

FAST TURNOVER OF STEREOCILIA TIP LINKS MEASURED WITH STABLE ISOTOPE RATIO IMAGING MASS SPECTROMETRY (MIMS)

Claude Lechene (cpl@harvard.edu)¹, Edmund Mroz², François Hillion³, Douglas Benson⁴

¹Harvard Medical School and Brigham and Women's Hospital, 65 Landsdowne Street, Room 535, Cambridge, MA, 02139, USA

²Massachusetts Eye Ear Infirmary, Eaton-Peabody Laboratory, 243 Charles Street, Boston, MA, 02114, USA

³Cameca, 103 blvd. St. Denis, Courbevoie, 92403, France

⁴NSee, Inc., 106 Greenhaven Lane, Cary, NC, 27511, USA

The study of molecular turnover of cochlear structure is essential to understanding both deafness and cochlear damage and repair. This is now possible using ¹⁵N as a molecular marker and the recently developed secondary ion mass spectrometer, which is able to measure several ion masses simultaneously and to provide high spatial resolution and high mass resolution at high transmission (Slodzian et al. 1992).

Mice were fed an experimental diet in which ¹⁵N-L-leucine replaced a fraction of L-leucine in the control diet. Cochlea were prepared with the method of Liberman (1987). Sections 0.5 mm thick were placed on silicon chips. They were analyzed with multi-isotope imaging mass spectrometry, using a cesium primary ion beam accelerated at 16kV that was focused at 35 nm and scanned over a 3- μ m field for a total of 256x256 pixels. Each species of secondary ion was detected with an electron multiplier. High mass resolution was obtained by adjusting deflection plates positioned just in front of each electron multiplier. Overall detection efficiencies were matched to within a few percent for all secondary ions. ¹²C⁻, ¹³C⁻, ¹²C¹⁴N⁻ and ¹²C¹⁵N⁻ (or ¹³C¹⁴N⁻) mass images were recorded in parallel. Ratio images ¹³C/¹²C and ¹²C¹⁵N/¹²C¹⁴N (or ¹³C¹⁴N/¹²C¹⁴N) were derived from the raw data. In order to show high dynamic range ratio images and to de-emphasize values resulting from data with few counts, a method of displaying the data as a hue-saturation-intensity transform was developed. These transformed images enable us to outline and quantitatively analyze regions of interest that were unsuspected based on the histological mass image. The percent nitrogen turnover was calculated for each region of interest as: 100*(Tissue ¹⁵N/¹⁴N ratio' - Control diet ratio)/(Experimental diet ratio -Control diet ratio).

We found that after 9 days of experimental diet, the tip links' ¹⁵N (protein) turnover was 57.7% (3.8% S.E., n=9) in an area approximately 500Åx500Å and was no more than 7.5% (1.7% S.E., n=9) in the stereocilia at a distance less than 2000 Å.

Such an unexpected finding has an important bearing on the gating of the mechano-transduction channels, which are the key for hearing.

References

- [1] M.C. Liberman, Chronic ultrastructural changes in acoustic trauma: serial-section reconstruction of stereocilia and cuticular plates. *Hear. Res.* 26(1) (1987), p.65-88.
- [2] G. Slodzian, B. Daigne, F. Girard, F. Boust and F. Hillion, Scanning secondary ion analytical microscopy with parallel detection. *Biol. Cell* 74 (1992), p.43-50.

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