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Molecular basis of human Usher syndrome: Deciphering the meshes of the Usher protein network provides insights into the pathomechanisms of the Usher disease

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Abstract

Usher syndrome (USH) is the most frequent cause of combined deaf-blindness in man. It is clinically and genetically heterogeneous and at least 12 chromosomal loci are assigned to three clinical USH types, namely *USH1A-G, USH2A-C, USH3A* (Davenport, S.L.H., Omenn, G.S., 1977. The heterogeneity of Usher syndrome. Vth Int. Conf. Birth Defects, Montreal; Petit, C., 2001. Usher syndrome: from genetics to pathogenesis. Annu. Rev. Genomics Hum. Genet. 2, 271–297). Mutations in USH type 1 genes cause the most severe form of USH. In USH1 patients, congenital deafness is combined with a pre-pubertal onset of retinitis pigmentosa (RP) and severe vestibular dysfunctions. Those with USH2 have moderate to severe congenital hearing loss, non-vestibular dysfunction and a later onset of RP. USH3 is characterized by variable RP and vestibular dysfunction combined with progressive hearing loss. The gene products of eight identified USH genes belong to different protein classes and families. There are five known USH1 molecules: the molecular motor myosin VIIa (USH1B); the two cell–cell adhesion cadherin proteins, cadherin 23 (USH1D) and protocadherin 15, (USH1F) and the scaffold proteins, harmonin (USH1C) and SANS (USH1G). In addition, two USH2 genes and one *USH3A* gene have been identified. The two USH2 genes code for the transmembrane protein USH2A, also termed USH2A (''usherin'') and the G-protein-coupled 7-transmembrane receptor VLGR1b (USH2C), respectively, whereas the USH3A gene encodes clarin-1, a member of the clarin family which exhibits 4-transmembrane domains. Molecular analysis of USH1 protein function revealed that all five USH1 proteins are integrated into a protein network via binding to PDZ domains in the USH1C protein harmonin. Furthermore, this scaffold function of harmonin is supported by the USH1G protein SANS. Recently, we have shown that the USH2 proteins USH2A and VLGR1b as well as the candidate for USH2B, the sodium bicarbonate co-transporter NBC3, are also integrated into this USH protein network. In the inner ear, these interactions are essential for the differentiation of hair cell stereocilia but may also participate in the mechano-electrical signal transduction and the synaptic function of maturated hair cells. In the retina, the co-expression of all USH1 and USH2 proteins at the synapse of photoreceptor cells indicates that they are organized in an USH protein network there. The identification of the USH protein network indicates a common pathophysiological pathway in USH. Dysfunction or absence of any of the molecules in the mutual ''interactome'' related to the USH disease may lead to disruption of the network causing senso-neuronal degeneration in the inner ear and the retina, the clinical symptoms of USH.

Keywords: senso-neuronal disease; retinal degeneration; retinitis pigmentosa; deafness; supramolecular network; interactome

1. Introduction

Human communication and perception of the environment are mainly formulated on information imported through the ear and the eye. Chronic diseases affecting the inner ear and the retina cause severe impairments of our communication systems. There are about 40 known human syndromes which include the symptoms of blindness in combination with deafness. In more than half of the cases, the Usher syndrome is the origin of this defect (Gorlin, 1995; Vernon, 1969). The human Usher syndrome (USH) is defined by congenital, bilateral deafness and a later onset of the loss of the visual field, caused

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by *retinitis pigmentosa* (RP). In RP, retinal degeneration is based on photoreceptor cell death which occurs from the periphery to the macula of the retina. Night blindness is the first symptom of RP, followed by narrowing of the visual field (''tunnel vision'') and later to complete blindness (van Soest et al., 1999; Wang et al., 2005). These visual deficits are triggered by any one of over 130 mutated genes (Tschernutter et al., 2005). USH is the most common cause of combined deaf-blindness (Vernon, 1969) and the most frequent form of recessive RP (Keats and Corey, 1999).

One of the earliest descriptions of USH was given by Albrecht von Graefe, a pioneer of modern ophthalmology. He reported a case of a deaf and dumb male patient with retinal degeneration who had two equally affected brothers (von Graefe, 1858). Subsequently his student Richard Liebreich, screened the population of Berlin for syndromes including RP and reported similar observations (Liebreich, 1861). He emphasized the recessive nature of the disease by commenting on the combination of congenital deafness with RP in several siblings from either consanguineous marriages or families with several members affected in different generations. The disease was eventually named after Charles Usher, a Scottish ophthalmologist who described the hereditary nature of this disorder in 19 cases out of 69 RP patients (Usher, 1914).

Based on the heterogenic clinical course of the disease described by Bell (1922) and Hallgren (1959) and co-workers, USH was subdivided into three clinical types, namely USH1, USH2 and USH3 (Davenport and Omenn, 1977). USH type 1 is the most severe form of this disease. USH1 patients are deaf at birth and the onset of RP is pre-pubertal. Most, but not all USH1 patients exhibit severe dysfunction of the vestibular system which leads to a further subdivision of USH type 1 (Otterstedde et al., 2001). USH type 2 is characterized by a constant moderate to severe hearing impairment from birth on and RP can be diagnosed during puberty (Reisser et al., 2002). USH type 3 (USH3) is distinguished from USH1 and USH2 by the later initiation of deafness combined with variable RP and vestibular dysfunction. In USH3 patients, the hearing impairment is progressive starting post-lingual and RP is diagnosed in most cases between the 2nd and 4th decade of life (Pakarinen et al., 1995; Petit, 2001). The classification into three USH types is still being used, although the increasing scientific knowledge through the end of the last century has revealed an even larger genetic heterogeneity to USH. To date, 12 independent loci on different chromosomes have been identified whose inherited defects lead to the development of USH. The loci defect dictates the subdivision into further subtypes, USH1A-G, USH2A-C, and USH3A as summarized in Table 1. Currently, an affected gene has been determined for eight different USH loci (Ahmed et al., 2003; Petit, 2001; Weil et al., 2003; Weston et al., 2004). However, in at least four of these genes, some mutations cause USH while others result in non-syndromic hearing loss. These USH genes are *MYO7A* for USH1B and DFNB2/DFNA11 (Liu et al., 1997b,c) and *CDH23* for USH1D and DFNB12 (Astuto et al., 2002; Bork et al., 2001), *PCDH15* for USH1F and DFNB23 (Ahmed et al., 2003) and *USH1C* for USH1C and DFNB18 (Ahmed et al., 2002; Ouyang et al., 2002). Some mutations in the *USH2A* gene cause isolated RP (Rivolta et al., 2000).

Epidemiological studies of USH show a prevalence of 3–6 patients per 100,000 inhabitants of the developed world (Boughman and Fishman, 1983; Forsius et al., 1971; Grondahl, 1987; Hope et al., 1997; Rosenberg et al., 1997; Spandau and Rohrschneider, 2002). Since false diagnosis of RP occurs frequently in infants, the prevalence is more likely to be 1/10,000 (Hope et al., 1997). The numbers of patients affected by the three distinct USH types is unequal. Studies in Europe show a proportion of 25–44% of USH1 patients and 56–75% of USH2 patients (Grondahl, 1987; Hope et al., 1997; Rosenberg et al., 1997; Spandau and Rohrschneider, 2002). Regional founder effects contribute to the described wide bandwidth of subtype prevalence. For example, USH3 in total accounts for a very low percentage ($\sim$ 2%), but in contrast contributes in

Table 1
Usher syndrome types: genes, proteins, functions, mouse models

Type	Gene locus	Gene	Protein	Cellular function	Mouse model
1A	14q32	*HEMAP ?*	EMAP	MAP (cytoskeleton)	–
1B	11q13.5	*MYO7A*	Myosin VIIa	Molecular motor	Shaker-1 (sh1)
1C	11q15.1	*USH1C*	Harmonin	Scaffold protein	*Deaf circler (dfcr)*
1D	10q21-q22	*CDH23*	Cadherin 23	Cell–cell adhesion	*Waltzer (v)*
1E	21q21	–	–	–	–
1F	10q11.2-q21	*PCDH15*	Pcdh15	Cell–cell adhesion	*Ames waltzer (av)*
1G	17q24-25	*SANS*	SANS	Scaffold protein	*Jackson circler (js)*
2A	1q41	*USH2A*	USH2A (usherin)	Matrix, cell adhesion	Knock outs
2B	3p23-24.2	*SLC4A7 ?*	NBC3	Ion co-transporter	k.o.: *Slc4a7 -/-*
2C	5q14.3-21.3	*VLGR1b*	VLGR1b	GPCR, cell adhesion	*Mass1 (frings), k.o.*
3A	3q21-25	*USH3A*	Clarin-1	Cell adhesion	k.o., in prep.
3B	20q	–	–	–	–

Description see text. MAP, microtubule associated protein. References: USH1A (Eudy et al., 1998; Kaplan et al., 1992), USH1B (Gibson et al., 1995; Kimberling et al., 1992; Weil et al., 1995), USH1C (Bitner-Glindzicz et al., 2000; Johnson et al., 2003; Smith et al., 1992; Verpy et al., 2000), USH1D (Bolz et al., 2001; Bork et al., 2001; Di Palma et al., 2001a, 2001b;Wayne et al., 1996; Wilson et al., 2001) USH1E (Chaib et al., 1997), USH1F (Ahmed et al., 2001; Alagramam et al., 2001a), USH1G (Kikkawa et al., 2003; Mustapha et al., 2002; Weil et al., 2003), USH2A (Eudy et al., 1998; Huang et al., 2002; Weston et al., 2000; Cosgrove et al., 2004; Liu et al., 2005); USH2B candidate (Bok et al., 2003; Hmani et al., 1999), USH2C (Johnson et al., 2005; Weston et al., 2004; Yagi et al., 2005), USH3A (Adato et al., 2002; Joensuu et al., 2001).

Birmingham (UK) for 20% of all USH cases and even higher percentage, 42%, in Finland (Hope et al., 1997; Pakarinen et al., 1995).

In addition to the characteristic senso-neuronal degeneration in the eye and the inner ear of USH, several reports indicate that USH affects other tissues and organs. This is confirmed by the rather wide expression profiles of all USH gene products obtained (see below). Functional studies on USH1 and USH2 patients indicate lower odor identification ability (Zrada et al., 1996). However, a study by Seeliger et al. (1999) on olfaction in USH did not confirm differences between USH patients and the control group. Nevertheless, recent expression analyses revealed that all analyzed USH molecules are expressed in the olfactory epithelium (Mikosz, 2005; Wolfrum et al., 1998 and Mikoz and Wolfrum, unpublished). Thus, the analysis of biopsies from the nasal epithelium of patients may be useful for USH diagnosis (Cohn et al., 2004). Furthermore, USH may also be related to brain dysfunction. An increase of mental deficiencies, cerebral atrophies, and ataxies are reported for USH patients (Drouet et al., 2003; Hess-Röver et al., 1999; Koizumi et al., 1988; Mangotich and Misiaszek, 1983). Despite the expression of all known USH proteins in the brain (Wolfrum et al., unpublished), often USH patients are highly educated and intelligent.

Sparse histopathological data from patients are often related to undefined USH subtypes. In some cases, ciliary abnormalities have been reported in patients of undefined USH subtype in retinal photoreceptors (connecting cilium) and the nasal epithelium, the trachea, and sperm cells (e.g. Arden and Fox, 1979; Baris et al., 1994; Barrong et al., 1992; Hunter et al., 1986; Tosi et al., 2003; Petrozza et al., 1991; van Aarem et al., 1999). Based on these observations, it has been suggested that USH is related to cilia dysfunction. When myosin VIIa, the product of the first identified USH gene, was localized in the connecting cilium of photoreceptor cells (Liu et al., 1997a), the latter suggestion warrants further attention.

