# INVESTIGATING THE ROLE OF GENOMIC POSITIONING IN DIRECTING MEIOTIC DOUBLE-STRAND DNA BREAK REPAIR

by

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During meiosis, the specialized form of cell division that produces gametes, cells utilize recombination to maintain genomic integrity and promote proper chromosome segregation, all of which are critical for fertility. Double-strand DNA breaks (DSBs), which serve as substrates for homologous recombination, are intentionally induced during meiosis. A single DSB per pair of homologous chromosomes must be repaired as a crossover recombination event in order to forge a physical connection, called a chiasma, required for proper chromosome segregation at the first meiotic division. Although DSBs are induced across the genome, crossovers in *C. elegans* preferentially form at the chromosome arms and avoid forming near the center of chromosomes. The mechanisms that underlie crossover preference at specific genomic locations is not well known. Previous studies indicate that the crossover landscape is not determined by chromatin marks or specific sequence motifs. However, proximity of a DSB to the synaptonemal complex (SC), a meiosis-specific proteinaceous structure that connects homologous chromosomes together and houses "recombination nodules" of DSB repair proteins, has been suggested to influence DSB

repair outcomes. To determine how genomic positioning of a DSB affects its repair outcome, I have taken advantage of a genetic assay that enables controlled induction of a single DSB at a known genomic location and assesses how that induced DSB was repaired. I designed and constructed plasmids that target the assay to four unique loci that differ in position both along the chromosome length and in proximity to the SC. Utilizing CRISPR/Cas9 genome editing, I have integrated the assay to one of four locations and have set up the experimental design to perform the assay. Together, the assays will elucidate how the position of a DSB within the genome influences how it is repaired to maintain genome integrity.

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# Introduction

## Meiosis

Most eukaryotic cells proliferate via mitosis, an asexual reproductive cell division that produces two genetically identical daughter cells. Gametes, or reproductive cells, are unique in that they are haploid, containing only half the number chromosomes as other cells. This halving of genetic material in each daughter cell is achieved via a specialized form of cell division called meiosis. Meiotic division ensures that offspring inherit the correct amount of genetic material since most sexually reproducing organism procreate via the fusion of two gamete cells: a sperm and an egg. Thus, offspring will inherit one copy of each chromosome from each parent, called homologous chromosomes, which encode different alleles of the same genes. Furthermore, meiosis creates new combinations of genetic material and historically, this enhancement of genetic variability has been crucial to species adaptation.

Meiosis begins with the replication of each of the homologous chromosomes, resulting in two sets of sister chromatids. Next, two separate rounds of division occur. In meiosis I, homologous chromosomes separate. Then, in meiosis II, sister chromatids divide. In sperm, this process results in four haploid gametes, but for eggs, it results in one gamete and two polar bodies, which do not develop into eggs (Figure 1).

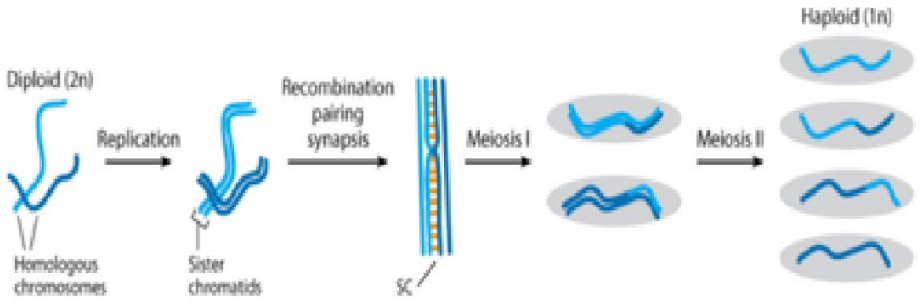


Figure 1. Overview of Meiosis

Prior to cell division, the organism possesses two copies of each chromosome and is diploid. Replication of each chromosome occurs followed by pairing of homologous chromosomes via the synaptonemal complex. The cell then undergoes the first round of division by separating homologous chromosomes in meiosis I followed by another round of division which separates sister chromatids and results in four haploid gametes. Image by Keeney et. al. 2014.

Meiosis I can be further broken down into four different phases. The first phase of meiosis I, prophase I, can be split into five sub-steps: leptotene, zygotene, pachytene, diplotene, and diakinesis (Figure 2). Within prophase I of meiosis I, two major events occur. First, the synaptonemal complex (SC), a zipper-like protein structure, physically connects paired homologous chromosomes. Assembly of the SC begins during zygotene and is complete in pachytene (Figure 2c). Second, recombination events result in the exchange of genetic material between homologous chromosomes. The SC is comprised of two lateral elements along with a central region whose proteins lie perpendicular to the lateral parts. Previous studies implicate that the SC has an important and complex role in regulating meiotic recombination events (Garcia-Muse and Boulton 2007).

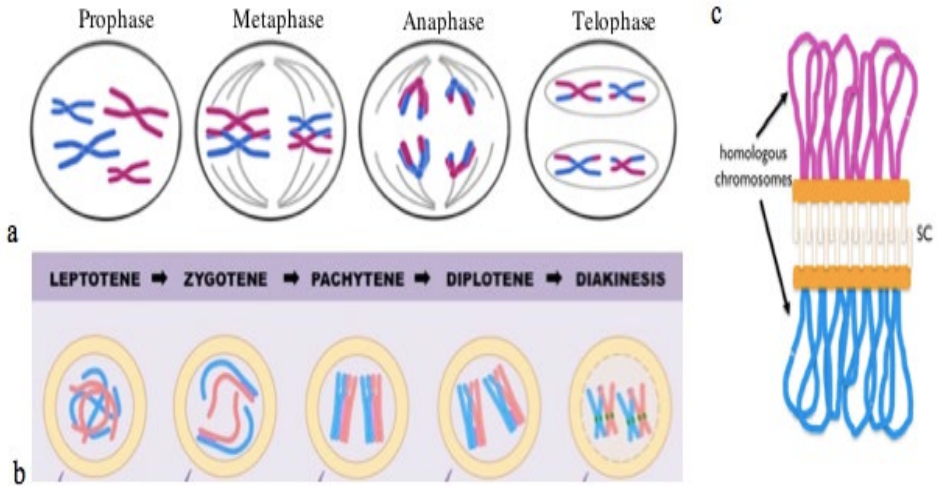


Figure 2. Stages of Meiosis I and Prophase I

**a.** Meiosis I is broken down into four phases. Recombination occurs in prophase I, homologous pairs align at the metaphase plate in metaphase I, homologs move to opposite poles in anaphase I and the cytoplasm begins dividing in telophase I.
**b.** Meiotic prophase I is subdivided into five stages. In leptotene, chromosomes condense. In zygotene, the synaptonemal complex (SC) begins to form. Crossovers are formed in pachytene and chiasma forms by diplotene. Diakinesis marks the end of prophase I.
**c.** Cartoon image depicting homologous chromosomes paired via the synaptonemal complex, marked in orange. DNA forms chromatin loops where DNA attached to the lateral elements, the horizontal bars of darker orange, of the synaptonemal complex are likely more constrained then DNA in the far loop ends.

## Double-Strand DNA Breaks

### *Break Induction and Repair*

Strikingly, given the importance of maintaining genomic integrity, meiotic cells deliberately induce double-strand DNA breaks (DSBs) in their genomes in order to initiate these integral recombination events. The conserved endonuclease SPO-11 cleaves the phosphate backbone of both strands of a DNA molecule, generating a DSB. DSBs are processed to generate 3’ single-strand overhangs, which act as scaffolds for subsequent proteins to load onto and promote repair (Dernburg *et. al.* 1998). One such

protein is RAD-51, which is a recombinase that loads to DSBs and is used as a cytological marker for DSB formation (Garcia-Muse and Boulton 2007).

