Aldicarb Exposure, and the Effects of the *GBB-1* Gene in *Caenorhabditis elegans*

Haley Parks, Hope Brown, Kylie Curtis, Alex Romer, Rebecca Thiemann-Siepelt
Middle Tennessee State University

Introduction

- In all living creatures that reside on the planet earth, gene expression plays a critical part in keeping that species alive and fully functional to survive within its ecosystem. Gene expression plays its part by expressing certain proteins that allow full functionality of multiple life essential internal systems in a living body.
- A neurotransmitter labeled GABA serves as a vital role in the development and the overall function within the nervous system. GABA uses synaptic transmission through both ionotropic receptors and G-protein-coupled receptors. Ionotropic receptors are made using ion channels gated through ligand to create small, incredibly fast signals between the neurons, while the GPCRs are gated by neurotransmitters and they prefer to make longer, yet much slower, signals between neurons (Dittman and Kaplan 2008).
- Acetylcholine is used in the process of contracting muscular tissue. The acetylcholine that is released from cholinergic moto neurons, the neurons that help send signals to the muscle fibers in the muscular system, is used to stimulate postsynaptic receptors located in the muscles making the body wall, thus allowing these muscles to contract and the organism can perform locomotion (Mahoney et al. 2006). The gene *GBB-1* is one of the subunits that makes up a GABAb receptor, and it aids in the expression of the cholinergic moto neurons (Schultheis et al. 2011).
- Aldicarb is an inhibitor to acetylcholine, and it is commonly used as a pesticide against unwanted nematodes. If a nematode is exposed to aldicarb, it prevents the acetylcholinase from breaking down the acetylcholine, making a large buildup of acetylcholine within the synapse. This leads to hyper-contractions within the muscles before gradually leading to paralysis.
- When exposed to both 2.628 millimolar and 5.256 millimolar of aldicarb, the RNA from the *GBB-1* gene will be alternatively spliced to form different isoforms in regions of the C. elegans nervous system, compared to those not associated with the exposure of aldicarb.

Methodology

Mapped out the reference sequence, predicted possible isoforms due to treatment, designed the PCR primers, and calculated the expected PCR sizes for the gene *GBB-1*.

Cultivated and harvested *C. elegans* on plates of bacteria, separating the nematodes into three separate groups. The first group would be the control, and the second and third would be the two experimental groups.

Treated the control group with 900 microliters of saline followed by 100 microliters of deionized water. The first experimental group was treated with 900 microliters of saline, followed with 100 microliters of 5.256 millimolar (mM) of Aldicarb. The second experimental group was treated with 900 microliters of saline, then followed by 50 microliters of deionized water and 50 microliters of 5.256 millimolar of aldicarb. Since the second experimental sample had its treatment solution diluted, the dosage would become 2.628 mM of Aldicarb. All three groups of nematodes were kept in solution for 25 minutes before stored in freezer at −80C.

Isolated and extracted RNA from nematode pellets and performed reverse transcription using the thermocycler, producing cDNA. Gradient PCR was run on produced cDNAs using Phusion Master Mix, then after incubating at annealing temperature, was used to perform agarose gel electrophoresis.

Results

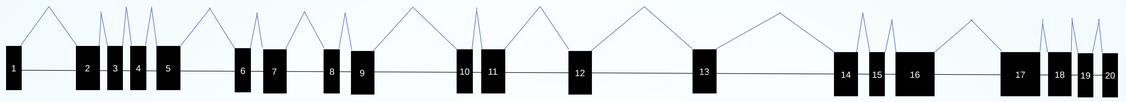

Figure 1. Gene structure of gene *GBB-1* in Caenorhabditis elegans. Since exon 16 is shown to be one of the largest, we will conduct alternative splicing on that exon.

