How important is enzymatic function? Determining which traits of the tumor suppressor BRCA1 rely on nucleosome ubiquitylation.

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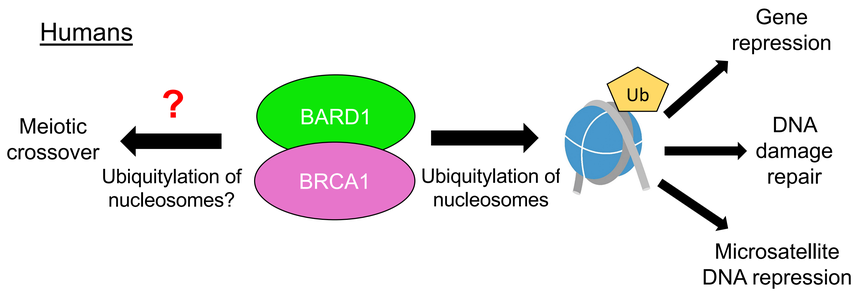
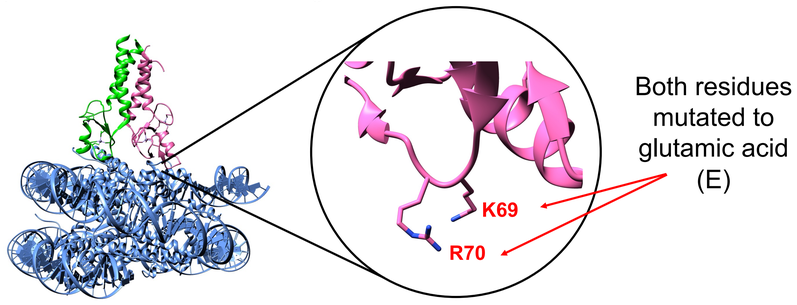
Introduction

BRCA-1 and BARD-1 play roles in various essential cell functions via nucleosome ubiquitylation.



Above: In human cells, the BRCA-1/BARD-1 protein complex is involved in several essential cellular functions, many of which are known to occur via ubiquitylation of the nucleosome by the complex. In the *Caenorhabditis elegans*, the BRC-1/BRD-1 protein complex, a homolog to the human BRCA-1/BARD-1 complex, is also known to be involved with these same essential functions. However, it has yet to be determined whether nucleosome ubiquitylation is also the mechanism used to perform these functions in *C. elegans*.

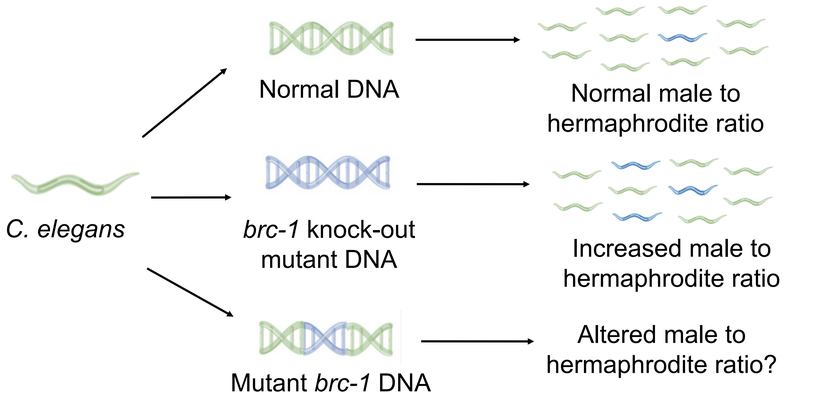


Above: Residues K69 and R70 of the *C. elegans brc-1* gene (pink) were both mutated to glutamic acid (E) residues. This mutation prevents the BRC-1/BRD-1 protein complex from ubiquitylating the nucleosome (blue).

Objectives

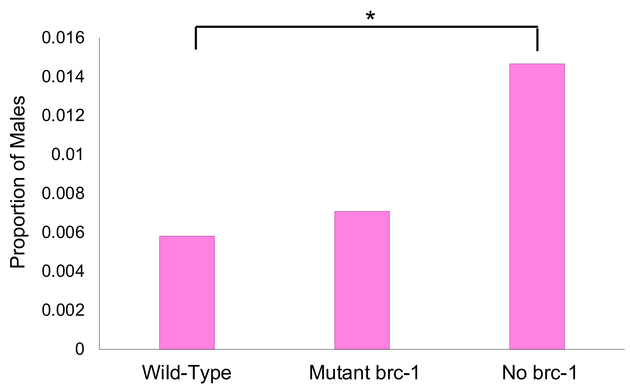
- Measure male ratio of *C. elegans* in strain that cannot ubiquitylate nucleosomes
- Compare levels of reactive oxygen species (ROS) in *C. elegans* strain that can and cannot ubiquitylate nucleosomes
- Determine which functions of *brc-1/brd-1* are dependent on nucleosome ubiquitylation

Meiotic crossover partially relies on nucleosome ubiquitylation



Above: The mutant *brc-1* strain cannot ubiquitylate the nucleosome. The ratio of adult males to hermaphrodites was counted to determine whether BRC-1/BRD-1 nucleosome ubiquitylation impacts meiotic crossover.

