Nanos Proteins Play a Minimal Role in *C. elegans* Dendrite Development

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

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Bachelor of Arts Degree in Molecular and Cellular Biology
May 17, 2020

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ABSTRACT

The complex network of dendritic branches plays a vital role within neurons, and their regulation is critical for nervous system function. Due to the role of the neuron in the neural network, several neurological disorders have been linked to aberrant branching patterns in dendrites. This connection necessitates the investigation into the mechanisms by which dendrites form their arbors. Because of their role in posttranscriptional regulation, RNA-binding proteins emerge as likely candidates for the regulation of dendrite morphogenesis. In a previous screen of different RNA-binding proteins, Nanos was identified as important for dendrite morphogenesis in *Drosophila melanogaster*. To provide a cross species comparison, homologs of *Drosophila nanos* in *Caenorhabditis elegans* were deleted and investigated. *C. elegans* has three paralogs of *nanos*: *nos-1, nos-2, and nos-3.* All three of these paralogs are linked on the same chromosome. Single mutants of all three paralogs and a double mutant with *nos-1* and *nos-2* knocked out had already been generated, but no triple mutant had ever been constructed. Therefore, in order to reveal possible genetic redundancy of the three paralogs, CRISPR-Cas9 was used to delete *nos-3* in a strain already containing *nos-1; nos-2* deletions. Sequence data indicated that CRISPR had successfully deleted the intended segment of DNA. However, *nos-1* single mutants*, nos-2* single mutants*, nos-3* single mutants*, nos-1; nos-2* double mutants*,* and *nos-1; nos-2; nos-3* triple mutants' dendrite morphology did not show differences that led to a clear and obvious conclusion when compared to a control strain. We conclude that *nanos* does not play a significant role in dendrite morphogenesis in *C. elegans*.

INTRODUCTION

Dendrite Morphology and Brain Disorder

Neurons are generally comprised of an axon, responsible for transmitting messages, dendrites, responsible for receiving messages, and a cell body, where the nucleus resides. The neural network relies on many series of neurons, connecting presynaptic axons with dendrites. To form the intended connections, dendrites must grow far from the cell body from which it extends (Scott & Luo, 2001). Because of this, dendrites' ability to accurately receive and process sensory information from their receptive field is dictated by their morphology. If dendrite morphology is altered, effective signal processing can be hindered. Dendrite morphology, due to the crucial role it plays within the nervous system, has been implicated in various neurological diseases and disorders (**Figure 1**) (Jan & Jan, 2010; Kulkarni & Firestein, 2012). For example, changes in dendrite branching patterns, fragmentation of dendrites, decreased branching, and changes in spine morphology or number have all been implicated in neurological disorders such as autism, Schizophrenia, and Parkinson's (Jan & Jan, 2010; Kulkarni & Firestein *et al.,* 2012).

The complexity of the dendritic arbor necessitates a complex network of factors involved in their regulation. The different cellular compartments – cell bodies, dendrites, and axons – each require specialized regulation of mRNA by RNA-Binding Proteins (RBPs). Because of their important role as post transcriptional regulators, RBPs have been extensively implicated in neurological diseases such as Fragile X syndrome, Schizophrenia, and Epilepsy, among others (Zhou et al. 2014). Taken together, the importance of dendrite morphology in the function of

neural networks makes elucidating the underlying roles RBPs play in dendrite morphology an important topic of inquiry.

RNA-Binding Proteins

Dendrites respond quickly and independently to signals and stimuli, which suggests local, post-transcriptional regulation plays a role in dendrite development. It is for this reason that post-transcription regulators, like RNA-binding proteins, have been implicated in dendrite morphogenesis. RNA-binding proteins bind mRNAs to form ribonucleoprotein (RNP) complexes, carrying out different functions such as mRNA transport, splicing, and translational regulation (Keene, 2007). RBPs typically contain one or more RNA-binding domains, or motifs, with which they bind mRNA. The motifs confer their specificity, dictating which mRNAs they bind to (Keene, 2007; Glisovic *et al*., 2008).

Because RBPs are capable of controlling when and where specific mRNAs are translated, they offer an intriguing point of departure for investigation deeper into the process of dendrite morphogenesis. Different studies have highlighted RBPs' role in local protein synthesis in neurons (Wells, 2006). For example, it has been shown in *Xenopus* and mouse neurons that mRNA localization is implicated in the direction of axon growth (Medioni *et al.,* 2012). Furthermore, the RBPs ZBP1, hnRNP A2, FMRP, and Saufen 2 have all been shown to be involved in transportation of mRNAs from nucleus, to cytoplasm, to dendrites of neurons (Eberhart *et al.*, 1996; Shan *et al*., 2003; Kiebler *et al.*, 2005). Studies such as these provide the scaffolding required for the creation of models of the local protein synthesis that is known to be crucial for dendrite form and function. For example, a screen of genes in *Drosophila* found 63

genes encoding RBPs that are implicated in dendrite morphology – 54 of which have a human homolog (Olesnicky *et al.*, 2014). These genes were then tested for their importance in dendrite morphogenesis in *C. elegans,* however, only 12 of the 63 showed significant abnormality when deleted. (Antonacci *et al.*, 2015). While this disparity between *Drosophila* and *C. elegans* may seem unexpected, there are a variety of reasons why this might be, including the differences between the two genomes. For example, one of the genes screened, *nanos,* exists as only one gene in *Drosophila* but as three separate paralogs in *C. elegans*. Therefore, the three paralogs could have the same function and therefore not show a phenotype upon deletion of one or two of them. This difference makes comparing the two difficult and requires a knockdown or knockout of the three *C. elegans* paralogs. However, a triple mutant knockout of all three *nanos* paralogs did not previously exist in *C. elegans*, complicating the comparison that was made between the two species.

