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Cochlear molecules and hereditary deafness

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1. ABSTRACT

Remarkable progress has been made in the past decade in identifying genes involved with deafness in man and mouse. The identification of these genes and functional analysis of the proteins they encode are paving the way towards a better understanding of the physiology and pathophysiology of the auditory system. Given the complexity of auditory transduction and diversity of cochlear structures, it is not surprising that an estimate of at least 1% of human protein-coding genes are involved in perception of sound. Over 400 distinct syndromes of which hearing loss is a component have been reported (www.ncbi.nlm.nih.gov/omim). Approximately 113 loci for monogenic disorders for which hearing loss is the only manifestation and therefore is nonsyndromic, have been mapped to the human genome (http:webhost.ua.ac.be/hhh/). As of August 2007, there are approximately 46 genes identified from these loci. Here, we review some of the major advances in our knowledge of auditory function within an evolving understanding of the structure and regulation of the machinery of hearing.

2. INTRODUCTION

Deafness is the most common sensory deficit in humans affecting 1 in 1,000 new-borns, and the likelihood of developing age-related hearing impairments increases during middle age. Hearing loss affects half of people older than 80 years. When present in an infant, it causes a delay in the development of receptive and expressive communication skills (speech and language). Hearing loss which becomes apparent in later childhood or in adult life often leads to social isolation and may have dramatic effects on many aspects of life, with many psychological ramifications. Hearing loss can be acquired or inherited and can also be either syndromic or non-syndromic. It has been estimated that well over half of all cases of childhood deafness have a genetic cause and non-syndromic forms are responsible for 70% of the cases of inherited deafness. Hearing loss is typically classified as conductive, sensorineural, or mixed, based on the anatomical defects of the structures involved. Conductive forms of deafness lead to mild or moderate impairment, whereas the degree of auditory impairment for sensorineural forms can vary from

mild, moderate to severe or profound. Most forms of non-syndromic autosomal recessive hearing loss are pre-lingual and almost always due to cochlear defects (sensorineural deafness). In contrast, the syndromic forms may be conductive, sensorineural or both (mixed loss) and account for 30% of pre-lingual genetic deafness (1). Some of the identified genes associated with syndromic forms of deafness are also responsible for isolated forms of hearing loss.

For many years, genetic studies of deafness have been hampered by problems arising from phenotypic and genotypic heterogeneity, as many different genetic forms of hearing loss can produce similar clinical phenotypes. Mapping strategies have circumvented some of these drawbacks by using, large, consanguineous families, and populations isolates, in which there is likely to be greater genetic homogeneity. The large screen of spontaneous deaf mouse mutants and those generated from mutagenesis programs, in conjunction with a number of gene targeting experiments, have provided valuable mouse mutants for investigation. The Human-Mouse conserved synteny maps are now better defined, with the completion of the human and mouse DNA sequencing projects. It will likely become easier to identify potential mouse models for mapped human deafness disorders. The identification of hereditary deafness genes and functional analysis of the proteins they encode, have advanced our understanding of the basic mechanism of the hearing machinery. They belong to a wide variety of classes of proteins, reflecting both the biological properties of the molecules and their likely function in the inner ear. The aim of this chapter is not to provide an exhaustive coverage of the genes involved in hereditary hearing loss, but to describe the function of some of the proteins in the context of basic molecular mechanisms of hearing.

3. MECHANISM OF HEARING AND COCHLEAR PHYSIOLOGY

Our major challenge today is to understand the mechanisms by which the genes mutations lead to hearing loss. This has to begin with a basic understanding of normal cochlear physiology.

