Stress Induced Remodeling in the Nematode *C. elegans*

Becky Rose,[1] Rebecca Androwski,[2] and Nathan E. Schroeder[2,3]

Lincoln Land Community College, Springfield, Illinois[1]
Neuroscience Program, University of Illinois at Urbana-Champaign[2]
Department of Crop Sciences, University of Illinois at Urbana-Champaign[3]

PRECS Phenotypic Plasticity Research Experience for Community College Students

Caenorhabditis elegans is a model organism for studying genetics and neuroscience

C. elegans is frequently studied to understand **how genes and the environment interact to produce new phenotypes**. We take advantage of an organism-wide stress response and genetic tools that provide an excellent model for studying how phenotypes are impacted by stress.

Stress-resistant dauer stage

Crowded conditions and lack of food cause *C. elegans* to enter an alternative stress-resistant stage called dauer.

Dauers undergo multiple **morphological changes including, thickening of the cuticle, radial shrinkage, and neuroplasticity**.[1]

Dauer morphology provides **resistance to 1% sodium dodecyl sulfate (SDS)**.[2]

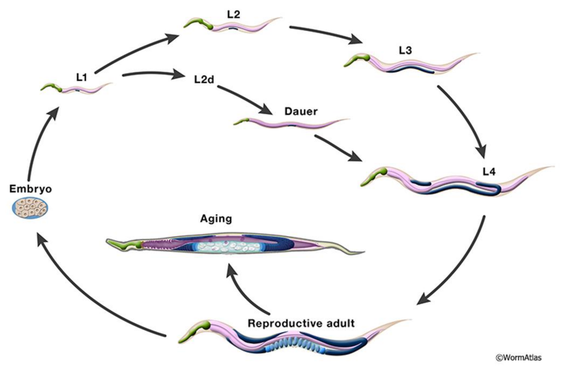


Figure 1: Environmental stress causes an alternate larval stage called dauer. Upon return to favorable environmental conditions the animal recovers from dauer and resumes normal development.

There is extensive remodeling of the IL2 neurons during dauer

Outside of dauer the six IL2 dendrites are unbranched. During dauer they form elaborate branches that cover the head of the animal. Once the animal recovers from dauer, the branches resorb and return to their unbranched morphology.

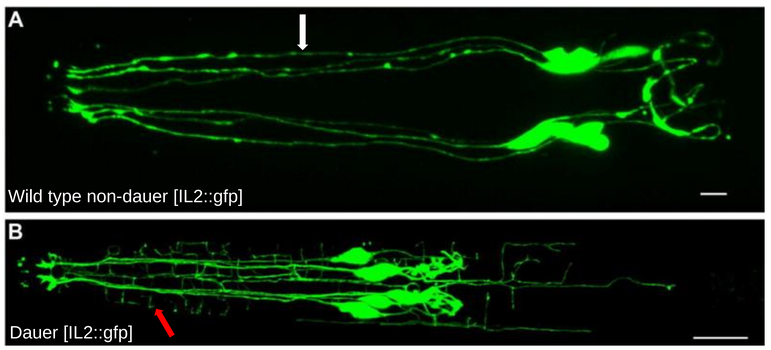


Figure 2: Neuroplasticity of IL2 neurons during dauer The IL2 dendrites (A) are unbranched in non-dauer animals. During dauer, the dendrites (B) branch extensively. White arrow indicates primary dendrite. Red arrow indicates body wall branch during dauer. The IL2s are labeled using *klp-6p::gfp*. Scale bar is 10 μm.[3]

daf-18 is required for IL2 branching

The candidate gene, *daf-18,* encodes a PTEN homolog in *C. elegans.*[4] PTEN is a critical component of human neurological health. Mutations in PTEN lead to abnormal neuronal growth and neurological dysfunction and are hypothesized to be a cause of autism spectrum disorder.[5] When C. elegans *daf-18*/PTEN is disrupted, we found IL2 dendrite branching defects.

