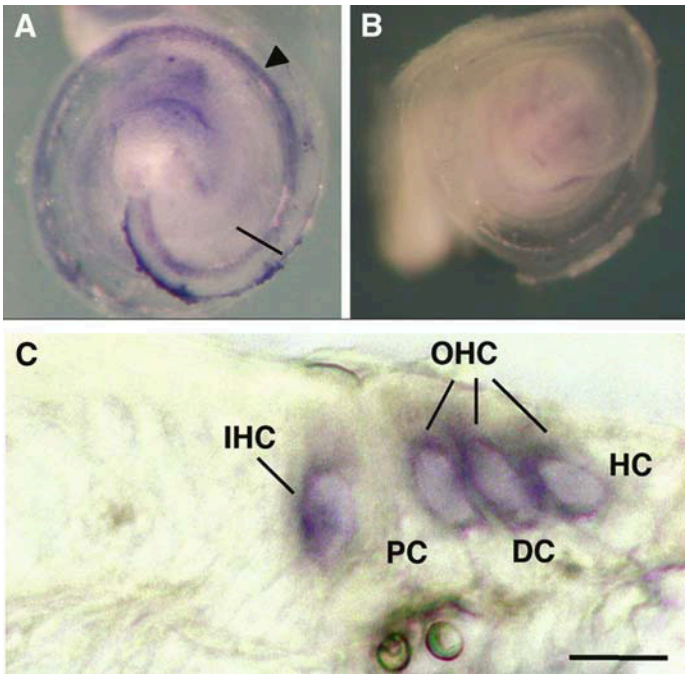


FIGURE 2.3. Detection of *Ush3a* mRNA expression by in situ hybridization. (**A**) Whole mount of a cochlea at embryonic (E) day 16, with staining along the region of the hair cells (*arrowhead*). (**B**) Sense probe demonstrates that staining in **A** is specific. (**C**) Sectioning through the cochleae (line in **A**) revealed specific hybridization in the inner (IHC) and outer hair cells (OHC) of the organ of Corti, but not in the Deiters cells (DC), pillar cells (PC), or the Hensen cells (HC). (Modified from Adato et al. 2002.)

and SOURCE, Table 2.2). There are fewer data about inner ear expression in these general expression databases.

Experiments using cell culture techniques may also reveal the function of a protein. One can overexpress the wild-type and mutant form of the gene that corresponds to the deafness phenotype. For example, to determine the mechanism for *POU4F3 DFNA15*-associated hearing loss in an Israeli kindred, the human gene was cloned into an expression vector and overexpressed in HEK293, COS-7, and the established cochlear cell line UB/OC-2 cells (Weiss et al. 2003). While the wild-type form of the gene was localized to the nucleus, as expected for a transcription factor, the mutant form was also expressed in the cytoplasm. Subsequent bioinformatics and experimental analysis revealed that a bipartite nuclear localization signal (NLS) was removed due to the truncation caused by the 8-bp deletion, leading to partial loss of nuclear localization (Fig. 2.5). To examine connexin mutations and proper localization of gap junction formation, the gene has been cloned in expression vectors and fused to a reporter, GFP, to enable localization of the wild-type and mutant proteins. In this way, many

