Personal Statement

Shortly before he died, physicist and renowned teacher Richard Feynman wrote on his blackboard: “What I cannot create, I do not understand.” If I had known two years ago that I were applying for the chance to spend sixteen weeks working with worms, I would have been certain that I had fallen deep into the rabbit hole. However, two years ago I was only just beginning to fathom the extent of what I did not know about science. It was not until I started working at a plant genomics laboratory at Louisiana State University that I began to understand Feynman’s last words and take an interest in turning research into a career. Once I arrived at Northeastern’s campus, I persistently heard references to the model organism *Caenorhabditis elegans*, a transparent nematode used in genetics studies. When *C. elegans* found its way into a conversation about undergraduate research with Professor Strauss, I took her advice and applied to work in Professor Javier Apfeld’s aging lab. This summer, I hope to continue strengthening my ability to understand science for my own enjoyment and to be a more competitive applicant for internships, fellowships, and graduate school.

Project Background and Objectives

A general interest with longevity and aging has been sewn through the entire history of our species, from the Sumerian kings who each, according to legend, lived for tens of thousands of years to Ponce de Leon’s obsession with the fountain of youth. More recently, an increasing number of scientists have begun to formulate concrete methods for exploring the biological mechanisms that underlie aging. One early theory, published in 1956, speculated that the byproducts of cellular oxidation-reduction processes were the primary cause of aging (1). This 1956 theory later developed into the oxidative stress theory of cellular aging, which remains prominent both in general aging research and within research focused on *C. elegans* (2, 3). Thus, when Dr. Cynthia Kenyon’s team at the University of California San Francisco discovered certain genes directly regulate the lifespan of *C. elegans* (4), the scientific community quickly began to speculate about the link between oxidative stress and the function of specific lifespan-regulating genes. While correlations between life expectancy and oxidative stress in *C. elegans* has been documented, the relationships between the different genes that regulate lifespan are more poorly understood (5).The goal of my proposed project is to test the lifespans of different mutant combinations and then compare those lifespans to the mutant’s degree of stress resistance. To obtain preliminary results in a timely manner, I will focus on short-lived mutants. In doing so, I hope to further clarify the interactions between longevity genes and the role of such interactions in responses to oxidative stress.

Project design and Methodology

This project will consist of four major stages. First, I will perform a literature review related to short-lived *C. elegans* mutants, focusing especially on those mutations on genes which encode transcription factors. Throughout this process, I will create a detailed annotated bibliography documenting my discoveries. I will then obtain necessary mutant strains, cross those to make novel mutants, and begin lifespan assays. I will document the behavior of the

worms under oxidative stress, and then repeat the process with long-lived mutants. The following paragraphs elaborate on each of the four stages individually.

The initial data collection is currently ongoing. Since the aging literature is extensive, this project will focus more narrowly on genes encoding transcription factors, which tend to be unique in biochemical pathway, but often overlap in mechanism (6).Transcription factor genes that I have currently identified include *daf-16*, *xbp-1*, *daf-12*, and *nhr-49*. *Daf-16*, which codes for the FOXO-transcription factor and primary mediator of the insulin/IGF-1 signaling pathway, is widely studied, highly evolutionarily conserved, and relevant to research on age-related disease in humans (5, 7). *Xbp-1* acts along the HSP-4 endoplasmic reticulum stress pathway (8). *Daf-12* is a well-documented gene which acts in conjunction with *daf-16* in the IGF-1 signaling pathway (9). Lastly, *Nhr-49* encodes a nuclear hormone receptor that also acts as a transcription factor (10). While the four genes I have identified thus far are well documented individually, their interactions are poorly understood. I will identify further genes of interest and focus on transcription factors, short-lived mutants, and those which interact with already-identified factors.

Crossing mutant strains will follow relatively straightforward procedures outlined by references such as "Wormbook" and methods from previous studies. Significant aspects for consideration include the amount a strain has been outcrossed and whether a null-mutation has been identified in previous studies. Most of these aspects will be addressed during the first step of this project. After mutants are obtained, their genotypes will be confirmed using allele-specific PCR.

After setting up the lifespan assays, I will begin to measure oxidative stress resistance on each strain in microtiter plates as outlined by researchers at McGill University in 2015 (11). The oxidative stress assays will use late-L4 worms and therefore be staggered based on availability.

If all of the previous sections of this proposal proceed as planned, I will use my remaining time to repeat this process using long-lived mutant combinations, once again focusing on the influence of transcription factors. This final stage will serve as a platform from which I will continue my research into future years.

