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17. The role of cadherins in sensory cell function

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Abstract. In vertebrate sensory cells of the eye and the inner ear a group of non-classical cadherins, namely cadherin 23 (CDH23), protocadherin 15 (PCDH15) and protocadherin 21 (PCDH21) are expressed, and are essential for sensory cell development and/or sensory function. Defects in these cadherins can cause nonsyndromic blindness or deafness or leads to deaf-blindness known as Usher syndrome (USH). As all cadherin superfamily members, the “sensory” “S”-cadherins possess the common EC domain signature in their extracellular part, but the number of repetitive ECs is much more variable and the molecular interactions of the extracellular parts and the cytoplasmic protein arrangements associated with these cadherins are less uniform. CDH23 is a member of the FAT cadherins subfamily containing up to 27 EC domains. In contrast, PCDH15 and PCDH21 are typical protocadherins containing 11 or 6 EC domains, respectively.

The USH cadherins, CDH23 (USH1D) and PCDH15 (USH1F) are integrated into the protein interactome related to the USH disease by binding with their cytoplasmic part to other USH proteins. In mechanosensitive hair cells, both cadherins are part of fibrous links connecting the membranes of neighbouring stereovilli, the mechano-sensory organelles during hair bundle differentiation. In mature hair cells both USH cadherins persist as tip links. In the vertebrate photoreceptor cells the two USH cadherins are thought to contribute to membrane-membrane adhesion between the inner and outer segment, but predominately at the ribbon synapses. PCDH21 is mainly found in photoreceptor cells of vertebrate retinae and seems not to be expressed in hair cells. In rod and cone cells, PCDH21 is localized in the rim of the newly synthesized disk membranes at the base of the outer segment. For its essential

role in disk neogenesis and stacking of newly formed disks PCDH21 is part of an actin associated adhesion complex containing prominin 1. For maturation of the outer segment disks it is necessary that the extracellular part of PCDH21 is proteolytically cleaved at the proximal EC6.

The present article provides an overview of current knowledge on the function of “S”-cadherins in sensory cells and thereby their role associated with sensorineuronal degenerations underlying human diseases.

Introduction

The cadherin superfamily consists of more than 100 members responsible for a variety of roles in differentiation, maintenance and function of tissues and cells. This superfamily involves: i) classical cadherins that are the major component of cell-cell adhesive junctions; ii) desmosomal cadherins (desmocollins and desmogleins); iii) protocadherins; iv) cadherin-related molecules like the *fat* protein of *Drosophila*; v) cadherins with tyrosine-kinase domains (pointing towards a role of cadherins in signal transduction pathways), and vi) cadherins with seven-transmembrane domains sharing additional sequence homology with G-protein coupled receptors, e.g. the *flamingo* protein originally identified in *Drosophila* where it is involved in planar cell polarity (1). The common, defining feature of the diverse cadherins is the cadherin or extracellular domain (EC) of their extracellular N-terminal part. ECs are compact domains of approximately 110 amino acid residues containing evolutionary highly conserved, negatively charged motifs. Classical cadherin molecules (CCDH) (C-cadherin, e.g. E-cadherins or N-cadherins) contain five ECs (EC1-EC5) joined by flexible hinge regions (Fig. 1a). Binding of Ca^{2+} -ions in the neighborhood of each hinge stiffens the extracellular part and thereby triggers homophilic interaction between the N-terminal EC1 domains of cadherin molecules which are located at the membranes of neighbouring cells (2). A short transmembrane domain links the extracellular part with the intracellular cytoplasmic domain which anchors the cell-cell adhesion complex through linker protein complexes (e.g. β/α -catenin) to the cytoskeleton (e.g. actin filaments). The cytoplasmic domain of cadherins also connects cell-cell adhesion complexes to intracellular signaling pathways (3). Although classical C-cadherins are expressed in sensory organs of vertebrates, recent studies revealed that non-classical cadherins - namely cadherin 23 (CDH23), protocadherin 15 (PCDH15), and protocadherin 21 (PCDH21) - are essential for the differentiation, the maintenance and the sensory function of sensory cells in the inner ear and the eye. As other cadherins, these “sensory” S-cadherins possess the common EC domain signature, but the number of repetitive ECs is much more variable and the molecular interactions of the extracellular parts and the cytoplasmic protein arrangements associated with these cadherins are less uniform (Fig. 1). In human patients, mutations in S-cadherins can cause non-syndromic deafness and blindness, but also combined deaf-blindness. The present article aims to present an overview of current knowledge on the function of S-cadherins in sensory cells and thereby their role associated with sensorineuronal degenerations underlying human diseases.

