Effects of heat stress on heat shock gene expression in *C. elegans*

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Summary

- The experiment tests for changes in gene regulation of certain genes of *C. elegans* under heat stress
- The gene expressions for the *swan-2*, *ragc-1*, *epg-3, dnj-13*, and *gcs-1* genes were assessed using qRT-PCR and the change was calculated through delta delta Ct
- The genes *dnj-13* and *ragc-1* from *C. elegans* up regulate from heat stress while the genes *epg-3*, *gcs-1*, and *swan-2* down regulate from heat stress.

Abstract

Heat stress (HS) is the physiological stress that occurs when the body heat load is greater than its capacity to lose heat. The cellular response to heat stress is caused by a group of genes coding for heat shock proteins. In this study, we investigated the effect of heat stress on the expression of five heat shock genes (swan-2, ragc-1, epg-3, dnj-13, and gcs-1) We extracted RNA from C. elegans using acid guanidinium thiocyanate-phenol-chloroform extraction (AGPC) extraction and ran our samples through qRT-PCR to measure the amount of RNA of each of the heat shock genes. We used the 2^-ΔΔCT method to analyze our data and found that the swan-2, ragc-1, and gcs-1 genes downregulate when exposed to heat while the epg-3 and dnj-13 genes upregulate when exposed to heat.

Introduction

When C. elegans are exposed to heat stress, proteins in the body are denatured which causes the dnj-13 and epg-3 genes to upregulate because they bind to and destroy unfolded proteins, while the gene gcs-1, ragc-1, and swan-2 found in C. elegans downregulate because they promote enzymatic activity.

- Studying the effects of heat stress on heat shock proteins
- *swan-2* codes for the Seven WD repeats protein of the AN11 family, which is involved in kinase signaling. Responds to heat stress.
- *gcs-1* codes for the Glutamate--cysteine ligase protein, which is involved in ATP binding and glutamate-cysteine ligase activity. May play a role in oxidative stress response
- *dnj-13* codes for a J domain-containing protein, which is involved in unfolded protein binding. Responds to heat stress.
- *epg-3* codes for the Ectopic P Granules protein, which is involved in the macroautophagy and the negative regulation of autophagosome assembly.
- *ragc-1* codes for the RAs-related GTP binding protein C, which is involved in GTP binding. Responds to starvation.
- Controlled laboratory conditions were used to establish baseline expression

Significance

Studying the heat shock proteins in *C. elegans* and its response to heat stress will enable us to understand the effects of heat stress of proteins in *H. sapiens* because certain proteins in *C. elegans* are comparable to proteins in *H. sapiens*. Heat stress is a very common stressor, making it an important topic to understand. It helps regulate gene expression.

Why *C. elegans?*

C. elegans have a brief lifespan of about two weeks. Therefore, both in physiological and genetic ways, the organism's phases of its life cycle and evolution process could be fully observed.

Methodology

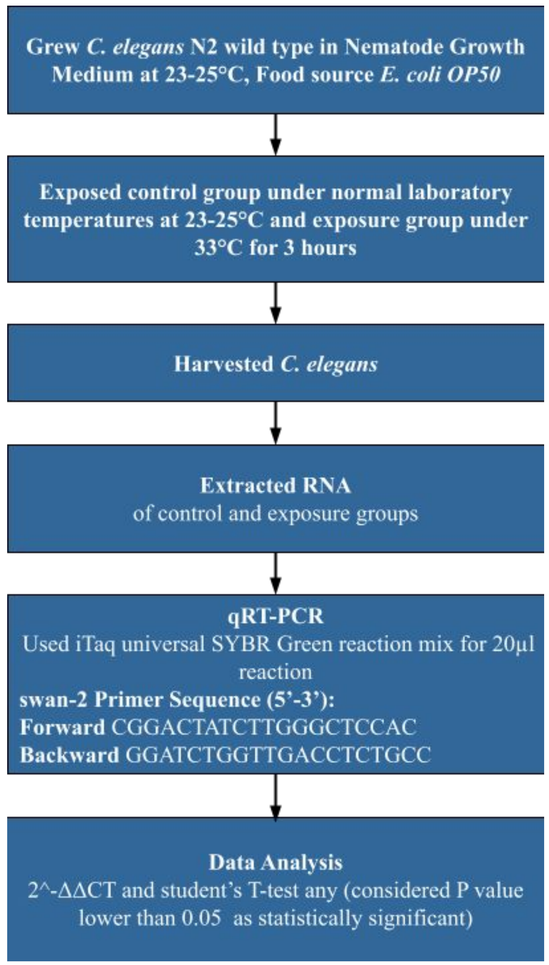

Figure 1: Flow chart of methods.

