RNA interference screen to identify epigenetic regulators of dietary restriction mediated endoplasmic reticulum hormesis and longevity

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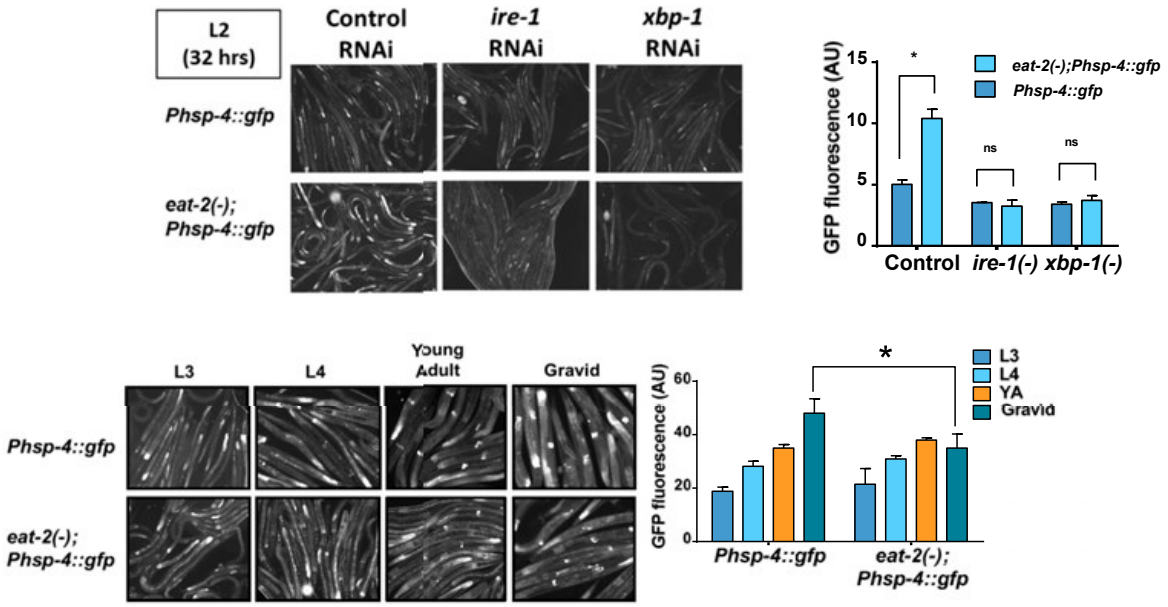
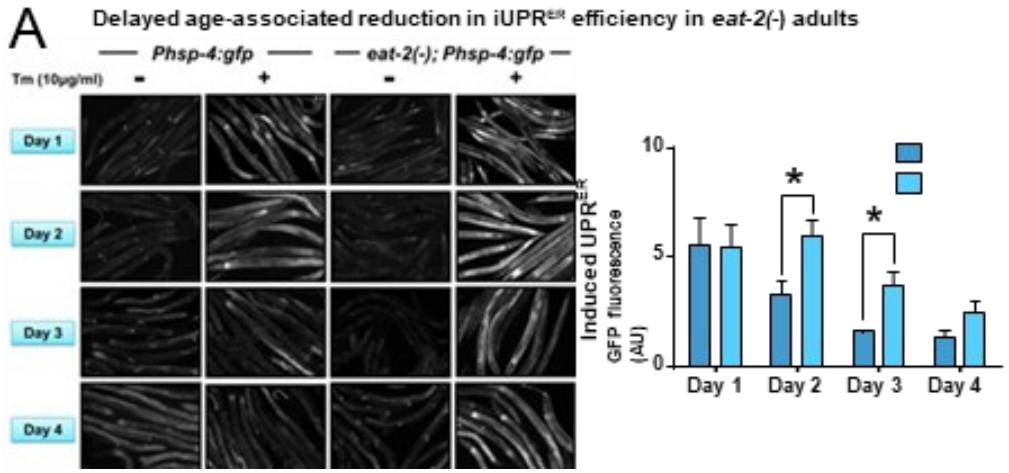
Team – Dr. Arnab Mukhopadhyay, Shivay Parihar, Simran Motwani, Jasleen Kaur

background





Hypothesis : Dietary restriction preconditions the endoplasmic reticulum to increased protein folding stress via the creation of an epigenetic memory

