# *SLO-1* Gene Expression is Regulated by Ethanol Exposure in *C. elegans*

Morgan Hill, Kailee Gatewood, Alex Romer, Rebecca Seipelt-Thiemann

MIDDLE TENNESSEE STATE UNIVERSITY

MTSU BIOLOGY

## INTRODUCTION

- *SLO-1* is a gene in *C. elegans* that affects the organism's behavioral response to ethanol. *SLO-1* deletion mutants display a higher resistance to the ethanol; whereas, SLO-1 overexpression mutants are hypersensitive to ethanol intoxication (Davies et al. 2003).
- *SLO-1* encodes a calcium-activated large conductance BK potassium channel (Davies et al. 2003). Ethanol exposure is known to inhibit the SLO-1 channels, but its effect on expression of the gene is unknown. The purpose of this research was to better understand whether *SLO-1* gene expression also changes with ethanol exposure.

## HYPOTHESIS

*C. elegans* placed into a 0.4275 M ethanol solution for 30 minutes will be show altered *SLO-1* alternative splicing of exon 4 compared to *C. elegans* exposed to a saline solution.

## METHODS




**Figure 1: Methods and Procedures.** The blue boxes represent steps carried out during the experiment. The arrows show the flow of steps by continually pointing to the step that followed the one before.

## RESULTS

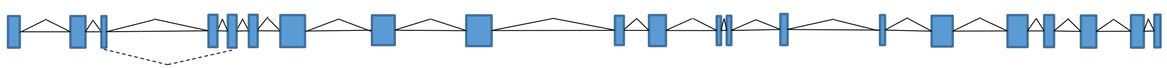

**Figure 2: *SLO-1* Gene Structure.** The blue boxes represent the exons. Horizontal lines represent introns. Angled lines above the image represent constitutive splicing pattern for the reference sequence. Angled dashed lines below the image represent the alternative splicing pattern if exon 4 was skipped. Feature sizes are relative to their actual length in basepairs (bp). This figure was designed using information obtained from EnsemblMetazoa (Kersey et al, 2018).

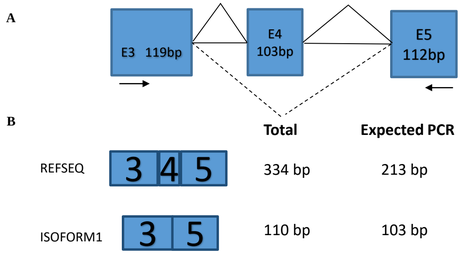


**Figure 3: *SLO-1* Alternative Splicing Assay Design. A. Splicing pattern in the region of interest (exon 3-5).** The blue boxes represent exons and horizontal lines represent introns. Angled lines above the image represent constitutive splicing pattern for *SLO-1* including exon 4. Angled dashed lines below the image represent the alternative splicing pattern for *SLO-1* if exon 4 was skipped. Features are labeled with their actual length in basepairs (bp). **B. Predicted Region of Interest Splicing Products.** REFSEQ represents the transcript region if exons are joined using the constitutive splicing pattern. ISOFORM1 represents the transcript region if exons are joined using the alternative splicing pattern. Expected total sizes and RT-PCR sizes are noted at the right of each transcript based on primers (arrows) designed using Primer3plus (Untergrasser A. et al. 2006.).

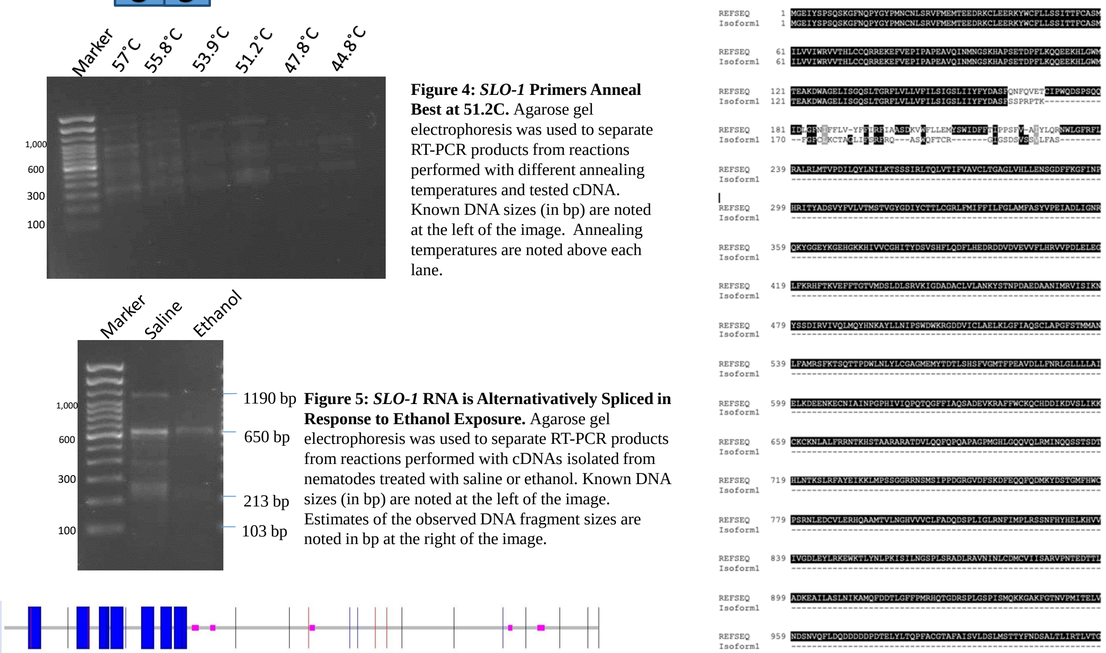


**Figure 6: Comparison of Predicted SLO-1 Protein Isoforms Alternative Splicing Results in Protein Truncation.** Transcripts were reconstructed using genomic sequences from ENSEMBL (Kersey et al, 2018). , translated using ExPaSy Translate (Artimo P., et al. 2012.), aligned using CLUSTAL Omega (Letunic L., et al. 2017), and shaded using Boxshade (Artimo P., et al. 2012).