The inner ear is a mechanoreceptive organ, comprised of two main parts; the auditory system for hearing and the vestibular system for spatial orientation and equilibrium. The cochlea, the auditory portion of the inner ear, is involved in the perception of sound, whereby the physical phenomena of sound waves are converted into nerve impulses that are transmitted to the hearing centers in the brain. This mechanism of sound translation is found in the organ of Corti that contains two types of sensory cells: 1) the inner hair cells (IHCs) are primarily responsible for transmission of signals to the acoustic nerve and the auditory cortex; and 2) the outer hair cells (OHCs) which have both sensory and motor capabilities that contribute to cochlear frequency selectivity (2). The apical surfaces of both types of hair cell contains an array of actin-filled stereocilia and are bathed by the potassium (K^+)-rich endolymph of the scala media. The sensitive stereocilia of the inner hair cells are embedded in the tectorial membrane, a sheet of extracellular matrix that overlies the cochlea's sensory epithelium. The organ of Corti with its hair cells lies on top of the basilar membrane. Deflection of the stereocilia in response to sound waves opens ion channels at the tip of the stereocilia, resulting in current flow (K^+) into the sensory cells. Depolarization of the hair cells activates voltage-sensitive calcium channels, causing a calcium influx into the hair cells. This calcium inflow triggers the release of neurotransmitters into postsynaptic terminals that in turn activate the acoustic nerve. The hair cells are repolarized when the potassium ions leave these cells via potassium channels and are taken up by the supporting cells. The potassium ions are then passively diffused to the stria vascularis through gap junctions and are actively pumped back into the endolymph through potassium channels, thereby resetting the mechanical and electrochemical properties of the acoustic transduction system (3-6). Malfunction of either of these auditory processing components leads to hearing impairment.

4. MOLECULES AND THEIR LIKELY FUNCTION IN HEARING

4.1. Cochlear extracellular matrix proteins

Extracellular matrix proteins play a critical role in the biomechanical properties of the propagation and detection of sound within the cochlea. The tectorial membrane that contains several types of collagen and three non-collagenous glycoproteins (α-tectorin, β-tectorin and otogelin) (7-11) which are key components of this structure. Mutations in the α-tectorin gene TECTA are known to cause non-syndromic recessive deafness DFNB21 (12), as well as an autosomal dominant hearing loss at the DFNA8/A12 loci (11). Defects in collagen structure of the tectorial membrane can also lead to deafness. Both autosomal and X-linked forms of Alport syndrome are due to mutations in collagen type IV genes (13). Alport syndrome is characterized by the association of renal dysfunction with the hearing loss. Symptoms of this syndrome include high frequency sensorineural hearing loss, hematuria with progressive renal failure, and ocular abnormalities. Mutations in COL2A1, COL11A1, and COL11A2 are associated with Stickler syndrome, which is characterized by ophthalmic, articular, orofacial, and auditory defects (14). Mutations in COL11A2 cause autosomal dominant hearing loss at the DFNA13 locus (15), as well as in the autosomal recessive deafness at DFNB53 (16). The DFNA9 protein COCH is ubiquitously present in the inner ear. It is expressed in cochlea as well as vestibular organs (17, 18). Patients with COCH mutation exhibit symptoms of Meniere's syndrome, including vertigo, tinnitus and a feeling of pressure in the ear (17-19). The translated product of the COCH gene, cochlin, is predicted to contain a domain homologous to Limulus factor C (a serine protease involved in host defense). This domain is known as the LCCL domain and is thought to mediate lipopolysaccharide binding and activation in Limulus factor C. Cochlin also contains two von Willebrand Factor A (vWFA) domains, which are also present in nonfibrillar collagens, and are proposed to mediate protein-protein interactions in the extracellular

matrix (17,18). Otoancorin, encoded by the OTOA gene, is thought to anchor the apical surface of the hair cells to the tectorial membrane, and is implicated in non-syndromic recessive deafness DFNB22 (20). Stereocilin is expressed only in the sensory hair cells and is responsible for the autosomal recessive nonsyndromal sensorineural deafness linked to the DFNB16 locus (21). Sequence similarity between stereocilin and otoancorin, may define a new protein family responsible for anchoring the tectorial membrane to the sensory hair bundles (22). OTOG encode Otogelin, an N-glycosylated protein that is present in the acellular membranes of the inner ear. In Otog -/- mice, both the vestibular and the auditory functions were impaired (23). OTOG is mapped to human chromosome 11p14.3 that also contains USH1C and DFNA18 loci.

