Extending the Neuron Navigator Pathway: Employing Genetic Screens to Identify Novel unc-53/Nav2 Interacting Genes in Caenorhabditis elegans
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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 (abnormal interaction-1), a modulator 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 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 C-terminal homology domain (CH, Red), LKK, LKK actin-binding motifs (LKK, Blue), polyglutamine-rich SH3 binding motifs (SH3B, Purple), Coiled-Coil domains (CC, Green), and a AAA ATPase associated with diverse cellular activities (AAA, Yellow) (Strickland and Schmidt, 2008). 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 cell 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 (EC) and associated cells are in green, and the sex myoblasts (SM) are shown in blue. 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 (Dorsal/ventral view). (C-F) Wild-type animals have correctly positioned vulva and uterine muscle attachments to the hypodermis while unc-53 (n166) mutants (D-F) are incorrectly attached, leading to an Egg-laying defect (from Strightman et al., 2002).

Figure 2. Model for UNC-53 function with other mediators of actin polymerization. UNC-53 is a TIP protein (shown binding (B1) that also binds ABI-1, a regulator of actin filament assembly through the WAVE complex (WAVEs not shown for simplicity) and ARP2/3 complex. UNC-53/NAV may function to bind branched actin filament assembly in protrusions at the leading edge of migrating cells to the plus ends of microtubules. SEM-5 is an inhibitor of UNC-53 but the upstream receptor linking UNC-53 and SEM-5 is not known. ABI-1 also binds to MID-15 which interacts with PIP2 and Flac. UNC-73 is also known to function with UNC-53 through a RhoGAP domain (Marcus-Gueant et al., 2013), while UNC-73 also possesses a Rho-GAP domain that can interact with Flac.

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 stage animals were mutagenized using EMS at a concentration of 50 mM in M3 buffer and rotating for 4 hrs. Mutated 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. HAP plating were examined for the presence of eggs and the presence of animals not appearing grand. unc-53 (n152) is 100% Egl (data not shown). (B) Life cycle of C. elegans at 25 degrees. (C-D) Egg-laying defective animal is shown in C while Egg-laying animal is shown in D (image from Landsberg and Ruvkun, 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).

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) (Strightman E.G. and Schmidt K.L., 2009). Image from Huwert et al., 2009.

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 cell-5 independent role for unc-53 in DTC migration. Future studies will combine unc-53 (n152) and cell-5 (n1812) and will also examine a role for cell-5 in cell corpse engulfment.

References
Schmidt K.L. et al., 2009. UNC-53 is linked to the Arp2/3 complex through ABI-1. Development.

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

Table 1. abl-1 (ma) but not unc-53 (ma) enhances the cell-5 (n1812) DTC defect

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<th></th>
<th>N2</th>
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<tr>
<td></td>
<td>% Abnormal</td>
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<tr>
<td>ppD129.3t</td>
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<td>20</td>
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<tr>
<td>abl-1 (ma)</td>
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</tr>
<tr>
<td>unc-53 (ma)</td>
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*Abnormal refers to the percentage of gene arms exhibiting altered DTC migration (including features such as abnormal trajectories or bizarre tails) compared to the total number of gene arms scored. **P<0.001, Chi-squared testing comparing cell-5 (n1812) treated with abl-1 (ma) to cell-5 (n1812) empty vector RNAi (ppD129.3t) treated animals. abl-1 (ma) also exhibited synthetic lethality alongside cell-5 (n1812) background (data not shown).