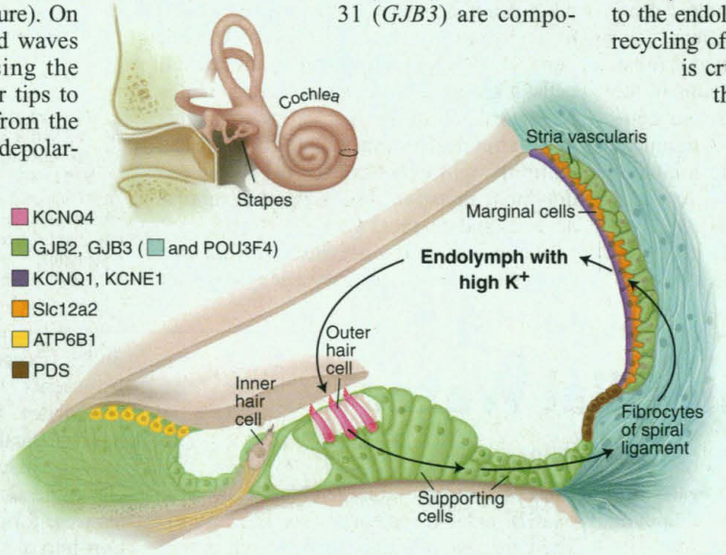
PERSPECTIVES: BIOMEDICINE

The Benefits of Recycling

Karen P. Steel

Nestled inside the inner ear is the cochlea, the snail-like organ that houses the sensory hair cells responsible for transducing sound waves into electrical impulses. Hairlike stereocilia on the upper surface of these sensory cells project into a cavity filled with a fluid called endolymph (see the figure). On reaching the inner ear, sound waves deflect the stereocilia causing the transducer channels near their tips to open. Potassium ions flood from the endolymph into the hair cells, depolarizing their cell membranes and initiating the electrical signal that is carried along the auditory nerve to the brain. The cation balance in the endolymph is such that the K^+ concentration is high and the Na^+ concentration is low. Cochlear endolymph is maintained at a high resting potential (the endocochlear potential). The voltage gradient—from the positive endocochlear potential to the negative potential inside the hair cell—drives the K^+ flow, but must be continuously maintained by rapid recycling of K^+ back into the endolymph. In the latest of a series of studies showing the importance of K^+ recycling for normal hearing, Minowa and colleagues (*1*) report on page 1408 that an abnormality in cochlear fibrocyte cells that recycle K^+ contributes to hearing impairment in a mouse model of one form of human deafness.

Recycling of K^+ in the cochlear duct has long been thought to be important for hearing (*2*). At least six of the many proteins associated with deafness in humans and mice are probably directly involved in K^+ recycling (see the figure). A potassium channel (encoded by the *KCNQ4* gene, which is mutated in a form of dominant, progressive hearing loss) in outer hair cells is thought to transport K^+ ions out of the cell (*3*). The K^+ ions are then taken up by the supporting hair cells below. From here they pass through a network of gap junctions that extends from the epithelial supporting cells to the mesenchymal fibrocytes that form the spiral ligament and then to the epithelial marginal cells of the stria vascularis, which secrete endolymph. Connexin 26 (*GJB2*) and connexin 31 (*GJB3*) are components of these gap junctions that when mutated lead to dominant and recessive forms of hearing impairment in humans (*4*). A Na-K-Cl cotransporter protein (encoded by *Slc12a2*), expressed on the basolateral membrane of marginal cells, is part of the K^+ pumping machinery that pumps K^+ into the marginal cells to high levels. Mutations in the cotransporter lead to a failure to produce endolymph in mice (*5*). Finally, channels at the apical surface of marginal cells—composed of the products of the *KCNQ1* (*KvLQT1*) and *KCNE1* (*ISK*) genes, which are mutated in Jervell and Lange-Nielsen syndrome (characterized by recessive congenital deafness with cardiac defects)—allow K^+ accumulating in the marginal cells to flow back into the endolymph, so regenerating the high K^+ concentration and the endocochlear potential (*4*). Inactivation of the *Isk* gene in mice results in a failure of the marginal cells to produce endolymph (*6*).