In the vertebrate eye, two types of photoreceptor cells, cones and rods, adapted to photopic vision allowing color perception and scotopic vision at low-light conditions, respectively, are arranged in the innermost layer of the neuronal retina. Both types are highly polarized sensory neurons consisting of morphological and functional distinct cellular compartments. From their cell body an axon projects to the synaptic terminus, where ribbon synapses connect the photoreceptor cells with the 2nd retinal neurons (4). At the other pole, a short dendrite named inner segment, terminates in a light sensitive outer

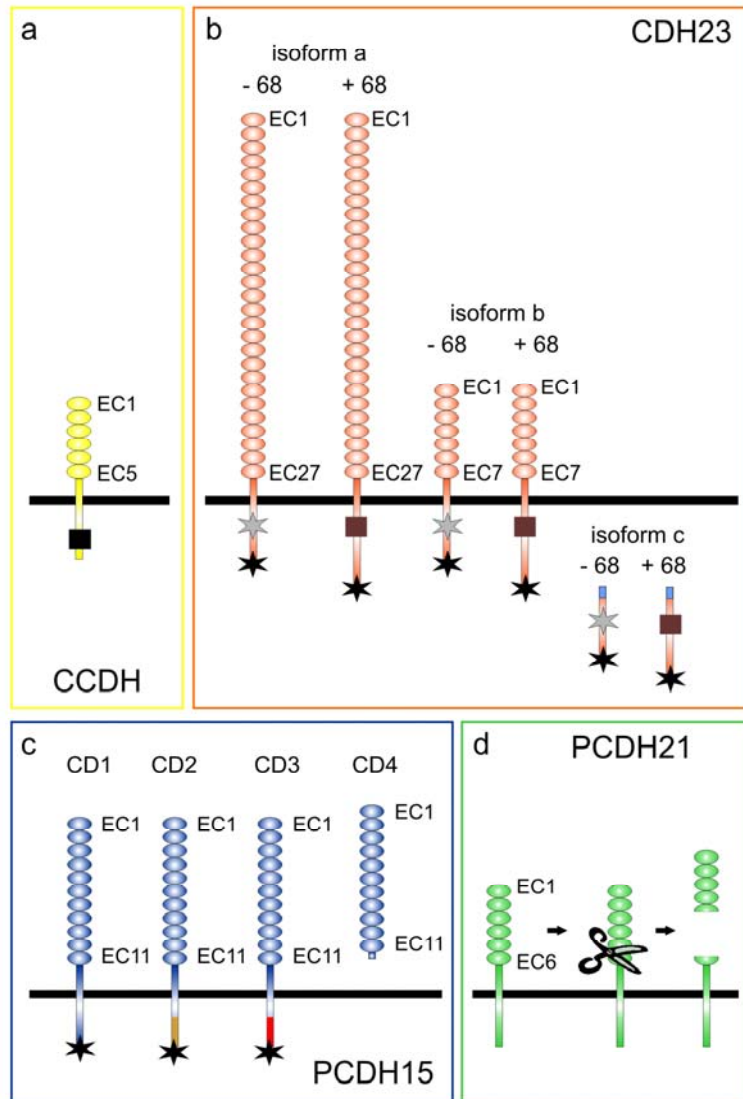


Figure 1. Domain structure of certain cadherins. **(a)** Structure of classical cadherin (CCDH) composed of 5 EC domains, a single transmembrane domain and the cytoplasmic domain containing a motif (black rectangle) mediating the association with the cytoskeleton through β -catenin. **(b)** Cadherin 23 (CDH23) encompasses three groups, a, b and c which differ in the number of EC domains. Isoform c contains an N-terminal unique seven amino acid sequence (light blue rectangles) but lacks any EC or transmembrane domain. All isoforms can alternatively splice leading to the inclusion or exclusion of exon 68 (brown rectangle) generating an internal PDZ binding domains (grey asterisk). C-terminal class 1 PDZ binding motifs (PBM) indicated by black asterisks. **(c)** Proteocadherin 15 (PCDH15) has four known isoforms CD1-CD4. All contain 11 EC domains. CD1, CD2 and CD3 contain PBMs (black asterisks) but differ in their cytoplasmic domain (indicated by different colors). CD4 lacks the transmembrane and cytoplasmic domain. **(d)** PCDH21 contains six EC domains, a single transmembrane domain and a cytoplasmic domain. Scissors indicates the internal proteolytic cleavage site in EC6.

segment (5;6) (Fig. 2a). This outer segment is similar to other sensory cilia (7), additionally characterized by specialized flattened disk-like membranes, where all components of the visual transduction cascade are arranged (8). The visual signal transduction cascade in vertebrates is one of the best studied examples of a G-protein transduction cascade. Photoexcitation leads to photoisomerisation of the visual pigment rhodopsin (Rh^*) which catalyses GDP/GTP exchange at the visual heterotrimeric G-protein transducin, which in turn activates a phosphodiesterase (PDE), catalyzing cGMP hydrolysis in the cytoplasm which leads to the closure of cGMP-gated channels in the plasma membrane, finally resulting in photoreceptor cell hyperpolarization. The phototransductive membranes of the outer segment are continually renewed throughout lifetime. Newly synthesized membranes are added at the base of the outer segment, whereas aged disks at the outer segment apex are phagocytosed by cells of the retinal pigment epithelium (9). Outer segment molecules are continually synthesized in the inner segment and transported through the slender connecting cilium to their destinations in the outer segments (6).

