# Using the Auxin Inducible Degron (AID) system to understand tissue-specific longevity signaling in *C. elegans*.

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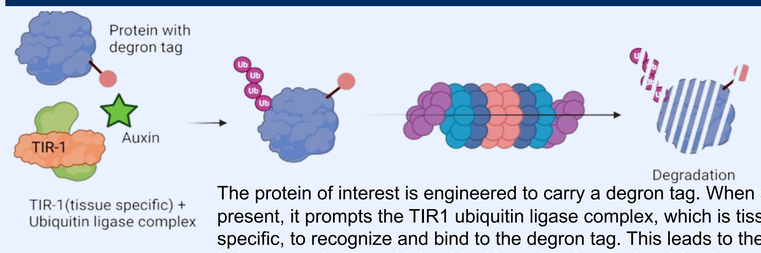
## Abstract

Previous research conducted by our laboratory has revealed that exposure to reactive oxygen species, which inactivate the Histone 3 Lysine 4 trimethylating complex (COMPASS complex), can trigger stress response pathways and increase the lifespan of *C. Elegans*. Our current goal is to gain a deeper understanding of how and when depletion of H3K4me3 leads to enhanced health span and lifespan benefits, specifically in which tissues this occurs.

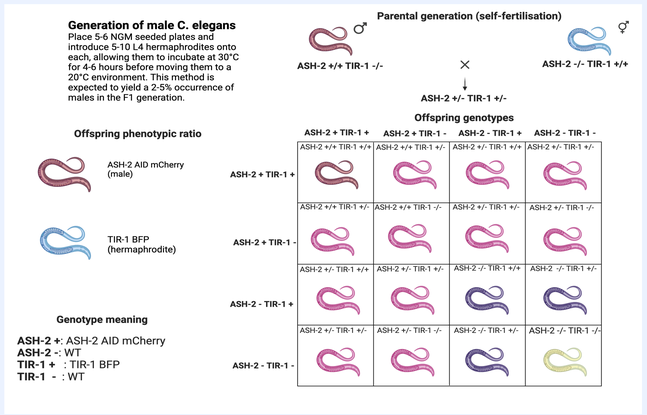
To achieve this, we utilized the AID system to create *C. elegans* strains that target the COMPASS complex component ASH-2 in several tissues. By controlling the timing of auxin addition, we can regulate ASH-2 degradation.

Our findings indicate that degradation of ASH-2 in somatic tissues during development is sufficient to extend lifespan. We have developed somatic cell-specific strains to further explore this connection. Ultimately, our study will provide insight into tissue-specific crosstalk between epigenetic modifications and longevity pathways.

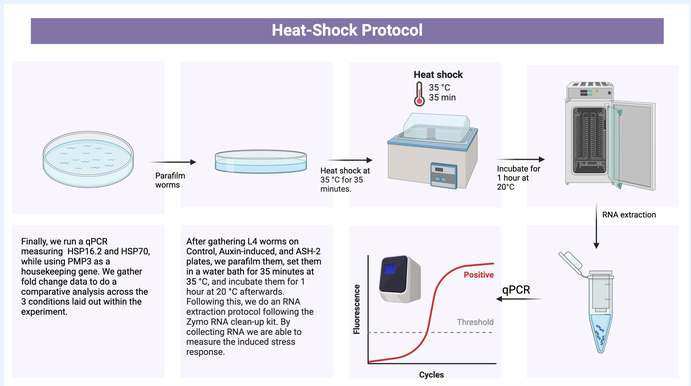
## Methods




The protein of interest is engineered to carry a degron tag. When auxin is present, it prompts the TIR1 ubiquitin ligase complex, which is tissue-specific, to recognize and bind to the degron tag. This leads to the protein being tagged with multiple ubiquitin molecules, a signal for degradation. Subsequently, the polyubiquitinated protein is recognized by the proteasome, a protein complex responsible for degradation, leading to the protein being broken down, which allows for the controlled removal of specific proteins within cells.




