Gene expression of *swan-2*, involvement in response to heat stressors, is expressed ~ 1.2 fold higher in *C. elegans* exposed to heat shock than those that were not, under controlled laboratory conditions

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SUMMARY

- Gene expression of our target genes, *swan-2, ragc-1, dnj-13, epg-3,* and *gcs-1,* were assessed using qRT-PCR
- Gene expression level of the *swan-2* gene isn't significantly different between genes exposed to heat shock in comparison to those that were not due to our t-value of 0.000006

ABSTRACT

Our target genes all have predicted functions of aiding the *C. elegan* under heat stress. This project looked at the difference in the gene expression levels of our target genes between C. *elegans* in our experimental group exposed to 33°C and the control group exposed to 25°C to better understand the effects of heat stress to their lives. Extracting RNA using the Guanidine Thiocyanate-Phenol-Chloroform extraction protocol, allowed us to perform qRT-PCR in order to analyze gene expression differences. The quantified relative expression is ~1.2 which we don't find significantly different due to a t-value of 0.000006. Therefore this result demonstrated a insignificant difference in gene expression levels between our target genes under heat stress.

INTRODUCTION

Essential Question: Does heat exposure in *C. elegans* increase the chances of newly-synthesized and preexisting proteins aggregating during gene expression?

Hypothesis: When the *C. elegans* are exposed to 33°C, changes to newly-synthesized and preexisting proteins affect gene expression and their lifespans. (6)

Heat Exposure:
- Heat stress restrains the transcription and translation of most proteins. (3)
- In contrast, heat shock proteins unregulated in the response to heat stress and that allows an organism to survive heat stress that otherwise may be lethal. (4)
- Heat stress also poses affects on metabolism and the reproductive system. (12-13)
- There is a tendency for longer lifespans and an organism's ability to withstand heat stressors to be positively correlated. (5)
- *C. elegans* that are thermotolerant (80%), having the ability to survive past certain heat levels, have shown greater than 15% longevity rates. (5)

The Relevance of C. *elegans*:
- Its genome shares 83% (15,344 sequences) of functional human counterparts, making it a great model for the identification of human disease genes (7&14)
- High sensitivity to temperature, with a 0.2°C change being sensed by the worm can alter its behavior (8&14)
- Rapid life cycle of 12-18 days (15)
- Inexpensive and convenient maintenance

Target Genes: The heat stress target genes consist of *swan-2, ragc-1, dnj-13, epg-3,* and *gcs-1* which are all involved with changes experienced during stress from heat exposure.
- The swan-2 gene was chosen due to its involvement in response to heat stressors.
- The *ragc-1* gene was looked at for its involvement in determining an organism's lifespan and regulating autophagosome assembly, which is a key step in autophagy. Autophagy is a process that aids in cell survival by eliminating protein aggregates and promotes homeostasis.
- The *dnj-13* gene is related to unfolded protein binding activities.
- The *epg-3* gene is related to protein kinase binding activities, enzymes that regulate protein biological activity, and is involved in helping to counteract aggregation.
- The *gcs-1* gene encodes a member of the bicoid subfamily - bicoid proteins repress mRNA translation and enhance genes that code for transcription - and is a part of the homeobox family of proteins. The family is transcription factors that regulate target genes. (9)

By examining these target genes, we can understand how heat exposure affects the *C-elegans.*

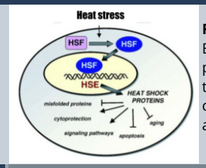

Figure 1 Heatshock Protein Reaction to Heat Stress Exposure to heat shock resembles similar effects in plants, bacteria, and mammal species. (1) The resistance to heat stress is carried out by heat shock proteins, chaperones that maintain protein homeostasis and assisting protein folding and wanted conformation. (2)

METHODOLOGY

C. *elegan* Collection
The control group consists of 1,000 *C. elegans* exposed to 25°C in MGM Medium and the experimental group consists of 1,000 *C. elegans* exposed to 33°C in MGM Medium

RNA Extraction
use the Guanidine Thiocyanate-Phenol-Chloroform extraction protocol,

qRT-PCR

- Usage of iTaq Universal SYBR Green One-Step Kit
- Primers were made using NCBI Blast

