Expression of *epg-3* gene, a negative regulator of autophagosome assembly, changes inconsistently in heat-stressed *Caenorhabditis elegans*

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Abstract

We wanted to examine the correlation between protein aggregation and biological aging using stress response pathways. Specifically in this study, we studied the changes of *epg-3* expression levels in heated *C. elegans.* Interestingly, we obtained conflicting results in the two experiments performed, with each experiment using technical replicate samples of each other.

Introduction

- Protein aggregation, a process in which misfolded proteins form aggregates in organisms, is observed in aged *C. elegans*[1]. It is also observed in cases of age-related diseases like Alzheimer's disease and Parkinson's disease[2].
- *C. elegans*' genome has been fully sequenced. Many of their genes are homologous to human genes, making them a useful model. *C. elegans* are also easy to maintain in the lab. Their lifespans are relatively short, usually about 3-4 weeks.
- *epg-3* is a gene that negatively regulates the assembly of autophagosomes. *epg-3* is an ortholog of human vacuole membrane protein 1 *VMP-1*[3].
- Autophagosome is a structure that can take in protein aggregates labelled for degradation, fuse with a lysosome and then degrade the aggregates.
- We hypothesized that protein aggregation increases under heated condition, and *epg-3* is downregulated as a response to heat stress to increase autophagosome assembly.

Figure 1: Mature *C. elegans.* Photo taken at 10X *magnification.*

Materials and Methods

Heat Exposure and Sample Collection

- 8 plates of worms were initially maintained at 23 ℃ on NGM medium with *E.coli OP50* lawn. Then 4 plates (H1, H2, H3, H4) were transferred to an incubator set to 33 ℃ and incubated for 3 hours. The other 4 plates (C1, C2, C3, C4) were kept at 23 ℃ until the 33 ℃ incubation was finished.
- Worms on each plate were flushed down and washed with 1X PBS. Worm suspensions were then frozen at -80 ℃ for 1 day. QIAGEN RNeasy Plus Mini Kit was used to extract total RNA from the worm samples.

qRT-PCR and Data Analysis

- Primers were designed with the Primer-BLAST tool from NCBI.
  epg-3 primers (5'-3')
  Forward: GGAGCAGAGCACATCCTACC
  Reverse : TTTGTCGCCGTTTTCGTTCC
- iTaq™ Universal SYBR® Green One-Step Kit was used for qRT-PCR setup.
- We used sample H1, H2, C1, C3 for the first qRT-PCR experiment (Experiment 1).
- The above experiment was repeated for Sample H3, H4, C2, C4 (Experiment 2).
- Unpaired t-test was performed for the ΔC_T values. When sample C4 was excluded (see Discussion), we replicated C3 ΔC_T for the t-test.

Results

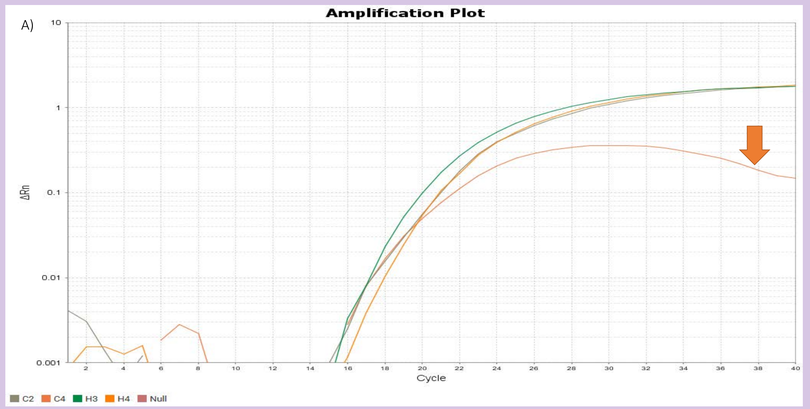

Figure 2. *epg-3* qRT-PCR readings. The plots were created by QuantStudio™ Design & Analysis Software v1.5.1. A) The amplification plot was generated based on the readings on QuantStudio3. ROX was used as the passive reference dye. The arrow indicates the amplification curve for sample C4. B) The melt curve plot was generated with the default melt curve analysis protocol on QuantStudio3. The arrow indicates the melt curve for sample C4.

