Spatial and Temporal Expression of *Fam3c* in the Developing Murine Inner Ear.

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Fam3c was reported to be highly expressed in the nonsensory epithelium of the developing semicircular canals at embryonic day (E) 15.5 (Pilipenko et al, 2004). This pattern of expression resembles the expression of the developmental regulatory genes *Nkx5.1* and *Nkx5.2*, referred to as *Nkx5* (Rinkwitz-Brandt, et al., 1996). Sequence analysis of the *Fam3c* promoter region suggested a role for *Nkx5.1* in Fam3c function. This finding, together with the finding of overlapping patterns of expression suggests that *Fam3c* may be a downstream target gene for the *Nkx5* transcription factor in semicircular canal development. By studying the expression of *Fam3c* in the inner ear at the otocyst stage, further insight into the molecules involved in the developmental pathways of inner ear development can be gained.

In situ hybridization was carried out on the mouse E9-E12, E18, and postnatal day (P) 2 inner ear cryosections using RNA probe of *Fam3c* subcloned into a pGEM plasmid. A similar probe was used for *Nkx5.1*. Pendrin was used as a positive control probe. Hybridizations were performed as described by Choo et al.(1998), but with some minor modifications. These include the tissue sections being permeabilized using 10 mg/ml proteinase K for 2 min and hybridization performed overnight at 68°C with a probe concentration of 2 mg/ml. Humidified boxes rather than sealed bags were used for hybridization. The hybridization signal was visualized using BCIP-NBT chromogen (Roche).

At E9, *Fam3c* showed expression in the periotic mesenchyme surrounding the newly formed otocyst. A similar pattern was found at E10 with signal also shown in the otocyst epithelium. E11 and E12 showed similar patterns to E10 with *Fam3c* expression in the otocyst epithelium and periotic mesenchyme. *Fam3c* expression was detected again at P2 in the cochlear epithelium. Interestingly, strong expression of *Fam3c* was also noted in the developing hair follicles in the dermis.

Nkx5.1 and *Fam3c* showed similar expression patterns in both location and time in the confines of the otic cyst and its derivatives. *Fam3c* shows mesenchymal expression, where as *Nkx5.1* does not. Our current data suggest that *Nkx5.1* is not the sole upstream activator of Fam3c. Further studies are underway to delineate the role of *Fam3c* in inner ear development and the specific relationship with other related molecules, such as *Nkx5.1*.