Defining the roles of conserved DNA repair complexes in maintenance of *C. elegans* meiotic genome integrity

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Abstract

Most organisms utilize meiosis, a specialized form of cell division, to produce haploid gametes such as sperm and eggs. Failure to maintain genomic integrity during meiosis can result in infertility and serious diseases, such as cancer and birth defects. Despite these risks, double strand DNA breaks (DSBs) are intentionally induced during meiotic prophase I. Meiotic cells must repair a specific subset of DSBs through interhomolog crossover recombination to ensure accurate chromosome segregation, while the remainder are resolved through alternative repair pathways to maintain genome integrity. Interhomolog recombination has been studied extensively, but the mechanisms of alternative meiotic DNA repair remain elusive. The Structural Maintenance of Chromosomes 5/6 complex (SMC-5/6), its E3 SUMO ligase subunit NSE-2, and the BRC-1/BRD-1 heterodimer are conserved proteins required for homolog-independent meiotic DSB repair and have been shown to genetically interact. However, the specific mechanisms by which these proteins function together to preserve meiotic genome integrity is unknown. To determine the NSE-2 specific and NSE-2 independent meiotic functions of the SMC-5/6 complex in meiotic DSB repair, we utilized immunofluorescence imaging and a mortal germline phenotype assay to assess *smc-5* and *nse-2 C. elegans* mutants. Both *smc-5* and *nse-2* mutants exhibit persistent DNA damage, suggesting that both SMC-5/6 and NSE-2 are required for efficient meiotic DSB repair. However, we find that SMC-5/6, but not NSE-2, is required for germline immortality. These data suggest a separation of function for SMC-5/6, which performs NSE-2 dependent and independent functions to maintain meiotic genome integrity. Finally, to define epistatic relationships between BRC-1/BRD-1, SMC-5/6, and NSE-2 in DNA repair, we assessed the germline sensitivity to ionizing radiation by brood viability of pairwise *brc-1*, *smc-5*, and *nse-2* double mutants . These data suggest that exogenous DSB repair is differentially regulated within meiotic prophase I and implicate SMC-5/6 as a central regulator of both NSE-2 and BRC-1 dependent DSB repair. Taken together, our research defines fundamental genetic mechanisms and interactions preserving genomic integrity.

How is genome integrity maintained across generations?

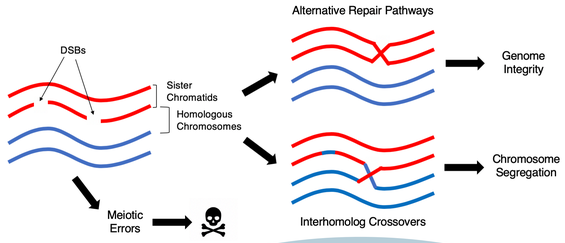

Figure 1. Meiotic double-strand DNA breaks (DSBs) are resolved via interhomolog recombination or through alternative repair pathways (intersister recombination, NHEJ, TMEJ, and SSA).

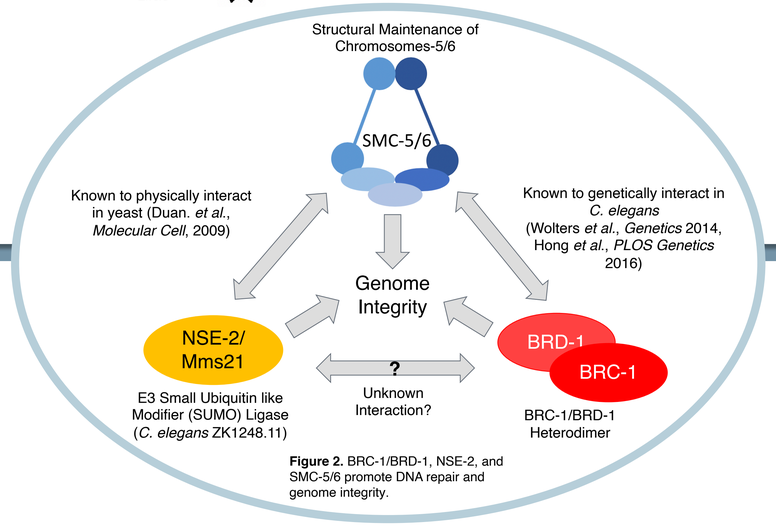

Figure 2. BRC-1/BRD-1, NSE-2, and SMC-5/6 promote DNA repair and genome integrity.