Matai, L., Sarkar, G. C., Chamoli, M., Malik, Y., Kumar, S. S., Rautela, U., Jana, N. R., Chakraborty, K., & Mukhopadhyay, A. (2019). Dietary restriction improves proteostasis and increases life span through endoplasmic reticulum hormesis. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(35), 17383-17392. https://doi.org/10.1073/pnas.1900055116

Methods

C. elegans strains used:
Phsp-4::gfp *(wild type)*
eat-2(-);Phsp-4::gfp *(DR-model)*

Measurement of Endoplasmic reticulum(ER) unfolded protein response(UPR)

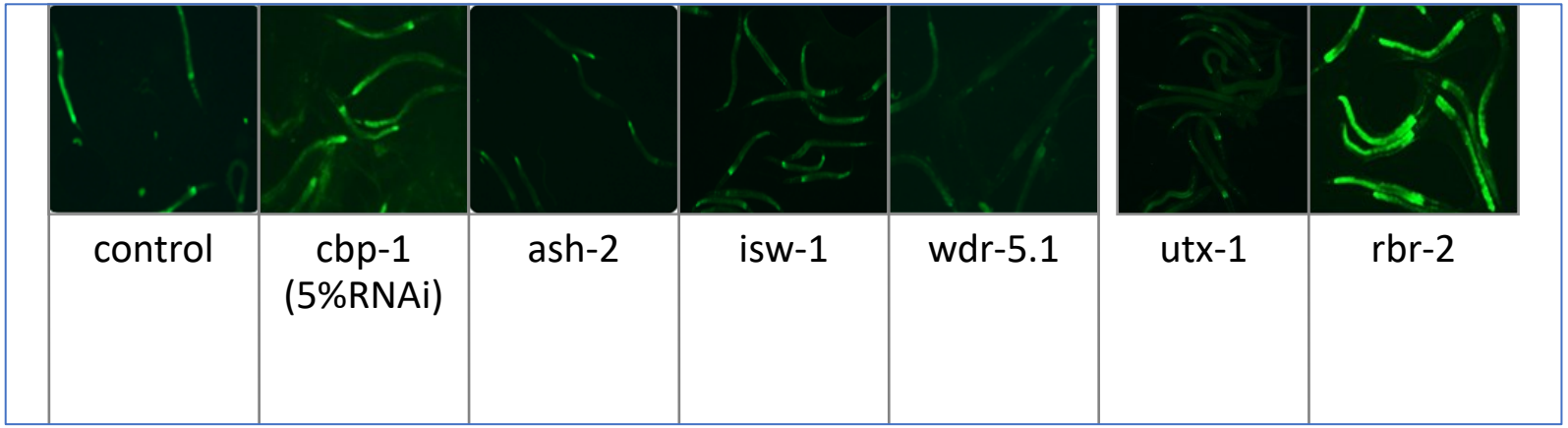
GFP fused with promoter of ER chaperone hsp-4 → Fluorescence microscopy → Quantification of fluorescence intensity

