Thesis Prospectus

A. Specific Aims

Serotonin is a conserved neurotransmitter that modulates a diverse array of behaviors and physiological processes such as feeding, movement, reproduction, respiration, sleep and affect (Murphy et al., 2008). Significantly, dysfunction of the serotonin system is associated with a variety of human mental illnesses including autism, depression, and anxiety (Bethea and Sikich, 2007; Hornung, 2003). Despite their far-reaching effects, serotonin neurons are relatively few in number within the CNS. These neurons achieve their vast influence through two neurodevelopmental strategies: the elaboration of axonal arbors and the formation of neurosecretory serotonin synapses (Jacobs and Azmitia, 1992). Although the appropriate morphological development of serotonin neurons is crucial for their function, the molecular and genetic underpinnings of these processes are not yet known.

Serotonin axon arborization and synaptogenesis occur within precise spatial coordinates, and with stereotyped developmental timing. Although serotonergic projections are widely distributed in the brain, examination of the serotonergic system has revealed that these neurons form terminal arbors within precise regions with characteristic physiological effects on specific targets. The timing of terminal arborization is also tightly controlled. For example, serotonin neurons in the rat are born and extend unbranched neurites in the embryo. The neurons remain unbranched until the post-natal period, when they extend synapse-containing axonal arbors (Lidov and Molliver, 1982). These precisely timed neurodevelopmental strategies are conserved in the nematode *C. elegans*. Here, the main serotonergic neurons (a bilateral pair of neurons called the NSM neurons) extend an unbranched neurite in the embryo. This neurite remains unbranched until the final larval stage, when synaptic axonal arbors extend in a precise neuroanatomical coordinate overlying the nerve ring (Axang et al., 2008). The genetic programs controlling the spatial and temporal regulation of these conserved neurodevelopmental processes are not yet known.

I have conducted a candidate screen for genes required for axonal arborization in the serotonergic *C. elegans* NSM neuron. I have identified a novel role for the canonical axon guidance molecules UNC-6 (Netrin) and its receptor UNC-40 (DCC) in localization and morphology of axon arbors. In NSM, mutants lacking the genes for these axon guidance cues display wild-type guidance of the main axons, but do not correctly execute the distinct process of axon arborization. I have preliminary evidence that UNC-40 also plays a role in synapse distribution along the main NSM axon. I propose to investigate the hypothesis that UNC-40 may represent one element of a developmental program to regulate the spatial and temporal specificity of serotonergic axon arborization. In this proposal I will also outline my plans to probe the relationship between the developmental processes of synapse assembly and axon arborization. Finally, I also propose to conduct a forward genetic screen to identify and characterize novel genes required for the temporal control of axon arborization.

Specific Aim 1. Characterize the role of UNC-40/DCC in synapse distribution. I have uncovered a new role for the axon guidance receptor UNC-40/DCC in synapse organization and axon arbor morphology and position. I propose to use genetic and cell biological tools to investigate UNC-40's novel developmental roles in synapse distribution and arbor formation. My preliminary results suggest a potential role for UNC-40/DCC in the timing of serotonergic axon arbor assembly. To investigate this possibility, I will conduct a panel of experiments to examine the temporal sequence of serotonergic synapse assembly and distribution as they relate to axon arborization. I will then use this information to probe UNC-40/DCC's role in these processes.

Specific Aim 2. Identify genes required for temporal specificity of axon arborization. In the NSM neuron, like in vertebrate serotonin neurons, axon outgrowth, synaptogenesis and axon arborization occur within distinct developmental windows. The mechanisms underlying this developmental regulation are not known. I will conduct a genetic screen to identify genes required for the temporal control of NSM arborization. I will then map these genes using single nucleotide polymorphism (SNP) and three-factor mapping strategies. Transgenic rescue and genomic DNA sequencing will then be used to identify gene mutations.

Specific Aim 3. Determine expression and characterize function for genes identified in the screen. Once I have identified the genetic lesions for each mutant of interest, I will identify the specific tissues in which these genes are acting. I will then determine the temporal and spatial expression patterns for each gene. I will also observe the dynamic subcellular localization patterns for these genes of interest. Additional experiments will be conducted to test potential gene functions and probe temporal requirements for genes of interest. This will allow me to place novel regulators of serotonergic neurodevelopment within a new mechanistic model.

B. Significance:

Although serotonin neurons constitute less than one millionth of the total number of neurons in the brain, their neuromodulatory influence is immense, with a single neuron modulating as many as half a million synapses throughout the CNS. This extraordinary influence is made possible by the unique anatomy and morphology of serotonin neurons (Jacobs and Azmitia, 1992). The cell bodies of serotonin neurons reside within one evolutionarily ancient structure in the brain stem called the raphe nucleus. They elaborate complex axon arbors that allow serotonergic release and neuromodulation within almost every part of the CNS. This network of axonal arbors is the most expansive neurochemical system in the brain (Hornung, 2003). Still, terminal arbors form with spatial precision in specific areas of the CNS.