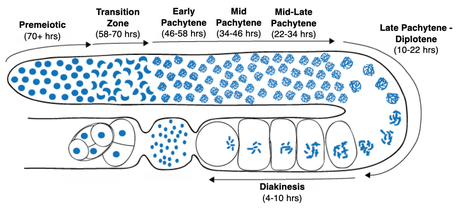

Figure 3. The *C. elegans* hermaphrodite gonad is spatially and temporally organized, which enables a reverse time-course analysis of meiotic prophase I.

SMC-5/6 performs NSE-2 dependent and independent functions

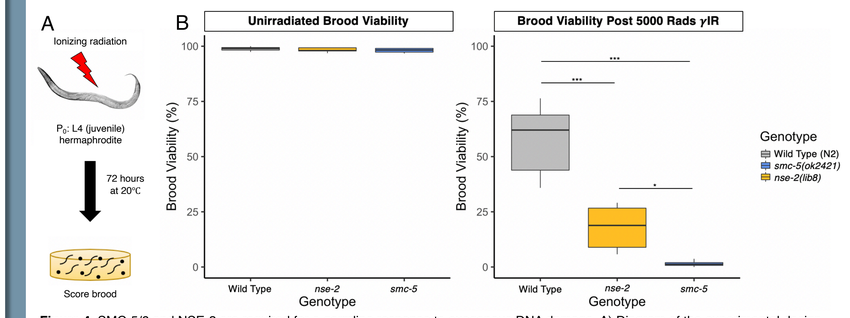

Figure 4. SMC-5/6 and NSE-2 are required for a germline response to exogenous DNA damage. A) Diagram of the experimental design. B) Boxplots of the brood viability by treatment and genotype show that *nse-2* and *smc-5* display a greater sensitivity to DNA damage than the wild-type strain (p values calculated by Tukey's HSD test, * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$). Brood viability is calculated as the proportion of live progeny of the total brood size (live and dead progeny).

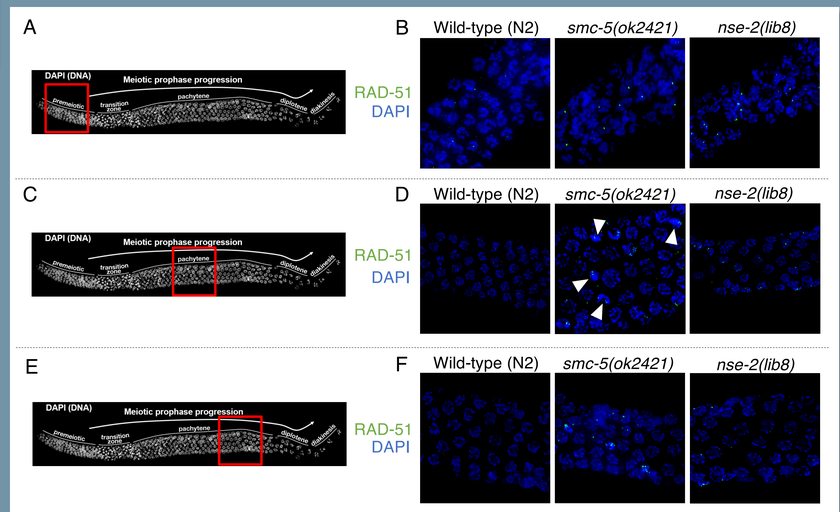

Figure 5. Preliminary immunofluorescence images of dissected *C. elegans* gonads suggest both NSE-2 and SMC-5/6 are required for efficient meiotic DNA repair and replication, but only *smc-5* mutants exhibit elevated putative apoptotic nuclei. A-B) Both *nse-2* and *smc-5* mutants exhibit elevated RAD-51 foci in the premeiotic tip (indicated by red box in A), indicative of errors in DNA replication. C-D) *smc-5* mutants, but not *nse-2* mutants, display elevated mid-pachytene (indicated by red box in C) nuclei with chromosome morphology indicative of apoptosis (white arrows). E-F) *B*oth *smc-5* and *nse-2* mutants display unresolved DNA damage in late pachytene (indicated by red box in E).

