Assessing the role of the SMC-5/6 complex in meiotic double strand DNA break repair

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Abstract

Meiosis is the specialized cell division used to form haploid gametes. During meiosis, endogenous double strand DNA breaks (DSBs) are induced. A subset of these DSBs must be repaired as crossovers with the homologous chromosome to ensure proper chromosome segregation. Although repair is required for proper chromosome segregation, use of the homologs as a repair template for DSB repair is restricted to a specific time window during meiotic prophase I. DSBs incurred outside of this window must be repaired to ensure genomic integrity. Multiple lines of evidence have suggested that these homolog-independent repair events utilize the sister chromatid as a template in repairing DSBs. Utilizing *Caenorhabidits elegans*, the Libuda lab has developed a genetic assay for intersister repair, directly demonstrating the occurrence of intersister repair events during meiosis; however, the molecular mechanism of intersister repair remains unknown. Previous studies have implicated multiple proteins in promoting homolog-independent DNA repair during meiosis, including the structural maintenance of chromosomes (SMC) 5/6 complex. Utilizing this assay, I will determine whether the SMC-5/6 complex is required for intersister repair during meiosis. Specifically, I will place an *smc-5* null mutation in the intersister repair assay and examine the frequency of intersister repair events at a specific locus in the genome. If SMC-5 is required for intersister repair, I expect to observe a lowered frequency or elimination of intersister repair events compared to wild type controls. Determining the precise role of *smc-5* and other candidate genes in DSB repair will provide insight into the mechanisms underlying DNA repair decisions during meiosis.

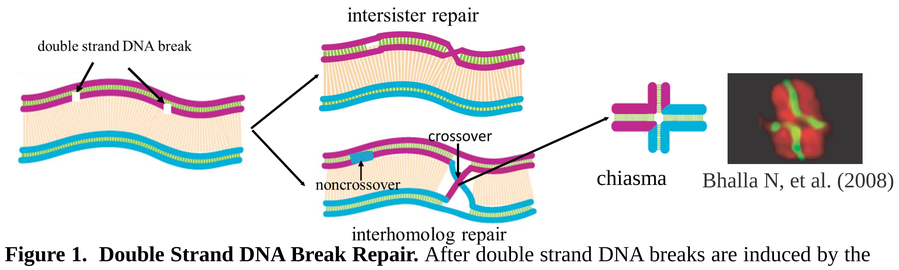


Figure 1. Double Strand DNA Break Repair. After double strand DNA breaks are induced by the endonuclease SPO-11, they must be repaired to ensure genomic integrity. These breaks can be repaired as a crossover or a non-crossover, using the homologous chromosome or the sister chromatid as a repair template.

How do cells choose which template is utilized to repair a double strand DNA breaks?

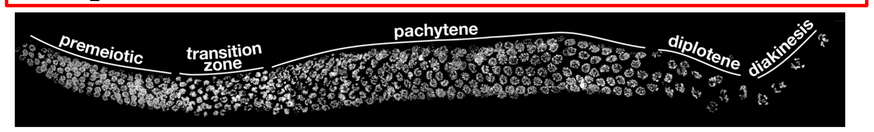


Figure 2. Organization of the *C. elegans* germ line. The nematode *Caenorhabditis elegans* provides us with an excellent model to study meiosis. Oocytes in The *C.elegans* gonad are arranged in a spatial temporal gradient, allowing us to simultaneously view each stage of meiotic prophase I.

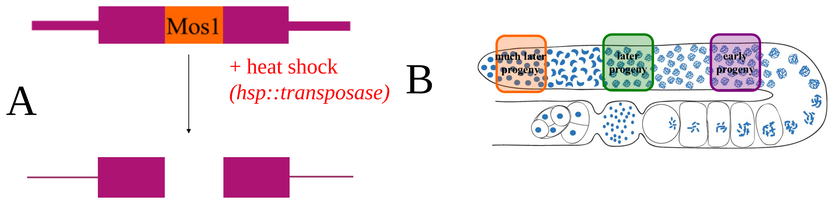
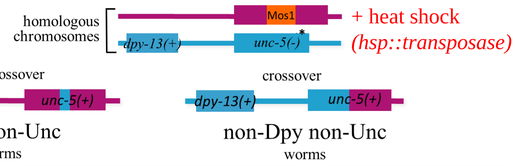


Figure 3. Exploiting *C. elegans*. A. Using a heat induced Mos1 transposon excision, the Libuda lab has developed novel assays to assess repair outcomes of breaks induced at a specific genomic location. **B.** With the arrangement of the *C. elegans* gonad, we can further exploit this system to not only view the repair outcome of a break, but how these outcomes change through the progression of meiotic prophase I.

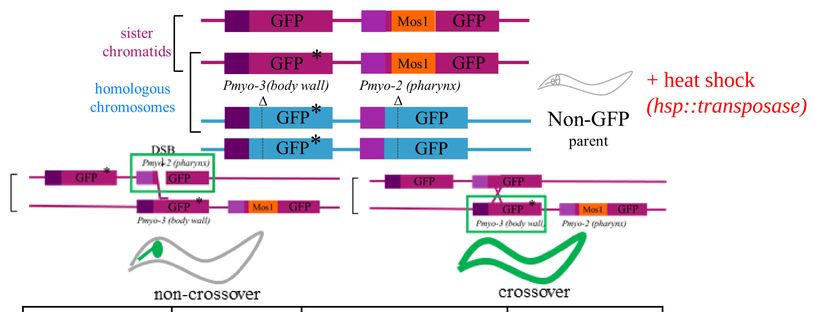
Interhomolog (IH) Repair Assay



Stage of Meiotic Prophase I	Time Point Post HS	Unc-5(+) Recombinant: Total Progeny	% IH Repair
Diakinesis/Post-fertilization	4-10 hr	0:517	0%
Late pachytene/diplotene	10-22 hr	0:1239	0%
Mid-pachytene	22-34 hr	69:1420	5%
Mid/early pachytene	34-46 hr	82:1326	6%
Early pachytene	46-58 hr	59:1092	5%
Leptotene/zygotene	58-70 hr	19:629	3%
Pre-meiotic	70+ hr	4:306	1%
	Total:	233:6529	4%

