AUDITORY AND VESTIBULAR MOUSE MUTANTS: MODELS FOR HUMAN DEAFNESS

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INTRODUCTION

Human deafness is extremely heterogeneous, and mutations in over 100 genes may be associated with this common form of sensory loss. The chromosomal location for over 60 loci is already known, but only 18 of the genes have been cloned /1/. Genes that cause non-syndromic hearing loss (NSHL) and vestibular dysfunction are difficult to identify in the human population for several reasons, including absence of characteristic clinical signs in deaf individuals, assortive mating in deaf communities, genetic heterogeneity and small family populations /2/. Geographically isolated families have been invaluable for mapping deafness loci, but the long, arduous process of identifying the genes continues. The critical region is often large since the number of informative recombinations is limited when obtained from a family. Furthermore, in order to understand the mechanisms involved in auditory function, in both the normal and pathological state, we need to be able to study parameters such as morphology, development and electrophysiology, which impossible to perform in humans. Fortunately, these complications can be circumvented by using mice to study human deafness. There are over 60 naturally occurring mouse mutations with hearing impairment that may serve as models for human deafness, facilitating research in this area. The human and mouse auditory systems are remarkably similar. Furthermore, manipulative genetic tools in the mouse, such as transgenic technologies and mutagenesis, allow scientists to introduce or remove genetic mutations at will, and genes for deafness are no

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exception. Due to similarities between humans and mice, phenotypic studies of hearing loss in mice can teach us a great deal about the human auditory and vestibular systems. Techniques such as electron microscopy (Fig. 1), *in situ* hybridization, immunochemical studies, electrophysiology and more, allow us to study the different functions of proteins in the mouse auditory and vestibular systems. High resolution genetic and physical maps have been developed for the mouse, and critical regions can be defined at a higher resolution than in humans since many matings can be generated, providing a large number of informative recombinations. At least five genes responsible for human hearing loss have been cloned as a direct result of the discovery of the mutated homologous mouse gene, and the identification of additional genes in the auditory pathway has been elucidated by use of these manipulative tools in the mouse.

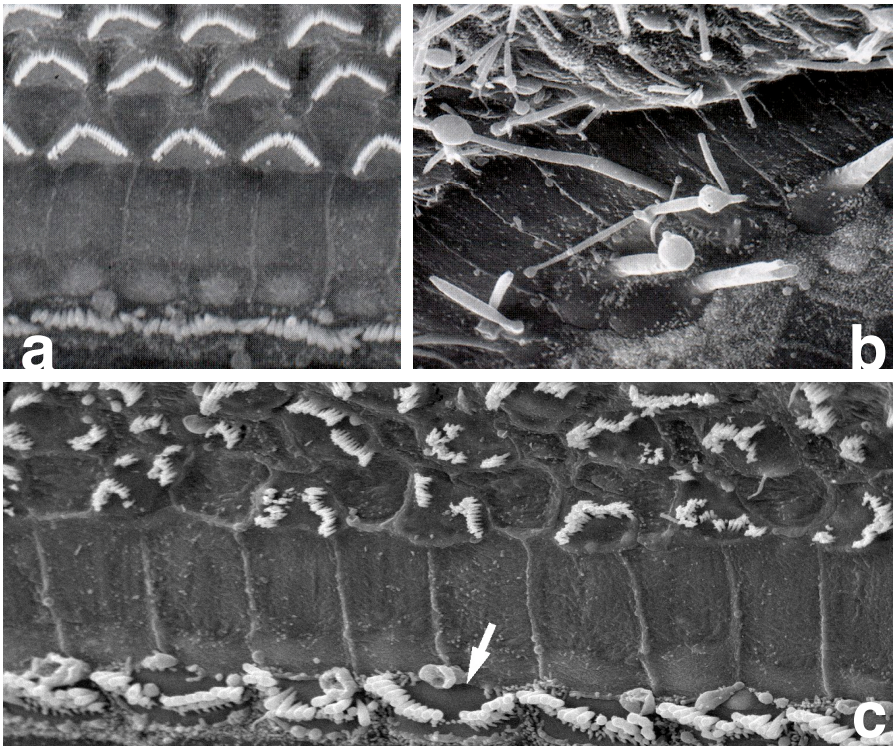

Fig. 1: Scanning electron microsopy of the organ of Corti derived from 20 day-old controls and mutants reveals a variety of phenotypes. **a.** control, **b.** Snell's waltzer /13/, Tailchaser /26/. Reprinted with permission.