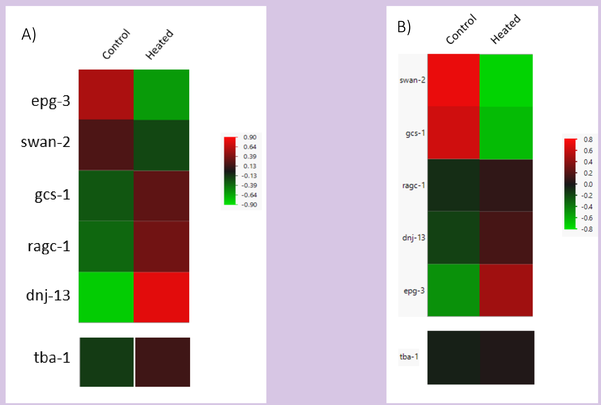

Figure 3. Differential expression of genes in Experiment 1 and Experiment 2. The heatmaps were generated with JMP Pro 14 based on ΔC_T values. *tba-1* , a housekeeping gene, was used for normalizing the C_T values. Red indicates relative higher expression levels and green indicates relative lower expression levels. A) The heatmap representing qRT-PCR results from Experiment 1. B) Heatmap representing qRT-PCR results from Experiment 2. ΔC_T value for sample C4 was not used.

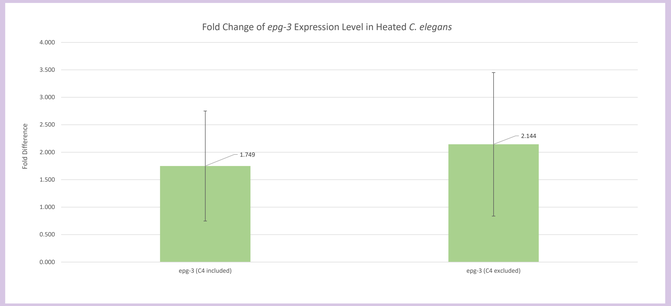

Figure 4. Fold change of *epg-3* expression level in heated *C. elegans*. The fold difference was calculated using the $2^{-\Delta\Delta CT}$ method. A different fold difference was obtained when the data for sample C4 was excluded from the calculation. The error bars represents the standard deviations. A 2- fold difference is considered as significant in this study.

Discussion

- The qRT-PCR reaction for sample C4 was unsuccessful. The signal of DNA products amplified from sample C4 went down before the plateau phase was reached (Fig. 2A). The melt curve plot also suggests that the DNA products of C4 are different from those of the other samples (Fig. 2B). Data point from sample C4 was then excluded when generating the heatmap (Fig. 3B).

- 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 expression pattern from Experiment 1 was therefore expected (Fig. 3A), but that from Experiment 2 was unexpected (Fig. 3B). This result was unexpected as the samples were technical replicates of each other.

- We performed t-test both when the fold difference is included and excluded (see Materials and Methods). Both p-values were larger than 0.2, so the data we obtained lack statistical significance. This is further demonstrated by the error bars of the fold differences (Fig. 4).

- The fold difference calculated when C4 data was excluded was larger than 2, but the error bar and the conflicting results from Experiment 1 and Experiment 2 prevented us from concluding any directional expression changes of *epg-3* under heated condition.

Study Limitations/Future Directions

- There might have been loading errors during the experiment, which could be a reason for the conflicting results.

- It is also possible that *epg-3* expression levels are affected by factors that we were not aware of. More repeats and larger sample size of this experiment is then required for drawing a conclusion about the *epg-3* expression level changes in heated *C. elegans*.

- We will also repeat this experiment while exposing the worms under other conditions like oxidative stress with paraquat and hyperoxia condition to study the expression level of *epg-3* when the worms are exposed to other stressors.

References

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