Session B:

Mechanics I

Chairpersons: J. Ashmore
J. Santos-Sacchi

O8 Advanced optical techniques for investigating the outer hair cell protein Prestin

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Prestin, a transmembrane protein, confers electromotility to cochlear outer hair cells (OHCs) by converting changes in membrane potential into mechanical forces manifested as axial length changes. Although the complete mechanism of electromotility is not presently known, this somatic deformation is responsible for the remarkable sensitivity and frequency selectivity of the mammalian auditory system. In order to characterize the electromotile process and its role in OHC function, a complete understanding of the constituents of the plasma membrane, both lipids and proteins, as well as their interactions, is crucial. Unfortunately, ex vivo, OHCs have a short lifetime making it necessary to study prestin in cellular model systems such as human embryonic kidney cells (HEKs). We have implemented fluorescence recovery after photobleaching (FRAP) to evaluate the lateral diffusion of prestin and voltage-sensitive dyes in HEK cells. Mobility data provides information on the extent of interaction between membrane components and the effect of the membrane environment on prestin. We are also utilizing fluorescence resonance energy transfer (FRET) to assay the degree of prestin self-association. Preliminary FRET experiments reveal significant prestin-prestin interactions, consistent with the gradual recovery observed in FRAP experiments. The use of these two advanced techniques in tandem allows for more sophisticated and quantitative studies of the activity of prestin and the mechanism of electromotility than through use of each technique alone.

O9 Developmental expression of the outer hair cell motor in the mouse

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The development of motor protein activity in the lateral membrane of the mouse outer hair cell (OHC) from P5 to P18 was investigated under whole cell voltage clamp. Voltage-dependent, non-linear capacitance (Cv), which represents the conformational fluctuations of the motor molecule, progressively increased during development. At P12, the onset of hearing in the mouse, Cv was about 70 % of the mature level. Cv saturated at P18 when hearing shows full maturation. On the other hand, C_{lin}, which represents the membrane area of the OHC, showed a relatively small increase with development, reaching steady-state at P10. This early maturation of linear capacitance is further supported by morphologic estimates of surface area during development. These results, in light of recent prestin knockout experiments, show that rather than the incorporation of new motors into the lateral membrane after P10, molecular motors mature to augment nonlinear capacitance. Thus, current estimates of motor protein density based on charge movement may be exaggerated. A corresponding indicator of motor maturation, the motor's operating voltage midpoint, V_{pkcm}, tended to shift to depolarized potentials during postnatal development, although it was unstable prior to P10. However, after P14, V_{pkcm} reached a steady state level near –67 mV, suggesting that intrinsic membrane tension or intracellular chloride, each of which can modulate V_{pkcm}, may mature at P14. These developmental data significantly alter our understanding of the cellular mechanisms that control cochlear amplification.

O10 Prestin is an oligomer

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Introduction: Prestin has 744 amino acids and provides the molecular basis for outer hair cell (OHC) electromotility, which is crucial for sensitivity and frequency selectivity in the mammalian cochlea. It is thought that these abundantly expressed motor proteins (prestin) constitute the 11-nm particles present in the OHC's lateral membrane. Because the estimated size of a prestin monomer is too small to form an 11-nm particle, the possibility of prestin's oligomerization is studied.

Materials and Methods: Prestin cDNA was transfected into different expression systems including yeast and mammalian cell lines. Prestin protein was studied in LDS-PAGE/Western blot or PFO-PAGE/Western blot. Various reducing reagents including β-mercaptoethanol, dithiothreitol and ethanedithiol, different detergents, 6 M urea and chemical cross-linking reagents were used to treat prestin-expressing cells.

Results and Conclusion: Two bands were consistently found in different host cells including prestin-expressing OHCs and prestin-expressing yeast in LDS-PAGE/Western blot. The size of the higher molecular-weight band is about twice that of the lower prestin band. These data imply that prestin may exist as a dimer or multimer. Prestin dimer is resistant to various reagents, detergents and 6 M urea, indicating that prestin dimer is very stable structure. We also used PFO (perfluo octanoiate) to study prestin's oligomerization. PFO was reported as a "biological detergent," less disruptive of interactions within protein oligomers, and thus permitting molecular mass determination of multimeric proteins. In prestin-transfected yeast and mammalian cell lines, four prestin bands were consistently observed in PFO-PAGE/Western blots. These bands probably correspond to prestin's monomer, dimer, trimer and tetramer. Finally, by using chemical cross-linking reagents, we demonstrated that prestin in OHCs is also present as an oligomer. [Supported by Grant DC00089, DC006412 and The Hugh Knowles Center].

O11 OHC-induced motion of the organ of Corti

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The somatic electromotility of the outer hair cells (OHCs) plays an essential role in amplification of the motion of the organ of Corti (OoC) (LIBERMAN *et al.*, 2002). Electromechanical force produced by the OHCs amplifies the motion of the OoC, which is somehow coupled to the hair bundle of the inner hair cells (IHCs). To investigate the role of OHC somatic electromotility, we developed an *in vitro* preparation in which the electrically induced vibration pattern of the reticular lamina (RL) and tectorial membrane (TM), upper and lower surface, could be measured.