Nanos

Nanos, an RNA-binding protein, contains a carboxy-terminal zinc finger motif, $(CCHC)_2$, which allows it to associate with RNA and interaction partners, such as Pumilio (Curtis *et* al., 1997; De Keuckelaere *et al*., 2018). As this domain is vital to the function of Nanos, it is evolutionarily conserved between all homologs of Nanos, from mammals to lower organisms, such as *C. elegans* (Bhandari *et al*., 2014). Nanos has been shown to bind Pumilio, part of the PUF protein family, to form a post-transcriptional repressor complex. Together, Nanos and Pumilio bind *cis*-elements in the 3'-untranslated region of target mRNAs, repressing them (Tsuda *et al*., 2003; Lai & King, 2013). All animals have one to four *nanos* genes. For example,

Drosophila melanogaster has one, *Fugu rubripes* has 4, and humans and *C. elegans* have 3 paralogs. This is likely due to a duplication event after which the different *nanos* paralogs evolved different roles (De Keuckelaere *et al*., 2018). There has been extensive research on Nanos in *Drosophila*, in which it has been shown to function as a translational repressor to establish body-axis polarity in early embryos and as a promoter of dendrite growth and branching in class III and IV neurons (Wang & Lehmann, 1991; Ye *et al*., 2004). As Nanos is important for proliferation and survival of germline stem cells and development of cyst stem cells, *nanos* mutants have been shown to produce few eggs. These mutants also exhibited a lack of germ cells in ovaries and testes (Kobayashi *et al*., 1996; Forbes & Lehmann, 1998).

Nanos in *C. elegans*

Similar to *Drosophila, nanos* was found to be important for germ cell development and survival in *C. elegans* (Subramaniam & Seydoux, 1999; Keuckelaere *et al*., 2018). Furthermore, it was found that *nos-1* and *nos-2* are not required for primordial germ cell fate specification but for certain aspects of germ cell development. The *nos-1 (RNAi)* single mutants appeared wild type, 37% of the *nos-2 (RNAi)* animals lacked germ cells, but 99% of the *nos-1 (RNAi); nos-2 (RNAi)* animals lacked germ cells. This additive effect suggests that *nos-1* and *nos-2* function redundantly in germline development. *nos-3* had no effect on the presence or absence of germ cells (Subramaniam & Seydoux, 1999). Furthermore, as *C. elegans* normally exist as hermaphrodites, a switch from spermatogenesis to oogenesis is necessary, and *nos-3* has been shown to be participate in this sperm-oocyte switch (Kraemer *et al*., 1999). (The similar findings in *Drosophila* and *C. elegans* suggest that *nanos* may play an evolutionarily conserved role in all

animals, but its nuanced roles in different organisms suggests that certain other functions have developed for *nanos* paralogs within species (Keuckelaere *et al*., 2018).

Nanos and Neuron Development

Along with germ cell development and proliferation, *nanos* has been shown to play an important role in dendrite morphogenesis. In *Drosophila*, mutation of *nanos* causes a decrease in higher order branching in dendrites of class IV dopaminergic neurons but does not affect the main branches. Also, *nanos* mRNA particles travel to dendritic termini and that this transport is crucial in the spatial regulation of *nanos* mRNA. It is this spatial regulation that is required for Nanos to function normally (Brechbiel & Gavis, 2008; Ye *et al*., 2004). Furthermore, mutation of *nanos* and *pumilio* cause similar dendritic defects, supporting the idea that they work together in the translational repression causing said defects (Ye *et al*., 2004).

CRISPR and *C. elegans*

To truly view *nanos'* effects in *C. elegans,* a triple mutant needed to be created. To do this we planned on using CRISPR-Cas9, which has recently become a trusted gene-editing technique. CRISPR-Cas9 allows precise edits to be made to DNA (Ran *et al*., 2013). In *C.* elegans, there existed already a double mutant strain containing *nos-1; nos-2* deletions and a single mutant containing a *nos-3* deletion. Due to *nos-3'*s proximity on chromosome II to *nos-1* and *nos-2*, recombining the *nos-3* deletion onto the double mutant chromosome would have been highly unlikely. Because of the low probability of a recombination event, CRISPR-Cas9's precise editing capabilities would be especially useful in the deletion of *nos-3* from the *nos-1; nos-2*

double mutant strain. Established methods for CRISPR editing in *C. elegans* simplified the protocol (Paix *et al*., 2017) and allowed for confidence in the feasibility and results.

PVD

Once a triple mutant was created, the phenotypes seen would need to be quantified somehow. In *C. elegans,* a large neuron called the PVD is well suited for quantification of defects. *C. elegans* has two PVD neurons – PVDL and PVDR – that are on either side of the adult worm, serving as mechano-receptors, nociceptors, proprioceptors, and cold temperature receptors. The PVD serves as a particularly effective model for measuring dendrite morphogenesis because of its regular branching pattern. The PVD axon grows ventrally from the cell body, connecting with the ventral nerve cord. The dendrites, however, branch first along the anterior/posterior axis, then orthogonally for each subsequent branch, forming a menorah shape (Oren-Suissa *et al*. 2010; Smith *et al.* 2010). This regularity allows for simple and controlled quantification of dendritic branching by secondary, tertiary, and terminal categories (**Figure 2**). As an RBP that has previously been implicated in dendrite morphogenesis in *Drosophila*, Nanos' effect on dendrites in *C. elegans* was an intriguing research question, with potentially far reaching impacts on human neurological disease.