The importance of this neuromodulator in the proper functioning of the nervous system is perhaps best exemplified by the multiplicity of human disorders that have their roots in serotonergic dysfunction (Murphy et al., 2008). For instance, disrupted developmental regulation of serotonin levels has been observed in a subpopulation of autistic patients. In fact, two animal models for autism feature both decreased serotonergic terminals in the brain and autism-like behaviors (Daubert, 2010).

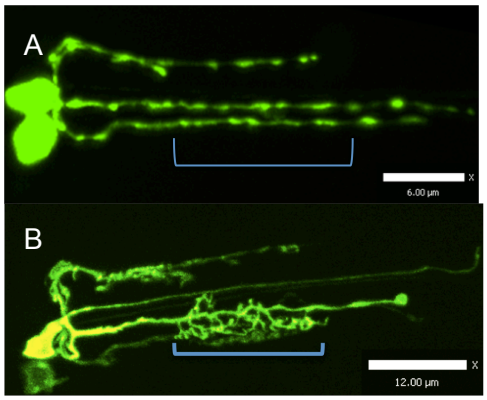


Figure 1. Development of the NSM serotonergic neuron.
In all images, use of cell specific promoter (*tph-1*) allows visualization of NSM in live animals, with single cell resolution. **A**. Cell-specific promoter driving GFP in the NSM neuron in a juvenile animal. Arborization has not yet occurred. **B**. As (**A**), but in an adult animal. Axon arbors form over the nerve ring (bracketed).

Serotonin's role as a neuromodulator is also crucial for proper functioning of the adult nervous system. Altered serotonin signaling is thought to play a significant role in the development of mood disorders such as depression and anxiety. Indeed, drugs that impinge on serotonin signaling (SSRIs and atypical antipsychotics) are used for the treatment of a multitude of mental disorders including clinical depression, anxiety, obsessive-compulsive disorder, eating disorders, post-traumatic stress disorder, and schizophrenia (Vaswani *et al.*, 2003; Lieberman *et al.*, 1998). Serotonin's role as a neuromodulator aside, this neurotransmitter is also crucial for regulating the early wiring of the limbic system. Studies have shown that genetic risk factors for depression, such as SERT polymorphisms that reduce serotonin availability, are correlated to volume reductions in limbic brain regions that likely precede the onset of depression (Hamann, 2005). Understanding the fine terminal development of serotonin neurons may help to advance our understanding of serotonin's role in development and disease (Gaspar *et al.*, 2003). In spite of the clear importance of serotonergic circuit neurodevelopment in physiology and disease, the mechanisms that control the precise delivery of serotonin to the appropriate targets remain largely unknown.

The *C. elegans* model system offers a unique opportunity to investigate the neurodevelopmental programs underlying serotonergic circuit assembly *in vivo*, in real time, and with single-cell resolution. Furthermore, the serotonergic pathways, ranging from neurotransmitter biogenesis to signaling, are well conserved across evolution (Ranganathan *et al.*, 2001; Sawin *et al.*, 2000; Harris *et al.*, 2009; Tanis *et al.*, 2008). *C. elegans*, like vertebrates, have only a handful of neurons that produce serotonin, but their influence on nematode behaviors is profound (Chase and Koelle, 2007). Interestingly, serotonergic neurons in nematodes exhibit morphological features similar to those seen in vertebrate serotonergic neurons. For instance, the main serotonergic neuron in *C. elegans* (called NSM) elaborates axonal arbors within a specific region (the basal membrane adjacent to the nerve ring) and within a stereotypical developmental window (Albertson and Thomson, 1976). Like in vertebrates, these NSM axon arbors contain neurosecretory serotonin synapses (Axang et al., 2008).

Here I propose to use *C. elegans* to examine the cellular and molecular mechanisms that regulate serotonergic axon arborization and synapse development *in vivo*. I have established an experimental system that allows me to probe these neurodevelopmental questions with single cell resolution and in real time, while in the context of the intact nematode nervous system. The proposed research is significant for the following reasons.

First, I will characterize a novel role for UNC-40/DCC in regulating serotonergic axon arbor position and morphology. I will also probe UNC-40's role in the distribution of neurosecretory serotonin release sites along the NSM axon. In both vertebrates and nematodes, neurosecretory synapses form in the axon arbors of serotonergic neurons. My proposed research will uncover the developmental relationship between axon

arborization and synaptogenesis. Questions I will address include: are axon arbors specified first, providing positional information to direct synapse formation? Alternatively, do serotonergic synapses along the axon shaft direct the positioning of axon arbors? Finally, my proposed research will provide information about UNC-40's potential role in regulating the precise developmental timing of serotonergic axon arbor formation and synapse development.