Primers:
Target genes:
gc-1_F_q : 5' GCAGGTGAATGCGATGCTTG 3'
gcs-1_R_q : 5' GCAAGCGATGAGACCTCCGT 3'
swan-2_F_q: 5' CGGACTATCTTGGGCTCCAC 3'
swan-2_R_q: 5' GGATCTGGTTGACCTCTGCC 3'
ragc-1_F_q: 5' TAATGGGACACAAGAGAAGCGG 3'
ragc-1_R_q: 5' TCTTGTGATTCGGGCCGTG 3'
dnj-13_F_q: 5' TCAAGGATAAGCCACACCCG 3'
dnj-13_R_q: 5' TCCAGTCAGCCGTCTTGTAG 3'
epg-3_F_q: 5' GGAGCAGAGCACATCCTACC 3'
epg-3_R_q: 5' TTTGTCGCCGTTTTCGTTCC 3
Housekeeping gene:
GAPDH_F_q: 5' GTCTCCTCTGACTTCAACAGCG 3'
GAPDH_R_q: 5' ACCACCCTGTTGCTGTAGCCA& 3'
tba-1
tba-1_F_q: 5' AGACCAACAAGCCGATGGAG 3'
tba-1_R_q: 5' TCCAGTGCGGATCTCATCAAC 3'

Data Analysis
Used ΔΔ CT to calculate fold change in RNA expression in a specific gene. Used t-test to ensure significance in data. P-value significance threshold was set to 0.5

RESULTS

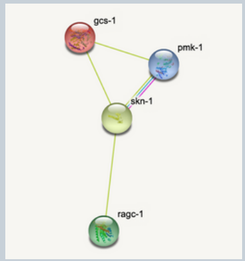

Figure 2: STRING Diagram: Target genes *gcs-1* and *ragc-1* share predicted functional partners *skn-1* and *pmk1. Pmk-1* responds to environmental stressors by phosphorylating downstream stressors. Phosphorylates *skn-1* regulates responses to oxidative stressors and is key in up-regulating *gcs-1* which shares the same function. *Ragc-1* also works to fight against stressors by promoting homeostasis and eliminating protein aggregation.

Figure 3: Heat exposed and control *C. elegans*. Red shows higher gene expression levels, while green shows lower gene expression levels. *dnj-13* and *ragc-1* gene show an up-regulation in gene expression after being exposed to heat stress. *epg-3, gcs-1,* and *swan-2* gene down-regulate after being esposed to heat stress.

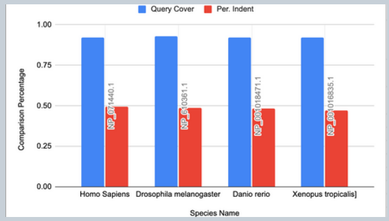

Figure 4: Gene Homology Graph. *gcs-1* gene and its homologous genes query cover represent their overlap in length. Their per. indent shows the matchup of the *gcs-1* gene and its homologous genes.

DISCUSSION/ CONCLUSION

With standard controlled laboratory conditions in observing gene expression of our 5 target genes (*swan-2, ragc-1, dnj-13, epg-3,* and *gcs-1*) exposed to heat shock, our results set a baseline for comparison. This result is consistent with each target gene's role in stabilizing the body during heat shock.

- *Dnj-13* and *ragc-1* in Figure 2 show an up-regulation during heat shock due to their predicted involvement with aiding in cell survival and combatting protein aggregation

- Epg-3, gcs-1, and swan-2 in Figure 2 show a down-regulation during heat shock due to a response in adaptation to new temperature conditions

STUDY LIMITATIONS

Limitations:
- PCR is capable of being influenced to inhibitors present in biological samples

- We don't know the *C. elegan's* survival rate and the process of qRT-PCR cannot tell between live or dead organisms

Follow up Experiments:
- The majority of our target genes are involved with combating general oxidative stress, so we can perform an experiment observing gene expression when put under exposure of a different stressor. Some examples can be radiation or smoke from pollution and we will analyze the difference in gene expression levels using qRT-PCR.

SOURCES