

Statistically Significant Upregulation (3.2-fold) of heat shock protein coding gene, *dnj-13* in heat-stressed *C. elegans*

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Abstract

C. elegans were tested under a control condition, maintained at 23 C°, and a stress condition, maintained at 33 C° for 3 hours. RNA was extracted from the control and stress samples and mRNA coding for gene *dnj-13* was measured by qRT-PCR. The *dnj-13* gene codes for a heat shock protein produced in response to stress exposure with the function of re-fold misfolded proteins. A statistically significant 3.2 fold increase was measured under the stress condition.

Introduction

Hypothesis:

Heat-stressing *C. elegans* will cause significant differences in the expression level of gene *dnj-13*.

Why *C. elegans*?

- They have a temperature sensitive lifespan
- They are inexpensive to maintain
- They grow in vast quantities in the lab
- They have a simple body/model



Figure I. *Caenorhabditis elegans* under microscope. Photo taken at 20X magnification.

About *dnj-13*

- *dnj-13* is in the **heat shock protein** family, a family of proteins that are produced by cells in response to exposure to stressful conditions¹.

Function:

- The protein product of *dnj-13* is predicted to have chaperone binding activity and unfolded protein binding activity.
- This protein assists in maintaining the proper structure of proteins and in the prevention of unwanted protein aggregation².

Background & Relevance:

- **Aging** is an inevitable biological process that occurs in most animals as well as in humans.
- By studying genes with a role in protein aggregation, it can be determined to what extent protein aggregation is potentially involved in the aging process.
- Applications of the data conducted can be made to other species, such as humans, due to the existence of human homologs of the gene *dnj-13*.

Materials and Methods

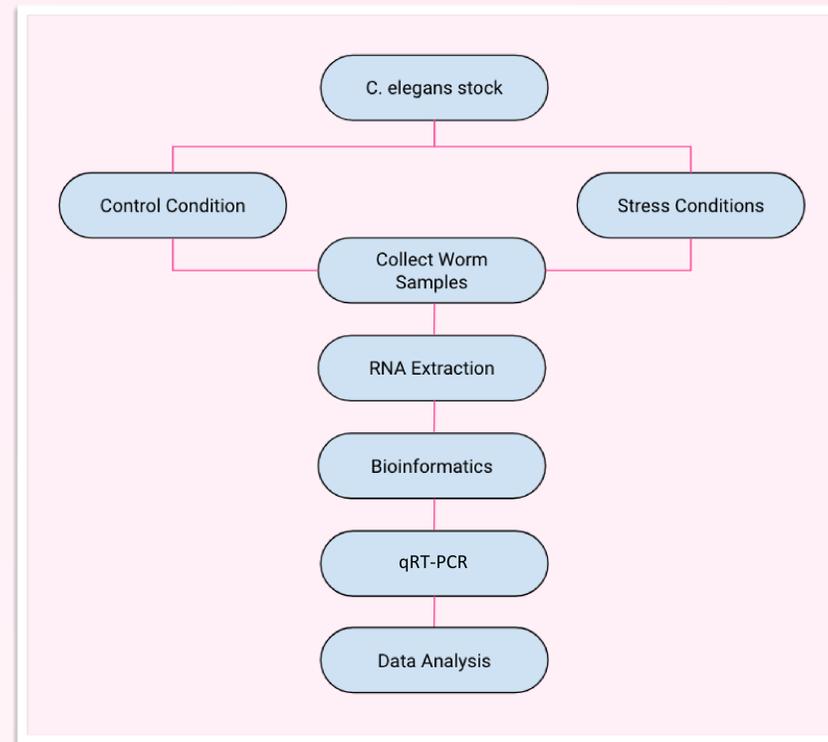


Figure II. Flowchart of steps performed in the experimental process.

Maintaining *C. elegans* stock

- LBM Liquid Medium
- NGM Medium

RNA Extraction

- QIAGEN RNeasy Plus Mini Kit

qRT-PCR

- Bio-Rad iTaq Universal SYBR Green One-Step Kit
- qRT-PCR data was normalized using the housekeeping gene *tba-1*. $\Delta\Delta C_t$ method was used for calculating the fold change.
- Data was statistically analyzed with unpaired t-test. A fold change of larger than 2 with $p \leq 0.1$ was considered as statistically significant in this study.