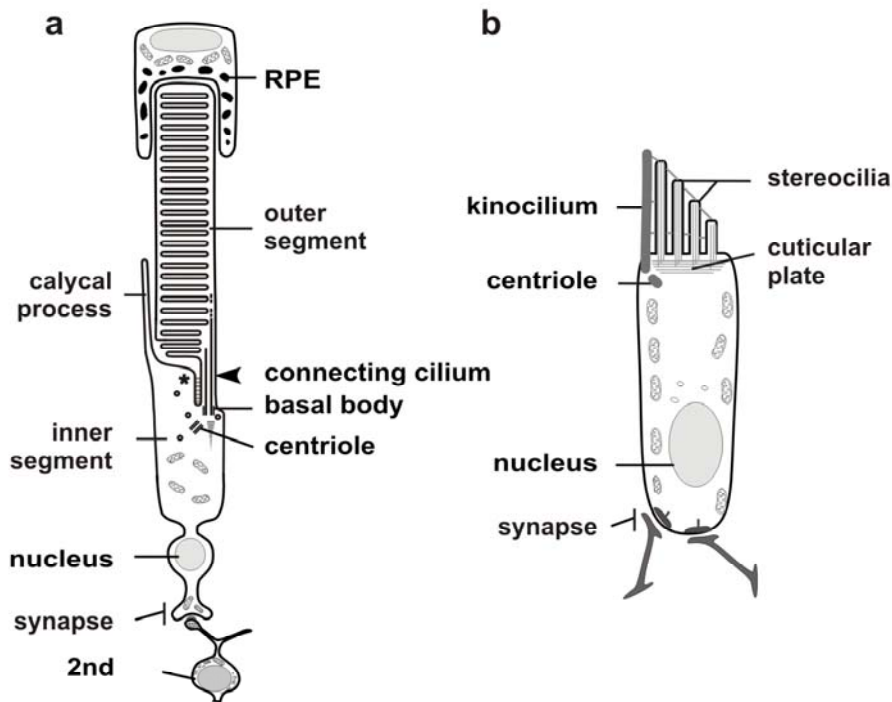


Figure 2. Schematic representation of the sensory cells in the retina and the inner ear. **(a)** Vertebrate rod photoreceptor cells are composed of distinct morphological and functional compartments. Photosensitive outer segment is connected with the biosynthetic active inner segment via the connecting cilium. At the ciliary base a basal body and the adjacent centriole are present. The proximal outer segment and the connecting cilium are enclosed by the periciliary specialization of the apical inner segment (asterisk). Calycal processes extend from the apical inner segment and project parallel to the outer segment. Ribbon synapses link photoreceptor cells and bipolar and horizontal cells. RPE: retinal pigment epithelium. **(b)** The mechanotransduction in hair cells takes place at the stereovilli (= stereocilia), at the apical part of the hair cell. Stereovilli are rigid microvilli-like structures that are organized in a staircase like manner of decreasing height.

In the inner ear, mechanosensitive sensory hair cells are found in the utricle and the saccule of the vestibular system that provides the dominant input about movement and equilibrium and are part of the auditory system in the Organ of Corti of the cochlea. Furthermore, sensory hair cells are elemental sensory units in the lateral line of fishes and amphibians. Hair cells are specialized mechanosensitive neurons that carry out the conversion from incoming mechanical stimuli into the intrinsic electrical signals. The schematic representation in figure 2b shows the structure of a mechanosensitive hair cell. The mechanosensitive structures of hair cells are the eponymous hair-like apical extensions, the so-called stereocilia. These stereocilia are actually modified microvilli and a term change from stereocilia to stereovilli is ongoing in the literature. The stereovilli contain a cytoskeletal core of densely packed actin filaments which are anchored in the actin filament meshwork of the cuticular plate by extensions of a few central actin filaments. On the apical surface of the hair cell, rows of stereovilli form a staircase of increasing height with the tallest row of stereovilli abutting the single kinocilium, an authentic cilium containing a microtubule-based cytoskeleton. The kinocilium is not required for mechanotransduction and can be absent in matured hair cells, but it is probably important for hair bundle development and polarity. Deflection of the hair bundle in the direction of the longest stereocilia increases the open probability of mechanotransduction channels localized towards the stereocilia tips (10).

The plasma membranes of adjacent stereovilli and the membranes of the longest stereovilli and of the kinocilium are connected by extracellular linker filaments with distinct morphological and molecular features (Figs. 2b, 4) (11). During development mouse cochlear hair cells exhibit transient lateral links, ankle links, and kinociliary links which are thought to abet the shape of differentiating hair bundles. In contrast, mature hair cells possess horizontal top connectors and tip links. The tip links are assumed to be physically connected to the mechanotransduction cation channels directly participating in the gating mechanism of the mechanosensitive channel (10). However, in the absence of molecular data the function of the linkages in the hair bundles remained speculative mostly inferred by indirect means. Only recent molecular and cellular analyses of deafness causing molecules, e.g. S-cadherins, enlightened molecular compositions of the extracellular links and thereby provide important founded insights into their function. The molecular decipherment of the link components also revealed molecular parallels between mechanosensitive hair cells and photoreceptor cells; the temporary ankle links between stereovilli and the fibres between the adjacent membranes of the periciliary inner segment and the connecting cilium of photoreceptor cells are composed of the extracellular domains of GPR98 (VLGR1), vezatin, and USH2A isoform B (Figs. 4, 5) (12-15). Further parallels are the ribbon synapses present in hair cells and photoreceptor cells. In the present review we will provide a current view of the putative function of non-classical cadherins, cadherin 23 (CDH23), protocadherin 15 (PCDH15), and protocadherin 21 (PCDH21) found in the sensory hair cells and the photoreceptor cells.

