

gcs-1 was upregulated by 1.5-fold, and *epg-3* was downregulated by 2-fold in heat stressed *Caenorhabditis elegans*

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Summary

- How does gene expression of *epg-3* and *gcs-1* change when *C. elegans* is put under heat stress?
- Quantified gene expression level changes when *Caenorhabditis elegans* (*C. elegans*) were exposed to heat stress.
- *gcs-1* was upregulated by about 1.5 fold in the heated worms, and *epg-3* was downregulated by about 2 fold in the heated worms.

Abstract

We wanted to explore the effect of protein aggregation. By checking *C. elegans*' gene expression changes during heat stress, we studied the mechanisms behind the worm's stress response pathways. Specifically, we quantified the expression level of *gcs-1* and *epg-3*, 2 genes that are possibly involved in the cellular response to protein aggregation. We found a 1.5 fold increase in *gcs-1* expression level and a 2 fold decrease in *epg-3* expression level.

Introduction

Hypothesis:

- Heat-stressing *C. elegans* will cause significant decrease in *epg-3* and increase in *gcs-1* expression.
- We are trying to find the genes that are responsive to heat stress and those that are not

Model organism:

- *C. elegans* were used because of their relatively short average lifespan (2-3 weeks), and they have many genes homologous to humans'.



Figure 1. *C. elegans* under microscope. This photo was taken at 10X magnification.

Gene targets:

- *epg-3* is involved in macroautophagy and negative regulation of autophagosome assembly. Autophagosome is a key component of the cell's recycling mechanism, as it collects the materials to be degraded and fuses with the lysosome to break them down. *epg-3* is an ortholog of human *VMP1* (vacuole membrane protein 1)¹.
- *gcs-1* is involved in cellular response to heat, and may play a role in oxidative stress response. The *gcs-1* gene is an ortholog of human gene *GCLC*^{2,3}.

Methodology

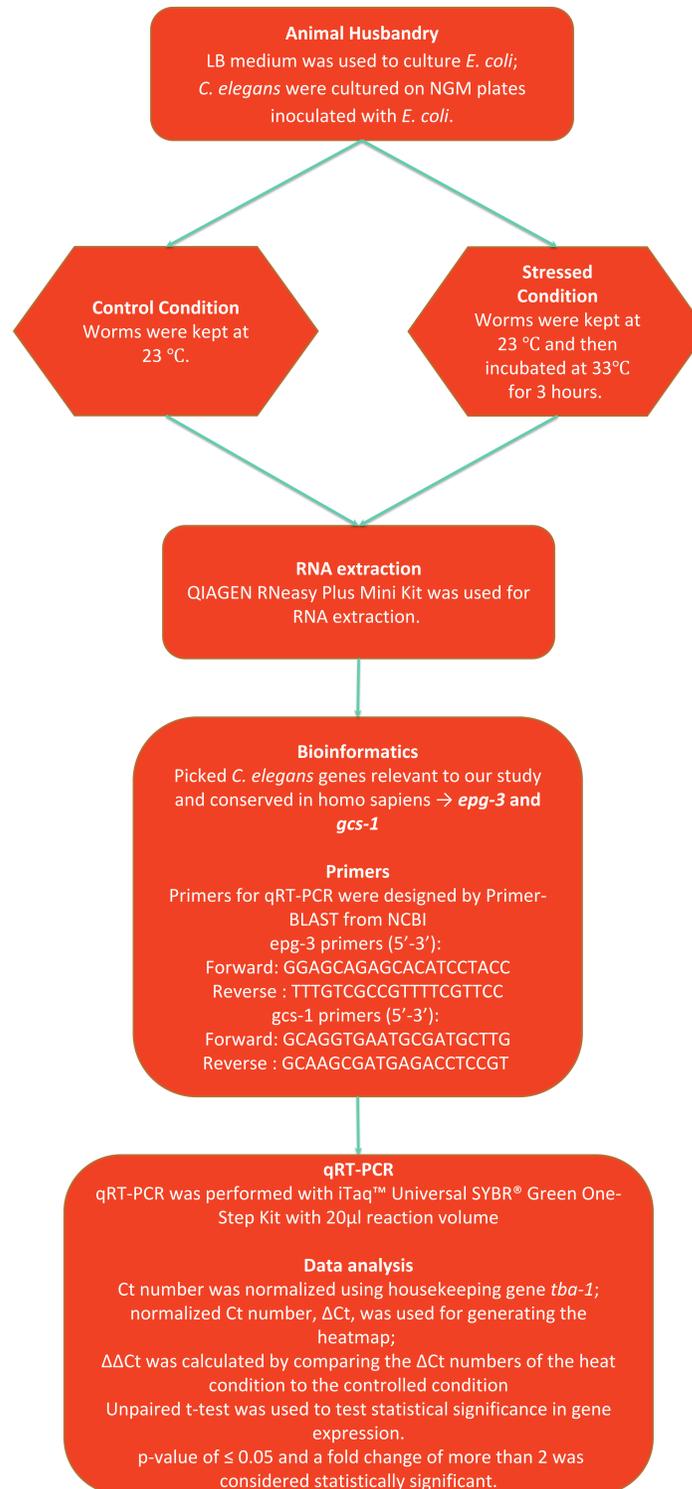


Figure 2. The flowchart of experiment progression.