Figure 4. Interhomolog repair is active until mid-pachytene phase of meiosis and not active during late meiosis. Previous work conducted in the Libuda Lab utilizing the Mos1 transposon to visualize homolog dependent repair events suggests that repair template choice switches during the later stages of meiotic prophase I (Rosu, Libuda, and Villeneuve, *Science 2011)*.

Intersister (IS) Repair Assay



Stage of Meiotic Prophase 1	Time point post HS	GFP Recombinant: Total Progeny	% IS Repair
Diakenisis	4-10 hrs	9:699	1.29%
Diplotene	10-22 hrs	11:1593	0.69%
Late Pachytene	22-34 hrs	8:1165	0.69%
Mid Pachytene	34-46 hrs	3:828	0.36%
Early Pachytene	46-58 hrs	3:709	0.42%
Transition Zone	58-70 hrs	3:365	0.82%
Premeiotic	70 + hrs	1:331	0.30%
Total		38:5690	0.67%

Figure 5. Intersister repair is active during the late stages of meiosis. Just as the IH assay has suggested that interhomolog repair is inactive during the later stages of meiotic prophase I, progeny from earlier time points (later in meiotic prophase I) Showed a higher frequency of GFP expression. This indicates that during the later stages of meiotic prophase I, the sister chromatid is being utilized as a repair template to facilitate double strand DNA break repair.

What proteins facilitate intersister repair?

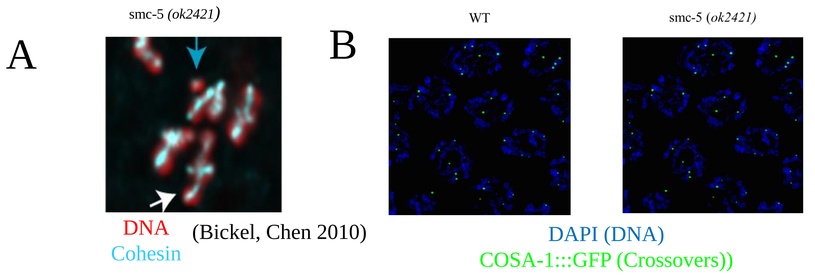


Figure 6. The role of SMC-5 in homolog independent repair. The SMC-5/6 complex has been demonstrated to have a role in the DNA damage response, and recent data implicate a role in homolog-independent repair. **A.** *smc-5* mutants exhibit chromosome fragmentation, indicating double strand incomplete repair of double strand DNA breaks. **B.** SMC-5/6 is dispensable for interhomolog repair. This is supported by *smc-5* mutant nuclei containing the proper number of crossovers.

$$♂/♀\ \frac{smc-5\ (ok2421)}{mln1}(II)\ \times ♂/♀\ \frac{unc-5(IS\ GFP\Delta)}{nT1}(IV)$$

$$\downarrow$$

$$♂\ \frac{mln1}{+}(II);\frac{+}{unc-5(IS\ GFP\Delta)}(IV)\ \times ♀\ \frac{smc-5\ (ok2421)}{+}(II);\frac{+}{unc-5(IS\ GFP\Delta)}(IV)$$

$$\downarrow$$

$$♀\ \frac{smc-5\ (ok2421)}{mln1}(II);\frac{unc-5(IS\ GFP\Delta)}{unc-5(IS\ GFP\Delta)}(IV)$$

Fig. 7. Strain construction to generate an *smc-5 (ok2421)* mutant that can be used in the intersister assay. To ensure that our assay is not biasing towards intersister repair while still providing a clear readout of whether or not an intersister repair event has occurred, we have generated mutants that contain a defective GFP allele in the intersister assay. This allows for unbiased homology search upon DSB induction and detection of intersister repair events when screening progeny.

Future Directions

- Repeat control assay
- Perform the intersister assay on *smc-5 (ok2421)* mutants.
- Perform immunofluorescence experiments to examine the kinetics of early stage repair markers in *smc-5 (ok2421)* mutants.
- Perform restriction digests and Southern blots on progeny that displayed body wall GFP to distinguish between crossover events and gene conversion events.
- Perform an interhomolog assay to confirm that *smc-5* is dispensable for interhomolog repair.
- Sequence GFP+ progeny from the intersister assay to analyze the gene conversion tract length using engineered SNPs.

Sources

Bickel JS, Chen L, Hayward J, Yeap SL, Alkers AE, Chan RC (2010) Structural Maintenance of Chromosomes (SMC) Proteins Promote Homolog-Independent Recombination Repair in Meiosis Crucial for Germ Cell Genomic Stability. PLoS Genet 6(7): e1001028. https://doi.org/10.1371/journal.pgen.1001028V

Bhalla N, et al. (2008) ZHP-3 Acts at Crossovers to Couple Meiotic Recombination with Synaptonemal Complex Disassembly and Bivalent Formation in *C. elegans*. PLoS Genet 4(10): e1000235. https://doi.org/10.1371/journal.pgen.1000235

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