1. Cadherin 23 and protocadherin 15 are related to non-syndromic deafness or the human Usher syndrome

In human, mutations in the genes for the cadherins cadherin 23 (*CDH23*) and protocadherin 15 (*PCDH15*) lead to non-syndromic deafness (DFNB) or Usher syndrome (USH), the most common form of hereditary deaf-blindness. Genotype-phenotype correlations for *CDH23* and *PCDH15* indicated that missense or inframe alterations result in nonsyndromic deafness DFNB12 and DFNB23, whereas truncating mutations

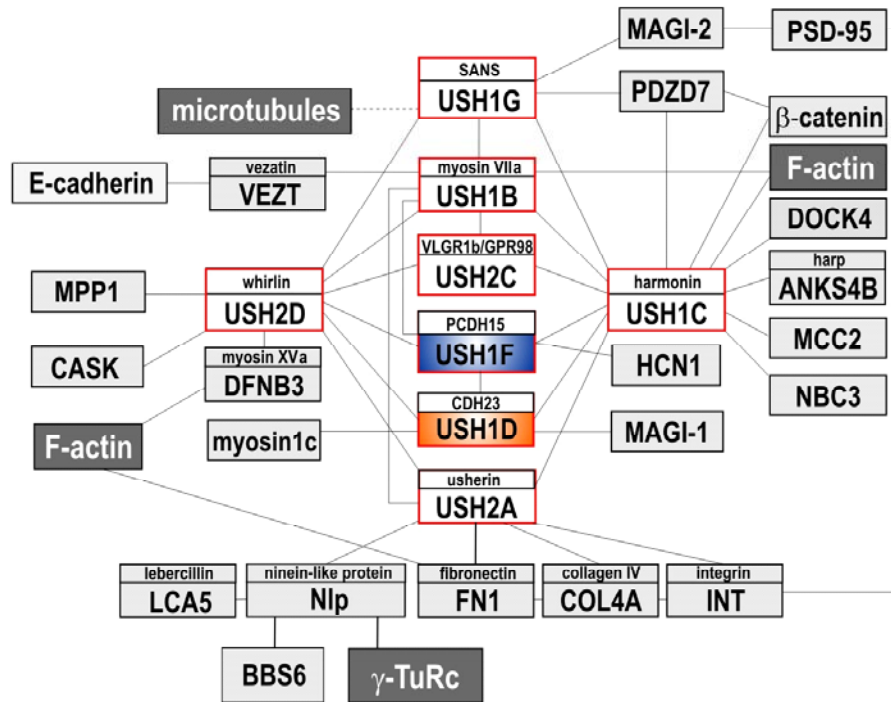


Figure 3. Scheme of the protein interactome related to the human Usher syndrome (USH). USH proteins are indicated by red boxes, CDH23 (USH1D) and PCDH15 (USH1F) are further highlighted in orange and blue, respectively; cytoskeleton components are shown in grey; other interaction partners in light grey. Confirmed interaction partners (by two or more independent methods) are indicated by solid lines; putative associations by dotted lines. Details of the interactome are reviewed in Kremer *et al.* (2006) and Reiners *et al.* (2006), currently updated in Nagel-Wolfrum *et al.*, in prep.

are causing USH1D and USH1F, respectively (16). USH is a complex disease divided in three clinical types (USH1-3). USH1 is the most severe form characterized by congenital deafness, vestibular dysfunction and progressive retinal degeneration, *retinitis pigmentosa* (RP). All three types additionally show genetic heterogeneity and from the identified 13 genes, so far five genes have been linked to USH1, three to USH2 and one to USH3 (16). Although mutations in these genes lead to a similar phenotype, they encode for proteins of different protein families exhibiting diverse cellular functions (17). Recent studies revealed that all USH1 and USH2 molecules are integrated mainly by PDZ-containing scaffold proteins harmonin (USH1C) and whirlin (USH2D) but also by SANS (USH1G) in protein networks - the protein interactome related to the USH disease (Fig. 3) (15;17-19).