During the repair of a DSB, meiotic cells may engage repair multiple repair templates and pathways (Boulton and Youds 2011). First, cells may choose to perform interhomolog repair (IH) or intersister repair (IS); the difference being whether the homologous chromosome or the sister chromatid is used as the repair template. Next, either template can then be repaired as a crossover (CO) or noncrossover (NCO) recombination event. A CO repair results in the reciprocal exchange of genetic material while nonreciprocal exchange occurs during a NCO repair (Boulton and Youds 2011). Both IH CO and IH NCO repair enhance genetic diversity in the daughter cells since homologous chromosomes may differ slightly in sequence (Figure 2a). However, IS repair maintains the original sequence of the chromosome, since sister chromatids have identical sequences, and is thus an invisible form of repair. While repair with the sister chromatid has been hypothesized to ensure genome integrity by repairing extraneous DSBs, homologous repair via IH COs is uniquely important. The crossing over of homologous chromosome arms creates the physical connection between chromosomes known as a chiasma (Figure 2b), which is vital for promoting proper chromosomal segregation in later stages of meiosis (Boulton and Youds 2011). As such, cells promote the establishment of a CO through crossover assurance. Despite the requirement of forming a chiasma, research also indicates that the formation of one CO represses the creation of additional crossovers via crossover interference, which likely acts to preserve the integrity of the genome (Rosu *et. al* 2013). These processes demonstrate

that meiotic cells carefully regulate DSB repair outcomes through a variety of mechanisms in order to ensure faithful genome inheritance.

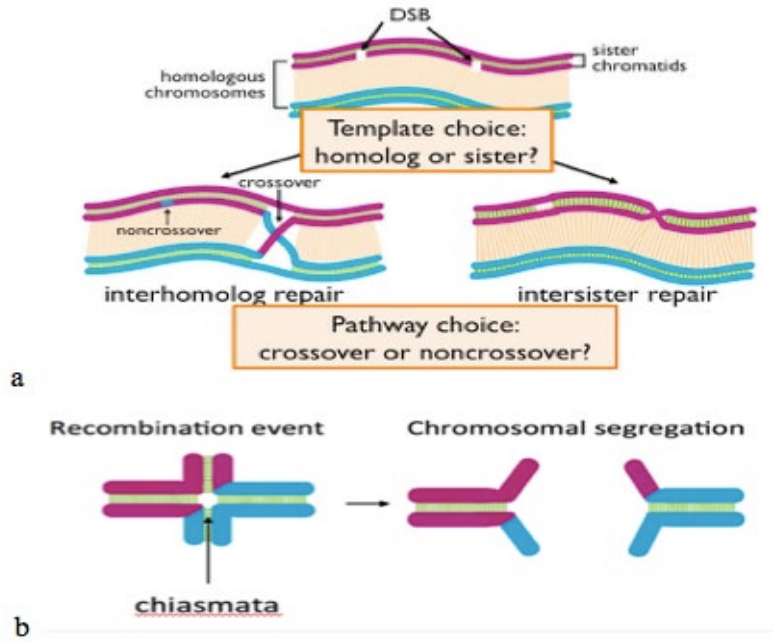


Figure 3. Double-strand DNA break repair during meiosis

**a.** Double-strand DNA break repair pathways. Once a break is made, it can repair using the homologous chromosome or sister chromatid and by a crossover or noncrossover. **b.** Interhomolog crossovers form a physical connection between chromosomes known as a chiasma which allows for proper chromosomal segregation.

*Consequences of Improper Repair*

Failure to establish a CO leads to improper chromosomal segregation in meiosis (Moore *et. al.* 1997). Aneuploidy, or the inheritance of an incorrect number of chromosomes, is the leading cause of miscarriages and birth defects. For example, the most common form of viable aneuploidy is trisomy of chromosome 21, which is known as Down Syndrome (Witters *et. al.* 2011). Improper DSB repair can additionally impact normal cellular processes, potentially even resulting in cancer. Given the health consequences of improper DSB repair, it is important to understand the underlying

mechanisms of meiotic DNA repair that are necessary for faithful genomic inheritance and healthy reproduction.

### *Caenorhabditis elegans*: a model organism

*Caenorhabditis elegans*, a species of roundworm, is the model organism used in the Libuda Lab to study meiotic processes. *C. elegans* shares 60-80% of the same genes with humans, including many implicated in human disease (Corsi). Most importantly for this study, core meiotic components are highly conserved between the two species, making *C. elegans* an excellent model organism in which to study meiotic DSB repair. *C. elegans* has two sexes: males and hermaphrodites, of which the latter self-fertilize in the absence of a male. Both forms of the worms contain five autosomal chromosomes, but hermaphrodites have two sex chromosomes (XX) while the males only inherit one (X0). A number of features make *C. elegans* an advantageous model in which to study DNA repair during meiosis (Garcia-Muse and Boulton 2007). *C. elegans* are very small, measuring approximately 1mm in length and reproduce quickly, making them easy to culture in a laboratory setting (Corsi). Second, these worms have a number of well-classified, fully penetrative, and distinctive morphological phenotypes (Figure 4a). The worms are also amenable to genetic manipulation, so while a massive variety of genetic strains are already available to researchers, new ones are also relatively easily created. Especially important for this study, the *C. elegans* gonad is laid out in a spatial-temporal gradient (Figure 4b), acting as a pipeline for meiosis. As the developing nuclei progress through the phases of meiosis, they are physically moving through the gonad. The gonadal organization of the worm permits the use of reverse time course analysis. Based on the time an egg is laid after a discrete condition was imposed on the parent worm,

the specific meiotic phase at which that developing nuclei was at during the treatment can be determined (Rosu *et. al.* 2011). Thus the first progeny laid will have been from late meiosis while the last cohort of progeny would have been in the earliest phases of meiosis.

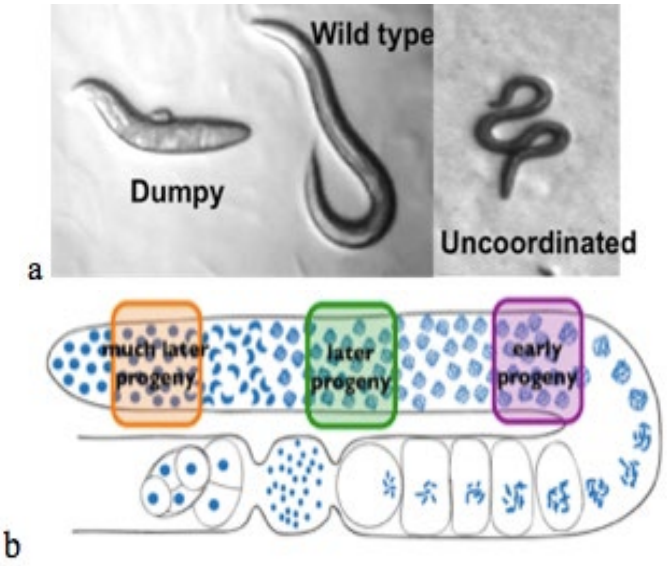


Figure 4. Features of *C. elegans*

**a.** Examples of common phenotypes of worms. Wild type worms move in a sinusoidal manor; dumpy (dpy) are shorter and fatter; uncoordinated (unc) worms exhibit abnormal movement.
**b.** Cartoon image of *C. elegans* gonad displaying the pipeline of meiosis and illustrating the ability for reverse time course analysis.

**The Interhomolog (IH) Assay**

In prior work, Dr. Libuda and colleagues wanted to investigate the mechanisms that govern repair pathways choice and specifically, to assess the repair outcomes between homologous chromosomes of DSBs induced at a particular chromosomal site (Rosu *et. al.* 2011). Analysis of interhomolog recombination events in *C. elegans* was previously limited to sequencing-based approaches with poor temporal resolution. Exploiting the biology of *C. elegans*, Dr. Libuda and colleagues designed a genetic

assay which allowed them to easily visualize IH repair outcomes (IH Assay). With the IH assay, frequency of interhomolog repair and CO:NCO pathway utilization can be directly assessed at all stages of meiotic prophase via assessment of phenotypic markers (Figure 5). The IH assay exploits two genes endogenously linked on chromosome IV: *dpy-13* and *unc-5.* The worms utilized in the assay were heterozygous for both the *dpy-13* and *unc-5* genes. One chromosome carries a mutant allele of *dpy-13* along with a copy of *unc-5* disrupted by a Mos1 transposon. The other chromosome carries a functional *dpy-13* gene and a mutated copy of *unc-5* (Fig 5). The worms also carry an exogenous heat-shock promoter-driven Mos1 transposase (*hsp::transposase*). A single DSB per developing nucleus is induced upon heat shock, and the haplotype of the resultant progeny is determined by the interhomolog repair pathway engaged to repair the DSB.

