Analyzing the role of the *rbm-39* gene in *C. elegans* neuronal morphology

A Senior Thesis Presented to
The Faculty of the Department of Molecular Biology,
Colorado College

By

Cade Thumann
Bachelor of Arts
Colorado College
May 2022

Supervisor: Darrell Killian, Associate Professor
Molecular Biology Department

Acknowledgements

I would like to first express gratitude to my advisor and mentor, Dr. Darrell Killian, for accepting me into his lab this previous summer and taking the time to guide me through every stage of the research. His vast knowledge combined with his patience and kindness have made this research a delightful and rewarding experience. I am also extremely grateful for all the professors and colleagues I have had throughout the academic years. I would also like to thank Dr. Eugenia Olesnicky Killian for opening her lab up to me and sharing insights on RBM-39 roles in *Drosophila*, and Matt Bowers who took his time to engage with my research and be my secondary reader. This paper would not be the same without their supports.

I thank Colorado College Molecular Biology Department for all the supports inside and outside of the lab. I would also like to extend my thanks to the Alfred W. Alberts family for setting up the endowment for my summer research, for this opportunity allowed to further my knowledge in Molecular Biology and will set me up to succeed in the medical field. I genuinely appreciate it.

I also want to thank other members from Dr. Killian's lab, Duffy Doyle and Ana Musto, for working along side me in the lab and making summer research enjoyable. I am glad I was able to have this experience to get to know the both of you inside and outside of the lab.

Lastly, I would like to thank my parents for their constant love and support, as well as providing me with a great education. I would also like to thank my brother, sister, and girlfriend, Edie, for always being by my side and encouraging and motivating me during this process and throughout my life.

Abstract

Neuronal function is highly dependent upon dendritic and axonal processes that enable an extensive network of connections between neurons and is essential in the process of synaptic integration and signal transmission. The precise morphology of neurons allows the nervous system to operate functionally because of their established connectivity. Recent studies have identified many genes that regulate dendrite morphogenesis in genetic model systems such as *Drosophila melanogaster* (the fruit fly) and *Caenorhabditis elegans* (the worm). For example, *rbm-39*, which encodes an RNA-binding motif protein, has recently been revealed to play a role in dendrite development in worms, while the fruit fly homolog of this gene, *caper*, has been shown to be important for various aspects of nervous system development and function. The extent of the role of *rbm-39* in nervous system development, however, is still unknown. Using a strain of *C. elegans* that had the *rbm-39* gene removed by CRISPR genome-editing technology, we examined the role of *rbm-39* in various neuron and glia subtypes. Specific neurons/glia were quantitatively analyzed and compared between the *rbm-39* mutant and the control worm. Our results suggest that *rbm-39* has surprisingly minimal impacts on nervous system development compared to the role of its homolog in the fruit fly. Previous studies suggest that RBM-39 family proteins are involved in alternative splicing and other mechanisms of gene regulation. To learn more about the molecular role of the RBM-39 protein, we isolated and sequenced mRNA from *rbm-39* mutants and controls to identify differentially expressed and differentially spliced targets. The results from the sequencing strongly suggest that RBM-39 plays a role in the regulation of gene expression, and more specifically in the regulation of alternative splicing. Consistent with a role in nervous system development, our results show the *rbm*-39 regulates many genes with known specific functions in the nervous system and its morphology. Additionally, RBM-39 homologs are found in humans, so a deeper understanding of this protein in worms and flies could help further illuminate RBM-39 function in the context of human neuronal development.

Introduction

Dendrite Morphogenesis

Nervous system function relies on its complex morphology to process information from other cells or the environment. The dendritic and axonal processes, which create a network of connections between neurons, impact nervous system function and development based on their branching, growth, and maintenance (McFarlane et al., 2000). Understanding the molecular genetic mechanisms that underlie dendrite morphogenesis will allow us to better approach dendritic defects observed in neurological disorders such as autism, schizophrenia, and Alzheimer disease (Kulkarni et al., 2012). Molecular mechanisms underlying dendrite formation and morphogenesis have recently been studied and many are implicated in cell signaling within developmental processes (Gao et al., 2007). Studies have also found molecules known to play a role in neuron survival and axon guidance have an effect in dictating the shape, direction, and growth of dendrite branching. There are many different molecular and cell biological mechanisms that control dendrite morphogenesis, including transcription regulators, secreted proteins, cell surface receptors, and cell adhesion molecules. For example, the homeodomain transcription factor protein, CUT, acts as a multi-level regulator of type-specific dendrite morphology of larval dendritic arborization neurons (Jan et al., 2010). Scientists have recently identified specific genes that regulate dendrite morphogenesis in model systems including *D. melanogaster* and *C. elegans* (Olesnicky et al., 2014; Antonacci et al., 2015). A genetic screen of RNA-binding protein-encoding genes in the fruit fly revealed that many of these genes impact neuronal morphology. More recently, a core set of RNA-binding proteins (RBPs) that impact dendrite morphogenesis in the PVD sensory neuron were identified in *C. elegans* (Antonacci et al., 2015). These two studies identified several evolutionarily conserved RBPs that impact nervous system development across species. For example, *rbm-39*, which encodes an RNA-binding motif protein, has been shown to play a role in dendrite morphogenesis in worms, while the fruit fly homolog, *caper*, is essential for various aspects of nervous system development and function (Olesnicky et al., 2014; 2017; Antonacci et al., 2015; Titus et al., 2021).

RNA-Binding Proteins

RNA-Binding Proteins (RBPs) have important function in gene expression and regulation such as mRNA transport, mRNA stability, alternative splicing, mRNA localization, and translational control. There are a few examples of mutations in RBP-encoding genes that lead to neurologic disorders, including the mammalian Hu/embryonic lethal abnormal vision-like (ELAVL) family of RBPs. A sensory neuropathy syndrome is caused by the production of autoantibodies against the Hu family proteins triggered by their ectopic expression in small cell lung cancers (Dalmau et al., 1992). It is not certain, however, the exact

number of RBPs that specifically regulate dendrite morphogenesis (Zhou et al., 2014). An RNAi screen was run in a recent study to determine the contribution of post-transcriptional gene regulation to neuronal morphogenesis. It was found that 63 RBP-encoding genes in the *Drosophila* genome impact dendrite development in larval class IV dendritic arborization neurons (Olesnicky et al., 2014). It was not clear, however, the extent of which RBPs were conserved throughout animal species, so Antonacci *et* al (2015) identified a core set of RBPs that are conserved throughout species and are important for dendrite morphogenesis in the PVD sensory neuron. The core set of RBP genes include CGH-1, CPB-3, DCR-1, DDX-17, LARP-5, MBL-1, MTR-4, RSP-3, RSP-6, SET-2, SUP-26, and RBM-39. Six of these twelve RBPs (MBL-1, RSP-3, RSP-6, SET-2, RBM-39, and DDX-17) that were found to be important for PVD dendritic morphogenesis are also known or predicted to be involved with alternative splicing. This strongly suggests that a proper regulation of alternative splicing is important for the process of dendrite development, and/or that there are important genes that have splice form-specific roles in dendrite morphogenesis. Very little is known about the relationship between alternative splicing and neuron development despite the evidence suggesting an important connection.

Alternative Splicing

The process of alternative splicing is an important step in eukaryotic gene expression that involves the inclusion or exclusion of various sequences within a messenger mRNA precursor (pre-mRNA) to produce variably spliced mRNAs. There are many alternatives that lead to variable outcomes in mRNA isoforms, including exon skipping, intron retention, mutually exclusive exons, and 5' or 3' alternative splice usage (Zheng et al., 2005). Multiple studies predict that the percent of human genes that are alternatively spliced range from 40-60% (Croft et al., 2000; Hide et al., 2001; Kan et al., 2001). Alternative splicing plays an important role in *C. elegans* development, for it is approximated that about 25% of *C. elegans* genes undergo alternative splicing. The intron and exon structure of *C. elegans* is extremely similar when compared to higher eukaryotes, which is a prime reason why they serve as a good model for the study of alternative splicing (Zahler et al., 2005). An example of a gene found in *C. elegans* that is alternatively spliced is a curated coding gene encoded by *unc-32*, that can be alternatively spliced to yield 6 different isoforms (Oka et al., 2001). Another example of alternative splicing in *C. elegans* is the trans-splicing of operons, which occurs with the *cha-1* and *unc-17* genes. While these two genes encode for two separate functions, analysis of transcripts of the genes indicate they share a common promoter as well as a 5' untranslated region. In this case, alternative splicing is used to produce two different coding transcripts from a single promoter (Blumenthal et al., 2018)

Alternative splicing plays a large role in the nervous system by generating diversity and specificity. In fact, a recent study of human tissues found that out of 11 different tissue samples, the cerebellum displayed the highest degree of alternative splicing, containing about 50% more differentially expressed alternative exons than the testis, the tissue with the next highest level of splicing (de La Grange et al., 2010). This suggests that regulated splicing could potentially be an efficient mechanism for generating molecular and cellular diversity in the nervous system.