CDH23 and PCDH15 are atypical members of the large cadherin superfamily. In the cytoplasmic tails of both cadherins, the consensus R1 and R2 binding sites for β -catenins (20) are missing (21-24). But, in contrast to classical cadherins, they harbor class I PDZ-binding motifs (PBMs) in the C-terminus of their cytoplasmic tail (Fig. 1) (21-24). Through these PBMs both USH cadherins are integrated into the protein networks of the USH protein interactome via the PDZ domains-containing USH proteins harmonin

Table 1. Cadherin 23 (CDH23), protocadherin 15 (PCDH15) and protocadherin 21 (PCDH21) interaction partners

	Interacting protein	Function	ID	Reference
CDH23	CDH23 (DNFB12/USH2D)	membrane adhesion	EC	Siemens et al., 2004 (33)
	PCDH15 (DNFB23/USH1F)	membrane adhesion	EC	Kazmierczak et al., 2007 (36)
	harmonin (DNFB18/USH1C)	scaffold protein	CD	Boeda et al., 2002 (23) Siemens et al., 2002 (24) Pan et al., 2009 (31)
	whirlin (DNFB31/USH2D)	scaffold protein	CD	Kremer et al. 2006 (19)
	myosin 1c	molecular motor	CD	Siemens et al., 2004 (33)
	MAGI-1	scaffold protein	CD	Xu et al., 2008 (57)
PCDH15	PCDH15 (DNFB23/USH1F)	membrane adhesion	EC	Kazmierczak et al., 2007 (36)
	CDH23 (DNFB12/USH2D)	membrane adhesion	EC	Kazmierczak et al., 2007 (36)
	harmonin (DNFB18/USH1C)	scaffold protein	CD	Adato et al., 2005 (25) Reiners et al., 2005 (18)
	whirlin (DNFB31/USH2D)	scaffold protein	CD	Reiners et al., 2005 (40)
	myosin VIIa (USH1B)	molecular motor	CD	Senften et al., 2006 (26)
	HCN1	channel protein	CD	Ramakrishnan et al., 2009 (58)
PCDH21	prominin 1	transmembrane protein	CD	Yang et al., 2008 (52)
	cytoskeletal proteins?		CD	Rattner et al., 2004 (50)

ID: interaction domain; EC: extracellular cadherin domain; CD: cytoplasmic domain MAGI-1: membrane associated guanylate kinase; HCN1: Hyperpolarization activated cyclic nucleotide-gated potassium channel 1

(USH1C) (17;18;23;25) and whirlin (USH2D) (19). Furthermore, PCDH15 binds directly to the USH1B protein myosinVIIa through its C-terminal domain (26) (Tab.1; Fig.3). Nevertheless, since similarities in sequence and in domain structure in the two USH cadherins are very low, with the exception of the class I PBM in the extreme C-terminus, it does not surprise that they belong to different branches or subfamilies of the cadherin superfamily.

1.1. Cadherin 23 (USH1D, DFNB12) - the “fat” USH cadherin

Cadherin 23 (CDH23) previously known as Otocadherin is related to the *Drosophila fat* protein, the so-called FAT cadherins which contain many more EC domains than the typically five of classical cadherins. The full length CDH23 (isoform a) contains 27 EC domains (22). In contrast, the CDH23 isoform b contains only 7 EC and the isoform c lacks all ectodomains and contains no transmembrane domain, but possesses an unique seven amino acid sequence at its N-terminus (Fig. 1b) (22;24;27;28). Since cell-cell adhesion function can be excluded for CDH23 isoform c, this isoform is thought to compete for cytoplasmic binding partners with the other CDH23 isoforms which may play a role in the regulation in signaling pathways in the cytoplasm (29). All three different isoforms have in common that additional alternative splicing leads to the inclusion or exclusion of exon 68 named CDH23 (+68) or CDH23 (-68), respectively (24). Whereas CDH23 (+68) splice variants are expressed preferentially in the inner ear sensory epithelium (24;30), CDH23 (-68) splice variants are more ubiquitously expressed and are found in the neuronal retina (24). CDH23's exon 68 encodes for an insert in the

cytoplasmic domain which destroys an internal PDZ-binding motif (PBM) homolog to the internal PBM of the adaptor protein Ril present in CDH23 (-68) (24). Both PBMs of CDH23 (-68) participate in interactions with the PDZ-containing scaffold protein harmonin (USH1C) and whirlin (USH2D). The C-terminal PBM of CDH23 binds to the PDZ2 of harmonin while the internal PBM of the CDH23 (-68) isoform interacts with the PDZ1 of harmonin (23;24). Recent data indicated that in addition to the PDZ1 domain an internal N-terminal sequence of harmonin may be necessary for the interaction with the internal cytoplasmic domain of CDH23 (31).

In the inner ear, CDH23 expression was found in the sensory hair cells and in the Reissner's membrane (1;23;32). Analyses of the waltzer (*v*) mice deficient in CDH23 revealed that CDH23 is required for the normal development of the stereocilia of hair cells (30). 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 (Fig. 4) (23;28;29). In mature cochlear hair cells, CDH23 is localized at the centrosome, most probably in the form of CDH23 isoform c, in the cuticular plate, in the apical, vesicle-rich pericuticular region and at the stereovilli (23;28;29). In addition, the longer isoforms of CDH23 were proposed to be a component of the tip links (33), structures linking the apical tips of the hair bundles in mature hair cells (Fig. 4) which are proposed to serve in gating the mechanosensory channels (34). This remarkable hypothesis was confirmed by the absence of tip links in the hair cells of the lateral line of zebrafish *cdh23* mutants *sputnik* (35). Robust immunolabelling of CDH23 and PCDH15 in mature hair cells of different vertebrate species further support this finding and co-localization of both proteins at the stereovilli tips suggested the interaction of CDH23 and PCDH15 as components of the tip link (36). Further analyses indicated the heteromeric adhesion of CDH23 and PCDH15 homodimers through their N-termini

