Cochlear Mechanics

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Material Properties of the Tectorial Membrane

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Introduction

The tectorial membrane (TM) is a gelatinous structure that lies on top of the mechanically sensitive hair bundles of sensory cells in the inner ear. From its position alone, we know that the TM must play a key role in transforming sounds into the deflections of hair bundles. But the mechanisms are not clear, largely because the TM has proved to be a difficult target of study. It is 97% water, and is therefore fragile. It is small: the whole TM would roll up and fit into little more than an inch of one human hair. Finally, it is transparent.

We have developed methods to isolate the TM so that its properties can be studied. Results using this technique (reported in previous RLE Progress Reports) show that the TM behaves as a gel. The material properties of a gel are a direct consequence of its molecular architecture. Charge groups on gel macromolecules attract mobile counterions from the surrounding fluid. Thus gels concentrate ions, and thereby increase osmotic pressure. The increase in osmotic pressure induces water to move into the gel and cause it to swell. The swelling stretches the macromolecules and increases the stiffness of the gel. The important consequence is that gels have mechanical, electrical, osmotic, and chemical behaviors that are all linked by their common molecular basis. We are exploring these linkages by investigating the effect of genetic mutations on material properties of the TM.

TECTA Mutation Decreases the Shear Impedance of the Tectorial Membrane

The shear impedance of the tectorial membrane (TM) plays a critical role in the interaction between the TM and the hair bundles. To determine whether a change in shear impedance is the underlying cause of the 50–80 dB hearing loss associated with a Y1870C missense mutation in TECTA (Legan et al. 2000, Lukashkin et al. 2004), we measured the shear impedance of TMs from normal and mutant mice using a microfabricated shearing probe (Gu et al. 2005). The frequency range probed was 10–800 Hz in the radial direction and 10–9000 Hz in the longitudinal direction. The magnitude of radial shear impedance fell with a slope of −16 dB/decade over the measured frequency range for TMs from both normal and mutant mice (figure 1). The phase was near −80 degrees for both groups. However, the overall magnitude was about 4–8 dB lower for TMs from mutant mice. Results for longitudinal shear impedance were similar except that the slope fell with a slope of −19 dB/decade. These results show that one effect of the mutation is to alter the viscous and elastic components of TM shear impedance in equal proportion. Moreover, the results provide important constraints on the role of the TM in cochlear mechanics, suggesting two possible interpretations: The first is that this small change in shear impedance is sufficient to cause a 50−80 dB hearing loss, which implies that the shearing response of the TM is intimately involved in cochlear amplification. The second is that the hearing loss seen in the TECTA mutant is due to something other than a change in shear impedance, which points to the possibility of a second role for the TM in cochlear mechanics.

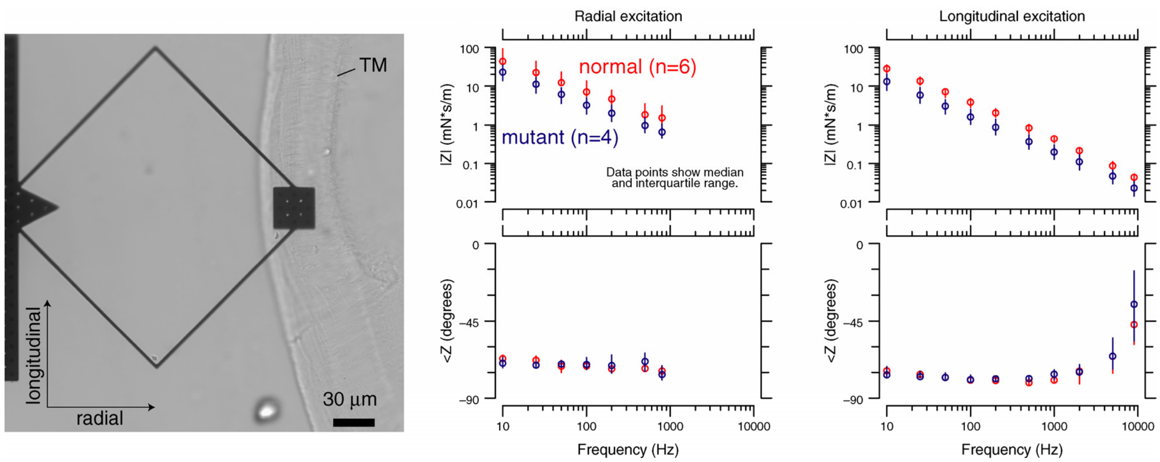

Figure 1. A microfabricated probe is placed on the TM (left). Forces applied to the probe base are coupled to the shearing plate by the springlike cantilever arms. Relative deflections of the base and shearing plate depend on the impedance of the TM and cantilever arms. The impedance of the TM has both viscous and elastic components that are proportional over a wide frequency range for both radial (center) and longitudinal (right) forces. A mutation in TECTA causes the shear impedance of the TM to fall by 4−8 dB in both directions.

