Heat-stressed induced gene expression of *swan-2* gene in *C. elegans*

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Abstract

We conducted our study to understand how the *swan-2* gene was affected in *C. elegans* under heat stress, and how these findings could help study the potential correlation between stress, protein aggregation and aging. According to our results, the gene *swan-2* was expressed slightly less in the stressed experimental group as opposed to the control group. However, this difference was determined to be statistically insignificant. Therefore, we concluded that the *swan-2* gene is not a factor that contributes to protein aggregation in response to heat.

Introduction

The overall purpose of this project was to determine if protein aggregation contributes to aging. We hypothesized that the excessive or insufficient expression of certain genes may contribute to protein aggregation[1]. Specifically, we performed this study to investigate how the *swan-2* gene expression level changes in response to heat in *Caenorhabditis elegans*. The *swan-2* gene is a regulator for heat and osmotic stress in *C. elegans* and is involved in kinase signaling[2,3].

Significance:

The basis behind the study of aging in *C. elegans* was to provide an explanation to how stress affects aging in human beings. In addition, aging causes extensive bodily changes, which need to be accounted for in personalized medicine.

Figure 1: Mature *C. elegans* hermaphrodite under microscope. This 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 (Fig. 2). In addition, they have very short lifespans of around two weeks, allowing us to easily study their development and survival rate at multiple different ages. Finally, the entire *C. elegans* genome has been sequenced and thoroughly studied, allowing gene selection to be very specific.

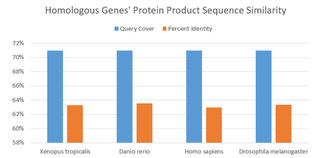
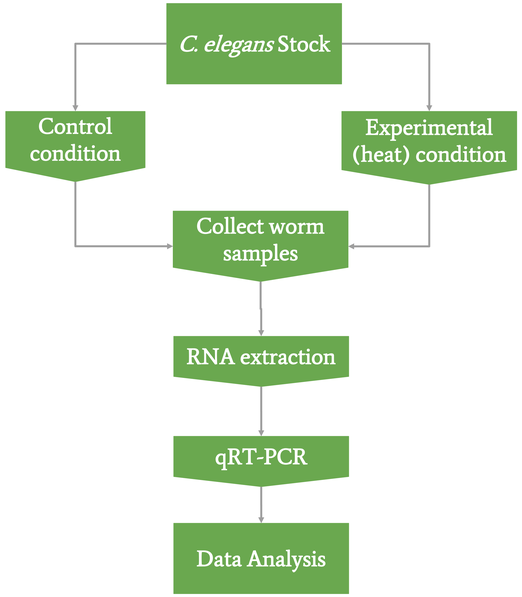


Figure 2: *swan-2* protein product homology. Query cover percentage indicates how long the protein product of the homologous genes.

Methodology



The roundworms were separated into two conditions: control and experimental.

Both groups of worms were incubated at 23°C on NGM plates; then the control group was kept at 23 °C, while the experimental group of *C. elegans* were moved to a higher temperature of 33°C for 3 hours.

All worms were collected for each plate, then their RNA was extracted and purified.

qRT-PCR primers for swan-2 (5'-3'):
Forward: CGGACTATCTTGGGCTCCAC
Reverse: GGATCTGGTTGACCTCTGCC

We used the $2^{-\Delta\Delta Ct}$ method to calculate the fold difference of gene expression from the qPCR data (Fig. 4).

Unpaired t-test was used for statistical analysis. More than two fold changes with $p \leq 0.05$ was considered as statistically significant.

Results

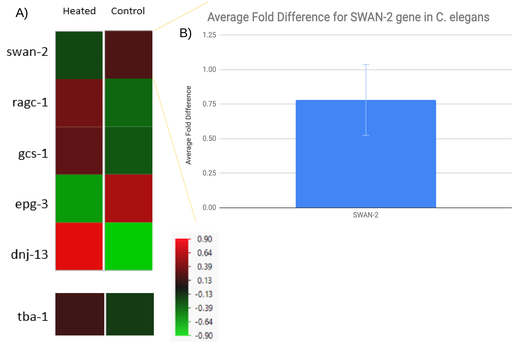


Figure 3: *C. elegans* gene expression changes in response to heat. *tba-1* was used as the control housekeeping gene to normalize the qRT-PCR data. A) We used JMP Pro to generate this figure. Green shows relative low expression level, red indicates relative high expression, and brighter colors indicate significant difference. B) The fold difference for heated to control is approximately 0.781 (p=0.425).

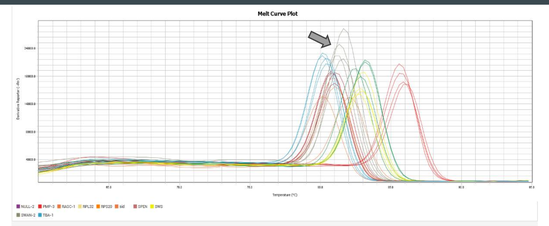


Figure 4: Melt curve plot for qRT-PCR: Melting temperature for *swan-2* qRT-PCR product is shown by the dark green peaks indicated by the arrow.

Discussion

- Considering Figure 3, the dark colors corresponding to expression levels of the *swan-2* gene in both experimental and control conditions suggest that there is little difference between the two groups.
- This conclusion was verified by the $2^{-\Delta\Delta Ct}$ test, the ratio value of which was 0.781 (Fig. 4).
- Because of this, there was not a statistically significant difference between the control group and the heat-stressed group. We concluded that the *swan-2* gene expression levels are not affected by heat.

Limitations

- Limitations for this study include the use of two plates 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[3].

Bibliography

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