Late life inhibition of insulin signalling removes age-associated protein aggregates

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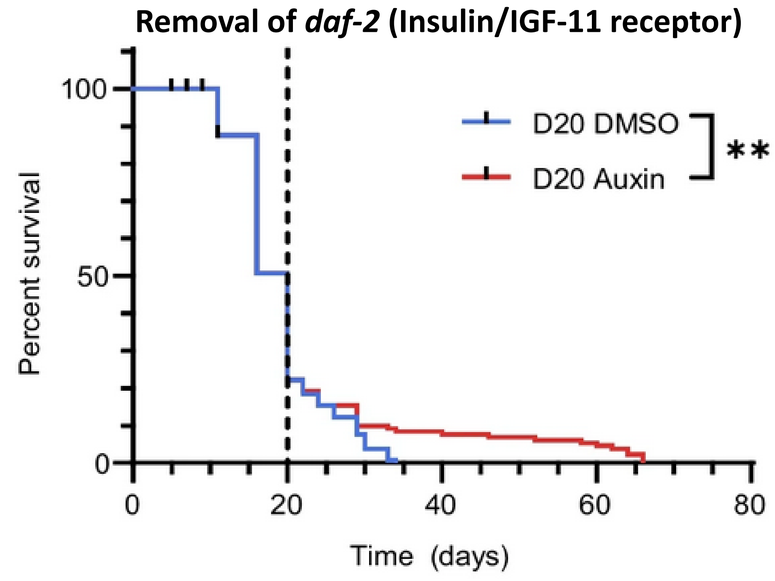
Introduction

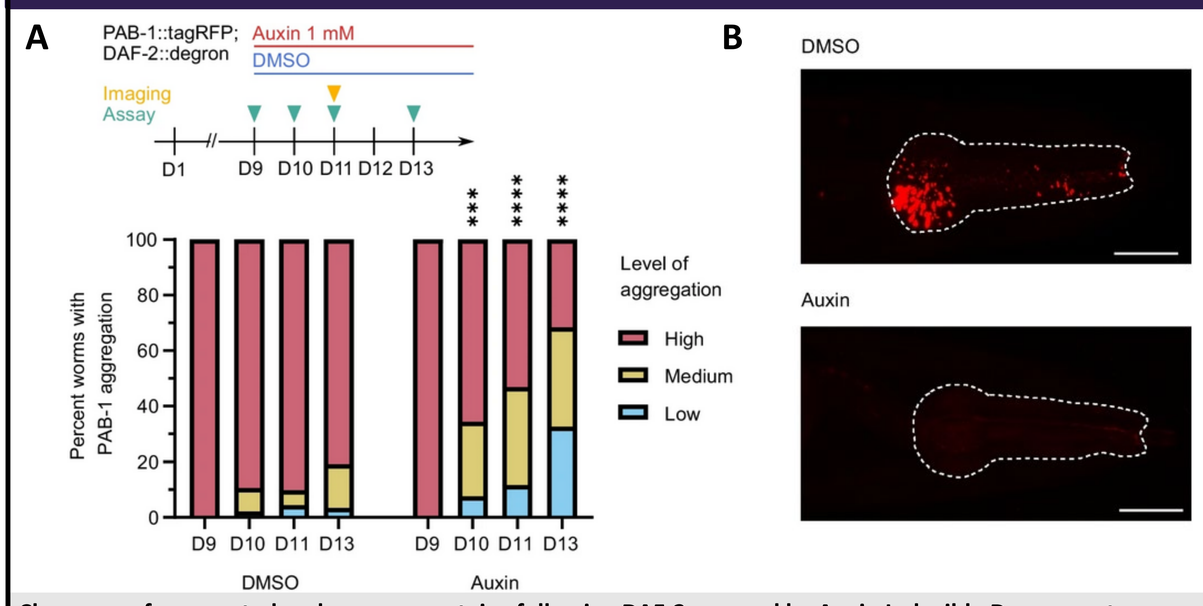
To develop effective longevity interventions, future therapies should target individuals already experiencing significant functional decline (that is, older people). Inhibition of the insulin/IGF-1 receptor is a well-characterized pro-longevity intervention in a variety of model organisms.

Late-life inhibition of insulin/IGF-1 signalling was recently shown to enhance stress resistance and extend lifespan in the nematode *Caenorhabditis elegans*. Fluorescent reporters of endogenous protein aggregates reveal that this intervention promotes the clearance of age-associated aggregates, reversing a key driver of functional decline. My current work focuses on identifying the effector mechanisms by which aggregates are removed, using a variety of approaches.



