Mouse Tales from Kresge:
The Deafness Mouse

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Abstract

Mouse models for human deafness have not only proven instrumental in the identification of genes for hereditary hearing loss, but are excellent model systems in which to examine gene function as well as the resulting pathophysiology. One mouse model for human nonsyndromic deafness is the *deafness* (*dn*) mouse, a spontaneous mutation in the *curly-tail* (*ct*) stock. The *dn* gene is on mouse Chromosome 19 and it was recently shown to be a novel gene called *Tmc1*. A mutation in *Tmc1* is also found in *Beethoven* (*Bth*), which is another deaf mouse mutant. In humans, one autosomal dominant form of nonsyndromic hearing loss (DFNA36) and two autosomal recessive forms (DFNB7 and DFNB11) are associated with mutations in *TMC1*, the human homologue of *Tmc1*. The transmembrane protein encoded by this gene is required for normal cochlear hair cell function and the mouse models will facilitate the elucidation of the molecular pathway that is disrupted when mutations are present.

Key Words: Nonsyndromic hereditary hearing loss, *dn* mouse, *Bth* mouse, *Tmc1* gene

Abbreviations: *dn*=deafness mouse, *ct*=curly-tail mouse, DFNA36=human autosomal dominant forms of nonsyndromic hearing loss, DFNB7 and DFNB11=two autosomal recessive forms of nonsyndromic hearing loss, *Bth*=mouse mutant, *Beethoven*, *Tmc1*=novel mouse gene that encodes a transmembrane protein, *TMC1*=novel human gene that encodes a transmembrane protein (human equivalent of *Tmc1*), *dn/dn*=homozygous deafness in *dn* mouse, *df/df*=deaf mouse observed by Charles Berlin, +/-=homozygous hearing mouse, +/dn=heterozygous hearing mouse, ABR=auditory brainstem response, DPOAEs=distortion-product otoacoustic emissions, CBA/J=an inbred mouse strain, DNA=deoxyribonucleic acid—the molecular basis of heredity in many organisms, RNA=ribonucleic acid—associated with the control of cellular chemical activities, BAC=bacterial artificial chromosome, FISH=fluorescent in situ hybridization, zrt216, Tmem2, Bem46=genes that are cochlear-expressed and in the same chromosomal region as the *dn* gene (but ruled out as the *dn* gene), ENU=N-ethyl-N-nitrosourea mutagenesis program, *Bth/Bth*=homozygous deafness in *Beethoven* mouse, *Bth/+*=heterozygous deafness in *Beethoven* mouse, tblASTx=computer program that allows comparison of nucleotide or protein sequences, cDNA=complementary DNA—DNA synthesized from an RNA template, using reverse transcriptase, RACE="Rapid amplification of cDNA ends," a method by which full-length cDNA clones can be generated from knowledge of a small portion of coding sequence, M412K=methionine changed to a lysine at amino acid number 412
HISTOLOGY AND ELECTROPHYSIOLOGY OF THE DEAFNESS MOUSE

In 1958 Deol and Kocher reported a naturally occurring deaf mouse in the curly-tail (ct) stock. While many mouse models of deafness exhibit aberrant movement behaviors such as circling, homozygous deafness (dn/dn) mice do not appear to have vestibular dysfunction. Breeding experiments suggested an autosomal recessive pattern of inheritance for the deafness phenotype, and histological studies showed degeneration of the organ of Corti, stria vascularis, and occasionally the saccular macula by ten days after birth. Extensive electrophysiological testing of deafness mice demonstrated that they have no cochlear microphonics, no VIII nerve compound action potential, and consequently they do not produce an auditory brainstem response (Steel and Bock, 1980). Also, as might be predicted, they have no spontaneous activity of cochlear origin (Durham et al., 1989), and they do not generate otoacoustic emissions (Horner et al., 1985). However, the endocochlear...
potential develops normally, and they do have preserved central auditory function upon direct electrical stimulation of the cochlear nerve (Bock et al., 1982; Frank et al., 1983). All of these findings point to a primary cochlear defect. Similar results were observed by Dr. Charles Berlin in his early work at Kresge on deaf mice referred to as df/df mice (Berlin et al., 1969). It is likely that these were in fact deafness (dn/dn) mice.

Following the original histological studies, Bock and Steel (1983) reported abnormalities of the extracellular spaces of the organ of Corti as well as loss of inner and outer hair cells by 15–20 days after birth, and using electron microscopy, Pujol and colleagues (1983) demonstrated that ultra-structural abnormalities of the inner hair cells and spiral ganglion cells are present at birth. Transmission electron microscopy studies showed shrunken and distorted inner and outer hair cells with vacuoles present in the cytoplasm, while scanning electron microscopy detected irregular arrangements and loss of most stereocilia (Bock and Steel, 1983). The dramatic changes in the hair cell structure and the abnormal stereocilia support the electrophysiologic findings in the dn/dn mice.

