PROPOSAL FOR SCHOLARS INDEPENDENT RESEARCH FELLOWSHIP

The TGFβ Pathway in Lifespan and Response to Oxidative Stress in *C. elegans*

Project Dates: April 30 2017 – July 2 2017

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Personal Statement

My long-term career goals are to contribute to the scientific community in a way that will improve the quality of life of a large segment of the population. I plan to meet those goals through working as a physician scientist and studying medically-relevant aspects of biochemistry and molecular biology. In the short-term, I am learning about scientific processes and preparing myself for graduate school by participating in a field of research that I believe will continue to be at the cutting edge of scientific discovery for the foreseeable future. One such field that has recently dominated my interest is the genetics and molecular biology of aging. My work over the past fifteen months with Dr. Javier Apfeld investigating aging's mechanisms in *C. elegans* continues to form the basis of my education as a scientist, and I am excited to continue to build on what I have learned throughout the remainder of my time as an undergraduate and beyond.

Previous SIRF Experience

Last summer, I conducted a broad inquiry into the cross-talk between transcription factors related to aging. In doing so, I learned vital techniques and created resources that will benefit me during this project. For example, I optimized lysing and genotyping procedures and learned how to effectively mate worms and analyze their survival under different conditions.

My previous SIRF experience gave me the necessary skills and resources to work towards a more specific and advanced project this summer. During this project I will narrow my focus and follow a detailed plan to produce a concrete finding supported by publication-quality data.

Project Background and Objectives

With the passage of time animals grow old, become susceptible to disease, and eventually die (Gardner 1980). Aging was previously thought to be an unavoidable biological process. Recently, researchers identified specific genes that play a significant role in regulating lifespan, driven by pioneering studies in the nematode *C. elegans*. Remarkably, many of these genes also regulate lifespan in other organisms such as flies, mice, and possibly humans (Kenyon 1993, Holzenberger 2003, Hesp 2015, Milman 2014). While dozens of aging-related genes have been identified, the interactions between such genes in determining the various aging-related phenotypes remain poorly understood (Zhou 2011). A complete understanding of the role of genetic interactions is crucial to understanding how lifespan is regulated.

The transforming growth factor-β (TGFβ) superfamily of growth factors are involved in a variety of cellular processes such as growth and immune suppression (Akhurst 2012). Although TGFβs are required for normal cellular regulation and tissue maintenance, they are also overexpressed in many disease states, including cancer and other age-related diseases such as Alzheimer's and osteoarthritis (Kraan 2014). TGFβ signaling is highly environment-specific *in-vivo*, and therefore drug development targeting the

pathway is challenging. Despite the difficulty, several drugs targeting the TGFβ pathway have been recently developed, including successful and ongoing Phase III clinical trials for fibrosis and oncology (Akhurst 2012, Junior 2015).

C. elegans is an ideal model in which to study the function of TGFβ signaling because the signaling process is highly conserved throughout the animal kingdom (Schmierer 2007). *C. elegans* is a simple animal model that allows for easy genetic manipulation and maintenance but also displays the complexity that comes from the interactions between cells and cellular environments in a multicellular organism.

In *C. elegans*, TGFβ signaling can be broken down into two streams: one that allows organisms to grow to a full size, and another that allows them to enter a hibernation-like state called dauer. The TGFβ dauer pathway involves a series of ligands and receptors that repress the production of a transcription factor that causes worms to enter dauer (Savage-Dunn 2005, Gumienny 2013). Interestingly, these genes interact in a similar manner in determining other phenotypes, including normal lifespan and lifespan under conditions where the population's food intake is significantly limited. In addition, previous studies have shown that the role of the TGFβ dauer-pathway genes is cell-specific in its control of the lifespan extension that worms experience under dietary restriction (Fletcher 2017).

Recently, the Apfeld lab has obtained data that suggests that, in addition to regulating dauer entry and some aspects of lifespan, TGFβ dauer genes also affect the survival of *C. elegans* upon exposure to oxidative stress (Figures 1 and 2). This is interesting because the response to oxidative conditions is commonly hypothesized to be a major contributing factor of age-related disease.

