Heat exposure to *C. elegans* at 33 °C results in induction of genes dnj-13-14 and ragc-1 and repression of genes gcs-1, swan-2 and epg-3.

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Summary

- *Null Hypothesis:* There is no significant difference between heat exposure to *C. elegans* at 33 degrees celsius and induction or repression of the genes gcs-1, swan-2, dnj-13-14, ragc-1, epg-3 in *C. elegans.*
- The gene expression for the gcs-1, swan-2, dnj-13-14, ragc-1, and epg-3 genes were expressed using qRT-PCR.
- The housekeeping gene used as the control is tba-1 gene for *C. elegans.*

Abstract

To research the effects of heat stress factors on RNA, *C. elegans* were exposed to high heat levels of up to 33 ℃. *C. elegans* were chosen as the model organism of the experiment because parts their genome have functional counterparts in humans; therefore, *C. elegans* can accurately represent human genes. Carrying out the experiment, a control group was created to have *C. elegans* N2 wild type placed in NGM at 23 degrees celsius, and for the exposure group *C. elegans* were placed in NGM at 33 degrees celsius for 3 hours. Then, the three distinct steps of RNA extraction, RNA quantitation, and RNA purification were taken to isolate the RNA. Lastly, the qRT-PCR method was employed to discern the induction or repression effects of heat exposure on the *C. elegans*. The genes dnj-13 and ragc-1 displayed an induction in gene expression, while genes swan-2, dnj-13-14, ragc-1, epg-3 genes were repressed compared to the housekeeping gene

Introduction

- Hypothesis: We expect heat exposure to *C. elegans* N2 wild type at 33 celsius for 3 hours to cause induction of genes gcs-1, swan-2, dnj-13-14, and a repression of genes ragc-1 and epg-3 gene, compared to the typical laboratory growth temperature of 25 degrees celsius. Each differential gene could have opposing reactions.
- The housekeeping gene tba-1 will serve as our control and will not be induced or repressed when exposed to heat.
- The effect of heat exposure on every differentially expressed gene shows upregulation and downregulation out of 1211 differentially expressed genes, which is possible in the selected genes of *C. elegans*. (Li, Lian et al.)
- In *C. elegans*, the target genes for gene regulation used during heat stress include:
  - **swan-2 gene** is should be induced because swan-2 responds to osmotic stress.
  - **gcs-1 gene** produces the Glutamate to help combat oxidative stress.
    - Should be induced: activates gene expression in oxidative stress.
  - **ragc-1 gene** is hypothesized to be repressed since the gene is not a heat shock protein.
    - An ortholog of the human gene RRAGC maintains cellular processes
  - **dnj-13-14 gene** aids negative regulation of transcription by RNA Polymerase II
    - An ortholog of human gene DNAJB1(DNAJ heat shock protein family)
    - Should be induced: a heat shock protein
  - **epg-3 gene** contributes to the negative regulation of autophagosome assembly
    - Hypothesized to be repressed: not a heat shock protein
  - **tba-1 gene** (housekeeping gene) is involved in the creation of the mitotic spindle during embryo development and helps regulate cytokinesis.
- The homology between *C. elegans* genes and human genes is about 41%. (Lai, Chou, Ch'ang, Liu, & Lin, 2000).
  - The similar homology of humans to *C. elegans* makes it ideal to better understand the effects of heat on *C. elegans* gene expression.
  - Heat exposure results will help more people understand the functions of human viruses and diseases by identifying *C. elegans* target genes affected by the heat stress factor (also found in humans).

Materials and Methods

Control Group: *C. elegans* N2 wild type are placed in NGM at 23 degrees celsius

Exposure Group: *C. elegans* N2 wild type are placed in NGM at 33 degrees celsius for 3 hours

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RNA Extraction: From the clear aqueous phase in tube A, precipitate the RNA with 100 percent isopropyl alcohol

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RNA Quantitation: Use the nanodrop to blank (take into account the blanking buffer) and calculate the amount of RNA in the sample

↓

RNA Purity: Ensure the RNA is pure by analyzing its ratio of absorbance at 260 nanometers and 280 nanometers. DNA is considered pure at a ratio of 1.8 and RNA at a ratio of 2.0

↓

qRT-PCR (iTaq Universal SYBR Green One-Step Kit): Reverse Transcriptase uses the RNA extracted to synthesize a new cDNA strand. The primers used to synthesize the cDNA include:

tba-1 (Housekeeping Gene): Primer Sequence Forward: AGACCAACAAGCCGATGGAG Reverse: TCCAGTGCGGATCTCATCAAC	**gcs-1 Primer Sequence** Forward: GCAGGTGAATGCGATGCTTG Reverse: GCAAGCGATGAGACCTCCGT
swan-2-11 Primer Sequence Forward: CGGACTATCTTGGGCTCCAC Reverse: GGTTACCTTGTTACGACTT GCC	**dnj-13-14 Primer Sequence** Forward: TCAAGGATAAGCCACACCCG Reverse: TCCAGTCAGCCGTCTTGTAG
ragc-1-15 Primer Sequence Forward: TAG ACACAAGAGAAGCGG Reverse: TCTTGTGATTCGGGCCGTG	**epg-3-8 Primer Sequence** Forward: GGAGCAGAGCACATCCTACC Reverse: TTTGTCGCCGTTTTCGTTCC

SYBR binds to double stranded DNA, creating the Ct number. The PCR products in the machine will increase temperature and the data will be analyzed to find the melt curve.

