Investigation of the role of the *ncl-1* gene in dendrite morphogenesis in *C. elegans*

A Senior Thesis

submitted to the

Department of Biology,

The Colorado College

by

Genevieve Kerr

Graduation Date: May 2012

Degree: Bachelor's of Arts Degree in Biology

Approved by:

Darrell Killian
Primary Thesis Advisor

Nancy Huang
Secondary Thesis Advisor

Table of Contents

I. Abstract

Neurons are polarized cells with specialized processes known as dendrites that receive environmental stimuli and transduce that input to the cell body, or soma. Dendrites are important in generating action potentials for cell-to-cell communication and, in the case of sensory neurons, for sensing the environment. Despite the important role that dendrites play, the molecular mechanisms that regulate dendrite development, or morphogenesis, are poorly understood. Recent research indicates that dendrite morphogenesis is regulated by the localized control of messenger RNA (mRNA) in dendrites. mRNA localization and translational regulation is often mediated by RNA-binding proteins (RBPs), which recognize and bind to specific mRNAs. It is thought that regulating protein translation in dendrites, which are located far from the nucleus where mRNAs originate, is a faster and more efficient way to regulate dendrite morphogenesis than changing gene expression.

A recent study has found that the RBP gene *brat* regulates dendrite morphogenesis in *Drosophila.* To determine if *brat* function is conserved, we studied the role of *ncl-1*, a *C. elegans* homolog of *brat,* in dendrite morphogenesis. Dendrite morphology in wild type and *ncl-1* mutants was compared using a fluorescent marker that is expressed in the PVD mechanosensory neurons in *C. elegans*. We find that *ncl-1* null mutant PVDs have fewer dendritic branches than wild type throughout development. Consistent with a role in PVD dendritic development, we find that *ncl-1* is expressed in most neurons during development, likely including the PVD. Since NCL-1 may be involved in regulating mRNAs in dendrites we wanted to see where

within neurons the NCL-1 protein is localized. We find that NCL-1 is localized to both axons and dendrites, but was excluded from the nucleus. Together, these results suggest that NCL-1 plays a conserved role as an RBP that regulates mRNAs important for dendrite elaboration and future studies will be aimed at learning which mRNAs NCL-1 binds and how it regulates them.

II. Introduction

A. Dendrite Morphogenesis

Cellular morphogenesis is made up of general morphological changes that are required by the cell to allow for its proper functioning, as it controls the organized spatial distribution of cells during the embryonic development of an organism. Part of this process involves breaking symmetry in which undifferentiated cells take on a rather spherical, shape while differentiated cells tend to adopt a more complex shape. Additionally, cellular morphogenesis relies on the dynamic interaction between several cytoskeletal elements, which each play their own unique role during morphogenesis, as controlled by their respective proteins. Furthermore, these proteins play a role in neuronal development, or morphogenesis, through which a neuron is elongated into its various components. This process underlies the establishment and plasticity of the several neuronal networks that make up the nervous system.

The nervous system is made up of glial cells and neurons, which have highly complex and asymmetric cellular morphologies. They are composed of the soma, or cell body, the axon which functions in the transmission of stimuli, and specialized branched projections known as dendrites that receive environmental stimuli. Although these dendrites have several important functions, as they generate action potentials for cell-to-cell communication, they are particularly important in sensing the environment. This is especially true for mechanosensory neurons, whose primary responsibility is to sense touch due to elaborate dendritic arborization patterns. These dendrites cover large fields of touch-sensitive tissue to guarantee

appropriate sensitivity to sensory stimuli, thus regions that lack these dendrites are insensitive to touch. Additionally, there are several other types of neurons, which are important for learning, behavior, and memory due to their elaborate dendritic branching. Despite the important role that dendrites play, the molecular mechanisms that regulate dendrite development, or morphogenesis, are poorly understood.

Due to recent research, it is becoming evident that post-transcriptional regulatory mechanisms such as mRNA localization and translational regulation play an essential role in dendrite morphogenesis, particularly in directing dendritic branch formation. As mRNAs can be locally activated or repressed within dendrites, they are able to influence local dendritic morphogenesis, maintenance, and function. Additionally, mRNA localization and translational regulation are often mediated by RNA-binding proteins (RBPs), which recognize and bind to specific mRNAs, as the genes that encode for RBPs are abundant within the eukaryotic genome. It is thought that regulating protein translation in dendrites, which are located far from the nucleus where mRNAs originate, is a faster and more efficient way to regulate dendrite morphogenesis than changing gene expression (Jan and Jan, 2010).

Understanding how dendritic development is genetically regulated is important because dendritic defects are known to be associated with several human disorders such as autism, schizophrenia, and Fragile X syndrome (Bassell and Warren, 2008; Fallini et al., 2011). In each of these disorders, synaptic and dendritic mechanisms are specifically targeted leading to impairments in cognition and perception. By uncovering the molecular mechanisms that regulate dendrite

morphogenesis, researchers could further their understanding of these various disorders to help find more appropriate treatments.

B. RNA Binding Proteins in *Drosophila*

Several studies aiming to determine the specific molecules and processes governing dendrite morphogenesis have been performed using the fruit fly, *Drosophila melanogaster (Drosophila)*, as the model organism. One study in particular has shown that the translational regulators *nanos* (*nos)* and *pumilio* (*pum)* in *Drosophila* play an essential role in dendrite morphogenesis in sensory neurons, particularly in the formation of high-order dendritic branches (Ye et al., 2004). Another study showed that loss-of-function mutations in either *nanos* or *pum* result in decreased dendritic branching, in addition to a failure to maintain existing branches within sensory neurons (Olesnicky et al., 2012).

