Effects of Cold Stress on Alternative Splicing of a Gene Implicated in Nematode Longevity, EGL-9

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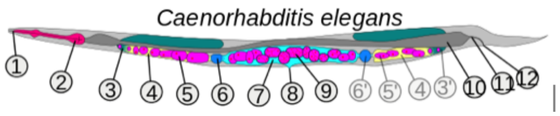


Figure 1, the hif-1 loss-of-work allele completely smothered the *EGL-9*-intervened obstruction phenotype. According to C elegans anatomy.svg.

Background

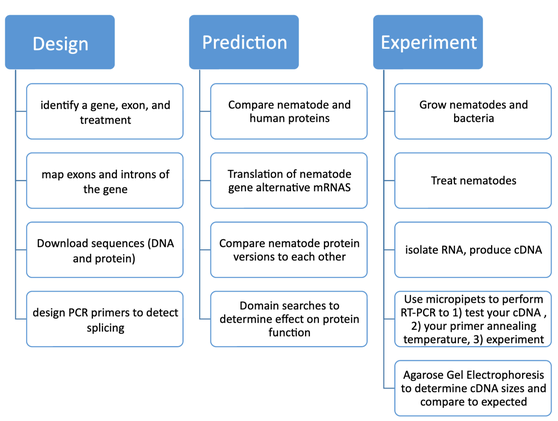
Environmental stressors are known to affect longevity. An excellent model for longevity studies is the soil nematode, C. elegans. One gene implicated in nematode longevity under a variety of environmental stresses is *EGL-9. EGL-9* is a 10-exon gene encodes an enzyme involved in protein localization and has two human homologs, *EGLN1* and *EGLN3*. Alternative splicing is a normal process that can produce multiple proteins from a single gene by selection of different exons in the mature RNA and can be altered in response to environmental stress.

Hypothesis

As of late, the nematode *Caenorhabditis elegans* has developed as an amazing hereditary framework to consider inborn invulnerability and protection from bacterial pathogens. Huge numbers of the qualities that add to *C. elegans* pathogen obstruction are developmentally moderated.

Materials and methods

To determine whether cold stress affects alternative splicing of *EGL-9* exon 9, gene structures and sequences were retrieved from a genomic database. Next, primers for reverse transcription-polymerase chain reaction were designed to detect both inclusion and skipping of exon 9. RNA was isolated from nematodes that were incubated at either room temperature or 4°C and used to produce cDNA.



Results

The *egl-9 (*sa307) solid loss-of-work transformation has been appeared to secure *C. elegans*. This cDNA is currently being used as a template in PCR and fragments will separated using agarose gel electrophoresis. The fragment sizes will indicate whether alternative splicing has occurred and whether it is different between room temperature or cold-treated nematodes.

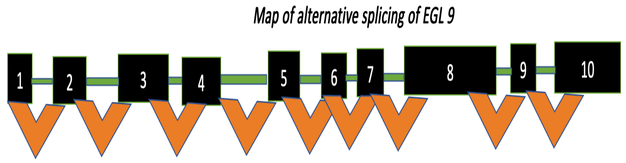


Figure2: *Map of alternative splicing of EGL 9.* displaying the exons and introns. The black boxes repesent each of the 10 exons present in EGL 9. The angled line below the boxes represent the constitutive splicing pattern foe EGL 9. Feature sizes are relative to their actual length in Basepairs (bp). This figure was designed using information obtained from EnsemblMetazoa (Kersey et al,2018).

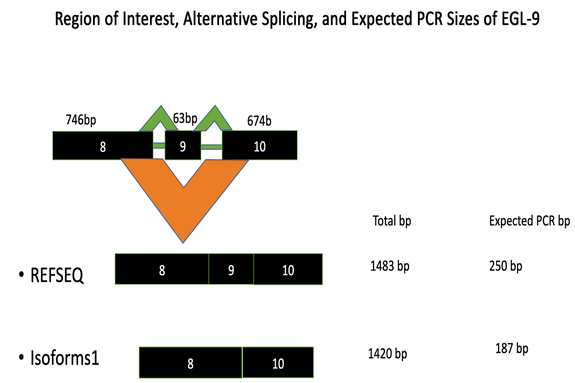


Figure 3. predicted isoforms and expected RT-PCR sizes. The region of interest shows that exon 9 was spliced. Total base pair numbers and expected PCR sizes are listed above for REFSEQ and Isoform1.

