Alterations in *NPR-19* Gene Expression in *C. elegans* following Anandamide Treatment

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Introduction:

- Anandamide (AEA) an endogenous chemical that interacts with cannabinoid 1 receptors (*CB1R*) appears to effect gene transcription (Lu and Mackie 2016).
- The use of the *CB1R* homologue *NPR-19*, found in *C. elegans*, (Oakes et al. 2017) could assist in identifying the potential transcriptional effects of AEA due to the full genomic mapping of the *C. elegans* (Kersey et al. 2018).
- Hypothesis: Exposure of *C. elegans* to AEA will affect the splicing of *NPR-19* to produced more functional *NPR-19* protein. Referring to the basis that some *CB1R*s are up-regulated in the presence of the ligand (AEA) (Brandman and Meyer 2008).

Methods Nematodes:

Worm Growth/Tx:
-Place chunk of *C. elegans* onto incubated nematode growth plate.
-Treatment (Tx): AEA (in ethanol)
-Control: Ethanol.

Sample Prep:
-Add Lysis & Proteinase K
-Add control RNA to master mix. Repeat Tx.
- Load into gel, compare to OGeneRuler.

Electrophoresis:
-Add F+R/nuclease free H_2O to RMM.
-Add RMM to control cDNA Repeat Tx cDNA.
-Load into gel, compare to ThermoFisher.

Methods Proteins:

Translation
-Use Expasy software (Artimo P. 2011), to translate Refseq DNA into proteins.
-Repeat for Isoform1 DNA.

Alignment
-Use the Protein Information Resource to compare the translated protein sequences.
-See figure 5. (Artimo P 2011).

Domain Search
-Put Refseq into Interpro software for protein domains.
-Repeat Isoform1.
-See figure 6. (Mitchell A.L. 2019).

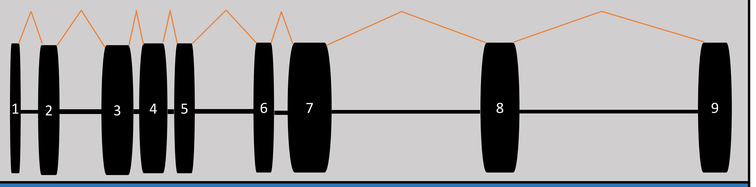

Figure 1. *NPR-19* Gene Structure. A homologue of the cannabinoid 1 receptor in *C. elegans.* The numbered boxes represent exons, the solid black lines represent introns, and the orange angled lines represent the normal splicing pattern. Information gathered from Ensembl Metazoa was used to design structure (Kersey et al. 2018).

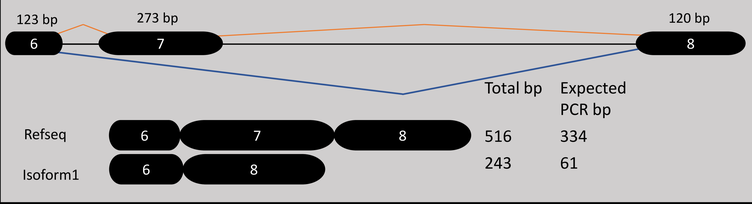

Figure 2. *NPR-19* Region of Interest Including the Alternative Splicing and Expected RT-PCR. At 273 base pairs (bp), exon 7 is the largest of the *NPR-19* exons. For this reason it was chosen as the focus of the experiment. By skipping exon 7 two different PCR sizes (334 and 61 bp) may be expected. The Refseq, which is the transcript that includes exon 7, results from the constitutive splicing pattern shown by the orange angled lines (above structure). Isoform1, which is the alternative transcript that skips exon 7, results from the alternative splicing pattern shown by the blue angled lines (below structure). The primers used to detect both were designed by Primer3 (Untergasser et al. 2007).

Results:

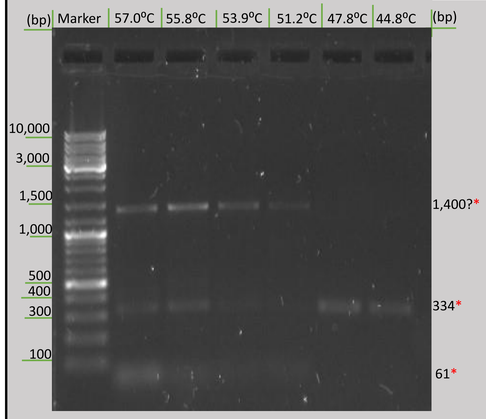

Figure 3. *NPR-19* RT-PCR Annealing Temperature Test. Agarose gel electrophoresis was used to separate RT-PCR products in which, PCR with *NPR-19* primers were tested at different annealing temperatures (listed above well). Sizes of the marker (OGeneRuler) are noted at left in base pairs (bp). Observed sizes are noted at right in bp.

