Crossover Regulation During *Caenorhabditis elegans* Meiosis

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Meiosis is essential for the generation of genetic diversity. All sexually-reproducing eukaryotes undergo this specialized cell division, consisting of one round of DNA replication followed by two rounds of chromosome segregation. Successful segregation requires crossover recombination, which is initiated by a programmed double strand break (DSB) that causes the reciprocal exchange of genetic information between homologous chromosomes. Crossovers (COs) provide physical links between homologs, but they also facilitate evolution by culling deleterious mutations and creating novel allelic combinations.

Due to their importance, COs are subject to strict regulation that guarantees at least one CO per homolog pair and ensures wide spacing of multiple COs. Additionally, COs preferentially occur on genomic intervals called "hotspots." These flank haplotype blocks, allelic combinations that tend to be inherited together and are evolutionarily more stable.[1] Hotspots determine the evolutionary genomic landscape, but efforts to predict their location have only been partially successful.[2] CO hotspots are also hotspots for DSBs, though not all DSBs become COs.[3] Therefore, CO regulation affects DSB distribution and DSB resolution into COs or noncrossovers (NCOs).[4,5] The nematode *C. elegans* provides an elegant system to study this control, for it exhibits complete CO interference: each homolog pair only has one CO per meiosis.[6]

Chromosomes are structured by a number of protein complexes, one being the highly-conserved condensin complex. *C. elegans* has three condensins involved in dosage compensation, chromosome compaction, and CO control.[7] Disruption of the meiotically-active condensin I complex causes chromosomal axis extension, which alters DSB distribution and thus CO distribution.[5] Previously, the condensin II complex was thought to act only in mitosis – but work in the Meyer lab has shown that it is also involved in meiosis, downstream of CO regulation.[8] Preliminary data from the lab implicates at least one condensin II subunit earlier in meiosis that affects CO distribution in a way that differs from condensin I.

Though CO control is widespread, its precise mechanism remains a mystery. I propose to use *C. elegans* as a model in which to deepen our understanding of CO regulation by examining how CO distribution is affected by both meiotic condensin complexes.

Hypothesis: Condensin II regulates crossover frequency at the level of DSB initiation by lengthening chromosome axes, which changes the binding of DNA to each axis. Mutations in condensin I or II will cause a change in CO frequency manifested by altered hotspot distribution.

Aim 1. Do changes in chromosome structure affect CO number by altering DSBs?

To determine when CO regulation occurs, I will identify the relationship between DSB formation, DSB resolution, and changes in chromosomal structure as revealed by a lengthened axis. For each experiment proposed below, I will test five condensin II subunit mutants, which we have in lab. Previously-characterized condensin I mutants will serve as a positive control and wild type animals as a negative control. I will also generate animals with mutations in both condensin I and II to uncover interactions between the two complexes. a. Measuring CO frequency. I will score six X chromosome SNPs (single nucleotide polymorphisms) in recombinant individuals generated from crosses between two divergent laboratory strains. In males, CO frequency and distribution can be ascertained along single X chromatids. Preliminary data leads me to expect increased CO frequencies in condensin II mutants, implying that condensin II limits CO formation, but a decreased CO frequency would indicate that condensin II acts to trigger CO formation. b. Measuring DSB frequency. To demonstrate that increased CO frequency is due to increased DSB frequency, I will label DSB position throughout meiosis by immunostaining with RAD-51 antibody, which marks recombination intermediates. Correlation of elevated DSB numbers with higher CO frequencies in condensin II mutants would indicate that additional DSBs provide further substrate for COs, while a lower DSB frequency would

implicate involvement at the level of DSB resolution. c. Measuring chromosomal axis length. To measure axis length of X chromosomes, I will use fluorescent *in situ* hybridization to sequences containing the SNPs from Aim 1a. After immunostaining for DSBs and an antibody to the chromosomal axis protein HTP-3, I can trace labeled X chromosome axes through deconvolved 3D image stacks. Computationally straightening these traces with software present in the lab will allow me to measure axis length within microns and analyze DSB foci on individual X chromatids. Unlike previous lower-resolution studies, this will identify whether sub-chromosomal axis expansions correlate with increased DSB frequency in condensin mutants, demonstrating that changes in chromosome structure affect CO number by creating more DSBs. However, any change in DSB frequencies on altered axis intervals would further bolster a relationship between chromosome structure, DSB initiation, and CO resolution.

Aim 2. How do condensins I and II exert effects on chromosome structure?

If the condensin complexes affect higher-order chromosome structure by modifying axis length (which I will have determined in Aim 1c), they must also change where DNA attaches to the chromosome axis. To examine whether condensin mutants have these structural changes, I will use ChIP-seq (chromatin immunoprecipation sequencing) to detect the binding of REC-8, a meiosis-specific cohesin that marks DNA-axis attachment, and the axis protein HTP-3. ChIP will isolate specific DNA sequences of protein binding to be identified by high-throughput Solexa sequencing. UC Berkeley has two Solexa sequencers readily available to graduate students in my department. I will analyze REC-8 and HTP-3 binding in condensin I mutants, condensin II mutants, and the double mutant, choosing the subunit mutant conditions that show the strongest CO effect from Aim 1a; wild type animals will serve as a control. Antibodies to both proteins suitable to ChIP have been generated in the lab. If condensin mutants exhibit no change in REC-8 or HTP-3 binding, CO regulation may change axis length without affecting DNA-axis attachment. However, differential DNA-axis binding and altered axis lengths in condensin mutants will reveal a direct association between chromosome structure and CO regulation.

Aim 3. What are genome-wide trends of CO in *C. elegans*?

To determine the relationship between DSBs and their resolution into COs or NCOs, I will use microarrays to generate recombination maps for wild type and animals mutant for condensin I, II, and both, again choosing mutant conditions with the strongest CO effect. Previous recombination studies have lacked the resolution to detect NCO formation. To address this, I will use hundreds of SNPs on each chromosome that cause differential hybridization between two divergent laboratory strains, choosing markers that are reproducibly observed on high-throughput tiling arrays.[9] Several studies in *S. cerevisiae* have utilized similar technology,[10,11] but few other metazoans will prove as tractable to a genome-wide analysis as *C. elegans*, due to its small genome, numerous SNPs, and clonal individuals. I will define CO hotspots, and therefore haplotype blocks, using the wild type recombination map. I will also uncover, for the first time, whether NCOs have an effect on overall CO regulation in *C. elegans*. Additionally, if hotspot architecture changes in condensin mutants, I will have identified a chromosome-wide mode of CO regulation consistent with global control of hotspot activity.

This project is fundamentally interesting because it will elucidate a conserved and universal phenomenon for generating diversity, but it will also have a significant impact on our understanding of a basic evolutionary mechanism. Condensins have the ability to exert global effects on chromosome architecture – permitting chromosome-wide communication that could explain the appearance and disappearance of CO hotspots within short spans of time. Determining the mechanism responsible for CO regulation and identifying CO hotspots will be crucial to our understanding of genome organization and evolution.

All proposed research is original and of my own design.

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