MTSU BIOLOGY

MTSU BIOL 3251 Presents-

The effects of H_2O_2 on the splicing mechanism of C. elegans' daf-16 mRNA.

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Introduction-

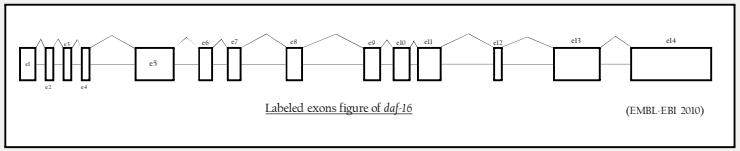
- It took one look to find the theme that ran among almost all the organism. The oxidative stress theory of aging, first proposed in 1950 by Denham Harman, took the scientific community by a storm. (Viviana et al. 2013) Using *C. elegans* as model organisms for the study, many papers suggested that increase in oxidative stress to lipids, DNA, and proteins increases aging. To reduce aging, bodies of researchers either increased the oxidative resistance of the model animal, which were either mice, *C. elegans*, by mutating certain genes and observing how it increased the animal's resistances to oxidative stress, reduced oxidative damage and increased life span. (Smith 2013; Viviana et al 2013)
- Among those genes in *C. elegans* was daf-16, a gene that has a variety of functions in the organism. daf-16, a FOXO transcription factor, and daf-12, a nuclear hormone receptor, work together to increase the lifespan of a *C. elegans* by 60% when its germline stem has been removed during adulthood. (Mark et al 2012) Though best known, daf-16 works in response to induced insulin/IGF-1 signaling to regulate the cell.
- This gene, Daf-16, is expressed in many organs including the muscular system, epithelial system, nervous system and reproductive system; daf-16 had been a popular gene of choice, reasonably so for its wide variety of tasks in the organism. More importantly, daf-16 has been the popular gene to study regarding Parkinson's disease and its involvement in mediating neuroprotection. (Yan et al 2019)

Hypothesis-

- The *C. elegans* will be put in an environment induced with hydrogen peroxide. The oxidative stress by the H_2O_2 will cause daf-16's mRNA to be alternatively spliced in such a way that it will function with a defect, either by increasing the worm's resistance to hydrogen peroxide or decreasing it, causing an increase or decrease in its life span.
- Our goal will be to mutate and observe the effects of the alternatively spliced daf-16. We hypothesize that the treatment will alter its function during an oxidative stress; alternatively, the mutated gene could increase the *C. elegans*' resistance to the ROS (Reactive Oxygen Species.).

Method-

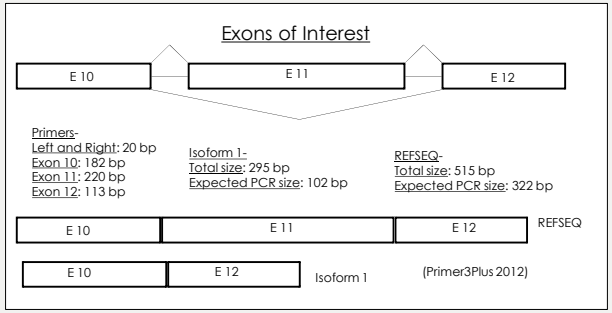
1. Retrieve the sequences of the *daf-16*.
2. Map out the entire gene including its intron and exons. (Information could be found in Wormbase.)
3. Retrieve the Amino Acid sequences.
4. Select the exon of study (exon 11) and purchase suitable primers to track the results of the treatment. (Find suitable primer from Primer3Plus.)
5. Prepare the chemical treatment. (5µM of H_2O_2 diluted for use to a 10x stock solution.)
6. Cultivate the worms. (Food source being OP50 Strain of *E. coli* incubated at 37°C.)
7. Harvest and treat the worm with prepared chemicals.
8. Extract the RNA and use reverse transcriptase to obtain the cDNA. (51µl and 52µl of the lysis was respectively used for the control and experimental to extract the RNA.)
9. Run a PCR annealing test on the primers to determine the best temperature. (Solvent used was 10mM of Tris + 1mM EDTA)
10. Map out and predict the PCR sizes of the experiment.
11. Run a PCR on the cDNA previously harvested.
12. Run an electrophoresis agarose gel test to observe the results.

