

**Stony Brook University
The Graduate School**

Doctoral Defense Announcement

Abstract

**Functional Comparison of Connexin26 Wild-type and Non-syndromic Recessive
Deafness Associated Mutant Channels**

By

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Gap junctions facilitate the exchange of ions, second messengers and small metabolites between adjacent cells. Gap junction channels are encoded by the connexin (Cx) gene family and show little selectivity to monovalent ions. However, gap junctions formed from different connexins are unique in terms of permselectivity to larger molecules. This phenomenon creates a problem in compensating for the loss of even one isoform in cases of disease causing mutations. Junctional communication plays an important role in cochlear homeostasis since mutations in the Cx26 gene (GJB2) are the leading cause of non-syndromic hereditary deafness. The exact function of intercellular communication in the inner ear is unknown. One view focuses on the importance of potassium recirculation through gap junctions while the other emphasizes the role of biochemical coupling between cochlear supporting cells. Partially active mutations would be an invaluable tool to differentiate the role of electrical coupling from metabolic coupling. Five Cx26 deafness mutants were screened in order to find channels that would retain some activity using a paired *Xenopus* oocyte expression system and dual whole-cell voltage clamp. Two of mutations (Thr8Met and Asn206Ser) formed active channels with properties different from wild-type Cx26. The single channel properties and permselectivity of wild-type and mutant Cx26 junctions were further characterized in transfected neuro-2A and HeLa cell pairs by dual whole-cell voltage clamp and intercellular fluorescent dye flux experiments. Protein expression and localization was verified by immunofluorescent staining. The average unitary conductance of wild-type and mutant channels was approximately 106pS in 120 mM potassium aspartate pipette solution, indicating comparable K⁺ permeability. To look for possible differences in their permselectivity to larger molecules, the permeability of mutant and wild-type channels to Lucifer Yellow (LY) and ethidium bromide (EtBr) was analyzed in individual cell pairs. Mutant channels were as permeable to LY as wild-type Cx26 while the EtBr transfer through the mutant channels was greatly reduced compared to wild-type junctions. These findings support the view that the biochemical coupling through gap junctions is important for normal cochlear functioning and might also provide insight into the etiology of the hereditary hearing loss at the molecular level.

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