MATERIALS AND METHODS

Imaging and Quantification of PVD Dendrite Morphology

Worms were picked at the life stages indicated, mounted on slides with 2% agarose pads, and immobilized with 600 µM levamisole. Initial screening, time-course studies, and rescue

experiments were conducted using a 40× or 63× objective on a Zeiss Axioskop. Dendrites were imaged with a Leica SP5 spectral confocal microscope at 63× with 0.5 µm per step and Leica LAS software. Secondary, tertiary, and terminal (quaternary and senary) dendrites were counted from the PVD cell body to the posterior end separately on the dorsal side, the ventral side, or both. Scoring of the different strains was performed by a blind test. Statistical tests were performed, and graphs created with Prism 6.0f software (GraphPad Software, Inc.).

CRISPR Injection and Screening Protocol

CRISPR editing was performed essentially as described in Paix, 2017. 1.5uL tracrRNA, 1.5uL crRNA L1 (**Table 1**), 1.5uL crRNA R1 (**Table 1**), and 0.5uL duplex buffer (all provided by IDT) were mixed and put at 95°C for 5 minutes, then RT for 5 minutes, then on ice. 1uL of this mix was combined with 1uL of Cas9 enzyme (IDT catalog # 1081059) and left at room temperature for 15 minutes. After 15 minutes, 0.5uL 2.5 ng/µL (final concentration) pCFJ90 [*Pmyo-2::mCherry*] plasmid (Frøkjær-Jensen *et al.,* 2008), 0.5 5 ng/µL (final concentration) pCFJ104 [*Pmyo-3::mCherry*] plasmid (Frøkjær-Jensen *et al.,* 2008), and 1uL ssDNA repair template (**Table 1**) were added to the 2uL RNP. This injection mix was microinjected into the gonad of ~20 adult hermaphrodite worms. 3-5 days later, F_1 worms were screened for red fluorescence in the pharynx and/or body wall muscle. Worms lysates were made from 20-30 fluorescence positive F_1 worms to then PCR amplify.

C. elegans Strains

PVD dendrites for all strains, including the control, were marked by *wdIs51[*$P_{F49H12.4}$*::GFP].*

C. elegans strains used were as follows (name – genotype): DJK93 - *nos-1(ok250); wdIs51,* DJK194 – *nos-2(ok230) unc-4(e120)/mIn1; wdIs51,* DJK203 – *nos-3(q650); wdIs51*, DJK204 – *nos-2(ok230) nos-1(gv5)/mIn1; wdIs51*, DJK309 – *nos-2(ok230) nos-1(gv5) nos-3(cnj3)/mIn1; wdIs51.*

RESULTS

nos-3 Was Successfully Deleted Using CRISPR-Cas9 Genome-editing Technology

All of the *nanos* paralogs (*nos-1, nos-2,* and *nos-3*) in *C. elegans* are located within 2.75 centiMorgans of each other on chromosome II. A strain with a *nos-3* deletion alone existed, and a strain with both a *nos-1* and a *nos-2* deletion existed. However, the proximity of these genes on chromosome II made any recombination unlikely and necessitated a precise DNA-editing technique. DJK204 a strain already containing deletions of *nos-1* and *nos*-2, a PVD neuron marker, and is heterozygous because it is homozygous sterile, was injected with the CRISPR-Cas9 machinery targeting the *nos-3* locus. Two different guide crRNAs targeted sequences near the start and end of the *nos-3* gene, enabling a large deletion. A repair template contained homology arms for the DNA on either side of the deletion (**Figure 3A**). As markers of successful injections, F_1 worms were screened for red fluorescence and then screened via PCR for the intended deletion (**Figure 3A**). PCR results were run on a gel and the band observed was consistent with the expected deletion band around 454 bp (**Figure 3A**). The other band observed was consistent with the expected wild type band around 660 bp (**Figure 3A**). As further confirmation of the intended deletion, the deletion amplicon was digested with NotI first, as a NotI site was placed in between the homology arms in the repair template (**Table 1**),

however the restriction digest did not work. As a DraI site was also present in the repair template, we next attempted a restriction digest with DraI and ran on a gel, showing the expected cut forming two bands at 268 bp and 185 bp (**Figure 3B**). Sequencing later showed that the NotI digest did not work because the edit to the genome deviated from the repair by a single base pair deletion within the NotI site. Aside from the unexpected mutation of the NotI site, the sequenced DNA otherwise showed the intended edit was made, and the repair template was incorporated (**Figure 3C**). We thus conclude that we successfully created a null allele that deletes from base pair 752 to base pair 3,789. Importantly, this deletes the region of the gene that encodes the conserved zinc finger motif that mediates RNA binding (Subramaniam & Seydoux, 1999).

nanos Paralogs Do Not Function Redundantly and Have a Negligible Effect on PVD Dendrite Morphology

