Worcester Polytechnic Institute

The Role of CSN-5 and ARX-3 in Neuronal Migration in *C. elegans*

Major Qualifying Project Report
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Abstract

MIG-10 is involved in the signal transduction of guidance cues during neuronal migration and has shown putative interactions with CSN-5 and ARX-3. CSN-5 is involved in protein degradation; ARX-3 is involved in actin polymerization. To determine whether these interactions are significant *in vivo*, RNAi was used to silence *csn-5* and *arx-3*. RNAi of *csn-5* exhibited truncation of specific neuron migration; meanwhile there is currently ongoing experimentation with *arx-3*. These results may suggest that CSN-5 regulates the degradation of MIG-10 during development.

Acknowledgements

Thanks to the Stringham lab for help with techniques and providing strains for the RNAi experiment. I would like to thank my advisor, Elizabeth Ryder, for all her help and guidance throughout this project. I have gained a lot of experience and practice with lab techniques. A special thanks to Erin Flaherty for assisting me in lab and collaborating with me on lab techniques. Also, I would like to thank Gabriela Nuñez for her peer-editing in writing the report.

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Introduction

Neurons are one of the most essential cell types in the human body and are the primary components of the nervous system. They are responsible for control of all other subordinate cells through a process of cellular signaling, fundamental to the regulation of the cells and their functions. Depending on specific cellular location and the spatial relationships between neurons, these signals may not be properly transmitted. During the development of the nervous system, neurons migrate from their place of origin to their final destination with the help of actin filaments, cytoskeletal elements important to the cell's motility. Netrins, a family of extracellular proteins, are another contributing factor to the direction of axon and cell migration. These proteins are bifunctional, causing a chemoattraction or chemorepulsion to some neurons. Netrins interact with receptors, such as the Deleted Colorectal Cancer (DCC) receptor, to facilitate a chemoattraction, and the UNC-5 receptor, to facilitate a chemorepellence, in order to direct the migration of the axon (Moore et al., 2007). In *C. elegans*, UNC-6 (netrin), has shown similar attractions of the axon to high netrin concentration, while UNC-5, another netrin receptor, mediates the repellent response of the axon to high netrin concentration (Hedgecock et al., 1990). Disruption of the microfilaments or netrin signaling can result in the disruption of the migration pathway.

As an ongoing focus of study, this migration pathway proves to be not only vital for the differentiation of neurons, but also influential to the neurological processes of the human body (Purves, 2001). Disruptions of neuronal migration have been proven to cause negative effects on the human brain, terminally giving rise to neuronal disorders. Both children and adults are affected by diseases such as pachgyria, fetal cocaine syndrome, depression, schizophrenia, bipolar disorder, or Alzheimer's disease (Valiente and Marín, 2010). In the past, migration

disorders were untreatable, but through modern science and technology, experiments have provided possibilities for therapeutic intervention. The complexity of the human body prevents efficient research of the crucial effects of neuronal migration, so it is more favorable to use a simpler model, such as *C. elegans*. A key player in *C. elegans* that mediates the neuronal migration during development is MIG-10. This protein is involved in signal transduction pathways downstream of guidance cues, and interacts in the actin polymerization mechanism. In this project, we investigate the effects of two candidate MIG-10 interactors that may participate in neuronal migration.

C. elegans as a model system

Scientific research utilizes worm models, specifically *Caenorhabditis elegans*, based on a variety of characteristics; their small size, transparency, rapid life cycle of three days to complete full development, and their non-parasitic nature, allowing for lab safety. Furthermore, only about a third of their cells collectively compose the nervous system, giving an anatomical simplicity of the model and a more accessible approach to conduct research. *C. elegans* has a total of 302 neurons, belonging to two independent nervous systems: the large somatic nervous system and the small pharyngeal nervous system (Riddle et al., 1997). Through the genomic sequencing project of *C. elegans*, about 40% of their genes were found to be homologous to genes in the human genome, thus making this species an ideal candidate as a model of study. Manipulation of genes specifically involved in neuronal migration has been shown to affect the worm's phenotype. For example, three genes, *unc-6*, *unc-5*, and *unc-40* encode for a cell guidance cue and two receptors, respectively, that assist in cell guidance of neurons (Hedgecock et al., 1990). Similarly in another study, mutations of the gene *mig-10* produced a complete loss of gene function, considerably truncating the migration of specific neurons (Manser et al., 1990). Using

methods of microscopy and fluorescent markers, scientists may observe these effects and identify genes regulating these migrations by measuring neurons in wild-type or mutant subjects.

MIG-10 and its vertebrate homologs Lamellipodin and RIAM

The *mig-10* gene is necessary for the migration of the CAN, ALM, and HSN neurons, and the normal development of the excretory canal. The *mig-10(ct41)* mutation was identified through a genetic screen; mutants displayed defective migrations of two embryonic canal-associated neurons (CANs). Hermaphrodite-specific neurons (HSNs) and anterior lateral microtubule cells (ALMs) were also affected by this mutation. In wild-type animals, CANs and ALMs migrate anterior to posterior, and HSNs migrate posterior to anterior. In m*ig-10* mutants, researchers observed a more anterior position of CANs and ALMs, a posterior position of the HSNs, and a truncation of the posterior excretory canal (Manser and Wood, 1990). Thus, the *mig-10* mutation is responsible for truncating the long-range anteroposterior migration in embryonic neurons. It is important to understand these truncations can disrupt cell-to-cell signaling, hence interrupting the functions of neighboring cells.

The vertebrate proteins, Lamellipodin (Lpd) and Lpd-related molecule, RIAM, are homologous to MIG-10. Lpd and RIAM are responsible for the stimulation of F-actin formation, lamellipodia formation, and integrin-dependent actin polymerization (Lafuente et al., 2004; Krause et al., 2004). These functions play critical roles in cell motility and cell-to-cell interactions.

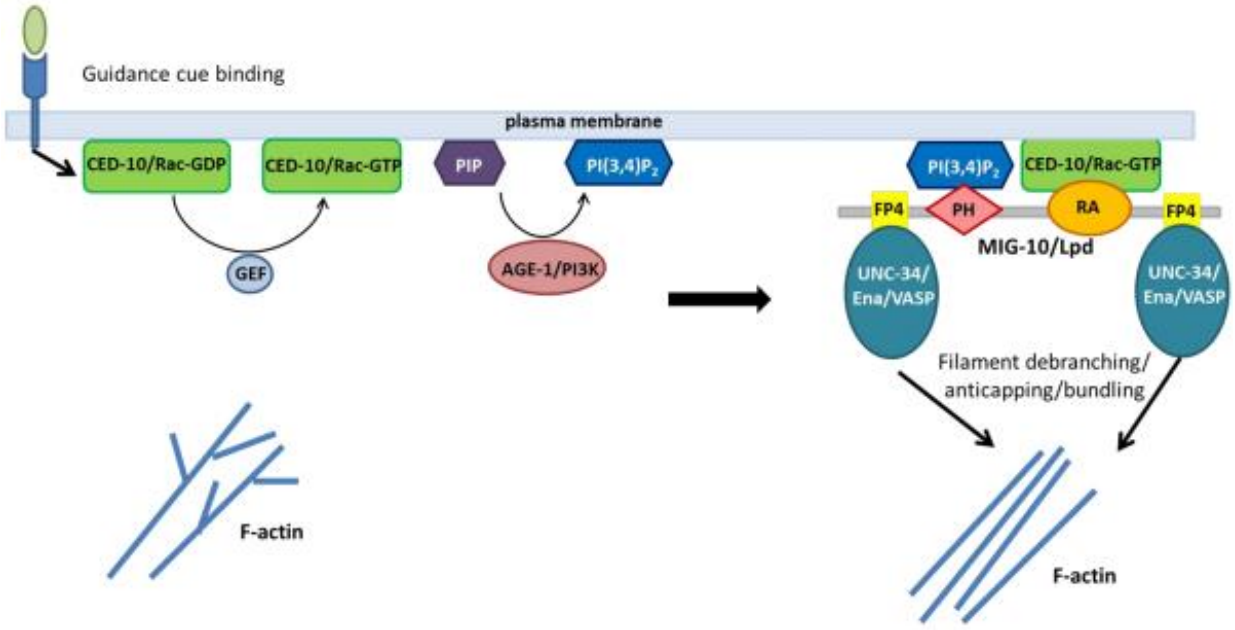

Figure 1: The Mig-10 Response to Guidance Signaling. Guidance receptors signal the Rac and AGE-1 molecules to activate. The phosphatidylinositol phosphate in the plasma membrane is then phosphorylated to phosphatidylinositol (3,4) bisphosphate, and along with activated Rac, engages MIG-10 through pleckstrin homology (PH) and Ras-association (RA) domain interactions. Along with MIG-10, UNC-34 is recruited through binding of the EVH-1 domain to FPPPP motifs in MIG-10 (McShea, 2009).

