Potential interactions between transcription factors that control axon outgrowth of serotonergic neurons in *C. elegans*

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Abstract

Hermaphrodite specific neurons (HSN) are serotonergic neurons that innervate muscles and stimulate egg-laying in hermaphroditic *C. elegans*.[1] HSN differentiation and cell fate are controlled by a network of transcription factors that together regulate neurotransmitter phenotype, cell migration, and axon outgrowth during development.[1] The goal of this project is to investigate how *sem-4*, *ast-1*, and *hlh-3* interact with *unc-86*, the master regulator of this transcriptional network, to control HSN outgrowth and pathfinding. To do this, fluorescent markers will be crossed into these mutants to track possible precocious outgrowth and pathfinding defects. Due to lack of access to the laboratory, the project focused on the initial steps of designing genetic crosses, bioinformatic analysis of the *sem-4* and *unc-86* genomic sequences, and designing PCR reagents and PCR genotyping experiments.

Background

HSN are generated in the tail of the embryo and migrate anteriorly to the middle of the animal during embryonic development. At a later larval stage, these cells extend axons that will synapse onto these muscles and form connections with interneurons in the primary nerve ring in the head. Previous studies have shown multiple transcription factors are responsible for HSN cell fate. A proposed regulatory hierarchy shows that *unc-86*, *hlh-3*, *sem-4*, and *ast-1* all contribute to controlling HSN effectors, directly and through the other proteins.[2] *unc-86*, a neuronally expressed POU homeodomain transcription factor, is the master regulator of this network.[1] POU proteins are required for various developmental events including the maintenance of vertebrate stem cell identity and temporal specification of neuroblasts. *unc-86* is part of one family that controls axon growth and pathfinding in *C. elegans, Drosophila,* and vertebrates.[2] In a previous study, loss of *unc-86* resulted in ventral axon growth defects in HSN including precocious outgrowth during differentiation.[2] HSN outgrowth is also influenced by a heterochronic gene network that regulates developmental timing in *C. elegans,* including *egl-18*.[1] *sem-4,* an ortholog of human SALL1 and SALL3 (spalt-like transcription factors), is responsible for the differentiation of neurons, muscle, and hypodermis.[3] *ast-1* has DNA-binding transcription factor activity and is involved in dopaminergic neuron differentiation and positive regulation of transcription by RNA polymerase II.[3] *hlh-3*, which has RNA polymerase II regulatory region sequence-specific DNA binding activity, is involved in several processes including generation of neurons and regulation of egg-laying.[3]

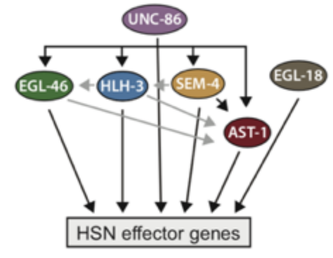

Fig. 1: Proposed regulatory gene hierarchy [1]

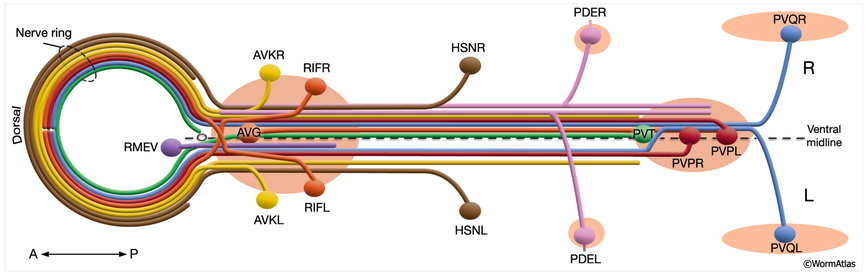

Fig. 2: Diagram of HSNs relative to associated interneurons in the ventral nerve cord[4]

Experimental Design

To determine how HSN axon outgrowth is regulated, I will test if *sem-4*, *ast-1*, and *hlh-3* mutants display the same precocious growth defects as *unc-86* mutants. To examine premature axon defects in these mutants, I will cross *Pegl-5*::GFP, a transgene marker for the HSN neuron, into *sem-4*, *ast-1*, and *hlh-3* mutants and examine HSN neurons by immunofluorescence microscopy. *C. elegans* undergo four larval stages. The HSN neurons extend neurons during the L4 larval stage. Mutants will be examined for outgrowth prior to the L4 stage. This experiment will help determine which of these genes are required for developmental timing of HSN outgrowth and accurate pathfinding and synaptic target selection.

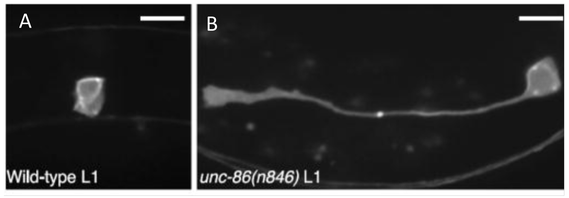

Fig. 3: Representative wildtype HSN (A) or *unc-86(n846)* HSN (B)[2]

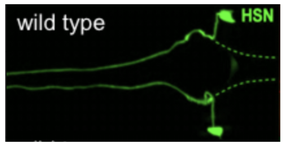

Fig. 4: HSN in wildtype adult (G. Garriga, personal communication)

Design of the Genetic Crosses

sem-4	**Males**		**Hermaphrodites**
P_0 5 males & 5 hermaphrodites	*Pegl-5*::GFP / *Pegl-5*::GFP	X	*sem-4* / *sem-4*
F_1 All progeny are heterozygous	*Pegl-5*::GFP / + ; *sem-4* / +		
F_2 Isolate homozygous progeny (1/16)	*Pegl-5*::GFP / *Pegl-5*::GFP ; *sem-4* / *sem-4*		

Homozygous *Pegl-5*::GFP animals are identifiable by fluorescence microscopy. *sem-4* homozygotes display uncoordination and egg retention visible via light microscopy.

