Gene Expression of *epg-3*, *gcs-1*, and *swan-2* were upregulated and *dnj-13* and *ragc-1* were downregulated in *C. elegans* under heat stress compared to *C. elegans* under normal conditions

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Summary

- Null hypothesis: Under a heat stressor, the expression of genes *swan-2, gcs-1, epg-3, ragc-1 and dnj-13* is not affected.
- The gene expression for the genes was assessed using qRT-PCR.
- Under a heat stressor, the expression of genes *epg-3*, *gcs-1*, and *swan-2* were upregulated, and *dnj-13* and *ragc-1* were downregulated.

Abstract

This study determined whether the expression of genes *epg-3*, *gcs-1*, *swan-2* ,*dnj-13* and *ragc-1* were upregulated or downregulated after being exposed to a heat stressor. The only gene that provided a statistically significant p-value was the *swan-2* gene. In Caenorhabditis elegans, *swan-2* is involved in response to osmotic stress. The gene expression for the *swan-2* gene was assessed using qRT-PCR and the fold difference between the control and heat shock gcs-1 protein fold was about 0.461. s*wan-2* gene expression in *C.elegans* was upregulated under a heat stressor as well as *epg-3* and *gcs-1* while *dnj-13* and *ragc-1* were downregulated.

Introduction

- Under a heat stressor, the expression of genes *swan-2, gcs-1*, and *epg*-3 are upregulated in *C. elegans,* and the control gene, *tba-1*, is downregulated due to extreme heat shock along with genes *ragc-1 and dnj-13.*
- This experiment is conducted on *C. elegans* because they are a specific type of nematode that can be experimented on and be given effective and accurate data, and despite their seemingly simple composition, *C. elegans* utilize molecular processes that have been found in more complex organisms.
- As a result, by doing two experiments, it was found that heat stressors on gene *swan-2* are ideal because their expression was shown more than when they were not under heat shock, and that heat shocks on the *tba-1* gene are not ideal because their expression is downregulated.
- The Tubulin Alpha Chain is studied because it codes for a protein that is fundamental as it plays many roles in the cell, one of which being in the cytoskeleton {1} and binding two moles of GTP. The cytoskeleton is disrupted by aging with loss of function in somatic cells, stem cells, and gametes {2}.
- *swan-1* was one of the genes studied because its main function is to code proteins {3}. By seeing if it could benefit or suffer from heat stress, it can be stated if proteins can keep being made.
- Glutamate cysteine ligase was studied because it plays a role in the oxidative stress response {4} which plays a role in important biological processes {5}. This can allow us to see how the cell functions if *gcs-1* is down or upregulated, which in this case, is upregulated.
- The *epg-3* gene has multiple functions including autophagy, Golgi organization, and more {6}. By being able to study this gene, we can see if under heat stress, it will be able to continue completing its functions thoroughly.
- *ragc-1* is a gene that binds and is active in the GTP which transfers energy throughout the cell {7}. By experimenting on this gene, we can see if energy can still be transferred and function with heat stress.
- The *dnj-13* gene binds and chaperones binding within the cell {8}. With this specific function, we were able to see if the binding can withstand heat stress allowing this gene to continue.

Significance:

By controlling the heat in this experiment, we can see if it has any effects on the function of *C. elegans*, or more specifically, gene expression. With this, we can see how effective the heat stressor is and further evaluate its role in aging .

We are using *C. elegans* because:

- They are small and can grow in large populations in small areas.
- They have a short lifespan which is good for aging studies.
- Has been researched a lot of times so there are additional resources for us to study.

Methodology

Maintained and Cultured *C. elegans*

- Nematode Growth Media
- Control Worms at 25°C and Heat-Exposed Worms at 33°C for 3 hours.

RNA Extraction

- Phenol, Guanidine, and Chloroform
- 1,000 worm eggs per 10 cm plate
- Use Nanodrop for RNA Quantitation
- Primer Sequence:
- *tba-1* (Housekeeping Gene 5'-3')

Forward: AGACCAACAAGCCGATGGAG
Reverse: TCCAGTGCGGATCTCATCAAC

- *gcs-1* (5'-3')

Forward: GCAGGTGAATGCGATGCTTG
Reverse: GCAAGCGATGAGACCTCCGT

- *swan-2* (5'-3')

Forward: CGGACTATCTTGGGCTCCAC
Reverse: GGATCTGGTTGACCTCTGCC

- *epg-3* (5'-3')