MIG-10, Lpd, and RIAM each contain multiple proline-rich FPPPP motifs, capable of binding to the Ena/VASP proteins in order to regulate the formation of actin filaments in the growth cones of the axon terminal; a Ras/Rap GTPase association domain; a lipid binding pleckstrin homology (PH) domain responsible for membrane targeting; and a profilin binding proline-rich domain (Krause et al., 2004; Michael et al., 2010). In response to guidance cues, MIG-10 acts to…. (Figure 1).

mig-10 affects axonal guidance in the anterior ventral mechanosensory neuron (AVM) in addition to cell migration (Quinn et al., 2006; Chang et al., 2006; Quinn et al., 2008). MIG-10 mediates axon guidance in attractive responses to high concentrations of UNC-6 (netrin), and repulsive responses to SLT-1 (Slit). The overexpression of MIG-10 in the AVMs enhances axon

outgrowth, similarly in HSNs, through these chemotropic characteristics. In the absence of UNC-6 and SLT-1, the axons of the AVM failed to migrate, but through an overexpression of MIG-10 in the absence of UNC-6 and SLT-1, a multipolar phenotype was expressed in 25% of the AVM. In response to UNC-6, the AVM axon failed to migrate in 39% of the neurons, but an overexpression of MIG-10 decreased these errors to 19%. In response to SLT-1, the AVM axon failed to migrate in 33% of the neurons, but an overexpression of MIG-10 decreased these errors to 16% (Quinn et al., 2006). These data suggest that *mig-10* is an essential player in the development of axons, demonstrating that the localization of MIG-10 by guidance cues allows the outgrowth to be in the correct direction..

MIG-10 In Complex with a Signaling Module

It is important to understand the signaling pathway of MIG-10 and its effects on the migration of neurons, and to further investigate key players also related to MIG-10.. Yeast two-hybrid screens have shown interactions *in vitro* between MIG-10 and two other molecules, CSN-5 and ARX-3 (Gosselin and O'Toole, 2008).

CSN-5, a COP-9 signalosome subunit

COP-9 signalosome complex subunit 5 (CSN-5) is one of eight subunits in the conserved nuclear protein complex involved in significant biochemical processes of both plants and animals. Identifying the subunits of the CSN complex reveals linkage to the 26S proteasome, the most common form of a proteasome. A yeast two-hybrid analysis shows direct interaction between COP-9 signalosome and cullin-based subunits of ubiquitin ligases (Schwechheimer et al., 2001). The interaction between COP-9 and the SCF-type E3 ubiquitin ligases, which regulate protein degradation, suggest a key role in eukaryotic development. CSN is found to associate with protein degradation, but is also associated with kinase activity responsible for de-

ubiquitylation of the E3 complexes. In several studies, E3 complexes possessed poly-ubiquitylation activity in the absence of CSN. These findings present an additional control of proteolysis by CSN (Schweichheimer, 2004). In a study of CSN-5's role in the regulation of UNC-96, research has shown diminished levels of UNC-96 in the loss-of-function mutation in *csn-5*. These results suggest that CSN-5 is involved in the stabilization of UNC-96 and may be responsible for the stabilization of other proteins, such as MIG-10.

The most studied subunit is CSN-5, or Jab-1. This protein is a c-Jun activation-domain binding protein, which stabilizes and co-activates the transcription factor. This interaction regulates intracellular signaling, and is involved in a number of pathways, such as, integrin signaling, cell cycle control, and other pathways key for cellular development (Shackleford and Claret, 2010). There is no research that has shown a direct interaction between MIG-10 and CSN-5, but if experiments show a dependent interaction within the same pathway, the silencing of CSN-5 may show effects on the migratory ability of neurons. Since it is also involved in the interactions of integrin signaling, the silencing could inhibit the interactions of cell-to-cell adhesions, resulting in loss of motility.

ARX-3, a component of the Arp2/3 complex

An essential part of neuronal migration is the proper development of the actin cytoskeleton. Arp2/3 complex component-3 (ARX-3) is one of several subunits of the Arp2/3 complex, which nucleates the formation of branched-filament-actin networks. These networks are configured in a y-branch arrangement at the junction of a new filament and pre-existing filament, most understood to be initiated by nucleation-promoting factors (NPFs) (Goley, 2006). ARX-3 is the ortholog of p41-ARC, subunit of the human homolog ARP2/3 complex. Loss of

ARX-3 was shown to exhibit defects in actin organization, more specifically in nucleating actin polymerization (Welch et al., 1997).

ARP2/3 complex is activated by WAVE proteins, also known as NPFs. The conserved C-terminal domain of the WAVE protein initiates the polymerization of actin by bringing together the ARP2/3 complex and actin monomers. Through electron microscopy, ARP2/3 complex has been shown to form F-actin networks localized at the leading edge of the cell, within the lamellipodia. This localization is consistent with its necessary function for cell migration and possible growth cone elongation. Upon studying the activation of ARP2/3 complex by WAVE proteins, ABI-1 (an essential player in the MIG-10 signaling pathway) is known to be an essential subunit of the WAVE regulatory complex (Disanza et al., 2005). It was proposed that ABI-1 shares a relationship to another protein, UNC-53, which is essential to axon migration and involved in the development of the actin cytoskeleton (Schmidt et al., 2009). Out of three human homologs to UNC-53, NAV2 (Neuron Navigator 2) is most similar. In C. elegans, UNC-53/NAV2 was proposed to act as a scaffold for signal transduction in the development of the actin cytoskeleton; possibly linking ABI-1 to the ARP2/3 complex. Experimental results display migration defects in the loss of *abi-1*, similar to the loss of *unc-53* or the ARP2/3 complex subunits (Schmidt et al., 2009).

RNAi

A popular and efficient approach to studying specific genes responsible for neuronal migration is the manipulation of genes using the introduction of double-stranded RNA (dsRNA). RNA interference (RNAi) inactivates specific gene sequences, enabling it to be used as a tool for reverse genetics. RNAi acts post-transcriptionally. The introduced dsRNA is degraded into smaller segments, commonly known as small interfering RNA (siRNA), by an endonuclease, a

dicer. The siRNA then binds to the Argonaute protein forming RNA-induced silencing complex, or RISC, which selectively targets sequences on the mRNA to form complementary binding with the sequence of a single-stranded siRNA. Once RISC has bound to mRNA, a hybrid is formed between the two strands, and RNase is activated to cleave the RNA. Specific genes are then silenced by these siRNAs (Fire et al., 1998).

RNAi can be implemented in *C. elegans* through three different methods: soaking the worms in dsRNA, injecting the dsRNA into the head or tail of the worm, or by feeding dsRNA, expressed in *Escherichia coli* bacteria (Kamath et al., 2003). The feeding method is favored by researchers because it produces results in a timely and inexpensive manner. To allow the feeding method to become a high-throughput tool, RNAi sensitized strains are used for experimentation. Normally the cells within the nervous system of *C. elegans* do not experience the systemic intake of dsRNA. A transmembrane protein, systemic RNAi-deficient (s*id-1*), must be expressed on the neuronal cells to allow uptake of the dsRNA. Research has tested strains against each other in order to detect the efficiency levels of dsRNA uptake in neurons by expressing the SID-1 protein (Calixto et al., 2010). Particularly effective was the TU3595 strain, a neuron-specific strain, specifically being used for this experiment to uptake dsRNA in the touch receptor neurons, AVM, ALML and ALMR.