Specific Aims

Aim 1: Systematically determine the genetic interactions between TGFβ Dauer pathway genes in the control of resilience to oxidative stress.
The main question I would like to answer over the summer is whether *C. elegans* responds to oxidative stress via interactions between the same genes that it utilizes when it promotes dauer entry and certain other lifespan-related phenotypes. These studies will build on the understanding genetic interactions in TGFβ signaling.

Aim 2: Investigate the role of cell-specific expression of *daf-7* in the control of resilience to oxidative stress.
I will further characterize the role of the TGFβ pathway by quantifying the effects of expressing the TGFβ ligand, *daf-7*, in ASI and ASJ neurons on resilience to oxidative stress. These studies will determine whether TGFβ acts in the same cell-dependent manner for the response to oxidants as it does in the response to dietary restriction.

Background to Project Design and Methodology

The TGFβ dauer pathway follows a well-characterized pathway that, at its most basic level, involves three components: one ligand, one receptor, and one transcription factor. In *C. elegans*, the genes corresponding to those three components are labeled *daf-7*, *daf-1,* and *daf-3.* The last gene, *daf-3*, is responsible for making dauers, while *daf-7* and *daf-1* work together to inhibit the dauer-making process. I will determine whether these three genes also function in a pathway to determine resilience to oxidative stress.

To determine whether three genes work in a pathway, one can compare the effects of deleting one gene to the effects of deleting two genes. For example: if A decreases lifespan and B increases lifespan, and A inhibits the action of B, then deleting both A and B will have the same effect as deleting only B. In other words: if B is absent, then A will have nothing to inhibit. Following the same logic, I will test double-mutant combinations of three components of the TGFβ pathway to determine how they interact.

Project Design and Methodology

Strains: All needed strains, except for *daf-7; daf-1*, will be provided by the Apfeld lab. I will create *daf-7; daf-1* using classical genetic techniques. Although I do not expect to have any problem creating the *daf-7; daf-1* double mutant, I can still form conclusions about pathway interactions using only the strains provided by the Apfeld lab.

Oxidative Stress: Strains will be grown to adulthood in normal conditions and then transferred to petri dishes that have, in addition to the normal environment of Nematode Growth Media (NGM) and *e. coli* food, a certain amount (between 2μM and 6μM) of the oxidant *tert*-butylhydroperoxide.

Preliminary Data: Rough survival data will be collected by monitoring the strains after exposure to *tert*-butylhydroperoxide and recording time of death at intervals determined by expected survival curves.

Final Data: Publication-quality data will be obtained via a high-throughput system that uses a series of flatbed scanners to automatically record lifespan. Although the Apfeld lab is comfortable using the “Lifespan Machine”, a significant portion of my time working on this project may be dedicated to ensuring that the machine’s data is accurate.

Project Timeline

Week 1: Grow out needed worm strains, confirm their genotypes using dauer-entry assays.

Week 2 - Week 4: Conduct oxidative stress assays by hand to obtain general findings.

Week 5 - Week 7: Conduct oxidative stress assays with the lifespan machine to obtain publication-quality findings.

Week 7 - Week 9: Analyze data, present findings at Genetic Society of America's International Worm Meeting.

Mentoring Plan

I will be in constant contact with Dr. Apfeld and much of the Apfeld Lab throughout the duration of this project. I will send Dr. Apfeld weekly summary emails of both the work that was completed in the previous week and the work that is to be done in the following week. I will also participate in summer lab meetings with both the Apfeld and Cram labs and schedule one-on-one meetings with Dr. Apfeld as needed.

Outcomes Statement

I will submit the findings of these studies to be presented in June at the Genetic Society of America's 21st International *C. elegans* Conference in Los Angeles. I will also pursue internal venues of presentation including the 3rd annual Active Site (the regional undergraduate poster session of the American Society of Biochemistry and Molecular Biology) and Northeastern's 2018 Research, Innovation, and Scholarship Exposition (RISE). The data collected through this project will likely contribute to a larger, formal publication within two years.

