Functional Testing of a Stress Adaptation Biosignature Observed in the Devil Worm

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Abstract:

Halicephalobus mephisto was discovered nearly 1.3 kilometers below Earth's surface in the Beatrix Gold Mine of South Africa. When underground, the organism was found to be residing in extreme environmental conditions that could prove to be very strenuous on the organism, such as heated (37 °C), methane-rich, hypoxic water. Genome sequencing of the nematode indicated that there were expansions of the 70 kilodalton heat shock protein (Hsp70) and *avrRpt2* -induced genes (AIG1), with both gene families having been previously identified as cellular survival genes. This expansion of both Hsp70 and AIG1 gene families were also found to be convergently expanded in distantly related Mollusks, with expansion specifically present in bivalves. The presence of these convergently expanded gene families within two distantly related animal phylum that both routinely endure environmental stressors such as hypoxia and elevated temperatures can indicate a biosignature response to environmental stress. However, to identify if the unique gene family expansion is truly a biosignature of environmental stress, the survival of the organism must be tested with the inactivation of the expanded gene families. In this work we are exploring the function of these genes, starting with a proposed master regulator gene: ARMET/MANF (Arginine-Rich Mutated in Early State Tumors / Mesencephalic Astrocyte Derived Neurotrophic Factor). RNA interference will allow the inactivation of suspected intermediate regulator proteins such as ARMET, which could then cause a change in either AIG1 or Hsp70 expression and ultimately lead to organismal impairment or death.

Introduction:

- Conditions at which the nematode indicate extremophilic abilities
- Gene Expansion of 70 kilodalton heat shock protein (Hsp70) and *avrRpt2* -induced genes (AIG1)
- Transcriptome analysis: 675 statistically downregulated transcripts and 285 statistically upregulated transcripts when induced by heat
- Upregulation of Hsp70 under heat, but no fold change in HSF-1
- ARMET/MANF proposed as the central regulator of Hsp70 and AIG1
- 30-fold statistically significant increase in the expression of ARMET/MANF when induced by heat

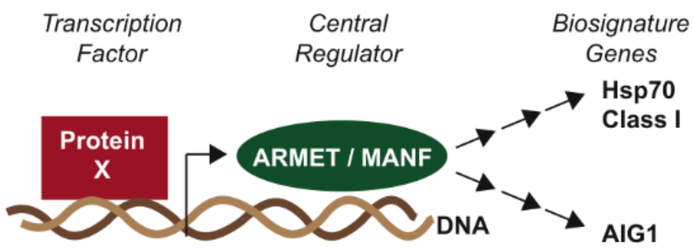


Figure 1. ARMET/MANF as a central regulator of Hsp70 & AIG1 biosignature genes

Methods:

- Knockdown of ARMET/MANF through RNAi feeding when exposed to a biological stressor such as Tunicamycin
  - Designed RNAi constructs for *H. mephisto*
  - Fed constructs to worms when exposed to tunicamycin
  - Quantification of worm length
  - qPCR to determine mRNA expression of ARMET/MANF

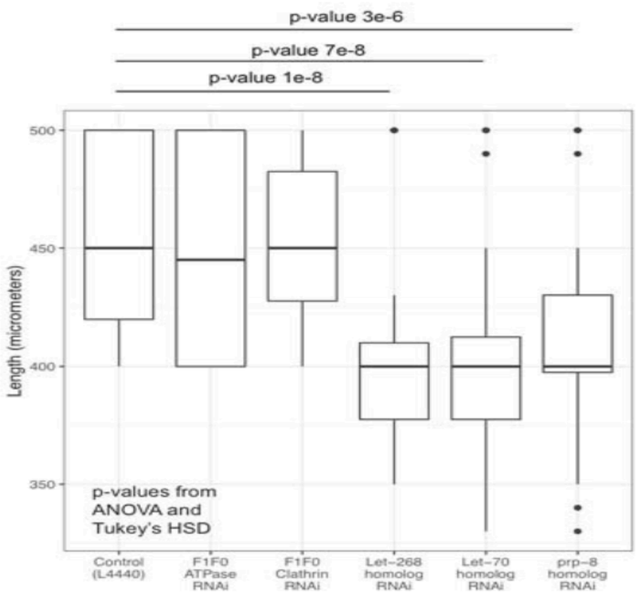


Figure 2. Proof-of-Concept RNAi knockdowns with 3 single copy core eukaryotic genes, 1 pre-mRNA splicing factor, and 2 *C. elegans* lethal genes