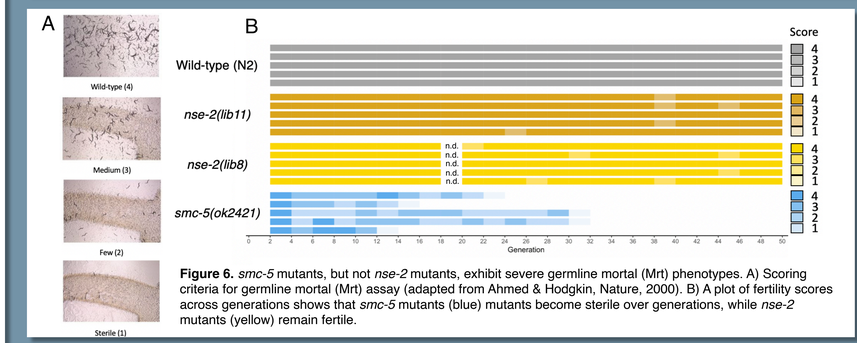

Figure 6. *smc-5* mutants, but not *nse-2* mutants, exhibit severe germline mortal (Mrt) phenotypes. A) Scoring criteria for germline mortal (Mrt) assay (adapted from Ahmed & Hodgkin, Nature, 2000). B) A plot of fertility scores across generations shows that *smc-5* mutants (blue) mutants become sterile over generations, while *nse-2* mutants (yellow) remain fertile.

- Both NSE-2 and SMC-5/6 are required for efficient meiotic DNA repair
- The SMC-5/6 complex has NSE-2 independent functions in meiotic apoptosis and the maintenance of fertility.

SMC-5/6, NSE-2, and BRC-1/BRD-1 are differentially engaged within meiotic prophase I to resolve exogenous DNA damage

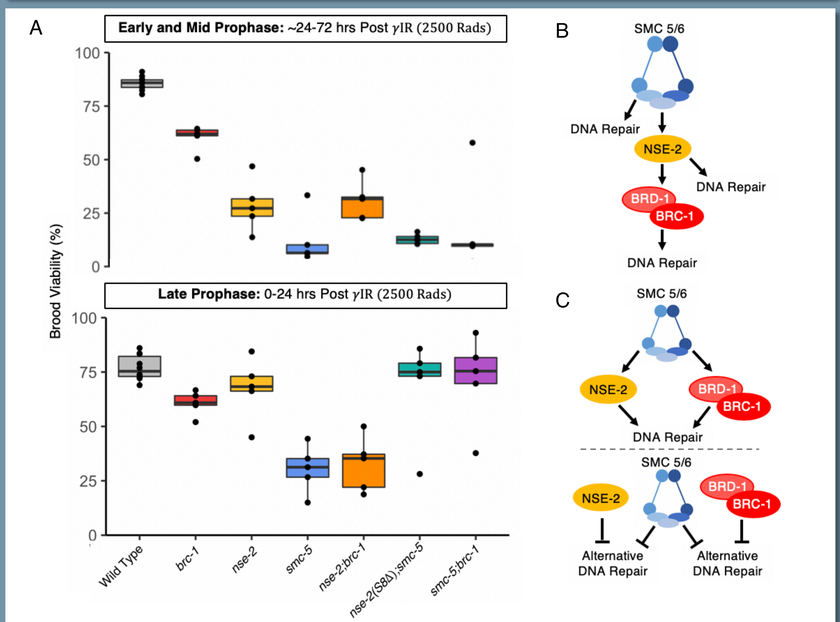

Figure 7. BRC-1/BRD-1, SMC-5/6, and NSE-2 are differentially engaged within meiotic prophase I to resolve exogenous DNA damage. A) Boxplots of the brood viability by genotype following 2,500 Rads of ionizing radiation and a reverse time course analysis of the meiotic prophase I stages at which exogenous DNA damage was induced in progeny. Irradiated *brc-1, smc-5/6,* and *nse-2* single and double mutants displayed varying sensitivity to exogenous DNA damage. B) In early to mid meiotic prophase I, exogenous DNA repair is regulated through a linear epistatic pathway. C) In late meiotic prophase I, exogenous DNA repair is regulated through a diverging epistatic pathway. *smc-5;brc-1* and *smc-5;nse-2* mutants partially suppressed the sensitivity to ionizing radiation observed in *smc-5* mutants, suggesting that SMC-5 and BRC-1 or NSE-2 act redundantly to suppress alternative and potentially mutagenic DNA repair pathways.

- SMC-5/6, NSE-2, and BRC-1/BRD-1 are differentially engaged within meiotic prophase I to resolve exogenous DNA damage.
- SMC-5/6 and BRC-1/BRD-1 or NSE-2 may act redundantly to suppress alternative and potentially mutagenic DNA repair pathways in late meiotic prophase I.

Future Directions

1. Define the contributions of NSE-2, and BRC-1 to sister chromatid repair.
   - SMC-5/6 promotes efficient sister chromatid repair – do NSE-2 and BRC-1?
   - Perform the sister chromatid repair assay in single and double mutants.
2. Quantify the dynamics of DNA repair.
   - Quantify RAD-51 foci in single and double mutant gonads.
3. Visualize repair complex localization.
   - Assess SMC-5, SMC-6, and NSE-2 localization in wild-type and mutant contexts.

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