swan-2 and *gcs-1* genes, regulators of heat stress response, are 2-fold downregulated in *Caenorhabditis elegans* under heat stress

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Abstract

Our study seeks to understand how five genes in *C. elegans* were affected under heat stress, and how these findings could contribute to studies of the potential correlations among stress, protein aggregation and aging. Our results indicated that *swan-2* and *gcs-1* were downregulated, while *ragc-1*, *dnj-13*, and *epg-3* were upregulated under heated condition. Among these changes, changes in *swan-2* and *gcs-1* expression level were considered as statistically significant.

Introduction

The over-arching goal of this project was to determine if protein aggregation contributes to aging. Our hypothesis is that the excessive or insufficient expression of certain genes may contribute to protein aggregation.

Significance:

The purpose of this study was to use *C. elegans* to study biological aging, and eventually be able to confer the results to human beings. Aging causes physiological changes that should be understood and taken into account in personalized medicine.

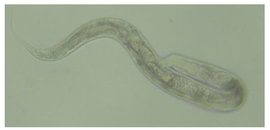

Figure 1: Matured *C. elegans* hermaphrodite under microscope. Photo was taken at 10X magnification.

Caenorhabditis elegans are a widely-used species of model organism because they share up to 71% of their genome with other organisms, making studies on their genes easily translatable to other organisms and studies. In addition, their life spans are only two weeks, which simplifies life-time and developmental studies in the worm. Finally, the entire *C. elegans* genome has been sequenced, allowing for very specific genetic studies to be done.

swan-2 is a regulator for heat and osmotic stress in *C. elegans* and is involved in kinase signaling[1,2].

gcs-1 is involved in cellular response to heat, and may play a role in oxidative stress response. The *gcs-1* gene is an ortholog of human gene *GCLC*[3,4].

Methodology

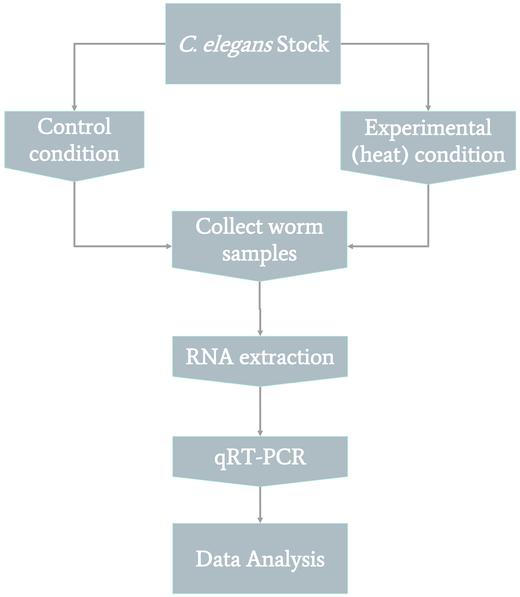

Figure 2: Experimental design flow chart.

C. Elegans were separated into two experimental conditions: Control and Heat stressed.

Control was incubated at 23°C on NGM plates.
Experimental group of *C. elegans* was incubated at 23°C initially, then heat stressed at 33°C for 3 hours.

All worms on plates were collected for RNA extractions.

$2^{-\Delta\Delta Ct}$ process was used to calculate the fold difference of gene expression from the qRT-PCR data (Fig. 3).

Unpaired t-test was performed on ΔC_T values. A p-value smaller than 0.1 was considered as statistically significant.

Results

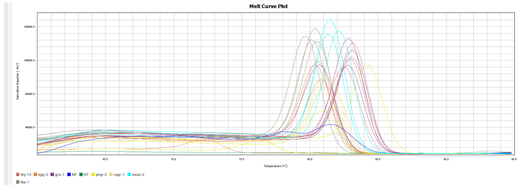

Figure 3: qRT-PCR melt curve plot: Melting temperatures for the five genes.

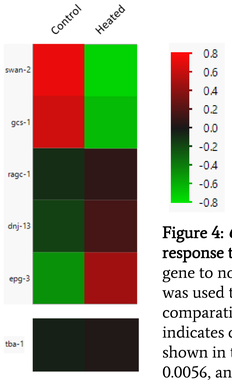

Figure 4: *C. elegans'* gene expression change in response to heat. *tba-1* was used as the reference gene to normalize the qRT-PCR data. JMP Pro was used to generate this figure. Green indicates comparatively lower expression level, red indicates comparatively higher expression, as shown in the ladder. The p-value for swan-2 was 0.0056, and that for gcs-1 was 0.0683.

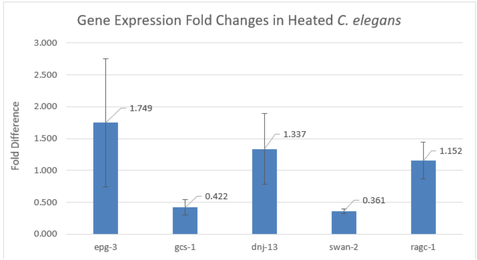

Figure 5: *C. elegans'* gene expression fold change in response to heat. Fold change values greater than 2 or less than 0.5 were considered significant.

Discussion

- Among the five genes tested, *swan-2* and *gcs-1* had the most significant changes (Fig. 4). Both of the genes were downregulated by more than two folds in heated worms.
- This conclusion was verified by the $2^{-\Delta\Delta Ct}$ test, both of the genes were downregulated by more than two folds in heated worms (Fig. 5).
- We considered a $p<0.1$ as statistically significant. Therefore, we concluded that there were a statistically significant 2-fold downregulation of *swan-2* and gcs-1.

Limitations

- Limitations for this study include the use of one plate of worms per condition, heat-stressed and controlled, which does not account for individual variations between worms.

Future Direction

- In future experiments, we will introduce different stressors, such as changes in light, food, temperature, and oxidative damage (limiting or increasing oxygen supply).
- We will conduct a lifetime assay, measuring the health status and survival rate at different ages of the *C. elegans* under these stressed conditions.
- Finally, we will conduct more comprehensive screening past qPCR, such as RNA sequencing and protein extraction.

Bibliography

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