In eukaryotic cells, the catalytic event of splicing is mediated by a large ribonucleoprotein, called the spliceosome, which recognizes and binds to splice sites to help excise introns and splice together exons. The spliceosome influences alternative splicing decisions, however, the regulation of alternative splicing, as well as where the spliceosome chooses splice sites is poorly understood (Saltzman et al., 2011). A recent study suggested sequence specific RBPs are heavily involved with controlling alternative splicing. Specifically, the protein quaking (QKI), a heterogenous nuclear ribonucleoprotein (hnRNP), was found to bind to intronic regions of DNA resulting in exon exclusion and skipping events (Fu et al., 2014). There are a few other studies where it is thought that RBM39 may be influencing the specific sites where the spliceosome splices (see below; Thomas et al., 2019).

RBM39

RNA Binding Motif Protein 39, also known as Caperα and hepatocellular carcinoma 1 (HCC1), is a highly conserved RBP found in animals and is involved in metabolism and sensory neuron development (Kang et al., 2015). Previous studies on human cell lines have also identified that it is a bifunctional RBP involved in transcriptional coregulation and RNA splicing (Dowhan et al., 2005; Jung et al., 2002). RBM39 is a member of U2AF-like family proteins, and is broadly expressed across tissues in humans, primarily found in the heart, skeletal muscle, liver, and placenta. It is also expressed maternally, in which the expression declines at a consistent rate across embryonic development (Fagerberg et al., 2014).

RBM39 contains one RS domain (arginine and serine rich), one non-canonical RRM domain (U2AF homology motif), and two canonical RRM domains – RRM1 and RRM2 (Loerch et al., 2014). These RRMs are essential for both RNA and protein interactions and have distinct functions. A paralog of RBM39 is RBM23, which contains homologous RRM1 and RRM2 domains and possesses a 95% sequence similarity. Although both proteins share similar components and functions, unlike RBM39, RBM23 is not as necessary for cancer cell survival. RBM39 is upregulated in numerous amounts of cancers, and its loss of function is lethal to cancers including acute myeloid leukemia (AML), colorectal cancer, breast cancer and lung cancer (Xu et al., 2020).

The role of RBM39 as a splicing factor began to pick up more research interest because it relates to two key splicing factors: U2 auxiliary factor 65 kDa subunit (U2AF65) and poly(U)-binding splicing factor 60 (PUF60). Both proteins were shown to cooperatively bind to RBM-39 with the recruitment of the U2 snRNP to the 3' splice site (Tari et al., 2014). Global transcriptomic studies show increasing evidence that RBM39 regulates alternative splicing for many genes, at least in part by promoting exon inclusion and intron exclusion. One specific study used idisulam, an anticancer drug that promotes the binding of an E3 ubiquitin ligase to RBM39, leading to the degradation of RBM39 by the proteosome. The inactivation of RBM39 through idisulam results in aberrant pre-mRNA splicing (Han et al., 2017).

Certain studies have suggested that RBM-39 plays a different role involved in transcriptional regulation and mitochondrial functions due to splicing defects (Song et al., 2021; Kang et al., 2015). In the Kang et. al study, it was found that integrating nutrient signals between the nucleus and mitochondria propagated by CAPER induces mitochondrial functions such as mitochondrial transcription and mitochondrial respiration via coactivation of nuclear receptor ERR-α-mediated Gabpa transcription. There was also a discovery that CAPER is a coactivator for NF-κB that in turn regulates c-Myc to coordinate transcriptome responses to mitochondrial stress. Although there are few experiments in *C. elegans*, Kang et al (2015) began to experiment with splicing factor CAPER in the mammalian system and discovered how it is used as a regulatory mechanism by which eukaryotic cells control homeostasis via CAPER-dependent coordinated control of mitochondrial transcriptomic programs and their metabolisms. By knocking down *rbm-39* (published as an unnamed gene *Y55F3AM.3* at the time), the worm ortholog of CAPER, they were able to examine long-term phenotypes, such as life span, in *C. elegans.*

RBM-39/Caper and Neuronal Phenotypes

Recent studies have identified RBPs that are involved in dendrite morphogenesis in both *C. elegans* and *Drosophila*, one of them being RBM-39/Caper (Olesnicky et al., 2014; Antonacci et al., 2015). Caper is an RNA recognition motif (RRM) containing protein shown to regulate many splicing events in *Drosophila* (Olesnicky et al., 2014). Adult *caper* mutant flies show sensory organ defects and behavioral phenotypes. These specific phenotypes include bristle sensory organs and chordotonal neurons, both which vary in expressivity. In the study led by Antonacci, an RNA interference (RNAi) screen was used to examine PVD dendritic defects, in which *rbm-39* was identified. Dendrite phenotypes of *C. elegans* were studied, specifically the dendritic branching and the number of dendritic termini. The screen used RNAi because no true null/deletion alleles were available at that time (Antonacci et al., 2015).

In recent research, to discover the full phenotypic effect in *C. elegans*, a null/deletion allele was created through CRISPR. This *rbm-39* knockout mutant was also used in our study to analyze and explore the *rbm-39* neuronal phenotypes in worm. This *rbm-39* mutant is sterile with germline development defects in gonadogenesis, proliferation, and production of functional oocytes (Judy Cheng, senior thesis 2021).

The human *caper* ortholog, *RBM39*, has not been studied extensively, and little is known about its function in neurons. Fly *caper* mutants, which have been found to have some defects in both neurons and glia, have many different nervous system phenotypes (Olesnicky et al., 2014; 2017; Antonacci et al., 2015; Titus et al., 2021) so we decided to examine a few phenotypes in the worm. In this study we looked for the cell presence or absence, and cellular morphology using GFP markers for various neuronal and glial subtypes.

The *C. elegans* GABAergic ventral cord neurons are subdivided into 6 DD motor neurons and 13 VD motor neurons, where the DD neurons synapse on the dorsal body wall muscles and the VD neurons synapse on the ventral body wall muscles (Donnelly et al., 2019). These motor neurons allow the animal to bend and steer in both ventral and dorsal directions. PVD neurons are multidendritic nociceptors that are known for their extensive branching both anteriorly and posteriorly along the worm. PVD neurons respond to harsh touch, requiring DEG/ENaC proteins MEC-10 and DEGT-1, and cold temperatures, requiring the TRPA-1 channel (Aguirre-Chen et al., 2016).

In this study, we aimed to analyze the impact that *rbm-39* has on nervous system development, specifically the role it plays in both neuronal and glia phenotypes. Also, in attempt to analyze the molecular role of theRBM-39 protein, specifically its function concerning splicing defects, we isolated and sequenced mRNA from *rbm-39* mutants.

Materials and Methods

C. elegans strains

Strains were derived from the Bristol strain N2, grown at 20°C, and constructed using standard procedures (Brenner et al., 1974). The *rbm-39(cnj4)* knockout mutation was generated in the Killian Lab (Judy Cheng Senior Thesis 2021) and was maintained as a heterozygous stock with the *tmC25* balancer chromosome, which carries both a recessive *unc-5* mutation as well as a dominant Venus fluorescent marker for the pharynx (Dejima et al., 2018). All other strains were obtained from the University of Minnesota Caenorhabditis Genetics Center. PVD dendrites were marked by *wdIs52* [*F49H12.4::GFP + unc-119(+)*] (Watson et al., 2008) for screening. DD and VD dendrites were marked by *juIs76* [*unc-*

25p::GFP + lin-15(+)] *II* for screening, in which there is GFP expression in GABAergic motor neurons (Kalichamy et al., 2016). CEP sheath glia were marked by *vprIs157* [*hlh-17p::GFP*] (Bowles et al., 2021).

Genetic crosses to create strains carrying the *rbm-39* mutation and fluorescent marker transgenes

We crossed *him-5* males with *tmC25* homozygotes and used F_1 cross progeny to further cross with worms homozygous for *wdIs52*. The male progeny with GFP/+; *tmc25*/+ were crossed with *rbm-39/tmc25* hermaphrodites. F_1 hermaphrodite progeny expressing GFP/+; *rbm-39/tmc25* were picked and singled onto individual plates. F_2 progeny carrying the GFP transgene as well as *rbm-39/tmC25* were singled and picked on new plates, to identify animals homozygous for the GFP transgene. Process was repeated with several other GFP strains, including *juIs76* [*unc-25p::GFP + lin-15(+)*] *II* and *vprIs157* [*hlh-17p::GFP*].

