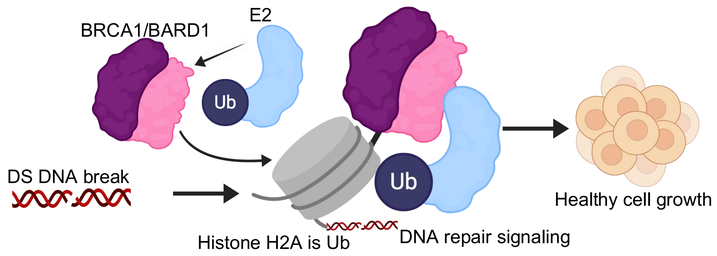
What in the worm!? Investigating nucleosome ubiquitylation by BRCA1 in *C. elegans*

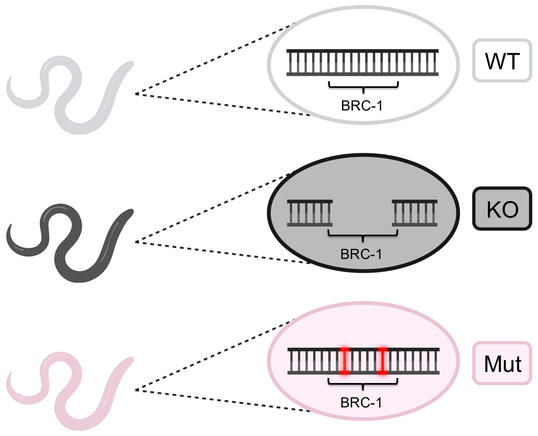
Meagan McMann-Cunningham, Lucy McCollum, and Nathalie Carlon, and Mikaela D. Stewart. Texas Christian University, Fort Worth, TX

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. 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 nucleosome monoubiquitylation is correlated with an increase in DNA damage repair through homologous recombination.

Generating a Nucleosome-Binding Deficient Mutant

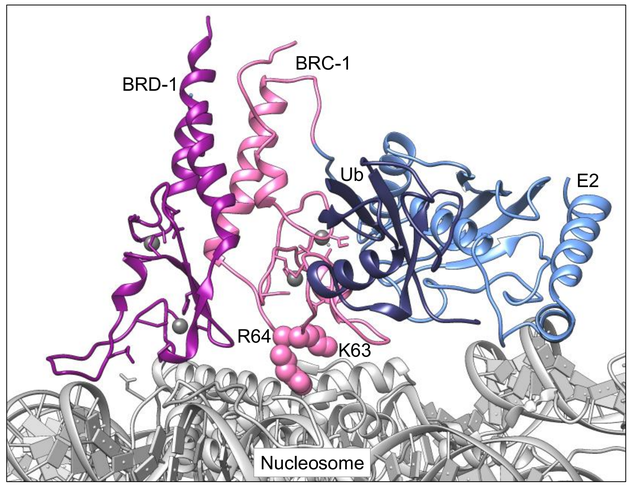


Above: To understand how DNA damage accumulation is related to BRC-1 variants, we tested the embryonic lethality across three worm strains. These are the three worm strains that our lab is using to determine the role of nucleosome monoubiquitylation in DNA damage accumulation. Shown in white, N2 Bristol strain is the wild-type (WT) worm with a fully functional BRC-1 protein. Shown in black is the strain *xoe4* (KO), which is the *brc-1* full genetic knockout. Shown in pink, 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 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.

Objectives

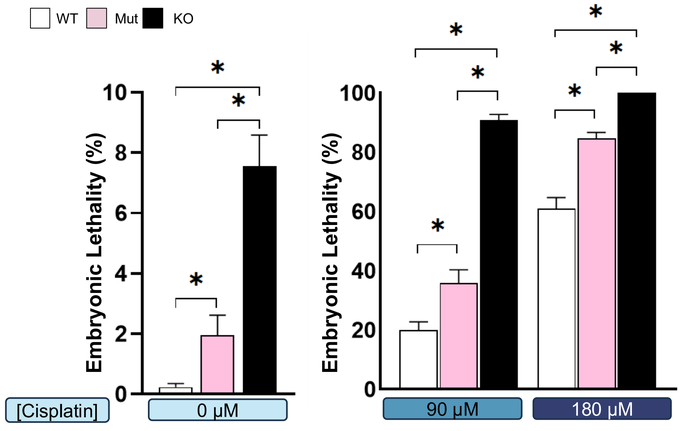
- Explore how nucleosome monoubiquitylation is related to DNA damage accumulation with mutations in BRC-1.
- Explore how BRC-1 variants respond to DNA damage in the presence of cisplatin, a DNA-damaging agent.
- Explore how nucleosome monoubiquitylation is related to cytochrome P450 and microsatellite repeat gene regulation.
- Further C. elegans as a model for understanding the genetic inheritance of cancer risk and conserved nuclear signaling pathways.