The *nos-1, nos-2,* and *nos-3* single mutants, the *nos-1, -2* double mutant, the *nos-1, -2, -3* triple mutant, and a control strain were imaged by confocal microscopy at the young adult stage to quantify the dendritic branching of the PVD neuron at secondary, tertiary, and terminal levels (see Materials and Methods; **Figures 4 and 5**). No significant difference was found among secondary order dendrite branches amongst the different strains (**Figure 5A**). When looking at the third order branches, the *nos-3* single mutant was found to have significantly more branches than the control strain, *nos-1, -2* double mutant, and *nos-1, -2, -3* triple mutant by a p value of less than 0.0001, and the *nos-1* and *nos-2* single mutants by a p value of less than 0.05 (**Figure 5B**). For the terminal dendrite branches, the three single mutants, *nos-1, nos-2,* and

nos-3 had significantly more branches than the control with a p value of less than 0.0001 (**Figure 5C**). The double mutant *nos-1,-2* was not significantly different than the control; however, the triple mutant *nos-1,-2,-3* had significantly more branching than the control with a p value of less than 0.05 (**Figure 5C**).

As *nanos* exists as three paralogs in *C. elegans*, it was a possibility that the paralogs would function redundantly. If this was true, it was probable that a phenotype would not be seen without deleting *nos-3* as well. As no additive effect was seen from deleting all three *nanos* paralogs (**Figure 5**), it does not appear that the three *nanos* paralogs perform redundant functions with regards to dendrite morphology in *C. elegans*.

DISCUSSION

Nanos Paralogs Are Not Genetically Redundant

As *nanos* mutants in *Drosophila* display dendrite morphology defects (Olesnicky *et al*., 2014), and *nanos* is a broadly conserved gene, it was a likely candidate for regulating dendrite development in *C. elegans* as well. In *Drosophila,* however, there is only one *nanos* gene, as opposed to the three paralogs present in *C. elegans* (Subramaniam & Seydoux 1999). In a previous screen based on genes shown to be important for dendrite morphogenesis in *Drosophila*, *nos-1* and *nos-2* mutants in *C. elegans* did not show significant dendrite defects. However, it was not a true comparison between *Drosophila* and *C. elegans* because, as there are 3 *nanos* paralogs in *C. elegans*, all three *nanos* genes would need to be knocked out to compare the null alleles. If there was redundancy in the functions of *nos-1, -2,* and *-3* then it is possible that there would be enough *nos-3* expression to make up for that lost by *nos-1* and

nos-2. To cleanly see if *nos-1, -2,* and *-3* are genetically redundant in *C. elegans*, then a triple mutant with all three paralogs deleted was required. When the dendrites of the triple mutant were analyzed in comparison to the single and double mutants, no additive effect was seen (**Figure 4**). Because deleting all three paralogs did not worsen the phenotype seen, the *nanos* paralogs in *C. elegans* do not appear to be redundant.

<u>*Nanos* Mutants Dendritic Phenotypes May Go Beyond Their Effects in the Neuron</u>

nos-3 mutants showed increased dendritic branching amongst third order branches, and all of the *nanos* mutants showed increased dendritic branching to some degree in terminal branches (**Figure 3B, C**). Given *nanos* has been shown to decrease dendritic branching in *Drosophila* (Brechbiel & Gavis, 2008; Olesnicky *et al.*, 2012; Ye *et al*., 2004), it is unexpected that *nanos* mutants are exhibiting increased dendritic branching in *C. elegans.* Furthermore, if *nanos* was a major factor in dendrite morphology in *C. elegans*, one would expect a synergistic effect, where deleting two genes creates a stronger phenotype than one, and deleting all three genes exacerbates the phenotype further. Instead, the double and triple mutants have almost returned to control levels. As this is the case, there are caveats that may explain why the data do not exhibit the expected pattern. First, all *nos-2* mutants are sterile, requiring the presence of a *mIn1* balancer chromosome to maintain the strain as a heterozygous fertile population and to assist with identifying the homozygotic offspring. Therefore, the homozygous worms observed from the *nos-2, nos-1, -2,* and *nos-1, -2, -3* mutants were all offspring of heterozygous worms. Because of this, it is possible that maternal wildtype messages or proteins could still be present in the offspring, allowing for maternal rescue of *nanos*.

Furthermore, there is no data to assure that the lack of Nanos' effect within the dendrites is what is directly causing the observed phenotype. It is plausible that the deletion of *nanos* is causing the worm's health to suffer on the whole, having non-specific and cell non-autonomous effects on its neurons. This may not explain the observed results on its own, but if it caused heterochronic differences in the worms, it is feasible it could have skewed the results. As worms were picked at approximately the same stage of development, it is plausible that the deletion of single *nanos* paralogs caused slower development of the vulva – a feature of *C. elegans* often used to determine its age – but not of its neurons. If the *nanos* single mutants were consequently picked at an older age, their neurons would be more developed, and therefore, would exhibit more branching than less developed neurons of a younger animal. Say, if two or more *nanos* paralogs were deleted then the whole worm was sick enough to slow all development – not just vulval – then double or triple mutants' neurons would appear less developed. This is an example of the sort of phenomenon that would explain the results seen. Regardless of the true cause of the observed results, it can be said that it is difficult to make sense of them, and that it is evident that *nanos* is not a major regulator of neuron morphology in *C. elegans*. The differences in dendritic branching amongst the mutant groups contrasted with the control group is too modest to conclude that *nanos* plays a large role.

