Investigating the role of hsf-1, skn-1, and daf-16 in regulating oxidative stress response in C. *elegans*

Grant Proposal

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Abstract (RQ) or Executive Summary (Eng)

Oxidative stress is an extremely detrimental condition caused by many free radicals in the body and a lack of antioxidants. As a result, cells can be extremely damaged, leading to deathly illnesses down the road. To address this, scientists can harness the power of transcription factors to combat oxidative stress. This project hypothesizes that the transcription factors daf-16, skn-1, and hsf-1 will be able aid C. *elegans* to combat oxidative stress, effectively extending their lifespans as opposed to if these TFs did not play a role. Using different strains of C. *elegans* and GFP reporters, data can be collected to analyze and draw conclusions about these transcription factors. Overall, this project is important, as it provides insight into how transcription factors can be used to combat conditions such as oxidative stress, which can improve the lives of many across the world.

Keywords: Oxidative stress, transcription factors, C. *elegans*, GFP, Stress Resistance

Investigating the Role of hsf-1, skn-1, and daf-16 in Regulating Oxidative Stress Response in *C. elegans*

Currently, 90 percent of the American population does not consume enough vegetables, and 80 percent of the population does not consume enough fruit (USDA, n.d.). As a result, many people lack enough antioxidants in their diet, which translates to oxidative stress (Cleveland Clinic, n.d.). Oxidative stress is a condition where there is an imbalance between free radicals and antioxidants in the body (Cleveland Clinic, n.d.). Free radicals are unstable molecules that are missing an electron in their outer shells, which are normal byproducts of metabolism that either occur naturally in our bodies or due to environmental factors such as air pollution (Cleveland Clinic, n.d.). Therefore, these radicals look for these electrons in our bodies, putting healthy molecules at risk of becoming unstable (ClevelandClinic, n.d.). To address this, the body utilizes antioxidant molecules that are designed to safely react with these free radicals to limit cellular damage (ClevelandClinic, n.d.). However, oxidative stress occurs when there are too many free radicals and not enough antioxidants for stabilization (ClevelandClinic, n.d.). Consequently, these free radicals harm tissues and cells in our bodies, damaging different parts of cells that allow them to work efficiently, including lipids and proteins (ClevelandClinic, n.d.).

Furthermore, oxidative stress plays a major role in the development of many chronic and degenerative conditions, including cancer, cardiovascular disease, kidney disease, neurological diseases, and respiratory diseases (ClevelandClinic, n.d.). Damage caused by oxidative stress at the molecular level can cause damage to proteins, lipids, and cells, leading to the conditions outlined above. (Cleveland Clinic, n.d.). diagnose oxidative stress, many doctors look for wrinkles, sunspots, and spider veins (Cleveland Clinic, n.d.). All in all, oxidative stress, which is caused by an excess of free radicals, is very detrimental to humans.

Transcription factors (TFs) could be utilized as a potential solution. TFs are proteins that regulate translation— the process where RNA is converted to proteins — in cells (ScienceDirect, n.d.). They usually contain a DNA-binding component and a regulation component, which will stimulate or repress transcription (ScienceDirect, n.d.). Generally, there are many families of TFs, and they are sectioned off by the type of DNA-binding domains they have (ScienceDirect, n.d.). These domains are important, as every DNA-binding domain in a protein has a specific target sequence (ScienceDirect, n.d.). TFs works through a process where they recognize and bind to specific DNA sequences named promoter and enhancer sequences (ScienceDirect, n.d.). Promoter sequences are typically upstream of a gene to initiate transcription (ScienceDirect, n.d.). Some TFs can bind to many promoters, while others are site-specific (ScienceDirect, n.d.). In the context of the experiment being proposed, three specific TFs can be experimented with. One such TF is Heat Shock Factor (hsf-1), a TF that can increase production of proteins that combat stress resistance caused in nematodes grown at constant or higher temperatures (Servello et al., 2020). Another is daf-16, a TF that can decrease oxidative stress, therefore increasing lifespan (ScienceDirect, n.d.). Finally, there is a TF called skn-1, which helps increase stress resistance in nematodes (Frankino et al., 2022). By implementing these TFs to regulate oxidative stress, a potential solution for oxidative stress can be found.

