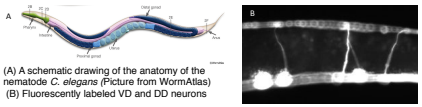
UNC-6/Netrin signaling inhibits growth cone protrusion underlying repulsive axon guidance

Dyan E. Morgan, Adam D. Norris, Zachary J. Roberts, and Erik A. Lundquist

Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045

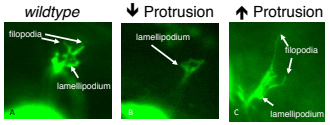
Introduction

We aim to better understand how the nervous system is formed and use the nematode *Caenorhabditis elegans* as a model system.



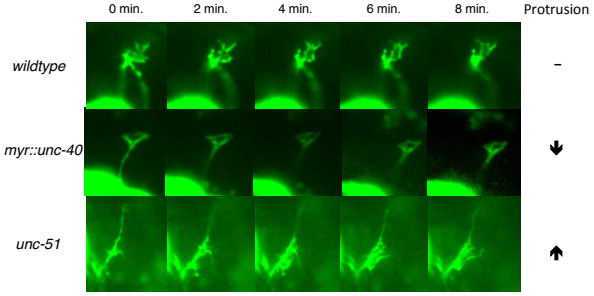
(A) A schematic drawing of the anatomy of the nematode *C. elegans* (Picture from WormAtlas)
(B) Fluorescently labeled VD and DD neurons

Past studies have relied on analysis of endpoint phenotypes. Our research focuses on filopodial dynamics in the growth cones of developing ventral D-type (VD) motor neurons.



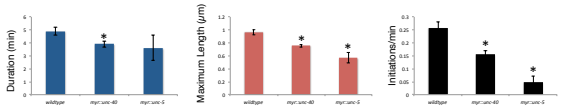
Filopodia length, number, and duration differ by genotype. Shown on the left are (A) wildtype, (B) *myr::unc-40*, and (C) *unc-51* growth cones with their filopodia and lamellipodia labeled.

Growth cones in mutant animals exhibit distinct and opposite phenotypes



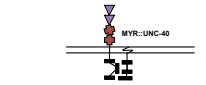
Shown above are time-lapse images of three different growth cone phenotypes. Wildtype growth cones have normal filopodia in both length and duration, growth cones expressing *myr::unc-40* have very short filopodia that retreat quickly, and *unc-51* mutants have long filopodia that persist for extended periods.

Activated *myr::unc-40* and *myr:unc-5* receptors inhibit growth cone protrusion



* $p < 0.05$, n ≥ 10 growth cones scored ≥ 20 minutes

Identification of downstream genes controlling growth cone dynamics



Other Regulators

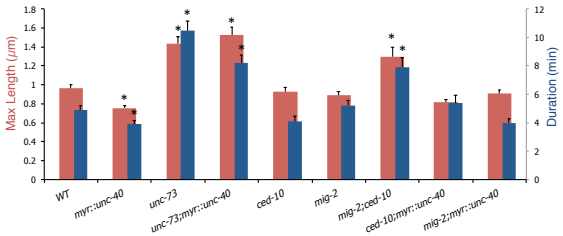
Cytoskeletal Rearrangements

Constitutive Growth Cone Inhibition

Constitutive Repulsive Guidance

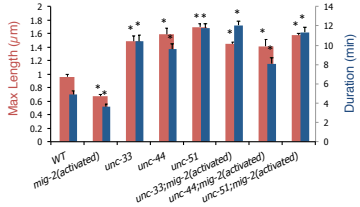
(1) Examine growth cones of mutants with axon branching defects
(2) Identify growth cones with increased protrusion
(3) Epistasis analysis with *myr::unc-40* and *myr::unc-5*

UNC-73 and the Rac GTPases are required for the inhibitory effects of *myr::unc-40*



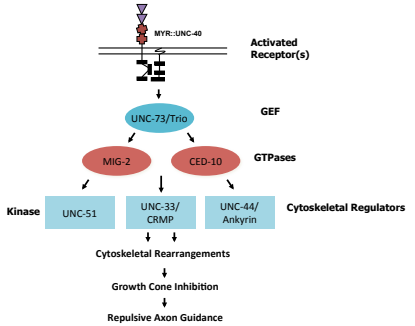
* $p < 0.05$, n ≥ 10 growth cones scored ≥ 20 minutes

UNC-33, UNC-44, and UNC-51 are required for the inhibitory effects of activated Rac GTPases



* $p < 0.05$, n ≥ 10 growth cones scored ≥ 20 minutes

Proposed model:
Netrin signaling inhibits growth cone protrusion via UNC-73/TRIO, the Rac GTPases, and UNC-33/ CRMP, a known Semaphorin effector protein



Future Directions

(1) Further test the model: Examine growth cones with activated *myr::unc-40* and *myr::unc-5*. Continue epistasis analysis using *myr::unc-5*.

(2) Track microtubule invasion into filopodia to test the relationship between growth cone phenotypes and endpoint phenotypes.

Acknowledgments

The authors thank the members of the Lundquist and Ackley labs for helpful discussions.

This work has been supported by grants from the National Center for Research Resources (P20 RR016475), the National Institute of General Medical Sciences (P20 GM103418 and K12GM63651), and the National Institutes of Health (N5040945).