Elucidating mechanisms directing meiotic DNA repair decisions

Erik Toraason, Marissa Glover, Cordell Clark, and Diana Libuda

Department of Biology Institute of Molecular Biology; Center for Genome Function

UNIVERSITY OF OREGON

Background

Our genomes are composed of DNA, the chemical sequence that codes for all of the genes required to generate a functioning organism. Most diploid organisms, including humans, carry two copies of the genome: one they inherited from their father, and one from their mother. To sexually reproduce, each organism must accurately generate a sex cell (gamete) with only one copy of each chromosome (Fig 1a). Failure to accurately segregate chromosomes can have severe consequences, including infertility.

During meiosis, the specialized form of cell division that produces gametes, double strand DNA breaks (DSBs) are intentionally induced across the genome. DSBs are substrates for the formation of crossovers with the homologous chromosome, forging a physical connection between chromosomes necessary for proper chromosome segregation (Fig 1b). DSBs are formed in excess to ensure that each pair of chromosome can form a crossover. However, the remaining endogenous DSBs, as well as exogenous damage incurred, must be repaired to ensure faithful inheritance of the genome.

Meiotic DNA repair utilizing the homologous chromosome as a template has been extensively studied. Access to the homolog is restricted to a specific window of meiotic prophase I (Fig 2b). Multiple lines of evidence have suggested that outside of this window developing germ cells employ 'mitotic-like' repair utilizing the sister chromatid. To directly assess meiotic sister chromatid recombination, the Libuda lab has developed a novel genetic assay that exploits the controlled excision of a transposable element to measure DNA repair with the sister chromatid (Fig 4). By using this assay in combination with an existing genetic assay measuring interhomolog repair, I am identifying the contributions of diverse elements to DSB repair decisions.

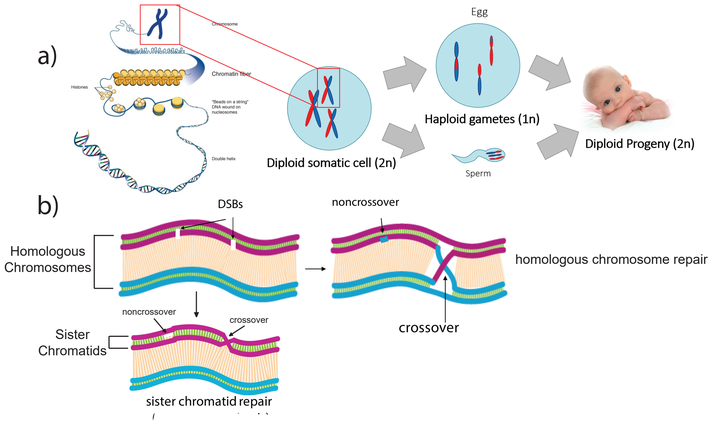


Fig 1. Meiosis generates haploid gametes. a) To generate diploid progeny, parents must accurately segregate their chromosomes. b) DSBs may be repaired as a crossover or noncrossover utilizing either the sister chromatid or homologous chromosome.

Question

How are DSB repair template and pathway decisions made in meiosis to ensure faithful inheritance of the genome?

Hypothesis

Chromosomal structural elements regulate engagement of specific meiotic recombination partners and pathways.

Funding

University of Oregon NIH Departmental Genetics Training Grant

National Institute of General Medical Sciences

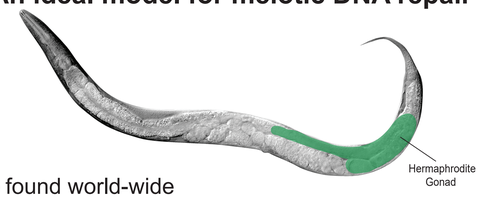
Eunice Kennedy Shriver National Institute of Child Health and Human Development

National Institute of General Medical Sciences

SEARLE SCHOLARS PROGRAM FUNDING EXCEPTIONAL YOUNG SCIENTISTS

ARCS ADVANCING SCIENCE IN AMERICA

Caenorhabditis elegans: An ideal model for meiotic DNA repair



- Soil nematode found world-wide
- 1mm in length and can be easiliy cultured in the laboratory
- Has two sexes: hermaphrodites and males
- ~70% of hermaphrodite body is dedicated to sperm and egg production
- Powerful genetic tools and resources

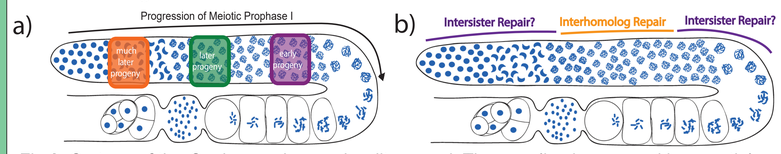


Fig 2. Cartoon of the *C. elegans* hermaphrodite gonad. The germline is arranged in a spatial temporal gradient. Each dot represents a single developing egg (oocyte). a) As oocytes move through the gonad, they also progressively move through meiosis. Resultant progeny are laid sequentially, allowing for retroactive correllation of the approximate position and meiotic stage of a nucleus at the time of a discrete treatment event. b) Repair utilizing the homologous chromosome is limited to a specific window of meiotic prophase I.

