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Examining the Role of RGS2 in the Maintenance of Diapause After Anoxic Stress in Embryos of the Annual Killifish Austrofundulus limnaeus

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Examining the Role of RGS2 in the Anoxic Stress Response of Diapausing Embryos of the Annual Killifish *Austrofundulus limnaeus*

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References

Abstract

Austrofundulus limnaeus live in ephemeral ponds of Venezuela. They have evolved a unique life history that includes embryonic diapause, a period of developmental dormancy, metabolic arrest, and reduced protein synthesis. Diapausing embryos are resistant to environmental stress and survive months without oxygen (anoxia). A. limnaeus' anoxia tolerance is an important survival mechanism as they can be buried in anoxic soil during development. Analysis of RNAseq data from diapausing A. limnaeus embryos show a significant down-regulation of RGS2 transcripts in embryos in anoxia. RGS2 regulates G-protein receptor signaling by inhibiting associated Gα-proteins and can inhibit protein synthesis[1]. I hypothesis that RGS2 acts as a "brake" for protein synthesis in diapausing embryos, allowing the embryos to respond to stress and aid in the maintenance of diapause post stress. To evaluate this hypothesis, I will use vivo-morpholinos to inhibit RGS2 in diapausing embryos. If RGS2 is the "brake" for protein synthesis, protein synthesis should continue after inhibition. If the inhibition of protein synthesis is required to maintain diapause, then RGS2-inhibited embryos should break diapause before non RGS2-inhibited embryos post anoxia. Understanding how A. limnaeus responds to anoxia and maintains dormancy could lead to a better understanding how stress is handled in dormancy.

Background: RNASeq Data From Annual Killifish Embryos

A	Control (normoxia)	Short Exposure (4 hours anoxia)	Long Anoxia (24 hours anoxia)	Short recovery (2 hours Normoxia)	Long recovery (24 hours Normoxia)	
0 days post diapause (dpd)	4	4	4	4	4	

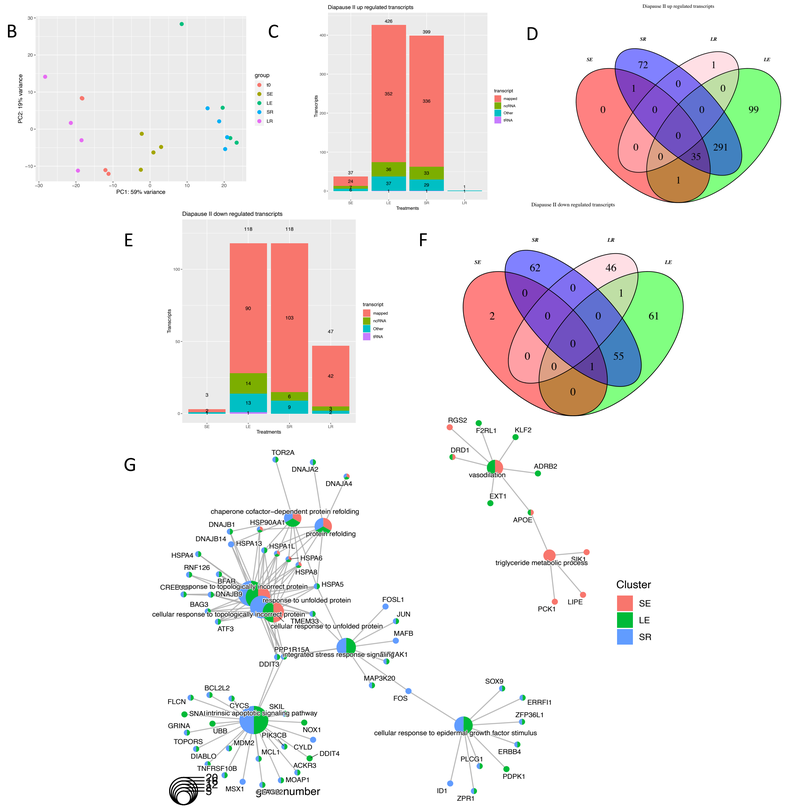
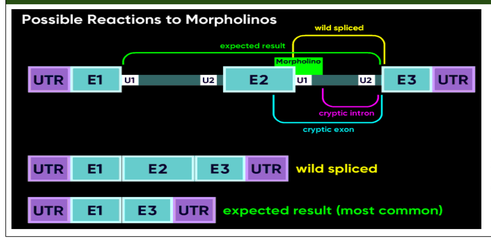


Figure 1: (A) The number of transcriptomes (RNA library) made at each of the 5 conditions and 4 stages of development. Each RNA library had approximately 27,000 Loci. The 0dpd developmental time point was used for the following analysis. **(B)** PCA analysis of diapause 2 RNA seq analysis (t0= normoxia, SE= short exposure, LE = long exposure, SR= short recovery, LR= long recovery[2]. **(C)** Significantly upregulated transcripts ($p < .05$, $\log_2$ fold change > .5) in all experimental conditions. (mapped = transcripts mapped to Homo sapiens ensembl ID, other = transcripts not fallen into the other listed categories[3]. **(D)** Venn diagram of all upregulated transcripts across experimental conditions. **(E)** Significantly downregulated transcripts ($p < .05$, $\log_2$ fold change < .5) in all experimental conditions. **(F)** Venn diagram of all downregulated transcripts across experimental conditions. **(G)** KEGG gene ontology analysis of all significantly expressed transcripts across all experimental conditions using cluster analysis[4,5].

Background: Vivo-morpholino



Vivo-Morpholinos are antisense RNA sequences with a linked moiety for use in in vivo experiments.