Future Directions

Though it appears as if *nanos* does not play as large of a role in neural morphology in *C. elegans* as it does in *Drosophila,* it is possible that is does play minor roles. It is known that *nanos* mRNA localizes in dendritic termini in flies. However, in this study it is possible that

nanos may have non-autonomous roles that impact dendrite development since we did not examine animals lacking *nanos* function specifically in PVD neurons. Creating conditional knockout alleles that only delete *nanos* paralogs in the PVDs would be needed to address this issue - a technically challenging proposition. Future studies could investigate mRNA targets of Nanos in *C. elegans* neurons. Does *C. elegans* Nanos have similar targets to those in *Drosophila*? Are its targets implicated in dendritic branching and morphology? What potentially unknown targets of Nanos exist in the two organisms? Answers to these questions would elucidate to what extent Nanos' autonomous activity in dendrites caused the results seen in this study and to what extent *nanos* is implicated in dendrite morphology in *C. elegans*. It is no longer a question of whether *nanos* is a major regulator of *C. elegans* neurons, but these experiments would elucidate to what extent, and via what pathways, *nanos* affects their neuronal morphology.

While it seemed plausible that *nanos'* role as a promoter of dendrite growth and branching in class III and IV neurons in *Drosophila* (Wang & Lehmann, 1991; Ye *et al*., 2004) would be conserved in *C. elegans,* it does not appear as if this is the case. The results from this paper seem to suggest that the role *nanos* plays in *Drosophila* neurons is a newly evolved role that is specific to fly and is not conserved in other species. However, with limited *nanos* research in other species, it is a difficult question to answer at this point in time.

FIGURES

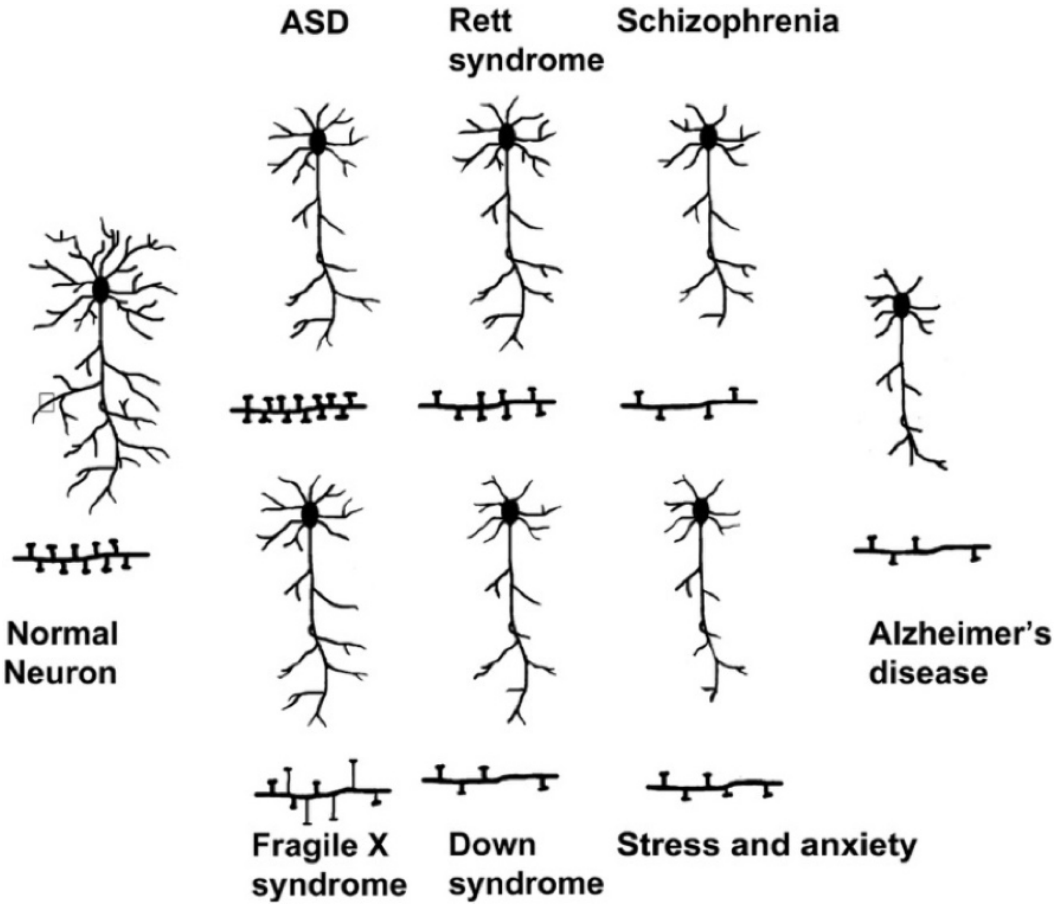


Figure 1. A visual representation of abnormal dendritic branching and the associated neurological disease and disorders.