4.2. Regulators of cochlear homeostasis

The endocochlear potential (EP) is generated by the stria vascularis. Both the EP and the high K + concentration of the endolymph are essential for the transduction of sound by the hair cells. Tremendous progress has been made in the past decade in identification of key molecules involved in regulation of the ionic composition of the fluid bathing the inner ear. Mutations in components implicated in the pathways for ions circulations, especially in K^+ ion recycling pathway via gap junction systems, and in the pH regulation of the endolymph are among the most frequent causes of genetic deafness in humans.

4.2.1. Gap junctions proteins and hearing loss

Potassium recycling pathway is postulated to start with an efflux of potassium from the outer hair cells through potassium channel. Through gap junctions between the supporting cells, the ions migrate to the stria vascularis and are secreted into the endolymph. Gap junctions are intercellular channels (connexons) that allows ions and small molecules to pass between adjacent cells. A connexon is a hemichannel comprised of a hexamer of connexin subunits and two connexons from opposing cells normally stick together to form the complete intercellular gap junction channel. Different connexins can assemble into homomeric or heteromeric hemichannels to form functional homotypic or heterotypic channels (25).

Several connexin (Cx) genes associated with autosomal dominant, autosomal recessive, and syndromic hearing loss have been identified (26-29). Four Cx have been reported to be expressed in the mammalian cochlea; Cx26 (GJB2), Cx31 (GJB3), Cx30 (GJB6), and Cx43 (GJA1). The expression of different gap junction channels in the cochlea suggests functional diversity. The most abundantly expressed connexin in the cochlear tissues are Cx26 and Cx30. They both colocalized in the spiral limbus, the spiral ligament, the stria vascularis and between supporting cells of the organ of Corti (30). Although it is not clear how Cxs contribute to the cochlear function, there is evidence that functional interactions of different subtypes of Cxs may be essential in normal hearing (31).

Mouse models of Cxs genes have provided some clues to the role of gap junctions in auditory function. In the inner ear, it has been shown that gap junctions assemble into two independent cellular networks; the connective tissue and epithelial gap junction networks (32, 33). The presence of Cx26 in all gap junctions of both networks may be the cause of the embryonic lethality of the Cx26 knockout mice (34). To overcome this difficulty, Cohen-Salmon *et al.* (35) generated mutant mice by performing targeted ablation of Cx26 specifically in the cochlear epithelial gap junction network. The homozygous mutant mice have hearing impairment, but do not exhibit vestibular dysfunction. Histological analysis showed cell death in the organ of Corti. However, because Cx26 was ablated only in a subset of cells in which the protein is normally expressed, the observed histopathologic changes may not accurately reflect those incurred by complete ablation of the GJB2 gene expression in the cochlea. A more complicated aspect of cochlear function of Cx26 was obtained using a mouse mutant generated based on the human dominant-negative R75W mutation of Cx26 (36). The dominant-negative effect of the mutation was observed only in the supporting cells of the organ of Corti, and the endocochlear potential of these mice was normal. Cx26 may also be involved in intercellular signaling (37, 38). Gap junctions have indeed also been shown to transmit signaling molecules, such as inositol (1,4,5) triphosphate (IP3), and calcium (Ca^{2+}), and several connexin mutations associated with deafness have been reported to cause disturbance in the permeability of the gap junctions to those molecules (39, 40, 41).

4.2.2. Ions channels and transporters

4.2.2.1. KCNQ1/KCNE1

Given the cochlea's need to generate and maintain the endocochlear potential and because of the unique ionic homeostasis of endolymph, it is therefore no surprise that several deafness genes encode ion channels, pumps, and transporters.

