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Review

Hearing molecules: contributions from genetic deafness

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Abstract. Considerable progress has been made over the past decade identifying many genes associated with deafness. With the identification of these hereditary deafness genes and the proteins they encode, molecular elements of basic hearing mechanisms emerge. As functional studies of these molecular elements become available, we can put together the pieces of the puzzle and begin to reach an understanding of the molecular mechanisms of hearing. The goal of this review is to discuss studies over the past decade that address the function of the proteins implicated in genetic deafness and to place them in the context of basic molecular mechanisms in hearing. The first part of this review highlights structural and functional features of the cochlea and auditory nerve. This background will provide a context for the second part, which addresses the molecular mechanisms underlying cochlear function as elucidated by genetic causes of deafness.

Keywords. Genetic deafness, auditory, cochlea, hearing, hair cell.

Introduction

Normal mammalian auditory function relies on two broad categories of function – mechanical and electrochemical. First, sound must be directed to the cochlea, analyzed into its frequency components, and then into oscillations of the cochlear hair cell hair bundle. Second, hair bundle oscillations must be converted into an electrochemical signal of the auditory neuronal pathway. Malfunction of either of these auditory processing components results in hearing loss, an affliction that affects an estimated 70 million people worldwide [1]. The hearing loss associated with these two broad components of auditory processing has considerably disparate clinical presentation, diagnosis, and management.

In the clinic, the two types of hearing loss are typically considered as separate entities. 'Conductive′ hearing loss refers to malfunction of the mechanical arm of auditory processing, whereas 'sensorineural′ hearing loss refers to malfunction of the electrochemical arm. Conductive hearing loss typically results from middle ear pathology – tympanic membrane perforation, ossicular discontinuity or fixation, or middle ear infections that are often amenable to improvement with either amplification (i.e. hearing aids) or surgical procedures – and is not addressed further in this review. Sensorineural hearing loss, on the other hand, can result from malfunction anywhere along the auditory pathway, from the hair cell to higher-order central auditory processing loci. Known etiologies of sensorineural hearing loss are abundant. Nearly half of the cases of congenital deafness can be attributed to genetic causes. One-third of these defects we accompanied by identified disorders in other systems, and we considered as 'syndromic′ deafness. The remaining two-thirds, however, are isolated to hearing loss and considered 'non-syndromic.′

Considerable progress has been made over the past decade identifying genetic loci and genes associated with mammalian deafness. The list of loci is well over 100 as of the submission of this review, and there are approximately 45 genes identified from these loci. An updated database of the non-syndromic deafness genes and loci is maintained at the Hereditary Hearing Loss Homepage (http://webhost.ua.ac.be/hhh/). With the identification of hereditary deafness genes and the proteins they encode, molecular elements of basic hearing mechanisms emerge. With studies of the function of these identified molecular elements we can put together the pieces of the puzzle and begin to reach an understanding of the molecular mechanisms of hearing. The goal of this review is to discuss studies of the function of the proteins implicated in genetic deafness in the context of basic molecular mechanisms in hearing.

Understanding molecular mechanisms of deafness begins with a basic understanding of the normal anatomy and physiology of the auditory pathway. The first part of this review thus briefly highlights structural and functional features of the cochlea and auditory nerve. This background will provide a context for the second part of this review that addresses the molecular mechanisms underlying cochlear function as elucidated by genetic causes of deafness. We are interested in cochlear function, rather than development, and as such will focus on the developed cochlea.

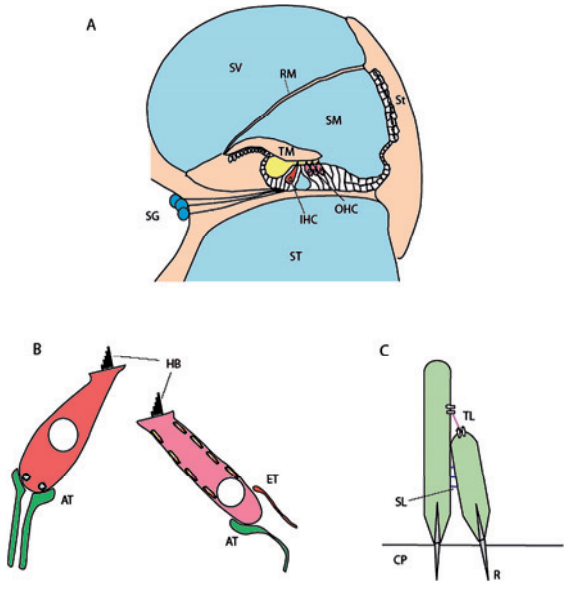

Figure 1. Schematic demonstrating the basic anatomy of the cochlea. A. Cross section through one turn of the cochlea. St = Stria vascularis, SM = scala media, SV = scala vestibuli, ST = scala tympani, IHC = inner hair cell, OHC = outer hair cell, TM = tympanic membrane, RM = Reisner's membrane, SG = spiral ganglion. B. Schematic of an inner (left) and outer (right) hair cell. AT = afferent terminal and synapse, HB = stereociliary (hair) bundle, ET = efferent terminal. C. Schematic of two stereocilia within a hair bundle. SL = side links, CP = cuticular plate, R = rootlet, TL = tip link.