The need arises for a model organism that can still provide applicable results to studying oxidative stress in humans. This model can be found in the form of C. *elegans*. C. *elegans* is classified as a Nematoda, which is a class of roundworms and threadworms with long cylindrical bodies that are tapered at the ends (CGC, n.d.). C. *elegans* is non-hazardous, non-infectious, non-pathogenic, and non-parasitic,

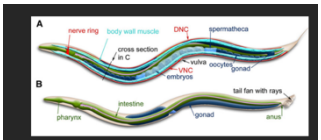


Figure 1.1 Anatomy of C. elegans. Figure from source [1].

making it very useful for studies (CGC, n.d.). C. *elegans* shares many biological characteristics that are central to human biology, including how the organs are structured, as well as how they can be manipulated easily (CGC, n.d.). As a result of the transparency, small size, and short life span of C. *elegans*, scientists can conduct a lot of research on these organisms (Moreno-Ariola et al., 2011).

This project aims to address oxidative stress through TFs. These specific TFs can help increase stress resistance in C. *elegans*. By doing this, oxidative stress can be neutralized, which can then translate to the eradication of future consequences that come with this condition.

Section II: Specific Aims

This proposal's objective is to determine the effects of different TFs on oxidative stress in C. *elegans*. Our long-term goal is to determine the interplay between these different TFs where the central hypothesis of this proposal is that these TFs will be able to reduce oxidative damage and improve organismal survival under stress conditions. The work we propose here can provide insight into how each TF can affect oxidative stress. Furthermore, it can show how these proteins interact with each other through different pathways.

Specific Aim 1: We aim to study how the absence of different TFs can affect oxidative stress resistance in C. *elegans*.

Specific Aim 2: We aim to utilize GFP to study how protein expression affects oxidative stress resistance in C. *elegans*.

Specific Aim 3: We aim to use the data collected above to analyze the relationship between these specific TFs and oxidative stress in C. *elegans*.

The expected outcome of this work is to analyze how different TFs will act to combat oxidative stress. Furthermore, this work will be able to uncover the relationships between these TFs.

Section III: Project Goals and Methodology

Relevance/Significance: This project is important for two major reasons. First~~,~~ as mentioned above, oxidative stress is detrimental to human health. For instance, oxidative stress is linked to cancer (Pizzino et al., 2017). Since oxidative stress can lead to chromosomal abnormalities and oncogene activation, cancer can be driven and promoted (Pizzino et al., 2017). Oxidative stress has a huge impact on society overall, which is why studies on this subject are crucial.

Secondly, there is a lack of studies that determine the relationship between different TFs. There are many studies that have determined the role that daf-16, hsf-1, and skn-1 play in C. *elegans*, but there has not been as much research done on the interplay between these TFs. GFP (green fluorescent protein) can help determine how each TF affects certain pathways.

Innovation: As mentioned above, this study is different, as we aim to determine how these TFs interact with each other. Therefore, we can analyze the relationships that each TF has with each other.

Methodology: Several methodologies need to be accounted for to determine the relationship between these TFs and oxidative stress. The methodologies used will be for preparing plates for C. *elegans,* inducing oxidative stress, and running a lifespan assay, a chemotaxis assay, and a chemotaxis assay. Finally, the results will be analyzed through devices such as flow cytometry.

Specific Aim #1: Study how TFs can affect oxidative stress resistance in C. *elegans*

Our aim is to determine the effectiveness of each TFs in combating oxidative stress. The objective is to evaluate these TFs to see what roles they play, as well as if some TFs are more effective than others.

Our methodologies are as follows:

Plates are prepared according to Chaudhuri et al. (2018).

To induce oxidative stress, add paraquat and water to create a stock solution (100 nM), and expose the C. *elegans* to this solution (Hu et al., 2018). Paraquat is used at a final concentration of 0.35 mM (Hu et al., 2018).

Furthermore, the following assays will be run:

Lifespan Assay (García-Garví et al., 2023) To run this assay, use the above methodology. Next, regularly observe and record the number of live worms over time. Use a microscope to assess their movement, which shows signs of life.