Methods and Materials

- RNA extraction using acid guanidinium thiocyanate-phenol-chloroform extraction (AGPC) extraction
- Used tba-1 gene, a housekeeping gene, to normalize qRT-PCR results
- ΔΔ Ct was used to find fold change

Results

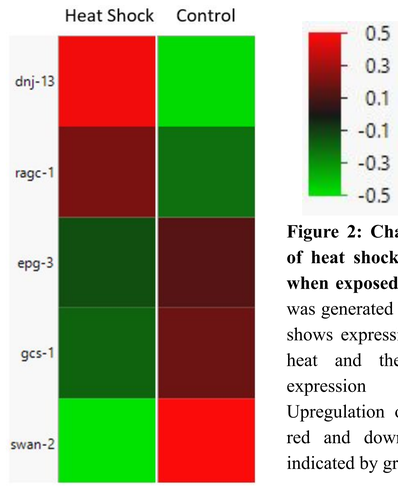

Figure 2: Change in gene expression of heat shock proteins in *C. elegans* when exposed to heat stress. Heatmap was generated by JMP Pro. Left column shows expression in worms exposed to heat and the right column shows expression in control worms. Upregulation of genes is indicated by red and downregulation of genes is indicated by green.

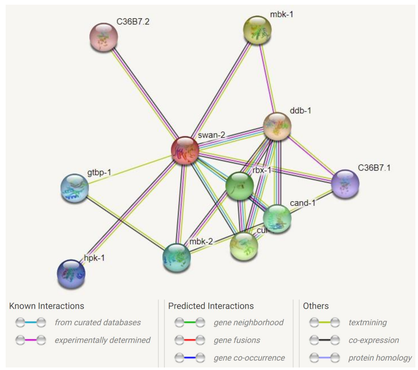

Figure 3: Association between the protein *swan-2* to other proteins in C. elegans. Protein-protein interaction map was generated by STRING.

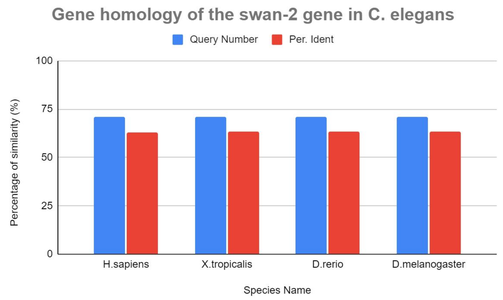

Figure 4: Homology of *swan-2* gene in *C. elegans* with other species. Information gathered from NCBI Database.

Discussion/Conclusion

Conclusion

From the study, we have found that the genes *dnj-13* and *ragc-1* in *C. elegans* up regulate under heat stress, while the *epg-3*, *gcs-1*, and *swan-2* genes down regulate under heat stress. This means that *C. elegans* have a multilayered response to maintain function under stress..

Study Limitations:

- The method we used to extract RNA, GTCP extraction, may have slight variations in purity or quality between samples.
- Due to restrictions placed by circumstances, the specificity of the product from qrt-PCR was not tested through creating a melt curve.
- Other methods, such as western blotting, could have been used to measure the upregulation or downregulation of the proteins translated from the genes. The use of a secondary method using the protein extracted from the RNA extraction could have provided more evidence and ensured the accuracy of our findings.

Further Discussion

This study enabled us to observe the connection between gene expression levels under prolonged heat exposure. From this study, it is shown that in response to stressful situations, some genes are upregulated while other genes are downregulated to maintain function for *C. elegans*. This is significant because the change in regulation of one gene, such as *swan -2* in *C. elegans*, can affect other proteins that are correlated to it, such as the *ddb-1, mbk-1,* and *gtbp-1* proteins (shown in figure 3). The *swan-2* gene is part of the signal transduction pathway, in which a disturbance in the *swan-2* gene may lead to neurotoxicity and cause mental diseases. Additionally, many proteins found in *C. elegans* are homologous to other species (shown in figure 4). Understanding how organisms respond to stress in a physiological way can encourage further discussion into how stress affects other processes such as aging seen in *C. elegans* and even humans.

Bibliography

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