Hear this. The cochlea of the inner ear showing the location of key proteins involved in the recycling of K^+ back into the cochlear fluid (endolymph). The transcription factor POU3F4 is expressed in the fibrocyte cells of the spiral ligament (blue); KCNQ4 is expressed in the outer sensory hair cells (red); connexins 26 (GJB2) and 31 (GJB3) are expressed in the supporting cells, spiral ligament fibrocytes and in cells of the spiral limbus.

Minowa *et al.* report a new mouse mutant in which the transcription factor Pou3f4 (also known as Brn-4), which may affect the expression of many different target genes, has been inactivated. The mice are profoundly deaf, have a reduced endocochlear potential and show ultrastructural abnormalities in the spiral ligament fibrocytes, which have reduced contacts with their neighbors. Normally *Pou3f4* expression is restricted to the mesenchymal cells (including fibrocytes) that surround the inner ear during development. The reduced contacts between fibrocytes may impede the flow of K^+ back to the endolymph, supporting the view that recycling of K^+ through the spiral ligament is crucial for normal hearing. But the *Pou3f4* mutant mice have a reduced endocochlear potential rather than a complete block in endolymph secretion (seen in the *Slc12a2* and *Isk* mouse mutants). Other mutant mice—including those with few or no melanocytes (pigment cells) in their stria vascularis—also show a decrease in endocochlear potential without a complete failure in endolymph production. This suggests that melanocytes too are needed to maintain the balance of ions in the cochlear duct (*7*).

Humans with X-linked deafness may also have mutations in *POU3F4* (*4*). Part of the hearing impairment in this form of deafness results from immobilization of the stapes, the last of the three middle ear bones that transmit sound to the inner ear. The inner ear is also malformed with a wide internal auditory meatus (the canal through which the cochlear and vestibular nerves run) and thin or absent bone between the meatus and the cochlea, allowing communication between the perilymph compartments of the inner ear and the cerebrospinal fluid that bathes the tissues of the brain. These patients also have defects in the sensorineural portion of the hearing machinery. The Minowa report of a primary deficit in the spiral ligament and a reduced endocochlear potential in the *Pou3f4* mouse mutant suggests that these defects may also underlie the sensorineural component of the human disorder.

No obvious middle or inner ear malformations were observed in the *Pou3f4* mutant mice (*1*). But, intriguingly, another group recently reported such defects in another *Pou3f4* mouse mutant (*8*). These mice

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have overt balance defects and only a mild hearing impairment but have a malformed stapes, shortened cochlear duct, an enlarged internal auditory meatus, thin bone in parts of the otic capsule, a constricted superior semicircular canal, and loosely packed spiral ligament fibrocytes. This phenotype resembles that of deaf individuals with mutations in human *POU3F4*, but, apart from the spiral ligament defects, is not at all like the phenotype of the Minowa *Pou3f4* mouse mutant (perhaps owing to differences in genetic background between the two mouse strains). Some phenotype variability is also found among X-linked deafness patients with *POU3F4* mutations.

At least four other human deafness genes may affect the balance of ions in the cochlear duct. The Norrie disease gene (*NDP*)—expressed in the spiral ligament and stria vascularis (*4*)—encodes a mucin-like protein that when mutated leads to late-onset progressive hearing loss. The *COCH* gene (thought to encode an extracellular matrix protein) is mutated in a form of dominant, nonsyndromic progressive hearing impairment associated with a loss of fibrocytes and increased extracellular deposits in the spiral ligament, sites that correspond to the route of K^+ recycling (*4*). The *COCH* gene has recently been implicated in some cases of Ménière's disease, the symptoms of which include fluctuating balance and hearing disruption thought to result from a fluid imbalance in the inner ear (*9*). A chloride and iodide transporter—encoded by the *PDS* gene and expressed by epithelial cells between the stria vascularis and the sensory hair cell region—is mutated in Pendred's syndrome as well as a form of nonsyndromic deafness (*4*, *10*). Another gene, *ATP6B1*, is expressed in interdental cells, a group of epithelial cells on the other (inner) side of the hair cell region. This gene encodes a component of a proton pump thought to control the pH of the endolymph; mutation results in renal tubular acidosis and deafness (*11*).