Second, I will identify new genes required for the temporal regulation of NSM arborization. In both vertebrates and in *C. elegans*, serotonin neurons undergo axon guidance long before axon arborization begins. The molecular mechanisms underlying this conserved temporal regulation are not known. I will take an unbiased approach to identify novel regulators of this process.

Third, I will characterize the functions for these novel developmental regulators. Once I have identified genes required for the temporal regulation of axon arbor formation in the NSM neuron, I will conduct experiments to uncover their specific functions.

Although my studies are focused on serotonergic synapses, this work will also contribute to our understanding of the molecular mechanisms that regulate synapse development as well as arbor morphology and positioning in other circuits. Based on the Colón-Ramos laboratory's previous experience using the nematode as a neurodevelopmental system, I believe that the mechanisms uncovered here will inform our understanding of how these conserved developmental programs are regulated in humans and how their dysregulation could result in disease.

C. Preliminary Studies

NSM forms serotonergic synapse-containing axon arbors

NSM is the main serotonergic neuron in *C. elegans*. Like serotonin neurons in vertebrates, NSM elaborates synapse-containing arbors within a stereotyped region and within a critical developmental time window (Axang *et al.*, 2008). I can use cell-specific promoters to visualize NSM development *in vivo* and with single cell resolution (Fig 1). Using these promoters, I observe that although NSM axon guidance is completed during embryogenesis, NSM does not elaborate axon arbors until the L4 stage (Fig 1). These same promoters can be used to drive a fluorescently tagged synaptic marker, cat-1::gfp, in order to visualize synaptic development.

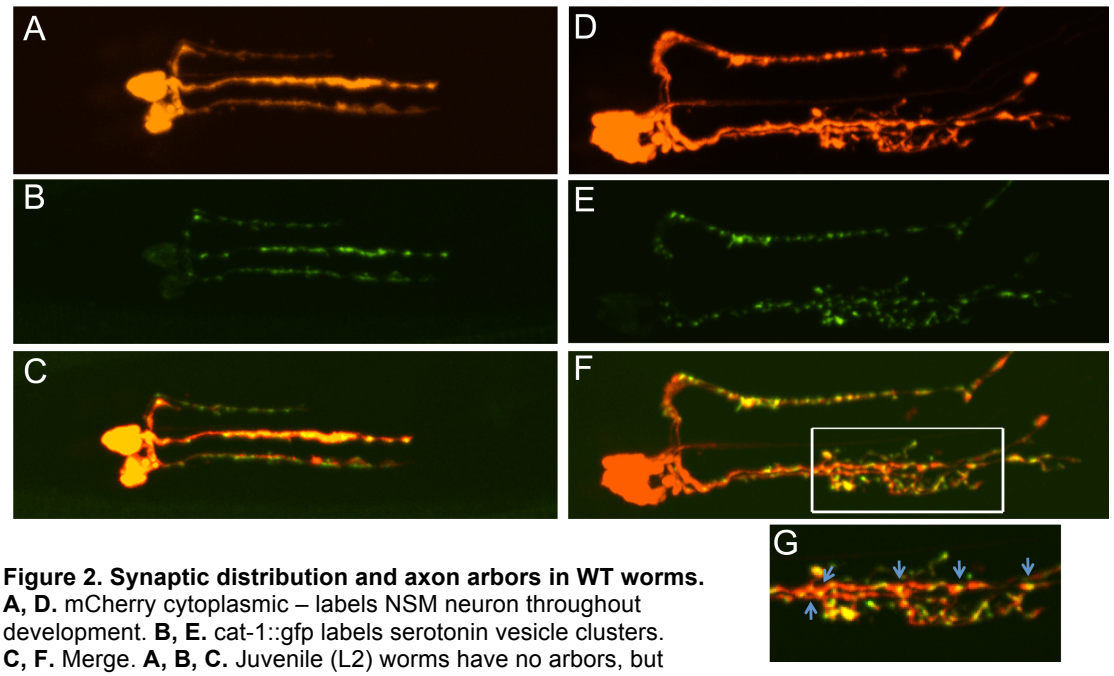


Figure 2. Synaptic distribution and axon arbors in WT worms. **A, D.** mCherry cytoplasmic – labels NSM neuron throughout development. **B, E.** cat-1::gfp labels serotonin vesicle clusters. **C, F.** Merge. **A, B, C.** Juvenile (L2) worms have no arbors, but already contain synapses. **D, E, F.** Adult worms have elaborate axon arbors, which contain synapses at arbor branch points. **G.** Rectangle in F expanded, arrows point to synapses that correspond to arbor branch points.

