The Role of Polycomb Group Proteins in Animal Activity

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Background

- Aging is a near-universal process among animals and chromatin dysfunction has been suggested as a possible source of the physical decline seen with aging and activity.
- There are two chromatin-based silencing systems in most eukaryotes, the HP1/H3K9me2 system and the Polycomb/H3K27me3 system. While the HP1 system has a documented impact on lifespan and aging, little is known about the role of the Polycomb system in aging.
- The Polycomb Group (PcG) proteins modify histones and repress developmentally regulated genes through the action of two complexes, PRC 1 and PRC 2.

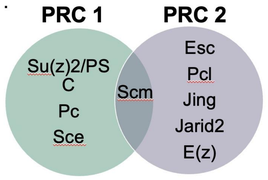


Figure 1: Polycomb Repressive Complexes 1 & 2. Binding of PRC 1 to a target gene recruits PRC 2, leading to the establishment of a repressive chromatin domain.

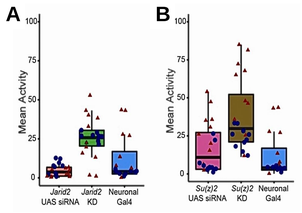
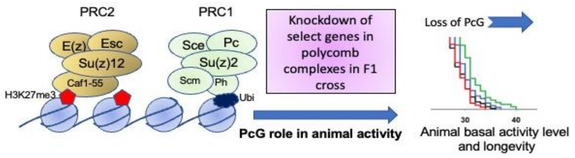


Figure 2: Jarid2 and Su(z)2 knockdown increases animal activity. A) Box plot showing the basal activity levels of animals with lower levels of Jarid2 in their neuronal tissues (Jarid2 KD, green) as well as their parents (UAS RNAi line, orange; elav-Gal4. line, blue). B) Box plot showing the basal activity levels of animals with lower levels of Su(z)2 in their neuronal tissues (Su(z)2 KD, brown) as well as their parents (UAS RNAi line, purple; elav-Gal4 line, blue).

In this study, we remove individual components of PRC 1 and PRC 2 in target tissues to identify the PcG protein involved in the control of animal activity levels. We seek to discover the specific tissues involved and used the UAS/Gal4 system to remove a gene product in a specific tissue within the animals.

Figure 3: Experimental overview. The experimental design to identify the role of PcG proteins in animal activity and lifespan.



Methodology

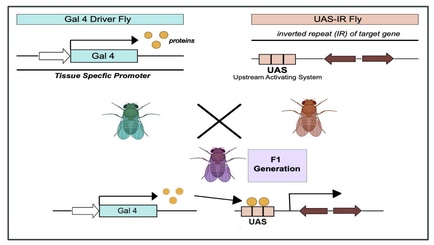


Figure 4: Knocking down a Polycomb protein. The UAS can induce transcription of a neighboring gene when Gal4 is bound to it. By transcribing a hairpin transcript that can form a double-stranded RNA using the UAS/Gal4 system, the cell destroys any mRNA in the cell leads to “knockdown” or loss of the target protein in the F1 generation of the cross(left).

Figure 5: Setting up a lifespan assay. Beginning with healthy stock bottles, all mature flies were removed (only larvae remain). Emerging virgin animals were collected and placed into vials (10 animals per vial; 10 vial per sex/genotype combination). From then on, fly deaths were recorded every 2 days. Flies were flipped onto new food every 4 days.

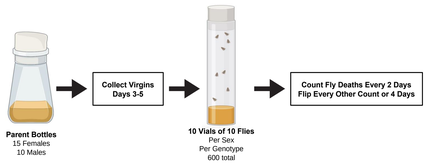


Figure 6: Measuring animal activity. The Drosophila Activity Monitoring System (DAM) was used to monitor activity over a 48-hour period. The system collects data in 5-minute intervals by using a laser that documents the number of times a fly passes through the laser.

Acknowledgments

I would like to thank everyone in the Riddle Lab for their suggestions and support in this project. I would like to thank Dr. Riddle for her help in developing my laboratory skill set and giving guidance where it was needed. I would also like to thank the National Science Foundation for their funding of my project through grant number #2021305.