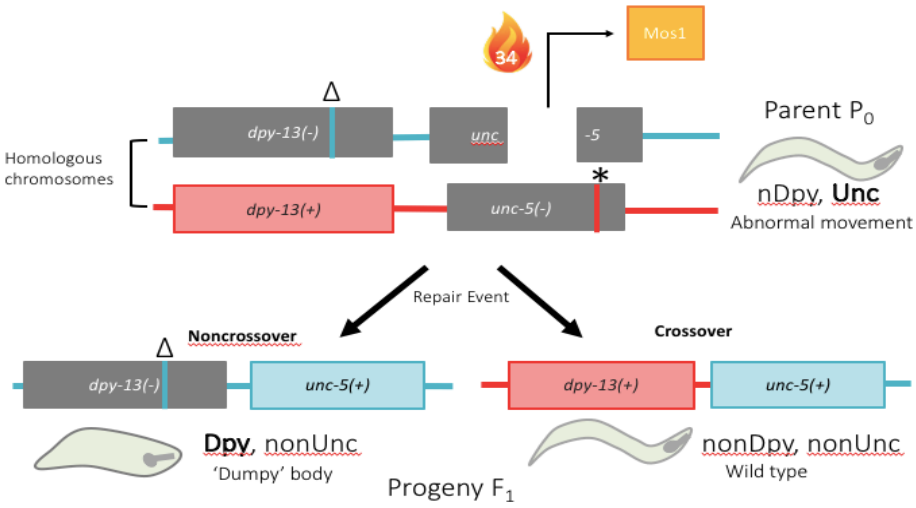


Figure 5. The *unc-5* Interhomolog Assay

A genetic assay developed to examine the repair outcomes of interhomolog repair. *Mos1* excision occurs upon heat shock, creating a single DSB in a desired location. A simple screen for phenotypes of progeny allow for the determination of crossover:noncrossover repair events.

The progeny exhibit a visually discernable phenotype that is dependent upon the pathway engaged during DSB repair. This phenomenon permits the screening for worm genotypes based on their phenotype. Thus, in this particular experiment, a dumpy (dpy), wild type moving worm arose from an oocyte in which the induced DSB was repaired via the NCO pathway, resulting in a chromatid maintaining the mutated *dpy-13* but acquiring a functional copy *unc-5.* Alternatively, CO repair with the homologous chromosome would produce a chromatid carrying functional alleles of both the *dpy-13* and *unc-5* genes, resulting in a phenotypically wild type progeny. Failure to excised *Mos1* or putative engagement of the sister chromatid as a repair template will result in progeny worms with an uncoordinated (Unc) phenotype, which can thus be easily discarded. Since parent worms are heterozygous for the *Mos1* construct, it only appears on one of the homologous chromosomes and repair only occurs on that chromosome.

Resulting progeny are diploid, inheriting one repaired chromosome from the egg and one chromosome from the sperm, which never formed a break. Due to diploid progeny, the two distinct morphologies arise from four possible genotypes. Thus, in an initial screen of F1 recombinant progeny, it is possible for the nDpy nUnc worms to carry either a CO or NCO recombinant chromosome. By observing the segregation of patterns of the $F_2$ generation, we can observe distinctive inheritance patterns and distinguish which recombination event took place (Table 1).

| Repair Outcome (NCO vs. CO) | Genotype (F1) | Phenotype (F1) | Diagnostic segregation pattern of progeny (F2) |
|---|---|---|---|
| NCO | $\frac{dpy-13(e184)unc-5(+)}{dpy-13(e184)unc-5(ox171::Mos1)}$ IV | **Dpy**, nUnc | 75% **Dpy** nUnc<br>25% **Dpy Unc** |
| NCO | $\frac{dpy-13(e184)unc-5(+)}{dpy-13(+)unc-5(e791)}$ IV | nDpy, nUnc | 25% **Dpy** nUnc<br>50% nDpy nUnc<br>25% nDpy **Unc** |
| CO | $\frac{dpy-13(+)unc-5(+)}{dpy-13(e184)unc-5(ox171::Mos1)}$ IV | nDpy, nUnc | 75% nDpy nUnc<br>25% **Dpy Unc** |
| CO | $\frac{dpy-13(+)unc-5(+)}{dpy-13(+)unc-5(e791)}$ IV | nDpy, nUnc | 75% nDpy nUnc<br>25% nDpy **Unc** |

Table 1. *unc-5* assay recombinants.

Listed are the four possible recombination outcomes for progeny of heat-shocked worms. Deviations from wildtype worm morphology and movement and noted in bold.

The results from Rosu et al. demonstrated that IH repair is restricted to a specific window of meiosis beginning in early pachytene and closing by late pachytene. CO:NCO ratios remain relatively consistent across throughout this IH repair window, at around 11%, suggesting that crossover designation is not restricted to a specific portion of the IH window (Rosu *et. al.* 2011). Additional replicate experiments done in the Libuda Lab have verified these results that IH repair shuts down during late pachytene

(Figure 6), corroborating research suggesting that DSB repair requirements switch during late prophase I (Hayashi *et. al.* 2017).

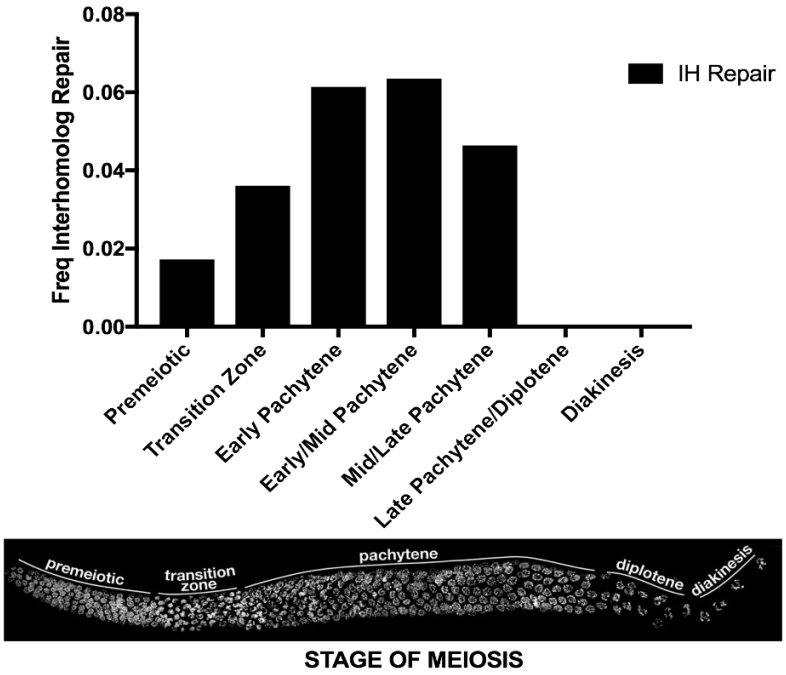


Figure 6 Data from *unc-5* IH assay.

Data from the *unc-5* IH assay experiments represented graphically with a worm gonad pictured below (nuclei of corresponding sub-steps of prophase I stained in white). Results indicate that IH repair is regulated to a specific range of meiosis during pachytene.