Consistent with EM studies, I observe that serotonin vesicles cluster at putative presynaptic sites present in the main axon shafts of NSM, as well as in its axon arbors (Fig 2 and (7)). These serotonin vesicle clusters are present throughout the development of the worm, from the larval through adult stages. Following arborization, however, I have observed a spatial relationship between synapses along the main axon shaft and points of arbor extension. Specifically, arbors often extend from regions of the axon shaft containing synapses (Fig 2G).

The Netrin pathway regulates axon arbor position and morphology

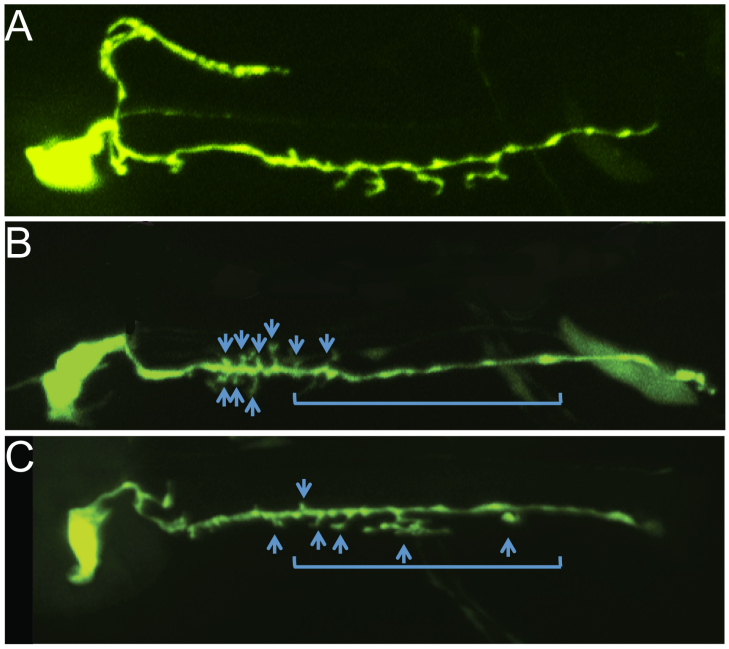
I have identified a novel role for the Netrin signaling pathway in controlling axon arborization in NSM. I found that Netrin is not required for the guidance of the main NSM axon shaft. Rather, in the absence of Netrin, or the

Netrin receptor UNC-40/DCC, the NSM axon fails to elaborate arbors correctly. Instead, NSM forms short, dysmorphic arbors proximal to the cell body (Fig 3). Thus, I hypothesize that Netrin and UNC-40/DCC are required for correct arbor localization and morphogenesis. I have also determined that UNC-40/DCC acts cell autonomously in NSM to instruct axon arborization (Fig 3). Furthermore, I have determined that this role for UNC-40/DCC is mediated through already known downstream effectors MIG-10/Lamellipodin and UNC-34/Ena/VASP (data not shown).

Interestingly, in vertebrates, the Netrin receptor UNC-40/DCC is highly expressed in developing serotonin neurons (Wylie *et al.*, 2010). Although the role of Netrin in vertebrate serotonergic arborization is not known, this expression pattern suggests that our findings on the role of Netrin in serotonergic neurons could be conserved.

UNC-40/DCC is dynamically regulated in NSM at the time of axon arbor formation

I have conducted transgenic rescue experiments to show that UNC-40 is sufficient in the NSM neuron to rescue the UNC-40 arborization defect. This led me to probe UNC-40's subcellular localization within the NSM neuron throughout development. I observed that UNC-40 is diffusely distributed in the NSM neuron early in development before arbor formation. Interestingly, UNC-40 later becomes localized to bright puncta along the main axon shaft, and within nascent axon arbors within the specific time window that coincides with axon arbor development. Briefly following this period of extensive arborization, the UNC-40 signal again becomes diffuse (Fig 4). Thus UNC-40's subcellular localization is dynamically regulated throughout development, and especially at the time of axon arborization.



