Exploring the enzymatic function of BRCA1 through BRC-1 nucleosome ubiquitylation in *C. elegans*

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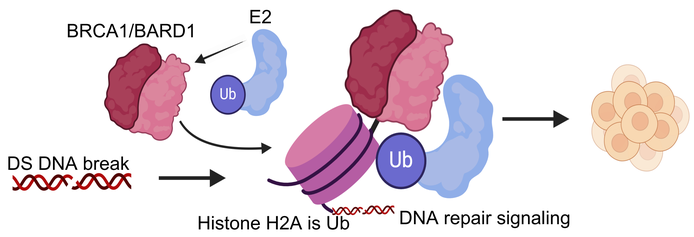
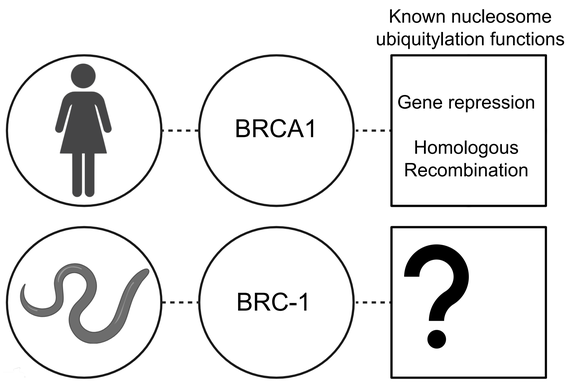
Introduction



Above: BRCA1 is a large, multifunctional gene that makes a protein involved in maintaining the genomic stability of cells. BRCA1 protein is an E3 ubiquitin ligase. BRCA1 protein forms a complex with BARD1 protein. The BRCA1/BARD1 protein complex binds with an E2 ubiquitin-conjugating enzyme protein that carries an activated ubiquitin protein. This large protein complex binds with the nucleosome to add ubiquitin onto histone H2A. This will cause DNA damage repair signaling, and the signaled repair pathway will decrease the likelihood of tumorigenesis. Mutations in BRCA1 involved in the nucleosome ubiquitylation function cause a higher predisposition towards cancer.

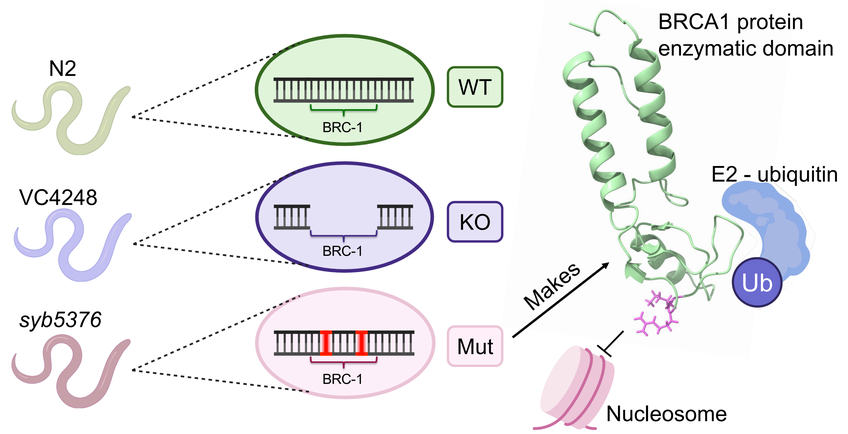


Above: BRCA1 active site is conserved in C. elegans as BRC-1. There are several known BRCA1 functions including DNA damage repair and gene repression. Our lab is exploring how the loss of mononucleosome ubiquitylation is correlated with an increase in DNA damage repair through homologous recombination.

Objectives

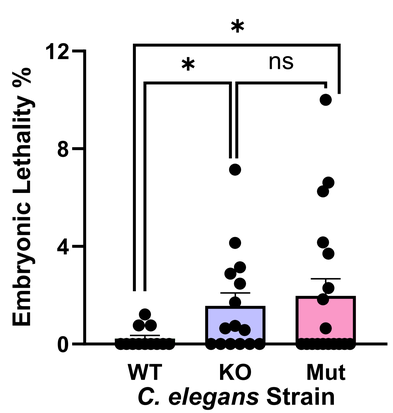
- Explore how mononucleosome ubiquitylation is related to DNA damage accumulation with mutations in BRC-1.
- Explore how BRC-1 variants respond to DNA damage in the presence of cisplatin, a DNA damage causing agent.
- Further *C. elegans* as a model for understanding genetic inheritability of cancer risk and conserved nuclear signaling pathways.