Cochlear anatomy and physiology

Figure 1 is a schematic diagram showing the anatomic details of the cochlea at different levels of magnification; it should be used to reference anatomical structures throughout the text. The cochlea is a spiraled bony tube housing three fluid-filled chambers that run along its length. Highly specialized cells within the cochlea regulate the ionic composition of these chambers. The outer chambers (scala tympani and scala vestibuli) contain perilymph, a filtrate of cerebrospinal fluid of similar composition to extracellular fluid (high sodium, low potassium). The middle chamber (scala media) contains endolymph, a high-potassium, low-sodium fluid of similar composition to intracellular fluid. The outer wall of the scala media is lined by the stria vascularis, a vascular epithelial layer that serves as the metabolic control center of the cochlea.

As a result of the differences in ionic composition between the compartments, the potential difference between endolymph and perilymph is about +80 mV. This positive potential is the largest found in the body. Since the intracellular resting potential of hair cell receptors is around −70 mV, the potential difference across the hair cell apex is a remarkable 150 mV. This large potential difference represents a tremendous ionic force and serves as the engine driving the mechanoelectrical transduction process of the hair cell.

Several cellular and extracellular specializations within the cochlea function to decompose the mechanical stimulus of sound into its frequency components and to convert these stimuli into an electrochemical signal conveyed by discharges along auditory nerve fibers. The stiffness gradient of the basilar membrane along the length of the cochlea functions like a bank of frequency band-pass filters aligned from the highest frequency at the cochlear base to the lowest frequency at its apex. Atop the basilar membrane rests the organ of Corti, which contains sensory hair cells and supporting cells. Specialized supporting cells in the organ of Corti complement the hair cells and play a vital role in maintaining the integrity and function of the hair cells. A final component of the cochlea functional apparatus is the tectorial membrane, a gelatinous ribbon of extracellular matrix attached medially and contacting the outer hair cell hair bundles.

Hair cells are polarized. From their apex protrude a bundle of sensory hairs called stereocilia, which are composed of actin filaments. The synaptic machinery populates the basolateral cell surface. Interconnecting links between the stereocilia have two functions – side-to-side links between stereocilia seem to provide structural stability to the sensory hair bundle, while links from the tip of a shorter stereocilia to the shaft of a longer neighbor (i.e. "tip links") attach to the mechanoelectric transduction channel [2, 3]. With mechanical oscillations of the basilar membrane, stereocilia within hair bundles are displaced relative to each other. This displacement puts the tip links under tension and pulls open cation channels. Driven by the endocochlear potential, the flow of cations into the hair bundle depolarizes the hair cell membrane potential. The intracellular processes that then respond to these changes in membrane potential are considerably different between the two types of auditory hair cells, inner and outer hair cells. Inner hair cells contain afferent synapses that relay membrane voltage changes into auditory nerve fiber action potentials; outer hair cells contain electromotile elements and generally serve as mechanical amplifiers of the sound stimuli [4].

For inner hair cells, membrane potential changes drive afferent synaptic activity localized to the hair cell's basolateral surface, and the pre-synaptic machinery is geared to generate graded release of neurotransmitter. Voltage-dependent calcium channels co-localized with neurotransmitter release sites open in response to membrane depolarization, which in turn results in the release of neurotransmitter. The amount of transmitter release is modulated by the magnitude of the membrane voltage changes. Neurotransmitter diffuses across the synaptic cleft and binds to post-synaptic receptors on afferent auditory nerve fiber dendrites. This process begins the generation and propagation of action potentials along the afferent fibers.

The peripheral processes (or dendrites) of primary neurons extend from the hair cells to their respective cell bodies that reside in Rosenthal's canal. This canal is carved within the internal bone of the cochlea and spirals medially and in parallel to the organ of Corti. The central axons of the spiral ganglion cells then coalesce within the central core of the cochlea to course through the internal auditory canal towards the brainstem as the auditory nerve.

Mechanisms of hereditary sensorineural hearing loss

In reviewing the identified genetic deafness genes, we will focus on the gene product and attempt to use the identified gene products to create a framework for a better understanding of basic mechanisms of hearing. Identification and characterization of genes associated with deafness is a progressive field. Our goal is to summarize advances made in the "post-genomic" aspects of hereditary deafness, and to create a framework so that new developments in deafness-associated genes and their products may be better understood in the context of auditory system function. Although the process that initiates hearing is a smooth continuum of multifaceted mechanisms, we have broken this continuum into a series of arbitrary general steps. The first is the mechanoelectric transduction process, which begins with oscillation of endolymph surrounding the sensory hair cell stereocilia and ends with hair cell membrane potential changes. The next step is hair cell synaptic transmission, which begins with voltage-gated hair cell channels and ends with the release of neurotransmitter from the afferent hair cell synapse. Conduction of auditory nerve fiber impulses completes the steps of afferent transmission. Homeostatic processes supporting the cochlea are then considered, and they include outer hair cells and maintenance of the endocochlear potential. A summary of the genes, proteins, their putative function in the cochlea, and any mouse models available appear in Table 1. Since the focus of this review is on molecular processes involved in the physiology of hearing, those genes whose mutations are implicated in absent or incomplete cochlear development are not included.