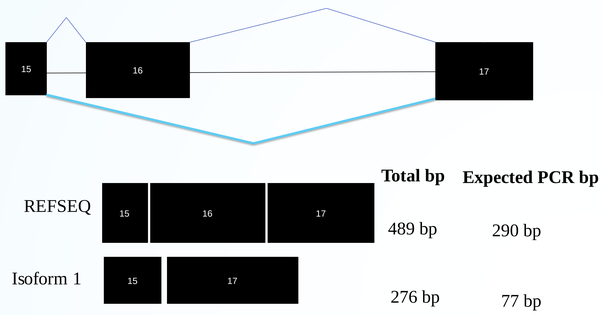

Figure 2. The region of interest, alternative splicing, and expected PCR size for the gene *GBB-1*. If alternative splicing occurs, then the total bp (base pairs) will decrease from 489 bp to 276 bp.

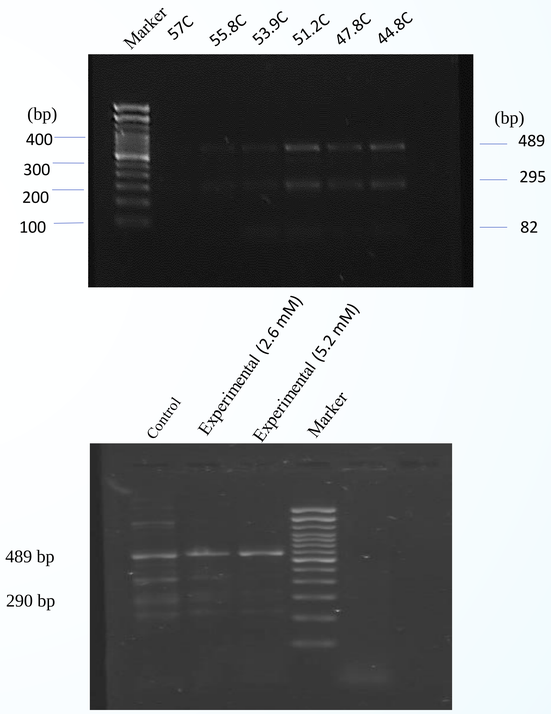

Figure 3. Agarose Gel Electrophoresis Results of Gradient PCR and Control/Experimental PCR for *GBB-1*. Figure shows that the best annealing temperature for our RNA samples will be 51.2 degrees Celsius (C).

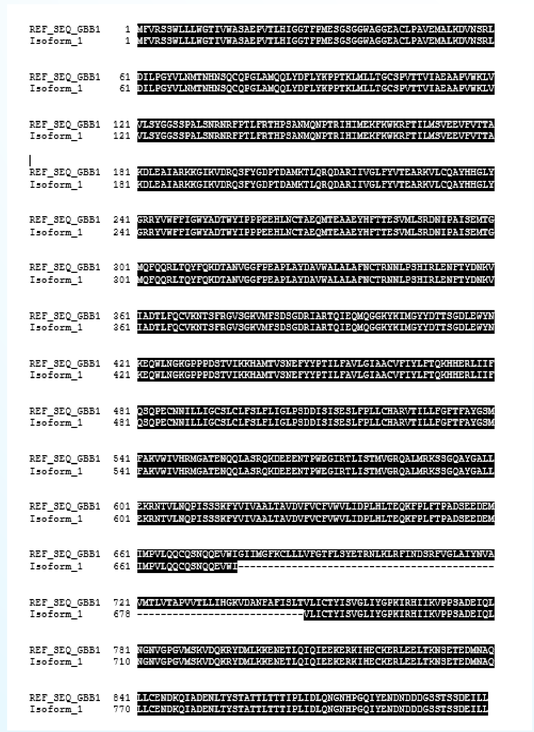

Figure 4. Translation Comparison between REFSEQ and Isoform 1 of *GBB-1*. (Artimo et al. 2012)

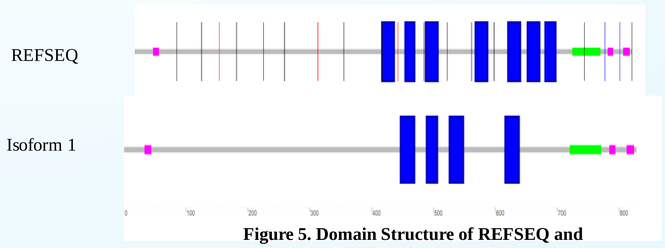

Figure 5. Domain Structure of REFSEQ and Isoform 1 of *GBB-1*. (Letunic and Bork, 2018)