The presence of cilia is one of the structural similarities between retinal photoreceptor cells and the mechanosensitive hair cells of the inner ear (Fig. 1). In photoreceptor cells, the connecting cilium, which is homologous to the transition zone found at the base of every motile cilium, links the biosynthetic and metabolic active inner segment with the outer segment, which is actually a modified cilium (Besharse and Horst, 1990). Each hair cell possesses one kinocilium which is responsible for organizing the array of stereocilia during hair cell differentiation (Kelley et al., 1992; Sobkowicz et al., 1995). In the mammalian cochlea, this "real" cilium disappears during hair cell maturation. The stereocilia, structures that take up the mechanical stimulus in hair cells, are not "real" cilia. On contrary, they are highly specialized microvilli ("stereovilli") characterized by a rigid actin filament core (Tilney et al., 1988). Microvillar-like, actin filament-supported structures are also found in photoreceptor cells in the form of calycal processes at the apical membrane of the inner segment (Nagle et al., 1986). These calyces sheath the base of the photoreceptor outer segment and thereby may stabilize it. Microvillar-like differentiations are more obvious on the

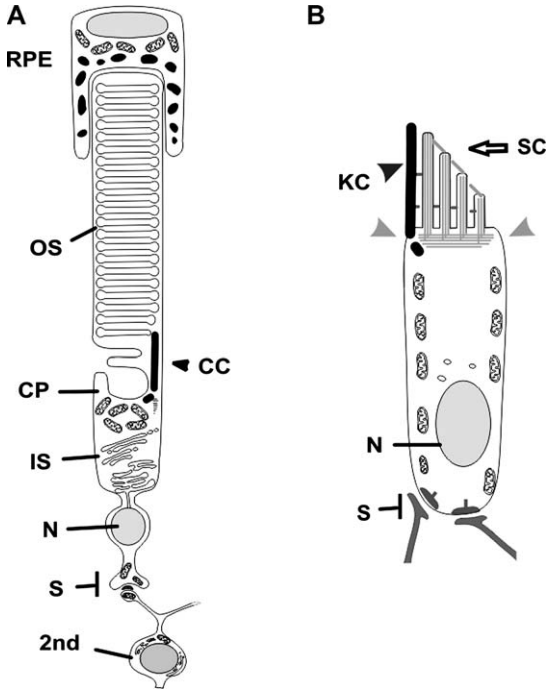

Fig. 1. Schematic representation of the sensory cells in the eye and the ear affected by USH. (A) Scheme of a rod photoreceptor cell. The apical extensions of cells of the retinal pigment epithelium (RPE) evolve the tips of light-sensitive outer segments (OS) of photoreceptor cells. The OS are linked via a connecting cilium (CC) to an inner segment (IS). Calycal processes (CP) ensheath the proximal outer segment. Nuclei (N) of photoreceptor cells are localized in the outer nuclear layer (ONL). Synaptic terminals (S) link photoreceptor cells and 2nd-order neurons, bipolar and horizontal cells. (B) Scheme of a mechanosensitive hair cell. The apical part of hair cells carries numerous rigid microvilli-like structures, improperly named stereocilia (SC, arrows), where the mechanotransduction takes place. They are anchored in the actin filament-rich cuticular plate (CP). Lateral to the longest stereocilum a kinocilium (black arrowhead) is present. Its basal body is localized in the pericuticular region (gray arrowheads). N, nucleus; S, synaptic junctions between hair cells and efferent and afferent neurons.

apical membrane of the cells of the retinal pigment epithelium. Furthermore, ribbon synapses are characteristic for both types of sensory cells. Their synapses are a unique type of chemical synapses structurally and functionally specialized for massive and sustained neurotransmitter release (Rao-Mirotznik et al., 1995; Wagner, 1997). Thus, the primary targets for defects caused by USH are probably molecules which are present in the subcellular compartments with the described similarities between both types of sensory cells. The molecular dissection of these subcellular compartments could reveal candidate genes for USH, while the study of the identified molecules related to USH should provide insights into novel processes shared by both types of sensory cells.

2. Characteristics and function of the proteins encoded by the identified USH genes

Of the three clinically characterized types of USH, eight genes on 12 USH loci have been cloned. The protein products of the eight USH genes, belong to different protein classes and therefore possess various cellular functions (Table 1).

2.1. USH1 proteins

Seven loci responsible for USH1 have been defined (USH1A-G) and five of the corresponding causative genes identified (Table 1). The USH1A gene was mapped to the chromosomal locus of the gene encoding the human homologue of a sea urchin microtubule-associated protein (EMAP) (Eudy et al., 1998), but had not yet been verified. In a search for a harmonin (USH1C) interacting partner, we identified a cell–cell adhesion molecule encoded by a gene mapping to the USH1E locus (Reiners, 2004). The candidate gene identified by this ''reverse genetic'' approach remains to be validated.

2.1.1. Myosin VIIa (USH1B), a membrane-associated actin-based molecular motor

The myosin VIIa gene (*MYO7A*) was the first USH gene identified (Weil et al., 1995) and at least half of the known USH1 cases are caused by mutations in *MYO7A* (Astuto et al., 2000). Myosin VIIa is an unconventional myosin composed of several functional domains (Fig. 2A). The highly conserved N-terminal motor or head domain classifies myosin VIIa as an actin-based molecular motor. As in other myosins, this domain contains the actin-binding site and the ATP-binding site (Sellers, 2000) which allows the protein to move along actin filaments towards their plus end in an ATP-dependent manner (Inoue and Ikebe, 2003; Udovichenko et al., 2002). In the neck region, five IQ (from the first two conserved *i*soleucine and *g*lutamine residues (Bahler and Rhoads, 2002)) motifs are expected to bind myosin-light chains, e.g. the Ca^{2+}-binding EF-hand protein calmodulin (Todorov et al., 2001; Udovichenko et al., 2002). The neck is followed by a long tail region that contains various functional domains and thereby determines, as in other myosin classes, the functional specificity of myosin VIIa (Sellers, 2000). The tail domain begins with

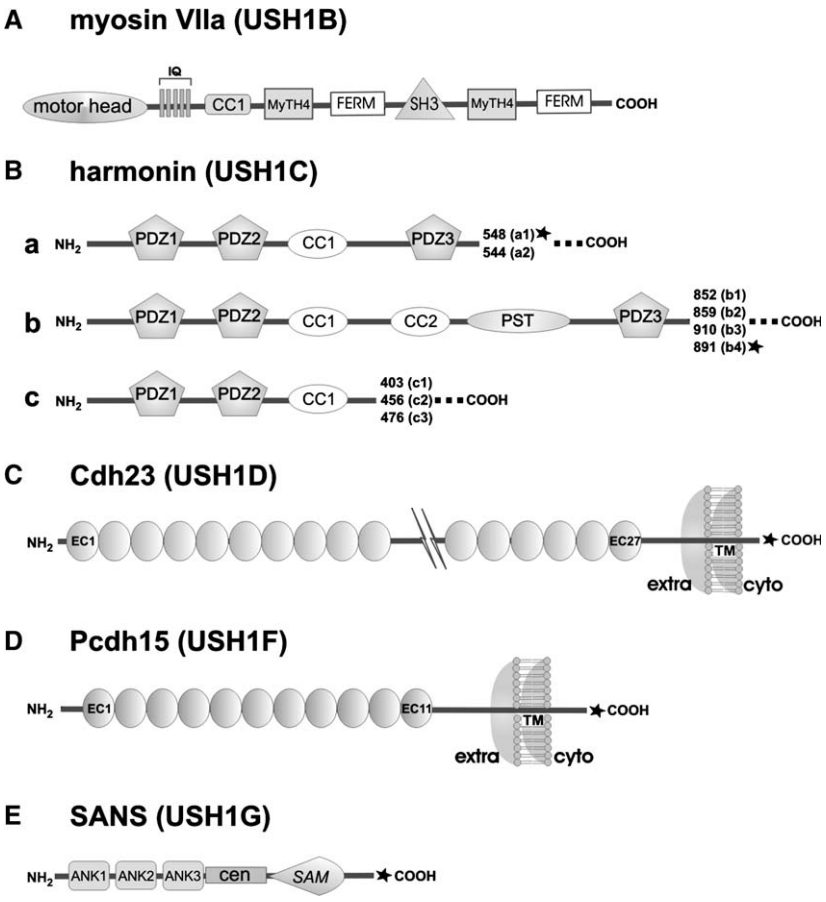

Fig. 2. Schematic representations of USH1 proteins. (A) The unconventional myosin VIIa (USH 1B) is composed of a motor head, a neck containing five isoleucine-glutamine (IQ) motifs, and a tail region. The tail region consists of a coil-coiled (CC1) domain, followed by two large repeats separated by a poorly conserved SH3 (*s*rc *h*omology-*3*) domain. These repeats include a MyTH4 (*my*osin *t*ail *h*omology *4*) and a FERM (*4*.1, *e*zrin, *r*adixin, *m*oesin) domain. (B) Harmonin (USH1C) isoforms can be divided into three classes (a–c). Common features of all isoforms are two PDZ (*P*SD95, *d*iscs large, *ZO*-1) domains (PDZ1–PDZ2) and one coiled-coil (CC1) domain. The harmonin class a isoforms consist of an additional PDZ (PDZ3) domain. The longest class b isoforms contain also a third PDZ domain (PDZ3), a second coiled-coil domain (CC2), and a *p*roline, *s*erine, *t*hreonine (PST)-rich region. Harmonin a1 and b4 possess a C-terminal class I *PDZ-b*inding *m*otif (PBM). (C) Cdh23 (Cadherin 23, USH1D) is composed of 27 extracellular cadherin repeats (EC1–EC27), a transmembrane domain (TM) and a short cytoplasmic domain with a class I PBM. (D) The non-classical cadherin Pcdh15 (Protocadherin 15, USH1F) comprises eleven ectodomains (EC1–EC11), one transmembrane domain (TM) and a C-terminal class I PBM. (E) The ''*s*caffold protein containing *an*kyrin repeats and *S*AM domain'' (SANS, USH1G) contains three N-terminal ankyrin repeats (ANK1–ANK3), a central region (cen), a *s*terile *a*lpha *m*otif (SAM) and a C-terminal class I PBM. extra, extracellular; intra, intracellular; asterisks indicate class I PBMs.

a coiled-coil domain which is predicted to mediate homodimer formation (Weil et al., 1995). Two large tandem repeats, separated by a poorly conserved SH3 (*s*rc *h*omology-3), follow. These repeats consists of a MyTH4 (*m*yosin *t*ail *h*omology *4*) domain and a FERM domain (*4*.1, *e*zrin, *r*eadixin, *m*oesin). The function of the MyTH4 domain of myosins is still unknown, however FERM domains are thought to be responsible for protein attachment to the plasma membrane (Chishti et al., 1998). Several myosin VIIa splice variants are mentioned in the literature (Sahly et al., 1997) which may be tissue specific, but detailed investigations of these variants are lacking. To date more than 90 different mutations have been identified in USH1B patients. These mutations are distributed along the full length of the gene with some clustering in the head domain (Petit, 2001). Mutations in the *MYO7A* gene result in not only USH1B but also USH3-like phenotypes, as well as in dominant or recessive isolated deafness with any degree of severity, congenital or progressive, with or without vestibular dysfunction (Petit, 2001). So far, it is difficult to determine whether the type of mutation accounts for the expression of USH or isolated deafness. A functional test for activity of the various mutated forms of myosin VIIa protein could help to link the individual effect of a given mutation to a phenotype. However, the phenotypic heterogeneity argues for a role of additional genetic and/or environmental factors.

Several ligands binding to the tail domain of myosin VIIa have been identified and specify myosin VIIa's role in e.g. intracellular transport, endocytosis and cell–cell adhesion (summarized in (Wolfrum, 2003)) (Table 2). The binding of myosin VIIa to the scaffold proteins harmonin (USH1C) and SANS (USH1G) unites the specific function of myosin VIIa to the USH protein network discussed in Section 4.

Although myosin VIIa is almost ubiquitously expressed, in human USH1B patients, dysfunctions are restricted to the inner ear and the retina. Expression of this unconventional myosin has been documented in all epithelial tissue containing ciliary processes or microvillar structures (Hasson et al., 1997; Sahly et al., 1997; Wolfrum et al., 1998). In the inner ear, myosin VIIa is expressed in the mechanosensory hair cells of the vestibular organ and cochlea where it is predominantly localized in the stereocilia, but is also found along the lateral membrane, in the cuticular plate, and in the synaptic region (El Amraoui et al., 1996; Hasson et al., 1997; Weil et al., 1996; Wolfrum et al., 1998). Studies on myosin VIIa deficient Shaker-1 mice indicate that myosin VIIa is essential for the differentiation and organization of hair cell stereocilia (Self et al., 1998; Boëda et al., 2002; Adato et al., 2005). In addition, there is evidence that myosin VIIa also participates in signal transduction in the hair cells (Kros et al., 2002; Siemens et al., 2004; Etournay et al., 2005). Interestingly, a recent study demonstrates that the *Drosophila* homolog of myosin VIIa, the *crinkled* gene product, is required for organization of the Johnston's organ (Todi et al., 2005). In this insect auditory organ, myosin VIIa is not only localized in the sensory cells of the mechanosensitive scolopidia, but is also necessary for the organization of the actin filament bundles of their innermost support cell (Todi et al., 2005) which have previously been described as arrangements analogous to the actin filament core of stereocilia (Wolfrum, 1990, 1991).

