dependent on FXa-activated FV. Once generated, thrombin would counter this effect of TFPIα by producing forms of activated FV that have been cleaved at R1545 and lack the potential TFPIα-binding site AR.

Is there a role for PS? PS is known to interact with FXa, FV(a), and TFPIα and is also released by stimulated platelets. Thus, its potential role in the reactions discussed above warrants investigation.

Most important to the affected members of the east Texas family, however, is that the elucidation of the underlying pathophysiology suggests that TFPI inhibitors currently in development may provide a means of treatment (14, 15).

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A "*Tric*" to tighten cell-cell junctions in the cochlea for hearing

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Tricellulin is a tricellular tight junction–associated membrane protein that controls movement of solutes at these specialized cell intersections. Mutations in the gene encoding tricellulin, *TRIC*, lead to nonsyndromic deafness. In this issue of the *JCI*, Nayak et al. created a gene-targeted knockin mouse in order to mimic the pathology of a human *TRIC* mutation. Deafness appears to be caused either by an increase in the K^+ ion concentration around the basolateral surfaces of the outer hair cells or, alternatively, by an increase in small molecules such as ATP around the hair bundle, leading to cellular dysfunction and degeneration. Furthermore, the mice have features suggestive of syndromic hearing loss, which may have implications for care and treatment of patients harboring *TRIC* mutations.

Millions worldwide suffer from a debilitating hearing loss (1). In many regions of the world in which health care conditions and public health are less developed or in populations with high rates of consanguinity, the number of people affected is extremely high. Understanding the mechanisms leading to hearing loss may help widen the current scope of therapeutic options, which are currently restricted to hearing aids and cochlear implants. The causes of hearing loss are manifold, including both genetic and environmental factors. Hereditary hearing loss is caused by mutations in a wide variety of genes that encode the proteins associated with the transduction of sound waves from the external ear to the middle and inner ear and finally to the brain. To date, hereditary hearing loss has been linked to over 60 genes (2).

The cochlea, located in the inner ear, is responsible for the conversion of sound to a neural electric excitatory signal (3). Two distinct fluids, the endolymph and perilymph, are contained within the cochlea. While the perilymph has an ionic composition similar to that of the general extracellular fluid, the endolymph is characterized by a high K^+ concentration and endocochlear potential (EP), which are essential for hearing (4). An epithelial cellular sheet covering the cochlea creates a barrier that allows these fluids to maintain their composition.

Tight junctions are essential for sound conduction

Tight junctions (TJs) provide a strong epithelial barrier function within the cochlea, preventing the leakage of solutes through

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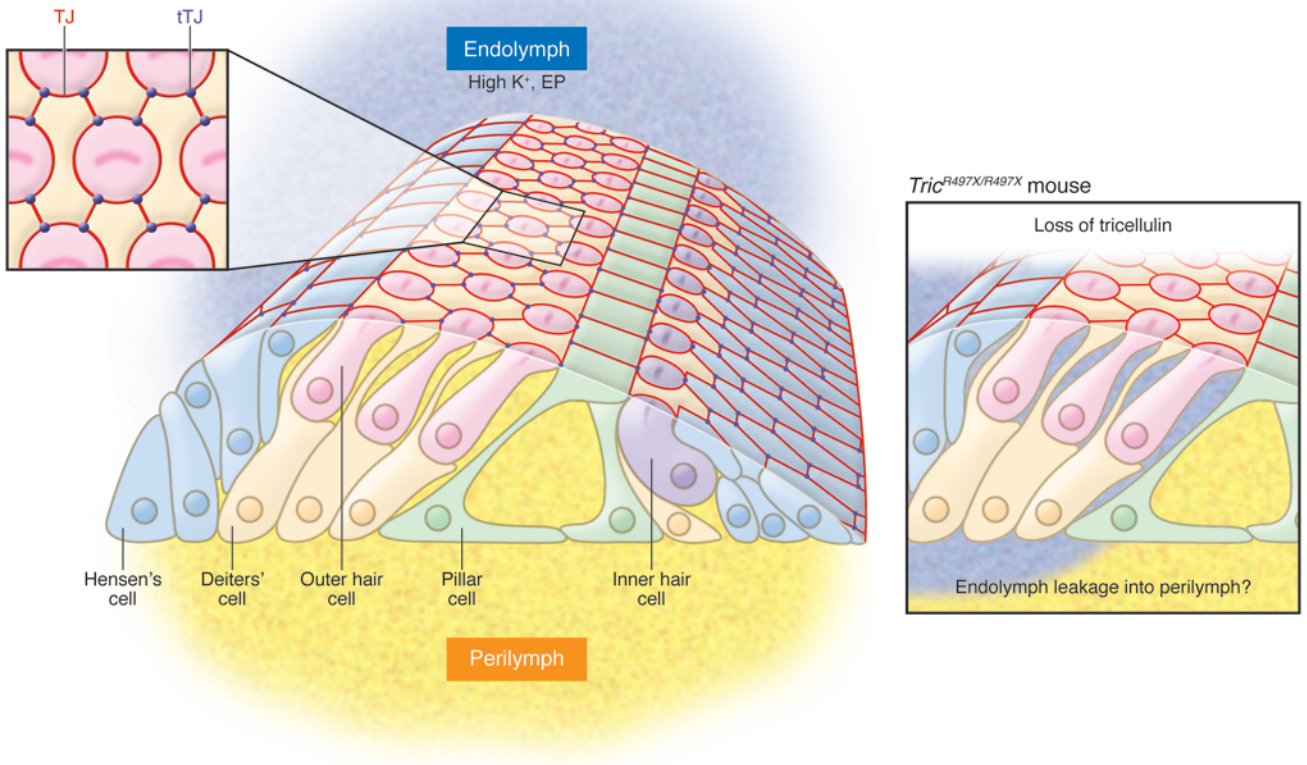