RNA extraction and RNA-Sequencing

Several 100mm plates of worms were washed off to collect a yield of 1µg of total RNA (Ly et al., 2018). 500µL of Trizol was added after worms were spun down in the microcentrifuge at 4k for 5 minutes multiple times. Worms were frozen, thawed and spun for 14k for 10 minutes at 4°C. Supernatant was removed and 100µL of chloroform was added. After spinning again at 12k for 10 minutes, the upper aqueous phase was removed to a new tube and spun down again with 250µL of isopropanol and 1µL of GlycoBlue. The supernatant was poured off, leaving a barely visible pellet, and 1 mL of 75% ethanol (mixed with RNase-free water) was added and spun at 7.5k for 5 minutes. The ethanol wash and spin step was repeated several times. 100µL of RNase-free water was added to dissolve the RNA, and the solution was heated at 60°C for 10 minutes. The concentration and purity of the RNA was measured with the nanodrop to obtain a total yield of ~0.8mg at a concentration of at least 20ng/µl for RNA-seq.

RNA was then sent for RNA-seq analysis at Novogene, in which a series of steps were performed to sequence the RNA in the highest quality possible. Steps include RNA integrity and purity analysis, library preparation, Ilumina sequencing, and data analysis. To ensure the reliability of the data, quality control (QC) is performed during every step of the procedure (Zhou et al., 2018).

Imaging of strains/ Quantification of strains

Worms were picked at the early adult stage, mounted on slides with 2% agarose pads, and immobilized with 500µM levamisole. Initial imaging and scoring were conducted using 40x objective on a Zeiss Axioscope or Leica M205 FA microscope. Secondary, tertiary, and terminal dendrites were counted from the PVD body to the posterior on both dorsal and ventral sides of the animal. For *juIs76*, DD and VD

neurons were counted throughout the ventral nerve cord of the adult animal. Commissures shown on the animals were also considered. Worms were screened through a Leica SP5 spectral confocal microscope at the UCCS facility to analyze both neural and glia subtypes.

Image Analysis

Analysis was done on Fiji Image J with .tiff images. The polygon selection tool was used to outline the GFP shown in the *vprIs157* control and *vprIs157; rbm-39* mutant strain (NIH, 2016). Once outlined, the measure tool was used to find the area, mean, min and max. In order to keep the area the same for each image, the same polygon outline was re-used and the placement of the outline was adjusted for each image. All images were measured and recorded. Unpaired t-tests assuming Gaussian distribution were performed on the number of secondary, tertiary, and terminal branches between *wdIs52* mutants and controls.

Results

Analysis of *rbm-39* Mutants Reveals Subtle Neurophenotypical Differences

Although *rbm-39* has previously been shown to play an important role in dendritic development (Antonacci et al., 2014), a null allele of *rbm-39* has yet to be used to examine different neuron and glia subtypes. In this study we constructed different strains of *C. elegans* carrying specific transgenes labeling various neuron and glia types and crossed them with the strain of *C. elegans* that had the *rbm-39* gene removed through CRISPR genome-editing technology to analyze the phenotypic changes quantitatively and qualitatively. We specifically analyzed the dendritic morphology and the glia subtypes in the *rbm-39* mutants that were created through these crosses.

The first transgene that we used is the *wdIs52* [F49H12.4::GFP + unc-119(+)] transgene, which carries a GFP marker for PVD neurons in the worms. The *C. elegans* PVD neuron is a great model to research dendrite morphogenesis due to its extensive yet stereotypical branching of dendrites. The bilateral PVDs run between the epidermis and the body wall muscles and have large dendritic branches that can function as mechanoreceptors, nociceptors, proprioceptors, and cold temperature receptors (Way and Chalfie 1989; Tsalik and Hobert 2003; Smith et al. 2010; Albeg et al. 2011). The PVD dendritic branches consist of primary (1°) branches that extend from the PVD cell body, and a sequence of orthogonal secondary (2°), tertiary (3°), and terminal (4°) branches (Figure 1A). Movement-dependent calcium transients are seen in PVD, a response that requires the subunit of the DEG/ENaC channel, MEC-10, which is needed to

maintain wild-type posture. In consequence, PVD senses specific signals to regulate *C. elegans* behavior, thus combining the functions of multiple mammalian somatosensory neurons (Albeg et al., 2011).

Branches were counted dorsally and ventrally starting from the PVD cell body and ending at the tail, for both control animals (ctl) and the *rbm-39* mutants (Figure 1B). The quantification of secondary branches between control and mutant worms resulted in a statistical difference between the two with a p-value between .001 and .01 with an unpaired t-test (Figure 1C). For the tertiary branches, although there was less of a difference with a p-value between .01 and .05, but still a significant difference. The quantification of terminal branches resulted in a p-value of less than .05, meaning there is no significant difference between the *rbm-39* mutants and control animals.

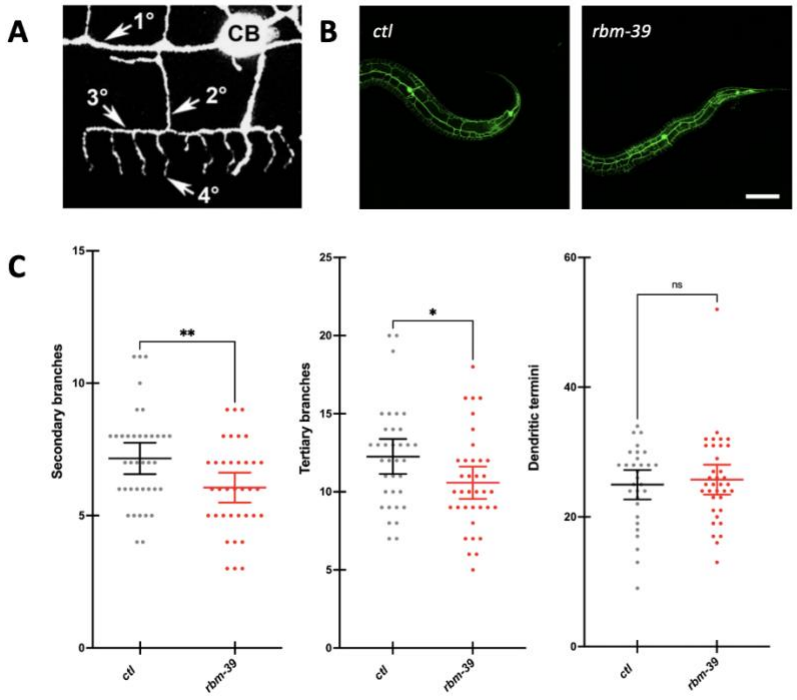
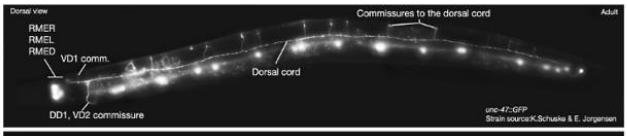


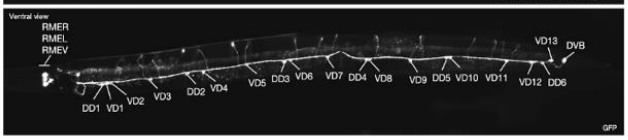
Figure 1. Quantification of PVD Dendrite Phenotypes in *rbm-39* mutants results in minimal statistical significance (A) PVD dendritic tree morphology consists of primary (1°) branches extending from the PVD cell body (CB) and a sequence of perpendicular secondary (2°), tertiary (3°), and quaternary (4°), or terminal, branches. (B) Animals carrying a green fluorescent protein (GFP) marker for PVD neurons (*wdIs52)* in control animals (*ctl*) and in animals carrying a mutation *rbm-39* gene. (C) Quantification and analysis of PVD dendrite phenotype in control animals and *rbm-39* mutants. Points within each scatter column represents counts of secondary branches, tertiary branches, and dendritic termini, respectively. Branches were counted starting from the PVD cell body to the tail end on both dorsal and ventral sides of the animal. Column lines shown in the graphs represent the means and the 95% confidence interval of the mean. ***rbm-39* mutants are significantly different from the control animals and have a p-value between 0.001 and 0.01. **rbm-39* mutants are significantly different from the control animals and have a p-value between 0.01 and 0.05. NS abbreviation denotes that *rbm-39* mutants are not significantly different from the control animals and have a p-value ≥ 0.05. All statistics are based on an unpaired t-test assuming Gaussian distribution and that both populations have the same SD.