In the vertebrate eye, the cell types affected by myosin VIIa defects are less clear than in the auditory organs. Myosin VIIa is expressed in the retinal pigment epithelium (RPE) as well as in rod and cone photoreceptor cells of the retina (Liu et al., 1997a). In RPE cells, myosin VIIa is highly concentrated in the apical microvilli-like processes (Hasson et al., 1995; Liu et al., 1997a). The identification of specific interaction partners (El Amraoui et al., 2002) together with analyses of the RPE of Shaker-1 mice revealed an active role for myosin VIIA in the migration of RPE melanosomes and a contribution to the phagocytosis of photoreceptor cell outer segment tips by RPE cells (El Amraoui et al., 2002; Gibbs et al., 2003; Liu et al., 1998; Williams and Gibbs 2004b). A recent study also suggests that myosin VIIa is a common lysosome-associated motor in general (Soni et al., 2005).

In mammalian rod and cone photoreceptor cells, myosin VIIa is predominantly localized in the connecting cilium (Liu et al., 1997a; Wolfrum and Schmitt, 2000; Wolfrum et al., 1998), but it is also found at photoreceptor synapses (El Amraoui et al., 1996; Reiners et al., 2003, 2005a; Wolfrum et al., 2004). Several lines of evidence indicate that myosin VIIa participates in the molecular transport through the photoreceptor cilium (Liu et al., 1997a; Williams and Gibbs 2004b; Wolfrum and Schmitt, 2000). Immunoelectron microscopy revealed that myosin VIIa is associated with the ciliary membrane (probably via its tail) and uses actin filaments in the cilium as a track for its movement through the cilium. The co-localization of myosin VIIa with opsin and the abnormal opsin accumulation in the membrane of the connecting cilium in photoreceptor cells of myosinVIIa-deficient Shaker-1 mice indicate participation of myosin VIIa in the opsin transport through the cilium (Liu et al., 1999; Wolfrum and Schmitt,

Table 2
Myosin VIIa binding proteins

Interacting protein	Myosin VIIa target domain	References
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 (kinase regulation)	MyTH4 (2), FERM (2)	Küssel-Andermann et al., 2000
Keap1 (actin-associated protein)	SH3	Velichkova et al., 2002
Vezatin (transmembrane protein)	FERM (2)	Küssel-Andermann et al., 2000
MyRIP (Rab-interacting protein)	MyTH4 (2), FERM (2)	El Amraoui et al., 2002
Harmonin (USH1C) (scaffold protein)	FERM (2)	Boëda et al., 2002
SANS (USH1G) (scaffold protein)	MyTH4/FERM (1,2)	Adato et al., 2005
PHR1 (transmembrane protein)	MyTH4/FERM (2)	Etournay et al., 2005

2000). In independent studies using different immunocytochemical and biochemical approaches, the localization of myosin VIIa has also been demonstrated in the synapses of mammalian photoreceptor cells (El Amraoui et al., 1996; Reiners et al., 2003, 2005a; Wolfrum and Reiners, 2004). Our recent studies indicate that partner proteins of myosin VIIa's organized in the USH protein network are also localized at the photoreceptor synapse (see Section 4) which further supports the synaptic expression of myosin VIIa. Nevertheless, this localization is still controversial (Williams and Gibbs, 2004a; Wolfrum and Reiners, 2004). However, functional roles of myosin VIIa at synapses are recently supported by findings on the neuromuscular junctions of *Drosophila* (Marella et al., unpublished) and by the morphological disorganization of the photoreceptor synapses in the myosin VIIa-deficient mariner zebrafish mutant, presented at ARVO in 2005 (Biehlmaier et al., 2005).

2.1.2. Harmonin (USH1C), a potent scaffold protein and key organizer of the USH protein network

USH type 1C is caused by defects in the harmonin *USH1C* gene (Bitner-Glindzicz et al., 2000; Verpy et al., 2000). The USH1C locus was first mapped in a small French-speaking, Acadian population of academics in southwest Louisiana and consequently named the academic USH (Kloepfer and Laguaite, 1966). The harmonin gene was originally identified as an autoimmune antigen upregulated in some forms of cancer and in cases of *a*uto*i*mmune *e*nteropathy (AIE) (Kobayashi et al., 1999; Scanlan et al., 1999). In these studies, two synonyms PDZ-73 and AIE-75 for the harmonin protein, in particular the human isoform harmonin a1 (see below), were introduced and are occasionally still in use. Genome screens and sequence comparisons revealed that harmonin genes are not present in invertebrates, e.g. *Drosophila* or *C. elegans*, but are highly conserved in genomes of vertebrates (Reiners, 2004; Reiners et al., 2003).

The harmonin *USH1C* gene consists of 28 coding exons of which eight are differentially spliced, generating a variety of alternatively spliced transcripts (Verpy et al., 2000). All elucidated mutations of *USH1C* that cause USH1 are localized in constitutively transcribed exons whereas mutations in alternatively transcribed exons are associated with non-syndromic deafness (Bitner-Glindzicz et al., 2000; Ouyang et al., 2002; Verpy et al., 2000; Zwaenepoel et al., 2001). So far, nine murine splice variants of harmonin have been described which are grouped in three classes a, b and c (Figs. 2B, 3). These isoforms differ in the composition of their domain structure, namely two or three PDZ domains, one or two coiled-coil domains and the presence or absence of a PST (*p*rolin, *s*erin, *t*hreonine-rich) domain. These three functional domains participate in protein–protein interactions, establishing harmonin as a potent scaffold protein (Fig. 4A). The PST domain of harmonin b isoforms mediates binding to actin filaments and introduces filament bundling (Boëda et al., 2002). The interaction between the 2nd coiled-coil domain (CC2) of harmonin b isoforms with the PDZ1 and/or PDZ2 domains of harmonin molecules provide the basis for homophilic interactions (Adato et al., 2005). The most important domains of the harmonin isoforms are the PDZ domains. PDZ domains are short, ~90 amino acid long, protein–protein interacting domains originally found in *P*SD95 (*p*ost-*s*ynaptic *d*ensity protein), *D*isk large (Dlg-A, tumor suppressor in *Drosophila*), and *ZO*-1 (*z*onula *o*ccludens-*1*, tight junction protein) and named after these proteins (Harris and Lim, 2001; Sheng and Sala, 2001). Proteins commonly interact with PDZ domains via a PDZ-binding motif (PBM) at their C-terminus. In some interacting proteins, internal β-hair pin loop structures mimic a C-terminal PBM and allow unpredicted protein-PDZ binding. Such loop structures are also found in PDZ domains which mediate PDZ-PDZ domain interactions (Nourry et al., 2003). In the cellular environment, PDZ domain proteins serve as organizers of supramolecular protein networks and complexes. They link, cluster, and coordinate the function of proteins at specific subcellular compartments, especially at the plasma membrane (Garner et al., 2000). The specific protein–protein interactions mediated by harmonin within a network of other USH proteins are examined in Section 4 (Fig. 7).

Harmonin is expressed in nearly all tissues analyzed (Bitner-Glindzicz et al., 2000; Harf, 2003; Kobayashi et al., 1999; Reiners et al., 2003; Reiners, 2004; Scanlan et al., 1999; Verpy et al., 2000), but surprisingly none of the harmonin isoforms are found in the RPE or in cultured cells derived from the RPE (see Fig. 6C,I) (Reiners et al., 2003; Reiners, 2004). In most tissues harmonin is expressed as isoform class a and/or c. Transcripts encoding harmonin isoforms b were previously thought to be exclusive for the cochlea (Boëda et al., 2002; Verpy et al., 2000), but more recently, harmonin b isoforms were also found in the neuronal retina, brain, pancreas, and testis (Harf, 2003; Johnston et al., 2004; Reiners et al., 2003; Reiners, 2004). Our analyses revealed the expression of a novel harmonin isoform b4 which is specific for the retina (Reiners et al., 2003; Reiners, 2004).

In the inner ear, harmonin is expressed in the sensory hair cells of the organ of Corti and in the vestibular organ. Harmonin b is detectable in differentiating hair cells and becomes concentrated at the apex of the maturing stereocilia. Although the expression of harmonin isoform b disappears in hair cells of adult mice, proteins of the other harmonin isoform classes remain localized in the stereocilia and in the cuticular plate as well as at the lateral plasma membrane and at the synapses throughout the life of the mice (Boëda et al., 2002; Reiners et al., 2005b). Studies on the harmonin deficient Deaf circler mice further indicate that harmonin b is essential for the proper development of the hair cell stereocilia (Johnson et al., 2003).

In the absence from the RPE, harmonin is localized in the ganglion cell layer, the inner and the outer plexiform layer, but predominantly in the photoreceptor layer of the neuronal retina (Fig. 6C,I) (Reiners et al., 2003). In the subcellular compartments of rod and cone photoreceptor cells, the scaffold protein harmonin is found in the outer and inner segments as well as at the ribbon synapses in the outer plexiform layer, but is absent from the connecting cilium (Fig. 6C,I) (Reiners et al., 2003). Our studies further revealed that the harmonin

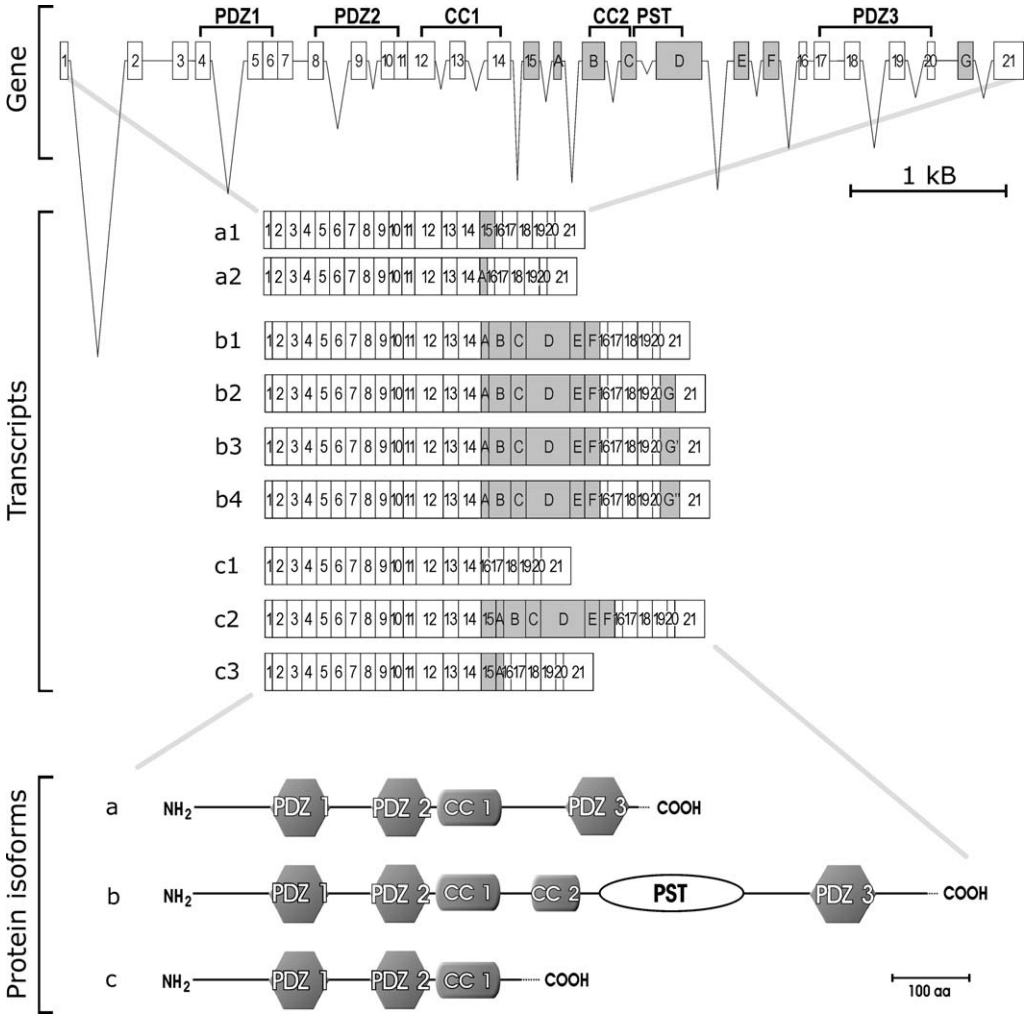

Fig. 3. The *USH1C* gene, USH1C transcripts and protein structures of harmonin isoforms. *USH1C* consists of 28 coding exons (white and gray squares) spaced by several introns (lines). So far, nine alternatively spliced transcripts that result from the differential use of eight exons (gray squares) are described. They are grouped into three subclasses according to their composition of functional domains. For description of the domain structures of harmonin isoforms see Fig. 2.

isoforms are differentially expressed in the subcellular compartments of the photoreceptor cell. Whereas the localization of harmonin b variants is restricted to the light-sensitive outer segment, isoforms a and c are detectable in all subcellular compartments including the synaptic terminals (Reiners et al., 2003). At photoreceptor synapses, harmonin co-localizes with all known USH molecules, actin filaments, and actin-associated cytoskeletal components of the USH protein network discussed in Section 4 (Figs. 6, 7).