The DFNA2 locus on chromosome 1q34 region contains at least two, and possibly three deafness genes; the Connexin 31 gene and the KCNQ4 gene, a component of a superfamily of genes encoding potassium channels. Mutations in two genes, KCNQ1 and KCNE1, belonging to this superfamily occur in Jervell and Lange-Nielsen Syndrome (JLNS). This disorder is characterized by long QT (LQT) cardiac arrhythmias and profound deafness (42). Patients heterozygous for KCNQ1 have Romano-Ward syndrome. JLNS is observed only when both alleles of the KCNQ1 or the KCNE1 genes are mutated, indicating that, although the LQT syndrome requires the presence of only one affected allele in the KCNQ1 gene, the mutation must be in the homozygous state to cause profound deafness characteristic of JLNS. In the cochlea, the potassium channels encoded by these three genes are though to be involved in the potassium recycling pathway. In the inner ear, the KCNQ1 channel is expressed at the apical surface of the marginal cells of the stria vascularis and in similar cells types of the vestibular system (43). Interestingly, a recent finding by Knipper et al. 2006 (44), suggest that the lysosomal integral membrane protein type 2 (LIMP2; a member of a family of proteins that includes cell adhesion molecules,

cell surface lipid receptors, and lysosomal membrane proteins) is involved in proper expression of the KCNQ1/KCNE1 channel to the cell surface membrane in the stria vascularis.

4.2.2.2. SLC26A4 (Pendrin gene)

Defects in the pendrin gene on chromosome 7q 31 lead to Pendred's syndrome (PDS), a syndromal form of deafness associated with structural abnormalities of the inner ear and goiter (45-48). The patients with PDS exhibit variable vestibular dysfunction. Enlargement of the vestibular aqueducts and endolymphatic sac and duct are found in nearly all PDS patients. Mutations in the pendrin gene are also responsible for the non-syndromic recessive deafness DFNB4 (49). The gene product, pendrin, encodes a chloride/anion transporter found in the inner ear, thyroid, and kidney (50). In the mouse cochlea, the pds transcript was localized to the endolymphatic sac and external sulcus suggesting a role in endolymphatic fluid resorption (51). Its expression in the supporting cells indicates its involved in endolymph ionic homeostasis (52).

4.2.2.3. TMC1

Mutations in a transmembrane channel-like gene 1 (TMC1) mapped on 9q13-q21 are the cause of autosomal dominant non- syndromic sensorineural deafness DFNA36 and of recessive deafness DFNB7/B11. The *TMC1* gene is predicted to encode a protein containing 6 transmembrane domains with no similarity to proteins of known function. The Tmc1 mouse ortholog transcript is expressed specifically in cochlear and vestibular hair cells (53, 54). *Tmc1* mutations were identified in the recessive deafness mouse (dn) and dominant Beethoven (Bth) mutant mice. They exhibit hearing loss and progressive hair cell degeneration and are deficient in the K+ currents which are necessary for normal functional maturation and survival of cochlear inner and outer hair cells (53).

4.2.2.4. Transmembrane protease, serine 3 (TMPRSS3)

Defects in TMPRSS3 (transmembrane protease, serine 3), which encodes a transmembrane serine protease, cause non-syndromic deafness DFNB8/10. TMPRSS3 is a type II transmembrane serine protease. Like the other members of this family, it has a transmembrane (TM) domain and a C-terminus characterized by: 1) a stem region containing LDLRA (low density lipoprotein receptor class A) and SRCR (scavenger receptor cysteine rich) domains; 2) a potential proteolytic activation cleavage site; and 3) a serine protease domain that has the catalytic triad signature. The structure of TMPRSS3 suggests that it could be active as a membrane-bound enzyme. The epithelial amiloride-sensitive sodium channel (ENaC), expressed in many sodium-reabsorbing tissues including the inner ear, is known to be regulated by membrane-bound channel activating serine proteases (CAPs). Interestingly, TMPRSS3 has recently been reported to interact with ENaC, that may play a role in the establishment and maintenance of the low sodium concentration of the endolymph (55, 56). In an in vitro expression system, proteolytic activity of wild type TMPRSS3 was associated with upregulation of ENaC-mediated Na current. In contrast, TMPRSS3 mutants causing deafness were defective in proteolytic activity and failed to activate EnaC (57).