Dr. Douglas Webster began his studies of the deafness mouse at Kresge in the late 1970s. He meticulously maintained inbred colonies of dn/dn and +/+ mice that were homozygous throughout their genomes, and he performed detailed and extensive comparisons of homozygous deaf (dn/dn) versus hearing (both homozygous [+/+] and heterozygous [+/dn]) mice. His histological experiments demonstrated that the dorsal and ventral cochlear nuclei of the auditory brainstem have smaller nuclear volumes and smaller cells in dn/dn mice than in the hearing mice (Webster, 1985). Another study measured the ABR in +/+ and +/dn mice and concluded that hearing thresholds are not significantly different (Kirsch et al., 1993). Huang and colleagues (1998) extended these studies at Kresge to examine distortion product otoacoustic emissions (DPOAEs) and suggested that there may be subtle differences between +/+ and +/dn mice. DPOAEs were also measured in +/dn mice that were the F1 hybrid offspring of dn/dn mice mated with Mus Musculus molossinus and CBA/J mice. In both cases, the hybrids had significantly larger DPOAE amplitudes than the hearing parental strains (Huang et al., 1996), an effect that is probably the result of the heterozygous genetic background in the hybrids.

One of the most intriguing findings by Dr. Webster was his observation of regeneration of non-hair cells in the organ of Corti of dn/dn mice between 45 and 90 days of age (Webster, 1992). He examined 45-day-old mice and found complete degeneration of the outer hair cells and no distinguishable cell types in the organ of Corti. However, identical experiments on 90-day-old mice showed evidence of regeneration of support cells, but not hair cells, in the apical turn. Faddis et al. (1998) were not able to confirm this finding, but the genetic backgrounds for their mice were quite heterogeneous and not consistent with the dn/dn genotype. These genetic differences probably explain why they could not replicate the findings of Webster (1992).

**THE ROLE OF MOUSE MODELS IN GENETICS**

The mouse has significant DNA sequence similarity to humans. This similarity extends not only to the sequences of the genome that encode proteins but also to regulatory sequences, DNA binding domains, and gene order and position. Regions of the mouse genome that contain a collection of specific genes often have a corresponding region in the human genome where the order of the genes is preserved. Thus, knowing the location of a gene in the mouse can often provide information about the location of the related human gene as well as surrounding genes.

Another important advantage of the mouse is the ability to study the RNA and protein expression in different tissues and at a range of developmental time points in both normal and mutant mice. While certain tissues in humans are easily accessible, such as peripheral blood, most tissues are only available as postmortem samples, and it becomes difficult to study the pathology in these samples. However, in mice, not only is the tissue accessible but we have the ability to see the disease progression at different developmental stages. By examining the histological pathology as well as the temporal and tissue specific expression of a gene, we gain important insight into the normal function of the encoded protein and its dysfunction when abnormal or missing.
Over 70 different genetic loci for human nonsyndromic hereditary deafness have been mapped (http://www.uia.ac.be/dnalab/hhh), highlighting the complexity of the auditory system. Similarly, studies of deaf mice have localized more than 25 genes within the mouse genome (Steel, 1995). Many of these deaf mice provide models for human deafness conditions (http://www.jax.org/research/hhim/documents/models.html).

GENETIC STUDIES OF THE DEAFNESS MOUSE

We began our efforts to identify the dn gene by setting up matings between dn/dn mice and a genetically diverse subspecies, Mus musculus molossinus. The heterozygous mice produced from these matings were backcrossed to dn/dn mice, and the backcross offspring were classified as sound-responsive or deaf using the Preyer reflex, ABR and DPOAE (Huang et al., 1995). DNA was then extracted from the kidneys of these offspring and genotyped for microsatellite markers spanning the mouse genome. Linkage analysis of the genotype and phenotype data localized the dn gene to mouse Chromosome 19 and was consistent with the presence of a chromosomal inversion in the region (Keats et al., 1995). The construction of a bacterial artificial chromosome (BAC) physical map of this interval identified probes for fluorescent in situ hybridization (FISH) experiments that provided additional evidence for a large chromosomal rearrangement, likely to be an inversion (Vinas et al., 1998). While we hypothesized that the inversion interrupted the dn gene sequence, thereby causing the deafness phenotype, it is possible that this is a neutral rearrangement in disequilibrium with the dn mutation, or that there is a position effect at a distance from the inversion that impacts the dn gene expression.

The dn region of mouse Chromosome 19 shows homology to human chromosome 9q13-q21, a region that contains two autosomal recessive nonsyndromic hearing loss genes, DFNB7 (Jain et al., 1995) and DFNB11 (Scott et al., 1996), and an autosomal dominant nonsyndromic hearing loss gene, DFNA36 (Kurima et al., 2002). Also, another mouse mutant, Beethoven (Bth), was mapped to the same region of mouse Chromosome 19 as dn (Vreugde et al., 2002). Several candidate genes that were cochlear-expressed and in the region, including znf216, Tmem2, and Bem46, were characterized and ruled out as the gene in both the mouse mutants and the three human forms of deafness (Scott et al., 1998, 2000). Recently, mutations in a novel gene (Tmc1) that encodes a transmembrane protein (Kurima et al., 2002; Vreugde et al., 2002) were found to be associated with dn, Bth, DFNB7, DFNB11, and DFNA36, providing convincing evidence that Tmc1 is the causative gene.