Syb BRC-1 mutant is unable to bind to nucleosome



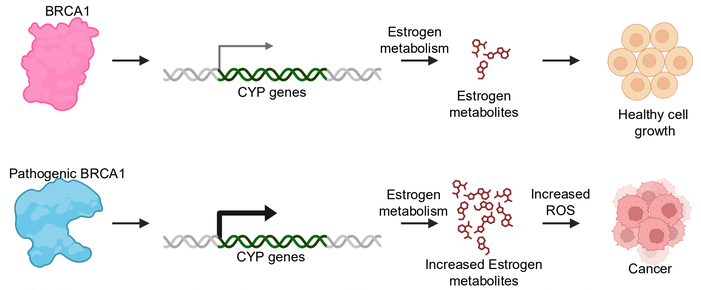
Above: Cryo-EM structure of interacting BRC-1, BRD-1 LET70, ubiquitin, and nucleosome. BRD-1 RING (magenta) is bound to BRC-1 RING (light pink). LET70 is an E2 conjugating enzyme (shown in light blue) that binds to BRC-1 to facilitate the addition of ubiquitin (Ub shown in navy) onto histone H2A (nucleosome shown in grey. Grey spheres show Zn. K63 and R64 are BRC-1 residues that interact with the nucleosome. In our *syb5376 C. elegans* mutant, these residues are mutated to glutamic acid residues through two point mutations. The glutamic acids will repel the nucleosome binding ability of BRC-1, thus hindering BRC-1 from acting as an E3 ligase but retaining all other functions of BRC-1. PDBID: 7JZV

Cisplatin Increases DNA Damage in Mutant Worms



Above: **Embryonic lethality of *C. elegans* strains exposed to 0, 90 µM, or 180 µM cisplatin.** **Above Left:** Embryonic lethality of worm strains without cisplatin exposure. Mean embryonic lethality plotted with SEM bars. Unpaired t-test with Welch's correction for unequal variance. All three strains, WT, KO, and Mut, were statistically significant from each other (*: p=<.0167 to account for multiple t-tests). **Above Right**: 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 exposure causes double-strand DNA breaks, which BRCA1 is recruited to correct. Cisplatin was used to induce DNA damage, as worms with BRC-1 mutations would be unable to repair DNA damage efficiently. Embryonic lethality was measured across three strains of *C. elegans* across three concentrations of cisplatin. Mean and standard error are shown for all strains across all concentrations. Ordinary two-way ANOVA with Tukey multiple comparison Test. *: WT (green), KO (purple), and Mut (pink) are different across cisplatin concentrations (p=<0.0001).

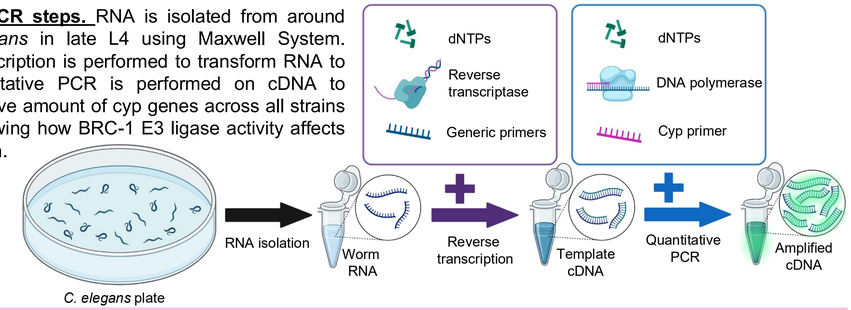
BRCA1 regulates Cytochrome p450 expression



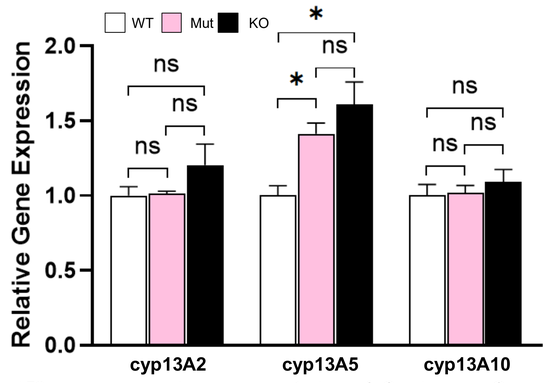
Left: Functional BRCA1 downregulates cytochrome p450 (cyp) genes that metabolize small molecules, drugs, and steroids (ie estrogen). Loss of function BRCA1 mutations cause an increase in estrogen metabolism, which causes an accumulation of DNA damaging estrogen metabolites with free radicals. We have previously shown BRC-1 to regulate cyp genes in worms, but it is unknown how BRC-1 E3 ligase activity affects cyp gene expression.

RT-qPCR determines gene expression

Right: RT-qPCR steps. RNA is isolated from around 2000 *C. elegans* in late L4 using Maxwell System. Reverse transcription is performed to transform RNA to cDNA. Quantitative PCR is performed on cDNA to measure relative amount of cyp genes across all strains of worms showing how BRC-1 E3 ligase activity affects cyp expression.



BRC-1 monoubiquitylation regulates cyp13A5 gene expression



Above: **Cytochrome p450 gene expression across strains of *C. elegans*.** Our lab has previously reported changes in cyp genes with brc-1 partial knockout. Cyp13A2, Cyp13A5, and Cyp13A10 were chosen because there were reported differences in gene expression using a different strain of KO brc-1. CQ values were standardized to a reference gene, Y45F10D.4, and averages of strains were standardized to the N2 worm strain to show differences between strains. The chosen cyp13A family previously showed differences in all tested genes above. Mean and standard error shown for all genes. Welches one-way ANOVA with Tukey multiple comparison Test. *: WT (green) is statistically different from both KO (purple) and Mut (pink) in cyp13A5 (p=<0.05). ns: strains are not significantly different than each other in cyp13A2 and cyp13A10 (p=>0.05).

Conclusions and future directions

- Loss of nucleosome monoubiquitylation enzymatic function of BRC-1 causes an intermediate effect between functional brc-1 and 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.

Funding & Thank You

Funding for this project came from SERC grants.
Thank you to Dr. Stewart and the Stewart Lab!
Thank you to TCU College of Science and Engineering and NIH for funding.
Thank you for being interested in my poster!