Following this cross, we were able to confirm the strains created through PCR and Gel electrophoresis.




The final method used included a lifespan assay where I was able to assess how tissue-specific degradation of ASH-2 affected lifespan. This was tested across multiple tissues and ASH-2 was degraded in different stages of development in each experiment.

## Results

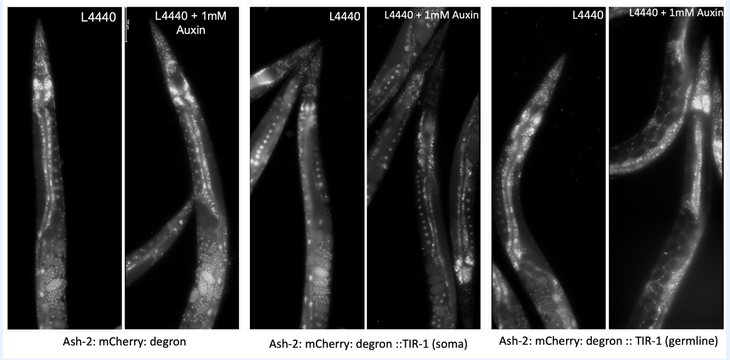



**Figure 1.** Tissue-specific degradation as observed through fluorescence microscopy to confirm auxin induced degradation of ASH-2.

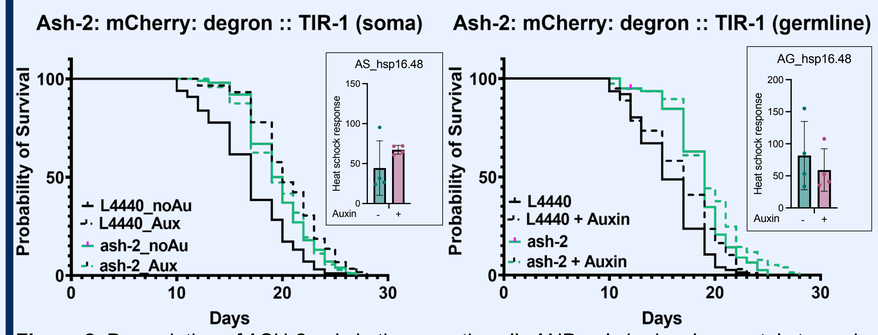



**Figure 2.** Degradation of ASH-2 only in the somatic cells AND only in developmental stages is sufficient to extend lifespan. Germline degradation of ASH-2 during development has no consequence.

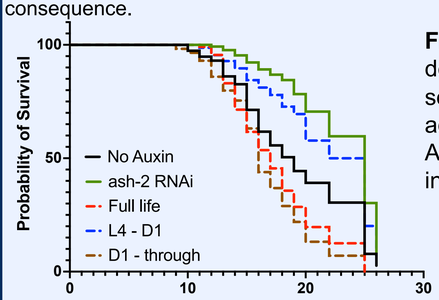



**Figure 3.** Survival curve showing degradation of ASH-2 only in the somatic tissue. Degradation during adulthood is detrimental to lifespan. ASH-2 might have important function in somatic cells during adulthood.

