Understanding the role of MANF / ARMET in the stress response of a subterrestrial nematode

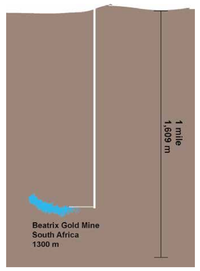
Megan N. Guerin[1], John R. Bracht[1]

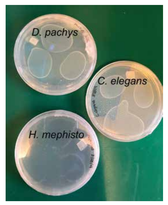
AFFILIATION: [1] Biology Department, American University, Washington DC 20016, USA

ABSTRACT

The nematode *Halicephalobus mephisto* was originally discovered inhabiting a deep terrestrial aquifer 1.3 km underground. This organism thrives under conditions of abiotic stress including heat and minimal oxygen, isolated from the surface biosphere and feeding on chemolithotrophic bacteria. The genome of this unique organism exhibits a dramatically expanded repertoire of 70 kilodalton heat-shock proteins (Hsp70) and avrRpt2 induced gene 1 (AIG1) proteins. While these genes are likely critical to the stress resistance of this organism, additional regulatory factors controlling the stress response in this organism remain undiscovered.Here show that the ER stress-activated gene mesencephalic astrocyte-derived neurotrophic factor (MANF), also known as Arginine-rich, mutated in early-stage tumors (ARMET), is one of the three most upregulated genes under heat stress. We further show that inactivation of MANF/ARMET inhibits growth of *H. mephisto* on the antibiotic tunicamycin, an inducer of ER stress. Therefore, we propose this is a central regulatory factor in the adaptation of *H. mephisto* to heat and other stresses.This work sheds light on the genomic basis of stress tolerance in a complete subterrestrial eukaryotic genome. Further, our work establishes H. mephisto as an emerging model organism for functionally testing the evolutionary adaptations of an extremophile metazoan.

Discovery of *Halicephalobus mephisto*

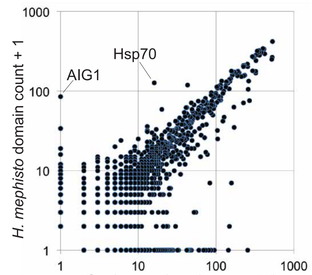


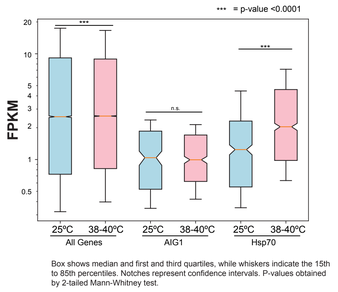


4-day culture at 37°C

Conditions in subterrestrial aquifer:
- warm (37°C)
- hypoxic (0.42 - 2.3mg/L dissolved O_2)
- high methane
- thriving microbial communities

A Signature of Stress Adaptation





Box shows median and first and third quartiles, while whiskers indicate the 15th to 85th percentiles. Notches represent confidence intervals. P-values obtained by 2-tailed Mann-Whitney test.

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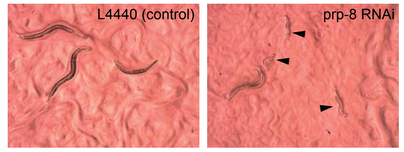
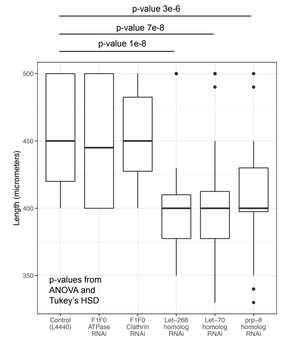
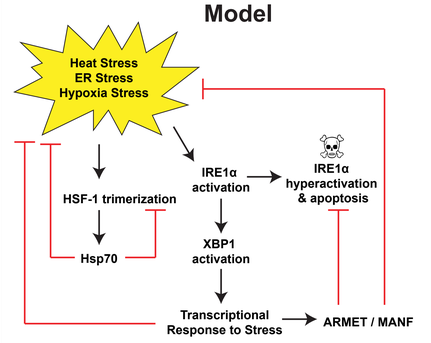
Test of feeding RNAi

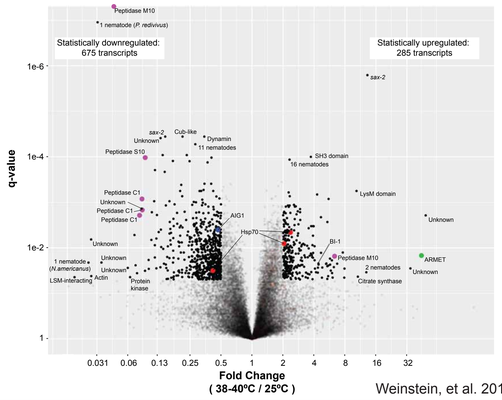




Model



Genome-wide expression changes



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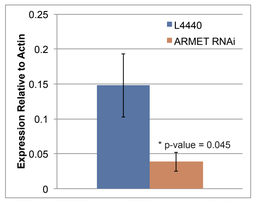
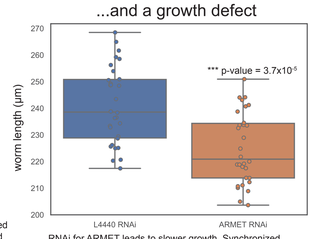
MANF / ARMET RNAi

MANF / ARMET RNAi leads to mRNA knockdown...