## Genome Editing by CRISPR/Cas9

The genome editing tool called CRISPR/Cas9 is composed of a protein-ribonucleic acid complex derived from the bacterial adaptive immune system (Ran *et. al.* 2013) which can be programmed to target specified genome locations. This technology is utilized to integrate the Mos1 construct into the worm genome. This targeted genome editing technology utilizes a specific and customizable guide RNA sequence that directs the Cas9 endonuclease to a designated location within the genome

that is adjacent to a Protospacer Adjacent Motif, or PAM, sequence, which allows the protein to latch onto the DNA. Once localized to the target site, Cas9 catalyzes the cleavage of the phosphodiester bonds within the DNA backbone, creating a DSB. By also introducing a designed repair sequence, or donor DNA, the DSB can be repaired via homologous recombination with that specific template, allowing for integration of exogenous sequences into the genome of the worm (Figure 7) (Waaijers *et. al.* 2013). In the Libuda lab, the CRISPR/Cas-9 system is now used to generate desired transgenic worms, like those used in the IH assay experiments.

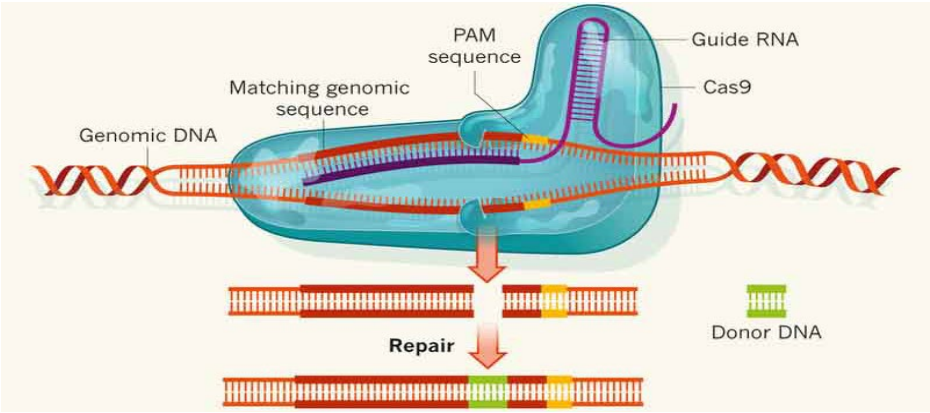


Figure 7. CRISPR/Cas-9 genome editing tool

A guide RNA directs the Cas9 protein to a specific target sequence within the genome and cuts the DNA. The cut site can repair using a new sequence of donor DNA.

**Mos1 Transposon**

In this experiment, the donor DNA for the CRISPR/Cas-9 system contains the *Mos1* transposon. Transposons are pieces of DNA that are able to move from one location of the genome to another due to the excision of the sequence by proteins known as transposases. *Mos1* is a member of the mariner family of transposons, a subset of mobile DNA sequences within the *Drosophila* genome (WormBase). Because *Mos1* is not endogenous to nematodes, the activity of its transposase does not target any

nematode transposons (Boulin *et. al.* 2007). Thus, integration of the *Mos1* element into the worm genome via the CRISPR/Cas-9 system allows for the creation of a single DSB at a chosen location within the genome independent of SPO-11 upon expression of its transposase (Bessereau *et. al.* 2001). My described experiments also utilized the heat-shock inducible promoter to drive expression of the *Mos1* transposase, allowing controlled expression and subsequent *Mos1* transposon excision.

**Factors Influencing Heterogeneous DSB Repair Outcomes**

Facilitating DSB initiation and repair requires the coordination of many different proteins and chromosome structure-related alterations. Many of the mechanisms that govern such repair events, including where DSBs are formed and how they are repaired, remain unknown. A conserved feature of meiotic DSBs is that their distribution is heterogeneous across the genome. In many species, this heterogeneity results in the creation of recombination "hot spots", frequently located within gene promoters (Boulton and Youds 2011). However, in *C. elegans* the uneven distribution of IH COs manifests as a pattern of low-recombination in the central region of the chromosome and high-recombination in the arms of chromosomes (Kaur and Rockman 2014). Research suggests that this division in CO abundance is a result of the unusual chromosome domain structure of *C. elegans* chromosomes. Unlike many species, *C. elegans* lacks centromeres and displays holocentric segregation. Unlike other species in which chromosome arms are distinguished by placement of the centromere, in *C. elegans* chromosome arms are instead defined by the aforementioned change in recombination rate (Kaur and Rockman 2014). However, it is uncertain whether the increased CO frequency in the arms is due to an increased generation of DSBs within

these domains, or if genomic positioning influences the nonrandom DSB repair pattern. Yu et. al. determined DSB location by RAD-51chromatin immunoprecipitation and high-throughput sequencing (ChIP-seq). DSB-bound RAD-51 protein is crosslinked to DNA and the chromatin is isolated from the nucleus and sheared via sonication. RAD-51 antibodies allow for immunoprecipitation of RAD-51 linked DNA. Finally, DNA is then unlinked, purified, and sequenced (Raha *et. al.* 2010). The meiotic DSB map created by RAD-51 ChIP-seq data demonstrated that RAD-51 was enriched in the arm domains of chromosomes, mirroring the pattern that COs produce (Yu *et. al.* 2016). Such data indicates that the CO distribution may be a result of heterogeneity in DSB formation. Alternatively, another study suggests that CO enrichment in the arms is due to preferential bias towards the CO repair pathway, independent of DSB distribution. This study was performed in mutant background incapable of CO formation, thereby potentially biasing results (Yu *et. al.* 2016). Though it is uncertain the exact mechanism behind observed heterogeneity in CO distribution, it is clear that recombination decisions are nonrandom and highly regulated. Due to regulation by CO assurance and interference previous studies have also shown that a single DSB is sufficient to ensure CO formation, and therefore for proper chromosome segregation (Rosu *et. al.* 2011).

To attempt to discern the mechanisms behind the heterogeneous spread of COs, many studies have focused on the role of DSB repair proteins in faciliating pathway choice. Research has implicated that the SC plays a dynamic role in DSB repair, specifically that it acts to regulate crossover control, both promoting and limiting CO formation (Libuda *et. al.* 2013). Mutations in either the lateral or central elements of the SC leads to failure to form a chiasmata (Engebrecht *et. al.* 2007). Furthermore, meiotic

DNA forms chromatin loops perpendicular to the lateral elements of the SC. DNA in the far ends of the loops may beless constrained, and therefore differentially processed, than the sequences bound by the SC (Garcia-Muse and Boulton 2007). It has been proposed that maintaining homologs in close proximity may facilitate COs, or conversely that the SC may present a physical barrier for repair proteins' access to certain breaks. Because research has demonstrated that the SC is a dynamic structure which changes with meiotic progession, it is conceivable that the altered structure allows the SC to perform both roles (Pattabiranam *et. al.* 2017). Likewise, another study found that temporal regulation of break processing occurs in meiotic prophase through another mechanism, MAP kinase phosphorylation. A study by Hayashi et. al. found a switch in DSB repair from a reliance on RAD-50 for RAD-51 loading to a RAD-50-independent repair mechanism, which coincided with a failure to repair via IH CO pathway (Hayashi *et. al.* 2007). Thus, recombination events vary temporally due to the dynamic nature of early meiosis. The switch in repair pathway also corresponds with a major remodeling of chromosome architecture, which has also been implicated as an influential factor of DSB repair in a number of other studies.

In *C. elegans,* recombination rate increases in areas of heterochromatin, a more closed structure of chromatin, in constrast to many other species. Furthermore, chromatin remodelers have been shown to play a role in directing DSB repair across species. A study performed by Mancera et. al. in yeast indicated that local chromosome factors largely influence repair outcomes of meiotic DSBs. They found that not only were DSBs distributed nonrandomly, but also that areas of the genome showed differences in CO:NCO ratios indicating that repair choice is also a controlled process

(Mancera *et. al.* 2008). The distinct bias for repair pathway implicates a role for local chromosomal context to influence DSB resolution.

Together, all these studies indicate the essential role of genomic context as a major overarching factor in directing meiotic DSB induction and repair choices. The heterogeneous pattern of distribution is present for both DSBs and COs. The ability of SPO-11 to access DNA is a major regulator of DSB formation, maintaining proper numbers, timing, and distributions of cuts (Yu *et. al.* 2016). However, the factors influencing CO landscape are not well understood, particularly whether their distribution is the direct cause of DSB distribution or whether the CO bias is imposed at a later step. To help distinguish the underlying causes of CO distribution, my work has focused on investigating how genomic positioning affects meiotic DSB repair outcomes.

