The effects of silencing insulins on the morphology and function of both healthy and degenerate mechanosensory neurons in the *C. elegans* aging model

Skyler Hunter[1,2], Heather Currey[1,2], Elena Vayndorf[2], Barbara Taylor[1,2]

UAF Dept. of Biology and Wildlife[1], UAF Institute of Arctic Biology[2]

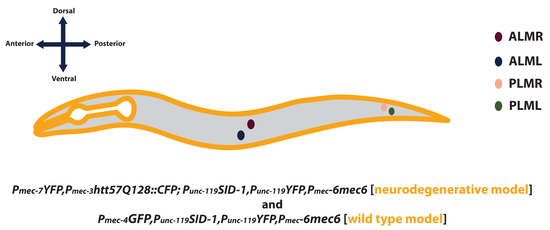
Introduction

The overall aim of this project is to establish how post-transcriptionally silencing members of the insulin gene family affects neuronal aberrations of mechanosensory neurons. Our overall goal is to assay aberrant neuronal morphology and touch response in wild type and neurodegenerative models, as well as hungtingtin protein aggregation in a neurodegeneration model of *C. elegans*.

Neurodegenerative diseases such as Huntington's, Alzheimer's and Parkinson's result in accelerated neuronal aging characterized by a gradual loss of structure and function of neurons, impaired motor control, and decreased cognitive ability. Huntington's disease (HD) is the result of a genetic mutation resulting in an expansion of CAG trinucleotide repeats in the first exon of the huntingtin (*HTT*) gene giving way to polyglutamine (polyQ) chains leading to aggregate formation, neuronal dystrophy and severe dysfunction.

In humans, more than 36 repeats may result in toxicity and the onset of Huntington's disease. Our HD model has 128 repeats and expresses the fluorescently labeled toxic (polyQ128) huntingtin in the mechanosensory neurons of the model system, *Caenorhabditis elegans*.

Models



P$_{mec-7}$*YFP,P*$_{mec-3}$*htt57Q128::CFP; P*$_{unc-119}$*SID-1,P*$_{unc-119}$*YFP,P*$_{mec-}$*6mec6* [neurodegenerative model]
and
P$_{mec-4}$*GFP,P*$_{unc-119}$*SID-1,P*$_{unc-119}$*YFP,P*$_{mec-}$*6mec6* [wild type model]

Figure 1 A simplified diagram of *Caenorhabditis elegans* with mechanosensory neurons of interest labeled. Models are transgenic mutants with fluorescently labeled mechanosensory neurons and aggregates. Overexpression of *SID-1* channels allows for RNAi sensitivity. Also listed are the genotypic nomenclature for the Huntington's disease and wild type models respectively.

Morphological Changes in Cytoskeletal Structure

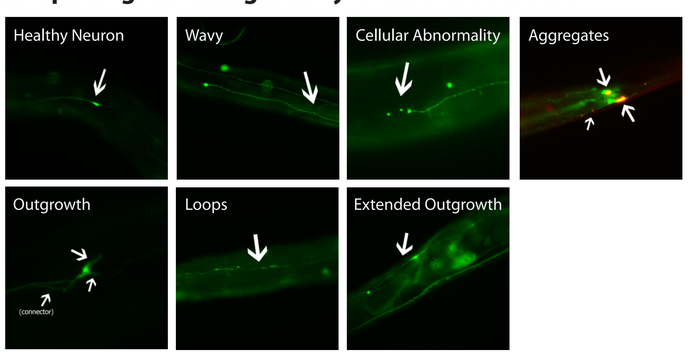


Figure 2 Examples of neuronal aberrations that appear in aging neurons. Normal brain aging is characterized by synaptic deterioration and changes in the cytoskeletal structures of neurons (B.A. Yankner et al. 2008). These structural changes may lead to an increase in cognitive decline and contribute to neurodegenerative diseases such as Alzheimer's and Huntington's disease (Knobloch and Mansuy, 2008).

Insulin Signaling

The *C. elegans* IIS pathway includes insulin-like peptides (ILP), a single receptor (DAF-2), and a downstream signaling cascade which influences the DAF-16 transcription factor.

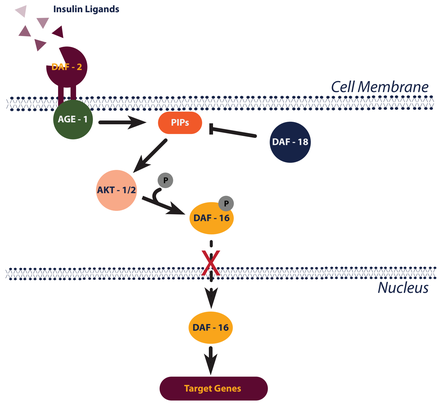


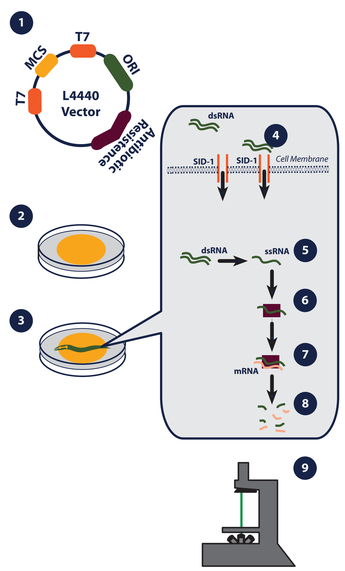
Figure 3 Insulin-like peptide signaling pathway in *C. elegans*. The *C. elegans* IIS pathway includes insulin-like peptides, a single receptor (DAF-2), and a downstream signaling cascade which influences the DAF-16 transcription factor. Loss-of-function mutations in daf-2 affect lifespan, reproduction,metabolism, stress and dauer formation, and a complete loss of the receptor is lethal
(Ritter, Ashlyn D et al. 2013).

