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Identification of Alternative Transcription Start Sites that Generate Neuron-Specific nhsl1b Isoform that Regulates Neuron Migration

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Identification of alternative transcription start sites that generate a neuron-specific *nhsl1b* isoform that regulates neuron migration

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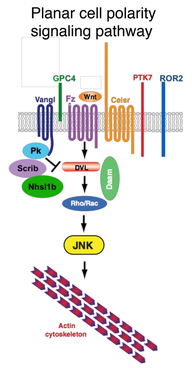
Introduction

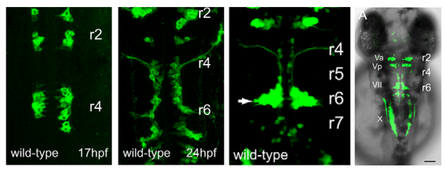
Neuron migration is a fundamental step in the assembly of neural circuits. The neuron cell body travels significant distances from where they are born to where the nerve cell is needed to function. Mutations in genes that regulate neuronal migration lead to human congenital disorders such as lissencephaly and epilepsy.

It is established that Planar cell polarity (PCP) signaling is required for the alignment of cells within an epithelium, as well as directional migration. *Whsl1b* is a novel, neuronal downstream effector of the PCP pathway, involved in the migration of neurons.

As a model system, we study the caudal migration of Facial Branchiomotor (FBM) neurons in the hindbrain of developing zebrafish. FBM neurons are born in rhombomere 4 (r4) at 16 hours post fertilization (hpf) and they migrate posteriorly into r6 by 48 hpf. They can be visualized in transgenic fish expressing GFP in cranial motor neurons under control of the islet1 promoter Tg(islet1:GFP).

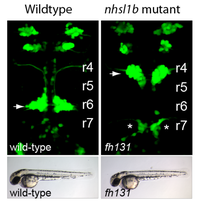
Planar cell polarity signaling pathway



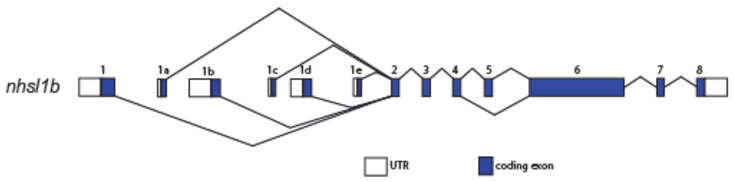


Mutations in a novel gene called Nance-Horan Syndrome-like 1b (Nhsl1b) lead to a dramatic loss of posterior migration of FBM neurons. In nshl1b mutants, FBM neurons remain unmigrated in r4 without affecting overall embryo morphology.

It remains unclear the function of Nhsl1b in neuron migration. Here we examine which isoform of Nhsl1b is responsible for regulating FBM neuron migration.

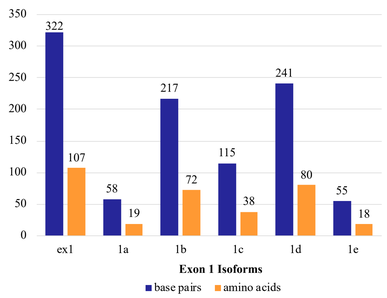


Genomic Architecture of nhsl1b



***Nhsl1b* in zebrafish:**

- 8 coding exons.
- Exon 5 is variably spliced
- 6 variants of exon 1, each with its own 5-prime UTR and transcriptional start site (TSS).



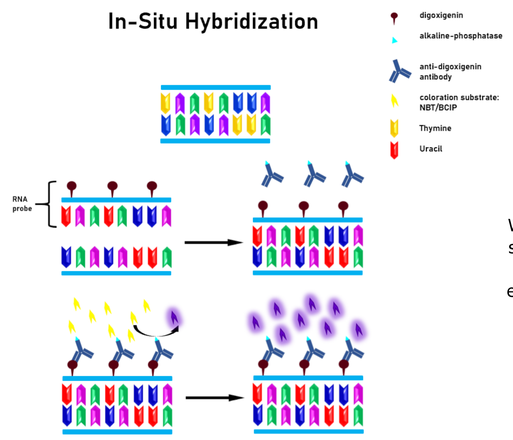
We used a bioinformatics approach to determine the protein domains encoded by Nhsl1b. No known protein domains were found except in isoforms derived from the first alternative transcription start site – encoding Nhsl1b^{ex1}.

Nhsl1b^{ex1} encodes a WAVE-homology that may regulate actin polymerization.