Mutation in TECTA Alters the Fixed Charge Concentration of the Tectorial Membrane

The tectorial membrane (TM) contains an abundance of charged macromolecules which are likely to contribute to the mechanical properties of the TM, as they do in similar tissues such as cartilage. To investigate the role of fixed charge, we excised TMs from a mouse model of a missense mutation in Tecta (a gene that encodes alpha-tectorin in the TM), which causes 50-80 dB hearing loss (Legan et al., 2005). We measured the fixed charge concentration (cf) of these TMs using a novel microfabricated planar patch clamp technique (Ghaffari & Freeman, 2005; Sigworth & Klemic, 2002). We found that the cf in mutant TMs was around 2.13±0.15 mmol/L, which is significantly smaller than the cf measured in normal mice (7.80±0.52 mmol/L). Based on a continuum model that relates electrostatic repulsion of fixed charge to mechanical stiffness (Ghaffari & Freeman, 2005), we estimate that 2.13 mmol/L of negative fixed charge will contribute 0.026 kPa to the TM bulk modulus in Tecta mice. This is approximately a 25-fold reduction in the contribution of fixed charge to TM's bulk modulus in Tecta mice compared to the normal mice. Therefore, a decrease in the fixed charge concentration can reduce the TM's compressive mechanical rigidity and is a candidate for the functional basis of hearing loss in Tecta mice.

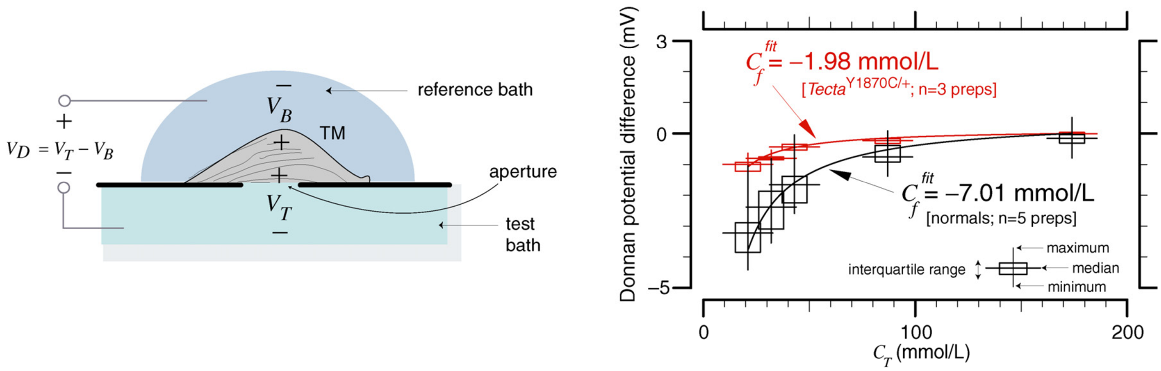

Figure 2. The TM is placed over a microfabricated hole separating dissimilar fluids (left). A Donnan potential is established between the TM and each fluid, so that the voltage between baths varies with bath composition and the concentration of fixed charge within the TM. A plot of voltage vs. bath ion concentration (right) can be fit with this theoretical relation to estimate the fixed charge concentration of the TM. These results imply that the fixed charge concentration of TMs from TECTA mutants is only 28% of that of wild-types.

Mechanical Properties of the Cochlea

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Introduction

The cochlea is responsible for turning the mechanical vibrations of sound into neural signals that are sent to the brain. This process starts with a traveling wave of vibration along the basilar membrane and ends with the deflection of stereocilia of hair cells. Along the way this traveling wave is amplified, and the sound signal is separated into individual frequency components. The mechanical properties of the cochlea that give rise to these complex motions are highly nonlinear and poorly understood. Understanding these properties more thoroughly will lead to a better understanding of hearing in general, including the development of more effective hearing aids and other assistive listening devices.

