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Molecular mechanisms of somatosensory neuron function and development in zebrafish

Sensory neurons enable appropriate responses to environmental stimuli, thereby allowing us avoid pain and danger [1]. Different neurons respond to various types of sensory stimuli such as touch, temperature, chemical, and pain. Dysfunction of these neurons can cause chronic pain; accordingly, improved understanding of sensory neuron development and function could enable the generation of treatments for chronic pain conditions [8]. I study the molecular mechanisms of sensory neuron development in larval zebrafish, and I am specifically interested in the genes that help these neurons grow, connect, and function. Sensory systems of zebrafish develop quickly and are easily visualized; moreover, zebrafish have a short regeneration time, lay many offspring at once, and have mostly translucent embryonic stages, making them amenable to live imaging and genetic manipulations. Importantly, zebrafish sensory systems and genes are comparable to ours -- meaning that information learned from this model could be translated to human conditions of interest [5]. These neurons grow by branching through the skin, and their goal is to maximize coverage of skin [7]. To do that, they interact with themselves, with other neurons, and with the skin itself [4]. We hypothesize that cell-surface adhesion proteins contribute to these interactions and are important for development [2]. Furthermore, we propose that regulators of the cytoskeleton help give these neurons their shape [3]. Previously, genes specifically expressed in genetically-identified subsets of somatosensory neurons were identified via a transcriptomic approach and candidates were filtered for cell-surface and cytoskeletal genes (Figure 1). First, I have worked to identify founders for CRISPR/Cas9-induced mutations and performed the husbandry to cross these mutations to homozygosity (Figure 2). Second, I have prepared assays to evaluate and analyze loss-of function mutations in genes that regulate development and activity of somatosensory neurons. Specifically, I have applied or developed assays to analyze cell fate, quantify growth and coverage of sensory arbors, and assess appropriate behavioral responses to sensory stimuli. Using PCR and gel electrophoresis, I determined if transmission through the germline occurred. For example, 2nd generation (f2) mutants in amigo3 demonstrated a transmitted deletion mutation (Figure 2). I have sequenced this mutation to determine its exact identity (Figure 2). While our initial amigo3 mutants, a01 and a02, appear not to disrupt the reading frame, we have 3 additional alleles growing. Once mutants have been crossed to homozygosity I plan to use behavioral assays to examine how each mutant respond differently to sensory stimuli. To assay changes in cell fate in our mutants, *in situ* hybridization can be used to localize markers of cell identity within neurons of the TGG. For example, the numbers of particular types of neurons such as p2x3b, tra1b, and trpv1 are well characterized in the TGG at multiple stages of development [1]. So, to test for differences in cell fate I will examine my mutants in comparison to published proportions. Similarly, the growth, shape, and coverage area of TGG or RB sensory axons can be quantified by tracing individual neurons in embryos that stably or transiently express GFP in different populations of these sensory neurons (Figure 3, [6]). For example, defects in

cytoskeletal and adhesion proteins may result in differences in number of branches within target field or changes in the angles between branches [9]. Both of the proposed phenotypes are easily detected via these neuron tracing experiments. I have begun testing my mutants for defects in somatosensory function using our video tracking setup. For example, larvae with disrupted development of chemosensory neurons may respond differently to mustard oil treatment compared to control siblings. Alternatively, larvae with disrupted development of thermosensory neurons may exhibit abnormal responses to hot or cold. These assays help to identify the specific phenotypes of subsets of sensory neurons, leading to a greater understanding of how they interact in somatosensory development. We use this knowledge to enhance our understanding of diseases causing sensory deficits in humans.

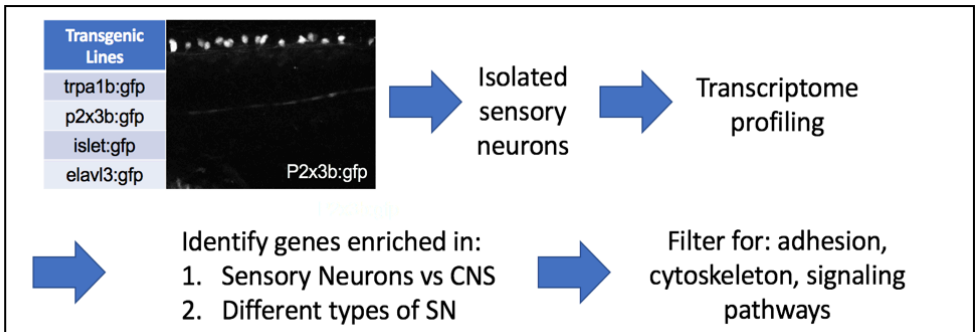

Figure 1. Identification of sensory enriched genes in zebrafish. Transgenic lines were identified that express GFP in different subsets of somatosensory neurons. GFP+ neurons were isolated, and transcriptome profiling was used for identification in each subset. We looked for genes enriched in sensory neurons vs. the Central Nervous System (CNS), and for genes specific for each subset. Candidate genes were then filtered for adhesion proteins and cytoskeletal regulators.

	# of founders	# of f1 lines	# of f2 allele lines
prph	6	6	2
gnb5	6	6	2
amig03	5	5	2
tppp3	6	6	9
cadm3	6	4	-
nptna	0	-	-

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CTCTGACTAGCTGCAGCAGGAAGGAGCTCAGCATTAGGG Wildtype
CTCTGACTAACTGCAGCAGGAACAA----------AGGG A01 allele: 10b deletion
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Figure 2. Sequencing results & current CRISPR lines I have identified for genes of interest in somatosensory neuron development. Values represent the number of alleles for a gene in a given part of the knockout process.

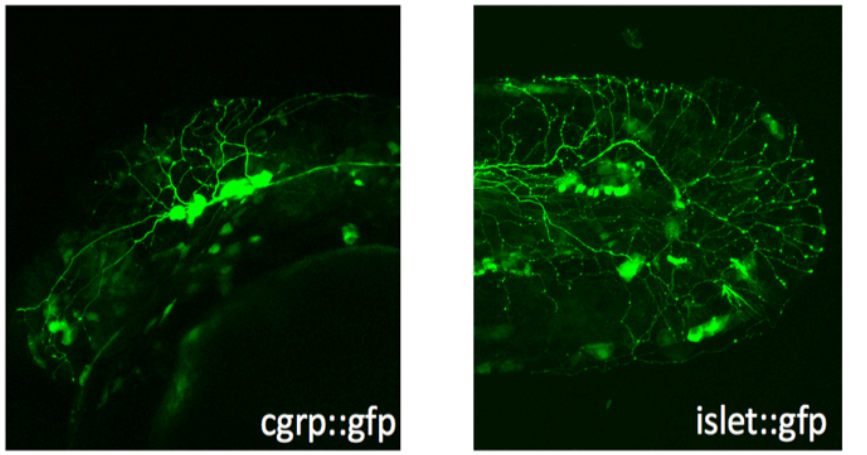

Figure 3. Sensory neuron innervation. TGG and RB neurons of the head and tail at 30hpf in cgrp::gfp and islet::gfp lines.

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