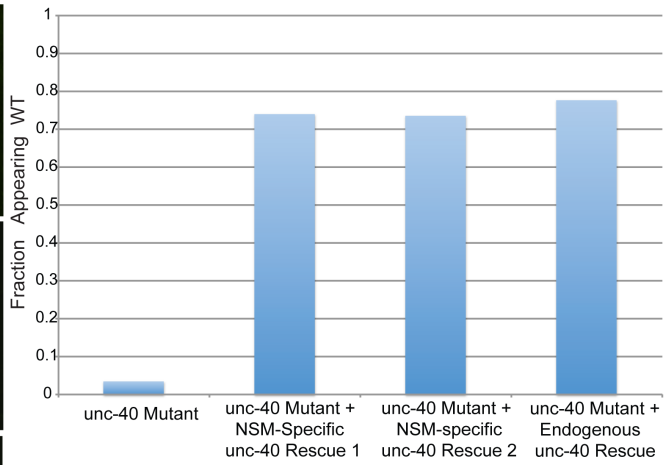


Figure 3. UNC-40/DCC cell autonomously regulates axon arbor position and morphology in NSM. A. WT. **B.** *unc-40* mutant has short, dysmorphic, proximally shifted arbors (bracket encloses WT arbor region.) **C.** *unc-40* mutant transgenically expressing UNC-40 cell-specifically in NSM has rescued arbor position and morphology. **D.** *unc-40* NSM-specific rescue, N>55 for each phenotype. Note that rescue with *unc-40* endogenous promoter is not significantly different from rescue with NSM-specific promoter (P=0.65).

UNC-40/DCC is required for appropriate synapse distribution

UNC-40/DCC was initially identified as an axon guidance molecule (Chan *et al.*, 1996). Recent work by the Colón-Ramos lab and others has identified a new role for UNC-40/DCC as a synaptogenic cue (Manitt *et al.*, 2009; Colon-Ramos *et al.*, 2007). I have found that while overall vesicle trafficking is unaffected in *unc-40,* as mutants contain serotonin vesicles both in the main NSM axon shaft and within the arbors, the synaptic pattern in *unc-40* mutant animals is different from the wild-type. In wild-type animals serotonergic vesicles are tightly clustered at presynaptic sites. Interestingly I do not observe these tight vesicle clusters in mutant animals. Instead, I observe a broader distribution of vesicles throughout the axon shaft (Fig 5). This observation suggests that *unc-40* is required for the correct distribution or clustering of synaptic vesicles. I will explore this role for UNC-40 and its potential connection to its arborization role in the specific aims outlined below.

D. Approach

Specific Aim 1. Characterize the role of UNC-40/DCC in synapse distribution. I have identified a new role for UNC-40/DCC in synapse distribution in the *C. elegans* serotonin neuron, NSM. In this same neuron, UNC-40 is required for appropriate axon arbor morphology and position. In order to understand the relationship

between these developmental roles, I propose to conduct a panel of experiments to examine the temporal sequence of synapse assembly, as it relates to axon arborization. I will then use this information to probe UNC-40/DCC's role in these processes.

1A. Examine synaptic pattern in early larval (L1) animals. I hypothesize that the role for UNC-40 in synaptic vesicle clustering is linked to its role in modulating arbor position. I have observed that early larval (L1) animals have NSM synapses, but do not form axon arbors. I have yet to carefully analyze the synaptic distribution pattern in L1 animals. I hypothesize that synaptic vesicles are diffusely distributed in L1 animals, and that later expression of UNC-40 is required for the clustering of these vesicles, concurrent with appropriate arbor formation. If this is the case, I can conclude that the processes of vesicle clustering and axon arbor formation are likely mechanistically linked and dependent on UNC-40.

1B. Probe the relationship between synapse assembly and axon arborization. First, using spinning disc confocal microscopy, I will collect three-dimensional images of adult NSM structures in order to quantify the relationship between shaft synapses and axon arbor extension points. Using a time course protocol, I will then determine whether synapses act as stable points for nascent arbor extension, or whether arbor extension points act as anchors for nascent synapses.

1C. Investigate temporal and spatial relationships between UNC-40/DCC expression and synaptic pattern. Using the cell-specific markers that I have developed, I will determine whether UNC-40 localizes to existing synapses. If this is the case, I will examine whether UNC-40 recruitment is followed by synaptic consolidation. It is also possible that UNC-40 localization will be followed by the de novo recruitment of nearby synaptic vesicles. Finally, using other synaptic markers currently in use in the lab (active zone markers ELKS-1, SYD-1, and SYD-2), I will probe the relationship between UNC-40 and a variety of synaptic components. This temporal information will help me to further probe the role of UNC-40 in synapse organization. A collaboration with the nanoscopy laboratory of Dr. Stefan Hell will allow us to visualize dynamic localization of these components with sub-diffraction resolution and, for the first time, *in vivo* and in real time.