Project Goal: Establishing an RNAi system in lab

Control experiments were used to establish the RNAi system. Three controls were performed: silencing genes *unc-22, abi-1,* and an empty vector control. The gene *unc-22* is responsible for regulation of muscle contractions in *C. elegans* and should express a twitching effect in the presence of RNAi. The gene *abi-1* is essential for proper actin polymerization.

Worms expressing a knockdown of *abi-1* should show similar neuron migration defects as *mig-10* mutants.

Project Goal: Analyzing the effects of gene silencing

Once the RNAi method was established it was used to silence the genes *csn-5,* and *arx-3*. We predicted that these genes would show similar phenotypes to *mig-10,* since we hypothesized they interact within the *mig-10* pathway. Two control strains were used in comparison to the RNAi-expressed strain: the strain, TU3595, is specific to neurons, whereas the strain, NY2054, is non-specific. Our goal was to determine which cell types the genes were functioning in.

In another experiment, past research using the strain *mig-10(ct41);pgp-12::gfp* has shown truncation of the excretory canal, and anteroposterior processes of the excretory cell. If CSN-5 normally stabilizes MIG-10, loss of *csn-5* would potentially cause truncation. If *csn-5(RNAi);mig-10(ct41);pgp-12::gfp* expresses a more severe truncation of either process, we can then suggest they are involved within the same signal transduction pathway. If no truncation is observed within either process, this is conclusive with the theory they belong to separate pathways.

Methods

Strains used in the project

NY2054 *ynIs54[Pflp-20::GFP] IV; him-5 (e1490) V.*
RY1211 *mig-10(ct41) III; ynIs54 IV*
TU3595 *sid-1(pk3321) him-5(e1490) V; lin-15(n744) X; uIs72*
uIs72 [pCFJ90(myo-2p::mCherry) + unc-119p::sid-1 + mec-18p::mec-18::GFP].

Preparation of RNAi Plates

The RNAi plates were prepared using NGM agar; IPTG and carbenicillin were added after autoclaving to final concentrations of 1mM IPTG and 25µg/mL carbenicillin. These plates were allowed to set under the tissue culture hood, with the blower on for the first 2-3 hours to facilitate drying, and remained in the hood overnight at room temperature, and stored at 4°C .

Inoculation and seeding of RNAi strains

RNAi strains were streaked onto Ampicillin/Tetracycline (Amp/Tet) plates (LB + 50 µg/ml Amp + 12.5 µg/ml Tet). Using sterile pipette tips, colonies were picked from each plate and inoculated into 5mL media (LB + 50 µg/ml Amp) in sterile 15 mL conical tubes. Tubes were placed in a rotating drum in a floor incubator at 37°C for 14 hours overnight.

After inoculation, the cultures were centrifuged in a balanced table top centrifuge at 2,000 x g for 2 minutes. About 4mL of the supernatant was discarded, and the pellet was resuspended in 1mL of supernatant by vortexing. Following sterile procedures, the RNAi plates were seeded under the tissue culture hood with the blower on. For each RNAi strain, 3 plates were seeded using 3-4 drops of the cultured RNAi bacteria using a sterile Pasteur pipette. These plates were allowed to set overnight for 24 hours under the tissue culture hood, with the blower

on for the first 2-3 hours. If the plates were still wet the following day, they were set to dry with the blower on for an additional 10-20 min. before plating.

Transfer of worms to RNAi plates

C. elegans were picked in the L4 stage from non-starved plates and transferred to primary RNAi plates (Table 1). 4-6 worms were transferred to each plate. The plates were stored at 20°C for 14-15 hours.

After about 15 hours, 4-5 gravid adults were transferred onto secondary RNAi plates. These plates were prepared following the same procedure as the primary plates. The primary and secondary RNAi plates were stored at 20°C for 45-50 hours. L4 progeny were then quantified.

Table 1: Timetable for RNAi Feeding.

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
8:00 AM							
9:00 AM							
10:00 AM			Seed Primary Plates	Seed Secondary Plates			
10:15 AM				Transfer P0 L4s to Primary Plates			
11:00 AM							Score Worms
12:00 PM							
1:00 PM					Transfer Parents on Primary Plates to Secondary Plates		
2:00 PM							
3:00 PM							
4:00 PM							
5:00 PM							
6:00 PM							
7:30 PM		Inoculate Primary Cultures	Inoculate Secondary Cultures				
8:00 PM							

Mounting Worms on Slides

Using a sterile Pasteur pipette, several drops of M9 were added to the secondary RNAi plate until completely covering the plate, and swirled gently. The liquid solution was then transferred to a 1.5 mL Eppendorf tube using a Pasteur pipette. The Eppendorf tube was vortexed in a microcentrifuge for 20 seconds.

Premade 2 ml aliquots of 2% agarose were heated to liquid in a microwave (1:30 minutes). An initial 20µL of 1M sodium azide was added to the liquid agarose. Two drops of the sodium azide and agarose solution were added to the center of a blank slide and another blank slide placed atop to form an agarose pad. Using a 20µL pipette, 4µL of the worm pellet was added to the agarose pad and a blank cover slip was quickly added.

Quantifying L4s from RNAi feeding

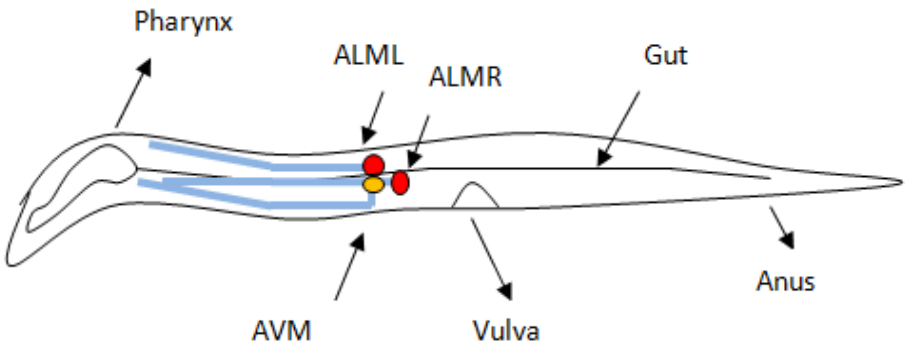

Figure 2: A schematic of the ALM and AVM neurons in C. elegans.

Under a microscope, the L4 progeny worms were examined using DIC and fluorescent imaging. Images of the neurons were used for measurements of the following: the posterior of the pharynx to the vulva (PV), the position of both ALM neurons from the posterior of the pharynx to the neuron cell body (ALMP), and the position of the AVM from the vulva to the

neuron cell body(AVMV). Normalized migratory distances were calculated as ALMP/PV and AVMV/PV.

Statistical analysis of different strains

Normalized migratory distances (NMD) were compared for various experimental strains using ANOVA. Using the program IBM SPSS Statistics 19, means were distinguished using the Tukey HSD post-hoc test.

Results

The gene CSN-5 has previously shown putative interactions with MIG-10 (Gosselin and O'Toole, 2008; Sullender, 2012). In order to test the effects of *csn-5* on the development of neurons and their migrations, we used RNAi to knockdown *csn-5*. In an attempt to measure cell autonomy of CSN-5, RNAi was used in two different strains: TU3595, an RNAi-sensitized strain that is specific to neurons, and the strain NY2054, a non-specific strain. Controls were used in order to establish the functionality of this method in lab.

Setting Up the RNAi System

Using the strains, TU3595 and NY2054, RNAi was tested using three controls: UNC-22, ABI-1 and an empty vector (Figure 3). Two different worm strains were used. NY2054 contains the marker gene *flp-20::GFP*, which labels the mechanosensory neurons, including ALML, ALMR, and AVM. TU3595 contains the *sid-1* mutation *sid-1(pk3321)* as well as a transgene array with the *unc-119::sid-1* construct; thus SID-1 is expressed specifically in neurons in this strain, and should allow RNAi to act only in the neurons. The array also contains *mec-18::GFP*, which labels the mechanosensory neurons. The gene *unc-22* is responsible for the regulation of muscle contractions in *C. elegans*. RNAi of *unc-22* displayed a twitching effect in the NY2054 strain, due to the loss of muscle contraction control (not shown), suggesting that the RNAi system was working.