Figure 1
The mammalian organ of Corti contains the sensory epithelium essential for hearing, including hair and supporting cells. The hair cells include both inner and outer hair cells, and the supporting cells include Hensen's cells, Deiters' cells, and pillar cells. A crucial parameter of the organ of Corti is the ionic composition of the perilymph and endolymph, held in balance by the epithelial sheet that contains TJs. A further demarcation of the TJs are the tTJs, which when impaired in *Tric* mutant mice are postulated to lead to endolymph leakage into the perilymph. This aberration would have dire consequences for hearing.

the intercellular space (5). TJs surround each epithelial cell at the boundary of the apical and basolateral membrane domains, sealing the intercellular space like a zipper (6). Consequently, the apical side of the cells faces a high potassium solution, the endolymphatic fluid, while the basolateral side contacts the perilymphatic fluid (ref. 7 and Figure 1).

TJ proteins have been localized to the inner ear, and a number of them have been implicated in hearing. These include the claudin family of membrane proteins, which are essential components of TJs. *CLDN14* was identified as the causal gene in human hereditary deafness at the DFNB29 locus (8), and *Cldn14* knockout mice exhibit hearing loss with degeneration of hair cells (9). *Cldn9* mutant mice also exhibit deafness with hair cell degeneration (10). Mice lacking the *Cldn11* gene, which is expressed in the stria vascularis, suffer from deafness and a loss of the EP (11, 12). In addition, overexpression of ZO-2 (also known as TJ protein 2 [TJP2]), a TJ-associated scaffold protein that binds to claudins, leads to nonsyndromic hearing loss DFNA51, mediated by the GSK-3β pathway (13). These observations indicate that the TJ-mediated permeability barrier in the cochlea is essential for hearing.

The TRIC to listening

Although it is not well recognized, the paracellular pathway within the cellular sheet can be spatially divided into two parts: one between two adjacent cells and the other at tricellular contacts (TCs) where the vertices of three cells meet. It is not easy to grasp how the "TJ zipper" seals the intercellular space at the TCs, because there are three plasma membranes. Electron microscopic studies have revealed that there are specialized structures of TJs at the TCs, named tricellular TJs (tTJs), which make the extracellular space at TCs reach their limit of narrowness (14). Previously, Saima Riazuddin and colleagues had reported that *TRIC*, which encodes a tTJ-associated membrane protein, tricellulin (15), is causally linked to nonsyndromic hereditary hearing loss, DFNB49 (16). In this issue of the *JCI*, in an elegant follow-up study to determine the mechanism of *TRIC*-associated deafness, Nayak et al. generated *Tric*$^{R497X/R497X}$ knockin mice, which mimic a human *DFNB49* mutation (17). Following the observation of a phenotype consistent with profound deafness by auditory brainstem response, Nayak et al. examined the morphology of the cells of the inner ear and noted hair cell degeneration, followed

by the demise of spiral ganglion cells by postnatal day 30. Freeze-fracture replica electron microscopy revealed that the tTJs were not normally formed among hair and supporting cells in the organ of Corti in *Tric*$^{R497X/R497X}$ mice; the sealing elements of TJs were not integrated into typical tTJs at the center of TC regions. A similar phenotype was seen at the TCs in the utricular macula (vestibular organ). These observations clearly demonstrate that tricellulin is essential for tTJ formation in the cochlea at the ultrastructural level, leading to impairment of epithelial barrier function at the TCs. Impairment of tTJ formation may thus lead to the leakage of small molecules through the TCs. Indeed, tricellulin knockdown in cultured epithelial cells results in a reduction of epithelial barrier function, as evaluated by the measurements of transepithelial electrical resistance as well as paracellular flux (15).