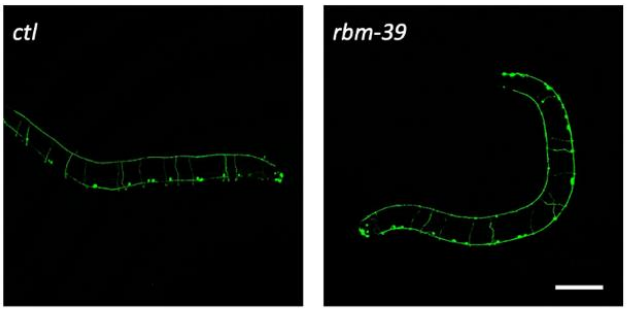
The next cell type that we analyzed through GFP were the ventral and dorsal class D motor neurons (DD and VD), marked by the *juIs76* [*unc-25p::GFP + lin-15(+)*] *II* transgene. VD neurons receive their synaptic input on the dorsal side and give neuromuscular synapses (NMJ) on the ventral side, whereas DD neurons counteract the ventral class D neurons and receive their synaptic input on the ventral side and give NMJs on the dorsal side (https://www.wormatlas.org/neurons/Individual%20Neurons/VDframeset.html). In *C. elegans* there are 6 DD neurons and 13 VD neurons from head to tail (Figure 2A). These GABAergic neurons allow for ventral and dorsal motor function in the worm. In our experiment, the DD and VD neurons were counted across the entire body of both control animals (*ctl)* and *rbm-39* mutants (Figure 2B). When the *juIs*76 control was compared to the *rbm-39* mutant, the average amount of DD neurons, across 20 animals, stayed the same at exactly 6 (Figure 2C). The average amount of VD neurons stayed about the same at 12.76 and 12.95 for the *juIs76* controls and *rbm-39* mutants respectively. Thus, there is no significant difference phenotypically for the two averages of DD and VD neurons between the control animals and mutants.

A



B





C

Strain	DD	VD	DD + VD
ctl	6.0	12.76	18.77
rbm-39	6.0	12.95	18.90

Figure 2. Quantification of DD and VD Dendrite phenotypes in *rbm-39* mutants results in nearly identical data (A) Motor Neurons of the DD and VD classes are located in the ventral nerve cord of an adult animal as shown with a green fluorescent protein marker (GFP); top is lateral view; bottom is ventral view. Commissures that extend across the animal to the dorsal cord are also visible. (B) Animals carrying a green fluorescent protein (GFP) marker for DD and VD neurons (*juIs76)* and a mutation in the *rbm-39* gene. (C) The average number of DD neurons, VD neurons, and both DD and VD neurons combined that are found in the control strain as well as the *rbm-39* mutants.

Glia are non-neuronal cells found in *C. elegans* that are similar to vertebrate glia due to their morphological, molecular and functional criteria, and have become a prevalent model in which to study glia and their neuronal interactions (Singhvi et al., 2019). Glial cells, once thought to be filler material in the brain, shape the function and development of the nervous system (Fung et al., 2020). The *C. elegans* hermaphrodite possess 50 ectoderm-derived glia and six mesoderm-derived glial-like cells (Zhang et al., 2021). Cell-type-promoters allow for the labeling of individual glial cell types which can aid in discovering the glial location and function. In order to mark the glial subtype, we acquired a strain of *C. elegans* expressing the transgene *vprIs157* [*hlh-17p::GFP*], which specifically uses the cell-specific promoter *hlh-17* to express GFP in the four sheath glial cells in the worm. In our study the glial subtypes were labeled in both the *vprIs157* control animals (*ctl)* as well as the *rbm-39* mutants (Figure 3C). Quantification and analysis on the relative brightness in arbitrary units (AU) of fluorescence in these cells was performed on the images that were taken of the animals. There is no actual unit of fluorescence since it is a function of multiple variables including illumination intensity, magnification level, and camera settings. When comparing relative brightness of the glia for both the control animal and the *rbm-39* mutant there was no significant difference.

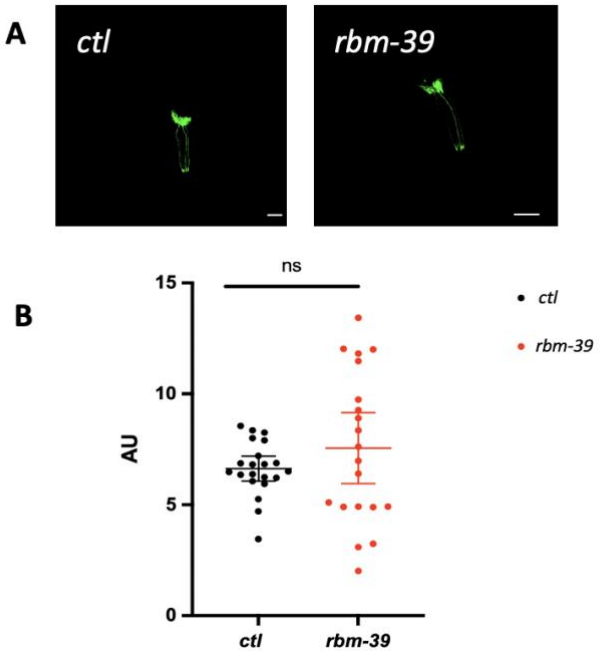


Figure 3. Quantification of glial phenotypes in *rbm-39* mutants results in no significant difference (A) Animals carrying a green fluorescent marker (GFP) for glia (*vprIs157*) in control animals and in animals carrying a mutation *rbm-39* gene. (B) Quantification and analysis of glia subtype in control animals and *rbm-39* mutants. Points within each scatter column represent arbitrary units (AU), and the mean relative brightness of the GFP-lit area is measured for both control strain (*ctl)* and *rbm-39* mutants. NS abbreviation denotes that *rbm-39* mutants are not significantly different from the control animals and have a p-value ≥ 0.05. All statistics are based on an unpaired t-test assuming Gaussian distribution and that both populations have the same SD.

Our results when comparing all the control strains to the *rbm-39* mutants only show very subtle defects. Moreover, the *rbm-39* mutant worms are at least superficially morphologically normal at the organismal level, and we did not observe any behavioral defects though no specific behavioral assays were performed. With our results from the neurophenotypical analysis we can conclude that the *rbm-39* gene plays a minor role in neural morphology. This may suggest that *rbm-39* could potentially have more of an effect on function rather than phenotype. Alternatively, it is possible that the effects of a loss of *rbm-39* on neurons and glia are masked by maternal rescue since all animals examined are homozygous for the *rbm-39* mutation but are derived from heterozygous mothers (see Discussion).

RNA Sequencing Analysis of *rbm-39* mutants reveals widespread defects in differential expression and alternative splicing

Several studies suggest that members of the RBM39 family of proteins are involved in alternative splicing while others suggest that it is involved in transcription (Xu et al., 2020; Song et al., 2021; Mai et al., 2016; Olesnicky et al., 2017; Antonacci et al., 2015).

To determine if both (or either) of these hypotheses for RBM-39 function are valid, we isolated and sequenced mRNA from *rbm-39* mutants and compared the results to controls. Specifically, RNA was extracted and isolated from both N2 animals (wild-type strain) and *rbm-39* mutants. The *rbm-39* mutants had to be picked from heterozygous mothers due to the fact they are sterile. The entire experiment was done in quadruplicate. A series of steps were involved for purification (see Materials and Methods), and RNA-Sequencing was performed so we could look for genes that are up or down regulated or differentially spliced (Figure 4).

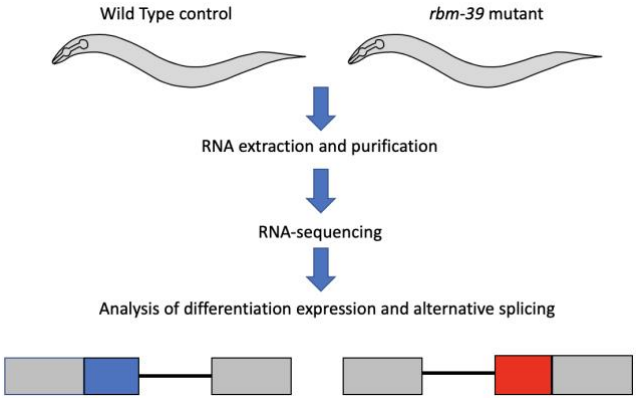


Figure 4: Image depicting methods used to extract and sequence mRNA from worms. The same gene is illustrated in this figure twice, shown after RNA-sequencing, in which it is alternatively spliced (depicted by the different colors blue and red).

RNA-Seq was outsourced to Novogene, which returned a series of significant data sets. One interesting data set is called RvsN DEG, which is a list of differentially expressed genes for *rbm-39* mutants versus N2 control worms. The list is in order of the most significant difference of expression levels between the mutant and control, where the adjusted P-value (pval) is given. Several of these genes that were annotated, after further research, were found to be heavily involved with nervous system development, including *bet-2*, T24E12.5, and *col-99* (Table 1). These specific genes are elaborated on in the discussion section. A volcano plot was also created displaying differential gene expression in both mutant and control worms (Figure 5). In total, 3,949 genes were found to be up regulated in the mutant, and 4,653 genes were shown to be down regulated in the mutant. In total, there are about 8,000 genes were shown to be mis-expressed in the mutant compared to the control based on the RvsN DEG data.