An Isolated Gerbil Cochlea Preparation for Measuring Sound-Induced Micromechanical Motions

The mechanical processes at work within the organ of Corti can be greatly elucidated by measuring both radial motions and traveling-wave behavior of structures within this organ in response to sound stimuli. To enable such measurements, we have developed a new preparation for observing three-dimensional motions of micromechanical structures in the apical region of an isolated gerbil cochlea. The cochlea is submerged in a low-chloride, low-calcium artificial perilymph solution and cemented to the bottom of a petri dish at an angle. The bone above scala vestibuli of one half of the apical turn is removed to allow optical imaging with a 40x, 0.8 NA water-immersion objective. Reissner's membrane is left intact. Illumination is provided with a blue LED coupled to an optical fiber. The fiber is positioned next to the bone surrounding scala tympani of the apical turn, so that the organ of Corti is illuminated from below. The resulting optical access allows imaging of a variety of structures that have been proposed to play a role in cochlear mechanics, including inner and outer hair cell bundles, the tectorial membrane, Deiters cells, inner and outer pillar cells, and efferent fibers in the tunnel of Corti. In some preparations, individual stereocilia of inner hair cell bundles can be resolved. Motions are stimulated by driving the stapes with a piezoelectric probe, and are measured using a computer microvision system. This system combines video microscopy, stroboscopic illumination, and computer vision to enable measurement of nanometer-scale motions of cochlear structures at audio frequencies. Initial measurements demonstrate that a variety of cochlear structures move relative to one another, suggesting that the cochlea exhibits multiple modes of motion. Moreover, the measurements have revealed a previously unknown and prominent longitudinal component of motion. Fibers that cross the fluid-filled tunnel of Corti exhibit a phase lead relative to other cochlear structures at the same longitudinal position. These measurements provide support for the idea that the longitudinal flow of fluid in intra-cochlear space may propagate energy along the cochlea and thus contribute to cochlear tuning (Hubbard et al, 2000).

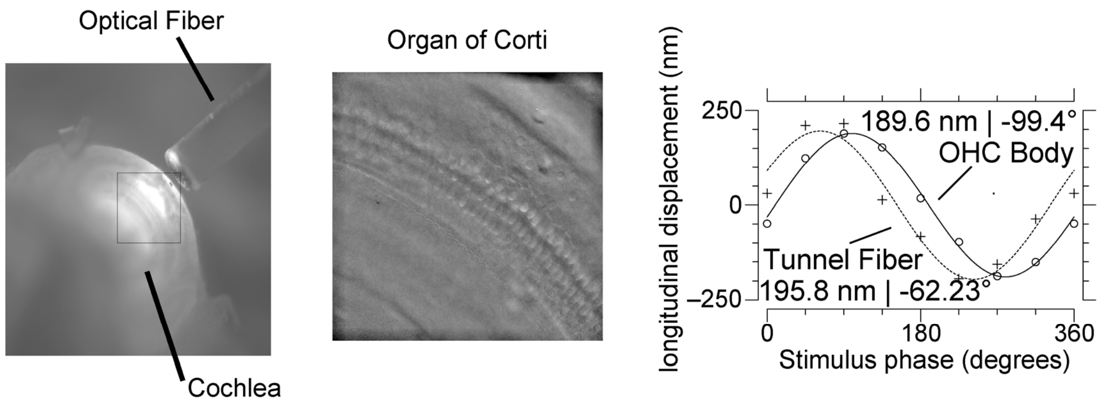

Figure 3. A gerbil cochlea is isolated and illuminated with an LED attached to an optical fiber (left). Sound stimuli are delivered with a displacement source attached to the stapes (not shown). A high-magnification objective allows imaging of cellular structures along a length of the cochlea (center). Strobing the LED at the frequency of the sound stimulus yields a series of stop-action images from which motions can be measured. The resulting measurements reveal longitudinal motion of the organ (right). Motion of fibers in the fluid-filled tunnel of Corti leads that of the bodies of outer hair cells.

Images and Motion Measurements of the Intact Mammalian Cochlea Using Doppler Optical Coherence Microscopy

Three-dimensional images and motion measurements have been obtained in an in vitro preparation of the gerbil cochlea using Doppler optical coherence microscopy (DOCM). The high optical sensitivity and coherence-gating capability of DOCM enable images and motion measurements to be obtained without the use of contrast agents and without wide optical access. The images possess sufficient dynamic range to image transparent structures such as the tectorial membrane and sufficient spatial resolution to image cellular structures such as outer hair cells. In addition, every pixel in the images yields a motion measurement comparable to that obtained using laser Doppler velocimetry. Consequently, motion measurements using DOCM are not restricted to reflective surfaces such as the basilar membrane and reticular lamina; the motions of all visible structures in the cochlea are measured including the tectorial membrane, tunnel of Corti, and outer hair cells.

