Transcriptional Regulation as a Conserved Function of BRCA1/ BARD1 in *Caenorhabditis elegans*

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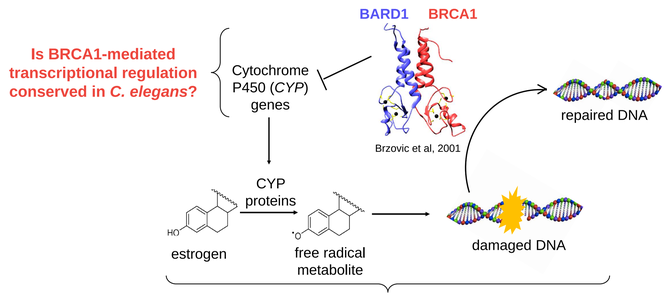
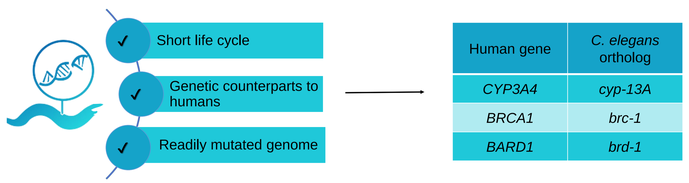
Introduction

BRCA1 contributes to genome stability in humans



Above: BRCA1 prevents the onset of tumorigenesis by heterodimerizing with BARD1 to silence cytochrome P450 enzymes. By preventing estrogen from metabolizing into free radicals, BRCA1 and BARD1 help prevent free-radical-induced DNA damage.

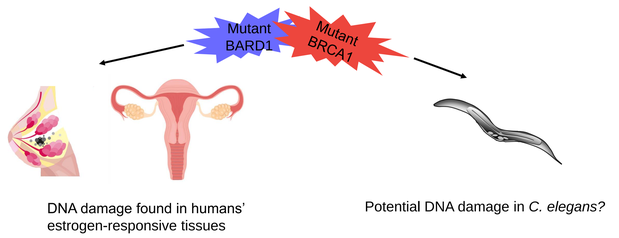
C. elegans as a model organism for BRCA1/BARD1 studies



Human gene	*C. elegans* ortholog
CYP3A4	*cyp-13A*
BRCA1	*brc-1*
BARD1	*brd-1*

Above: *C. elegans* may serve as a model organism for studying the many functions of BRCA1 and BARD1. It could allow for assessment of genetic risk due to inherited mutations.

Challenge of assessing of DNA damage in *C. elegans*



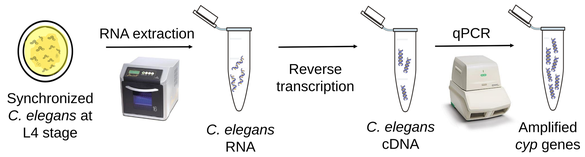
Above: Without epithelial breast tissues, the ovaries and their functions remain a viable target for studying the impact of DNA damage in *C. elegans* due to estrogen exposure.

Objectives

- Analyze differences in the expression of *cyp-13A* gene subfamily between wild-type and defective BRC-1/ BRD-1 *C. elegans* strains.
- Determine the impact of estrogen exposure on *C. elegans*' reproductive potential.

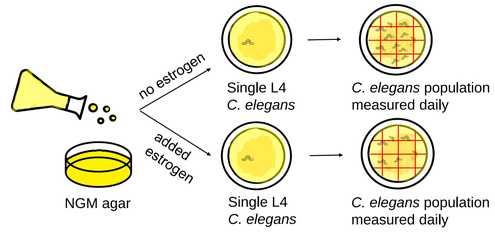
Methods

Gene expression analysis



Above: The population growth of each strain is synchronized to the fourth larval (L4) stage. RNA is extracted from the tissues and reverse transcribed to cDNA. Levels of expressed *cyp* genes are detected via qPCR.