4.2.2.5. Cochlear pigmentation genes: melanocytes and the Mitf transcriptional network

Melanocytes represent key components of the endocochlear potential generation and K^+ secretion. Defects in genes involved in melanocyte development and function result in 4 related syndromes that are defined and designed as Waardenburg syndromes (WS) types I-IV. The clinical features usually include sensorineural deafness, pigment abnormalities of the skin, iris, and hair. There is a tremendous variation in degree of hearing loss that may range from mild to profound and present as an unilateral or bilateral type of deafness. Six genes belonging to the family of transcription factors have been identified. Mutations of PAX3 (58), a gene encoding a DNA-binding protein, have been associated with WSI and III phenotypes. Individuals afflicted with WSII have been reported to carry mutations in the microphthalmia (MITF) gene (59). WSIV has been linked to the transcription factor Sox10, the cytokine Endothelin 3 (Edn3), or its receptor EDNRB gene mutations (60). Each of those genes has been implicated in determining the fate of neural crest cells for the normal development of melanocytes. In the cochlea, melanocytes are located in the stria vascularis, the structure responsible for generating the endolymph (33, 61, 62). Some clues on the role of melanocytes in auditory function come from the analysis of melanocyte-deficient mice. Mutant mice with no melanocytes in their stria vascularis have low K^+ composition in endolymph and no recordable endocochlear potential and exhibit severe hearing impairment (63). These observations suggest that melanocytes directly or indirectly facilitate K^+ transport. Such a lack of K^+ in the endolymph would be expected to affect the capacity of hair cells to respond to sound induced motion of the endolymph.

4.2.3. Tight Junctions

In the inner ear, tight junctions (TJ) proteins are thought to function as barriers that separate the K+-rich endolymph from the Na+-rich perilymph. Mutations in the gene encoding claudin 14 TJ protein are responsible for the recessive non-syndromic deafness DFNB29 (64). In the mouse cochlea, claudin 14 is expressed at the apical junctions of hair cells and supporting cells (65). Claudin 14-null mice are deaf due to the rapid degeneration of cochlear outer hair cells (OHCs). However, the mutant mice have a normal endocochlear potential (66). These findings suggest that claudin 14 may not be a key element in the maintenance of the endocochlear potential, or there may be some functional redundancy between claudin proteins where other claudins proteins can compensate for loss of claudin 14.

4.2.4. Other homeostatic genes

Defects in the wolframin (WFS1) gene are responsible for Wolfram syndrome (WS). WS is an autosomal recessive disorder characterized by diabetes mellitus, optic atrophy, a series of neurological deficits and often deafness (67). Low-frequency sensorineural hearing loss (LFSNHL) mapped to 4p16 (DFNA6, DFNA14, and DFNA38) has also been shown to be caused by mutations

in the *WFS1* gene (68, 69). The *WFS1* gene encodes wolframin, a putative multispanning membrane glycoprotein with no similarity to proteins of known function (70). Wolframin is expressed in the endoplasmic reticulum of a variety of inner ear cells, which include hair cells and supporting cells (71). These observations suggest that wolframin may play a role in inner ear K^+ and/or Ca^{2+} homeostasis as maintained by the canalicular reticulum of inner ear sensory and supporting cells.

4.3. Neural and synaptic gene

The OTOF gene mapped on chromosome 2p23.1 was identified as the gene responsible for recessive deafness DFNB9 (72). The gene encodes for otoferlin, a transmembrane protein, belonging to a mammalian gene family related to the *C. elegans* spermatogenesis factor gene fer-1. It is predicted to contain six cytoplasmic C2 domains. Sequence comparison suggests that four of the six C2 domains of otoferlin bind Ca^{2+}. Other C2 domain-containing proteins have been implicated in either lipid second messengers signal transduction pathways, or in membrane trafficking. This latter category includes several proteins that are involved in the docking and/or fusion of synaptic vesicles to the plasma membrane (73-75). In the mature cochlea, the murine Otof transcript is restricted to the IHCs (72). Based on the expression of Otof in the inner hair cells, and the impaired vesicle-plasma membrane fusion process in *C. elegans* fer-1 mutants, it has been hypothesized that otoferlin is involved in Ca^{2+}-triggered fusion of synaptic vesicles to the plasma membrane of inner hair cells. The character of innervation of cochlear inner hair cells and vestibular hair cells is primarily afferent and their synapses, termed ribbon synapses, have specific structural and functional characteristics (76). Interestingly, mutations in *OTOF* have been linked to a non-syndromic recessive deafness known as auditory neuropathy (77), in which the function of cochlear OHCs, (whose innervation is predominantly efferent) is normal but dysfunctional in the afferent auditory system, either at the level of the IHCs or at higher levels in the central auditory pathway.