As a convention, the nomenclature of non-syndromic deafness loci takes the form 'DFNA*X*' for autosomal

Table 1. Overview of genetic deafness genes.

Cochlear process	Functional class	Human gene	Protein	Human Deafness locus	Mouse model
Mechanoelectric transduction	tectorial membrane composition	TECTA	alpha-tectorin	DFNA8 DFNA12 DFNB21	targeted null [10] targeted missense [9]
Mechanoelectric transduction	tectorial membrane composition	COL11A2-	collagen XIa	DFNA13 DFNB53 Stickler syndrome type 3	targeted null [16]

Table 1. Overview of genetic deafness genes. *(Continued)*

Cochlear process	Functional class	Human gene	Protein	Human Deafness locus	Mouse model
Mechanoelectric transduction	tectorial membrane anchoring	OTOA	otoancorin	DFNB22	none
Mechanoelectric transduction	tectorial membrane anchoring	STRC	stereocilin	DFNB16	none
Mechanoelectric transduction	stereocilia	MYO15a	myosin XVa	DFNB3	shaker-2 [129]
Mechanoelectric transduction	stereocilia	WHRN	whirlin	DFNB31	whirl*er* [24]
Mechanoelectric transduction	stereocilia	MYO7A	myosin VIIa	DFNB2, DFNA11 USH1B	Shaker-1 [130]
Mechanoelectric transduction	stereocilia	USH1c	harmonin	DFNB18 USH1c	Deaf circler [131]
Mechanoelectric transduction	stereocilia	CDH23	cadherin23	DFNB12 USH1D	waltzer [29]
Mechanoelectric transduction	stereocilia	PCDH15	protocadherin	DFNB23 USH1F	Ames waltzer [30]
Mechanoelectric transduction	stereocilia	SANS	SANS	USH1G	Jackson shaker [132]
Mechanoelectric transduction	stereocilia	ESPN	espin	DFNB36	Jerker [51]
Mechanoelectric transduction	stereocilia	VLGR1b	Vlgr1b	USH2c	Mass1Frings in BUB/BnJ strain [133] targeted null [134]
Mechanoelectric transduction	outer hair cell electromotility	SLC26	prestin	DFNB61	targeted null [135]
Synapse and auditory nerve	hair cell exocytosis	DFNB9	otoferlin	DFNB9	none
Cochlear ionic homeostasis	gap junctions	GJB2	connexin26	DFNB1 DFNA3	targeted, conditional null [75] dominant negative transgene [76]
Cochlear ionic homeostasis	gap junctions	GJB3	connexin31	DFNA2	targeted null [136]
Cochlear ionic homeostasis	endolymph ion homeostasis	SLC26A4	pendrin	Pendred syn. DFNB4	targeted null [137]
Cochlear ionic homeostasis	hair cell ion homeostasis	TMC1	Tmc1	DFNB7 DFNB11 DFNA36	Deafness [89] Beethoven [90]
Cochlear ionic homeostasis	cell surface proteolytic enzyme	TMPRSS3	transmembrane serine protease ss3	DFNB8 BFNB10	none
Cochlear ionic homeostasis	endolymph potassium secretion	KCNQ1	KCNQ1	JLNS1	targeted null [138,139]
Cochlear ionic homeostasis	endolymph potassium secretion	KCNE1	KCNE1	JLNS2	targeted null [140,141]
Cochlear ionic homeostasis	unknown	WFS1	wolframin	DFNA6 DFNA14 DFNA38	none
Cochlear ionic homeostasis	tight junctions	CLDN14	Claudin14	DFNB29	targeted null [121]
Cochlear ionic homeostasis	melanocyte	PAX3	Pax-3	WS1	splotch [142]
Cochlear ionic homeostasis	melanocyte	MITF	Mitf	WS2	microphthalmia [143]

dominant loci, where *X* is an identifying integer assigned to the specific locus. Autosomal recessive loci are named 'DFNB*X*' and X-linked loci with 'DFN*X*,' again where *X* is the identifying integer.

Mechanoelectric transduction

The physical vibration of a sound stimulus causes the apical surface of the hair cell, where hair bundles are in contact with the overlying tectorial membrane, to oscillate and cause shearing forces between stereocilia. These shearing forces result in the opening and closing of mechanically gated ion channels and initiate the process of mechanoelectric transduction. The consequent ionic influx depolarizes the hair cell membrane potential with a magnitude commensurate with the frequency and amplitude of the mechanical oscillation [5]. The key structural elements responsible for mechanoelectric transduction are the tectorial membrane and the sensory hair bundle.

