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Title of Research *	Age-dependent meiotic chromosome fragmentation in <i>C. elegans</i>
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Introduction & Purpose *

Meiosis is a fundamental process of life in all sexually reproductive organisms. It is the specialized cell division that produces haploid (1n) gametes, such as sperm and eggs, from a diploid cell (2n). It is achieved by a round of DNA duplication, followed by a reductional division that divides the chromosome number in half, and an equational division where sister chromatids are pulled apart towards opposite poles. Errors that occur during the two divisions often lead to nondisjunction of chromosomes or chromosome abnormalities (Hassold T, Hunt P, 2001). In humans, these errors increase exponentially as women age, which can lead to infertility, miscarriages and children with developmental disabilities (Hunt P, Hassold T, 2008). Despite the importance of understanding these age-dependent processes, it is not clear what mechanisms are controlling them. Our lab is investigating the mechanism of chromosome segregation using genetic, cytological, and biochemical techniques.

The soil nematode *Caenorhabditis elegans* is a valuable model organism to use for meiosis studies. They are easy to manipulate and maintain. The large number of nuclei in the gonad in combination with various stains and markers such as DAPI, FISH, and tagged antibodies makes it practical to observe details of chromosome behavior and structure in meiosis (Garcia-Muse T, Boulton S, 2007). Also, meiotic prophase I nuclei are arranged in a temporal-spatial pattern that allows for specific stages of meiosis to be observed in dissected gonads.

We are currently investigating a mutant, known as 123, in the well-established model organism *Caenorhabditis elegans*. This strain was isolated in a chemical mutagenic screen, using UV/TMP, for mutants affecting chromosome behavior in meiotic prophase I. Trimethylpsoralen (TMP) is a chemical mutagen that is induced with UV light to crosslink to DNA and creates small deletions in the genome. Preliminary analysis of mutant 123 includes; abnormal SC assembly/disassembly, enlarged nuclei throughout the gonad, gonadal degradation, and chromosome fragmentation. All of these phenotypes progressively increase in severity as the nematodes age. These observations suggest that the mutation is affecting a very important gene needed for the successful completion of meiosis. Elucidating the pathway of this mutant has a strong possibility of revealing novel proteins that may be involved in the etiology of aneuploidy that increases with age in women.

Experimental Design *

The severity of the chromosome fragmentation of the 123 mutant increases with age. It is not clear, however, if this increased fragmentation is correlated with other problems associated with meiosis as the worm ages such as SC (synaptonemal complex) assembly and/or disassembly. Gonads of day 1, day 3, and day 5 123 homozygotes and wild-type worms were analyzed and compared using immunocytochemistry and high-resolution microscopy. To properly stain the gonads for visualization, the gonads were dissected out with razor blades and attracted to a charged superfrost

slide. Slides were bathed in cold methanol permeabilizing the outer membrane, which is crucial for penetration of tagged antibodies into the gonad. Gonads were incubated in a humid chamber in a solution of formaldehyde that fixed the gonad structure and minimized obstruction of the organ. The fixed gonads were washed in a PBST solution and stained with the immunofluorescent SYP-1 antibody (acquired from goat) and DAPI. DAPI allows chromosome structure to be visualized and SYP-1 is a component of the central region of the SC. Preserved gonads were sealed with a coverslip and the dynamics of the chromosomes and the SC throughout prophase I of meiosis were analyzed with immunohistochemistry cytology using the Deltavision, a high-resolution deconvolution microscope. Photographs of SYP-1 staining in 1 day, 3 day and 5 day 123 were analyzed and compared with the wild-type control.

Chromosome fragmentation phenotype can often be seen in mutants defective in double strand break repair of meiosis leading us to believe the 123 gene might play a role in DSB (double strand break) repair. Spo-11 is a gene necessary for induction of DSBS, and it is also located on chromosome IV. We created a double mutant, 123;spo-11, to test if DSBS were necessary for the fragmentation of 123 to occur. If 123 gene product is involved in double strand break repair then we would expect to see no fragmentation in a 123;spo-11 double mutant. We analyzed 1 day and 3 day old double mutants and compared them to spo-11, wild type, and 123 controls. We fixed the double mutants on a microscope slide and stained the gonads with DAPI. We looked for chromosome fragmentation in the -1 oocyte of diakinesis I of meiotic prophase I.

The nuclei in the gonad of the 123 mutant seemed to be irregular and enlarged compared to wild type (both 1 day and 3 day). This could indicate problems in mitosis that occurs in the premeiotic tip of the worm gonad that eventually leads to entry into meiosis- specifically replication not followed by proper division. To quantify this observation we stained the gonads with DAPI and calculated the area of each nuclei in both wild type and 123 gonads.

Young (day 1) 123 mutants do not display chromosome fragmentation in diakinesis I of meiosis; however, the nuclei in pachytene are very irregular probably indicating problems derived in the mitotic cell cycle prior to entry into meiosis. We hypothesize that day 1 123 mutants will show increased apoptosis of the nuclei indicating that the mutant is utilizing its apoptotic machinery to try and correct the defective nuclei. To test this we will stain live worms with acridine orange and visualize them under the Delta Vision. Acridine orange fluoresces orange in the acidic environment of an apoptotic cell.

Results *

123 did show abnormalities in SC formation compared to the wild type control. The syp-1 should be visualized as a continuous ribbon localized between the interface of paired chromosomes. Both 1 day and 3 day 123 showed irregular syp-1 staining that formed aggregates and did not localize correctly.

The 3 day double mutant displayed 60% chromosome fragmentation compared to 100% fragmentation in 3 day 123 mutants. This indicated that the fragmentation phenotype characteristic of the 123 mutant is partially dependent on meiotic double strand breaks. However, it might also be part of a pathway upstream of spo-11 double strand break induction.

Day 1 and 3 mutant worms showed a significant variation in the size of their meiotic nuclei.

Day 1 123 mutants showed a significant increase in apoptotic nuclei compared to wild type worms.

Conclusions *

The 123 mutant has great potential to aid in the discovery of the etiology of age dependent meiotic problems seen in human females. We have found out possible roles of the product encoded by the 123 gene such as participation in double strand break repair and the mitotic cell cycle preceding induction into meiosis. We also discovered potential mechanisms that may contribute to the age related problems such as involvement in apoptosis and synaptonemal

complex assembly and disassembly. Future plans include cloning the 123 mutant gene utilizing whole genome sequencing methods and discovering its interaction partners using yeast two hybrid techniques.

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