microPublication BIOLOGY

9/29/2026 - *Open Access*

Role of vitellogenins on the mitochondrial unfolded protein response

Nathanial A. Jordan[1], Suzanne Angeli[1§]

[1]Molecular and Biomedical Sciences, University of Maine

[§]To whom correspondence should be addressed: suzanne.angeli@maine.edu

Abstract

Sex-specific differences influence aging and disease onset. Using *Caenorhabditis elegans*, we previously found that hermaphroditic nematodes activate a robust mitochondrial unfolded protein response (UPR^{mt}) in the intestines, while female nematodes do not. We hypothesized that the accumulation of vitellogenins in females was suppressing the UPR^{mt}. Loss of vitellogenins via RNAi enhanced the UPR^{mt} in hermaphrodites but had no impact on the UPR^{mt} of females unless a combination of vitellogenins was simultaneously knocked down. Furthermore, combinatorial loss of vitellogenins did not restore the female UPR^{mt} to hermaphroditic levels. We conclude that additional factors besides vitellogenins are involved in suppressing the intestinal UPR^{mt} in female nematodes.

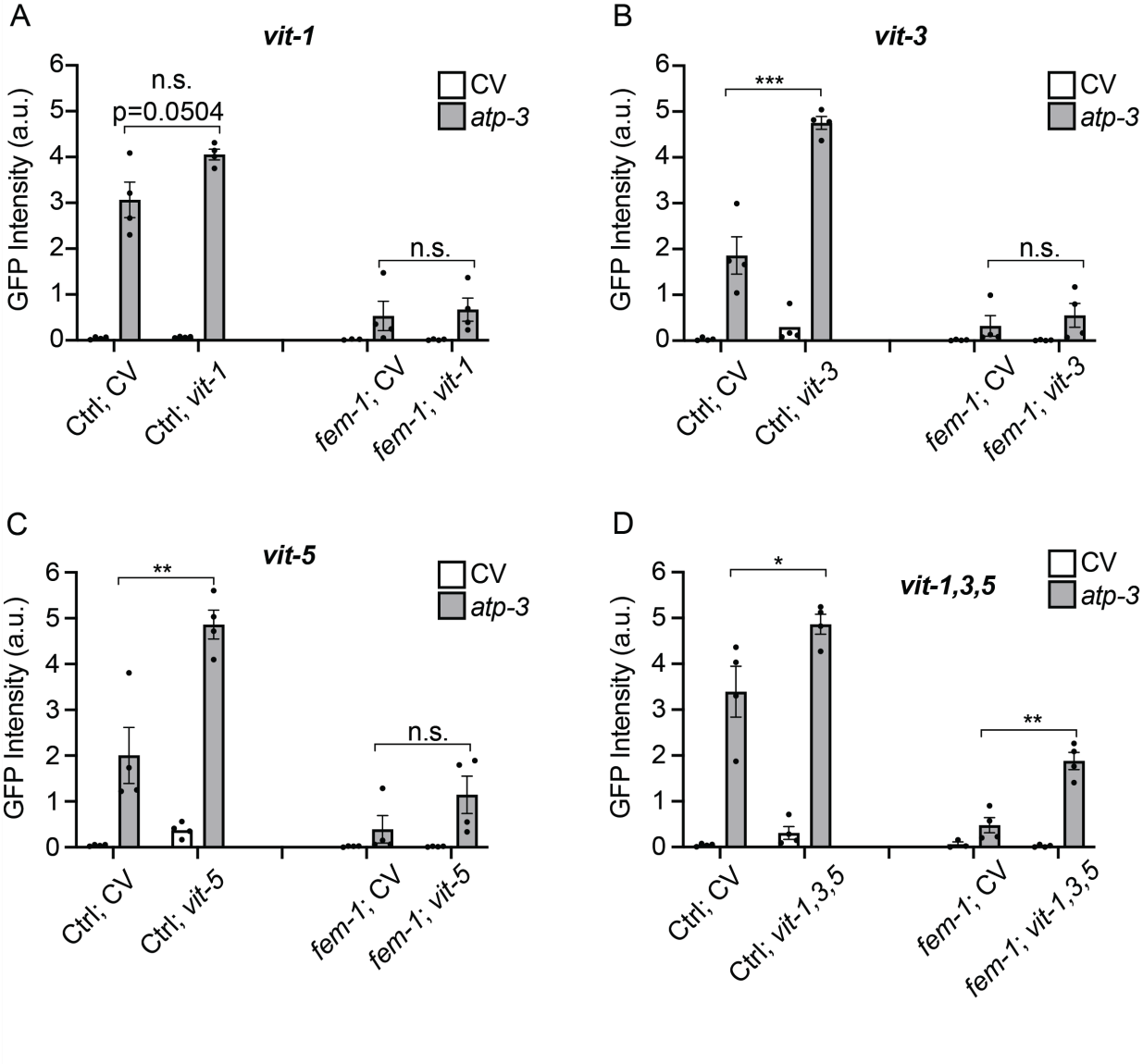

Figure 1. Impact of vitellogenin RNAi on the UPR^{mt}:

A-D. Quantification of GFP intensity from *phsp-6*::GFP or *fem-1*(*hc17*); p*hsp-6*::GFP nematodes. For single vitellogenin gene knockdown, nematodes were developed on either control vector (CV) RNAi or *vit-1* (*A*), *vit-3* (*B*), or *vit-5* (*C*) RNAi

at 25°C. At the young adult stage, control worms were shifted to 2o°C and onto RNAi plates seeded with CV or CV/*atp-3* (50%/50% RNAi mixture). Vitellogenin RNAi treated worms were shifted to 2o°C and onto RNAi plates seeded with the indicated CV/*vit* RNAi or *vit*/*atp-3* (50%/50% RNAi mixture). For the combinatorial vitellogenin gene knockdown (*D*), nematodes were developed on either control vector (CV) RNAi or *vit-1*, *vit-3*, *vit-5* (33%/33%/33% mixture) RNAi at 25°C. At the young adult stage, vitellogenin RNAi treated worms were shifted to 2o°C and onto RNAi plates seeded with the CV/*vit-1*,*3*,*5* RNAi (50%/50% combinatorial RNAi mixture) or *vit-1*,*3*,*5*/*atp-3* (50% combinatorial mixture/50% *atp-3* RNAi). Error bars represent ± SEM. ***$p \leq 0.001$, **$p \leq 0.01$, and *$p \leq 0.05$.