Results

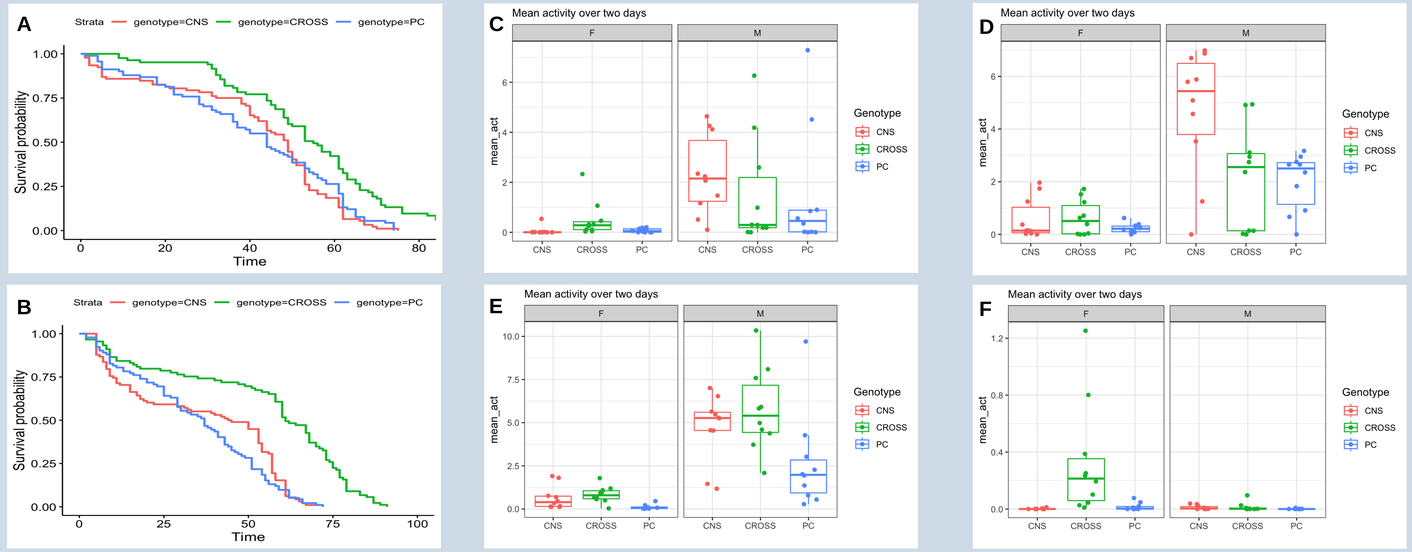


Figure 7: Knockdown of Pc (PRC1) in CNS increases lifespan but not animal activity
A) Lifespan of Males B) Lifespan of Females C) Activity Age 9-11 D) Activity Age 20-22 E) Activity Age 29-31 F) Activity Age 49-51

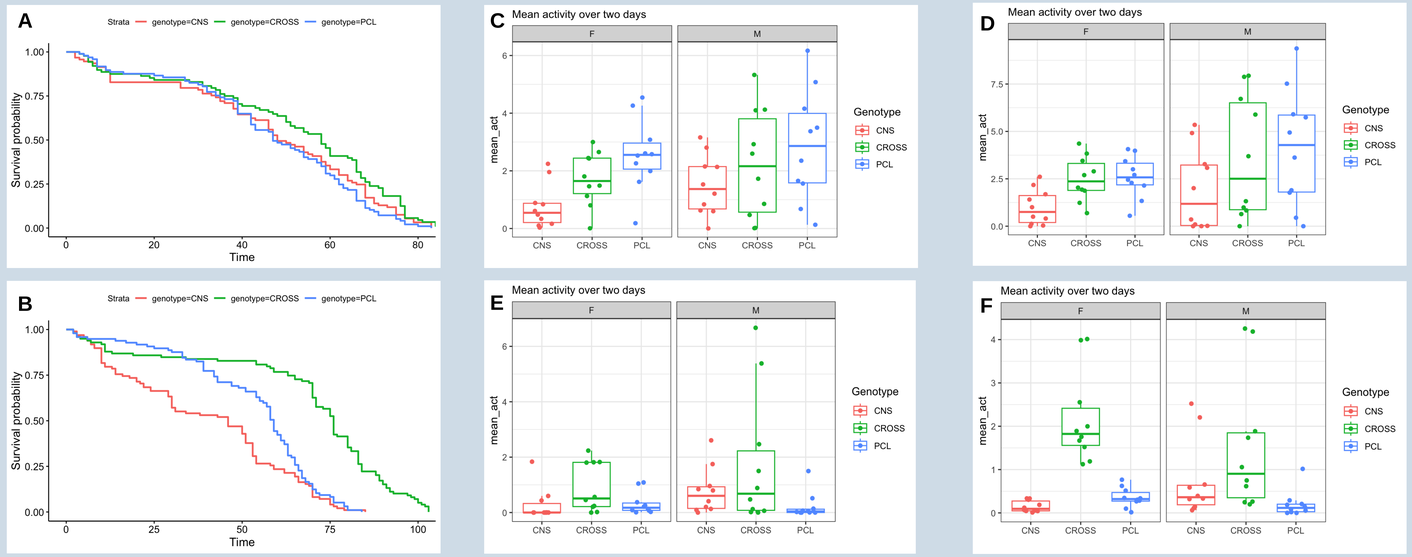


Figure 8: Knockdown of Pcl (PRC2) in CNS increases lifespan and animal activity
A) Lifespan of Males B) Lifespan of Females C) Activity Age 9-11 D) Activity Age 20-22 E) Activity Age 29-31 F) Activity Age 49-51

Conclusions

- Results reveal a significant increase in activity level comparing the knockdown to control genotypes.
- Our findings confirm the potential role of the PRC complexes in the control of animal activity.
- Even as the animals age, we still see a difference in between the knockdown versus the controls. This is also mirrored in the lifespan data.