Conclusions

- The best annealing temperature for the primers for *GBB-1* was 51.2° C.
- After running PCR on our samples, the image shows that alternative splicing did not occur in both the 5.256 mM and the 2.628 mM experimental samples, however it did show that the expression of *GBB-1* is inversely correlated with the concentration of aldicarb. It showed that as the concentration of aldicarb that was added to the nematode pellets increased, the expression of *GBB-1* in the nematode pellets decreased. No difference in band placements were shown between the control and the two experimental samples.
- Since the image showed the inverse correlation between the concentration of aldicarb and the expression of the *GBB-1* gene, it concludes that aldicarb reduces the expression of the cholinergic moto neurons (Schultheis et al. 2011), inhibiting the release of acetylcholine and preventing neural signals from reaching muscle fibers located in the muscular system that are vital to perform locomotion in *C. elegans* (Mahoney et al. 2006).

Future Directions

- Repeated testing is needed in order to confirm no other possible factors affected the two experimental samples.
  - Tests on electrophoresis would be wise to reassure assumption that there was no possible contamination among the three samples.
- Further testing on the *GBB-1* gene involving aldicarb would be recommended to see if other possible dependent variables are affected by the exposure of aldicarb .
  - Experiment using different primers to look for possible alternative splicing in other exons (such as exon 17, 9, or even 7).
  - Extending exposure time of aldicarb among the nematodes longer than 25 minutes.

References

Artimo P, Jonnalagedda M, Arnold K, Baratin D, Csardi G, de Castro E, Duvaud S, Flegel V, Fortier A, Gasteiger E, Grosdidier A, Hernandez C, Ioannidis V, Kuznetsov D, Liechti R, Moretti S, Mostaguir K, Redaschi N, Rossier G, Xenarios I, and Stockinger H. 2012. ExPASy: SIB bioinformatics resource portal, *Nucleic Acids Res* [Internet]. [cited 2019 Nov 21]; 40(W1):W597-W603. Available from: https://www.expasy.org/about

Cathy H. Wu, Lai-Su L. Yeh , Hongzhan Huang, Leslie Arminski , Jorge Castro-Alvear , Yongxing Chen , Zhangzhi Hu , Panagiotis Kourtesis , Robert S. Ledley , Baris E. Suzek , et al. 2003. The Protein Information Resource. Nucleic Acids Res [Internet]. [cited 2019 Nov 21]; Vol. 31, No. 1 345–347 Available from: https://proteininformationresource.org/pirwww/aboutpir/doc/nar03pir.pdf doi: 10.1093/nar/gkg040

Dittman JS, Kaplan JM. 2008. Behavioral impact of neurotransmitter-activated-g-protein-coupled receptors: muscarinic and GABA receptors regulate Caenorhabditis elegans. J. Neurosci [internet]. [cited 2019 Sept 3]; 28(28): 7104 – 12. Available from: https://www.jneurosci.org/content/jneuro/28/28/7104.full.pdf doi: 10.1523/jneurosci.0378-08.2008

Letunic I and Bork P. 2018. 20 Years of the SMART protein domain annotation resource. *Nucleic Acids Res* [Internet]. [cited 2019 Nov 21]; Volume 46, Issue D1, Pages D493–D496. Available at: https://doi.org/10.1093/nar/gkx922

Mahoney TR, Luo S, Nonet ML. 2006. Analysis of synaptic transmission in Caenorhabditis elegans using an aldicarb-sensitivity assay. Nat Protoc [Internet]. [cited 2019 Sept 3]; 1(4): 1772 – 1777. Available from: https://www.researchgate.net/publications/6344585 Analysis of synaptic transmission in Caenorhabditis elegans using aldicarb-sensitivity assay doi: 10.1038/nprot.2006.281

Schultheis C, Brauner M, Liewald JF, Gottschalk A. 2011. Optogenetic analysis of GABA receptor signaling in Caenorhabditis elegans. J Neurophysiol [Internet]. [cited 2019 Sept 3];106: 817 – 827. Available from: https://www.physiology.org/doi/pdf/10.1152/jn.00578.2010 doi: 10.1152/jn.00578.2010