Additional studies have looked at another translational co-factor *brain tumor* (*brat*), which functions with *nos* and *pum* to regulate dendrite morphogenesis. *brat* acts as an inhibitor of cell growth and represses ribosomal RNA synthesis (Frank et al., 2002). Furthermore, it has been shown that *brat* is required for elaborate dendrite branching in dendritic arborization neurons (Olesnicky et al., 2012).

To determine the contribution of post-transcriptional gene regulation in dendrite morphogenesis, an RNA interference (RNAi) screen was performed of all *Drosophila* RBPs for dendrite defects within the dendritic arborization sensory neurons (Olesnicky et al., 2012). This screen allowed researchers to specifically examine the role of each RBP gene in the da neurons to determine which are

required for a biological process of interest. Like most sensory neurons, dendritic arborization neurons have complex dendritic trees in non-overlapping receptive fields (Parrish et al., 2007). This RNAi screen uncovered approximately 90 RBP genes that function in dendrite morphogenesis including genes that encode translational repressors, translation initiation and elongation factors, splicing factors, and proteins involved in mRNA transport (Olesnicky et al., 2012). Interestingly, the vast majority of these RBP genes are highly conserved between *C. elegans, Drosophila,* and humans. This discovery that the RBPs required for dendrite morphogenesis in *Drosophila* (Olesnicky et al, 2012) are conserved in other animal species allows for researchers to investigate the extent to which post-transcriptional gene regulatory mechanisms contribute to neuronal morphogenesis across these species.

C. *Caenorhabditis elegans* PVD Neurons

Caenorhabditis elegans (C. elegans), a type of nematode worm, is an ideal model system for the study of gene regulation and function in living animals, including the molecular genetic dissection of dendrite morphogenesis. This is in part due to the fact that they are inexpensive and easy to maintain in the laboratory, and have a fast, convenient life cycle. Additionally, due to their transparency, it is easy to observe neurons and other cells in living animals. Lastly, they are available in a variety of mutant strains and are quite susceptible to genetic manipulations. These properties of *C. elegans* provide members of the scientific community with an attractive pool of resources.

PVD neurons, a type of nociceptive neuron with complex dendritic arbors, occupy posterior-lateral positions on either the left or the right side of the worm. Due to their dendrites, which cover the animal in a web-like arrangement, these neurons function in responding to pain-evoking stimuli. Although little is known about the molecular mechanisms that govern PVD dendrite morphogenesis, it has been shown that this complex dendritic branching is essential for sensory function (Tsalik and Hobert, 2003). It will be interesting to see if common mechanisms and conserved molecules are used in dendrite elaboration among a diverse group of animal species.

D. Homologue of *brat* in *C. elegans*

An investigation of the function of RBP candidate genes in the *C. elegans* PVD system will help to determine the extent to which the genes and mechanisms of dendrite morphogenesis are conserved between two distantly related animals. The *ncl-1* gene in *C. elegans* is homologous to *brat* in *Drosophila* (Frank et al., 2002). Brat/NCL-1 negatively regulates ribosome biogenesis, a process that helps to control cell growth, differentiation, and proliferation (Frank and Roth, 1998; Frank et al., 2002; Voutev et al., 2006). Additionally, loss-of-function mutations in *ncl-1* have previously been shown to result in enlarged nucleoli, increased rates of rRNA transcription, and enlarged cells (Frank and Roth, 1998). Since *brat* is known to function in *Drosophila* dendrite morphogenesis, I reasoned that *ncl-1* might also be involved in dendrite morphogenesis in *C. elegans*. By comparing the alignment of the amino acid sequences *ncl-1* and *brat*, they share 83% identity (Figure 1).

Additionally, the e-blast value, which is the chance that the amino acids would be the same based on chance alone and based on the number of sequences in the database, is close to zero suggesting that the match is significant and that the two proteins are orthologs, share a common ancestor, and likely share a common function.

Figure 1: Alignment of the amino acid sequences of *ncl-1* homologues in *Homo sapiens* (Hs), Danio rerio (Dr), *Drosophila melanogaster* (Dm), and *Hydra magnipapillata* (Hm)

Consistent with the idea that *ncl-1* functions in dendrite morphogenesis, a microarray experiment that was used to identify all genes enriched for expression in the PVD neuron found that *ncl-1* was expressed 2.02-fold higher in the PVD than in the lysate of all cells (Smith et al, 2010).

D. Specific Aims

In basic evolutionary relationships, a protein and/or gene has a similar function in each of the organisms in which a homolog exists. In the case of *ncl-1*, there is a homolog in each organism that has a nervous system; however, a homolog does not exist in those organisms that do not possess a nervous system (Figure 1). Through these studies, I investigated the role of *ncl-1*, as I aimed to determine if the role of this translational regulator is conserved in the development of dendrites, similar to the role of *brat* in *Drosophila.*

To study this, I compared dendrite morphology in wild type and *ncl-1* mutants using a green fluorescent protein (GFP) reporter that is expressed in the PVD mechanosensory neurons in *C. elegans.* For the purposes of this study, I compared dendritic morphology at three different points in development, 40 hours (prior to adult maturation), 46 hours (young adults), and 60 hours (old adults), by imaging neurons and quantifying the dendritic branches. Next, it is known that the *ncl*-1 gene is expressed in many cells in *C. elegans* but it is not known if *ncl-1* is specifically expressed in mechanosensory neurons. Because of this, I wanted to see where, temporally and spatially, *ncl-1* is expressed within developing worms by creating transgenic worms with the *ncl-1* promoter fluorescently tagged to drive

GFP expression. I then imaged the worms to determine where and when the *ncl-1* promoter drives gene expression. Lastly, I worked to determine where the NCL-1 protein is localized within developing worms by creating transgenic worms with fluorescently tagged NCL-1. Once again, I imaged the mechanosensory neurons to determine where the protein is present within the neurons.