↓

Data Analysis: After normalizing the Ct values of all genes, calculate the fold change in gene expression using the 2^(−ΔΔCT) method. This data is shown in the heat map.

Results

1.

Figure 1: Changes in gene expression after heat shock. Red indicates higher gene expression, while green indicates lower gene expression. Genes dnj-13 and ragc-1 depict higher gene expression (were induced). Genes epg-3, gcs-1, and swan-2 exhibit lower gene expression (were repressed).

2.

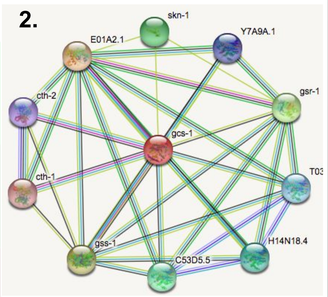

Figure 2: Network of proteins for gcs-1. The nodes represent proteins and the connections represent the different predicted and known interactions between these proteins.

3.

Gene	Species	Query	Per. Indent
gcs-1	Caenorhabditis elegans	100%	100%
gcs-1	Necator americanus	99%	79.97%
gclc	Ancylostoma ceylanicum	99%	79.51%
gcs	Haemonchus contortus	99%	79.42%
gcs-1	Dictyocaulus viviparus	99%	78.44%
Cbn-gcs-1	Caenorhabditis brenneri	100%	95.87%
FL82_05551	Caenorhabditis remanei	100%	95.87%
FL83_09269	Caenorhabditis latens	100%	95.72%

Figure 3: Gene gcs-1 Homology. The gene homology of gcs-1 represents the genes similarities, with query comparing the length of the gene and per. Indent comparing the sequences of the genes. The chart shows the different species' genes being nearly identical to gcs-1 in *C. elegans.*

Discussion and Conclusion

In *C. elegans*, the target genes for gene regulation used during heat stress include the swan-2, gcs-1, ragc-1, dnj-13-14, and epg-3 genes, while tba-1 was used as the housekeeping gene unaffected by heat exposure in regards to induction and repression. According to all outside studies referenced below, the swan-2 gene was shown to respond to heat stress and osmotic stress, the gcs-1 gene activates under gene expression, ragc-1 and epg-3 gene is not a heat shock protein, and dnj-13-14 is a heat shock protein. With that data, originally, we hypothesized that heat exposure would cause induction of genes gcs-1, swan-2, dnj-13-14, and a repression of genes ragc-1 and epg-3. However, the resulting data shown in a heat map suggests that genes dnj-13 and ragc-1 depict higher gene expression, while genes epg-3, gcs-1, and swan-2 exhibit lower gene expression. This is because the dnj-13gene is an ortholog of human gene DNAJB1 from the heat shock protein family. The ragc-1 gene contributes to cellular processes, so induction occurs in order to ensure that even with heat stress, the organism stays alive. Correspondingly, higher gene expression depicted from dnj-13 and ragc-1 represents induction, and lower gene expression depicted from epg-3, gcs-1, and swan-2 represents repression. This is because induction or repression is a process where cells increase or decrease production as an effect of a cell triggered by a stress factor (in this case, heat exposure), therefore depicting higher expression when a gene is induced, or lower expression when a gene is repressed.

The study on the induction and repression (up and down-regulation) results of heat exposure on selected genes found in *C. elegans* was for the purpose of finding a better understanding of the relationship between thermotolerance with heat stress and aging. In previous experiments regarding *C. elegans*, it has been evident that the induction of genes in response to heat stress has been linked to increased longevity (Lithgow, White, Melov, & Johnson, 1995). This is because the genes that are induced in response to heat stress produce either heat shock proteins or are involved in mediating stress. As a result, with the induction of these genes, they are able to withstand the stress and live longer. *C. elegans* was a relevant model organism in regards of heat stress and aging, as the similarity in the homology between *C. elegans* genes and human genes is about 41%. In addition, at least 83% of C. elegans proteome has human homologous genes (Lai, Chou, Ch'ang, Liu, & Lin, 2000).

Study Limitations and Future Directions

- Individual differences between C. elegans were not considered
- Due to reliable references that stated the tba-1 gene did not induce or repress under heat exposure, the gene was used as the housekeeping gene in this experiment. However, under the circumstances of an experimental lab, we were unable to know the results of doing a physical lab ourselves.
- During PCR, the presence of non-specific proteins could contribute to increased recorded fluorescence creating a false Ct value and affect the accuracy of the RNA quantification

References and Acknowledgements

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