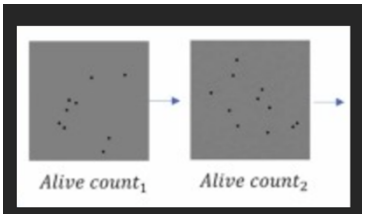


Figure 1.2 Count of alive C. *elegans*. This number serves as an input to predict the number of live worms in the future. This number can also be used for lifespan assays. Retrieved from [5].

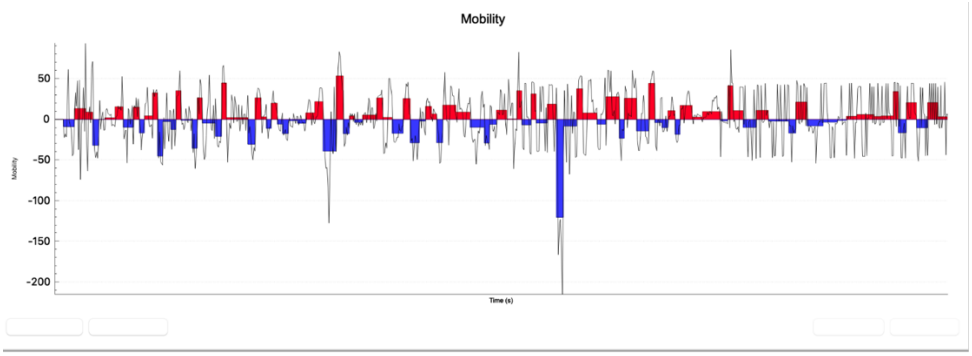
Locomotion Assay (Queirós et al., 2022) To run this assay, use the above methodology. Then, place an attractant on one side of the plate, and move the worms to the other side. Finally, observe changes in movement to determine changes in behavior.

Our rationale for this approach is that to test the effects of oxidative stress in C. *elegans*, they need to be prepared and set in normal growth conditions. Additionally, through these assays, data can be collected regarding the efficiency of these TFs.

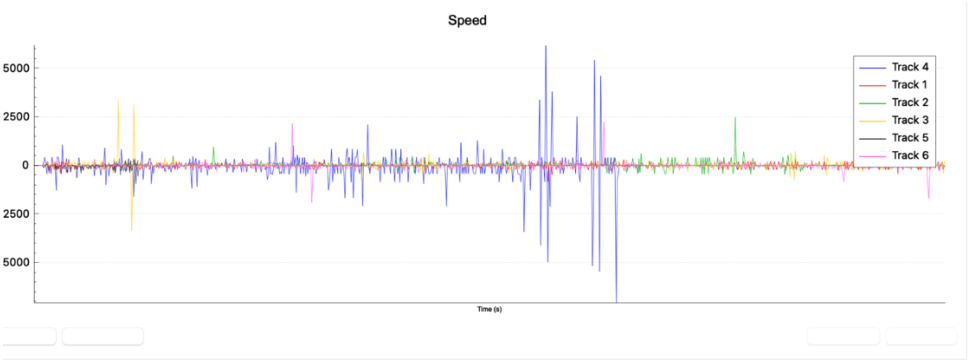
Justification and Feasibility: The methods in this section are relevant, as they have been used in past studies to achieve similar goals. Oxidative stress induces a reduction of movement and lifespan, which is why using the above assays allows for data that can accurately show the

effects of oxidative stress in this experiment. Furthermore, this can help determine how movement and lifespan is affected with the absence of different transcription factors.

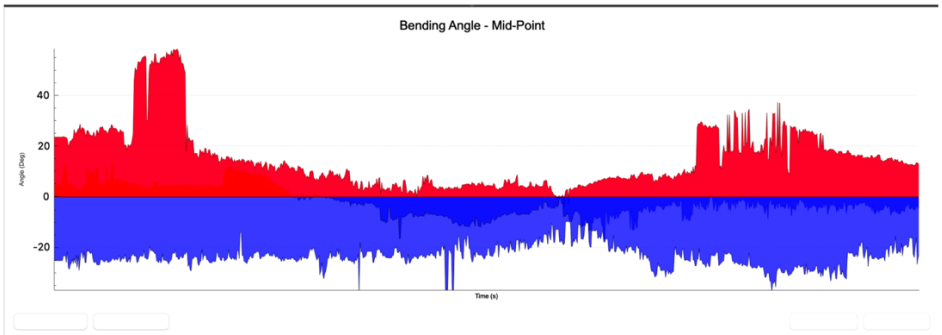
Summary of Preliminary Data:



Mobility graph of C. *elegans*. Red color shows forward movement, blue color shows rest or backwards movement. Obtained from WormLab analysis tool.