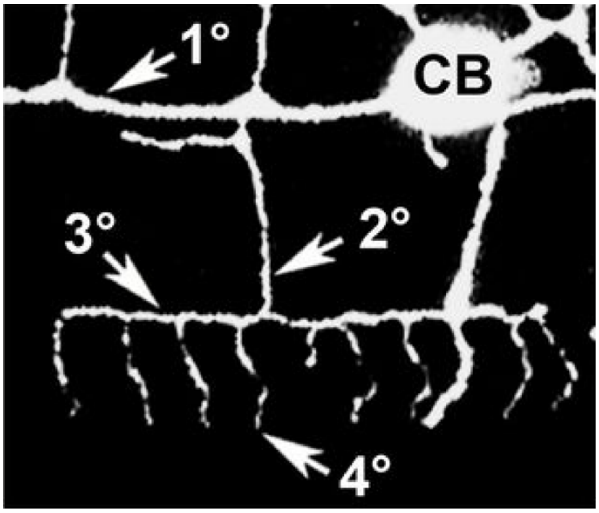
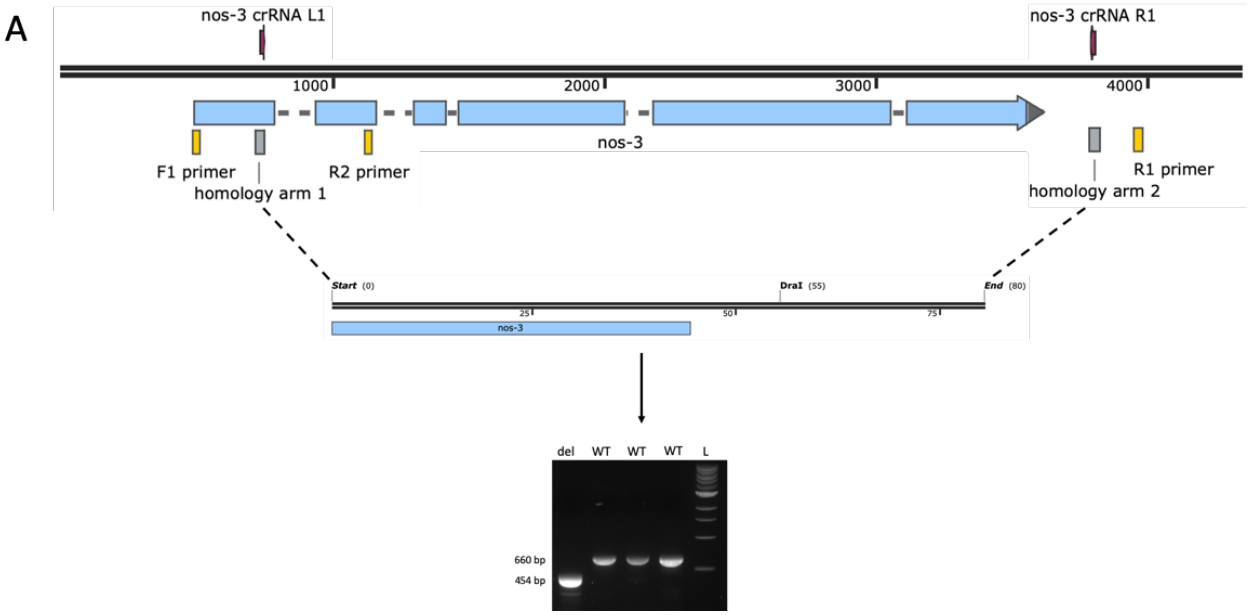
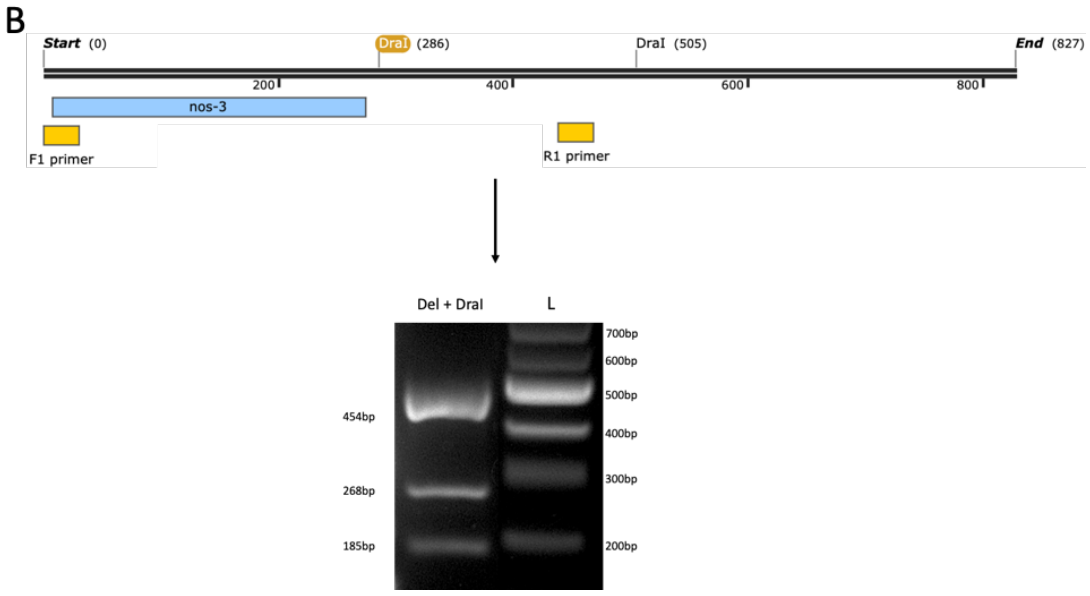


Figure 2. Schematic of dendritic branching orders. PVD dendritic tree morphology includes primary (1°) branches extending from the cell body (CB) and a series of perpendicular secondary (2°), tertiary (3°), and quaternary (4°) branches (taken from Antonacci *et al.,* 2015).


