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

Is there a role for PS? PS is known to interact with FXa, FV(a), and TFPI 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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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. 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.

**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.

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 TJs, 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<sup>R497X/R497X</sup> 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 cells and supporting cells in the organ of Corti in TricR497X/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 TJs 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 TJs. Impairment of tTJ formation may thus lead to the leakage of small molecules through the TJs. 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 TricR497X/R497X mouse 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<sup>−/−</sup>. 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 TricR497X/R497X mouse endolymph were able to rescue the hair cell degeneration in Pou3f4<sup>−/−</sup> mice. Remarkably, the double mutants had no hair cell degeneration, suggesting that eliminating the extracellular factors from the stria vasularis 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<sup>+</sup> ion concentration at the basolateral compartment of hair cells (Nuel’s space). The high concentration of K<sup>+</sup> has been reported to cause degeneration of hair cells in mice deficient in KCC3 or KCC4, which are responsible for the retrieval of K<sup>+</sup> ions from Nuel’s space (18, 19). On the other hand, the authors speculated that there may be minimal leakage of K<sup>+</sup> ion, based on the normal EP of the knockin mice, and proposed the possibility of leakage of other molecules, such as Na<sup>+</sup> and ATP. However, in Cldn9 mutant mice, which also exhibit an unaltered EP, the K<sup>+</sup> concentration in the perilymph increased significantly (10). Hence, the possibility of K<sup>+</sup> leakage from the Tric<sup>R497X/R497X</sup> mouse endolymph cannot be excluded. Judging from the fact that Cldn14-deficient mice, Cldn9 mutant mice, and Tric<sup>R497X/R497X</sup> 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<sup>+</sup> 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<sup>+</sup> 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 TricR497X/R497X mice.

The Tric<sup>R497X/R497X</sup> 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<sup>R497X/R497X</sup> 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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