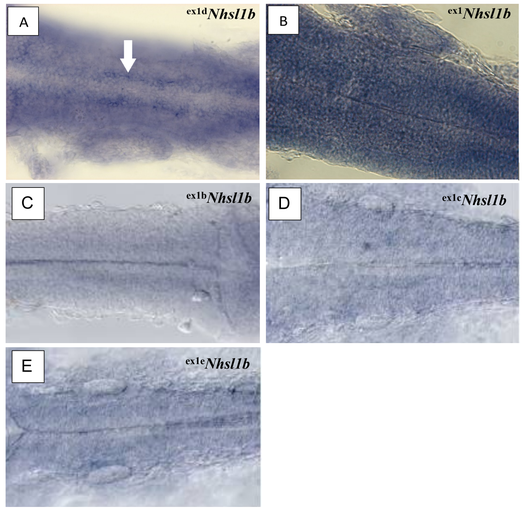
Hypothesis: Nhsl1b^{ex1} variant is the most important isoform to direct neuron migration because it has an N-terminal WAVE homology domain.

Which isoform is expressed in motor neurons?

In order to determine the spatial expression pattern of the Nhsl1b variants, we designed RNA probes directed against each of the exon1 sequences. Using these probes, we performed whole mount In situ hybridization to visualize which cells within the embryos express each transcript.



Whole mount RNA in-situ hybridization was performed for ex1, ex1b, ex1c, ex1d, and ex1e isoforms.



(A) Darkly stained FBMN cells, indicted by arrow, show enrichment of ex1d*Nhsl1b.*

(B-E) All variants are expressed uniformly throughout the entire nervous system.

Conclusion:

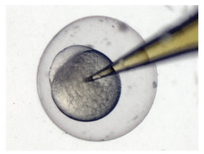
Our data suggests

1) Most Nhsl1b variants are generally expressed throughout the nervous system, particularly in neural progenitor cells.

2) That Nhsl1b^{ex1d} is the only Nhsl1b variant that is enriched in FBM neurons and may be a neuron-specific Nhsl1b isoform that is required for FBM neuron migration.

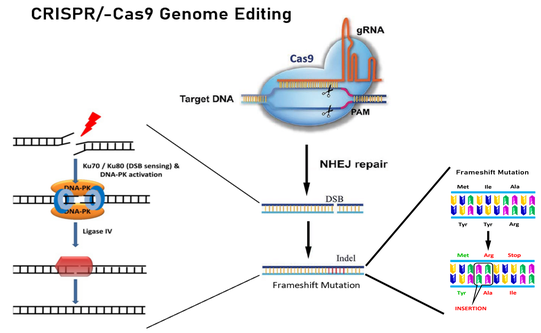
What happens to neuron migration in the absence of exon 1d?

In order to determine whether Nhsl1b^{ex1d} variant is required for FBM neuron migration, we generated guide RNAs (gRNA) targeting ex1d*nhsl1b* for co-injection with Cas9 mRNA in Tg(is1et1:GFP) embryos.



nhsl1b gRNA
Cas9 mRNA

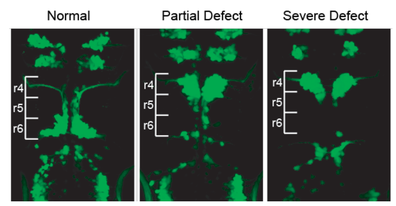
When CRISPR guide RNAs are injected in founder (F0) embryos, we can generate mosaic embryos carrying various mutations in different early stem cells. Some cells in the embryos carry predominantly heterozygous mutations, some cells carry mutations in both chromosomes (bi-allelic mutations). We inject high levels of gRNAs in order to promote as many cells carrying bi-allelic mutations as we can.



CRISPR/Cas9 genome editing generates DNA double strand breaks that is repaired by the imprecise non-homologous end-joining (NHEJ) repair mechanism. Due to the imprecise nature of NHEJ repair, insertion or deletion mutations lead to frameshifts in the coding sequence.

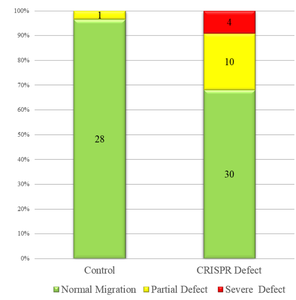
Injection of Nhsl1b-ex1d gRNAs led to variable defects in FBM migration. We scored these defects as normal, partial defects, or sever defects.

We quantified the number of injected embryos that displayed defects.



Conclusion:

Mutations in ex1d*Nhsl1b* leads to a severe migration defect of motor neurons consistent with the idea that ex1d*Nhsl1b* is a neuron-specific isoform that is essential for neuron migration.



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Future Direction

- Investigate activation of the ex1d promoter.
- Further study neuron expression in the absence of other exon 1 isoforms.