In the guinea-pig cochlea (n = 81), a transepithelial electrical stimulus was applied with electrodes placed in *scala vestibuli* and *scala tympani*. The stimulus was multi-tone and contained 81 frequencies (480 Hz - 70 kHz) with equal amplitude but random phase. Transversal motion was measured with a laser Doppler vibrometer, at three different places along the cochlea (characteristic frequency 0.8, 3 and 24 kHz).

A complex vibration pattern of the RL was found. The RL motion exhibited two pivot points - one at the pillar cells and the other at the Hensen´s cells. In contrast, TM motion was in-phase along its entire lower and upper surfaces. This leads to counterphasic motion of the RL and TM above the IHCs. This motion causes radial fluid motion inside the subtectorial space, which is theoretically capable of deflecting the IHC stereovilli.

The experiments showed an additional possibility of OHC-induced amplification - fluid motion in the subtectorial space which will add vectorially to the travelling wave-induced shearing motion between RL and TM.

Lieberman,M.C., Gao,J., He,D.Z., Wu,X., Jial,S., Zuo,J. Nature 419, 300-304 (2002).

O12 A mathematical model of the regulation of OHC basolateral permeability and transducer operating point

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The cochlea presumably possesses a number of regulatory mechanisms to maintain cochlear sensitivity in the face of disturbances to its function. Evidence for such mechanisms can be found in the time-course of the recovery of CAP thresholds during experimental manipulations, and in observations of slow oscillations in cochlear micromechanics following exposure to LF tones (the 'bounce phenomenon') and other perturbations. To increase our understanding of the regulatory processes within the cochlea, and OHCs in particular, we have developed a mathematical model of the OHC that takes into account its known electrical properties, and includes the effect of fast and slow-motility of the cell body on transducer operating point and apical conductance. Central to the operation of the model is a putative intracellular 2nd-messenger system based on cytosolic Ca^{2+} concentration. Cytosolic Ca^{2+} is involved in regulation of i) the operating point of OHC MET channels via slow motility and axial stiffness; ii) the permeability of the basolateral wall to potassium via Ca^{2+}-sensitive potassium channels; and iii) the cytosolic concentration of Ca^{2+} itself, via extrusion from the OHC (via the Ca^{2+}-ATPases in the plasma membrane) and Ca^{2+}-induced Ca^{2+}-release (CICR) from intracellular Ca^{2+} storage organelles. The permeability of the OHC basolateral wall determines the standing current through the OHCs (and therefore a component of EP regulation), and in the presence of sound, affects the magnitude of the AC receptor potential that drives the prestin-mediated somatic electromotility and active gain. The mathematical model we have developed provides a physiologically-plausible and internally-consistent explanation for the time-courses of the cochlear changes observed during a number of different perturbations. We show how much of the oscillatory behaviour can be attributed to oscillations in cytosolic calcium concentration, and present results from the model for a number of simulations, including DC current injection into scala media, perilymphatic perfusions, and exposure to LF tones, and compare the results of these simulations to experimental data recorded from the guinea pig.

O13 NompC TRP channel is a true mechanotransducer channel in the *Drosophila* ear

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Introduction: Mechano-electrical transducer channels (METs) are gated in the straightest way possible: directly by the forces of the mechanical stimuli themselves. As a consequence, the unequivocal identification of METs requires that their direct mechanical gating is demonstrated in a native environment. Only then, true METs can be distinguished from downstream channels involved in electrical signal amplification.

Materials and Methods: Using a computer controlled scanning laser Doppler vibrometer we recorded the displacement response of the fly's antennal sound receiver to electrostatically applied external forces.

Results: The response of wild type receivers to force steps was complex. When actuated by a force, the receiver displayed an initial, pronounced movement in the direction of the force that was followed by a fast movement in the opposite direction and a subsequent slow excursion to a steady-state position. The initial peak in the receiver's displacement response scaled nonlinearly with the applied force and coincided with the phasic electrical nerve activity. Loss-of-function mutations in *nompC* virtually abolished both the mechanical nonlinearity and the nerve response in the range of forces analyzed.

Conclusion: Here, we show that *Drosophila* NOMPC, a transient-receptor-potential (TRP) channel reportedly required for the sensation of touch and sound (1,2), constitutes a true MET in the fly's antennal ear. We found that the mechanical gating of METs impacts on the mechanics of the ear. Including a gating related, nonlinear compliance and mechanical correlates of transducer adaptation this impact accords with a tethered mechanism of channel activation via gating-springs (3). These findings establish *Drosophila* NOMPC as the first true, mechanically gated transducer channel for hearing and show that the gating of this channel governs the mechanical performance of the entire ear.

(1) Walker, R. G. et al., *Science* **287**, 2229 –2234 (2000)
(2) Eberl, D. F. et al., *J. Neurosci.* **20**, 5981-5988 (2000)
(3) Howard, J. and Hudspeth, A. J., *Neuron* **1**, 189 –199 (1988).

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