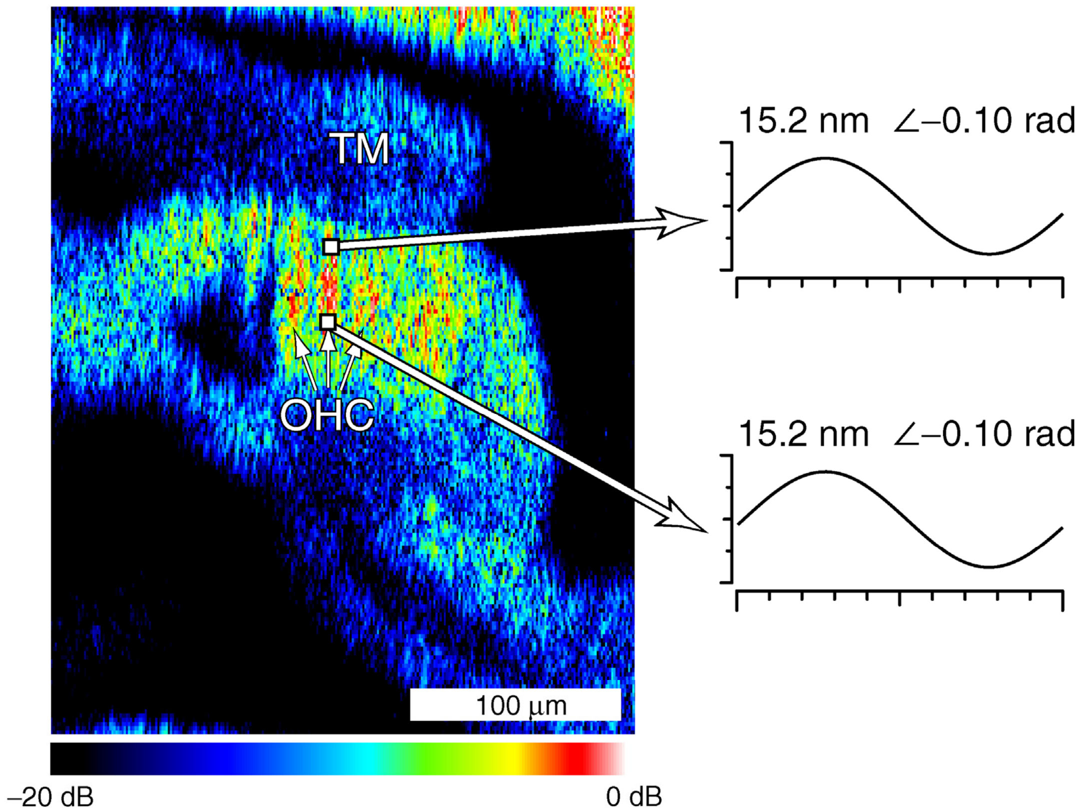

Figure 4. An image of a fixed gerbil cochlea taken with the DOCM system reveals a variety of structures, including the tectorial membrane (TM) and outer hair cells (OHC), with reflectivity mapped logarithmically to color (left). The DOCM system provides independent measurements of the transverse component of motion of each point in the image. In response to transverse motion of the entire cochlea, displacement of the apical and basal poles of one OHC had the same magnitude and phase, as expected for fixed tissue.

Lensless focusing with subwavelength resolution by direct synthesis of the angular spectrum

Imaging of microscopic structures is typically done with refractive optics, but there are many situations for which such optical elements are impractical or impossible to use. We have developed a method for the coherent superposition of unfocused wavefronts for lensless focusing of electromagnetic waves with subwavelength resolution. Near the focal point, intensity distributions generated using the approach approximate those generated using lenses. Far from the focal point, discretization of spatial frequencies results in a trade-off between the number of wavefronts and the accuracy of the approximation. We experimentally demonstrated the feasibility of the approach by generating an approximation of an azimuthally-polarized Bessel beam with a focal spot diameter of 0.37λ (FWHM).