Tectorial membrane

The tectorial membrane is necessary for focusing the mechanical oscillations of the sound stimulus onto the sensory hair bundle. Protein-protein interactions appear to anchor the tectorial membrane to sensory and supporting cells of the organ of Corti. Mutations in the genes encoding either the composition of the tectorial membrane or these anchoring proteins have been associated with hereditary deafness.

The composition of the tectorial membrane includes several types of collagen and three non-collagenous glycoproteins that are expressed at high levels only in the inner ear: alpha-tectorin, beta-tectorin, and otogelin [6–8]. The function of the tectorins appears to be both optimize the sensitivity of the basilar membrane to frequency-tuned mechanical oscillations [9] and to provide mechanical feedback to the outer hair cells in order to adjust the gain of the cochlear amplifier [10]. Mutation in the alpha-tectorin gene TECTA is the basis for two identified autosomal dominant non-syndromic deafness loci DFNA8 and DFNA12 [11], as well as an autosomal recessive non-syndromic deafness DFNB21 [12].

Disruption of the collagen composition of the tectorial membrane through mutation is also associated with deafness. Collagen types II, IX, and XI are critical elements in the tectorial membrane. Absence of type IX collagen in mouse knockouts reveals a deaf phenotype and disrupted tectorial membrane [13, 14]. Collagen types II and XI gene mutations are associated with Stickler syndrome, which includes craniofacial dysmorphism and ocular abnormalities in addition to sensorineural hearing loss [15]. Collagen type XI is implicated in the autosomal dominant, non-syndromic hearing loss DFNA13, which may affect the triple-helix domain of the collagen type XI protein [16], as well as in the autosomal recessive hearing loss DFNB53 [17].

The anchoring proteins include otoancorin and stereocilin. Otoancorin is an inner ear-specific glycosylphosphatidylinositol-anchored protein that is found on the apical surface of non-sensory cells, where they contact the tectorial membrane [18]. Otoancorin is the product of the OTOA gene whose mutation is associated with the non-syndromic recessive deafness DFNB22 [18]. Stereocilin is a protein expressed only on the hair cell bundles. Mutation of the stereocilin gene STRC is the root of the autosomal recessive non-syndromal sensorineural deafness linked to the DFNB16 locus [19]. Stereocilin and otoancorin share C-terminal sequence homology, suggesting that both may function to anchor the tectorial membrane to organ of Corti cell structures [20].

Stereociliary bundle

The hair bundle is an exquisitely sensitive oscillation detector, as movements as small as 1 nm result in hair cell potential changes [21]. Several elements of the machinery responsible for both the integrity of the hair bundle and the sensitivity of the transduction process have been elucidated through studies of hereditary deafness. Since the stereocilia are composed of polymerized actin filaments, it follows that members of the myosin family would be involved in the dynamic state of actin polymerization. Mutation in the gene encoding myosin XV is the basis for the non-syndromic recessive deafness DFNB3 [22]. Mutations of a homologous murine gene in the *shaker-2* mouse reveal abnormally short stereocilia and deafness. The human recessive deafness DFNB31 maps to the same gene as that altered in the deaf mouse mutant *whirler* [23]. Similar to the *shaker-2* mouse, *whirler* is also characterized by abnormally short stereocilia. The protein encoded by the mutated gene in *whirler* (i.e., whirlin) contains a PDZ domain characteristic of protein-protein interaction [24]. Subsequently, myosin XV has been shown to work by shuttling whirlin to the stereocilia tip [25]. Myosin XV and whirlin are thus implicated in establishing and maintaining the length of the individual stereocilia [26].

Usher syndrome is characterized primarily by congenital deafness and progressive blindness due to retinitis pigmentosum, but can also involve vestibular deficits [27]. Molecular genetic studies of Usher syndrome have yielded significant clues about the molecular function of the stereociliary bundle over the past decade. Nearly a dozen genetic loci have been implicated in the different clinical subtypes of Usher

syndrome, and several genes have been identified and studied. These include the unconventional myosin VIIa, harmonin, the cadherin CDH23 and related protocadherin PCDH15, and SANS. Mouse mutants of these proteins have been produced and have helped elucidate the function of the proteins. The corresponding mouse mutants are the *shaker-1* mouse for myosin VIIa [28], *waltzer* for cadherin 23 [29], and *Ames waltzer* for protocadherin 15 [30]. Mutations in mouse mutants of these proteins all share the common phenotype of hair bundle structural anomalies.

