1.72-fold upregulation of autophagosome-assembly regulator *ragc-1* gene in heat-stressed *Caenorhabditis elegans*

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Summary

- Does the expression of life span regulating protein *ragc-1* in *C. elegans* change under heat stress?
- *C. elegans* were exposed to heat stress; gene expression of *ragc-1* was analyzed using qRT-PCR.
- There was about 1.72-fold increase in expression *of ragc-1* under heat stress as opposed to under control condition.

Abstract

Protein aggregation is a potential cause of neurological diseases. Stressors such as heat can cause protein aggregation in the model organisms *C. elegans*. *ragc-1* is a gene conserved in humans that determines adult life span and regulates autophagosome assembly in *C. elegans*. In this study, we heat-stressed *C. elegans* and looked at the changes in *ragc-1* gene expression. Following qRT-PCR of the RNA extracted from control and heat stressed worms, we found that *ragc-1* expression is not significantly impacted by heat stress.

Introduction

- Previous research shows that the increase of protein aggregation is correlated to aging or diseases like Alzheimer's[1]. However, the causes of protein aggregation are unclear.
- Autophagosome is a structure that can take in protein aggregates and then fuse with a lysosome to degrade the aggregates.
- Cells respond to heat stress via gene expression regulations.
- We used the stress condition, heat, to induce expression level changes to investigate stress response pathways.

We used *C. elegans* to do the experiment because:

- *C. elegans* are easy to feed, grow from eggs to adults in about 2 days, and do not require much space.
- *C. elegans* genome is well documented.

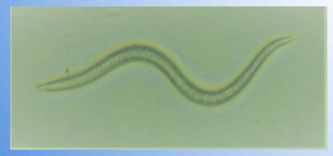

Figure 1. *C. elegans* larva under a microscope. The image (left) was captured at 20x magnification.

ragc-1: This gene is conserved in human and other common laboratory model organisms (Fig.2). It is involved in the determination of adult lifespan[2] and the regulation of autophagosome assembly[3].

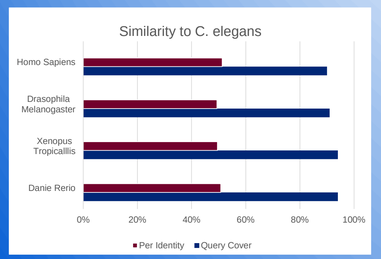

Figure 2. Conservation of protein structure of *C. elegans ragc-1* gene across species. Query Cover represent in percentage how much one species homologous protein is covering *C. elegans*' *ragc-1* protein. Percent Identity indicates how many amino acids are identical between the homologs of each species and the *C. elegans*.

Material & Method

***C. elegans* Stock**
LB medium was used to grow E. coli
NGM medium was used to grow *C. elegans*

RNA Extraction
Materials: *C. elegans*, QIAGEN RNeasy Plus Mini Kit
Method: All worms were frozen after the heat exposure, then RNeasy Kit protocol was followed for RNA extraction

Data analysis
Ct number for each gene was normalized against the housekeeping gene tba-1. Normalized Ct number, ΔCt was used to generate the gene expression heatmap (Fig. 4A)
$2^{-\Delta\Delta Ct}$ method was used to analyze qRT-PCR results (Fig. 4B)
Statistical significance is determined via unpaired t-test; a fold change of more than 2 with $p \leq 0.05$ is considered as statistically significant

Control condition: Worms were kept at 23℃

Heat exposure: Worms were incubated at 33 ℃ for 3 hours

Homology analysis:
Protein sequences were obtained through NCBI Protein database.
The percentage numbers were calculated by NCBI protein BLAST.

qRT-PCR
Materials: Samples from RNA extraction, iTaq Universal SYBR Green One-Step Kit
Method: iTaq Universal SYBR Green One-Step Kit protocol
ragc-1 primers (5'-3')
Forward primer: TAATGGGACACAAGAGAAGCGG
Reverse primer: TCTTGTGATTCGGGCCGTG

Results

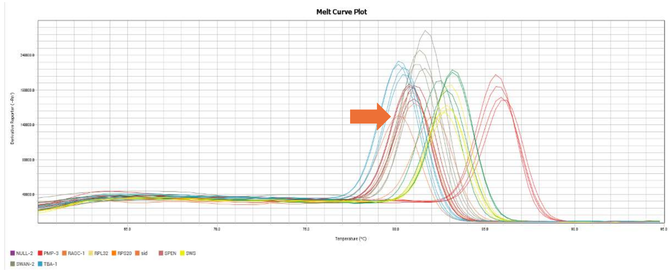

Figure 3. Melt curve for qRT-PCR. Orange peaks indicated by the arrow show melting temperature for the *ragc-1* qRT-PCR product.

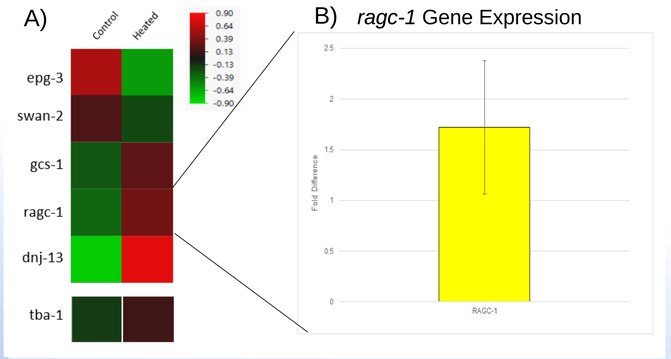

Figure 4. Relative gene expression levels in *C. elegans* under heat stress. A) Quantitative real time PCR results were normalized using the housekeeping gene tba-1. This heat map is generated by JMP Pro with the normalized data. Red indicates relative higher expression levels and green indicates relatively lower expression levels. B) *ragc-1* is expressed about 1.72-fold in the heat stress condition compared to the control condition (p=0.29).

Discussion

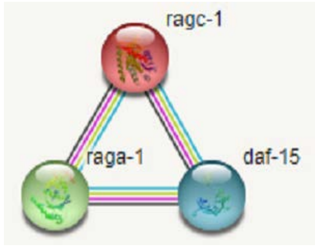

Figure 5. Proteins that interact with *ragc-1* protein. These proteins are co-expressed and related to adult life span. daf-15 is also involved in autophagosome assembly. *raga-1* plays a role in multicellular organism reproduction. This figure is generated by STRING (https://string-db.org/).

- We considered *ragc-1* to be involved in stress response pathways due to its function and interaction with other proteins (Fig. 5).
- About 1.72-fold change on *ragc-1* gene was observed according to the result of qRT-PCR (Fig. 4).
- With a conservative approach, we conclude that *ragc-1* is not statistically significantly upregulated in heat-stressed *C. elegans*.

Study Limitation

- Individual differences between worms were not taken considered.
- Different gene expression levels in *C. elegans* were not accounted for as RNA samples were extracted from entire plates of worms at different developmental stages.

Future Direction

- Repeat this experiment with more biological and technical replicates to confirm the results.
- Check the expression level of *ragc-1* in other stress conditions such as hypoxia, oxidative stress, and starvation.
- Quantify stress exposure effects within a specific developmental stage

References

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