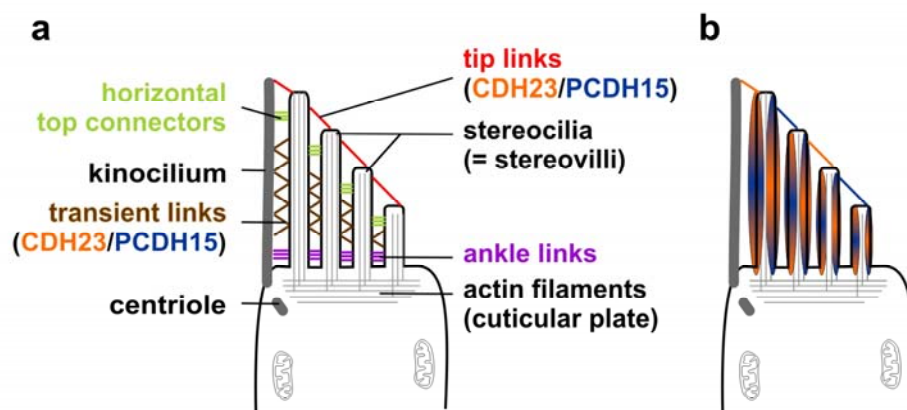


Figure 4. Localization of CDH23 and PCDH15 in inner ear hair cells. **(a)** Scheme of the apical region of an inner ear hair cell where the mechanotransduction takes place. Numerous links, interconnecting the growing stereovilli: transient links (brown), ankle links (purple) and tip links (red). During maturation transient lateral, ankle links and the kinocilium degenerate in the mammalian cochlear hair cells; horizontal top connectors (light green) and tip links (red) remain. **(b)** Localization of CDH23 (orange) and PCDH15 (blue) in the stereovilli of hair cells. During hair cell development CDH23 (orange) and PCDH15 (blue) are localized in the transient links. Isoforms of both cadherins are detected along the length of the stereovilli illustrated by the colored ovals and are components of the tip links. Similar distribution of CDH23 and PCDH15 is illustrated by means of the color gradient from orange to blue and *vice versa*.

forming fibres of an approximate length of 180 nm, which is consistent with the length of tip links. The asymmetric composition of the fibres where CDH23 and PCDH15 are localized to opposite ends of tip links indicates that other components of the mechanotransduction machinery, such as the transduction channel itself, may also be localized asymmetrically.

In contrast to the function of CDH23 in inner ear hair cells less molecular details are known about the role of CDH23 in vertebrate photoreceptor cells. In the retina, CDH23 is localized in the inner segment, the ciliary apparatus, and the ribbon synapses of rod and cone photoreceptor cells (Fig. 5) (37;38) (Lagziel et al., submitted). At the basal body complex of the photoreceptor cilium which is the structure homolog to the centrosome of non-ciliated cells the short cytoplasmic CDH23 isoform c is most likely expressed (Lagziel et al., submitted) and may contribute to ciliary functions (Fig. 5). The EC-domains of the transmembrane forms of CDH23 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 postsynaptic cytomatrices of a synaptic junction, and play an important role in synaptogenesis (39). Since, CDH23 and PCDH15 (see below) are co-expressed in the synaptic region they may also form asymmetric “tip link”-like fibers projecting through synaptic clefts between photoreceptor cells and retinal 2nd neurons.

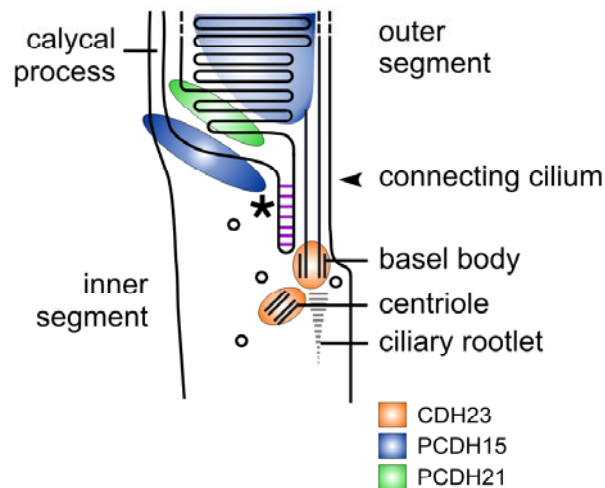


Figure 5. Localization of CDH23, PCDH15 and PCDH21 in the photoreceptor ciliary region. Schematic representation of the ciliary region of a rod photoreceptor cell: the photosensitive outer segment is linked by the connecting cilium to the biosynthetic active inner segment. A centriole is adjacent to the basal body. The membrane of the apical periciliary extension of the inner segment (asterisk) is linked by fibers (purple) to the connecting cilium membrane. Calycal processes extend from the apical inner segment and project parallel to the outer segment. In the ciliary region of vertebrate photoreceptor cells CDH23 isoforms (orange) are localized to the basal body and the centrioles. Isoforms of PCDH15 (blue) localize to the outer segment membranes and to the apical periciliary extension of the inner segment, opposing the base of the outer segment. PCDH21 (green) localization is restricted to the base of the outer segment where the nascent disks are formed.