Gene name	Data Set	P-value	Importance
pdk-1	3' Splice Site	1.24E-213	Enables protein kinase activity; regulates synaptic growth at neuromuscular junctions
sax-3	3' Splice Site	4.00E-94	Regulates sensory neuron axon guidance; involved in cell differentiation
sax-7	3' Splice Site	2.01E-88	Regulates dendrite extension; involved in cell differentiation
dli-1	3' Splice Site	3.07E-50	Regulates dendrite morphogenesis and retrograde axonal transport
MO1E5.3	3' Splice Site	2.77E-49	Enriched in PVD
math-3	3' Splice Site	1.51E-11	Enriched in PVD
Y41C4A.12	3' Splice Site	0.00328569	Enriched in GABAergic neurons
bet-2	Differential Expression	1.23E-149	Enriched in PVD
T24E12.5	Differential Expression	1.32E-88	Enriched in PVD and dopaminergic neurons
col-99	Differential Expression	1.61E-28	Involved in axon guidance

Table 1: Candidate genes involved in nervous system development

In addition to the differentially expressed genes identified in the analysis, there were also multiple genes that are spliced significantly differently between the mutant and N2 worms, including alternative 5' splice sites (A5SS), alternative 3' splice sites (A3SS), mutually exclusive exons (MXE), intron inclusion (RI), and skipped exons (SE). The total number of genes found to be significantly differentially alternatively spliced in mutants compared to controls was 1,397 genes, 287 of them coming from A3SS, 1,056 from SE, and 54 from A5SS. Compared to intron retention, which only 58 genes were found, alternative splicing seems to play much more of a dominant role. Numerous mis-spliced genes, also described further in the discussion section, have been implicated in nervous system and dendrite development in the alternative 3' splice site including *sax-3, sax-7,* and *dli-1* (Table 1).

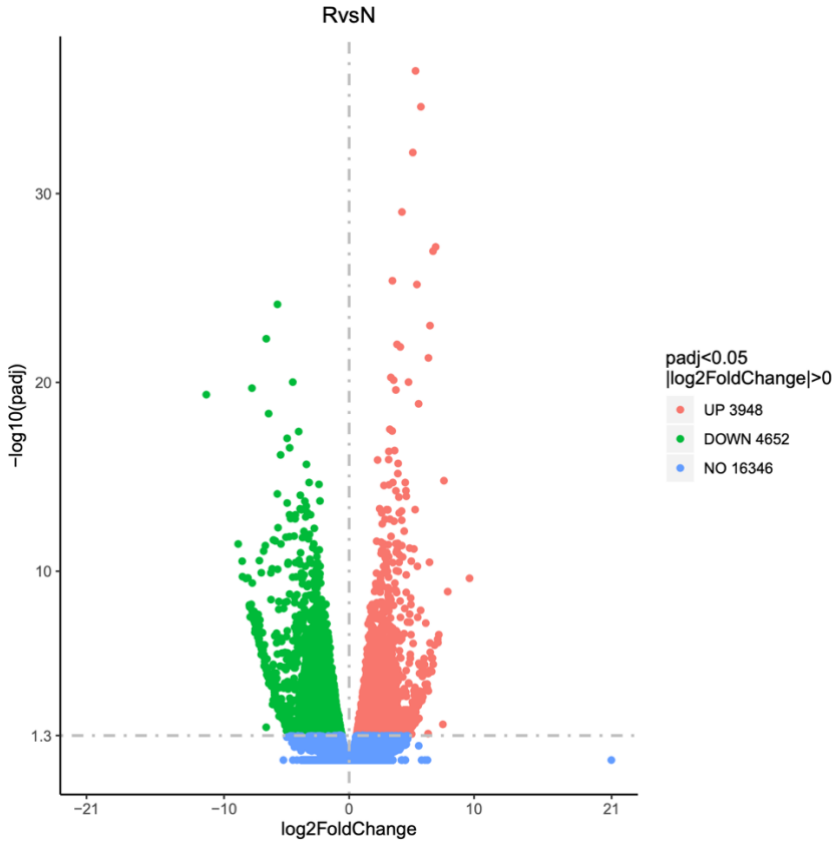


Figure 5. Differential gene expression shown in *rbm-39* mutants Volcano Plot depicting gene mis-regulation from the RvsN data sheet. In terms of -log10 (padj). Green dots represent genes that are being down regulated and pink dots represent genes being up regulated. Blue dots were not mis-regulated at all.

For the differentially expressed genes, a Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was performed, where KEGG is used to categorize genes based on their presumed function. A few KEGG terms that were pulled out from the RNA-Seq data include "axon regeneration" and "neuroactive ligand receptor interaction" (NLRI), both dealing with neuronal morphogenesis. A heat map was produced

depicting the expression of genes in specific categories where the *rbm-39* mutant has the genes downregulated or upregulated relative to the control (Table 2 and 3). The axon regeneration heat map resulted in a list of 83 genes and the NLRI heat map consisted of 29 genes. Many of these genes, especially in the axon regeneration list, were found to have specific impacts on the nervous system and its development, including *sax-3 and slt-1*.

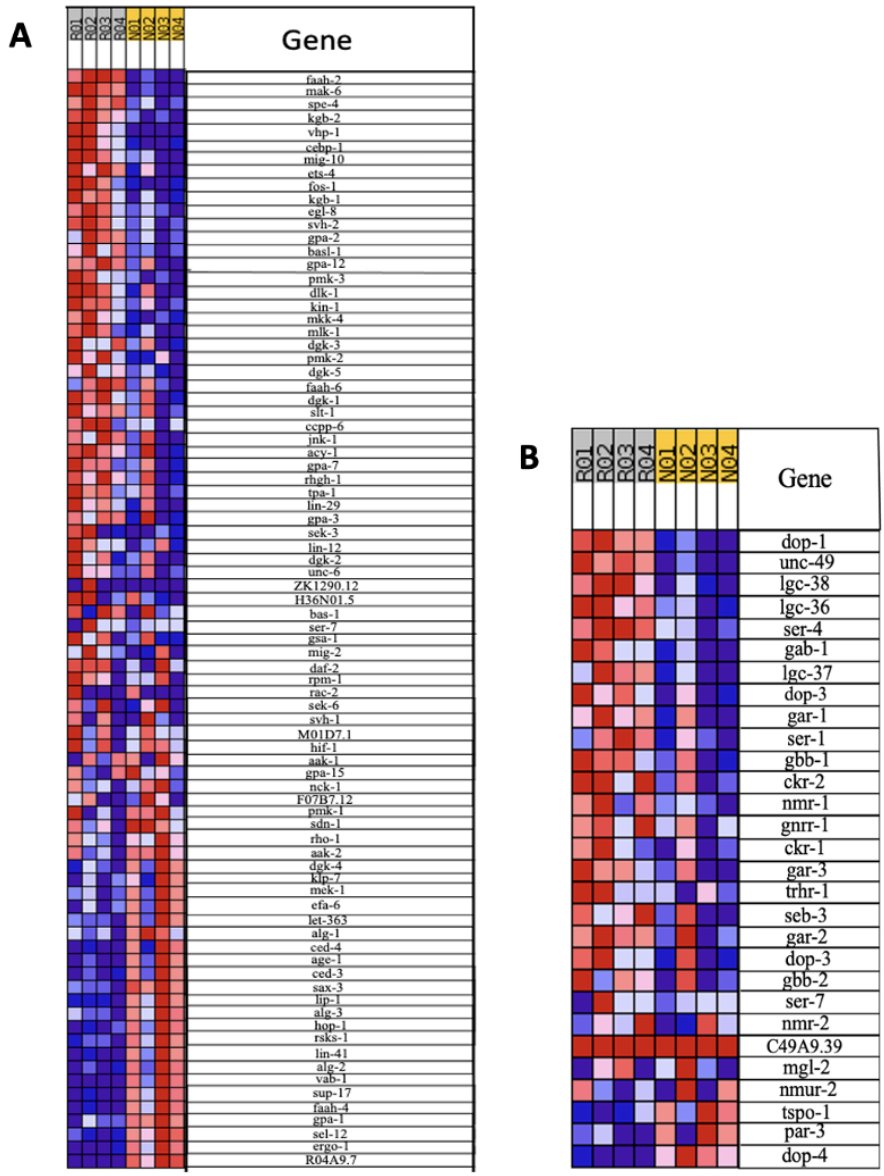


Table 2 and 3: KEGG Analysis Heat Map of upregulated and downregulated genes. (A) Axon regeneration. (B) Neuroactive ligan receptor interaction. R01-04 are mutant samples, in which red for R01-04 samples denotes upregulation in the mutant, and blue for R01-04 denotes downregulation in the mutant. The N samples are N2 controls and the trends of red and blue are opposite the mutant – if the mutant is high (red) the controls are low (blue) and vice versa.