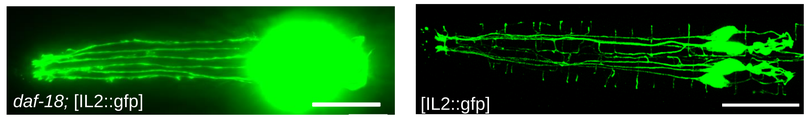


Figure 3 The daf-18 mutant showed a lack of IL2 branching. The IL2s are labeled using *klp-6p::gfp.* Scale bar is 10 μm.

IRE-1 activates a stress response that plays a critical role in regulating dendrite growth

We looked at two variations of ***ire-1*, *(v33) and (ok799)***.

Figure 4 *ire-1(ok799)* and *ire-1(v33)* are both large deletions.[6]

Previously both alleles have been considered to completely disrupt protein function.[7,8] *ire-1(v33)* and *ire-1(ok799)* have been linked separately to dauer formation and to neuronal defects, respectively.[7,8] **We found branching defects in both alleles**. However, we found these defects at low penetrance where only 20% of the animals had severe defects.

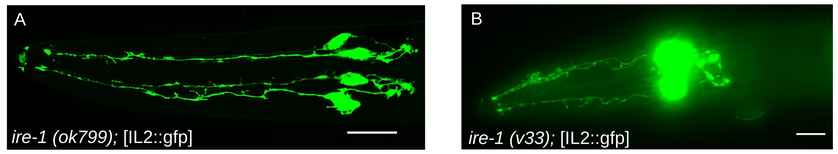


Figure 5 *ire-1(ok799)* and *ire-1(v33)* are representative of severe branching defects (A,B). The IL2s are labeled using *klp-6p::gfp.* Scale bar is 10 μm.

Are *ire-1* mutants true dauers?

We did a 1% SDS test to isolate dauers of each allele. Exposure to 1% SDS is a common way to isolate true dauers. None of the *(v33)* animals survived and only 3 of the *(ok799)* animals survived. Interestingly, these results contradicted literature that stated (v33) mutants form 100% dauers.[8] This led us to question whether these animals were true dauers. We then performed SDS dose response assay to assess susceptibility. We used the following SDS concentrations: 1%, 0.5%, 0.2%, 0.1%.

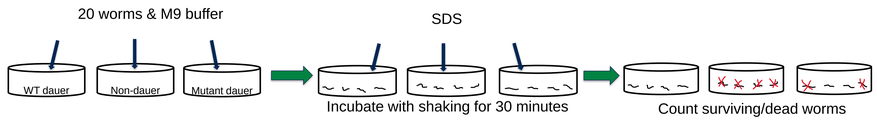
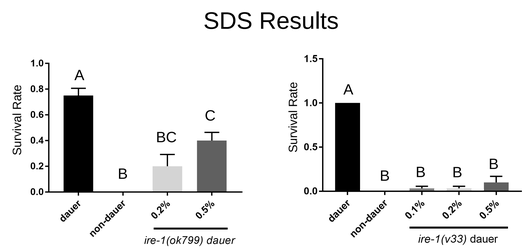


Figure 6 ANOVA and Tukey's Multiple Comparison Test were performed. *ire-1(ok799):* n=20 for 0.2% and n=60 for 0.5%. *Ire-1(v33):* n=60 for all treatments. Dauer is negative control; non-dauer is positive control. For *(ok799),* there was a significance between the non-dauer and 0.5% treatment but no significance between non-dauer and 0.2%. For *(v33),* there was no significance between non-dauer and any treatment. Different letters indicate statistical significance.



Conclusions

- ***daf-18*/PTEN** is required for IL2 branching
- ***ire-1*** is a critical component in the IL2 branching pathway
- We found allelic differences between ire-1(ok799) and ire-1(v33)
  - ***ire-1(ok799)*** appears to have an **intermediate resistance to SDS** as compared to non-dauer animals
  - ***ire-1(v33)*** appears to **lack resistance to SDS** as compared to non-dauer animals

Future Work

- Perform more SDS dose response replicates for *ire-1(ok799)*
- Perform SDS dose response replicate at lower concentration (0.01%) for *ire-1(v33)*
- Look at other highly branched neurons in *daf-18* adult mutants to determine effect of mutation outside of IL2s
- Look at IL2s in non-dauer *daf-18* mutants

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Acknowledgments

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