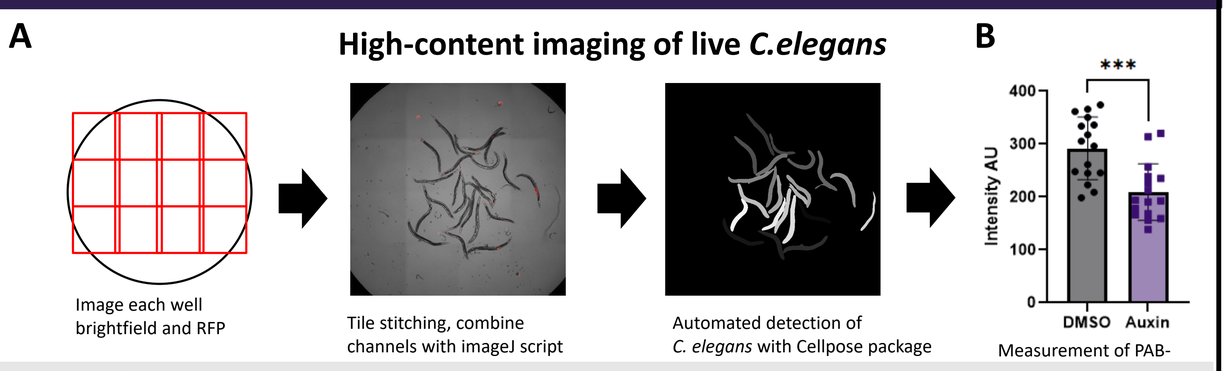
Molière et al., 2024

Late life *daf-2* inhibition clears protein aggregates



Clearance of aggregated endogenous proteins following DAF-2 removal by Auxin Inducible Degron system.
A DAF-2 AID on day 9 in whole-body DAF-2::degron; PAB-1::tagRFP. Semi-qualitative scoring of live animals. (****$p < 0.0001$; ***$p < 0.001$ Chi-Square test.) **B** Representative images of DAF-2::degron; PAB-1::tagRFP *C. elegans* on day. Procorpus and anterior pharyngeal bulb are outlined. Scale bar = 20 µm.

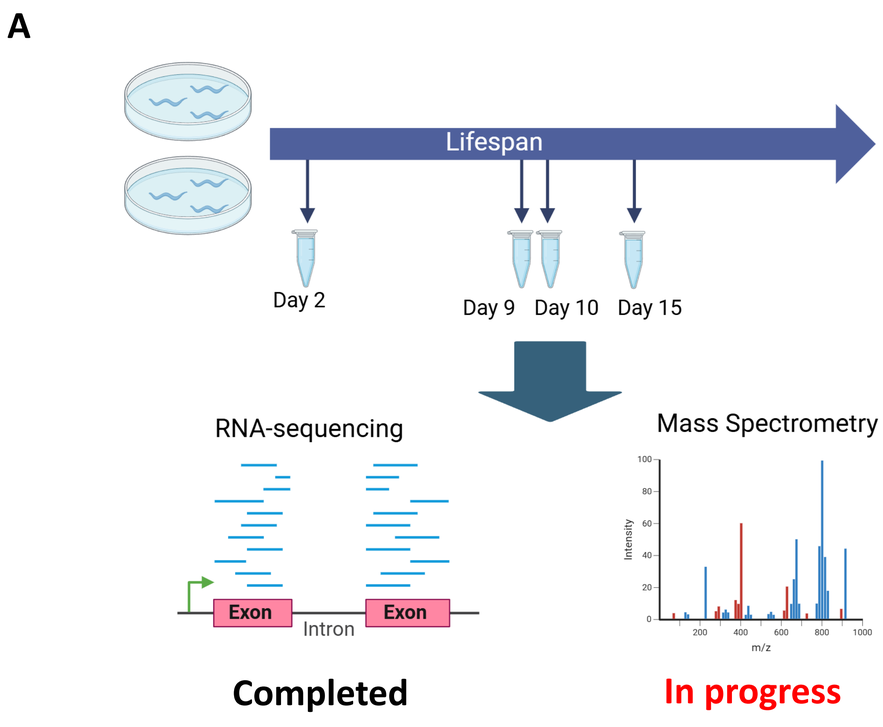
Establishing a high-throughput screening approach to validate effector mechanisms