Results:

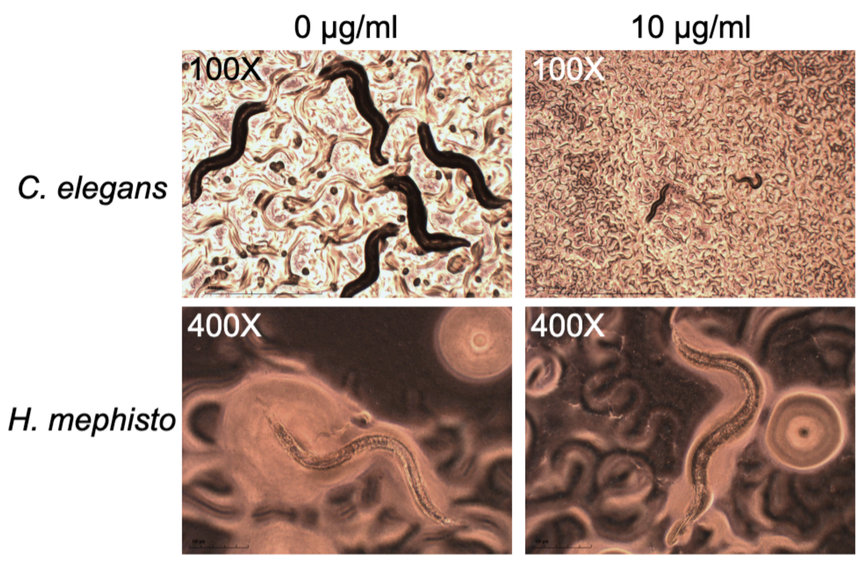


Figure 3. Response to tunicamycin in *C. elegans* vs. *H. mephisto*

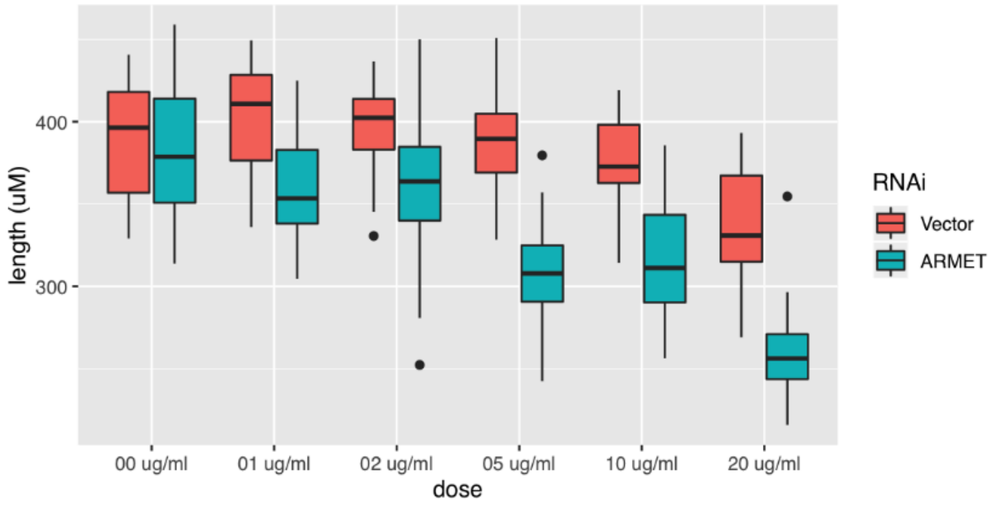


Figure 4.Quantifiction of worm lengths on feeding ARMET/MANF RNAi

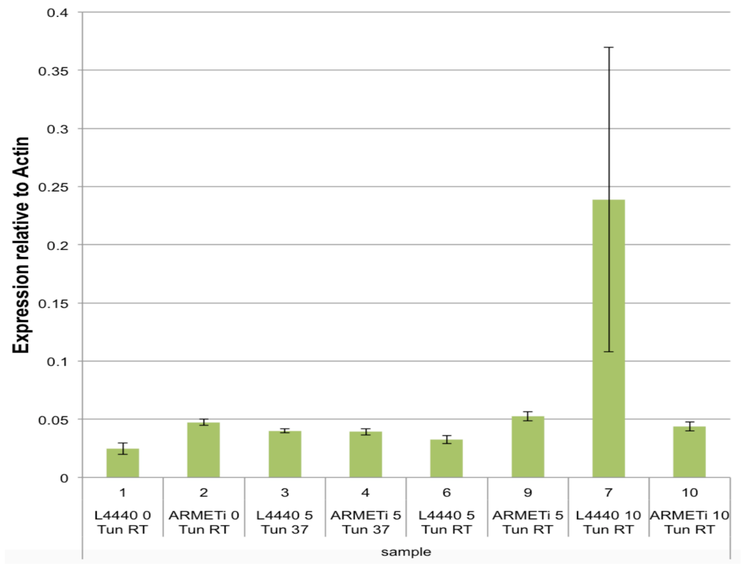
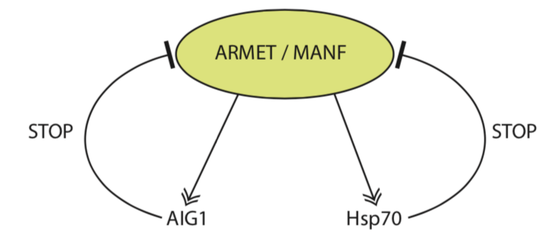


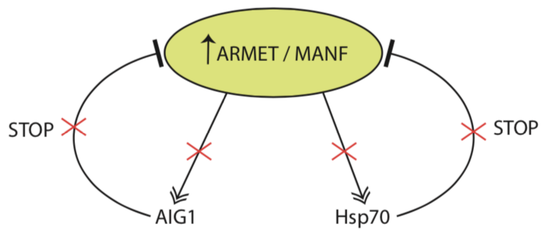
Figure 5. ARMET/MANF expression after RNAi knockdown relative to Actin expression

Proposed ARMET/MANF Function:

NORMAL FUNCTION:



KNOCKDOWN FUNCTION:



Future Directions

- Confirmation of increased AMRET/MANF expression after knockdown and biological stressor exposure
- ARMET, AIG1, and Hsp70 expression analysis to confirm or deny the proposed feedback mechanism
- ARMET, AIG1, and Hsp70 expression analysis on varying days – worms will be exposed to biological stressors/RNAi and RNA will be collected on different days to establish a "time frame" for the proposed feedback mechanism
- Observation of downstream effects due to molecular knockdown – are other genes statistically upregulated or downregulated in response to inhibition and manipulation of the ARMET feedback loop?

References:

Borgonie, G., Garcia-Moyano, A., Litthauer, D., Bert, W., Bester, A., van Heerden, E., Moller, C., Erasmus, M. & Onstott, T. C. Nematoda from the terrestrial deep subsurface of South Africa. *Nature* 474, 79-82, doi:10.1038/nature09974 (2011).

Weinstein, Deborah J., Sarah E. Allen, Maggie C. Y. Lau, Mariana Erasmus, Kathryn C. Asalone, Kathryn Walters-Conte, Gintaras Deikus, et al. "The Genome of a Subterrestrial Nematode Reveals Adaptations to Heat." *Nature Communications*10, no. 1 (November 21, 2019): 5268. https://doi.org/10.1038/s41467-019-13245-8.

Guerin, M. N., Weinstein, D. J. & Bracht, J. R. Stress Adapted Mollusca and Nematoda Exhibit Convergently Expanded Hsp70 and AIG1 Gene Families. *J Mol Evol*, doi:10.1007/s00239-019-09900-9 (2019).