The purpose of these studies was to determine if *ncl-1* in *C. elegans* like *brat* in *Drosophila* is involved in dendrite morphogenesis. As supporting evidence, I found that *ncl-1* null mutant PVDs have fewer dendritic branches than wild type throughout development. Additionally, I found that the *ncl-1* gene is expressed in most neurons during development, likely including the PVD. Lastly, I found that NCL-1 protein is localized to the axon, cell body, and dendrites, but excluded from the nucleus. Collectively, the results suggest that NCL-1 may regulate mRNAs important for dendrite elaboration and/or NCL-1 may be a conserved RBP involved in dendrite morphogenesis. Given that the homologous RBPs Brat and NCL-1 play important roles in dendrite morphogenesis in fly and worm respectively, it is possible that the human homolog Brain-Expressed Ring Finger Protein (Figure 1) plays a similar role in human neurons.

III. Materials & Methods

A. Strains

Strain NC1841 *rwIs1 [mec-7::RFP]; wdIs52 [F49H12.4::GFP]* (Smith et al., 2010) was used as the standard wild-type strain, which has a GFP marker for PVD neurons and an RFP marker for FLP neurons not used in this study. Strain DJK49 was used as the mutant strain, which has the same transgenes but also includes the *ncl-1 (e1492)* null mutation. *unc-76 (e911)* worms were used to create transgenic worms through microinjection. Worms lacking a functional copy of unc-76 are uncoordinated; however, injected DNA carrying a functional copy of unc-76 can rescue this uncoordinated phenotype. Worms were cultured using standard procedures (Brenner, 1974) and maintained at 20°C.

B. Synchronization

Thirty plus gravid worms from each strain were placed on fresh plates and allowed to lay eggs overnight at 20° C. M9 buffer was used to wash each plate by gently swirling to dislodge the worms so that only embryos remained on the plates. After waiting one hour, each plate was washed an additional time with M9 buffer, and worms that hatched in the one hour time period were collected in a 15 mL tube and centrifuged. The supernatant liquid was removed and the remaining liquid and worms were dispensed onto a new plate. Time was set to zero and the worms were allowed to develop until the desired time point was reached: 40, 46, and 60 hours.

C. Quantification of Dendritic Branching

A total of 20 *C. elegans* from each strain were mounted on 2% agarose pads and paralyzed with 800μM levamisole. Using a Zeiss AxioScope A1 fluorescence compound microscope at 40X magnification, the number of 4th order dendritic branches in the PVD neurons were counted and recorded. A student t-test was run on the results from each of the three different time points: 40 hours, 46 hours, and 60 hours.

D. Transgene Construction by Gateway Cloning

A *ncl-1* transcriptional fusion to nuclear GFP was created in several steps. The 1kb *ncl-1* promoter was PCR amplified using primers "*ncl-1* promoter Forward *att*B1" and "*ncl-1* up to ATG Reverse *att*B2" and was then cloned into the destination vector, pDONR221 using BP Clonase (Invitrogen). The entry clone containing the *ncl*-1 promoter was called pDJK185. The *ncl-1* promoter was then recombined from pDJK185 into pDJK81 (promoterless vector with a gateway site adjacent to 4xNLS::GFP) with LR Clonase (Invitrogen) to create pDJK186, a plasmid carrying the promoter of *ncl-1* driving 4xNLS::GFP.

To create a *ncl-1* translational fusion to GFP, the *ncl-1* cDNA from the C. elegans ORF-RNAi library (Open Biosystems) was PCR amplified using primers "*ncl-1* +2ATG Forward *att*B1" and "*ncl-1* STOP Reverse *att*B2" and was cloned into the destination vector, pDONR221 to create pDJK195. This plasmid was then recombined through an LR reaction into pDJK21 (ubiquitous promoter from *tra-*

5::GFP::gateway) to form pDJK196, a plasmid carrying the *tra-5* ubiquitous promoter driving *GFP::ncl-1*.

The primer sequences used for the above PCR amplifications are as follows:

ncl-1 promoter Forward *att*B1:

GGGGACAAGTTTGTACAAAAAAGCAGGCTTGTGGTTCATTTCAATGATCTCATCC

ncl-1 up to ATG Reverse *att*B2:

GGGGACCACTTTGTACAAGAAAGCTGGGTGCATGTACAAATAAGGAAATTATCAC

ncl-1 +2ATG Forward *att*B1:

GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGAGACTCAGTTAAGGTACAGAAG

ncl-1 Stop Reverse *att*B2:

GGGGACCACTTTGTACAAGAAAGCTGGGTCTAGATCTGGCTAGAAGCGGAAGAAGTTG

E. *C. elegans* Microinjection

unc-76 mutant worms were injected with an injection mix composed of 60ng/ul *unc-76* rescue DNA, 10 mM Tris pH 8.5, and 20-40ng/ul of pDJK186 or pDJK196 DNA for a total concentration of 100 ng/uL. After the microinjection took place in the syncytial gonad, each injected worm was placed onto its own plate so that it could produce progeny. After a few days F1 worms displaying the non-Unc phenotype were moved to new plates and allowed to reproduce. The F2 worms were then scored for the transgenic phenotype and imaged at 40X and 100X magnification at different stages of development to document GFP expression patterns and localization.

