Investigation of Conservation of BRD-1 Activity in C. elegans

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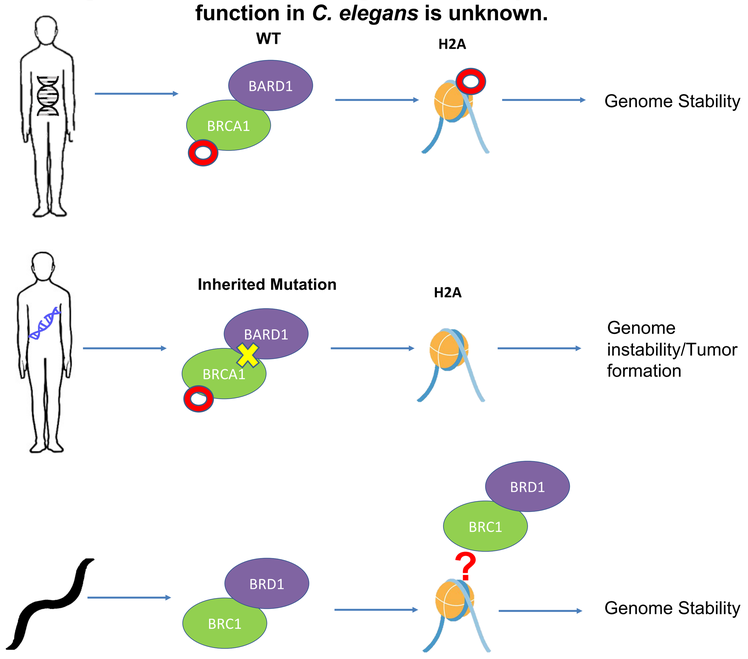
Introduction

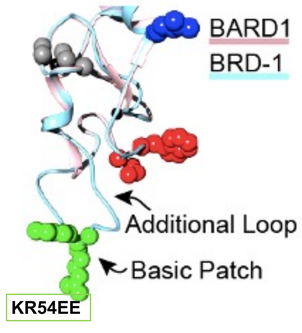
BARD1-BRCA1 is a protein complex seen in humans involved with tumor suppression[1,2] with ortholog proteins BRC-1 and BRD-1 seen in *C. elegans*. All are important for genome stability but mechanism of function in *C. elegans* is unknown.



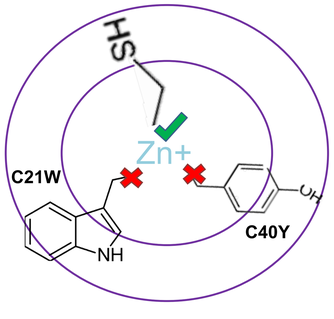
Visualizing Mutations under study

Right: Homolgy model shown in light blue is an estimation of the structure of BRD-1. It overlays the structural model of BARD1 shown in light red. BRD-1 has an additional loop that extends toward the nucleosome. This loop has a basic patch that was mutated to acidic amino acids and hypothesized to disrupt function and help show proof of binding to the nucleosome.

Structural Nuleosome Binding Mutation



Cancer Associated Mutants

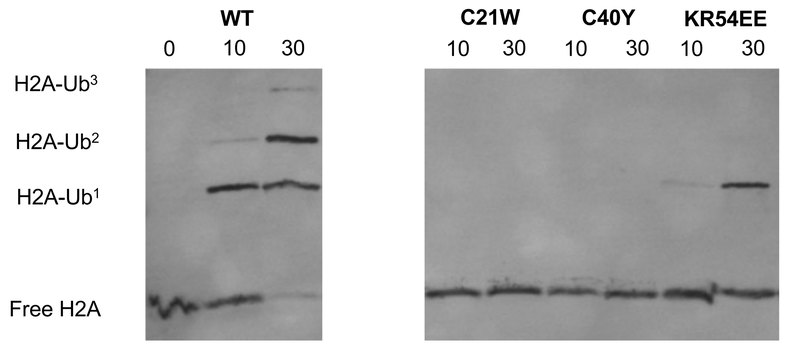


Left: Cartoon depicting loop of BRD-1 protein surrounding a positively charged zinc atom. Green check mark shows succesful chelation with the Zinc atom to assist in proper folding. Red X marks show mutations seen in human cancer lines that are hypothesized to disrupt protein folding.

