Final Narrative Report
New Jersey Commission on Spinal Cord Research

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1. Original aims of the project:

Netrins are well known as secreted guidance cues that are required to direct growth cones to their targets during the development of the vertebrate central nervous system (CNS). Recent studies also show that netrins and their receptors are expressed as well in the adult CNS. During injury the expression of netrin and its receptors are altered, possibly influencing the ability of adult neurons to regenerate proper connections. Understanding these effects and developing therapies based on them will require new insights into the molecular mechanisms through which netrins function in the extracellular environment. Much has been learned about netrin activities from studies using the model organism *C. elegans*. In fact, the *C. elegans* netrin ortholog, UNC-6, is the founding member of the family. We are using the powerful genetics and well-described neuroanatomy of *C. elegans* to study UNC-6/netrin interactions with other proteins that might help regulate UNC-6 functions.

AIM 1: Identify genes that are required for specific netrin UNC-6 guidance functions.

AIM 2: Characterize the *in vivo* association of netrin UNC-6 with the extracellular matrix.

2. Project successes:

We made great success in isolating and characterizing mutations that suppress the phenotypes of a netrin *unc-6* mutation. Extragenic suppressors have been uncovered that may define UNC-6-interacting proteins or molecules controlling UNC-6 activity. In addition, intragenic suppressors have been isolated that will be informative about the molecular properties of UNC-6 and its interactions with other proteins.

We have found mutations in a gene encoding RPM-1, a RING finger/E3 ubiquitin ligase, which is known to regulate the organization of presynaptic structure. We have also found mutations in a gene containing CLEC-38, a predicted protein containing two C-type lectin-like domains (CTLDs) and an N-terminal type-II transmembrane domain. Through genetic analysis we have shown that these genes help regulate the receptors that control UNC-6 signaling.

In addition, we have uncovered and characterized the sequence alteration of several intragenic suppressors and alleles of *unc-40*, which encodes one of the UNC-6 receptors. These results are important for improving our understanding of the molecular structures that are required for the function of these guidance molecules.

3. Project challenges:

We had proposed to carry out genetic suppressor screens using different reduction-of-function alleles of *unc-6.* This might have uncovered more allele-specific *unc-6* suppressors. However we found that suppressors were difficult to distinguish in the screens because of the lower penetrance and weaker phenotypes associated with reduction-of-function *unc-6* alleles. Moreover, we find that mutations in *rpm-1* and *clec-38* are not suppressors that are specific for different lunc-6 reduction-of-function alleles. Instead they can suppress the phenotypes associated with various *unc-6* reduction-of-function alleles, although not the phenotypes associated with the null allele. In light of this, the different screens are somewhat redundant for finding alleles of genes like *rpm-1* and *clec-38*.

Progress towards Aim 2 was slight. In part, this is because the success of Aim 1 triggered a decision to use the limited resources of the laboratory to focus on characterizing the genes that were isolated.

4. Implications for future research and/or clinical treatment:

Uncovering new genes that are involved in regulating the axon guidance process has lead to new models concerning the mechanisms through which the genes assert their influence. Experiments aimed at testing these models have begun. Further, the phenotypes associated with the new mutations have allowed new genetic screens designed to uncover more components of the system.

5. Plans to continue this research, including applications submitted to other sources for ongoing support.

We are continuing this research. As it turns out, the genetic interactions we observed with the various mutations have tied the results of the NJCSCR-funded study nicely to the results from other studies funded by our NIH RO1 grant. This has expanded the project beyond the original aims of the NIH grant and we are now planning to submit a new RO1 application that focuses on further characterizing the roles that CLEC-38 and RPM-1 play in regulating guidance receptors. We plan to submit this grant after the submission of a paper describing the current results.

6. List of publications

Kulkarni, G., Li, H. and Wadsworth, W.G. CLEC-38 and RPM-1 Regulate UNC-6/netrin- and SLT-1/slit-mediated Axon Guidance in *C. elegans*. Submitted (This paper acknowledges NJCSCR support)