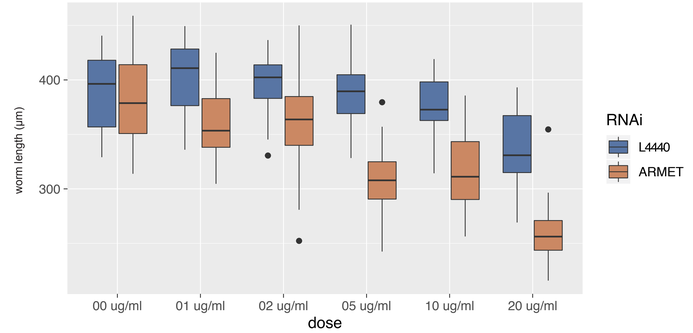
RNAi for ARMET leads to mRNA knockdown. Synchronized L1 *H. mephisto* worms were cultured on the indicated food source at 35ºC for 72 hr with 5mg/mL Tunicamycin included in the RNAi plates. Shown are RT-qPCR relative to actin for n=3 replicates, each measured in triplicate. Error bars represent standard error of the mean (SEM) and p-value derived from Student's T-Test.

...and a growth defect



RNAi for ARMET leads to slower growth. Synchronized L1 *H. mephisto* worms were cultured on the indicated food source at 35ºC for 72 hr with 5mg/mL Tunicamycin included in the RNAi plates. At the end of 72 hr n=29 worms from each treatment were measured. Boxplots indicate inter-quartile range, with median indicated by horizontal line and extreme values as whiskers. P-value derived from derived from Student's T-Test.

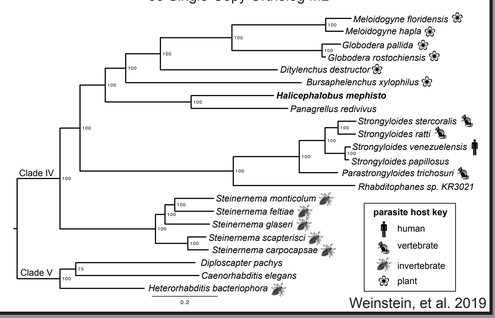
Tunicamycin x MANF / ARMET RNAi Interaction



ARMET RNAi interacts with Tunicamycin in generating a growth phenotype. Tunicamycin treatment indicated on X-axis (dose). Synchronized *H. mephisto* worms were plated as L1 and cultivated for 5 days at room temperature (22°C) prior to measurement of lengths. At least n=20 worms measured for each dose. By ANOVA Vector vs. ARMET are statistically different; p-value < 3e-14.

Nematode Phylogeny

99 Single-Copy Ortholog ML



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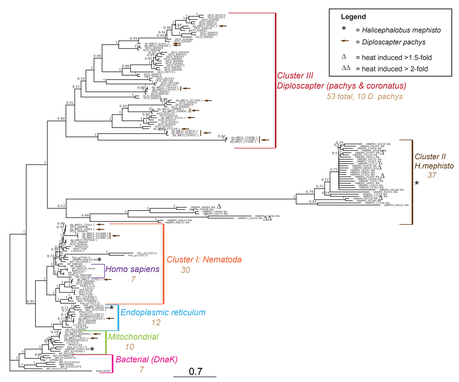
Hsp70 Bayesian tree



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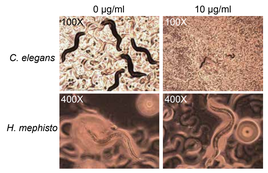
Tunicamycin Resistance of *H. mephisto*

A. Microsopy of tunicamycin-treated worms



B. Quantification of worm lengths

H. mephisto exhibits resistance to ER stress, which is induced with tunicamycin. A, Light microscopy images of *C. elegans* (control) and *H. mephisto*. Note differences in magnification because *H. mephisto* are much smaller than *C. elegans*. B. Quantification of worm lengths on tunicamycin. Note *H. mephisto* are consistently longer than *C. elegans* control worms, indicating they are less affected by the drug.

Future directions

- Test effect of knockdown of HSF-1, Hsp70, and AIG1 on survival at 37ºC.
- Test microinjection and transgenic array formation.
-Develop CRISPR technology in *H. mephisto.*
- Add *H. mephisto* genes to *C. elegans.*
- Test hypoxia stress.
- A new model system for stress adaptation research!

References

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Contact

John R. Bracht, Ph.D
Associate Professor of Biology
Email: jbracht@american.edu
Follow me on Twitter: @BrachtLab