THE BTH MOUSE

Unlike the dn mouse, the semi-dominant Bth mouse arose in a large-scale ENU (N-ethyl-N-nitrosourea) mutagenesis program. This approach is most likely to cause subtle DNA alterations and usually results in the detection of dominant or semidominant phenotypes. Standard mouse backcross matings localized the Bth mutation to a region of Chromosome 19 that overlaps with the dn critical interval. The homozygous (Bth/Bth) mice never show a Preyer reflex and have complete degeneration of inner hair cells by postnatal day 30, and the heterozygous (Bth/+) mice show progressive loss of the Preyer reflex beginning at about 30 days after birth. Histologically they appear to have grossly normal middle and inner ear structures, and scanning electron microscopy revealed progressive hair cell degeneration from about day 20, with greater loss of inner hair cells than outer hair cells. The stereocilia of the remaining hair cells appear normal. Whole-cell patch clamp hair cell analysis indicated that before degeneration begins, individual hair cells are able to generate normal transducer and basolateral calcium and potassium currents (Vreugde et al., 2002).

Tmc1

The human gene, TMC1, was identified as a candidate gene for DFNA36, DFNB7, and DFNB11 using direct sequence analysis of the human critical interval and the tBLASTx algorithm (www.ncbi.org). The entire cDNA was then amplified using a combination of cDNA libraries from fetal brain and human cochlea with 5' and 3' rapid amplification of cDNA ends (RACE) experiments. The corresponding mouse
cDNA sequence was determined in a similar fashion from a mouse inner ear cDNA library. While the human sequence contains 24 exons, with four upstream non-coding exons, the mouse sequence consists of 20 exons with only one upstream non-coding exon (Kurima et al., 2002).

Analysis of this gene demonstrated that it is expressed in fetal cochlear and inner ear tissue and in testis and placenta; no evidence of expression in any other tissues was found. Developmental analysis of Tmc1 in mice indicated that it is expressed embryonically at low static levels with increased expression during the early postnatal period. Tmc1 expression in the cochlea is localized to the inner and outer hair cells as well as the neurosensory epithelial cells in the vestibular apparatus. Sequence analysis of the predicted protein structure detects the presence of transmembrane domains suggesting that Tmc1 may be a channel or ion transporter (Kurima et al., 2002; Vreugde et al., 2002).

Mutation analysis has revealed multiple different mutations, including deletions and point mutations, in the different human deafness conditions. While the specific mutations in affected individuals fail to assist in the determination of the putative function of the protein, possible insight may be gained through the examination of the altered physiology in the dn and Bth mice.

Sequence analysis of Tmc1 in the dn/dn mice revealed a 1.6 kb genomic deletion that encompasses exon 14 and parts of introns 13 and 14. Analysis of Tmc1 cDNA confirmed an inframe deletion of exon 14 without alterations in other parts of the cDNA sequence or any quantitative changes in RNA level. The Bth mutation is a point mutation in exon 13 that changes a methionine to a lysine (M412K). This residue is located in a putative transmembrane domain, and the hydrophobic lysine would likely disrupt protein folding. The histological information from these mouse models suggests hypotheses about the function of Tmc1. As both models initially develop a normal configuration of hair cells and inner ear structures, but suffer early postnatal degeneration of the hair cells, it seems plausible that Tmc1 is required for the maintenance of the hair cells but not for their development and organization. Further experiments, such as investigating normal and mutant Tmc1 expression in Xenopus oocytes, may provide key information for understanding the function of this protein as well as the impact of the mutations (Vreugde et al., 2002). It will be exciting to determine the function of Tmc1 in the cochlea and potentially unravel its role in the survival of hair cells.

CONCLUSIONS AND FUTURE RESEARCH

While we still do not have a clear idea of the function of the protein encoded by Tmc1, important information has been gained by identifying the mutations in both the dn and the Bth mouse. Using these mouse models, we have shown that Tmc1 is expressed in the hair cells early in development and is increased in expression about the time that hearing first develops. However, many questions remain: For example, (1) Is Tmc1 expressed in the progenitor cells that give rise to both the hair cells and the support cells, or is its expression limited to the differentiated cells? (2) As there is an increase in Tmc1 expression at around postnatal day one, do histological changes occur at that stage in development? (3) What are the triggers for the increase of Tmc1 expression? (4) Are there alterations of Tmc1 expression levels in dn and Bth mice at different stages of development? (5) Are there changes in the expression of other genes that would suggest interactions with Tmc1? As we seek to answer these questions using the mouse models, we will discover important information about DFNA36 and DFNB7/11 human deafness conditions.

Mouse models for human hereditary deafness have proven to be extremely useful, not only in gene identification strategies and mapping but, perhaps more importantly, in allowing a thorough evaluation of the physiologic impact of these genes on the auditory system. Animal models allow characterization of gene structure and function at a level inaccessible in humans. They provide important clues not only for understanding the role of the protein in the inner ear but also will assist in the designing of effective therapies for affected individuals.

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REFERENCES