1.2. Protocadherin 15 (USH1F, DFNB23) – the outer segment cadherin

Protocadherin 15 (PCDH15) belongs to the protocadherin family. In mammals, alternative splicing of PCDH15 results in numerous isoforms which can be divided in four groups, CD1-CD4 (Fig. 1c). The isoform CD4 does not contain any transmembrane domain and is predicted to be secreted. The isoforms CD1, CD2 and CD3 contain 11 EC domains, a single transmembrane domain and differ in their cytoplasmic domain. The cytoplasmic domain of PCDH15 CD1 consists of two prolin rich regions and a class I C-terminal PBM in the cytoplasmic domain. Via the latter PBM, PCDH15 binds to PDZ domains of harmonin (USH1C) (PDZ2) (25;40) and whirlin (19) (van Wijk, unpublished data) and is thereby integrated in the USH protein interactome (Fig. 3).

In zebrafish *Danio rerio*, two closely related *pcdh15* genes, *pcdh15a* and *pcdh15b* originated by fish-specific whole genome duplication (41), frequently found in Danio species, were detected. Interestingly, the gene duplication also resulted in the division of functional profiles indicated by distinct phenotypes caused by defects in the two *pcdh15* genes (41). Mutations in the *pcdh15a* gene in the *orbiter* mutants (42) do not affect vision, but splaying of inner-ear hair bundles causes deafness and vestibular dysfunction. In contrast, morpholino knock downs of *pcdh15b* result in a visual defect and improperly arranged morphant photoreceptor outer segments (41). In the evolutionary more distant fruit fly *Drosophila melanogaster*, *Cad99C* has been identified and characterized as an orthologue of human *PCDH15* (43). Interestingly, in the fruit fly, the *Cad99C* protein also participates in the morphogenesis of microvilli, present in the fly photoreceptor cells and structurally related to stereovilli of vertebrate hair cells.

In adult mammals, PCDH15 is expressed in a wide range of tissues including the liver, spleen, brain, inner ear, and retina (44;45). In the fetal cochlea, PCDH15 was detected in supporting cells, outer sacculus cells and the spiral ganglion (46) while in the mature inner ear, PCDH15 is additionally present in the stereovilli of sensory hair cells of both the cochlea and the vestibular organ (26;36;45;47) (Wolfrum, unpublished data). Studies in Ames waltzer (*av*) mice bearing mutations in the murine *pcdh15* gene indicate an essential role of PCDH15 in morphogenesis of the hair cell stereovilli and that PCDH15 is important for the correct localization of its interaction partner myosin VIIa (26;44;45;48). However, the molecular motor myosin VIIa is also necessary for correct distribution of PCDH15 in the stereovilli (26). As described in chapter 1.2, homodimers of PCDH15 and CDH23 interact thereby forming asymmetric heteromeric fibers as a backbone of the tip link (36). A specific localization of PCDH15 isoform CD3 at the tips of stereovilli indicates that the tip links contains CD3. In contrast, the PCDH15 isoform CD1 is found along the length of the stereovilli, concentrated at the base and less prominent at the tip. The CD2 isoform localization shows a broad distribution in the entire hair bundle (26;47). This expression pattern suggests that the different PCDH15 isoforms may also participate in the formation of the transient lateral links and kinociliary links during hair cell differentiation which would explain the disorganization of the hair bundles and the displacement of the kinocilium in hair cells of PCDH15 deficient Ames waltzer mice (34). In conclusion, PCDH15 is essential for the differentiation of stereovilli of hair cells and their mechanosensitive function. Furthermore, PCDH15 localization at the ribbon synapses indicates a role in synaptogenesis and/or function of synapses (17;18).

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 (18;45). In rod and cone photoreceptor cells, PCDH15 was found to be present at the synaptic region, in the cell-cell adhesions of the outer limiting membrane,

in the apical region of the inner segment and in the outer segment (Fig.5) (18;45). The parallel localization of PCDH15 and scaffold protein harmonin at the outer segment membranes of the photoreceptor cell (18;37) makes their cellular interaction obvious and suggests that harmonin coordinates the outer segment function of PCDH15. Moreover, both interacting partners are also present at the photoreceptor synapses where they are integral components of the USH protein network.