## Experimental Question

The nature of this research project is hypothesis driven. Based on previous data, it has been hypothesized that DSBs are differentially repaired due to their different chromosomal contexts, which includes factors such as global location, relative distance to the SC and potentially chromosome architecture. I aimed to set up the preliminary experiments for assessing how genomic positioning affects DSB repair choice through the integration of the IH assay into four different genomic locations. The goal of this research is to determine the pattern of recombination outcomes, specifically frequency of IH repair, as well as the ratio of CO:NCO pathway choice, across the different points within the worm genome. Collectively, the data will provide insight into how DSB repair events differ by genomic location, allowing for a deeper analysis of the mechanisms underlying the variation in CO distribution observed across the genome.

### How Does DSB Repair Change in Response to Differing Genomic Location?

The original IH assay experiment was performed at the *unc-5* locus, which is located in the left arm of chromosome IV, a region of increased IH CO frequency, and at midrange distance (10kb) from the SC. However, the various positional and physical contexts of loci, which differ widely across the genome, may influence DSB repair outcomes. My research project adapts the original IH assay to function at four new locations within the *C. elegans* genome: *dpy-9, dpy-6, unc*-22 and *unc-1* (Table 2). Mutation of these four targets via insertion of *Mos1* will result in a distinct recessive mutant phenotype, enabling detection of IH repair outcomes by phenotypic changes in recombinant progeny similar to the original experiment. The four target loci lie on two

different chromosomes, IV and X, which allows us to determine how repair outcomes vary depending on the type of chromosome (autosomal or sex). Research has demonstrated considerable differences between autosomal and sex chromosomes, which are transcriptionally silenced during late meiosis and also display a more even distribution of recombination events (Garcia-Muse and Boulton 2007). Additionally, the target loci vary in their placement along the length of the chromosome, therefore both the chromosomal context and distance from the SC differs for each of the locations. Through the experimental assay, which permits us to control DSB formation for a given location, we can further investigate the role that genomic positioning, or context, plays in governing recombination events. By circumventing SPO-11-dependent DSB induction we can isolate the role that chromosome context plays in deciding repair choice and parse out the relationship between DSB induction-driven and genomic context-driven CO recombination landscapes.

| **Gene** | **Location along length, chromosome** | **Distance from synaptonemal complex** |
|---|---|---|
| *unc-5* | Middle, IV | 10 kb |
| *dpy-9* | End, IV | <2 kb |
| *unc-22* | Middle, IV | 25 kb |
| *dpy-6* | Middle, X | 6 kb |
| *unc-1* | End, X | <1 kb |

Table 2. Genomic locations examined in this study.

The interhomolog assay is being integrated into four genomic positions, *unc-5* was the location of the original assay. These locations differ both length along a chromosome as well as distance from the SC.

I successfully constructed guide sequence and repair template plasmids containing the genetic assays targeted to the four unique locations required to address my research question. Each location required the creation of a unique repair template and guide-RNA expressing plasmid. Utilizing the CRISPR/Cas9 genome editing system, one assay was successfully integrated into the *dpy-9* locus. Construction of worm strains necessary for completion of the experiment are in progress. Once strain constructions are complete, an IH assay at the *dpy-9* locus will be performed and the repair outcomes at this locus can be assessed.

Based on previous research and the current understanding of genomic positioning on DSB repair choices, the *dpy-9* locus is predicted to demonstrate a different pattern of CO:NCO than did the original *unc-5* locus. This is because *dpy-9* is located towards the end of chromosome IV, nearing the telomere region, which tend to be biased toward NCO repair (Yu *et. al.* 2016). However, based on ChIP-seq data, *dpy-9* is also closely associated with the SC, which also may affect CO:NCO repair patterns, either complementing putative inhibitory effects of its telomere-proximal chromosome position or potentially offsetting it, depending on the role of the SC in facilitating repair.

# Research Methods

## Plasmid Construction

The Cas9 guide RNA plasmid was constructed by replacing a portion of pJW1285, a plasmid gRNA(F+E) targeting pha-1, with the target-specific sequence via PCR. Note all primer sequences used for PCR amplification are listed in Table 3. The PCR product was then circularized into a plasmid via High Efficiency Transformation Protocol C2987H in competent α-5 *E. coli* cells (New England BioLabs (NEB) ). The *E. coli* was cultured overnight at 37°C, and the plasmid was subsequently extracted following QIAquick PCR Purification Kit protocols (Qiagen). To create the repair template plasmid, primers were designed for amplification of the *Mos1* gene from the DLB31 bacterial stock, which contains an ampicillin resistance vector, along with designed primers for the homology arm sections for each of the four target genes (Table 3). Each location requires two homology arms, specific to the target locus, flanking the *Mos1* segment in order properly anneal to the Cas9 induced break site. All four DNA segments per location were amplified by PCR and stitched together via Gibson Assembly Protocol E5510 (NEB). Each resulting repair segment was circularized into plasmids using competent α-5 *E. coli* cells (NEB) and the plasmid was then extracted via mini-prep technique (Qiagen). Gel electrophoresis was run at each stage to confirm expected product sizes. Final plasmids were sequenced to confirm successful creation of desired product. Samples were sent to Sequetech for sequence analysis. Samples contained 0.6 μL sequencing primer (Tables 4 &3), 1-2 μL plasmid, and deionized water for total sample volume of 10 μL.

| DLO # | Sequence (5' → 3') | Annealing Temperature (°C) | Use | Target Locus |
|---|---|---|---|---|
| 411 | ATCGGATCCCGGGCCCGT | 71.3 | Ampicillin backbone | all |
| 412 | ATCTAGATGCATTCGCGAGGTACCG | 71.3 | Ampicillin backbone | all |
| 428 | TGTGACATGCGTTGGTTCCTGGGTTTAAGAGCTATGCTGGAAACAG | 68.2 | sgRNA | *dpy-9* |
| 429 | AACCCAGGAACCAACGCATGTCACAAGACATCTCGCAATAGG | 68.2 | sgRNA | *dpy-9* |
| 421 | catgcgttggTACCAGGTGTACAAGTAGGG | 56.5 | *Mos1* | *dpy-9* |
| 422 | gaaagtatctTCAGGTGTACAAGTATGAAATG | 56.5 | *Mos1* | *dpy-9* |
| 412 | cctcgcgaatgcatctagatCTTTTCCACGATCGCCGG | 65.4 | Homology arm 1 | *dpy-9* |
| 413 | acacctggtaCCAACGCATGTCAGAAATGTC | 65.4 | Homology arm 1 | *dpy-9* |
| 414 | gtacacctgaAGATACTTTCCAGCTATACCATTG | 60.3 | Homology arm 2 | *dpy-9* |
| 415 | cgacgggcccgggatccgatCAGGAATAAGCCTAGATATTAAGAC | 60.3 | Homology arm 2 | *dpy-9* |
| 540 | TGAGTCACAAATGCAGCGTCGGGTTTAAGAGCTATGCTGGAAACAG | 68.1 | sgRNA | *dpy-6* |
| 541 | AACAGTCACAAATGCAGCGTCGGCAAGACATCTCGCAATAGG | 68.2 | sgRNA | *dpy-6* |
| 527 | agtcacaaatTACCAGGTGTACAAGTAGGG | 56.5 | *Mos1* | *dpy-6* |
| 528 | gattttcagaTCAGGTGTACAAGTATGAAATG | 56.5 | *Mos1* | *dpy-6* |
| 525 | cctcgcgaatgcatctagatTATCATTTCATTTCGAGCTTTTTC | 57.9 | Homology arm 1 | *dpy-6* |
| 526 | acacctggtaATTTGTGACTGCAACTGAAG | 57.9 | Homology arm 1 | *dpy-6* |
| 529 | gtacacctgaTCTGAAAATCAAAGATTAAATTAAATAATTTAG | 62.5 | Homology arm 2 | *dpy-6* |
| 530 | cgacgggcccgggatccgatTTCAGGCTGATAAATATTACCG | 62.5 | Homology arm 2 | *dpy-6* |
| 537 | TGTTATGCCAAGTAACAACTGGGTTTAAGAGCTATGCTGGAAACAG | 68.2 | sgRNA | *unc-22* |
| 538 | AACTTATGCCAAGTAACAACTGGCAAGACATCTCGCAATAGG | 68.2 | sgRNA | *unc-22* |
| 521 | ttatgccaagTACCAGGTGTACAAGTAGGG | 56.4 | *Mos1* | *unc-22* |
| 522 | gcagagttatTCAGGTGTACAAGTATGAAATG | 56.4 | *Mos1* | *dpy-9* |