In the following review, we highlight particularly interesting examples of mouse mutations, organized according to the methods by which they were generated. For a comprehensive list of mouse hearing and vestibular mutants known to date, refer to the Jackson Laboratory Hereditary Hearing Impairment in Mice Web site: http://www.jax.org/research/hhim/.

SPONTANEOUS MUTANTS

Spontaneous mutations arise without any intervention, due to natural causes. Some of these may affect the auditory and vestibular system and, over the years, many scientists have identified and characterized these mutations. These spontaneous mutations have contributed a great deal to the study of hearing, especially regarding the role of unconventional myosins in hearing loss.

Shaker1 (*sh1*), a spontaneously-induced mutant discovered in 1929 exhibiting deafness, hyperactivity, head-tossing and circling /3/, was the first circling mouse for whom its mutated gene was identified /4/. *sh1* encodes an unconventional myosin, myosin VIIA. Myosins are a superfamily of motor proteins defined by their ability to bind actin and hydrolyze adenosine triphosphate (ATP). Unconventional myosins have been implicated in many crucial cellular functions, including endocytosis, ion channel regulation, anchoring of stereocilia and vesicle movement /5/. Usher syndrome type 1B (*USH1B*) was mapped to the homologous chromosomal region in mice. Once the *sh1* gene was discovered, it quickly led to the finding that mutations in myosin VIIA are associated with *USH1B* and two forms of non-syndromic deafness, *DFNB2* and *DFNA11*, that map to the same region /6-8/. *In situ* hybridization studies revealed that myosin VIIA is expressed at embryonic day 14.5 in the sensory epithelia and at day 16.5 in the cochlea /9/. Different *sh1* mutations were examined, each showing a variable phenotype in the development of their stereocilia bundles by electron microscopy, with a prominent correlation between the severity of the genotype and phenotype. Furthermore, myosin VIIA was found to be required for aminoglycoside accumulation in cochlear hair cells /10/.

The Snell's waltzer (*sv*) mouse was found in a litter maintained by George Snell, winner of the 1980 Nobel Prize for his pioneering work in mouse genetics, at the Jackson Laboratory /11/. Thirty years later,

using a positional cloning approach, the gene causing deafness was discovered to also be an unconventional myosin, myosin VI (*Myo6*) /12/. Scanning and transmission electron microscopy on *sv* mice cochleas revealed severe stereociliar disorganization, with stereocilia fusing shortly after birth, suggesting a role for myosin VI in the anchoring of the apical hair cell membrane to the actin-rich cuticular plate /13/ (Fig. 1b). Yet another unconventional myosin, myosin XV, is associated with mutations in the shaker2 (*sh2*) mouse, as discussed below /14/. Unconventional myosins clearly have an essential role in normal cochlear function and the data accumulated on the *sh1*, *sv*, and *sh2* mice will be useful for understanding hair cell pathology in human hereditary deafness.

KNOCKOUTS

Gene targeting, or "knockouts", enable us to remove or mutate a specific gene in the mouse and thus study the effects of the gene due to its loss of function. Deafness and vestibular knockout mice are a resource to study specific auditory gene function and morphological characterization of the inner ear. Given here are a few examples of the strength of this technique, as well as its limitations, due to similarities and differences between human and mouse phenotypes.

POU3F4, the first gene found to be associated with human nonsyndromic deafness, is X-linked /15/. Mutations lead to stapes fixation, an enlarged internal auditory meatus and hypoplasia of the cochlea that causes progressive sensorineural deafness. In order to further study the function of Pou3f4 in the development of the inner ear, a Pou-domain knockout mouse was established /16/. In the knock-out mice, a malformation of the temporal bone was found due to the enlargement of the internal auditory meatus. Hypoplasia of the cochlea was also found and a reduction of coiling was found in 90% of the *Pou3f4* mutant mice. Histological analysis of the cochlea showed a smaller spiral limbus and thinner and less adherent fibrocytes of the spiral ligament. On the other hand, several differences were found between the mice and humans. The mice show only a mild hearing loss and instead of stapes fixation, a malformation in the footplate of the stapes was found. The *Pou3f4* mutant mouse whiskers were smaller and normal whisker sweeping motion was not seen. A head bobbing phenotype was also observed due to a

constriction in the bony labyrinth of the superior semicircular canals. In humans the brain compensates for vestibular malfunction and we obviously exhibit a null phenotype when it comes to whiskers!