IV. Results

A. Quantification of Dendritic Branching

To determine if *ncl-1* is required for dendrite morphogenesis in *C. elegans*, I analyzed the dendrite morphology in the PVD neuron at various time points in development using a GFP marker for the PVD and fluorescence microscopy (see Materials and Methods).

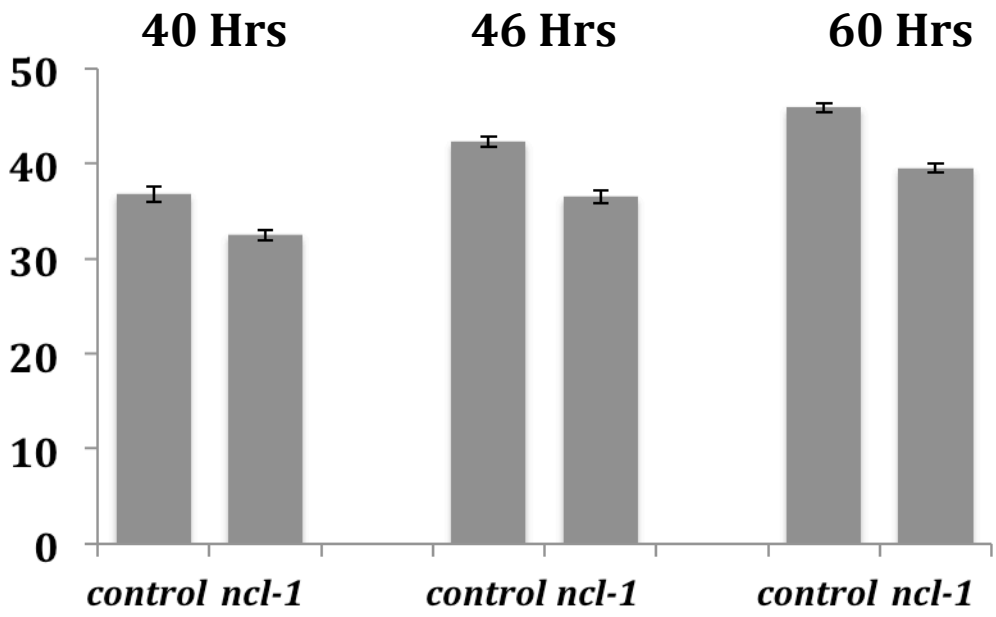

Figure 2: Quantification of 4th order dendritic branches at 40 hours, 46 hours, and 60 hours.

After 46 hours of development, the average number of dendritic termini was 42.3 for wildtype and 36.5 for *ncl-1* mutants (Figure 2). By running a student t-test on these numbers, this difference proved to be statistically significant ($t = 6.456$, p-value < 0.001, $df = 38$).

Looking at the 40-hour time point, the average number of dendritic termini was 36.8 for wildtype and 32.5 for n*cl-1* mutants (Figure 2). Once again, by running a student t-test, this difference proved to be statistically significant (t = 4.6532, p-value < 0.001, df = 38).

Looking at the 60-hour time point, the average number of dendritic termini was 45.9 for wildtype and 39.5 for *ncl-1* mutants (Figure 2). This difference proved to be statistically significant by running a student t-test (t = 9.566, p-value < 0.001, df = 38).

