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.

Methodology

Animal Husbandry
Leach medium was used to culture E. coli. C. elegans were cultured on NGM plates inoculated with E. coli.

Control Condition
Worms were kept at 23 °C.

Stressed Condition
Worms were kept at 23 °C and then incubated at 33°C for 3 hours.

RNA extraction
QIAgen RNAeasy Plus Mini Kit was used for RNA extraction.

Bioinformatic
Picked C. elegans genes relevant to our study and conserved in homo sapiens (A) epg-3 and gcs-1

Primer
Primer for qRT-PCR were designed by Primer-BLAST from NCBI.

qRT-PCR
qRT-PCR was performed with Taq™ Universal SYBR® Green One-Step Kit with 20μl reaction volume

Results

Figure 2. Flowchart of experiment progression.

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 was generated with STRING (https://string-db.org/). Protein-protein interactions regarding A) gcs-1 and B) epg-3 respectively.

Discussion and Conclusion

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

This article was generated with STRING (https://string-db.org/). Protein-protein interactions regarding A) gcs-1 and B) epg-3 respectively.