Project Timeline

May 8th – May 14th (Week 1):

- Create detailed project outline and annotated bibliography.
    - Include further strains of interest, especially those with altered TOR, IGF, or germline signaling pathways.
- Confirm that proposed crosses are novel.

May 15th-May 21st (Week 2):

- Order all needed resources, including worms and chemicals for oxidation assay.

May 21st – June 4th: Vacation

June 5th – June 11th (Week 3):

- Begin to maintain worms and set up genetic crosses between mutants.
    - This will create double and triple mutants used for initial assays.

June 12th – June 18th (Week 4):

- Continue to cross and maintain worms.
    - Confirm that crosses were successful by genotyping.

June 19th – June 25th (Week 5):

- Begin lifespan assays on all strains.
- Draft progress report.

June 26th-July 2nd (Week 6):

- Create more mutants for follow-up studies.
- Use JMP to construct graphs and tables from lifespan assays.

July 3rd- July 9th (Week 7):

- Continue lifespan analysis in JMP.
- Begin oxidation assays on L4 nematodes.

July 10th- July 16th (Week 8):

- Begin lifespan assays for follow-up mutants.
- Continue oxidation assays.

July 17th – July 23rd (Week 9):

- Use JMP to analyze original oxidation assays and follow-up lifespan assays.

July 24th – July 30th (Week 10):

- Draft final report on original mutants, include initial data on follow-up mutants.

July 31st – August 6th (Week 11):

- Perform literature search on long-lived mutants and create an annotated bibliography.

August 7th – August 13th (Week 12):

- Obtain all needed resources for long-lived mutant assays.

August 14th – August 20th (Week 13):

- Set up genetic crosses for long-lived mutant assays.

August 21st – August 27th (Week 14):

- Begin lifespan assays on long-lived mutants.

August 28th – September 3rd (Week 15):

- Revise and finalize final report.
- Prepare for poster presentation.

Mentoring Plan

I will be working similar hours to Professor Apfeld and our lab manager, William Heath, and will therefore be under direct supervision for most of this project. I will participate in lab meetings, and one-on-one meetings with Professor Apfeld will be scheduled on an as-needed basis.

If, for any reason, Professor Apfeld and William Heath are both unavailable to supervise an aspect of my project that requires supervision per Northeastern safety regulations, I have an invitation to work under supervision in Professor Cram's lab space.

Outcomes Statement

By the end of this project I will produce an extensive annotated bibliography of my sources, new strains of *C. elegans* to be sent for maintenance in the Caenorhabditis Genetics Center, and extensive data from observations about health, stress resistance, and reproduction of mutant worms. I will then continue this project into the fall of 2016 and the spring of 2017, and plan to submit my findings for presentations at the meeting of the American Society for Biochemistry and Molecular Biology (ASBMB) in April of 2017 and Genetics Society of America's 21st International *C. elegans* conference in June of 2017. I will also plan to apply to multiple Northeastern presentation opportunities, such as the RISE 2017 poster session, and undergraduate poster sessions, such as the ASBMB's undergraduate poster competition. Success in this project and the networking opportunities that it entails may give me the opportunity to be an extremely competitive candidate for future research fellowships. Further, I hope to eventually collect enough data to be the primary author of a publishable research paper.

Annotated Bibliography

1. **Citation:** Harman, D. 1956 Aging: a theory based on free radical and radiation chemistry. J Gerontol. 2:298–300.

   ***Commentary*:** In the context of my project, this paper is mostly interesting just for historical perspective concerning the origin of the ROS-hypothesis for cellular aging. Further, it is interesting to note the differences in scholarly writing and terminology in the span of just fifty years.

2. **Citation:** Correia-Melo, C., Hewitt, G. and Passos, J. 2014 Telomeres, oxidative stress and inflammatory factors: partners in cellular senescence? Longevity & Healthspan 3:1.

   Commentary: Basic review that overviews the main theories of cellular aging. For my purposes, this paper gives a discussion of the oxidative stress theory of aging in the context of the field as a whole. It focuses on cellular senescence, a general term for a cell becoming dysfunctional with age. In addition, the review has exceptional commentary on the Hayflick limit, the limit to the amount of times a specific cell or cell-type can divide.

3. **Citation:** Kregel, K. and Zhang, H. 2007 An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. American Journal of Physiology 292:18-36

   Commentary: Another review of cellular aging. In contrast to the Correia-Melo 2014 paper, this review focuses specially on oxidative stress. It talks about the sources of ROS, especially the mitochondria and the role of ROS in aging. Most importantly, this paper also contains a section of the effects of ROS on transcription factors, which is particularly relevant to this project.