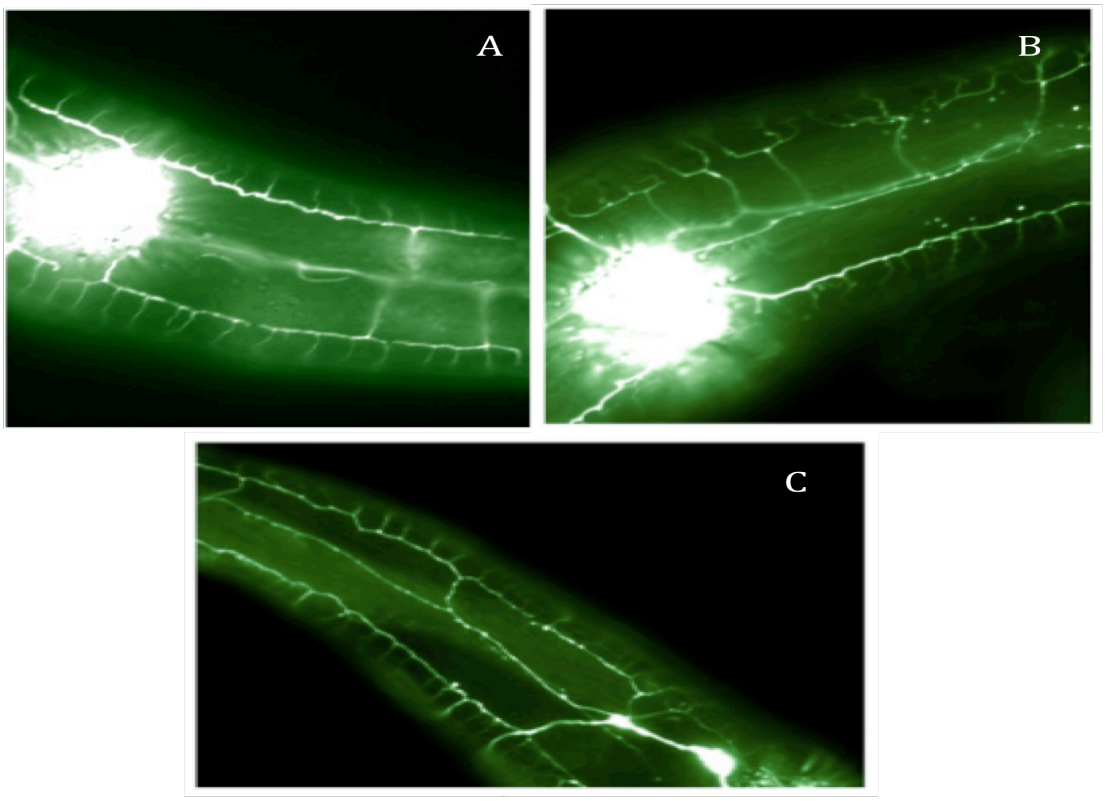

Figure 3: PVD neuron at various points in development. A.) Wildtype control at 40 hours. B.) *ncl-1* mutant at 46 hours. C.) *ncl-1* mutant at 60 hours.

Figure 3 shows representative images of the number of dendritic termini at the various points in development. Consistent with statistical analysis, it is visually evident that there are slightly fewer 4th order dendritic branches at 46 hours than at 60 hours in *ncl-1* mutants and even less at 40 hours than at 46 hours. Additionally, these images show the physical differences between the wildtype control and mutant strains. It is evident that the dendritic termini are much more regular and evenly spaced in the wildtype control, whereas in the *ncl-1* mutants, they are quite irregular and sporadic, even looking deformed in some cases.

B. Phenotypic Analysis of the Nucleolus

To provide further evidence of the function of *ncl-1* in PVD neurons, the PVD neurons in control and *ncl-1* mutant strains were examined under 1000X magnification.

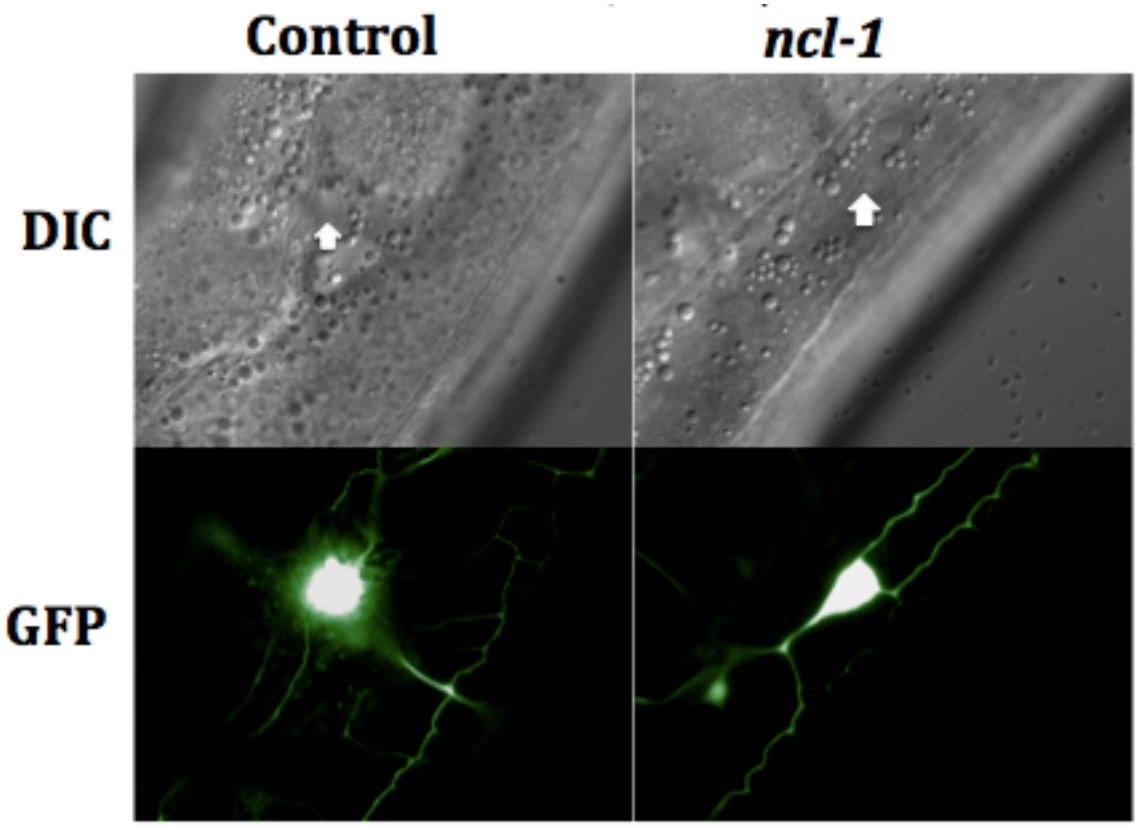

Figure 4: PVD neurons in wildtype control and *ncl-1* mutant strains. The arrow indicates the nucleolus. The images of the same sample taken using fluorescence microscopy verify that the cell is the PVD neuron.

