Effects of Caffeine on the Stress-Regulated Gene, *HSF-1*, in *Caenorhabditis elegans*

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Introduction

Caffeine is a chemical substance that is largely consumed globally, despite being associated as a psychoactive drug. Moderate intake of caffeine has been recorded to show a significant decrease in the risk of acquiring age-related diseases in humans, such as dementia and Alzheimer's (Eskelinen and Kivipelto 2010). As humans age, managing the heat becomes more difficult, and the elderly are more susceptible to illnesses regarding high temperatures. When dealing with stressors such as elevated temperatures, humans and other eukaryotic organisms respond by increasing the synthesis of heat shock proteins (Wu 1995). The increase of proteins initiates gene expression by *heat shock transcription factors*, or *HSF-1* (Wu 1995). This gene expression has been recorded to be found within *Caenorhabditis elegans*. As a result of the sharing of genes between the free-living nematodes and humans, *C. elegans* hold great significance due to being testable for treatments that can promote longevity.

Hypothesis

HSF-1 mRNA will be spliced differently in nematodes exposed to caffeine compared to no caffeine, and that these caffeine-induced RNAs will produce functional proteins

Methods

Selection of Gene in *C. elegans* Genome and treatment of interest

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Perform "Chunking" Method to Grow a *C. elegans* Population on Bacteria Culture Plate Seeded with OP50

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Create Serial Dilution of 100mM Caffeine Treatment into 3mM

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Expose *C. elegans* to Caffeine Treatment and Isolate the RNA to Use Reverse Transcription to Ultimately Produce cDNA Using RNA Protocol

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Perform Primer Resuspension and Polymerase Chain Reaction to Test Annealing Temperature on Control cDNA

Calculate Predicted PCR Sizes For the *HSF-1* Gene

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Run Gradient PCR Products on Agarose Gel Electrophoresis

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Construct a Multiple Alignment and Determine Regions That are Different or Missing in the Reference Sequence and Isoform

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Determine Best Annealing Temperature for Thermocycler Run and set up PCR on Control and Experiment cDNAs

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Agarose Gel Electrophoresis of Control and Experimental PCR and Analyze Gel

Results

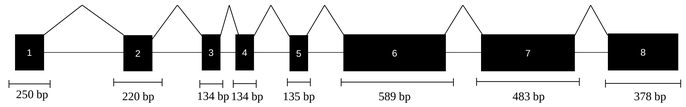

Figure 1. *HSF-1* Gene Structure. Eight exons are labeled below with relative sizes in base pairs (bp). Horizontal lines (introns) are spliced out (angled lines) to produce the *HSF-1* mRNA. Information to produce this gene structure was obtained from Ensembl Metazoa (Kersey et al. 2018).

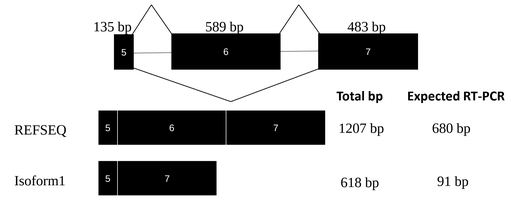

Figure 2. Comparison of *HSF-1* Transcripts in the Region of Interest. Exons in the region of interest are shown with calculated total base pair sizes and expected reverse transcription-polymerase chain reaction (RT-PCR) base pair sizes for the reference sequence (REFSEQ; top splicing pattern including exon 6) and Isoform1 (bottom splicing pattern skips exon 6). Primers to detect both transcripts were designed using Primer3Plus (Untergasser et al. 2007).

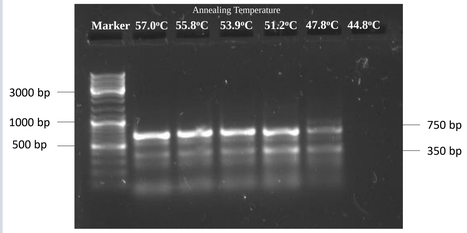

Figure 3. Annealing Temperature Test for *HSF-1* Primers. Gel electrophoresis was performed on products from reverse transcription-polymerase chain reaction (PT-PCR) using different annealing temperatures from 57.0°C-44.8°C. Marker (OGeneRuler;Thermofisher) sizes are noted at left. Estimates of observed DNA fragments sizes are noted at right, both in base pairs (bp). Marker information was acquired from Thermofisher (Thermo Fisher Scientific, 2018).