A
nos-3 crRNA L1
nos-3 crRNA R1
1000
2000
3000
4000
nos-3
F1 primer
homology arm 1
R2 primer
R1 primer
homology arm 2
Start (0)
DraI (55)
End (80)
25
50
75
nos-3
del
WT
WT
WT
L
660 bp
454 bp


B
Start (0)
DraI (286)
DraI (505)
End (827)
200
400
600
800
nos-3
F1 primer
R1 primer
Del + DraI
L
454bp
268bp
185bp
700bp
600bp
500bp
400bp
300bp
200bp

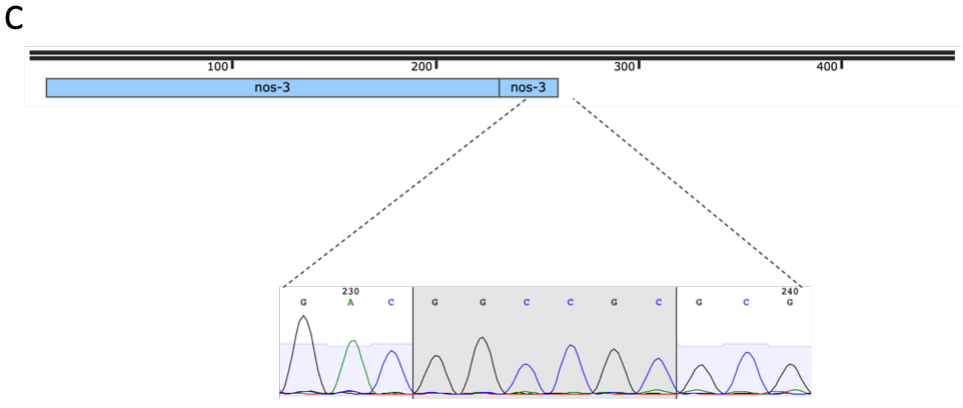

C
100
200
300
400
nos-3
nos-3
230
240

Figure 3. The CRISPR Cas9 editing strategy created the precise deletion desired. (A) The general strategy involved injection into hermaphrodite worms, followed by screening of the F_1 generation for the desired deletion at 454 base pairs on a gel. Lanes are worms containing the expected deletion (del), worms that are wild-type for *nos-3* (WT), and the ladder (L). (B) This was confirmed by cutting the deletion amplicon with DraI (Del + DraI), showing two expected bands at 268 and 185 base pairs. (C) Sequencing data confirmed that the correct deletion was made, and that homologous repair with the provided repair template had taken place. Repair template sequence is shown.

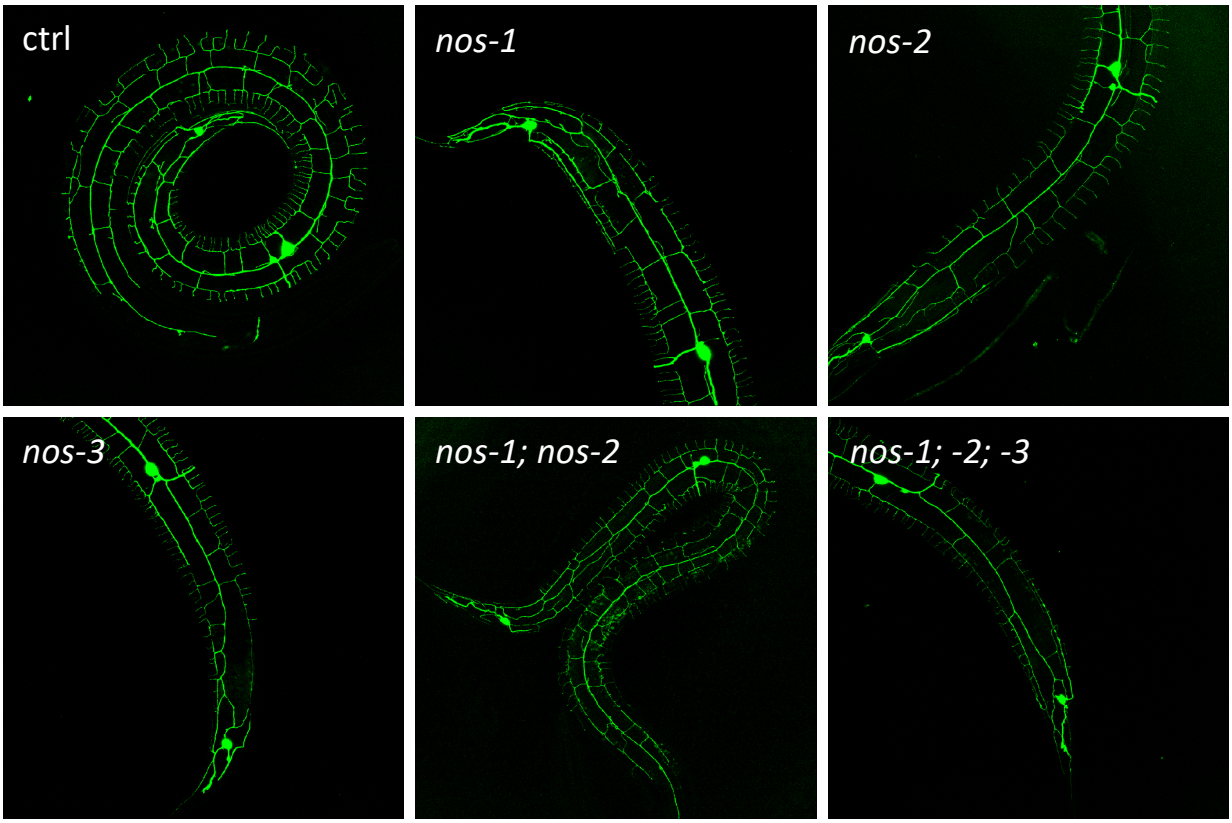


Figure 4. Large differences in dendrite morphology was not seen when different mutants were compared to a control (ctrl). Animals carried a GFP transgene marking their PVD neuron, allowing for visualization and quantification.