Interhomolog Repair Assay

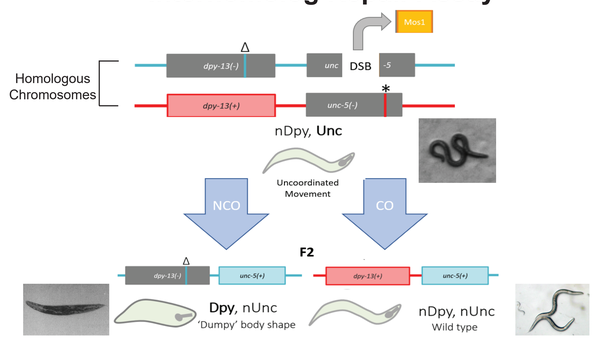


Fig 3. Interhomolog Assay. Upon induced excision of the Mos1 transposon, repair with the homologous chromosome will result in progeny of differentiable phenotypes. Noncrossover (NCO) and Crossover (CO) repair outcomes are distinguished by unique phenotypes.

Sister Chromatid Recombination Assay

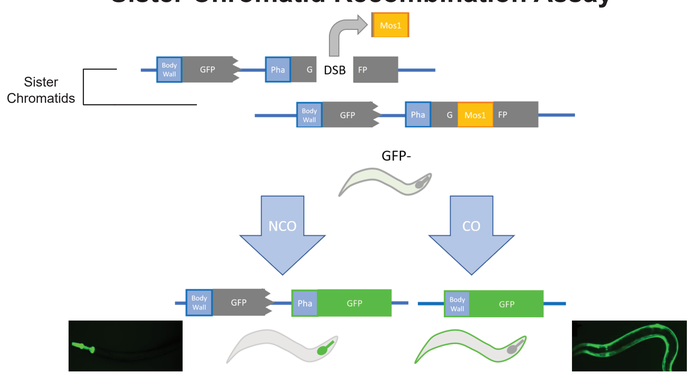


Fig 4. Sister Chromatid Recombinaton Assay. Upon induced excision of the Mos1 transposon, repair with the sister chromatid will result in progeny expressing a fluorescent transgene. Noncrossover (NCO) and Crossover (CO) repair outcomes are distinguished by unique fluorescent phenotypes. Fluorescent images adapted from Calarco *et al Bio-Protocols* 2018.

The Homologous Chromosome and Sister Chromatid are Differentially Utilized in Meiotic Prophase I

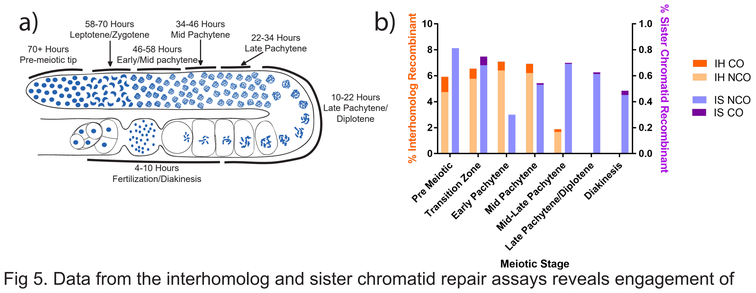


Fig 5. Data from the interhomolog and sister chromatid repair assays reveals engagement of repair templates throughout meiotic prophase. a) Isolating progeny at 12 hour timepoints after DSB induction allows for differentiation of different stages of meiotic prophase I. b) Together, my assays demonstrate that interhomolog and intersister repair are differentially utilized in meiotic prophase I. Access to the homolog for recombination is limited to a window of meiotic prophase I. In late meiotic prophase I, the sister chromatid is the only available repair template. Interhomolog assay data from Rosu *et al Science* 2011.

Structural Maintenance of Chromosomes (SMC-5/6) Complex

The SMC-5/6 complex is dispensible for the formation of interhomolog crossovers, but is necessary to complete meiotic DSB repair. This phentoype suggests that SMC-5/6 plays a role in directing intersister repair. Our preliminary data suggests that SMC-5/6 is necessary for efficient intersister recombination in late meiotic prophase I (Fig 6a). To dissect the mechanism by which intersister is recombination is promoted by SMC-5/6, I will next assess the putative SUMO ligase NSE-2 component of the SMC-5/6 complex (Fig 6b). SUMOylation is necessary to maintain meiotic proteostasis and form crossovers. My preliminary data from our lab demonstrates that *smo-1* SUMO deficient mutants also fail to repair DSBs in meiosis (Fig 6c).

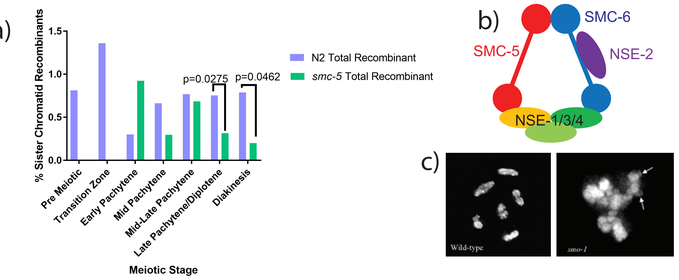


Fig 6. SMC-5/6 complex promotes intersister recombination. a) Intersister repair is reduced in a smc-5 mutant background. b) Cartoon of SMC-5/6 complex components. c) *smo-1* mutants exhibit chromosome fragmentation at diakinesis, indicative of failure to repair DSBs. Arrows indicate chromosome fragments. Wild-type image adapted from Zalevsky et al *Genetics* 1999.

Conclusions

- The sister chromatid is available as a repair template throughout meiotic prophase I
- The sister chromatid is the exclusive recombination partner in late meiotic prophase I
- The SMC-5/6 complex is required to promote efficient intersister recombination in late meiotic prophase I, and appears required for the formation of crossovers with the sister chromatid