# Role Of Transmembrane Protein Strabismus

# In Motor Neuron Migration In The Zebrafish Hindbrain

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## ABSTRACT

Nervous system development involves extensive cell migration, causing immature neurons to move from proliferative zones to specific locations to generate functional circuits. Defective in neuronal migration can cause severe anomalies including mental retardation and learning disabilities. Therefore, it is important to understand the molecular mechanisms underlying neuronal migration. We use zebrafish as a model to study one such migration. In the zebrafish and mouse hindbrain, Facial Branchiomotor Neurons (FBMNs), which mediate jaw and facial movements in mammals, migrate caudally (tangentially) from rhombomere 4 (r4) into r6 and r7. The transmembrane protein Strabismus (Stbm) is a component of the non-canonical Wnt/PCP pathway and is necessary for the normal migration of FBMNs. To understand the mechanisms by which *stbm* regulates neuronal migration, I sought (1) to identify the cell types where *stbm* function is required for FBMN migration (2) to analyze the various domains of

Stbm and their requirement for FBMN migration and (3) to analyze other genes interacting with *stbm* to regulate FBMN migration.

Previous analyses showed that *stbm* is expressed ubiquitously, and function non-cell autonomously during FBMN migration. Expression analysis of *stbm and* its interacting partner *prickle1a* (*pk1a*) raised the possibility that *stbm* and *pk1a* may function in non-neural tissues such as the paraxial mesoderm or endoderm to regulate FBMN migration. FBMN migration occurs normally in embryos lacking endoderm suggesting that endoderm-expressed *stbm* is not necessary for FBMN migration. Targeted transplantation of *stbm*-deficient cells into the mesoderm of wild-type host embryos does not affect FBMN migration indicating that mesoderm-expressed *stbm* is also not essential for FBMN migration. However, transplanted wild-type cells generating ventral neural tube cells including floorplate were able to rescue FBMN migration in *stbm*$^{-/-}$ mutants. Conversely, transplanted *stbm*-deficient cells generating ventral neural tube cells such as floorplate were able to block FBMN migration in wild-type zebrafish embryos, suggesting strongly that *stbm* expression in the floorplate is necessary and sufficient for FBMN migration. Strabismus (Stbm) is predicted to be a four pass transmembrane protein with N- and C- terminal cytoplasmic domains, and a PDZ domain binding motif at the C-terminus. To identify regions of Stbm that are essential for mediating FBMN migration, we tested the abilities of the cytosolic N- & C- terminal fragment to rescue

migration in the *stbm*$^{-/-}$ mutants. Surprisingly, both constructs rescued defective FBMN migration, suggesting that both N- and C- terminal domains of Stbm can independently facilitate downstream events mediating FBMN migration in zebrafish hindbrain.

Genetic mosaic analyses have indicated that *stbm* functions in the environment especially in the ventral neural tube cells such as floorplate to regulate FBMN migration. This result suggests that *stbm* expressed outside motor neurons genetically interact with other genes expressed in FBMNs and the ventral neural tube to mediate FBMN migration. To test this hypothesis, we examined the roles of *Transient axonal glycoprotein-1* (*Tag-1*) and *Laminin* $\alpha$*1*, which respectively encode cell adhesion and extracellular matrix protein, during FBMN migration. *Tag-1* is expressed in FBMNs and its knockdown using antisense morpholinos leads to loss of FBMN migration. It genetically interacts with *stbm* to regulate FBMN migration. *Laminin* $\alpha$*1* (*lama1*) also interact genetically with *stbm* to regulate FBMN migration. These results indicate that FBMN expressed *tag-1* may be interacting with *stbm* in adjacent cells and *lama1* to regulate FBMN migration.