RNA interference experiments

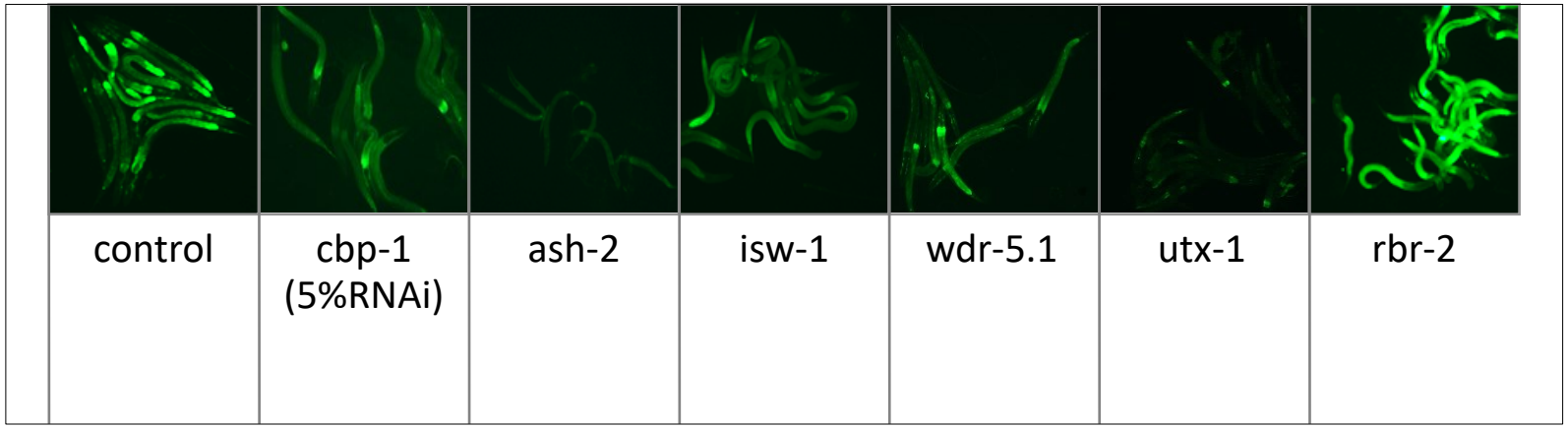
Bacteria expressing dsRNA against gene of interest chosen from RNAi library → Bacteria fed to C. elegans → dsRNA mediated knockdown of gene of interest

For induction of Unfolded protein response- 5ug/ml Tunicamycin treatment used

Results- Transient upregulation of basal UPR^{ER} during larval development with epigenetic knockdown



Phsp-4::gfp *(*wild type)

