Investigating the Role of BRCA1 in Regulating Reactive Oxygen Species and Maintaining Genomic Stability

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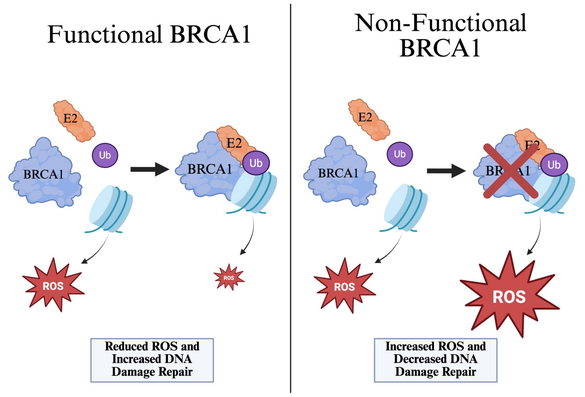
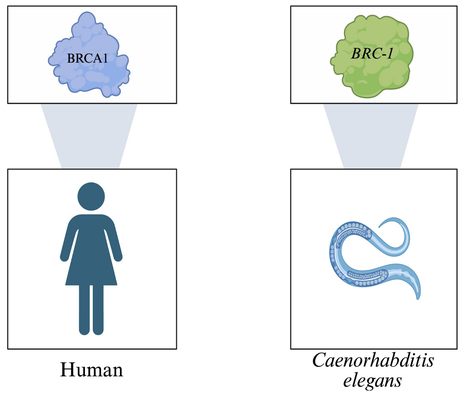
Introduction



Above: BRCA1, which is widely known for its link to hereditary breast and ovarian cancer, helps protect cells by repairing DNA damage and keeping oxidative stress under control. When reactive oxygen species, ROS, levels rise, they can damage DNA, proteins, and other cell structures, and BRCA1 responds by activating repair pathways while also helping regulate histone H2A monoubiquitylation through its E2 partner to keep chromatin stable under stress. Without functional BRCA1, ROS can build up, DNA damage increases, and disrupted H2A ubiquitylation weakens the cell's overall stress response.

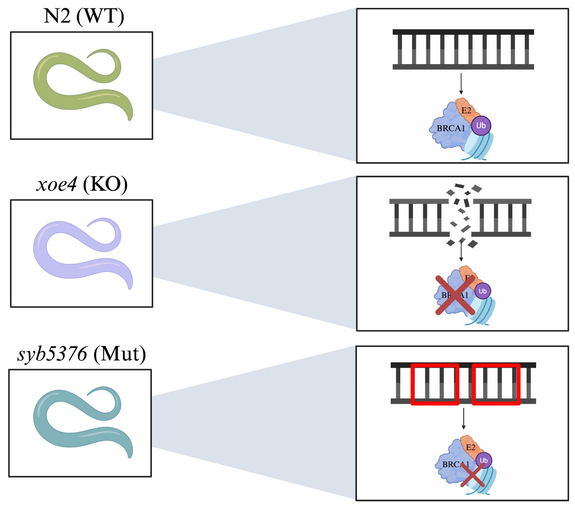


Above: *C. elegans* are a valuable model organism for studying how BRCA1 functions in protecting the genome from damage. The worm's BRC-1 protein closely mirrors human BRCA1, sharing similar roles in DNA repair, chromatin stability, and response to cellular stress. Research on the BRC-1 protein allows for the ability to trace how BRCA1's repair functions evolved and how disruptions to this pathway can lead to genomic instability.

Objectives

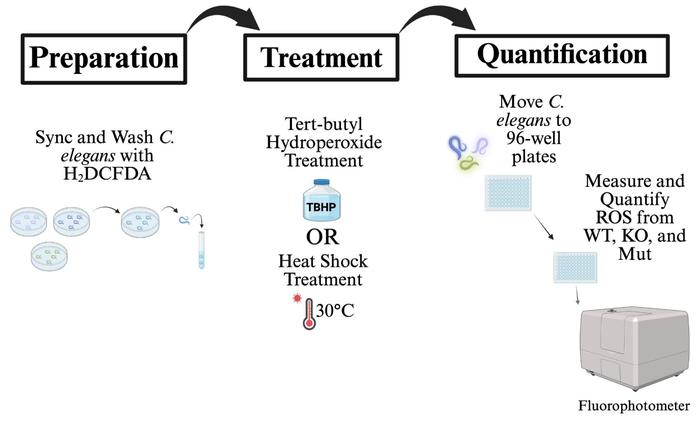
- **Develop Assay to Measure Oxidative Stress:** Experiment with different methods to establish an assay for measuring oxidative stress in *C. elegans* through intracellular ROS quantification
- **Examine Differences Between Different BRC-1 Genotypes:** Compare how oxidative stress responses differ WT, Mut, and KO to determine how BRC-1 status affects cellular response to stress
- **Evaluate Impact of Environmental Stressors:** Measure intracellular ROS levels following exposure to oxidative stressors, tert-butyl hydroperoxide (TBHP) and heat shock treatment