The gene *abi-1*, essential to actin polymerization, encodes for an adapter protein that interacts with *mig-10*. Since we know that *abi-1* mutants affect migration of the ALML neuron (McShea et al., 2013), we predicted that RNAi of *abi-1* would also truncate these migrations. As shown in Figure 3, *abi-1(RNAi)* exhibited a truncation phenotype in the NY2054 strain. As

expected, the extent of truncation was similar to that observed in *mig-10(ct41)* mutant animals (strain RY1211).

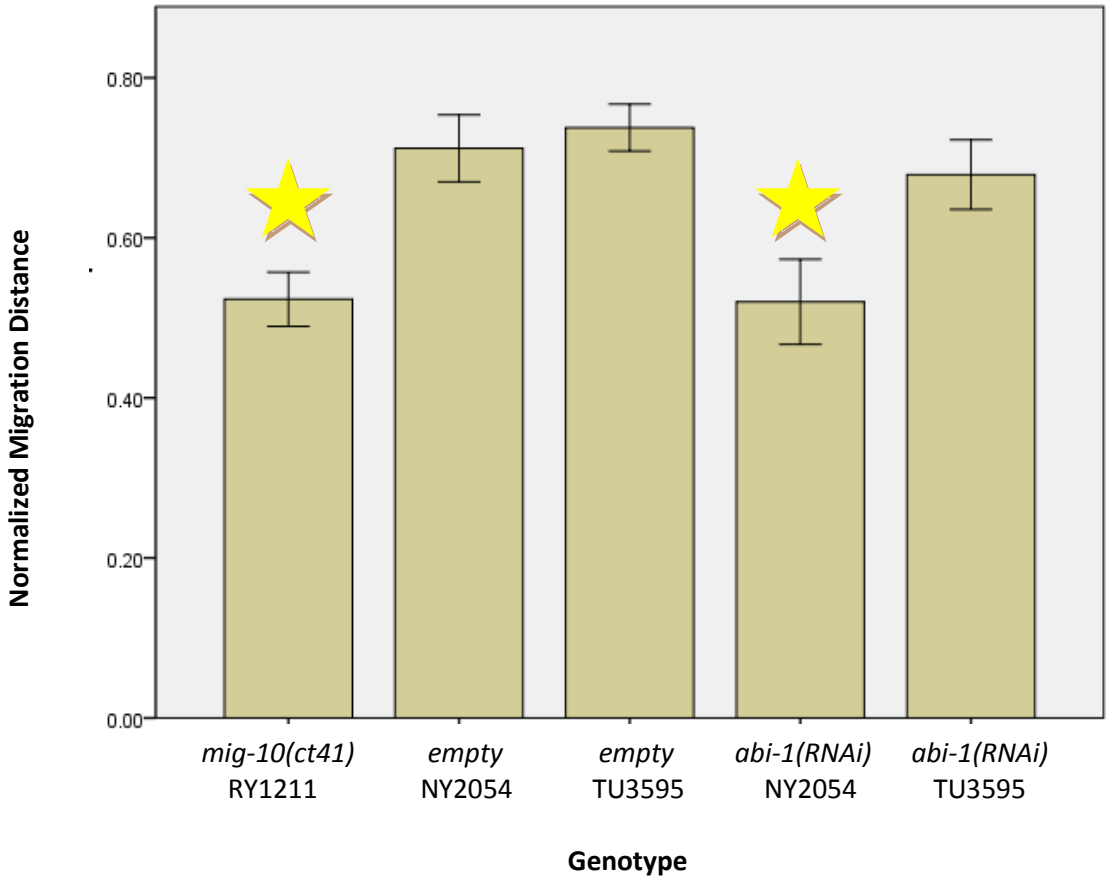

Figure 3: RNAi of *abi-1* in NY2054 and TU3595 Strains. Both RY1211 and *abi-1(RNAi)*, in the NY2054 strain, show similar migration phenotypes and are not significantly different from each other (indicated by stars) in the migration of the ALML neuron. These phenotypes are significantly different from the empty vector in the NY2054 strain, among the other non-starred graphs. The non-starred graphs are not significantly different from each other. Each strain collected data from a specific number of worms, n. RY1211, n=24 , empty vector NY2054, n=27 , empty vector TU3595, n= 22,*abi-1(RNAi)* NY2054, n= 21, and *abi-1(RNAi)* TU3595, n=20 . This analysis used ANOVA followed by a Tukey post-hoc test, with a p value of 0.05.

It is important to note that the migrations of the ALMR and AVM neurons showed no significant difference in either of the strains when comparing *abi-1(RNAi)* and empty vector, and therefore are not shown here. Since the TU3595 strain was not affected by *abi-1(RNAi)*, these results may suggest that *abi-1* does not function in the ALM neuron. In general, these results suggested that the RNAi system was working and could be applied to the gene of interest, CSN-5.

csn-5(RNAi) Shows An Effect on the Migration of the ALML Neuron

In order to test the hypothesis that CSN-5 is involved in the migrations of the AVM, ALML and AMLR neurons, we performed experiments of *csn-5(RNAi)* in comparison to an empty vector as a control, using both NY2054 and TU3595 strains. Representative phenotypes are shown (Figure 4).

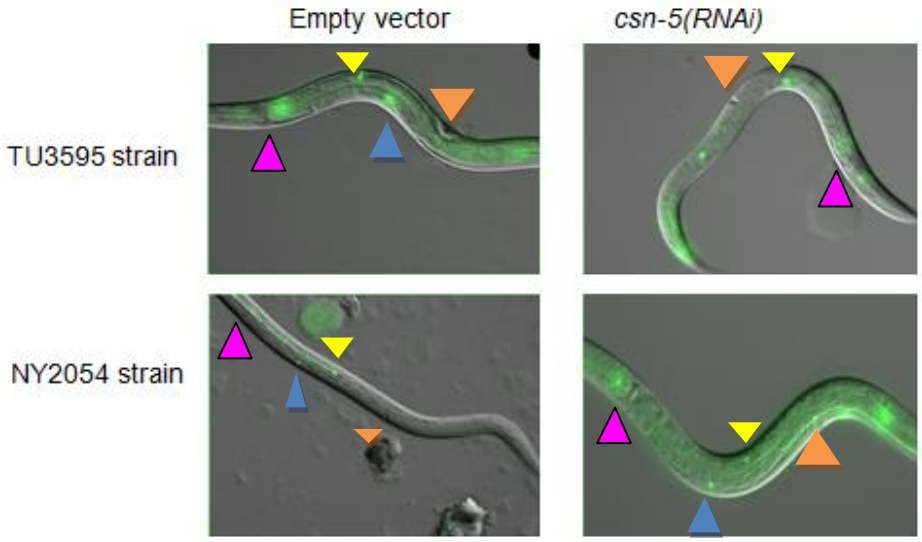

Figure 4: Migration phenotypes of csn-5(RNAi). An experimental design using strains, TU3595 (neuron-specific) and NY2054 (non-specific) in the presence of an empty vector and *csn-5(RNAi)*. The arrows indicate the posterior pharynx (pink arrowhead), the vulva (orange arrowhead), the AVM neuron (blue arrowhead), and the ALMR neuron (yellow arrowhead).