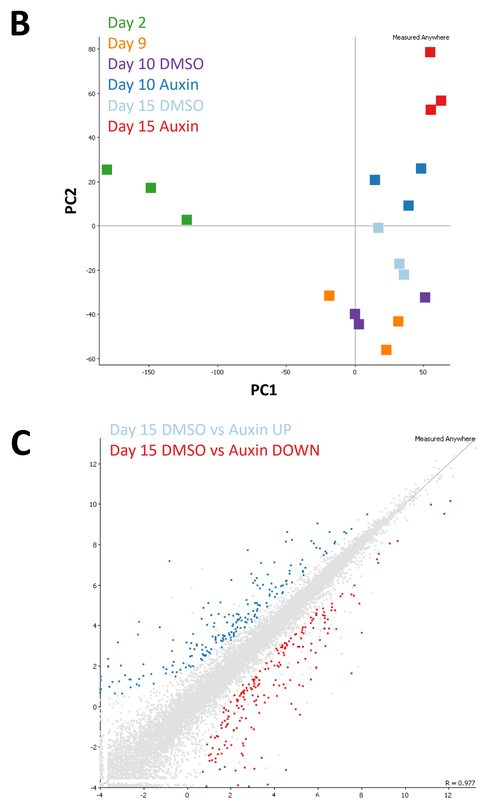


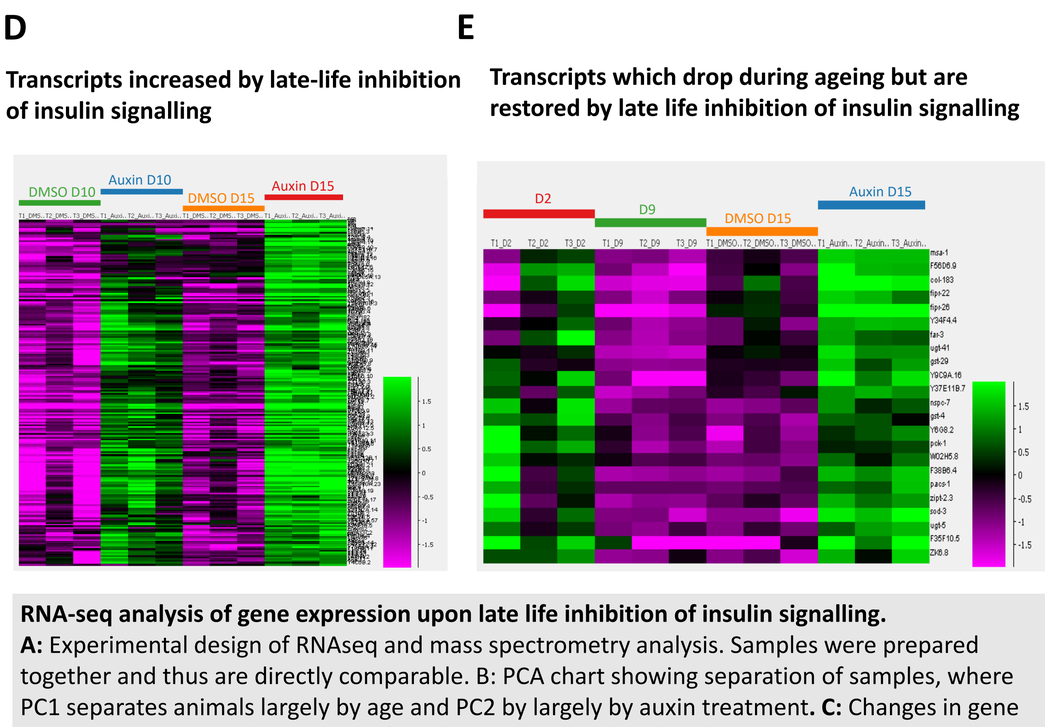
Screening of aggregate removal using high content imaging.
A: Workflow for high throughput measurement of aggregate clearance in live *C. elegans* **B:** Quantification of average intensity per well showing clearance of PAB-1::tagRFP aggregates. Each point represents one well containing approximately 20 animals ($p<0.001$, Students t-test).

- **Approach successfully detects removal of aggregates.**
- **However, effect size with PAB-1::tagRFP aggregates is too small for large scale screening.**
- **Evaluating more sensitive *C. elegans* models of aggregate removal, including temperature sensitive metastable reporters of protein aggregation and strains expressing fluorescently labelled polyglutamine repeats.**

RNA-seq and Mass Spectrometry analysis to identify mechanisms of clearance







RNA-seq analysis of gene expression upon late life inhibition of insulin signalling.
A: Experimental design of RNAseq and mass spectrometry analysis. Samples were prepared together and thus are directly comparable. B: PCA chart showing separation of samples, where PC1 separates animals largely by age and PC2 by largely by auxin treatment**. C:** Changes in gene expression at Day 15 showing significantly increased and depleted transcripts. **D:** Heat map showing genes increased at Day 15 with auxin treatment, with relative levels in controls and earlier timepoints. Transcripts increased at D10 almost all further increased at D15.

Future directions

Mass spectrometry of the aggregated fraction of the proteome to provide a proteome level view of aggregate clearance and identify the breadth of substrates which are cleared.

Correlative light electron microscopy to gain better insight into the aggregate clearance at the ultrastructural level.

Screening –omics hits with our high throughput pipeline

UKRI Biotechnology and Biological Sciences Research Council

References
1. Molière A, Park JYC, Goyala A, Vayndorf EM, Zhang B, Hsiung KC, Jung Y, Kwon S, Statzer C, Meyer D, Nguyen R, Chadwick J, Thompson MA, Schumacher B, Lee SV, Essmann CL, MacArthur MR, Kaeberlein M, David D, Gems D, Ewald CY. Improved resilience and proteostasis mediate longevity upon DAF-2 degradation in old age. Geroscience. 2024 Oct;46(5):5015-5036. doi: 10.1007/s11357-024-01232-x. Epub 2024 Jun 20. PMID: 38900346; PMCID: PMC11335714.