The quality of my work can be judged by the quality of the data that I will produce by the end of this project, which I plan to include in my final report.

Figures: Preliminary Data

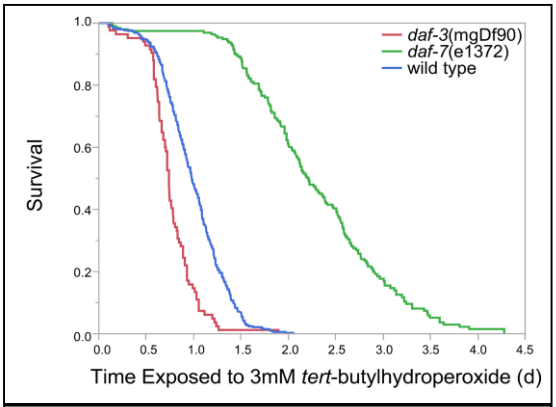

Figure 1: Survival of *daf-3*, *daf-7,* and wild-type at 25°C on 3mM *tert*-butylhydroperoxide. *daf-7* lived significantly longer than wild type; *daf-3* lived significantly shorter.

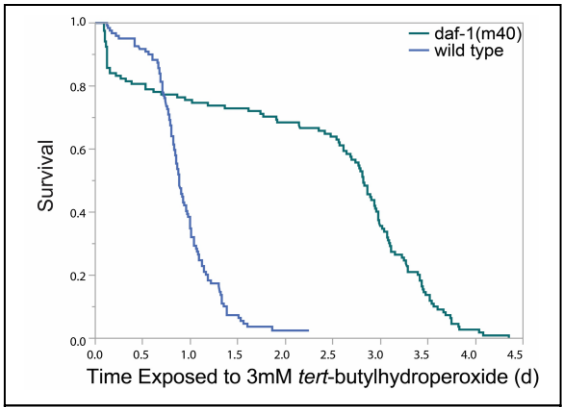

Figure 2: Survival of *daf-1* and wild type at 25°C on 3mM *tert*-butylhydroperoxide. *daf-1* lived significantly longer than wild type.

Annotated Bibliography

1. **Citation:** Gardner, ID. 1980 The effect of aging on susceptibility to infection. Rev Infect Dis. 2:801-810.
   Commentary: Early paper formally characterizing the risk of infection with age.

2. **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: Here, Dr. Kenyon reveals that *daf-2* mutants live 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*.

3. **Citation:** Holzenberger, M. et al. 2003 IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature 421:182-187.
   Commentary: One of the papers that reported the conservation of the basic lifespan-enhancing characteristic of daf-2 mutants in mice.

4. **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.

5. **Citation:** Milman, S., et al. 2014 Low insulin-like growth factor-1 level predicts survival in humans with exceptional longevity. Aging Cell 13:769-71.
   Commentary: Human trial suggesting that *daf-2's* function is conserved in humans.

6. **Citation:** Zhou, K., Pincus, Z. and Slack, F. 2011 Longevity and stress in Caenorhabditis elegans. Aging 3:733-753.
   Commentary: Explicitly states that the relationship between longevity phenotypes remains unclear.

7. **Citation:** Akhurst, R. and Hata, A. 2012 Targeting the TGFβ signaling pathway in disease. Nat Reviews 11: 790-811.
   Commentary: A review of the TGFβ signaling pathway in human drug development and pathology. Although it is not directly relevant to *C. elegans*, it was very helpful in enhancing my understanding of where my proposal fits in to the broad literature.

8. **Citation:** Junior, A. et al. 2015 Preoperative tranilast as adjunctive therapy to primary pterygium surgery with a 1-year follow-up
   Commentary: Paper describing a successful Phase III clinical trial, was in progress when cited by Akhurst in 2012.

