The Effect of the *age-1* gene on lifespan in *Caenorhabditis elegans*

Zachary Edwards

Plymouth State University, Department of Atmospheric Sciences and Chemistry

Introduction

How certain genes are involved in specifying the restricted life span that humans experience is still an ongoing question. There are many theories that suggest molecular and physiologic processes are responsible for this limitation. Aging is a central aspect of biology that triggers strong emotions with humans, because they experience and see others going through the process daily. Many people wish to delay or even modify the aging process. *Caenorhabditis elegans* have emerged as one of the premiere model systems for aging research because they grow in vast quantities in the lab, are cheap to maintain and have an accessible genome. Their genome is small compared to humans yet it encodes over 22,000 proteins, only slightly fewer than humans, while about 35% *of C. elegans* genes are related to humans[3].

Studies have identified hundreds of genes that control lifespan in *C. elegans*, but one of the most important is the *age-1* gene. The *age-1* gene codes for the phosphatidylinositol 3-kinase homolog, which regulates longevity and diapause of these *C. elegans* while it promotes cell survival during embryonic development[1]. An effective way to examine this regulatory process is to identify mutations that may alter it. If there are proteins that determine the lifespan of these nematodes, then there should be a possible way to modify the function of the gene and to alter the rate of aging. Creation of an RNAi feeding vector that was fed to *C. elegans* was responsible for silencing the age-1 gene function. The experimental worms were exposed to this feeding vector and without the function of the age-1 gene, the worm's life spans were decreased. The basis of this study focused on the percentage of nematodes that survived through an acute juglone sensitivity assay. These aspects together could provide vital understandings of aging in *C. elegans* that could be applicable to humans in the near future.

Hypothesis

The *age-1* gene codes for phosphatidylinositol 3-kinase, which is part of a pathway that regulates longevity. An RNAi feeding vector was created to silence the *age*-1 gene. My hypothesis is that the lifespan of the treated nematodes will decrease relative to the control. The effectiveness of my constructed RNAi feeding strain will be studied by performing an acute juglone sensitivity assay.

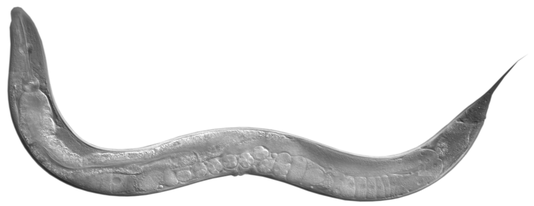

Figure 1. Model of an *C. elegan's* body structure.

Methods

Culturing the *C. elegans*

The worms were raised on NGM-lite plates with OP50 that served as the control. Also worms were transferred to experimental plates containing the RNAi feeding strain of *Escherichia coli* that targeted the *age-1* gene.

1. Use BLAST and Wormbase to find the *C. elegans* gene that was homologous to a human gene.
   - Use BLAST to identify gene sequence length (925 base pairs).
   - Use NCBI program to create primers for PCR amplification of the C. elegans gene with appropriate overlaps for cloning.

2. Clone *C. elegans* specific DNA
   - Isolate *C. elegans* DNA.
   - Run PCR to amplify the DNA.
   - Run gel electrophoresis to confirm DNA isolation and amplification.
   - Recombine DNA with vector pPR422.

3. Transform competent E. coli cells with the recombinant DNA vector.

4. Purify DNA
   - Preform QIAprep spin mini-prep kit on DNA to isolate and purify the plasmid.

5. Prepare RNAi feeding strain
   - Transform purified plasmid into RNAi feeding strain.

6. Prepare LB/Kanamycin plates along with LB plates
   - Seed all plates with the feeding strain.

7. Use feeding strain to induce RNAi and silence the *age-1* gene in Cl4176 *C. elegans*
   - Plate L4 larval stage worms onto plates containing the RNAi feeding strain for a few days, so they can ingest the feeding strain.

8. Age testing
   - Preform acute juglone sensitivity assay[5].

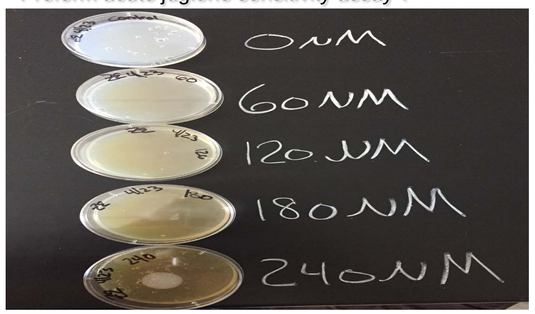

Figure 2. The various juglone concentrations used for the assay.