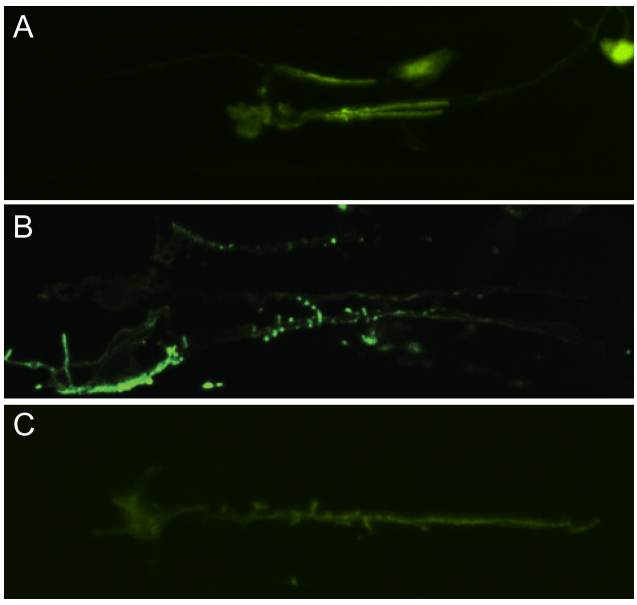


Figure 4. UNC-40::GFP dynamically localizes during the time of arbor formation. A. Juvenile animal before arborization. UNC-40 localizes diffusely. **B.** L4 animal, arbors beginning to form. UNC-40 localizes to discrete puncta in shaft and nascent arbors. **C.** Late L4 animal, arborization is almost complete. UNC-40 is again diffuse.

Specific Aim 2. Forward genetic screen for genes required for temporal specificity of axon arborization. Serotonergic axon arborization and axon guidance occur within distinct developmental windows in both vertebrate systems and *C. elegans.* This suggests the existence of conserved genetic mechanisms underlying the temporal control of these processes. I have identified UNC-40/DCC expression as one potential control element. **Specific Aim 1** will investigate this possibility. However, we have observed that early, NSM-specific expression of UNC-40 is insufficient to drive precocious arborization. Rather, arbor formation coincides with a dramatic change in UNC-40 localization within NSM. This suggests additional temporal control elements in NSM. An unbiased forward genetic screen will identify novel regulators of this temporal control.

2A. Identify L1 mutants containing precocious arbors in a visual forward genetic F2 EMS screen. I will use ethyl methanesulfonate (EMS) to mutagenize animals containing both my NSM-specific cytoplasmic and synaptic markers (Fig 2). L1, or early larval animals displaying precocious arbors will be recovered. If I am able to identify differences in synaptic organization between young wild-type animals and adult wild-type animals (see Aim **1A**), I will also recover any L1 animals displaying precociously clustered synapses. Recovered mutants will then be cloned out onto individual plates and scored in the F3 generation. Next, mutants will be out-crossed to the wild-type (N2) strain to confirm phenotype dependence on a single genetic lesion. Finally, complementation tests will be conducted to further categorize mutants. Mutants identified will likely lack genes required for the temporal regulation of axon arborization.

2B. Identify adult mutants containing no arbors in a visual forward genetic F2 EMS screen. In the same round of EMS mutagenesis, I will seek adult worms containing no arbors. These potential mutants will be

confirmed as in **2A**. If I am able to identify differences in synaptic organization between young wild-type animals and adult wild-type animals (see **Aim 1A**), I will also recover any adult animals with the immature synaptic organization pattern. Mutants containing no axon arbors will either be deficient in genes required for the assembly of axon arbors, or potential master switch genes required for the temporal activation of the arborization program. Both classes of genes will be of interest for understanding the assembly of serotonergic axon arbors.

2C. Map individual mutations. Each mutant will be mated to the CB4856 mapping strain, which contains single-nucleotide polymorphisms (SNPs) throughout the genome that are distinct from the standard wild-type strain (N2). I will use SNP and three-factor mapping techniques as previously described to restrict the mutation to a relatively small (~100kb) genomic interval (Davis and Hammarlund, 2006). Once I have mapped each mutation to a manageable interval, I will perform rescue experiments to identify the gene of interest. To this end, I will microinject large fragments of wild-type genomic DNA contained within the interval of interest. (These fragments will be obtained from the *C. elegans* genome-spanning fosmid library in use in our laboratory). I will then examine the progeny of these microinjected parents for rescue of the observed arborization timing phenotypes. Once I have identified rescuing fragments, I will isolate genomic DNA from the mutant worms and perform direct DNA sequencing to identify the specific molecular lesion.