Neuronal morphology is affected by the insulin-IGF signaling pathway, specifically through differential insulin-like peptide expression.

Methodology

Figure 4 Schematic and description of screen methodology used in this project.

1. L4440 vector contains regions for specific insulin peptide, antibiotic resistance, and other useful tools.
2. The vector is incorporated into *E. coli* (HT115).
3. IPTG in the plate media activates the synthesis of dsRNA as the bacteria grows into a lawn.
4. RNAi sensitive worms take up the vector while feeding on *E. coli*.
5. Over expression of *SID-1* channels results in dsRNA entering the cell. Inside the cell dsRNA is converted into ssRNA.
6. The ssRNA is complexed into the RNA Induced Silencing Complex (RISC).
7. RISC pairs to complementary mRNA strand targeting it for destruction.
8. RISC degrades mRNA - peptide never forms.
9. We score and image 5 day old adult worms under fluorescent microscope.



Preliminary Results

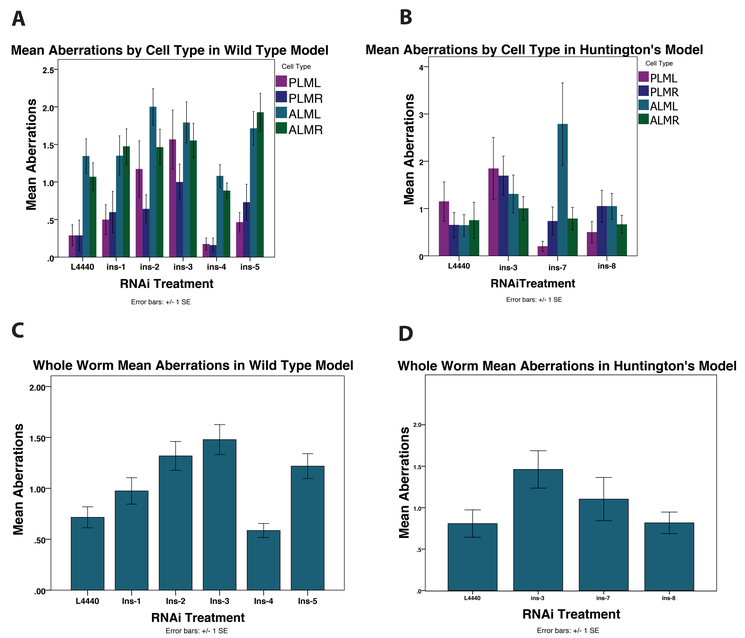


Figure 5 Mean aberrations in whole worm and cell type in (A/C) wild type and (B/D) neurodegenerative models.
A) Mean aberrations of cell types versus treatment in wild type (overall $P < 0.001$, ANOVA PLML $P = 0.001$, ANOVA ALMR $P = 0.012$).
B) Mean aberrations of cell types versus treatment in neurodegenerative model (overall $P < 0.014$, ANOVA ALML cell type L4440 against ins-7 $P < 0.011$).
C) Mean aberrations of whole worm versus treatment in wild type ($P < 0.001$).
D) Mean aberrations of whole worm versus treatment in neurodegenerative model ($P = 0.067$).

Preliminary Discussion and Future Directions

So far we have seen a significant impact on neuronal morphology by knocking down insulin like peptides 2, 3, 5 and 7. Our primary goal is to complete this screen for all 40 insulin like peptides in *C. elegans*. After the completion of the screen, more in depth study on specific and interesting peptides. We are also interested in how these insulins like peptides interact with each other.

References

1. M. Knobloch, I. M. Mansuy, Changes 37, 73-82 (2008).
2. C.-L. Pan, C.-Y. Peng, C.-H. Chen, S. McIntire, Proceedings of the National Academy of Sciences of the United States of America 108, 9274-9279 (2011).
3. A. -D. Ritter et al., Genome Res. gr.150466.112, Published in Advance (2013).
4. E. M. H. Tank, K. E. Rodgers, C. Kenyon, Journal of Neuroscience 31, 9279-9288 (2011).
5. L. Timmons, D. L. Court, A. Fire, Gene 263, 103-112 (2001).
6. M. L. Toth et al., Journal of Neuroscience 32, 8778-90 (2012).
7. B. A. Yankner, T. Lu, P. Loerch, Annual Review of Pathology 3, 41-66 (2008).

Acknowledgements

We would like to thank URSA and INBRE for funding this project and Elena Vanydorf, Courtney Nichols, Barbara Taylor, Gary Laursen and Kerry Dufseth for their personal support.

To Contact Team Regarding Project

Heather Currey hncurrey@alaska.edu
Skyler Hunter schunter@alaska.edu