| | | | | |
|---|---|---|---|---|
| 519 | cctcgcgaatgcatctagatTCTTCAACTCTTGGTGGAC | 57.6 | Homology arm 1 | *unc-22* |
| 520 | acacctggtaCTTGGCATAAGGATGATAGAG | 57.6 | Homology arm 1 | *unc-22* |
| 523 | gtacacctgaATAACTCTGCAATAGAAATTAGCAG | 58.7 | Homology arm 2 | *unc-22* |
| 524 | cgacgggcccgggatccgatCTCAAATTCCAAGCAGCAG | 58.7 | Homology arm 2 | *unc-22* |
| 543 | TGAGTTTGCGCTAGCAGTTTGGGTTTAAGAGCTATGCTGGAAACAG | 68.2 | sgRNA | *unc-1* |
| 544 | AACAGTTTGCGCTAGCAGTTTGGCAAGACATCTCGCAATAGG | 68.2 | sgRNA | *unc-1* |
| 533 | agtttgcgctTACCAGGTGTACAAGTAGGG | 56.5 | *Mos1* | *unc-1* |
| 534 | tgttgacgatTCAGGTGTACAAGTATGAAATG | 56.5 | *Mos1* | *unc-1* |
| 531 | cctcgcgaatgcatctagatGTTTGTTTGGGTTACGAAATTG | 61.8 | Homology arm 1 | *unc-1* |
| 532 | acacctggtaAGCGCAAACTACGCTGAG | 61.8 | Homology arm 1 | *unc-1* |
| 534 | gtacacctgaATCGTCAACATTGTTGACGGAAG | 69.9 | Homology arm 2 | *unc-1* |
| 536 | cgacgggcccgggatccgatGGAGCTCGTGGTCCTGGAATG | 69.9 | Homology arm 2 | *unc-1* |

Table 3. Primers used to construct CRISPR/Cas9 plasmids.

Listed is name, the sequence, annealing temperatures, use, and target locus for each of the primers used.

| DLO # | Sequence (5' → 3') | Annealing Temperature (°C) | Confirmation of PCR | Confirmation of sequence | Target Locus |
|---|---|---|---|---|---|
| 229 | ctctgacacatgcagctcccgg | 62.3 | Guide sequence insertion | sgRNA plasmid | all |
| 430 | TGACATGCGTTGGTTCCTGG | 68.1 | Guide sequence insertion | N/A | *dpy-9* |
| 542 | AGTCACAAATGCAGCGTCGG | 58.4 | Guide sequence insertion | N/A | *dpy-6* |
| 539 | TTATGCCAAGTAACAACTGG | 50.4 | Guide sequence insertion | N/A | *unc-22* |
| 545 | AGTTTGCGCTAGCAGTTTGG | 56.2 | Guide sequence insertion | N/A | *unc-1* |

Table 4. Additional primers for confirming PCR insertion & final plasmid sequences

Listed is name, sequence, annealing temperature, use, and locus for each of the primers used. Note, additional sequencing primers included those listed in Table 3 above.

**Microinjections**

The EN909 worm strain, which carries the *hsp::transposase* transgene necessary for *Mos1* excision, was used in all experiments. L4 progeny were picked the day before. The following day the young adult hermaphrodites were laid on an agar pad in a droplet of halocarbon oil. A needle was used to microinject a mixture containing the CRISPR/Cas-9 plasmids (repair template and guide RNA) and a co-conversion plasmid, to help identify injection success, directly into the gonad of the worm. The worms were pipetted onto an agar plate to recover before being transferred onto individual plates and allowed to reproduce. Progeny of injected worms were screened for phenotypes indicating integration of the Mos1 construct into the genome.

**Worm strains**

Worm strains were maintained primarily at 20°C with occasional periods at 15°C, which only slows maturation and does not impair worm development. All strains and corresponding genotypes are listed in Table 7 under Supplementary information. All crosses consisted of 3 males (♂) x 1 hermaphrodite (☿).

*Strain Constructions: IH dpy-9 Assay*

Strains for the IH Assay at the dpy-9 locus were created via multiple crosses. EN909 ♂ x DLW28 ☿ allowed regaining of the *hsp::transposase* needed to excise *Mos1*. Progeny ☿ worms were then backcrossed twelve times to CB4856 ♂ to generate worms with the integrated *Mos1* gene in the Hawaiian background to produce the parent ☿ for the assay. Parent males for the IH assay at *dpy-9* were produced by crossing

FX30203 ♂ with MT628 ♀ to produce males containing the mutated *dpy-9* and *unc-17* mutant alleles. Mating MT628 ♂ x HA-DLW28 ♀ produced the worms that were then heat-shocked for the assay.

*SNP Analysis to verify repair outcome in the* dpy-9 *IH assay*

After twelve backcrosses of DLW28 worms with the CB4856 strain, dumpy progeny were lysed following established protocols in order to obtain genomic DNA (WormBook). PCRs were completed to amplify extracted DNA regions corresponding to each of the SNPs, primers used for PCR are indicated in Table 5. Samples were then subject to restriction enzyme digests with the appropriate RE and conditions depending on the SNP (Supplementary Info Table 8). Digests were then run via electrophoresis on a 1% agarose gels to visual bands of DNA fragments and confirm expected digests sizes. Controls using N2 and CB4846 DNA were tested first to confirm that restriction enzyme digests could reliably differentiate between SNPs of the two wildtype worm strains. Centimorgan distances of SNPs relative to the *dpy-9* gene locus were estimated based their locations along Chromosome IV (WormBase).

| DLO # | Sequence (5' → 3') | Annealing Temperature (°C) | Target SNP |
|---|---|---|---|
| 762 | CCAAGCTGAAAACGGATATAAC | 51.7 | 1 |
| 763 | ATTGGGTTCACTGAGAGCTTC | 51.7 | 1 |
| 764 | GTCATTGTCAGCAGGTTTCCC | 55 | 2 |
| 765 | TTCAAAGTCTTGATGGCTTCTG | 55 | 2 |
| 707 | CACAGTGTTCCCAGGTTGTAG | 50 | 3 |
| 708 | CCAAAATTCAAGTTGATGGC | 50 | 3 |
| 709 | TCCGCTCTGGTTGACATTAC | 59 | 4 |

| | | | |
|---|---|---|---|
| 710 | AAATCTACATGGACGCGC | 59 | 4 |
| 711 | ACTTTCAGCTGCTCGTACTCTC | 55 | 5 |
| 712 | TCTGCCTTTTCACTTGCC | 55 | 5 |
| 713 | AGAAGAAGCTATGGGAGTGCC | 55 | 6 |
| 714 | TCCCTAAGCCAAGTGGTTTC | 55 | 6 |

Table 5. Primers used for SNP analysis

Listed is the name, the sequence, annealing temperatures, use, and target locus for each of the primers used to amplify each target SNP.