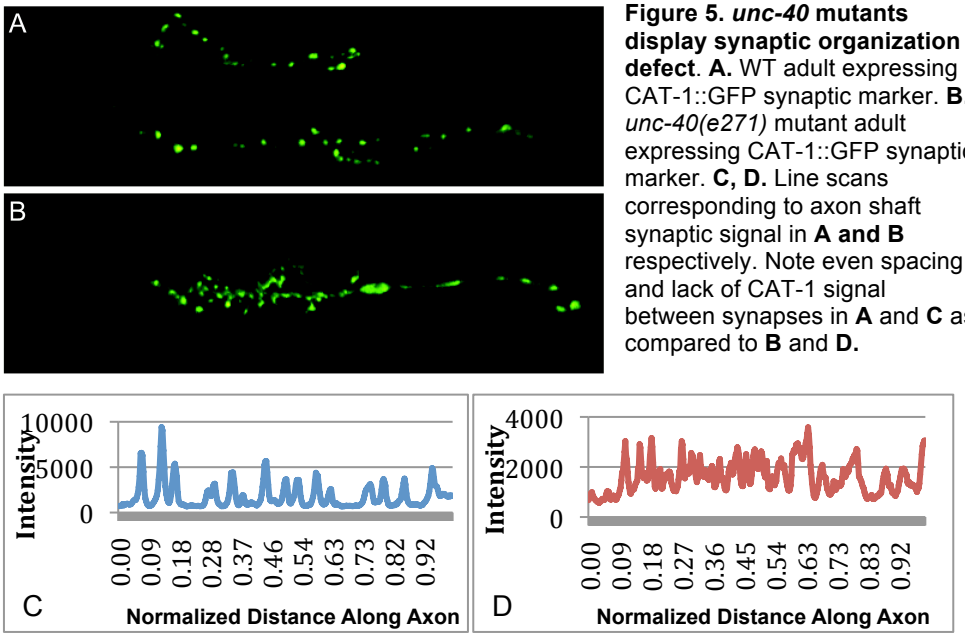


Figure 5. *unc-40* mutants display synaptic organization defect. **A.** WT adult expressing CAT-1::GFP synaptic marker. **B.** *unc-40(e271)* mutant adult expressing CAT-1::GFP synaptic marker. **C, D.** Line scans corresponding to axon shaft synaptic signal in **A and B** respectively. Note even spacing and lack of CAT-1 signal between synapses in **A** and **C** as compared to **B** and **D.**

Specific Aim 3. Determine expression and characterize function for genes identified in the screen. Once I have identified the specific genes required for appropriate timing of axon arborization, I will probe the precise modes of action of these individual genes.

3A. Identify sites of action and characterize temporal and spatial expression patterns for each gene of interest. I will first determine whether genes of interest are required in NSM by conducting mosaic rescue analysis. I will achieve this by generating a transgenic *C. elegans* line in my NSM-labeled background by co-expressing a rescuing fosmid and an NSM tag. Mosaic analysis takes advantage of the mitotic instability of extrachromosomal arrays in *C. elegans.* I will use my NSM tag as a marker for retention of the rescue construct. I will then determine whether phenotype rescue correlates to array retention, by scoring rescue in animals in which the rescue construct has been lost from one or both NSM neurons. Concurrent with these experiments, I will generate promoter::gfp fusions for each gene. I will then microinject these constructs and use microscopy to identify tissues that express each gene. Once I have identified cells/tissues of interest, I will use tissue-specific promoters to conduct rescue experiments, in order to identify tissues in which gene expression is sufficient to rescue the mutant phenotype. Using these same tissue-specific promoters, I will drive translational fluorophore fusions in order to characterize dynamics in the subcellular localization and expression patterns of the gene products. Using my cytoplasmic and synaptic markers, I will visualize the relationship between these gene products and axon arbors/synapses over time.

3B. Examine functions for novel regulators of axon arborization. Using markers for synaptic components (CAT-1, ELKS-1, SYD-1, SYD-2) and cytoskeletal elements (UtrCH labels f-Actin, data not shown), I will probe potential functions for novel genes (Burkel *et al.*, 2007). Furthermore, I will perform pathway analysis by generating double mutants of novel genes and already known effectors of axon arborization (*unc-40, mig-10, unc-34*), and observing effects of novel gene mutations on UNC-40, MIG-10, and UNC-34 localization.

3C. Probe temporal requirement and sufficiency of novel regulators of axon arborization. Adult mutants containing no arbors, or displaying the immature synaptic phenotype may lack genes required for the physical assembly of arbors, or master switch genes required for the temporal selectivity of the arborization program.

One way to distinguish between these two possibilities is to closely examine the results of rescue experiments. For example, a transcriptionally regulated "master switch" gene may be transcribed only within the arborization critical period. However, when conducting tissue-specific rescue experiments, I will likely employ promoters (such as the NSM-specific tph-1 promoter), which activate gene transcription from embryogenesis through adulthood. Thus, I will be able to determine whether early activation of these adult mutant genes is sufficient to induce early arborization. Such genes are likely to represent "master switches." Similarly, I will use early-transcribing tissue-specific promoters to drive conditional heat-shock constructs available in the lab to test the sufficiency of putative early inhibitors of the arborization program, isolated from L1s (Fig 6, (Poon *et al.*, 2008)). For example, I will raise animals at the permissive temperature (arbors should not form as long as gene is intact), and switch animals to the restrictive temperature (arbors should form when gene function is eliminated), in order to test whether these temporal manipulations of gene activity can create temporal shifts in the arborization critical period.