Gene	Accession	Primer Sequence	Direction
<i>dnj-13</i>	NM_064067.6	TCAAGGATAAGCCACCCCG	Forward
		TCCAGTCAGCCGCTCTGTAG	Reverse

Table I. *dnj-13* primers for qRT-PCR. The pair of primers used in qPCR was chosen based on the Primer Melting Temperature (T_m) and self-complementation rating

Results

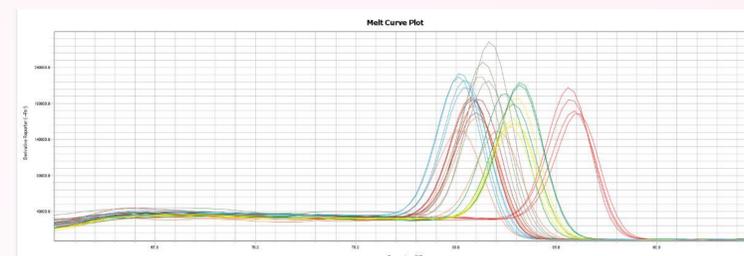


Figure III. Melt curve plot. Peaks indicating melting temperature of *dnj-13* are in yellow.

Results

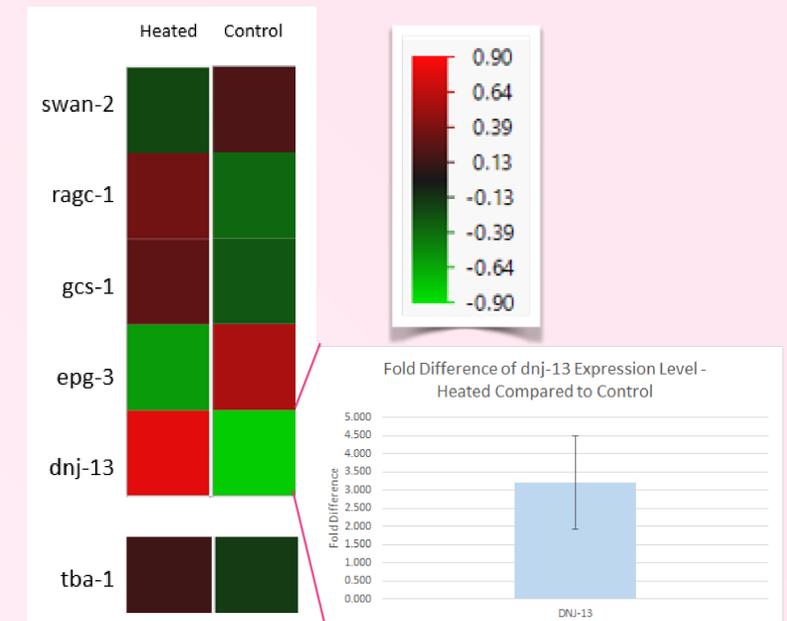


Figure IV. Gene expression differences. A) *tba-1* was used as a housekeeping gene to normalize the results. A green color signified lower gene expression levels while a red color signified higher gene expression levels. B) About 3.21 fold change of *dnj-13* expression level in the heated sample is observed ($p=0.0887$).

Conclusion

Discussion

dnj-13 had a statistically significant 3.2 fold upregulation of expression level in heat-shocked *C. elegans* when compared to *C. elegans* in controlled conditions. This result correlates with *dnj-13*'s role as a heat-shock protein, and its function as a chaperonin. This study confirms that *dnj-13* is a viable candidate for future studies on protein aggression and aging in *C. elegans*.

Study Limitations

Lack of individual variation: The *C. elegans* used in the heat-stress and control samples each consisted of one plate.

Future Directions

This project can be propelled forward and developed by exposing *C. elegans* to other stressors, such as oxidative stress, and observing gene expression in *dnj-13*.

Bibliography