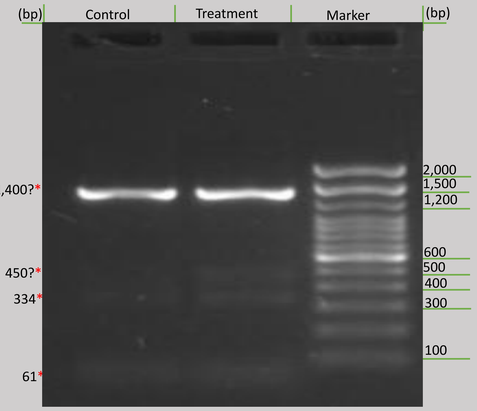

Figure 4. *NPR-19* RT-PCR Ethanol Control and Anandamide Treatment. Agarose gel electrophoresis was used to separate RT-PCR products in which, PCR with *NPR-19* primers containing either control cDNA (ethanol) or the treatment cDNA (Anandamide & Ethanol) were tested at 57.0°C. Sizes of the marker (ThermoFisher) are noted right in base pairs (bp). Observed sizes are noted at left in bp.

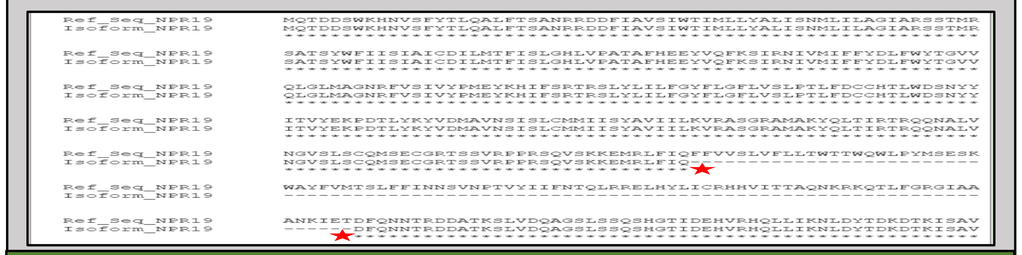

Figure 5. Multiple Alignment. The reference protein sequence of *NPR-19* ("Ref_Seq_NPR19") is aligned with the isoform protein sequence of *NPR-19* ("Isoform_NPR19") to compare the two translated protein sequences. Differences between the reference and the isoform sequences are marked by red stars. Used Expasy software for protein sequence translations (Artimo P 2011).

Figure 6. Reference Protein Domains and Isoform Protein Domains. A visual representation of the reference sequence protein domains (above) and a visual representation of the isoform sequence protein (below). Domains marked with a star represent statistically significant *e*-values. Red symbols represent similarities between the reference and isoform sequences. Green symbols represent differences between the reference and isoform sequences. "SF8" highlighted in yellow to accentuate the specific difference between the reference and the isoform. Information about protein domains was gathered from InterPro (Mitchell A.L. 2019).

Conclusions:

- Best annealing temperature of 57.0°C.
- Alternative splicing is occurring in both the control and treatment.
- The control shows the same alternative splicing as the isoform tested for annealing with PCR fragments at 61, 334 (expected PCR) and about 1,400 bp.
- The treatment additionally shows the same alternative splicing. However, it also shows an additional PCR product at roughly 450 bp.
- Overall, the reference and the isoform appear to make proteins with the same domains. However, the reference has an unnamed domain (labeled PTHR22718) with an *e*-value=0, that the isoform does not have. The function of the domain is unknown. Additionally, the isoform has a neuropeptide receptor family domain (labeled PTHR22718:SF8) with an *e*-value=1.4E-181, that the reference does not have. It also has no known function but, appears to be in the G-protein coupled receptor (GPCR) class.
- Another difference between the reference and the isoform occurs with the GPCR rhodopsin-like domain. This particular domain is represented along the reference and isoform sequences in four separate locations. The location of the first three GPCRs are identical for both the reference and the isoform (30-54; 64-85; 113-133 bp). The location of the last GPCR is located in completely different regions along the sequence between the reference and isoform (reference: 303-329; Isoform: 147-168).
- The GPCR domain represents a plethora of different proteins including neurotransmitters, light receptors, and hormones (Mitchell A.L. 2019). These proteins can allow for function in different systems including endocrine, autocriine, and paracrine processes (Mitchell A.L. 2019). The difference in location of the fourth GPCR rhodopsin-like domain may indicate a slight alteration in function of the proteins in these systems.
- Additionally, the GPCRs are expected given that the *NPR-19* gene is a homologue of the CB1R gene in humans, as cannabinoid receptors are G-protein coupled receptors (Oakes et al. 2017).

Future Directions:

- Investigate the unpredicted PCR product at 1,400 bp to determine if it is a fully included intron or genomic DNA.
- Run a qRT-PCR to identify the quantity of PCR products being produced between the control and the treatment cDNAs.
- Examine the importance of the GPCR movement between the reference and the isoform sequences (if any).
- Attempt to determine the difference between the unnamed domain (PTHR22718) from the reference sequence and the neuropeptide receptor family domain (PTHR22718:SF8) from the isoform.
- Explore the physiological effects of the treatment on the endocrine system compared to the control. (Research on polycystic ovarian syndrome, an endocrine disorder, has revealed increased levels of AEA within the system (Juan et al. 2015). Could this be caused by similar alternative splicing?)
- Investigate the alternative splicing PCR product at 450 bp caused by treatment of AEA

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