Figure 2: Vivo-morpholinos that target exon/intron splice junctions can cause exon skipping in preprocessed RNA transcripts that can lead to a complete knock down or nonfunctional proteins[6]

Methods: Microinjection of Vivo-morpholinos

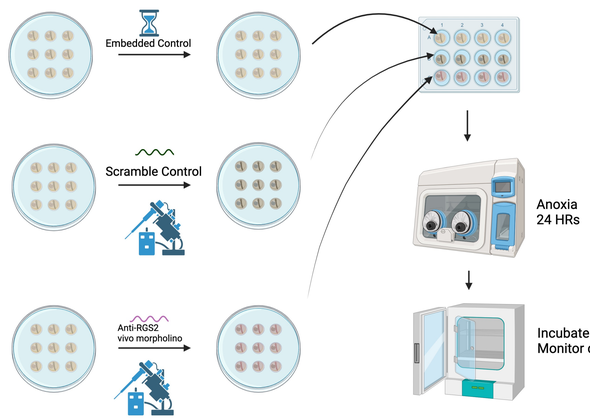


Figure 3: Three groups of diapause 2 embryos are embedded in agarose; control embryos are only embedded, scramble control embryos are microinjected with a non target vivo-morpholino, and the treated group in micro injected with anti RGS2 vivo morpholino. After injection embryos are placed into a plate and exposed to anoxia for 24 hours. After exposure embryos are monitored daily to detect breaking from diapause[7].

Methods: RNA extraction and RT PCR

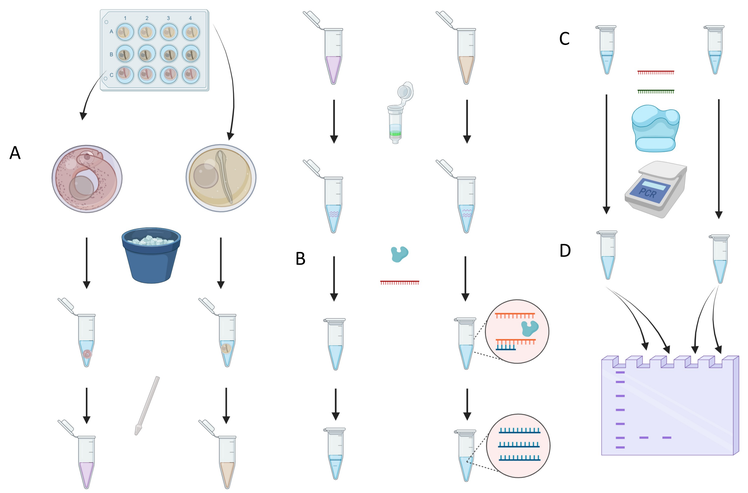


Figure 4: To confirm that treated embryos did receive a dose of the anti-RGS2 vivo- morpholino (A) Embryos that break diapause and those that do not are flash frozen in liquid nitrogen and undergo RNA extraction using Qiagen's total RNA extraction kit. (B) The total RNA is converted to cDNA libraries using Qiagen's reverse transcriptase kit. (C) The cDNA libraries then undergo a PCR reaction with custom IDT primers that start in exon 2 and end in exon 4 of RGS2. (D) After PCR, the products are used in gel electrophoresis , samples derived from embryos treated with anti-RGS2 vivo-morpholinos should show no banding or light bands below the 500 bp while control embryos should produce bands around 600 bp[7].

Results

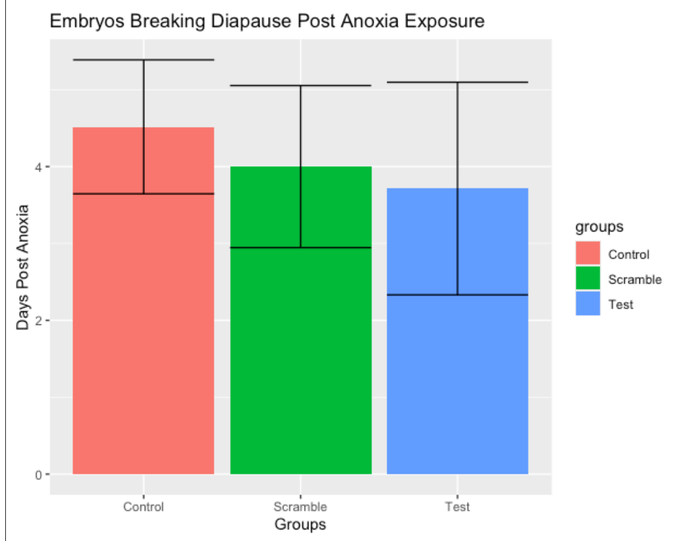


Figure 6: Bar plot of days until diapause 2 was broken in control and test embryos exposed to 30 hours of anoxia. Data current as of 5/7/2024. Control (N=30, mean = 4.52 days, SD = .87) Scramble (N=10, mean = 4 days, SD = 1.05) Test (N=14, mean = 3.71 days, SD = 1.38)

Discussion

- Transcriptomic sequencing as a tool is helpful for researchers looking for genetic pathways and/or genes of interests in various experimental conditions.
- Vivo-morpholinos allow for knock down/out of target proteins in vivo via interruptions in RNA transcripts.
- According to the results knock down/ out of RGS2 has no significant effect on the maintenance of diapause 2 in embryos that have been challenged with anoxia.

Future Directions

- Complete more replicates of the experiment to avoid low Ns due to initial die off of embryos, and to confirm results.
- Find additional targets to investigate anoxia tolerance in diapause 2 embryos using the RNAseq data.
- Compare these data to other vertebrate models of anoxia tolerance.
- Reverse transcriptase PCR to confirmed knock down/out of RGS2 in anti-RGS2 vivo-morpholino treated embryos.

Acknowledgements

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