4.4. Molecular components of the stereociliary bundle

The sensory hair bundle, a precisely determined array of actin-filled stereocilia (SC) is located on the hair cell's apical surface. In response to sound or acceleration stimuli, these specialized structures deflect and directly lead to gating of auditory hair cells ion channels, potassium influx and cell depolarization (3). A number of genes whose products are involved in both the integrity of the hair bundle and the sensitivity of the mechanical transduction process are implicated in deafness. Because of the organization of actin filaments in the SCs of cochlea, it is not surprising that several members of the myosin family of actin filament-based molecular motors have been implicated in hearing impairment. Myosins bind cytoskeletal actin and use the energy of adenosine triphosphate (ATP) hydrolysis to move along actin filaments. Mutations in an unconventional myosin, Myosin15 (myo15), cause the non-syndromic recessive deafness DFNB3 (78). The mouse mutant shaker-2 (sh2) defective in myo15 is deaf and exhibit abnormally short stereocilia on the auditory hair cells (78). Defects in whirlin, a PDZ (postsynaptic density 95, Disc large, Zonula occludens-1) scaffold protein, cause deafness in the mutant mouse strain whirler and families with DFNB31 (79). Whirlin is known to also be involved in the process of the stereocilia elongation. It has subsequently been shown that myosin-XVa, through its carboxy-terminal PDZ-ligand, interacts with the third PDZ domain of whirlin and then carries it to the tips of stereocilia (80). The interaction between myosin-XVa and whirlin is therefore important for a normal pattern of hair bundle growth and morphogenesis.

The genes that underlie deaf-blindness in the various forms of Usher syndrome (USH) have provided particular insights into stereocilia development and/or maintenance. At least 11 independent genetic loci are assigned to three distinct clinical subtypes, referred to as USH1, USH2, and USH3. Currently, 8 USH underlying genes have been identified. These include the actin-based motor protein myosin VIIa (MYO7A, USH1B); two cadherin-related proteins, otocadherin 23 (CDH23, USH1D) and protocadherin 15 (PCDH15, USH1F); and two scaffold proteins, USH1C and USH1G. A mouse mutant has been reported for each of the known *Ush1* genes; shaker1 (*sh1*) for *Myo7a*, waltzer (*v*) for *Cdh23*, Ames waltzer (*av*) for *Pcdh15*, deaf circler (*dfcr*) for *Ush1c*, and Jackson shaker (*js*) for *Ush1g*. All of these mice are deaf, exhibit vestibular dysfunction. In all, the stereocilia of cochlear hair cells are disorganized and splayed (81, 82), suggesting that they participate in formation of lateral links that connect adjacent cilia. Myosin VIIa is a motor protein that moves along the tracks of actin filaments (83) and may also play a role in controlling tip-link tension and maintaining the sensitivity of the hair-cell transduction channel (84). Defects in harmonin (the USH1C gene product, a PDZ-domain-containing protein**)** underlie the DFNB18 form of isolated deafness (85-87). Analysis of the transcripts of Ush1c predicts that there are at least ten protein isoforms present in the mouse inner ear (88), which can be grouped into three subclasses (a, b, c) according to their protein domain composition (89). The harmonin b isoforms are expressed specifically in the inner ear, whereas other isoforms have broader patterns of tissue distribution (89, 90). Previous studies have shown that harmonin can bundle actin filaments *in vitro* (91). The F-actin-bundling property of harmonin suggests that it is implicated in the dynamics of the developing SCs, by stabilizing the elongating actin filaments and contributing to the stiffness of SCs. It has been suggested that Myosin VIIA (USH1B) in association with harmonin b, ensures the structural integrity of SC bundles, probably by mediating the anchorage of Cadherin-23 (USH1D) and PCDH15 (USH1F) crosslinks between adjacent SC to their actin bundles. SANS (scaffold protein containing ankyrin repeats and SAM domain), the protein encoded by USH1G, is mainly expressed below the cuticular plate of IHCs and OHC, and has been suggested to function in the trafficking of the Usher proteins along microtubules and actin filaments towards the stereocilia (82). As members of the cadherin superfamily, CDH23 and PCDH15 are candidates to form extracellular linkages in hair bundles. In the adult inner ear, cadherin 23 has been proposed as a candidate for hair cell stereocilia tip links