Generating a Nucleosome-Binding Deficient Mutant



Above: To understand how DNA damage accumulation is related to BRC-1 variants, we tested the embryonic lethality across three worm strains. These are the three worm strains that our lab is using to determine the role of mononucleosome ubiquitylation in DNA damage accumulation. N2 Bristol strain is the wild-type (WT) worm with a fully functional BRC-1 protein. VC4248 (KO) is the *brc-1* full genetic knockout. Our lab has used CRISPR to generate *syb5376* (Mut), a BRC-1 mutant that is nucleosome ubiquitylation deficient but retains all other BRC-1 enzymatic functions. The Mut worm strain has two point mutations that cause two amino acid changes in the nucleosome binding site of BRC-1. The changes in nucleosome binding site will repel the nucleosome, which does not allow a ubiquitin to be added to histone H2A. These nucleosome binding residues are shown by the two pink amino acids on the protein above right. The embryonic lethality of each of these strains will show accumulation of DNA damage. Embryonic lethality is compared between all three strains of worms, as an increase in DNA damage is associated with higher embryonic lethality.

Embryonic Lethality Depends on Nucleosome Ubiquitylation



Left: **Embryonic lethality of three strains of *C. elegans***. Mean embryonic lethality plotted with SEM bars. Unpaired t-test with Welch's correction for unequal variance. WT was statistically significant from KO and Mut strains, but KO and Mut were not statistically significant from each other (*: p=<.016 to account for multiple t-tests; ns= not significant). This allows us to conclude that nucleosome ubiquitylation is the BRC-1 driving mechanism that promotes embryonic survival.

Funding & Thank You

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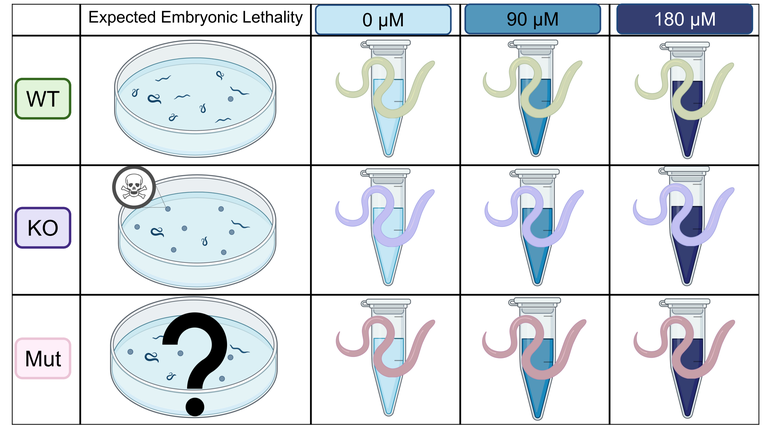
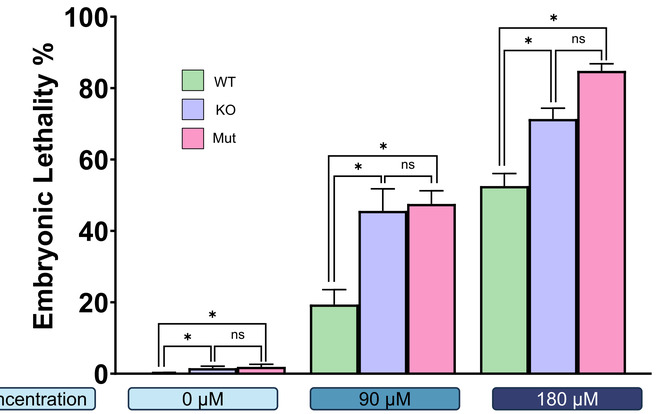
Thank you for being interested in my poster!

Cisplatin Increases DNA Damage in Mutant Worms



Above: The same strains of worms were exposed to a low and high concentration of a DNA damage causing agent, cisplatin. Cisplatin is a platinum chemotherapy drug used to treat people who develop breast cancer with BRCA1 mutations. Cisplatin was used to induce DNA damage, as worms with BRC-1 mutations would be unable to efficiently repair DNA damage.



Above: **Embryonic lethality of *C. elegans* strains exposed to 0, 90 µM, or 180 µM cisplatin.** Embryonic lethality measured across three strains of *C. elegans* across three concentrations of cisplatin. Mean and standard error shown for all strains across all concentrations. Ordinary two-way ANOVA using strain effects only with Tukey multiple comparison Test. *: WT (green) is statistically different from both KO (purple) and Mut (pink) across cisplatin concentrations (p=<0.0001). ns: No statistical difference is predicted between KO and Mut strains across cisplatin concentrations (p=0.73).

Conclusions & Future Directions

- Loss of mononucleosome ubiquitylation enzymatic function of BRC-1 increases embryonic lethality in *C. elegans* statistically equivalent to knock-out BRC-1. This trend is seen with and without cisplatin, a DNA damage inducing agent.
- Ongoing research is probing DNA damage through more direct mechanisms.