Extending the Neuron Navigator Pathway: Employing Genetic Screens to Identify Novel *unc-53/Nav2* Interacting Genes in *Caenorhabditis elegans*

Julz Quiroz, Leah Amstutz, Ali Hochstetler, Caleb Longenecker, David Zehr and Kristopher Schmidt
Department of Biological Sciences, Goshen College, Goshen, IN. Contact: kschmidt@goshen.edu

Abstract

unc-53 (*uncoordinated-53*) is the *C. elegans* homolog of human *Nav2* (*Neuron navigator-2*), and a member of the Neuron Navigator (NAV) protein family, a group of cytoskeletal binding proteins with conserved roles in the guidance and outgrowth of cells and cellular processes. In *C. elegans*, *unc-53* controls the migration of several cells, including the mechanosensory neurons, the excretory canals, and the sex muscles, the latter resulting in an egg laying defective phenotype in hermaphrodites. Previous studies have revealed that *unc-53* interacts both genetically and physically with *abi-1* (*abelson interactor-1*), a modifier of Arp2/3 mediated actin polymerization. We are interested in identifying novel genetic interactors of *unc-53* using a combination of forward genetic and candidate screens. A forward F2 genetic screen with a current coverage of approximately 6000 haploid genomes is targeting suppressors of the egg-laying phenotype of the null allele *unc-53 (n152)*. A candidate approach is being used to identify a role for *unc-53* in known *abi-1* mediated processes, including the dorsoventral migration of mechanosensory axons and the complex migration of the distal tip cell.