***Crosses will be repeated for *unc-86*, *ast-1*, and *hlh-3* strains**

Designing Primers for ARMS-PCR Genotyping

ARMS-PCR is used for the genotyping of a single nucleotide polymorphism (SNP) without sequencing. To detect SNPs, specific primers for each allele are designed. APE, a DNA analysis program, was used to select primer sequences from the *sem*-4 sequence to generate an annotated sequence file for *sem-4*.

General criteria for selecting primers:

- 18-25 nucleotides in length
- TM above 55 °C
- GC content between 40-60%
- 3' ending in C or G
- 200-500 base pair amplicon

Additional criteria for selecting primers for ARMS-PCR:

- Forward primer must incorporate mutation site
- Additional mutations are added to increase specificity of the primer

FWD WT 1: cgaaaTtccacAgcttgcaTtgC (BP 23, TM 59, GC 48%)
FWD MT 1: cgaaaTtccacAgcttgcaTtgT (BP 23, TM 58, GC 43%)

FWD WT 2: cgaaaaAccactgcAtgcaaAgC (BP 23, TM 60, GC 43%)
FWD MT 2: cgaaaaAccactgcAtgcaaAgT (BP 23, TM 59, GC 43%)

FWD WT 3: cgaaTatccactgcAtgcaTtgC (BP 23, TM 59, GC 48%)
FWD MT 3: cgaaTatccactgcAtgcaTtgT(BP 23, TM 58, GC 43%)

REV 1: agagaattaattgtaggcggggc (BP 23, TM 59, GC 48%)
REV 2: gctttgttgccacgaatgagttc (BP 23, TM 59, GC 48%)

Fig. 5: Selected primers for ARMS-PCR of *sem-4(n1378)*

Sequencing *unc*-86 Alleles

Because the *unc-86(n946)* strain is not curated, primers that generate 400 base pair overlapping segments were chosen to sequence the allele via PCR. Each primer pair is indicated in the same color. The reverse primer is the reverse complement of the indicated sequence.

ATGAGACCACATGATATGTTCAGGtaatttctttttttttcactctgaccatctttcaggacttcattctatttatttttcagCTATAGCAACTACT
TTTCGGGAATCCACGACACATCCGCCGCCACAAACATCTACCAAGGACTCCCAAGCTCTTCAGAACCATTCGATGCATCTGTAGTTGTGCCAACAT
CCTCAGATGATCAAATGACACCCTTACAACAAGTAATGGCAATGCAACAATCATATGGAGCACCTCCACCATTTCAATACAATATGACTCATCCAT
TTTCAACAACATCCATTGCCAGTTCTAATAATCTTGCTCGTTACCCAATTGCCCCGCCAACTTCAGATATGGACACTGATCCGAGACAATTGGAGA
CGTTTGCAGAGCATTTCAAGCAGAGAAGAATAAAATTAGGAGTCACACAGgttagtataaaagggttgacaactgggtgttaactcttatattatc
ttaattatcttaattatctatctatcttaattatgagtaaacttaggttttttgaaaaacaaaaacgttcgggagtacgtagccacttctcaatc
tacttaggcttctgccaccttacaaacaattatcaccaacttatattctgctaaatttatctacatatttcagtaaaagccaccggagaaatggct
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caaaagtaagttagttcaacttttagtttcagaatgttatacatatattccaataaaagtgattgcaatccagaaatagtccaaaagttaattttaa
tacttcagGCGGACGTTGGAAAAGCACTTGCTCATCTGAAAATGCCTGGTGTCGGATCATTATCCCAGTCCACGATCTGCCGCTTCGAATCTCTCA
CCCTTTGCCATAACAACATGGTCGCACTGAAACCAATTCTACATTCCTGGCTGGAGAAAGCTGAAGAGGCTATGAAACAAAAAGATACAATTGGAG
ATATCAATGGAATTTTGCCAAATACTGATAAGAAACGGAAGAGAACTAGTATTGCTGCTCCTGAGAAAAGAGAACTAGAACAGTTTTCAAgtgag
tttgagtaggggagagtataaattcaaaatcatacatgctaaaattatgttaaaattaaacaaatttgcttttcactcaatactttagagatcaac
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GCGTCTGGTTTTGCAATCAACGGCAGAAACAGAAAAGgtaatatttaatttttttagcaagacttatcaaataaaatttcagAGATTTCCGTTCCCA
ATTCCGTGCAAGAAGTGCAGCAGCAGTAATGGGTCCACGTGTGATGCCAGTTTTAAATGGAAATAATTCAAATAATAATCTGAAACAGGGTCAGAC
AACATACAATGGGCTACCTGGATTCTTTGATTAG

Fig. 6: Sequences used for primer choice for PCR sequencing of *unc-86(n946)*

References

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This work was supported by the Occidental College Office of Undergraduate Research

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