SPATIAL DYNAMICS OF COCHLEAR PROTEIN TURNOVER MEASURED WITH MULTI-ISOTOPE IMAGING MASS SPECTROMETRY (MIMS)

Edmund Mroz (eam@epl.meei.harvard.edu)[1], Claude Lechene[2]

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

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

The human sense of hearing depends on a small number of highly specialized, terminally differentiated cells. Once lost, these specialized cells in the cochlea are not regenerated. If hearing is not to be lost, these cells must stay healthy throughout a lifetime.

Regulation of protein turnover is expected to be a crucial part of keeping cochlear cells healthy. The small amount of tissue available had previously made it extraordinarily difficult to examine the dynamics of protein turnover in the cochlea. Furthermore, purely biochemical measures of protein turnover would blur distinctions among the more than a dozen specialized cochlear cell types and differences in protein turnover among different regions within individual cells.

We have shown that combining MIMS analysis with heavy-nitrogen labeling of mouse cochleae provides a direct way to quantitate cochlear protein turnover. Mice are fed diets including amino acids enriched in nonradioactive ^{15}N; the rate of appearance of this isotopic label in tissue over time provides a measure of protein turnover rate. MIMS allows us to quantify this marker in sub-cellular regions on the order of 100 nanometers.

Initially, we found continuing turnover of the proteins in the stereocilia of the hair cells, with newly-synthesized proteins preferentially added at the base of those organelles. This result strengthens our hypothesis that dysfunction of protein turnover may play a role in hearing loss, since the "rootlets" at the base of the stereocilia are an initial site of damage during acoustic over-stimulation. There is marked protein turnover at the apical surface of the organ of Corti, a region known to have high levels of proteins involved in ion transport. And we have also found addition of new protein to the acellular tectorial membrane, a major mechanical structure in mammalian hearing, particularly on the margin facing the hair cells. We have performed the MIMS analysis of these cochlear structures after 3 days, 9 days, 22 days and 50 days of ^{15}N leucine diet so as to measure the time course of protein turnover in the varied cellular structure of the cochlea.

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