(92), which are involved in gating the sound transduction ion channels (93). Based on the results of immunolocalization studies, protocadherin 15 has been proposed as a component of adult hair cell lateral stereocilia links (94). Mutations in the gene encoding usherin cause USH2A, the most common genetic form of USH syndrome (95, 96). Usherin is an extracellular matrix protein localized to the hair bundles of cochlear and vestibular hair cells (HCs). Several lines of evidence indicate that Usherin is also a component of inters-tereocilia ankle links of inner ear sensory cells and it can bind to two PDZ domain-containing proteins, i.e. harmonin and whirlin. These PDZ proteins could anchor the inter-stereocilia links to the actin core of stereocilia. The G-protein-coupled 7-transmembrane receptor VLGR1b (USH2C) that contains Ca^{2+} binding domains, has been associated with tip links (97). Both usherin and VLGR1b have also been localized to the sensory hair cell synaptic region (88, 98), suggesting their possible involvement in hair cell afferent synapses.

In summary, molecular analyses of USH1 protein function have shown that the identified USH1 proteins are integrated in a protein-protein interaction network, that involves binding to multi-PDZ protein, harmonin. In its function as a potent scaffold protein, harmonin is supported by SANS (USH1G) and whirlin. Furthermore, the USH2 proteins USH2A and VLGR1b as well as the candidate for USH2B (i.e. sodium bicarbonate co-transporter NBC3) have been shown to also be integrated into this supramolecular-protein network. In the inner ear, these interactions within the stereocillary bundle are essential for the proper organization of growing hair bundles and may also participate in the mechano-electrical signal transduction and the synaptic function of mature hair cells.

Both recessive and dominant mutant alleles of the espin (ESPN) gene have been associated with deafness in humans (99, 100). The espins are actin-bundling proteins that are produced in multiple isoforms from a single gene. All known espin isoforms contain a C-terminal actin-bundling module (ABM), responsible for actin-bundling (101, 102) and have microvillar parallel actin bundle (PAB) elongating activities (103). They also contain a Wiskott-Aldrich Syndrome protein homology 2 (WH2) domain that binds actin monomer and is necessary for espin-mediated actin bundle formation (103-105). The espin isoforms have a variable N-terminus resulting from a differential transcription start-site selection (104). The findings that mutations in Espin in the deaf jerker mouse mutant lead to a shortened and degenerated stereocilia, suggest that espins play critical roles in length regulation and the integrity of the stereocilium (105). The jerker mutation ($Espn^{je}$) occurs in the espin ABM domain and therefore is expected to affect all known espin isoforms. The pathophysiology seen in the jerker mouse mutant may be due to the loss of the C-terminal half of the ABM, which would result in poor actin – bundling. The molecular basis for the espin defect in jerker mice is not yet established.