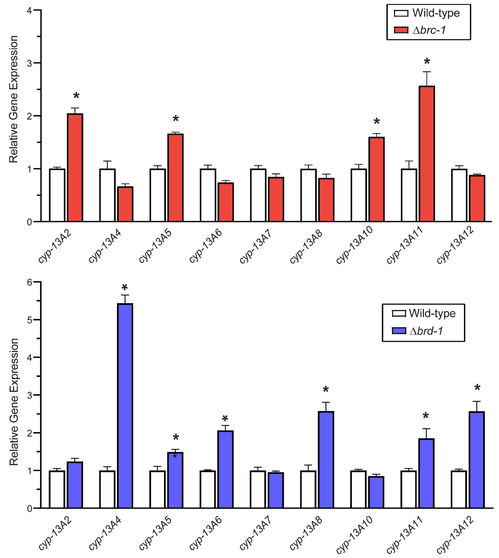
Estrogen exposure assay



Above: *C. elegans* are cultured on nematode growth medium (NGM) agar. Estrogen is mixed with *E. coli* OP50 for exposure. A single *C. elegans* from each strain at the fourth larval (L4) stage is transferred to each plate and let grow at 20°C. The population of each plate is monitored and counted over four days.

Results

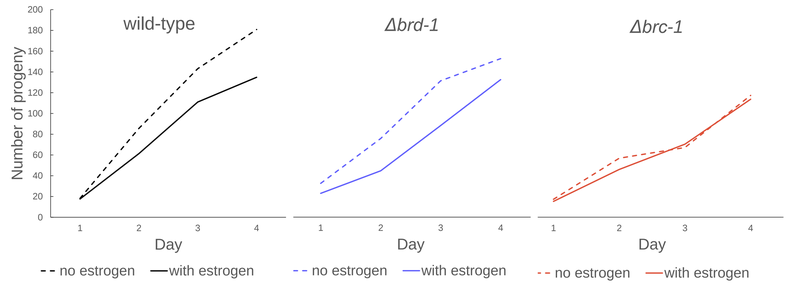
BRCA1- mediated transcriptional regulation is conserved.



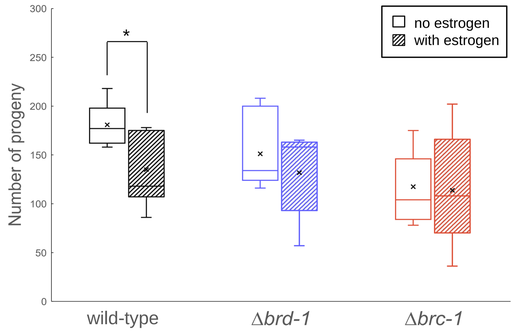
Above: *Δbrc-1* and *Δbrd-1* strains showed significant upregulation of *cyp-13A* genes. Data are normalized to the reference gene, *tba-1*, and presented relative to wild-type.

Results

Fecundity measurement suggests estrogen-induced DNA damage is not increased in the absence of *brd-1* and *brc-1*.



Above: Mean number of progeny of each strain over four days. Parent *C. elegans* is either exposed or not exposed to estrogen.



Above: Number of progeny on day four. Mean difference between wild-type *C. elegans* exposed to estrogen and not exposed to estrogen is statistically significant. This difference is not observed for Δ*brd-1* and Δ*brc-1* strains. A difference in fecundity between wild-type and Δ*brc-1* was noted in the absence of estrogen.

Conclusions and Future Directions

- BRC-1 and BRD-1 regulate the expression of *cyp-13A* genes in *C. elegans*.
  - *C. elegans* is a viable model organism to explore BRCA1 biochemical functions.
  - *C. elegans* shows the potential for testing human BRCA1 mutations.
- Functional BRC-1 and BRD-1 do not protect *C. elegans* from estrogen-induced DNA damage as measured by fecundity.
  - Measure reactive oxygen species directly using fluorescence-based assays.
  - Use comet assay to directly quantify DNA damage.
  - Monitor alternate biomarkers over generations.

References

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