The nucleolus is significantly enlarged in the *ncl-1* mutant, as it is clearly detectable, whereas the nucleolus in the wildtype control isn't visible at 1000x magnification. (Figure 4).

C. *ncl-1* is expressed in neurons

Since *ncl-1* mutant worms have defects in dendritic elaboration, I wanted to see where, temporally and spatially, *ncl-1* is expressed within developing worms. To test for *ncl-1* expression, I created a *ncl-1* transcriptional fusion by fusing the regulatory region of *ncl-1* to the GFP gene with four tandem nuclear localization signals so that cells expressing *ncl-1* will have GFP-labeled nuclei (see Materials and Methods).

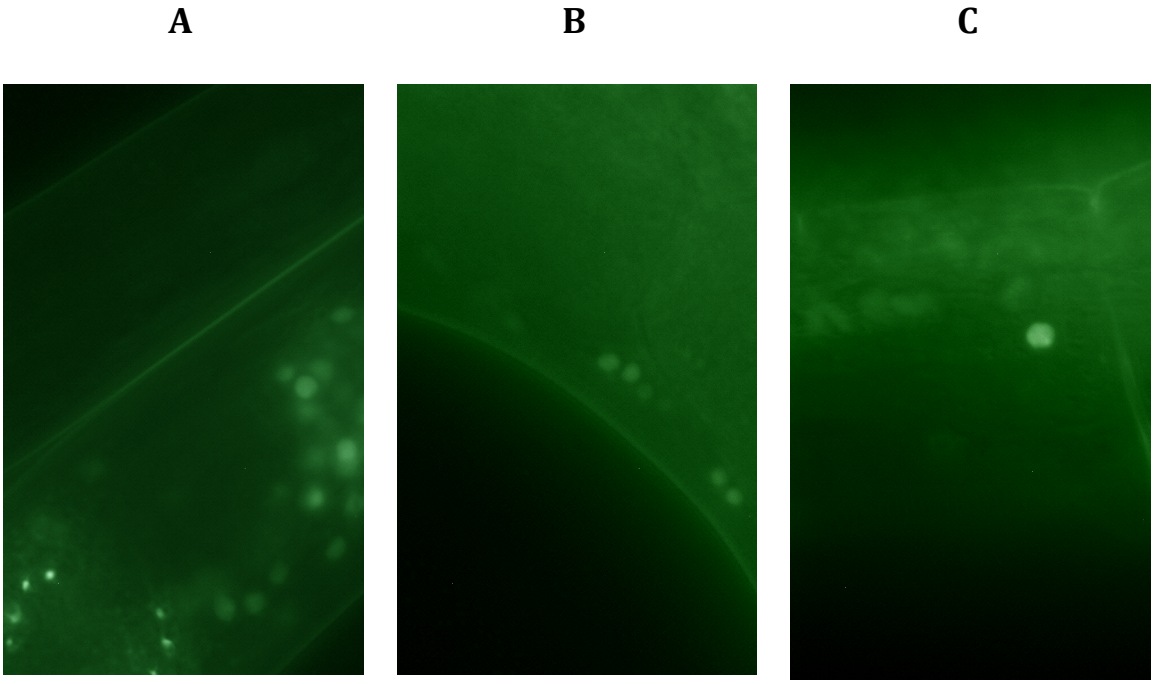

Figure 5: A *ncl-1* transcriptional fusion with the *ncl-1* promoter driving nuclear GFP expression. A.) Nerve Ring B.) Neurons within the Ventral Nerve Cord (VNC) C.) HSN

Figure 5 shows that the injected worms did indeed the express the pDJK186 transgene with each visible dot corresponding to the nucleus of one of several neurons. In Figure 5A, the largest collection of neurons in the head of the worm, the

nerve ring, is expressing GFP. In Figure 5B, the neurons that make up the ventral nerve cord (VNC), which contains most *C. elegans* motor neurons, are expressing GFP. Lastly, in Figure 5C, another motor neuron, the HSN, or hermaphrodite specific neuron, is shown to be expressing GFP.

D. NCL-1 protein is localized to axons, dendrites, and the cytoplasm

Since *ncl-1* is expressed in most, if not all, cells during development, I wanted to see where within neurons the NCL-1 protein is localized. To test this, I created a *ncl-1* translational fusion with the *ncl-1* coding region fused to GFP driven by the *tra-5* promoter, which is ubiquitously expressed (Killian and Xue, unpublished; see Materials and Methods). This transgene causes a GFP:NCL-1 fusion protein to be expressed in all cells so that its localization within cells can be determined.