**Interhomolog Assays**

After respective crosses were complete, 40-50 hermaphrodites containing the proper assay genotype were picked as L4s 18 hours before intended time of heat shock and allowed to incubate overnight at 15°C until reaching the young adult stage. Those worms were then heat shocked at 34°C for 1 hour in an incubator and then moved to 20°C for the rest of the experiment. Heat shocked worms transferred to individual plates four hours post-heat shock. Worms were then transferred to fresh plates ten hours after shock, after which worms were transferred another five times at 12 hour intervals. Plates were screened for phenotypes of patterns of $F_1$ progeny once progeny reached L4 stage, and CO:NCO ratios were determined based on their phenotypes. Wild-type moving worms were then self-ed and the $F_2$ segregation pattern observed to verify which type of repair event occurred in the $F_1$ parent.

# Results

## Interhomolog assay *dpy-9* Assay

Following successful integration of the Mos1 construct targeting the *dpy-9* locus, nearby genes were examined to determine which could serve as a linked marker to help assess recombination events. The *unc-17* gene was selected as it was the nearest gene in proximity to *dpy-9* that would produce a distinctive phenotype of abnormal movement distinguishable in Dpy or nDpy individuals.

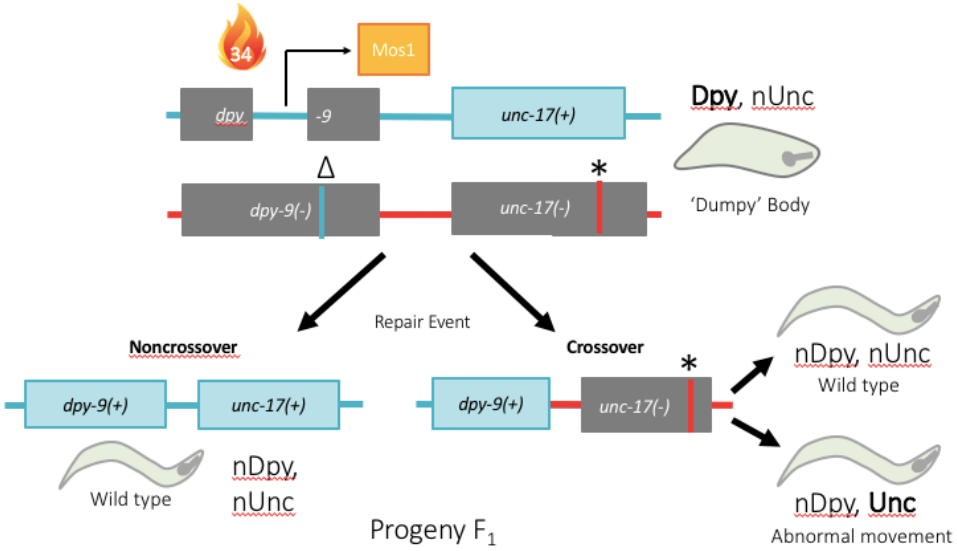


Figure 8. Schematic for the IH *dpy-9* assay

The original IH assay adapted for the *dpy-9* locus. *Mos1* excision occurs upon heat shock, creating a single DSB in a desired location. A simple screen for phenotypes of progeny allow for the determination of CO:NCO repair events.

In addition to the two new gene loci employed in this version of the assay, the phenotypic readout for the corresponding recombination events also differ compared to the original assay. However, similar to the original *unc-5* IH assay, in this experiment some of the CO repair outcomes will be phenotypically indistinguishable from NCO

recombinants in the F1 generation. In following, the subsequent segregation patterns of the $F_2$ progeny must be observed in order to assess which pathway was used to repair the DSB left by *Mos1* excision.

| Repair Type (NCO vs. CO) | Genotype (F1) | Phenotype (F1) | Diagnostic segregation pattern of progeny (F2) |
|---|---|---|---|
| $NCO_1$ | $\frac{dpy-9(+)unc-17(+)}{dpy-9(ox171::Mos1)unc-17(+)}$ IV | **Dpy**, nUnc | 75% nDpy nUnc<br>25% **Dpy** nUnc |
| $NCO_2$ | $\frac{dpy-9(+)unc-17(+)}{dpy-9(e12)unc-17(e245)}$ IV | nDpy, nUnc | 75% nDpy nUnc<br>25% **Dpy Unc** |
| $CO_1$ | $\frac{dpy-9(+)unc-17(e245)}{dpy-9(ox171::Mos1)unc-17(+)}$ IV | nDpy, nUnc | 25% nDpy **Unc**<br>50% nDpy nUnc<br>25% **Dpy** nUnc |
| $CO_2$ | $\frac{dpy-9(+)unc-17(e245)}{dpy-9(ox171::Mos1)unc-17(e245)}$ IV | nDpy, **Unc** | 75% nDpy **Unc**<br>25% nDpy **Unc** |

Table 6. IH *dpy-9* assay recombinants.

Listed are the four possible recombination outcomes for progeny of heat-shocked worms. Deviations from wildtype worm morphology and movement are noted in bold.

The new *dpy-9* IH assay has an added complication in that the two marker genes, *dpy-9* and *unc-17,* are farther apart on the genetic map than *dpy-13* and *unc-5* were in the original assay. In the *dpy-9* IH assay, the two loci are spaced 26.00 centimorgans (cM) apart, while genes in the original assay were only 1.9 cM in distance (Rosu *et. al.* 2011). This poses an issue because relative distances along a chromosome are measures of recombination frequencies: a 1 cM length indicates a 1% frequency of recombination between loci. This means that there is a 26% chance of a recombination event occurring between the two gene loci of interest to the *dpy-9* IH assay independent of the programmed DSB created by excision of *Mos1*. If a crossover formed between *dpy-9* and *unc-17* in a recombinant worm that has also undergone an IH NCO at *dpy-9*,

it would produce a false IH CO phenotype, thereby confounding the screening of recombinant progeny.

To circumvent the obstacle of potential recombination events independent of the Mos1 DSB, we exploited existing *C. elegans* background strains with a high density of well-mapped single nucleotide polymorphism (SNPs). Specific SNPs alter the recognition sequence of a restriction enzyme at that locus, referred to as 'snip-SNPs'. The Bristol N2 and Hawaiian worm strains, both of which are wildtype, differ genetically only due to SNPs. Thus, the DLW28 strain, which contains the transgenic *Mos1* insertion in the N2 background, was crossed with HA worms so that the *dpy-9* IH assay was introgressed into the HA background. Six specific 'snip-SNPs' spanning the region between the two loci were selected (Figure 9 and Table 5).

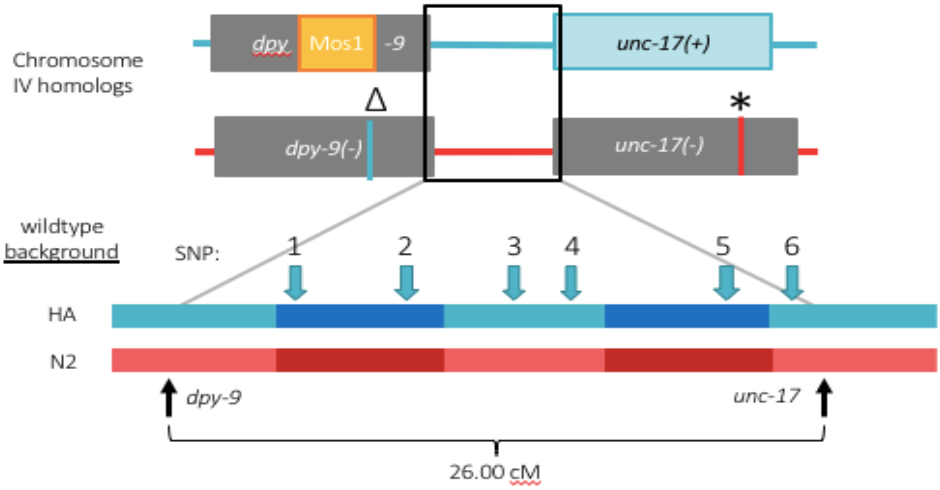


Figure 9. Locations of target genes and SNPs along homologous Chromosome IVs

The *dpy-9* and *unc-17* genes are mapped to a distance of 26.00 cM, corresponding to a 26% chance of a recombination event between the two loci. Relative positions of the six chosen SNPs in the first five segments of Chromosome IV are illustrated above.