Another possible consequence of generating a knockout is the discovery that a specific gene is involved in deafness. Such was the case with the winged helix transcription factor *Fkh10*. The *Fkh10* knockout mice are deaf due to the fact that instead of an inner ear, the homozygous mutant mice contain an irregular and continuous cavity /17/. These mice also show circling and head tilting behaviour due to vestibular malfunction. The human *Fkh10* gene was mapped to chromosome 5q34, which implicates it as a possible deafness-causing gene if a non-syndromic hearing loss family should be found linked to this region.

Gene targeted mutagenesis of the *Pou4f3* gene also led to its discovery as a deafness-causing gene /18/. Of even greater significance, the knockout helped lead to the subsequent identification of mutations in *DFNA15*, an autosomal dominant locus in an Israeli Jewish family with progressive hearing loss /19/. Genetic linkage analysis placed *DFNA15* on human chromosome 5q31, in a region homologous to mouse chromosome 18 which contains the gene for a transcription factor, *Pou4f3* (Fig. 2). Since the *Pou4f3* null mouse is deaf, this gene was an ideal candidate for deafness in humans. Sequencing of the human homolog revealed a mutation in this gene in deaf individuals of the Israeli family.

At times, when a gene that causes deafness in humans is knocked out in mice, we observe a totally different phenotype. Connexin 26 (*GJB2*) mutations hae been shown to account for about 50% of autosomal recessive non-syndromic deafness /20/. Connexin 26 is a gap junction protein that is thought to have a role in the recycling of potassium ions back to the endolymph of the cochlear duct after the stimulation of the sensory hair cells. *GJB2* knockout homozygous mice showed a lethal phenotype, dying at embryonic day 11 due to a dysfunction of the placenta, thought to be caused by decreased transplacental uptake of glucose /21/. The difference in phenotype might be explained by the different morphology of the mouse and human placenta. The mouse placenta consists of two syncytiotrophoblast layers that are interconnected by gap junctions in order to support the transport of nutrients and removal of waste products, whereas the human placenta consists of only one large layer. A

knockout directed only to the inner ear might address human connexin 26 pathology better.

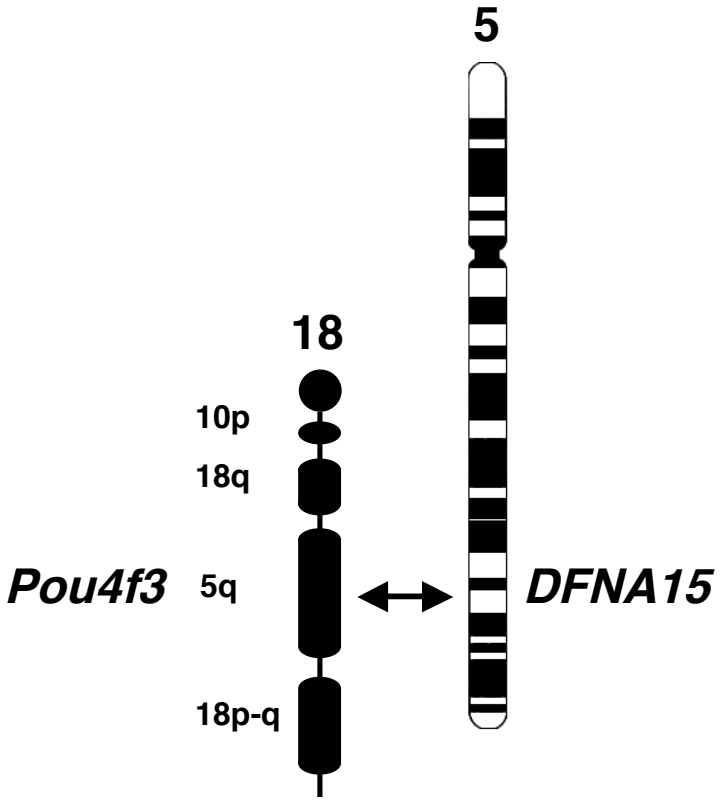

Fig. 2: Homology between human (right) and mouse (left) chromosomes and transgenic technology has facilitated gene discovery in deafness. Human chromosome 5 shares homology with different mouse chromosomes, including mouse chromosome 18, which was known to contain the *Pou4f3* gene. The mouse knockout of *Pou4f3* is deaf and circling and once the human deafness locus DFNA15 was discovered on 5q31, *POU4F3* became a perfect candidate for this form of human deafness. This led to the mapping of the human *POU4F3* gene on 5q31 and identification of a mutation in this gene in human deafness /19/.