Alteration of Nucleosome Ubiquitination in *C. elegans*



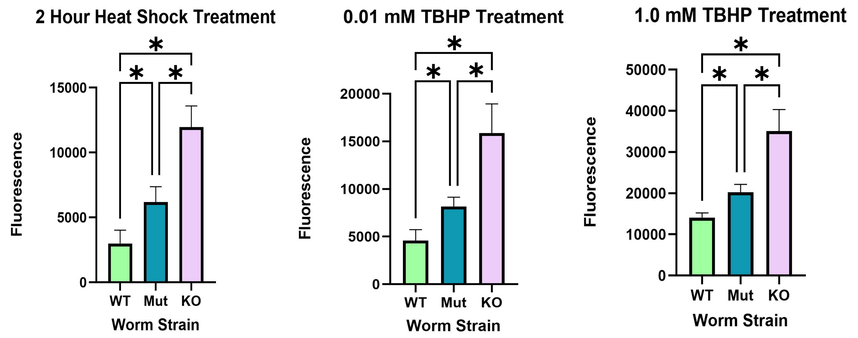
Above: The strains shown here represent the differences in nucleosome monoubiquitylation across the three *C. elegans* strains used in our experiments. N2 is the wild-type strain (WT) , carrying functional BRC-1 that binds nucleosomes and supports normal H2A monoubiquitylation. *xoe4* is the *brc-1* full genetic knockout (KO), which lacks BRC-1 and cannot perform nucleosome-associated ubiquitylation. The *syb5376* mutant (Mut) contains two CRISPR-engineered point mutations in the nucleosome binding region of BRC-1, keeping other enzymatic functions intact but preventing proper nucleosome interaction and blocking ubiquitin addition to histone H2A. Comparing these strains allows us to isolate how the loss of nucleosome monoubiquitylation, rather than total loss of BRC-1, shapes DNA damage accumulation in vivo.

Quantification of ROS levels in *C. elegans*



Above: This figure shows the workflow used to measure ROS levels in *C. elegans*. The process starts by synchronizing the worm populations so all strains are at the same developmental stage, followed by washing the worms to remove bacteria before any treatment. C. elegans are then washed with M9 and H_2DCFDA to allow for fluorescence of the worms and detection of ROS. Once the worms are washed and synced, they are exposed to the stress conditions being tested. After treatment, the worms are placed into a 96-well plate in order to quantify ROS. A fluorophotometer then reads the fluorescence from each well, giving a direct measurement of ROS levels across the various strains and conditions. Overall, the figure captures the full pipeline from synchronization and worm handling to quantitative ROS data.

Loss of BRCA1 Histone H2A Ubiquitination Increases Oxidative Stress in *C. elegans*



Above: Stress-induced ROS levels across the three *C. elegans* strains are linked to differences in H2A monoubiquitination and BRC-1 function. After a 30°C heat shock for two hours, WT worms showed the lowest ROS accumulation. Mut produced higher ROS than WT, while the KO showed the highest fluorescence due to complete loss of BRC-1. This intermediate phenotype in Mut suggests that nucleosome monoubiquitylation contributes to controlling ROS during stress, while other BRC-1 functions still provide partial protection. A similar pattern was observed in the tert-butyl hydroperoxide (TBHP) experiments, with WT showing the lowest ROS, KO the highest, and Mut again displaying an intermediate response between the two other phenotypes. ROS levels were higher for both TBHP experiments due to the direct creation of free radicals while heat shock did not. An ordinary one-way ANOVA with Tukey's multiple comparisons test was used. WT (green) differs significantly from Mut (purple) and KO (cyan), and KO also differs from Mut. * ($p < 0.0001$).

Conclusions and Future Directions

- Loss of BRC-1 alters stress responses in *C. elegans*, with disrupted H2A monoubiquitylation leading to increased ROS and reduced cellular stability under oxidative and heat stress.
- Comparison of WT, Mut, KO shows that BRCA1 status strongly influences stress responses, with greatest vulnerability in strains lacking BRC-1 nucleosome regulation and BRC-1 in general.
- Under oxidative and heat stress, BRC-1–deficient *C. elegans* exhibit elevated ROS levels, suggesting a role for BRC-1 in coordinating DNA repair during cellular stress.
- These findings suggest that BRCA1 loss may heighten vulnerability to environmental stress rather than directly causing immediate pathology, potentially contributing to variable outcomes among individuals with similar mutations.

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