Restriction enzymes digest PCR-amplified segments of the worm genome into fragments which can be visualized on a gel. Each SNP will be digested either in N2 or

the HA sequence, but not both. Because the parent worms will be heterozygous, with one N2 chromosome and one HA, we can determine where recombination events occurred based on how each SNP digests in recombinant progeny. For the example given above, we could distinguish if the CO occurred at the *dpy-9* site, each SNP would digest according to the N2 background, or if the CO happened somewhere in between, SNPs before the CO would digest as HA. So, based on the results of the restriction enzyme digest analysis of each of the six SNPs for the progeny on heat-shocked worms, we can determine approximately where the recombination events occurred: whether it was at the assayed DSB site, between the two genes, or potentially both if two recombination events occurred.

# Discussion

## Relevance of future findings

A crucial first step to understanding the forces shaping the meiotic recombination landscape is identifying sources of variation in DSB repair outcomes at multiple locations within the genome. To address this question, I successfully created the repair plasmids for each of the four target loci and have integrated the assay into the *dpy-9* locus. Moving forward, the assays I constructed will be incorporated into each of the other three genomic locations. Once all four assays are complete and their respective patterns of CO:NCO repair are calculated, composite data will reveal how DSB repair choice varies among the four chosen positions. Potentially, relative to the original IH assay at *unc*-5, we could observe a similar rate of COs at both *unc-22* and *unc-1*, which are within chromosome arms regions, and a decreased CO frequency at *dpy-9* and *dpy*-6, both of which lie in outside the CO-enriched areas of the chromosome. These results would indicate that global genomic positioning is the primary determinant in the regulation of CO distribution. Alternately, the IH assay at *dpy-9* could reveal an increased frequency of COs relative to *unc*-5 which, along with a relative decrease in COs at *unc*-22, would indicate that DSB proximity to the SC plays a major role in directing repair outcomes. As the SC is required for CO formation, it is intuitive that the SC might facilitate CO formation preferentially at loci that are closest to the protein structure. A third outcome could be that CO frequency is maintained throughout the four locations, but the total number of interhomolog recombination events varies, which

would indicate that chromosome context influences recombination template choice to the homologous chromosome or sister chromatid.

It is possible that no pattern may arise in the frequency of interhomolog repair or in CO:NCO ratios between loci when comparing outcomes to global genomic position or proximity to the SC. It is possible that additional factors, such as differences in chromosome architecture and chromatin structure, instead are promoting specific recombination outcomes. By comparing the data we collect with ChIP-seq data from the *C. elegans* modENCODE project, we can correlate recombination trends with specific features such as histone modifications, proximity to SC proteins, and other elements of chromosome architecture to assess for correlations that might underly any observed variation between loci.

The reagents I have built will directly contribute to experiments that will improve our understanding of the underlying factors shaping the recombination landscape. If specific candidate repair proteins or genomic elements are correlated with specific repair outcomes, the IH assays I built will be performed in mutant worm strains deficient in the candidate element. Using mutants deficient for proposed repair component will enable us to directly test the role those candidates play in governing DSB repair. For instance, my data may produce results demonstrating that CO frequency is actually increased at *dpy-9*, despite its proximity to the end of the chromosome which is a low frequency area, while COs are also increased at *unc-22*. By examining data from the modENCODE project, we may find that the *dpy-9* locus is enriched for a histone modification associated with repression of gene transcription. We can then perform the IH assay at *dpy-9* in a mutant worm deficient in the enzyme

responsible for catalyzing that histone modification. If the CO frequency is shown to decrease in that mutant, it would support a role for the histone mark in promoting CO recombination.

**Conclusion**

Though discerning the mechanisms which regulate meiotic DSB repair has been the focus of a considerable amount of studies, much is remains unknown as to exact causes that shape DSB distribution and recombination pathway choice. Given the current understanding of interhomolog repair, genomic context appears to play a complex though integral role in determining DSB repair outcomes. This research provides the foundation for continued exploration of the effects of genomic location on meiotic DSB repair. Upon completion of the experiments, we will have directly assessed how DSB repair decisions vary genome wide as well as the role of chromosome context in governing DSB repair.

Meiotic DSBs are crucial to proper genomic inheritance and yet, malfunctions in the repair process result in serious health consequences for potential offspring. Gaining a more comprehensive understanding of the basic mechanisms that dictate meiotic DSB repair is fundamental to our ability to diagnose and potentially remedy meiotic errors.

# Supplementary Information

| Strain Name | Genotype | Phenotype |
|---|---|---|
| N2 | Bristol wildtype | WT |
| EN909 | ***KrIs14****[(hsp)::transposase; lin-15B; cc::GFP]V* | coelomocyte GFP$^{+}$ |
| AV554 | $\frac{\boldsymbol{dpy-13}(e184sd)\boldsymbol{unc-5}(ox171::Mos1)}{nT1(qIs51)}IV;$ $\frac{\boldsymbol{KrIs14}[(hsp)::transposase;\ lin-15B;\ cc::GFP]}{nT1(qIs51)}V;$ | Wild type, pharynx/coelomocyte GFP$^{+}$ |
| CB791 | ***unc-5****(e791) IV* | unc |
| DLW28 | ***dpy-9****[Mos1]IV;* ***kris14****(hsp::transposase)III* | dpy |
| CB4865 | Hawaiian wildtype | WT |
| FX302030 | *tmC25* [*unc-5*(tmIs1241, *myo-2p::Venus*)] IV. | pharynx::venus$^{+}$ |
| MT628 | ***dpy-9****(e12)/****unc-17****(e245)IV* | dpy, unc |

Table 7. Worm strains used in this study.

Strains are identified in the above table with specific information regarding their genotypes and phenotypes. Strains are provided by the Caenorhabditis Genetics Center.

| SNP # | Chromosome Segment | RE Used | Strain digested | Approximate distance from *dpy-9* locus (cM) |
|---|---|---|---|---|
| 1 | 2 | DraI | N2 | 3 |
| 2 | 2 | HpaII | N2 | 8 |
| 3 | 3 | ApoI | N2 | 17 |
| 4 | 3 | DraI | HA | 19 |
| 5 | 4 | DraI | N2 | 23 |
| 6 | 5 | AflIII | HA | 25 |

Table 8. List of specific SNPs utilized in the *dpy-9* IH assay.

All SNPs are located on Chromosome IV. SNPs were selected in order to span the region between *dpy-9* and *unc-17* genes so that CO location could be determined.

## Glossary

Double-strand break (DSB): damage resulting from cleavage of the phosphate backbone in the both strands of a double-strand DNA molecule.

Coelomocyte: a phagocytic leukocyte appearing in the coelom of an animal.

Chiasma(ta): A specialized chromatin structure which physically links paired chromosomes.

CRISPR/Cas-9: A genome-editing tool derived from prokaryotic immune systems.

Chromosome: A threadlike structure of genes comprised of a single DNA molecule and associated proteins.

Crossover: Homologous recombination outcome resulting in the reciprocal exchange of genetic material between DNA molecules.

Green Fluorescent Protein (GFP): a protein that exhibits green fluorescence used as a marker for expression.

Genotype: Set of genes that comprise the genome of an organism.

Gonad: The organ that produces gametes, either sperm or eggs.

Homologous chromosome: Pairs of chromosome coding the same genes but with different alleles. One set is from the sperm and one the egg.

Locus/loci: The specific position of a gene one a chromosome.

Mos-1: A transposon derived from drosophila capable of excision by heat shock.

PCR: A genetic tool to amplify gene segments.

Pharynx: a part of an epithelial tube which makes up a portion of the *C. elegans* digestive tract.

Phenotype: Physical characteristics of an organism influenced by its genotype and environment.

Plasmid: A small piece of circular DNA, separate from chromosomal DNA.

Restriction Enzyme (RE): A RE cleaves DNA molecules at particular sequences.

Sister chromatid: Identical copies of a replicated chromosomes.

Synaptonemal Complex: The protein structure which connects homologous chromosomes during meiosis I.

Transposon: Small DNA segments capable of moving locations in the genome.

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