PCDH15 seems obviously associated with the membranes of the entire outer segment (18), but recent results of pre-embedding labeling immunoelectron microscopy revealed that PCDH15 is most prominent in the apical membrane of the inner segment exactly in the region which faces the rim of the newly formed membrane disks at the base of the outer segment (Fig. 6). Interestingly, in this open disk region of the outer segment PCDH21, a photoreceptor specific cadherin, is distinctively found (see chapter 2. below) (49;50). Therefore, there is evidence for an intriguing hypothesis that PCDH15 and PCDH21 may also interact through their extracellular domains forming heterogenic asymmetric fiber complexes (40) as evident in the tip link arrangement of PCDH15 and CDH23 (see above, summarized in (51)). In such an adhesion complex between the membranes of the inner and outer segment, PCDH21 is thought to be positioned in rims of the “open” disks by its interaction with prominin 1 (52), whereas PCDH15 is probably anchored in the cytoplasm of the inner segment by its binding to the scaffold protein whirlin which is specifically localized there (15). Anyway, such an adhesion complex including both protocadherins would perfectly fit to stabilize the fragile newly formed disk membranes. Furthermore, more recent data on amphibian and primate photoreceptor cells have indicated that PCDH15 molecules are localized in the calycal processes

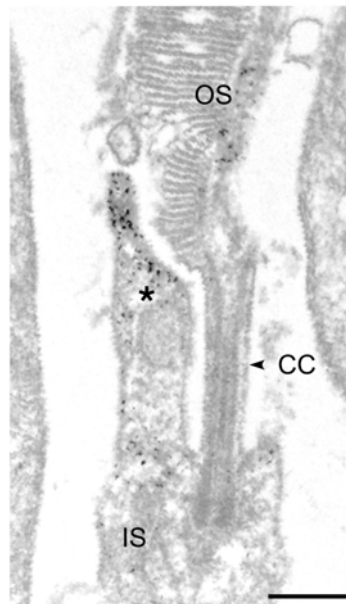


Figure 6. Subcellular localization of PCDH15 in a mouse rod photoreceptor cell by pre-embedding immunoelectron microscopy. Electron micrograph of anti-PCDH15 labeling in a longitudinal section through the ciliary region of a mouse photoreceptor cell reveals PCDH15 localization in the apical part of periciliary extension (asterisk) of the inner segment (IS) and in the outer segment (OS). CC: connecting cilium. Scale bar: 500 nm

(Nagel-Wolfrum *et al.*, unpublished). Calycal processes are apical microvilli-like extensions of the inner segment which are thought to mechanically stabilize the outer segment, and PCDH15 may contribute to the adhesion of the membrane of calycal processes and the plasma membrane of the photoreceptor outer segment.

2. Protocadherin 21 (PCDH21) - the photoreceptor specific protocadherin

Protocadherin 21 (PCDH21), also known as MT-PCDH, KIAA1775, and prCAD, is a member of the protocadherin subfamily of cadherins (49;50;53). PCDH21 is a single pass transmembrane protein containing 6 extracellular cadherin (EC) domains and a short cytoplasmic domain (Fig. 1d) (49). PCDH21 orthologues have so far only been identified in vertebrates, but not in invertebrates. However, due to their significant similarity between PCDH21 of vertebrate species to CAD74A of *Drosophila*, CAD74A has been recently suggested as a putative invertebrate orthologue (53). In contrast to the broad tissue expression of most cadherins, PCDH21 expression is mainly found in rod and cone photoreceptor cells of vertebrate retinas. Although, the *PCDH 21* gene has been suggested as a candidate gene for a USH locus by mapping to chromosome 10q32 in the vicinity of the USH1D and USH1F loci (53), PCDH21 seems not to be expressed in hair cells. As mentioned above, PCDH21 is localized in the rim of the newly synthesized disk membranes at the base of the outer segment (49). This distinctive localization together with the obvious phenotype of disorganized outer segments in *Pcdh*^{-/-} knockout mice strongly suggests that PCDH21 is essential for correct *de novo* assembly of disks at the base of rod and cone outer segments (49;50). Absence of PCDH21 from photoreceptor cells leads to retinal degeneration in *Pcdh*^{-/-} knockout mice.

For its function PCDH21 may be integrated in the adhesion complex described above (chapter 1.2), interacting with prominin 1 (PROM1) (52). PROM1 is a 5-transmembrane protein (54) specifically associated with membrane protrusions and - like PCDH21 - it is localized to the nascent disks of rod and cone photoreceptor cells and essential for their formation (52). PROM1 may anchor the proposed adhesion complex to the actin cytoskeleton.

During maturation of the outer segment disks the extracellular domain of PCDH21 is proteolytically cleaved (50). This proteolytic cleavage in EC6 of PCDH21 drives the release of a soluble N-terminal fragment while the transmembrane C-terminal fragment remains associated with the outer segment. The shedding of PCDH21's ectodomain should ultimately degrade the adhesion function of PCDH21 at the base of the outer segment and the rim complex proteins may take over the stabilization of disk membranes (55;56). Interestingly, in the absence of the rim complex proteins the cleavage of the PCDH21 is partially inhibited (50).

Although the role of PCDH21 in disk morphogenesis is not fully understood it certainly contributes to adhesive processes essential for photoreceptor cell disk neogenesis and stacking of outer segment disks and thereby for photoreceptor cell differentiation and maintenance.

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