CHEMICAL AND RADIATION INDUCED MUTANTS

Mouse mutations can be induced by radiation or by treating mice with chemicals, such as the popular N-ethyl-N-nitrosourea (ENU). Mice are then screened for a specific phenotype, such as deafness or

vestibular malfunction, and the genes can then be mapped and identified using different cloning approaches.

Shaker2 (*sh2*) mice, which exhibit recessive deafness, head-tossing and circling behavior, were identified in 1928 as a consequence of X-ray irradiation /22/. Phenotypically they show shorter stereocilia and abnormal actin structures in the inner hair cells /14/. Using an *in vivo* complementation approach by injecting bacterial artificial clones (BAC) that spanned the mapped mutated region into *sh2/sh2* fertilized eggs, the disease-causing gene, unconventional myosin XV, was discovered. The human deafness locus, *DFNB3*, lies in the homologous region, 17p11.2. Mutations in myosin XV were discovered in *DFNB3* deaf individuals /23/, again showing the strength of mouse models in finding human deafness-causing genes.

By injecting the alkylating agent ENU into mice, point mutations or small intragenic lesions can be created in a single gene to produce a large variety of phenotypes, including mouse mutants with abnormalities in sense organs, limbs, and the cental nervous system /24-25/ (for catalogue of ENU mouse mutants, see the GSF ENU-Mouse Mutagenesis Screen Project, http://www.gsf.de/ieg/groups/enu/mutants/index.html; and the UK Mouse Genome Centre ENU Mutagenesis Programme, http://www.mgu.har.mrc.ac.uk/shirpa_data/). One such example is the mutant mouse, Tailchaser (*Tlc*) /26/. *Tlc* mice show a hearing loss and head-shaking phenotype that is progressive with age. Using scanning electron microscopy, it was shown that *Tlc* mice show structural disorganization of the outer hair cells, while the inner hair cells look normal but begin degenerating at around 4 months of age (Fig. 1c). The vestibular hair cells look normal in younger mice, but around 2.5 months there are fewer stereocilia found in the cristae as compared to wild-type mice and the utricle shows fused bundles of hair cells. The *Tlc* locus was mapped to a 12 cM region on mouse chromosome 2 by an intraspecific backcross. This region shares homology with several human chromosomal regions associated with human deafness loci, so that the isolation of the *Tlc* gene might lead to the discovery of a human deafness gene. In any event, the morphological characterization of *Tlc* revealed a novel mouse mutation affecting hair cell survival and stereocilia bundle development, demonstrating the power of ENU to generate inportant auditory phenotypes.

SUMMARY

We have shown here several examples of how hearing and vestibular impaired mouse mutants are generated and the insight that they provide in the study of auditory and vestibular function. These types of genetic studies may also lead to the identification of disease-susceptibility genes, perhaps the most critical element in presbyacusis (age-related hearing loss). Some individuals may be more prone to hearing loss with increasing age or upon exposure to severe noise, and susceptibility genes may be involved. Different inbred mice show a variety of age-related and noise-induced hearing loss that varies between normal hearing and severe deafness throughout their life span /27/. Genetic diversity between inbred mouse strains has been shown to be a powerful tool for the discovery of modifier genes. Already two studies have found regions in which these genes may reside /28-29/. Future studies will hopefully lead to the identification of genes that modify hearing loss and will help us understand the variability that exists in human hearing, a crucial component in developing successful treatment strategies.

The first human non-syndromic deafness-causing gene was identified in 1995, and since then, additional genes have been discovered. Much of the credit for this boom is due to deaf and vestibular mouse mutants. Their study has led to great insight regarding the development and function of the mammalian inner ear, and correlations with human deafness can now be made since mutations in the same genes have been found in these two mammals. As deafness is the most common form of sensory impairment and affects individuals of all ages, elucidating the function of the auditory and vestibular systems through genetic approaches is essential in inproving and designing effective treatments for hearing loss.

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