In sensory pathways of cells, PDZ-containing scaffold proteins are commonly found to coordinate the organization of signaling molecules into macromolecular complexes (‘‘transducisomes’’) providing specificity, sensitivity and speed in intracellular signaling (Montell, 1999; Tsunoda et al., 1997; Zuker and Ranganathan, 1999). It has been previously emphasized that harmonin may play an important role in the mechano-signal transduction in the stereocilia of mechanosensitive hair cells (Boëda et al., 2002; Gillespie and Walker, 2001; Montell, 2000). In the subcellular compartment of visual transduction of vertebrate photoreceptor cells, the outer segment, harmonin likely also participates in the assembly of protein networks or complexes associated with the actin cytoskeleton of the outer segment (Kajimura et al., 2000; Körschen et al., 1999; Reiners et al., 2003). Harmonin may function as a vertebrate analogue of INAD which is known to cluster the components of the visual signal transduction cascade into a signal complex in the rhabdomeric photoreceptor cell of invertebrates (Montell, 1999).

2.1.3. USH cadherins, cadherin 23 (USH1D) and protocadherin 15 (USH1F) mediate membrane–membrane adhesion

The USH cadherins, cadherin 23 (USH1D) and protocadherin 15 (USH1F), are atypical members of the large cadherin superfamily of transmembrane proteins defined by the presence of a variable number of extracellular cadherin domains termed ‘‘EC’’ (Fig. 2C,D) (Bolz et al., 2002). These ECs, five in classical cadherins, mediate the Ca^{2+}-dependent dimerization of cadherin molecules and the trans-extracellular linkages between cadherin dimers of two neighboring cells. In cell–cell adhesion complexes, in general, these intercellular contacts are indirectly coupled to the actin cytoskeleton by

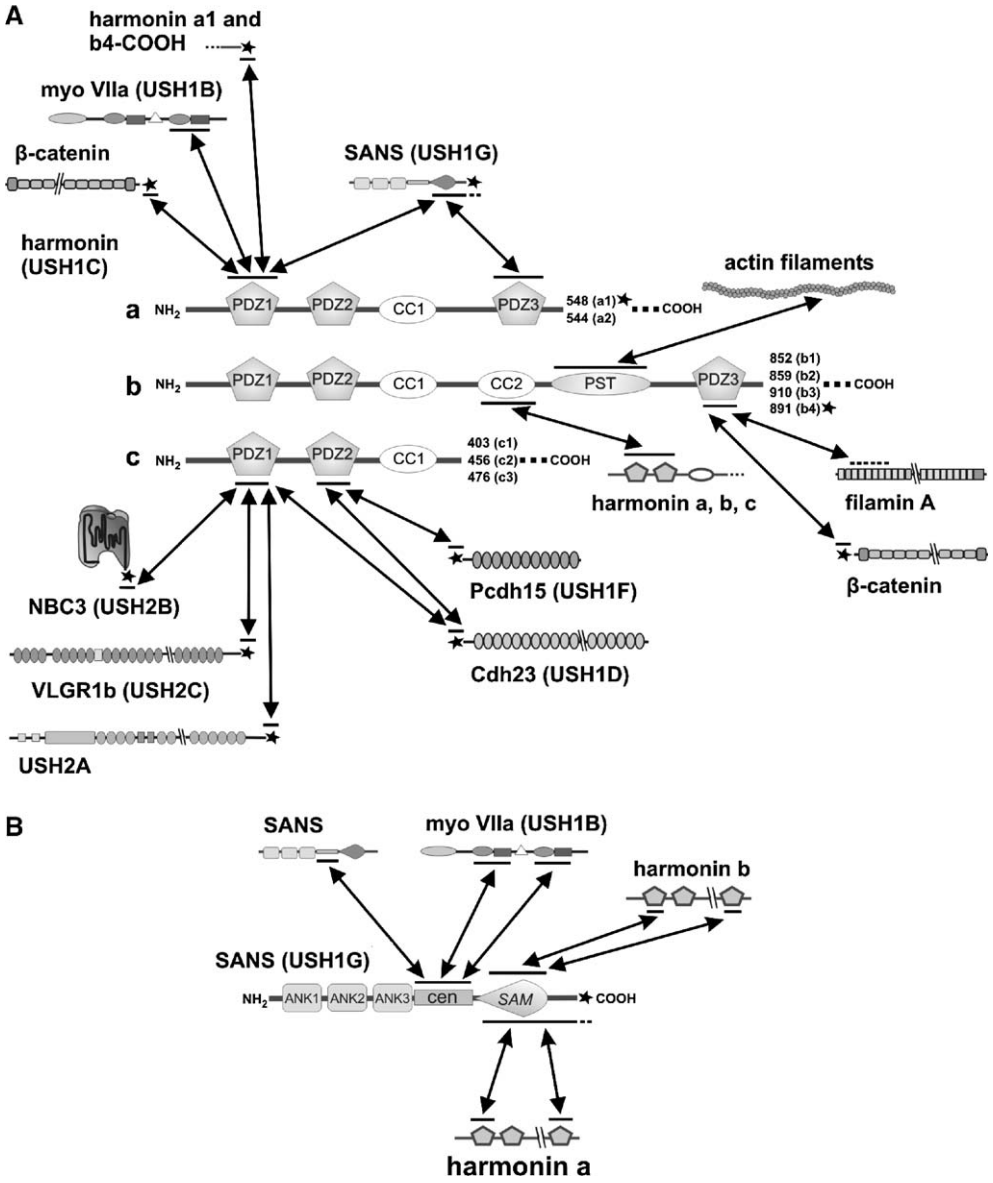

Fig. 4. Schematic representation illustrating protein–protein interactions mediated by the two USH1 scaffold proteins harmonin (A) and SANS (B). (A) Harmonin isoforms homophilically interact, bind to all USH proteins, and mediate links to the actin cytoskeleton. All harmonin isoforms can dimerize via their PDZ1 and/or PDZ2 domains with the CC2 domain of harmonin b and/or with C-terminal PBMs (asterisk) of the isoforms a1 and b4. USH2 proteins, VLGR1b, USH2A, and NBC3 bind via their C-terminal PBM to the harmonin's PDZ1 which also interacts with the tails of myosin VIIa dimers and with the C-terminal PBM (asterisk) and/or the SAM domain of SANS. SANS's SAM domain also binds to PDZ3. The USH1 cadherins, Cdh23 and Pcdh15 bind via their C-terminal PBM to PDZ2. The splice variant Cdh23 (+68) isoform A also binds via an internal PBM to PDZ1. Actin filaments are linked to harmonin directly via the PST domain of harmonin b and indirectly through myosin VIIa (see above) and the actin-associated protein filamin A and β-catenin which interact with PDZ3 or with PDZ1 and PDZ3, respectively. (B) SANS (*s*caffold protein containing ankyrin repeats and *S*AM domain) homodimerizes via its central cen region. SANS's cen region also interact with the MyTH4-FERM of myosin VIIa. Molecular interactions of SANS with harmonin are described in (A). (For references please see text.)

the anchor proteins α-catenin and β-catenin via binding to the cytoplasmic tail of classical cadherins. The cytoplasmic domain in cadherins also connects cell–cell adhesion complexes to intracellular signaling pathways (Goodwin and Yap, 2004). In the cytoplasmic tails of the two USH cadherins, the consensus R1 and R2 binding sites for β-catenins (Imamura et al., 1999) are missing (Ahmed et al., 2001; Boëda et al., 2002; Bork et al., 2001; Siemens et al., 2002). But in contrast to classical cadherins, they harbor class I PDZ-binding motifs (PBMs) in the C-terminus of their cytoplasmic tail (Fig. 2C,D) (Ahmed et al., 2001; Boëda et al., 2002; Bork et al., 2001; Siemens et al., 2002). Via these PBMs both USH cadherins are capable of being anchored to the actin cytoskeleton via the USH1C PDZ-containing protein harmonin (Figs. 4A, 7) (Adato et al., 2005; Boëda et al., 2002; Reiners et al., 2005a).

2.1.4. Cadherin 23 (USH1D), the long-tailed USH cadherin

Mutations in the *CDH23* gene are linked to USH1D and the non-syndromic deafness DFN12, as well as deafness in Walzer

mice (Bolz et al., 2001; Bork et al., 2001; Di Palma et al., 2001a). Cadherin 23 (Cdh23), also known as Otocadherin (Di Palma et al., 2001a), is characterized by a short intracellular domain and an extremely long extracellular domain of 27 ECs. So far three isoforms of Cdh23, A, B, and C, have been described (Bork et al., 2001; Di Palma et al., 2001b; Michel et al., 2005; Siemens et al., 2002). Splice variants Cdh23 A and B differ in the presence (A, Cdh23 (+68)) or absence (B, Cdh23 (−68)) of exon 68 which encodes for an insert in the cytoplasmic domain (Bork et al., 2001; Di Palma et al., 2001b; Siemens et al., 2002). The Cdh23 (+68) A isoform harbors, in addition to its C-terminal PBM, an internal PBM with homology to the internal PBM of the adaptor protein Ril (Siemens et al., 2002). Both PBMs of Cdh23 (+68) participate in interactions with the PDZ-containing scaffold protein harmonin (USH1C). As discussed in Section 4, Cdh23's C-terminal PBM binds to the PDZ2 while the internal PBM of the Cdh23 (+68) A isoform interacts with the PDZ1 of harmonin (Figs. 4A, 7) (Boëda et al., 2002; Siemens et al., 2002). Whereas the Cdh23 (+68) A isoform is expressed preferentially in the inner ear sensory epithelium (Di Palma et al., 2001a; Siemens et al., 2002), the Cdh23 (−68) B isoform is more ubiquitously expressed in the heart, kidney and spleen, as well as the brain and the neuronal retina (Siemens et al., 2002). The more recently identified short Cdh23 isoform C consists of only the cytoplasmic domain, and a cell−cell adhesion function can therefore be excluded. Competition for cytoplasmic binding partners between Cdh23 C and the other Cdh23 isoforms may play a role in the regulation in signaling pathways in the cytoplasm (Lagziel et al., 2005).

In the inner ear, Cdh23 expression was found in the sensory hair cells and in the Reissner's membrane (Boëda et al., 2002; Bolz et al., 2002; Wilson et al., 2001). Analyses of the Waltzer mice revealed that Cdh23 is required for the normal development of the stereocilia of hair cells (Di Palma et al., 2001a). During the differentiation of hair cells, Cdh23 is localized at transient lateral links between the membranes of neighboring stereocilia, which are absent in mature cochlear hair cells (Boëda et al., 2002; Lagziel et al., 2005; Michel et al., 2005). In mature cochlear hair cells, Cdh23 is localized at the centrosome, probably in the form of Cdh23 isoform C and in the apical, vesicle-rich percuticular region (Lagziel et al., 2005; Michel et al., 2005). Nevertheless, Cdh23 was suggested to be a component of the tip links (Siemens et al., 2004; Söllner et al., 2004), structures linking the tips of the hair bundles in mature hair cells which are proposed to serve in gating the mechanosensory channel (Furness and Hackney, 1985; Pickles et al., 1984, 1991). Since these findings could not be confirmed by independent studies (Boëda et al., 2002; Lagziel et al., 2005; Michel et al., 2005) they are still controversial.

In the retina, Cdh23 is not expressed in the RPE, but is localized in the inner segment, the connecting cilium, and the basal body complex, as well as the ribbon synapses of rod and cone photoreceptor cells (Fig. 6D,I) (Reiners et al., 2003). Latter localizations were recently confirmed by immunoelectron microscopy (Lillo et al., 2005). In the basal body complex of the connecting cilium, the structure homolog to the centrosome of none-ciliated cells, the short cytoplasmic Cdh23 C isoform is the most likely expressed isoform. The EC-domains of the transmembrane form of Cdh23 (+68) expressed in the retina have been suggested to mediate membrane−membrane adhesions between the inner segment membranes of neighboring photoreceptor cells, and between the pre- and post-synaptic membranes of photoreceptor cells and 2nd order retinal neurons. At synapses, it is assumed that cadherins keep the synaptic cleft in close proximity, contribute to the organization of the pre- and post-synaptic cytomatrices of a synaptic junction, and play an important role in synaptogenesis (Bruses, 2000). Embedded in the USH protein network of the photoreceptor synapse (see Section 4, Fig. 7), Cdh23 and the 2nd USH cadherin, protocadherin 15 (see below) may play roles in membrane adhesion at the specialized ribbon synapse of photoreceptor cells (Reiners et al., 2003).