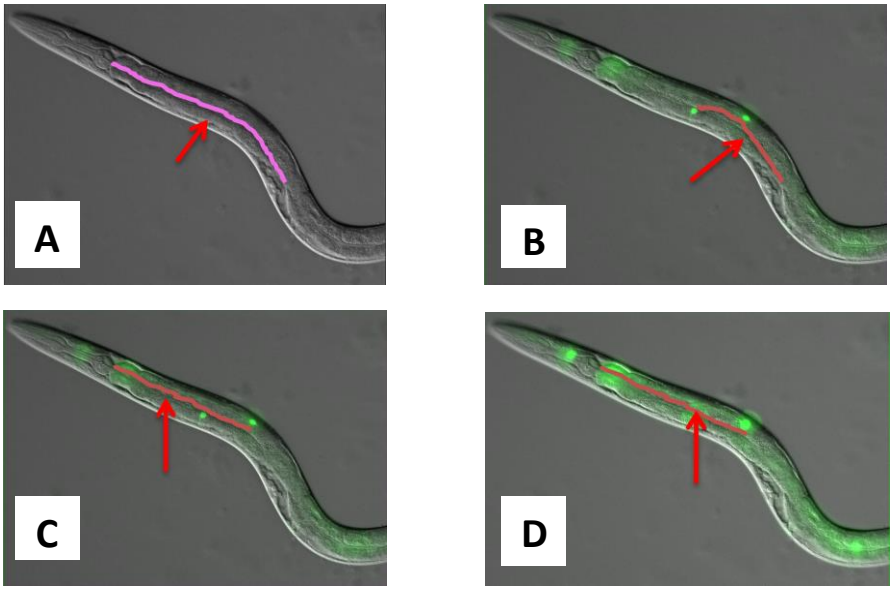

Figure 5: Measurements of the AVM, ALML, ALMR at the L4 Stage. *A*, The distance between the posterior pharynx and vulva. *B*, The distance of the AVM position in regards to the vulva. *C*, The distance of the ALMR position in regards to the posterior pharynx. *D*, The distance of the ALML position in regards to the posterior pharynx. *B-D* are divided by *A* in order to normalize the migration distance. This figure displays the migration of the neurons in the wild-type strain, NY2054.

We measured the distance of each neuron position from either the posterior pharynx (ALM) or the vulva (AVM) and normalized it by dividing by the total distance between the posterior pharynx and the vulva (Figure 5). *csn-5(RNAi)* of the NY2054 strain caused truncation of the migration of the ALML neuron, similar to the phenotype of RY1211 (Figure 6).

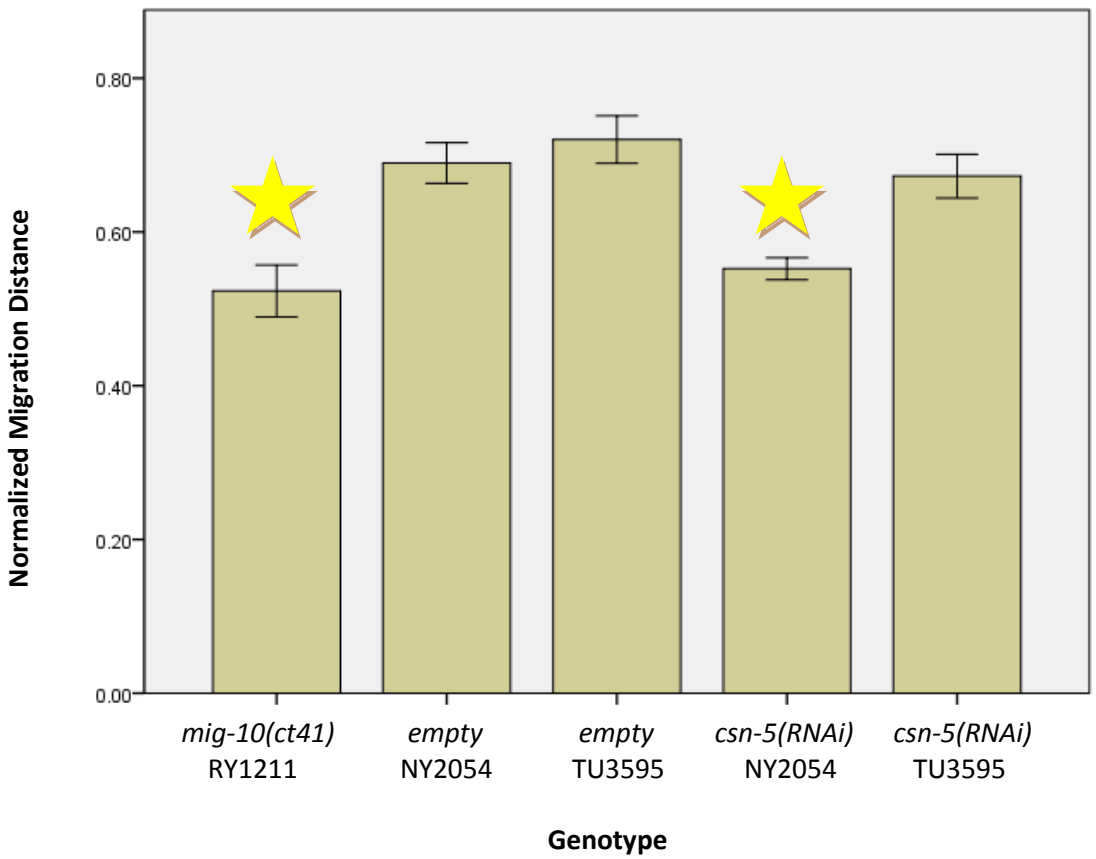

Figure 6: csn-5(RNAi) Shortens the Normalized Migration Distance of the ALML Neuron. Both RY1211 and *csn-5(RNAi),* in the NY2054 strain, show similar phenotypes and are not significantly different from each other (indicated by stars) in the migration of the ALML neuron. These phenotypes are significantly different from the empty vector in the NY2054 strain, among the other non-starred graphs. The non-starred graphs are not significantly different from each other. Each strain collected data from a specific number of worms, n. RY1211, n= 51 , empty vector NY2054, n= 42 , empty vector TU3595, n= 25, *csn-5(RNAi)* NY2054, n= 41, and *csn-5(RNAi)* TU3595, n= 34 . This analysis used ANOVA followed by a Tukey post-hoc test, with a p value of 0.05.

The migration of the ALMR neuron showed no significant difference in migration distance in *csn-5(RNAi)* animals compared to the empty vector in both strains, TU3595 and NY2054, (Figure 7).

