are specifically involved in the anchoring of Usher proteins in the synaptic region or also have a scaffolding function for other components of the large network of synaptic proteins, such as several types of Ca^{2+}- and K^{+}-channels or neurotransmitter receptors, remains to be determined. The association and colocalization of harmonin and NBC3 at the synaptic regions of both hair cells and photoreceptor cells suggest this to be the case (14). As PDZ protein scaffolds are also emerging as regulators of dynamic synaptic processes controlling its strength, structure and plasticity (reviewed in 60), a harmonin/whirlin organized scaffold might be involved in similar processes. They might also play a role in the transport of cargo vesicles to the synapse by contacting molecular motors, myosin VIIa, for example, as suggested for other PDZ proteins (60), and comparable with the function that has already been suggested for SANS and myosin VIIa in the sub/pericuticular region.

THE USHER PROTEIN COMPLEX FUNCTIONS IN THE CALYCAL PROCESSES OF PHOTORECEPTOR CELLS

Cadherin 23 and the ankle link antigen, recently shown to be VLGR1 (46,47,53), were previously described to be localized in the fibrous links that connect the calycal processes of photoreceptor cells to the connecting cilium membrane and outer segments. The calycal processes are microvilli-like extensions from the apical region of inner segments that surround the basis of the outer segments and actin filaments extend into these calycal processes (61,62). Analogous to the stereociliar links, VLGR1 and also USH2A and whirlin are located at the more proximal part of the calycal processes in the so-called periciliary ridge complex of mammalian photoreceptor cells (Fig. 4 and Märker *et al*., manuscript in preparation). This periciliary ridge region corresponds to the docking side of post-Golgi vesicles (e.g. opsin transport carriers), which are translocated through the inner segment to the apical membrane, for further delivery through the connecting cilium to the outer segment (reviewed in 63). In contrast, recent investigations indicate the localization of protocadherin 15 more distally in the calycal processes (Märker and Wolfrum, unpublished observations). Myosin VIIa was not reported to be present in these structures, but myosin IIIa might be its functional homolog there (64). Mutations in the *MYO3A* gene cause hearing loss, but not visual impairment (65). Not much is known about the function of the calycal processes, but a role in rod outer segment disk morphogenesis has been suggested (66). The localization of several members of the Usher protein complex in the fibrous links associated with the calycal processes suggests a structural role of the complex analogous to that in the stereocilia.

THE USHER PROTEIN COMPLEX AT CROSSROADS OF CELLULAR FUNCTION

Many of the reported interactions at different sites in the inner ear and retina have functional implications for these organs. Some important links need to be highlighted that connect the Usher complex to basic cellular pathways. These are indicative of a conserved role in the eye and the ear. Very prominently present in the complex are multiple links to the actin cytoskeleton, i.e. through binding of actin to harmonin (21), to myosin XVa (67), to the USH2A interactors fibronectin (68,69) and integrin (70), and to myosin VIIa (33,71) as well as to its interactors MyRIP/Slac2-c (72,73) and KEAP1 (74,75). The actin cytoskeleton is essential in cellular morphology, polarity, motility and in cell-to-cell interaction (76). Actin renewal in stereocilia was found to follow a treadmill mechanism, shaping the functional architecture of the stereocilia bundle and thus regulating its length (67), a process that also involves the unconventional myosins VI, VIIa, XVa and X (77). Similar actin- and myosin-guided processes are also essential in neuronal morphogenesis and structural plasticity of adult neurons, and these processes are subserved by signaling of the Rho family of small GTPases (78). This signaling was recently also connected to the Usher interactome through the harmonin interactor DOCK4, a guanine nucleotide exchange factor (GEF) for Rho GTPase and a potent Rac activator (26). The presence of the Usher protein complex in the photoreceptor synaptic region of the retina, the OPL, and in the ribbon synapses in the inner ear (14,15,18,45) is fully in line with these findings.

Besides playing an important role in the architecture of the actin cytoskeleton, the Usher protein network can also be connected to the cadherins/catenins in the adherens junction-associated protein complexes. Although the direct interaction of the members of the cadherin superfamily in this complex, protocadherin 15 and cadherin 23, has not yet been demonstrated, harmonin (7,25) and the USH2A interactor integrin (79,80) were found to interact with β-catenin,

whereas vezatin was found to link myosin VIIa to the cadherins/catenins in the adherens junctional protein complexes in inner ear hair bundles (54). In view of the recently revised model of cadherin–catenin–actin association (81,82), the Usher proteome may be involved in this process in multiple ways, both by participating in the actin renewal, as described before, and by providing the direct actin links with other junction proteins, which could be one or more of the transmembrane proteins present in the network. The stimulation of the formation of adherens junctions by DOCK4 activation of the Rap GTPase (83) is fully in line with such a connection. The identification of the transmembrane proteins USH2A and VLGR1, as well as whirlin at the actin-rich outer limiting membrane of the retina (15), the site of the photoreceptor-Müller cell junctions, would match with a role of the Usher protein complex in the adherens junctions and associated processes in the retina. The binding of USH2A to fibronectin, integrin (84) and collagen IV (85) would enhance the actin–catenin–cadherin connection and anchor it to the extracellular matrix.

The presence of integrin in the network would also provide means for bidirectional signaling through the cell membrane as a response to adhesive ligands (86), as well as a role in Rho signaling-directed microtubule stabilization (87). Although it is unknown what the specific implications are of the link of the Usher protein complex with the microtubuli, it physically links to this important cytoskeletal structure through binding to the microtubule associated protein-2B (88). Its interacting motor protein, myosin VIIa, has also been shown to enable opsin transport through the microtubuli (89,90) and although this was indicated to be an actin-based transport mode (89), cooperation between actin and microtubule-based motor proteins has been described (91).

Finally, already in the absence of any knowledge on molecular defects in Usher syndrome, it was suggested to be due to defects in cilia structure or function (92), and RP is a characteristic of several of the disorders, such as Bardet-Biedl syndrome, which are caused by defects in proteins localized to cilia or basal bodies (93). Recently, it was shown that disruption of several of the ciliary proteins involved in Bardet-Biedl syndrome perturbs planar cell polarity illustrated by the disrupted orientation of the stereocilia bundles and a genetic interaction between BBS genes and the planar cell polarity gene Vangl2 in mouse and zebrafish. In accordance with this, the Vangl2 protein was found to be present in the basal body and axoneme of ciliated cells (94). Some signs of disturbed planar cell polarity have also been described for mice that have a mutation in one of the Usher genes, and cadherin 23, probably isoform c, is present in the basal body/centrosomes of cochlear and vestibular hair cells already during development and in the greater epithelial ridge of the cochlea (42). Preliminary data indicate that also other Usher proteins are present in the basal body, in this case, the basal body of the connecting cilium. The localization of whirlin, protocadherin 15, USH2A and VLGR1 in the outer limiting membrane (15,18) also suggests a connection of the Usher protein complex to apico-basal cell polarity. This might be supported by the localization of members of the crumbs protein family in the basal body of hair cell kinocilia and the presence of other apico-basal cell polarity determinants in cilia (95).

In summary, the outline of the Usher interactome, as shown in this review, reveals important common denominators that could account for the common phenotypic characteristics in the inner ear and retina of Usher syndrome patients. In addition, it points out that the genes encoding different partners in the complex are, by association, candidate genes for Usher syndrome, neurosensory deafness and retinal degeneration.

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