The hair cells of the cochlea are exquisitely tuned sensory receptors that depend for their survival on maintenance of a suitable environment. Mutations in one of the many proteins that maintain this environment result in a gradual loss of hair cell function leading to progressive hearing loss and eventual hair cell death. Therapeutic intervention to bypass the dysfunctional protein and to restore a benevolent environment before the hair cells die might halt the progression of deafness.

References

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PERSPECTIVES: PLANETARY SCIENCE

Primordial Water

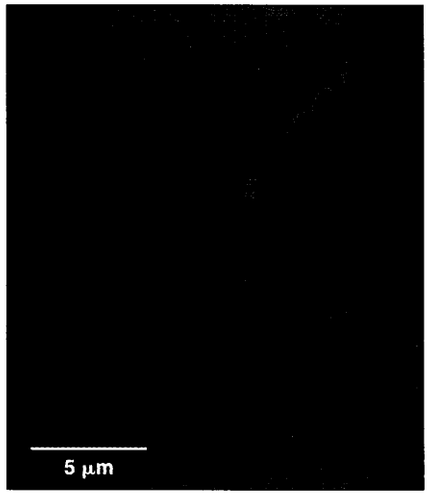
Robert N. Clayton

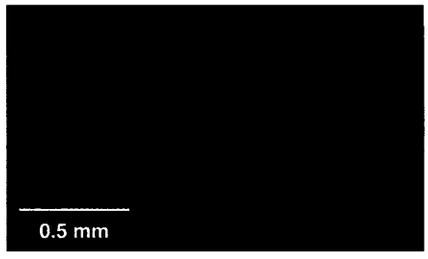
The most abundant element in the sun is hydrogen; the third most abundant element is oxygen. It therefore follows that water molecules must have been a major constituent of the solar nebula from which the planets formed. Most water condensed on the "giant" planets in the outer parts of the solar nebula, but some water remained in the inner regions of the solar nebula, where it was acquired by Earth and other rocky "terrestrial" planets, by processes that remain largely unknown.

Clues come from meteorites, which can provide evidence for the chemical behavior of water at the time when planetesimals—small rocky bodies from which planets accreted—grew in the early solar system. Two reports in this issue deal with chemical processes involving liquid water within asteroids or their planetesimal precursors. On page 1380, Brearley (*1*) presents transmission electron microscope evidence for the formation of iron-rich olivine at low temperatures and argues that these observations support an earlier proposal that this ubiquitous phase was formed by a hydration-dehydration sequence. And on page 1377, Zolensky *et al.* (*2*) present direct evidence for meteoritic water: tiny inclusions of brine within large crystals of halite (NaCl) inside a meteorite (see the figure). The existence of a water-soluble salt in this meteorite is astonishing. Also, this sample of aqueous solution trapped within the meteorite provides the first opportunity to study solar nebular water directly.

Ordinary chondrites and carbonaceous chondrites are two major classes of primitive stony meteorites. Both are characterized by abundant chondrules—millimeter-sized silicate spheroids that were once molten droplets in the solar nebula. In ordinary chondrites, the matrix between these chondrules is composed primarily of chondrule fragments. In contrast, in carbonaceous chondrites, the matrix is chemically and mineralogically distinct from chondrules and in many cases consists of hydroxyl-bearing clay minerals. Whether these hydrous minerals were formed in the nebula before accretion into a planetesimal, or within a planetesimal after accretion, remains controversial. It is generally assumed that the parent bodies of ordinary chondrites were dry and those of carbonaceous chondrites were wet.

Most ordinary chondrites are metamorphic rocks and show evidence of closed-system heating to temperatures of several hundred degrees Celsius. Under these conditions, nonvolatile element transport occurs only on a millimeter scale (*3*). Mobilization of water-soluble phases is well-





It just fell out of the sky. (Left) Fluid inclusions in halite crystals in the Monahans meteorite. The small bubble contains both a low-viscosity liquid and vapor and is mobile at room temperature. **(Right)** True color image of the halite crystals. The purple color may be caused by exposure to solar and galactic cosmic rays, exposure to decaying ^{40}K in small sylvite crystals within the halite, or both. See (*2*).

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