**Figure 4: *SLO-1* Primers Anneal Best at 51.2C.** Agarose gel electrophoresis was used to separate RT-PCR products from reactions performed with different annealing temperatures and tested cDNA. Known DNA sizes (in bp) are noted at the left of the image. Annealing temperatures are noted above each lane.




**Figure 5: *SLO-1* RNA is Alternativatively Spliced in Response to Ethanol Exposure.** Agarose gel electrophoresis was used to separate RT-PCR products from reactions performed with cDNAs isolated from nematodes treated with saline or ethanol. Known DNA sizes (in bp) are noted at the left of the image. Estimates of the observed DNA fragment sizes are noted in bp at the right of the image.




**Figure 7: SLO-1 Functional Domain Structure.** The amino acid reference sequence was used to search for known protein domains using SMART domain analysis (Wu CH, et al. 2003.). The blue boxes represent the seven transmembrane regions. Seven membrane passes are common for channel proteins. The purple boxes represent low complexity regions. The protein isoform generated by alternative splicing would be truncated such that half of the membrane regions would be missing,

## CONCLUSIONS

- *SLO-1* primer annealing occurred best at the annealing temperature of 51.2 ˚C.
- Alternative splicing occurred in nematodes exposed to saline and to ethanol and was different between the two treatments.
- Saline-treated nematodes produced five cDNA fragments, one of which corresponds to the reference sequence (213 bp). The remaining fragments are larger and unpredicted, and thus, represent new alternative splicing patterns.
- Ethanol-treated nematodes produced two cDNA fragments, one of which corresponds to the reference sequence (213 bp) and one other larger unpredicted fragment that is in common with saline-treated nematodes.
- The reference sequence RNA is predicted to produce a large protein with 7 transmembrane regions typical of channel proteins, but the isoform is predicted to produce a truncated protein lacking several of these characteristic domains, thus, it is likely non-functional. It is unknown whether the unpredicted, observed isoforms would produce functional protein.
- Based on these analyses, ethanol treatment induces a switch in *SLO-1* alternative splicing such that more functional channels may be produced.

## FUTURE DIRECTIONS

- The next step in this research would be to determine the exact sequence of the unexpected isoforms and whether they may protein functional or non-functional protein.
- Variation in the exposure time and concentration would be interesting to examine as well as other alcohol types.

## LITERATURE CITED

Alaimo JT, Bettinger JC, Burnette CR, Davies AG, Davis SJ, Grotewiel M, Pierce-Shimomura JT, Shelton KL, Song SS. 2012. Ethanol metabolism and osmolarity modify behavioral responses to ethanol in *C. elegans*. Alcohol Clin Exp Res. 36(11): 1840-1850

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. ExPASy: SIB bioinformatics resource portal, *Nucleic Acids Res*, 40(W1):W597-W603, 2012.

Bargmann CI, Bonci A, Davies AG, Kim H, McIntire SL, Pierce-Shimomura JT, Thiele TR, VanHoven MK. 2003. A central role of the BK potassium channel in behavioral responses to ethanol in C. elegans. Cell. 115: 655-666.

Bull K, Glautier S, Nolden-Dye L, Hopper NA, Mitchell PH, O'Connor V. 2007. The concentration-dependent effects of ethanol on *Caenorhabditis elegans* behavior. Pharmacogenomics J. 7: 411-417.

Davies AG. 2012. Different genes influence toluene- and- ethanol-induced locomotor impairment in C. elegans. Drug Alcohol Depend. 122(1-2): 47-54.

Dhawn R, Dunsenbery DB, Williams PL. 1999. Comparison of lethality, reproduction, and behavior as toxicological end points in the nematode *Caenorhabditis Elegans*. J Toxicol Environ Health. 58:451-462.

Letunic I., Bork P., 20 years of the SMART protein domain annotation resource, *Nucleic Acids Research*, Volume 46, Issue D1, 4 January 2018, Pages D493–D496, https://doi.org/10.1093/nar/gkx922

Nonet ML, Saifee O, Salkoff L, Wang ZW. 2001. Slo-1 potassium channels control quantal content of neurotransmitter release at the *C. elegans* neuromuscular junction. Neuron. 32: 867-881.

R. J. Kinsella, A. Kähäri, S. Haider, J. Zamora, G. Proctor, G. Spudich, J. Almeida-King, D. Staines, P. Derwent, A. Kerhornou, P. Kersey, P. Flicek. 2011.Ensembl BioMarts: a hub for data retrieval across taxonomic space*Database: the journal of biological databases and curation.* doi:10.1093/database/bar030

Salkoff, L., Butler, A., Ferreira, G. *et al.* High-conductance potassium channels of the SLO family. *Nat Rev Neurosci* **7,** 921–931 (2006) doi:10.1038/nrn1992

Untergasser A., Nijveen H., Rao X., Bisseling T., René G., and Leunissen J.A.M. 2006. Primer3Plus, an enhanced web interface to Primer3 Nucleic Acids Research. doi:10.1093/nar/gkm306

Wu CH, Lai-Su LY, Hongzhan H, Arminski L, Castro-Alvear J, Chen Y, Hu Z, Kourtesis P, Ledley RS, Suzek BE, Vinayaka CR, Zhang J, Barker WC. 2003. The protein information resource. Nucleic Acids Res [internet]. [cited 2019 Nov 20]; 31(1) 345-347. available from doi: 10.1093/nar/gkg040