SUMO-Mediated Recruitment of DNA Damage Response Proteins to Repair Sites

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Background

- DNA double-strand breaks (DSBs) are among the most dangerous forms of DNA damage. If improperly repaired, they can lead to chromosomal instability and mutations that drive cancer. In response to DSBs, cells assemble DNA damage response (DDR) condensates that concentrate repair proteins at sites of damage, otherwise known as DDR SUMO substrates.
- The role of SUMOylation in organizing these condensates remains incompletely defined. SUMOylation involves the covalent attachment of Small Ubiquitin-like Modifier (SUMO) proteins to target substrates. This can occur as monoSUMOylation (a single SUMO moiety) or polySUMOylation (formation of SUMO chains). Emerging evidence suggests that SUMO modifications may coordinate both assembly and turnover of repair complexes, but how distinct SUMO states regulate condensate dynamics is unclear.
- We hypothesize that monoSUMOylation promotes the formation of DDR condensates, whereas polySUMOylation facilitates their disassembly through the polySUMO axis.. Thus, distinct SUMOylation states may function as a regulatory timer controlling the formation and resolution of DNA protein complexes.
- Despite increasing recognition of SUMO involvement in DNA repair, the field lacks a clear mechanistic model distinguishing how mono- versus polySUMOylation differentially control condensate assembly and disassembly at damage sites. Defining this distinction is essential for understanding how post-translational modifications regulate genome stability.

Methods

- This study uses budding yeast (*Saccharomyces cerevisiae*) with an inducible HO endonuclease system to generate a site-specific DNA DSB adjacent to a fluorescent (mApple) reporter locus. GFP-tagged DNA repair proteins are monitored using live-cell fluorescence microscopy to quantify condensate dynamics in wild-type and SUMO pathway mutant strains.

Recruitment Timing

- Recruitment is measured as the time from DSB induction to the first detectable accumulation of GFP-tagged repair proteins at the break site. Strains with reduced Ubc9 activity are used to decrease global SUMO conjugation. Delayed or impaired focus formation in these strains would support a requirement for monoSUMOylation in condensate initiation.

Condensate Persistence

- Persistence is quantified as the duration that repair foci remain detectable following formation. SUMO mutants that prevent chain elongation while preserving single SUMO attachment are used to distinguish mono- from polySUMOylation effects. Changes in focus stability under these conditions indicate how distinct SUMO states influence condensate maintenance.

Disassembly Kinetics

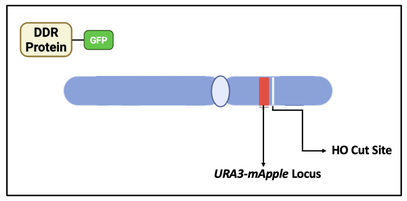
- Resolution is measured as the rate of condensate disappearance following repair. Strains lacking functional Slx5 or Slx8, which recognize SUMO chains for downstream protein turnover, are analyzed to determine whether polySUMOylation promotes condensate clearance. Prolonged persistence in these strains would support a role for SUMO chains in disassembly.

Comparative Quantitative Analysis

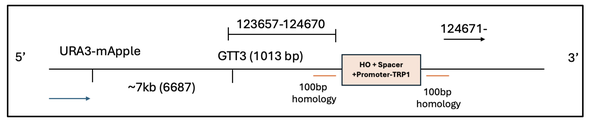
- Time-course imaging enables direct comparison of recruitment timing, persistence duration, and disassembly rates between wild-type and SUMO pathway mutants, allowing mechanistic distinction between monoSUMOylation-driven assembly and polySUMOylation-mediated turnover.