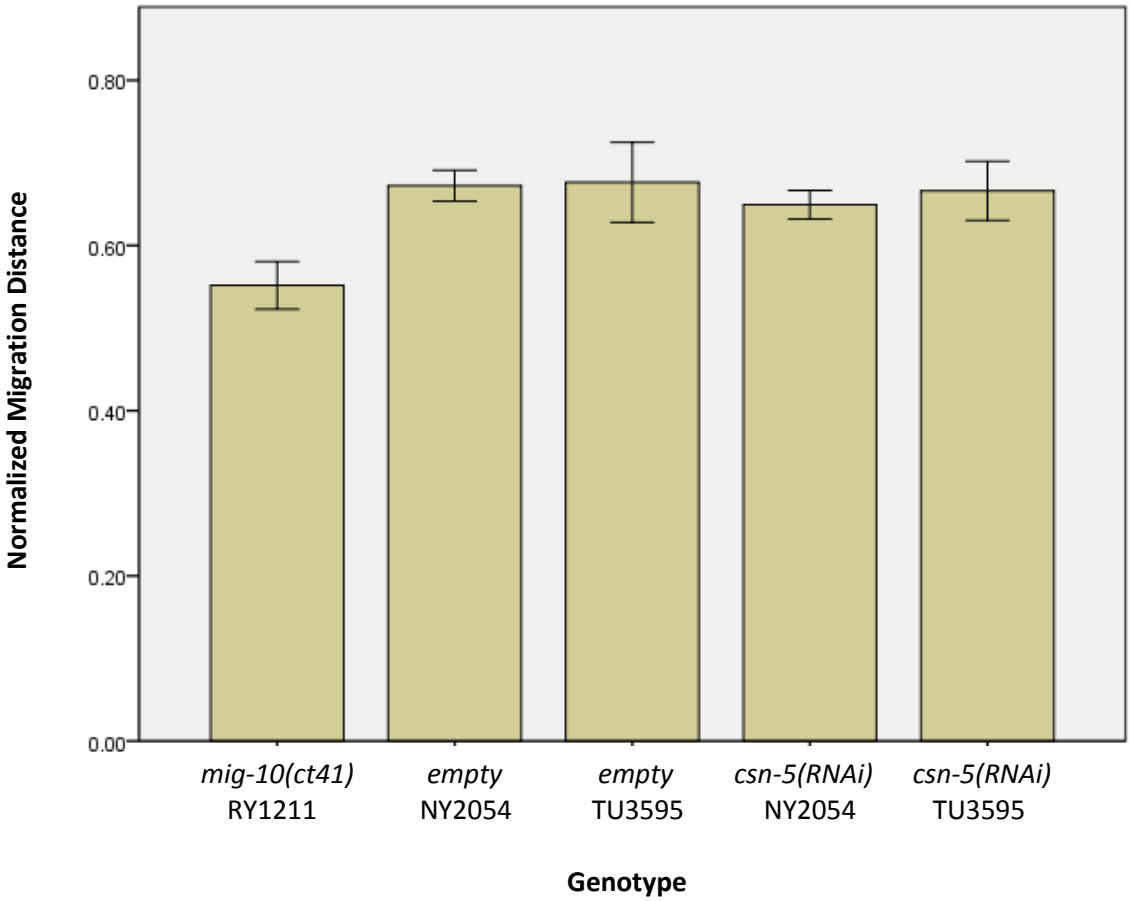

Figure 7: csn-5(RNAi) Does Not Shorten the Normalized Migration Distance of the ALMR Neuron. Phenotypes displaying the migration of the ALMR neuron show no significant difference in either strain in the presence of *csn-5(RNAi).* Notice the RY1211 strain is the only strain mutation showing an effect on the neuron's migration, which is expected considering it was used as a control. Each strain collected data from a specific number of worms, n. RY1211, n= 51 , empty vector NY2054, n= 42 , empty vector TU3595, n= 25, *csn-5(RNAi)* NY2054, n= 41, and *csn-5(RNAi)* TU3595, n= 34 . This analysis used ANOVA followed by a Tukey post-hoc test, with a p value of 0.05.

These results suggest that *csn-5(RNAi)* disrupts the migration of one of these neurons, consistent with the model that this gene displays important interactions with MIG-10 *in vivo.* The fact that ALML migration was truncated in the NY2054 and not the TU3595 strain may suggest that *csn-5* does not function within the neuron, considering TU3595 is a neuron-specific strain.

Results regarding the migration of the AVM neuron are inconclusive due to little or no visibility of the neurons in some of the subjects, especially in the TU3595 strain. Further

experiments should be performed to test the significance of CSN-5 in regards to the AVM neuron.

csn-5 data suggests it belongs within the same pathway as *mig-10*

Further experiments were done to show if *csn-5(RNAi)* had an effect on another phenotype experienced with *mig-10(ct41)*. *mig-10(ct41)* mutant animals display truncation of the posterior and anterior processes of the excretory cell; this phenotype can be observed using the *pgp-12::gfp* marker, which is expressed in the excretory cell. Although the excretory cell is not a neuron, its outgrowth is controlled by many of the same genes as those that control neuronal migration, including *mig-10*. We measure the subjects using a similar method (Figure 8) as described in the previous experiment with the AVM, ALMR, and ALML neurons.

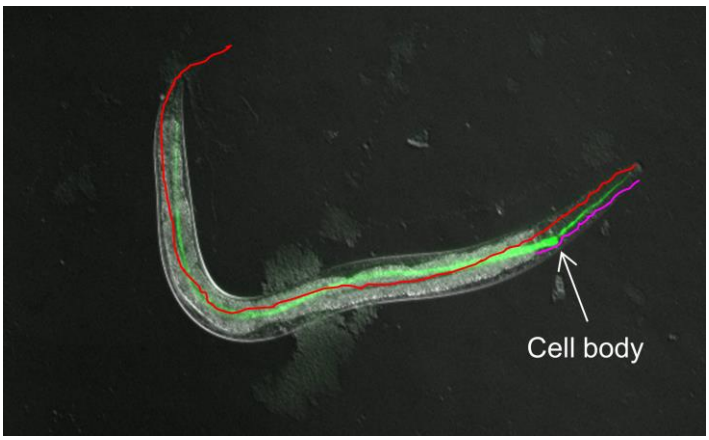

Figure 8: Measurement of the Anteroposterior Processes of the Excretory Cell. The anterior process of the excretory cell is measured from the middle of the cell body to the end of the process (pink line). This measurement is then divided by the red line from the posterior pharynx to the tip of the nose. The posterior process of the excretory cell is measured from the middle of the cell body to the end of the green process located posterior of the pharynx (green line). This measurement is then divided by the length of the body, indicated by the red line, measuring from the tip of the tail to the tip of the nose. These measurements are done to normalize the anteroposterior processes.

Considering we know that *mig-10(ct41)* exhibits a truncation in these processes and excretory canal, we tested *csn-5* to observe if it also functioned during the development of the

excretory cell. If *csn-5* and *mig-10* belonged in separate signal transduction pathways we would observe a more severe truncation when we performed *csn-5(RNAi)* in a *mig-10(ct41)* background, but if we do not see a more severe truncation, we can conclude they belong in the same pathway. Below (Figure 9) the anterior proportions of the excretory cell are displayed using *csn-5(RNAi)*.

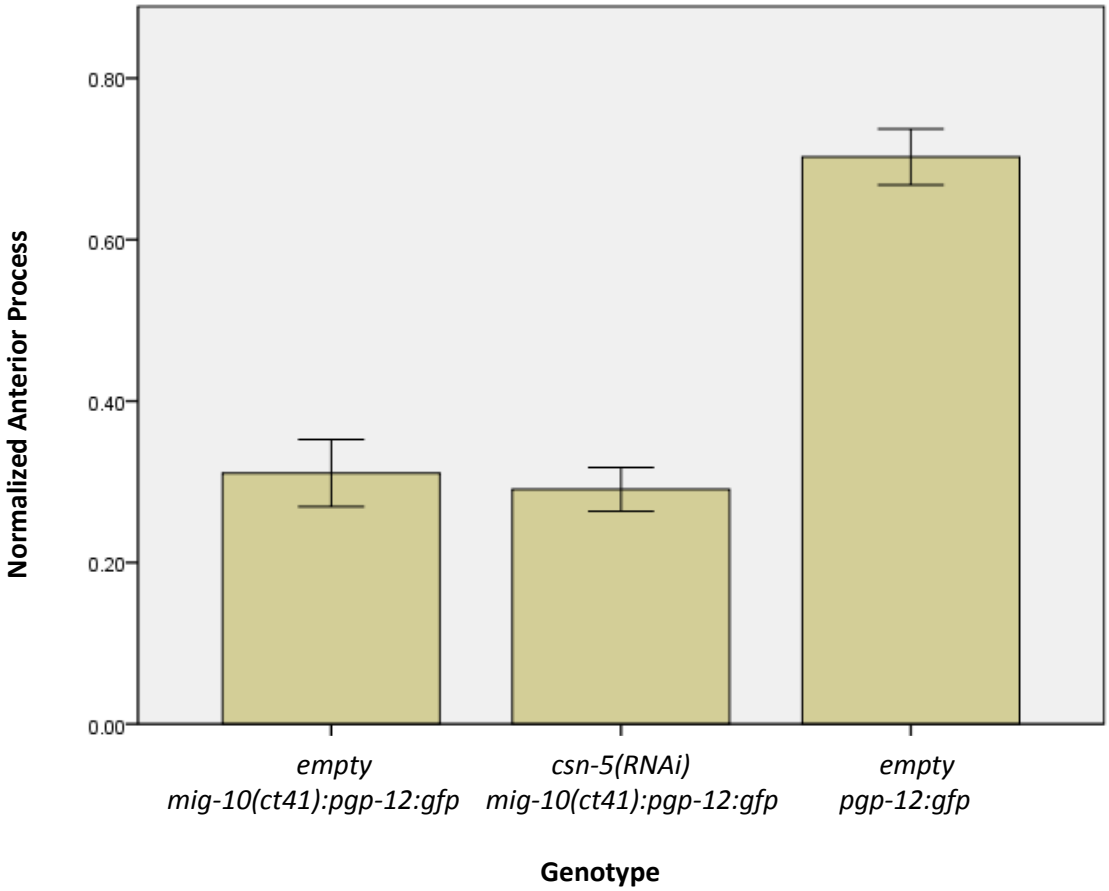

Figure 9: Normalized Anterior Process of the Excretory Cell in C. elegans. Data was collected and analyzed using ANOVA to display any effects on the anterior process of the excretory cell in a *mig-10(ct41);pgp-12::gfp* strain. *Left*, shows the truncation of the anterior process in the *mig-10* mutant using an empty vector. *Middle*, displays *csn-5(RNAi)* in a *mig-10(ct41)* background. *Right*, the empty vector in a wild-type strain.