Discussion

Although Caper/RBM-39 is known to participate in both neuronal development and germline development, as well as been implicated in cancers, its full phenotypic effect is still relatively unknown (Judy Cheng senior thesis 2021, Wright, 2016; Olesnicky et al., 2017; Mercier et al., 2014; Antonacci et al., 2015). Previous studies of the evolutionary conserved roles of RBM-39 homologs were hindered due to the fact that there was not a knockout mutant available in *C. elegans.* To study the *rbm-39* role in neuronal development, we used the *rbm-39* knockout mutant *(cnj4)* in the *C. elegans* model generated by Judy Cheng.

We found, to our surprise, that *rbm-39* has very little impact on nervous system development unlike the role of its homolog in the fruit fly. After sequencing mRNA from *rbm-39* mutants, however, we discovered that RBM-39 plays a role in the regulation of gene expression as well as alternative splicing. Our results suggest though that the loss of *rbm-39* may result in both differential expression and mis-splicing of important genes in the nervous system. Keeping in mind, however, that although these results may be valid, the mutant is sterile and therefore every animal's mother is heterozygous and could contribute wildtype mRNA for *rbm-39* and/or functional RBM-39 protein into the oocytes, which could obscure some roles of *rbm-39* in development. The half-life of *rbm*-39 mRNA and RBM-39 protein are unknown. This is a confounding issue in our study and is one that is very difficult to resolve. This may also be a reason for the discrepancy for PVD dendrite phenotypes observed in *rbm-39(RNAi)* (Antonacci et al., 2015) and *rbm-39(cnj4)* (this study). If the maternal contribution of mRNA or protein is conserved from worms to mammals, it suggests a good chance that the homozygotes from the heterozygous mothers would be less severely affected, especially in tissues that develop early on, such as neurons. This could potentially be looked at in future studies, in which specific post-embryonic developing neurons are carefully examined.

Morphology of the *rbm-39* mutants

Our research consisted of the investigation of the effects of *rbm-39* deletion in the neuron development by crossing *rbm-39* with animals possessing neuronal markers. With that being said, we only observed a particular set of neurons and how they specifically affect the morphology of the worm. The morphological features we observed consisted of dendritic shape, structure, pattern, and cell size. In our study, the cells may have been morphologically "normal", however, they very well could be dysfunctional, and we would not have known. In the future, it is important that the cell type and function are accounted for, because there are countless cell types, such as neurons and glia, that could be further observed in studies down the line. Another important thing to account for in future studies would be to

look at neurons that develop later in the development period of the worm to observe if they are less likely to be maternally rescued. In this case, the later developing tissue could possibly contain less RBM-39 protein or RNA leftover from the maternal contribution.

Genes that were found to be differentially expressed or mis-spliced

The RNA sequencing data point to a handful of genes that are either mis-spliced or differentially expressed, and which have been annotated or implicated in nervous system development or function.

Three differentially expressed genes of interest in nervous system development/function are *bet-2*, T24E12.5, and *col-99.* Both *bet-2* and T24E12.5 are enriched in PVD, based on RNA-seq studies and microarray (https://wormbase.org/species/c_elegans/gene/WBGene00010199#0-9fd-10). The *col-99* gene is expressed in neurons and located in both the neuromuscular junction and extracellular space. This protein coding gene is specifically involved in axon guidance and is predicted to be a structural constituent of cuticle (https://wormbase.org/species/c_elegans/gene/WBGene00000674#0-9fd-10).

The next several genes were also found in the RvsN data, and all have changes to alternative 3' splice site compared with controls. These genes include the *pdk-1, sax-3, sax-7, dli-1,* M01E5.3, *math-3* and Y41C4A.12.

The *pdk-1* gene is expressed in multiple structures including neurons and the ventral nerve cord and its protein is found in axons and neuronal cell bodies (Paradis et al., 1999). The p-value recorded for *pdk*-1 from the 3' splice site data set was extraordinarily high at 1.24E-213 (Table 1). This gene specifically enables protein kinase activity as well as regulates synaptic growth at neuromuscular junctions. In mammals, PDK1 has shown to be involved in several biological processes including cell proliferation and migration and plays a large role in the neurogenesis of cortical excitatory neurons (Xu et al., 2019). In a recent study, it was shown that PDK1 is also involved in the regulation of the survival cortical interneurons in development (Wei et al., 2020).

The *sax-3* gene is expressed in various structures including neuroblasts and ventral cord neurons and is a protein coding gene that can regulate sensory neuron axon guidance (https://wormbase.org/species/c_elegans/gene/WBGene00004729#0-9fd-10). It is also involved in cell differentiation. The p-value for *sax*-3 was also very high at 4.00E-94 (Table 1). In a recent study, it was found that the *sax-3* gene mediates cell interactions during axon guidance decisions (Zallen et al., 1998).

It was shown that mutations in the *sax-3* gene lead to repeated midline crossing of ventral cord axons, a phenotype very similar to that of Robo (the fly homolog) mutants in *Drosophila.*

The *sax-7* gene, or L1CAM (L1 Cell Adhesion Molecule), is expressed in several structures including neurons and the somatic nerve system and is a protein coding gene that regulates the extension of dendrites (https://wormbase.org/species/c_elegans/gene/WBGene00004732#0-9fd-10). It is also involved in cell differentiation as well as the establishment of mitotic spindle orientation. In a recent study of L1CAM expression, it was shown to regulate axon outgrowth and facilitate the guidance of motor neurons (Sherry et al., 2020). It was also found to facilitate the maturation and growth of a single neuron. Another recent study on the *sax-7* gene discovered its role in the long-term neuronal maintenance and organization (Desse et al., 2021).

The *dli-1* gene is a protein coding gene that is expressed in ciliated neurons, hypodermis, and the pharynx of *C. elegans*. This protein is a dynein light intermediate chain, which is predicted to link cargo to microtubule motor proteins. It involved in the regulation of dendrite morphogenesis as well as retrograde axonal transport (https://wormbase.org/species/c_elegans/gene/WBGene00001007#0-9fd-10). In recent studies on the *dli-1* gene, it was shown to work directly with the *sax-7* gene, which regulates and instructs PVD dendrite branching. It was shown that DLI-1 regulates the localization pattern of SAX-7 in epidermal cells, thus controlling dendrite morphology (Zhu et al., 2017).

The M01E5.3 and *math-3* genes are enriched in PVD based on RNA-seq studies and microarray (https://wormbase.org/species/c_elegans/gene/WBGene00010806#0-9fd-10). Whereas the Y41C4A.12 gene is enriched in GABAergic neurons (produce GABA, or *gamma*-Aminobutyric acid), where GABA is the chief inhibitory neurotransmitter in the mammalian nervous system (https://wormbase.org/species/c_elegans/gene/WBGene00012758#0-9fd-10). This is based off tiling array and RNA-seq studies. While there are no compelling functional studies of these genes in neuron morphology or function, the expression patterns suggest they may be important for neuronal processes.

Other genes that were found in the KEGG analysis, specifically the axon regeneration list, were found to have significant impacts on axon guidance defects. These two genes are *sax*-3 and *slt-1*, in which both Robo receptor SAX-3 and slit protein SLT-1 work together to promote ventral axon guidance in *C. elegans* (Hao et al., 2001). SLT-1 acts through the SAX-3 receptor, along with the ventral attractant UNC-6 (Netrin) in which both give guidance clues for axons. When both SLT-1 and UNC-6 are removed, all ventral guidance information for specific axons is eliminated, in turn displaying an underlying

longitudinal guidance pathway. In this study, it was found that *slt-1* has a complex expression pattern that explains the observed phenotypes of *sax-3* mutants. These results are consistent with SLT-1 functioning as a repellent for *C. elegans* cells and axons that express *sax-3,* with necessary roles both in anterior-posterior guidance in embryos and dorsal-ventral guidance in larvae.

Because both genes are shown to be mis-expressed, we could expect to see *rbm-39* mutants to have some sort of phenotypical similarities when it comes down to axon phenotype. The *sax-3* gene, in fact, is found to be both mis-expressed and mis-spliced and could be a vital component in future research on neuronal function in *C. elegans.* All these genes shown to be differentially expressed or mis-spliced have potential implications in nervous system function and could be studied further with future research.

Glia results and implications

When obtaining the glia results, it is important to note that originally, we were thinking about the morphology of the glial cells rather than the relative brightness. No morphological differences were observed (unpublished), however, so variation in brightness was analyzed. Because the imaging that was done through the confocal microscope adjusted each image independently, it is hard to compare the brightness between glia with the images we obtained. Due to these conditions, we had high variability so more quantitative studies may be necessary in the future.

