INVESTIGATING DFNA20 MUTATIONS IN y-ACTIN:

STUDIES IN YEAST, CELL CULTURE, AND MOUSE

By

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Ten dominant missense mutations in gamma-actin (ACTG1) have been reported as the cause of hearing loss in DFNA20 families. Although the mutations are located in different functional domains of y-actin, the end result is a progressive form of non-syndromic sensorineural hearing loss beginning in the high frequencies with an onset in the second to third decade of life. This shared phenotype is indicative of a common functional deficit in mutant gamma-actin protein function. To address questions pertaining to the unique function of yactin in the inner ear, I implemented a yeast 2-hybrid screen of an inner ear library. Surprisingly, given then number of proteins in the inner ear known to interact with actin, only identified six proteins were identified more than once in the screens: y-actin, (3-actin, cyclase associated protein 2, cofilin 1, cofilin 2, and a novel actin binding protein, ubiquitin E2i ligase. Furthermore, I used a directed yeast 2-hybrid to show deficits in the interaction of P264L mutant y-actin with four of the actin binding proteins from the initial library screens.

Next I evaluated the localization of a y-actin specific binding protein, annexin 5a (ANXA5), in the inner ear of the mouse. My data demonstrate that in the postnatal mouse ear, annexin 5a is differentially localized to the stereocilia, cell body, and nuclear membrane of developing hair cells. Anxa5 knock-out mice do not show hearing loss by 3 months of age. Furthermore, y-actin is

appropriately localized to the periphery of the stereocilia and F-actin gaps in these mice. Using a GST-pulldown assay, I confirmed that annexin 5a interacts exclusively with the y-isoform of cytoplasmic actin. Therefore, the interaction of annexin 5a and y-actin in the inner ear is not critical for establishing or maintaining proper hearing in mice.

Finally, to address questions regarding the effects of these mutations on the structure and function of the inner ear and the molecular mode of action, we generated a knock-in mouse model for the p.P264L mutation. In the process, I identified a novel Actgl transcript, enriched in skeletal muscle-containing tissues. Splicing of this alternative transcript creates a premature termination codon and is concurrent with down-regulation of Actgl. A protein product corresponding to the use of this stop codon was not found. I provide evidence that inclusion of exon 3a is means of post-transcriptionally down-regulating Actgl via the nonsense mediated decay pathway. The knock-in mouse model recapitulates aspects of the DFNA20 deafness phenotype observed in humans. Mice homozygous for this mutation have early onset hearing loss which progresses rapidly in adolescent mice. My data demonstrate that the mutant P264L protein is stably expressed and localized properly to the stereocilia. Scanning electron micrographs support the hypothesis that hearing loss involves outer hair cell dysfunction, and provides evidence that degeneration of the stereocilia occurs in the two rows of stereocilia uniquely responsible for mechanotransduction.