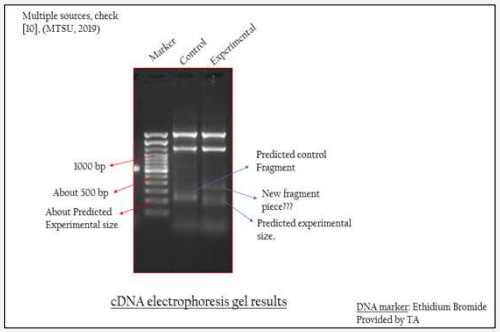


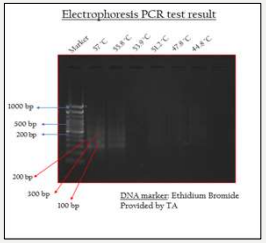
Labeled exons figure of *daf-16* (EMBL-EBI 2010)

Results-

After the experiments, we collected various data and compared it to our predicted outcome. Our Null hypothesis was that no difference would be observed after the treatment and we gathered results from different sources to cross examine our hypothesis.







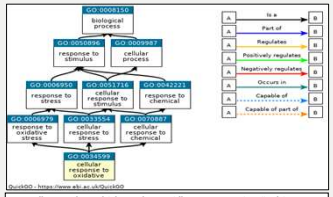
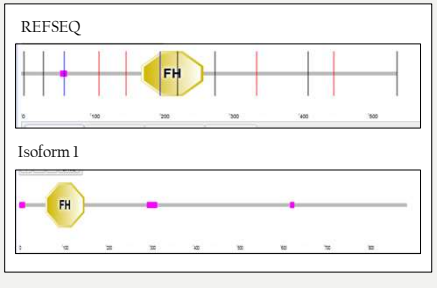


Fig I. Different isoforms of daf-16 performing different actions in the cells of the nematode. (image from QuickGo -unspecified date-)



Conclusion-

- After testing out the primers on random DNA, the results indicated two identical annealing temperatures, we chose 57°C as our annealing temperature for our *C. elegans*' cDNA. The annealing temperatures chosen worked with our cDNA giving us 2 almost identical results in our electrophoresis. That didn't line up with our prediction so upon closer inspection there is an alternative splicing band alongside our predicted outcome, larger than our predicted size, indicating that alternative splicing did occur. (MTSU 2019.)
- The proteins observed after the splicing are different and surprisingly larger than the original reference sequence. Two prevailing conclusions could be made from this observation. It is known in well-established literature that H_2O_2 not only introduces double strand breaks but also inhibits/significantly decreases the activity of the DNA repair system. (Driessens N., et al 2009.) First, H_2O_2 could have introduced double breaks and broke the DNA in such a way that when electrophorized, a new fragment was repaired/stitched together compared to the control cDNA.(refer to the cDNA electrophoresis result image). Secondly, the disruption caused by H_2O_2 altered the function of the spliceosome in such a way that when exon 11 was "spliced", it alternatively spliced in some introns with other exons, that led to larger proteins being formed. The new fragment had, as a result, a different splicing pattern.
- The new proteins would have new functions; it could be a defective protein as a result of the shock that would explain the small tandem proteins observed in the predicted isoform. It could also be a protein that functions as a membrane efflux to remove H_2O_2, a response to the chemical shock.
- Whichever product the new mRNA might translate to, there are clear literatures establishing that treatment with H_2O_2 will result in colossus damages to the DNA (as observed) that decreases the overall lifespan of the *C. elegans*. (Xinmiao F., et al 2015) This experiment didn't deal or observe the overall behaviour of the nematode so further research is needed to truly finalize on the effects of the treatment on the organism.

Future Directions-

Obviously, there is so much more to discuss and analyse than just the mRNA splicing pattern of *daf-16*. As a Pleiotropy gene, the alteration of *daf-16* could have more drastic measures on the overall behavior and internal systems of the Nematode. A future research to observe and analyze the macroscopic results of such a treatment would be beneficial to the overall knowledge of H_2O_2's effects as well as observing how the organism's internal responses are. Furthermore, alternatively splicing out other genes could also yield useful information as to how different isoforms work and how the Nematode responds to such changes in *daf-16*'s protein translation.

The oxidative stress theory of aging is a very prevalent research area so macroscopic observation of the worm's overall behavior and life span could really help add to the bank of knowledge against humanity's age-old enemy, the Reaper.

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