Future Direction with *rbm-39*

For future studies it would be interesting to try and use CRISPR to insert GFP immediately upstream of the stop codon for *rbm-39* so that we have a native translational fusion. This can then be followed by an immunoprecipitation from worm lysate to purify the RBM-39 protein. Next, we could sequence the RNAs directly associated with it, and use mass spectroscopy to identify specific RNA transcripts that are bound to *rbm-39* to directly implicate a given splicing. The expectation is that *rbm-39* binds to proteins that are involved in the spliceosome or other splicing regulators.

New information surrounding the *rbm-39* gene could lead to an understanding of how splicing is regulated more broadly in eukaryotes, given that there is a *rbm-39* homolog in flies and humans. RBM39 proteins are widespread in animals and seem to be involved in splicing as well as potentially being able to regulate how the spliceosome recognizes (or does not recognize) specific splice sites. Splicing still regularly occurs in the mutants, however, the patterns and proportions of different splice forms are changed. Thus, it is not clear if RBM39 plays a specific, special role in neurons and glia from any species, such that we see the phenotypes that we do. The fact that splicing is used a lot in neurons may also simply

means that neurons are disproportionally impacted. Splicing is also shown to be regulated in germ cells, which is another avenue that has been explored.

It is important to note that in this study, a lot of alterations at the transcription and splicing levels were recorded, but very little in the way of phenotypic changes. Thus, it may be due to the function of the animals that differs. Future research could include specific behavioral tests for the neuronal subtypes, for example, analyzing the motor behavior of the worms.

References

Albeg, A., C. J. Smith, M. Chatzigeorgiou, D. G. Feitelson, D. H. Hall, et al. (2011). C. elegans multi-dendritic sensory neurons: morphology and function. Mol. Cell. Neurosci. 46: 308–317. https://doi.org/10.1016/j.mcn.2010.10.001

Aguirre-Chen, Cristina, et al. (2016). "C. Elegans PVD Neurons: A Platform for Functionally Validating and Characterizing Neuropsychiatric Risk Genes." *BioRxiv*, Cold Spring Harbor Laboratory. https://www.biorxiv.org/content/10.1101/053900v1.full

Blumenthal T, Davis P, Garrido-Lecca A. (2018). Operon and non-operon gene clusters in the C. elegans genome. WormBook; 2005-2018. https://www.ncbi.nlm.nih.gov/books/NBK293639/

Bowles, S. N., & Johnson, C. M. (2021). Inferences of glia-mediated control in Caenorhabditis elegans. *Journal of neuroscience research*, *99*(5), 1191–1206. https://doi.org/10.1002/jnr.24803

Brenner, S. (1994). "The Genetics of Caenorhabditis Elegans." *Genetics*, Genetics https://www.genetics.org/content/77/1/71?ijkey=ea38f884ecfa2b1f5a144207a93c97e205c7aec5&keytype2=tf_ipsecsha

Croft, L., Schandorff, S., Clark, F. *et al.* (2000). ISIS, the intron information system, reveals the high frequency of alternative splicing in the human genome. *Nat Genet* **24,** 340–341. https://doi.org/10.1038/74153

Dalmau, J., Furneaux, H. M., Cordon-Cardo, C., & Posner, J. B. (1992). The expression of the Hu (paraneoplastic encephalomyelitis/sensory neuronopathy) antigen in human normal and tumor tissues. *The American journal of pathology*, *141*(4), 881–886

Dejima, K., Hori, S., Iwata, S., Suehiro, Y., Yoshina, S., Motohashi, T., & Mitani, S. (2018). An Aneuploidy-Free and Structurally Defined Balancer Chromosome Toolkit for Caenorhabditis elegans. *Cell reports*, *22*(1), 232–241. https://doi.org/10.1016/j.celrep.2017.12.024

Desse, V. E., Blanchette, C. R., Nadour, M., Perrat, P., Rivollet, L., Khandekar, A., & Bénard, C. Y. (2021). Neuronal postdevelopmentally acting SAX-7S/L1CAM can function as cleaved fragments to maintain neuronal architecture in Caenorhabditis elegans. *Genetics*, *218*(4), iyab086.

Donnelly, J. L., Clark, C. M., Leifer, A. M., Pirri, J. K., Haburcak, M., Francis, M. M., Samuel, A. D., & Alkema, M. J. (2013). Monoaminergic orchestration of motor programs in a complex C. elegans behavior. *PLoS biology*, *11*(4), e1001529. https://doi.org/10.1371/journal.pbio.1001529

Dowhan, D. H., Hong, E. P., Auboeuf, D., Dennis, A. P., Wilson, M. M., Berget, S. M., & O'Malley, B. W. (2005). Steroid hormone receptor coactivation and alternative RNA splicing by U2AF65-related proteins CAPERalpha and CAPERbeta. *Molecular cell*, *17*(3), 429–439. https://doi.org/10.1016/j.molcel.2004.12.025

Fagerberg, L., Hallström, B. M., Oksvold, P., Kampf, C., Djureinovic, D., Odeberg, J., Habuka, M., Tahmasebpoor, S., Danielsson, A., Edlund, K., Asplund, A., Sjöstedt, E., Lundberg, E., Szigyarto, C. A., Skogs, M., Takanen, J. O., Berling, H., Tegel, H., Mulder, J., Nilsson, P., … Uhlén, M. (2014). Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Molecular & cellular proteomics : MCP*, *13*(2), 397–406. https://doi.org/10.1074/mcp.M113.035600

Fung, W., Wexler, L., & Heiman, M. G. (2020). Cell-type-specific promoters for *C. elegans* glia. *Journal of neurogenetics*, *34*(3-4), 335–346. https://doi.org/10.1080/01677063.2020.1781851

Fu, X. D., & Ares, M., Jr (2014). Context-dependent control of alternative splicing by RNA-binding proteins. *Nature reviews. Genetics*, *15*(10), 689–701. https://doi.org/10.1038/nrg3778

Gao F. B. (2007). Molecular and cellular mechanisms of dendritic morphogenesis. *Current opinion in neurobiology*, *17*(5), 525–532. https://doi.org/10.1016/j.conb.2007.08.004

Han, T., Goralski, M., Gaskill, N., Capota, E., Kim, J., Ting, T. C., Xie, Y., Williams, N. S., & Nijhawan, D. (2017). Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15. *Science (New York, N.Y.)*, *356*(6336), eaal3755. https://doi.org/10.1126/science.aal3755

Hao, J. C., Yu, T. W., Fujisawa, K., Culotti, J. G., Gengyo-Ando, K., Mitani, S., Moulder, G., Barstead, R., Tessier-Lavigne, M., & Bargmann, C. I. (2001). C. elegans slit acts in midline, dorsal-ventral, and anterior-posterior guidance via the SAX-3/Robo receptor. *Neuron*, *32*(1), 25–38. https://doi.org/10.1016/s0896-6273(01)00448-2

Hide, W. A., Babenko, V. N., van Heusden, P. A., Seoighe, C., & Kelso, J. F. (2001). The contribution of exon-skipping events on chromosome 22 to protein coding diversity. *Genome research*, *11*(11), 1848–1853. https://doi.org/10.1101/gr.188001

Jan, Y. N., & Jan, L. Y. (2010). Branching out: mechanisms of dendritic arborization. *Nature reviews. Neuroscience*, *11*(5), 316–328. https://doi.org/10.1038/nrn2836

Jung, D. J., Na, S. Y., Na, D. S., & Lee, J. W. (2002). Molecular cloning and characterization of CAPER, a novel coactivator of activating protein-1 and estrogen receptors. *The Journal of biological chemistry*, *277*(2), 1229–1234. https://doi.org/10.1074/jbc.M110417200

Kalichamy, S. S., Lee, T. Y., Yoon, K. H., & Lee, J. I. (2016). Hypergravity hinders axonal development of motor neurons in *Caenorhabditis elegans*. *PeerJ*, *4*, e2666. https://doi.org/10.7717/peerj.2666

Kan, Z., Rouchka, E. C., Gish, W. R., & States, D. J. (2001). Gene structure prediction and alternative splicing analysis using genomically aligned ESTs. *Genome research*, *11*(5), 889–900. https://doi.org/10.1101/gr.155001

Kang, Y. K., Putluri, N., Maity, S., Tsimelzon, A., Ilkayeva, O., Mo, Q., Lonard, D., Michailidis, G., Sreekumar, A., Newgard, C. B., Wang, M., Tsai, S. Y., Tsai, M. J., & O'Malley, B. W. (2015). CAPER is vital for energy and redox homeostasis by integrating glucose-induced mitochondrial functions via ERR-α-Gabpa and stress-induced adaptive responses via NF-κB-cMYC. *PLoS genetics*, *11*(4), e1005116. https://doi.org/10.1371/journal.pgen.1005116

Kulkarni, V. A., & Firestein, B. L. (2012). The dendritic tree and brain disorders. *Molecular and cellular neurosciences*, *50*(1), 10–20. https://doi.org/10.1016/j.mcn.2012.03.005