MyosinVIIa was the first of these hair bundle-specific elements identified from Usher syndrome mutations [31]. Studies have since shown that myosin VIIa is a motor protein that translates along actin filaments [32] and is responsible for maintaining the sensitivity of the mechanoelectric transduction channel [33]. Harmonin was identified as a hair cell-specific protein that contained a protein-protein binding region PDZ (post-synaptic density, disc large, zonula occludens [34]) [35]. Several isoforms of harmonin have been identified, and various harmonin mutations have been found to cause syndromic as well as non-syndromic deafness. One of these isoforms, harmonin b, and cadherin 23 appear together and bind in the developing stereocilia, and both disappear from the adult hair bundle [36]. Harmonin b also binds F-actin and myosin VIIa. These results therefore suggest that harmonin b bridges cadherin 23 to the cytoskeletal actin core of the developing stereocilia via the myosin VIIa motor [36]. Other isoforms of harmonin persist in the adult cochlear hair cells. Another element of the Usher syndrome-related protein complex is SANS (*s*caffold protein containing *an*kyrin repeats and *S*AM domain). SANS was identified in the site of the Usher 1G mutation [37]. SANS has been localized to the apical portion of the hair cell rather than the stereocilia, and interacts with harmonin [37]. Cadherins CDH23 and PCDH15 form extracellular linkages within the hair bundle. CDH23 localizes to both lateral links and tip links [36, 38–43]. Protocadherin 15 (PCDH15) is a cadherin-related protein whose gene is mutated in Usher syndrome type 1F and the non-syndromic deafness DNFNB23 [44]. PCDH15 binds myosin VIIa and localizes to the hair bundle of mature animals [45]. The mechanism common to all of these proteins interacting within or around the stereociliary bundle is beginning to be understood. Harmonin is the 'glue' that binds them all together. The multi-protein unit is involved at least in the maintenance of the stereociliary bundle [44, 46] by shuttling the Usher-related proteins along actin filaments to their sites of action within the stereocilia [47, 48].

The ESPN gene has been identified as a mutation in several families with non-syndromic deafness [49, 50]. The product of the ESPN gene is the actin-binding protein espin, which has been localized to the stereocilia [51]. Espin consists of a C terminus responsible for bundling actin, a WH-2 actin monomer-binding domain [52], and a variable N terminus determined by the specific isoform. Functions of espin in stereocilia have been recently reviewed [53]. Mutations in the mouse homolog of espin were found to be responsible for the phenotype of the mouse mutant *jerker* [51]. *jerker* mice are characterized by deafness and circling behavior associated with vestibular dysfunction [54]. Hair cell morphology in *jerker* mice demonstrates shortened and degenerated stereocilia [55]. The pathophysiology of espin mutation in the *jerker* mouse may be that the lack of a C-terminal actin-binding domain leads to poor actin bundling. Mutated espin may also be targeted for early degradation [53].

Two other genes implicated in causing Usher syndrome, albeit a milder form of the disease, are USH2A (also called Usherin) and VLGR1b. USH2A is an extracellular matrix protein localized to the hair cell stereocilia that participates in this Usher syndrome protein unit through binding to a harmonin PDZ domain [56]. VLGR1b (very large G protein-coupled receptor-1b) is a member of G protein-coupled receptors with a large extracellular domain. This extracellular domain contains Ca^{2+}-binding domains [57], similar to that associated with tip links. In this context, both USH2a and VLGR1b are likely to be involved in the scaffold maintaining the function of the stereociliary bundle. However, USH2A and VLGR1b have also been localized to the sensory hair cell synaptic region [56], and here involvement in the hair cell afferent synapse may yet be uncovered.

Outer hair cell electromotility

Outer hair cells are responsible for modulating the sensitivity and fine tuning of the cochlea's response to the mechanical stimulus of sound. Although the basic phenomenon of electromotility of the outer hair cell has been well characterized [4, 58], the molecular machinery involved has only recently begun to be understood. The gene encoding the outer hair cell voltage-sensitive electromotility motor has been identified as prestin [59], a member of the anion transporter family, solute carrier protein 26 (SLC26). Prestin is abundant on the membrane of outer hair cells [60] and is highly conserved among mammalian species, including humans. Mutations in the prestin gene were discovered segregating with deafness in several families [61]. The details of the structure and function of prestin and its interaction with other molecular components are an active source of investigation [58].

Synapse and auditory nerve

Otoferlin was identified as the gene responsible for the recessive deafness DFNB9. Its mRNA is expressed strongly in inner hair cells in mature animals, but not in supporting cells [62]. Otoferlin is a transmembrane protein with a cytoplasmic domain that resembles synaptotagmin, a member of Ca^{2+}-binding synaptic proteins involved in membrane trafficking and exocytosis [63]. Given its expression in inner hair cells and resemblance to a synaptic protein, otoferlin is implicated in afferent hair cell neurotransmitter release [62]. If otoferlin were involved in afferent hair cell synapse, defects in otoferlin should be manifest as a functional mechano-electric transduction apparatus, but dysfunctional auditory nerve fiber neurotransmission. Such a profile is purported to underlie the clinical phenomenon of auditory neuropathy, an entity defined by deafness in the face of intact outer hair cell function [64]. Further clinical evidence supports the involvement of otoferlin in auditory neuropathy, namely that deaf patients with mutations in otoferlin tend to meet criteria for auditory neuropathy [65–67].