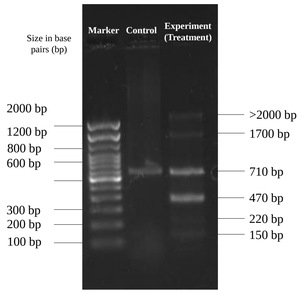

Figure 4. Comparison of *HSF-1* Control and Experiment cDNA. Agarose gel electrophoresis was performed on control cDNA and experimental cDNA treated with caffeine. Marker (TrackIt;Thermofisher) sizes are noted at left. Approximations of observed DNA fragments sizes are noted at right, both in base pairs (bp). Marker information was obtained from Thermofisher (Thermo Fisher Scientific, 2018).

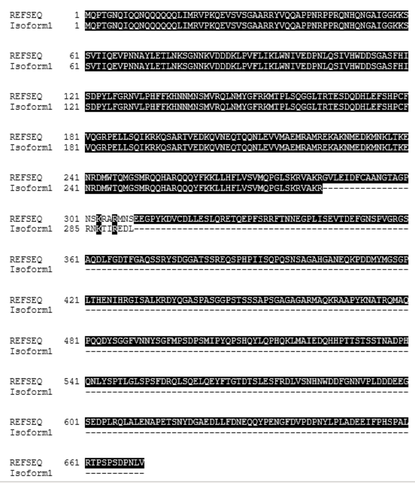

Figure 5. Alignment of Reference Sequence (REFSEQ) and Isoform1. Comparison of HSF-1 protein sequence using ExPasy translation software (Wu et al, 2003). BoxShade alignment software was used to align the REFSEQ and Isoform1 (Artimo et al, 2011).

Results

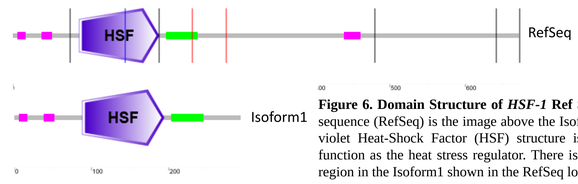

Figure 6. Domain Structure of *HSF-1* Ref Seq and Isoform1. The reference sequence (RefSeq) is the image above the Isoform1 domain structure. The blue-violet Heat-Shock Factor (HSF) structure is depicted largely and serves to function as the heat stress regulator. There is a deletion of the low complexity region in the Isoform1 shown in the RefSeq located at 439-461aa.

Conclusions

- The best annealing temperature was 51.2°C.
- Alternative splicing is present in the experimental treatment at 47.8°C.
- The experimental treatment has more alternatively spliced versions than the control treatment, therefore, the caffeine stressor does allow gene expression changes in the HSF-1 mRNA.
- Based on the domains that are present, the alternate proteins that are produced by caffeine-induced RNAs are likely to be functional proteins.
- Functional proteins indicate that gene expression of the *HSF-1* gene is increased, therefore promoting regulation of heat-induced stressors.
- A recent study suggests that caffeine enhances the regulation of heat stressors and promotes proteostasis in *C. elegans* (Brunquell et al. 2017).
- Taken together, these results suggest that caffeine treatment may be used to promote longevity in *C. elegans* because it has significant effects on gene expression of the *HSF-1* gene.

Future Directions

- Further research is needed to test functionality of the proteins that were produced from the alternative splicing and identify those that were not predicted.
- Additional research is also needed to test *C. elegans'* longevity and its mechanism after being treated with caffeine.
- Caffeine treatment effects on other species, including the effects on the HSF-1 gene should be undertaken. Further research will answer the question of if it allows humans, or other organisms, to regulate stressors more efficiently after the consumption (or usage) of caffeine.

Literature Cited

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