Loerch, S., Maucuer, A., Manceau, V., Green, M. R., & Kielkopf, C. L. (2014). Cancer-relevant splicing factor CAPERα engages the essential splicing factor SF3b155 in a specific ternary complex. *The Journal of biological chemistry*, *289*(25), 17325–17337. https://doi.org/10.1074/jbc.M114.558825

Ly, K., Reid, S. J., & Snell, R. G. (2015). Rapid RNA analysis of individual Caenorhabditis elegans. *MethodsX*, *2*, 59–63. https://doi.org/10.1016/j.mex.2015.02.002

Mercier, I., Gonzales, D. M., Quann, K., Pestell, T. G., Molchansky, A., Sotgia, F., Hulit, J., Gandara, R., Wang, C., Pestell, R. G., Lisanti, M. P., & Jasmin, J. F. (2014). CAPER, a novel regulator of human breast cancer progression. *Cell Cycle*, *13*(8), 1256–1264. https://doi.org/10.4161/cc.28156

Mai, S., Qu, X., Li, P., Ma, Q., Cao, C., & Liu, X. (2016). Global regulation of alternative RNA splicing by the SR-rich protein RBM39. *Biochimica et biophysica acta*, *1859*(8), 1014–1024. https://doi.org/10.1016/j.bbagrm.2016.06.007

McFarlane S. (2000). Dendritic morphogenesis: building an arbor. *Molecular neurobiology*, *22*(1-3), 1–9. https://doi.org/10.1385/MN:22:1-3:001

Olesnicky, E. C., Killian, D. J., Garcia, E., Morton, M. C., Rathjen, A. R., Sola, I. E., & Gavis, E. R. (2014). Extensive use of RNA-binding proteins in Drosophila sensory neuron dendrite morphogenesis. *G3 (Bethesda, Md.)*, *4*(2), 297–306. https://doi.org/10.1534/g3.113.009795

Oka, T., Toyomura, T., Honjo, K., Wada, Y., & Futai, M. (2001). Four subunit a isoforms of Caenorhabditis elegans vacuolar H+-ATPase. Cell-specific expression during development. *The Journal of biological chemistry*, *276*(35), 33079–33085. https://doi.org/10.1074/jbc.M101652200

Paradis, S., Ailion, M., Toker, A., Thomas, J. H., & Ruvkun, G. (1999). A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in Caenorhabditis elegans. *Genes & development*, *13*(11), 1438–1452. https://doi.org/10.1101/gad.13.11.1438

Pierre de la Grange, Lise Gratadou, Marc Delord, Martin Dutertre, Didier Auboeuf. (2010). Splicing factor and exon profiling across human tissues, *Nucleic Acids Research*, Volume 38, Issue 9, 1 May 2010, Pages 2825–2838, https://doi.org/10.1093/nar/gkq008

Saltzman, A. L., Pan, Q., & Blencowe, B. J. (2011). Regulation of alternative splicing by the core spliceosomal machinery. *Genes & development*, *25*(4), 373–384. https://doi.org/10.1101/gad.2004811

Singhvi, A., & Shaham, S. (2019). Glia-Neuron Interactions in *Caenorhabditis elegans*. *Annual review of neuroscience*, *42*, 149–168. https://doi.org/10.1146/annurev-neuro-070918-050314

Smith, C. J., J. D. Watson, W. C. Spencer, T. O'Brien, B. Cha et al. (2010). Time-lapse imaging and cell-specific expression profiling reveal dynamic branching and molecular determinants of a multi-dendritic nociceptor in C. elegans. Dev. Biol. 345: 18–33.

Song, Y., Guo, Y., Li, X., Sun, R., Zhu, M., Shi, J., Tan, Z., Zhang, L., & Huang, J. (2021). RBM39 Alters Phosphorylation of c-Jun and Binds to Viral RNA to Promote PRRSV Proliferation. *Frontiers in immunology*, *12*, 664417. https://doi.org/10.3389/fimmu.2021.664417

Tari, M., Manceau, V., de Matha Salone, J., Kobayashi, A., Pastré, D., & Maucuer, A. (2019). U2AF^{65} assemblies drive sequence-specific splice site recognition. *EMBO reports*, *20*(8), e47604. https://doi.org/10.15252/embr.201847604

Tessa Sherry, Ava Handley, Hannah R. Nicholas, Roger Pocock. (2020). Harmonization of L1CAM expression facilitates axon outgrowth and guidance of a motor neuron. https://journals.biologists.com/dev/article/147/20/dev193805/225944/Harmonization-of-L1CAM-expression-facilitates-axon

Thomas, R., & Majeti, R. (2019). No Matter How You Splice It, RBM39 Inhibition Targets Spliceosome Mutant AML. *Cancer cell*, *35*(3), 337–339. https://doi.org/10.1016/j.ccell.2019.02.013

Ting Zhu, Xing Liang, Xiang-Ming Wang, Kang Shen. (2017). Dynein and EFF-1 control dendrite morphology by regulating the localization pattern of SAX-7 in epidermal cells. *J Cell Sci*; 130 (23): 4063–4071. doi: https://doi.org/10.1242/jcs.201699

Titus, M. B., Wright, E. G., Bono, J. M., Poliakon, A. K., Goldstein, B. R., Super, M. K., Young, L. A., Manaj, M., Litchford, M., Reist, N. E., Killian, D. J., & Olesnicky, E. C. (2021). The conserved alternative splicing factor caper regulates neuromuscular phenotypes during development and aging. *Developmental biology*, *473*, 15–32. https://doi.org/10.1016/j.ydbio.2021.01.011

"Tools." *National Institutes of Health*, U.S. Department of Health and Human Services, https://imagej.nih.gov/ij/docs/tools.html

Tsalik, E. L., and O. Hobert. (2003). Functional mapping of neurons that control locomotory behavior in Caenorhabditis elegans. J. Neurobiol. 56: 178–197.

Watson, J.D., Wang, S., Von Stetina, S.E. *et al.* (2008). Complementary RNA amplification methods enhance microarray identification of transcripts expressed in the *C. elegans* nervous system. *BMC Genomics* **9,** 84. https://doi.org/10.1186/1471-2164-9-84

Way, C., and M. Chalfie. (1989). The mec-3 gene of Caenorhabditis elegans requires its own product for maintained expression and is expressed in three neuronal cell types. Genes Dev. 3: 1823–1833.

Wei, Y., Han, X. & Zhao, C. (2020). PDK1 regulates the survival of the developing cortical interneurons. *Mol Brain* **13,** 65. https://doi.org/10.1186/s13041-020-00604-6

Wright, E. G. (2019). *How Does the RNA Binding Protein Caper Influence Drosophila behavior and Longevity?* (Document No. 13878043) [Master thesis, University of Colorado, Colorado Springs] ProQuest Dissertations Publishing.

Xu, M., Han, X., Liu, R., Li, Y., Qi, C., Yang, Z., Zhao, C., & Gao, J. (2019). PDK1 Deficit Impairs the Development of the Dentate Gyrus in Mice. *Cerebral cortex (New York, N.Y. : 1991)*, *29*(3), 1185–1198. https://doi.org/10.1093/cercor/bhy024

Xu, Y., Nijhuis, A., & Keun, H. C. (2020). RNA-binding motif protein 39 (RBM39): An emerging cancer target. *British journal of pharmacology*, 10.1111/bph.15331. Advance online publication. https://doi.org/10.1111/bph.15331

Zahler A. M. (2005). Alternative splicing in C. elegans. *WormBook : the online review of C. elegans biology*, 1–13. https://doi.org/10.1895/wormbook.1.31.1

Zallen, J., Yi, A., Bargmann, C. (1998). The Conserved Immunoglobulin Superfamily Member SAX-3/Robo Directs Multiple Aspects of Axon Guidance in C. elegans. *Cell 92(2)*, 217-222. https://www.sciencedirect.com/science/article/pii/S0092867400809162?via%3Dihub

Zheng, C. L., Fu, X. D., & Gribskov, M. (2005). Characteristics and regulatory elements defining constitutive splicing and different modes of alternative splicing in human and mouse. *RNA (New York, N.Y.)*, *11*(12), 1777–1787. https://doi.org/10.1261/rna.2660805

Zhang, A., & Yan, D. (2021). C. elegans as a model to study glial development. *The FEBS journal*, 10.1111/febs.15758. Advance online publication. https://doi.org/10.1111/febs.15758

Zhou, H., Mangelsdorf, M., Liu, J. *et al.* (2014). RNA-binding proteins in neurological diseases. *Sci. China Life Sci.* **57,** 432–444. https://doi.org/10.1007/s11427-014-4647-9

Zhou, Q., Su, X., Jing, G. *et al.* (2018). RNA-QC-chain: comprehensive and fast quality control for RNA-Seq data. *BMC Genomics* **19,** 144. https://doi.org/10.1186/s12864-018-4503-6