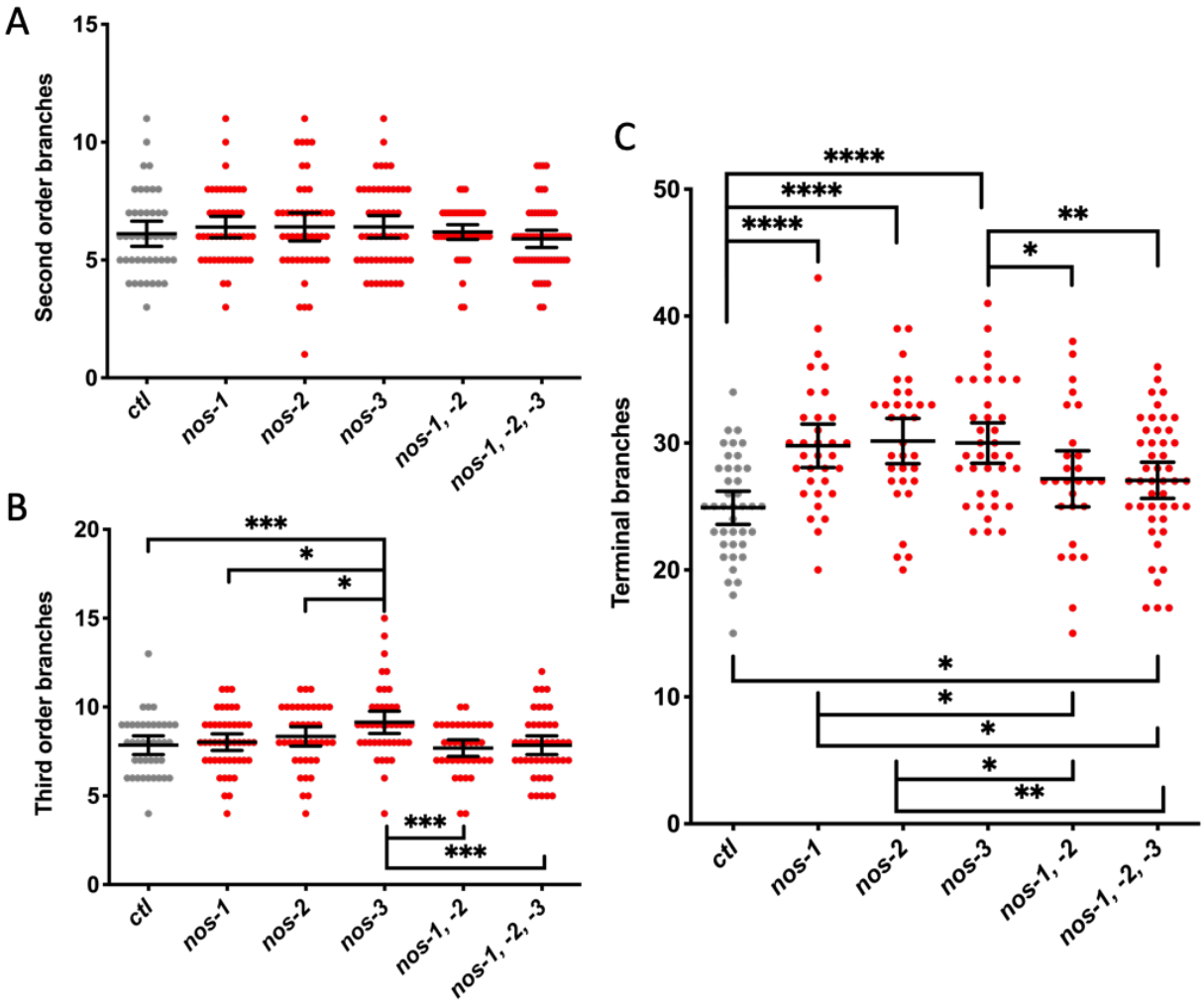


Figure 5. Quantification of the PVD dendrite phenotypes in the different *nanos* mutants. Points in the plots represent counts of (A) second order branches, (B) third order branches, or (C) terminal branches from the PVD cell body to the tail on either the dorsal or ventral side of the worm. Lines within the separate plots represent the mean and the 95% confidence interval around the mean. Statistics were performed using a one-way ANOVA test with a Fisher's Least Significant Difference multiple comparisons test with a 95% confidence interval. Bars indicate that results are significantly different between columns with a p value<0.05(*), <0.005(**), <0.0005(***),or <0.0001 (****). Other comparisons are not significant.

TABLES

Table 1. Sequences of the crRNA L1, crRNA R1, and repair template. Exons are upper case and introns are lower case. The repair template contains two homology arms on either side of a NotI site. In the sequenced DNA from the CRISPR edit, the NotI site in the repair template was missing a guanine for an unknown reason.

crRNA L1	GCTAGGGACTGACGACCAAA
crRNA R1	TCTTTTAAAGCTTTCGCAGT
Repair template	GGACAACACCAGATTCTGGGCTAGGGACTGACGACCgcggccgcgcgaaagctttaaaagaaaccc cctcatgtctatcc

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