

Engineering a C-1 mutation in the Prestin gene of Outer Hair Cells

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It is known that outer hair cells (OHCs) of the mammalian cochlea actively change their cell length in response to changes in membrane potential.¹ This electromotility, thought to be the basis of cochlear amplification, is mediated by a voltage sensitive motor molecule recently identified as the membrane protein prestin.¹⁻³ The cell loss and reduction in soma length of OHCs seen in prestin-knockout mice suggests that other active mechanism(s) (such as that conferred by the active movements of hair bundles, observed in lower vertebrates) might have been compromised. To provide definitive evidence that no active forces other than OHC electromotility exist in OHCs, we will generate and characterize prestin-knockin mice in which a crucial mutation (C1) is introduced in the endogenous prestin gene. The C1 mutation in the prestin gene results in a reduced or non-motile yet structurally intact protein in vitro.² We will determine whether this mutation abolishes OHC electromotility and cochlear amplification without causing cell loss and reduction of soma length of OHCs, to confirm that prestin-mediated OHC electromotility is the only active mechanism by which cochlear amplification is generated. In this research the first stages of the knock-in procedure was accomplished in which a stem cell clone was produced with the C1 mutation. This mutation was accomplished by stem-cell transfection of an engineered vector with the C1 mutation. Genomic southern analysis confirmed the knock-in procedure.

Supported by 5 R25 CA23944 and P30 CA-21765 from the National Cancer Institute and by the American Lebanese Syrian Associated Charities (ALSAC)