Forward: GGAGCAGAGCACATCCTACC
Reverse: TTTGTCGCCGTTTTCGTTCC

- *ragc-1*(5'-3')

Forward: TAATGGGACACAAGAGAAGCGG
Reverse: TCTTGTGATTCGGGCCGTG

- *dnj-13* (5'-3')

Forward: TCAAGGATAAGCCACACCCG
Reverse: TCCAGTCAGCCGTCTTGTAG

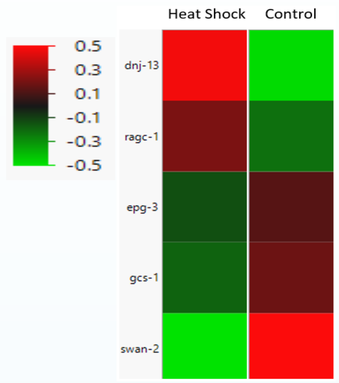
qRT-PCR

- iTAQ Universal SYBR green one-step kit used for RNA extraction.
- One reaction tube- that contains both reverse transcription (cDNA from RNA) and PCR

Data Analysis

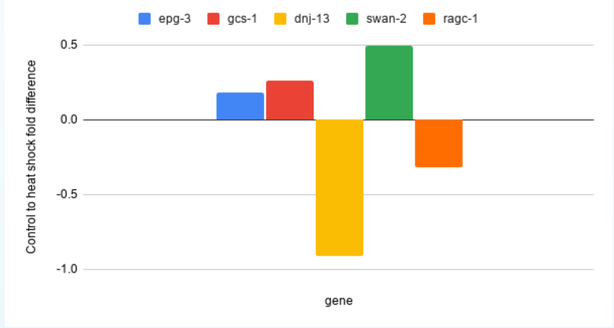
- The gene tba-1, a housekeeping gene was used to normalize qRT-PCR results. Normalized Ct number, ΔCT was used to generate heatmap.
- ΔΔCT analysis was used to generate the fold difference.
- P-value less than 0.05 was considered statistically significant.

Results



Figures 1: Differential gene expression in *C. elegans* due to heat shock. Higher expression levels are indicated by red color and lower expression levels are indicated by green color. *gcs-1, epg-3* and *swan-2* expression level was up-regulated whereas *ragc-1* and *dnj-13* expression level was down-regulated.

Figure 2: The control to heat shock fold difference for *epg-3, gcs-1, dnj-13, swan-2* and *ragc-1.*



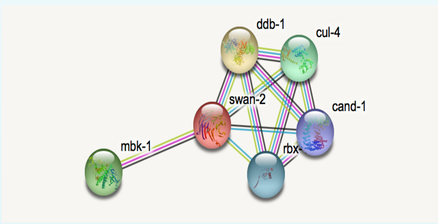


Figure 3: STRING Figure of *swan-2* that demonstrates protein-protein interactions.

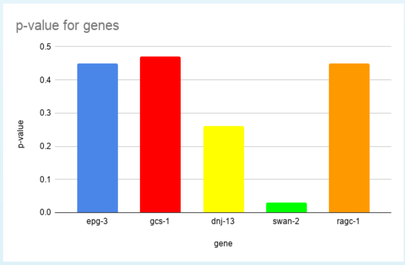


Figure 4: p value for *gcs-1, epg-3, swan-2, ragc-1 and dnj-13.* P value of 0.03 for gene *swan-2* was considered statistically significant.

Discussion/Conclusion

Conclusion/Discussion:

Gene expression as result of heat shock varied from gene to gene. *epg-3*, *gcs-1*, and *swan-2* were upregulated and *dnj-13* and *ragc-1* were downregulated. However, the only p-value that was statistically significant (using the cut-off of .05) was the *swan-2* gene. The swan-2 gene affects other proteins such as *ddb-1* gene that is a DNA damage-binding protein that plays a significant role in DNA repair as well as the *mbk-1* gene that affects olfactory neurons.

Study Limitations:

- The specific genders of the *C. elegans* were not considered in the experiment and therefore could have an impact of protein regulation.
- The age of the *C. elegans* also were not considered when testing the effect of heat shock on gene expression, which means that the different *C. elegans* depending on where they were in their life cycle could have expressed this gene differently.

Future Directions:

- In a future experiment, perhaps the *C. elegans* could be sorted based on gender and exposed to the heat shock to determine whether the proteins in males and females are upregulated to the same extent.
- The age of the *C. elegans* could also be a factor that affects the amount of gene expression and comparing the protein fold difference between *C. elegans* of different ages could provide more information on heat shock protein expression.

References

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