Mobility graph of C. *elegans*. Different colors indicate movement of different worms. Obtained from WormLab analysis tool.



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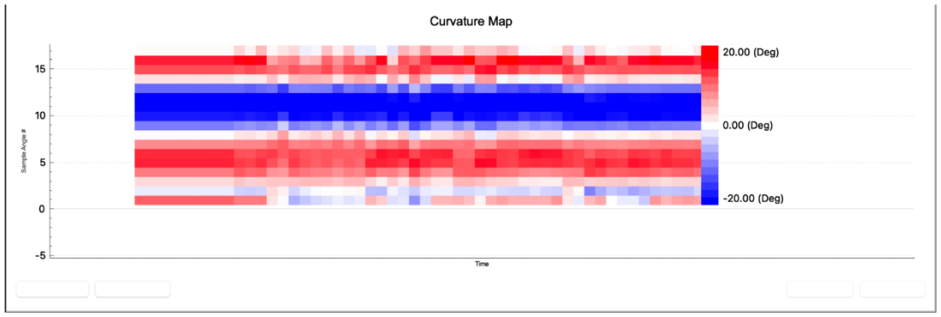

Mobility graph of C. *elegans*. Red color shows forward movement, blue color shows rest or backwards movement. Obtained from WormLab analysis tool.

This preliminary data was obtained from a locomotion assay. It shows that the wildtype worms (the control group) were behaving "normally", which means that this data could be used as a control point for the other experimental groups.

Expected Outcomes: The overall outcome of this aim is to study the effectiveness of different TFs. This knowledge will be used to determine how these TFs play a role in defending against oxidative stress.

Potential Pitfalls and Alternative Strategies: We expect that getting this data may provide complications through interference in experimentation. To combat this, we can use data from

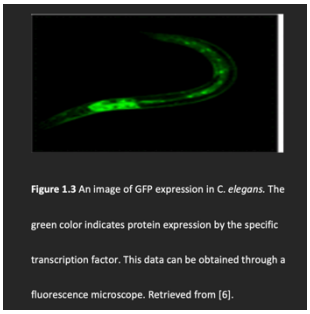

Figure 1.3 An image of GFP expression in C. *elegans*. The green color indicates protein expression by the specific transcription factor. This data can be obtained through a fluorescence microscope. Retrieved from [6].

past studies to determine how specific groups may be behaving in terms of locomotion and lifespan, which can help us analyze and cross-check data.

Specific Aim #2: We aim to utilize GFP to study how protein expression affects oxidative stress resistance in C. *elegans*.

Our aim is to determine how these TFs interact with each other through GFP expression. The objective is to analyze protein expression through different pathways to determine how they interact with each other.

Add the worms to the desired plate to run the GFP reporter assay. Next, observe over a period and analyze the results, which will be explained later.

Our rationale for this approach is that by using GFP, we can determine how these TFs interact with each other.

Justification and Feasibility: These methods used are very relevant, which can be seen in past studies. They have provided ways to determine how GFP is expressed, which is also the role that GFP will play in this experiment. By using these methods, we will be able to fulfill our aim.

Summary of Preliminary Data: The data acquired will show the protein expression in different pathways in C. *elegans*.

Expected Outcomes: The overall outcome of this aim is to study the interaction between different TFs. This knowledge can determine how these TFs work together on a molecular level.

Potential Pitfalls and Alternative Strategies: We expect that getting this data may provide complications through interference in experimentation. To combat this, we can use historical data to provide points for analysis.

Specific Aim #3: We aim to use the data collected above to analyze the relationship between these specific TFs and oxidative stress in C. *elegans*.