9. **Citation:** Kraan, PM 2014 Age-related alterations in TGF beta signaling as a casual factor of cartilage degeneration in osteoarthritis. Biomed Mater Eng. 24: 75-80.
Commentary: Further commentary on the relevance of TGFβ in human pathology.

10. **Citation:** Schmierer, B. and Hill, C. 2007 TGFβ-SMAD signal transduction: molecular specificity and functional flexibility Nat Reviews 8: 970-982.
Commentary: A well-written review describing the specific molecular mechanisms and interactions of the TGFβ superfamily.

11. **Citation:** Savage-Dunn, C. 2005 TGF-β signaling. *Wormbook,* ed. The *C. elegans* Research Community, WormBook,
doi: 10.1895/wormbook.1.22.1, http://www.wormbook.org
Commentary: A simple overview of TGFβ pathways in *C. elegans*.

12. **Citation:** Gumienny, T. and Savage, Dunn, C. 2013 TGF-β signaling in *C. elegans*. *Wormbook,* ed. The *C. elegans* Research Community, Wormbook,
Doi/10.1895/wormbook.1.22.2, http://www.wormbook.org
Commentary: Perhaps the most informative and up-to-date review of TGFβ signaling in *C. elegans*.

13. **Citation:** Fletcher, M. and Kim, D. 2017 Age-Dependent Neuroendocrine Signaling from Sensory Neurons Modulates the Effect of Dietary Restriction on Longevity of *Caenorhabditis elegans*. PLOS Genetics 13(1): e10065444.
Commentary: Describes the effect of TGFβ on the well-characterized response to dietary restriction. Concludes that daf-7 is specific to ASI and ASJ neurons and also finds tangential data suggesting an interaction between TGFβ and insulin signaling in the response to dietary restriction. This is a great paper and many of the strains I am testing in this experiment have been given to us by the Kim lab.

14. **Citation:** Shaw, W. et al. 2007 The *C. elegans* TGF-β Dauer Pathway Regulates Longevity via Insulin Signaling. Curr Biol. 17:1635-1645.
Commentary: This paper has clear lifespan data for *daf-3* and shows that it is both independently short lived and independent of the longevity induced by *daf-2* mutations.

15. **Citation:** Finkel, T. and Holbrook, N. 2000 Oxidants, oxidative stress and the biology of ageing. Nat Reviews 408: 239-247.
Commentary: A somewhat dated but easy-to-read and comprehensive review of the role of oxidative stress in aging.

16. **Citation:** Stroustrup, N. et al. 2013 The *C. elegans* Lifespan Machine. Nat Methods 10:665-670.
Commentary: This paper describes in great detail the workings of the lifespan machine. The Apfeld lab worked closely with the authors of this paper to build and begin using the machine.

Funding Request: $5000.00

Details:

Item	Unit Cost	Estimation Source	Units	Gross Cost	Provided by Apfeld Lab?	Real Cost	Funding Request
FUDR	62.89/25mg	Fisher (0210555125)	1	62.89	Y	0	0
NGM	128.65/1KG	Fisher (50488872)	1	128.65	Y	0	0
6cm Petri Dishes	275.82/500	Fisher (FB087513)	2	551.64	Y	0	0
Lifespan Machine Plates	339.77/500	VMR (25369-022)	1	339.77	Y	0	0
Platinum Wire Pick	129.73/3ft	Tritech (PT-90110)	1	129.73	Y	0	0
tert-butylhydroperoxide stock	29.07/250g	Fisher (AC180342500)	1	29.07	Y	0	0
LB Media	28/500mL	Fisher (10-855-021)	1	28	Y	0	0
Housing	3240/Semester	Northeastern Housing	2	3240	N	3240	3240
Food	1800/semester	Northeastern Meal Plan Cost	1	1800	N	1800	1760
Transportation	84.50/month	Monthly Charliecard	2	169	N	169	0
Total Gross Cost				**6478.75**			
Total Real Cost						**5209**	
Total Funding Request							**5000**