4. **Citation:** Kenyon, C., Chang, J., Genesch, A. R., and Tabtlang, R. 1993 A *C. elegans* mutant that lives twice as long as wild type. Nature 366:481-464.

   Commentary: The paper that started it all. Here, Dr. Kenyon reveals that *daf-2* mutants life twice as long as wild type, and shows that it requires the activity of *daf-16*, the transcription-factor-encoding-gene that we now know is repressed by *daf-2*.

5. **Citation:** Zhou, K., Pincus, Z. and Slack, F. 2011 Longevity and stress in Caenorhabditis elegans. Aging 3:733-753.

   Commentary: For this proposal, this paper serves as a credible source which explicitly states that the relationship between longevity phenotypes remains unclear. It will also be very important as this project progresses. It highlights problems in replicability in oxidation

studies and, most significantly, describes multiple short-lived mutants, such as *daf-18*, and is organized based on chemical pathways.

6. **Citation:** Lapierre, L. and Hansen, M. 2012 Lessons from C. elegans: signaling pathways for longevity. Trends Endocrinol Metab 23:637-644.

   Commentary: This paper elegantly illustrates the distinct yet interconnected qualities of age-determining signaling pathways. It overviews the IGF, TOR, and germline signaling pathways.

7. **Citation:** Hesp, K., Smant, G. and Kammenga, J. 2015 *Caenorhabditis elegans* DAF-16/FOXO transcription factor and its mammalian homologs associated with age-related disease. Experimental Gerontology 72:1-7.

   Commentary: This paper describes *daf-16* and FOXO and goes further to highlight the relevance of *daf-16* research to humans that gives a compelling argument in favor of the importance of *C. elegans* research.

8. **Citation:** Taylor, R. and Dillin, A. 2013 XBP-1 Is a Cell-Nonautonomos Regulator of Stress Resistance and Longevity. Cell 153:1435-1447.

   Commentary: I found this article while trying to learn about ER stress, a concept that was ill-defined, or not defined at all, in other papers. The introduction provides crystal-clear background on the endoplasmic reticulum (ER) unfolded protein response and the role of *xbp-1* on ER stress and therefore lifespan.

9. **Citation:** Lithgow, G. and Fisher, A. 2006 The nuclear hormone receptor DAF-12 has opposing effects on Caenorhabditis elegans lifespan and regulates genes repressed in multiple long-lived worms. Aging Cell 5:127-138.

   Commentary: This paper is the basis on which I propose making mutant combinations with *daf-12* mutants. It describes *daf-12*'s interactions with other key genes and describes *daf-12* as the first gene with loss-of-function and gain-of-function alleles that shorten and lengthen lifespan, respectively.

10. **Citation:** Gilst, M., Hadjivassiliou, H., Jolly, A., and Yamamoto, K. 2005 Nuclear Hormone Receptor NHR-49 Controls Fat Consumption and Fatty Acid Composition in *C. elegans*. PLoS Biol 3:e53.

    Commentary: Gilst et. al. make a handful of key observations about genes with mutated *nhr-49.* They found two main phenotypes of the knockout: high-fat content and shortened lifespan. Interestingly, they find that those two phenotypes could be attributed to distinct pathways, and *nhr-49* more broadly influences the expression of thirteen genes.

11. **Citation:** Possik, E., Pause, A. 2015 Measuring Oxidative Stress Resistance of *Caenorhabditis elegans* in 96-well Microtiter Plates. J. Vis. Exp. 99:52746

    Commentary: This paper provides a detailed report and accompanying instructional video that gives a comprehensive and simple procedure to measure the resistance to oxidative stress of large quantities of nematodes. My project will follow this procedure carefully and it will be cited in the final report.

12. **Citation:** Apfeld, J. and Kenyon, C. 1999 Regulation of lifespan by sensory perception in Caenorhabditis elegans. Nature 402:804-809.

    Commentary: Great paper written by Dr. Apfeld seventeen years ago. For the purposes of this project, it is most valuable for its simple and informative graphs of lifespans of mutant worms, including *daf-10, daf-1, osm-3,* and *daf-16.*

Budget

Expected Expenses	Unit Cost	# of Units in Summer	Total Cost
Housing	750/Month	4	3000
Food	1615/Semester	2	3230
Transportation	4.20/RoundTrip	123	516.6
Personal Contribution			-1746.6
Total Funding Request			5000