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Multi-Isotope Imaging Mass Spectrometry (MIMS) Mapping of Protein Turnover in Hair Cells Reveals Highly Stable Stereocilia

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Hair cells are not replaced during the life of an animal, so they need to degrade and replace their proteins on a regular basis. Tip links, for instance, can be replaced in 5- 10 hours, and actin in the bundles of neonatal hair cells in culture is thought to turn over in two days. We have used a new method, multi-isotope imaging mass spectrometry (MIMS), to quantify protein turnover in defined subcellular compartments. This method can detect atoms of specific isotopic mass and has spatial resolution near that of electron microscopy. We fed frogs and mice precursor amino acids labeled with the stable isotope ¹⁵N, we sacrificed after times of days to months, and we recorded quantitative images at mass ¹²C, ¹³C, ¹²C¹⁴N and ¹²C¹⁵N. The ¹⁵N/¹⁴N ratios in the inner ear revealed regions of high and low protein turnover.

Adult frogs fed ¹⁵N-enriched food were sacrificed at 1, 2, 4, 8, 16, and 32 days. Two control frogs were sacrificed at 1 and 32 days. After a few days, there was a small but statistically significant incorporation of ¹⁵N in frog saccular tissue. After 32 days, total protein turnover in hair cells and supporting cells was ~20%, but stereocilia had <10% turnover. Otolithic membrane turnover was very low. Adult mice were fed ¹⁵N food and sacrificed at 1, 2, 4, 8, 16, 22, 32, 50, and 150 days. Cochlear hair cell cytoplasm underwent 100% renewal in 3-5 months but stereocilia had <30% of their protein replaced in 5 months. Other highly stable regions included the shafts of pillar cells, reticular lamina, and tectorial membrane. In both species, small domains of higher turnover appeared towards the tips of stereocilia. These results, obtained in adult animals in vivo, suggest that the normal turnover of protein in stereocilia may be slower than previously suggested. Indeed, the most stable structures in cochlea are stiff elements carrying the mechanical stimulus: the tectorial membrane, the pillar cells, the reticular lamina and the stereocilia.