E. Potential Pitfalls

Aim 2 proposes a screen that is relatively labor-intensive in comparison to other *C. elegans* screens. Besides the mutagenesis protocol, these screens also require the following additional steps: mounting the mutagenized animals on microscope slides, anesthetizing the animals, observing their synaptic and arborization pattern using a compound microscope, and recovering mutants under a dissecting microscope. Although these screens require a great deal of effort, screens like these have contributed significantly to our understanding of synaptic biology. For instance, similar screens led to the discovery of immunoglobulin superfamily members and synaptic specificity receptors SYG-1 and SYG-2, as well as synaptic assembly molecules SYD-1, SYD-2/liprin-alpha, RPM-1/Highwire, SAD-1 and RSY-1 (Shen and Bargmann, 2003; Shen *et al.*, 2004; Crump *et al.*, 2001; Schaefer *et al.*, 2000; Patel and Shen, 2009; Hallam *et al.*, 2002). All of these molecules are conserved throughout evolution and have been shown to play a critical role in synaptic biology. Furthermore, my sponsor, Dr. Daniel Colón-Ramos performed a similar screen to the one described here to identify a new role for UNC-40 in directing presynaptic targeting (Colon-Ramos et al., 2007). Most significantly, I myself have completed a productive screen like the one proposed. Therefore I am confident that I can successfully complete this screen, and that it will be productive.

Aims 2 and 3 are reliant on the assumption that the timing of the conserved neurodevelopmental processes of axon arborization and synapse formation are genetically encoded. Given the conservation of serotonergic axon arborization and neurosecretory synapse formation in vertebrates and invertebrates, I expect these processes to be regulated by conserved and genetically encoded programs (Budnik *et al.*, 1989; French *et al.*, 1992; Axang *et al.*, 2008). Preliminary data presented here confirms that aspects of these processes such as arbor positioning and synapse distribution are genetically encoded.

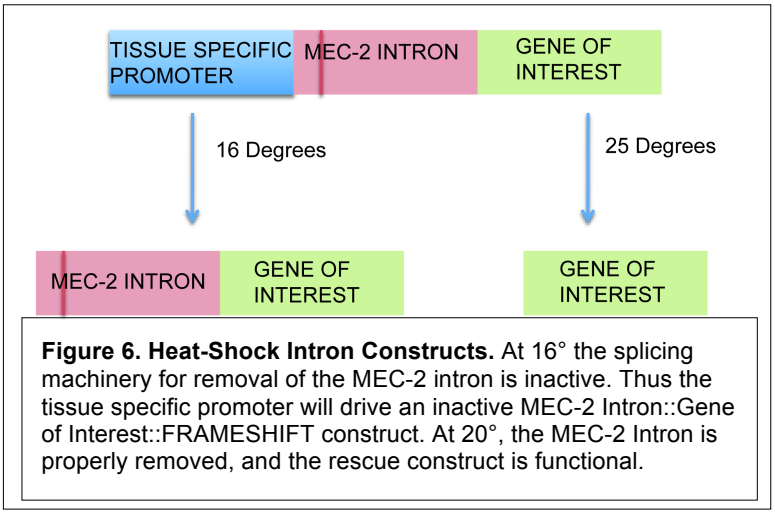


Figure 6. Heat-Shock Intron Constructs. At 16° the splicing machinery for removal of the MEC-2 intron is inactive. Thus the tissue specific promoter will drive an inactive MEC-2 Intron::Gene of Interest::FRAMESHIFT construct. At 20°, the MEC-2 Intron is properly removed, and the rescue construct is functional.

The proposed experiments also require the generation of multiple transgenic strains and the execution of rescue experiments. Though these are labor-intensive experiments, I have been very successful in conducting such work in the Colón-Ramos laboratory, and in the two years since beginning my thesis, I have successfully generated well over one hundred transgenic strains.

F. Overall Expected Outcome

This work will provide molecular insights into the neurodevelopment of serotonin neurons. I will provide new insights regarding UNC-40/DCC's novel role in synapse organization and axon arbor morphogenesis with single cell resolution and *in vivo*. UNC-40/DCC expression is highly dynamic, especially at the time of axon arborization. This suggests the existence of potential UNC-40 regulators that are required for the conserved temporal control of serotonergic axon arborization and synaptogenesis. My forward genetic screen will uncover some of these novel regulators. Considering the lack of information regarding the highly stereotyped development of serotonin neuron morphology, and the importance of this unique morphology for serotonin neuron function, I expect this work to result in new insights on serotonin neurodevelopment and disease.

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