Regulation of cochlear ionic homeostasis

Connexins

Mutations in genes encoding the connexin family of proteins comprise the most common loci of non-syndromic deafness. Several connexin genes associated with deafness have been identified, and include autosomal dominant, autosomal recessive, and syndromic hearing loss [68–72]. Given the commonality of connexin mutations in hereditary deafness, considerable interest in their function has been generated. Connexin molecules bind to each other as intramembranous hexameric assemblies and appose similar assemblies with adjacent cells to create intercellular channel particles. Clusters of closely aggregated channel particles comprise gap junctions. Several different connexin (Cx) subunits are reported to be expressed in the mammalian inner ear, including Cx26, Cx30, Cx31, and Cx43. However, the predominant connexins expressed in cochlear tissues are Cx26 and Cx30. Gap junctions mediate ionic intercellular communication in both epithelial and connective tissues within the spiral limbus, the organ of Corti, and the stria vascularis [73]. Individual gap junctions appear to harbor different combinations of Cx26 and Cx30 heteromers rather than just one of the isoforms [74].

Mouse mutants of connexin genes have yielded clues about the function of the connexins in the cochlea. Cohen-Salmon et al. [75] demonstrated that targeted ablation of Cx26 in the epithelial cell network of the cochlea resulted in normal cochlear development, but following the onset of hearing, cell death of the supporting cells ensued. The timing of this cell death coincided with decreases in the potassium concentration driving the endolymphatic potential. They hypothesized that the loss of Cx26 prevented recycling of K+ ions after sound stimulation and that elevated K^+ in the extracellular perilymph inhibited uptake of the neurotransmitter glutamate, which ultimately resulted in cell death within the hair cell population. This 'K^+ recycling theory' is the leading hypothesis for Cx26 function in the cochlea.

Other evidence paints a more complicated picture of Cx26 cochlear function. A mouse mutant of Cx26 was generated based on the human autosomal dominant Cx26 mutation (R75W) that inhibited the ability of native Cx26 to form channels, thus functioning as a dominant-negative mutation [76]. The dominant-negative effect was only seen in the supporting cells of the organ of Corti, rather than in the stria vascularis or spiral ligament, and the endocochlear potential was *preserved* in these mice. The authors concluded that the Cx26 mutation affects the fluid surrounding the inner hair cells (i.e., 'cortilymph') rather than the endolymph. Furthermore, the role of gap junctions in the cochlea may extend beyond channeling basic ions. Gap junctions have also been shown to transmit second-messenger molecules such as inositol (1,4,5)triphosphate (IP_3), the calcium-mobilizing messenger [77]. Several connexin mutations associated with deafness reduce the permeability of gap junctions to IP_3 [78]. One of these mutants (V84L) represses an intercellular calcium wave that is otherwise seen with non-mutated Cx26 [79]. Cx26 is thus likely to have several roles in intercellular signaling [80, 81].

Pendred syndrome

Pendred syndrome, which was first described in 1896 [82], is an autosomal recessive disorder characterized as the co-existence of sensorineural deafness and enlarged thyroid goiter. The cochleae of patients with Pendred syndrome can be dysplastic, and the vestibular aqueduct is typically dilated [83]. The gene for Pendred syndrome (PDS) has been identified on chromosome 7q31 [84], and mutations in the gene are also responsible for the non-syndromic recessive deafness DFNB4 [85]. The gene product, pendrin, encodes a chloride/anion (e.g., iodide) transporter [86], which explains its involvement in thyroid goiter. With respect to the inner ear, the pds gene product mRNA was localized to the endolymphatic sac and external sulcus of the cochlea [87]. The endolymphatic sac regulates the resorption of endolymph in the cochlea. Further studies have also found pendrin in several supporting cells of the cochlea [88]. These results suggest that pendrin is involved in endolymph ionic homeostasis.

TMC1

Three loci of non-syndromic deafness, two recessive (DFNB7 and DFNB11) and one dominant (DFNA36), involve a newly identified gene on 9q13-21 called transmembrane cochlear-expressed gene 1 (TMC1). The gene is expressed specifically on inner and outer hair cells [89]. The locus of the mouse ortholog of TMC1 was found to be the site of mutation in recessive deafness mouse strain '*dn*' [89]. Another mouse model for a TMC1 mutation has since been developed (the 'Beethoven,' or *Bth* strain), for which heterozygotes demonstrate deafness and progressive hair cell loss [90]. An understanding of TMC1 function, however, is in its infancy. With six transmembrane domains, the TMC1 gene product appears to be a transmembrane protein. Both TMC1 mutant mouse strains have recently demonstrated deficits in the K^+ currents that contribute to normal functional maturation of inner and outer hair cells. Although mutant mice exhibit progressive hair cell damage and loss, the pre-synaptic function of remaining hair cells also appears disrupted: the Ca^{2+} current remains immature into adulthood, and the compound action potential response of the auditory nerve is absent even at ages where many hair cells are still present [91].