Description

Age-related diseases, such as cardiovascular disease, cancer, and neurodegenerative syndromes can display sex-specific differences. Since mitochondrial dysfunction is central to most age-related diseases, we examined the mitochondrial unfolded protein response (UPR^{mt}), a conserved, broad-range transcriptional response that, among other functions, aids in the refolding of mitochondrial matrix proteins (Kim, Ramalho, and Haynes 2024). In *C. elegans*, UPR^{mt} activation is largely localized to the intestine, the major metabolic tissue in the nematode. In addition to digestion, the intestine also carries out many functions similar to the liver, such as detoxification, immunity, and fat metabolism (Dimov and Maduro 2019). We and others recently discovered that the activation of the intestinal UPR^{mt} depends on the function of another tissue: the germline (Foulger et al. 2025; Charmpilas et al. 2024; Shen et al. 2024; Zhou et al. 2024)· Specifically, we found that adult nematodes with actively proliferating germlines, such as hermaphrodites or mated females, can activate a robust UPR^{mt} in the intestines when challenged with either a high dose of the metal manganese (Mn) or RNAi of the OSCP/*atp-3* subunit of complex V (Foulger et al. 2025). Conversely, adult nematodes with mutations that lead to a lack germline stem cells or lack of sperm, such *glp-1* or *fem-1* mutants, cannot activate the intestinal UPR^{mt}. Additionally, we found that loss of the transcription factor FOXO/*daf-16* fully restored the UPR^{mt} in *fem-1* mutants (Foulger et al. 2025). Here, we probe whether vitellogenesis, which occurs in the intestines and is dependent on FOXO/*daf-16*, impacts the UPR^{mt}.