2.1.5. Protocadherin 15 (Pcdh15) (USH1F), the outer segment cadherin

Mutations in the *PCDH15* gene encoding for protocadherin 15 (Pcdh15) are responsible for USH1F (Ahmed et al., 2001). Pcdh15 consists of 11 EC motifs, a single transmembrane domain, and two prolin rich regions and a class I C-terminal PBM in the cytoplasmic domain (Fig. 2D). Via the latter PBM, Pcdh15 binds to harmonin's PDZ2 domain (Fig. 4A) (see also Section 4, Fig. 7) (Adato et al., 2005; Reiners et al., 2005a). In adult mice and humans, Pcdh15 is expressed in a wide range of tissues including the liver, spleen, brain, inner ear, and retina (Ahmed et al., 2003; Alagramam et al., 2001a). In the fetal cochlea, Pcdh15 was detected in supporting cells, outer sulcus cells and the spiral ganglion (Alagramam et al., 2001b) while in the mature inner ear, Pcdh15 is also localized in stereocilia of sensory hair cells of both the cochlea and the vestibular organ (Ahmed et al., 2003; Wolfrum, unpublished observations by immunoelectron microscopy). Studies in Ames waltzer (*av*) mice bearing mutations in the *Pcdh15* gene indicate an important role of Pcdh15 in morphogenesis and/or maintenance of the microvilli-like stereocilia of hair cells (Ahmed et al., 2003; Alagramam et al., 2001a; El Amraoui and Petit, 2005). An orthologue of human Usher cadherin *PCDH15, Cad99C* has recently been identified and characterized in *Drosophila melanogaster* (D'Alterio et al., 2005). Interestingly, in the fruitfly, the Cad99C protein also participates in the morphogenesis of stereocilia-like structures, regular microvilli.

In the mammalian eye, Pcdh15 expression has been described in the photoreceptor layer, the outer plexiform layer, and the ganglion cell layer of the neuronal retina, but not in the RPE layer (Fig. 6E,I) (Ahmed et al., 2003; Reiners et al., 2005a). In rod and cone photoreceptor cells, Pcdh15 is localized in the synaptic region, in the cell−cell adhesions of the outer limiting membrane, and in the outer segment (Ahmed et al., 2003; Reiners et al., 2005a). Pcdh15 is associated with the membranes of the entire outer segment, and is particularly concentrated in the proximal outer segment of cone cells, distinct from the connecting cilium (Reiners

et al., 2005a,b). The latter localization of Pcdh15 corresponds with the localization of prCAD, a photoreceptor specific cadherin described previously (Rattner et al., 2001; Reiners et al., 2005a). Both cadherins are suggested to contribute to the de novo formation of membrane disks, which occurs at the base of the photoreceptor outer segment. The parallel localization of Pcdh15 and harmonin in the outer segment (Fig. 6C,E,I) (Reiners et al., 2003, 2005a) makes their interaction obvious and suggests that harmonin coordinates the outer segment function of Pcdh15. Moreover, both interacting partners are also present at the photoreceptor synapses (Fig. 6C,E,I) where they are integral components of the USH protein network (Section 4, Fig. 7).

2.1.6. SANS (USH1G), a scaffold protein associated with the microtubule system

The gene product underlying USH1G was identified as the scaffold protein SANS (*s*caffold protein containing *an*kyrin repeats and *S*AM domain) (Weil et al., 2003) (Table 1). SANS consists of several putative protein–protein interaction domains, three ankyrin domains (ANK1-3) at the N-terminus, a central region followed by a sterile alpha motif (SAM), and a class I PBM at its C-terminus (Fig. 2E) (Nourry et al., 2003; Sedgwick and Smerdon, 1999; Stapleton et al., 1999). Although ankyrin repeats mediate protein-binding in other proteins, they do not appear to participate in the identified SANS interactions (Adato et al., 2005). The central domain is responsible for SANS homodimerization and its interaction with myosin VIIa's FERM domain while the SAM domain mediates binding to the PDZ1 and PDZ3 domain of harmonin (Adato et al., 2005). In contrast, previous analysis demonstrates the binding of harmonin's PDZ1 to the C-terminal PBM of SANS (Weil et al., 2003) and from present experimental data in Adato et al. (2005) this form of interaction cannot be excluded. The known protein–protein interactions of SANS are summarized in Fig. 4B.

SANS shares its domain structure with Harp (*h*armonin-interacting, *a*nkyrin *r*epeat-containing *p*rotein) (Johnston et al., 2004; Weil et al., 2003). The similar proteins display 41% sequence identity and 65% sequence similarity (Weil et al., 2003). The most similar parts are ANK repeats and SAM domains while the central region is more divergent (26% identity) than the terminal regions (Johnston et al., 2004; Weil et al., 2003). The C-terminus of Harp also harbors the PBM consensus sequence for binding to harmonin's PDZ1 (Johnston et al., 2004; Reiners et al., 2005b). Expression analyses of both proteins indicate differential tissue expression (Johnston et al., 2004; Weil et al., 2003). While Harp is expressed in several tissues, the expression of SANS is probably restricted to tissues in the inner ear and eye.

In the ear, SANS is expressed in the inner ear hair cells as well as some supporting cells (Adato et al., 2005). During hair cell differentiation, SANS is localized in the apical hair cell bodies underneath the cuticular plate of cochlear and vestibular hair cells, but not in the stereocilia. In cochlear outer hair cells, this apical labeling of SANS is more concentrated beneath the kinocilium basal body, which becomes more prominent in mature outer hair cells (Adato et al., 2005). SANS localization is also found in the kinocilium which is in contrast to the microvilli-like stereocilia a "real" cilium containing the axonemal $9 \times 2 + 2$ arrangement of microtubules. Finally, SANS is also present in the region of the hair cell synapses (Adato et al., 2005) where it co-localizes with its binding partner harmonin and other components of an USH protein network (Kikkawa et al., 2003; Reiners et al., 2005b; Weil et al., 2003).

Preliminary data on the subcellular expression of SANS in the rodent eye indicate, that SANS is localized in retinal photoreceptor cells, but it is not expressed in the RPE (Fig. 6I) (Märker et al., unpublished). In rod and cone cells, SANS expression is found in the inner segment, but is more concentrated in the ciliary basal apparatus (including the basal body), the connecting cilium, and at the synapses. In conclusion, the SANS localization is present in principally the same subcellular compartments of mechanosensitive hair cells and photoreceptor cells. These cellular compartments are known to contain mircotubules rather than actin filaments, indicating an association of SANS with the microtubule cytoskeleton.

2.2. The USH2 and USH3 proteins

Two of the three genes responsible for USH2 have been definitely identified, namely *USH2A* and *VLGR1b* (USH2C) (Eudy et al., 1998; van Wijk et al., 2004; Weston et al., 2004). With the 3rd USH2 gene, USH2B, the *SLC4A* gene encoding the ion-co-transporter NBC3 has been implicated (Bok et al., 2003) but has been not yet verified (Table 1, Fig. 5).

2.2.1. USH2 proteins

2.2.1.1. USH2A (''Usherin'') is connected to the extracellular matrix. Mutations in the *USH2A* gene are responsible for USH2A the most common USH subtype (Eudy et al., 1998; van Wijk et al., 2004). The *USH2A* gene codes for two alternatively spliced isoforms, a short ~170 kDa USH2A isoform a, previously termed usherin, and a much longer ~580 kDa USH2A isoform b (Fig. 5A) (Bhattacharya et al., 2002; Eudy et al., 1998; van Wijk et al., 2004). The USH2A isoform a is thought to be an extracellular matrix protein as it is composed of protein domains that are commonly seen in extracellular proteins or in extracellular domains of proteins that are involved in protein–protein or protein–matrix interactions (Eudy et al., 1998; Huang et al., 2002; Weston et al., 2000). The domain structure of USH2A isoform a molecules start with a N-terminal signal peptide followed by one laminin G-like domain (LamGL), one laminin N-terminal (LamNT), 10 laminin-type EGF-like modules, and two sets of fibronectin type III (FN3) repeats spaced by two laminin G domains (LamG) (Fig. 5A). More recently, while searching for additional mutations, 51 novel exons at the 3′ end of the *USH2A* gene were identified (van Wijk et al., 2004). These encode in addition to the known functional domains for two laminin G domains, 28 fibronectin type III repeats, a transmembrane region, and a cytoplasmic domain (Fig. 5A) (van Wijk et al.,

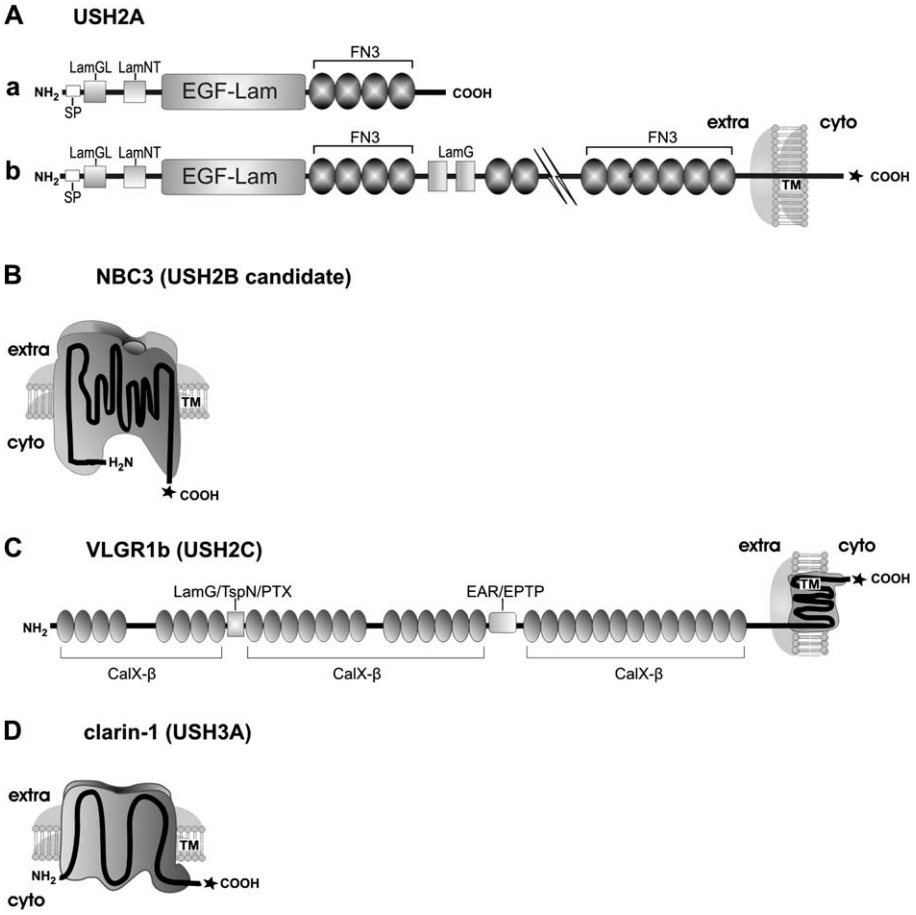

Fig. 5. Schematic representation of USH2 and USH3 proteins. (A) The extracellular domain of the USH2A isoform b (USH2A) contains a laminin G-like domain (LamGL), a N-terminal laminin domain (LamNT), 10 laminin-type EGF-like modules (EGF-Lam), 32 fibronectin type III (FN3) repeats (4 + 28), spaced by two laminin G domains (LamG) followed by a transmembrane region (TM) and the intracellular C-terminal domain containing a PBM. (B) The transmembrane protein NBC3 (USH2B candidate) possesses 12 transmembrane regions and a C-terminal PBM. (C) VLGR1b (*v*ery *l*arge *G*-protein coupled *r*eceptor *1b*, USH2C) consists of extracellular N-terminal extension with a LamG/TspN/PTX-homologous domain, seven EAR/EPTP repeats, 35 Ca^{2+}-binding *cal*cium e*x*changer β (CalX-β) modules, one 7-transmembrane domain (TM) as well as a short intracellular domain containing a PBM. (D) Clarin-1 (USH3A) is built up by four transmembrane domains and contains a glycosylation consensus site. A C-terminal "TNV" signature may serve as a PBM (asterisk). extra, extracellular; intra, intracellular; asterisks indicate class I PBMs.

2004). The cytoplasmic tail contains a C-terminal class I PBM which interacts with the PDZ1 domain of harmonin, (Fig. 4A) integrating USH2A into the USH protein network discussed below (Section 4, Fig. 7) (Reiners et al., 2005b; Reiners and Wolfrum, 2006).