Transmembrane serine protease

The gene responsible for the recessive, non-syndromic deafnesses DFNB8 and DFNB10 was identified on chromosome 21q22.3 as the transmembrane serine protease TMPRSS3 [92–94]. TMPRSS3 is expressed in spiral ganglion, cochlear supporting cells, and stria vascularis [95]. TMPRSS3 is a member of a class of cell surface proteolytic enzymes. Serine proteases have classically been described as secreted enzymes, whose signal cascades are involved in blood coagulation, wound healing, immune responses, and tumor invasion. Unlike secreted serine proteases, TMPRSS3 is a transmembrane protein with an extracellular serine protease domain, suggesting that it can play a role in intracellular, cell surface, and intercellular signaling [96]. TMPRSS3 was recently found to interact with the epithelial amiloride-sensitive sodium channel (ENaC), which is also found in the cochlea. While wild-type TMPRSS3 was able to activate ENaC in vitro, mutants of TMPRSS3 known to be associated with deafness were unable to activate ENaC [95]. Interestingly, ENaC is implicated in maintaining the low-sodium concentration of endolymph [97]. However, patients who have a defect in ENaC (pseudohypoaldosteronism type I) have normal hearing [98].

Potassium secretion and the endolymphatic potential

Jervell and Lange-Neilsen first identified a child with prolonged QT interval, congenital deafness, and sudden death [99]. This constellation has been termed Jervell and Lange-Neilsen syndrome. The gene responsible for the syndrome has been identified as KCNQ1, which encodes a delayed rectifier K^+ channel that localizes to the stria vascularis [100]. A second gene associated with Jervell and Lange-Neilsen syndrome was also identified (KCNE1) that encodes the beta subunit of the KCNQ1/KCNE1 channel complex [101]. The genes encode two proteins that form a channel complex that serves as the final phase of active transport from plasma and perilymph through the stria vascularis. The cycle of active K^+ transport begins as basal cells absorb the ions from the perilymph and transport them through gap junctions to intrastrial fluids. Melanocytes (i.e., intermediate cells) then pump K^+ across their cell membranes through the inward rectifier K^+ channel Kir4.1 [102], creating high intracellular K^+. This concentration gradient is responsible for the endocochlear potential [103]. Potassium ions are transported from the intrastrial fluid across the basolateral membrane of strial marginal cells via an Na^+- K^+-ATPase [104], and are then eventually secreted at the apical membrane through the KCNQ1/KCNE1 channel complex [104]. Disruptions in this K^+ secretion with mutations of KCNQ1 prevents the endocochlear potential from being established and result in atrophy of the stria vascularis, degeneration of the organ of Corti, and deafness [105]. Recently, new clues have emerged regarding the pathway of KCNQ1/KCNE1 channel function. Targeting transmembrane proteins to the cell surface requires proper packaging and turnover by lysosomes. Lysosomal integral membrane protein type 2 (LIMP2) is a member of a family of proteins that includes cell adhesion molecules, cell surface lipid receptors, and lysosomal membrane proteins. A LIMP2 knockout mouse has shown a significantly reduced endocochlear potential that was linked to the loss of the KCNQ1/KCNE1 channel. The LIMP2 knockout mice demonstrate a progressive high-frequency hearing loss and decreased otoacoustic emissions as early as 4 weeks of age. This result suggests that LIMP2 is involved in proper expression of the KCNQ1/KCNE1 channel to the cell surface membrane in the stria vascularis [106].

Cochlear melanocytes

Melanocytes are a key component of K^+ secretion and the endolymphatic potential. Disruption of melanocyte differentiation is the etiology of Waardenburg syndrome, which includes sensorineural hearing loss and pigmentary abnormalities of the skin, hair, and eyes [107]. Several clinical subtypes of Waardenburg syndrome have been described, with different genes being identified as sites of mutation in these patients. These genes encode a group of transcription factors

involved in melanocyte differentiation, and include PAX3 [108], MITF [109], SOX10, EDN3, and EDNRB [110].

Other homeostatic mechanisms

Mutations in a gene located at 4p16 have been associated with genetic deafness and with the autosomal recessive neurodegenerative disorder Wolfram syndrome (juvenile onset diabetes mellitus and optic atrophy, as well as various degrees of progressive hearing impairment [111]). Other mutations in the same genetic locus are associated with the non-syndromic progressive deafness involving low and mid frequencies called 'autosomal dominant progressive low frequency sensorineural hearing loss.' Two separate loci associated with 4p16 mutations have been denoted, DFNA6 [112] and DFNA14 [113]. The gene responsible for these genetic deafness phenotypes has been identified as 'wolframin' The wolframin gene encodes a membrane glycoprotein with no functional homology with any known family of proteins [114]. It has been localized to the endoplasmic reticulum of various cells in the cochlea, including hair cells and supporting cells [115]. As a component of the endoplasmic reticulum, wolframin may be involved in homeostasis of K^+ and/or Ca^{2+} [115].

Several clues about the function of wolframin have surfaced from investigations in other systems. Expression of wolframin in *Xenopus* oocytes resulted in intracellular calcium release from the endoplasmic reticulum and suggested that the protein functions as a cation channel or regulator of cation channels in the endoplasmic reticulum [116]. In conditional knockout mice lacking wolframin in pancreatic β cells, a dilated endoplasmic reticulum and increased apoptosis of affected cells was seen [117]. This finding was corroborated *in vitro* in β cells [118]. Nonetheless, the function of wolframin remains to be determined.