There is no significant difference between *csn-5(RNAi);mig-10(ct41);pgp-12::gfp* and *mig-10(ct41);pgp-12::gfp.* This suggests that *csn-5* functions within the same pathway as *mig-10.* This can also be observed for the posterior process shown below in Figure 10.

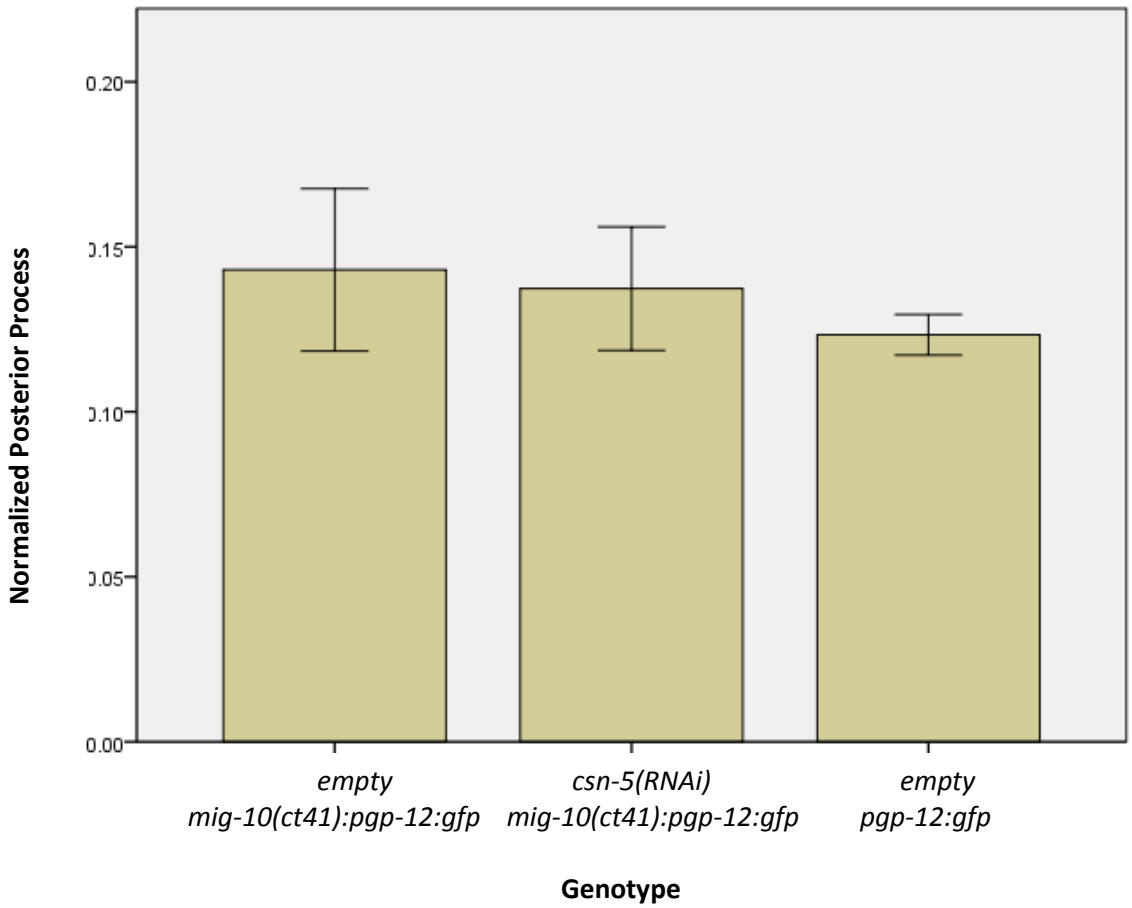

Figure 10: Normalized Posterior Process of the Excretory Cell in C. elegans. Data was collected and analyzed using ANOVA to display effects on the posterior process of the excretory cell in a *mig-10(ct41);pgp-12::gfp* strain. *Left,* shows the truncation of the posterior process in the *mig-10* mutant using an empty vector. *Middle*, displays *csn-5(RNAi)* in a *mig-10(ct41)* background. *Right,* the empty vector in a wild-type strain.

Results exhibit that there is no significant difference from the empty vector and *csn-5(RNAi)* in the *mig-10(ct41)* background. It is interesting to note that the wild-type strain (shown on the *right)* shows a severely truncated posterior process of the cell. This data shows no significant difference between the *mig-10(ct41)* phenotype and wild-type, inconsistent with results from previous experiments. Something may have gone wrong with the strain at some point during the experiment and further experiments should be done with this strain to make further conclusions.

Discussion

CSN-5 is the *C. elegans* ortholog of subunit 5 in the COP9 signalosome (CSN), which regulates the process of protein degradation. This signalosome can either stabilize proteins or promote degradation of protein (Millet et al., 2009). Researchers have also identified CSN subunits as critical components in integrin signaling, which play crucial roles in the interaction of the cell and its environment (Shackleford and Claret, 2010). Previous experiments had shown *in vitro* interactions of MIG-10 and CSN-5 through a yeast-two hybrid screen. We were interested in observing any interactions *in vivo,* and searching for similar phenotypes shown between mutations of *mig-10* and *csn-5.*

Through the method of RNAi, our results concluded that *csn-5(RNAi)* in the NY2054 strain displayed a similar phenotype in the migration of the ALML neuron as is observed in *mig-10(ct41)* mutant animals. The strain TU3595, which allows neuron-specific RNAi, showed no truncation in the ALML migration, which leads us to believe that CSN-5 may not function in the neurons. This conclusion cannot be made, however, because our control expressing *mig-10 (RNAi)* in the TU3595 strain did not show a truncation, as well. Research has shown us that MIG-10 does function within the neuron, leaving us with the theory that something may have gone wrong with the TU3595 strain at some point during experimentation. Results did not show any significant difference of *csn-5(RNAi)* between the wild-type strains in the migration of ALMR or AVM neurons; the GFP marker in the TU3595 strain was very faint in the observation of the AVM migration. Since we saw the truncation in one of these neurons, this model suggests that CSN-5 normally stabilizes MIG-10. We believe that CSN-5 may play a critical role in the stabilization of the MIG-10 protein from being degraded.