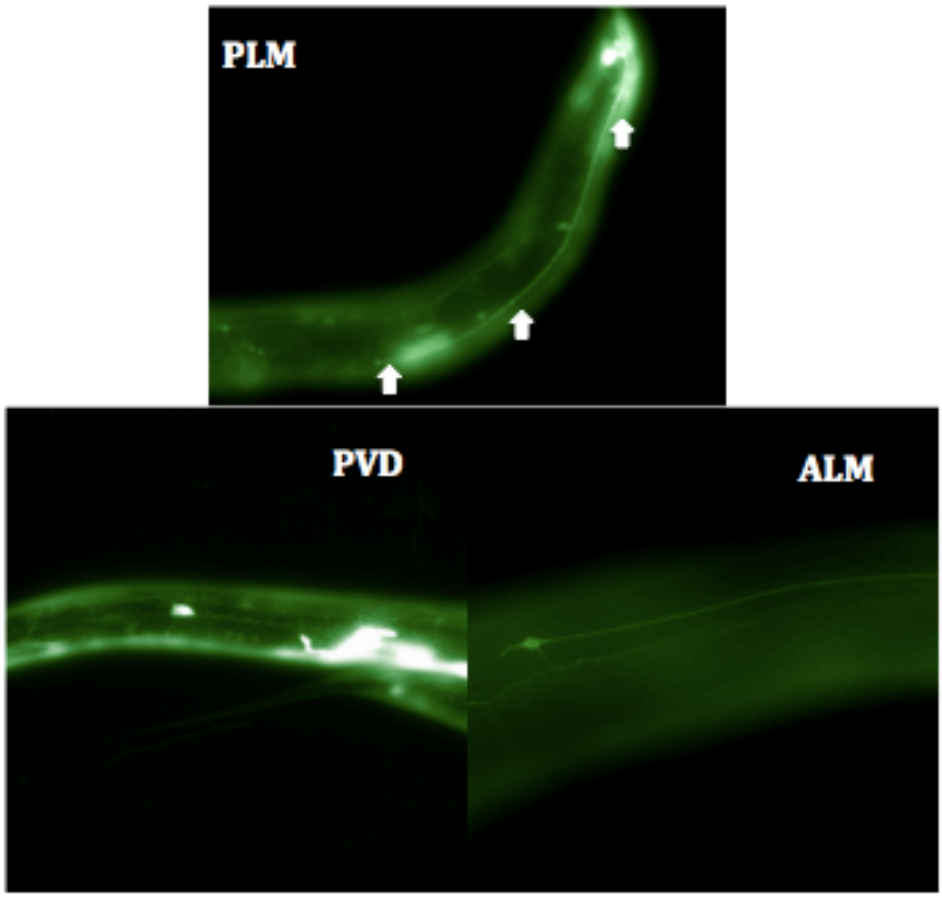

Figure 6: Localization of GFP::NCL-1 fusion protein in the PLM, PVD, and ALM neurons.

I found that the GFP::NCL-1 fusion protein was expressed much more brightly in the touch neurons, including the PLM, ALM, and PVD neurons (Figure 6) than in other cells. Within these neurons, GFP::NCL-1 is localized to the dendrites, axons, and the cell body, but excluded from the nucleus, as the center of the cell body is slightly dimmer than the rest of the neuron. Additionally, it appears as though the protein is potentially enriched at the terminus and branch points, as these select areas are slightly brighter than the rest of the neuron, particularly in the PLM neuron.

V. Discussion

A. Phenotypic Analysis of the PVD Neuron

The results found at the 46-hour time point provided evidence that *ncl-1* mutants do show reduced dendritic branching. Because of these results, I wanted to see if this difference held true at an earlier and a later time point, so to demonstrate that the number of dendritic branches in the *ncl-1* mutants was reduced throughout development rather than increasing or decreasing at a certain point. I reasoned that if the number of dendritic termini in the *ncl-1* mutant did not match that seen in the wildtype control by 60-hours, then they would be continue to be reduced even later in development. By looking at the results for both 40 hours and 60 hours, in addition to 46 hours, I find that, consistent with the *brat* mutant phenotype, *ncl-1* null mutant PVDs have reduced 4th order dendritic branching throughout development (Figure 2). These results are important because they showed that there is at least one conserved component in dendrite elaboration between *Drosophila* and *C. elegans*, a reduction in dendritic branching. It will be interesting to see if other *brat* neuronal phenotypes are present in *ncl-1* mutant neurons.

Additionally, by looking at the nucleolus of the PVD neuron in both wildtype controls and ncl-1 *mutants*, it was evident that *ncl-1* functions in the PVD due to the increase in the size of the nucleolus in the *ncl-1* mutant. These results helped to supplement what was found in the previous study by providing further evidence that *ncl-1* does function in the PVD to result in decreased dendritic branching throughout development.

B. *ncl-1* is expressed in most if not all neurons

Using a *ncl-1* transcriptional fusion to nuclear GFP it is apparent that GFP is expressed in most, if not all, neurons (Figure 5). Due to the nuclear localization signals, each of the fluorescent signals in Figure 5 corresponds to the nucleus of one of several neurons. This shows that, consistent with a role in PVD dendritic development, *ncl-1* is expressed in most neurons during development, likely including the PVD, with the promoter of *ncl-1* driving GFP expression.

Although *ncl-1* wasn't found to be precisely located within the PVD, a microarray experiment that was used to identify all genes enriched for expression in the PVD neuron found that *ncl-1* was expressed 2.02-fold higher in the PVD than in the lysate of all cells (Smith et al, 2010). This suggests that *ncl-1* is expressed within the PVD. In order to determine if *ncl-1* is actually expressed in the PVD, the transgene pDJK186 could be co-injected with a red fluorescent protein (RFP) marker for PVDs. If GFP and RFP expression were to be found within the same cell, it would strongly suggest that *ncl-1* is expressed in the PVD to corroborate the aforementioned results.

C. Localization of NCL-1 Protein

From the *ncl-1* transcriptional fusion, we saw that the *ncl-1* promoter was essentially expressed everywhere throughout developing worms; here, however, it appears as though the NCL-1 protein is differentially upregulated in different cell types. In this way, these results suggest that NCL-1 protein may be degraded in

other cells, but translationally upregulated in neurons, which serves as a possible explanation to why the neurons may be fluorescing more brightly than other cells (Figure 6). These results also suggest that NCL-1 plays a conserved role as an RBP that is expressed in a variety of neurons. Furthermore, NCL-1 may be localized to dendrites because, as an RNA-binding protein, it may regulate the localization or translation of mRNAs important for dendrite elaboration (see below).