Objectives

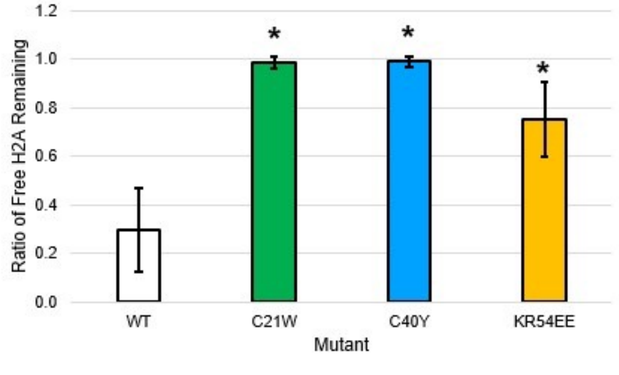
- Create variants of unknown significance within the *C. elegans* BCBD binding interface and BRD-1, nucleosome binding interface
- Use in vitro ubiquitylation assay to examine the enzymatic effects of variants of unknown significance

Enzyme Activity Assay



Above: Western blot created using primary rabbit antibody, secondary goat antibody, and visualized using an alkaline phosphatase mixture. Western blot was cropped to highlight mutations under study. WT has a 0, 10, and 30 minute time point while the other mutants only visualized a 10 and 30 minute time point respectively. The lowest lane visualizes the amount of free H2A protein while the upper lanes show ubiquitylated H2A because they are slightly bigger, depending on how many ubiquitin molecules are placed.

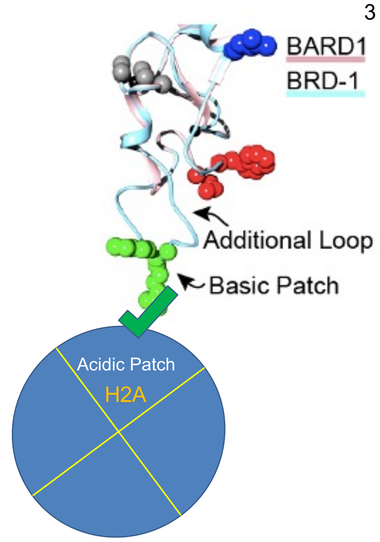
Quantification of Western Blot



Above: All three mutants remaining free H2A was averaged at the 30-minute time point and graphed. Ratios were calculated from WT at time 0. Error bars show standard deviation and asterisks denote significance utilizing Student's T-test with p-value <0.05

BRD-1 activity is conserved and binds the nucleosome in *C. elegans*

Right: Cartoon depicting the BRD-1 homology model binding the H2A octamer portion of the nucleosome. Reduced ubiquitylation activity as evidenced from the western blot shows the basic amino acids are important for binding and the mutation was disrupting. Confirms hypothesis through reduced ubiquitylation abilities, quantified on the statistical analysis bar graph.



Conclusions and Future Directions

- BRD-1 activity in *C. elegans* is conserved
- The *C. elegans* BCBD complex does bind the nucleosome
- *C. elegans* can now be confidently used to study in vivo interactions of the BCBD complex

References and Funding

1. Witus, S.R., Burrell, A.L., Farrell, D.P. *et al.* BRCA1/BARD1 site-specific ubiquitylation of nucleosomal H2A is directed by BARD1. *Nat Struct Mol Biol* **28,** 268–277 (2021).
2. Hashizume, R., Fukuda, M., Maeda, I., Nishikawa, H., Oyake, D., Yabuki, Y., Ogata, H., & Ohta, T. (2001). The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. *The Journal of biological chemistry*, *276*(18), 14537–14540.
3. Stewart, M.D., Klevit, R.E., Jeffries, M.K., Falkenberg, O., Lightle, Caitlin., Witus, S.R., Vahrenkamp, R., Thapa, I. (2022). Conservation of transcriptional regulation by BRCA1 and BARD1 in *Caenorhabditis elegans*. Pending review and approval.

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