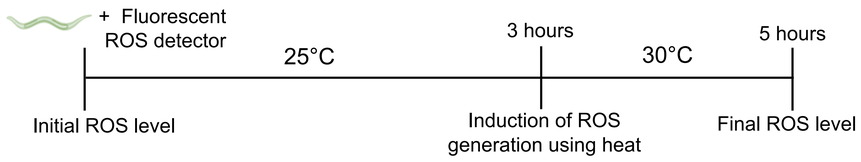
C. elegans lacking ubiquitylation ability produce an intermediate proportion of male offspring.



Left: Proportions of male progeny of three *C. elegans* strains. A significant difference was observed between wild-type and *brc-1* knock-out strains, but not between the mutant *brc-1* strain lacking ubiquitylation activity and the other strains.

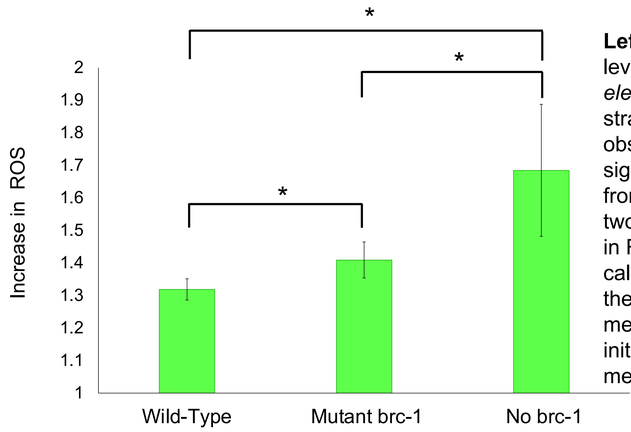
DNA damage is partially impacted by nucleosome ubiquitylation

Fluorescence assay was used to measure *C. elegans* ROS levels as a proxy for DNA damage.



Above: To measure DNA damage through ROS, fluorescent compound H_2DCFDA was added to well plates each containing 50 L1 stage worms and M9 buffer. Three strains of worms were used. Fluorescence measurements were recorded hourly using a plate reader. After three hours, heat was added as an oxidative stressor for two more hours. Higher fluorescence levels indicated greater production of ROS.

C. elegans without ubiquitylation ability generate intermediate ROS levels under oxidative stress.



Left: Increase in ROS levels of three *C. elegans* strains. Each strain's increase was observed to be significantly different from both of the other two strains. Increase in ROS was calculated by dividing the final ROS measurement by the initial ROS measurement.

Conclusion and Future Directions

BRC-1/BRD-1 functions are partially dependent on nucleosome ubiquitylation capability.

- Meiotic crossover is partially affected in *C. elegans* that cannot ubiquitylate the nucleosome.
  - Greater proportion of male worms indicates altered meiotic crossover.
  - *brc-1* KO strain contained significantly greater proportion of males than the wild-type strain.
  - Male proportion of mutant *brc-1* strain did not differ compared to either wild-type or *brc-1* KO strains.
  - This indicates BRC-1/BRD-1 has an intermediate dependence on nucleosome ubiquitylation to control meiotic crossover events.
- DNA damage is moderately increased in *C. elegans* lacking nucleosome ubiquitylation ability.
  - Increased ROS levels indicate greater DNA damage.
  - Mutant *brc-1* strain produced significantly more ROS than wild-type strain, but significantly less ROS than *brc-1* KO strain.
  - This indicates BRC-1/BRD-1 protein complex partially relies on nucleosome ubiquitylation to regulate DNA damage.

Further studies will be done to determine which other functions of BRC-1/BRD-1 are dependent on nucleosome ubiquitylation.

- BRCA1/BARD1 is known to repress genes through nucleosome ubiquitylation in humans, but it is unknown if *C. elegans* BRC-1/BRD-1 also depends on this mechanism for gene repression.
  - Using RT-PCR, we will measure levels of gene repression in mutant *brc-1* worms and compare them to wild-type and *brc-1* KO levels.

References and Acknowledgements

1. Boulton, S. J., Martin, J. P., Polanowska, J., Hill, D. J., Gartner, A., & Vidal, M. (2004). *Current Biology*, *14*(1), 33–39.
2. Li, Q., Kaur, A., Okada, K., McKenney, R. J., & Engebrecht, J. (2023). *PLoS genetics*, *19*(1), e1010457.
3. Thapa, I., Vahrenkamp, R., Witus, S. R., Lightle, C., Falkenberg, O., Jeffries, M. K. S., Klevit, R. E., & Stewart, M. D. (2022). *Nucleic Acids Research*.
4. Yoon, D. K., Lee, M., & Cha, D. S. (2018). *Bio-protocol*, *8*(6).

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