Previous expression analyses with tools which did not discriminate between both USH2A isoforms demonstrate USH2A expression in the basement membrane of several tissues in addition to the cochlea and the retina (Bhattacharya et al., 2002; Pearsall et al., 2002). RT-PCR using isoform specific primers revealed USH2A isoform b expression predominantly in the retina, but also in the heart and the kidney (van Wijk et al., 2004). Biochemical analysis identified the USH2A isoform a as a component of the extracellular basement membrane where it interacts via its EGF-like modules with type IV collagen and fibronectin (Bhattacharya et al., 2002, 2004; Bhattacharya and Cosgrove, 2005).

A more recent subcellular analysis confirmed the localization of USH2A in the basement membrane of the cochlea and the retina (e.g. Bruch's membrane), but revealed additional expression sites in both sensory epithelia (Fig. 6F,I) (Liu et al., 2005; Reiners et al., 2005b). In cochlear hair cells, USH2A was additionally localized in their stereocilia and at their synaptic region. Retinal USH2A localization was observed in the connecting cilium and at the synaptic terminals of cone and rod photoreceptor cells (Fig. 6F,I). At the synapses of both sensory cell types, USH2A is believed to participate in adhesion of pre- and post-synaptic membranes integrated into the USH protein network (Reiners et al., 2005b; Reiners and Wolfrum, 2006) (see Section 4). The synaptic localization of USH2A, together with the homology of USH2A's laminin domains to the axonal attractant matrix molecule netrine-1, suggests a role for USH2A in nerve fiber guidance (Bhattacharya et al., 2002).

2.2.1.2. USH2B—the candidate NBC3—an ion co-transporter involved in pH regulation. The USH2B gene was mapped to chromosome 3 in the region p23-24.2 in a single Tunisian consanguineous family (Hmani et al., 1999; Hmani-Aifa et al., 2002) (Table 1; Fig. 5B). Although its locus is slightly outside

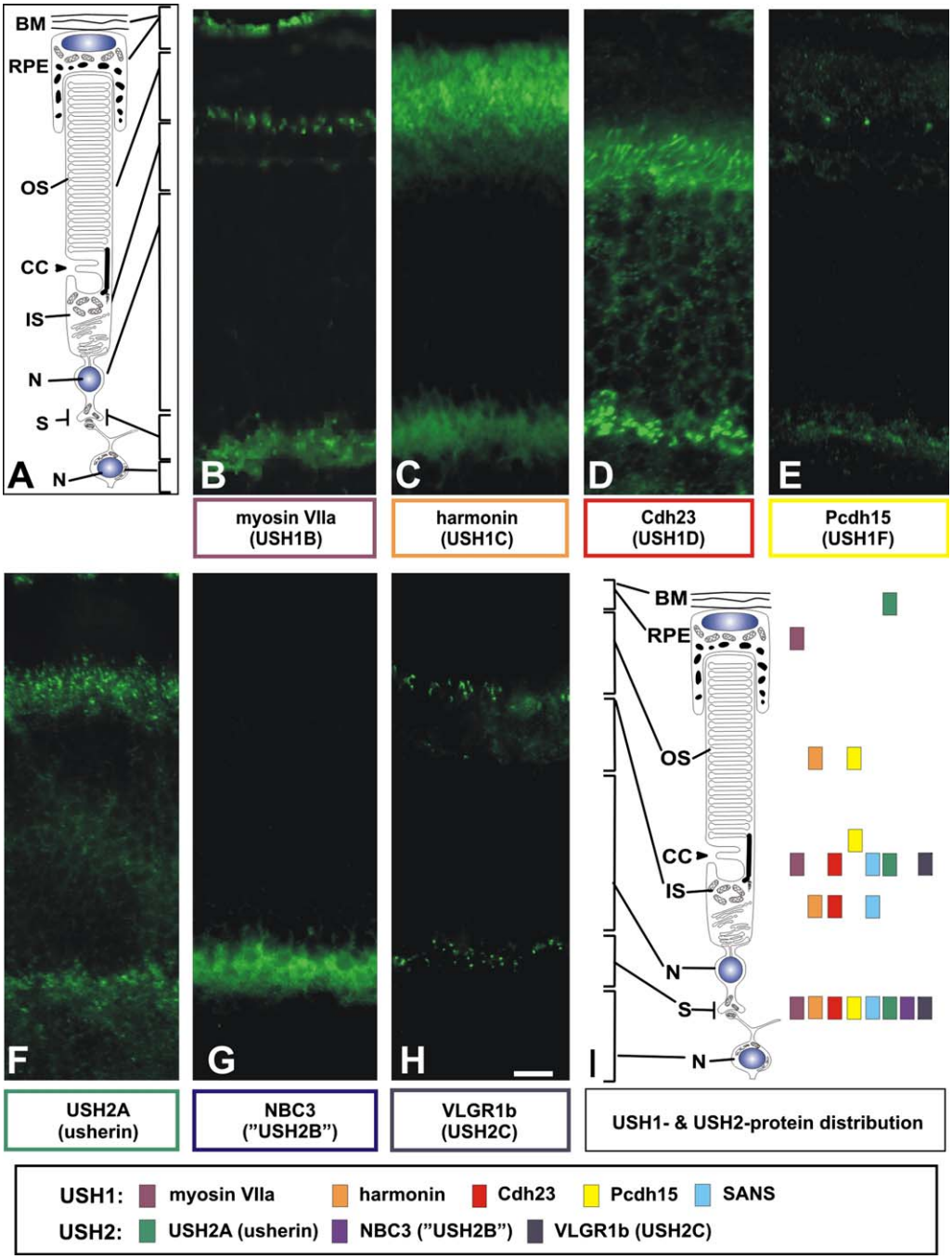

Fig. 6. Localization of USH1 and USH2 proteins in rod photoreceptor cells. (A) Scheme of a rod photoreceptor cell. The apical extensions of cells of the retinal pigment epithelium (RPE) evolve the tips of the photoreceptors light-sensitive outer segments (OS). The OS are linked via a connecting cilium (CC) to an inner segment (IS), which contains the biosynthetic machinery. Photoreceptor nuclei (N) are localized in the outer nuclear layer (ONL). The synaptic terminals (S) between photoreceptor cells and bipolar and horizontal cells are located in the outer plexiform layer (OPL) of the retina. The *B*ruch's *m*embrane (BM) is located above the RPE. (B–H) Indirect immunofluorescence of antibodies against myosin VIIa (B), harmonin (C), Cdh23 (D), Pcdh15 (E), USH2A (F), NBC3 (G), and VLGR1b (H) in parallel longitudinal cryosections through a mouse retina. (B) Myosin is found in RPE cells, at the CC, and in photoreceptor synapses in the OPL. (C) Harmonin is stained in the OS, IS and OPL (D) Cdh23 is localized in the CC, the IS and OPL (E) Pcdh15 is present in dot-like structures of distinct sizes in the OS concentrated at its base, as well as in the OPL. (F) USH2A is stained in the BM, in the CC, and in the OPL. (G) NBC3 is only found in the OPL (H) VLGR1b is detectable in the CC and with punctuated structures in the OPL. (I) Schematic summary of the USH1 and USH2 protein localization in the different subcellular compartments of a photoreceptor cell, represented by the color code in the legend. Note: all eight analyzed USH proteins are localized in the OPL where the ribbon synapses of photoreceptor cells are localized. Scale bar: 5 μm.

this region, the gene for a sodium bicarbonate co-transporter NBC3, SLC4A, was suggested as a candidate gene for USH2B (Bok et al., 2003; Pushkin et al., 1999). Mice lacking NBC3 (*Slc4a7*$^{-/-}$) develop a combined deaf-blindness phenotype similar to that observed in USH patients (Bok et al., 2003). Recently this hypothesis was supported by our finding that NBC3, like other identified USH1 and USH2 proteins, interacts with harmonin (Fig. 4A) and is in turn integrated in the USH protein network (Section 4, Fig. 7) (Reiners et al., 2005b; Reiners and Wolfrum, 2006).

NBC3 is expressed in a variety of alternatively spliced transcripts which are often tissue specific (Choi et al., 2000; Ishikawa et al., 1998; Pushkin et al., 1999). In the inner ear, NBC3 expression was detected in regions beneath the stria vascularis (Bok et al., 2003; Reiners et al., 2005b), but also in the stereocilia, the lateral membrane, and in the synapses of cochlear hair cells (Reiners et al., 2005b). An association of NBC3 with synapses was also described for the retinal neurons, in particular for photoreceptor synapses (Fig. 6G,I) (Bok et al., 2003; Reiners et al., 2005b). NBC3 plays an important role in the pH regulation of cellular compartments, especially at synapses (Bok et al., 2003; Krizaj et al., 2002). NBC3-mediated bicarbonate-flux is thought to be essential for an efficient buffering of H^+-loads, necessary for the maintenance of a normal rate of the plasma membrane Ca^{2+}-ATPase (PMCA)-mediated Ca^{2+}-efflux (Soleimani, 2003). Especially in photoreceptor synapses, spatial integration of NBC3 and PMCA1 may enhance the efficiency of H^+-buffering, possibly via binding of the C-terminal PBM of the PMCA1 (Krizaj et al., 2002) to one of harmonin's PDZ domains. Analogous to this, a functional association between NBC3 and PMCAs can be postulated to occur in the stereocilia, the synaptic region, and lateral membrane of hair cells where major Ca^{2+}-fluxes take place (Dumont et al., 2001).

2.2.1.3. VLGR1b (USH2C), the ''Goliath'' USH molecule and largest cell surface receptor ever found. Mutations in the *v*ery *l*arge *G*-coupled *r*eceptor 1b (*VLGR1b*) gene are responsible for USH2C (Table 1) (Burgess, 2001; Staub et al., 2002; Weston et al., 2004), febrile and afebrile seizures in humans (Skradski et al., 2001), and is linked with susceptibility to audiogenic seizures in mice (McMillan and White, 2004). The previously identified *Mass* (*m*onogenic *a*udiogenic *s*eizure *s*usceptibility *1*) gene is thought to be a splice-variant of VLGR1b (McMillan and White, 2004). In mammals, five isoforms of VLGR1 (VLGR1a to 1e) have been identified so far (Weston et al., 2004;Yagi et al., 2005). They belong to the 33-member subgroup of the large N-terminal family B of seven *trans*membrane receptors. Their motif architectures are similar to that of the cadherin superfamily of integral membrane proteins involved in membrane–membrane adhesion (Fig. 5C). The isoform VLGR1b is the largest cell surface receptor known (McMillan et al., 2002).

The N-terminus of VLGR1b starts with a signal peptide followed by repeated units of CalX-β (*cal*cium e*x*changer β) domains named for the homology shared between the regulatory domain of Na^+/Ca^{2+}-exchanger proteins and the cytoplasmic domain of integrin β4 (McMillan et al., 2002). These modules bind Ca^{2+} and other cations, and therefore might function as an extracellular Ca^{2+}-sink, in Ca^{2+}-dependent cell–cell adhesion, or as an extracellular Ca^{2+}-monitor sensitive to extra- and intracellular Ca^{2+} trafficking (Nikkila et al., 2000; Weston et al., 2004). These features of VLGR1b might be the molecular basis for the failure of normal development of stereocilia and an early onset of hearing impairment in VLGR1b deficient mice carrying the *Mass1*^{frings} mutation. In addition to the CalX-β motifs, the ectodomain of VLGR1b consists of one LamG/TspN/PTX and a repeated set of EAR/EPTP domains. The pentraxin (PTX) homology domain shares high homology with USH2A, suggesting that VLGR1b and USH2A may share either common binding partners or may interact with each other. The seven EAR/EPTP repeats form a putative seven-bladed-β-propeller folding domain (Scheel et al., 2002; Staub et al., 2002). From other proteins it is known that this structure acts as highly specific receptor (Pons et al., 2003). Interestingly, the extracellular region of USH2A has been discussed as a possible ligand to the EAR/EPTP domain of VLGR1b (Weston et al., 2004). The C-terminal part of VLGR1b bears a class I PBM with the consensus sequence responsible for binding to harmonin's PDZ1 domain (Fig. 4A) (Reiners et al., 2005b; Reiners and Wolfrum, 2006).