Results

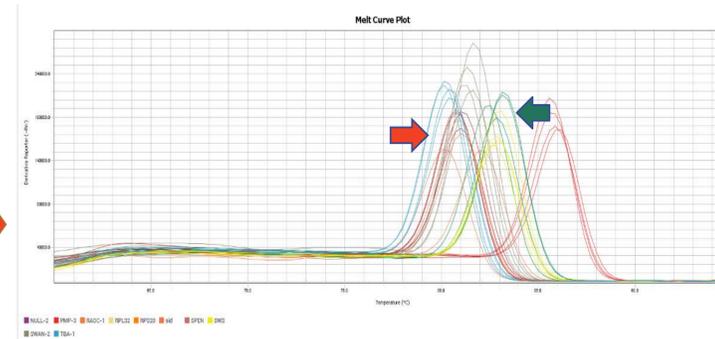


Figure 3. Melt curve plot. The dark green peaks indicated by the green arrow show the melting temperature of *gcs-1* qRT-PCR product. The dark red peaks indicated by the red arrow show the melting temperature of *epg-3* qRT-PCR product.

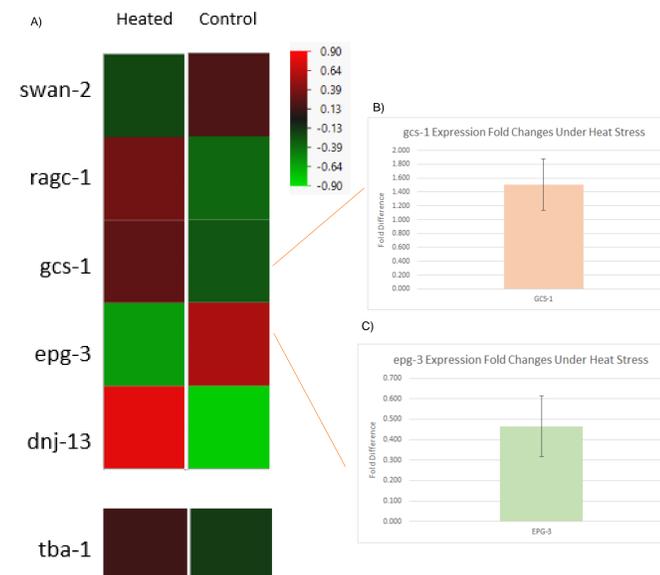


Figure 4. Gene expression differences. *tba-1* was used as a housekeeping gene to normalize the expression levels of the other genes. A) The heatmap is generated with JMP Pro. Red indicates relative higher expression level and green indicates relative lower expression level. B) *gcs-1* was expressed about 1.502 fold higher in the heat-stressed worms ($p=0.2184$). C) *epg-3* was expressed about 0.464 fold lower in the heat-stressed worms ($p=0.1018$).

Discussion and Conclusion

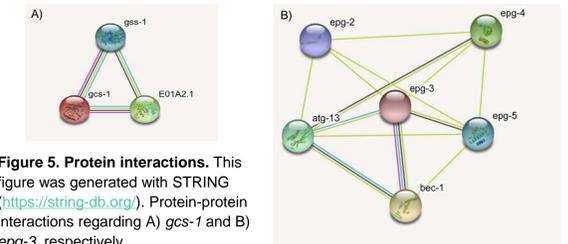


Figure 5. Protein interactions. This figure was generated with STRING (<https://string-db.org>). Protein-protein interactions regarding A) *gcs-1* and B) *epg-3*, respectively.

Based on the genes' functions and their protein products' interactions with other proteins (Fig. 5), we had the following expectations:

- Worm metabolism is expected to increase when the environmental temperature is higher, resulting in increased oxidative stress, which should increase *gcs-1* expression.
- The amount of misfolded protein should increase during the heat stress period due to the increased temperature, causing the cell to activate autophagy pathways and downregulate *epg-3*.
- The upregulation of *gcs-1* was expected but the 1.5 fold increase was not considered significant (Fig. 4B).
- *epg-3* expressed twice as much in the controlled condition as in the heated condition (Fig. 4C). We concluded that there is a significant down-regulation of *epg-3* with heat stress.

Study Limitations

- Individual variation was not measured as all worms on each plate were used for RNA extraction.
- Different developmental stages and adult worm age were not considered.

Future Directions

- Expose the worms to different stress conditions such as starvation and oxidation, and observe the expression level of *epg-3*.
- Perform lifespan and health assays on *C. elegans* under different stress conditions.

Bibliography

