Investigating the ubiquitin ligase activity of BRCA1 from *C. elegans*

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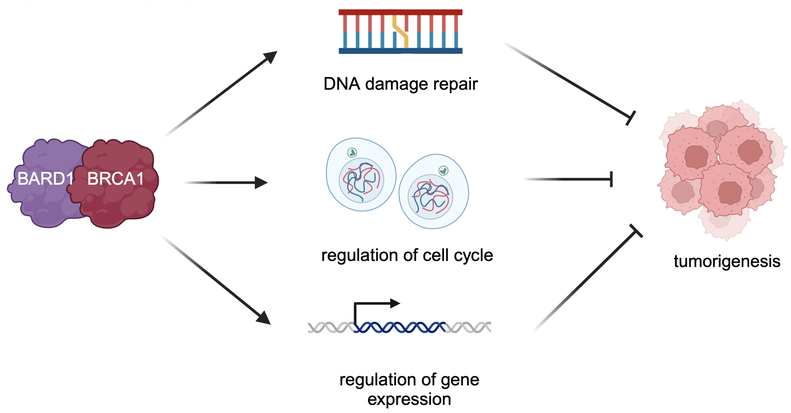
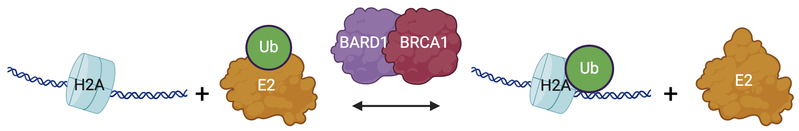
Introduction

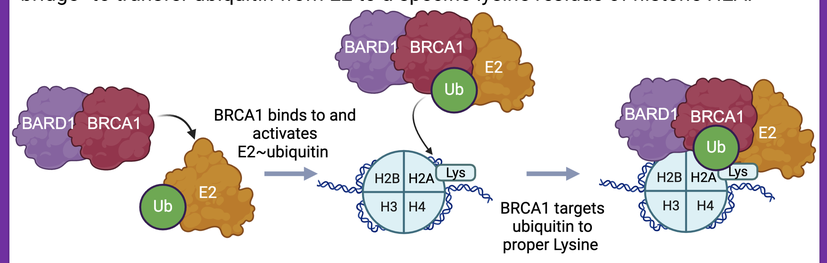
Below: BRCA1 binds to its partner, BARD1, to form a complex that suppresses tumor development by facilitating DNA damage repair, regulating the cell cycle, and regulating gene expression.[1] These functions of BRCA1 are retained by BRC-1, the *C. elegans* homolog of human BRCA1.[2] For simplicity, we will refer to BRC-1 and BRD-1 as BRCA1 and BARD1.



Below: BRCA1 catalyzes the transfer of ubiquitin, a small regulatory protein, onto histone H2A, a component of nucleosomes.[3] Ubiquitination of histone H2A by BRCA1 may be a specific mechanism by which BRCA1 carries out tumor suppression.



Below: In the reaction mechanism for BRCA1-mediated ubiquitination of histone H2A, BRCA1 first binds to and activates the E2~ubiquitin conjugate. BRCA1 then acts as a "bridge" to transfer ubiquitin from E2 to a specific lysine residue of histone H2A.[4]



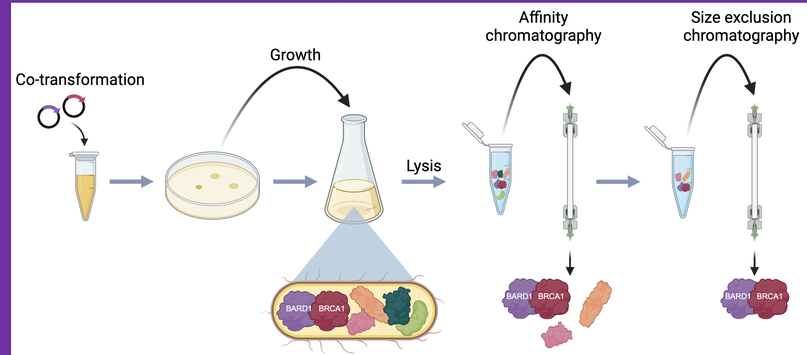
Objectives

Goal: Generate a mutant of BRCA1 that has no ubiquitin ligase activity towards histone H2A but retains all other functions in order to determine the role of ubiquitin ligase activity in tumor suppression.

To accomplish this, we:

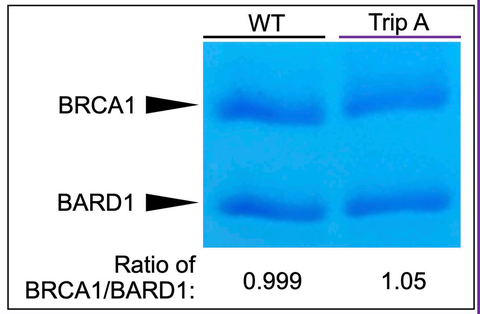
- Created and isolated the Trip A mutant (I23A, I59A, R61A)
- Performed ubiquitination assays using Trip A, WT, and a "no E3" control group
- Quantified ubiquitin ligase activity of Trip A vs WT vs "no E3" using western blotting
- Used SDS-PAGE to determine if BRCA1:BARD1 binding was retained in the Trip A mutant

BRCA1:BARD1 binding is retained in Trip A mutant



Above: WT or Trip A BRCA1 and WT BARD1 were co-transformed into *E. coli.* Both WT and Trip A BRCA1 contained a histidine tag, but WT BARD1 did not. BARD1 binds to BRCA1 in vivo. Affinity chromatography and size exclusion chromatography were performed to isolate BRCA1.