UNC-53 controls longitudinal migration in *C. elegans*

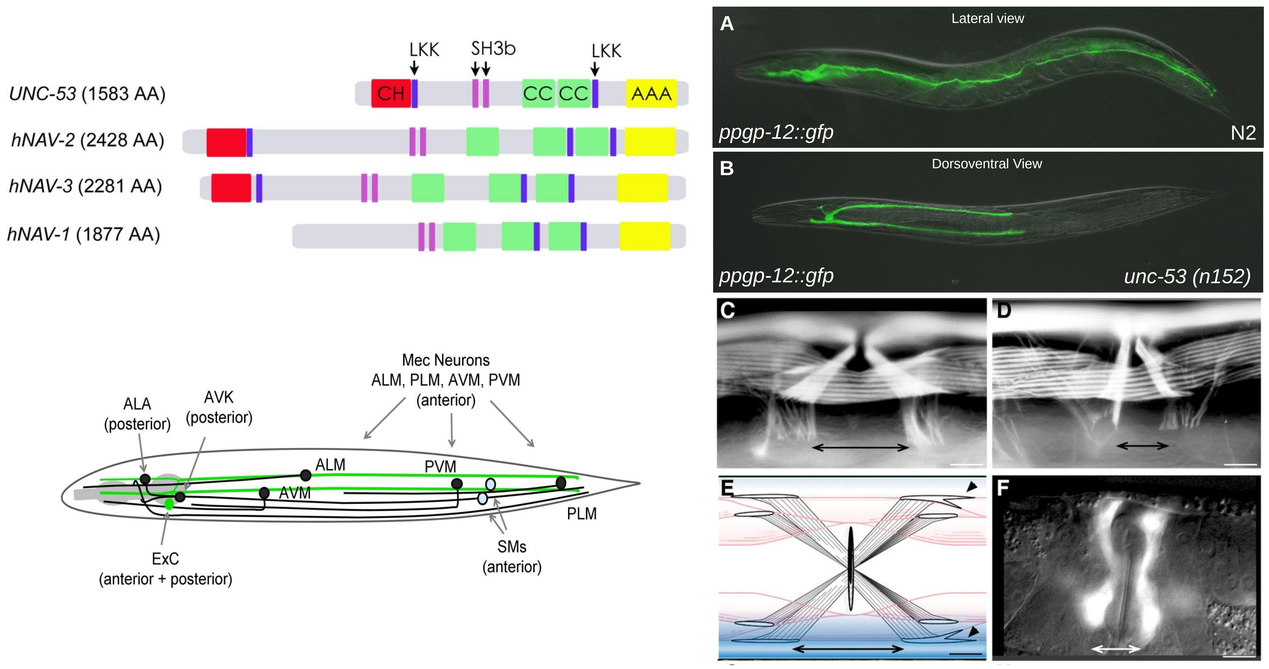

Figure 1. UNC-53 controls longitudinal migration in *C. elegans*. (**Left, Top**) General domain organization of the NAVs. NAVs display a highly conserved domain organization containing multiple domains involved in signal transduction and cytoskeletal binding. Domains include a Calponin Homology domain (CH, Red), LKK actin-binding motifs (LKK, Blue), polyproline rich SH3 binding motifs (SH3b, Purple), Coiled Coil domains (CC, Green), and a AAA ATPase associated with diverse cellular activities (AAA, Yellow) (Stringham and Schmidt, 2009). Note that NAV1 differs substantially from NAV2/3 and UNC-53 because it lacks an N-terminal CH domain. (**Left, Bottom**) Examples of some of the cells and cellular processes controlled by *unc-53* in *C. elegans* are shown. *unc-53* affects the migration of cells and cellular processes along the longitudinal axis. Neuronal cell bodies and axons are in black, the excretory cell (ExC) and associated canals are in green, and the sex myoblasts (SMs) are shown in blue. Note that the migrations and trajectories are all oriented along the anteroposterior axis, and *unc-53* controls both anterior and posterior migration. (**A**) Wild-type animals have excretory canals that extend the entire length of the animal (Lateral view). (**B**) *unc-53 (n152)* animals have excretory canals that stop short both anteriorly and posteriorly (Dorsoventral view). (**C-F**) Wild-type animals have correctly positioned vulval and uterine muscle attachments to the hypodermis while *unc-53 (n166)* mutants (D+F) are incorrectly attached, leading to a Egg laying defect (from Stringham *et al.*, 2002).

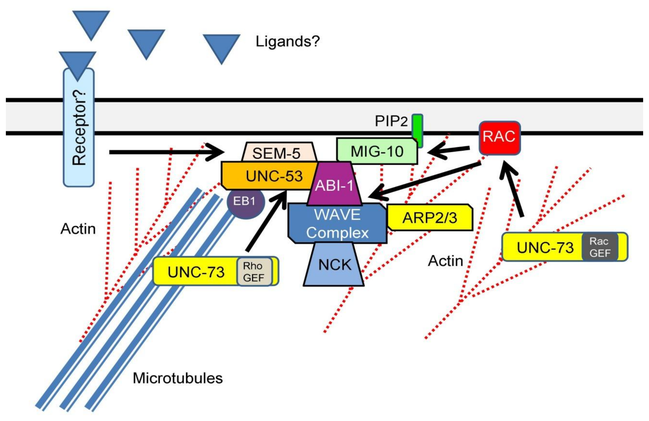

Figure 2. Model for UNC-53 function with other mediators of actin polymerization. UNC-53 is +TIP protein (shown binding EB1) that also binds ABI-1, a regulator of actin filament assembly through the WAVE complex (GEXs not shown for simplicity) and ARP2/3 complex. UNC-53/NAV2 may function to link branched actin filament assembly in protrusions at the leading edge of migrating cells to the plus ends of microtubules. SEM-5 is an interactor of UNC-53 but the upstream receptor linking UNC-53 and SEM-5 is not known. ABI-1 also binds to MIG-10 which interacts with PIP_2 and Rac. UNC-73 is also known to function with UNC-53 through a RhoGEF domain (Marcus-Gueret et al., 2012) while UNC-73 also possesses a Rac-GEF domain that can interact with Rac.

Forward genetic screens to identify suppressors of the egg laying (Egl) defect of *unc-53*