VLGR1 is expressed in high levels in the developing nervous system of mice and zebrafish and is suggested to play a conserved role in neural proliferation (Gibert et al., 2005; McMillan et al., 2002). In the inner ear, VLGR1 expression is detected in the synaptic region and in the stereocilia of the sensory hair cells (Reiners et al., 2005b). This sterocilia expression, together with its long N-terminus, suggest that VLGR1b may serve as a platform for extracellular filaments which harbors numerous of Ca^{2+}-binding motifs (Weston et al., 2004). This feature of VLGR1b is shared with tip links, elastic structures between neighboring stereocilia which are thought to be involved in gating the mechanosensitive channels in the stereocilia membrane (Pickles et al., 1991). In the retina, VLGR1b is localized in the synaptic terminals and in the connecting cilium of photoreceptor cells (Fig. 6H,I) (Reiners et al., 2005b). At synapses, the large G-protein coupled receptor, VLGR1b may play a cadherin-like role in adhesion of synaptic membranes and may, in particular, participate in cell adhesion mediated G-protein signaling which is known to regulate the organization of the synaptic cytomatrix especially during synaptogenesis (Bruses, 2000; Neubig and Siderovski, 2002; Togashi et al., 2002). Although precursors of retinal pigment epithelium (RPE) cells express VLGR1b (McMillan et al., 2002), no protein is found in mature RPE cells (Fig. 6H,I) (Reiners et al., 2005b).

2.2.2. USH3-proteins

To date, only a single USH3-gene has been identified. A second locus on the long arm of chromosome 20 is not yet defined (Table 1) (Petit, 2001).

2.2.2.1. USH3A (clarin-1), a synaptic protein not expressed in photoreceptor synaptic terminals. The *USH3A* gene was shown to encode a novel transmembrane protein (Joensuu

et al., 2001) which is expressed as alternatively spliced transcripts (Adato et al., 2002). The longest isoform clarin-1 is a member of the novel vertebrate-specific clarin protein family. Characteristics of all clarins are 4-transmembrane domains, a single glycosylation site between transmembrane domain 1 and 2, and several conserved sequence motifs (Fig. 5D). The C-termini of human and murine clarin-1 bear a ''TNV'' signature that might serve as a class I PBM (Adato et al., 2002) (Fig. 5D).

Clarin-1 is expressed in several tissues (e. g. heart, skeleton muscle, testis), in the olfactory epithelium, and in the sensory epithelia of auditory and visual system (Adato et al., 2002; Joensuu et al., 2001). In the inner ear, clarin-1 expression is present in spiral ganglion cells and cochlear hair cells where it is associated with pre- and post-synaptic membranes (Adato et al., 2002). Based on its sequence homology to the synaptic protein stargazin, a synaptic protein described in the cerebellum a role for clarin-1 in the ribbon synapses of the inner ear and the retina has been suggested (Adato et al., 2002). Preliminary immunohistochemical localization of clarin-1 in the inner plexiform layer of the mouse retina (Geller et al., 2004) was recently confirmed by mRNA expression analysis in a rat model with degenerated photoreceptor cells (Geller and Flannery, 2005). The photoreceptor cell loss did not change the mRNA-level of clarin-1 in retina indicating the absence of expression of clarin-1 in photoreceptor cells.

3. Animal models for USH

Mouse models that mimic the mutant phenotypes of human diseases are important in the development of therapies. At least one mouse model exists or is currently under generation for all USH types (Table 1).

Mouse mutants defective for USH1 proteins myosin VIIa (Shaker-1) (Gibson et al., 1995; Mburu et al., 1997), cadherin 23 (Waltzer) (Di Palma et al., 2001a), protocadherin 15 (Ames waltzer) (Alagramam et al., 2001a), harmonin (Deaf circler), harmonin isoform b (Deaf circler-2J) (Johnson et al., 2003), and SANS (Jackson waltzer) (Kikkawa et al., 2003) have been reported (Table 1). More recently, rat and mouse models for myosin VIIa were generated by mutagenesis (Rhodes et al., 2004; Smits et al., 2005). All rodent USH1 models are deaf and exhibit vestibular dysfunction. The mechanosensitive hair cells of the inner ear display anomalies in the development of their stereocilia, indicating the essential function for USH1 proteins in stereocilia differentiation (see recent review by El Amraoui and Petit, 2005).

Although all identified USH1 proteins are also expressed in the rodent retina, none of the current USH1 rodent models for USH1 proteins undergo progressive retinal degenerations characteristic for USH1 patients. So far, only two cases, Deaf circler mice and Shaker-1/Waltzer double-mutant mice, display a slight retinal degeneration noticed in 9 or 12 month old mice, respectively (Johnson et al., 2003; Lillo et al., 2003). Although Shaker-1 mouse alleles develop a phenotype, namely anomalies in outer segment phagcytosis and melanosome motility in the RPE as well as in the transport of opsin molecules through the connecting cilium of photoreceptor cells, they do not lead to retinal degeneration. In addition, electrophysiologic studies on USH1 mouse models (e.g. Shaker-1 and Waltzer mice) reveal a slight reduction of electroretinograms that is consistent with the dysfunction of synapses in neuronal retina (Libby and Steel, 2001; Libby et al., 2003). It is still not clear why retinal degeneration in USH1 occurs in humans but not in mice. There are several possibilities including differences in specific mutations between the USH1 mouse models and human patients, differences in the genetic background, differences between the species in the extent of the molecular redundancy. Finally, for USH1 patients, RP is typically first diagnosed at 10 years of age and if the progress of the development of the defects by the mutated USH1 proteins is as slow in mice as it is in humans, photoreceptor cell death will never be evident during the maximal life span ($\sim$2 years) of mice.

The phenotypes observed in the photoreceptor cells and the neuronal retina of the USH1 mouse models are not suitable for evaluation of therapeutic strategies to cure retinal defects caused by the USH1. There is need for alternative animal models. Currently, defects caused by homologue USH1 genes are studied in the zebrafish (*Danio*) (Biehlmaier et al., 2005; Ernest et al., 2000). Interestingly, in contrast to human and mice, the zebrafish expresses two closely related *Pcdh15* genes with independent roles in hearing and vision (Seiler et al., 2005). Whereas mutations in *Pcdh15a* cause vestibular dysfunction and deafness, reduction of *Pcdh15b* activity results in a visual defect.

The Frings mouse and a recently generated *Vlgr1* knock out mouse are animal models for USH2C (Johnson et al., 2005; Weston et al., 2004). Based on its early onset of hearing loss, the Frings mouse was introduced as a model for the pathology of USH2C. In contrast, *Vlgr1* knock out mice respond to auditory stimuli even at 6 months of age. Nothing is known about visual impairments in either animal model. USH2A knock out mice were independently generated by the laboratories of D. Cosgrove (Boys Town National Research Hospital, Omaha, USA) and T. Li (Harvard Medical School, Boston, USA). Contradictory preliminary reports on both mice were independently presented at ARVO meetings. Of the previously characterized USH2A knock out mice, the first has no hearing impairment but undergoes retinal degeneration (Cosgrove et al., 2004) whereas the other knock out strain displays no phenotype in the retina but seems to exhibit abnormal hearing (Liu et al., 2005). The laboratory of J. Flannery (University of California, Berkeley, USA) has developed a clarin-1 (USH3A) knock out which has been not yet characterized (John Flannery, personal communication). Finally, based on the combined deaf-blindness phenotype of NBC3 deficient *Slc4a7*$^{-/-}$ mice, the sodium bicarbonate co-transporter gene *SLC4A* was suggested to be a candidate gene for USH2B (Bok et al., 2003).

4. USH protein network is co-ordinated by the key organizer harmonin, an interactome related to USH

From growing evidence emerges the picture that all proteins related to the currently identified USH genes are

organized in a protein network. Several studies describe protein–protein interactions between USH1 protein suggesting an USH1 protein network (Boëda et al., 2002; Siemens et al., 2002; Adato et al., 2005; Reiners et al., 2005a). More recently, we have shown that USH2 proteins are also integrated within this USH1/2 protein network (Reiners et al., 2005b; Reiners and Wolfrum, 2006). Moreover, there are indications that the USH3A protein clarin-1 may also participate in the USH protein network (Adato et al., 2002; Reiners et al., 2005b). Finally, there is evidence that this USH protein network is connected to the actin cytoskeleton and probably also to the microtubules (Boëda et al., 2002; Adato et al., 2005; Reiners and Wolfrum, 2006).

The USH protein network is coordinated and integrated by the USH1 gene products harmonin and SANS. Both scaffold proteins possess protein domains which are responsible for the specific interactions between themselves and other proteins (Fig. 4). SANS forms homodimers, which interact with myosin VIIa and harmonin, and seems to be associated with microtubules (Adato et al., 2005). The three different isoforms of harmonin are capable of generating a harmonin network by homophilic interactions. Within this harmonin network the two or three PDZ domains of the harmonin molecules provide a platform for further interactions with other USH proteins. The USH2 proteins, USH2A isoform b and VLGR1b, and the USH2B candidate NBC3, as well as SANS and the SANS relative Harp, contain in their C-terminus a consensus sequence class I PBM specific for binding to harmonin PDZ1 (Weil et al., 2003; Reiners and Wolfrum, 2006; Reiners et al., 2005b). Myosin VIIa, SANS and Cdh23 also interact through an internal PBM with PDZ1 (Boëda et al., 2002; Adato et al., 2005). The binding of Cdh23 and Pcdh15 to harmonin's PDZ2 occurs through a C-terminal class I PBM present in both USH1 cadherins (Boëda et al., 2002; Adato et al., 2005; Reiners et al., 2005a). Finally, harmonin also connects the network to the actin cytoskeleton. Direct interaction of harmonin b isofoms with actin filaments is mediated by the PST domain (Boëda et al., 2002). Additional indirect linkages of the protein network to the actin cytoskeleton are provided by binding to harmonin's PDZ domains of the actin-associated protein filamin A, the actin-based molecular motor myosin VIIa, and β-catenin, which is anchored via α-catenin to actin filaments (Boëda et al., 2002; Johnston, et al., 2004; Reiners, 2004; Reiners and Wolfrum, 2006; Jürgens et al., unpublished).

The experimentally verified protein–protein interactions in the entire USH protein network, the ''interactome'' of proteins related to USH, is schematically illustrated in Fig. 7. The USH protein network integrates the function of cell adhesion molecules (the two USH1 cadherins and two USH2 proteins) and other USH molecules (e.g. myosin VIIa, NBC3) via the USH scaffold molecules, SANS and predominantly harmonin. Furthermore, harmonin provides linkages to the cytoskeleton, especially to the actin cytoskeleton and the motile activities associated with the latter.

Where do these protein–protein interactions between the USH molecules occur in the cellular environment? There are several lines of evidence that in the inner ear, the USH protein network is essential for the coordinated differentiation of the ''actin filament-based'' stereocilia during the hair cell development (e.g. Boëda et al., 2002; Adato et al., 2005; Reiners et al., 2005b; and recent review by El Amraoui and Petit, 2005). One scenario might be that myosin VIIa conveys harmonin b to the stereocilia where it stabilizes the actin filament core of stereocilia and connects the actin filament bundles to the transmembrane proteins of the network (e.g. USH1 cadherins and USH2 molecules). The latter may cooperate in the formation transient lateral links between adjacent differentiation stereocilia. In mature hair cells, the USH protein network may also participate in processes involved mechano-electrical transduction in the stereocilia (Kros et al., 2002; Siemens et al., 2004; Etournay et al., 2005) and may additionally be important for the function of the ribbon synapses of hair cells (Adato et al., 2005; Reiners et al., 2005b).