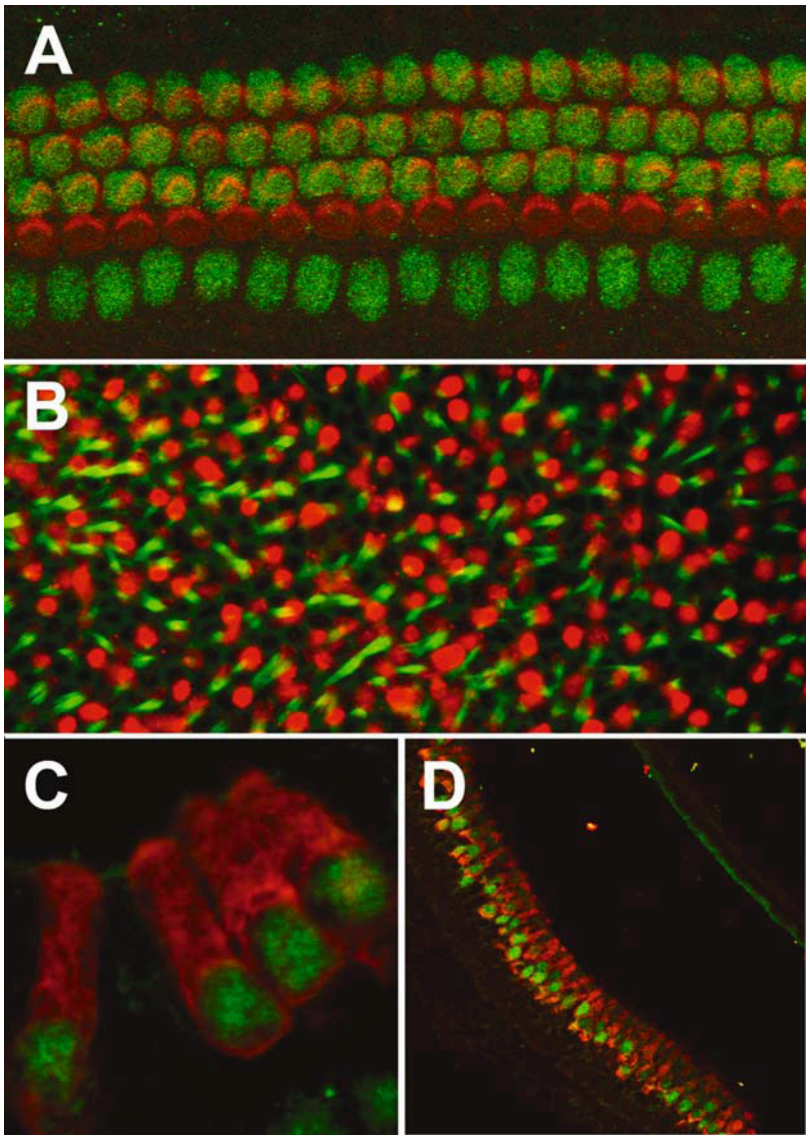


FIGURE 2.4. Expression of Pou4f3, myosin VI, and Lhx3 in the auditory and vestibular systems, demonstrated by immunohistochemistry with antibodies against each protein. (**A**)Whole-mount immunohistochemistry shows that Pou4f3 is expressed in the nuclei of inner and outer hair cells at E18.5 (green in online version). Actin can be visualized with phalloidin (red in online version). (Modified from Hertzano et al. 2004) (**B**) Whole-mount immunohistochemistry shows that the unconventional myosin VI (red in online version) is expressed in the cytoplasm of utricular hair cells at P10, while actin demonstrates the presence of stereocilia, stained with phalloidin (green in online version). (**C**) Cryosections shows the expression of Lhx3 (green in online version) and myosin VI (red in online version) in the cochleae and (**D**) the vestibular system neuroepithelial cells in the utricle from inner ears of E18.5 mice. Lhx3 is expressed in the nuclei of all hair cells. (**B**, **C**, and **D** provided by Amiel Dror and Karen Avraham; Hertzano et al., 2007.)

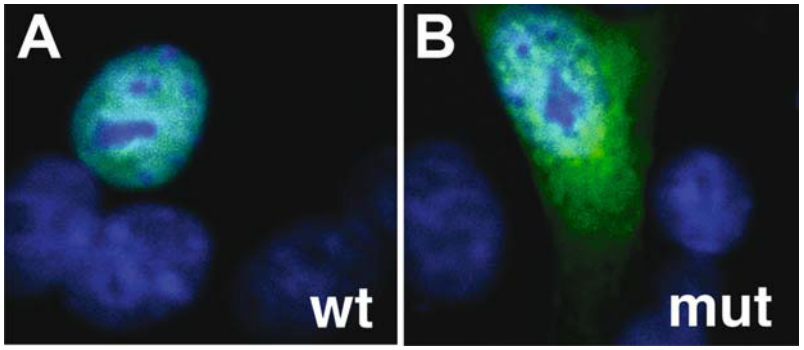


FIGURE 2.5. The dominant mutation in POU4F3 changes the subcellular localization of the protein in transfected COS-7 cells. (**A**) The wild-type form of the protein is localized to the nucleus. (**B**) The mutant form of the protein is localized to both the cytoplasm and nucleus. (Modified from Weiss et al. 2003.)

deafness causing connexin 26 mutations have been studied. For example, not only is the abnormality revealed by these experiments (Fig. 2.6), but determining whether the mutation is pathogenenic or not may be answered. For example, the M34T connexin 26 mutation has been the subject of debate for years. Though originally identified in a family with deafness (Kelsell et al. 1997), reports from other investigators revealed that this mutation exists in normal hearing individuals (Scott et al. 1998). One study suggests that the mutation is pathogenic, because

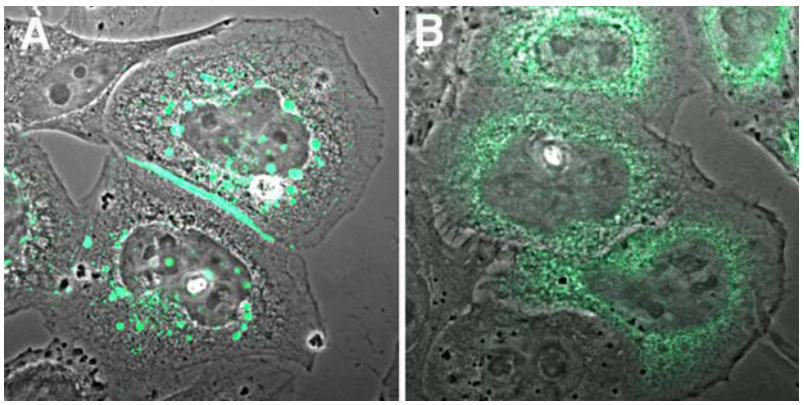


FIGURE 2.6. Connexin-GFP fused protein expressed in transfected HeLa cells. (**A**) Wild-type Cx26 is expressed in the plasma membrane, creating gap junction plaques between adjacent cells. (**B**) When expressing Cx26 carrying the deafness-causing mutation Ser139Asn in transfected cells, the protein fails to reach the plasma membrane and no gap junction plaques are formed. (Courtesy of Adi Sabag and Karen Avraham; Fleishman et al. 2006.)

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