D. Human homologue: BERP

The mammalian homologs of *ncl*-1 and *brat* belong to a group of proteins known as the tripartite motif proteins and include brain-expressed RING finger protein (BERP). One study in particular indicated that BERP is able to interact with two distinct proteins associated with the actin cytoskeleton: α-actinin-4 and myosin V (El-Husseini et al., 2000), both of which are involved in muscle contraction. Furthermore, in *C. elegans*, a Yeast 2-hybrid assay was performed that showed that NCL-1 binds ALP-1, a type of worm actinin (Worm Interactome Database). This suggests that there is a direct link between NCL-1 and the actin cytoskeleton similar to the link between BERP and the actin cytoskeleton. If there exists at least this one conserved component between *C. elegans* and humans, it would be interesting to see if other conserved components are present.

E. Next Steps

An antibody stain could be performed in order to confirm the subcellular localization seen by the GFP::NCL-1 fusion protein, which suggested that it is

localized to branch points and the terminus. If an antibody stain shows the same result, we could be more certain that the GFP::NCL-1 fusion protein is localized to these areas. Frank and Roth (1998) produced an antibody that recognizes the NCL-1 protein. The purified antibodies can be used to determine where the NCL-1 protein is in worms by fixing worms and applying the antibody so it binds to the NCL-1 protein in a variety of cells. Lastly, another antibody can be used, which will bind to the mouse anti-NCL-1 antibodies. This so-called secondary antibody will have a covalently bound fluorescent molecule such as fluorescein to determine where the protein is localized.

More simply, the worms that expressed the GFP::NCL-1 fusion protein could be looked at more closely using a confocal microscope in order to get higher resolution images of the localization. This could help us learn if the GFP::NCL-1 fusion protein is generally cytoplasmic or enriched in certain areas.

Another future study that would help determine how *ncl-1* affects dendrite morphogenesis would be to determine which mRNAs NCL-1 binds. This could be done by performing a cross-linked immunoprecipitation of NCL-1, using the NCL-1::GFP fusion protein and an anti-GFP antibody. By targeting the protein with an anti-GFP antibody, it becomes possible to pull down the entire protein complex in order to identify previously unidentified components, such as RNAs, which allows for their sequencing. Target mRNAs will then be analyzed by genetic epistasis analyses and the conservation of these targets will be assessed using similar biochemical and genetic epistasis experiments in *Drosophila* dendritic arborization neurons.

VI. Acknowledgements

I would like to thank my research and thesis advisor, Darrell Killian, for his support and guidance throughout this process. I'd also like to thank Nancy Huang and Eugenia Olesnicky for their encouragement and helpful insight. Lastly, I'd like to thank Colorado College for providing me with a Venture Grant that funded the laboratory supplies needed for my research.

VII. References

Bassell GJ, Warren ST (2008). Fragile X Syndrome: loss of local mRNA regulation alters synaptic development and function. *Neuron* 60, 201–214.

El-Husseini AED, Kwasnicka D, Hirohashi TYS, Vincent SR (2000). BERP, a Novel Ring Finger Protein, Binds to a-Actinin-4. *Biochemical and Biophysical Research Communications* 267, 906–911.

Fallini C, Zhang H, Su Y, Silani V, Singer RH, Rossoll W, Bassell GJ (2011). The survival of motor neuron (SMN) protein interacts with the mRNA-binding protein HuD and regulates localization of poly(A) mRNA in primary motor neuron axons. *J. Neurosci.* 31, 3914–3925.

Frank DJ, Roth MB (1998). *ncl-1* is required for the regulation of cell size and ribosomal RNA synthesis in *Caenorhabditis elegans*. *J Cell Biol* 140(6), 1321-9.

Frank DJ, Edgar BA, Roth MB (2002). The *Drosophila melanogaster* gene *brain tumor* negatively regulates cell growth and ribosomal RNA synthesis. *Development* 129, 399-407.

Jan YN, Jan LY, (2010). Branching out: mechanisms of dendritic arborization. *Nature Reviews Neuroscience* 11, 316-328.

Olesnicky EC, Gavis ER, Bhoga B. Combinatorial use of translational co-factors for cell type specific regulation during neuronal morphogenesis in *Drosophila. Developmental Biology*. 2012 May 365(1):208-18.

Parrish JZ, Emoto K, Kim MD, Jan YN (2007). Mechanisms that regulate establishment, maintenance, and remodeling of dendritic fields. *Annu Rev Neurosci 30*, 399-423.

Smith CJ, Watson JD, Spencer WC, O'Brien T, Cha B, Albeg A, Treinin M, Miller DM 3rd (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(1), 18-33.

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

Voutev RV, Killian DJ, Ahn JH, Hubbard EJA (2006) Alterations in ribosome biogenesis cause specific defects in *C. elegans* hermaphrodite gonadogenesis. *Dev Biol* 298, 45-58.

Ye B, Petritsch C, Clark IE, Gavis ER, Jan LY, Jan YN (2004). Nanos and pumilio are essential for dendrite morphogenesis in *Drosophila* peripheral neurons. *Current Biology* 14(4), 314-21.