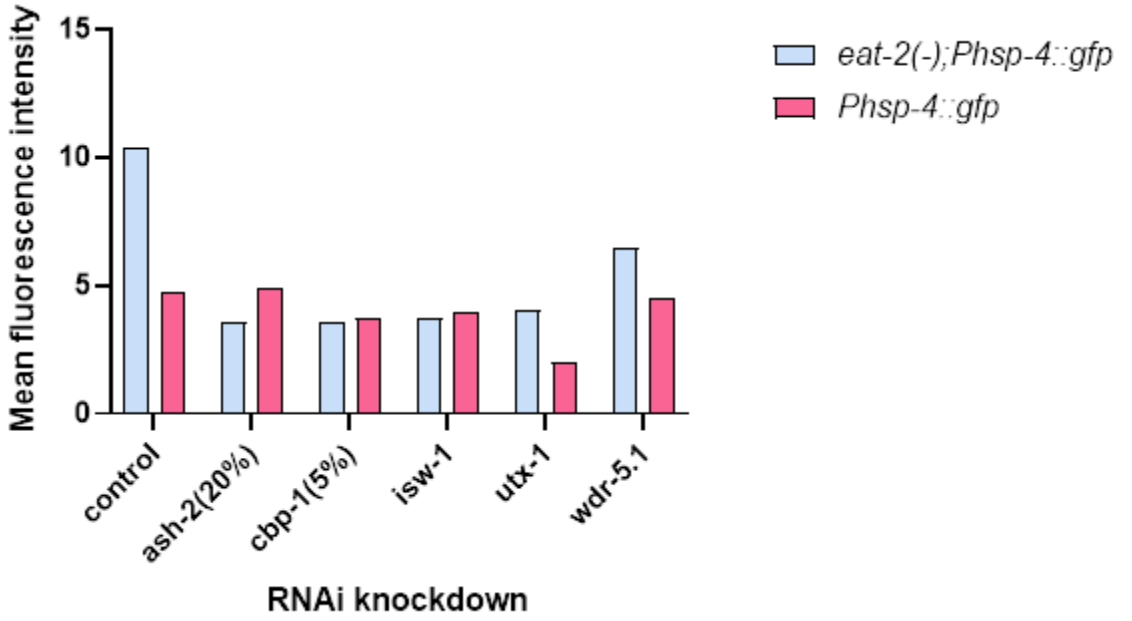


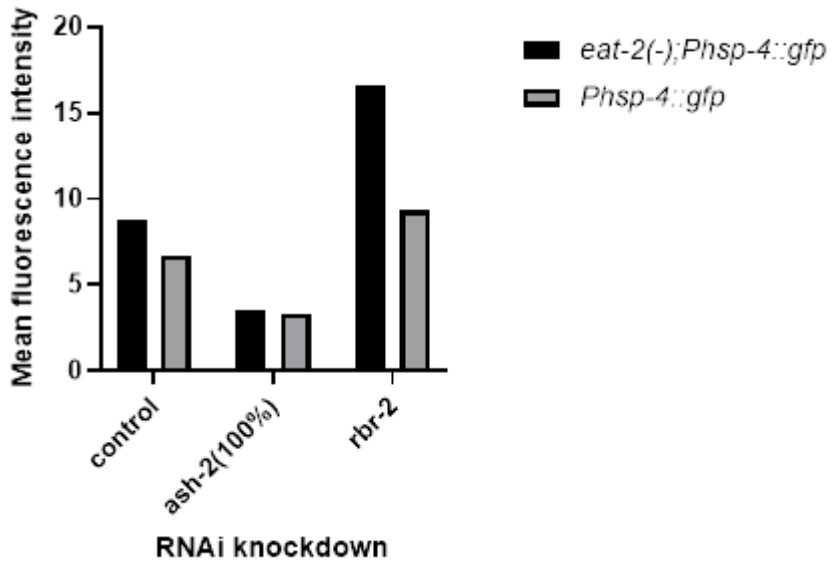
eat-2(-);Phsp-4::gfp (Dietary restriction model)

Epigenetic regulators identified from primary screen

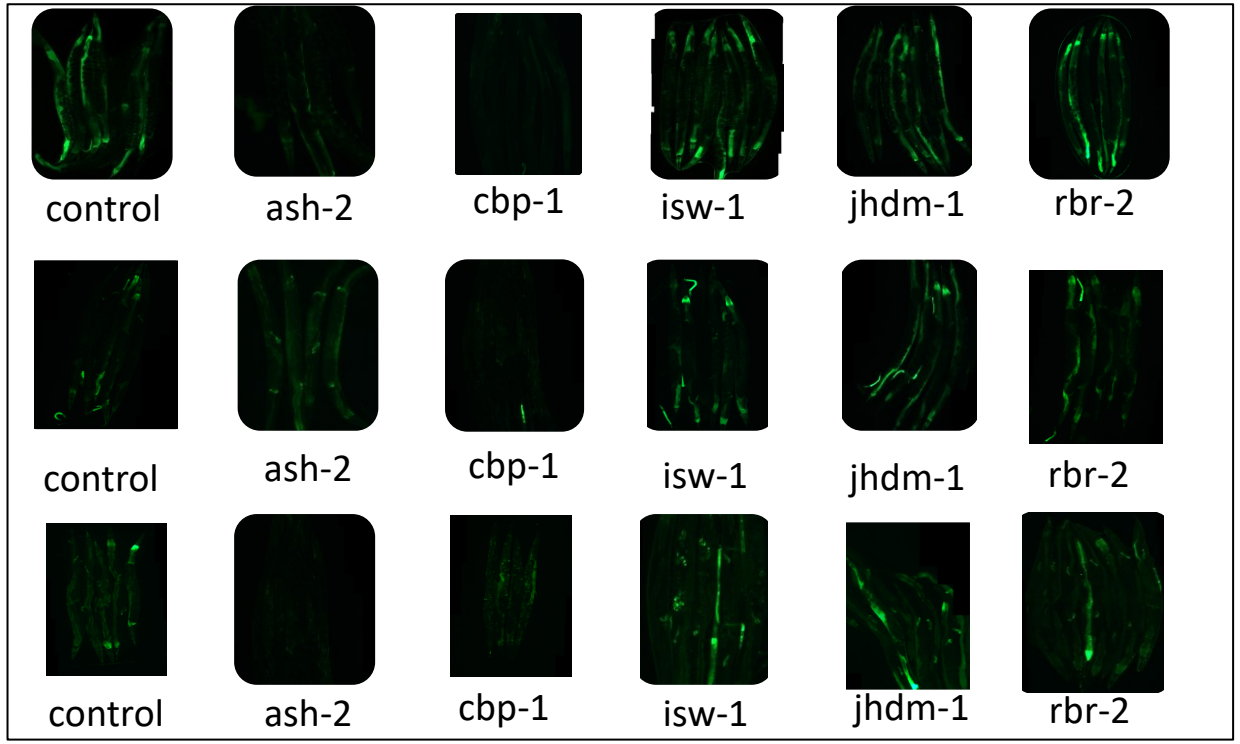
Gene	Modification	Effect on transcription
ash-2	H3K4 trimethylation	Activation
cbp-1	Histone lysine acetylation	Activation
isw-1	Chromatin remodeling	Activation
jhdm-1	H3K9 demethylase	Activation/Repression
rbr-2	H3K4 demethylase	Repression
utx-1	H3K27 demethylase	Activation
wdr-5.1	H3K4 trimethylation	Activation