Our aim is to analyze the data collected from previous aims. The objective is to draw conclusions about these TFs and their involvement with C. *elegans*.

We will be able to use fluorescence microscopy to analyze the protein expression in C. *elegans*. Furthermore, by using tools such as Fiji, we can analyze our results. Additionally, we can use tools such as Kaplan-Meier survival analysis to determine how TFs play a role in combating oxidative stress.

Justification and Feasibility: These methods are crucial, as they have been used in past studies for similar aims. These methods have been used in various studies that have analyzed the data in similar ways. Therefore, by using these methods, we can complete our goals.

Summary of Preliminary Data: There will not be any preliminary data, as this aim focuses on analysis.

Expected Outcomes: The overall outcome is to analyze links between these TFs and oxidative stress in C. *elegans*, which can help us determine how these TFs affect oxidative stress resistance.

Potential Pitfalls and Alternative Strategies: The complications can be easily addressed by using other methods of analysis.

Section IV: Resources/ Equipment

The materials needed to carry out the experiment are the NGM growth plates, mutant strains, bacteria, paraquat, as well as picks to transfer the nematodes. Furthermore, the equipment needed consists of a microscope, flow cytometry, and a device to analyze the collected data.

Section V: Ethical Considerations

One ethical consideration would be when using paraquat, as large amount of this chemical can be extremely detrimental. However, this can be addressed through proper use of safety equipment,

as well as by using a fume hood to make solutions. Another consideration has to do with E. coli, but this can also be addressed with safety equipment.

Section VI: Timeline

We aim to finish up data collection around mid-December. After this, we plan to finish data analysis around mid- to late January. By following this timeline, we will be able to complete this research.

Section VII: Appendix

There are no further terms that need to be defined; all information has been previously addressed above.

Section VIII: References

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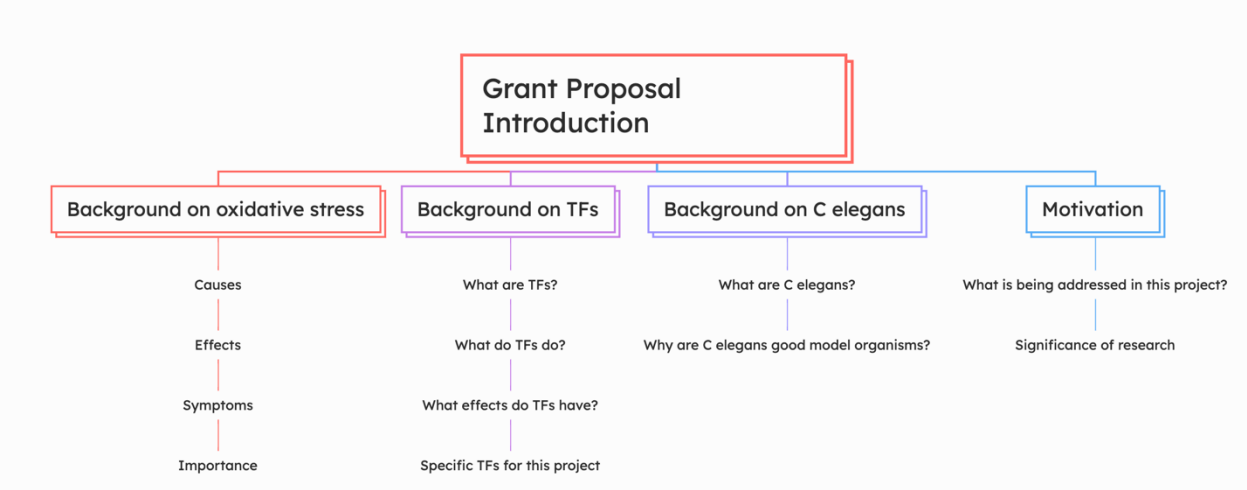
Mind map:


Grant Proposal Introduction
Background on oxidative stress
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Symptoms
Importance
Background on TFs
What are TFs?
What do TFs do?
What effects do TFs have?
Specific TFs for this project
Background on C elegans
What are C elegans?
Why are C elegans good model organisms?
Motivation
What is being addressed in this project?
Significance of research