Vitellogenins are precursor yolk proteins that provide nutrients to developing embryos. In *C. elegans*, the highly conserved genes *vit-1* through *vit-6* are responsible for encoding these proteins (Perez and Lehner 2019). In hermaphrodites, they are synthesized in the intestines and transported into the pseudocoelom and to maturing oocytes (Perez and Lehner 2019). Young adult *fem-1* mutants display elevated yolk content compared to wild-type hermaphrodites at Day 1 of adulthood (DePina et al. 2011), presumably due to a lack of vitellogenin transport out of the intestines to maturing oocytes. We used RNAi to knockdown *vit-1*, *vit-3*, *vit-5*, or all three genes simultaneously (*vit-1*,*3*,*5*) and monitored intestinal activation of the UPR^{mt} in young adult nematodes. We used the UPR^{mt} reporter in which the GFP expression is driven by the promoter of the mitochondrial chaperone mtHsp70/*hsp-6* (Yoneda et al. 2004). We additionally used RNAi of OSCP/*atp-3* to induce the UPR^{mt} in young adult nematodes as previously described (Angeli et al. 2021). We found that in hermaphroditic nematodes, knockdown of *vit-1* trended toward elevating the intestinal UPR^{mt} (p=0.0504) and knockdown of *vit-3*, *vit-5*, or *vit-1*,*3*,*5* all significantly elevated the UPR^{mt} (**Figure 1A-1D**). In contrast, knockdown of *vit-1*, *vit-3*, or *vit-5* did not significantly alter the UPR^{mt} of *fem-1* mutants (**Figure 1A-1D**). The knockdown of *vit-1*,*3*,*5* did significantly enhance the UPR^{mt} of the *fem-1* mutants, but not to wild-types levels (**Figure 1D**). One caveat of this study is that we were not able to procure RNAi clones for *vit-2*, *vit-4*, or *vit-6*. However, since *vit-2* is over 80% identical to *vit-1* and *vit-3*/*vit-4* are duplicated genes that are 99% identical (Perez and Lehner 2019), we would expect to observe more impact from individual RNAi knockdown on females if these vitellogenins played an outsized role on the UPR^{mt}. The *vit-6* gene is the most divergent vitellogenin, so it remains possible that it plays an untested role in the UPR^{mt}. Overall, while it does appear that vitellogenins can mildly suppress the adult UPR^{mt}, especially in hermaphroditic nematodes, we conclude that additional germline-to-intestinal signals are responsible for the potent suppression of the UPR^{mt} in female nematodes.

Methods

Strains. Bristol N2 (wild type) nematodes were obtained from the *Caenorhabditis* Genetics Center (CGC, University of Minnesota) and cultured using standard conditions (Sulston J 1988). The following strains were used: GL347 (SJ4100 p*hsp-6*::GFP backcrossed 6× to N2) and GL364 (*fem-1*(*hc17*); p*hsp-6*::GFP).

Nematode and bacterial culture conditions. Nematodes were maintained on nematode growth medium (NGM) plates. NGM plates were seeded with *Escherichia coli* OP50 obtained from CGC that was grown in LB at 37°C for 18 hours shaking at 225 rpm. Seeded plates were dried for 48 hours at room temperature before use. For RNAi experiments, HT115 (DE3) bacteria obtained from the Horizon Biosciences RNAi library were used. All RNAi clones were verified via sequencing. RNAi plates were prepared by cooling NGM to 55°C and supplementing with a final concentration of 50µg/ml carbenicillin and 1 mM isopropyl β-d-1-thiogalactopyranoside (IPTG). RNAi bacteria were inoculated with one

colony of RNAi bacteria into LB with 50µg/ml carbenicillin and were grown shaking overnight for 18 hours at 37° at 225 rpm. RNAi cultures were seeded on RNAi plates and allowed to grow for 48 hours at room temperature. Plates were stored at 4 °C for no longer than 2 weeks.

Microscopy. Nematodes were anesthetized with 2 - 5mM levamisole and mounted on 2% agarose pads on glass slides. Fluorescence micrographs of GFP were taken using a Zeiss Axioscope 5 fluorescent compound microscope equipped with Zen microscopy imaging program. GFP expression was enhanced using the brightness/contrast tool in Adobe Photoshop. The same parameters were used for all images. GFP intensity of nematodes was quantified using ImageJ 1.54G. The “integrated density” of GFP expression and length of nematodes was measured using ImageJ tools. Integrated density value was normalized by number of nematodes and average length of nematodes. The final value is in arbitrary units.