Results

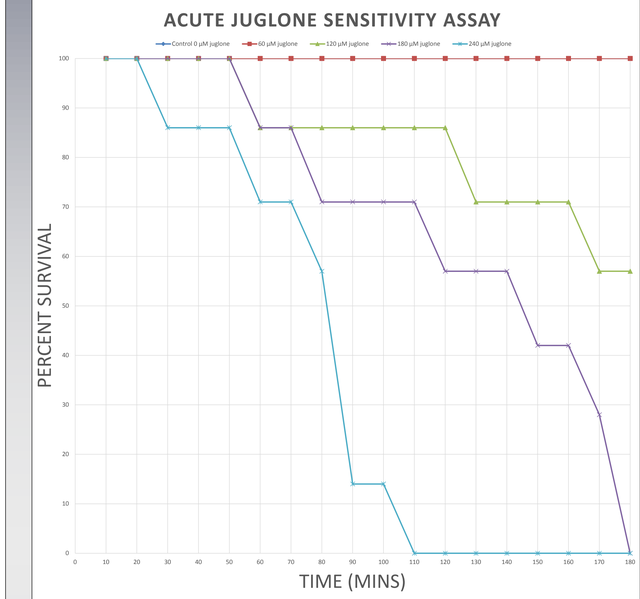

Figure 3. Lifespan of *C. elegans* in the various concentrations of juglone.

time (mins)	Control 0 µM juglone	60 µM juglone	120 µM juglone	180 µM juglone	240 µM juglone
10	100%	100%	100%	100%	100%
20	100%	100%	100%	100%	100%
30	100%	100%	100%	100%	86%
40	100%	100%	100%	100%	86%
50	100%	100%	100%	100%	86%
60	100%	100%	86%	86%	71%
70	100%	100%	86%	86%	71%
80	100%	100%	86%	71%	57%
90	100%	100%	86%	71%	14%
100	100%	100%	86%	71%	14%
110	100%	100%	86%	71%	0%
120	100%	100%	86%	57%	0%
130	100%	100%	71%	57%	0%
140	100%	100%	71%	57%	0%
150	100%	100%	71%	42%	0%
160	100%	100%	71%	42%	0%
170	100%	100%	57%	28%	0%
180	100%	100%	57%	0%	0%

Table 1. Percent of *C. elegans* alive every 10 minutes.

Figure 4. Group of *C. elegans* on the control plate.

Conclusions

Oxidative stress has been proposed to be one of the main causes of aging and has been implicated in the pathogenesis of many diseases. Sensitivity to oxidative stress was measured by quantifying survival following exposure to a reactive oxygen species (ROS)-generating compound, in this case juglone. Sensitivity to oxidative stress is a balance between basal levels of ROS, the ability to detoxify ROS, and the ability to repair ROS-mediated damage. As expected, the worms that were exposed to higher concentrations of juglone resulted in lower lifespans. The control worms that were exposed to no juglone survived past a full day compared to the worms that were exposed to 240 µM of juglone which died within less than two hours. This experiment proved that the *age-1* gene plays a role in lifespan of *C. elegans.* Silencing the *age-1* gene would be expected to decrease the survival of the worms exposed to juglone, compared to worms fed with OP50.

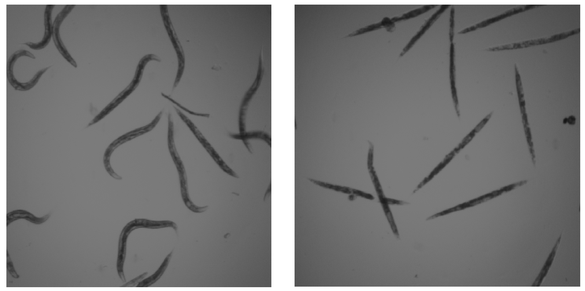

Figure 5. Difference between alive versus dead *C. elegans.*

Future Work

- I will extend my research on the *Age-1* gene by researching the affects on aging from natural products like purple corn powder. This natural product contains anthocyanins that are known to promote longevity in *C. elegans.*

References

1. Dorman, Jennie B. "The Age-1 and d a f 2 Genes Function in a Common Pathway to Control the Lifespan of Caenorhabditis Elegans." *The Age-1 and d a f 2 Genes Function in a Common Pathway to Control the Lifespan of Caenorhabditis Elegan*, 1995, pdfs.semanticscholar.org/6f51/71edb9ae0a46e746b395e673d73ba79a9bb7.pdf.
2. Friedman, David B. *A Mutation in the Age-1 Gene in Caenorhabditis Elegans Lengthens Life and Reduces Hermaphrodite Fertilit*. 1987, www.bing.com/cr?IG=0D9207D1766D473DA37524202F614DCF&CID=3A360CFA95BB608C259107279414619D&rd=1&h=KjQYm4q0oiiqdA98kj4oJGte98EEzlEq1gONhq2EnAU&v=1&r=http%3a%2f%2fwww.genetics.org%2fcontent%2fgenetics%2f118%2f1%2f75.full.pdf&p=DevEx.LB.1,5064.1.
3. Johnson, Thomas E. "25 Years after Age-1: Genes, Interventions and the Revolution in Aging Research." *Experimental Gerontology*, U.S. National Library of Medicine, July 2013, www.ncbi.nlm.nih.gov/pmc/articles/PMC3686982/.
4. Tissenbaum, Heidi A. "Using C. Elegans for Aging Research." *Invertebrate Reproduction & Development*, Taylor & Francis, 30 Jan. 2015.
5. Senchuk, Megan M., et al. "Measuring Oxidative Stress in Caenorhabditis Elegans: Paraquat and Juglone Sensitivity Assays." *Bio-Protocol*, U.S. National Library of Medicine, 5 Jan. 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5739066/.

Acknowledgments

- All C. elegans strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).
- Dr. Susan Swope