Currently the picture of the USH protein in the eye is more diverse. In the retina, the proteins related to USH and components of the USH protein network are expressed in different cell types or are localized within diverse subcellular compartments of the photoreceptor cells. Since myosin VIIa is the only USH protein expressed in the cells of the RPE (Reiners and Wolfrum, 2006; Reiners et al., 2003, 2005b), the existence of an USH protein network in the RPE can be excluded. If the network is responsible for the development of the disease in the retina (see below), the origin of USH would not be expected to be located in the RPE. To date, with the exception of clarin-1 (USH3A) for which there are no data published, all identified USH1 and USH2 proteins including the USH2B candidate NBC3 are expressed in the retinal photoreceptor cells. It is obvious that the USH protein network can only be generated where the expression sites of USH proteins overlap. The USH proteins are concentrated in several different subcellular compartments, namely the outer segment, the connecting cilium, and the synaptic region (see above). The assembly of a network of outer segment molecules, including molecules of the visual transduction cascade and USH1F-protein Pcdh15, may be mediated by harmonin (Reiners et al., 2003, 2005a). The co-localization of SANS with the molecular motor myosin VIIa and the transmembrane proteins Cdh23, USH2A, and VLGR1b in the connecting cilium indicates a specific role in the ciliary function of photoreceptor cells (Besharse and Horst, 1990). So far all identified USH proteins (except clarin-1) are localized in the inner plexiform layer at the specialized synaptic junctions between photoreceptor cells and bipolar cells as well as horizontal cells. Both USH network scaffold proteins, SANS and harmonin may target the network components and their physiologic function to the specialized ribbon synapses. It has previously been stressed that molecular motors like myosin VIIa play an important role in the physiology of ribbon synapses (Prescott and Zenisek, 2005). As in other cellular environments, myosin VIIa may participate in synaptic molecule trafficking and/or endocytosis (see above). The transmembrane proteins related to USH have been suggested to be involved in synaptic adhesion and in regulation of the ionic homeostasis of the synapse. In synaptic

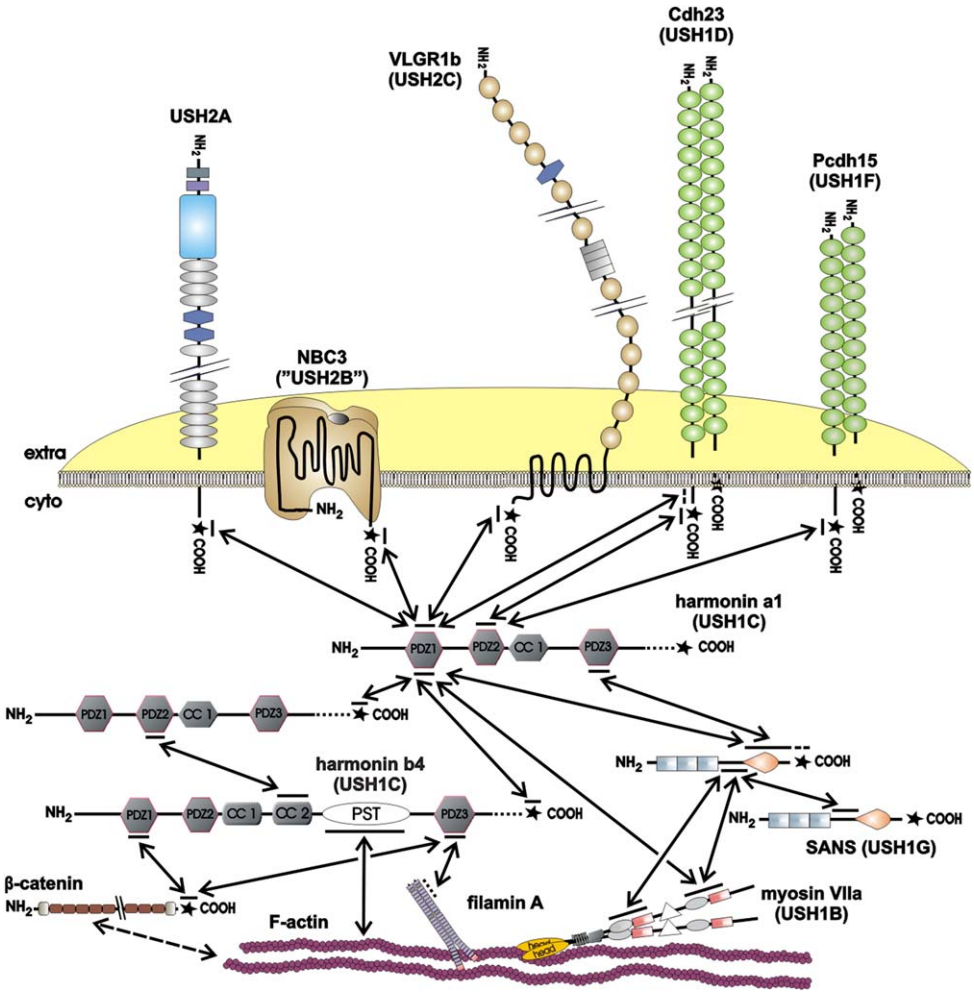

Fig. 7. Schematic diagram illustrating the deciphered interactions within the USH protein network, "interactome". The three USH2 related transmembrane proteins USH2A, NBC3 ("USH2B" candidate) and VLGR1b (USH2C) bind via their C-terminal PBM to PDZ1 of harmonin. The cadherins Cdh23 (USH1D) and Pcdh15 (USH1F) interact via their C-terminal PBM with PDZ2 of harmonin whereas the splice variant Cdh23 (+68) (isoform A) binds through an internal PBM to PDZ1. The scaffold protein SANS (USH1G) is suitable to form homomers via its central domain. It interacts with its C-terminal part with PDZ1 and PDZ3 of harmonin, whereas its central domain also binds to the MyTH-FERM domains in the tail of myosin VIIa (USH1B). Homo- and hetromeric interactions between the harmonin isoforms can occur via binding of PDZ1 to the C-terminal PBM of some splice variants (e.g. harmonin a1 and b4) and/or through interactions of PDZ1 and PDZ2 with the second coiled-coil (CC2) domain restricted to harmonin b isoforms. The USH protein network is directly connected to the actin cytoskeleton through the PST domain present in harmonin b isoforms and/or indirectly via the actin-based molecular motor myosin VIIa, the actin-associated protein filamin A. β-Catenin which is anchored through α-catenin to actin filaments, provides a further indirect connection of the USH network to actin filaments. (For references please see text.)

terminals of neuronal cells in general, cell adhesion molecules in the pre- and post-synaptic membrane interact via their extracellular domains and keep the synaptic cleft in close proximity (Garner et al., 2000). Therefore, in the specialized ribbon synapses, the cadherins, Cadh23, and Pcdh15, as well as the transmembrane proteins USH2A and VLGR1b could fulfill comparable functions. Although, the localization of clarin-1 in the retina remains elusive, the homology of clarin-1 to stargazin suggests a role of clarin-1 in forming protein–protein contacts across the synaptic cleft. An important role of the sodium bicarbonate co-transporter NBC3 in the pH regulation at synaptic terminals was previously discussed (see above (Bok et al., 2003)). The localization of the USH molecules in the region of specialized ribbon synapses in both the retinal photoreceptor cells and the inner ear hair cells also indicates that the synapses in both cell types are not only morphologically similar, but also have a similar molecular composition and function.

Phylogenetic analysis of components of the USH network indicates that the network is restricted to the vertebrate phylum. While myosin VIIa and protcadherin 15 genes already exist in genomes of rather low eukaryotes, the orthologous genes to the USH1 molecules harmonin, cadherin 23, SANS, USH2A, VLGR1b and clarin-1 are found only in higher vertebrates (e.g. Adato et al., 2002; Ahmed et al., 2001; Bhattacharya et al., 2002; D'Alterio et al., 2005; Reiners, 2004; Reiners et al., 2003; Seiler et al., 2005; Weil et al., 2002).

The USH subtypes show clinical similarities which find their molecular basis in the integration of the gene products of all identified members of the USH subtypes within

a USH protein network. Although, causes of the retinal degeneration include defects in the RPE or in the photoreceptor cells (Kalloniatis and Fletcher, 2004), the functional analysis of the USH proteins provides evidence that in the case of USH, the origin of the retinal degeneration is the photoreceptor synapse. The elucidation of the USH protein network further suggests a common pathophysiological pathway for the senso-neuronal degeneration in USH. Dysfunction or absence of any of the proteins in the mutual ''interactome'' related to the USH disease may lead to the disruption of the network causing degeneration of the sensory epithelium of the inner ear and the neuronal retina, the clinical symptoms characteristic for USH patients. Further unraveling of this ''interactome'' in the future will provide candidate genes for syndromic and non-syndromic forms of deafness and retinal degeneration.

5. Perspectives in therapy of USH

USH patients suffer from congenital hearing loss and RP starting in childhood or even later in the 2nd to 4th decade of life. Gene therapy shows great promise for the treatment of monogenic diseases. In most forms of USH, with exception of USH3, the hearing impairment of USH patients originates in developmental failures of the inner ear hair cells (see above). Therefore prenatal inner ear treatments are necessary. However, gene therapy strategies based on prenatal treatment are currently not practical. Nevertheless, cochlear implants are successfully used to solve some of the problems of the hearing deficiency in USH patients.

Although research on visual implants is improving (Zrenner, 2002), this technology is currently not applicable for the cure of disabilities in vision. In the absence of any other effective treatment for inherited retinal degenerations, strategies for gene therapy approaches have emerged over the last decade. Gene therapy strategies are attractive for the treatment of retinal degeneration in general. Due to the longevity of retinal cells a permanent correction of the mutated gene is eligible. Furthermore, the retina is easily accessible and allows local application of therapeutic vectors with the reduced risk of systemic effects (Rolling, 2004).

The most common and effective vectors for delivering genes into retinal cells in vitro and in vivo are adenovirus, adeno-associated viruses, and lentiviral vectors (Cheng et al., 2005 and citations therein). Between these delivery systems, the lentiviruses have several advantages: (i) lentiviruses can infect post-mitotic cells (Lewis and Emerman, 1994), (ii) they stably integrate their reverse-transcribed DNA into the host cell genome, and (iii) they thereby allow a rapid onset as well as (iv) a high level of persistent transgene expression. Subretinal injection of lentivirus results in specific and efficient infection of RPE cells and long term expression of lentiviral mediated gene expression (Naldini et al., 1996; Tschernutter et al., 2005). Moreover, using cell-specific promoters, lentiviral vectors have been shown to mediate photoreceptor-specific gene expression (Ikawa et al., 2003; Miyoshi et al., 1997). Furthermore, they have a respectable cloning capacity which is close to 10 kb and induce little host immune response (Sinn et al., 2005; Tschernutter et al., 2005).

To our knowledge, data of only one gene therapeutic approach for USH defects has been presented. In D.S. Williams' laboratory (University of California, San Diego, USA), the gene replacement of myosin VIIa mediated by lentivirus has been validated and preliminary results were recently shown at the ARVO meeting 2005 (Gibbs et al., 2005; Yang et al., 2005). In these studies, the lentiviral mediated transduction of myosin VIIa in cultured RPE cells derived from Shaker-1 mice revealed a high transduction efficiency and the correction of the phenotype previously observed in the myosin VIIa deficient RPE (Gibbs et al., 2005). After transduction, degradation of phagocyted outer segments and melanosome transport occurs as in wild type RPE cells. Furthermore, in vivo experiments with Shaker-1 mice revealed myosin VIIa expression after subretinal injection of these viruses in the RPE of the Shaker-1 mice (Yang et al., 2005), but so far nothing is known about the rescuing effect of the RPE function in vivo.

Despite of all the advantages of lentiviral vectors over other delivery systems, one disadvantage of all viral transfer vectors is the limitation the cloning capacity. Although genes up to 10 kb can be cloned into lentiviral vectors, this is still not sufficient for the coding regions of some USH genes, e.g. the *VLGR1b* and *CDH23* genes. Furthermore, a previous study indicated that lentiviruses preferentially transduce RPE cells and only to a minor extent photoreceptor cells (Miyoshi et al., 1997). Successful gene transfer to photoreceptor cells is important for effective gene therapy of USH because degeneration primarily affects photoreceptor cells and all identified USH genes are expressed specifically in photoreceptor cells (see above). To enhance tissue-specific expression, at least part of a photoreceptor cell specific promoter would have to be inserted into the vector. This in turn restricts the size of coding sequence which can be integrated into the vector. Therefore, alternative therapeutic strategies have to be developed. One option would be the precise evaluation of the protein domains which are necessary for the protein function. The nucleotide coding sequence of such a minimized peptide could more easily delivered by using one of the specific transfer vectors.

There are still some problems concerning gene therapy, for example, randomized integration of viral DNA into the host genome and precise regulation of gene expression or expression of alternative splice-variants. The latter is an important issue for gene therapy of USH. As described above, several USH genes are known to be alternatively spliced and in particular, the key organizer of the USH protein network harmonin is expressed as various splice forms which are differentially localized in functional compartments of photoreceptor cells. A therapeutic approach which overcomes these problems is gene repair (Sangiuolo and Novelli, 2004). In contrast to gene replacement, the alternative therapeutic nucleic acid repair approach corrects a mutation by targeting oligonucleotides to the genomic DNA sequence

where alteration is required. Consequently, the relationship between the coding sequences and regulatory elements remains intact, and therefore the corrected gene is expressed at physiological levels in the appropriate cell type (Liu et al., 2002; Sangiuolo and Novelli, 2004). However, until gene repair strategies can be clinically applied, several steps to improve the efficiency of oligonucleotide delivery will be necessary.

Whatever therapeutic approaches for USH will be chosen, it is currently necessary to validate them in an animal model that mimics the mutant phenotype of human USH disease. At present, no appropriate rodent model which provides a phenotype comparable to the retinal dysfunction in USH patients is available for USH1 and USH2, or USH3A (see Section 3). Therefore, there is a need for alternative animal models for USH to proceed in the development of USH therapies. In conclusion, although no curative therapy of the vision impairment of USH currently exists, some promising approaches have been initiated.

After acceptation of our manuscript, the PDZ domain protein whirlin was identified to be connected to the dynamic USH protein interactom (van Wijk et al 2006).

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