Statistics. Significance between control and experimental groups was determined by using a two-tailed Student's *t*-test. Asterisks denote corresponding statistical significance: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. Error bars were generated using the standard error of the mean (SEM), typically from three or more pooled biological replicates.

Acknowledgements: We thank the *Caenorhabditis* Genetics Center (CGC), which is funded by the NIH Office of Research Infrastructure Programs (P40 OD010440).

References

Angeli S, Foulger A, Chamoli M, Peiris TH, Gerencser A, Shahmirzadi AA, Andersen J, Lithgow G. 2021. The mitochondrial permeability transition pore activates the mitochondrial unfolded protein response and promotes aging. eLife 10: 10.7554/elife.63453. DOI: 10.7554/eLife.63453

Charmpilas N, Sotiriou A, Axarlis K, Tavernarakis N, Hoppe T. 2024. Reproductive regulation of the mitochondrial stress response in Caenorhabditis elegans. Cell Reports 43: 114336. DOI: 10.1016/j.celrep.2024.114336

DePina AS, Iser WB, Park SS, Maudsley S, Wilson MA, Wolkow CA. 2011. Regulation of Caenorhabditis elegans vitellogenesis by DAF-2/IIS through separable transcriptional and posttranscriptional mechanisms. BMC Physiology 11: 10.1186/1472-6793-11-11. DOI: 10.1186/1472-6793-11-11

Dimov I, Maduro MF. 2019. The C. elegans intestine: organogenesis, digestion, and physiology. Cell and Tissue Research 377: 383-396. DOI: 10.1007/s00441-019-03036-4

Foulger AC, Jordan NA, Castle A, Bhaumik D, Andersen JK, Lithgow GJ, Angeli S. 2025. Germline regulation of the intestinal mitochondrial unfolded protein response. GeroScience : 10.1007/s11357-025-01890-5. DOI: 10.1007/s11357-025-01890-5

Kim S, Ramalho TR, Haynes CM. 2024. Regulation of proteostasis and innate immunity via mitochondria-nuclear communication. Journal of Cell Biology 223: 10.1083/jcb.202310005. DOI: 10.1083/jcb.202310005

Perez MF, Lehner B. 2019. Vitellogenins - Yolk Gene Function and Regulation in Caenorhabditis elegans. Frontiers in Physiology 10: 10.3389/fphys.2019.01067. DOI: 10.3389/fphys.2019.01067

Shen K, Durieux J, Mena CG, Webster BM, Tsui CK, Zhang H, et al., Dillin. 2024. The germline coordinates mitokine signaling. Cell 187: 4605-4620.e17. DOI: 10.1016/j.cell.2024.06.010

Sulston J, H.J., *Methods*, in *The Nematode Caenorhabditis elegans*, W.B. W., Editor. 1988, Cold Spring Harbor Laboratory Press Cold Spring Harbor. p. 587-606

Yoneda T, Benedetti C, Urano F, Clark SG, Harding HP, Ron D. 2004. Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. Journal of Cell Science 117: 4055-4066. DOI: 10.1242/jcs.01275

Zhou L, Jiang L, Li L, Ma C, Xia P, Ding W, Liu Y. 2024. A germline-to-soma signal triggers an age-related decline of mitochondrial stress response. Nature Communications 15: 10.1038/s41467-024-53064-0. DOI: 10.1038/s41467-024-53064-0

Funding: NIGMS COBRE Research Pilot Project Subaward 5P20GM144265 - 03 and the UMaine Institute of Medicine. Supported by National Institute of General Medical Sciences (United States) 5P20GM144265 - 03 to Suzanne Angeli.

Conflicts of Interest: The authors declare that there are no conflicts of interest present.

Author Contributions: Nathanial A. Jordan: data curation, methodology, validation, writing - original draft, formal analysis. Suzanne Angeli: conceptualization, funding acquisition, supervision, writing - review editing.

Reviewed By: Anonymous

Nomenclature Validated By: Anonymous

WormBase Paper ID: WBPaper00070205

9/29/2026 - *Open Access*

History: Received August 19, 2026 **Revision Received** September 25, 2026 **Accepted** September 26, 2026 **Published Online** September 29, 2026 **Indexed** October 13, 2026

Citation: Jordan NA, Angeli S. 2026. Role of vitellogenins on the mitochondrial unfolded protein response. microPublication Biology. 10.17912/micropub.biology.002360