4.5. Hair cell motor molecule

Cochlear OHCs are responsible for the exquisite sensitivity and frequency-resolving capacity of the cochlea. Changes in membrane potential result in an OHC length change. This electromotility is thought to be the basis of cochlear amplification. The molecular machinery of OHC electromotility is formed by the unconventional motor protein known as prestin (106, 107). Prestin, is a member of the solute carrier (SLC) family SLC26A and is densely packed into the lateral membrane of the OHC, where it may be part of a supramolecular motor complex (108). Although prestin is the key player in electromotitlity, evidence suggests that the motor does not normally function in isolation and the association of prestin with other proteins within the OHC may importantly affect the activity and function of prestin, with a direct influence OHC electromotility. Identification of prestin-associated proteins therefore is of great interest to scientists studying OHC electromotility.

4.6. Mitochondrial deafness

Mitochondria, present in every cell of the body except red blood cells, are complex organelles that are responsible for cellular energy production through ATP synthesis and oxidative phosphorylation (OXPHOS). These cellular organelles also have other specific functions, including oxidative stress control and apoptosis. It therefore came as no surprise that mitochondrial DNA (mtDNA)-associated disorders often present with sensorineural hearing loss (SNHL) either in isolation or as a part of a multisystem disorder. Many mtDNA mutations leading to non-syndromic hearing impairment have been reported. Hot spot mutation regions are genes encoding the 12S ribosomal RNA (rRNA) (MTRNR1), and the MTTS1 gene encoding the tRNA for Ser $^{(UCN)}$. Several reviews on mitochondrial hearing loss have previously been published (109-115). Different mutations (961delT/insC, T1095C, C1494T, A1555G, and possibly A827G, T1005C and A1116G) in the MTRNR1 gene can lead to maternally inherited, non-syndromic hearing loss, which in most cases is induced or aggravated by exposure to aminoglycoside antibiotics. Individuals with these mutations show variable severity of deafness, suggesting that the nuclear background may play a determinant role in the observed phenotypic variation (112). Several mutations in the MTTS1 gene have also been associated with SNHL: these include the A7445G; 7472insC; T7510C; and T7511C mutations. The pathogenic mechanism underlying mitochondrial dysfunction that causes hearing loss is still unclear. However, this mechanism may very well involve not only OXPHOS deficiency, but also apoptosis and oxidative damage due to inherited or acquired mutations of either mitochondrial or nuclear genes encoding mitochondrial proteins that are implicated in the mitochondrial functions.

5. PERSPECTIVES

Just as the identification of deafness genes and characterization of the proteins they encode have enabled us to better understand the molecular pathogenesis of various forms of deafness, we have gained much insight into the basic biology and structure-function of the auditory system. They have provided a significant increase in our knowledge of the ion-homeostasis processes and hair cell stereocilia development and stucture in the inner ear, and

cochlear mechanics as well. We have reviewed some of the major milestones in the basic investigation of cochlear function, but we have not considered the mechanisms of hearing loss associated with ageing. There is a general consensus that it is a complex disorder, caused by environment/lifestyle and stochastic factors and influenced by inter-individual differences in susceptibility genes. Although breakthroughs have been made in identifying genes involved in monogenic forms of deafness in both human and mice, correlating the functions of those genes with specific auditory processes or pathways remains difficult. In addition, the phenotypic variability in patients carrying an identical mutation, and phenotypic discrepancies between mice and humans carrying the same gene defect, suggest environmental factors and interacting genes in producing the clinical outcome. The challenge we face in the post-genome era is to understand the molecular interactions and the cell-biological processes that result from mutations and deficits in these genes and proteins. This knowledge will help guide concrete predictions of the hearing status of unborn children and give clues to potential avenues for corrective treatments.

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Abbreviations: IHCs: inner hair cells; OHCs: outer hair cells; K^+: potassium; vWFA: von Willebrand Factor A; EP: endocochlear potential; Cx: connexin; IP: inositol triphosphate; TJ: tight junctions; SC: stereocilia; ATP: adenosine triphosphase; PDZ: postsynaptic density 95, Disc large, Zonula occludens-1; ABM: actin-bundling module; PAB: parallel actin bundle; SLC: solute carrier; OXPHOS: oxidative phosphorylation; mtDNA: mitochondrial DNA; SNHL: sensorineural hearing loss

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