Cochlear hair cell degeneration

Perhaps the most compelling experiment performed by Nayak et al. was the attempt to discern why the hair cells of the *Tric*$^{R497X/R497X}$ mice die. Was hair cell death due to disrupted hair cell function or to an altered microenvironment around these cells? To address this question, they used a mouse model for the human form of X-linked deafness, *Pou3f4*^{delJ}. While inner ear endolymph in this model has a reduced EP, mice harboring this allele have no organ of Corti defects. The authors asked whether changes induced in the *Tric*$^{R497X/R497X}$ mouse endolymph were able to rescue the hair cell degeneration in *Pou3f4*^{delJ} mice. Remarkably, the double mutants had no hair cell degeneration, suggesting that eliminating the extracellular factors from the stria vascularis and endolymph, not intracellular signaling between the hair cells, led to rescue of the phenotype (17). A most likely candidate for being this extracellular factor is the elevated K^+ ion concentration at the basolateral compartment of hair cells (Nuel's space). The high concentration of K^+ has been reported to cause degeneration of hair cells in mice deficient in KCC3 or KCC4, which are responsible for the retrieval of K^+ ions from Nuel's space (18, 19). On the other hand, the authors speculated that there may be minimal leakage of K^+ ion, based on the normal EP of the knockin mice, and proposed the possibility of leakage of other molecules, such as Na^+ and ATP. However, in *Cldn9* mutant mice, which also exhibit an unaltered EP, the K^+ concentration in the perilymph increased significantly (10). Hence, the possibility of K^+ leakage from the *Tric*$^{R497X/R497X}$ mouse endolymph cannot be excluded. Judging from the fact that *Cldn14*-deficient mice, *Cldn9* mutant mice, and *Tric*$^{R497X/R497X}$ mice share the deafness phenotypes with unchanged EP and hair cell degeneration all within the same time frame, a common mechanism may underlie deafness in these models. Future studies may be able to clarify whether only subtle K^+ leakage, which does not affect the EP or endolymphatic ion compositions, can induce hair cell degeneration and whether the leakage of other molecules, such as Na^+ or ATP, is involved in the viability of hair cells.

Localizing the problem

Is tTJ localization of tricellulin required for its function? Recently, it has been reported that tricellulin is recruited to tTJs by the angulin family of membrane proteins, which consists of LSR, ILDR1, or ILDR2 (20). Interestingly, *ILDR1* is causally linked to familial deafness DFNB42 (21). ILDR1 is the major type of angulin family protein in the inner ear, suggesting that the ILDR1-tricellulin system may play a role at tTJs for hearing. Indeed, some DFNB42-associated ILDR1 mutant proteins are defective in tricellulin recruitment to tTJ, and all DFNB49-associated tricellulin mutant proteins are defective in tTJ localization (22). It would be of interest to determine whether *Ildr1*-deficient mice exhibit a deafness phenotype accompanied by early hair cell degeneration, as was observed in the *Tric*$^{R497X/R497X}$ mice.

The *Tric*$^{R497X/R497X}$ mouse may also provide new insights into the human condition it was created to model. Although DFNB49 was previously reported as a form of nonsyndromic deafness, *Tric*$^{R497X/R497X}$ mice exhibited morphological changes in several tissues, including the mandibular salivary gland, thyroid follicles, heart, and olfactory epithelium (17). Indeed, tricellulin is expressed ubiquitously and is predicted to be involved in establishing tricellular barriers in almost all epithelia. In particular, myocardial hypertrophy may be an issue, since patients with this problem are asymptomatic and only excessive exercise may bring on sudden cardiac death (23). The possibility that patients with DFNB49 hearing loss should have more frequent cardiology exams or perhaps avoid extreme exercise should be examined further.

Conclusions

Although tTJs were identified by electron microscopy about 40 years ago, many issues remain elusive regarding their structure and function. Further detailed analysis could clarify the molecular basis of tTJs and lead to the elucidation of their involvement in deafness pathogenesis. Understanding the structure and function of the inner ear and the details of its components is crucial for deciphering the senses of hearing and balance: how they perform normally, why they fail at times, and how we might be able to restore them in the future. The work of Riazuddin's team is an excellent step in this direction.

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