Right: Since only BRCA1 contained the histidine tag, any BARD1 present after purification must exist bound to BRCA1. SDS-PAGE performed after purification demonstrates that the ratio of BRCA1:BARD1 is similar for WT and Trip A. This suggests that the Trip A mutant of BRCA1 retains the ability to bind BARD1 in vivo, which is critical to maintenance of its other key functions.



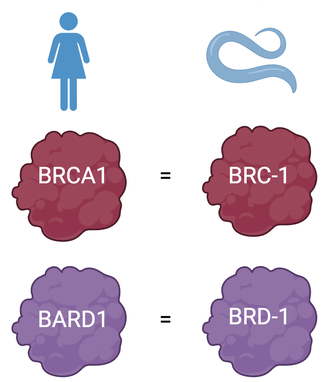
Conclusions and Future Directions

Conclusions:

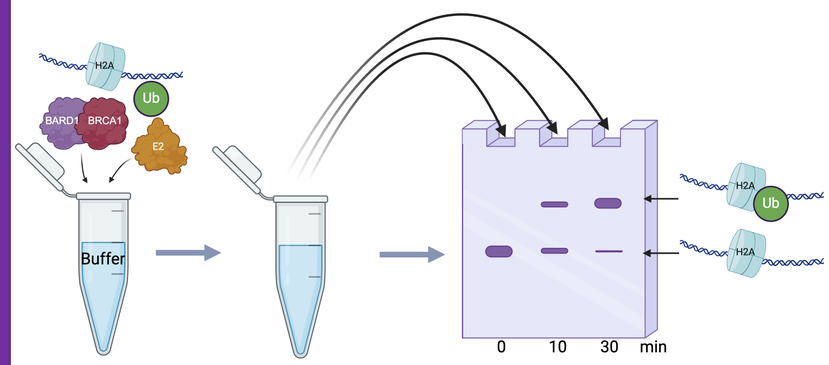
- The Trip A mutant of BRC-1 lacks E3 ubiquitin ligase activity towards histone H2A of nucleosomes in vivo.
- Trip A retains the ability to bind BRD-1, which is critical to maintenance of all other BRC-1 functions.
- Trip A is thus a suitable ligase-dead mutant for in vivo experiments in *C. elegans.*

Future Directions:

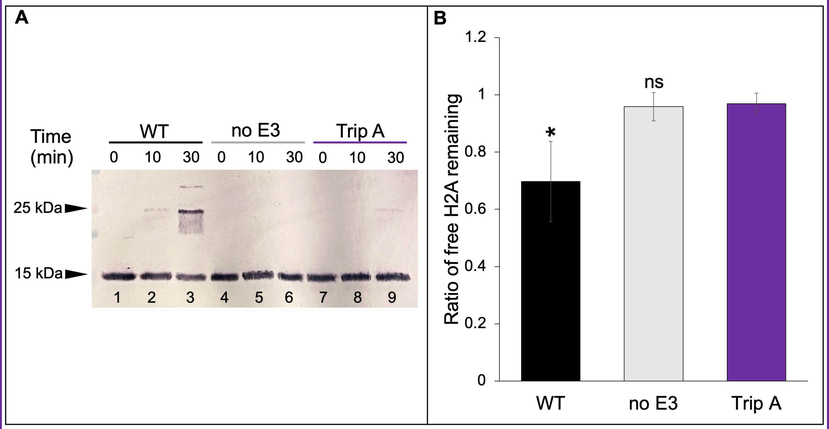
- In vivo experiments using Trip A in *C. elegans* will allow for identification of which functions of BRC-1 depend on E3 ubiquitin ligase activity.
- Characterizing the role of BRC-1's E3 ubiquitin ligase activity may provide insight into the role of BRCA1's E3 ubiquitin ligase activity, since BRCA1 is the human homolog of BRC-1 of *C. elegans.*
- Understanding BRCA1's E3 ubiquitin ligase activity opens additional avenues to the advancement of breast and ovarian cancer treatment



Trip A mutant lacks E3 ubiquitin ligase activity



Above: To measure the activity of Trip A and WT BRCA1, samples were taken from the reaction mixture at 0, 10, and 30 minutes after the reaction began. Western blotting for histone H2A was performed to visualize the change in the amount of ubiquitinated histone H2A vs the amount of free histone H2A.



Above: A) Western blotting of the ubiquitination assays performed on WT, no E3, and Trip A samples of BRCA1 suggest that the no E3 control and Trip A mutant decreased ubiquitin ligase activity compared to WT. **B)** Quantification and statistical analysis of the western blots confirmed that there was no significant difference between the ratio of free histone H2A remaining for the no E3 control vs the Trip A mutant, but there was a significant difference between the of the Trip A mutant vs the WT. These results suggest that the activity of the Trip A mutant resembles the "no E3" control, and thus that Trip A lacks ligase activity. To determine significance, the mean amount of H2A remaining (n=3) after 30 minutes was quantified using ImageJ, and Welch's t-test for data with equal variances was used with $p < 0.05$.

References and Acknowledgements

- Scan for references:

- Scan for Stewart Lab Website:

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