Results- Quantified basal UPR^{ER} levels

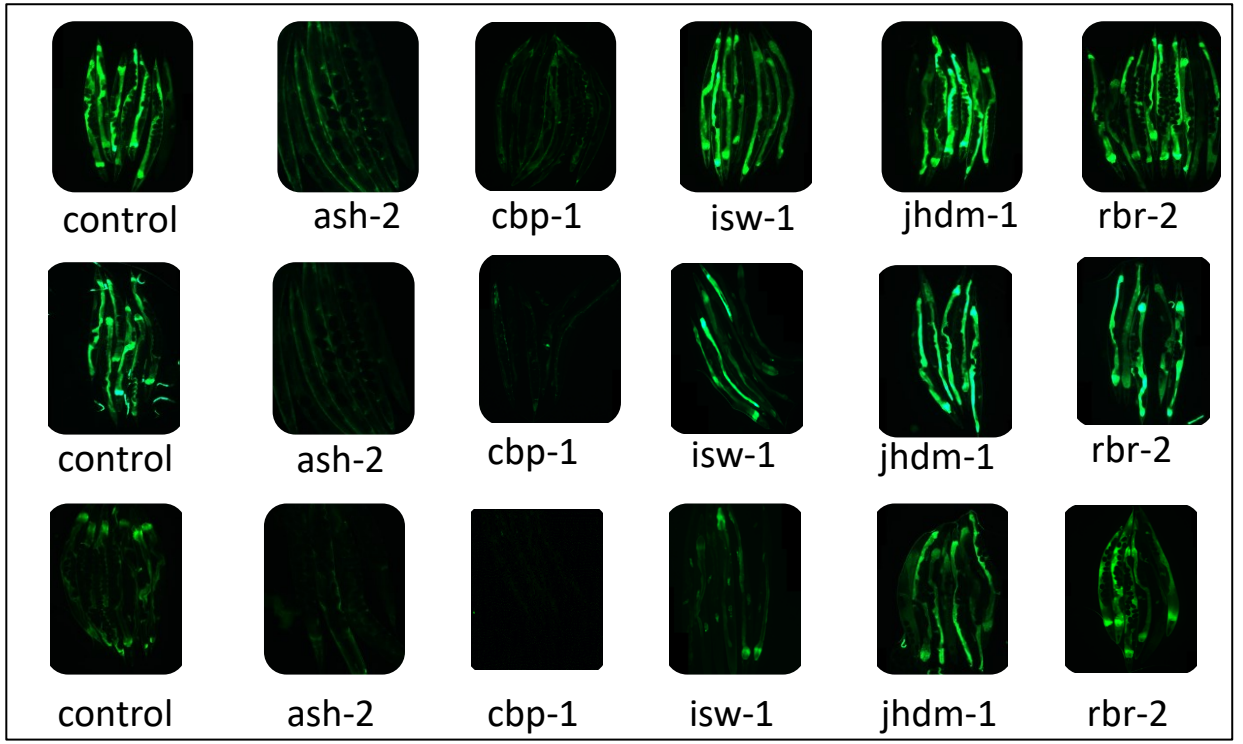




Induced UPR^{ER} efficiency on day 1, 2 and 3 of adulthood with epigenetic knockdown



Phsp-4::gfp *(*wild type)



eat-2(-);Phsp-4::gfp (Dietary restriction model)

Contributions

- This study provides insights into the epigenetic effects of dietary restriction.
- This study also explores epigenetic regulation of Endoplasmic reticulum mediated proteostasis.
- Paves way for investigation of similar epigenetic regulation of the ER in higher animals such as mammals.