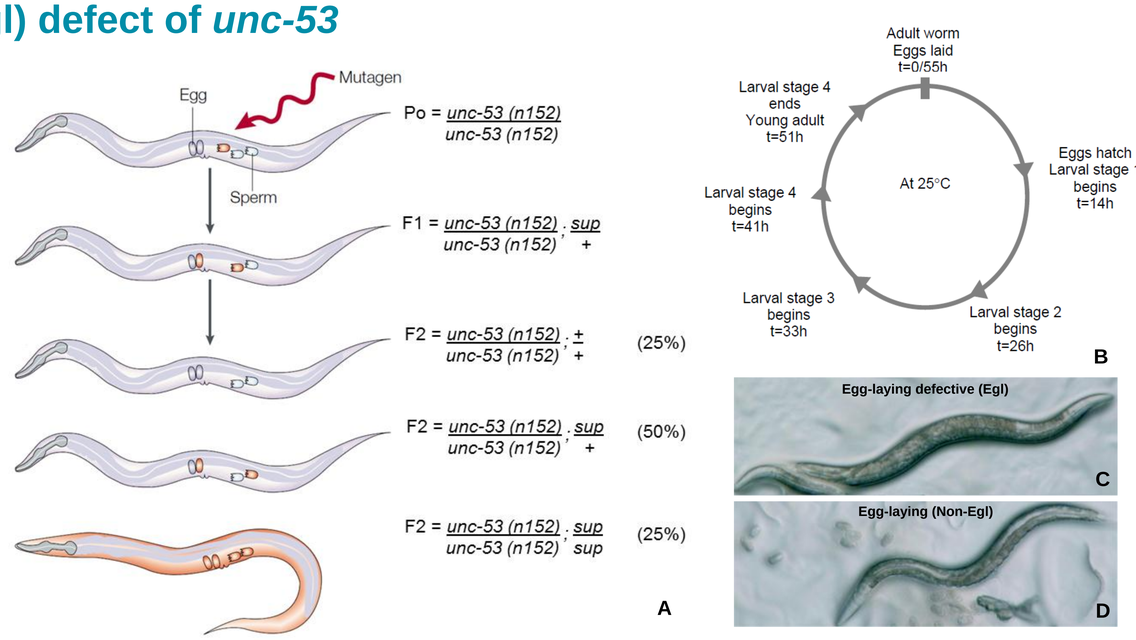

Figure 3. Genetic screen used to identify suppressors of *unc-53*. (**A**) Schematic of the suppressor screen approach used to identify suppressor of the null mutant *unc-53 (n152)* (image from Jorgensen and Mango, 2002). L4 staged animals were mutagenized using EMS at a concentration of 50mM in M9 buffer and rotating for 4 hrs. Mutagenized worms were plated in pairs and progeny of both the F1 generation (for dominant suppressors) and F2 (for recessive suppressors) were screened using a dissecting microscope. NGM plates were examined for the presence of eggs and the presence of animals not appearing gravid. *unc-53 (n152)* is 100% Egl (data not shown). (**B**) Lifecycle of *C. elegans* at 25 degrees (**C+D**) Egg-laying defective animal is shown in C while and Egg-laying animal is shown in D (image from Lakowski and Roelens 2006). Approximately 6000 haploid genomes were screened and no suppressor has been found. The efficacy of the mutagenesis was confirmed by the presence of many animals with classic dissecting microscope phenotypes (e.g. Embryonic lethal, Larval lethal, Gut on Exterior, Dumpy).

Is *unc-53* required for distal tip cell (DTC) migration?

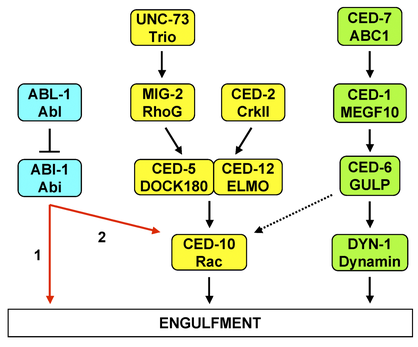

Figure 4. Known genetic pathways controlling cell corpse engulfment. At least three genetic pathways play redundant roles in cell corpse engulfment and DTC migration. UNC-53 interacts with ABI-1 and functions in endocytosis (not shown) (Stringham E.G. and Schmidt K.L., 2009). Image from *Hurwitz* et al., 2009.

Table 1. *abi-1 (rnai)* but not *unc-53 (rnai)* enhances the *ced-5 (n1812)* DTC defect

RNAi Treatment	N2	N=	*ced-5 (n1812)*	N=
	% Abnormal		% Abnormal	
pPD129.36	0%	20	25.9%	27
abi-1 (rnai)	7.7%	26	45.5%**	22
unc-53 (rnai)	0%	23	16.0%	25

% Abnormal refers to the percentage of gonad arms exhibiting altered DTC migration (including features such as abnormal trajectories or bizarre twists) compared to the total number of gonad arms scored. **P<0.001, Chi-squared tested comparing *ced-5 (n1812)* treated with *abi-1 (rnai)* to *ced-5 (n1812)* empty vector RNAi (*pPD129.36)* treated animals. *abi-1 (rnai)* also exhibited synthetic lethality alongside *ced-5 (n1812)* background (data not shown).

Conclusions & Future Directions

- *unc-53* is a member of the NAV family of genes and controls the longitudinal migration of cells and cellular processes in *C. elegans*, with the Egg-laying defective phenotype being readily identifiable.
- We have used EMS mutagenesis to target suppressors of the Egl defect of *unc-53* with a coverage of ~6000 haploid genomes. Further animals will be screened to identify suppressors.
- Using an RNAi approach we have so far not observed a *ced-5* independent role for *unc-53* in DTC migration. Future studies will combine *unc-53 (n152)* and *ced-5 (n1812)* and will also examine a role for *unc-53* in cell corpse engulfment.

References

Jorgensen E. and Mango S, 2002. The Art and Design of Genetic Screens: Caenorhabditis elegans. *Nat Rev Genetics*.
Hurwitz *et al.*, 2009. Abl kinase inhibits the engulfment of apoptotic cells in *C. elegans*. *PLOS Biology*.
Schmidt K.L., *et al.,* 2009. UNC-53 is linked to the Arp2/3 Complex through ABI-1. *Development*.
Stringham E.G., *et al.*, 2002. unc-53 controls longitudinal migration in *C. elegans*. *Development.*
Stringham E.G. and Schmidt K.L., 2009. Navigating the Cell: UNC-53 and the navigators, a family of cytoskeletal regulators with multiple roles in cell migration, outgrowth and trafficking. *Cell Adh Migr*.