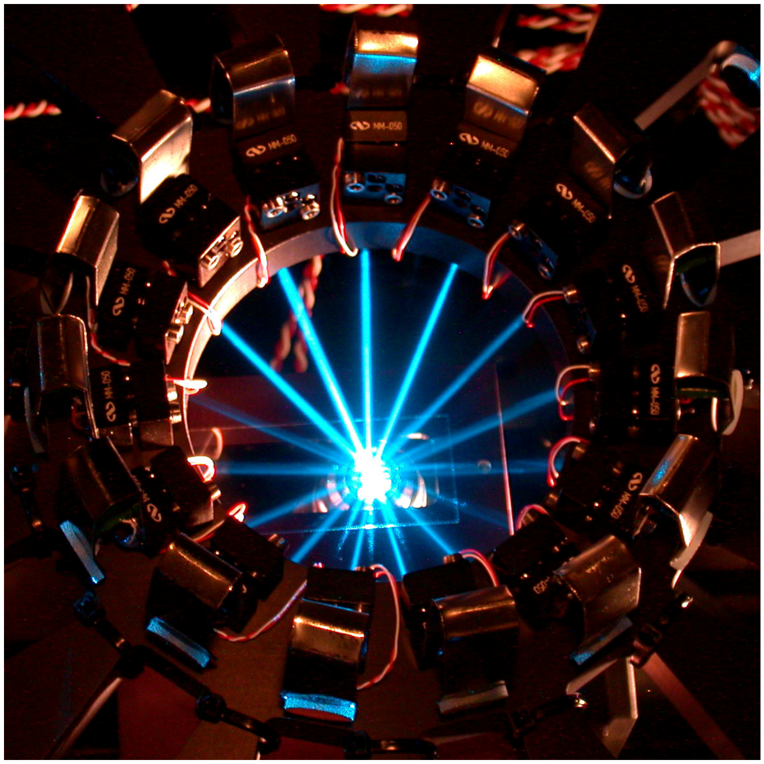

Figure 5. A single laser beam is split into fifteen individual beams which are directed into a cone-shaped pattern to recombine at a target. The resulting interference pattern generates a Bessel beam which approximates the point spread function of a lens with a high numerical aperture. Unlike the lens approach, this technique uses no refractive optics and should be scaleable to a wider range of the electromagnetic spectrum.

Publication

Meeting Papers Published

Gu, J., K. Masaki, G. P. Richardson, and D. M. Freeman, "TECTA mutation decreases the shear impedance of the tectorial membrane," poster at *Twenty-Ninth Midwinter Research Meeting of the Assoc. for Research in Otolaryngology*, February 2006.

Ghaffari, R., K. Masaki, G. P. Richardson, and D. M. Freeman, "Mutation in TECTA alters the fixed charge concentration of the tectorial membrane," poster at *Twenty-Ninth Midwinter Research Meeting of the Assoc. for Research in Otolaryngology*, February 2006.

Page, S., A. J. Aranyosi, and D. M. Freeman, "An isolated gerbil cochlea preparation for measuring sound-induced micromechanical motions," poster at *Twenty-Ninth Midwinter Research Meeting of the Assoc. for Research in Otolaryngology*, February 2006.

Hong, S. and D. M. Freeman, "Images and motion measurements of the intact mammalian cochlea using Doppler optical coherence microscopy," podium presentation at *Twenty-Ninth Midwinter Research Meeting of the Assoc. for Research in Otolaryngology*, February 2006.

Masaki, K., D. M. Freeman, G. P. Richardson, and R. Smith, "Comparing the equilibrium stress/strain relations of tectorial membranes from TectaY1870C/+ and Col11a2 -/- mouse mutants," podium presentation at the Ninth International Mechanics of Hearing Workshop, July 2005.

Aranyosi, A.J., "A 'twin-engine' model of level-dependent cochlear motion," poster at the Ninth International Mechanics of Hearing Workshop, July 2005.

Bergevin, C., D. M. Freeman, and C. A. Shera, "Comparing otoacoustic emissions in the gecko and the human," poster at the Ninth International Mechanics of Hearing Workshop, July 2005.

Papers Published

Aranyosi, A.J. and D. M. Freeman (2005), "Two modes of motion of the alligator lizard cochlea: measurements and model predictions," *J Acoust Soc Am* **118**(3), 1585-1592.

Hong, S. S., B. K. P. Horn, D. M. Freeman, and M. S. Mermelstein, "Lensless focusing with subwavelength resolution by direct synthesis of the angular spectrum," *Appl Phys Lett*, in press.

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Ryu, J., S. S. Hong, B. K. P. Horn, D. M. Freeman, and M. S. Mermelstein (2006), "Multi-beam interferometric illumination as the primary source of resolution in optical microscopy," *Appl Phys Lett* **88**, 171112.

Undergraduate Projects

Scott, Mark, "Design of a MEMS transducer for use on the tectorial membrane," Undergraduate Research Opportunity (UROP) in EECS, June 2006.

Masters Theses

Page, Scott, "Sound-induced micromechanical motions in an isolated cochlea preparation," M.S. in EECS, June 2006.

Doctoral Theses

Masaki, Kinuko, "Measuring material properties of tectorial membranes from normal and genetically modified mice," Ph.D. in HST, June 2006.