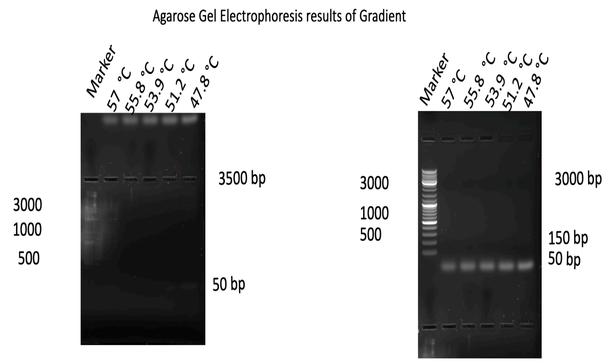


Figure 4. Gradient PCR gel was run to obtain electrophoresis results pictured above. Results were not clear for the gradient on the left (bottom) compare to the right gradient PCR gel (top).

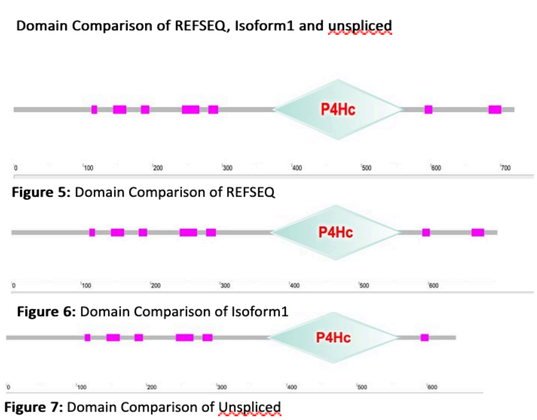


Figure 5: Domain Comparison of REFSEQ

Figure 6: Domain Comparison of Isoform1

Figure 7: Domain Comparison of Unspliced

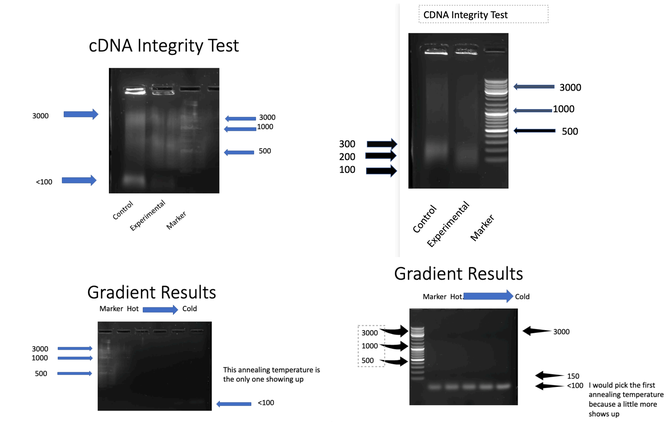


Figure 8: Gel Analysis cDNA integrity test and g

Table 1. Summary of Alternative Splicing of Exon 9 in Response to Cold Stress Treatment

RT-PCR size (bp)	transcript	Predicted protein length (aa)	Protein domains[1] missing?	Predicted functional ?	Present in control?
1483	Unspliced/ genomic DNA	641	yes	yes	no
251	Reference Sequence	721	no	yes	no
188	Isoform 1	699	no	yes	no

bp = base pairs; aa = amino acids; [1]The protein domain found was the PINT domain at amino acids 600-700 in unspliced protein

Discussion

The fragment sizes will indicate whether alternative splicing has occurred and whether it is different between room temperature or cold-treated nematodes.

Conclusion

Based on the results with the *EGL-9* primers, the cDNA is ok to use in the experiment. The control sample had some damages to it, but the results showed that alternative splicing occurred in the experimental sample. The domain search showed no result for *EGL-9.* The gradient PCR analysis shows that alternative splicing occurred in the experimental sample at the optimal annealing temperature 57.0 °C.

Future direction

We suggest that over-articulation of HIF-1 focuses past a limit level secures *C. elegans* from the cyanide delivered by P. aeruginosa PAO1. It is likewise conceivable that change of *EGL-9* permits HIF-1-interceded interpretation in explicit cells or tissues that are particularly imperative to this obstruction phenotype.

References

File:C elegans anatomy.svg. File:C elegans anatomy.svg - Wikimedia Commons. [accessed 2020 Apr 9]. https://commons.wikimedia.org/wiki/File:C_elegans_anatomy.svg

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