Tight junctions

Whereas gap junctions mediate intercellular electrochemical communication, tight junctions mediate communication between extracellular compartments, as well as separating apical from basolateral portions of polarized epithelial cells. One role of tight junctions in the cochlea is to help maintain the separation of endolymph and perilymph, thus supporting the endocochlear potential. The gene responsible for the recessive non-syndromic deafness DFNB29 was identified as claudin-14 (Cldn14), a member of the claudin family, a known tight junction component. *Cldn14* is expressed in the supporting cells of the organ of Corti, but not in the stria vascularis or Reissner's membrane. Expression in mouse is seen as early as post-natal day 4, but increases through post-natal day 8, coincident with the establishment of the endocochlear potential [119]. Mutations in claudin14 known to be associated with human deafness prevent the protein from forming tight junctions [120]. In *Cldn14* knockout mice, however, the endocochlear potential is preserved. These mice lacking *Cldn14* demonstrated outer hair cell loss, followed by inner hair cell loss, and the animals were deaf [121]. Claudin14 may therefore not be a key component for maintaining the endocochlear potential, or other claudin components may be able to compensate for its loss. These results suggest, however, that claudin14 is necessary for maintaining the paracellular barriers surrounding the inner and outer hair cells.

Mitochondrial function

Mitochondria are responsible for cellular energy production as an integral component of the oxidative phosphorylation pathway. Several mitochondrial DNA mutations are associated with deafness, either in isolation or associated with symptoms in other organ systems. One of these encodes the mitochondrial 12S ribosomal RNA gene. Mutations in this gene are associated with both non-syndromic hearing loss as well as abnormal aminoglycoside susceptibility to ototoxicity [122]. Individuals with these mutations have widely varying severities of deafness. This variable penetrance of deafness severity suggests the involvement of different modifier genes that contribute to the observed phenotypes [123]. Another gene whose identified mutations have been associated with deafness is tRNASer(UCN). Mutations in different families have demonstrated phenotypes of isolated hearing loss as well as deafness associated with symptoms such as myoclonus epilepsy, ataxia, severe psychomotor retardation, and diabetes mellitus. The mechanism by which mitochondrial mutations specifically cause deafness is poorly understood. However, it is likely that cochlear processing, which requires substantial energy consumption, is especially susceptible to inefficiencies in the energy-producing apparatus that mitochondrial mutations would cause.

Conclusions: clinical implications of understanding molecular hearing

Rapid progress has been made in the past decade toward understanding the complexity of the peripheral auditory system, and genetic deafness has been responsible for much of that progress. As a result, there have been both short- and long-term advances for the treatment of deafness. In the short term,

identification of the gene mutations that result in deafness provides the opportunity for genetic testing as a means of determining both diagnosis and prognosis of hearing loss, especially in newborns and infants where behavioral testing is difficult. Congenital hearing loss is often discovered with newborn hearing screening. For the children diagnosed with hearing loss, the etiology of the loss is often difficult to determine. However, with genetic testing, identifying the mutation involved could yield an accurate prognosis of further progression of hearing loss. This knowledge will help guide therapy. For the Cx26 mutation, for example, hearing acuity does not improve with time, but is not associated with other neurologic sequelae. These children are therefore outstanding candidates for early cochlear implantation. In the long term, identifying the mutation responsible for hearing loss offers the opportunity for early and specific pharmacologic therapy, either to halt the progression of hearing loss or, better, to correct it.

Understanding hearing at a molecular level has another important long-term clinical application. Deafness associated with lesions anywhere in the cochlea, regardless of the specific etiology, typically leads to sensory hair cell degeneration. One approach to treating hearing loss associated with the loss of sensory hair cells is to regenerate the hair cells. Hair cell regeneration is a topic of active investigation, for which progress has already been made. A multipotent cell line, for example, has been described whose cells were derived from the developing otocyst of a transgenic mouse (H-2Kb-tsA58). The cells can be maintained in a proliferating, undifferentiated state in vitro in the presence of γ-interferon [124, 125]. These cells can then be manipulated to differentiate into sensory hair cells when transfected to over-express the mammalian 'atonal' homolog (Math-1) gene product [126, 127]. "New" hair cells that have differentiated in vitro have been shown to demonstrate an ability to respond to mechanical stimulation and form connections with spiral ganglion cells in co-culture [128]. If hair cells can be regenerated, however, the underlying cochlear dysfunction may preclude establishing a cochlear environment in which the regenerated hair cells could function. The ability to correct specific molecular abnormalities of the cochlea as well as replace lost sensory hair cells offers much more promise than hair cell regeneration alone.

One of the goals of research in auditory neurobiology is to discern the genetic basis of anatomical and physiological processes of hearing and deafness. Because deafness can be caused by any of a wide variety of genetic defects, it is important to realize that there is no single cure. Ultimately, knowledge of the gene involved in a specific form of deafness will suggest a pharmaceutical treatment plan, a change in lifestyle, or a particular type of surgical intervention.

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