Now that we know *csn-5* functions in the development of the ALML neuron, we were interested in observing if *csn-5(RNAi)* expressed similar phenotypes as *mig-10(ct41)* in the excretory cell. *mig-10(ct41)* shows truncation in the anterior and posterior process of the excretory cell (Manser and Wood, 1990). If the two proteins, CSN-5 and MIG-10, were working together in a simple linear pathway, *csn-5(RNAi)* in a *mig-10* mutant background should have no additional effect on the truncation. In contrast, if the two genes function within different pathways, an enhancement (greater truncation) would be expected. We concluded that there was no significant difference between our control, an empty vector in a *mig-10(ct41)* background, and the *csn-5(RNAi)* in a *mig-10(ct41)* strain. This phenotype is consistent with the theory that the two genes are in the same pathway, because the double knockdown displays no further truncation in either processes. These theories cannot be made into conclusions for a couple of reasons: the posterior process shows an unclear observation in the wild-type strain, and *csn-5(RNAi)* has not been tested independently. The posterior process expressed in the control, an empty vector expressing the *pgp-12::gfp* marker, shows a phenotype similar to the *mig-10(ct41).* This wild type strain should not have a truncated process; thus, this experiment needs to be repeated. We are also unsure if CSN-5 has any function within this pathway until we test *csn-5(RNAi)* independent of *mig-10(ct41).*

Further experiments should be performed to examine the validity of the strain expressing *pgp-12::gfp,* as well as performing further experiments expressing *csn-5(RNAi)* within this strain, independent of *mig-10(ct41).* It is also essential to further test the RNAi method with a positive control RNAi that produces a phenotype in the TU3595 strain. We did not see any effect on the TU3595 strain with *mig-10(RNAi),* which is inconsistent with the fact that MIG-10 does function within the neuron. We should observe a truncation in the migrations of these neurons

within the neuron-specific strain. For future research, it may be noted that the TU3595 strain does not grow at the same rate as the NY2054 strain, and may take an extra day to reach the L4 stage when following the schedule outlined in the methods section.

The study of neuronal migration in model animals is essential to understanding the processes within the human body. Neurons interact with one another through neurotransmission; neuronal migration is essential to the proper transmission of neurotransmitters. If this migration is disrupted, neurons cannot reach their destination and interact with other cells in order to send signals throughout the body. In this study, we have determined that CSN-5 may play a role in regulating the stability of the MIG-10 protein. This result may shed light on the control of homologous proteins in vertebrates that are important for development of the nervous system.

References

Calixto, Andrea, Dattananda Chelur, et al. "Enhanced Neuronal RNAi in C. elegans Using SID-1." Nature Methods. 7.7 (2010): 554-559.

Chang, Chieh, Carolyn E. Adler, et al. "MIG-10/Lamellipodin and AGE-1/PI3K Promote Axon Guidance and Outgrowth in Response to Slit and Netrin." Current Biology. 16. (2006): 854-862.

Cui, M., Chen, J., et al. SynMuv Genes Redundantly Inhibit lin-3/EGF Expression to Prevent Inappropriate Vulval Induction in C. elegans. Dev. Cell 10 (2006): 667–672.

Disanza, A., A. Steffen, et al. "Cellular and Molecular Life Sciences." Cellular and Molecular Life Sciences. 62.9 (2005): 955-970.

Fire, A, S Xu, MK Montgomery, SA Kostas, SE Driver, and CC Mello. "Potent and Specific Genetic Interference by Double-stranded RNA In *Caenorhabditis elegans*." Nature. 391.6669 (1998): 806-811.

Goley, Erin D., and Matthew D. Welch. "Nature Reviews Molecular Cell Biology." Nature Reviews Molecular Cell Biology. 7. (2006): 713-726.

Gossellin, Jennifer and O'Toole, Sean. "Mig-10, an Adapter Protein, Interacts with ABI-1, a Component of Actin Polymerization Machinery." Major Qualifying Project. (2008).

Hedgecock, EM, JG Culotti, et al. "The unc-5, unc-6, and unc-40 Genes Guide Circumferential Migrations of Pioneer Axons and Mesodermal Cells on the Epidermis in C. elegans." Neuron. 4.1 (1990): 61-85.

Huang, S., Lee, W.H., et al. "A Cellular Protein that Competes with SV40 T Antigen for Binding to The Retinoblastoma Gene Product." Nature. 350. (1991): 160–162.

Kamath, Ravi S., and Julie Ahringer. "Genome-wide RNAi screening in *Caenorhabditis elegans*." Methods. 30.4 (2003): 313-321.

Krause M, Leslie JD, Stewart M, Lafuente EM, Valderrama F, et al. Lamellipodin, an Ena/VASP ligand, is implicated in the regulation of lamellipodial dynamics. Developmental Cell. 7. (2004): 571–583.

Lu, Xaiowei and Horvitz, Robert. "*lin-35* and *lin-53*, Two Genes that Antagonize a *C. elegans* Ras Pathway, Encode Proteins Similar to Rb and Its Binding Protein RbAp48." Cell. 95. (1998): 981–991.

Manser, James, and William B. Wood. "Mutations Affecting Embryonic Cell Migrations in *Caenorhabditis elegans*." Developmental Genetics. 11. (1990): 49-64.

McShea, Molly A. "Evidence of An Anteraction Between the Actin Cytoskeletal Regulators MIG-10 and ABI-1." Major Qualifying Project (2009): 10-12.

Michael, Magdalene, Anne Vehlow, et al. "c-Abl, Lamellipodin, and Ena/VASP Proteins Cooperate in Dorsal Ruffling of Fibroblasts and Axonal Morphogenesis." Current Biology. 20. (2010): 783-791.

Miller, Rachel K., Hiroshi Qadota, et al. "CSN-5, a Component of the COP9 Signalosome Complex, Regulates the Levels of UNC-96 and UNC-98, Two Components of M-lines in *Caenorhabditis elegans* Muscle." Molecular Biology of the Cell. 20. (2009): 3610-3615.

Moore, SW, M Tessier-Lavigne, et al. "Netrins and their Receptors." *Advances in Experimental Medicine and Biology*. 621. (2007): 17-31.

Purves, Dale, George J Augustine, et al. Neuroscience. 2nd ed. Sunderland: Sinauer Associates Inc, 2001.

Quinn, CC, DS Pfeil, et al. "UNC-6/netrin and SLT-1/slit Guidance Cues Orient Axon Outgrowth Mediated by MIG-10/RIAM/lamellipodin." Current Biology. 16.9 (2006): 845-853.

Quinn CC, Pfeil DS, Wadsworth WG. CED-10/Rac1 Mediates Axon Guidance by Regulating the Asymmetric Distribution of MIG-10/lamellipodin. Current Biology 18. (2008): 808–813.

Riddle, DL, T Blumenthal, Barbara J Meyer, and James R Priess. C. elegans II. 2. 33. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1997.

Schmidt, Kristopher L., Nancy Marcus-Gueret, et al. "Development." Development. 136. (2009): 563-574.

Schwechheimer C. "The COP9 signalosome (CSN): An Evolutionary Conserved Proteolysis Regulator in Eukaryotic Development." Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 1695.1-3. (2004):45-54.

Schwechheimer C., and Deng XW. "COP9 Signalosome Revisited: A Novel Mediator of Protein Degradation." Trends Cell Biol. 11. (2001):420–426.

Shackleford, Terry J, and Francois X Claret. "JAB1/CSN5: A New Player in Cell Cycle Control and Cancer." Cell Division. 5.26 (2010).

Shannon, Adam J, Trevor Tyson, et al. "Systemic RNAi Mediated Gene Silencing In The Anhydrobiotic Nematode Panagrolaimus Superbus." BMC Molecular Biology. 9.58 (2008).

Stufflebeam, Robert. "Neurons, Synapses, Action Potentials, and Neurotransmission." The Mind Project. Consortium of Cognitive Science Instruction, 2008. Web. 30 Sep 2012. <http://www.mind.ilstu.edu/curriculum/neurons_intro/neurons_intro.php>.

Takai, Yoshimi, Jun Miyoshi, Wataru Ikeda, and Hisakazu Ogita. "Nectins and Nectin-like Molecules: Roles in Contact Inhibition of Cell Movement and Proliferation." Nature. 9. (2008): 603-605.

Thien, Christine B. F., Wallace Y. Langdon, et al. "CBL: Many Adaptations to Regulate Protein Tyrosine Kinases." Nature Reviews Molecular Cell Biology. 2. (2001): 294-307.

Valiente, Manuel, and Oscar Marín. "Neuronal Migration Mechanisms in Development and Disease." Current Opinion Neurobiology. 20.1 (2010): 68-78.

Welch, Matthew D., Angela H. DePace, et al. "The Journal of Cell Biology." Journal of Cell Biology. 138.2 (1997): 375-384.

Wilson, R., Ainscough, R., et al. "2.2 Mb of Contiguous Nucleotide Sequence from Chromosome III of *C. elegans*." Nature. 368. (1994): 32–38.