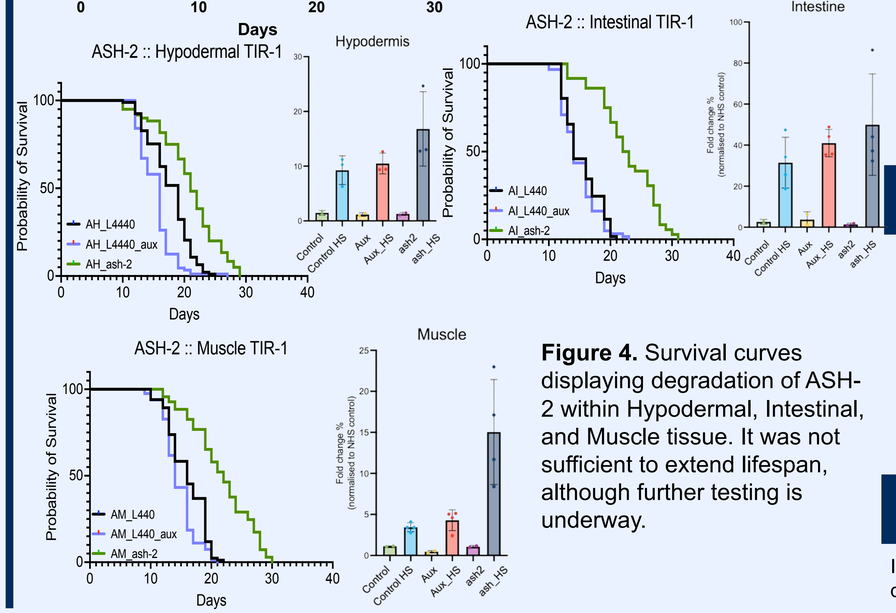



**Figure 4.** Survival curves displaying degradation of ASH-2 within Hypodermal, Intestinal, and Muscle tissue. It was not sufficient to extend lifespan, although further testing is underway.

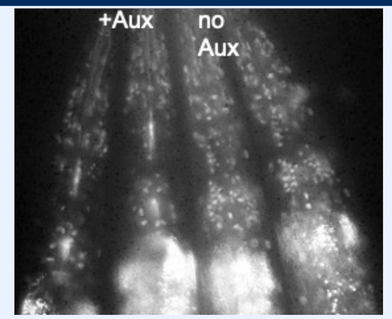



**Figure 5.** Tissue specific ASH-2 degradation upon auxin treatment within neuronal tissue of *C. elegans* as shown through fluorescence microscopy.

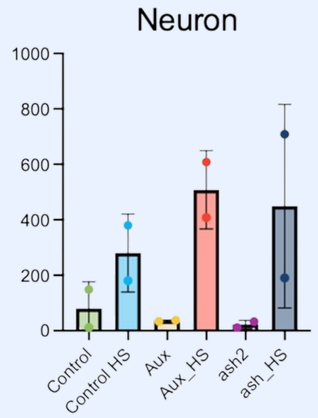



**Figure 6.** Heat stress response within the neuronal tissue of *C. elegans*. Higher level of HSR within neuronal tissue displayed, which leads us to believe that degradation of Ash-2 within neurons of *C. elegans* may cause an increase in lifespan.

## Conclusions

We find a spatiotemporal regulation of longevity pathways via COMPASS complex depletion. By harnessing the AID system to induce tissue-specific degradation of ASH-2 at different developmental stages, we have discovered that its depletion in somatic tissues during development notably extends the lifespan of these organisms. These findings not only deepen our understanding of the interaction between epigenetic regulation and longevity but also highlight the significance of tissue-specific responses in the overall lifespan of *C. elegans*. Our ongoing research with somatic and cell-specific and neuronal strains aims to unravel the intricacies of this epigenetic influence on life extension. Through this, we anticipate contributing to a broader framework for developing potential tissue specific interventions in aging and age-related diseases, where modulation of epigenetic factors could pave the way for novel therapeutic strategies.

## Future Work

We are working towards testing if depletion of ASH-2 in the neurons is sufficient for lifespan extension. Work in the lab has shown that ASH-2 depletion induces the longevity factor HSF-1. Upregulation of HSF-1 is neuronal cells is sufficient to extend lifespan. We would also like to induce ASH-2 degradation in smaller doses in our somatic tissue specific strains. We will compliment our lifespan experiments with health span and stress response tests.

## Acknowledgements

I would like to acknowledge my mentor, Janakraj Bhattrai, my PI, Ursula Jakob, the MCDB department for their support, and Suny Biotech for generating the parental ASH-2 AID strain.