Results

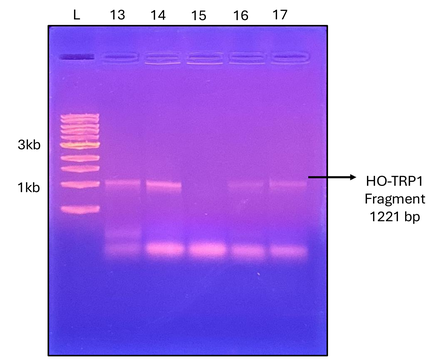
- Preliminary work has focused on strain development and validation to ensure robust fluorescent signal detection and reliable induction of DNA damage. Optimization efforts aim to enhance visualization of repair foci and improve experimental reproducibility.
- Based on our proposed model, we anticipate that disruption of SUMO conjugation will impair repair focus formation, reflecting a requirement for monoSUMOylation in condensate assembly. We further anticipate that defects in the polySUMO axis will result in prolonged persistence of DDR condensates, suggesting a role for polySUMOylation in promoting condensate disassembly. These findings would support a regulatory framework in which SUMOylation coordinates both the initiation and resolution phases of the DNA damage response.



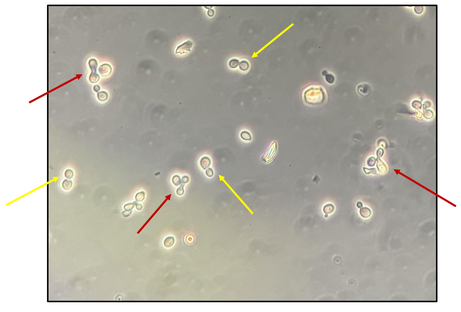
Chromosome V



HO-Induced Double Strand Break Construct Design



Using PCR to Verify Insertion of HO Site



Double Strand Break Induced by β-Estradiol Causes Cell Cycle Arrest as Large Budded (yellow) and Sick Cells (red)

Conclusion & Discussion

- This study investigates how SUMOylation regulates the assembly and resolution of DNA damage response (DDR) condensates. To test this mechanism, we are developing yeast strains with targeted disruptions in SUMO conjugation and SUMO-chain formation. These strains provide a controlled system to isolate how different SUMO states influence the recruitment and persistence of repair proteins at DNA DSBs.
- If the model is supported, strains with reduced SUMO conjugation should exhibit delayed or weakened recruitment of DDR proteins, suggesting a requirement for monoSUMOylation during condensate assembly. In contrast, strains deficient in SUMO-chain formation may show prolonged persistence of repair foci, indicating that polySUMOylation contributes to condensate turnover and disassembly. Comparing condensate dynamics across these engineered strains will allow the functional roles of distinct SUMOylation states to be evaluated. Together, the development and analysis of these strains establishes a framework for examining how SUMO-mediated post-translational modifications regulate condensate behavior during DNA repair.
- Understanding how post-translational modifications govern biomolecular condensate dynamics provides broader insight into mechanisms that preserve genome stability. Because dysregulated DNA repair contributes to oncogenesis, defining how SUMOylation modulates repair complex behavior may reveal molecular vulnerabilities in cancers characterized by defective DNA damage response pathways.

Future Directions

- Following strain optimization and validation of reproducible HO-induced DNA damage, future work will focus on detailed quantitative time-course analyses comparing wild-type and SUMO pathway mutant strains. Statistical evaluation of recruitment timing, persistence duration, and disassembly rates will clarify how altered SUMOylation states affect condensate dynamics and repair efficiency.
- Additional studies will examine whether prolonged or defective condensate behavior correlates with impaired genome stability, including increased sensitivity to stress or accumulation of repair defects. Expanding this work to additional repair proteins may further define whether SUMO-dependent regulation is conserved across multiple DDR pathways.
- Ultimately, this research aims to establish a mechanistic model for how SUMO-regulated modifications coordinate DNA repair complex dynamics and to explore the translational relevance of these pathways in cancer biology and therapeutic intervention strategies.

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Acknowledgements

I would like to extend my gratitude to Dr. Yanchang Wang for his mentorship, guidance, and support throughout this project. I am especially grateful to Sophia Owutey and members of the Wang Lab for their training and collaboration. This work was conducted in and supported by the Department of Biomedical Sciences at the Florida State University College of Medicine.